UNIVERSITY OF SOUTH BOHEMIA
IN CESKE BUDEJOVICE
FACULTY OF AGRICULTURE

DISSERTATION THESIS

RESEARCH ON THE AGRONOMIC AND QUALITY CHARACTERISTICS OF MODERN WHEAT CULTIVARS IN ORGANIC FARMING

2021 **KHOA DANG TRAN**

UNIVERSITY OF SOUTH BOHEMIA
IN CESKE BUDEJOVICE
FACULTY OF AGRICULTURE

Department of Agroecosystems

Study Program: P4102 / Phytotechnology

Field of Study: 4102V008 / Special Plant Production

Head of Department: Petr Konvalina, Assoc. Prof., Ph. D.

DISSERTATION THESIS

RESEARCH ON THE AGRONOMIC AND QUALITY CHARACTERISTICS OF MODERN WHEAT CULTIVARS IN ORGANIC FARMING

Supervisor: Petr Konvalina, Assoc. Prof., Ph. D.

Author of Dissertation: Khoa Dang Tran

Ceske Budejovice, 2021

I declare that in accordance with § 47b of Act No. 111/1998 Coll. as amended, I agree to publish my dissertation with full form by the Faculty of Agriculture - electronically in a publicly accessible part of the STAG database operated by the University of South Bohemia in Ceske Budejovice, while my copyright of the submitted text of this qualification thesis is maintained. I further agree that similar electronic means must be in accordance with the above provision of Act No. 111/1998 Coll. published opinions of the supervisor and opponents of the thesis as well as a record of the course and result of the defense of the qualification thesis. I also agree with the comparison of the text of my thesis with the database of qualification theses Theses.cz operated by the National Register of University Qualification Theses and the plagiarism detection system.

In Ceske Budejovice, April 21st, 2021.

....

Khoa Dang Tran

Acknowledgement

I would first like to express my great appreciation to my supervisor Associate Professor Petr Konvalina, who always has assisted me with the valuable guidance throughout the research process and the writing of this dissertation.

I would like to thank Dr. Karel Suchy, and Dr. Marek Kopecky for never-ending support both the material and spiritual I was given. I would also like to thank Josef Safar and Martina Zemanova, who supported me collect data on the field trials.

Last but not least, this dissertation is dedicated to beloved people such as my mother, my wife, two daughters Bao Ha and Bao Anh, and my big family.

Abstrakt

Pšenice setá je jednou z hlavních tržních plodin ekologického zemědělství. Přetrvávajícím problémem je nedostatek informací o reakci odrůd na pěstitelský systém ekologického zemědělství. Z tohoto důvodu je potřebné uvést na trh odrůdy pšenice, které byly buď přímo šlechtěny pro systém ekologického zemědělství, nebo se jedná o odrůdy šlechtěné v konvenčních šlechtitelských postupech, ale pro ekologické zemědělce jsou k dispozici informace o agronomicky významných znacích a jakosti zrna. Dostatek informací o dostupných odrůdách tak dále přispěje k většímu zájmu o pěstování tržních plodin na orné půdě v ekologickém zemědělství.

Cílem disertační práce bylo posoudit a analyzovat agronomicky významné znaky a kvalitativní parametry zrna devíti odrůd pšenice seté, ozimé formy, pěstované v systému ekologického zemědělství. Přesné maloparcelkové pokusy byly založeny na dvou lokalitách, ve třech ročnících v Českých Budějovicích a ve Zvíkově. Během vegetačního období byly hodnoceny vybrané agronomicky významné znaky. Vybrané jakostní parametry zrna byly analyzovány v laboratoři podle standardních metodik.

Ze získaných výsledků byl patrný vliv lokality a průběhu ročníku na devět hodnocených odrůd pšenice seté, ozimé formy. Rozdíly v agronomicky významných parametrech a hodnocených znacích jakosti byly statisticky průkazné. Na základě analýzy hlavních komponent (PCA) dle těsnosti jejich pozic ke kvantitativním a kvalitativním parametrům v analýze hlavních složek, jako je výnos, obsah bílkovin, SDS test, číslo poklesu, gluten index, obsah mokrého lepku a reologické vlastnosti, zejména pak stabilita těsta, čas C2 doba vývoje těsta, torzní síla C2, torzní síla C3, torzní síla C4 a torzní síla C5, byly identifikovány odrůdy s vysokým výnosem nebo odrůdy s vysokou pekařskou jakostí, dále pak odrůdy s vysokým výnosem a současně odpovídající jakostí. Vyhodnoceny byly také korelační vztahy mezi jednotlivými znaky.

Klíčová slova: ekologické zemědělství, pšenice ozimá, agronomické významné znaky, pekařská jakost zrna, reologické vlastnosti, Mixolab.

Abstract

The wheat cultivar demand for organic farming has been increasing rapidly in the recent year, which requires breeders applying new selection methodologies to release new varieties suitable for organic agriculture. This has been fostering not only the expansion of wheat production organic farming areas, but also the rise market demand, growing interest in low-input agriculture for ecological reasons. In this dissertation, the main goal study was to assess and analyze the agronomic and quality characteristics of nine bread wheat cultivars cultivated in organic farming system in the south region of Czech Republic. Field experiments were set up in two locations namely Ceske Budejovice and Zvikov in three years to measure all agronomic traits, whilst the quality parameters were analyzed in the laboratory according to the standard methods. From the collected data, results revealed the effects of location, year, and the weather condition on nine varieties studied as well as the statistically significant differences of agronomic traits and quality parameters among nine varieties. Based on the proximity of their positions to the parameter quantitative and quality in the principal component analysis, such as yield, protein content, SDS test, the falling number, gluten index, wet gluten and the rheological properties namely the stability of dough, time C2, the dough development time, torque C2, torque C3, torque C4, and torque C5, either the cultivars having high yield or the good baking performance cultivars, or the cultivar having both high yield and good quality were identified, and depicted the correlation among them.

Key words: winter wheat, agronomic traits, quality traits, rheological properties, Mixolab, organic farming.

Contents

List of tables

List of figures

Figure 8: The line graph of four cultivars throughout Mixolab II. Red line: Wiwa; Green line: Bernstein: Yellow line: Sultan; Blue line: Gordian; Light red: Block temperature; Purple line: Dough temperature

Figure 9: The projection of study variables based on correlations

Figure 10: Principal component analysis biplot based upon various quantitative and quality traits of nine wheat cultivars using mean value of two locations in three years

List of Brands and Abbreviations

AACC	American Association of Cereal Chemists
ANOVA	Analysis of Variance
BP	Before Present
CB	Ceske Budejovice
cm	centimeter
DP	Degree of Polymerization
DDT	Dough Development Time
EU	European Union
FN	Falling Number
FAO	Food and Agriculture Organization
FiBL	Forschungsinstitut für biologischen Landbau - Research Institute of Organic Agriculture
GS	Glutenins
GI	Gluten Index
ha	hectare
HI	Harvest Index
HMW	High Molecular Weight
HSD	Honest Significant Difference
HC	Hydrothermal Coefficient
IFOAM	International Federation of Organic Agriculture Movements
ICC	International Association for Cereal Science and Technology
kDa	Kilodaltons
LMG	Low Molecular Weight
min	minute
MW	Molecular Weights
Nm	Newton meter
N	Nitrogen
No.	number
OA	Organic Agriculture
PC	Permaculture
PG	Permanent Grassland

PCA	Principal Component Analysis
PC	Protein Content
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
TKW	Thousand Kernel Weight
$ton.ha^{-1}$	ton per hectare
VCU	Value for Cultivation and Use
wt	weight
WG	Wet Gluten
yr	year
ZV	Zvikov

1 Introduction

Wheat is plant grown on more land area than any other commercial crop. It is also one of three main cereal crops grown all over the world, together with rice and maize. *Triticum aestivum* L. belonging to the grass family *Poaceae*, domesticated over 9,000 years ago, is a cultigen. Wheat is often considered primarily as a source of energy (carbohydrate) and it is certainly important in this respect. However, it also contains significant amount of other important nutrients including proteins, fiber, and minor components including lipids, vitamins, minerals, and phytochemicals which may contribute to a healthy diet (Mpofu *et al.*, 2006). Wheat kernel is composed of endosperm (81 - 84 %), bran (14 - 16 %), and germ (2 - 3 %) (Pomeranz, 1988). Endosperm is the inner part playing a role as storage of energy and functioning protein. Bran is outer layer protecting the grain and germ is the kernel's reproduction system. Whereas wheat endosperm contains mostly starch and protein, bran and germ are rich in dietary fiber, vitamins, minerals and phytochemicals playing an important role in nutrition and health benefits for humans (Pomeranz, 1988). The customers are, therefore, strongly recommended to consume whole-grain foods with at least three servings per day. The recent studies have showed that regular consumption whole wheat has been found to be associated with reduced total mortality, as well as reduced risk of coronary heart disease, ischemic stroke, type two diabetes (Mpofu *et al.*, 2006), hypertension in women and colorectal cancer (Schatzkin *et al.*, 2007). Wheat species are the most frequently grown crops in the organic farming system. However, all of them are not suitable for organic farming, the ones grown in marginal regions. Currently, a lot of alternative crops, including the marginal wheat species, have become attractive too. Such species have been bred neither to increase the yield rate nor for the intensive farming system. Although the baking quality is not high from the conventional point of view, these species have a lot of specific characteristics such as higher proportion of proteins, favorable composition of amino acids, and a high proportion of mineral elements. Products made of such wheat species are considered as the specialty productions with a higher added value at the market and they may be applied well (Konvalina *et al.*, 2011).

The last decade has witnessed a boom in organic farming, both in term of market growth and societal recognition in EU particular and all over the world in general. The success of organic agriculture section is marked by ever-growing consumer support and public recognition - the steady growth of organically farmed land and sales of organic food is backed by dedicated operators and consumers all over the world. Europe has played a key role in the development of organic food and farming. In the early 1980s, the organic sector began to emerge in the number of European countries, and then had spread across the whole EU by the 1990s. Recent figures underline this clear development. The area under organic farming in Europe had over doubled from 6.8 million hectares in 2005 to 15.6 million hectares in 2018. In the same period, the number of producers has increased from 187.780 to over 327.000 (Willer *et al.*, 2020). Obviously, the organic movement is beyond a niche market and has a wide variety of strengths in many fields such as agriculture and food production, direct marketing, international relations, and process auditing. Fueled by strong global consumer demand for ethically produced goods, the organic movement is expected to continue growing and diversifying (Kristiansen *et al.*, 2006).

As organic farming has undergone a significant development and it is still unevenly set up in various countries, there is a deficiency of suitable varieties for the sustainable farming systems. High costs for breeding, weak and uneven representation of the organic farming are the main reasons of such a state. Almost any modern bred varieties of bread wheat which are conventionally grown are not suitable for organic farming. Different selective criteria from the ones applied to the selection of varieties for organic farming are among the main reasons, for instance, less efficient root system, low competitiveness to weeds, low resistance to usual diseases or reduced baking quality provoked by a reduction of the proportion of nitrogen in the soil (Konvalina *et al.*, 2011). In addition, organic farmers have largely depended on cultivars bred for conventional systems, although not all traits are optimal for organic systems because traits associated with independence from external inputs have not received high priority in current breeding programs (Bueren and Myers, 2012).

Along with the development of organic farming area, the demand for organic varieties have been developing fast. This requires breeders applying new selection methodologies and logistics to isolate competitive genotypes for organic systems, and make sure that the best key traits such as weed competitiveness, nutrient efficiency and resistance to seed borne diseases are evaluated. This has been promoted increasing market demand, growing interest in low-input agriculture for ecological reasons, and going up organic wheat production as well (Arterburn *et al.*, 2012).

2 Literature Review

2.1 Overview of Organic Agriculture

Primarily in Europe, organic agriculture began in the early part of the twentieth century. With a desire to deal with the perennial trouble of agriculture, such as erosion, soil depletion, decrease of crop cultivars, low quality food and live-stock feed, and rural poverty, the pioneers of the early organic movement embraced a holistic concept that the long-term vitality of soil will decide the health of agriculture sector of its nation. It is believed that the soil's health and vitality were embodied by its biology and humus (Kuepper, 2010a). Nowadays, organic farming has become a notable aspect of the agriculture worldwide. During the recent years, organic farming has been developing not only in the scale, but only in the scope. This results in increasing the demands from market and consumers, particularly in Europe, Oceania, and North America.

2.1.1 The History of Organic Agriculture

Organic agriculture (OA) has a history of being contentious. It is considered as an inefficient method to food production by some. However, organic section has been developing rapidly in the global food industry (Reganold and Wachter, 2016).

The natural way of farming appeared very soon about 10,000 years ago as humans started farming with very natural and handling methods to grow crops and to feed the animal. During all this time, most of our plants depended on the natural resources such as rainfall. The major nutrients supplying for crops came from soil organic matter, animal manures, plant residues, and small amounts from rainfall (Francis and Wart, 2015). Organic farming, hence, could be considered as the oldest form of global agriculture and it was the only agricultural method lasting until the 1920's. From the 1930's, with new technical inventions such as machines, chemical fertilizers and pesticides applied led to the change of farming methods substantially, which marked to the birth of conventional farming (Darnhofer et al., 2010).

Besides the benefits of industrial farming such as high yield, high economy profit, the drawbacks of this model are unavoidable. More details, it has resulted in negative effects on the environment as well as society because of application chemical fertilizers and pesticides to protect the crops. This leads to toxic residues in agricultural products and environment, pesticide pollution and pest resistance, reduction storage life of products, and alteration taste after fertilization with fresh manure (Sarapatka and Urban, 2009). These problems became more apparent in the 1970's, growing the return of public interest on organic farming (Kuepper, 2010).

The founder of organic farming in English-speaking countries was Sir Albert Howard, who mentioned the organic farming techniques to create healthy soil, plants, and animals in his book the waste products of agriculture; and Lady Eve Balfour with Haughley Experiment compared the difference between organic and non-organic production over long time. She also wrote the high influence book with title "The Living Soil" (Darnhofer et al., 2010; Kristiansen et al., 2006), and was instrumental in the foundation of the soil association in Great Britain in 1946. In Switzerland, Hans Mueller, Maria Biegler, and Hans Peter Rusch played an important role in encouraging and developing organic farming techniques (Kristiansen et al., 2006). Hans Mueller founded a movement to protect traditional farm-life in the industrialization world, using the motto: Healthy soil, healthy food, and healthy people (Sarapatka and Urban, 2009).

In the United State, J.I. Rodale found out the importance of the natural health of soil and its influence on human health, leading to the foundation of *the Rodale Institute* or *Soil and Health Foundation* in 1947 with a wide range of organic gardening and farming publication (Kristiansen *et al.*, 2006).

In 1936, Mokichi Okada practiced natural farming in Japan, applying spiritual and well as agronomic aspects to improve humanity. One pioneer appearing the same time with Okada is Masanobu Fukuoka. His method had a spiritual underpinning like Okada and highlighted the importance of organic farming as a worldwide phenomenon (Kristiansen *et al.*, 2006).

In the 1960s and 1970s, organic farming was re-emerged and focused upon the process of certification of farmers and growers. Although there was growing interest in organic agriculture, it was still clearly outside of mainstream agriculture and national politics (Kristiansen *et al.*, 2006). At the same time, a system of voluntary inspection and certification of farms was established by organic farmers to check on the farming system and its production methods (not measuring outputs) (Sarapatka and Urban, 2009).

From 1972, the pioneers of OA all over the world began to associate the foundation of the international federation of organic agriculture movements (IFOAM). This marked a new step of organic movement as the first and only global organic non-government organization remaining to this day (Kristiansen *et al.*, 2006). IFOAM was established by five OA organizations from south Africa, the USA, and Europe. The aims of IFOAM was to set the principles of organic farming, international basic standards for organic production and processing; to provide information about organic farming worldwide; promote its global application and exchange knowledge; to build relationships and cooperation between all stakeholders in global organic sector; and to stimulate the development of international organic production and market (Luttikholt, 2007).

Organic farming had a large growth from the 1980s, occurring in many regions around the world in the rising interest and awareness of scientists and governments which supported a wide range of research and projects in successfully adopting organic agriculture (Kristiansen *et al.*, 2006).

The 1990s marked a most boisterous period of OA development, reaching a peak at the turn of the millennium. OA had professional structures about advisory services, product manufacturing, marketing, etc. The OA principles promoted by the founders were right as a whole (Sarapatka and Urban, 2009).

In 2018, organic farming was practiced in 178 countries and has been expanded. 57.8 million hectares of agricultural land was cultivated organically with roughly 2.7 million farmers. The worldwide sales of organic products reached over 90 billion us dollars (Willer *et al.*, 2020).

2.1.2 The Definition of Organic Agriculture

In 1999, the Codex Alimentarius commission stated: Organic agriculture is a holistic production management system which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles, and soil biological activity. It emphasizes the use of management practices in preference to the use of off-farm inputs, which takes into account that regional conditions require locally adapted systems. This is accomplished by using, where possible, agronomic, biological, and mechanical methods, as opposed to using synthetic materials, to fulfill any specific function within the system (FAO, 1999). After about 8 years, a concise definition of organic agriculture was released in Vignola, Italy as follows:

"Organic agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved." (IFOAM, 2008).

2.1.3 Principles of Organic Agriculture

Principles of Organic Agriculture based upon the principles of health, ecology, fairness, and care, were built by a designated task force of IFOAM. These ones are the roots so as to organic farming grows and develops (Luttikholt, 2007). As below are the statements of organic agriculture:

- Principle of Health: Organic Agriculture should sustain and enhance the health of soil, plant, animal, human and planet as one and indivisible (IFOAM, 2014).

- Principle of Ecology: Organic Agriculture should be based on living ecological systems and cycles, work with them, emulate them and help sustain them (IFOAM, 2014).

- Principle of Fairness: Organic Agriculture should build on relationships that ensure fairness with regard to the common environment and life opportunities (IFOAM, 2014).

- Principle of Care: Organic Agriculture should be managed in a precautionary and responsible manner to protect the health and well-being of current and future generations and the environment (IFOAM, 2014).

2.1.4 Organic Agriculture Worldwide

According to the latest FiBL survey on the world of organic agriculture, the year 2018 was a good year for global organic agriculture. The data from 186 countries, as of the end 2018, show that more than 71.5 million hectares of organic agricultural land, including in-conversion areas. The largest area of organic farmland is Oceania with 36 million hectares, occupying a half of the world of organic farmland, followed by Europe (15.6 million hectares, 22 %), Latin America (8 million hectares, 11 %), Asia (6.5 million hectares, 9 %), North America (3.3 million hectares, 3.3 %), and Africa (2 million hectares, 3 %). The country with the highest share of the total agricultural land all over the world is Australia (35.7 million hectares). The second and the third place belong to Argentina and China with 3.6 million hectare and 3.1 million hectares, respectively (Willer *et al.*, 2020).

Currently, the organic farmland has been reached 1.5 % of the world's agricultural land. The highest organic shares of the total agricultural land, by region, are in Oceania (8.6 %), and Europe (3.1 %). In the European Union, 7.7 % of the agricultural land is organic. However, some counties reach far higher shares such as Liechtenstein (38.5 %) and Samoa (34.5 %). In sixteen countries, 10 % or more of the agricultural land is organic. In 2018 witnessed a growth in organic farmland by 2.02 million hectares or 2.9 %. Many countries had a remarkable increase, for instance France (16.7 % increase; over 0.27 million hectares more) and Uruguay (14.1 % increase; around 0.24 million hectares more). In all regions, there was a growth in the organic farmland. The area of Europe increased by approximately 1.25 million hectares (8.7 %), while the area of Asia grew by 0.54 million hectares; the area of Africa grew by 4,000 hectares; the area of Latin America grew by 13,000 hectares; the area of North America grew by 0.1 million hectares; the area of Oceania grew by 0.1 million hectares (Willer *et al.*, 2020).

Apart from the organic agricultural land, there are further organic areas, most of which are areas for wild collection and beekeeping. Other areas including aquaculture, forests, and grazing areas on non-agricultural land, constitute more than 35.7 million hectares.

Although global market has reached over 95 billion euros, there are still persistent challenges. For instance, demand for organic food remains concentrated in North America and Europe. Although the share of these two regions is decreasing, they still make up a large part of global sales. Conversely, it has been challenging for strong local markets to develop in Asian, Latin America, and African countries (Willer *et al.*, 2020).

According to FiBL in 2018, the countries with the largest organic markets were the United States (40.6 billion euros), Germany (10.9 billion euros), and France (9.1 billion euros). The largest single market was the United States (42 % of the global market), followed by the European Union (37.3 billion euros, 38.5 %), and China (8.1 billion euros, 8.3 %). The highest per-capita consumption in 2018, with 312 euros, was found in Switzerland and Denmark. The highest organic market shares were reached in Denmark (11.5 %), the first country to reach an organic market share of over ten %, Switzerland (9.9 %) and Sweden (9.6 %) (Willer *et al.*, 2020).

2.1.5 Organic Agriculture in Europe

The latest data, as of the end of 2018, 15.6 million hectares of agricultural land in Europe (European Union 13.8 million hectares) were managed organically by more than 418,000 producers (EU over 327,000). In Europe, 3.1 % of the agricultural land area were organic (European Union 7.7 %). This increased by over 1.25 million hectares compared 2017. Spain was the countries with the largest organic farmland (2.2 million hectares), followed by France (2.0 million hectares), and Italy (2.0 million hectares). In ten countries, at least 10 % of the farmland is organic: Liechtenstein had the lead (38.5 %), followed by Austria (24.7 %) and Estonia (21.6 %). Retail sales of organic products totaled 40.7 billion euros in 2018 (European Union 37.4 billion euros), an increase of 7.8 % as compared 2017. Germany was the biggest market organic products in 2018 was Germany, with retail sales of 10.9 billion euros, followed by France (9.1 billion euros), and Italy (3.5 billion euros) (Willer *et al.*, 2020).

The European Union imported a total of 3.3 million ton of organic agri-food products. Imports of tropical fruit (fresh or dried), nuts and spices represented the single biggest category, totaling 793,597 ton or 24.4 % of total imports, followed by oilcakes, cereals other than wheat, as well as rice, and wheat. China is the biggest supplier of organic agri-food products to the EU, with 415,243 ton of produce; that is 12.7 % of the total organic import volume. Ecuador, the Dominica Republic, Ukraine, and Turkey each have an 8 % share of the total organic import volume (Willer *et al.*, 2020).

In Europe, cereals were the biggest group cultivated made up 2.6 million hectares or 2 % of the cereal area. In European Union, cereals stood at the second-largest group with 2.2 million hectares or 3.9 % of the total cereal area. Of these, wheat, the most important of cereals, was grown with 1 million hectares. Italia had the largest cereal area with roughly 326,000 hectares, followed by France (roughly 311,000 hectares), and Germany (roughly 302,000 hectares). The countries with the highest organic shares of the total cereal area were Austria, Estonia, and Sweden with 15.5, 13.8, and 11.4 %, respectively (Willer *et al.*, 2020).

2.1.6 Organic Agriculture in the Czech Republic

According to the latest data updated to 31.12.2018, the Czech Republic had 4,606 organic farms accounting for approximately 9.5 % of agricultural enterprises with a total organic area of 538,223 hectares, which represents a 12.8 % share of total agricultural land. In the last 10 years, organic area increased to 1.6-fold (341,000 hectares in 2008), and the number of organic farms also increased more than 2-fold (1,946 farms in 2008). The year-on-year increase in the total organic area was 18,191 ha. During 2018, roughly 9,500 hectares of arable land (increase by 13.2 %) and 7,500 hectares of permanent grassland (increase by 1.8 %) were added to organic farming. The share of area under the conversion period was 8.8 %, which corresponds to 9.3 % in 2017 and 12.6 % in 2016 and showed the potential for the organic farming development in the coming years (Yearbook, 2018).

From a long-term point of view, permanent grassland (PG) was the dominant form within OA, representing over 435,000 ha in 2018. Grassland area within total organic area, nonetheless, was not significantly increase, and its percentage share remained approximately 80 % of total organic farming land. PG area rose 1.5-fold in the last decade, from 281,000 ha in 2008, while arable land increased doubled to the current nearly 81,000 ha, which was 15 % of total organic acreage - the highest percentage to date. Permaculture (PC) constituted about 1 % of organic area. Their area increased more than 2-fold in 2018 (about 3,105 ha in 2008). Orchards accounted for the dominant proportion of permaculture with 85 % of the total PC area). Vineyards made up 15 % of PC area (roughly 935 ha), while hop-field area levelled off at approximately11 hectares (0.2 % of PC area) (Yearbook, 2018).

The Czech Republic belonging to countries, whose have had the average size of organic farm about 40 ha. This exceeds the average size of EU. Within the EU, the Czech Republic is one of the countries having the largest average size of organic farm. In 2018, the average size of organic farm was 117 ha and constantly decreased. In terms of the pattern of farm-size, the largest category of organic farms with the area of 10 - 50 ha increased year-on-year to 40.9 % of organic land. The largest scale of organic farmland was from 100 to 500 ha. A comparison showed that approximately a quarter of farms had over 100 ha, and 5 % of farms had over 500 ha of the total organic area. Hence, it was believed that large farms with mostly grassland was predominant in organic farming (Yearbook, 2018).

The main crops on arable land were cereals accounting for 46 % and fodder with 41 %. Of these, wheat and oats were again predominant, and the most grown cereals, together representing about 53 % of entire organic cereal area. These were followed by triticale, barley, and spelt with a share of about 10 %. Year-on-year, there was an increase area in durum wheat, rye, grain, maize, and triticale. In 2018, organic cereal production reached over 77,000 tons, making up 1.1 % of total cereal production in the Czech Republic. Approximately 70 % of this amount was sold on the market; the rest was kept on farms as feedstuff and seed (Yearbook, 2018). The data from yearbook also indicates that the yield of common wheat under organic farming system was 3.04 ton.ha^{-1}. This figure has been low, therefore, the requirement of collection new common wheat varieties that having both high yield and high quality was pressing and necessary.

2.2 Overview of Bread Wheat

Bread wheat (*Triticum aestivum* L. em Thell; AABBDD 2n = 6x = 42), also known as common wheat, is an annual plant belonging to the grass family *Poaceae*, a member of tribe *Triticeae* and subtribe *Triticineae* (Arzani and Ashraf, 2017; Haider, 2013). The polyploid of *Triticum. aestivum* AABBDD is derived from three homoeologous genomes. Of these genomes, A, B, and D contribute seven pairs of chromosomes to wheat total genome (Haider, 2013; Kihara, 1924).

2.2.1 Phylogeny of Cultivated Wheats

There are six species in the genus Triticum, namely *Triticum monococcum* L., *Triticum urartu* Tumanian ex Gandilyan, *Triticum turgidum* L., *Triticum timopheevii* (Zhuk.) Zhuk., *Triticum aestivum* L., and *Triticum zhukovskyi* Menabde & Ericz. These species are divided into three sections described in the Table 1: Section Monococcon consists of diploid species; Section Dicoccoidea consists of tetraploid species; and Section Triticum consists of hexaploidy species. Of these species, while *Triticum aestivum* and *Triticum zhukovskyi* exists only as cultivated forms, *Triticum urartu* exists only as wild form. The rest species exist both forms, a wild and a domesticated form (Kihara, 1924; Matsuoka, 2011).

Table 1 (part 1): The nomenclature of wild and cultivated *Triticum* wheats (after Van Slageren 1994) (Slageren, 1994)

Section	Species and subspecies	Genome constitution	Examples of common names
Monococcon	*Triticum monococcum* L.	AA	
	subsp. *aegilopoides* (Link) Thell		Wild einkorn
	subsp. *monococcum*		Cultivated einkorn
	Triticum urartu Tumanian *ex* Gandilyan	AA	
Dicoccoidea	*Triticum turgidum* L.	AABB	
	subsp. *dicoccoides* (Körn. *ex* Asch. & Graebn.)		Wild emmer
	subsp. *dicoccon* (Schrank) Thell.		Cultivated emmer
	subsp. *durum* (Desf.) Husn.		Durum or macaroni wheat
	subsp. *polonicum* (L.) Thell.		Polish wheat
	subsp. *turanicum* (Jakubz.) A. Löve & D. Löve		Khorassan wheat
	subsp. *turgidum*		Rivet wheat
	subsp. *carthlicum* (Nevski) A. Löve & D. Löve		Persian wheat
	subsp. *paleocolchicum* (Menabde) A. Löve & D. Löve		Georgian wheat

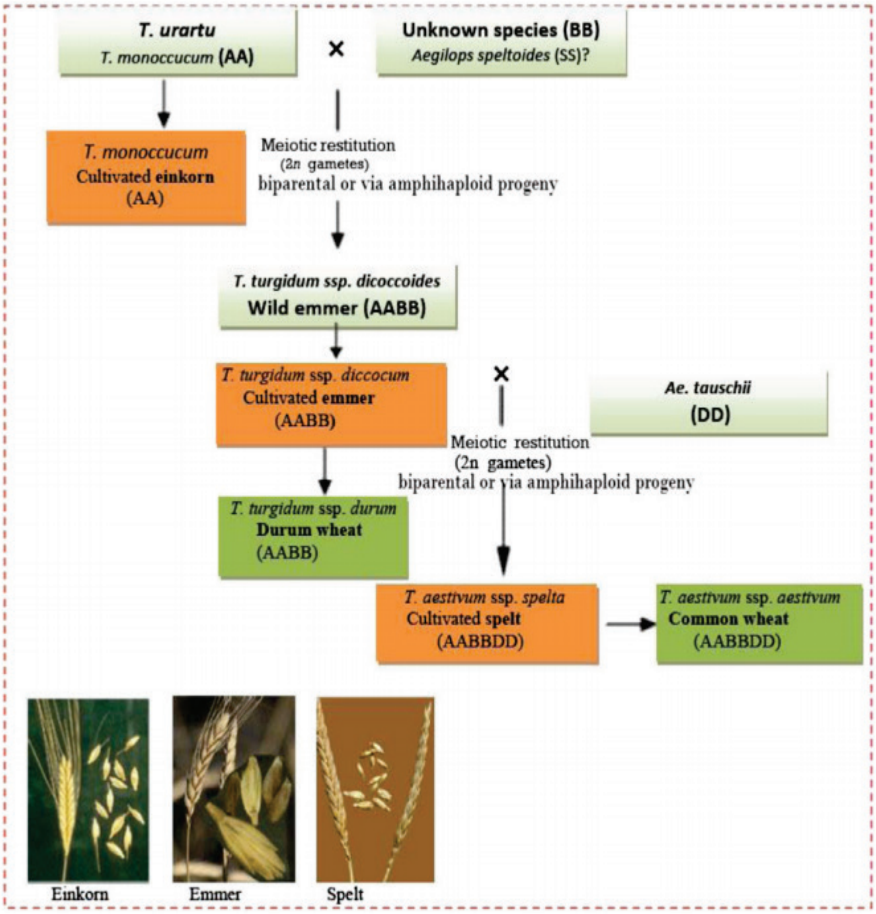
Table 1 (part 2): The nomenclature of wild and cultivated *Triticum* wheats (after Van Slageren 1994) (Slageren, 1994)

	Triticum timopheevii (Zhuk.) Zhuk.	AAGG	
	subsp. *armeniacum* (Jakubz.) van Slageren		Wild timopheevii
	subsp. *timopheevii*		Cultivated timopheevii
Triticum	*Triticum aestivum* L.	AABBDD	Common wheat
	subsp. *aestivum*		Bread wheat
	subsp. *compactum* (Host) MacKey		Club wheat
	subsp. *sphaerococcum* (Percival) MacKey		Indian dwarf wheat
	subsp. *macha* (Dekapr. & Manabde) MacKey		
	subsp. *spelta* (L.) Thell.		Spelt
	Triticum zhukovskyi Menabde & Ericz	AAAAGG	

2.2.2 Origin of Hexaploidy Wheat

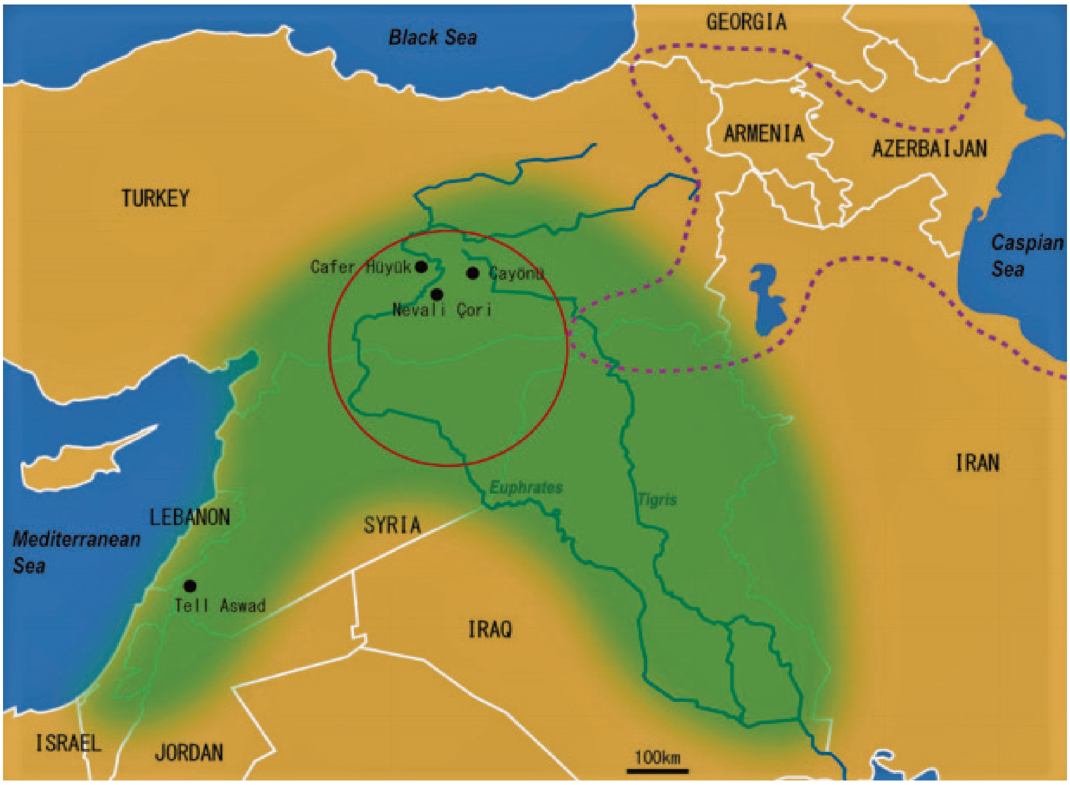
The appearance of *Triticum aestivum* were probably only after the domestication of diploid and tetraploid wheats. The scenario of hybridization among the genera within *Triticeae* is widely accepted for the allopolyploid speciation of hexaploidy bread wheat *Triticum aestivum* L.. The scenario shown that the cultivated forms of *Triticum turgidum* migrated north-eastward, then across and beyond the Fertile Crescent region association with the spread of agriculture. Finally, *Triticum turgidum* (AABB genome) came into connect with *Aegilops tauschii* (DD gennome), a critical natural hybrid cross (Figure 1), marking the birth of hexaploid wheat (AABBDD genome) (Kihara, 1944; McFadden and Sears, 1944). This also indicated that no wild hexaploid ancestor of *Triticum aestivum* has ever been found (Hsam *et al.*, 2001; Kihara, 1966).

Figure 1: The diagram of domesticated species of the *Triticum* spp., consisting of einkorn, emmer, spelt, durum, and bread wheat (Arzani and Ashraf, 2017)



It is presumed that the origin of hexaploidy wheat have come from in northwestern Iran or northeastern Turkey (Bonjean and Angus, 2001). The scientists in genetic, biochemical, and molecular field indicated that the hometown of hexaploidy wheat was in Iran, southwest to the Caspian Sea (Figure 2). The evidence strongly supports the genesis of hexaploid wheat from the cultivated tetraploid wheat, rather than from wild emmer ssp. *dicoccoides*. In addition, when hexaploid types first emerged in the middle of the 9th millennium BP, there was no evidence for geographical contact between wild emmer and *Aegilops tauschii*. By the time hexaploid wheat was produced, the cultivated tetraploid wheat had grown in western Iran and eastern Turkey, and it contacted with *Aegilops tauschii*, which probably was growing as a weed within and at the edges of wheat field (Bonjean and Angus, 2001).

Figure 2: The Levantine corridor (shaded in green). The dashed purple line and the red circle are thought where agriculture have emerged (Lev-Yadun *et al.*, 2000) and the western peripheral area of the natural range of *Aegilops tauschii* where the allopolyploid speciation of *Triticum aestivum* supposedly occurred (Matsuoka, 2011)



2.2.3 Spread of Wheat Cultivation

The diffusion of the wheat culture from the Fertile Crescent to Europe, Africa, and Asia was described by (Evans *et al.* 1981, Kislev 1984, and Bar-Yosef 1998), together with the evidence of archeological. The Fertile Crescent, which includes 10 countries, is surrounded by the Syrio-Arabian desert in the south, the Mediterranean in the west, and chains of large and high mountain ranges in the east and north. Located between mountains, the desert, and the sea, this area is influenced by several different climates. The Fertile Crescent is known as one of the most diversified regions. Archaeological evidence substantiates that the Fertile Crescent is the hometown of the wild taxa of four species of wheat, and it is most likely the area where wheat was first domesticated (Bonjean and Angus, 2001). The spread of wheat started in the 8th and 7th millennia BP. There were two routes of expansion of the wheat to Europe. The first route, seen as the mainstream, was from Anatolia to Greece (ca. 8,000 BP). From there the route divided into two branches. One of these branches, wheat was brought to Italy, southern France, and Spain (ca. 7,000 BP). This branch contained mainly emmer, a significant quantity of einkorn, and small amount of tetraploid and hexaploidy wheat; hulled hexaploidy wheat was missing. The other branch, wheat was carried across the Balkans, via the Danube Valley to the Rhine Valley, from where wheat diffuse to central, western, and northern Europe. The second route was through Transcaucasia and Caucasus (ca. 7,000 BP), southern Russia, to central Europe. This route carried mainly bread and club wheat, and small amount of spelt wheat (Bonjean and Angus, 2001).

In Africa, the wheats were spread by several routes. The first route started at ca. 6,000 BP in Egypt, from where the wheats expand southward to Sudan, Ethiopia, and westward to Libya. The second route was across the Mediterranean from Greece to Crete and to Libya, and from southern Italy through Sicily to Tunis, Algeria, and Morocco. In this journey, majority of emmer was spread to Africa. A few samples of einkorn were found in Morocco, while no evidence of appearance of einkorn was found in Egypt, Ethiopia, and North Africa. *Triticum parvicoccum* (naked tetraploid wheat) was in admixture with emmer. Durum wheat was established as an important plant in Egypt only during the Greek period (ca. 2,300 BP) (Bonjean and Angus, 2001).

In Asia, the diffusion of wheat came from northern Iran. The eastward spread of wheat reached western Pakistan only ca. 6,500 BP. This later 1,500 compared to Iran. Wheats were cultivated in Baluchistan, Pakistan at approximately 6,000 BP, and in prehistoric site Mehrgarh in the Indus plain, southwestern Pakistan, ca. 5,300 BP (Jarrige and Meadow, 1980). The samples of wheat grown in Mehrgarh were domestic einkorn, emmer, and free-threshing form. The free-threshing form appeared in Mehrgarh in the beginning of the 5th millennium BP. In India, wheat was found in northwestern at ca. 5,000 BP (Hutchinson, 1974). After that 2,000 years, bread wheat was the dominant crop in India (Harris, 1998).

Wheats expanded to east Asia via the Silk Road, run from Turkestan through Sinkiang to northern China (Zeven, 1980). The white-grained bread was migrated with this route, while the red-grained bread was migrated with the other route that started in Pakistan and Afghanistan, then went on through Khyber Pass crossing the Punjab plain, from where it branched to the Indian sub-continent, and to Burma, crossing Yunnan and Szechman to reach the Yangtse Valley. Wheat reached western China at ca. 4,000 BP (Zeven, 1980). In Japan, bread wheat presented at ca. 2,300 years ago (Zeven, 1980). Bread wheat was the dominant plant in central and eastern Asia. Emmer and naked tetraploid wheat were only small amount of the mixture, apart from India, while einkorn was rare.

Mainly bread wheat was introduced to the New World in 1529, when Spaniards brought it to Mexico.In 1788, bread wheat was taken to Australia (Bonjean and Angus, 2001).

2.2.4 Agronomic Traits

Numerous studies have reported that agronomic characteristics such as plant height, thousand kernel weight, harvest index (HI), etc. are positively associated with grain yield improvement in wheat (Beche *et al.*, 2014; Foulkes *et al.*, 2007; Gao *et al.*, 2017; Lopes *et al.*, 2012). In order to accelerate further grain yield improvement, hence, the application of trait-based breeding using high performing and genetically complementary genotypes is play an important role. (Bustos *et al.*, 2013; Chen *et al.*, 2012; Liu *et al.*, 2015; Reynolds and Tuberosa, 2008; Reynolds *et al.*, 2017).

2.2.4.1 Plant Height

Plant height of cereals is one of the important morphological characteristics. It is also an important index to assess the lodging resistance of wheat. Plant height is measured in all plots, during the milk-waxy maturation when the maximum height level is achieved (Curna, 2016).

Several studies worldwide have suggested that wheat plant height has reached the limit of its theoretical at about 70 to 80 cm. Hence, the further reduction in plant height has not been feasible (Shearman *et al.*, 2005). The main reason of plant height is not able to be decreased any further to avoid risking reductions in biomass and grain yield (Berry *et al.*, 2015). Consequently, Gao *et al.* suggested the strategic breeding should combine both plant height and grain yield to maximize yield potential and lodging resistance (Gao *et al.*, 2017).

Results of reduction plant height via breeding progress to improve lodging resistance and grain yield indicated that there were a decrease in the height of plant from 120 to 57 cm in Italy (De Vita *et al.*, 2007), from 125 to 65 cm in Spain (Royo *et al.*, 2007), from 110 to 95 cm in the UK (Berry *et al.*, 2015), and from 130 to 60 cm in China and Brazil (Beche *et al.*, 2014; Gao *et al.*, 2017) when replacing old cultivars by recent and short plant height wheat ones.

According to Graybosch and Peterson, the genetic progress resulting in reduction plant height ranged from - 0.32 to - 0.33 % yr^{-1} across varied environments in the USA (Graybosch and Peterson, 2010). The other results from China and Brazil showed the higher percentage of reduction plant height by - 0.69 and - 0.74 % yr^{-1}, respectively (Beche *et al.*, 2014; Zhou *et al.*, 2007). In Table 2 and Table 3, the plant height varies from 75.97 to 115.75 cm in conventional farming, from 78.7 to 99.67 cm in organic farming.

Table 2: The agronomic traits of winter wheat in conventional farming according to different researchers (Akhtar *et al.*, 2001; Assefa, 2017; Cox *et al.*, 2019; Gevrek and Atasoy, 2012; Seleiman *et al.*, 2010)

	Akhtar *et al.*	Seleiman *et al.*	Assefa *et al.*	Gevrek and Atasoy	Cox *et al.*
Plant height (cm)	102.00	115.75	75.97	88.7	-
TGW (g)	41.88	51.77	39.97	-	34.18
Grain yield (ton.ha^{-1})	6.10	2.92	3.19	3.26	4.73
Harvest index (%)	45.41	35.50	36.09	19.00	-

The data in the table was took an average value either from the listed references or from the genotypes reported in a reference.

Table 3: The agronomic traits of winter wheat in organic farming according to different researchers (Ceseviciene *et al.*, 2009; Cox *et al.*, 2019; Gevrek and Atasoy, 2012; Konvalina *et al.*, 2011; Tran *et al.*, 2020)

	Ceseviciene *et al.*	Konvalina *et al.*	Tran *et al.*	Gevrek and Atasoy	Cox *et al.*
Plant height (cm)	-	99.67	-	78.7	-
TGW (g)	-	39.55	39.99	-	32.80
Grain yield (ton.ha^{-1})	4.27	4.24	3.81	1.60	4.69
Harvest index (%)	-	40.00	-	20.00	-

The data in the table was took an average value either from the listed references or from the genotypes reported in a reference.

2.2.4.2 Thousand Kernel Weight

Thousand kernel weight (TKW) is a crucial component of grain yield, which defines not only the improvement of grain yield but also the improvement in milling yield. Thousand kernel weight have associated with increasing grain yield (Morgounov *et al.*, 2010; Qin *et al.*, 2015; Zheng *et al.*, 2011). On the other hand, grain yield improvement was reportedly high correlation with thousand kernel weight (Aisawi *et al.*, 2015; Lopes *et al.*, 2012; Morgounov *et al.*, 2010; Tian *et al.*, 2011; Zheng *et al.*, 2011; Zhou *et al.*, 2007). The improvement of TKW varied from 39 to 55 g (Gao *et al.*, 2017), and 29 to 49 g (Zhang *et al.*, 2016) between old landrace varieties and modern wheat ones in China. In the study of Giunta *et al.* reported that, likewise, TKW ranged from 33 to 55 g in old varieties, and from 41 to 57 g in modern wheat ones (Giunta *et al.*, 2007). Also, Underdahl *et al.* reported improvement in TKW being 20.4 g for old varieties and 33.6 g for newly released varieties (Underdahl *et al.*, 2008). In addition, reporting about genetic gains, Underdahl *et al.*, and Gao *et al.* found that there was an increase of 0.30 % yr^{-1} and 0.35 % yr^{-1} for TKW between Chinese and American wheat genotypes, respectively (Gao *et al.*, 2017; Underdahl *et al.*, 2008). Likewise, reported increasing TKW of 0.03 g yr^{-1} among Brazilian wheat genotypes was reported by Beche *et al.* (Beche *et al.*, 2014).

To improve grain yield, numerous authors suggested that the selection of heavier grains could be highly effective to grain yield (Gao *et al.*, 2017; Morgounov *et al.*, 2010; Qin *et al.*, 2015; Zheng *et al.*, 2011). However, the breeding process for high grain number and TKW in the same genotype has been reported to be difficult due to trade-offs. According to Gaju *et al.*, trade-off can be minimized by selecting genotypes with higher number spikelet per spike, which resulted in spikes with higher grain number and heavier TKW (Gaju *et al.*, 2009). An alternative approach might be a useful strategy to increase yield potential in wheat is that combining both traits (grain number and grain weight) in the progeny. This was proposed by Bustos *et al.*, who indicates that there was an increase in grain yield with crossing genotypes expressing high grain number with those expressing high TKW (Bustos *et al.*, 2013).

Comparing the data between Table 2 and Table 3 show that TGW in conventional is higher or equal than that one in organic farming exception with the data of Cox *et al.* (Cox et al., 2019).

2.2.4.3 Grain Yield

Wheat grain yield is obviously influenced by agronomic characteristics such as plant height, total biomass, number of productive tillers, harvest index, grain number per spike, spike length, number of kernels per spike, thousand grain weight, and grain weight per spike; and physiological characteristics such as canopy temperature, chlorophyll content, photosynthetic rate, and water-soluble carbohydrates (Chen *et al.*, 2012; Chen and Hao, 2015; Foulkes *et al.*, 2007; Liu *et al.*, 2015). Agronomic characteristics are used as indirect selection criteria during breeding and cultivar development because of their high heritability and correlation with grain yield (Abdolshahi *et al.*, 2015). What is more, they have been explored to accelerate the cultivar development in wheat improvement programs. For example, several traits conferring better agronomic and physiological performance with biotic and abiotic stress tolerance are simultaneously selected and introgression to a single variety (Lopes *et al.*, 2012).

According to (Akhtar *et al.*, 2001; Assefa, 2017; Cox *et al.*, 2019; Gevrek and Atasoy, 2012; Seleiman *et al.*, 2010), the grain yield of winter wheat ranged from 2.92 to 6.10 ton.ha^{-1} in the conventional farming. The study findings of winter wheat grown in organic farming showed that the grain yield ranged from 1.60 to 4.27 ton.ha^{-1} (Ceseviciene *et al.*, 2009; Cox *et al.*, 2019; Gevrek and Atasoy, 2012; Konvalina *et al.*, 2011; Tran *et al.*, 2020).

2.2.4.4 Harvest Index

Harvest index is the ratio of harvested product to total above-ground biological yield. It is usually calculated from unit area yield and dry matter data (machine-harvested yield/ total plant dry weight at maturity) (Unkovich *et al.*, 2010).

HI has gone up over time due to breeding for higher yield (Perry and D'Antuono, 1989; Whitehead *et al.*, 2000). Results of recent studies showed that the rate of HI increases due to wheat breeding in Australia is about 21 - 43 % (Flohr *et al.*, 2018); in the USA is roughly 42 - 46 % (Green *et al.*, 2012); in Italia is about 41 - 43 % (Giunta *et al.*, 2007); in Spain is about 26 - 42 % (Royo *et al.*, 2007); in Turkey is approximately 28 - 36 % (Gummadov *et al.*, 2015).

The variation of HI is presented in Table 2 and Table 3. Here, HI of winter wheat ranges from 19 - 45.41 % in conventional farming, and from 20 - 40 % in organic farming.

2.2.5 Nutritional Composition of Ancient and Modern Wheats

Along with the secondary components such as carotenoids and starch, protein plays a crucial role in the functional food ingredients.

2.2.5.1 Proteins

Mulder and Berzelius published the word protein in 1838, meaning *primary substance* (Malik, 2009). Proteins in wheat grain are contained in endosperm, germ, and aleurone layer. Wheat flour consists starch, water and proteins with the percentage (70 - 75 %), (14 %), and (10 - 12 %) respectively (Goesaert *et al.*, 2005). The major types of protein can be divided into three categories: Simple, conjugated, and derived. However, only simple protein is found in wheat plants, consisting of four major types: Albumins (soluble in water and dilute buffers), globulins, prolamins, and glutelins. Gluten, the remainder of wheat flour after removing starch, non-starchy polysaccharides, and water-soluble constituents, comprises alcohol-soluble gliadins and alcohol-insoluble glutenins (Shewry and Halford, 2002). Wheat storage proteins have two basic fraction groups: Gliadins and glutenins. Glutenins are known as being the larger polymers in nature and are measured as high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS). These are used as protein markers for predicting the quality of bread and identifying wheat varieties (Bradová and Šašek, 2005; Branlard *et al.*, 2001).

2.2.5.2 Albumins and Globulins

Albumins and globulins of wheat is made up of 15 - 20 % of total wheat flour proteins. While albumins are soluble in water, globulins are soluble in salts (Pence *et al.*, 1954; Singh and Skerritt, 2001). Although the molecular weights of the proteins are normally from 60,000 to 70,000, the molecular weights (MW) of albumins and globulins are mostly lower than 25,000, (Veraverbeke and Delcour, 2002). Albumins and globulins are mainly found in the aleurone layer. They are considered as nutritionally better amino acid compositions because of their higher lysine and methionine contents as compared to the remainder of the proteins in the wheat grain

(Lásztity, 1984). According to Galterio (Galterio *et al.*, 1999), the content of the albumin and globulin fractions in durum and bread wheat are about 15 - 25 % of total protein, lower than that one of emmer (30 - 39 % total proteins).

2.2.5.3 Gluten

Gluten accounting for 80 - 85 % protein and 5 % lipids is the last part after washing with water to remove starch, non-starchy polysaccharides, and water-soluble constituents (Wall, 1979; Wieser, 2007). Gluten proteins are present in the mature wheat grain endosperm where they form a continuous matrix around the starch granules. Gluten contains hundreds of protein components which are present either as monomers or, linked by inter-chain disulphide bonds, as oligo- and polymers (Wrigley and Bietz, 1988). Typically, gluten proteins have been divided into gliadins and glutenins based upon their solubility in alcohol-water solutions of gluten (Table 4). They play an important to the rheological properties of dough, but their functions are divergent. Hydrated gliadins have little elasticity and are less cohesive than glutenins; they contribute mainly to the viscosity and extensibility of the dough system. In contrast, hydrated glutenins are both cohesive and elastic and are responsible for dough strength and elasticity (Wieser, 2007). In the amino acid composition of gluten, although cysteine is comprised approximately 2 %, it is crucial important for the structure and functionality of gluten. Although this little amount is very crucial for the structure and functionality of gluten. Cysteine (oxidized form) normally form inter-chain disulphide bonds between proteins or intrachain disulphide bonds within a protein (Grosch and Wieser, 1999; Wieser, 2012).

2.2.5.4 Gliadins

Gliadins of wheat belong to the proline-and glutamine rich prolamin family, and have been characterized by many authors (Cunsolo *et al.*, 2004; Dworschak *et al.*, 1998; Haraszi *et al.*, 2011; Shewry, 2003). Most of gliadins are monomeric proteins that consist of single chain polypeptides and constitute from 30 to 40 % of total flour protein content. Gliadins are polymorphic mixture of proteins soluble in 70 % alcohol (Anderson and Greene, 1997; Wieser, 2007). Gliadins have been further classified into three groups based upon their mobilities in sodium dodecyl sulphatepolyacrylamide gel electrophoresis (SDS-PAGE). First group is α-/β-gliadins with the highest mobility, followed by γ-gliadins and ω-gliadins (Cunsolo *et al.*, 2004). The molecular weights (MW) of gliadins range from 30 to 80 kDa. Most of them have MW from 30 to 40 kDa, exception with some ω-gliadins having 80 kDa MW (Cunsolo *et al.*, 2004; Shewry *et al.*, 1986). Based upon sequences and composition of amino acids, and molecular weights of different classes of gliadins. α, β and γ gliadins contain inter-chain disulfide bonds, while ω-gliadins lack cysteine 12 residues and do not form disulphide bonds, Wieser divided gliadins into four different groups: ω5-gliadins, ω1 and 2-gliadins, α/β-gliadins, and γ-gliadins (MacRitchie and Lafiandra, 1997; Wieser, 2007). The α/β and γ- gliadins are the major components, whereas the ω-gliadins occur in much lower proportions of wheat varieties (Wieser and Kieffer, 2001).

Table 4: Classification of proteins according to Osborne, modified from Goesaert *et al.*, (2005)

Osborne fraction	Solubility behavior	Composition	Biological role	Functional role
Albumin	Water and dilute buffers	Non-gluten proteins (mainly monomeric)	Metabolic and structural proteins	Protection from pathogens
Globulin	Dilute salt	Non-gluten proteins (mainly monomeric)	Metabolic and structural proteins	Providing food reserve to embryo
Gliadin	Aqueous alcohols	Gluten proteins (mainly monomeric gliadins and low molecular weight glutenin polymers	Prolamins-type seed storage protein	Dough viscosity/plasticity
Glutenin	Dilute acetic acid	Gluten proteins (mainly HMW glutenin polymers)	Prolamins-type seed storage proteins	Dough viscosity/plasticity
Residue	Unextractable in water and dilute buffers but extractable with Urea+ DTT+SDS SDS+ Phosphate buffers+ sonication etc	Gluten proteins (high molecular weight polymers) and polymeric non-gluten proteins (triticins)	Prolamins-type (gluten) and globulin-type (triticin) seed storage proteins	

DDT: Dithiothreitol; SDS: Sodium dodecylsulfat.

2.2.5.5 Glutenins

Wheat glutenins, polymeric proteins of wheat gluten, are extractable in dilute acetic acid (Table 4) (Field *et al.*, 1983; Goesaert *et al.*, 2005). A part of glutenins belongs to the largest proteins in nature, more than 10 million MW, making wheat glutenins outstanding proteins in the plant kingdom. The MW distribution of glutenins has been recognized as one of the main determinants of dough properties and baking performance (Wieser, 2007; Wrigley, 1996). Wheat glutenins can be divided into gluten subunits: High molecular weight (HMW) and low molecular weight (LMW) glutenins after reduction of the disulphide bonds. The separation of the gluten subunits based upon their sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) mobility (Cunsolo *et al.*, 2004; Haraszi *et al.*, 2011; Payne *et al.*, 1984; Wieser, 2007). The released glutenin subunits were further classified into four subgroups (A - D) by electrophoretic mobility on SDS-PAGE. Subgroup A was determined to be HMW-GS, and subgroups B, C, and D were referred to as LMW-GS (Gianibelli *et al.*, 2001; Shewry and Tatham, 1990; Wang *et al.*, 2006; Wrigley *et al.*, 2006). It was also observed that once reduced and separated by SDS-PAGE, high molecular weight gliadins had a mobility like those of the B and C subunits of LMW-GS (Payne and Corfield, 1979). Having molecular weights between 65 and 90 kDa, based on derived amino acid sequences, and 80 - 130 kDa on SDS-PAGE, HMW-GS are encoded at complex loci on the long arms of chromosomes 1A, 1B, and 1D of hexaploid wheat, which are the Glu-A1, Glu-B1, and Glu-D1 loci, respectively (Gianibelli *et al.*, 2001). HMW-GSs are usually used to indicate the genetic potential of bread wheat varieties, from the baking quality perspective using the Glu-1 score reported by Payne *et al.* (Payne *et al.*, 1987), which allocates scores for the subunits (alleles) in each of the wheat's three genomes (Glu-A1, Glu-B1, and Glu-D1).

2.2.5.5.1 Protein Content

Some studies recently suggested that the emphasis on bread making quality has resulted in modern wheats having higher contents of protein than older types. However, when comparisons of modern and old types grown under the same conditions in Europe, Shewry *et al.* did not support this because of the primary target of wheat breeders over the past century has been increased yield. Higher yield results mainly from increased accumulation of starch, which dilutes other grain components including protein (Shewry *et al.*, 2020).

Not only does vary protein content within and across species, but it is strongly influenced by the environment (Arzani and Ashraf, 2016; Ashraf, 2014). Table 5 reveals that grain protein contents (whole grain flour) of the ancient wheat are commonly superior to those of the modern counterpart (Abdel-All *et al.*, 1995; Brandolini *et al.*, 2008; Grausgruber *et al.*, 2004; Løje *et al.*, 2003; Shewry *et al.*, 2013; Tran *et al.*, 2020).

A wheat grain consists of three main components: Germ (embryo), endosperm, and outer layers (pericarp, seed coat and, aleurone). The endosperm is made up of roughly 90 % of the dry weight of the grain. Grain size and weight are important determinants and affect in many qualitative and compositional constituents. For instance, if a grain is a big and heavy, it yields a larger endosperm as well as smaller proportions of aleuronic layers and external pericarp. Hence, the protein content of modern wheat is lower than the ancient wheat, which may be explained by its bigger and heavier grain that yields a larger starchy endosperm (Arzani and Ashraf, 2017). In other words, the percentage of protein content wheat has negative correlation with the one of starch (Shewry *et al.*, 2020).

2.2.5.6 Carbohydrates

Carbohydrates constitute the majority of the wheat grain from 65 % to 75 %, with starch being the main constituent of wheat grain and flour at roughly 60 % to 70 % (Lineback and Rasper, 1988). Amylose and amylopectin are the major components of starch which are α-D-glucose polymers of different structures intertwined to form a starch granule. Amylose consisting of a-(1,4)-linked D-glucopyranosyl units with a degree of polymerisation (DP) in the range of 500 - 6,000 glucose residues, is an essentially linear molecule. It is now well recognized that a fraction of the amylose molecules is slightly branched by a-(1,6)-linkages (Hizukuri *et al.*, 1981; Shibanuma *et al.*, 1994). On the other hand, amylopectin is a very large and highly branched polysaccharide with a DP ranging from $3*10^5$ to $3*10^6$ glucose units. It is composed of chains of a-(1,4)-linked D-glucopyranosyl residues which are interlinked by a-(1,6)-bonds (Zobel, 1988). While amylose is easily hydrolyzed by α-amylase and β-amylase to maltose, amylopectin is degraded to maltose and dextrins (roughly 60 %, and roughly 40 %, respectively). Although amylose accounting for only around one-quarter of the starch granule, the frequency of its molecules is almost 150 times greater than that of amylopectin ones. This is due to the much smaller size of amylose compared to amylopectin. In contrast, its higher resistance to hydrolytic enzymes, because of the more tightly packed amylose chains than amylopectin ones, leads to the low postprandial levels of glycemic and insulinemic responses which, in turn, give rise to reduced post-meal blood insulin and glucose, yielding longer satiety (Arzani and Ashraf, 2017). In the nutritional aspects, total starch can be divided into rapidly digestible starch, slowly digestible starch, and resistant starch. The latter starch fraction is not susceptible to enzymatic hydrolysis and can be included in the dietary fiber (Marconi and Cubbada, 2005). During the processing, It is known that starch digestibility may be reduced because of the formation of enzyme resistant starch (Galterio *et al.*, 1999). A study of Massaux *et al.* reveals that the starch content varies inter-species, which could be explained by the genotype and environment conditions (Massaux *et al.*, 2008).

The results from Table 5 show that the amount of digestible carbohydrate and starch of bread wheat are higher than those of ancient counterpart (Abdel-All *et al.*, 1995; Brandolini *et al.*, 2008; Branlard *et al.*, 2001; Davis *et al.*, 1981; Lacko-Bartošová and Čurná, 2015). The enhanced grain yield of modern wheat results in the so-called "yield dilution phenomenon" coupled with the higher ploidy level may explain the higher starch content of modern wheat (Arzani and Ashraf, 2017).

Table 5: Mean of chemical compounds of whole-grain flour in modern and ancient wheats according to Arzani and Ashraf, modified from Tran. (Arzani and Ashraf, 2017)

Component	Bread Wheat	Ref.	Spelt	Ref.	Emmer	Ref.	Einkorn	Ref.
Digestible carbohydrate (% or g per 100g)	73 *	1	65.9 *	2	71	3	64.5	2
Protein (%)	12.9 - 19.9	4;5	16.3 - 17.5	2	13.5 - 19.1	6;7	15.5 - 22.8	7;8
Starch (%)	68.5	8	63.8 *	2	65	3	62.3 *	2;8;9
Amylose (% starch)	28.4 *	10	-	-	25.1	11	23.8 *	8;11
Dietary fiber (%)	13.4	12	12	13	9.8	13	9.8 *	6;13
Lipid (%)	2.8	14	2.39 *	2;15	2.16 *	7;15	3.5 *	14;15
Ash (%)	1.9	6	2.1	6	2.3	6	2.3	6;14

Ref.: Reference. 1. (Davis *et al.*, 1981); 2. (Abdel-All *et al.*, 1995); 3. (Lacko-Bartošová and Čurná, 2015); 4. (Shewry *et al.*, 2013); 5. (Tran *et al.*, 2020); 6. (Løje *et al.*, 2003); 7. (Grausgruber *et al.*, 2004); 8. (Brandolini *et al.*, 2008); 9. (Brandolini *et al.*, 2011); 10. (Regina *et al.*, 2015); 11. (Mohammadkhani *et al.*, 1998); 12. (Andersson *et al.*, 2013); 13. (Gebruers *et al.*, 2008); 14. (Hidalgo *et al.*, 2009); 15. (Suchowilska *et al.*, 2012).

* By taking an average value either from the listed references or from the genotypes reported in a reference.

2.2.5.7 Dietary Fiber

Wheat fiber, concentrated in the bran layers, and wholewheat flour has a higher fiber content than white flour, is an important source of fiber in the Western diet, with bread alone providing between 17 % and 21 % (depending on age group) of the daily intake in the UK (Lockyer and Spiro, 2020).

The contents of individual dietary fiber components in wholewheat flours in Table 5 vary from 9.8 % to 13.4%. Of those, bread wheat has the highest value, followed by spelt (12 %). Emmer and Einkorn have the lowest value with 9.8 % dietary fiber (Andersson *et al.*, 2013; Gebruers *et al.*, 2008; Løje *et al.*, 2003). When research 129 of the winter wheat varieties in the HEATHGRAIN sample set, Andersson *et al.* reported that total dietary fiber ranged from 11.5 - 15.5 % dry wt. and arabinoxylan (the major component) from 5.53 to 7.42 % dry wt. Other components were cellulose (1.67 - 3.05 % dry wt.), Klason lignin (0.74 - 2.03 % dry wt.), fructans (0.84 - 1.85 %) and b-glucan (0.51 - 0.96 %) (Andersson *et al.*, 2013; Gebruers *et al.*, 2008). According to Hazard *et al.*, the concentration of dietary fibre is higher in wholemeal than in white flour. The major component is again arabinoxylan (up to about 3 % dry wt.) with lower concentrations of b-glucan (about 0.5 % dry wt.), fructans (about 1.5 % dry wt.) and arabinogalactan peptide (up to 0.4 % dry wt.) (Hazard *et al.*, 2020). From a nutritional point of view, the difference between soluble and insoluble fibre is very important, since each form is considered to have physiological effects and benefits as a constituent of the human diet. Soluble fiber is known for its hypocholesterolaemic effect (Jenkins *et al.*, 1995). In wheat, whole grain flour and its bran fraction are a reliable source of fiber, especially the water-insoluble type (Bushuk and Rasper, 1994). On the other hand, white flour is not rich in total fiber, but it is relatively rich in soluble fiber.

2.2.5.8 Lipids

Lipids are minor components of wheat flour, but it has an essential function in wheat end-use quality. Total lipids account for 3 - 4 % of the wheat kernels and about 45 % of these are located in the starchy endosperm (Chung, 1986). They are more concentrated in germ, which contains 28.5 % of lipids, and in the aleurone layer about 8.0 %, in endosperm (1.5 %) (Delcour and Hoseney, 2010). What is more, wheat lipids have large structural diversity and comprise neutral (acylglycerols and free fatty acids) and polar (glycolipids and phospholipids) components. In seed tissues, triacylglycerols are the main storage lipids, they are contained in subcellular organelles called oil bodies. Even though being a minor constituent of wheat flour (about 2 - 2.5 % dry weight), they are considered to have significant impacts on flour and dough functionality, by interacting with gluten proteins and starch and by stabilizing gas cells in breadmaking (Pareyt *et al.*, 2011). Moreover, lipids have also been shown to vary in amount and composition between millstreams and varieties (Bekes *et al.*, 1986; Prabhasankar *et al.*, 2000; Prabhasankar and Haridas Rao, 1999).

Most studies related to lipid functionality in wheat flour have focused on bread-baking quality (Crosbie, 1991; Huang *et al.*, 1996; Konik *et al.*, 1994; Rho *et al.*, 1989, 1989; Toyokawa *et al.*, 1989), for which protein is the most essential ingredient. A good deal of evidence has shown that starch quality is also very important for oriental flour foods, like steamed bread and noodles. In addition, wheat lipids are quite a complex family of components, present both as free and bound to various other constituents in the cereal, including proteins and starch (Ruibal-Mendieta *et al.*, 2002).

As reviewed by Arzani and Ashraf (2017), einkorn had the highest content of lipids (3.5 %), compared to bread wheat, spelt, and emmer with 2.8 %, 2.39 %, and 2.16 %, respectively (Abdel-All *et al.*, 1995; Grausgruber *et al.*, 2004; Hidalgo *et al.*, 2009; Suchowilska *et al.*, 2012).

2.2.6 Technological Parameters

2.2.6.1 Gluten Content and Gluten Index

Gluten proteins are among the most complex protein networks in nature because of reasons such as numerous different components and different size; the variability due to genotype, growing conditions, and technological processes. They play an important role in determining the unique rheological dough properties and baking quality of wheat by conferring water absorption capacity, cohesivity, viscosity, and elasticity on dough (Wieser 2007; Oikonomou *et al.* 2015).

According to Grobelnik Mlakar *et al.*, the wet gluten content and gluten index are indicators that closely relates to the baking quality of flour (Grobelnik Mlakar *et al.*, 2009). A complex mixture of proteins occurring when flour is mixed with water. It has a unique property of being able to form a viscoelastic dough (Oikonomou *et al.*, 2015). Wet gluten is defined as a cohesive viscoelastic proteinaceous substance obtained after washing out starch granules from dough. Gluten quality was described by the degrees of strength and extensibility, which allows a sufficient expansion, good distribution, and retention of the gas cells within fermenting dough (Grobelnik Mlakar *et al.*, 2009). The gluten index is a measurement of wheat protein that provides a simultaneous determination of gluten quality and quantity (AACC 38 - 12 A, 2000a). Its value expresses the weight percentage of the wet gluten remaining on a sieve after automatic washing with salt solution and centrifugation (Bonfil and Posner, 2012). The criterion of gluten index was evaluated whether the gluten quality is weak (GI < 30 %), normal (GI = 30 - 80 %), or strong (GI > 80 %) (Cubadda *et al.*, 1992). Wheat with similar protein contents can be classified according to GI values. In addition, it is evident that GI has been correlated with protein strength variable (Edwards *et al.*, 2007; Wang and Kovacs, 2002). Also, GI is an important index of gluten quality, seen to be an indicator of the status of the protein and often used to specify its technological usefulness (Oikonomou *et al.*, 2015).

In the research on genetic improvement of grain yield and bread-making quality of winter wheat from 1930 to 2015, Mirosavljevic *et al.* pointed out changes in gluten index indicated positive bi-linear trend of increase. There was an increase in GI with rate of 0.463 yr^{-1} until 1975, while GI improvement during the period (1975 - 2015) was 0.285 yr^{-1}. Comparison of growing seasons revealed that wheat cultivars achieved the highest GI in 2019, and the lowest in 2018. The average GI was 71.5 %, and among cultivars increased from 48.4 to 91.3 % (Mirosavljević *et al.*, 2020). In the Table 6, wet gluten and gluten index of bread wheat vary from 27.13 to 34.07 %, from 76.42 to 95.56 %, respectively.

Table 6: Means of some quality parameters of bread wheat

	Dhaka and Khatkar (1)	Makawi *et al.* (2)	Janczak-Pieniazek *et al.* (3)	Oelofse *et al.* (4)	Karaman (5)
WG (%)	30.32	34.07	27.13	-	33.50
GI (%)	76.42	81.86	95.56	-	-
SDS (ml)	47.10	-	-	63.64	-
FN (s)	539.80	520.35	332.83	389.18	-

1. (Dhaka and Khatkar, 2013); 2. (Makawi *et al.*, 2013); 3. (Jańczak-Pieniążek *et al.*, 2020); 4. (Oelofse *et al.*, 2010); 5. (Karaman, 2020). The data in the table was took an average value from the genotypes reported in each reference.

2.2.6.2 SDS Test

SDS sedimentation test was considered be a reliable, highly reproducible quality test that generally gives a good indication of the end-use quality of wheat (Blackman and Gill, 1980; Carter *et al.*, 1999). This method is correct in cases that wheat has a low to medium protein content, and have used to forecast protein quality and quantity (Carter *et al.*, 1999). Further, Galterio *et al.* reported that high SDS values are positively correlated with gluten quality (Galterio *et al.*, 2003, 2001). In their study, Oelofse *et al.* pointed out that there was highly an association significant between SDS sedimentation and protein content with mean value being 63.64 ml.

2.2.6.3 Falling Number

Falling number (FN) is the indicator of enzymatic activity contained in the flours. It has been used to detect the damage of storage matter of grain wheat endosperm by hydrolytic enzymes which are not only synthetized in consequence of start germination before harvest in grain (Konvalina *et al.,* 2008), but also substrates for the dough fermentation process (Kindred *et al.*, 2005; Ma *et al.*, 2009). A high falling number indicates minimal enzyme activity. Conversely, a low falling number indicates more substantial enzyme activity (Hrušková *et al.*, 2011).

According to Linina and Ruza, the falling number value depends significantly upon the weather conditions, grain storage time and the applied nitrogen dose (Liniņa and Ruža, 2015), especially the weather condition during harvest time (Konvalina *et al.* 2008). One of main factors impact on the falling number when the weather change is that α-amylase activity (Triboi 2003; Mašauskienė and Ceseviciene 2005; Skudra and Linina 2011). Either under rainy conditions or pre-harvest sprouting or sprouting during storage at a high temperature and humidity increases the level of α-amylase enzyme on wheat grains. As a result, wheat has a low FN and lower values of other quality elements (Kettlewell, 1999; Kondhare *et al.*, 2015; Krupnova and Svistunov, 2014; Lan *et al.*, 2005).

Another factor influencing to the falling number is the cultivar genetic characteristics (Liniņa and Ruža, 2012; Raza *et al.*, 2010). Numerous authors reported the variation of falling number of different varieties in the same growing conditions (Johansson, 2002; Liatukas *et al.*, 2012). For example, Gooding *et al.* found the difference of wheat cultivars as compared the data in middle of the 1970s with that one in the 1990s, although the climatic conditions and nitrogen fertilizer level were similar. Their results showed that cultivars planted in the middle of the 1970s had lower FN value than those cultivars planted in the 1990s (Gooding *et al.*, 1997).

Nitrogen (N) fertilizer is last factor influenced the falling number. Grain of winter wheat without nitrogen fertilizer tended to have a lower falling number (Clarke *et al.*, 2004; Knapowski and Ralcewicz, 2004). According to Knapowski and Ralcewics, the application of N 120 kg ha^{-1} rose considerably FN in winter wheat grains, as compared with the application of N 0 kg ha^{-1} (control treatment) and the object treated with N 80 kg ha^{-1} (Knapowski and Ralcewicz, 2004). In contrast, Kindred *et al.* totally disagreed with the above idea and pointed out that increasing nitrogen amount leads to lodging and associated sprouting in the ear. As a consequence, the falling number went down (Kindred *et al.*, 2005). Researching wheat in organic farming in Lithuania indicated that it had a lower α-amylase activity in comparison with conventional farming applying 120 kg N ha^{-1} (Basinskiene *et al.*, 2011). However, the influence of nitrogen fertilizer on falling number was lower than the one of variety and climatic conditions (Smith and Gooding, 1996).

2.2.6.4 The Rheological and Mixolab Parameters

Rheological traits are crucial for processing flour in the baking industry. This index is used for predicting dough-processing parameters and the end-product of quality. To investigate flour and dough characteristics, such as elasticity, viscosity, and extensibility, traditional rheological instruments such as farinograph, extensograph, and alveograph can be used. Nonetheless, with Mixolab II (Chopin Technologies, Paris, France), a new rheological device, researchers can measure the physicochemical behavior of dough during heating and cooling processes (Švec and Hrušková, 2015). During five stages in the process, Mixolab parameters are measured as the change of torque when mixing and heating wheat flour and water. It provides information about maximum torque, protein quality, starch characteristics, enzyme activity, and starch retrogradation (Harati *et al.*, 2020). In addition, other parameters such as water absorption, dough development time, stability, amplitude, slope α, β, and γ also appear as Mixolab analyzed.

From the point of material science view, glutens and doughs are most unique because of its complex behaviors (Abang Zaidel *et al.*, 2010). During the Mixolab analysis, complex chemical and physical transformations occur and proteins are considered as the important for bread-making quality (Payne *et al.*, 1987). The mixing disrupts the discrete masses of gluten proteins in wheat flour, hydrates them and the other flour constituents, and transfers elastic energy to dough (Belton, 2005). Many studies indicated that the flour composition, processing parameters, and ingredients affected directly to rheological properties of dough and gluten during the mixing process. According to Janssen *et al.*, Tronsmo *et al.*, Sliwinski *et al.*, and Chiang *et al.*, strong flour creates a better gluten and dough quality than the weak flour in comparison of giving a higher response in extensibility, bread loaf volume and height volume expansion (Chiang *et al.*, 2006; Janssen *et al.*, 1996; Sliwinski *et al.*, 2004; Tronsmo *et al.*, 2003). Besides, strong flour usually has a longer mixing time is expected for mixing dough from due to the dense particles of and slower water penetration (Hoseney, 1985). Cuq *et al.* pointed out that the mixing time and work input above the optimum level increase during mixing leading to the changes in mechanical properties of dough (Cuq *et al.*, 2002). Another factor influencing dough and gluten rheological properties is water. Adding too much water to the flour makes it slurry. In contrast, too little water results in slightly cohesive powder (Faubion and Hoseney, 1990). The optimum water level is different between flour and flour. The strong flours require more water level than weak flours because it has a higher protein content and dense particles (Sliwinski *et al.*, 2004).

2.2.7 The Pressing and Necessary Requirements of Value for Cultivation and Use for Organic Breeding Process

Although modern breeding process has achieved extraordinary successes in rising quantity and quality of cereal production by creating the modern varieties, however, modern varieties are suitable for conventional farming not for organic farming and low-input farming systems (Fischer and Edmeades, 2010; Löschenberger *et al.*, 2008). In general, organic grain yield is always lower than conventional agriculture by 20 - 30 %, even though this yield gap is greater, up to 50 % due to the absence of suitable varieties in the Czech Republic (Bueren *et al.*, 2002; de Ponti *et al.*, 2012; Konvalina *et al.*, 2011a; Mäder *et al.*, 2007). According to Konvalina *et al.*, although organic farming has undergone a significant development, it is still unevenly set up in various countries, and lack of suitable varieties for the sustainable farming systems. The main reasons of such a state are high costs for breeding, the weak and uneven representation of the organic farming. Almost any modern bred varieties of bread wheat being conventionally grown are not suitable for organic agriculture. The main reasons explained for this situation are the different selective criteria from the ones applied to the selection of varieties for organic farming. For example, less efficient root system, low competitiveness to weeds, low resistance to usual diseases or reducing baking quality provoked by a reduction of the proportion of nitrogen in the soil. Thus, in practice requires a new method to make sure that the best key traits such as weed competitiveness, nutrient efficiency and resistance to seed borne diseases are included. That the reason Value for Cultivation and Use (VCU) testing for organic farming was set up (Konvalina *et al.*, 2011b).

According to council directive 2002/53/EC, the European legislation for VCU approval says that “The value of a variety for cultivation or use shall be regarded as satisfactory if, compared to other varieties accepted in the catalogue of the Member State in question, its qualities, taken as a whole, offer, at least as far as production in any given region is concerned, a clear improvement either for cultivation or as regards the uses which can be made of the crops or the products derived therefrom. Where other, superior characteristics are present, individual inferior characteristics may be disregarded” (Council Directive 2002/53/EC). What is more, the EU seed legislation requires the VCU testing as one of the obligatory steps required for the registration of new varieties of arable crops. Therefore, new varieties to the market are only admitted when they have a clear improvement compared to the existing varieties.

The assessment of the suitability of new cultivars for organic farmers and the necessity to adapt the VCU protocol for cereals to the specific requirements of the organic sector has been conducted during the last decade by a number of EU countries such as Germany, Austria, Netherlands, and Switzerland. These needs include the evaluation of varieties for plant traits, which are not regularly observed in VCU, but plays an important role for organic farmers, such as weed competitiveness, resistance to seed borne diseases, and conducting the trials in organic fields (Rey *et al.*, 2008).

Regarding to the cost for breeding process, to release a winter wheat variety for organic farming of course, it has to be tested under conventional high input growing conditions for 11.605 €. Afterwards, it can be tested under organic farming conditions with a cost of 5.700 €. Thus, the breeders will break even when an area of 1.000 ha has to be planted by the customers of the seed. During VCU test process, the new cultivar has to be better just under organic growing conditions and the conventional testing is used to help the staff of the Federal Seed Office easier to describe the variety related to the conventional released varieties in the recommended list. It would be, however, much cheaper to make an additional page, leaflet, or website with the describing parameters, which were received under organic testing. This would also be of more interest for organic seed multipliers and growers (Mueller, 2008). Also, Mueller reported that to select the best adapted cultivar for the organic sector the trials should conduct under different regional organic farming conditions with seed from a certified organic production. A conventional variety testing could be avoided and given up. For the same cost, it would be better to have one or two more potential lines to be tested in order to release for organic farmers use than one variety tested additional under conventional farming only for tables of the Seed Office (Mueller, 2008).

In a VCU test to evaluate the diseases of winter wheat in organic farming conditions, Vija Strazdina V. and Sturite L. indicated that snow mould (*Fusarium nivale*), hard smut (*Tilletia tritici*), powdery mildew (*Blumeria graminis f.sp. tritici*), brown rust (*Puccinia triticina*), *Fusarium spp.*, *Mycosphaerella graminicola*, *Stagonosphora nodorum*, and *Pyrenosphora tritici- repentis* were main diseases. The economy losses might rise high infection with hard smut. Oat varieties had a good resistance to diseases such as (*Puccnia coronifera Cda.f.sp.avenae*, *Ustilago avenae*, *Erysphe graminis DC.f,sp.avenae*. For spring barley varieties, the most observed diseases were loose smut (*Ustilago nuda*), powdery mildew (*Blumeria graminis*), leaf rust (*Puccinia hordei*), net bloch (*Phyrenosphora teres*), leaf stripe (*Phyrenosphora graminea*). Especially, loose smut was the most dangerous disease and affect the yield and quality. In addition, based upon theirs results, they recommended that the duration of testing period have to be not less than three years due to the high variation between years and the capacity of tillering of barley varieties as well as its root systems, should pay more attention (Strazlina and Sturite, 2008).

3 Aims and Hypothesis

3.1 Aims of Thesis

The main goal of this dissertation was study on agronomic and quality characteristics of bread wheat varieties cultivated in organic farming system in the Czech Republic. The aims are presented in detail as below:

- To summarize knowledge about bread wheat cultivation, agronomic traits, nutritional composition, and technological quality parameters by using the available scientific literature data.

- To set up field experiments in three consecutive years to investigate and evaluate the agronomic traits.

- To analyze the basic parameters of baking quality such as protein content, wet gluten content, gluten index, SDS test, falling number, and rheological characteristics.

- To statistically analyze the data of field experiments.

- To highlight the potential wheat varieties in organic farming in the Czech Republic.

3.2 Hypothesis

- During field experiments will be possible to distinguish more and less perspective varieties of wheat for organic farming.

- During field experiments will be possible to distinguish more and less quality varieties of wheat for organic farming.

- The statistical analysis of data will find potential wheat cultivars for organic farming.

4 Materials and Methods

4.1 Field Experiment

The experimental material consisted of nine cultivars presented in the Table 7. The experiments were conducted from 2016 to 2019 (three growing seasons) in the southern Czech Republic at two sites, namely Ceske Budejovice (CB) and Zvikov (ZV). In Ceske Budejovice (Figure 3), the soil of experimental site was pseudo gley cambisols, kind of soil-loamy sand soil; the weather condition was mild warm climate; altitude of 388 m. In Zvikov (Figure 4), the soil of experimental site was loamy soil; altitude of 460 m. These experiments were carried out by using randomized complete block design with four replicates at each of the two sites. All cultivars were sown on the organic certified research area of the University of South Bohemia in Ceske Budejovice and Zvikov, the Czech Republic. Crop rotation belongs to legume family, with broad bean (*Vicia faba*) grown in Ceske Budejovice, and common pea (*Pisum sativum*) grown in Zvikov. The seeding rate was adjusted with a density of 350 germinal grains per m^2. The crop standards were treated in compliance with the European legislation (the European Council Regulation (EC) No. 834/2007, the European Commission Regulation (EC) No. 889/2008.

Table 7: List of all nine winter wheat varieties used in this study

No	Name of variety
1	Sultan
2	Zeppelin
3	Annie
4	Gordian
5	Penelope
6	Bernstein
7	Balitus
8	Wiwa
9	KM 15 - 17

Figure 3: The field trial in Ceske Budejovice under organic farming system (48.974790N, 14.444783E). Source: Google Map

Figure 4: The field stationary in Zvikov under organic farming system (48.9758531N, 14.6245594E). Source: Google Map

4.2 Evaluation of Agronomy Characteristics

Agronomy traits were evaluated according to the breeding and variety testing of bread wheat - *Triticum aestivum* L. for organic and low input farming of Agriculture Faculty, South Bohemia University (Konvalina *et al.*, 2007).

In this study, indexes were analysed including: The length of plant, the number of spikelets per m^2, thousand kernel weight (TKW), yield per hectare. Thousand kernel weight and yield per hectare were calculated at a humidity of 14 % (Curna, 2016).

4.3 Evaluation of Qualitative Parameters

4.3.1 Milling Flour

Common wheat samples were milled into white flours using a PSY MP 20 (Mezos, Hradec Kralove, Czech Republic) and Quadrumat Junior machine (Brabender, Duisburg, Germany).

4.3.2 The Baking Quality

4.3.2.1 The Gluten Index (AACC 38 - 12 A, 2000a)

The Gluten Index (GI) is defined as the percentage of wet gluten remaining on a special sieve when it is prepared and centrifuged to the prescribed standardized method. Gluten Index were measured by Glutomatic 2200 and Centrifuge 2015 (Perten Instruments, Hagersten, Sweden) according to AACC 38 - 12A method (AACC 38 - 12 A, 2000a), ICC (International Association for Cereal Chemistry) 155 (ICC - Standard No. 155, 1994), and ICC 158 (ICC - Standard No. 158, 1995).

$$Gluten\ Index = \frac{Wet\ Gluten\ remained\ on\ the\ sieve\ (g)}{Total\ Wet\ Gluten\ (g)} \times 100\ (\%)$$

The criterion of gluten index was evaluated as the Table 8 below.

Table 8: The evaluation of gluten index according to Cubadda *et al.* (Cubadda *et al.*, 1992)

Parameter	Weak flour	Normal flour	Strong flour
Gluten Index (%)	< 30	30 - 80	> 80

4.3.2.2 The Wet Gluten Content

Wet Gluten Content (WGC), the amount of wet gluten contained at 14 % moisture of each 100 g flour, was measured by Glutomatic 2200 and Centrifuge 2015 (Perten Instruments, Hagersten, Sweden).

$$Wet\ Gluten\ Content\ = Total\ Wet\ Gluten\ (g) \times 10$$

4.3.2.3 Protein Content

Protein content (PC) was determined by the Kjeltec 1002 System (Tecator AB, Hoganas, Sweden), based upon N * 5.7 (in dry matter).

4.3.2.4 Sodium Dodecyl Sulphate Test

Sodium dodecyl sulphate (SDS) was analyzed in flour samples according to the method of Axford *et al.* (Axford *et al.*, 1978).

4.3.2.5 The Falling Number

The falling number was determined by Perten Falling Number 1310 (Perten Instruments, Hagersten, Sweden) according to AACC 56 - 81 B and ICC Standard 107/1(AACC 56 - 81 B, 2000b; ICC - Standard No. 107/1, 1995). The falling number was evaluated as the table below.

Table 9: The evaluation of falling number

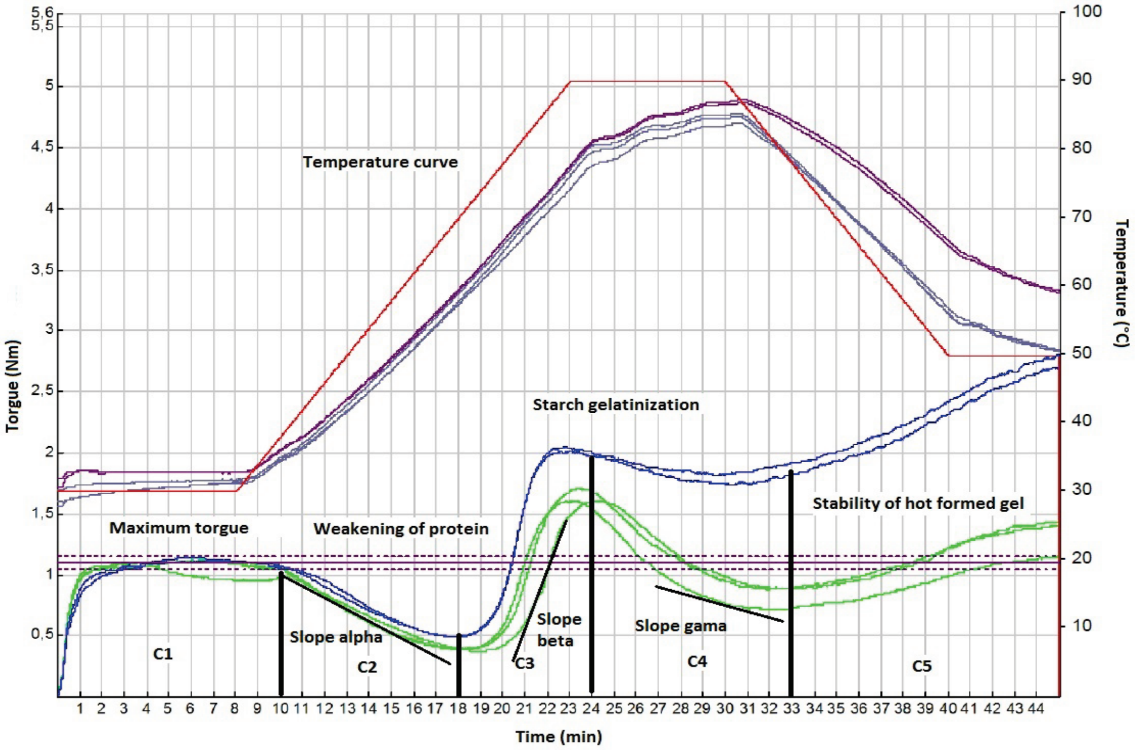
Value (s)	Enzymatic activity	Baking properties
< 150	High	Viscous crumb of bread
200 - 300	Optimal	Very good crumb of bread
> 300	Low	Dry crumb of bread, reduced loaf volume

4.3.2.6 The Dough Rheological Parameters

Mixolab II was used to evaluate baking quality according to the ICC standard method No. 173 - ICC 2006 (ICC - Standard No. 173, 2006), which allowed us to evaluate physical dough properties, such as dough stability or weakening, and starch characteristics in one measurement. For the rheological test, the varieties with four replicates from three years and two locations were analyzed. The evaluated parameters in Mixolab are illustrated in Figure 5, in which five stages can be distinguished.

In the first stage, hydration of the flour compounds occurs at 30 °C together with the stretching and alignment of the proteins, which leads to formation of the viscoelastic structure. An increase in the torque was observed during this stage until it reached the maximum value (1.10 Nm). The torque decreased to a minimum value in the second stage, which was attributed to the weakening of the protein network for mechanical shear stress and protein destabilization (Haros *et al.*, 2006; Rosell *et al.*, 2007). The third stage demonstrates an increased temperature and gelatinization of starch. The granules absorb the water available in the medium and they swell, so the viscosity increases. In the fourth stage, the amylase activity and the physical breakdown of the granules are associated with a reduction in the viscosity. A decrease in the temperature resulted in an increase in torque, which is referred to as setback and corresponds to the gelation process. The last stage is related to retrogradation (Rosell *et al.*, 2007). Temperature regime in Mixolab was as follows: 8 min at 30 °C, heating at a rate of 4 °C min^{-1} for 15 min, holding at 90 °C for 7 min, cooling to 50 °C at a rate of 4 °C min^{-1} for 10 min, and holding at 50 °C for 5 min (Schmiele *et al.*, 2017).

Figure 5: The Mixolab curves from wheat flour. Time for C1: The time evolution of the dough. The stronger the flour, the longer the time evolution (time to reach C1); Amplitude: The elasticity of the dough. The higher the value, the more flexible the flour; Stability: Resistance against kneaded dough. The longer the duration, the stronger the flour; C2: Attenuation of protein due to mechanical work and temperature; C3: The gelling starch; C4: The stability of the hot gel; C5: Measured starch retrogradation in the cooling phase; Guideline α (C1 - C2): Attenuation rate of protein in warming; Guideline β (C3 - C4): Speed starch gelatinization; Guideline γ (C5 - C4): The rate of enzymatic degradation (Konvalina *et al.*, 2017)



4.4 Statistical Analysis

Data were analyzed by using the Statistica 13 program (StatSoft. Inc., California, USA). Comparisons of mean varieties and their division into statistically different categories were conducted using the Tukey's honest significant difference (HSD) test with *p-value* < 0.01 and < 0.05 considered statistically significant. One-way ANOVA and factorial ANOVA were applied for variance analysis. Principal component analysis was used to assess the association between groups of variables and the differences among the study parameters.

5 Results and Discussion

Based upon the data from field stationary experiments being set up in two locations namely Ceske Budejovice and Zvikov in three years and the figures being analyzed in the laboratory as well as the weather data, the results and discussion are presented as below:

5.1 Agrometeorological Conditions

The weather condition data were collected during three years from September 2016 to August 2019 at Ceske Budejovice and Zvikov. The source of average data of thirty years (1989 - 2019) in South Bohemia region was from Czech Hydrometeorological Institute.

5.1.1 The Temperature Condition at the Research Places from 2016 to 2019

The temperature had variable for three years. In general, the temperature in Ceske Budejovice is higher than that one in Zvikov (Table 10), and the temperature of 2018 - 2019 was higher than the rest ones. As comparison with the average temperature of thirty years, the figures from Table 10 indicates that the temperature had increased in recent years and higher the average data, apart from the data of 2016 in September, the data of 2017 in January, the data of 2018 in March, and the data of 2019 in May.

Table 10: Monthly temperatures during three years at Ceske Budejovice and Zvikov compared to the average monthly temperature of thirty years

Month	Year						
	2016 - 2017		2017 - 2018		2018 - 2019		1989 - 2019
	CB	ZV	CB	ZV	CB	ZV	
September	11.34	10.96	12.61	11.21	14.92	14.31	12.37
October	8.10	7.65	10.63	10.06	10.06	9.78	7.71
November	3.20	2.78	4.54	4.08	4.43	3.92	2.85
December	- 0.10	- 2.26	1.23	0.55	2.80	1.93	- 0.73
January	- 4.85	- 4.92	3.34	2.83	0.04	- 1.04	- 1.59
February	2.57	2.41	- 2.30	- 3.51	1.91	1.81	- 0.61
March	6.89	6.11	2.25	1.66	6.88	6.05	3.03
April	7.95	6.97	13.80	12.62	10.03	9.12	7.70
May	14.51	13.43	16.61	15.58	11.26	10.67	12.55
June	19.53	18.66	17.99	17.18	21.55	20.73	15.95
July	19.67	18.94	19.65	18.95	19.78	19.03	17.66
August	19.71	19.12	21.03	20.21	20.95	19.14	17.22

CB: Ceske Budejovice. ZV: Zvikov.

5.1.2 The Precipitation at the Research Places from 2016 to 2019

The precipitation had a downtrend from September and hit the bottom in February before an uptrend to July. In contrast with the temperature, the precipitation in Ceske Budejovice was lower than that one in Zvikov. In the 2016 - 2017, the total precipitation was highest, followed by the 2018 - 2019, and the 2017 - 2018 (Table 11). In Zvikov, the total precipitation in the 2016 - 2017 and the 2018 - 2019 were higher than the total average precipitation of region. The data of the 2018 - 2019 was more fluctuation than the average data.

Table 11: Monthly and total precipitation during three years at Ceske Budejovice and Zvikov compared to the average monthly precipitation of thirty years

Month	Year						
	2016 - 2017		2017 - 2018		2018 - 2019		1989 - 2019
	CB	ZV	CB	ZV	CB	ZV	
September	24.20	37.20	31.80	24.80	73.00	67.20	56.87
October	52.00	61.80	38.60	35.20	28.60	41.80	47.26
November	36.60	39.20	34.00	39.90	31.20	39.20	42.35
December	16.20	18.20	22.40	31.80	54.80	73.00	40.65
January	5.60	10.80	20.20	24.00	30.20	61.80	40.29
February	16.80	23.20	15.80	19.80	22.20	33.20	32.58
March	26.60	28.80	22.00	25.20	20.80	36.80	45.77
April	90.00	122.20	12.80	20.60	40.20	42.00	41.00
May	33.60	52.00	106.00	156.60	61.60	62.00	73.00
June	64.40	86.20	104.80	96.20	64.40	76.20	88.87
July	104.60	124.60	32.80	86.80	83.40	104.20	92.48
August	157.20	171.20	55.80	35.20	60.20	71.20	82.94
$\sum P$	624.80	775.40	497.00	596.10	570.60	698.60	684.06

CB: Ceske Budejovice. ZV: Zvikov.

5.1.3 The Effect of Temperature and Precipitation on Growth and Development of Wheat

The hydrothermal coefficient (*HC*) was calculated according Selyaninov formula (Selyaninov, 1928) for projection the effect of temperature and precipitation on growth and development of winter wheat:

$$HC = \frac{Pm \times 10}{n \times Tm} \tag{1}$$

Where *Pm* is the monthly total precipitation (mm), n is the number of days in a month, and *Tm* is the monthly average of air temperatures (^{0}C).

Table 12: The hydrothermal coefficient of Selyaninov during the vegetation periods in three years at Ceske Budejovice and Zvikov

Month	Year					
	2017		2018		2019	
	HC in CB	*HC* in ZV	*HC* in CB	*HC* in ZV	*HC* in CB	*HC* in ZV
March	1.24	1.52	3.16	5.08	0.98	1.98
April	3.78	4.89	0.31	0.54	1.34	1.54
May	0.75	1.01	2.06	3.26	1.77	1.89
June	1.10	1.36	1.94	1.88	1.00	1.23
July	1.72	1.90	0.54	1.48	1.36	1.77
August	2.57	2.89	0.86	0.56	0.93	1.03

CB: Ceske Budejovice. ZV: Zvikov.

Table 13: Scale of the hydrothermal coefficient of Selyaninov and corresponding weather conditions (Rachoń *et al.*, 2020; Selyaninov, 1928)

$HC \leq 0.4$	Extremely Dry
$0.4 < HC \leq 0.7$	Very Dry
$0.7 < HC \leq 1.0$	Dry
$1.0 < HC \leq 1.3$	Fairly Dry
$1.3 < HC \leq 1.6$	Optimal
$1.6 < HC \leq 2.0$	Fairly Wet
$2.0 < HC \leq 2.5$	Wet
$2.5 < HC \leq 3.0$	Fairly Wet
$HC > 3.0$	Extremely Wet

The figures from Table 12 and Table 13 indicate that the growing season 2016 - 2017 started with optimal weather in March. However, in the stem elongation period, the weather was dry condition in CB and fairly dry in Zvikov. In the anthesis period (in June), the weather was optimal in Zvikov and fairly dry in CB. The ripening and harvest period was wet and fairly wet condition. For the growing season 2017 - 2018, starting with the extremely wet condition in March, the weather was very dry in April. The stem elongation stage met the wet weather. Fairly wet condition appeared in the heading stage. The ripening and harvest stage was very dry in CB but optimal condition in Zvikov. In the third growing season, the HC was different between CB and Zvikov in March. While the dry condition was in CB, the fairly wet condition was in Zvikov. The stem elongation period met the reasonable weather in both places. Starting heading period was fairly dry. The final stage met the fair-weather condition.

To sum up, the weather condition in the growing season 2018 - 2019 was more favorable than these one in 2016 - 2017, and 2017 - 2018 with the fairly steady precipitation distribution during growing season. In addition, the temperature and precipitation in May and June were more advantageous condition during the elongation phase, the heading and flower phase compared to the previous years. The weather in Zvikov was better than in Ceske Budejovice.

5.2 The Quantitative Parameters

Table 14 and Table 15 lists results of selected qualitative indicators of nine winter wheat varieties. The agronomical important indicators included plant height, the number of spikes per m^2, TKW, and yield were collected and analyzed for three years (growing season 2016 - 2017, growing season 2017 - 2018, and growing season 2018 - 2019) in Ceske Budejovice and Zvikov. The mean values and standard error of four replicates for the qualitative parameters are also displayed in Table 14 and Table 15 for each cultivar.

5.2.1 The Impact of Environment Effects on Plant Height, the Number of Spikes per m^2, Thousand Kernel Weight, and Yield

In the Table 14, the mean value of varieties significantly differed from year-to-year and locations. In details, an upward trend of plant height was witnessed from 2017 to 2019 by 18.87 cm. Also, the data in Zvikov was higher than in Ceske Budejovice. Analysis of variance of plant height (harvest year, $F = 695.29$, $p < 0.001$; location, $F = 119.43$, $p < 0.001$, species, $F = 179.25$, $p < 0.001$) was more influenced by harvest year, followed by species and location. The interaction of two factors (year × location, year × species, location × species), and the interaction of three factors (year × location × species) were significant at *p value* < 0.001 for Y × L and Y × S, and *p value* < 0.05 for L × S and Y × L × S. Similarly, an increasing tendency of the number of spikes per m^2 and yield were observed for three years. While the number of spikes per m^2 was more affected by harvest year ($F = 106.79$, $p < 0.001$, yield was more affected by location ($F = 1327.41$, $p < 0.001$). The yield in Zvikov was 1.72 ton higher than in Ceske Budejovice. This could be explained by the different environment between two locations. The ANOVA of the number of spikes per m^2 showed that there was more interaction between Y × L ($F = 23.94$, $p < 0.001$) than the others. The ANOVA of yield was not significant in the interaction of Y × L, and L × S, but significant for Y × L and

Y × L × S with $p < 0.001$. Thousand kernel weight was a little different trend when compared to others in quantitative parameters. TKW increased in 2018 before witnessing a decrease in the next year. The analysis of variance of TKW were significant difference with *p value* < 0.001 for main effects, the interaction of two factors and three factors. Results from Table 14 indicated that location with F = 128.55 was more impacted than harvest year and species with F = 117.88, F = 107.00, respectively.

Table 14: Means ± standard error (SE) and ANOVA F-value (p-value) for the effects of harvest year, location, and wheat species on plant height, the number of spikes per m^2, thousand grain weight, and yield

	Plant height (cm)	Number of spikes per m^2	TKW (g)	Yield ($ton.ha^{-1}$)
Harvest year				
2017	64.90 ± 0.74 c	296.38 ± 6.51 c	41.01 ± 0.32 c	2.82 ± 0.12 c
2018	71.94 ± 0.95 b	327.29 ± 5.98 b	43.88 ± 0.42 a	3.78 ± 0.11 b
2019	83.77 ± 1.52 a	391.47 ± 8.85 a	42.00 ± 0.34 b	4.55 ± 0.13 a
Location				
ZV	75.82 ± 1.25 a	362.66 ± 7.24 a	43.18 ± 0.32 a	4.58 ± 0.09 a
CB	71.25 ± 1.06 b	314.10 ± 5.89 b	41.42 ± 0.29 b	2.86 ± 0.08 b
ANOVA				
Main effects				
Harvest year (Y)	695.29 (<0.001)	106.79 (<0.001)	117.88 (<0.001)	450.17 (<0.001)
Location (L)	119.43 (<0.001)	80.30 (<0.001)	128.55 (<0.001)	1327.41 (<0.001)
Species (S)	179.25 (<0.001)	14.92 (<0.001)	107.00 (<0.001)	23.31 (<0.001)
Interactions				
Y × L	27.14 (<0.001)	23.94 (<0.001)	29.79 (<0.001)	2.48 (ns)
Y × S	12.47 (<0.001)	2.60 (<0.05)	9.86 (<0.001)	2.79 (<0.001)
L × S	3.47 (<0.05)	2.07 (<0.05)	7.66 (<0.001)	0.78 (ns)
Y × L × S	2.63 (<0.05)	2.32 (<0.05)	7.26 (<0.001)	3.61 (<0.001)

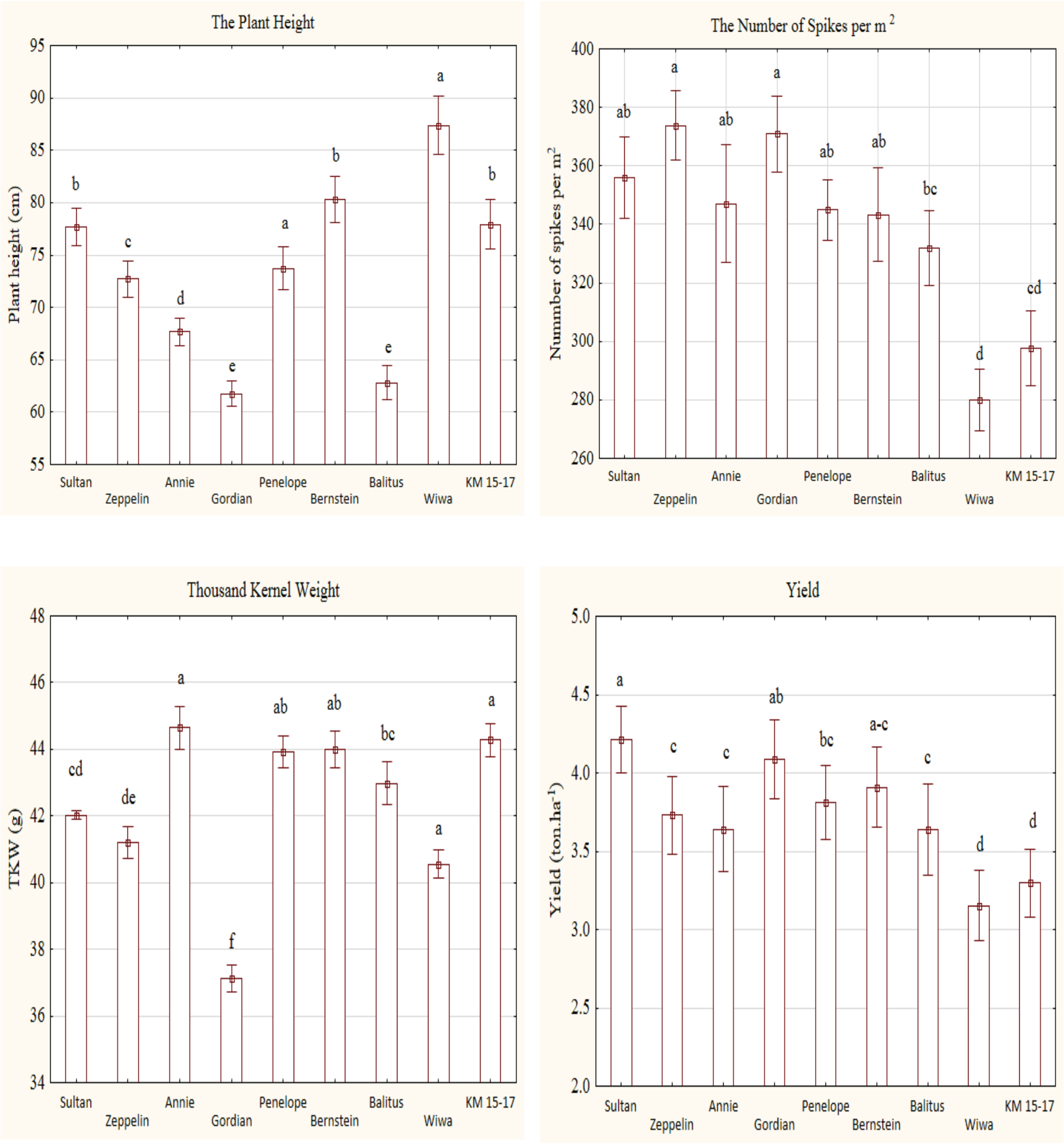
Mean values associated with different lowercase letters are significant different within each column analyzed by one-way ANOVA, Turkey's test, *p value* < 0.05. ns: Not significant. CB: Ceske Budejovice. ZV: Zvikov.

Table 15: Agronomic traits of different wheat varieties for three years (from 2016 to 2019)

Genotype	Plant height (cm)	Number of spike per m^2	TKW (g)	Yield ($ton.ha^{-1}$)
Sultan	77.66 ± 1.74 b	355.87 ± 13.93 ab	42.03 ± 0.13 cd	4.21 ± 0.21 a
Zeppelin	72.70 ± 1.70 c	373.83 ± 11.94 a	41.20 ± 0.46 de	3.73 ± 0.24 c
Annie	67.66 ± 1.32 d	347.12 ± 20.17 ab	44.64 ± 0.64 a	3.64 ± 0.27 c
Gordian	61.75 ± 1.22 e	370.87 ± 13.12 a	37.12 ± 0.41 f	4.09 ± 0.25 ab
Penelope	73.70 ± 2.06 c	344.91 ± 10.26 ab	43.91 ± 0.48 ab	3.81 ± 0.23 bc
Bernstein	80.29 ± 2.16 b	343.37 ± 16.04 ab	43.99 ± 0.55 ab	3.90 ± 0.25 a-c
Balitus	62.79 ± 1.63 e	331.95 ± 12.76 bc	42.97 ± 0.64 bc	3.63 ± 0.29 c
Wiwa	87.37 ± 2.77 a	279.87 ± 10.58 d	40.54 ± 0.42 e	3.15 ± 0.22 d
KM 15 - 17	77.91 ± 2.35 b	297.62 ± 12.94 cd	44.28 ± 0.49 a	3.29 ± 0.21 d

Mean values associated with different lowercase letters are significant different within each column analyzed by one-way ANOVA, Turkey’s test, *p value* < 0.05. ns: Not significant.

Figure 6: The agronomic characteristics of nine wheat varieties at two locations, four replicates, in three years (N = 216). Box plots represent the interquartile range, the columns in the box symbolize the mean value, and whiskers designate the minimum and maximum of the box plot



The independent of year and growing location, Table 15, and Figure 6 point out the comparison of nine varieties. The plant height ranged from 61.75 ± 1.22 cm to 87.37 ± 2.77 cm and was statistically different among varieties. Wiwa cultivar had the highest plant height with 87.37 cm followed by Bernstein, KM 15 - 17, and Sultan, whereas Balitus and Gordian had the lowest one with 62.79 cm and 61.75 cm, respectively. To compare with the results of plant height in organic farming (Table 3), our results is lower than that one of Konvalina *et al.*; and is nearly similar to the results of Gevrek and Atasoy (Gevrek and Atasoy, 2012; Konvalina *et al.*, 2011).

The number of spikes per m^2 of different varieties was not likewise the plant height. The group of cultivars having the highest number were Zeppelin, Gordian, Sultan, Penelope, and Bernstein with the number of spikes being 373.83 ± 11.94, 370.87 ± 13.12, 355.87 ± 13.93, 344.91 ± 10.26, and 343.37 ± 16.04, respectively. The lowest number of spikes per m^2 belonged to KM 15 - 17 (297.62 ± 12.94) and Wiwa (279.87 ± 10.58). In a study reported by Cox *et al.*, the number of spikes per m^2 of winter wheat ranged from 503 to 585 in organic farming, these findings are almost double higher than our results (Cox *et al.*, 2019).

In spite of belonging to the lowest number of spikes per m^2, KM 15 - 17 cultivar together with Annie, Bernstein, and Penelope had the highest mean of TKW ranging from 44.28 ± 0.49 to 43.91 ± 0.48 g and were significantly difference, while Zeppelin and Wiwa had the lowest mean of TKW with 41.20 ± 0.46 g, 40.54 ± 0.42 g, respectively. These figures in our study are higher as compared to the results of other authors namely Konvalina *et al.*, Tran *et al.*, and Cox *et al.* (Cox *et al.*, 2019; Konvalina *et al.*, 2011; Tran *et al.*, 2020).

The yield of studied cultivar varied between 3.15 ± 0.22 $ton.ha^{-1}$ and 4.21 ± 0.21 $ton.ha^{-1}$. Sultan, Gordian, and Bernstein had the highest yield with 4.21 ± 0.21 $ton.ha^{-1}$, 4.09 ± 0.25 $ton.ha^{-1}$, and 3.90 ± 0.25 $ton.ha^{-1}$, respectively, vice versa was true for KM 15 - 17 (3.29 ± 0.21 $ton.ha^{-1}$) and Wiwa (3.15 ± 0.22 $ton.ha^{-1}$). The comparison of grain yield with different authors depicted that the yield of wheat cultivars in our experiments are lower than the findings of Cox *et al.* Ceseviciene *et al.*, and Konvalina *et al.*, whilst it is higher the results in previous study and over double higher than these one of Gevrek and Atasoy (Ceseviciene *et al.*, 2009; Cox *et al.*, 2019; Gevrek and Atasoy, 2012; Konvalina *et al.*, 2011; Tran *et al.*, 2020).

From the results and discussion above, there was an increase trend in all agronomical traits for three years. This could be explained by the impact of weather condition on these parameters.

5.3 Flour Quality Parameters

The flour quality parameters assessment was completed in the laboratory for indexes such as protein content, SDS test, falling number, gluten index, and wet gluten according to standardized tests for three years, eight replicates per cultivar (exception Falling number index with four replicates per cultivar) with nine cultivars in two locations, (N = 432).

5.3.1 The Protein Content

Protein content was analyzed by the Kjeltec 1002 System (Tecator AB, Hoganas, Sweden), based upon N * 5.7 (in dry matter) according to Kjeldahl method. Results from Table 16, Table 17, and Figure 7 illustrated that the amount of protein content went down during three years from 11.80 % to 10.78 %, decreasing by 1.02 %. The significant differences in statistic were found for this index between years, locations, and varieties. Analysis of variance of protein content (harvest year, $F = 114.49$, $p < 0.001$; location, $F = 40.51$, $p < 0.001$, species, $F = 98.33$, $p < 0.001$) indicated that obviously, harvested year and species were more influenced than location. The interaction of two factors (year × location, location × species) were significant with *p value* < 0.001, while the interaction of three factors (year × location × species) and the interaction between year and location were insignificant. Compared to all cultivar, Wiwa had the highest amount of protein content with 12.92 ± 0.16 %, followed by Bernstein, KM 15 - 17, and Annie with 11.72 ± 0.12, 11.70 ± 0.12, and 11.47 ± 0.07, respectively. The lowest protein content was found in the flour of Zeppelin with 10.32 ± 0.16 %. These results are out of ranging of protein content (12.9 - 19.9 %) in the research of Shewry *et al.*, and Tran *et al.* exception Wiwa cultivar (Shewry *et al.*, 2013; Tran *et al.*, 2020). Unlike the yield parameter, protein content gradually went down in the year by year. This is suitable with the Shewery *et al.* citation, who wrote that higher yield results mainly from increased accumulation of starch, which dilutes other grain components including protein (Shewry *et al.*, 2020).

Table 16: Means ± standard error (SE) and ANOVA F-value (p-value) for the effects of harvest year, location, and wheat species on protein content, SDS test, falling number, gluten index, and wet gluten

	Protein (%)	SDS test (ml)	FN (s)	GI (%)	WG (%)
Harvest year					
2017	11.80 ± 0.11 a	57.57 ± 1.17 ns	321.37 ± 6.26 b	86.91 ± 1.42 c	22.72 ± 0.36 a
2018	11.21 ± 0.10 b	57.29 ± 1.37 ns	321.30 ± 6.86 b	91.81 ± 0.76 b	20.91 ± 0.37 b
2019	10.78 ± 0.11 c	57.29 ± 1.48 ns	384.98 ± 6.02 a	96.72 ± 0.35 a	19.52 ± 0.28 c
Location					
ZV	11.09 ± 0.09 b	57.24 ± 1.03 ns	338.00 ± 5.54 b	95.82 ± 0.31 a	20.41 ± 0.26 b
CB	11.44 ± 0.09 a	57.53 ± 1.16 ns	347.11 ± 6.32 a	87.81 ± 1.05 b	21.68 ± 0.33 a
ANOVA					
Main effects					
Harvest year (Y)	114.49 (<0.001)	0.31 (ns)	139.7 (<0.001)	86.28 (<0.001)	113.82 (<0.001)
Location (L)	40.51 (<0.001)	0.72 (ns)	135.2 (<0.001)	172.57 (<0.001)	53.97 (<0.001)
Species (S)	98.33 (<0.001)	468.64 (<0.001)	112.6 (<0.001)	19.33(<0.001)	92.34 (<0.001)
Interactions					
Y × L	1.51 (ns)	85.22 (<0.001)	34.3 (<0.001)	45.13 (<0.001)	6.75 (<0.001)
Y × S	2.45 (<0.001)	10.63 (<0.001)	9.1 (<0.001)	1.22 (ns)	4.69 (<0.001)
L × S	2.96 (<0.001)	21.19 (<0.001)	7.2 (<0.001)	7.48 (<0.001)	1.68 (ns)
Y × L × S	1.24 (ns)	6.20 (<0.001)	7.7 (<0.001)	2.10 (<0.05)	4.03 (<0.001)

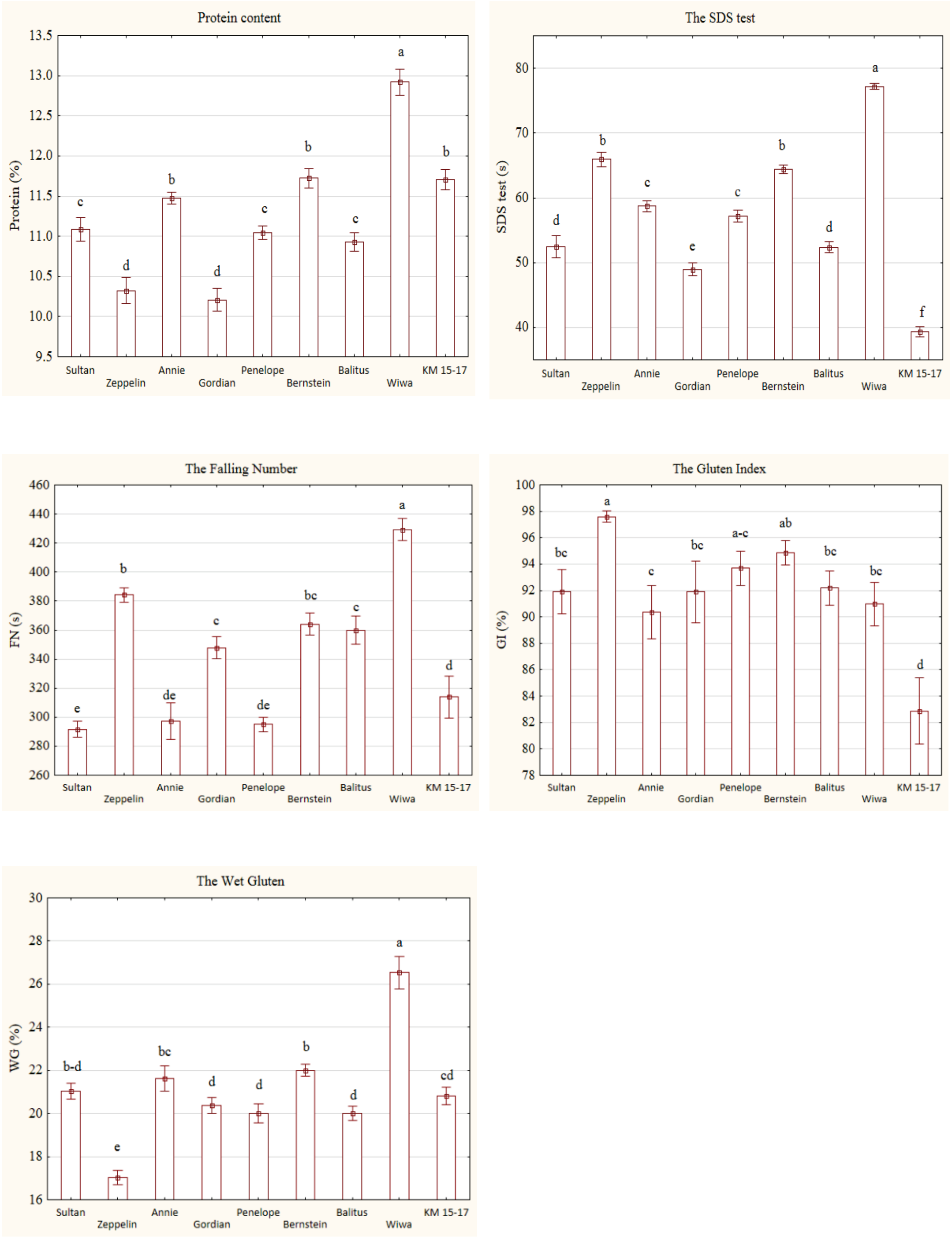
Mean values associated with different lowercase letters are significant different within each column analyzed by one-way ANOVA, Turkey's test, *p value* < 0.05. ns: Not significant. CB: Ceske Budejovice. ZV: Zvikov.

Table 17: Quality traits of different wheat varieties for three years

Genotype	Protein (%)	SDS test (ml)	FN (s)	GI (%)	WG (%)
Sultan	11.08 ± 0.15 c	52.44 ± 1.72 d	291.75 ± 5.41 e	91.91 ± 1.66 bc	21.04 ± 0.36 b-d
Zeppelin	10.32 ± 0.16 d	65.94 ± 1.13 b	384.16 ± 4.95 b	97.59 ± 0.43 a	17.02 ± 0.32 e
Annie	11.47 ± 0.07 b	58.70 ± 0.80 c	297.29 ± 12.67 de	90.34 ± 2.01 c	21.63 ± 0.58 bc
Gordian	10.21 ± 0.14 d	48.97 ± 0.92 e	347.83 ± 7.68 c	91.90 ± 2.34 bc	20.37 ± 0.35 d
Penelope	11.04 ± 0.08 c	57.18 ± 0.90 c	295.00 ± 5.09 de	93.68 ± 1.32 a-c	20.01 ± 0.44 d
Bernstein	11.72 ± 0.12 b	64.38 ± 0.62 b	364.08 ± 7.69 bc	94.87 ± 0.91 ab	22.00 ± 0.27 b
Balitus	10.93 ± 0.11 c	52.36 ± 0.87 d	359.87 ± 9.81 c	92.19 ± 1.29 bc	20.01 ± 0.32 d
Wiwa	12.92 ± 0.16 a	77.17 ± 0.51 a	429.16 ± 7.49 a	90.96 ± 1.67 bc	26.53 ± 0.76 a
KM 15 - 17	11.70 ± 0.12 b	39.30 ± 0.78 f	313.83 ± 14.30 d	82.86 ± 2.50 d	20.80 ± 0.39 cd

Mean values associated with different lowercase letters are significant different within each column analyzed by one-way ANOVA, Turkey's test, *p value* < 0.05. ns: Not significant.

Figure 7: Contents of protein, SDS test, the falling number, gluten index, and wet gluten. Figures are presented as the mean of nine wheat species grown at two locations, four replications, in three years. Box plots represent the interquartile range, the columns in the box symbolize the mean value, and whiskers designate the minimum and maximum of the box plot



5.3.2 The SDS Test

Sodium dodecyl sulphate (SDS) test was determined in flour samples according to the method of Axford *et al.* This method quoted the link with superior baking quality and stronger gluten (Axford *et al.*, 1978). The mean values of SDS test were not significant difference among years and locations. The data from Table 16 indicated that species had more effect on SDS test parameter with F = 468.64, $p < 0.001$, while locations and harvest year was not an effect on this index. In addition, the interaction between two factors and three factors were found with *p value* < 0.001. The SDS test of all varieties ranged from 39.30 ± 0.78 ml to 77.17 ± 0.51 ml. The highest sedimentation volume was observed in the Wiwa variety whilst the lowest one belonged to KM 15 - 17 variety. The second highest SDS test included Zeppelin and Bernstein with 65.94 ± 1.13 ml, and 64.38 ± 0.62 ml, respectively. In comparison to other results, the sedimentation volume in our study is higher than the results of Dhaka and Khatkar (47.10 ml), apart from KM 15 - 17 variety; and lower than the ones of Oelofse *et al.* (63.64 ml), exception in wheat cultivar Wiwa, Zeppelin, and Bernstein. According to approved methods of analysis for sedimentation test for wheat, wheat flour having SDS test volume from 45 to 65 ml belongs to the good bread making. Thus, the cultivars in our study are classified as good bread making variety, exception KM 15 - 17 (AACC 56 - 61.02, 1999).

5.3.3 The Falling Number

According to ICC Standard No. 107/1, the time in seconds required to stir and to allow a viscometer stirrer to fall a measured distance through a hot aqueous meal, flour of starch gel undergoing liquefaction as a result of alpha-amylase activity is called the falling number (ICC - Standard No. 107/1, 1995). The impact of years, locations, and varieties as well as the interaction were found in the falling number. What is more, the falling number mean values were almost similar between 2017 and 2018, but it was significant difference from the data of 2019 with approximately 63.65 second less. The figures also show that the falling number in Zvikov was longer 9,11 s than in Ceske Budejovice. Regarding to the interactions (Table 16), the influence of environment and variety on the mean values of the falling number was highly statistically significant with *p value* < 0.001. Compared to all cultivars, Wiwa had the highest falling number with 429.16 ± 7.49 s, followed by Zeppelin (384.16 ± 4.95 s) and Bernstein (364.08 ± 7.69 s). The lowest falling number group was Annie, Penelope, and Sultan with 297.29 ± 12.67 s, 295.00 ± 5.09 s, and 291.75 ± 5.41 s, respectively. According to the findings of Dhaka and Khatkar, and Makawi *et al.*, the mean values of falling number were 539.80 s, and 520.35 s, respectively, which is higher than our results, while the figures of Janczak-Pieniazek *et al.*, and Oelofse ranging from 332.83 s to 389.18 s were located in the middle of our data ranging from 291.75 to 429.16 s. To evaluate the baking properties and the enzymatic activity of wheat flour, the AACC 56 - 81 B and ICC Standard 107/1 were used (AACC 56 - 81 B, 2000b; ICC - Standard No. 107/1, 1995). Thus, the varieties having falling number are less than 300 s such as Sultan, Gordian, and Annie have the optimal activity as well as very good crumb of bread. The remainder varieties have the low enzymatic activity and are dry crumb of bread and reducing loaf volume.

5.3.4 The Gluten Index

The gluten index, related to physicochemical traits of wheat flour and its quality, is a measurement of wheat protein that provides a simultaneous determination of gluten quality and quantity (AACC 38 - 12 A, 2000a). From the Table 16, Table 17, and Figure 7, an increasing tendency of GI was observed via the different year study from 86.91 % to 96.72 %. Thus, GI increased by 9.81 % for three years. The figures pointed out that the impact of location was higher than harvest year and species, but the interaction between year and species was not found. Three years analysis of nine wheat genotypes confirmed the statistically significant difference. The group having the highest GI was Zeppelin (97.59 ± 0.43 %), Bernstein (94.87 ± 0.91 %), and Penelope (93.68 ± 1.32 %). Conversely, KM 15 - 17 had the lowest GI with 82.86 ± 2.50 %. According to Cubadda *et al.*, gluten index was classified as three groups: Less than 30 % belonging to the weak flours; from 30 to 80 % belonging to the normal flour; greater than 80 % belonging to the strong flour (Cubadda *et al.*, 1992). In comparison with the results of Cubadda *et al.*, the varieties in our experiments are higher 80 % gluten index, therefore, they are categorized the strong flour. Furthermore, the findings of the present study are higher these ones reported by Makawi *et al.*, and Dhaka and Khatkar, who wrote out the variation in gluten index of winter wheat ranging from 64.53 to 93.34 %, and from 59.2 to 99.3 %, respectively (Dhaka and Khatkar, 2013;

Makawi *et al.*, 2013). However, the results reported by Janczak-Pieniazek *et al.* are higher than that of our results (Jańczak-Pieniążek *et al.*, 2020).

5.3.5 The Wet Gluten

The wet gluten content was measured by Glutomatic 2200 and Centrifuge 2015 (Perten Instruments, Hagersten, Sweden). Unlike with the GI trend, wet gluten declined gradually for three years by 3.2 % and was influenced by harvest year with $F = 113.82$, $p < 0.001$. The interaction of two factors and three factors were found with *p value* < 0.001, apart from the interaction between location and year. However, the wet gluten had the same tendency with protein content. The highest wet gluten was observed in the Wiwa cultivar (26.53 ± 0.76 %) in comparison to other wheat cultivars. In contrast, KM 15 - 17 and Zeppelin had the lowest one with 20.80 ± 0.39 % and 17.02 ± 0.32 %, respectively. In general, although all varieties in this study have higher the percentage of gluten index, the amount of wet gluten is lower than the data studied by Dhaka and Khatkar, Makawi *et al.*, and Janczak-Pieniazek *et al.* (Dhaka and Khatkar, 2013; Jańczak-Pieniążek *et al.*, 2020; Makawi *et al.*, 2013).

5.4 The Rheological Evaluation of Different Wheat Flour

Mixolab II was used to evaluate baking quality according to the ICC standard method No. 173 - ICC 2006 (ICC - Standard No. 173, 2006), which allows to the characterization of physico-chemical dough behavior properties as submitted to a dual mixing and temperature constraints. It is, therefore, possible to record the mechanical changes as a result of mixing and heating simulating the mechanical work and the heat conditions that might be expected through the baking process (Rosell *et al.*, 2007).

Mixolab parameters were measured in four replicates per sample (9 varieties in three years at two locations, N = 216). The mean values of each variety for water absorption, time of C1, amplitude, the stability, torque C1, torque C2, torque C3, torque C4, torque C5, and slope α, β, and γ are summarized in Table 18.

The water absorption of all wheats varied in a relatively narrow, ranging from 55.24 to 62.05 % and were significant difference. In comparison to all cultivar, Annie had the highest mean values of water absorption, whereas Sultan had the lowest one with 55.24 %. It is said that this parameter was affected by the protein quality and starch properties. According to Mixolab manual, a wheat flour sample is considered strong when its value presented water absorption over 55 g.100 g^{-1} or 55 %. To compare with the results of Banfalvi *et al.* (Bánfalvi *et al.*, 2020) with water absorption varying from 57.5 to 64.1 %, our results are a little lower, but they also are over 55 % water absorption, an indicator for good baking quality, which is in agreement in the results of Voicu *et al.*, who reported that the water absorption of wheat flour is less than 54 % producing dough that forms fast, but quickly degrading in the final fermentation and showing a poorer-quality bread making at the end (Voicu *et al.*, 2017).

The first stage of Mixolab analysis supplies the information about the mixing properties related to mainly to gluten quality and the behavior of the flour protein complex which is impacted by thermomechanical changes (Bánfalvi *et al.*, 2020; Magdaléna Lacko-Bartošová *et al.*, 2019; Rosell *et al.*, 2007). The dough development time (C1 time) is an essential index, which is known as the dough development or the gluten development time. Longer C1 time was observed for three varieties namely Wiwa, Annie, Sultan, and Balitus with mean value respectively being 3.18 s, 2.96 s, 2.78 s, and 2.74 s. Similarly, the stability plays an important role in the first stage. This index reflects the resistance of dough against intensive mixing. From the Table 18 shows that Wiwa, Penelope, and Bernstein cultivar belonged the group having the longest stability with 8.76 minutes, 8.47 minutes, and 8.36 minutes, respectively. In contrast, Annie (6.99 minutes), Sultan (6.74 minutes), Gordian (6.45 minutes), and KM 15 - 17 (6.34 minutes) had the shortest stability. As expected, torque C1 was insignificant difference among varieties and varied in a small narrow, which ranged from 1.09 to 1.11 Nm. In term of amplitude, only Annie variety was statistically significant difference as compared with other varieties and had the lowest number (0.06). In general, the dough development time ranges from 0.99 to 7.36 minutes. Our results were categorized this ranging. In comparison with the results of Banfalvi *et al.*, C1 time in our study was shorten than the mean value of Banfalvi *et al.* (4.71 minutes), however, the stability of some varieties in our study were higher 8.12 minutes. The previous our study showed that the C1 time of Vanek and SW Kardili are nearly double longer time than all varieties in this study. The stability is also higher than these ones. Normally, wheat flour having longer dough development time and stability are related to better gluten quality (Rasper and Walker, 2000). As reported by Lundh and Macritchie (Lundh and Macritchie, 1989), the difference in proportion of glutenin results in the differences of dough development time. The greater proportion of gluten wheat flour gets, the better baking performance and stronger dough properties wheat flour has. The results of Moreira *et al.*, was confirmed the cite above and pointed that longer dough stability are associated with strong flour (Moreira *et al.*, 2011). Thus, a stronger flour having the longer dough development time (≥ 3 minutes) longer dough stability (≥ 4 minutes) was suitable for Wiwa, Annie, Sultan and Balitus.

In the second stage where torque C2 and slope α were evaluated. This stage occurs when the temperature is about 52 - 57 ^{0}C. This phase gives the information about the weakening of proteins due to protein denaturation through the changes on their quaternary, tertiary, and secondary structures during mechanical processing and temperature (Schmiele *et al.*, 2017). Figures in Table 18 confirmed the differences among studied cultivars for torque C2 and slope α parameters. The highest value of torque C2 was recorded for Gordian with 0.52 Nm, followed by Bernstein and Wiwa with 0.48 Nm, vice versa was true for Sultan, Penelope, Balitus, and Annie, ranging from 0.44 to 0.42 Nm. The slope α implying the speed of the protein network attenuating varied from - 0.09 to - 0.07 and was significant difference. According to Lacko-Bartosova *et al.*, Banfalvi *et al.*, Svec and Hruskova, Schmiele *et al.*, Wiwart *et al.*, and Dhaka *et al.*, strong wheat flour has the C2 value higher than 0.4 Nm. If this value ranges from 0.5 to 0.6 Nm, it refers to good quality protein, the higher ability resistance of gluten to heating, and stronger gluten network (Bánfalvi *et al.*, 2020; M. Lacko-Bartošová *et al.*, 2019; Schmiele *et al.*, 2017; Švec and Hrušková, 2015; Dhaka *et al.*, 2014; Wiwart *et al.*, 2017). Like that, accompanying with the dough development time, and the dough stability, the torque C2 is also related to the properties of gluten. The results of the value C2 torque analyzed in our study was higher 0.4 Nm (exception KM 15 - 17), especially, Gordian cultivar had the value higher than 0.5 Nm. The explanation for KM 15 - 17 was due to the fact that it had the lowest gluten index as compared with other varieties. To sum up, all wheat samples in this study were strong flour and generally satisfactory for bread making, apart from KM 15 - 17. In term of slope α, the findings in this study varying from - 0.09 to - 0.07 are higher than the results of Wiwart *et al.* (- 0.096) (Wiwart *et al.*, 2017).

The third stage, characterized by torque C3 and slope β parameters, has the heating until reaching 70 ^{0}C. Although dough remained under constant mixing, the process of protein denaturation and starch gelatinization stopped (Schmiele *et al.*, 2017). Results from Table 18 confirmed that torque C3 ranged from 1.50 to 1.83 Nm. Maximum torque C3 was recorded for Batilus, Wiwa, Bernstein, and Sultan with 1.83 Nm, 1.82. Nm, 1.81 Nm, and 1.76 Nm, respectively.

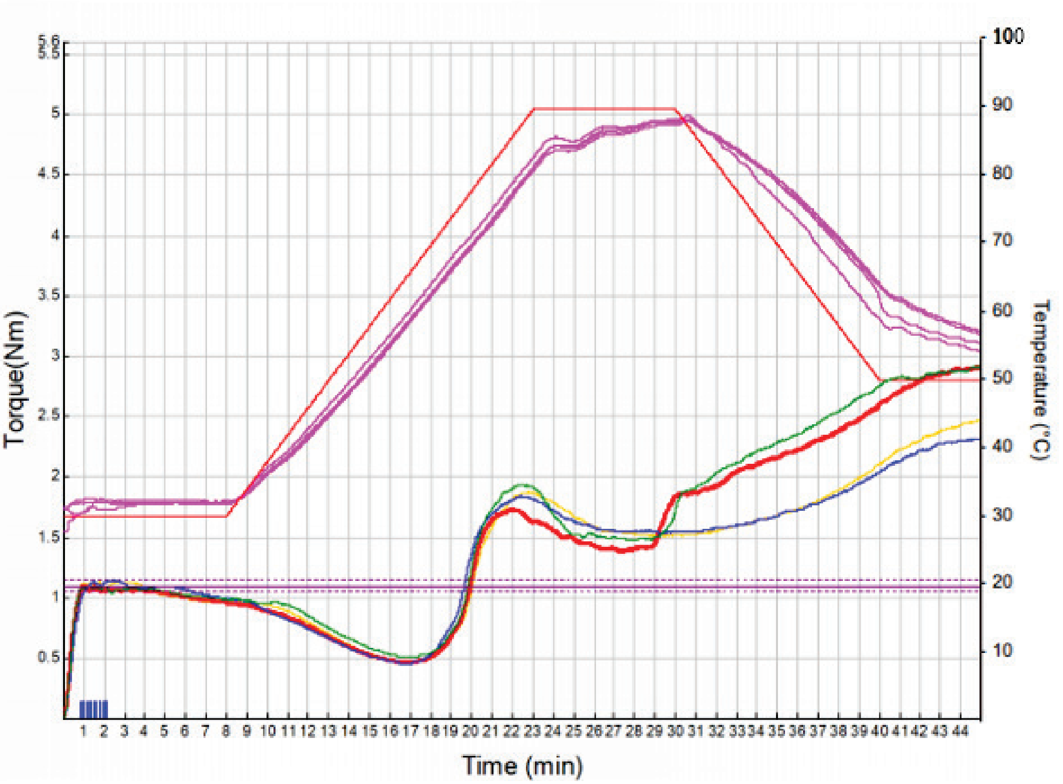
Table 18: Data obtained from Mixolab parameters for the rheological evaluation of nine wheat varieties

Genotype	Water absorption (%)	Time of C1 (min)	Torque C1 (Nm)	Alfa	Amplitude	Stability (min)
Sultan	55.24 ± 0.13 h	2.78 ± 0.12 a-c	1.11 ± 0.00 (ns)	-0.08 ± 0.00 ab	0.08 ± 0.00 a	6.74 ± 0.30 e
Zeppelin	59.23 ± 0.13 d	2.38 ± 0.08 cd	1.10 ± 0.00 (ns)	-0.08 ± 0.00 ab	0.08 ± 0.00 a	7.60 ± 0.40 cd
Annie	62.05 ± 0.25 a	2.96 ± 0.09 ab	1.09 ± 0.00 (ns)	-0.07 ± 0.00 a	0.06 ± 0.00 b	6.99 ± 0.27 de
Gordian	56.90 ± 0.16 f	2.35 ± 0.08 cd	1.10 ± 0.00 (ns)	-0.07 ± 0.00 a	0.08 ± 0.00 a	6.45 ± 0.27 e
Penelope	58.07 ± 0.22 e	2.59 ± 0.10 bc	1.09 ± 0.00 (ns)	-0.09 ± 0.00 cd	0.08 ± 0.00 a	8.47 ± 0.25 ab
Bernstein	58.36 ± 0.11 e	2.60 ± 0.15 c	1.09 ± 0.00 (ns)	-0.09 ± 0.00 cd	0.09 ± 0.00 a	8.36 ± 0.25 a-c
Balitus	55.83 ± 0.11 g	2.74 ± 0.22 a-c	1.09 ± 0.00 (ns)	-0.08 ± 0.00 bc	0.08 ± 0.00 a	7.68 ± 0.28 b-d
Wiwa	59.76 ± 0.18 c	3.18 ± 0.18 a	1.10 ± 0.00 (ns)	-0.09 ± 0.00 d	0.09 ± 0.00 a	8.76 ± 0.13 a
KM 15 - 17	60.49 ± 0.14 b	1.89 ± 0.10 d	1.09 ± 0.00 (ns)	-0.08 ± 0.00 b	0.08 ± 0.00 a	6.34 ± 0.24 e
Genotype	Torque C2 (Nm)	Beta	Torque C3 (Nm)	Gamma	Torque C4 (Nm)	Torque C5 (Nm)
Sultan	0.44 ± 0.01 cd	0.53 ± 0.03 b-d	1.76 ± 0.04 a-c	-0.09 ± 0.02 b	1.39 ± 0.05 b	2.48 ± 0.05 b
Zeppelin	0.45 ± 0.01 c	0.44 ± 0.01 e	1.73 ± 0.01 b-d	-0.04 ± 0.01 a	1.50 ± 0.02 ab	2.30 ± 0.05 c
Annie	0.42 ± 0.01 d	0.40 ± 0.01 e	1.50 ± 0.03 f	-0.07 ± 0.00 b	0.94 ± 0.05 d	1.57 ± 0.08 f
Gordian	0.52 ± 0.01 a	0.47 ± 0.05 c-e	1.60 ± 0.08 e	-0.06 ± 0.01 ab	1.46 ± 0.02 ab	2.60 ± 0.04 ab
Penelope	0.44 ± 0.01 cd	0.46 ± 0.02 de	1.69 ± 0.03 cd	-0.06 ± 0.01 ab	1.24 ± 0.06 c	2.10 ± 0.09 d
Bernstein	0.48 ± 0.01 b	0.63 ± 0.02 a	1.81 ± 0.01 ab	-0.07 ± 0.01 ab	1.48 ± 0.02 ab	2.60 ± 0.05 ab
Balitus	0.44 ± 0.01 cd	0.60 ± 0.02 ab	1.83 ± 0.02 a	-0.07 ± 0.01 b	1.53 ± 0.04 a	2.70 ± 0.07 a
Wiwa	0.48 ± 0.01 b	0.56 ± 0.02 ab	1.82 ± 0.01 a	-0.06 ± 0.01 ab	1.55 ± 0.02 a	2.61 ± 0.04 a
KM 15 - 17	0.39 ± 0.00 e	0.55 ± 0.03 a-c	1.67 ± 0.04 de	-0.07 ± 0.01 ab	1.16 ± 0.08 c	1.81 ± 0.11 e

Mean values associated with different lowercase letters are significant different within each column analyzed by one-way ANOVA, Turkey's test, *p value* < 0.05. ns: Not significant.

Conversely, minimum torque C3 was recorded for Annie with 1.50 Nm. These results are similar to with our previous study (Tran *et al.*, 2020), are lower than the results of Wiwart *et al.*, but are higher than the ones of Banfalvi *et al.* (Bánfalvi *et al.*, 2020; Wiwart *et al.*, 2017). Regarding to the slope β parameter, an indicator for the starch gelatinization speed, the mean values of Bernstein, Balitus, Wiwa, and KM 15 - 17 were found to be significantly higher than other varieties and higher the findings of Wiwart *et al.* (0.57). This means that they had faster gelatinization process.

Figure 8: The line graph of four cultivars throughout Mixolab II. Red line: Wiwa; Green line: Bernstein: Yellow line: Sultan; Blue line: Gordian; Light red: Block temperature; Purple line: Dough temperature



In the fourth stage, the parameters torque C4 and slope γ were assessed. This phase relates to the heat stability of the starch gel as the temperatures increasing over 80 ^{0}C and the resistance of starch against the enzymatic hydrolysis due to amylase agent. The results of the assessment of different cultivar in Table 18 indicate that the torque C4 fluctuated from 0.94 to 1.55 Nm. A statistically significant difference was witnessed for all varieties with the highest value belonging to Wiwa (1.55 Nm), Balitus (1.53 Nm), Zeppelin (1.50 Nm), Bernstein (1.48 Nm), and Gordian (1.46 Nm). In contrast, the minimum value was recorded for Annie with 0.94 Nm. In comparison with the findings other authors, our results were lower, especially for Annie variety. This implied that the resistance of starch against the enzymatic hydrolysis by amylose and the hot gel stability of our sample were lower than the counterpart results. The slope γ displays the speed of enzymatic degradation of starch and the heating stability of starch gel. Unlike the slope α and β, the slope γ was more variation from - 0.09 to - 0.04. However, only Zeppelin was significant difference with Balitus, Annie, and Sultan, the remainder varieties was similar when compared together. The results of Wiwart *et al.* ranged from - 0.06 to - 0.10 was a little lower than our results.

In the final stage, the ability of retrogradation of starch granules during the cooling phase at 58 - 60 ^{0}C was assessed. The values of torque C5 varied from 1.57 to 2.70 Nm. Significantly, the higher value of torque C5 was noted in reference to Balitus (2.70 Nm), Wiwa (2.61 Nm), Gordian (2.60 Nm), and Bernstein (2.60 Nm), whereas Annie had the lowest value of torque C5 with 1.57 Nm. These results, higher than our previous study and higher the results of standard sample flour reported by Svec and Hruskova (Švec and Hrušková, 2015), indicated a high level of starch retrogradation.

5.5 The Correlation among Agronomical Traits, Quality Parameters and Mixolab Parameters and Statistics Analyzed by Principal Component Analysis

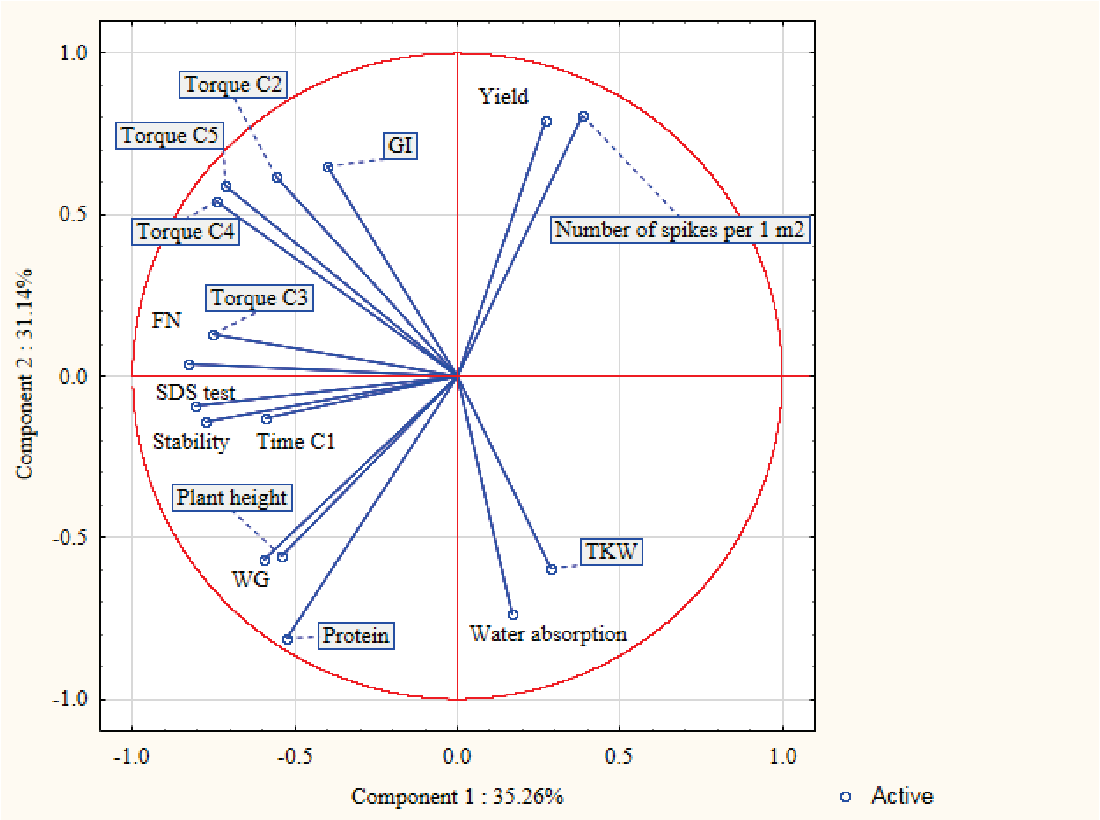
Analyzing the quantity and quality parameters of nine varieties in order to find the relationships among them, principal component analysis was used. The first five principal components accounted for 93.88 % of the variability of all variables. The first two principal components constituting 66.40 % was used to explain the correlation among parameters. The loading plots of PCA are displayed in Table 19 and Figure 9.

Table 19: Factor coordinates of the variables based on correlations

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Plant height	- 0.54	**- 0.56**	- 0.06	0.23	- 0.03
Number of spikes per 1 m^2	0.39	**0.81**	0.41	0.02	0.02
TGW	0.29	**- 0.59**	0.25	**0.66**	- 0.04
Yield	0.27	**0.79**	0.16	0.14	- 0.42
FN	**- 0.83**	0.04	- 0.10	- 0.26	0.45
SDS test	**- 0.81**	- 0.09	**0.55**	- 0.07	0.12
GI	- 0.40	**0.65**	**0.59**	0.20	0.16
WG	**- 0.60**	- 0.57	- 0.10	- 0.31	- 0.45
Protein	- 0.52	**- 0.81**	- 0.07	- 0.01	- 0.21
Time C1	**- 0.59**	- 0.13	**0.50**	- 0.10	- 0.48
Stability	**- 0.77**	- 0.14	0.39	0.32	0.09
Torque C3	**- 0.75**	0.13	- 0.35	0.53	0.06
Torque C2	-0.56	**0.62**	0.07	- 0.46	- 0.11
Torque C4	**- 0.74**	0.54	- 0.33	0.08	0.17
Torque C5	**- 0.71**	0.59	- 0.34	0.07	- 0.11
Water absorption	0.17	**- 0.74**	0.38	- 0.33	0.38

The first component, accounting for 35.26 % of the variation, was negatively associated to the parameters such as the falling number, SDS test, the stability of dough, torque C4, torque C3, torque C5, the wet gluten, and time C1 with loading factors - 0.83, - 0.81, - 0.77, - 0.75, - 0.74, - 0.71, - 0.60, and - 0.59, respectively. The second principal component, representing 31.14 % of the variability, was positively associated to the number of spikes per m^2, yield, gluten index, and torque C2 with loading factor being 0.81, 0.79, 0.65, and 0.62, respectively. In contrast, negatively loading factors were observed for protein, water absorption, thousand kernel weight, and plan height with - 0.81, - 0.74, - 0.59, and - 0.56, respectively. The third component constituted 11.50 % with positively loading factors for gluten index (0.59), SDS test (0.55), and time C1 (0.50). The fourth component accounted for 9.00 % with positively loading factor for the thousand kernel weight (0.66). The torque C1, the slope α, β, and γ were not presented in Table 19 and Figure 9 due to the results of PCA depicted that they were not important variable.

Figure 9: The projection of study variables based on correlations



From the Figure 9, strong correlations were seen among the falling number, SDS test, the stability, time C1, and torque C3; among Protein, wet gluten, and plant height; among gluten index, torque C2, torque C5, and torque C4; between yield and the number of spikes per m^2. What is more, SDS test was found closely with the stability, time C1, and torque C3, and gluten index was positive correlation with torque C2, which are in agreement with the results of Dhaka *et al.* and Collar *et al.* reported the correlations among SDS test, time C1, the stability and torque C3 and between gluten index with torque C2 (Collar *et al.*, 2007; Dhaka *et al.*, 2014). Also, the figures showed that the yield variable was strongly negative correlation with protein, this implies higher yield are relation to the lower protein content as the results reported by Jablonskyte-Rasce *et al.* (Jablonskytė-Raščė *et al.*, 2013). Similar to the results of Spanic *et al.*, our results indicated the strong correlation between protein and wet gluten, but no correlation was seen among protein, wet gluten and gluten index (Spanic *et al.*, 2021). Regarding to agronomic traits, plant height and TKW had a negative correlation with the yield, while the number of spikes per m^2 showed an opposite trend. This indicates that the number of spikes per m^2 in our experiments was a decisive factor to the yield, which is in concordance with Mason *et al.* and

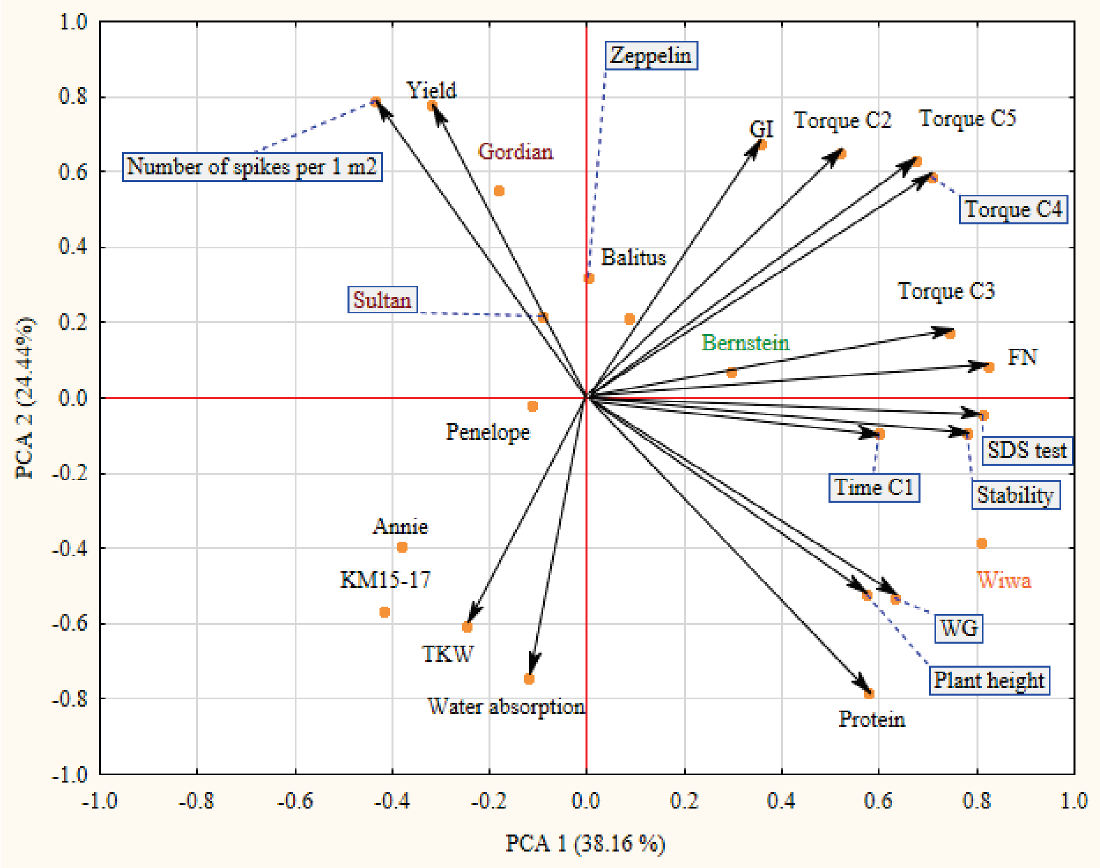
Mayer *et al.*, who reported that the number of spikes per m^2 was the important factor of yield component responsible for the wheat yield in organic farming (Mason *et al.*, 2007; Mayer *et al.*, 2015).

5.6 The Identification of Varieties Association with High Yield and Good Baking Quality

The principal component analysis of quantitative and quality attributes of wheat flours accounting for 62.6 % of the variability was explained by the first two components shown in Figure 10.

Obviously, the Sultan and Gordian variety were located near the loading of yield. This mean that they have the highest yield than other varieties. In contrast, the position of Wiwa cultivar was in close proximity to the protein, WG, and the quality parameters such as time C1 the stability, SDS test, the falling number, and torque C3. Thus, Wiwa cultivar was seen a potential cultivar for good baking performance. For KM 15 - 17, Annie, and Penelope, an opposite direction with the loading of quality parameters was observed in biplots because they had the lowest ones. In our study, a successful cultivar having both high yield and good baking performance is that Bernstein because it is situated in the middle of loading yield and loading quality traits in PCA 1.

Figure 10: Principal component analysis biplot based upon various quantitative and quality traits of nine wheat cultivars using mean value of two locations in three years



6 Conclusions

The wheat cultivar demand for organic farming has been increasing rapidly in the recent year, which requires breeders applying new selection methodologies to release new varieties suitable for organic agriculture. In this study, nine winter wheat varieties were cultivated under the conditions of organic agriculture system at two locations in the south region of Czech Republic and were analyze and assessed according to the standard methods. Having discussed the results in relation to the scope of research, there are some main conclusions drawn from this dissertation with regard to research on the agronomic and quality characteristics of modern wheat cultivars in organic farming.

6.1 The Agronomical Parameters

- The results of analyses depict that the environment affected to the quantitative parameters for different years and locations study. In detail, the plant height, and the number spikes per m^2 were impacted by different years more than locations and species. The factors such as harvest year, location, and species are the same effect on TWK. Yield is strongly affected by location more than harvest year and species.

- The weather condition in 2018 - 2019 was favor with the vegetation and development phase of wheat with the precipitation steadily distribution during growing season and the temperature suitable for wheat growing in sensitive periods.

- The Sultan, Gordian, and Bernstein cultivar having the highest yield are selected for the potential high yield cultivar.

- There is a strong relationship between the number of spikes per m^2 and yield.

6.2 The Quality Parameters

- The effects of harvest year, location, and species on protein content, SDS test, the falling number, gluten index, and wet gluten are found. The harvest of different years is more effect on protein content and wet gluten than species and location. The gluten index is affected by location more than harvest year and location. SDS test is only impacted by species.

- All varieties are classified as strong flour with high SDS test and Gluten index, apart from KM 15 - 17.

- A strong correlation is observed between SDS test and falling number and between wet gluten and protein, fair correlation is true between SDS test and wet gluten.

- The best quality is detected for Wiwa cultivar, which is characterized the highest protein content. SDS test and wet gluten.

6.3 The Rheological Properties

- The water absorption of all wheats, ranging from 55.24 to 62.05 %, belongs to strong flour group.

- The best rheological behavior is found for Wiwa, Annie, Sultan and Balitus cultivar with the longer dough development time (≥ 3 minutes), longer dough stability (≥ 4 minutes), and higher C2 value (≥ 0.4 Nm).

- The high level of starch retrogradation is found in Balitus, Wiwa, Gordian, and Bernstein cultivar.

- A strong correlation is seen between the time C1 and the stability dough, and between torque C4 and torque C5. Fair strong is observed between GI and torque C2.

6.4 The Conclusions Based upon PCA

- Sultan and Gordian are the potential high yield cultivar.

- Wiwa is the good bread making cultivar.

- Bernstein is a successful cultivar having both high yield and good baking performance.

7 Recommendations

- Further research in different regions is necessary to assesses the adaptation of all varieties in this study.

- Further agronomical traits should research such as the number of grains per spike, the weight of grains per spike, the weed competitiveness, and the resistance to usual diseases to be able to take appropriate conclusion.

- To expand the growing area for Bernstein cultivar.

- Wiwa cultivar has the potential bread making, and Sultan and Gordian have the potential high yield that should use for the breeding process in organic farming in the near future.

8 References

1. AACC 38 - 12 A, 2000a. Approved Methods of the American Association of Cereal Chemists, 10th edn. St. Paul : American Association of Cereal Chemists.

2. AACC 56 - 61.02, 1999. Approved Methods of Analysis, 11th Edition - AACC Method 56 - 61.02. Sedimentation Test for Wheat [WWW Document]. URL http://methods.aaccnet.org/summaries/56 - 61 - 02.aspx (accessed 4.13.21).

3. AACC 56 - 81 B, 2000b. Approved Methods of The American Association of Cereal Chemists, 10th edn. St. Paul : American Association of Cereal Chemists.

4. Abang Zaidel, D.N., Chin, N., Yusof, Y.A., 2010. A Review on Rheological Properties and Measurements of Dough and Gluten. Journal of Applied Sciences 10. https://doi.org/10.3923/jas.2010.2478.2490.

5. Abdel-All, E.S.M., Hucl, P., Sosulski, F., 1995. Compositional and Nutritional Characteristics of Spring Einkorn and Spelt Wheats. Cereal Chemistry 62, 621 - 624. https://www.cerealsgrains.org/publications/cc/backissues/1995/Documents/72_621.pdf.

6. Abdolshahi, R., Nazari, M., Safarian, A., Sadathossini, T.S., Salarpour, M., Amiri, H., 2015. Integrated Selection Criteria for Drought Tolerance in Wheat (*Triticum aestivum* L.) Breeding Programs Using Discriminant Analysis. Field Crops Research 174, 20 - 29. https://doi.org/10.1016/j.fcr.2015.01.009.

7. Aisawi, K. a. B., Reynolds, M.P., Singh, R.P., Foulkes, M.J., 2015. The Physiological Basis of the Genetic Progress in Yield Potential of CIMMYT Spring Wheat Cultivars from 1966 to 2009. Crop Science 55, 1749 - 1764. https://doi.org/10.2135/cropsci2014.09.0601.

8. Akhtar, M.Z., Khan, M., Ahmad, K., Alam, M., 2001. Evaluation of Wheat (T*riticum aestivum* L.) Varieties for Their Potential Grain Yield under the Agro-ecological Conditions of D.I.Khan. Biology Journal of Biological Sciences. https://doi.org/10.3923/JBS.2001.568.570.

9. Anderson, O.D., Greene, F.C., 1997. The α-gliadin Gene Family.II. DNA and Protein Sequence Variation, Subfamily Structure, and Origins of Pseudogenes. Theoretical and Applied Genetics 95, 59 - 65. https://doi.org/10.1007/s001220050532.

10. Andersson, A.A.M., Andersson, R., Piironen, V., Lampi, A.-M., Nyström, L., Boros, D., Fraś, A., Gebruers, K., Courtin, C.M., Delcour, J.A., Rakszegi, M., Bedo, Z., Ward, J.L., Shewry, P.R., Åman, P., 2013. Contents of Dietary Fibre Components and Their Relation to Associated Bioactive Components in Whole Grain Wheat Samples from the HEALTHGRAIN Diversity Screen. Food Chemistry, ASSET 2011 136, 1243 - 1248. https://doi.org/10.1016/j.foodchem.2012.09.074.

11. Arterburn, M., Murphy, K., Jones, S.S., 2012. Wheat: Breeding for Organic Farming Systems, Bueren E. T. L. and Myers J. R. ed. Wiley-Blackwell. ISBN: 978-0-470-95858-2.

12. Arzani, A., Ashraf, M., 2017. Cultivated Ancient Wheats (*Triticum* spp.): A Potential Source of Health-Beneficial Food Products: Ancient Wheats for Healthy Foods…. Comprehensive Reviews in Food Science and Food Safety 16, 477 - 488. https://doi.org/10.1111/1541-4337.12262.

13. Arzani, A., Ashraf, M., 2016. Smart Engineering of Genetic Resources for Enhanced Salinity Tolerance in Crop Plants. Critical Reviews in Plant Sciences 35, 146 - 189. https://doi.org/10.1080/07352689.2016.1245056.

14. Ashraf, M., 2014. Stress-induced Changes in Wheat Grain Composition and Quality. Critical Reviews in Food Science and Nutrition 54, 1576 - 1583. https://doi.org/10.1080/10408398.2011.644354.

15. Assefa, A., 2017. Response of Bread Wheat (*Triticum aestivum* L.) Varieties to Different Seeding Rate for Growth, Yield and Yield Components in Kombolcha District, North-Eastern Ethiopia. Journal of Biology, Agriculture and Healthcare 7, 79. https://www.iiste.org/Journals/index.php/JBAH/article/view/40190.

16. Axford, D., Mcdermott, E., Redman, D., 1978. Small-Scale Tests of Bread-Making Quality. Cereal Chemistry 18 - 20.

17. Bánfalvi, Á., Németh, R., Bagdi, A., Gergely, S., Rakszegi, M., Bedő, Z., Láng, L., Vida, G., Tömösközi, S., 2020. A Novel Approach to the Characterization of Old Wheat (*Triticum aestivum* L.) Varieties by Complex Rheological Analysis. Journal of the Science of Food and Agriculture 100, 4409 - 4417. https://doi.org/10.1002/jsfa.10479.

18. Bar-Yosef, O., 1998. On the Nature of Transitions: The Middle to Upper Palaeolithic and the Neolithic Revolution. Cambridge Archaeological Journal. https://doi.org/10.1017/S0959774300000986.

19. Basinskiene, L., Gražina, J., Kalvaityte, V., Ceseviciene, J., Leistrumaite, A., 2011. Enzyme Activity of Different Cereals Grown Using Organic and Conventional Agricultural Practices, in: Foodbalt-2011, Innovations for Food Science and Production. Presented at the The Baltic Conference on Food Science and Technology, Latvia, pp. 27 - 32.

20. Beche, E., Benin, G., da Silva, C.L., Munaro, L.B., Marchese, J.A., 2014. Genetic Gain in Yield and Changes Associated with Physiological Traits in Brazilian Wheat during the 20th century. European Journal of Agronomy 61, 49 - 59. https://doi.org/10.1016/j.eja.2014.08.005.

21. Bekes, F., Zawistowska, U., Zillman, R.R., Bushuk, W., 1986. Relationship Between Lipid-content and Composition and Loaf Volume of 26 Common Spring Wheats. Cereal Chemistry 63, 327 - 331.

22. Belton, P.S., 2005. New Approaches to Study the Molecular Basis of The Mechanical Properties of Gluten. Journal of Cereal Science, New Approaches in Cereal Science 41, 203 - 211. https://doi.org/10.1016/j.jcs.2004.06.003.

23. Berry, P.M., Kendall, S., Rutterford, Z., Orford, S., Griffiths, S., 2015. Historical Analysis of The Effects of Breeding on The Height of Winter Wheat (*Triticum aestivum*) and Consequences for Lodging. Euphytica 203, 375 - 383. https://doi.org/10.1007/s10681-014-1286-y.

24. Bonfil, D.J., Posner, E.S., 2012. Can Bread Wheat Quality Be Determined by Gluten Index? Journal of Cereal Science 56, 115 - 118. https://doi.org/10.1016/j.jcs.2012.07.003.

25. Bonjean, A.P., Angus, W.J. (Eds.), 2001. The World Wheat Book: A History of Wheat Breeding, 1st edition. ed. Lavoisier, Londres.

26. Bradová, J., Šašek, A., 2005. Diversity of Gliadins and HMW Glutenin Subunits in Czech Registered Wheat Varieties. Czech Journal of Genetics and Plant Breeding 41, 160 - 163. https://doi.org/10.17221/6159-CJGPB.

27. Brandolini, A., Hidalgo, A., Moscaritolo, S., 2008. Chemical Composition and Pasting Properties of Einkorn (*Triticum monococcum* L. subsp. *monococcum*) Whole Meal Flour. Journal of Cereal Science 47, 599 - 609. https://doi.org/10.1016/j.jcs.2007.07.005.

28. Brandolini, A., Hidalgo, A., Plizzari, L., Erba, D., 2011. Impact of Genetic and Environmental Factors on Einkorn Wheat (*Triticum monococcum* L. subsp. *monococcum*) Polysaccharides. Journal of Cereal Science 53, 65 - 72. https://doi.org/10.1016/j.jcs.2010.09.008.

29. Branlard, G., Dardevet, M., Saccomano, R., Lagoutte, F., Gourdon, J., 2001. Genetic Diversity of Wheat Storage Proteins and Bread Wheat Quality, in: Bedö, Z., Láng, L. (Eds.), Wheat in a Global Environment: Proceedings of the 6th International Wheat Conference, 5 - 9 June 2000, Budapest, Hungary, Developments in Plant Breeding. Springer Netherlands, Dordrecht, pp. 157 - 169. https://doi.org/10.1007/978-94-017-3674-9_18.

30. Bueren, E.L.V., Myers, J.R. (Eds.), 2012. Organic Crop Breeding. Wiley-Blackwell. https://research.wur.nl/en/publications/organic-crop-breeding.

31. Bueren, E.T.L.V., Struik, P.C., Jacobsen, E., 2002. Ecological Concepts in Organic Farming and Their Consequences for an Organic Crop Ideotype. NJAS 50, 1 - 26. https://library.wur.nl/ojs/index.php/njas/article/view/401.

32. Bushuk, W., Rasper, V.F., 1994. Wheat: Production, Properties and Quality. Springer US. https://doi.org/10.1007/978-1-4615-2672-8.

33. Bustos, D.V., Hasan, A.K., Reynolds, M.P., Calderini, D.F., 2013. Combining High Grain Number and Weight Through a DH-population to Improve Grain Yield Potential of Wheat in High-yielding Environments. Field Crops Research 145, 106 - 115. https://doi.org/10.1016/j.fcr.2013.01.015.

34. Carter, B.P., Morris, C.F., Anderson, J.A., 1999. Optimizing the SDS Sedimentation Test for End-Use Quality Selection in a Soft White and Club Wheat Breeding Program. Cereal Chemistry 76, 907 - 911. https://doi.org/10.1094/CCHEM.1999.76.6.907.

35. Ceseviciene, J., Leistrumaite, A., Paplauskienė, V., 2009. Grain Yield and Quality of Winter Wheat Varieties in Organic Agriculture. Agronomy Research 7, 217 - 223. https://agronomy.emu.ee/vol07Spec1/p7sI12.pdf.

36. Chen, X., Hao, M.-D., 2015. Low Contribution of Photosynthesis and Water-use Efficiency to Improvement of Grain Yield in Chinese Wheat. Photosynthetica 53, 519 - 526. https://doi.org/10.1007/s11099-015-0147-9.

37. Chen, X., Min, D., Yasir, T.A., Hu, Y.-G., 2012. Evaluation of 14 Morphological, Yield-related and Physiological Traits as Indicators of Drought Tolerance in Chinese Winter Bread Wheat Revealed by Analysis of the Membership Function Value of

Drought Tolerance (MFVD). Field Crops Research 137, 195 - 201. https://doi.org/10.1016/j.fcr.2012.09.008.

38. Chiang, S.-H., Chen, C.-S., Chang, C.-Y., 2006. Effect of Wheat Flour Protein Compositions on the Quality of Deep-fried Gluten Balls. Food Chemistry 97, 666 - 673. https://doi.org/10.1016/j.foodchem.2005.04.030.

39. Chung, O.K., 1986. Lipid-protein Interactions in Wheat Flour, Dough, Gluten, and Protein Fractions. Cereal Foods World 31, 242 - 256.

40. Clarke, M., Gooding, M., Jones, S., 2004. The Effects of Irrigation, Nitrogen Fertilizer and Grain Size on Hagberg Falling Number, Specific Weight and Blackpoint of Winter Wheat. Journal of the Science of Food and Agriculture 84, 227 - 236. https://doi.org/10.1002/jsfa.1657.

41. Collar, C., Bollain, C., Rosell, C.M., 2007. Rheological Behaviour of Formulated Bread Doughs During Mixing and Heating. Food Science and Technology International 99 - 107. https://journals.sagepub.com/doi/10.1177/1082013207078341.

42. Council Directive 2002/53/EC, n.d. Council Directive 2002/53/EC on the Common Catalogue of Varieties of Agricultural Plant Species. [WWW Document]. URL https://www.ecolex.org/details/legislation/council-directive-200253ec-on-the-common-catalogue-of-varieties-of-agricultural-plant-species-lex-faoc038667/ (accessed 4.16.21).

43. Cox, W., Cherney, J., Sorrells, M., 2019. Organic Compared with Conventional Wheat Had Competitive Yields during the Early Years of Organic Production in the Northeast USA. Agronomy 9, 380. https://doi.org/10.3390/agronomy9070380.

44. Crosbie, G.B., 1991. The Relationship between Starch Swelling Properties, Paste Viscosity and Boiled Noodle Quality in Wheat Flours. Journal of Cereal Science 13, 145 - 150. https://doi.org/10.1016/S0733-5210(09)80031-3.

45. Cubadda, R. (Molise U., Carcea, M., Pasqui, L.A., 1992. Suitability of the Gluten Index Method for Assessing Gluten Strength in Durum Wheat and Semolina. Cereal Foods World (USA).

46. Cunsolo, V., Foti, S., Saletti, R., 2004. Mass Spectrometry in the Characterisation of Cereal Seed Proteins. European Journal of Mass Spectrometry (Chichester) 10, 359 - 370. https://doi.org/10.1255/ejms.609.

47. Cuq, B., Yildiz, E., Kokini, J., 2002. Influence of Mixing Conditions and Rest Time on Capillary Flow Behavior of Wheat Flour Dough. Cereal Chemistry 79. https://doi.org/10.1094/CCHEM.2002.79.1.129.

48. Curna, V., 2016. Agronomic Performance and Quality Parameters. The Dissertation.

49. Darnhofer, I., Lindenthal, T., Bartel-Kratochvil, R., Zollitsch, W., 2010. Conventionalisation of Organic Farming Practices: from Structural Criteria towards an Assessment Based on Organic Principles. A review. Agronomy for Sustainable Development 30, 67 - 81. https://doi.org/10.1051/agro/2009011.

50. Davis, K., Cain, R.F., Peters, L., Tourneau, D.L., Mcginnis, J., 1981. Evaluation of the Nutrient Composition of Wheat. II. Proximate Analysis, Thiamin, Riboflavin,

Niacin, and Pyridoxine [Varieties from 49 growing locations]. https://www.semanticscholar.org/paper/Evaluation-of-the-nutrient-composition-of-wheat.-49-Davis-Cain/07d0f6ec15a621ed875cbdc67c9e85ce98a19c64.

51. De Ponti, T., Rijk, B., Van Ittersum, M.K., 2012. The Crop Yield Gap between Organic and Conventional Agriculture. Agricultural Systems 108, 1 - 9. https://doi.org/10.1016/j.agsy.2011.12.004.

52. De Vita, P., Nicosia, O.L.D., Nigro, F., Platani, C., Riefolo, C., Di Fonzo, N., Cattivelli, L., 2007. Breeding Progress in Morpho-physiological, Agronomical and Qualitative Traits of Durum Wheat Cultivars Released in Italy during the 20th Century. European Journal of Agronomy 26, 39 - 53. https://doi.org/10.1016/j.eja.2006.08.009.

53. Delcour, J.A., Hoseney, R.C., 2010. Principles of Cereal Science and Technology. St. Paul, MN, USA: American Association of Cereal Chemists. ISBN: 978 - 1891127632.

54. Dhaka, V., Gulia, N., Khatkar, B., 2014. Application of Mixolab to Assess the Bread Making Quality of Wheat Varieties. https://doi.org/10.4172/scientificreports.183.

55. Dhaka, V., Khatkar, B. s., 2013. Mixolab Thermomechanical Characteristics of Dough and Bread Making Quality of Indian Wheat Varieties. Quality Assurance and Safety of Crops & Foods 5, 311 - 323. https://doi.org/10.3920/QAS2012.0166.

56. Dworschak, R.G., Ens, W., Standing, K.G., Preston, K.R., Marchylo, B.A., Nightingale, M.J., Stevenson, S.G., Hatcher, D.W., 1998. Analysis of Wheat Gluten Proteins by Matrix-assisted Laser Desorption/ionization Mass Spectrometry. Journal of Mass Spectrometry 33, 429 - 435. https://doi.org/10.1002/(SICI)1096-9888(199805)33:5<429::AID-JMS645>3.0.CO;2-O.

57. Edwards, N.M., Gianibelli, M.C., McCaig, T.N., Clarke, J.M., Ames, N.P., Larroque, O.R., Dexter, J.E., 2007. Relationships between Dough Strength, Polymeric Protein Quantity and Composition for Diverse Durum Wheat Genotypes. Journal of Cereal Science 45, 140 - 149. https://doi.org/10.1016/j.jcs.2006.07.012.

58. Evans, Lloyd Thomas, Evans, L. T., Peacock, W.J., 1981. Wheat Science - Today and Tomorrow. Cambridge University Press.

59. FAO, 1999. Definition of Organic Agriculture. http://www.fao.org/unfao/bodies/COAG/COAG15/X0075E.htm#P86_4004.

60. Faubion, J. M., Hoseney, R.C., 1990. The Viscoelastic Properties of Wheat Flour Doughs, in: Faridi, H., Faubion, Jon M. (Eds.), Dough Rheology and Baked Product Texture. Springer US, Boston, MA, pp. 29 - 66. https://doi.org/10.1007/978-1-4613-0861-4_2.

61. Field, J.M., Shewry, P.R., Miflin, B.J., 1983. Solubilisation and Characterisation of Wheat Gluten Proteins: Correlations between the Amount of Aggregated Proteins and Baking Quality. Journal of the Science of Food and Agriculture. 34, 370 - 377. https://doi.org/10.1002/jsfa.2740340409.

62. Fischer, R.A. (Tony), Edmeades, G.O., 2010. Breeding and Cereal Yield Progress. Crop Science 50, S-85-S-98. https://doi.org/10.2135/cropsci2009.10.0564.

63. Flohr, B.M., Hunt, J.R., Kirkegaard, J.A., Evans, J.R., Swan, A., Rheinheimer, B., 2018. Genetic Gains in NSW Wheat Cultivars from 1901 to 2014 as Revealed from Synchronous Flowering during the Optimum Period. European Journal of Agronomy 98, 1 - 13. https://doi.org/10.1016/j.eja.2018.03.009.

64. Foulkes, M., Sylvester-Bradley, R., Weightman, R., Snape, J., 2007. Identifying Physiological Traits Associated with Improved Drought Resistance in Winter Wheat. Field Crops Research 103, 11 - 24. https://doi.org/10.1016/j.fcr.2007.04.007.

65. Francis, C., Wart, J.V., 2015. History of Organic Farming and Certification, in: Organic Farming: The Ecological System. John Wiley & Sons, Ltd, pp. 1 - 17. https://doi.org/10.2134/agronmonogr54.c1.

66. Gaju, O., Reynolds, M.P., Sparkes, D.L., Foulkes, M.J., 2009. Relationships between Large-Spike Phenotype, Grain Number, and Yield Potential in Spring Wheat. Crop Science 49, 961 - 973. https://doi.org/10.2135/cropsci2008.05.0285.

67. Galterio, G., Cardarilli, D., Codianni, P., Acquistucci, R., 2001. Evaluation of Chemical and Technological Characteristics of New Lines of *Triticum turgidum* ssp *dicoccum*. Nahrung 45, 263 - 266. https://doi.org/10.1002/1521-3803(20010801)45:4<263: AID-FOOD263>3.0.CO;2-J.

68. Galterio, G., Codianni, P., Giusti, A.M., Pezzarossa, B., Cannella, C., 2003. Assessment of the Agronomic and Technological Characteristics of *Triticum turgidum* ssp. *dicoccum* Schrank and *T. spelta* L. Food / Nahrung 47, 54 - 59. https://doi.org/10.1002/food.200390012.

69. Galterio, G., Colonna, M., Mariani, G., Novembre, G., 1999. Technological and Nutritional Characteristics of *Triticum turgidum* ssp. *dicoccum* and *T. spelta* grown in the Molise Region. Journal of Agricultural Medicine and Community Health, 129, 227 - 234.

70. Gao, F., Ma, D., Yin, G., Rasheed, A., Dong, Y., Xiao, Y., Xia, X., Wu, X., He, Z., 2017. Genetic Progress in Grain Yield and Physiological Traits in Chinese Wheat Cultivars of Southern Yellow and Huai Valley since 1950. Crop Science 57, 760 - 773. https://doi.org/10.2135/cropsci2016.05.0362.

71. Gebruers, K., Dornez, E., Boros, D., Anna Fraś, Dynkowska, W., Bedő, Z., Rakszegi, M., Delcour, J.A., Courtin, C.M., 2008. Variation in the Content of Dietary Fiber and Components Thereof in Wheats in the HEALTHGRAIN Diversity Screen. Journal of Agricultural and Food Chemistry 56, 9740 - 9749. https://doi.org/10.1021/jf800975w.

72. Gevrek, M.N., Atasoy, G.D., 2012. Performance of Some Bread Wheat Genotypes under Organic and Conventional Production Systems. International Journal of Agriculture and Biology (Pakistan). https://www.researchgate.net/publication/285940073_Performance_of_some_Bread_Wheat_Genotypes_under_Organic_and_Conventional_Production_Systems.

73. Gianibelli, M.C., Larroque, O.R., MacRitchie, F., Wrigley, C.W., 2001. Biochemical, Genetic, and Molecular Characterization of Wheat Glutenin and Its Component Subunits. Cereal Chemistry Journal 78, 635 - 646. https://doi.org/10.1094/CCHEM.2001.78.6.635.

74. Giunta, F., Motzo, R., Pruneddu, G., 2007. Trends since 1900 in the Yield Potential of Italian-bred Durum Wheat Cultivars. European Journal of Agronomy 27, 12 - 24. https://doi.org/10.1016/j.eja.2007.01.009.

75. Goesaert, H., Brijs, K., Veraverbeke, W.S., Courtin, C.M., Gebruers, K., Delcour, J.A., 2005. Wheat Flour Constituents: How They Impact Bread Quality, and How to Impact Their Functionality. Trends in Food Science & Technology 16, 12 - 30. https://doi.org/10.1016/j.tifs.2004.02.011.

76. Gooding, M.J., Smith, G., Davies, W.P., Kettlewell, P.S., 1997. The Use of Residual Maximum Likelihood to Model Grain Quality Characters of Wheat with Variety, Climatic and Nitrogen Fertilizer Effects. The Journal of Agricultural Science 128, 135 - 142. https://doi.org/10.1017/S0021859696004054.

77. Grausgruber, H., Sailer, C., Ghambashidze, G., Bolyos, L., Ruckenbauer, P., 2004. Genetic Variation in Agronomic and Qualitative Traits of Ancient Wheats. Genetic Variation for Plant Breeding. Proceedings of the 17th EUCARPIA General Congress, Tulln, Austria, 8 - 11 September 2004 19 - 22.

78. Graybosch, R.A., Peterson, C.J., 2010. Genetic Improvement in Winter Wheat Yields in the Great Plains of North America, 1959 - 2008. Crop Science 50, 1882 - 1890. https://doi.org/10.2135/cropsci2009.11.0685.

79. Green, A., Berger, G., Griff, C., Pitman, R., Thomason, W., Balota, M., Ahmed, A., 2012. Genetic Yield Improvement in Soft Red Winter Wheat in the Eastern United States from 1919 to 2009. Crop Science 52, 2097 - 2108. https://doi.org/10.2135/cropsci2012.01.0026.

80. Grobelnik Mlakar, S., Bavec, M., Turinek, M., Bavec, F., 2009. Rheological Properties of Dough Made from Grain Amaranth-cereal Composite Flours Based on Wheat and Spelt. Czech Journal of Food Sciences 27, 309 - 319. https://doi.org/10.17221/61/2009-CJFS.

81. Grosch, W., Wieser, H., 1999. Redox Reactions in Wheat Dough as Affected by Ascorbic Acid. undefined 29, 1 - 16. https://doi.org/10.1006/jcrs.1998.0218.

82. Gummadov, N., Keser, M., Akin, B., Cakmak, M., Mert, Z., Taner, S., Ozturk, I., Topal, A., Yazar, S., Morgounov, A., 2015. Genetic Gains in Wheat in Turkey: Winter Wheat for Irrigated Conditions. The Crop Journal 3, 507 - 516. https://doi.org/10.1016/j.cj.2015.07.007

83. Haider, N., 2013. The Origin of the B-genome of Bread Wheat (*Triticum aestivum* L.). Russian Journal of Genetics 49, 263 - 274. https://doi.org/10.1134/S1022795413030071.

84. Haraszi, R., Chassaigne, H., Maquet, A., Ulberth, F., 2011. Analytical Methods for Detection of Gluten in Food-Method Developments in Support of Food Labeling Legislation. Journal of AOAC International 94, 1006 - 25. https://doi.org/10.1093/jaoac/94.4.1006.

85. Harati, H., Bekes, F., Howell, K., Noonan, S., Florides, C., Beasley, J., Diepeveen, D., Appels, R., 2020. Signatures for Torque Variation in Wheat Dough Structure Are Affected by Enzymatic Treatments and Heating. Food Chemistry 316, 126357. https://doi.org/10.1016/j.foodchem.2020.126357.

86. Haros, M., Ferrer, A., Rosell, C., 2006. Rheological Behaviour of Whole Wheat Flour 681. https://doi.org/10.1051/IUFoST:20060681.

87. Harris, DR., 1998. The Origins of Agriculture in Southwest Asia. Review Archaeology 5 - 11.

88. Hazard, B., Trafford, K., Lovegrove, A., Griffiths, S., Uauy, C., Shewry, P., 2020. Strategies to Improve Wheat for Human Health. Nature Food 1, 475 - 480. https://doi.org/10.1038/s43016-020-0134-6.

89. Hidalgo, A., Brandolini, A., Ratti, S., 2009. Influence of Genetic and Environmental Factors on Selected Nutritional Traits of *Triticum monococcum*. Journal of Agricultural and Food Chemistry 57, 6342 - 6348. https://doi.org/10.1021/jf901180q.

90. Hizukuri, S., Takeda, Y., Yasuda, M., Suzuki, A., 1981. Multi-branched Nature of Amylose and the Action of Debranching Enzymes. Carbohydrate Research 94, 205 - 213. https://doi.org/10.1016/S0008-6215(00)80718-1.

91. Hoseney, R.C., 1985. Mixing Phenomenon. Cereal Foods World (USA) 30, 453 - 457.

92. Hrušková, M., Škodová, V., Blažek, J., 2011. Wheat Sedimentation Values and Falling Number. Czech Journal of Food Sciences 22, 51 - 57. https://doi.org/10.17221/3406-CJFS.

93. Hsam, S., Kieffer, R., Zeller, F., 2001. Significance of *Aegilops tauschii* Glutenin Genes on Breadmaking Properties of Wheat. Cereal Chemistry 78, 521 - 525. https://doi.org/10.1094/CCHEM.2001.78.5.521.

94. Huang, S., Yun, S.-H., Quail, K., Moss, R., 1996. Establishment of Flour Quality Guidelines for Northern Style Chinese Steamed Bread. Journal of Cereal Science 24, 179 - 185. https://doi.org/10.1006/jcrs.1996.0051.

95. Hutchinson, J., 1974. Evolutionary Studies in World Crops: Diversity and Change in the Indian Subcontinent. CUP Archive.

96. ICC - Standard No. 107/1, 1995. Determination of the “Falling Number” According to Hagberg - as a Measure of the Degree of Alpha-Amylase Activity in Grain and Flour.

97. ICC - Standard No. 155, 1994. Determination of Wet Gluten Quantity and Quality (Gluten Index ac. to Perten) of Whole Wheat Meal and Wheat Flour (*Triticum aestivum*).

98. ICC - Standard No. 158, 1995. Gluten Index Method for Assessing Gluten Strength in Durum Wheat (*Triticum durum*).

99. ICC - Standard No. 173, 2006. Whole Meal and Flour from *T. aestivum* - Determination of Rheological Behavior as a Function of Mixing and Temperature Increase.

100. IFOAM, 2014. The IFOAM NORMS for Organic Production and Processing Version 2014. https://www.unipapress.it/_entomologia/ifoam_norms_july_2014_t.pdf.

101. IFOAM, 2008. Definition of Organic Agriculture. https://www.ifoam.bio/why-organic/organic-landmarks/definition-organic.

102. Jablonskytė-Raščė, D., Maikštėnienė, S., Mankevičienė, A., 2013. Evaluation of Productivity and Quality of Common Wheat (*Triticum aestivum* L.) and Spelt (*Triticum spelta* L.) in Relation to Nutrition Conditions. Zemdirbyste-Agriculture 100, 45 - 56. https://doi.org/10.13080/z-a.2013.100.007.

103. Jańczak-Pieniążek, M., Buczek, J., Kaszuba, J., Szpunar-Krok, E., Bobrecka-Jamro, D., Jaworska, G., 2020. A Comparative Assessment of the Baking Quality of Hybrid and Population Wheat Cultivars. Applied Sciences 10, 7104. https://doi.org/10.3390/app10207104.

104. Janssen, A.M., van Vliet, T., Vereijken, J.M., 1996. Fundamental and Empirical Rheological Behaviour of Wheat Flour Doughs and Comparison with Bread Making Performance. Journal of Cereal Science 23, 43 - 54. https://doi.org/10.1006/jcrs.1996.0004.

105. Jarrige, J.-F., Meadow, R.H., 1980. The Antecedents of Civilization in the Indus Valley. Scientific American 243, 122 - 137.

106. Jenkins, D.J., Jenkins, A.L., Wolever, T.M., Vuksan, V., Rao, A.V., Thompson, L.U., Josse, R.G., 1995. Effect of Reduced Rate of Carbohydrate Absorption on Carbohydrate and Lipid Metabolism. European Journal of Clinical Nutrition 49 Supplement 3, S68-73.

107. Johansson, E., 2002. Effect of Two Wheat Genotypes and Swedish Environment on Falling Number, Amylase Activities, and Protein Concentration and Composition. Euphytica 126, 143 - 149. https://doi.org/10.1023/A:1019646916905.

108. Karaman, M., 2020. Evaluation of the Physiological and Agricultural Properties of Some of the Bread Wheat (*Triticum aestivum* L.) Genotypes Registered in Turkey Using Biplot Analysis. Pakistan Journal of Botany. 52. https://doi.org/10.30848/PJB2020-6(5).

109. Kettlewell, P.S., 1999. The Response of Alpha-amylase Activity during Wheat Grain Development to Nitrogen Fertiliser. Annals of Applied Biology 134, 241 - 249. https://doi.org/10.1111/j.1744-7348.1999.tb05260.x.

110. Kihara, H., 1966. Factors Affecting the Evolution of Common Wheat. Indian Journal of Genetics 26A, 14 - 28.

111. Kihara, H., 1944. Discovery of the DD-Analyser, One of the Ancestors of *Triticum vulgare*. Agriculture and Horticulture 19, 889 - 890.

112. Kihara, H., 1924. Cytologische Und Genetische Studien Bei Wichtigen Getreidearten Mit Besonderer Rucksicht Auf Das Verhalten Der Chromosomen Und Die Sterilität In Den Bastarden. https://repository.kulib.kyoto-u.ac.jp/dspace/bitstream/2433/257667/1/mcskiu-b_01_1_1.pdf.

113. Kindred, D.R., Gooding, M.J., Ellis, R.H., 2005. Nitrogen Fertilizer and Seed Rate Effects on Hagberg Falling Number of Hybrid Wheats and Their Parents Are Associated with α-amylase Activity, Grain Cavity Size and Dormancy. Journal of the Science of Food and Agriculture 85, 727 - 742. https://doi.org/10.1002/jsfa.2025.

114. Kislev, M.E., 1984. Emergence of Wheat Agriculture. Paléorient 10, 61 - 70. https://www.jstor.org/stable/41489606.

115. Knapowski, T., Ralcewicz, M., 2004. Evaluation of Qualitative Features of Mikon Cultivar Winter Wheat Grain and Flour Depending on Selected Agronomic Factors. Electronic Journal of Polish Agricultural Universities 7. http://www.ejpau.media.pl/volume7/issue1/agronomy/art-01.html.

116. Kondhare, K.R., Farrell, A.D., Kettlewell, P.S., Hedden, P., Monaghan, J.M., 2015. Pre-maturity α-amylase in Wheat: The Role of Abscisic Acid and Gibberellins. Journal of Cereal Science 63, 95 - 108. https://doi.org/10.1016/j.jcs.2015.03.004.

117. Konik, C.M., Mikkelsen, L.M., Moss, R., Gore, P.J., 1994. Relationships between Physical Starch Properties and Yellow Alkaline Noodle Quality. Starch - Stärke 46, 292 - 299. https://doi.org/10.1002/star.19940460804.

118. Konvalina, p., Grausgruber, H., Tran, D.K., Capouchova, I., Vlasek, O., Sterba, Z., Suchy, K., Stolickova, M., Kyptova, M., 2017. Rheological and Technological Quality of Minor Wheat Species and Common Wheat | IntechOpen [WWW Document]. URL https://www.intechopen.com/books/wheat-improvement-management-and-utilization/rheological-and-technological-quality-of-minor-wheat-species-and-common-wheat (accessed 12.15.20).

119. Konvalina, P., Capouchova, I., Stehno, Z., Moudry, J. (Jr), Moudry, J., 2011a. Spike Productivity in Relation to Yield as Criterion for Emmer Wheat Breeding. Romanian Agricultural Research. URL https://orgprints.org/id/eprint/20760/ (accessed 4.15.21).

120. Konvalina, P., Moudrý jr., J., Moudrý, J., 2008. Quality Parametres of Emmer Wheat Landraces. Journal of Central European Agriculture 9, 539 - 546. https://www.researchgate.net/publication/228663423_Quality_parametres_of_emmer_wheat_landraces.

121. Konvalina, P., Stehno, Z., Capouchova, I., Moudry, J., 2011b. Wheat Growing and Quality in Organic Farming, in: Nokkoul, R. (Ed.), Research in Organic Farming. InTech. https://doi.org/10.5772/29330.

122. Konvalina, P., Zechner, E., Moudry, J., 2007. Breeding and Variety Testing of Bread Wheat - *Triticum aestivum* L. For Organic and Low Input Farming. ISBN: 978-80-7394-039-3.

123. Kristiansen, P., Taji, A., Reganold, J., 2006. Organic Agriculture: A Global Perspective. CSIRO Publishing, Collingwood / CABI, Wallingford / Cornell University Press, Ithaca / Manaaki Whenua Press, Lincoln, Australia.

124. Krupnova, O.V., Svistunov, Yu.S., 2014. Preharvest Sprouting Resistance and Falling Number of Winter Bread Wheat in the Volga Region. Russian Agricultural Sciences 40, 395 - 398. https://doi.org/10.3103/S1068367414060147.

125. Kuepper, G., 2010. A Brief Overview of the History and Philosophy of Organic Agriculture 26. https://kerrcenter.com/publication/brief-overview-history-philosophy-organic-agriculture/.

126. Lacko-Bartošová, M., Čurná, V., 2015. Nutritional Characteristics of Emmer Wheat Varieties. JMBFS 4, 95 - 98. https://doi.org/10.15414/jmbfs.2015.4.special3.95-98.

127. Lacko-Bartošová, M., Konvalina, P., Lacko-Bartošová, L., 2019. Baking Quality Prediction of Spelt Wheat Based on Rheological and Mixolab Parameters. Acta Alimentaria 48, 213 - 220. https://doi.org/10.1556/066.2019.0002.

128. Lacko-Bartošová, Magdaléna, Konvalina, P., Lacko-Bartošová, L., Štěrba, Z., 2019. Quality Evaluation of Emmer Wheat Genotypes Based on Rheological and Mixolab Parameters. Czech Journal of Food Sciences 37 (2019), 192 - 198. https://doi.org/10.17221/101/2018-CJFS.

129. Lan, X.-J., Wei, Y.-M., Liu, D., Yan, Z., Zheng, Y.-L., 2005. Inheritance of Seed Dormancy in Tibetan Semi-wild Wheat Accession Q1028. Journal of Applied Genetics 46, 133 - 8.

130. Lásztity, R., 1984. Wheat Proteins, The Chemistry of Cereal Proteins. https://doi.org/10.1201/9780203737057-3.

131. Lev-Yadun, S., Gopher, A., Abbo, S., 2000. The Cradle of Agriculture. Science 288, 1602 - 1603. https://doi.org/10.1126/science.288.5471.1602.

132. Liatukas, Ž., Ruzgas, V., Razbadauskiene, K., Brazauskas, G., Koppel, R., 2012. Winter Wheat Cultivars “kovas ds”, “zunda ds”, “vikaras ds”, “kaskada ds” for High Input Farming: Development and Characterization. Zemdirbyste 99, 255 - 264.

133. Lineback, DR., Rasper, VF., 1988. Wheat Carbohydrates. Paul, Minn.: American Association of Cereal Chemists. ISBN: 0-913250-65-1.

134. Liniņa, A., Ruža, A., 2015. Impact of Agroecological Conditions on the Hagberg Falling Number of Winter Wheat Grain. Volume 1. https://llufb.llu.lv/conference/Research-for-Rural-Development/2015/LatviaResearchRuralDevel21st_volume1-19-26.pdf.

135. Liniņa, A., Ruža, A., 2012. Cultivar and Nitrogen Fertiliser Effects on Fresh and Stored Winter Wheat Grain Quality Indices. Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences. 66, 177 - 184. https://doi.org/10.2478/v10046-012-0026-8.

136. Liu, H., Searle, I., Mather, D., Able, A., Able, J., 2015. Morphological, Physiological and Yield Responses of Durum Wheat to Pre-anthesis Water Deficit Stress Are Genotype-dependent. Crop and Pasture Science In Press. https://doi.org/10.1071/CP15013.

137. Lockyer, S., Spiro, A., 2020. The Role of Bread in the UK Diet: An Update. Nutrition Bulletin 45, 133 - 164. https://doi.org/10.1111/nbu.12435.

138. Løje, H., Møller, B., Laustsen, A.M., Hansen, Å., 2003. Chemical Composition, Functional Properties and Sensory Profiling of Einkorn (*Triticum monococcum* L.). Journal of Cereal Science 37, 231 - 240. https://doi.org/10.1006/jcrs.2002.0498.

139. Lopes, M.S., Reynolds, M.P., Manes, Y., Singh, R.P., Crossa, J., Braun, H.J., 2012. Genetic Yield Gains and Changes in Associated Traits of CIMMYT Spring

Bread Wheat in a “Historic” Set Representing 30 Years of Breeding. Crop Science 52, 1123 - 1131. https://doi.org/10.2135/cropsci2011.09.0467.

140. Löschenberger, F., Fleck, A., Grausgruber, H., Hetzendorfer, H., Hof, G., Lafferty, J., Marn, M., Neumayer, A., Pfaffinger, G., Birschitzky, J., 2008. Breeding for Organic Agriculture: The Example of Winter Wheat in Austria. Euphytica 163, 469. https://doi.org/10.1007/s10681-008-9709-2.

141. Lundh, G., Macritchie, F., 1989. Size Exclusion HPLC Characterisation of Gluten Protein Fractions Varying in Breadmaking Potential. Journal of Cereal Science 10, 247 - 253. https://doi.org/10.1016/S0733-5210(89)80054-2.

142. Luttikholt, L., 2007. Principles of Organic Agriculture as Formulated by the International Federation of Organic Agriculture Movements. NJAS - Wageningen Journal of Life Sciences 54, 347 - 360. https://doi.org/10.1016/S1573-5214(07)80008-X.

143. Ma, D., Guo, T., Wang, Z., Wang, C., Zhu, Y., Wang, Y., 2009. Influence of Nitrogen Fertilizer Application Rate on Winter Wheat (*Triticum aestivum* L.) Flour Quality and Chinese Noodle Quality. Journal of the Science of Food and Agriculture 89, 1213 - 1220. https://doi.org/10.1002/jsfa.3578.

144. MacRitchie, F., Lafiandra, D., 1997. Structure-function Relationships of Wheat Proteins. https://publications.csiro.au/publications/publication/PIprocite:ca2e133d-59ff-4265-b8da-5e216b6bac83.

145. Mäder, P., Hahn, D., Dubois, D., Gunst, L., Alföldi, T., Bergmann, H., Oehme, M., Amadò, R., Schneider, H., Graf, U., Velimirov, A., Fließbach, A., Niggli, U., 2007. Wheat Quality in Organic and Conventional Farming: Results of a 21 Year Field Experiment. Journal of the Science of Food and Agriculture 87, 1826 - 1835. https://doi.org/10.1002/jsfa.2866.

146. Makawi, A.B., Mustafa, A.I., Mohamed Ahmed, I.A., 2013. Characterization and Improvement of Flours of Three Sudanese Wheat Cultivars for Loaf Bread Making. Innovative Romanian Food Biotechnology 30 - 44. https://www.gup.ugal.ro/ugaljournals/index.php/IFRB/article/view/3483.

147. Malik, A.H., 2009. Nutrient Uptake, Transport and Translocation in Cereals: Influences of Environmental and Farming Conditions (Report No. 2009:1). Alnarp. https://pub.epsilon.slu.se/4083/.

148. Marconi, E., Cubbada, R., 2005. Emmer Wheat. St. Paul, Minnesota: American Association of Cereal Chemistry, Inc., pp. 63 - 108. ISBN 1 - 891127-41-1.

149. Mason, H.E., Navabi, A., Frick, B.L., O’Donovan, J.T., Spaner, D.M., 2007. The Weed-Competitive Ability of Canada Western Red Spring Wheat Cultivars Grown under Organic Management. Crop Science 47, 1167 - 1176. https://doi.org/10.2135/cropsci2006.09.0566.

150. Massaux, C., Sindic, M., Lenartz, J., Sinnaeve, G., Bodson, B., Falisse, A., Dardenne, P., Deroanne, C., 2008. Variations in Physicochemical and Functional Properties of Starches Extracted from European Soft Wheat (*Triticum aestivum* L.): The Importance to Preserve the Varietal Identity. Carbohydrate Polymers 71, 32 - 41. https://doi.org/10.1016/j.carbpol.2007.05.012.

151. Matsuoka, Y., 2011. Evolution of Polyploid Triticum Wheats under Cultivation: The Role of Domestication, Natural Hybridization and Allopolyploid Speciation in their Diversification. Plant and Cell Physiology 52, 750 - 764. https://doi.org/10.1093/pcp/pcr018.

152. Mayer, J., Gunst, L., Mäder, P., Samson, M.-F., Carcea, M., Narducci, V., Thomsen, I.K., Dubois, D., 2015. "Productivity, Quality and Sustainability of Winter Wheat under Long-term Conventional and Organic Management in Switzerland." European Journal of Agronomy 65, 27 - 39. https://doi.org/10.1016/j.eja.2015.01.002.

153. McFadden, E.S., Sears, E.R., 1944. The Artificial Synthesis of *Triticum spelta*. Records of the Genetics Society of America 26 - 27.

154. Mirosavljević, M., Momčilović, V., Živančev, D., Aćin, V., Jocković, B., Mikić, S., Takač, V., Denčić, S., 2020. Genetic Improvement of Grain Yield and Bread-making Quality of Winter Wheat over the Past 90 Years under the Pannonian Plain Conditions. Euphytica 216, 184. https://doi.org/10.1007/s10681-020-02724-5.

155. Mohammadkhani, A., Stoddard, F.L., Marshall, D.R., 1998. Survey of Amylose Content in Secale Cereale, *Triticum monococcum*, *T. turgidum* and *T. tauschii.* Journal of Cereal Science 28, 273 - 280. https://doi.org/10.1016/S0733-5210(98)90007-8.

156. Moreira, R., Chenlo, F., Torres, M.D., 2011. Effect of Sodium Chloride, Sucrose and Chestnut Starch on Rheological Properties of Chestnut Flour Doughs. Food Hydrocolloids 25, 1041 - 1050. https://doi.org/10.1016/j.foodhyd.2010.09.025.

157. Morgounov, A., Zykin, V., Belan, I., Roseeva, L., Zelenskiy, Yu., Gomez-Becerra, H.F., Budak, H., Bekes, F., 2010. Genetic Gains for Grain Yield in High Latitude Spring Wheat Grown in Western Siberia in 1900 - 2008. Field Crops Research 117, 101 - 112. https://doi.org/10.1016/j.fcr.2010.02.001.

158. Mpofu, A., Sapirstein, H.D., Beta, T., 2006. Genotype and Environmental Variation in Phenolic Content, Phenolic Acid Composition, and Antioxidant Activity of Hard Spring Wheat. Journal of Agricultural and Food Chemistry 54, 1265 - 1270. https://doi.org/10.1021/jf052683d.

159. Mueller, K.M., 2008. Requirements of Organic Cereal Breeders for VCU Testing in Germany. https://www.eco-pb.org/fileadmin/eco-pb/documents/reports_proceedings/Proceedings_Brussel_08022829.pdf.

160. Oelofse, R.M., Labuschagne, M.T., Van Deventer, C.S., 2010. Influencing Factors of Sodium Dodecyl Sulfate Sedimentation in Bread Wheat. Journal of Cereal Science 52, 96 - 99. https://doi.org/10.1016/j.jcs.2010.03.010.

161. Oikonomou, N.A., Bakalis, S., Rahman, M.S., Krokida, M.K., 2015. Gluten Index for Wheat Products: Main Variables in Affecting the Value and Nonlinear Regression Model. International Journal of Food Properties 18, 1 - 11. https://doi.org/10.1080/10942912.2013.772198.

162. Pareyt, B., Finnie, S.M., Putseys, J.A., Delcour, J.A., 2011. Lipids in Bread Making: Sources, Interactions, and Impact on Bread Quality. Journal of Cereal Science 54, 266 - 279. https://doi.org/10.1016/j.jcs.2011.08.011.

163. Payne, P.I., Corfield, K.G., 1979. Subunit Composition of Wheat Glutenin Proteins, Isolated by Gel Filtration in a Dissociating Medium. Planta 145, 83 - 88. https://doi.org/10.1007/BF00379931.

164. Payne, P.I., Holt, L.M., Hutchinson, J., Bennett, M.D., 1984. Development and Characterization of a Line of Bread Wheat, *Triticum aestivum*, Which Lacks the Short-arm Satellite of Chromosome 1B and the Gli-B1 Locus. Theoretical and Applied Genetics 68, 327 - 334. https://doi.org/10.1007/BF00267886.

165. Payne, P.I., Nightingale, M.A., Krattiger, A.F., Holt, L.M., 1987. The Relationship between HMW Glutenin Subunit Composition and the Bread-making Quality of British-grown Wheat Varieties. Journal of the Science of Food and Agriculture 40, 51 - 65. https://doi.org/10.1002/jsfa.2740400108.

166. Pence, J.W., Weinstein, N.E., Mecham, D.K., 1954. The Albumin and Globulin Contents of Wheat Flour and Their Relationship to Protein Quality. Cereal Chemistry 31, 303 - 311.

167. Perry, M.W., D'Antuono, M.F., 1989. Yield Improvement and Associated Characteristics of Some Australian Spring Wheat Cultivars Introduced between 1860 and 1982. Australian Journal of Agricultural Research 40, 457 - 472. https://doi.org/10.1071/ar9890457.

168. Pomeranz, Y., 1988. Wheat, Chemistry and Technology. Starch - Stärke 42, 243 - 243. https://doi.org/10.1002/star.19900420612.

169. Prabhasankar, P., Haridas Rao, P., 1999. Lipids in Wheat Flour Streams. Journal of Cereal Science 30, 315 - 322. https://doi.org/10.1006/jcrs.1999.0289.

170. Prabhasankar, P., Vijaya Kumar, M., Lokesh, B.R., Haridas Rao, P., 2000. Distribution of Free Lipids and Their Fractions in Wheat Flour Milled Streams. Food Chemistry 71, 97 - 103. https://doi.org/10.1016/S0308-8146(00)00149-7.

171. Qin, X., Zhang, F., Liu, C., Yu, H., Cao, B., Tian, S., Liao, Y., Siddique, K.H.M., 2015. Wheat Yield Improvements in China: Past Trends and Future Directions. Field Crops Research 177, 117 - 124. https://doi.org/10.1016/j.fcr.2015.03.013.

172. Rachoń, L., Bobryk-Mamczarz, A., Kiełtyka-Dadasiewicz, A., 2020. Hulled Wheat Productivity and Quality in Modern Agriculture Against Conventional Wheat Species. Agriculture 10, 275. https://doi.org/10.3390/agriculture10070275.

173. Rasper, V.E.., Walker, C.E., 2000. Quality Evaluation of Cereals and Cereal Products, Handbook of Cereal Science and Technology, Revised and Expanded. CRC Press. https://doi.org/10.1201/9781420027228-51.

174. Raza, S., Khalil, S., Naseem, K., Gilani, M.A., Amjad, M., Masud, T., Naqvi, S.M.S., 2010. Effect of House Hold Storage Receptacles on Physico Chemical Characteristics of Wheat. Sarhad Journal of Agriculture 26, 275 - 287.

175. Reganold, J.P., Wachter, J.M., 2016. Organic Agriculture in the Twenty-first Century. Nature Plants 2, 15221. https://doi.org/10.1038/NPLANTS.2015.221.

176. Regina, A., Berbezy, P., Kosar-Hashemi, B., Li, S., Cmiel, M., Larroque, O., Bird, A.R., Swain, S.M., Cavanagh, C., Jobling, S.A., Li, Z., Morell, M., 2015. A

Genetic Strategy Generating Wheat with Very High Amylose Content. Plant Biotechnology Journal 13, 1276 - 1286. https://doi.org/10.1111/pbi.12345.

177. Rey, F., Fontaine, L., Osman, A., Waes, J.V., 2008. Value for Cultivation and Use Testing of Organic Cereal Varieties: What Are the Key Issues? p. 58. https://www.eco-pb.org/fileadmin/eco-pb/documents/reports_proceedings/Proceedings_Brussel_08022829.pdf.

178. Reynolds, M., Tuberosa, R., 2008. Translational Research Impacting on Crop Productivity in Drought-prone Environments. Current Opinion in Plant Biology, Genome Studies and Molecular Genetics, Edited by Juliette de Meaux and Maarten Koornneef / Plant Biotechnology, Edited by Andy Greenland and Jan Leach 11, 171 - 179. https://doi.org/10.1016/j.pbi.2008.02.005.

179. Reynolds, M.P., Pask, A.J.D., Hoppitt, W.J.E., Sonder, K., Sukumaran, S., Molero, G., Pierre, C.S., Payne, T., Singh, R.P., Braun, H.J., Gonzalez, F.G., Terrile, I.I., Barma, N.C.D., Hakim, A., He, Z., Fan, Z., Novoselovic, D., Maghraby, M., Gad, K.I.M., Galal, E.G., Hagras, A., Mohamed, M.M., Morad, A.F.A., Kumar, U., Singh, G.P., Naik, R., Kalappanavar, I.K., Biradar, S., Sai Prasad, S.V., Chatrath, R., Sharma, I., Panchabhai, K., Sohu, V.S., Mavi, G.S., Mishra, V.K., Balasubramaniam, A., Jalal-Kamali, M.R., Khodarahmi, M., Dastfal, M., Tabib-Ghaffari, S.M., Jafarby, J., Nikzad, A.R., Moghaddam, H.A., Ghojogh, H., Mehraban, A., Solís-Moya, E., Camacho-Casas, M.A., Figueroa-López, P., Ireta-Moreno, J., Alvarado-Padilla, J.I., Borbón-Gracia, A., Torres, A., Quiche, Y.N., Upadhyay, S.R., Pandey, D., Imtiaz, M., Rehman, M.U., Hussain, Manzoor, Hussain, Makhdoom, Ud-Din, R., Qamar, M., Sohail, M., Mujahid, M.Y., Ahmad, G., Khan, A.J., Sial, M.A., Mustatea, P., von Well, E., Ncala, M., de Groot, S., Hussein, A.H.A., Tahir, I.S.A., Idris, A.A.M., Elamein, H.M.M., Manes, Y., Joshi, A.K., 2017. Strategic Crossing of Biomass and Harvest Index - Source and Sink - Achieves Genetic Gains in Wheat. Euphytica 213, 257. https://doi.org/10.1007/s10681-017-2040-z.

180. Rho, K.L., Chung, O.K., Seib, P.A., 1989. Noodles. VIII. The Effect of Wheat Flour Lipids, Gluten, and Several Starches and Surfactants on the Quality of Oriental Dry Noodles.

181. Rosell, C.M., Collar, C., Haros, M., 2007. Assessment of Hydrocolloid Effects on the Thermo-mechanical Properties of Wheat Using the Mixolab. Food Hydrocolloids 21, 452 - 462. https://doi.org/10.1016/j.foodhyd.2006.05.004.

182. Royo, C., Álvaro, F., Martos, V., Ramdani, A., Isidro, J., Villegas, D., García del Moral, L.F., 2007. Genetic Changes in Durum Wheat Yield Components and Associated Traits in Italian and Spanish Varieties during the 20th Century. Euphytica 155, 259 - 270. https://doi.org/10.1007/s10681-006-9327-9.

183. Ruibal-Mendieta, N.L., Delacroix, D.L., Meurens, M., 2002. A Comparative Analysis of Free, Bound and Total Lipid Content on Spelt and Winter Wheat Wholemeal. Journal of Cereal Science 35, 337 - 342. https://doi.org/10.1006/jcrs.2001.0434.

184. Sarapatka, B., Urban, J., 2009. Organic Agriculture. Institute of Agricultural Economics and Information, the Czech Ministry of Agriculture, the PRO-BIO Association of Organic Farmers, Bioinstitut o.p.s. and Faculty os Science of Palacky University Olomuc.

185. Schatzkin, A., Mouw, T., Park, Y., Subar, A.F., Kipnis, V., Hollenbeck, A., Leitzmann, M.F., Thompson, F.E., 2007. Dietary Fiber and Whole-grain Consumption in Relation to Colorectal Cancer in the NIH-AARP Diet and Health Study. The American Journal of Clinical Nutrition 85, 1353 - 1360. https://doi.org/10.1093/ajcn/85.5.1353.

186. Schmiele, M., Ferrari Felisberto, M.H., Pedrosa Silva Clerici, M.T., Chang, Y.K., 2017. Mixolab^{TM} for Rheological Evaluation of Wheat Flour Partially Replaced by Soy Protein Hydrolysate and Fructooligosaccharides for Bread Production. LWT - Food Science and Technology, SLACA 2015: "Food Science for Quality of Life and Health Ageing" 76, 259 - 269. https://doi.org/10.1016/j.lwt.2016.07.014.

187. Seleiman, M.F., Ibrahim, M.E., Abdel-Aal, S.M., Zahran, G.A., 2010. Effect of Seeding Rates on Productivity, Technological and Rheological Characteristics of Bread Wheat (*Triticum aestivum* L.). International Journal of Current Research 4, 75 - 81.

188. Selyaninov, G.T., 1928. Agricultural Assessment of Climate. Tr. S-kh. Meteorol. 165 - 172.

189. Shearman, V.J., Sylvester-Bradley, R., Scott, R.K., Foulkes, M.J., 2005. Physiological Processes Associated with Wheat Yield Progress in the UK. Crop Science 45, cropsci2005.0175. https://doi.org/10.2135/cropsci2005.0175a.

190. Shewry, P.R., 2003. Wheat Gluten Protein Analysis. American Association of Cereal Chemists (AACC), St Paul, MN.

191. Shewry, P.R., Halford, N.G., 2002. Cereal Seed Storage Proteins: Structures, Properties and Role in Grain Utilization. Journal of Experimental Botany 53, 947 - 958. https://doi.org/10.1093/jexbot/53.370.947.

192. Shewry, P.R., Hassall, K.L., Grausgruber, H., Andersson, A.A.M., Piironen, V., Rakszegi, M., Ward, J.L., Lovegrove, A., 2020. Do Modern Types of Wheat Have Lower Quality for Human Health? Nutrition Bulletin 45, 362 - 373 https://onlinelibrary.wiley.com/doi/abs/10.1111/nbu.12461.

193. Shewry, P.R., Hawkesford, M.J., Piironen, V., Lampi, A.-M., Gebruers, K., Boros, D., Andersson, A.A.M., Åman, P., Rakszegi, M., Bedo, Z., Ward, J.L., 2013. Natural Variation in Grain Composition of Wheat and Related Cereals. Journal of Agricultural and Food Chemistry 61, 8295 - 8303. https://doi.org/10.1021/jf3054092.

194. Shewry, P.R., Tatham, A.S., 1990. The Prolamin Storage Proteins of Cereal Seeds: Structure and Evolution. Biochemical Journal 267, 1 - 12. https://doi.org/10.1042/bj2670001.

195. Shewry, P.R., Tatham, A.S., Forde, J., Kreis, M., Miflin, B.J., 1986. The Classification and Nomenclature of Wheat Gluten Proteins: A Reassessment. Journal of Cereal Science 4, 97 - 106. https://doi.org/10.1016/S0733-5210(86)80012-1.

196. Shibanuma, K., Takeda, Y., Hizukuri, S., Shibata, S., 1994. Molecular Structures of Some Wheat Starches. Carbohydrate Polymers 25, 111 - 116. https://doi.org/10.1016/0144-8617(94)90146-5.

197. Singh, J., Skerritt, J.H., 2001. Chromosomal Control of Albumins and Globulins in Wheat Grain Assessed using Different Fractionation Procedures. Journal of Cereal Science 33, 163 - 181. https://doi.org/10.1006/jcrs.2000.0351.

198. Slageren, M.W.S.J.M. Van, 1994. Wild Wheats: A Monograph of Aegilops L. and Amblyopyrum (Jaub. & Spach) Eig (Poaceae): A Revision of All Taxa Closely Related to Wheat, Excluding Wild *Triticum* species, with Notes on Other Genera in the Tribe Triticcae, Especially Triticum. Wageningen Agricultural University; International Center for Agricultural Research in the Dry Areas, Wageningen, The Netherlands; Aleppo, Syria.

199. Sliwinski, E.L., Kolster, P., van Vliet, T., 2004. On the Relationship between Large-deformation Properties of Wheat Flour Dough and Baking Quality. Journal of Cereal Science 39, 231 - 245. https://doi.org/10.1016/j.jcs.2003.10.005.

200. Smith, G.P., Gooding, M.J., 1996. Relationships of Wheat Quality with Climate and Nitrogen Application in Regions of England (1974 - 1993). Annals of Applied Biology 129, 97 - 108. https://doi.org/10.1111/j.1744-7348.1996.tb05735.x.

201. Spanic, V., Cosic, J., Zdunic, Z., Drezner, G., 2021. Characterization of Agronomical and Quality Traits of Winter Wheat (*Triticum aestivum* L.) for Fusarium Head Blight Pressure in Different Environments. Agronomy 11, 213. https://doi.org/10.3390/agronomy11020213.

202. Strazlina, V., Sturite, L., 2008. The Evaluation of Main Traits of Cereal Varieties for Organic Farming in Latvia. https://www.eco-pb.org/fileadmin/eco-pb/documents/reports_proceedings/Proceedings_Brussel_08022829.pdf.

203. Suchowilska, E., Wiwart, M., Kandler, W., Krska, R., 2012. A Comparison of Macro- and Microelement Concentrations in the Whole Grain of Four *Triticum* species. Plant Soil Environment 58, 141 - 147. https://doi.org/10.17221/688/2011-PSE.

204. Švec, I., Hrušková, M., 2015. The Mixolab Parameters of Composite Wheat/hemp Flour and Their Relation to Quality Features. LWT - Food Science and Technology 60, 623 - 629. https://doi.org/10.1016/j.lwt.2014.07.034.

205. Tian, Z., Jing, Q., Dai, T., Jiang, D., Cao, W., 2011. Effects of Genetic Improvements on Grain Yield and Agronomic Traits of Winter Wheat in the Yangtze River Basin of China. Field Crops Research 124, 417 - 425. https://doi.org/10.1016/j.fcr.2011.07.012.

206. Toyokawa, H., Rubenthaler, G.L., Powers, J.R., Schanus, E.G., 1989. Japanese Noddle Qualities. II. Starch Components. Cereal Chemistry 66, 387 - 391.

207. Tran, K.D., Konvalina, P., Capouchova, I., Janovska, D., Lacko-Bartosova, M., Kopecky, M., Tran, P.X.T., 2020. Comparative Study on Protein Quality and Rheological Behavior of Different Wheat Species. Agronomy 10, 1763. https://doi.org/10.3390/agronomy10111763.

208. Tronsmo, K.M., Magnus, E.M., Baardseth, P., Schofield, J.D., Aamodt, A., Færgestad, E.M., 2003. Comparison of Small and Large Deformation Rheological Properties of Wheat Dough and Gluten. Cereal Chemistry 80, 587 - 595. https://doi.org/10.1094/CCHEM.2003.80.5.587.

209. Underdahl, J.L., Mergoum, M., Ransom, J.K., Schatz, B.G., 2008. Agronomic Traits Improvement and Associations in Hard Red Spring Wheat Cultivars Released in North Dakota from 1968 to 2006. Crop Science 48, 158 - 166. https://doi.org/10.2135/cropsci2007.01.0018.

210. Unkovich, M., Baldock, J., Forbes, M., 2010. Variability in Harvest Index of Grain Crops and Potential Significance for Carbon Accounting, in: Advances in Agronomy. Elsevier, pp. 173 - 219. https://doi.org/10.1016/S0065-2113(10)05005-4.

211. Veraverbeke, W.S., Delcour, J.A., 2002. Wheat Protein Composition and Properties of Wheat Glutenin in Relation to Breadmaking Functionality. Critical Reviews in Food Science and Nutrition 42, 179 - 208. https://doi.org/10.1080/10408690290825510.

212. Voicu, G., Constantin, G., Ipate, G., Tudor, P., 2017. Farinographic Parameter Variation of Doughs from Wheat Flour with Amount of Water Added. Presented at the 16th International Scientific Conference Engineering for Rural Development. https://doi.org/10.22616/ERDev2017.16.N200.

213. Wall, J.S., 1979. The Role of Wheat Proteins in Determining Baking Quality, in: Recent Advances in the Biochemistry of Cereals. London, New York: Academic, pp. 275 - 311.

214. Wang, C., Kovacs, M.I.P., 2002. Swelling Index of Glutenin Test. II. Application in Prediction of Dough Properties and End-Use Quality. Cereal Chemistry 79, 190 - 196. https://doi.org/10.1094/CCHEM.2002.79.2.190.

215. Wang, Y.G., Khan, K., Hareland, G., Nygard, G., 2006. Quantitative Glutenin Composition from Gel Electrophoresis of Flour Mill Streams and Relationship to Breadmaking Quality. Cereal Chemistry 83, 293 - 299. https://doi.org/10.1094/CC-83-0293.

216. Whitehead, S.J., Summerfield, R.J., Muehlbauer, F.J., Coyne, C.J., Ellis, R.H., Wheeler, T.R., 2000. Crop Improvement and the Accumulation and Partitioning of Biomass and Nitrogen in Lentil. Crop Science 40, 110 - 120. https://doi.org/10.2135/cropsci2000.401110x.

217. Wieser, H., 2012. The Use of Redox Agents in Breadmaking, in: Cauvain, S.P. (Ed.), Breadmaking (Second Edition), Woodhead Publishing Series in Food Science, Technology and Nutrition. Woodhead Publishing, pp. 447 - 469. https://doi.org/10.1533/9780857095695.2.447.

218. Wieser, H., 2007. Chemistry of Gluten Proteins. Food Microbiology, 3rd International Symposium on Sourdough 24, 115 - 119. https://doi.org/10.1016/j.fm.2006.07.004.

219. Wieser, H., Kieffer, R., 2001. Correlations of the Amount of Gluten Protein Types to the Technological Properties of Wheat Flours Determined on a Micro-scale. Journal of Cereal Science 34, 19 - 27. https://doi.org/10.1006/jcrs.2000.0385.

220. Willer, E.H., Schlatter, B., Trávnícek, J., Kemper, L., Lernoud, J. (Eds.), 2020. The World of Organic Agriculture Statistics and Emerging Trends 2020. Reasearch Institute of Organic Agriculture FiBL IFOAM - Organics International, p. 337.

221. Wiwart, M., Szafrańska, A., Wachowska, U., Suchowilska, E., 2017. Quality Parameters and Rheological Dough Properties of 15 Spelt (*Triticum spelta* L.) Varieties Cultivated Today. Cereal Chemistry 94, 1037 - 1044. https://doi.org/10.1094/CCHEM-05-17-0097-R.

222. Wrigley, C., Bekes, F., Bushuk, W., 2006. A Balance of Gliadin and Glutenin. pp. 3 - 32. https://doi.org/10.1094/9781891127519.002.

223. Wrigley, C.W., 1996. Giant Proteins with Flour Power. Nature 381, 738 - 739. https://doi.org/10.1038/381738a0.

224. Wrigley, C.W., Bietz, J.A., 1988. Proteins and Amino Acids. Wheat Chemistry and Technology. American Association of Cereal Chemists, St. Paul, Inc., St. Paul, 1988, pp. 159-275.

225. Yearbook, 2018. Organic Farming in the Czech Republic. http://eagri.cz/public/web/file/643739/Rocenka_ekologickeho_zemedelstvi_2018_WEB.pdf.

226. Zeven, A.C., 1980. The Spread of Bread Wheat over the Old World since the Neolithicum as Indicated by Its Genotype for Hybrid Necrosis. Journal D'agriculture Traditionnelle et de Botanique Appliquée 27, 19 - 53. https://doi.org/10.3406/jatba.1980.3807.

227. Zhang, Y., Xu, W., Wang, H., Dong, H., Qi, X., Zhao, M., Fang, Y., Gao, C., Hu, L., 2016. Progress in Genetic Improvement of Grain Yield and Related Physiological Traits of Chinese Wheat in Henan Province. Field Crops Research 199, 117 - 128. https://doi.org/10.1016/j.fcr.2016.09.022.

228. Zheng, T.C., Zhang, X.K., Yin, G.H., Wang, L.N., Han, Y.L., Chen, L., Huang, F., Tang, J.W., Xia, X.C., He, Z.H., 2011. Genetic Gains in Grain Yield, Net Photosynthesis and Stomatal Conductance Achieved in Henan Province of China between 1981 and 2008. Field Crops Research 122, 225 - 233. https://doi.org/10.1016/j.fcr.2011.03.015.

229 Zhou, Y., Zhu, H.Z., Cai, S.B., He, Z.H., Zhang, X.K., Xia, X.C., Zhang, G.S., 2007. Genetic Improvement of Grain Yield and Associated Traits in the Southern China Winter Wheat Region: 1949 to 2000. Euphytica 157, 465 - 473. https://doi.org/10.1007/s10681-007-9376-8.

230. Zobel, H.F., 1988. Starch Crystal Transformations and Their Industrial Importance. Starch - Stärke 40, 1 - 7. https://doi.org/10.1002/star.19880400102.

9 Appendixes

Appendix A: The field trials in Zvikov and Ceske Budejovice.

Appendix B: The Mixolab standard protocol of studied varieties.

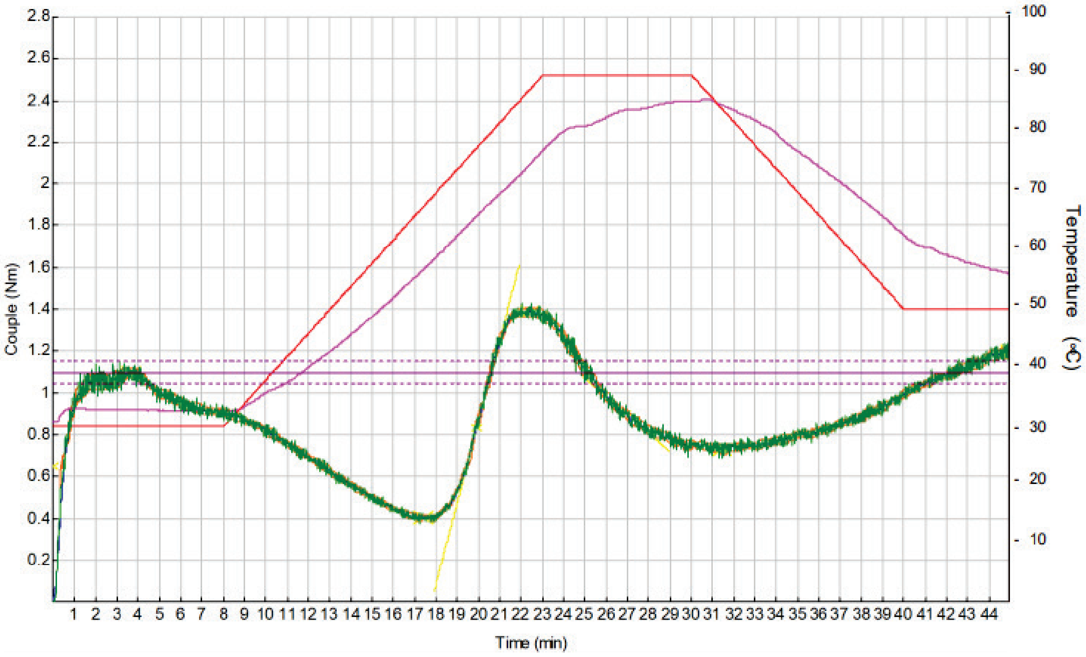
Appendix C: The list of articles publication.

Appendix A: The field trials in Zvikov and Ceske Budejovice

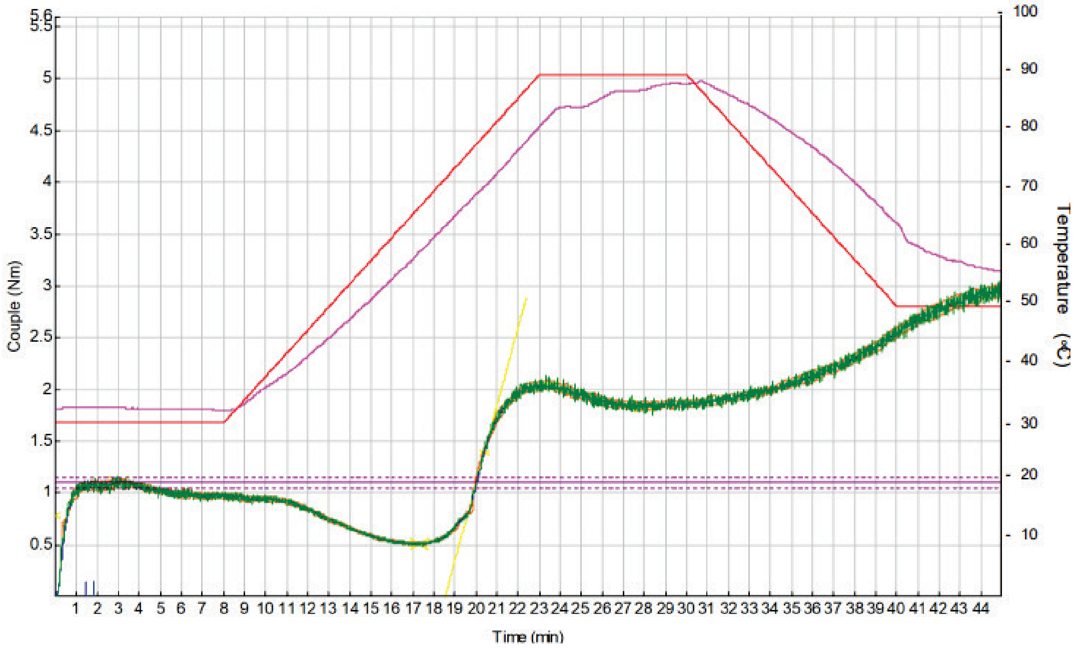
The field trials in Zvikov

The field trials in Ceske Budejovice

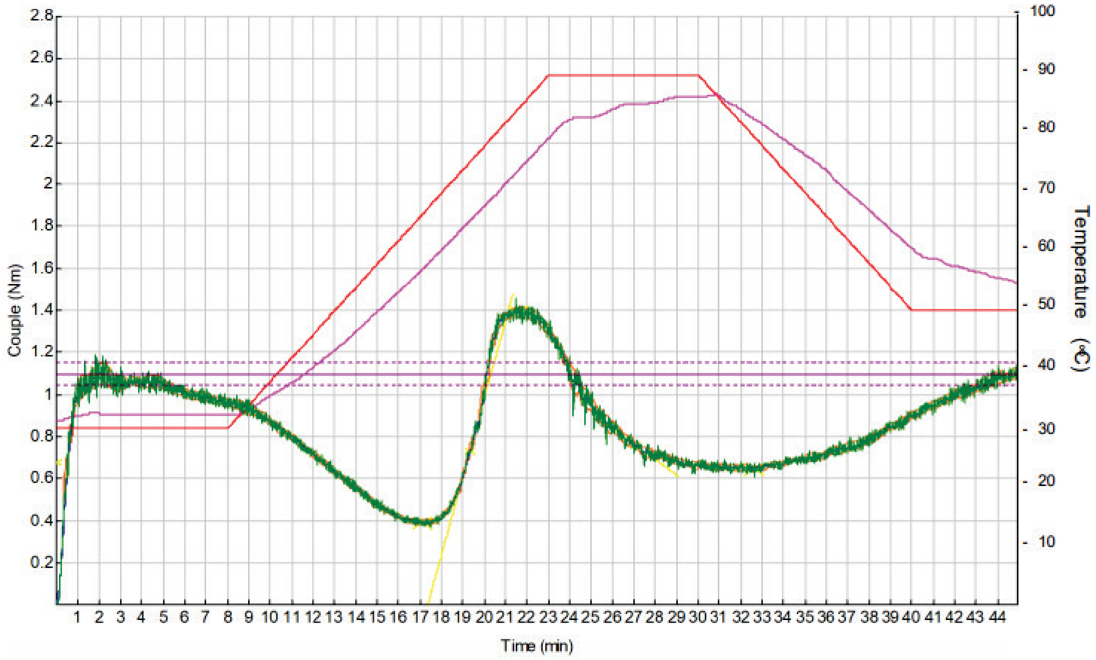
Appendix B: The Mixolab standard protocol of studied varieties



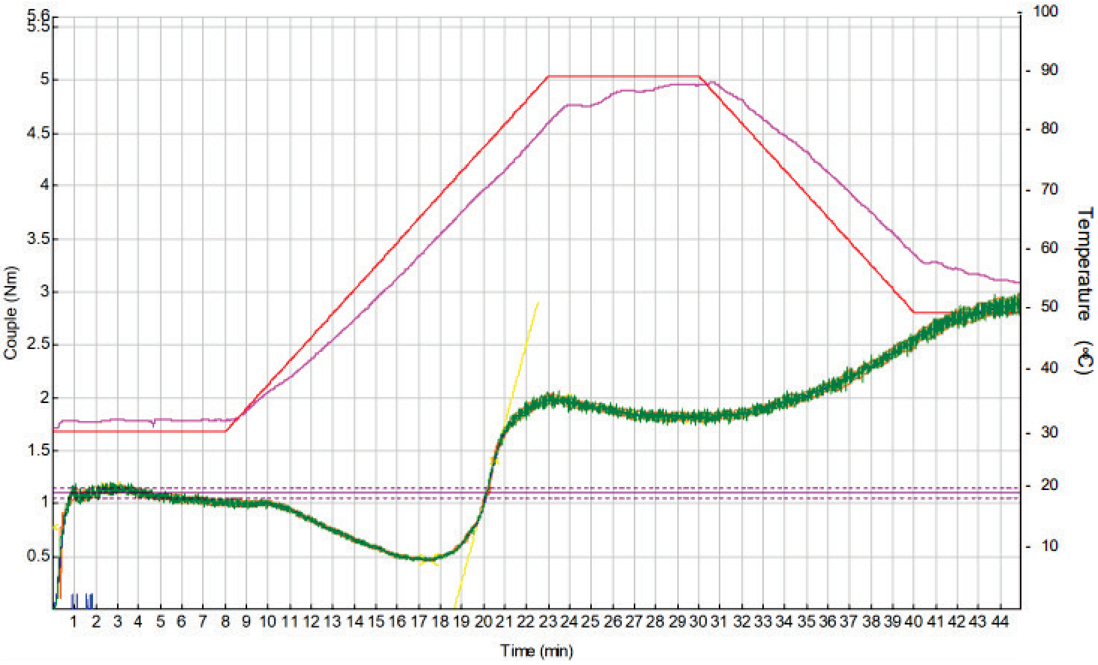
The Mixolab standard protocol of Annie variety



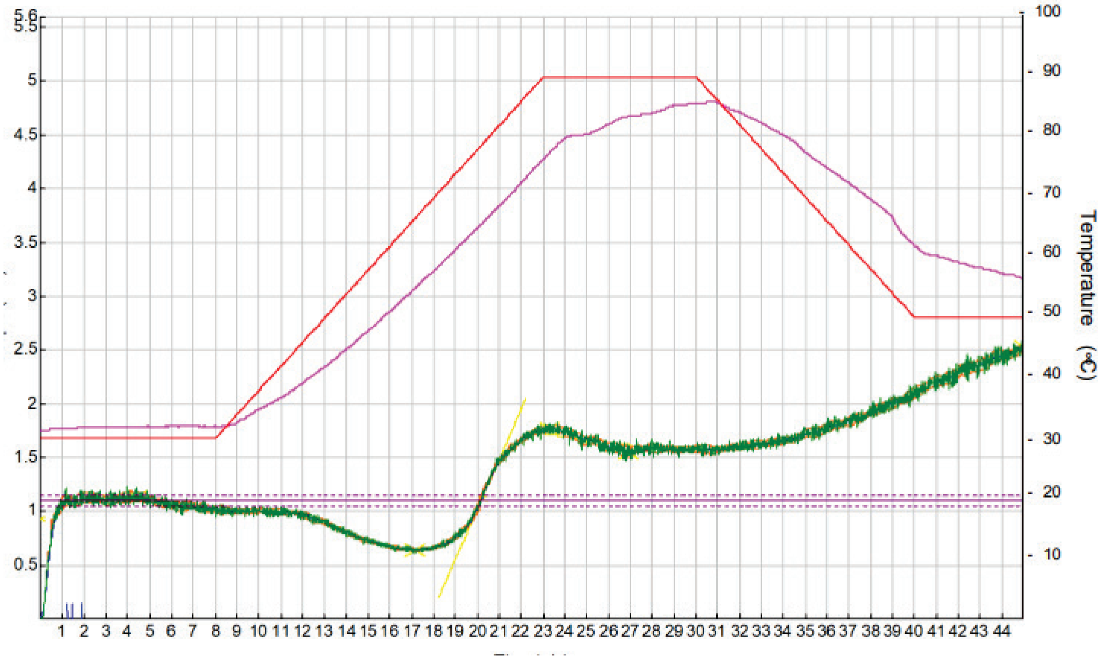
The Mixolab standard protocol of Balitus variety



The Mixolab standard protocol of KM 15 - 17 variety



The Mixolab standard protocol of Penelope variety



The Mixolab standard protocol of Zeppelin variety

Appendix C: The list of articles publication.

No	Genre and Impact Factor (IF)	First author/co-author	Published	Year
Journal				
1	Comparative Study on Protein Quality and Rheological Behavior of Different Wheat Species **IF = 2.603**	First author	Agronomy https://www.mdpi.com/2073-4395/10/11/1763	2020
Conference article				
2	The Role of Genetic Resources for the Development of Organic Farming and Decentralized Food Production	Second author	Proceedings of the International Scientific Congress "Life Sciences, a Challenge for the Future" (17th - 18th October 2019, Iasi, Romania)	2019
3	The Quality of Hulled Wheat Species in Organic Farming	First author	Proceeding of 24th International Ph.D. Students Conference in Mendel University in Brno. https://mendelnet.cz/pdfs/mnt/2017/01/142.pdf	2017
4	The Comparative Research on Rice Production between Low Input Farming System and Conventional Farming System from Life Cycle Assessment in Central Vietnam	First author	Proceeding of ORGATROP International Conference at Universitas Gadjah Mada Yogyakarta	2017

5	The Antioxidant Activity of Ancient Wheat Varieties and Modern Wheat Varieties	First author	Proceeding of 23th International Ph.D. Students Conference in Mendel University in Brno. https://mendelnet.cz/pdfs/mnt/2016/01/26.pdf	2016
Journal				
6	Influence of Selected Maize Cultivation Technologies on Changes in the Labile Fraction of Soil Organic Matter Sandy-loam Cambisol Soil Structure **IF = 4.601**	Coauthor	Soil and Tillage Research Influence of Selected Maize Cultivation Technologies on Changes in the Labile Fraction of Soil Organic Matter Sandy-loam Cambisol Soil Structure - ScienceDirect	2021
7	Influence of Husk on Grain Contamination by *Fusarium* spp. and *Alternaria* spp. in Hulled Spelt (*Triticum spelta* L.). **IF = 0.936**	Coauthor	Environmental Engineering and Management Journal March 2002 Vol (tuiasi.ro)	2018
Conference article				
8	The Importance of Soil Organic Matter	Coauthor	Proceeding of ORGATROP International Conference at Universitas Gadjah Mada Yogyakarta	2017
9	LCA - Tool for Evaluation of Impact of Organic Agriculture on Greenhouse Gases Emissions	Coauthor	Proceeding of ORGATROP International Conference at Universitas Gadjah Mada Yogyakarta	2017

10	Technological and Sensory Quality of Grain and Baking Products from Spelt Wheat	Coauthor	Conference: Research for Rural Development in Litva Technological and Sensory Quality of Grain and Baking Products from Spelt Wheat (llu.lv)	2017
Chapter				
11	Rheological and Technological Quality of Minor Wheat Species and Common Wheat	Coauthor	INTECH DOI: 10.5772/67229	2017

agronomy

Article

Comparative Study on Protein Quality and Rheological Behavior of Different Wheat Species

Khoa Dang Tran [1,2], Petr Konvalina [1,*], Ivana Capouchova [3], Dagmar Janovska [4], Magdalena Lacko-Bartosova [5], Marek Kopecky [1] and Phuong Xuan Thi Tran [2]

[1] Faculty of Agriculture, University of South Bohemia in Ceske Budejovice, Studentska 1668, 37005 Ceske Budejovice, Czech Republic; trandangkhoa@hueuni.edu.vn (K.D.T.); mkopecky@zf.jcu.cz (M.K.)
[2] Faculty of Agronomy, University of Agriculture and Forestry, Hue University, 102 Phung Hung street, Hue City 530000, Vietnam; ttxphuong@hueuni.edu.vn
[3] Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Kamycka 129, 16500 Praha 6 Suchdol, Czech Republic; capouchova@af.czu.cz
[4] Crop Research Institute, Drnovska 507/73, 16106 Praha 6 Ruzyně, Czech Republic; janovska@vurv.cz
[5] Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Tr A. Hlinku 2, 94976 Nitra, Slovakia; magdalena.lacko-bartosova@uniag.sk
* Correspondence: konvalina@zf.jcu.cz; Tel.: +420-387-772-547

Received: 15 October 2020; Accepted: 9 November 2020; Published: 12 November 2020

Abstract: The quantity and quality of protein and the rheological traits of wheat are crucial for processing flour in the baking industry, but there are few comparisons in the literature between old and modern wheat species. To help fill this gap, the baking quality characterization, gluten content, protein fraction composition, high molecular weight glutenin subunits, and rheological properties of ancient and modern wheat were determined and compared. These varieties were collected by the gene bank of the Crop Research Institute in Prague-Ruzyne and were grown in organically certified research areas in the Czech Republic. Results revealed differences in protein content and composition between varieties with different ploidy levels, as well as differences in development time and stability between einkorn and bread wheat varieties. Based on the proximity of their positions to the parameter quality in the principal components analysis, such as gluten content, gluten index (GI), Zeleny test, stability, dough development time (C1) and gliadin, the baking performances of cultivars were identified.

Keywords: einkorn; emmer; spelt; bread wheat; protein composition; nutritional and technological quality; mixolab

1. Introduction

Originating in the Fertile Crescent, known as "the cradle of civilization", wheat initially migrated to North Africa and then spread to Europe and Asia. Parts of Western Europe cultivated wheat in the early 15th century. The wheat-cultivating regions continuously expanded to other continents from the 17th to 19th centuries, apart from Antarctica.

The Fertile Crescent, which includes 10 countries, is surrounded by the Syrio-Arabian desert in the south, the Mediterranean in the west, and chains of large and high mountain ranges in the east and north. Located between mountains, the desert, and the sea, this area is influenced by several different climates. The Fertile Crescent is known as one of the most diversified regions. Archaeological evidence substantiates that the Fertile Crescent is the hometown of the wild taxa of four species of wheat, and it is most likely the area where wheat was first domesticated [1].

Einkorn wheat (*Triticum monococcum* L.) is the oldest domesticated wheat species. It is a diploid ($2n = 2x = 14$ chromosomes; AA genome) wheat [2]. Diploid wheat domestication occurred in the northeastern part of the Levantine Corridor (ca. 10,000 BP) [1]. In the 20th century, einkorn was mainly cultivated on marginal lands and under low input conditions. The production of einkorn wheat was limited to local areas.

Triticum dicoccum (Schrank) Schuebl is a tetraploid wheat ($2n = 4x = 28$ chromosomes; AABB genome). The earliest archeological evidence of wild emmer is from the first half of the 10th millennium BP in the Jordan Valley and Damascus basin [3]. Other evidence indicated that wild emmer originated from the second half of the 10th millennium in Cayonu, East Anatolia, Alikos, and Southwestern Iran [3]. Emmer was cultivated from the beginning of agriculture until it was replaced by free-threshing wheat in Graeco-Roman times [4]. In recent years, emmer was reestablished in several countries in Europe such as Austria, Germany, and Switzerland [2].

Spelt wheat (*Triticum spelta* L., hexaploid: $2n = 6x = 42$ chromosomes; AABBDD genome) has been well-documented by archeologists in Europe. Spelt was discovered at Neolithic sites (from 2500 to 1700 BC) in Germany, Poland, and Denmark [2], and has been cultivated until modern times in Central Europe. In German-speaking countries (Switzerland, Southern Germany, and Austria), spelt was the dominant cereal until the end of the 19th century. After World War II, spelt cultivation areas diminished to a few thousand hectares.

Einkorn, emmer, and spelt are hulled wheat. The disadvantage of this group is its low yield and difficulties with threshing because the hulls remain attached upon threshing [5]. This is why it has been superseded by bread wheat (*Triticum aestivum* L. hexaploid: $2n = 6x = 42$ chromosomes; AABBDD genome).

In wheat, both the quantity and quality of protein are crucial. The major types of protein can be divided into three categories: simple, conjugated and derived. However, only simple protein is found in wheat plants, consisting of four major types: albumins (soluble in water and dilute buffers), globulins, prolamins, and glutelins. Gluten, the remainder of wheat flour after removing starch, non-starchy polysaccharides, and water-soluble constituents, comprises alcohol-soluble gliadins and alcohol-insoluble glutenins [6].

Wheat storage proteins have two basic fraction groups: gliadins and glutenins. Glutenins are known as being the larger polymers in nature and are measured as high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS). These are used as protein markers for predicting the quality of bread and identifying wheat varieties [7,8].

Rheological traits are important for processing flour in the baking industry. This index is used for predicting dough-processing parameters and the quality of the end product. To investigate flour and dough characteristics, such as elasticity, viscosity, and extensibility, traditional rheological instruments such as farinograph, extensograph, and alveograph can be used. However, with Mixolab II (Chopin Technologies, Paris, France), a new rheological device, researchers are able to measure the physico-chemical behavior of dough during heating and cooling processes [9]. During five stages in the process, Mixolab parameters are measured as the change of torque when mixing and heating wheat flour and water. They provide information about maximum torque, protein quality, starch characteristics, enzyme activity, and starch retrogradation [10].

The aim of our research was to evaluate the differences in proteins and their technological quality between *Triticum aestivum* L. varieties and other less common species, such as diploid *Triticum monococcum* L., tetraploid *Triticum diccocum* Schrank (Schuebl), and hexaploid *Triticum spelta* (L.).

2. Materials and Methods

2.1. Field Trial and Sampling

All varieties used in this study originated from the gene bank of the Crop Research Institute in Prague-Ruzyne, Czech Republic. Four *Triticum monococcum* L. (einkorn), eight *Triticum diccocum* Schrank (Schuebl) (emmer), seven *Triticum spelta* L. (spelt), and seven *Triticum aestivum* L. varieties were chosen (Table S1). Crops were cultivated in a random complete block design trial with four field replications (subplots) under certified organic management. The field trials were conducted in three locations at the University of South Bohemia in Ceske Budejovice (USB), Czech University of Life Sciences in Uhrineves (CULS), and Crop Research Institute in Prague, the Czech Republic (CRIP) during vegetation seasons 2012–2015. The seeding rate was adjusted to a density of 350 grains per m^2. The samples were grown in four replicates. The harvested plot size was 10 m^2. All varieties were spring forms. The crop stands were treated in compliance with European legislation (European Council (EC) Regulation No. 834/2007, the EC Regulation No. 889/2008).

Characteristics of the conditions at the University of South Bohemia in the Ceske Budejovice research area were as follows: mild warm climate, pseudo gley cambisols soil, with loamy sand soil, and an altitude of 388 m. The conditions at the Czech University of Life Sciences in Prague were as follows: warm and mid-dry climate, brown soil, loamy clay soil, and altitude of 295 m. The Crop Research Institute in Prague-Ruzyne has a warm mid-dry climate, degraded chernozem soil, clay and loamy soil, and an altitude of 340 m.

2.2. Baking Quality Characterization

2.2.1. Wheat Flour Samples

In this study, 26 wheat varieties were assessed. Each variety was determined in four replicates. The wheat samples were milled in to white flours using a PSY MP 20 (Mezos, Hradec Kralove, Czech Republic) and Quadrumat Junior machine (Brabender, Duisburg, Germany). Protein content (PC) was determined by the Kjeltec 1002 System (Tecator AB, Hoganas, Sweden), based upon N * 5.7 (in dry matter). Gluten content and gluten index (GI) were estimated by Glutomatic 2200 and Centrifuge 2015 (Perten Instruments, Hägersten, Sweden according to ICC (International Association for Cereal Chemistry) 155 (ICC, 1994a)). Sodium dodecyl sulphate (SDS) was analyzed in flour samples according to the method of Axford et al. [11]. Zeleny index (ZI) was measured by using a SDZT4 apparatus (Santec, Vydrany, Slovakia) according to the ICC 116/1 (ICC, 1994b).

2.2.2. Protein Fractions

Hulled grains were used for the analysis. Albumins + globulins were determined by extraction with 10% NaCl, prolamins by extraction with 70% ethanol, and glutelins by extraction with 0.2% NaOH. All samples were analyzed during 45 min at 20 °C and repeated three times [12].

2.2.3. High Molecular Weight Glutenin Subunits (HMW-GS) Analysis

Glutenins were extracted from single crushed wheat kernels using a 0.25 M Tris-HCl buffer (pH: 6.8) containing 5% (*v*/*v*) β-mercaptoethanol, 2% (*w*/*v*) SDS, 10% (*v*/*v*) glycerol, and 0.02 (*w*/*v*) bromophenol blue. The extract was heated at 100 °C for 2 min and centrifuged for 2 min at 15,000 rpm. The electrophoretic patterns of HMW-GSs were determined by using one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), using the Laemmli buffer system [13]. The OWL Separation System P9DS (Thermo Scientific, Waltham, MA, USA) was used to run gels. The acrylamide/bisacrylamide concentration (T) and the cross linker (C) used were as follows: T = 10% and C = 2.60%. Electrophoresis was performed at a constant current (30 mA/gel), at 10 °C for the time required for the tracking marker dye to migrate off the gel. Proteins in the gels were fixed for 1 h with 10% (*w*/*v*) trichloroacetic acid solution and subsequently stained with 0.5% (*w*/*v*) Coomassie Brilliant Blue R-250 solution, 25% (*v*/*v*) methanol, and 10% (*v*/*v*) acetic acid. Destaining was conducted with running water. Particular alleles of HMW-GSs were identified according to the catalog published by Payne and Lawrence [14,15].

2.3. Mixolab Analysis

Mixolab II was used to evaluate baking quality according to the ICC standard method No. 173-ICC 2006, which allowed us to evaluate physical dough properties, such as dough stability or weakening, and starch characteristics in one measurement. For the rheological test, only selected varieties with three replications from three years and three locations were analyzed. The evaluated parameters in Mixolab are illustrated in Figure 1, in which five stages can be distinguished.

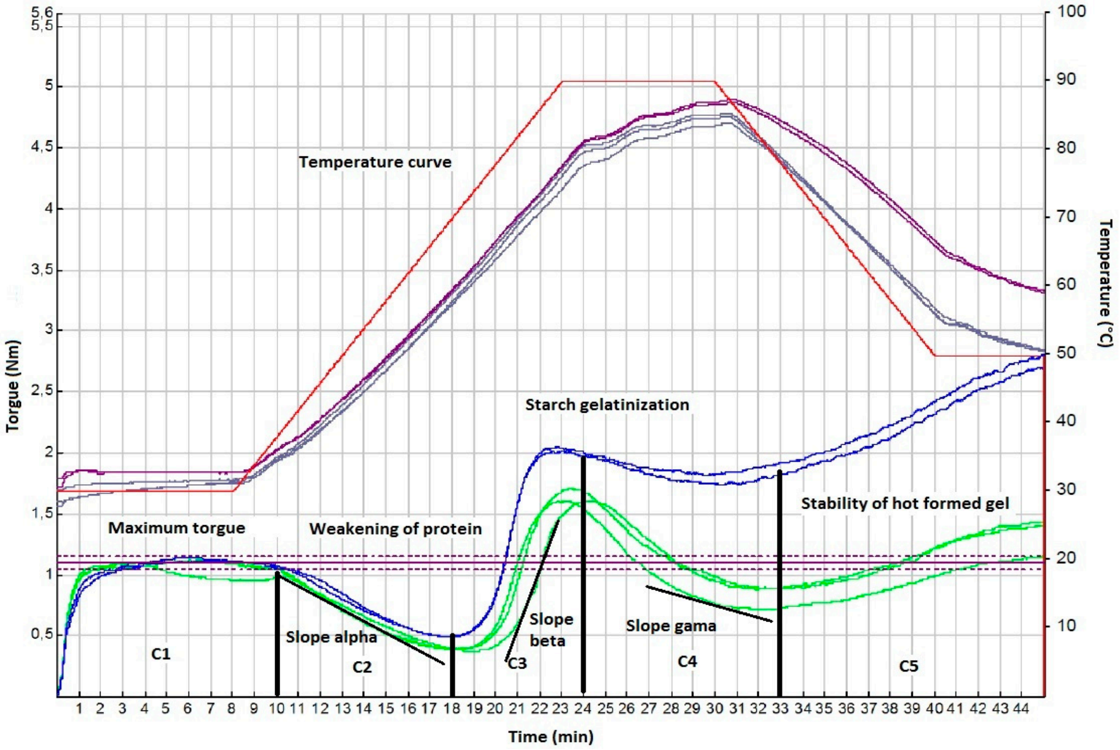


Figure 1. The Mixolab curves from wheat flour. Time for C1: The time evolution of the dough. The stronger the flour, the longer the time evolution (time to reach C1); Amplitude: The elasticity of the dough. The higher the value, the more flexible the flour; Stability: Resistance against kneaded dough. The longer the duration, the stronger the flour; C2: Attenuation of protein due to mechanical work and temperature; C3: The gelling starch; C4: The stability of the hot gel; C5: Measured starch retrogradation in the cooling phase; Guideline α (C1–C2): Attenuation rate of protein in warming; Guideline β (C3–C4): Speed starch gelatinization; Guideline γ (C5–C4): The rate of enzymatic degradation. [16].

In the first stage, hydration of the flour compounds occurs at 30 °C together with the stretching and alignment of the proteins, which leads to formation of the viscoelastic structure. An increase in the torque was observed during this stage until it reached the maximum value (1.10 Nm). The torque decreased to a minimum value in the second stage, which was attributed to the weakening of the protein network for mechanical shear stress and protein destabilization [17,18]. The third stage demonstrates an increased temperature and gelatinization of starch. The granules absorb the water available in the medium and they swell, so the viscosity increases. In the fourth stage, the amylase activity and the physical breakdown of the granules are associated with a reduction in the viscosity. A decrease in the temperature resulted in an increase in torque, which is referred to as setback and corresponds to the gelation process. The last stage is related to retrogradation [17]. Temperature regime in Mixolab was as follows: 8 min at 30 °C, heating at a rate of 4 °C min^{-1} for 15 min, holding at 90 °C for 7 min, cooling to 50 °C at a rate of 4 °C min^{-1} for 10 min, and holding at 50 °C for 5 min [19].

2.4. Statistical Analysis

Data were analyzed using the Statistica 9.0 program (StatSoft. Inc., Palo Alto, CA, USA). Comparisons of mean varieties and their division into statistically different categories were conducted using the Tukey's honest significant difference (HSD) test with p-values <0.01 and <0.05 considered statistically significant. One-way analysis of variance (ANOVA) and combined ANOVA were applied for variance analysis. Principal component analysis was used to assess the association between groups of variables and the differences between ancient and modern wheat varieties.

3. Results

3.1. Thousand Grain Weight and Grain Yield of Wheat Varieties

Thousand grain weight (TGW) is a crucial component of grain yield, which defines not only the improvement of grain yield but also the improvement in milling yield. The data in Figure 2 indicate that the SP4 variety had the maximum TGW (41.05 g), while the minimum value (24.87 g) was observed for variety J4. Analysis of variance (Table S2) shows that the groups with the highest TGW (from 39.71 g to 41.05 g) belong to the spelt species and common wheat, whereas the einkorn species had the lowest TGW at approximately 25.5 g.

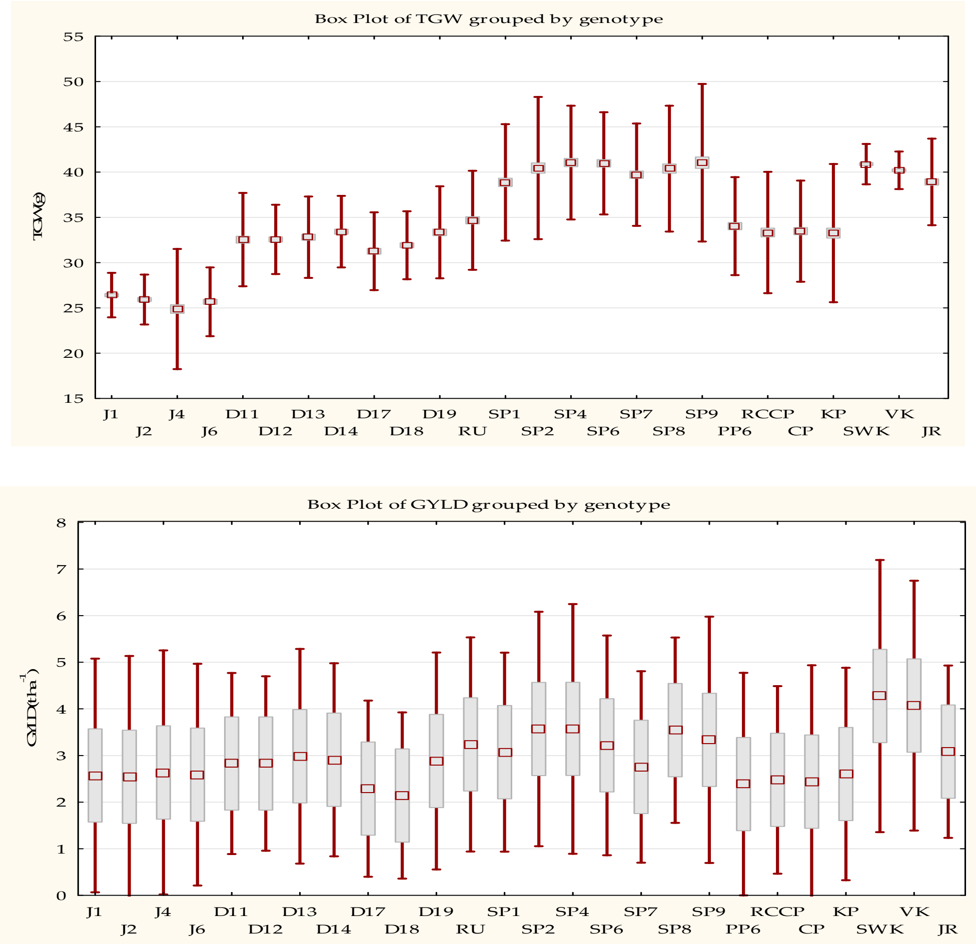


Figure 2. The data show the box plots of thousand grain weight (TGW) and grain yield GYLD, grouped by genotype. The figures are presented as means of 26 wheat varieties grown at three locations, four replications, in four years ($n = 48$). Box plots represent the interquartile range; the rectangles in the box symbolize the mean value ± 1, and whiskers designate the minimum and maximum of the box plot.

Grain yield of all wheat varieties was recorded and summarized in Figure 1 and Table S2. As expected, common wheat had the highest yield (from 4.07 t ha^{-1} to 4.28 t ha^{-1}), with the exception of Jara variety (3.08 t ha^{-1}), compared to the other four wheat species. Einkorn, emmer, and bread wheat landraces fell into the lowest yield group. Interestingly, SP8, SP2 and SP4 had the highest yields (3.54 t ha^{-1}, 3.57 t ha^{-1}, and 3.58 t ha^{-1}, respectively) and were not significantly different from the VK and SWK cultivars in organic farming conditions.

3.2. Basic Baking Quality

Baking Quality Characterization

Analysis of variance for protein content, wet gluten content, gluten index, SDS test, and Zeleny test was performed in four replicates per sample with 26 varieties each of five wheat species cultivated in three locations, (n = 1248). The results were significant at p-value <0.05 for the environments (four years cultivated and three locations) for all indexes and indicated highly significant variance among the species with regard to the protein content, wet gluten content, gluten index, SDS text, and Zeleny test. From Figure 3, Tables S3 and S4, it is apparent that common wheat and bread wheat landraces had the lowest protein content (12.76%, 13.15%, respectively) compared to the other three wheat species. The bread wheat varieties SWK and VK had the lowest protein content at 12.13% and 12.87%, respectively, whereas emmer variety D17 had the highest at 17.50%. In general, all varieties of einkorn, emmer, and spelt were not statistically different. A remarkable increase was seen for gluten in the five wheat species. Spelt species had the highest gluten content (42.19%) and common wheat the lowest (30.87%), with bread wheat landraces (33.07%), emmer wheat (37.96%), and einkorn (36.35%) in between. The SP9 genotype had the highest gluten content (45.29%), whilst the SWK had the lowest gluten content (27.52%). In contrast, common wheat had the highest gluten index (mean 68.78), followed by bread wheat landraces (mean 41.46) and spelt (mean 35.68). The gluten index of einkorn wheat and emmer wheat ranked at the bottom of the table with 14.4 and 15.4, respectively. SDS and Zeleny tests showed similar trends for the five species, with the highest value for common wheat, the second highest value for bread wheat landraces and spelt, and the lowest for the rest of the species. The highest values from SDS and Zeleny tests were seen for the SWK variety with 71.83 mL and 46.89 mL, respectively. J4, on the other hand, had the lowest values (21.67 mL and 9.59 mL, respectively).

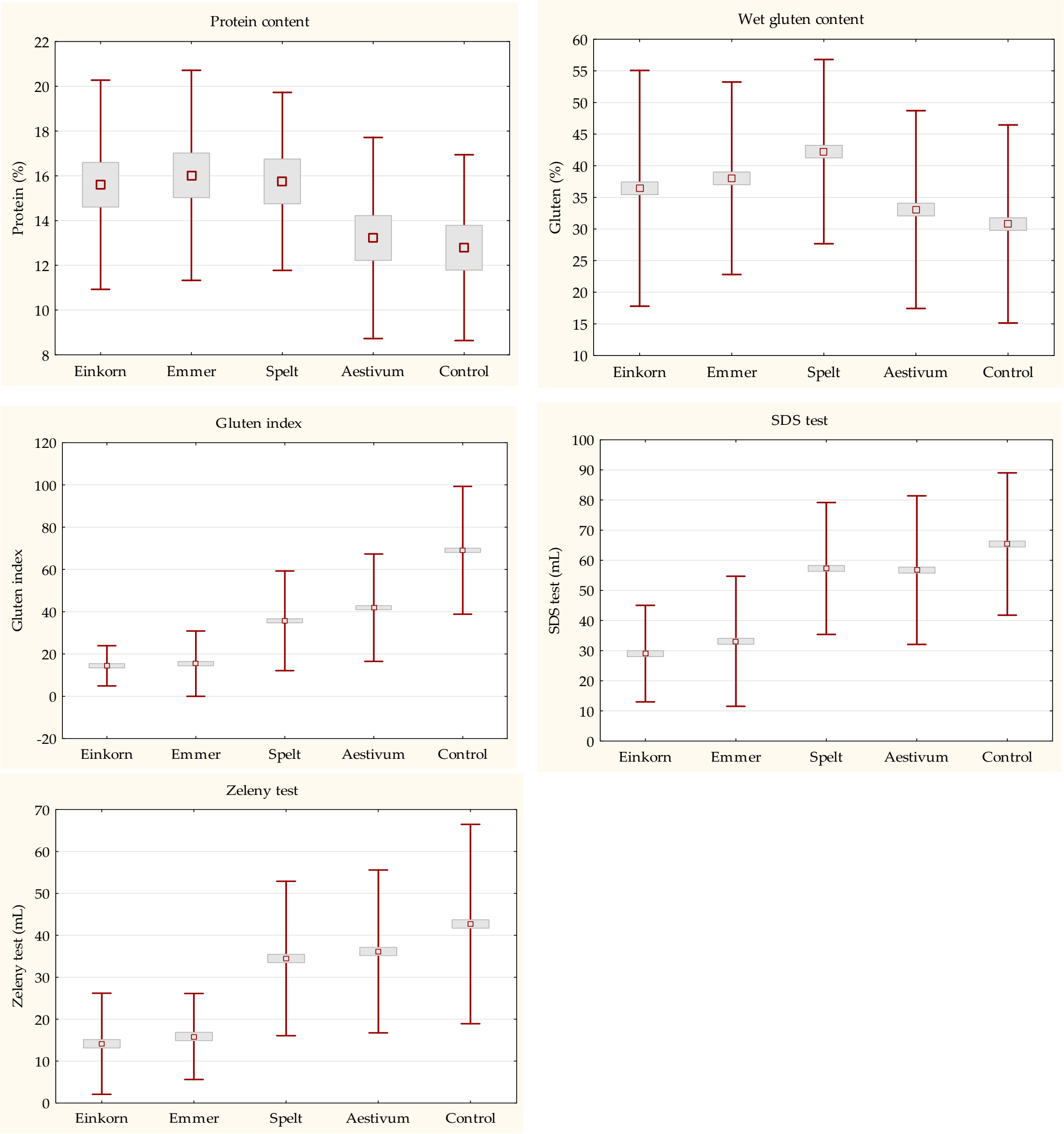


Figure 3. Contents of protein and gluten, gluten index, sodium dodecyl sulphate (SDS) test, and Zeleny test in einkorn, emmer, spelt, aestivum, and control varieties. Figures are presented as the mean of five wheat species grown at three locations, four replications, in four years (*n* = 1248). Bbox plots represent the interquartile range, the rectangles in the box symbolize the mean value ±1, and whiskers designate minimum and maximum of the box plot.

Regarding two-way interactions, the entire index of protein fractions was significantly different for year × location, year × species, and location × species. This was also true for the analysis of three-way interactions.

3.3. *Quantitaion of Gluten Content and Protein Fractions Composition*

The analysis of variance for gluten content, albumins + globulins, gliadins, glutenins, and insoluble remainder is presented in Figure 4, Tables S5–S7. The results reveal a highly significant effect with a *p*-value < 0.01 for the environments and varieties for all traits. Similarly, the data revealed a highly remarkable effect on all characteristics, with a *p*-value <0.01 for the interaction between two and three factors.

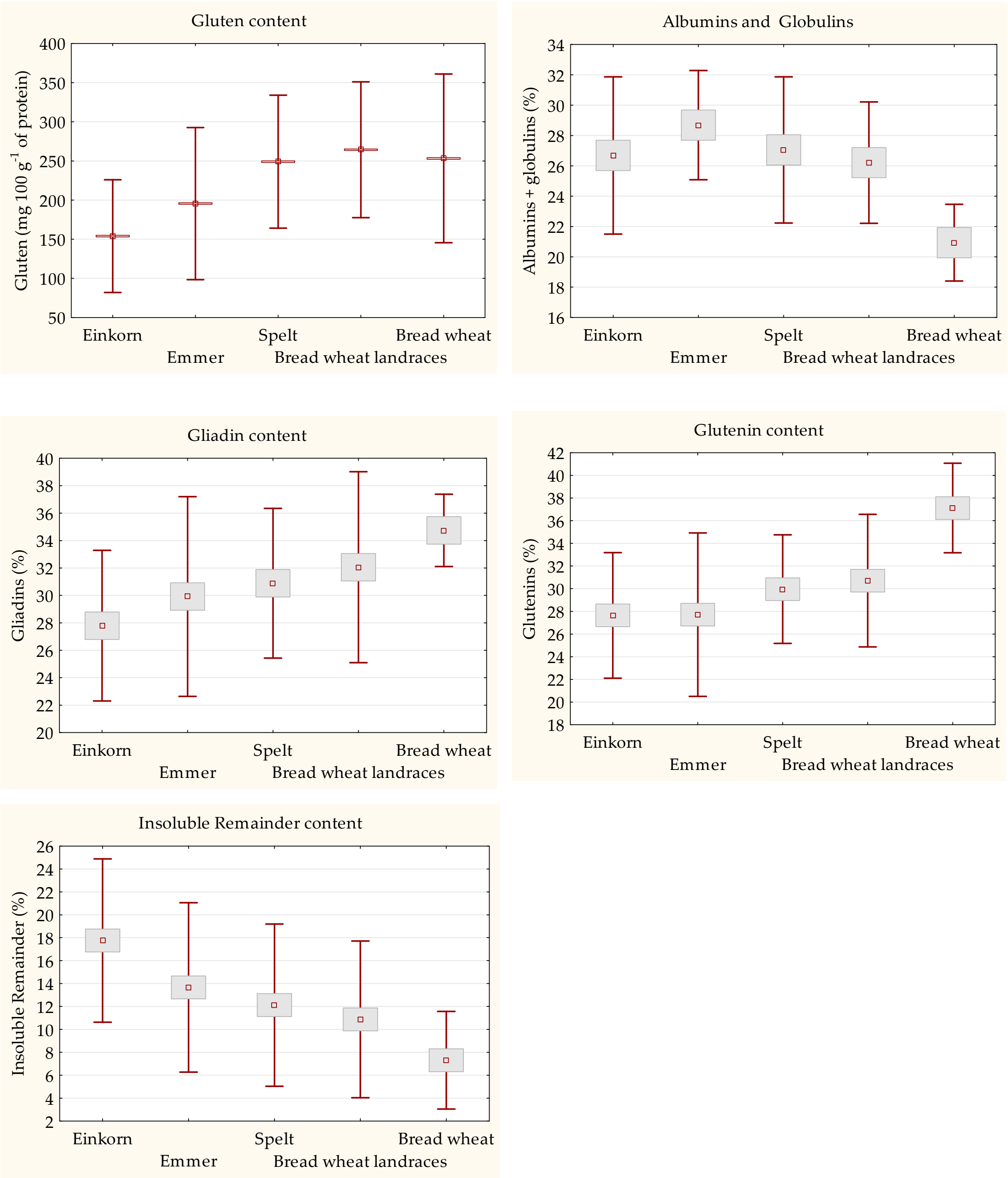


Figure 4. Contents of gluten, albumins and globulins, gliadins, glutenins, and insoluble remainder. The figures are presented as means of five wheat species grown at three locations, three replications, in four years (*n* = 936). Box plots represent the interquartile range, the rectangles in the box symbolize the mean value ± 1, and whiskers designate the minimum and maximum of the box plot.

Gluten content was the highest in the control varieties and landraces of *Triticum aestivum* L., followed by spelt with no statistical difference. The lowest value was found in einkorn wheat and emmer wheat (confirmed also as statistically different using Tukey's HSD test). The values for gluten content ranged from 146.41 to 273.78 mg 100 g^{-1}. The highest amount of gluten content was found in bread wheat landraces of wheat variety CP, whereas the lowest one was found in the J2 variety. Globulins were highest in hulled wheat (26.67–28.68%), while the control wheat lagged considerably in this parameter (20.93%). The mean values of albumins and globulins ranged from 20.12 to 30.02%, with the lowest value for the VK variety and highest one for the RU variety. Einkorn had nearly three times as much insoluble remainder as bread wheat. In contrast, for gliadins and glutenins, the control variant of common wheat showed the highest values. Highly significant differences were reported with respect to gliadins and glutenins among species as well as varieties. The highest gliadin amount was observed in wheat variety KP (35.53%), whereas the J1 variety showed the lowest amount (27.01%). The mean values of glutenins ranged from 20.43% to 38.67%. Wheat variety D14 exhibited the lowest glutenin amount (20.43%); conversely, wheat variety SWK had the highest glutenin (38.67%). The highest amount of insoluble remainder was found in the J1 variety (20.48%), whereas the lowest was found in the JR variety (6.91%).

3.4. Characterization of HMW-GSs from the Technological Viewpoint

Although HMW-GS constitutes approximately 10% of total flour protein, it is the most essential determinant of bread-making quality [20]. Two classes of glutenin subunits, HMW-GS and LMW-GS, are present in wheat, and they are released during the reduction of disulfide bonds with reducing agents and determined when analyzed by electrophoresis. The released glutenin subunits were further classified into four subgroups (A–D) by electrophoretic mobility on SDS-PAGE. Subgroup A was determined to be HMW-GS, and subgroups B, C, and D were referred to as LMW-GS [21–24]. It was also observed that once reduced and separated by SDS-PAGE, high molecular weight gliadins had a mobility similar to those of the B and C subunits of LMW-GS [25]. Having molecular weights between 65 and 90 kDa, based on derived amino acid sequences, and 80–130 kDa on SDS-PAGE, HMW-GS are encoded at complex loci on the long arms of chromosomes 1A, 1B, and 1D of hexaploid wheat, which are the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci, respectively [23].

HMW-GSs are usually used to indicate the genetic potential of bread wheat varieties, from the baking quality perspective using the Glu-1 score reported by Payne et al. [26], which allocates scores for the subunits (alleles) in each of the wheat's three genomes (Glu-A1, Glu-B1, and Glu-D1). This is mainly associated with the HMW glutenin subunits encoded at the Glu-D1 locus. The absence of the D genome in emmer wheat and einkorn wheat might be one reason for the lower bread making quality compared to spelt, bread wheat landraces, and bread wheat. The results from Table 1 showed that the spelt wheat varieties differed from the bread wheat varieties in HMW-GSs polymorphism. Different HMW-GS alleles for Glu-B1 and Glu-D1 were found in the studied spelt wheat varieties. Glu-D1 subunit 2 + 12 predominated instead of 5 + 10 at the HMW-GS alleles of *Triticum aestivum* L.

Table 1. Allelic frequency for Glu-A1, Glu-B1, and Glu-D1 loci of the evaluated varieties.

Species	**Variety**	**HMW-GSs**		
		Glu-A1	***Glu-B1***	***Glu-1D***
Einkorn	J1	0		
	J2	0		
	J4	0		
	J6	0		
Emmer	D11	1	(7 + 8)	
	D12	1	(7 + 8)	
	D13	2 *	6 (21)	
	D14	0	0	
	D17	1	6 (21)	
	D18	1	(7 + unk)	
	D19	1	(7+8)	
	RU	1	(7+8)	
Spelt	SP1	1	7+8	2+12
	SP2	1	6 + 8	2 + 12
	SP4	1	6 + 8	2 + 12
	SP6	1	7 + 8	2 + 12
	SP7	1	7 + 8	2 + 12
	SP8	1	7 + 8	2 + 12
	SP9	1	6 + 8	2 + 12
Landraces of bread wheat	P1	2 *	7	2 + 12
	P2	0	7 + 9	5 + 10
	P3	0	7 + 9	5 + 10
	P4	0	7 + 9	5 + 10
Bread wheat	JR	2 *	7 + 9	5 + 10
	VK	1	7 + 9	5 + 10
	SWK	1	14 + 15	5 + 10

Note: unk = unknown; * is used to discriminate between 2* and 2.

3.5. Rheological Properties of Einkorn, Emmer, Spelt, and Bread Wheat

Mixolab parameters were represented in triplicate per sample (12 varieties in three years at three locations, $n = 324$). The mean values of each variety for Time C1, The stability, Torque C1, Torque C2, Torque C3, Torque C4, Torque C5, and slope α, β, and γ are displayed in Figure 5, Tables S8 and S9.

In the first stage of Mixolab analysis, dough development time (Time C1) is an essential index, known as the dough development or the gluten development time. For wheat, this period is usually a long time from 0.99 to 7.36 min. Better flour has a longer dough development time. C1 is influenced mainly by the quality of protein, the size of starch granules, and level of starch degradation.

In general, bread wheat had the longest dough development time (from 5.37 min to 6.38 min), with the exception of the Jara variety (2.53 min), compared to the other three wheat species, and variety J4 had the shortest time (1.66 min). The SP8 variety had quite a long development time (4.23 min). In more detail, from Table S8, it is clear that the SW Kadrilj variety (*Triticum aestivum*) had the longest dough development time (DDT). The development time was short for einkorn. Emmer (tetraploid species) seemed to be better, and no significant differences from spelt (hexaploid) were found. The torque of all varieties reached 1.1 ± 0.05; however, the data from Table S8 do not support statistical differences among the varieties studied. Amplitude is responsible for dough elasticity: the more elastic the dough is, the higher the amplitude. Similarly to Torque C1 values, the findings indicated no differences between varieties and species.

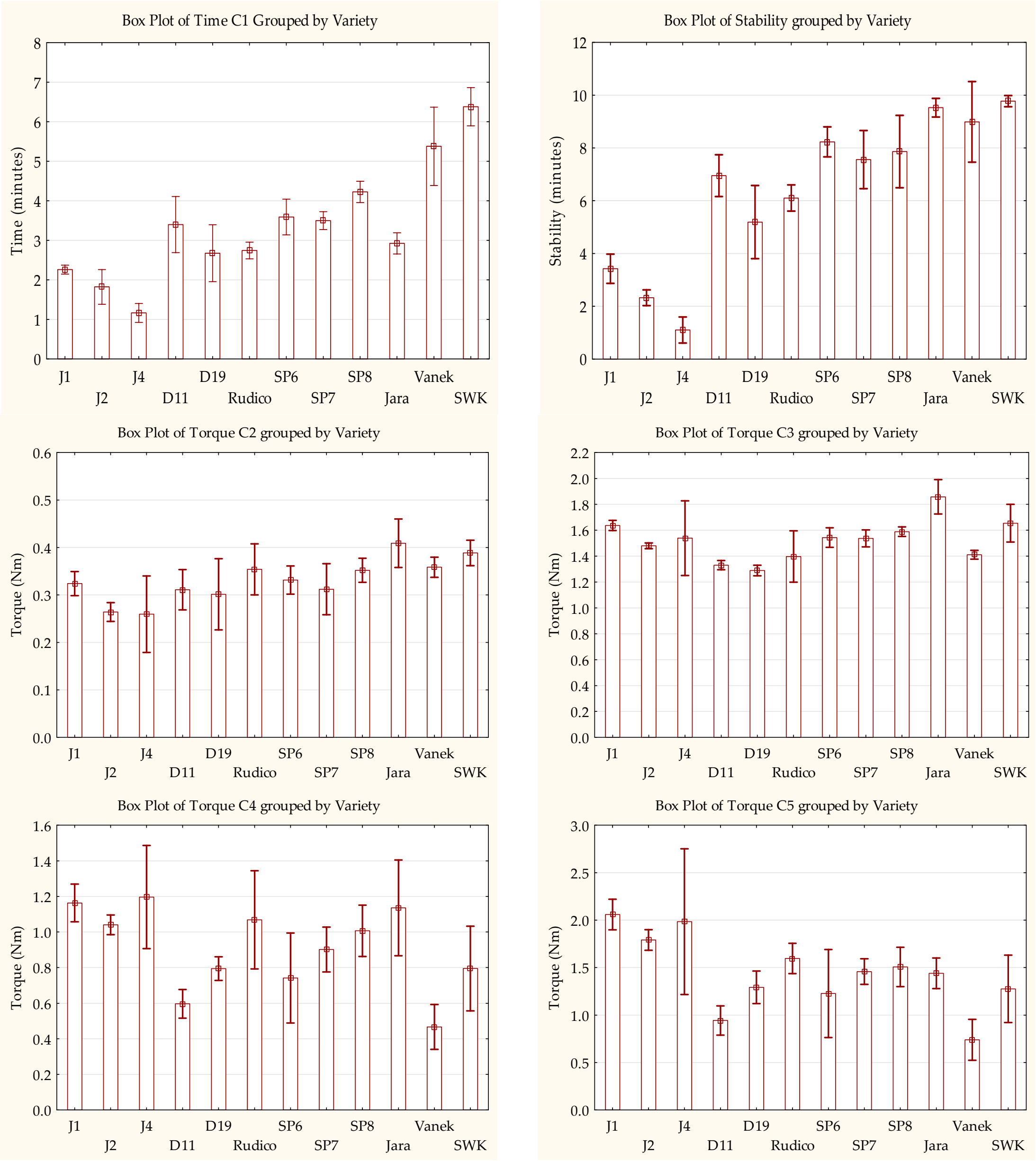


Figure 5. Parameters of Time C1, Stability, Torque C2, Torque C3, Torque 4, and Torque C5 in the measured Mixolab. Data are the means of 12 wheat varieties at three locations, three replicates, in three years ($n = 36$). Box plots represent the interquartile range, the columns in the box symbolize the mean value, and whiskers designate the minimum and maximum of the box plot.

One of the vital parameters in phase C1 of Mixolab is stability, which is the resistance of dough against intensive mixing. This parameter and DDT are frequently associated with better gluten quality [27]. Usually, this value ranges between 4.69 and 11.42 min. Figure 5 and Table S8 show that einkorn had a low stability and needed from 1.11 min to 3.43 min to form a dough structure, while those most resistant to intensive mechanical processing were hexaploid spelt wheat and bread wheat. Additionally, the group of spelt and *T. aestivum* had a higher stability than the other ones, with the time fluctuating from 7.56 to 9.76 min. According to Bonet et al., 2006 [28], slope α is related to attenuating protein, recorded when heating reaches 52–57 °C at the beginning of the second stage. This was significantly different among species, with lower values for Einkorn species (0.04–0.05 Nm.min^{-1}) than for spelt and bread wheat (0.08–0.12 Nm.min^{-1}). In the second phase, protein attenuation occurs by the change of temperature and mechanical work. The C2 parameter indicates the weakening of proteins during mechanical processing and temperature. The longer the processing time, the higher the value. The values of torque C2 usually range between 0.37 and 0.63 Nm. In this experiment, the small differences between varieties in the torque C2 parameter were found, apart from J1 (*Triticum monococum* 38), RU, SP6 (VIR St. Petersburg, Russian Federation), and SP8 in comparison to *T. aestivum*. The third phase of the Mixolab II curve was evaluated via the C3 and slope β parameters. Maximum torque C3 of hot dough at 90 °C, ranged from 1.29 to 1.86 Nm and was significantly different between varieties. However, the same species did not show differences. Slope β, an indicator of pasting speed, was statistically different for four species. In the einkorn group, the data of the J2 variety were significantly different from the tetraploid emmer varieties D19 (*Triticum dicoccum*—Tabor) and RU. D19 and RU also had the lowest of slope β (0.32 Nm.min^{-1}–0.34 Nm.min^{-1}) compared to other cultivars such as J1, SP6, SP7, JR, and SWK. The stability of hot gel in the starch evaluation phase characterizes the C4 stage. This phase relates to the resistance of starch against the enzymatic hydrolysis by amylase [27] and provides similar information as the falling number [29]. Figure 3 shows that diploid einkorn had a high value of C4. Low C4 values were also found for two tetraploid emmer wheat varieties: D11 (Weisser Sommer) and D19 (*Triticum dicoccum*—Tabor). In the group of common wheat there was a significant difference between the Vanek (0.47 Nm) with Jara varieties (1.14 Nm), and vice versa was true for spelt varieties. Slope γ is used to determine the speed of enzymatic degradation of starch and heat stability of the starch gel at temperatures over 80 °C. The γ indexes ranged from 0.05 Nm.min^{-1} to 0.11 Nm.min^{-1} and were not significantly different in the data. In the final stage, the C5 parameters were assessed by characterizing the retrogradation of starch granules during the cooling phase. Higher C5 torque, in general, was usually associated with higher amylose content, which indicates that wheat flour has strong starch gels [27]. The values varied from 0.74 Nm to 2.06 Nm. All the evaluated values fell within the aforementioned range.

3.6. Principal Component Analysis

The relationships among the quality and quantity parameters of 12 wheat varieties are shown in Figure 6 by principal component analysis (PCA). The first two principals account for 76.25% of the variation. Accounting for 51.04% of the variability, the first principal component was positively related to protein content, insoluble remainder (IR), torque C5, TGW, and wet gluten with loading factors 0.68, 0.75, 0.73, 0.65, and 0.53, respectively. The second principal component constituted 25.21% of the variability with quite high loading factors for gluten content (0.79). Biplots of quantitative traits and quality traits are also presented in Figure 6. In Figure 6, all einkorn and emmer varieties were located in the area of the loading of wet gluten, protein content, Torque C3, Torque C4, and Torque C5, due to their high protein and Nm, compared to bread wheat and spelt wheat. In contrast, common wheat and spelt wheat were located in the area of the loading of quantity traits (GYLD, TGW) and quality traits (gluten content, gliadins, time C1, stability, GI, Zeleny test, SDS test, glutenins, AG, torque C1, and torque C2). The data point of SP8 variety was positioned among gluten content, time C1, stability, GI, and Zeleny test. This means that SP8 could be a good baking performance cultivar and is a suitable candidate for breeders in the future.

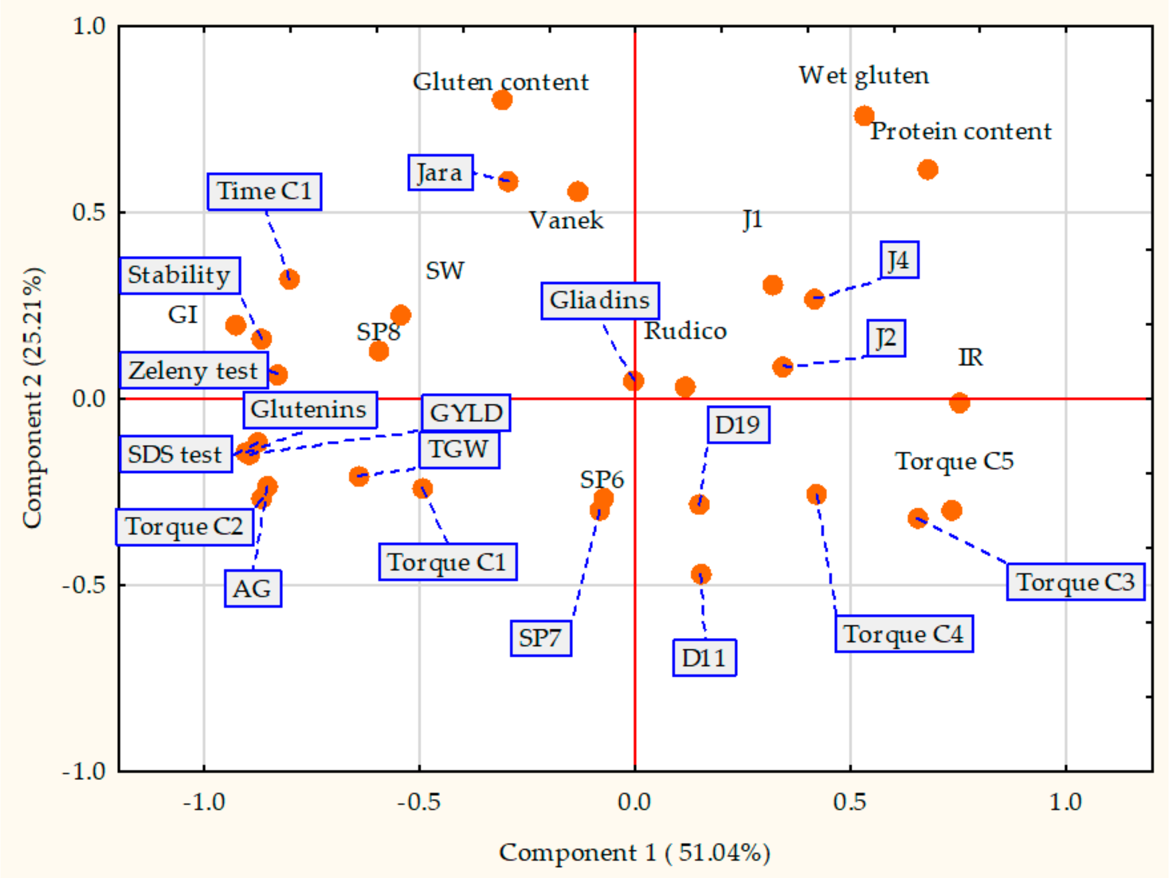


Figure 6. Principal component analysis biplot based on various physicochemical parameters of wheat varieties using mean value of three locations. AG: Albumins and globulins; IR: Insoluble remainder; FN: Falling number.

4. Discussion

Protein content plays an important role in both the nutritional and technological values of wheat. The protein contents at the diploid level and tetraploid levels were higher than those of hexaploids. In our study, the protein content of the diploid level was 15.59% and that of tetraploid was 16.04%. These results are higher than those reported by Hidalgo and Brandolini [30] by roughly 2% of protein. With our findings confirmed by the data reported by Grausgruber [31], the protein content of the diploid group can exceed 20%. These values were higher than our results indicated. The indexes for the baking quality of einkorn were lower than those of hexaploids. Hence, although the diploid group protein values were superior to those of the rest of the group, the diploid storage proteins might contribute to poor bread manufacturing properties.

Through domestication and modern breeding, the protein content and protein composition of wheat have been changed [32]. The composition of protein fractions can be described by analysis. A good example is einkorn wheat, a diploid species that has a higher protein content but a very different protein fraction composition in comparison to hexaploid wheat [33]. Protein content and total (grain and straw) yield are strongly correlated. The old varieties had a higher protein content but lower yield [34]. In the case of modern varieties, the nutritionally important protein is replaced by starch. Similarly, the protein fractions, nutritionally important albumins and globulins and the insoluble remainder were present in smaller quantities. This comparison is relative, because albumin and globulin are mostly present in the outer layer of einkorn and emmer varieties; therefore, they are relatively low in the endosperm of wheat kernels [35]. In contrast, common wheat cultivars used in this study had lower protein contents, which could be less interesting from a nutritional point of view.

HMW-GSs generally play a major role in determining the viscoelastic properties of dough, according to Sramkova et al. [36]. The content, presence, and variation of HMW-GSs affect the baking quality, notably high bread volume, as well as crumb structure [37]. Favorable effects on dough properties are created by the HMW-GS alleles Glu-A1 (subunits 1, 2*) [38] and Glu-B1, (subunits 7 + 8, 7 + 9) [22]. Subunit 2*, encoded at Glu-A1, which is common in modern common wheat, was found in the case of one landrace, Postoloprtska presivka (P1 variety), and one control variety (Jara variety). This subunit can be used in breeding to increase the glutenin diversity of common wheat and is usually connected with better baking quality [39]. The composition of HMW-GS in other species is typical and is connected to ploidy level. Diploid einkorn has no bands of the Glu-A1 locus while tetraploid emmer wheat has a high variability of subunits on Glu-A1 and Glu-B1, which is related to inferior bread-making quality [36]. Generally, the cultivars in our study having HMG-GS, 5 + 10, 2*, and 7 + 9 positively impacted bread-making quality, which is in agreement with the study of Dhana et al. and Khatkar et al. [40,41]. The subunit 2 + 12 (Glu-1D), present in all spelt wheat cultivars is not as good as the subunit 5 + 10 and is associated with poor baking quality. This was confirmed in the study of Shewry et al. [20]. Although the SP8 variety has the subunit 2 + 12, it has good bread-making qualities because of the long C1 time and stability. The HMW-GS results showed a considerable change in the composition during wheat domestication and intensive breeding.

In order to measure the rheological properties of dough, we used Mixolab to analyze the two stressors of mixing and temperature changes. Mixolab characterizes torque (in Nm) produced by the dough during two-blade mixing. Our results indicated that the torques during mixing in the C1 stage were not significantly different for these varieties. The reverse was true for the dough development time and stability. SWK, VK, and SP8 varieties had longer dough development times, indicating that they produce stronger flours. This relates to the high gluten and glutenin content of their cultivars. In the second and third stages, we found statistically significant differences in the data of the different varieties. However, the differences between groups were unclear. Some of our findings were confusing, especially when the majority of torque C2 and C3 parameters were lower than the usual limits of the range (C2: 0.37–0.63 Nm; C3 1.59–2.27 Nm), apart from Jara and SWK at C2 and J1, Jara and SWK at C3. In the phase of starch evaluation, the lower the torque, the lower the Falling number. The α-amylase complex could be broken in the control variety of the hexaploid wheat Vanek. This explains why Vanek had the lowest torque. Generally, based on our previous results, the less common varieties usually do not meet the falling number criteria. In the last phase, starch retrogradation in the cooling phase, the lowest value was measured for the bread wheat variety Vanek. The starch granules of this hexaploid variety were of low quality, whereas the diploid einkorn wheat had a higher quality of starch measured at the C5 parameter. As reported by Svec and Hruskova [9], wheat flour used as a standard sample during the Mixolab test has torques from C1 to C5 of 1.10, 0.05, 2.06, 1.69, and 2.54 Nm, respectively. Similarly, wheat flour standard torques ranging from C1 to C5 are 1.10, 0.60, 1.98, 1.90, and 2.97 Nm, respectively, according to Schmiele et al. [19]. In comparison, the figures in our research were lower than the two findings quoted above. Only the rheological behaviors of wheat flour dough of Jara and SWK were nearly equivalent to the standard sample, apart from torque C4 and torque C5. This showed that the stability of starch gel formed and the retrogradation stage of starch for Jara and SWK were not as good as those of the wheat flour standard.

PCA was presented with all data in Figure 4, and the corresponding biplot is indicated for component scores and variable loadings. This is a useful tool to choose varieties from other wheat species that have similar characteristics as bread wheat. For instance, in this study it is understood that SWK, VK, and SP8 varieties have good baking performances. This conclusion is based upon the proximity of their positions to the parameters quality such as gluten content, GI, Zeleny test, stability, time C1 and gliadin. J1, J2, and J4 cultivars had the highest protein contents.

5. Conclusions

The objective of this paper was to compare the protein composition and rheological characterization of einkorn, emmer, spelt, bread wheat landraces and common wheat species.

In this study, differences between the diploid level group and hexaploid level groups of wheat were found, not only in terms of the basic baking quality but also from a nutritional point of view. Domestication and modern breeding processes have changed the composition of the grain. The less common and older forms of wheat contain more protein compared to modern varieties. In the less common species, the share of nutritionally important protein fractions is higher, including albumins, globulins, and the insoluble remainder. However, this higher possible nutritional value is compensated for by the lower technological quality for baking, as demonstrated by the high molecular weight of glutenin subunits of protein. The same results were provided via the rheological analysis in Mixolab. Some of the varieties from the group of genetic resources have the potential to be used during the breeding process. To our knowledge, this is the first study comparing the protein fractions and rheological characterization of five wheat species grown at three locations. However, further work is required to expand the information obtained in the present study using a large number of wheat varieties when analyzing protein composition and Mixolab. PCA with all parameters was suitable to determine the good baking performance of cultivars. Ancient wheat species, such as diploid einkorn and tetraploid emmer, had high possible nutritional value, but their technological quality was lower. Therefore, to improve the dough and baking properties of einkorn and emmer, a mixture of einkorn, emmer and common wheat should be created.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/11/1763/s1. Table S1: List of all 26 wheat varieties used in this study and their abbreviations, Table S2: Thousand grain weight and grain yield of wheat varieties, Table S3: Means ± standard error (SE) and ANOVA *F*-value (*p*-value) for the effects of harvest year, location, and wheat species on protein content, gluten content, gluten index (GI), sedimentation index (SDS test), and Zeleny test (ZT), Table S4: Quality characteristics of varieties of different wheat species. Table S5: Analysis of variance for protein fractions under three locations, four years, and different varieties, Table S6: Contents of gluten, albumins + globulins, gliadins, glutenins, and insoluble remainder of different wheat species, Table S7: Contents of gluten, albumins + globulins, gliadins, glutenins, and insoluble remainder of different wheat cultivars, Table S8: Gluten development time at the C1 in Mixolab, Table S9: Flour quality parameters of wheat varieties at C2, C3, C4, and C5 stage.

Author Contributions: Conceptualization, K.D.T. and P.K.; Formal Analysis, K.D.T., P.X.T.T. and P.K.; Methodology, K.D.T. and P.K., Writing original draft, K.D.T.; Funding acquisition, P.K. and D.J.; Resources, P.K. and D.J.; Writing review and editing, P.K., I.C., and M.L.-B., Supervision, P.K.; Validation; K.D.T., Visualization, M.K. and P.X.T.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the University of South Bohemia in Ceske Budejovice, research project GAJU 059/2019/Z and by the Ministry of Agriculture of the Czech Republic, research project NAZV QK1910046. The National Program for the Conservation and Use of Plant Genetic Resources and Agrobiodiversity, Ministry of Agriculture of the Czech Republic, 6.2.5/51834/2017-MZE-17253.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. *The World Wheat Book a History of Wheat Breeding*, 1st ed.; William, J., Angus, A.P.B., Eds.; Lavoisier: Cachan, France, 2001; ISBN 1-898298-72-6.
2. Zrcková, M.; Capouchová, I.; Paznocht, L.; Eliášová, M.; Dvořák, P.; Konvalina, P.; Janovská, D.; Orsák, M.; Bečková, L. Variation of the total content of polyphenols and phenolic acids in einkorn, emmer, spelt and common wheat grain as a function of genotype, wheat species and crop year. *Plant Soil Environ.* **2019**, *65*, 260–266. [CrossRef]
3. Van Zeist, W.; Bakker-Heeres, J.A.H. Archaeobotanical studies in the Levant: I. Neolithic sites in the Damascus basin: Aswad, Ghoraife, Ramad. *Palaeohistoria* **2015**, *24*, 165–256.
4. Crawford, D.J. Food: Tradition and change in Hellenistic Egypt. *World Archaeol.* **1979**, *11*, 136–146. [CrossRef]
5. Arzani, A.; Ashraf, M. Cultivated Ancient Wheats (*Triticum* spp.): A Potential Source of Health-Beneficial Food Products. *Compr. Rev. Food Sci. Food Saf.* **2017**, *16*, 477–488. [CrossRef]

6. Shewry, P.R.; Halford, N.G. Cereal seed storage proteins: Structures, properties and role in grain utilization. *J. Exp. Bot.* **2002**, *53*, 947–958. [CrossRef] [PubMed]
7. Branlard, G.; Dardevet, M.; Saccomano, R.; Lagoutte, F.; Gourdon, J. Genetic diversity of wheat storage proteins and bread wheat quality. *Euphytica* **2001**, *119*, 59–67. [CrossRef]
8. Bradová, J.; Šašek, A. Diversity of gliadins and HMW glutenin subunits in Czech registered wheat varieties. *Czech J. Genet. Plant Breed.* **2012**, *41*, 160–163. [CrossRef]
9. Švec, I.; Hrušková, M. The Mixolab parameters of composite wheat/hemp flour and their relation to quality features. *LWT Food Sci. Technol.* **2015**, *60*, 623–629. [CrossRef]
10. Harati, H.; Bekes, F.; Howell, K.; Noonan, S.; Florides, C.; Beasley, J.; Diepeveen, D.; Appels, R. Signatures for torque variation in wheat dough structure are affected by enzymatic treatments and heating. *Food Chem.* **2020**, *316*, 126357. [CrossRef]
11. Axford, D.; Mcdermott, E.; Redman, D. Small-Scale Tests of Bread-Making Quality. *Milling Feed Fertil.* **1978**, *161*, 18–20.
12. Capouchová, I.; Petr, J.; Tlaskalová-Hogenová, H.; Michalík, I.; Faměra, O.; Urminská, D.; Tučková, L.; Knoblochová, H.; Borovská, D. Protein fractions of oats and possibilities of oat utilisation for patients with coeliac disease. *Czech J. Food Sci.* **2011**, *22*, 151–162. [CrossRef]
13. Laemmli, U.K. Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. *Nature* **1970**, *227*, 680–685. [CrossRef] [PubMed]
14. Payne, P.I.; Lawrence, G.J. Catalogue of alleles for the complex gene loci, Glu-A1, Glu-B1, and Glu-D1 which code for high-molecular-weight subunits of glutenin in hexaploid wheat. *Cereal Res. Commun.* **1983**, *11*, 29–35.
15. Bradová, J.; Matějová, E. Comparison of the Results of SDS PAGE and Chip Electrophoresis of Wheat Storage Proteins. *Chroma* **2008**, *67*, 83–88. [CrossRef]
16. Konvalina, P.; Grausgruber, H.; Tran Dang, K.; Vlasek, O.; Capouchova, I.; Sterba, Z.; Suchy, K.; Stolickova, M.; Kyptova, M.; Bernas, J.; et al. Rheological and Technological Quality of Minor Wheat Species and Common Wheat. In *Wheat Improvement, Management and Utilization*; Wanyera, R., Owuoche, J., Eds.; BoD–Books on Demand: Norderstedt, Germany, 2017; ISBN 978-953-51-3151-9.
17. Rosell, C.M.; Collar, C.; Haros, M. Assessment of hydrocolloid effects on the thermo-mechanical properties of wheat using the Mixolab. *Food Hydrocoll.* **2007**, *21*, 452–462. [CrossRef]
18. Ferrer, A.; Rosell, C.M. Rheological Behaviour of Whole Wheat Flour. In Proceedings of the IUFOST—13th World Congress of Food Science & Technology: Food Is Life, Nantes, France, 17–21 September 2006. [CrossRef]
19. Schmiele, M.; Ferrari Felisberto, M.H.; Pedrosa Silva Clerici, M.T.; Chang, Y.K. Mixolab^{TM} for rheological evaluation of wheat flour partially replaced by soy protein hydrolysate and fructooligosaccharides for bread production. *LWT Food Sci. Technol.* **2017**, *76*, 259–269. [CrossRef]
20. Shewry, P.R.; Halford, N.G.; Tatham, A.S. High molecular weight subunits of wheat glutenin. *J. Cereal Sci.* **1992**, *15*, 105–120. [CrossRef]
21. Shewry, P.R.; Tatham, A.S. The prolamin storage proteins of cereal seeds: Structure and evolution. *Biochem. J.* **1990**, *267*, 1–12. [CrossRef]
22. *Gliadin and Glutenin: The Unique Balance of Wheat Quality*; Wrigley, C.W., Békés, F., Bushuk, W., Eds.; AACC International: St. Paul, MN, USA, 2006; ISBN 978-1-891127-51-9.
23. Gianibelli, M.C.; Larroque, O.R.; MacRitchie, F.; Wrigley, C.W. Biochemical, Genetic, and Molecular Characterization of Wheat Glutenin and Its Component Subunits. *Cereal Chem.* **2001**, *78*, 635–646. [CrossRef]
24. Wang, Y.G.; Khan, K.; Hareland, G.; Nygard, G. Quantitative Glutenin Composition from Gel Electrophoresis of Flour Mill Streams and Relationship to Breadmaking Quality. *Cereal Chem.* **2006**, *83*, 293–299. [CrossRef]
25. Payne, P.I.; Corfield, K.G. Subunit composition of wheat glutenin proteins, isolated by gel filtration in a dissociating medium. *Planta* **1979**, *145*, 83–88. [CrossRef] [PubMed]
26. Payne, P.I.; Nightingale, M.A.; Krattiger, A.F.; Holt, L.M. The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. *J. Sci. Food Agric.* **1987**, *40*, 51–56. [CrossRef]
27. Banfalvi, A.; Nemeth, R.; Bagdi, A.; Gergely, S.; Rakszegi, M.; Bedo, Z.; Lang, L.; Vida, G.; Tomoskozi, S. A novel approach to the characterization of old wheat (*Triticum aestivum* L.) varieties by complex rheological analysis. *J. Sci. Food Agric.* **2020**, *100*, 4409–4417. [CrossRef] [PubMed]

28. Koksel, H.; Kahraman, K.; Sanal, T.; Ozay, D.S.; Dubat, A. Potential Utilization of Mixolab for Quality Evaluation of Bread Wheat Genotypes. *Cereal Chem.* **2009**, *86*, 522–526. [CrossRef]
29. Codina, G.G.; Mironeasa, S.; Bordei, D.; Leahu, A. Mixolab versus Alveograph and Falling Number. *Czech J. Food Sci.* **2010**, *28*, 185–191. [CrossRef]
30. McKevith, B. Nutritional aspects of cereals. *Nutr. Bull.* **2004**, *29*, 111–142. [CrossRef]
31. Grausgruber, H.; Sailer, C.; Ghambashidze, G.; Bolyos, L.; Ruckenbauer, P. Genetic variation in agronomic and qualitative traits of ancient wheats. In Proceedings of the 17th EUCARPIA General Congress, Tulln, Austria, 8–11 September 2004; pp. 19–22.
32. Kiszonas, A.; Morris, C.F. Wheat Breeding for Quality: A Historical Review. *Cereal Chem. J.* **2017**, *95*, 17–34. [CrossRef]
33. Konvalina, P.; Capouchova, I.; Stehno, Z.; Moudry, J., Jr.; Moudry, J. Fusarium Identification by PCR and DON Content in Grain of Ancient Wheat. *J. Food Agric. Environ.* **2009**, *9*, 321–325. Available online: http://orgprints.org/20762/ (accessed on 3 May 2012).
34. Konvalina, P.; Stehno, Z.; Capouchová, I.; Zechner, E.; Berger, S.; Grausgruber, H.; Janovská, D.; Moudrý, J. Differences in grain/straw ratio, protein content and yield in landraces and modern varieties of different wheat species under organic farming. *Euphytica* **2014**, *199*, 31–40. [CrossRef]
35. Goesaert, H.; Brijs, K.; Veraverbeke, W.S.; Courtin, C.M.; Gebruers, K.; Delcour, J.A. Wheat flour constituents: How they impact bread quality, and how to impact their functionality. *Trends Food Sci. Technol.* **2005**, *16*, 12–30. [CrossRef]
36. Šramková, Z.; Gregová, E.; Šturdík, E. Genetic improvement of wheat—A review. *Nova Biotechnol.* **2009**, *9*, 27–51.
37. Jiang, P.; Xue, J.; Duan, L.; Gu, Y.; Mu, J.; Han, S.; Chen, L.; Li, Y.; Ma, W.; Yan, Y.; et al. Effects of high-molecular-weight glutenin subunit combination in common wheat on the quality of crumb structure. *J. Sci. Food Agric.* **2019**, *99*, 1501–1508. [CrossRef] [PubMed]
38. Vasil, I.K.; Anderson, O.D. Genetic engineering of wheat gluten. *Trends Plant Sci.* **1997**, *2*, 292–297. [CrossRef]
39. Stehno, Z.; Bradová, J.; Dotlačil, L.; Konvalina, P. Landraces and obsolete cultivars of minor wheat species in the Czech collection of wheat genetic resources. *Czech J. Genet. Plant Breed.* **2010**, *46*, 100–105. [CrossRef]
40. Dhaka, V.; Gulia, N. Application of Mixolab to Assess the Bread Making Quality of Wheat Varieties. *J. Food Process. Technol.* **2012**, *1*. [CrossRef]
41. Khatkar, B. Effects of high Mr glutenin subunits on dynamic rheological properties and bread making qualities of wheat gluten. *J. Food Sci. Technol.* **2006**, *43*, 382–387.

LATE ARRIVALS

SECTION 1
AGRICULTURE AND FOOD ENGINEERING

The Role of Genetic Resources for the Development of Organic Farming and Decentralized Food Production

KONVALINA Petr[1], **TRAN Dang Khoa**[1,2]

[1] *University of South Bohemia in Ceske Budejovice, Faculty of Agriculture, Studentska 1668, 37005 Ceske Budejovice (CZECH REPUBLIC)*
[2] *University of Agriculture and Forestry, Hue University, Faculty of Agronomy, Phung Hung 102, Hue city (VIETNAM)*
Email: konvalina@zf.jcu.cz

Abstract

Nowadays, modern varieties of crops have been bred over a short period they have not respected local environmental conditions. The varieties are usually adapted to farming technologies and they are not able to respond to unfavourable environmental conditions.

Therefore, the most valuable varieties of genetics resources should be conserving by the on-farm method which assures a dynamic process. Our results are composed from more studies made in organic farming system from 2006 till now. We have been working with many varieties of cereals. Evaluation of landraces was oriented to analysis of its technological and nutritional quality and possibility of processing their products by small scale processing methods. Our results show potential of some landraces of cereals to be grown in organic farming system – high resistance to common diseases and competition ability against weed plant. Landraces had lower yield potential than modern varieties of cereals. Different situation was on less quality soils or low level of plant nutrition. The main advantage of landraces was quality of grain and they have potential to be grown in organic farming and used for the preparation of local high-quality products. In this case there will be combination of two important aspects – unique value of genetics resources and added value of organic growing methods.

Keywords: organic farming, genetics resources, cereals, quality, processing

Introduction

In the history of agriculture, the cropping pattern changed considerably. In a majority of agricultural crops, intensive and one-sided breeding results in narrowing the genetic base of the current range of varieties. This process is called genetic erosion and was confirmed by a number of studies [1]. Losses or damage of the genetic base of those resources are connected with reduction of possibilities of further genetic improvement of agricultural crops and their adaptation to the changing conditions and needs [2]. The modern varieties which are resistant to diseases and other stresses [3], give high yields that were mostly obtained by improving the current properties by the genes of wild species [4], and have the high antioxidant activity [5].

The protection of the gene pool takes place on two levels – in situ (on site preservation) and ex situ (off-side preservation) [6].

Cereals are good example of management and use of genetics resources in organic farming.

There is a group of hulled wheat species – *Triticum monococcum* L., *Triticum dicoccum* Schrank (Schuebl) and *Triticum spelta* L. [7]. Hulled wheats rank among often overlooked crops which, however, have a potential for future utilization in food industry. Due to their low demands on the environmental conditions, they are suitable for cultivation in areas less favourable for agriculture. Generally, hulled wheats give lower but stable yields of high-quality production [8]. The high quality of the production is interesting particularly with regard to healthy nutrition. In general, not only have hulled wheat a thicker aleurone layer [9] but also more the insoluble rest fractions than modern common wheat [10].

Over the last decades, the humankind has been increasingly addressing the matter of sustainable development in connection with the protection of the environment and its components. The most common sustainable farming method is organic farming. Organic farming often grows hulled wheat species, due to their low yield potentials in conventional farming, replaced by hybrids with higher yields [6]. Despite the lower yields, these species as example are interesting for farmers for several reasons. Given the complicated legislation regulating organic farming, the farmers find it more convenient to grow species from the group of genetics resources showing greater ecological plasticity. Their cultivation has a lower negative impact on the environment [11], and they give lower but stable yields [12].

The aim of the study is to evaluate the combination of yield and quality parameters of genetics resources of wheat and assessment of their value for use as source for the preparation of local food products. The second objective was also to evaluate its value for sustainable development of rural regions if local product was replaced by global product.

Methodology

Grain of genetics resources of hulled wheat species were produced in organic farming. Used varieties: mean values of four varieties of einkorn, eight varieties of spelt and seven varieties on spelt. As control we used two varieties of bread wheat. We use small plot trials in randomized plot design. Experiments were carrying out in organic farming system. We use four varieties of einkorn. The quality analysis of harvested grain was tested by The International Association for Cereal Chemistry (ICC) methods. Protein fractions were measured according methodology developed by Osborn.

Results and Discussion

According to CSN 46 1100-2 (Czech standard of quality), an amount of N-substances in wheat for food use should reach 10.8-13.7 %, which corresponds with the sample of bread wheat flour. The fact that hulled wheat is higher in protein has been confirmed within this study "Fig. 1". The tested wheat varieties contained 16% of N-substances. The most important than protein content is also composition of protein fractions in the grain (Table 1). It is one from the main advantages of hulled wheat species from the group of genetics resources.

The results of determination of baking quality are summarized in Figure 1. The lowest protein content was measured in bread wheat flour. This fact is also confirmed by the classification in a statistically different group ($p<0.05$). Similarly, the lowest protein content was detected in white spelt flour. The observed result is consistent with the data published in the literature, where the protein content is normally referred above the limit of 15%.

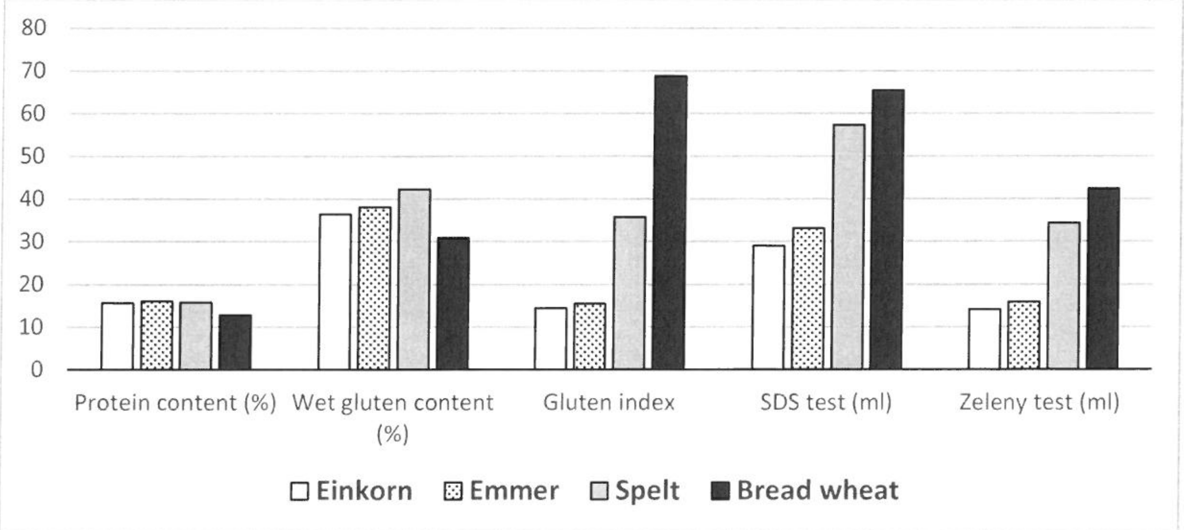


Fig. 1. Baking quality characteristics of different wheat species (mean and SD, three localities; four years

Table 1. Baking quality characteristics of different wheat species (mean and SD, three localities; four years)

Species	Gluten content (mg.100g^{-1})	Albumins + Globulins (%)	Gliadins (%)	Glutenins (%)	Insoluble rest (%)
Einkorn	153,45a	26,67b	27,93a	27,64a	17,76d
Emmer	194,86b	28,67c	30,06b	27,70a	13,67c
Spelt	248,17c	27,04c	31,03bc	29,95b	12,12b
Bread wheat	248,52c	21,38a	34,67c	36,96c	6,99a

Remark: Within column values followed by the same letter are not significantly different at P < 0.05 (Tukey HSD test).

The amount of wet gluten in the samples was in optimum quantity excluding white bread wheat flour that showed a statistically significant difference from white spelt flour. The values resulting from the Zeleny's test were generally low. Only bread wheat reached higher values.

Low values of sedimentation are general problems of hulled wheat, which are due to the genetic background to some extent. The gluten index was determined to assess the gluten quality. The highest amount of gluten was found in bread wheat flour. However, the value of Gluten index in whole-wheat bread flour was surprisingly low. A partial explanation was found based on the correlation analysis, because the flour had low Zeleny values. The correlation between these values and the values of Gluten index was statistically significant (r=0.89). Flour of both wheat varieties showed high values of the falling number – an indicator of damage to the starch grains due to the pre-harvest sprouting. The values are very high (exceed the standard). Such a high falling number may have negative effects on loaf volume, as well as the sensory evaluation of bread crumb. The most objective parameter of the baking quality is determination of the loaf volume. Whole-wheat bread showed the highest values. Conversely, the lowest values were found in whole-wheat spelt flour. The results did not fully correspond with the values regarding the Gluten index and Zeleny's test "Fig. 1". Correlation analysis results indicate negative correlation (r=-0.69) between the bread volume and protein content. A possible explanation is that spelt is higher protein content, but it is of lower baking quality than bread wheat "Fig. 2".

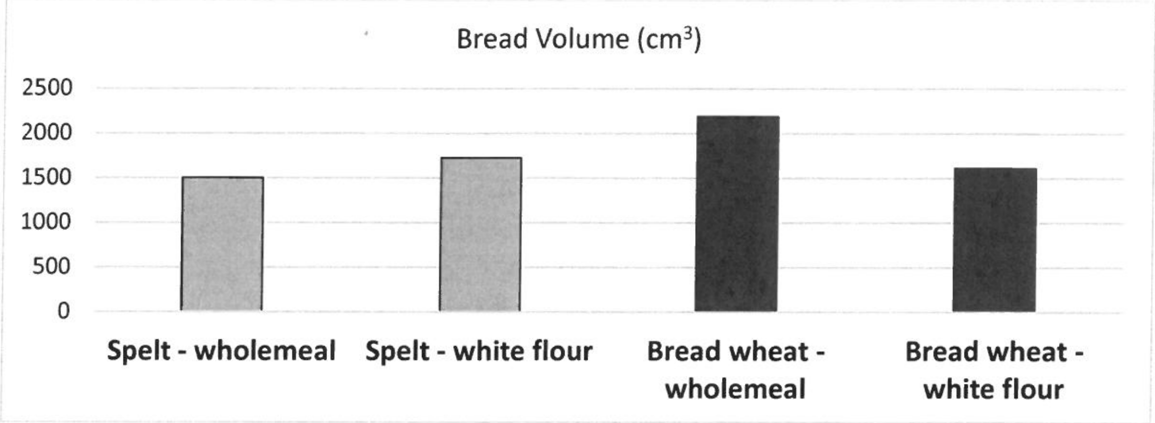


Fig. 2. Comparison of volume of different kinds of bread

Generally, the sedimentation index was low in hulled wheat samples. It may be therefore stated that these varieties are not suitable for baking purpose. Other values did not affect the quality of pasta to a great extent. However, Gluten index and the amount of sediment have an impact on the quality of pasta. Negative correlation indicates that increased Gluten index decreases an amount of sediment with 99.9% probability when cooking pasta. Gluten index of einkorn wheat is very low, which consequently resulted in the relatively large amount of sediment. The interesting is the dependence of wet gluten on the amount of water bound by pasta during boiling. The amount of water absorbed by pasta thus increases due to the higher wet gluten content together with the weight of pasta. Spelt showed the highest values of wet gluten and thereby the highest binding, conversely, the lowest amounts were found in bread wheat.

Discussions

It has been proven that hulled wheats have higher protein content (Figure. 1) than common wheat when grown in the same agronomic conditions [13]. Spelt wheat grains have high protein content (13-20%). The results from Italy [7] show that spelt wheats with a high protein content of grains can also be used for the production of pasta. The protein content of emmer wheat ranges widely from 9 to 18% [14]. However, according to Cubadda and Marconi [15], emmer's protein even reaches up to 20.6-21.9%. The protein content of emmer wheat is very variable and depends on the given site conditions. Einkorn wheat contains 13.2-22.8% proteins [16].

Because of the lower quality of gluten, which is rather running, the einkorn wheat flour is not suitable for the production of yeast doughs and yeast products [17]. Low values of SDS and Zeleny test and worse rheological properties are also typical of emmer wheat (this is also true for einkorn wheat) [18]. For example, einkorn wheat is suitable for the production of children's and special food due to the good transmission of properties connected with high protein and carotenoid content [17]. Einkorn wheat can also be used for the production of non-yeast products, biscuits and flakes. In macrobiotic diet, its germinated grains are used [19]. The only exception is spelt wheat, which could be used for the production of yeast products in a mixture with common wheat. In the study of Cubadda and Marconi [15] described a high baking quality of spelt wheat despite that fact that the spelt gluten is less firm and the dough has worse rheological properties.

The important aspect of hulled wheat growing and processing in organic farming is also its dynamic management as source of genes for the future. In case of perspective and most valuable genetics resources is supported conservation.

Conclusions

Hulled wheat from the genetics resources constitutes an interesting alternative for the preparation of organic food. Due to the lower requirements for growing conditions, they may also be grown in less favourable areas for the cultivation of cereals. In addition to the production aspect itself, the cultivation of hulled wheats in organic farming shows a large number of other positives which are further studied. The use of hulled wheats in nature-like farming systems not only contributes to sustainable development but can also help the regional economy due to the processing of products right at the place of origin. So, a part of the profit can remain with the local farmers. At the same time, it is an interesting alternative e.g. in the current development of agritourism at farms (a tourist is also a purchaser of products). In this respect, organic farming, including the cultivation of hulled wheats, is an irreplaceable element of this large chain.

Ackowledgement

Our work was supported by the research project No. NAZV QK1910046 of the Ministry of Agriculture of the Czech Republic and the University of South Bohemia in České Budějovice (project No. GAJU 059/2019/Z).

REFERENCES

1. Dotlačil, L., Stehno, Z., Faberová, I. and Michalová, A. (2002). Research, Conservation and Utilisation of Plant Genetic Resources and Agro-Biodiversity Enhancement – Contribution of the Research Institute of Crop Production Prague-Ruzyně. Czech Journal of Genetics and Plant Breeding. 38. pp. 3-15.
2. Brumlop, S., Reichenbecher, W., Tappeser, B. and Finckh, MR. (2013). What is the SMAR Test way to breed plants and increase agrobiodiversity? Euphytica. 194. pp. 53-66.
3. Konvalina, P., Stehno, Z., Capouchová, I., Zechner, E., Berger, S., Grausgruber, H., Janovská, D. and Moudrý, J. (2014a). Differences in Grain/Straw Ratio, Protein Content and Yield in Landraces and Modern Varieties of Different Wheat Species Under Organic Farming. Euphytica. 199. pp. 31-40.
4. Yumurtaci, A. (2015). Utilization of wild relatives of wheat, barley, maize and oat in developing abiotic and biotic stress tolerant new varieties. Emirates Journal of Food and Agriculture. 27 (1). pp. 1-23.
5. Tran, D. K., Konvalina, P., Vlasek, O., Sterba, Z., Suchy, K. (2016). The antioxidant activity of ancient wheat varieties and modern wheat varieties. Proceeding of International PhD. Students conference, pp. 158-162.
6. Serpolay, E., Dawson, JC., Chable, V., Lammerts Van Bueren, ET., Osman, A., Pino, S., Silveri, D. and Goldringer, I. (2011). Diversity of different farmer and modern wheat varieties cultivated in contrasting organic farming conditions in western Europe and implications for European seed and variety legislation. Organic Agriculture. 1. pp. 127-145.
7. Marconi, M. and Cubadda, R. (2005). Emmer wheat. In: Abdel-Aal E-S. M, Wood P (Eds.). Speciality grains for food and feed. American Association of Cereal Chemists, Inc. St. Paul, Minesota, USA., pp. 63-108.
8. Stehno, Z., Bradová, J., Dotlačil, L. and Konvalina, P. (2010). Landraces and Obsolete Cultivars of Minor Wheat Species in the Czech Collection of Wheat Genetic Resources. Czech Journal of Genetics and Plant Breeding. 46 (Special issue). pp. 100-105.
9. Konvalina, P., Capouchová, I., Stehno, Z., Moudrý, J. jr. and Moudrý, J. (2011). Fusarium Identification by PCR and DON Content in Grain of Ancient Wheat. Journal of Food, Agriculture & Environment. 9(3-4). pp. 321-325.
10. Tran, D. K., Konvalina, P., Sterba, Z., Capouchova, I., Janovska, D., Suchy, K. (2017). The quality of hulled wheat species in organic farming. Proceeding of International PhD. Students conference, pp 580-583.
11. Moudrý, J. jr., Bernas, J., Kopecký, M., Konvalina, P., Bucur, D., Moudrý, J., Kolář, L., Štěrba, Z. and Jelínková, Z. (2018). Influence of Farming System on Greenhouse Gas Emissions Within Cereal Cultivation. Environmental Engineering & Management Journal. 17(4). pp. 905-914.
12. Konvalina, P., Moudry, J. srov., Suchy, K., Capouchova, I., Janovska, D. (2014b). Diversity of carbon isotope discrimination in genetic resources of wheat. Cereal Research Communications. 42(4). Pp. 687-699.

13. Blanco, A., Giorgi, B., Perrino, R. and Simeone, R. (1990). Risorse genetiche e miglioramento della qualità del frumento duro. Agricoltura Ricerca. 114. pp. 41-58.
14. Borghi, B., Castagna, R., Corbellini, M., Heun, M. and Salamini, F. (1996). Breadmaking quality of einkorn wheat (Triticum monococcum ssp. monococcum). Cereal chemistry. 73(2). pp. 208-214.
15. Cubadda, R., and Marconi, E. (1996). Technological and nutritional aspects in emmer and spelt S. Padulosi, K. Hammer, J. Heller (Eds.), Hulled wheats, International Plant Genetic Resources Institute, Rome, pp. 203-211.
16. Brandolini, A., Hidalgo, A. and Moscaritolo, S. (2008). Chemical composition and pasting properties of einkorn (Triticum monococcum L. subsp. monococcum) whole meal flour. Journal of Cereal Science. 47. pp. 599-609.
17. Grausgruber, H., Scheiblauer, J., Schonlechner, R., Ruckenbauer, P. and Berghofer, E. (2004). Variability in chemical composition and biologically active constituents of cereals. In: Vollmann J, Grausgruber H, Ruckenbauer P (Eds.). Genetic variation for plant breeding, Proc. 17th EUCARPIA General Congress, 8-11 September, Tulln, Austria, pp. 23-26. BOKU University, Vienna, Austria.
18. Watanabe, S., Hirakawa, A., Nishijima, C., *et al.*, (2016). Food as medicine: The new concept of "medical rice". Advanced Food Technology and Nutrition Sciences Open Journal. 2(2). pp. 38-50.

THE COMPARATIVE RESEARCH ON RICE PRODUCTION BETWEEN LOW INPUT FARMING SYSTEM AND CONVENTIONAL FARMING SYSTEM FROM LIFE CYCLE ASSESSMENT IN CENTRAL VIETNAM

D. K. Tran[1,2*], J. Bernas[2],P. Konvalina[2],T. X. P. Tran [1], M. Kopecky[2], J. Moudryjr.[2]

[1]University of Agriculture and Forestry, Hue University 102 Phung Hung, Hue, Vietnam,
Email: trandangkhoa@huaf.edu.vn
[2]University of South Bohemia, Studentska 1668, 37005, Ceske Budejovice, Czech Republic,
Email: petr.konvalina@gmail.com

ABSTRACT

Global warming has been the main topic in the news in recently. This phenomenon is caused greenhouse gas emission from human activities. One of these activities comes from the rice cultivation practices contributing to over 65% gas emission. In order to assess the environmental issues of the rice production, life cycle assessment is a popular method. In this study, the rice production activities were compared to organic farming and conventional one by analysing the inputs. The research was also to assess the life cycle greenhouse gas emissions of the comparative rice production systems. The results show that in the rice production of the organic farming condition reduces other negative aspects to the environment although it is lower yield than the rice production of the conventional farming condition. The findings indicate that there were significant differences GHG emissions between two systems.

Keywords: Life cycle assessment, rice production, organic farming, conventional farming, greenhouse gas emission.

INTRODUCTION

Rice is a crucial food source in the diet of over three billion people in the globe. Most of the paddy rice areas focus on Asia, in which total rice production accounts for over 90% of the world total. This continent also has the highest of rice used per capita with 78.6 kg. year^{-1}. (FAO 2016). Currently, rice is grown more than 100 countries and it was projected that total annual production was about 480 million ton (USDA 2015). Despite the fact that rice makes up 21% per caput human energy intake, it contents an important protein source in the kernels varying from 4.3 to 18.2 % (mass fraction of grain) (Walter et al. 2008). Its total food protein

---

production per hectare is second only to that of wheat, although the yield of utilizable protein is actually higher for rice than for wheat, due to the superior quality of rice proteins (Childs 2004). Rice represents, therefore, an interesting source of proteins for the development of protein-enriched ingredients for the formulation and manufacture of nutritional products. (Amagliani et al. 2017).

Vietnam represents one of the leading rice producer, ranged the fifth largest country for producing rice with 29.4 million ton (FAO 2016). Also, following the data of FAO in 2016, Vietnam is the second biggest exporter in the globe with an export of 8.4 million ton, made up roughly 20% of the total rice export in world trade. This country has, moreover, the third highest food use per capita approximately 160kg.year^{-1}.

With high rice production and export, the agriculture sector in Vietnam has been contributing to the global greenhouse gases (GHG) emissions. Because rice production emits both methane (CH_4) and nitrous oxide (N_20), two GHG that are more potent than carbon dioxide (CO_2) in driving climate change. Most of the GHG emissions from rice field derive from CH_4 which was generated under anaerobic conditions from organic matter in the flooded fields (Brodt et al. 2014)

Rice cultivation is the largest source of agriculture's greenhouse gas (GHG) emissions in Vietnam, with estimated emissions of 37.4 Tg CO_2 equivalents, accounting for 58 % of the total agricultural GHGs (MoNRE 2014).

In recent years, Vietnam has been developing organic agriculture, especially organic rice in order to increase the value products, be friendly with environment and decrease the environmental impacts in the form of greenhouse gases. In this context, the goal of this research is to compare the GHG emissions on rice production between organic farming system and conventional farming system by life cycle assessment (LCA).

METHODOLOGY

Research location and the data of rice cultivation

A survey was performed in 2013 with a sample of 180 farmersfrom most representative communes for the different production systems. The choice of representative communes fromwhich farmers were randomly chosen, was based on interviewswith the questionnaire in the conventional farming system at HuongAn, ThuyThanh, Phu Da in the organic farming system at Nham, Hong Thai and Hong Quang. A comprehensive questionnaire was appliedto farmers including sections on: 1) rice yield,2) inputs of crop production.

In the conventional farming system, the data were collected from two seasons (Winter-Spring season and Summer-Autumn season). The average yield comes from three varieties, grown popular in Central Vietnam namely Khang Dan, HT1 and BT7.

In the organic farming system, Rice is cultivated only one crop per year in upland at the mountainous areas. The varieties used are Can Nguon, Radu and Nep Than with the vegetation and development about six months.

Life Cycle Assessment

This study used the simplified method of Life Cycle Assessment, defined by the international standards of ISO 14 040 (ISO, 2006a) and ISO 14 044 (ISO, 2006b), to calculate the emission load. The system boundaries are “cradle-to-farmgate” including land preparation andpaddy harvesting.

The results of the study were related to *the Climate change* impact category expressed in the carbon dioxide equivalent ($CO_2e = 1x\ CO_2; 23x\ CH_4; 298x\ N_2O$). The SIMAPro software and the ReCiPe Midpoint (H) method were used for the calculations. The system functional unit represented 1 kg of the final product (1 kg of paddy rice).

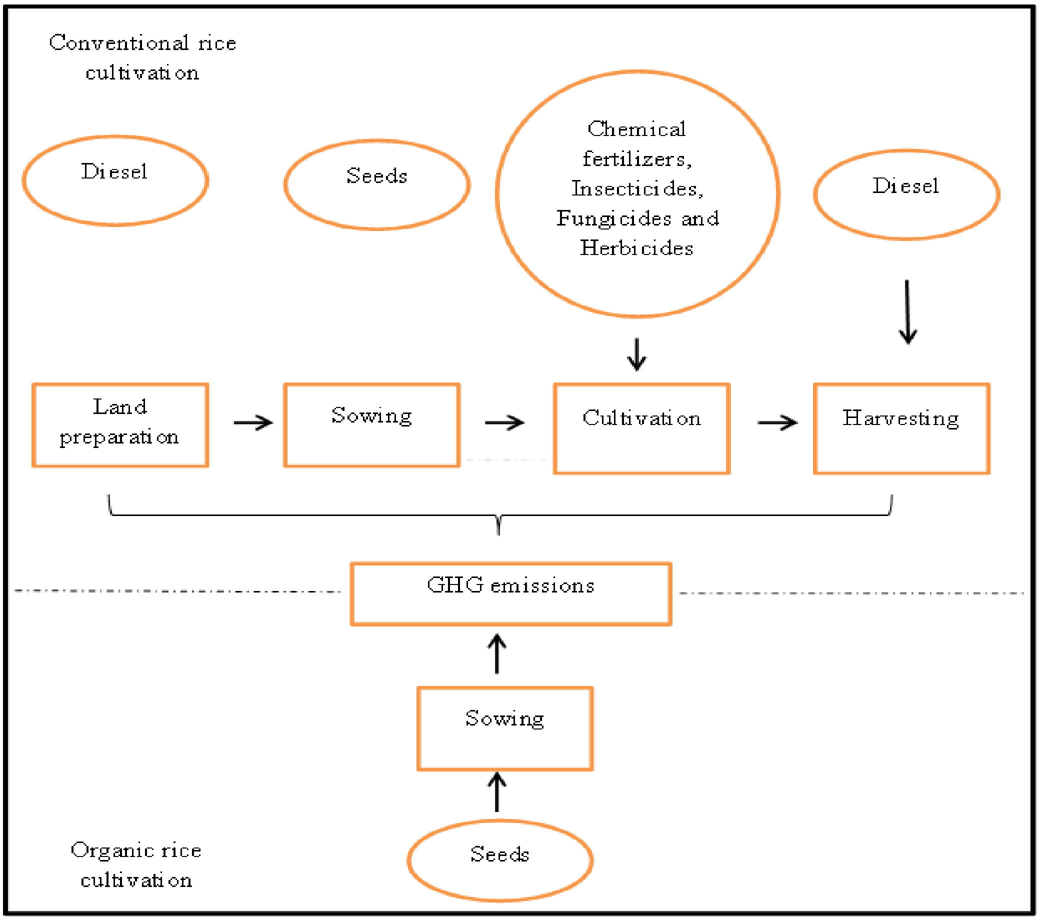


Fig. 1. The system boundary of both systems

The data selected for the modelling is based on the average of commonly applied technologies. Agrotechnical operations from seedbed preparation, a number of seeds, the use of plant protection products, production and application of fertilizers, etc., to harvesting the main product was included into the model system. Besides the emissions arising from the inputs mentioned above, so-called field emissions (N_2O emissions) are also produced after the application of nitrogen fertilizers. The IPCC methodology (*Intergovernmental Panel on Climate Change*) is used to quantify them (De Klein et al. 2006).

RESULTS AND DISCUSSION

Rice production inventory analysis in both systems

The production process of rice conventional system in Thua Thien Hue typically occurs on two seasons, including four stages; land preparation, sowing, cultivation and harvesting. Land preparation begins on the middle December and May by the tractor. Chemical fertilizers are applied three times; the first time is done before sowing with N:P:K fertilizer; the second time is from 12 days to 15 days after sowing with Urea and N:P:K fertilizer; the last time is applied before head emergence from 18 to 20 days with Urea and potassium. The herbicides are used before sowing 1 day. The fungicides and pesticides commonly used in conventional farming to control diseases and pests. Farmers often apply a fungicide and pesticide to control the rice blast and rice leaffolder as well as other diseases, pests. In the Winter-Spring season, water pumps are not used because the paddy fields are rain-fed. Before harvesting 10-day, the paddy field water is drained. The final stage is harvesting. The reap process is done by harvesting machines. Apart from land preparation and harvesting using machines, other activities are usually human labor.

In the organic system, farmers use the varieties that are longer cultivation period than these ones in conventional farming and cultivate 1 crop per year. They are sown in May and harvested in November. The cultivation techniques are completely human labor and do not apply fertilizer both organic and inorganic ones.

Table 1. Input of conventional rice cultivation and organic rice cultivation

Rice cultivation stage	Input	Unit	Conventional farming			Organic farming		
			Huong An	Thuy Thanh	Phu Da	Hong Thai	Hong Quang	Nham
Sowing	Seeds	$kg.ha^{-1}$	99.17	97.5	91.96	100.6	93.23	106.32
Land preparation	Diesel	$kg\ ha^{-1}$	50	50	50	-	-	-
Cultivation	Urea	$kg\ ha^{-1}$	129.3	76.865	108.775	-	-	-

	Superphosphate	kg ha^{-1}	28.35	0	0	-	-	-
	KCl	kg ha^{-1}	55.85	52	40.91	-	-	-
	N:P:K (16:16:8)	kg ha^{-1}	337.85	428.76	346.205	-	-	-
	Dylan 2 EC	l. ha^{-1}	0.15	0	0.15	-	-	-
	Tungcydan 55EC	l. ha^{-1}	0	0.75	0	-	-	-
	Applaud 10WP	kg ha^{-1}	0	0.8	0.8	-	-	-
	Bassa 50EC	l. ha^{-1}	1	0	0	-	-	-
	Sofit 300 EC	l. ha^{-1}	1	1	1	-	-	-
	Beam 75WP	kg ha^{-1}	0.25	0.25	0.25	-	-	-
	Map super 300EC	l. ha^{-1}	0.3	0.3	0	-	-	-
	Validacin 5L	l. ha^{-1}	0.8	0.8	0.8	-	-	-
Harvesting	Diesel	l. ha^{-1}	40	40	40	-	-	-

The GHG emissions per one kilogram rice in conventional and organic rice cultivation

The whole life cycle of both systems including of four stages such as land preparation, sowing, cultivation and harvesting and field emissions are given in Figs. 2. From these figures it can be seen that while the total GHG emissions of conventional rice production were 0.68 kg CO_2-eq per kg of paddy rice in Huong An; 0.69 kg CO_2-eq per kg of paddy rice in both ThuyThanh and Phu Da, the data in organic farming were very low ranging from 0.07 to 0.09 kg CO_2-eq per kg of paddy rice in Nham, Hong Quang and Hong Thai, respectively. Obviously, the GHG emissions in organic rice cultivation were approximately 10 timeslower than these ones in conventional farming. Compared with Yan's result (Yan et al. 2015), our result in conventional farming is slightly higher about 0.02 kg CO_2-eq per kg of paddy rice. However, another research was published by Thanawong et al. 2014 shows that the life cycle GHG emissions of conventional farming in North-Eastern Thailand was 2.97 kg CO_2-eq per kg of paddy rice which is approximately 4 times higher than our findings. In the organic farming, our GHG emission result is much lower than the result ofS. Yodkhum et al. 2017, at 0.84kg CO_2-eq per kg of paddy rice and 0.58 kg CO_2-eq per kg of paddy rice, respectively. This is because organic rice cultivation in this study does not use any input aside from seeds.

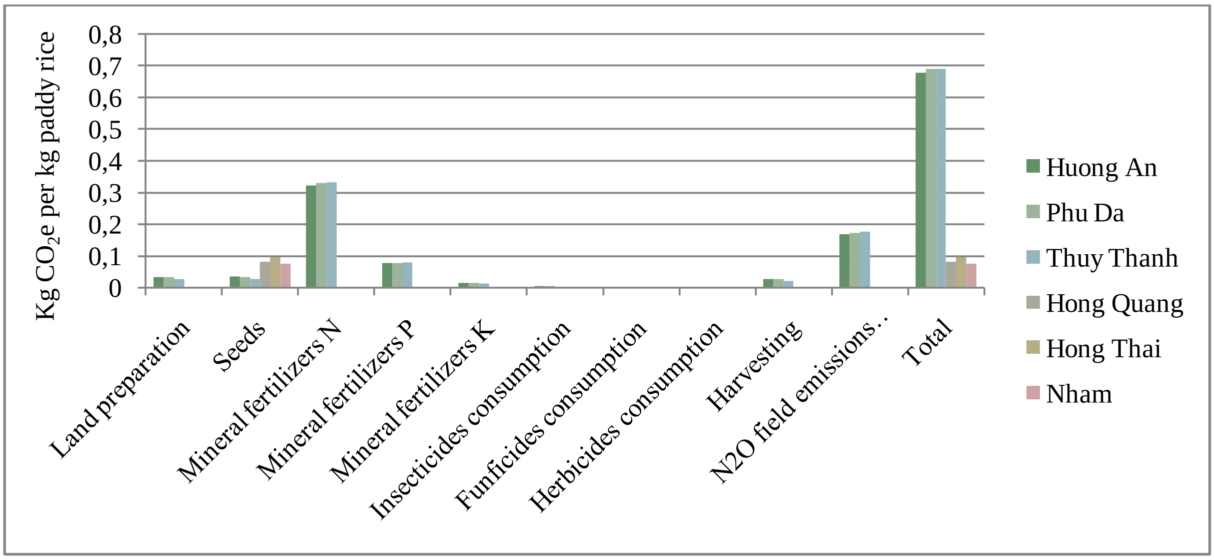


Fig.2. GHG emissions per 1 kg rice from both farming systems

Regarding field emissions, the graph above indicates that about of 0.17 kg CO_2-eq per kg of paddy rice field emissions in the conventional farming account for roughly 25 % of total life cycle GHG emissions of rice production. Our results are different to those of Soni et al. 2013; Koga and Tajima 2011; Xu et al. 2013; Wang et al. 2010. According to these results of them, field emissions of rice cultivation contributed from 65% to 89% of the total GHG emissions. It is apparent that our result is two-time and three times lower. This may have been because the rice cultivation in our study used less chemical fertilizers and no application farm yard manure and straw incorporated removed after harvesting.

The amount of GHG emission of both farming systems per 1 ha

Fig.2. illustrates GHG emissions in conventional rice production and organic rice production. As can be seen from the graph below, the total GHG emissions in Thuy Thanh is the highest with approximately 4000 kg CO_2-eq per ha, followed by Phu Da and Huong An with about 3333kg CO_2-eq per ha and 3308 kg CO_2-eq per ha, respectively, whereas in organic rice cultivation is roughly 179 kg CO_2-eq per ha. These findings are comparable to the research of Konga and Tajima 2011 reported that the GHG emissions in Northern Japan contributed 7.67 ton CO_2 ha^{-1}. However, according to the study of Yan et al. 2015, the GHG emission in rice production is about 6 ton CO_2-eq ha^{-1}.

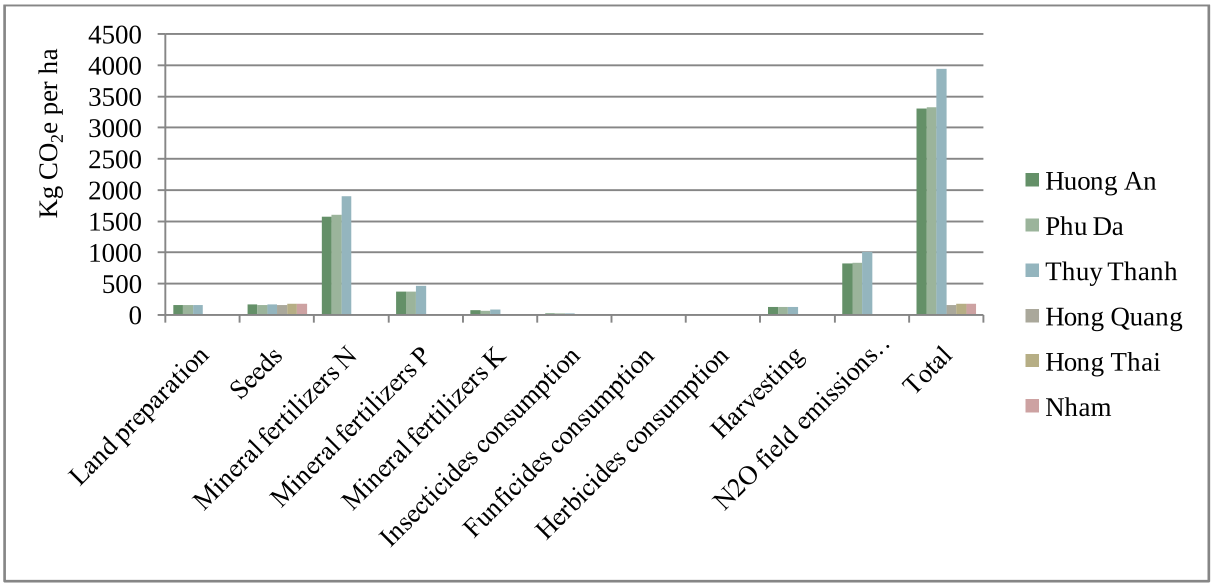


Fig. 3. GHG emission from both farming systems per 1ha

Also, Fig. 3.points out the differences of field emission among three study sites. The field emission in ThuyThanh is leading group at just over 1000 kg CO_2-eq per ha. This datum is slightly higher than these ones in Phu Da and HuongAn because of the fact that farmers in ThuyThanh use more fertilize.

CONCLUSION

From the research that has been performed, it is possible to conclude that cultivating rice in organic farming has the lower GHG emissions from 0.07 to 0.09 kg CO_2-eq per kg of paddy rice and from 157.27 to 179.36 kg CO_2-eq per ha. In the case of conventional farming, the GHG emissions were evaluated at 0.69 09 kg CO_2-eq per kg of paddy rice and from 3308 to 3941 kg CO_2-eq per ha. The findings obviously show that field emissions in conventional farming constitute nearly 25 % of total life cycle GHG emissions of rice production. This result study also confirms that in the rice production of the organic farming condition reduces GHG emission although it is lower yield than the rice production of the conventional farming condition.

ACKNOWLEDGEMENT

This work was supported by the research project of the University of South Bohemia in České Budějovice (project No. GAJU 094/2016/Z).

REFERENCES

Amagliani L., O'Regan J. Kelly A., and O'Mahony (2017). The composition, extraction, functionality and applications of rice proteins: A review. Journal Trands in Food Science and Technology 64, pp. 1-12

Brodt, S., Kendall, A., Mohammadi, Y., Arslan, A., Yuan, J., Lee, I.S., and Linquist, B. (2014).Life cycle greenhouse gas emissions in California rice production. Field Crops Research 169, pp. 89-98.

Childs N. W. (2004). Production and utilization of rice. In E. T. Champagne (Ed.),Rice: Chemistry and technology 3rd edition, pp. 1-23

De Klein C., Novoa R. S., Smith K. A., Rochette P., Wirth T. C., and Williams S.A. (2006). N_2O emissions from managed soils, and CO2 emissions from lime and urea application. IPCC Guidelines for National Greenhouse Gas Inventories, Prepared by the National Greenhouse Gas Inventories Programme, 4.

FAO – Food and Agriculture Organization of the United Nations, (2016).Food outlook biannual report on global food markets.Available at. www.fao.org/3/a-i6198e.pdf

ISO–International Organization for Standardization, (2006a). Environment Management - Life Cycle Assessment - Principle and Framework, second ed. ISOCopyright Office, Switzerland.

ISO– International Organization for Standardization, (2006b).Environment Management - Life Cycle Assessment e Requirements and Guideline (ISO 14044:2006), second ed. ISO Copyright Office, Switzerland.

Koga N., Tajima, R. (2011). Assessing energy efficiencies and greenhouse gas emissionsunder bioethanol-oriented paddy rice production in northern Japan. Journal of Environmental Management 92, pp. 967 - 973.

MoNRE–Ministry of Natural Resources and Environment (2014). The initial biennial updated report of Viet Nam to the UnitedNations framework convention on climate change, Viet NamPublishing House of Natural Resources, Environment andCartography, Hanoi. http://unfccc.int/resource/docs/natc/vnmbur1.pdf.

Thanawong K., Perret S.R., and Mens C.B. (2014). Eco-efficiency of paddy rice inNortheastern Thailand: a comparison of rain-fed and irrigated cropping systems.Journal Cleaner Production 73. pp. 204-217.

USDA. (2015). Grain: World markets and trade. Washington, DC: UnitedStates Department of Agriculture, Foreign Agricultural Service.

Walter, M., Marchezan, E., de Avila, L.A. (2008). Rice: composition and nutritional characteristics. Cienc.Rural. 38, pp. 1184-1192.

Wang M., Xia X., Zhang Q., and Liu J.(2010). Life cycle assessment of a rice productionsystem in Taihu region, China. International Journal of Sustainable Development and World Ecology 17. pp. 157-161.

Xu X., Zhang B., Liu Y., Xue Y., and Di B. (2013). Carbon footprints of rice production infive typical rice districts in China. Journal of ActaEcologicaSinica 33.pp.227-232.

Yan M., Cheng, K., Luo, T., Yan, Y., Pan, G., and Rees, R.M. (2015). Carbon footprint of graincrop production in China -based on farm survey data. Journal Cleaner Production 104, pp. 130-138.

Yodkhum S., gheewala S. H., and Sampattatagul S. (2017).Life cycle GHG evaluation of organic rice production in northern Thailand. Journal of Environmental Management 196, pp. 217-223.

THE ANTIOXIDANT ACTIVITY OF ANCIENT WHEAT VARIETIES AND MODERN WHEAT VARIETIES

DANG KHOA TRAN[1], PETR KONVALINA[1], ONDREJ VLASEK[1], ZDENEK STERBA[2], KAREL SUCHY[3]
[1]Department of Agroecosystems
[2]Department of Plant production
[3]Department of Biological Disciplines
University of South Bohemia in Ceske Budejovice
Studentska 1668, 37005 Ceske Budejovice
CZECH REPUBLIC

trandangkhoa@huaf.edu.vn

Abstract: Wheat is a crucial dietary stable and economic commodity around the globe. It plays an important role in health benefits to combat oxidative stress in the human body by maintaining a balance between antioxidants and oxidants. The objective of this study was to determine the contents of antioxidant activity (tocopherols) in varieties of einkorn, emmer, spelt and *Triticum aestivum* L. and identify the richest sources for improving the nutritional value of bread, pasta and other wheat products. The field experiment were arranged in Ceske Budejovice from 2010 to 2012 with 26 wheat varieties. 2,2-diphenyl-1-pycrylhydrazyl assay was used to evaluate the level of antioxidant activity. The results revealed that antioxidant activity (AOA) ranged from 225.45 mg/kg Trolox DM to 400.83 mg/kg Trolox DM and its values were significantly different among varieties, ploidy level and wheat accessions. Also, modern wheat varieties showed higher AOA than ancient wheat varieties apart from emmer varieties.

Key Words: Antioxidant activity, wheat, food grain sources, phytochemical, reactive oxygen species.

INTRODUCTION

Wheat is plant grown on more land area than any other commercial crop. It is also one of the most important food grain sources for people all over the world because of the universal use of wheat for a wide variety of products such as bread, noodles, cakes, biscuits, etc. Wheat kernel is composed of endosperm (81–84%), bran (14–16%), and germ (2–3%) (Pomeranz 1988). Endosperm is the inner part playing a role as storage of energy and functioning protein. Bran is outer layer protecting the grain and germ is the kernel's reproduction system. Whereas wheat endosperm contains mostly starch and protein, bran and germ are rich in dietary fiber, vitamins, minerals and phytochemicals playing an important role in nutrition and health benefits for humans (Pomeranz 1988). The customers are, therefore, strongly recommended to consume whole-grain foods with at least three servings per day. The recent studies have showed that regular consumption whole wheat grain has been found to be associated with reduced total mortality, as well as reduced risk of coronary heart disease, ischemic stroke, type 2 diabetes (Archie et al. 2006), hypertension in women and colorectal cancer (Schatzkin 2007).

The aim of this study was to determine the level of antioxidant activity (tocopherols) in varieties of einkorn (*T. monococcum* L.), emmer (*T. dicoccum* Schuebl [Schrank]), spelt (*T. spelta* L.) and *T. aestivum* L. and identify the richest sources for improving the nutritional value of bread, pasta and other wheat products.

MATERIAL AND METHODS

Used varieties

The varieties came from the Gene bank of the Crop Research Institute in Prague-Ruzyne. In the precise three-year field experiments in 2010, 2011 and 2012 four varieties of wheat einkorn, eight varieties of emmer, seven varities of spelt, four varieties of landraces of bread wheat and three varieties of spring wheat as control (SW Kadrilj, Vanek, Jara) were used.

Field Trials

The field experiments were arranged in a randomized complete design with two replications. Plot size of this treatment was 10 m^2. Varieties were sown on the organic certified research area of the University of South Bohemia in Ceske Budejovice, the Czech Republic. The seeding rate was adjusted for a density of 350 germinable grains per m^2. The crop stands were treated in compliance with the European legislation (the European Council Regulation (EC) No. 834/2007, the European Commission Regulation (EC) No. 889/2008. Characteristics of the conditions of the University of South Bohemia in Ceske Budejovice research area: Mild warm climate, soil type – pseudo gley cambisols, kind of soil – loamy sandsoil, altitude of 388 m.

Laboratory analysis

Finely ground wheat samples (ca 5.0 g) were weighed into 100 mL volumetric flasks and dissolved in methanol. The flasks were filled up with methanol to volume of 100 mL. For AOA determination, 100 µL aliquots of sample solutions were pipetted. Determination of AOA with DPPH assay. Indirect method described by Roginsky and Lissi (2005) was used. Sample containing antioxidants reacts with a solution of stable synthetic radical being converted to a colourless product (DPPH assay). Methanolic DPPH solution [absorbance (t$_0$) 0.600 ± 0.01] was prepared and 100 µL of the sample were added. Reaction time was 20 min. Absorbency was measured at wavelength $\lambda = 515$ nm. AOA was calculated as the decrease of absorbency according to the equation (1): $\text{AOA}\ (\%) = 100 - [(A_{t20}/A_{t0}) \times 100]$ (1)Where: At$_{20}$– absorbency in time 20 min; A$_{t0}$– absorbency in time 0 min. Calculated AOA was expressed in mg Trolox/kg DM. At$_0$ and A$_{t20}$ were determined from the standard calibration curve ($r^2 \geq 0.9945$). Calibration curves were prepared using working solutions of Troloxin methanol between 5-25 µg Trolox/mL (LOD = 0.601 µg Trolox/mL, LOQ = 2.000 µg Trolox/mL, RSD = 1.83%). All samples were analysed in duplicates.

Statistical analysis

The data were subjected to analysis by using software Minitab 17.0. Specifically, ANOVA multiple factorial analysis, Turkey's HSD test and t-test were used for analyzing the parametric data and non-parametric data.

RESULTS AND DISCUSSION

Whole grain phytochemicals have antioxidant activity, the ability to scavenge free radicals that may oxidise biologically relevant molecules (Liu 2007). Thank to this, whole wheat foods could to contribute to the health benefits of people such as reducing the risk of heart disease, diabetes type 2, cancer and etc. In the present study, there were highly significant differences ($p < 0.05$) among 26 varieties for antioxidant activity (Table 1).

Table 1 Content of antioxidant activity in different wheat grains.

Variety	D11*	D12*	D13*	D14*	D17*	D18*
AOA (mg Trolox/kg DM)	400.83^{a}	364.15^{ab}	341.60^{bc}	288.36 $^{e-g}$	304.56^{c-f}	351.62^{b}
Variety	D19*	RUDICO*	J1**	J2**	J4**	J6**
AOA (mg Trolox/kg DM)	339.92^{bc}	332.90 $^{b-d}$	247.42 ^{gh}	306.16^{c-f}	293.23 ^{df}	327.73^{b-e}
Variety	P1***	P2***	P3***	P4***	SP1****	SP2****
AOA (mg Trolox/kg DM)	345.88 ^{bc}	362.25^{ab}	365.26^{ab}	360.95^{ab}	225.45 ^{h}	226.55 ^{h}
Variety	SP3****	SP6****	SP7****	SP8****	SP9****	JARA
AOA (mg Trolox/kg DM)	232.63 ^{h}	265.56 $^{f-h}$	280.63^{fg}	281.10 ^{fg}	248.82^{gh}	357.36^{ab}
Variety	SW	VANEK				
AOA (mg Trolox/kg DM)	336.98 $^{b-d}$	353.70^{b}				

Values marked with different small letters are significantly different at $P \leq 0.05$

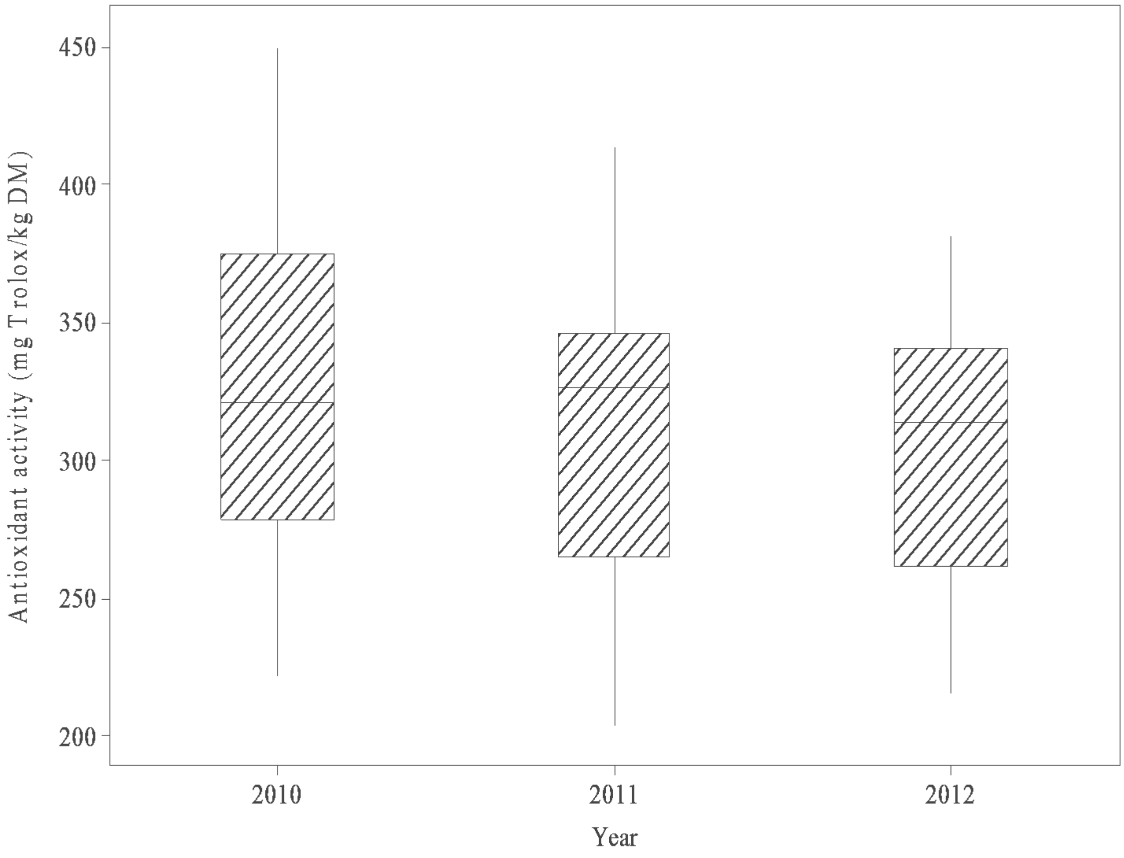
** Emmer varieties; ** Einkorn varieties; *** Landrace of T. aestivum; **** Spelt varieties*

Mean antioxidant activity among varieties ranged from 225.45 mg Trolox/kg DM to 400.83 mg Trolox/kg DM. This demonstrates a broad range of antioxidant content in wheat species. There were eight groups in which the means were not significantly different from one another. Having 400.83 mg Trolox/kg DM, D11 variety belonged to lead group and was significantly different from all other varieties except P3, D12, P2, P4 and JARA. In contrast, the varieties containing the lowest content of antioxidant were SP6, SP9, J1, SP3, SP2 and SP1 with 266.57 mg Trolox/kg DM, 248.82 mg Trolox/kg DM, 247.42 mg Trolox/kg DM, 232.63 mg Trolox/kg DM, 226.55 mg Trolox/kg DM and 225.45 mg Trolox/kg DM, respectively.

According to the findings of Lachman et al. (2012) the antioxidant activity content of 7 varieties ranged between 134.0 and 197.5 mg Trolox/kg DM. Obviously, our results are approximately two-time higher than these ones. This means that the varieties in our experiment are potential to breeding new wheat varieties, as well as its essential as a source of functional food ingredients.

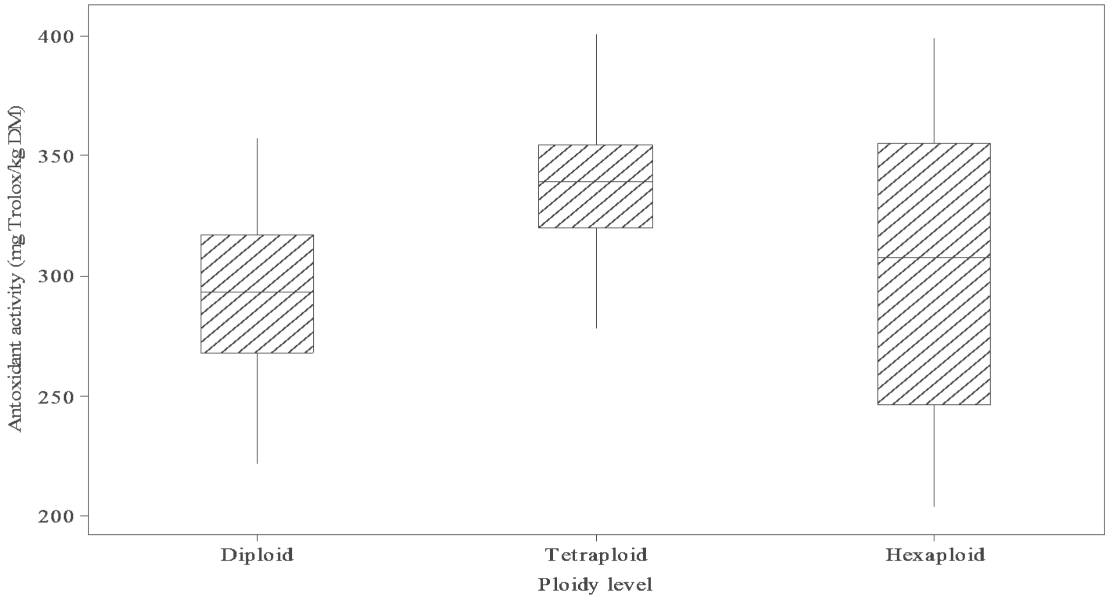
It is known that antioxidant activity content can be influenced by stress factors of the weather conditions during the vegetation period and genotype effects. Comparing the data collected from 2010 to 2012 of four species (Figure 1) show that there is a decrease gradually the mean of antioxidant during the three-year period with 23.26 mg Trolox/kg DM. These differences are, however, not statistically significant.

Figure 1 Antioxidant activity in 26 varieties harvested in 2010, 2011 and 2012



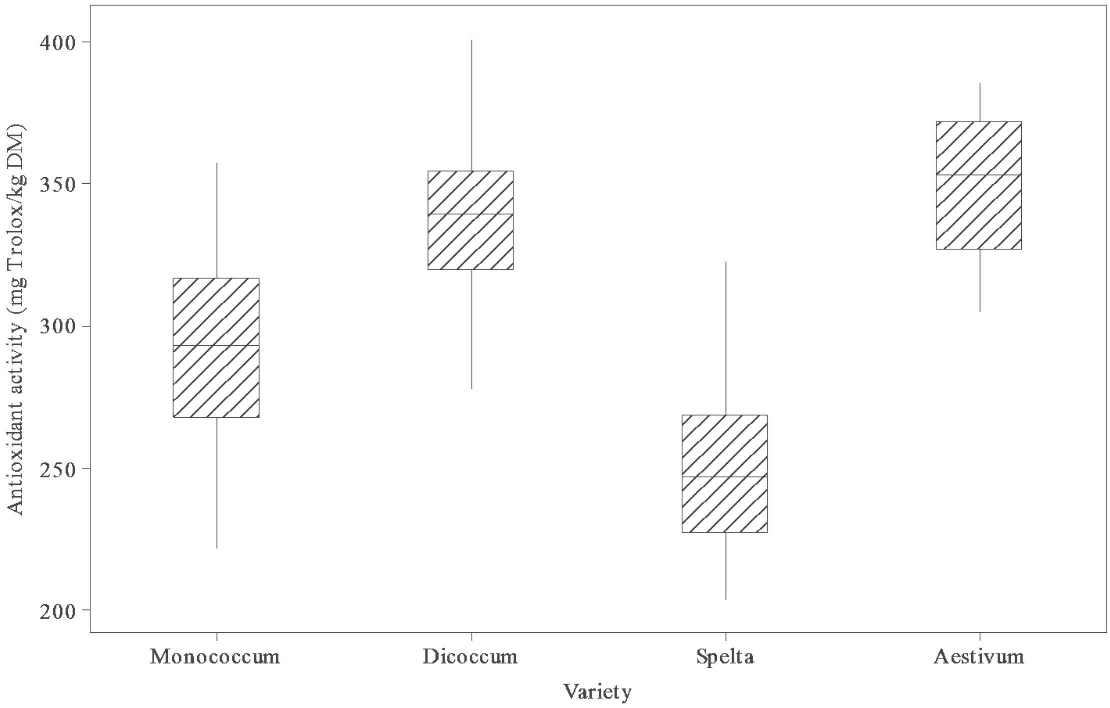
The cultivated diploid (einkorn), tetraploid (durum wheat), hexaploid (bread wheat) and varieties possess antioxidant activity due to their content of hydrophilic (phenolics, selenium) and lipophilic (carotenoids, tocopherols) antioxidants (Hidalgo et al. 2008).

Figure 2 Content of antioxidants in wheat grains from the harvests 2010, 2011 and 2012



Analysing ANOVA Tukey's HSD revealed statistically significant differences between Tetraploid and Diploid as well as between Tetraploid and Hexaploid (Figure 2). The mean antioxidant activity of tetraploid from 2010 to 2012 (340.49 ± 39.11 mg Trolox/kg DM) was higher than the value of diploid and hexaploid (293.64 ± 34.82 mg Trolox/kg DM) and (303.08 mg Trolox/kg DM), respectively. Our results are different to those of Lachman (2012). While antioxidant values in our findings increase from diploid (einkorn) to tetraploid, the reverse is true for Lachman's results. This is because our experiment used 26 varieties in three years compared to 7 varieties in two years of Lachman's experiment.

Figure 3 Antioxidant activity values of four species



The figure 3 illustrates the differences of four varieties. *T. aestivum* and emmer wheat shared the highest value with 354.44 ± 24.97 mg Trolox/kg DM) and 340.49 ± 39.11 mg Trolox/kg DM, respectively. The second high value belonged to *T. monococcum* (293.64±34.82 mg Trolox/kg DM). With 251.54 ± 29.60 mg Trolox/kg DM), *T. spelta* had the lowest value in total four species ($P < 0.05$)

CONCLUSION

Wheat contains a huge essential antioxidants such as dietary fiber, tocopherols, tocotrienols, and etc. The consumption of wheat is associated with reducing risk of chronic diseases including type 2 diabetes, obesity, and cardiovascular disease. In this study, the content antioxidant activity of 26 varieties of whole wheat are reported. Antioxidant activity ranged from 225.45 mg Trolox/kg DM to 400.83 mg Trolox/kg DM. The antioxidant activity values were significantly different among varieties, ploidy level and wheat accessions. Also, this study showed a genotypical variation in the antioxidant activity of einkorn, emmer, spelta and *T. aestivum*.

ACKNOWLEDGEMENTS

The research was financially supported by the National Agency for Agricultural Research of the Ministry of Agriculture of the Czech Republic (Project No. NAZV QJ1310072) and the University of South Bohemia in Ceske Budejovice (Project No. GAJU 094/2016/Z).

REFERENCES

Archie, M., Harry, D., Trust, B. 2006. Genotype and Environmental variation in phenolic content, phenolic acid composition, and Antioxidant activity of Hard Spring Wheat. *Journal Agriculture Food Chemistry,* 54: 1265–1270.

Hidalgo, A., Brandolini, A., Gazza, L. 2008. Influence of steaming treatment on chemical and technological characteristics of einkorn (Triticum monococcum L. ssp monococcum) wholemeal flour. *Food Chemistry,* 111: 549–555.

Lachman, J., Orsák, M., Pivec, V., Jiru, K. 2012. Antioxidant activity of grain of einkorn (Triticum monococcum L.), emmer (Triticum dicoccum Schuebl [Schrank]) and spring wheat (Triticum aestivum L.) varieties. *Plant Soil Environment,* 58: 15–21.

Liu, R. 2007. Whole grain phytochemicals and health. *Journal of Cereal Science,* 46: 207–219.

Pomeranz, Y. 1988. Chemical composition of kernel structures in wheat. *Chemistry and Technology,* 1: 97–158.

Roginsky V., Lissi E.A. 2005: Review of methods to determine chain-breaking antioxidant activity in food. *Food Chemistry*, 92: 235–254.

Schatzkin, A., Mouw, T., Park, Y. 2007. Dietery fiber and whole-grain consumption in relation to colorectal cancer. *NIH-AARP Diet and Health Study,* 85: 1353–1360.

Contents lists available at ScienceDirect

Soil & Tillage Research

journal homepage: www.elsevier.com/locate/still

Influence of selected maize cultivation technologies on changes in the labile fraction of soil organic matter sandy-loam cambisol soil structure

Marek Kopecký [a,*], Jiří Peterka [a], Ladislav Kolář [a], Petr Konvalina [a], Josef Maroušek [b], Radka Váchalová [a], Marcel Herout [c], Otakar Strunecký [d], Jana Batt [a], Dang Khoa Tran [a]

[a] University of South Bohemia in České Budějovice, Faculty of Agriculture, Studentská 1668, 370 05 České Budějovice, Czech Republic
[b] The Institute of Technology and Business in České Budějovice, Faculty of Technology, Okružní 517/10, 370 01 České Budějovice, Czech Republic
[c] ZD Krásná Hora nad Vltavou a.s., Krásná Hora nad Vltavou 172, 262 56, Krásná Hora nad Vltavou, Czech Republic
[d] University of South Bohemia in České Budějovice, Faculty of Science, Branišovská 1760, 370 05 České Budějovice, Czech Republic

ARTICLE INFO

Keywords:
Indicators
Labile fractions
Soil organic matter
Soil structure

ABSTRACT

Disc cultivators are a commonly used method in soil processing when growing maize in Central Europe. However, the slope of the land leads to soil losses through water erosion. Therefore, conservation technologies for soil treatment—strip-till and no-till—are recommended. The aim of this research was to assess these technologies in terms of the labile fractions of soil organic matter and the structural state of the soil. Another goal of the work was to find the most sensitive indicators of change in the labile fractions of soil organic matter, which would indicate changes even over a relatively short-term experiment (three years). The experiment was conducted in Central Bohemia, Czech Republic on plots of sandy-loam cambisol. Changes in soil structure and carbon and nitrogen content in various soil fractions were monitored. The obtained results of two soil conservation technologies (strip-till, no-till) were compared with the results for a commonly used technology (disc cultivation). The strip-till technology led to the highest accumulation of a very labile fraction of organic soil matter and the most sensitive indicator of change was the content of water-extractable organic carbon. The no-till technology protected the soil organic matter from decomposition by physical protection in soil aggregates. Most of the soil organic matter remained un-decomposed. Sensitive indicators of change were the nitrogen content in particulate organic soil matter and the content of water-extractable organic nitrogen. It was found that changes in the labile fraction of soil organic matter can be monitored through suitable indicators during a short-term experiment. Furthermore, we found that no-till technology contributes to the protection of unstable soil organic matter against decomposition, especially through physical protection in soil aggregates. In terms of the content of labile fractions of soil organic matter and their possible effect on the potential soil fertility, it was shown in this short-term experiment that strip-till technology was optimal.

1. Introduction

Soil organic matter (SOM) consists of functional pools that differ in their rate of decomposition. They are referred to as labile (active), slow (medium) and stable (resistant, passive, inert) (von Lützow et al., 2007v). The most labile water-extractable portion constitutes only a very small part of the total SOM. Nevertheless, it is a major source of energy and substrate for soil microorganisms and contributes to the nutrient regime of soils (Fiedler et al., 2015). Labile fractions of soil organic matter directly affect soil microbial activity and consequently the potential soil fertility (Haynes, 2005). In contrast, long-term carbon storage in soil is enabled by stable and particularly resistant SOM

Abbreviations: C_{org}, Organic carbon; DC, Disc cultivation; fLFOM, Free light fraction of soil organic matter; LFOM, Light fraction of soil organic matter; NT, No-till technology; N_{tot}, Total soil nitrogen; oLFOM, Occluded light fraction of soil organic matter; POM, Particulate organic matter; POMC, Particulate organic matter carbon; POMN, Particulate organic matter nitrogen; SOM, Soil organic matter; ST, Strip-till technology; WEOC, Water-extractable organic carbon; WEON, Water-extractable organic nitrogen.

* Corresponding author at: University of South Bohemia in České Budějovice, Faculty of Agriculture, Branišovská 1457, 370 05 České Budějovice, Czech Republic.
E-mail addresses: mkopecky@zf.jcu.cz (M. Kopecký), peterj03@zf.jcu.cz (J. Peterka), kolar@zf.jcu.cz (L. Kolář), konvalina@zf.jcu.cz (P. Konvalina), marousek@mail.vstecb.cz (J. Maroušek), vachalr@zf.jcu.cz (R. Váchalová), heroutzdkh@seznam.cz (M. Herout), ostrunecky@prf.jcu.cz (O. Strunecký), janabatt@volny.cz (J. Batt), trandangkhoa@hueuni.edu.vn (D.K. Tran).

https://doi.org/10.1016/j.still.2020.104865
Received 25 October 2019; Received in revised form 30 October 2020; Accepted 31 October 2020

fractions (Wiesmeier et al., 2020).

In the past, soil fractionation focused on the isolation of humic substances from the soil and their division into humic acids, fulvic acids and humins. This humification model has been criticized. This is because it has been found that, contrary to the earlier conception of high stability of humic substances, some can be decomposed at surprisingly fast rate. Another contradiction is the fact that the large molecular masses of humic acids (of hundreds to millions of daltons) are in fact only aggregates of small compounds, mimicking large molecules. The fact that the chemical structures of polyaromatic carbon compounds often observed in extracts are commonly produced by plants and microorganisms also leads to criticism of this model. However, these compounds have a clear physiological purpose and are therefore not the products of a random decomposition process (Lehmann and Kleber, 2015). Criticism of the humification model is therefore justified.

In recent years, the humification model has been replaced by fractionation of SOM according to specific stabilization mechanisms. The selective protection against SOM biodegradation is spatial inaccessibility and interaction with mineral surfaces and metal ions (von Lützow et al., 2006v). There are two main mechanisms of physical stabilization: Physical protection in soil aggregates and stabilization of organic matter by formation of organo-mineral complexes (Llorente et al., 2010). According to hierarchical theory (Six et al., 2004), stable micro-aggregates ($<$250 μm) form macro-aggregates ($>$250 μm) by combining with organic substances of different origin and stability. There is a higher concentration of SOM in macro-aggregates than in micro-aggregates (Yamashita et al., 2006), mainly because, in addition to persistent sealants (microbial and plant polysaccharides and glucoproteins), macro-aggregates contain other organic binding agents (Jensen et al., 2020). The role of decomposed organic material as an intermediate binding substance in aggregates has been confirmed (Abiven et al., 2009). It has been proven, that humic substances are a heterogeneous pool of substances with very different rates of decomposition (von Lützow et al., 2007v). Great heterogeneity of humic carbon in clays, organic sediments, soils and single soil fractions has been found using instrumental methods including thermal analysis, nuclear magnetic resonance and pyrolysis (Mao et al., 2007).

Soil organic matter fractionation using ultrasonic dispersion and density determination enables its separation into three different fractions according to different physical protection mechanisms (Curtin et al., 2019). The free SOM fraction is isolated prior to ultrasonic disintegration of stable aggregates. The occluded SOM fraction is then isolated after ultrasonic disintegration, leaving the organo-mineral fraction, the so-called “heavy” fraction (John et al., 2005; Six et al., 2002). The free fraction is a labile fraction of SOM with a fast decomposition rate, unlike the occluded and organo-mineral fractions, which are more stable and have conversion times ranging from decades to centuries (Llorente et al., 2010).

Particulate organic matter (POM) is a significant fraction of SOM. Organic material which corresponds to a particle size of 53–2000 μm is defined as POM (Carter and Gregorich, 2006). The free fraction can be divided by density fractionation, i.e., a solution with a density of $1.6–2.0 \cdot 10^{-3}$ kg m^{-3} (Gregorich et al., 2006). In the first step of the reaction, the sample is finely dispersed in the solution and the supernatant is filtered as a free light fraction (fLFOM). In the second step, the remaining matter is vigorously shaken in the solution of the same density or higher. The supernatant is again filtered as an occluded light fraction (oLFOM) (Sequeira et al., 2011). The free light fraction of soil organic matter comes from the organic matter of the outer surface of soil aggregates or pseudo-aggregates, while oLFOM comes from stable aggregates (Bird et al., 2008). The free light fraction is considered to be a more labile SOM fraction than oLFOM. And it is because the chemical composition and structure of fLFOM is still close to the original material, and it exists outside the aggregates. The occluded light fraction of soil organic matter fraction is also considered to be degradable, but must be released from the inside of soil aggregates (Gulde et al., 2008). Therefore, the oLFOM is more stable when the quanitity of water-stable aggregates in the soil is greater (Sequeira et al., 2011).

The labile fraction of SOM, especially POM and the light fraction of organic matter (LFOM), are potentially sensitive indicators of changes in soil caused by management practices (cultivation, fertilization, crop rotation) and indicate changes in soil quality over the short term (Sharifi et al., 2008; Yoo and Wander, 2008). However, Sequeira et al. (2011) found that POM and oLFOM were more sensitive to changes in soil management than fLFOM, especially when monitoring tillage and no-till technology. In particular, the organic matter and light fraction of soil organic matter are associated with changes in the mineralizable organic nitrogen pool in soils. Organic carbon belonging to the POM fraction (POMC) was found to be a very sensitive indicator of changes in the mineralizable nitrogen pool in the study of soil treatment (conventional tillage and no-till) effects in loam and clay soils (Sharifi et al., 2008). Experiments have shown that POMC and nitrogen of the POM fraction (POMN) were closely related to the potentially mineralizable nitrogen of many soils including spodosols, molisols, alphisols, inceptisols (Sharifi et al., 2007).

Soil microbial biomass is a sensitive indicator of changes in SOM that corresponds to changes in land use and vegetation (Krüger et al., 2018). The recommended indicators of SOM quality are total soil nitrogen (N_{tot}), C_{org} and C/N ratio (Li et al., 2019). More sensitive indicators of change induced by soil management are water-extractable organic nitrogen (WEON) and POMN (Haynes, 2005). Water-extractable organic nitrogen determination is often used as a substitute for the determination of soluble nitrogen in soil solution (Surey et al., 2020), providing information related to soil microbial activity and dynamics, as well as helping assess the quantity and quality of SOM (Luce et al., 2014). The particulate organic matter nitrogen fraction is composed of partially decomposed plant residues and microbial products and is a major nitrogen source for soil microbes (Gregorich et al., 2006). Stable fractions of soil organic matter are also a source of other plant nutrients after mineralization (Elbl et al., 2019).

Conventional soil tillage in long-term experiments (Andruschkewitsch et al., 2013), as well as reduced soil tillage (rotationally, to a depth of 0.05–0.08 m), reduces the amount of macro-aggregates in soils compared to no-till technology. In addition to the physical impact on soil, tillage affects the dynamics of organic matter decomposition (Jacobs et al., 2010).

No-till technologies reduce the contact of plant residues with soil, and therefore in conventionally moldboard plowed soils the decomposition of organic matter is faster and greater (Oorts et al., 2007). Changes in soil C_{org} are regulated by selective protection, that is, by the formation of insoluble substances and spatial inaccessibility in biogenic aggregation, as well as interaction with mineral surfaces. Therefore, the chemical composition of organic substances in the soil is less important for C_{org} dynamics than its physical protection and location (von Lützow et al., 2008v). Fractionation of water-stable aggregates and density measurement of SOM fractions are more sensitive indicators of changes in soil management than C_{org} determination (von Lützow et al., 2006v). Li et al. (2020) found that water-stable macro-aggregates are enriched with younger organic material and have a higher rate of decomposition than the organic matter of micro-aggregates. Reduced movement of macro-aggregates in no-till systems (Álvaro-Fuentes et al., 2009) causes stabilization of LFOM in aggregates in both temperate and tropical climates (Buurman and Roscoe, 2011; Yoo and Wander, 2008).

Identifying early indicators of SOM dynamics will allow early management decisions and quick remedial action. In this work, the following hypothesis will be verified: The content of aggregates of the labile and particulate fractions of soil organic matter, the nitrogen content in the particulate organic matter (POMN) and the amount of water-extractable carbon (WEOC) and nitrogen (WEON) in the soil differ among the three tested technologies of tillage (strip-till, no-till, disking) for maize.

2. Material and methods

2.1. Field experiments

Field experiments were carried out in 2014–2016 on agricultural land of the cadastral territory Krásná Hora nad Vltavou (434 m above sea level), Czech Republic (49°60′44.89’’ N, 14°27′82.38’’ E). The experimental site belongs to the climate region, which is characterized as slightly warm and humid, with an average annual temperature of 7 °C and average annual rainfall of 650 mm. The soils are sandy-loam cambisols.

The field on which the pilot experiment was carried out had a total area of 18.6 ha. It was divided into three equal-sized areas and in each section the crop rotation described below was applied (three-year repetition). In a given year, one third of the experimental field was divided into three parts to apply the three experimental variants described below.

Winter oilseed rape (*Brassica napus* subsp. *napus*) was grown on the plot in the first year, and in the second year, winter wheat (*Triticum aestivum* L.) was grown. After its harvest, digestate from a biogas plant (Table 1) was applied to the field at a rate of 40 m^3 ha^{-1} (approximately $5 \cdot 10^4$ kg ha^{-1}), which was incorporated by a Horsch Terrano cultivator. A non-frost-tolerant cover crop of *Phacelia tanacetifolia* Benth. was sown in the field at a dose of 10 kg ha^{-1} with a Pottinger Terrasem C6 machine. In the following year, maize (*Zea mays* L.) was planted.

The experiments were organized in three variants according to the cultivation technology. These were the DC variant (disking), the ST variant (strip-till) and the NT variant (no-till technology). For the DC variant, the soil was prepared to a depth of 0.08 m with a Lemken Rubin disc stubble cultivator before sowing. The strip-till variant consisted of separate processed strips (depth 0.2 m) with a Kuhn Striger machine. For the NT variant, maize was sown directly into untreated soil and the soil surface was protected by a frozen cover crop.

Sowing of corn (90,000 seeds ha^{-1}, row spacing 0.75 m, Silvinio variety, FAO number 210) with fertilization at a dose of 100 kg ha^{-1} of Amofos, ammonium phosphate fertilizer (12 kg N ha^{-1}, 23 kg P ha^{-1}), was performed after reaching the required soil temperature each year (10 April 2014, 15 April 2015, 7 April 2016) with a Kinze 3500 seeding-machine.

After corn sowing, a mixture of Gardoprim plus gold (4 L ha^{-1}) and Roundup flex (2.3 L ha^{-1}) herbicides with a carrier medium of 200 L of DAM 390 liquid fertilizer (60 kg N ha^{-1}) was pre-emergently applied to kill the rest of the *Phacelia tanacetifolia* Benth. and to control weeds. The residues of plants were left on the surface.

2.2. Processing of soil samples

After harvesting corn for silage (7 October 2014, 11 October 2015, 4 October 2016), soil samples were taken from all cultivation variants. Sampling was performed using a pedological sampling rod from a depth of 0–0.2 m. Twenty samples were taken from each variant each year, after which they were dried at 60 °C and composited. Material from the composited samples was used for subsequent analyses. Each analysis was repeated ten times for each individual sample.

Determination of the structural condition of soil samples was performed by sieving and sedimentation (DIN ISO 11277, 2002). Water-stable aggregates were fractionated into micro-aggregates (<250 μm) and macro-aggregates (>250 μm) according to John et al. (2005): 50 g of sieved soil (≤ 10 mm) was dried at 40 °C for 48 h and sieved in deionized water in a sieving machine for 10 min. After 50 vertical lifts of the sieve (38 mm), the water-stable aggregates (>250 μm) were sprayed onto a vacuum filter, the water was aspirated and the aggregates dried at 40 °C. Particles that passed through the sieve (<250 μm) were isolated by the addition of 2.5 mL of 0.5 M $AlCl_3$ solution per 1000 mL of supernatant, after which they were decanted and dried for 48 h at 40 °C.

Table 1
Nutrient content and dry matter content of the used digestate (%).

	N_{min}	N_{org}	P	K	Ca	Mg	DM
2014	0.23	0.14	0.08	0.37	0.23	0.05	7.55
2015	0.23	0.13	0.08	0.35	0.25	0.06	7.80
2016	0.21	0.15	0.07	0.36	0.26	0.06	7.50

N_{min} – mineral nitrogen; N_{org} – organic nitrogen; DM – dry matter.

Particulate organic matter (2000–53 μm) was determined according to Carter and Gregorich (2006): 20 g of air-dried soil which passed through the sieve (≤2 mm) was dispersed in 100 mL of a 0.0082 mol·L^{-1} sodium hexametaphosphate solution at 180 rpm min^{-1} in a radial shaker for 12 h. The suspension was then shaken under deionized water through a 53 μm sieve until the clay fraction was completely removed. The sand on the sieve together with coarse organic particles were then dried at 60 °C for 1 h. The residue on the sieve was collected in an aluminium container, dewatered and dried for 24 h at 60 °C. Particulate organic matter was determined by subtracting the weight of sand after burning the mixture in a muffle furnace at 600 °C for 4 h.

2.3. Chemical analyses

Particulate organic matter carbon and particulate organic matter nitrogen were determined in the POM fractions and in the original samples using an Elementar CN Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

Water-extractable organic carbon and water-extractable organic nitrogen were determined according to Zhao et al. (2008) by shaking 75 g of sieved soil through a 2 mm sieve with 150 mL of a 10 mmol·L^{-1} $CaCl_2$ solution on an orbital shaker for 30 min at 175 rpm min^{-1}. After sedimentation for 1 h, the supernatant was vacuum filtered through a cellulose acetate filter (<0.45 μm) (OE 67, Schleicher and Schüll). The filtrate was then frozen at –20 °C. Water-extractable organic carbon was then determined by infrared detection of CO_2 after burning at 850 °C using a DIMA-TOC 100 device (Essen, Germany). Water-extractable organic nitrogen was determined on the same device using chemoluminescence detection.

Data obtained from the analysis were analyzed using STATISTICA 13 (Statsoft, Inc., USA). One-way analysis of variance (ANOVA) with a post-hoc Tukey test was used to test for differences between soil samples. The level of significance for all analyses was $p \leq 0.05$.

3. Results and discussion

The distribution of soil aggregates in sand-free soil samples (<2000 μm) in ST, NT and DC variants of cultivation technologies is shown in Fig. 1. The differences in the representation of the aggregates of the three classes (2000–250 μm, 250–53 μm and fraction <53 μm) between the three technologies are negligible. This is probably due to the short timeframe of the experiment and because the same level of organic fertilization was used. Organic fertilization increases the microbial activity of the soil and thus the production of binding agents, which in particular affects macro-aggregation (Six et al., 2004). Mineral nitrogen (Mustafa et al., 2020), roots (Miao et al., 2017) and soil fungal hyphae (Jastrow, 1996) also play an important role in macro-aggregation.

It has been proven that the amount of macro-aggregates positively correlates with soil fertility (Six et al., 2000). The macro-aggregates in all three treatments significantly exceed the amount of micro-aggregates and fraction <53 μm; thus, it can be said that in this respect all three technologies are equivalent. The situation in ST seems optimal, but the differences are relatively small. Since there is a higher concentration of SOM in macro-aggregates than in micro-aggregates (Yamashita et al., 2006), it can be stated that all three technologies support the protection of SOM from biodegradation. However, it was found (Table 2) that the amount of macro-aggregates in the ST variant differed significantly from the DC variant ($F_{(2,\ 27)} = 8.2064$; $p = 0.00164$). The difference between

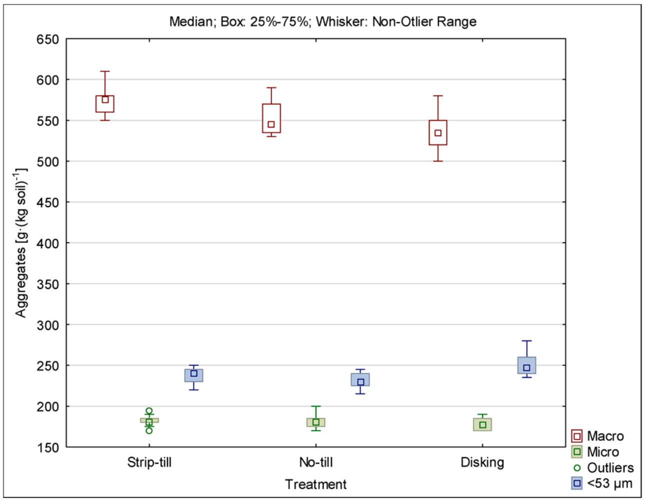


Fig. 1. Division of water-stable aggregates (after sand subtraction) into macro-aggregates (250–2000 μm), micro-aggregates (53–250 μm) and fraction <53 μm in variants strip-till, no-till and disking.

Table 2
Average content of water-stable macro-aggregates (250–2000 μm), micro-aggregates (53–250 μm) and fraction <53 μm in strip-till, no-till and disking technologies of tillage [g aggregates·(kg soil after sand subtraction)$^{-1}$].

	Macro-aggregates	Micro-aggregates	Fraction <53 μm
Strip-till	567.00 ± 17.44^{b}	182.00 ± 6.78	238.50 ± 9.23^{a}
No-till	551.50 ± 20.25^{ab}	181.50 ± 9.50	231.00 ± 8.89^{a}
Disking	536.00 ± 24.98^{a}	178.50 ± 7.43	251.50 ± 13.61^{b}
$HSD_{0.05}$	495.65	NS	129.44

HSD – different letters indicate statistically significant differences between evaluated files at the significance level $p \leq 0.05$ - Tukey HSD test; NS – no significant differences.

the NT variant and the other two variants was not statistically significant. There were no statistically significant differences between technologies in the group of micro-aggregates ($F_{(2,\ 27)} = 0.50500$; $p = 0.60896$). In the fraction <53 μm, it was found that the DC variant differs statistically from the remaining two ($F_{(2,\ 27)} = 8.3112$; $p = 0.00154$). Many authors argue that the movement of soil particles in conventional tillage results in partial destruction of macro-aggregates, whose organic matter is then released by microbial decomposition (von Lützow et al., 2008v; Tan et al., 2007; Zotarelli et al., 2007), although others are of the opposite opinion (Jacobs et al., 2010; Six et al., 2000). The results of this study favour the latter group and Fernández et al. (2015) achieved the same results. In conclusion, the aggregate analysis does not appear to be a sensitive indicator with which to assess the differences between the monitored technologies, although some authors (Doran, 2002; Su et al., 2010) consider the state of the soil structure as an indicator of soil condition.

The amount of organic carbon and nitrogen is shown in Table 3. The content of C_{org} was almost equal in the soils of all variants and the subsamples were comparable ($F_{(2,\ 27)} = 2.5020$; $p = 0.10072$). Some authors find the highest values in the NT variant (Awale et al., 2013; Oorts et al., 2007), while others find higher carbon concentrations in the ST variant at greater sampling depths, or even in the classic tillage variant (Andruschkewitsch et al., 2013; Fernández et al., 2015; Sainju et al., 2013; Sequeira et al., 2011). Furthermore, there were no significant differences in N_{tot} content among the three variants ($F_{(2,\ 27)} = 0.83887$; $p = 0.44315$). Consequently, N_{tot} seems inappropriate to use as a sensitive indicator of changes in SOM.

Table 3 also shows the C_{org} content in the macro- and micro-aggregates and the C_{org}/N_{tot} ratio in the above-mentioned aggregate groups. The content of C_{org} in macro-aggregates is highest in the NT variant and differs significantly from the ST and DC variants ($F_{(2,\ 27)} = 9.8276$; $p = 0.00062$); there was no difference between the latter two variants. This is in line with the findings of previous research (von Lützow et al., 2008v; Tan et al., 2007). Andruschkewitsch et al. (2013) found the C_{org} contents in the macro-aggregates in 0–5 cm depth were significantly higher in soils of the DC and NT treatment, than the contents in the soils of the CT treatment. With increasing depth, the C_{org} contents in the macroaggregates of the DC and NT treatments decreased to the level of the CT treatment. The C_{org} content in micro-aggregates was significantly higher in the NT variant than in the ST and DC variants ($F_{(2,\ 27)} = 7.6491$; $p = 0.00233$).

The C_{org} content in micro-aggregates was slightly lower than in macro-aggregates for the NT and DC variants, consistent with the results of other studies (Six et al., 2000; Yamashita et al., 2006). The opposite situation was observed in the ST variant.

A difference in the C_{org}/N_{tot} ratio was found for both macro-aggregates ($F_{(2,\ 27)} = 6.9595$; $p = 0.00365$) and micro-aggregates ($F_{(2,\ 27)} = 7.7097$; $p = 0.00225$). In both cases, the highest value was achieved in the NT variant. On the contrary, in a significant work by Andruschkewitsch et al. (2013), the C_{org}/N_{tot} ratio was found to be lowest in both fractions of the NT variant.

Table 2 shows the amount of particulate organic matter (2000–53 μm) as the sum of the amounts of macro-aggregates and micro-aggregates. From the perspective of indicating differences between technologies, the nitrogen content of this fraction (POMN) is significant. It is listed together with POMC, WEOC and WEON in Table 4.

Particulate organic matter nitrogen is a sensitive indicator of changes in SOM induced by different soil management types (Haynes, 2005; Luce et al., 2014). This was confirmed by our experiment. Statistically significant differences in POMN content were found between all variants ($F_{(2,\ 27)} = 139.04$; $p < 0.00001$). Although Liang et al. (2004) found that soil-protection technologies do not always lead to an increase in the mineralizable nitrogen content, in our case the highest POMN value was found in the NT variant, which is considered a soil-protection technology (Vilček et al., 2019). Higher values in the NT variant were also observed by Sharifi et al. (2008), who compared this technology with classical tillage. They also found that having more POMN in the soil does not necessarily mean greater nitrogen supply to plants, and even if the

Table 3
The content of organic carbon, the content of total nitrogen and the content of organic carbon in macro-aggregates (250–2000 μm) and micro-aggregates (53–250 μm), organic carbon/total nitrogen ratio in macro-aggregates and micro-aggregates in strip-till, no-till and disking technologies of tillage (± SD).

	C_{org} [g C·(kg soil)$^{-1}$]	N_{tot} [g N·(kg soil)$^{-1}$]	C_{org} [g C·(kg macro-aggregates)$^{-1}$]	C_{org} [g C·(kg micro-aggregates)$^{-1}$]	C_{org}/N_{tot} ratio in macro-aggregates	C_{org}/N_{tot} ratio in micro-aggregates
Strip-till	11.80 ± 0.11	1.29 ± 0.12	13.00 ± 1.90^{a}	13.50 ± 1.36^{a}	10.17 ± 1.79^{a}	10.54 ± 1.27^{a}
No-till	11.72 ± 0.15	1.32 ± 0.17	18.00 ± 2.72^{b}	16.70 ± 2.10^{b}	13.80 ± 2.64^{b}	12.69 ± 1.24^{b}
Disking	11.91 ± 0.25	1.37 ± 0.12	15.00 ± 2.53^{a}	14.00 ± 2.05^{a}	11.00 ± 1.97^{a}	10.28 ± 1.72^{a}
$HSD_{0.05}$	NS	NS	6.4444	3.8741	5.1998	2.2616

HSD – different letters indicate statistically significant differences between evaluated files at the significance level $p \leq 0.05$ - Tukey HSD test; NS – no significant differences; C_{org} – organic carbon; N_{tot} – total nitrogen.

Table 4
The content of particulate organic matter carbon, particulate organic matter nitrogen, water-extractable organic carbon and water-extractable organic nitrogen in soil from strip-till, no-till and disking technologies of tillage (± SD).

	POMC [g C·(kg soil)$^{-1}$]	POMN [g N·(kg soil)$^{-1}$]	WEOC [g C·(kg soil)$^{-1}$]	WEON [g N·(kg soil)$^{-1}$]
Strip-till	5.28 ± 0.74^{b}	0.29 ± 0.03^{a}	12.11 ± 0.72^{c}	4.20 ± 0.30^{a}
No-till	4.40 ± 0.76^{a}	0.59 ± 0.04^{c}	11.01 ± 0.64^{b}	7.52 ± 0.46^{c}
Disc cultivation	4.76 ± 0.47^{ab}	0.41 ± 0.03^{b}	8.98 ± 0.49^{a}	6.10 ± 0.46^{b}
$HSD_{0.05}$	0.49556	0.00156	0.43533	0.19096

HSD – different letters indicate statistically significant differences between evaluated files at the significance level $p \leq 0.05$ - Tukey HSD test; POMC – particulate organic matter carbon; POMN – particulate organic matter nitrogen; WEON – water-extractable organic nitrogen; WEOC – water-extractable organic carbon.

actual supply of N to the soil is higher, this increase can be offset by increased losses of nitrogen by denitrification and leaching of NO_3 anion.

The no-till variant had not only the highest POMN content, but also the most C_{org} in both macro-aggregates and micro-aggregates. This shows that SOM is protected from decomposition by physical protection in soil aggregates in this variant. The high nitrogen contents of the POM fractions prove that most of the organic matter was not decomposed. However, it must be taken into account that SOM in aggregates degrades slowly.

Water-extractable organic nitrogen resulting from the decomposition of microbial biomass and underground and aboveground organic residues may be related to the quality and quantity of soil organic matter and microbial activity in soil (Wijanarko and Rahmianna, 2019). In our study, it was closely related to POMN. The differences of water-extractable organic nitrogen content in ST, NT and DC variants were statistically significant ($F_{(2,\ 27)} = 145.31$; $p < 0.00001$). Thus, it also proved to be a relatively sensitive indicator of changes in soil management.

The highest POMC value was found in the ST variant and the lowest value was found in the NT variant. The differences between these variants were significant ($F_{(2,\ 27)} = 3.9498$; $p = 0.03129$). The low content of POMC in the NT variant is surprising. For example, the results of Sharifi et al. (2008) present a positive impact of no-till technology on POMC growth. Bongiorno et al. (2019) reported an increase in POMC in the reduced tillage variant (tillage to 0–10 cm) compared to classical tillage (ploughing to 20–25 cm depth). However, this difference was only proven in soil samples taken from a depth of 0–10 cm; at greater depths, the differences were not significant.

Like POMC, the largest quantity of WEOC was found in the ST variant. Water-extractable organic carbon content analysis showed statistically significant differences in WEOC between all variants ($F_{(2,\ 27)} = 57.917$; $p < 0.00001$). This indicator appears to be more sensitive than POMC content. Awale et al. (2017) investigated soil organic carbon pools as early indicators for soil organic matter stock changes under different tillage practices. They found out that WEOC is an optimal indicator in the early detection of SOM trends for the purposes of adjusting management practices to enhance SOC accretion and improving soil health.

In the ST variant, most of the primary organic matter was formed. However, this labile SOM was more rapidly decomposed in the ST variant than in NT. Organic carbon content in soil is therefore an unreliable indicator of changes in SOM due to soil management. The suggestion by some authors, that conservation agrotechnology and soil-protection technologies reduce carbon loss from soil by CO_2 emissions and store carbon in soil (Alam et al., 2018), does not apply to the ST variant in this short-term experiment.

4. Conclusion

The differences in the representation of the aggregates (2000–250 μm, 250–53 μm and fraction <53 μm) between the three technologies were negligible. Therefore, aggregate analysis cannot be considered an accurate indicator with which to assess differences between the monitored technologies. On the contrary, this work confirmed the finding that the nitrogen content of particulate organic matter (POMN) is a very sensitive indicator of changes in SOM induced by different soil management types. Water-extractable carbon and water-extractable nitrogen were also dependable indicators. Most C_{org} in macro- and micro-aggregates and the highest POMN content were found in the NT variant (the amount was almost double in NT compared to ST). Thus, in this variant, the organic matter is best protected from decomposition. On the other hand, the high content of labile fractions of soil organic matter supports the biological activity of the soil and, therefore, the high potential soil fertility. From this perspective, the best management variant was strip-till. The highest content of POMC and WEOC was found in this variant, which is important from an agronomic aspect. Selected sensitive indicators of the state of labile fractions of soil organic matter confirmed this in a short-term experiment.

Funding

This work was supported by the University of South Bohemia in České Budějovice, Czech Republic, research project GAJU 059/2019/Z.

Declaration of Competing Interest

The authors report no declarations of interest.

References

Abiven, S., Menasseri, S., Chenu, C., 2009. The effects of organic inputs over time on soil aggregate stability - a literature analysis. Soil Biol. Biochem. 41, 1–12. https://doi.org/10.1016/j.soilbio.2008.09.015.

Alam, M.K., Bell, R.W., Haque, M.E., Kader, M.A., 2018. Minimal soil disturbance and increased residue retention increase soil carbon in rice-based cropping systems on the Eastern Gangetic Plain. Soil Tillage Res. https://doi.org/10.1016/j.still.2018.05.009.

Álvaro-Fuentes, J., Cantero-Martínez, C., López, M.V., Paustian, K., Denef, K., Stewart, C. E., Arrúe, J.L., 2009. Soil aggregation and soil organic carbon stabilization: effects of management in semiarid mediterranean agroecosystems. Soil Sci. Soc. Am. J. https://doi.org/10.2136/sssaj2008.0333.

Andruschkewitsch, R., Geisseler, D., Koch, H.J., Ludwig, B., 2013. Effects of tillage on contents of organic carbon, nitrogen, water-stable aggregates and light fraction for four different long-term trials. Geoderma. https://doi.org/10.1016/j.geoderma.2012.07.005.

Awale, R., Chatterjee, A., Franzen, D., 2013. Tillage and N-fertilizer influences on selected organic carbon fractions in a North Dakota silty clay soil. Soil Tillage Res. https://doi.org/10.1016/j.still.2013.08.006.

Awale, R., Emeson, M.A., Machado, S., 2017. Soil organic carbon pools as early indicators for soil organic matter stock changes under different tillage practices in Inland Pacific Northwest. Front. Ecol. Evol. https://doi.org/10.3389/fevo.2017.00096.

Bird, J.A., Kleber, M., Torn, M.S., 2008. 13C and 15N stabilization dynamics in soil organic matter fractions during needle and fine root decomposition. Org. Geochem. https://doi.org/10.1016/j.orggeochem.2007.12.003.

Bongiorno, G., Bünemann, E.K., Oguejiofor, C.U., Meier, J., Gort, G., Comans, R., Mäder, P., Brussaard, L., de Goede, R., 2019. Sensitivity of labile carbon fractions to tillage and organic matter management and their potential as comprehensive soil quality indicators across pedoclimatic conditions in Europe. Ecol. Indic. https://doi.org/10.1016/j.ecolind.2018.12.008.

Buurman, P., Roscoe, R., 2011. Different chemical composition of free light, occluded light and extractable SOM fractions in soils of Cerrado and tilled and untilled fields, Minas Gerais, Brazil: a pyrolysis-GC/MS study. Eur. J. Soil Sci. https://doi.org/10.1111/j.1365-2389.2010.01327.x.

Carter, M.R., Gregorich, E., 2006. Soil sampling and methods of analysis. Measurement. https://doi.org/10.1017/S0014479708006546.

Curtin, D., Beare, M.H., Qiu, W., Sharp, J., 2019. Does particulate organic matter fraction meet the criteria for a model soil organic matter pool? Pedosphere. https://doi.org/10.1016/S1002-0160(18)60049-9.

DIN ISO 11277, 2002. Bodenbeschaffenheit–Bestimmung Der Partikelgrößenverteilung in Mineralböden–Verfahren Mittels Siebung Und Sedimentation. Beuth-Verlag GmbH Berlin.

Doran, J.W., 2002. Soil health and global sustainability: translating science into practice. Agriculture, Ecosystems and Environment. https://doi.org/10.1016/S0167-8809(01)00246-8.

Elbl, J., Maková, J., Javoreková, S., Medo, J., Kintl, A., Lošák, T., Lukas, V., 2019. Response of microbial activities in soil to various organic and mineral amendments as an indicator of soil quality. Agronomy. https://doi.org/10.3390/agronomy9090485.

Fernández, F.G., Sorensen, B.A., Villamil, M.B., 2015. A comparison of soil properties after five years of no-till and strip-till. Agron. J. https://doi.org/10.2134/agronj14.0549.

Fiedler, S.R., Buczko, U., Jurasinski, G., Glatzel, S., 2015. Soil respiration after tillage under different fertiliser treatments - implications for modelling and balancing. Soil Tillage Res. https://doi.org/10.1016/j.still.2014.12.015.

Gregorich, E.G., Beare, M.H., McKim, U.F., Skjemstad, J.O., 2006. Chemical and biological characteristics of physically uncomplexed organic matter. Soil Sci. Soc. Am. J. https://doi.org/10.2136/sssaj2005.0116.

Gulde, S., Chung, H., Amelung, W., Chang, C., Six, J., 2008. Soil carbon saturation controls labile and stable carbon pool dynamics. Soil Sci. Soc. Am. J. https://doi.org/10.2136/sssaj2007.0251.

Haynes, R.J., 2005. Labile organic matter fractions as central components of the quality of agricultural soils: an overview. Adv. Agron. https://doi.org/10.1016/S0065-2113(04)85005-3.

Jacobs, A., Helfrich, M., Hanisch, S., Quendt, U., Rauber, R., Ludwig, B., 2010. Effect of conventional and minimum tillage on physical and biochemical stabilization of soil organic matter. Biol. Fertil. Soils. https://doi.org/10.1007/s00374-010-0472-x.

Jastrow, J.D., 1996. Soil aggregate formation and the accrual of particulate and mineral-associated organic matter. Soil Biol. Biochem. https://doi.org/10.1016/0038-0717(95)00159-X.

Jensen, J.L., Schjønning, P., Watts, C.W., Christensen, B.T., Obour, P.B., Munkholm, L.J., 2020. Soil degradation and recovery – changes in organic matter fractions and structural stability. Geoderma. https://doi.org/10.1016/j.geoderma.2020.114181.

John, B., Yamashita, T., Ludwig, B., Flessa, H., 2005. Storage of organic carbon in aggregate and density fractions of silty soils under different types of land use. in: Geoderma. https://doi.org/10.1016/j.geoderma.2004.12.013.

Krüger, I., Chartin, C., van Wesemael, B., Carnol, M., 2018. Defining a reference system for biological indicators of agricultural soil quality in Wallonia. Belgium. Ecol. Indic. https://doi.org/10.1016/j.ecolind.2018.08.010.

Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. Nature. https://doi.org/10.1038/nature16069.

Li, P., Shi, K., Wang, Y., Kong, D., Liu, T., Jiao, J., Liu, M., Li, H., Hu, F., 2019. Soil quality assessment of wheat-maize cropping system with different productivities in China: establishing a minimum data set. Soil Tillage Res. https://doi.org/10.1016/j.still.2019.02.019.

Li, N., Long, J., Han, X., Yuan, Y., Sheng, M., 2020. Molecular characterization of soil organic carbon in water-stable aggregate fractions during the early pedogenesis from parent material of Mollisols. Int. J. Soil Sediment Water. https://doi.org/10.1007/s11368-020-02563-w.

Liang, B.C., McConkey, B.G., Campbell, C.A., Curtin, D., Lafond, G.P., Brandt, S.A., Moulin, A.P., 2004. Total and labile soil organic nitrogen as influenced by crop rotations and tillage in Canadian prairie soils. Biol. Fertil. Soils. https://doi.org/10.1007/s00374-003-0712-4.

Llorente, M., Glaser, B., Turrión, M.B., 2010. Storage of organic carbon and black carbon in density fractions of calcareous soils under different land uses. Geoderma. https://doi.org/10.1016/j.geoderma.2010.06.011.

Luce, St.M., Ziadi, N., Zebarth, B.J., Grant, C.A., Tremblay, G.F., Gregorich, E.G., 2014. Rapid determination of soil organic matter quality indicators using visible near infrared reflectance spectroscopy. Geoderma. https://doi.org/10.1016/j.geoderma.2014.05.023.

Mao, J., Fang, X., Schmidt-Rohr, K., Carmo, A.M., Hundal, L.S., Thompson, M.L., 2007. Molecular-scale heterogeneity of humic acid in particle-size fractions of two Iowa soils. Geoderma. https://doi.org/10.1016/j.geoderma.2007.03.014.

Miao, S., Qiao, Y., Li, P., Han, X., Tang, C., 2017. Fallow associated with autumn-plough favors structure stability and storage of soil organic carbon compared to continuous maize cropping in Mollisols. Plant Soil. https://doi.org/10.1007/s11104-017-3187-z.

Mustafa, A., Minggang, X., Shah, S.A.A., Abrar, M.M., Nan, S., Baoren, W., Zeijang, C., Saeed, Q., Naveed, M., Mehmood, K., Núñez-Delgado, A., 2020. Soil aggregation and soil aggregate stability regulate organic carbon and nitrogen storage in a red soil of southern China. J. Environ. Manage. https://doi.org/10.1016/j.jenvman.2020.110894.

Oorts, K., Garnier, P., Findeling, A., Mary, B., Richard, G., Nicolardot, B., 2007. Modeling soil carbon and nitrogen dynamics in no-till and conventional tillage using PASTIS model. Soil Sci. Soc. Am. J. https://doi.org/10.2136/sssaj2006.0203.

Sainju, U.M., Stevens, W.B., Evans, R.G., Iversen, W.M., 2013. Irrigation system and tillage effects on soil carbon and nitrogen fractions. Soil Sci. Soc. Am. J. https://doi.org/10.2136/sssaj2012.0412.

Sequeira, C.H., Alley, M.M., Jones, B.P., 2011. Evaluation of potentially labile soil organic carbon and nitrogen fractionation procedures. Soil Biol. Biochem. https://doi.org/10.1016/j.soilbio.2010.11.014.

Sharifi, M., Zebarth, B.J., Burton, D.L., Grant, C.A., Cooper, J.M., 2007. Evaluation of some indices of potentially mineralizable nitrogen in soil. Soil Sci. Soc. Am. J. https://doi.org/10.2136/sssaj2006.0265.

Sharifi, M., Zebarth, B.J., Burton, D.L., Grant, C.A., Bittman, S., Drury, C.F., McConkey, B.G., Ziadi, N., 2008. Response of potentially mineralizable soil nitrogen and indices of nitrogen availability to tillage system. Soil Sci. Soc. Am. J. https://doi.org/10.2136/sssaj2007.0243.

Six, J., Elliott, E.T., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biol. Biochem. https://doi.org/10.1016/S0038-0717(00)00179-6.

Six, J., Feller, C., Denef, K., Ogle, S.M., De Moraes Sa, J.C., Albrecht, A., 2002. Soil organic matter, biota and aggregation in temperate and tropical soils - effects of no-tillage. in: Agronomie. https://doi.org/10.1051/agro:2002043.

Six, J., Bossuyt, H., Degryze, S., Denef, K., 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Tillage Res. https://doi.org/10.1016/j.still.2004.03.008.

Su, Y.Z., Yang, R., Liu, W.J., Wang, X.F., 2010. Evolution of soil structure and fertility after conversion of native sandy desert soil to irrigated cropland in arid region. China. Soil Sci. https://doi.org/10.1097/SS.0b013e3181e04a2d.

Surey, R., Lippold, E., Heilek, S., Sauheitl, L., Henjes, S., Horn, M.A., Mueller, C.W., Merbach, I., Kaiser, K., Böttcher, J., Mikutta, R., 2020. Differences in labile soil organic matter explain potential denitrification and denitrifying communities in a long-term fertilization experiment. Appl. Soil Ecol. https://doi.org/10.1016/j.apsoil.2020.103630.

Tan, Z., Lal, R., Owens, L., Izaurralde, R.C., 2007. Distribution of light and heavy fractions of soil organic carbon as related to land use and tillage practice. Soil Tillage Res. https://doi.org/10.1016/j.still.2006.01.003.

Vilček, J., Koco, Š., Torma, S., Lošák, T., Antonkiewicz, J., 2019. Identifying soils for reduced tillage and no-till farming using GIS. Polish J. Environ. Stud. https://doi.org/10.15244/pjoes/90787.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H., 2006v. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review. Eur. J. Soil Sci. https://doi.org/10.1111/j.1365-2389.2006.00809.x.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007v. SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. Soil Biol. Biochem. https://doi.org/10.1016/j.soilbio.2007.03.007.

von Lützow, M., Kögel-Knabner, I., Ludwig, B., Matzner, E., Flessa, H., Ekschmitt, K., Guggenberger, G., Marschner, B., Kalbitz, K., 2008v. Stabilization mechanisms of organic matter in four temperate soils: development and application of a conceptual model. J. Plant Nutr. Soil Sci. https://doi.org/10.1002/jpln.200700047.

Wiesmeier, M., Mayer, S., Burmeister, J., Hübner, R., Kögel-Knabner, I., 2020. Feasibility of the 4 per 1000 initiative in Bavaria: a reality check of agricultural soil management and carbon sequestration scenarios. Geoderma. https://doi.org/10.1016/j.geoderma.2020.114333.

Wijanarko, A., Rahmianna, A.A., 2019. Labile fraction changes of soil organic matter along the gradients of altitude in drylands with dry climate. J. Degrad. Min. Lands Manag. https://doi.org/10.15243/jdmlm.2019.063.1755.

Yamashita, T., Flessa, H., John, B., Helfrich, M., Ludwig, B., 2006. Organic matter in density fractions of water-stable aggregates in silty soils: effect of land use. Soil Biol. Biochem. https://doi.org/10.1016/j.soilbio.2006.04.013.

Yoo, G., Wander, M.M., 2008. Tillage effects on aggregate turnover and sequestration of particulate and humified soil organic carbon. Soil Sci. Soc. Am. J. https://doi.org/10.2136/sssaj2007.0110.

Zhao, M., Zhou, J., Kalbitz, K., 2008. Carbon mineralization and properties of water-extractable organic carbon in soils of the south Loess Plateau in China. Eur. J. Soil Biol. https://doi.org/10.1016/j.ejsobi.2007.09.007.

Zotarelli, L., Alves, B.J.R., Urquiaga, S., Boddey, R.M., Six, J., 2007. Impact of tillage and crop rotation on light fraction and intra-aggregate soil organic matter in two Oxisols. Soil Tillage Res. https://doi.org/10.1016/j.still.2007.01.002.

Environmental Engineering and Management Journal April 2018, Vol.17, No. 4, 885-895
http://www.eemj.icpm.tuiasi.ro/; http://www.eemj.eu

"Gheorghe Asachi" Technical University of Iasi, Romania

INFLUENCE OF HUSK ON GRAIN CONTAMINATION BY *Fusarium* spp. AND *Alternaria* spp. IN HULLED SPELT (*Triticum spelta L.*)

Karel Suchý[1], Petr Konvalina[1*], Ivana Capouchová[2], Dagmar Janovská[3], Leona Leišová-Svobodová[3], Zdeněk Štěrba[1], Jan Moudrý jr.[1], Daniel Bucur[4], Jaroslav Bernas[1], Marek Kopecký[1], Dang Khoa Tran[1]

[1]"University of South Bohemia in Ceske Budejovice, Faculty of Agriculture, Studentska 1668,
370 05 Ceske Budejovice, Czech Republic
[2]Czech University of Life Sciences in Prague, Kamýcká 129, Prague, Czech Republic
[3]Crop Research Institute in Prague, Drnovska 507/73. 161 06 Praha 6 – Ruzyne, Czech Republic
[4]The University of Agricultural Sciences and Veterinary Medicine Iaşi, Faculty of Agriculture,
Aleea Mihail Sadoveanu no.3, 700490Iasi, Romania

Abstract

Fusarium Head Blight is caused by several *Fusarium* species. Infections can result in mycotoxin contamination on cereals and associated foods. Harvested products are contaminated due to its secondary metabolites. The aim was to analyse the occurrence of spike *Fusarium* and *Alternaria* spp. in hulled *Triticum spelta* L. wheat species via polymerase chain reaction (PCR) method and the deoxynivalenol (DON) content analysis. Three varieties of spelt were used (Ceralio and Rubiota – winter and one spring form variety from genetics resources). Grains were sown in a randomized complete block design on organic certified experimental parcels during the years of 2011 and 2013. During the vegetation period plants were artificially inoculated with *Fusarium* spp. The occurrence of spike *Fusarium* and *Alternaria* spp. was assessed by the PCR method - DNA extracting and determination of *Fusarium* species and *Alternaria* spp. by the DNA markers and PCR method. DON content was analysed by ROSA®-DON Quantitative test. Strong infestation of grains with *Fusarium* spp. led to low contamination of grains with *Alternaria* spp. The technological operation of grain dehulling was performed and it was highly efficient there. The grain contamination by *Fusarium* spp. and *Alternaria* spp. decreased. Hulls protect grains to a certain point because of protection against *Fusarium* spp. and *Alternaria* spp. occurrence which produce harmful secondary metabolites. On the other hand the protection of grain by hulls only partly works. It is also important to pay attention to chemism of secondary metabolites in grain.

Key words: Alternaria spp., contamination, *Fusarium* spp., husk, wheat

Received: May, 2017; Revised final: February, 2018; Accepted: March, 2018; Published in final edited form: April 2018

1. Introduction

Microorganisms commonly found in grain include filamentous fungi, primarily those from the genera *Aspergillus*, *Fusarium* and *Penicillium* (Ostrowska-Kołodziejczak et al., 2016). They are responsible for the accumulation of mycotoxins in grain (Zain, 2011). Currently, over 31,000 known metabolites produced by fungi occur in cereals. Some of these chemicals called mycotoxins are harmful to both humans and animals (AntiBase, 2005; Kuzdralinski et al., 2013). Mycotoxins produced by those pathogens lead to several health problems in humans and animals, and high mycotoxin concentrations in grain cause mycotoxicoses (Richard, 2007). Currently, there have been more than 400

---

[*] Author to whom all correspondence should be addressed: e-mail: konvalina@zf.jcu.cz; Phone: +420 387 772 547

known mycotoxins (Kuzdralinski et al., 2013). The most studied mycotoxins are aflatoxins, trichothecenes, zearalenone, and ochratoxins (Binder et al., 2007; Filtenborg et al., 2000). Biological and chemical properties of mycotoxins vary, as well as their toxicity (Salem and Ahmad, 2010). Many of these compounds have oestrogenic, teratogenic, mutagenic and carcinogenic effects (Binder et al., 2007; D'Mello et al., 1999; Prelusky et al., 1993). Mycotoxins can enter the human body in different ways, mainly through the consumption of contaminated food (Berthiller et al., 2005; Salem and Ahmad, 2010).

Due to the great economic significance of wheat, particular attention should be paid to its health management as well as to the threat posed by fungal pathogens, including species of the genus *Fusarium* causing Fusarium head blight (Ostrowska-Kołodziejczak et al., 2016). *Fusarium* head blight is one of the most severe diseases of small grain cereals. Four *Fusarium* species, *F. graminearum* Schwabe, *F. culmorum* (W.G. Smith) Sacc., *F. avenaceum* (Fr.) Sacc and *F. poae* (Peck) Wollenw, have been identified as important toxigenic pathogens that affect the heads of small grain cereals (Miedaner et al., 2008; Pasquali and Migheli, 2014).

Cereals produced in temperate zone climatic conditions can be frequently contaminated with mycotoxins (Zachariasova et al., 2014). Due to the fact that in the Central Europe climatic zone the most toxigenic fungi are *Fusarium culmorum* and *F. graminearum,* the presence of these fungi and also of chemotypes 3-acetyl-deoxynivalenol (3-AcDON) and 15-acetyl-deoxynivalenol (15-AcDON) in grain is increasingly often analysed (Ostrowska-Kołodziejczak et al., 2016). In particular *F. culmorum* has been a prevalent species in several countries in Europe and the species has been predominating especially in regions that have cooler climatic conditions while *F. graminearum* has been predominant in relatively warmer regions. However, generalisation about geographic region distribution of *Fusarium* spp. has been interrupted by several investigations in Europe (Yli-Mattila et al., 2013; Yörük et al., 2016). Formally in Central and Central-Eastern Europe, Fusarium head blight was caused primarily by *Fusarium culmorum* (W.G.Smith) Sacc. (Parry et al., 1995). Nowadays *Fusarium graminearum* predominates (Yli-Mattila et al., 2013) which attacks wheat spikes in the flowering stage. As a result, the grain may be shrivelled and discoloured, with a high toxin content, especially trichothecenes of group B (Chelkowski, 1989; Perkowski et al., 2002).

Organic farming is an alternative to the conventional cultivation system providing farm products of high quality, referred to as organic (Jelínková et al., 2016; Maeder et al., 2002). In organic farming, fungicides are not used as prevention against fungal diseases (Janovská et al., 2015). The FAO report (FAO, 2000) concerning the content of mycotoxins in agricultural crops showed no clear differences between organic and conventional farming systems. It was stated however that in certain circumstances, such differences might occur. Most studies published after the FAO report (FAO, 2000) also failed to identify significant differences between organic and conventional cropping systems (Champeil et al., 2004; Cirillo et al., 2003; Jestoi et al., 2004). In some cases, the differences of mycotoxin levels between organic and conventional production systems, nevertheless, were reported (Knudsen et al., 1995; Kuzdralinski et al., 2013; Skaug, 1999; Woese et al., 1997).

The growing of resistant varieties is the best way to reduce *Fusarium* infection (Scholten et al., 2007). Bread wheat (*Triticum aestivum* L.) is the most frequent cereal species grown within the Czech organic farming system. Because in the Czech Republic are only available varieties bred in conventional breeding programmes, organic farmers use different species and crops (Konvalina et al., 2014). The information about the reaction of spikes of ancient wheat species to *Fusarium* spp. infection and toxin accumulation in grain is very important for growing systems limiting chemical plant protection. Hulled spelt wheat (*Triticum spelta* L.) is one from less bred among the *Triticeae* grown by farmers (Suchowilska et al., 2010). Information on the response of spelt cultivars to the infection by pathogens causing FHB is scant. Previous results (Wiwart et al., 2004) show that the response of this cereal to spike infection is slightly stronger than that of common wheat. Probably it is caused because of coverage of grain by hulls. Hulls can create more favourable conditions for *Fusarium* growth. But husks are removed from the grain before use and also an important part of contamination could be solved (Konvalina et al., 2011). There are many conflicting claims related to the role of husk as a protective factor against the secondary metabolites of *Fusarium* contamination. The example of *Alternaria* spp. contamination indicated significantly higher concentrations of Alternariatoxins in hulls than in dehulled kernels which implicate the possible protective effect of spelt wheat hulls (Vuckovic et al., 2013). Therefore, the contamination of the grain product, hulled wheat, by *Fusarium* and *Alternaria* toxins is an important question because of the safety of food and feed.

This study aimed to analyse the occurence of *Fusarium* and *Alternaria* species in organic and conventionally produced hulled and dehulled grain of spelt wheat by DNA markers and PCR (polymerase chain reaction) method and deoxynivalenol (DON) content analysis by ROSA®-DON Quantitative test. The second aim was to analyse the role of the hull factor as potential protection against *Fusarium* spp. and *Alternaria* spp. contamination.

2. Material and methods

2.1. Plant material and field experiments

There were used two varieties of winter spelt (Rubiota and Ceralio) and one variety (spring form) from the collection of genetic resources. The organic field trials were carried out from 2011 to 2013 as randomized complete block designs with three replications. The seeding rate was adjusted to a density of 350 germinating seeds per 1 m^2. Plot size was 12 m^2. Crop stands were treated in compliance with European Council regulations EC No. 834/2007 and EC No. 889/2008. The weather conditions of the years 2011 and 2012 were favourable for the *Fusarium* spp. development due to high temperatures and precipitation in comparison to the long term mean. The year 2013 was deficient in precipitation. All materials were harvested at maturation stage from small-scale field plots at the Experimental and Research Station of the Department of Crop Production, Czech University of Life Sciences, Prague and at the experimental areas of the University of South Bohemia in České Budějovice.

2.2. Artificial inoculation

The isolates of *F.* c*ulmorum* and *F. graminearum* used for the artificial inoculation were obtained from the mycological collection of the Crop Research Institute in Prague and cultivated on sterile wheat grains. More detailed information on isolates can be found in Leišová et al. (2006). The preparation of inoculums for the application: wheat grains with the cultures of *F. culmorum* and *F. graminearum* were put into a vessel with water and shaken for 15 minutes in a laboratory shaker to release the spores into the water. The obtained suspension was filtered through the gauze. Then artificial inoculation was done with the suspension of *F. culmorum* and *F. graminearum* spores in the ratio of 1:1, 10^7 of spores/mL (Bürkerchamber was used for the verification of inoculum density), 2 litres of suspension per experimental plot (12 m^2). The suspension was dosed according to the list of variants with a hand sprayer at the beginning and at the end of the wheat flowering.

During experiments we also inoculated spikes by inoculum contained *Alternaria* spp. The ability of *Alternaria* spp. isolates to produce mycotoxine was proved before. Inoculation was made during milky rippeness, but was not sucessful. There were no differences between inoculated plots and plots only after natural infection.

2.3. Processing of samples

For the dehulling of hulled grain, samples were used Wintersteiger LG 180 (Wintersteiger, Ried, Austria) laboratory thresher. For the analysis were milled both – grain covered by hulls and dehulled (naked) grain. For the milling was used laboratory mill PSY MP 40 (holes in the sieve 0.8 mm).

2.4. Evaluation of spike Fusarium occurence by the PCR method

DNA both from a mycelium of all tested fungi and from infected grain samples was extracted using DNeasy Plant Mini Kit (QIAGEN, Germany) according to the manufacturer instructions. The quality and the concentration of extracted DNA were verified electrophoretically in 0.8% agarose gel. DNA was visualized by ethidium-bromide and detected under a UV lamp. DNA extracted from infected seed samples was diluted to a concentration of 50 ng/µL using a GeneQuantPro spectrophotometer (Amersham, Cambridge, UK).

2.5. Species specific amplification

The markers specific to the species: *F. culmorum* (Fc), *F. graminearum* (Fg), *F. pseudograminearum* (Fpse), *F. poae* (Fp), *F. sporotrichioides* (Fsp), *F. equiseti* (Fe) and *F. avenaceum* (Fa) were borrowed from the literature (Aoki and O´Donnell, 1999; Demeke et al., 2005; Doohan et al., 1998; Leišová et al., 2006; Parry and Nicholson, 1996). Primers were designed based on the sequence of the ITS region of about 5 isolates of *Alternaria* spp. The sequence of primers is in Table 1.

PCR reactions were performed in a 15 µL reaction mixture (0.3 µM of each primer, 170 µM dNTP, 1x PCR buffer, 2 mM $MgCl_2$, 1U *Tth* DNA polymerase Biotools (DYNEX) and 50 ng of template DNA) in the cycler SensoQuest (Goettingen, Germany). The amplification products were separated in 1.6% agarose gel, stained with ethidium bromide and visualised under UV light. The size of the product was verified by comparing it with the size standard GeneRulerTM 100bp DNA Ladder (Thermo Fisher Scientific, USA).

Fusarium culmorum assay was used from a previous project (Leišová et al., 2006). *Fusarium graminearum* and *Alternaria* spp. specific primers for Real-time PCR were designed on the base of the sequences for elongation factor obtained from public databases using the Primer Express for Windows NT 1.5 software (Applied Biosystems, Foster City, CA, USA).

Table 1. The primer list

Name	***Forward***	***Reverse***
Fc92s1	TTCACTAGATCGTCCGGCAG	GAGCCCTCCAAGCGAGAAG
Fg	TTCCCTGGGCGCTCATC	GGCTTCCTATTGACAGGTGGTT
Fpse	CGGGGTAGTTTCACATTTCYG	GAGAATGTGATGAGGACAATA
Fp	CAAGCAAACAGGCTCTTCACC	TGTTCCACCTCAGTGACAGGTT
Fsp	AAAAGCCCAAATTGCTGATG	TGGCATGTTCATTGTCACCT
Fe	CATACCTATACGTTGCCTCG	TTACCAGTAACGAGGTGTATG
Fa	CAAGCATTGTCGCCACTCTC	GTTTGGCTCTACCGGGACTG
Asp	TGGTGTTGGGCGTCTTGTC	TAGGCCGGCTGCCAATTAC

In case of *F. graminearum* the assay contained also a MGB (minor groove binder) probe; in *Alternaria* spp. SYBR Green detection system was used because all *Alternaria* species should have been detected in one reaction without the necessity of species determination. The specificity of all primers and probes was verified in silico by blast analysis. After optimization, PCR reactions were carried out in a 25 µL volume consisting of either 1x TaqMan Universal PCR Master Mix (Life Technologies, Foster City, CA, USA; in case MGB probe was used) or SYBR Green master mix (Life Technologies, Foster City, CA, USA; for *Alternaria* spp. detection), 0.3 µM of each primer, 0.3 µM Taq Man MGB probe and 250 ng of template DNA. Real-time quantitative PCR was performed using the cycler ABI PRISM 7900 (Life Technologies, Foster City, CA, USA) in MicroAmp optical 96-well plates. Reaction consisted of 2 min at 50°C, 10 min at 95°C and 40 cycles of 95°C for 15 s and 60°C for 1 min. followed only in SYBR Green detection system by dissociation stage (95°C for 15 s, 60°C for 15 s and then a slow increase to 95°C). The Sequence Detection Software (Life Technologies, Foster City, USA) collected data for the reported dye every 7 seconds from each well, generating a fluorescence profile for each amplification. The threshold cycle (Ct) was recorded for each dye as the cycle at which the fluorescent signal, associated with an exponential growth of PCR product, exceeded the background fluorescence. Dilution series of *F. culmorum*, *F. graminearum* and *A. alternata* isolates - DNA (from 0.1 pg to 100 ng) was included in triplicate as standard in every real-time PCR experiment. Standard curves for all assayed fungi were generated by plotting the known DNA amounts against the Ct values calculated by the SDS software. Unknown samples were quantified from measured Ct values by interpolation using the regression equation derived from standard curves. Final results of fungal content in samples were expressed in micrograms per 100 mg of groats.

2.6. Deoxynivalenol (DON) content

At first, toxin was extracted from the sample (deionized water was used as a solvent). 100 µL of the extract was diluted in 1 mL of buffer. 300 µL of the diluted extract was applied on the strip (ROSA®-DON Quantitative test). Incubation of the strip - 10 minutes at the temperature of 45°C (ROSA®-M Incubator). Assessment of the test – by ROSA®-M Reader (results in ppb).

2.7. Statistical analysis

In case of evaluation of the presence of DNA of pathogen – based on quantification of the intensity of the luminous band in relation to the marker for the statistical analysis, we used the following scale: 0 = no infection, 1 = weak infection, 2 = medium infection, 3 = strong infection.

The results were statistically evaluated by the Mann-Whitney Test (by variable organic system vs. conventional system, winter variety vs. spring variety, *Alternaria*+natural infection vs. *Fusarium* spp. inoculation, dehulled grain vs. hulled spikelets) and Kruskal-Wallis ANOVA evaluation of factor of variety. The selected factors are displayed in figures with the statistical significance expression on the level $p \leq 0.05$. The calculation was done by the software STATISTICA 12.0 CZ (StatSoft, Inc. USA).

3. Results and discussion

Degree of grain contamination (qualitative) with various *Fusarium* spp. (F.) and relationships between the contamination degree and other evaluated factors were evaluated at first (Tables 2 and 3). Farming system (organic or conventional one) is a statistically non-significant factor there. *Fusarium avenaceae*, *culmorum*, *equiseti* and *poae* infected the grains but they were statistically non-significant in our research. *Alternaria* spp. also infected the grains (Table 3) grown under both farming systems. The difference in DNA of *F. culmorum*, *F. graminearum* and *Alternaria* spp. was also statistically non-significant. Results of the Mann-Whitney test are shown in Table 4.

The difference in the degree of grain contamination with *Fusarium* spp. and *Alternaria* spp. toxins between the organic and conventional farming systems has already been noticed and discussed by many authors. Numerous surveys comparing mycotoxin content in organic and conventional production systems have already been conducted. The mycotoxin contamination of organic food products was reported to be either more or less equal to that of conventional systems (Magkos et al., 2006). Birzele et al. (2000) showed that contamination of conventional samples of winter wheat with DON and ochratoxin A (OTA) was comparable to that of the organic samples. In another study in Germany, comparable results for DON were obtained (Lücke et al., 2003). A study conducted in France revealed that organic cereals were contaminated with a higher level of mycotoxins but less frequently than cereals from conventional production (Malmauret et al., 2002). However, findings are not unequivocal in general. Works giving evidence of organic plants being as infected with Fusarium Head Blight as the conventional ones prevail.

The factor of variety has already been studied and evaluated with various methods. Table 6 (results of Kruskal-Wallis ANOVA) shows the factor of variety did not have any impact on the grain contamination with toxins in our research. Results were statistically non-significant. There was a minimum difference in the degree of grain contamination with toxins between winter and spring varieties (Tables 2 and 3). The same result was shown in the analysis of DON content in grain (Table 5).

Table 2. Grain contamination by different *Fusarium* species related to different factors

Factor		***Fusarium***			
		avenaceae	***culmorum***	***equiseti***	***graminearum***
System	Organic	1.9 ± 0.9	1.7 ± 1.0	0.7 ± 1.0	1.2 ± 1.2
	Conventional	2.4 ± 0.7	1.8 ± 1.0	0.8 ± 1.1	1.2 ± 1.2
Type of variety	Spring	1.5 ± 0.8	1.3 ± 1.1	0.3 ± 0.7	0.8 ± 0.9
	Winter	2.4 ± 0.7	2.0 ± 0.8	1.0 ± 1.1	1.4 ± 1.2
Year	2011	2.3 ± 0.5	1.8 ± 0.7	0.8 ± 1.0	1.3 ± 1.4
	2012	2.5 ± 0.8	1.9 ± 1.1	0.8 ± 1.1	1.5 ± 1.1
	2013	1.7 ± 1.0	1.7 ± 1.1	0.8 ± 1.1	0.9 ± 0.9
Processing	Dehulled	1.9 ± 0.8	1.7 ± 1.0	0.4 ± 0.8	1.1 ± 1.2
	Hulled	2.7 ± 0.6	2.0 ± 0.8	1.6 ± 1.1	1.4 ± 1.3
Inoculation	Natural	2.0 ± 0.9	1.2 ± 0.8	0.4 ± 0.8	0.5 ± 0.7
	Fusarium spp.	2.0 ± 0.8	2.5 ± 0.8	1.0 ± 1.2	2.1 ± 1.0
	Alternaria spp.	2.5 ± 0.7	1.5 ± 0.5	1.0 ± 1.1	0.9 ± 1.1

Note: 0 = no infection, 1 = weak infection, 2 = medium infection, 3 = strong infection

Table 3. Grain contamination by different *Fusarium* species and *Alternaria* spp. related to different factors

Factor		***Fusarium***			***Alternaria spp.***
		poae	***pseudo-graminearum***	***sporotrichioides***	
System	Organic	1.9±0.9	0.0±0.0	1.1±1.1	2.4±0.5
	Conventional	2.1±1.0	0.0±0.0	0.9±0.9	2.4±0.5
Type of variety	Spring	2.3±0.9	0.0±0.0	0.9±0.9	2.3±0.5
	Winter	1.9±0.9	0.0±0.0	1.0±1.1	2.4±0.5
Year	2011	2.3±0.6	0.0±0.0	1.4±1.0	2.0±0.2
	2012	2.0±1.2	0.0±0.0	0.5±0.8	2.0±0.0
	2013	1.7±1.0	0.0±0.0	0.8±1.0	3.0±0.0
Processing	Dehulled	1.8±1.0	0.0±0.0	0.6±0.8	2.3±0.5
	Hulled	2.5±0.7	0.0±0.0	1.8±1.0	2.5±0.6
Inoculation	Natural	1.9±1.0	0.0±0.0	0.6±0.8	2.3±0.5
	Fusarium spp.	2.1±0.9	0.0±0.0	1.2±1.1	2.4±0.5
	Alternaria spp.	2.0±1.0	0.0±0.0	1.1±1.1	2.4±0.5

Note: 0=no infection, 1=weak infection, 2 = medium infection, 3 = strong infection

Table 4. Mann-Whitney Test by variable Organic system vs. Conventional system of growing (concentration of DNA of pathogen in µg/100mg of grain)

Variable	***Rank sum***	***Valid N***	***Rank sum***	***Valid N***	***U***	***Z***	***p-value***	***Z adjus-ted***	***2*1 sided exact p***
	ORGANIC		***CONVENTIONAL***						
F. culmorum	919	30	911	30	446	0.052	0.959	0.052	0.959
F. graminearum	922	30	908	30	443	0.096	0.923	0.096	0.923
Alternaria spp.	906	30	924	30	441	-0.126	0.900	-0.126	0.900

Note: marked tests are significant at p<0.05; U = U value, Z = Z value

Table 5. Concentration of DNA of *F. culmorum*, *graminearum* and *Alternaria* spp. and DON content in grain

Factor		***Fusarium culmorum (µg Fc DNA /100 mg of grain)***	***Fusarium graminearum (µg DNA Fg/100 mg of grain)***	***Alternaria spp. (µg DNA Asp/ 100 mg of grain)***	***DON (ppb)***
System	Organic	5.39±16.68	0.54±1.61	0.04±0.05	2327±2774
	Conventional	3.69±8.66	0.72±2.21	0.04±0.05	2070±2606
Processing	Dehulled	2.07±4.85	0.29±0.87	0.02±0.02	2170±2780
	Hulled	10.72±22.72	1.47±3.23	0.09±0.06	2210±2660
Year	2011	6.72 ±12.09	0.73±1.67	0.06±0.04	2268±2459
	2012	2.05±2.60	0.31 ±0.52	0.01 ±0.01	2514±2924
	2013	3.92±17.59	0.73±2.62	0.03±0.06	1933±2788
Type of variety	Spring	1.47±2.39	0.05±0.09	0.02±0.02	2196±2614
	Winter	5.77±15.46	0.86±2.24	0.05±0.05	2199±2725
Inoculation	Natural	0.036±0.07	0.01±0.02	0.04 ±0.05	2660 ±358
	Fusarium spp.	11.801±19.53	1.63±2.87	0.03±0.05	5337±1613
	Alternaria spp.	0.136±0.23	0.02±0.03	0.05±0.06	2700 ±285

Note: Mean ± Standard deviation

There was practically the same mean but within the samples were differences (high standard deviation). *F. avenaceae*, *culmorum*, *equiseti and graminearum* infected more winter varieties (statistically non-significant occurrence). Winter wheat varieties were more infected by Fusarium spp. than spring wheat ones – it was registered by Etzeriod et al. (2016), for instance. Table 3 shows winter varieties were more seriously infected with *Fusarium culmorum* and *Fusarium graminearum* in our case (they contained more DNA of these two toxins). As there was a high variability between tested samples, it was a statistically non-significant result and finding (Mann-Whitney Test – Table 7).

All spikes were seriously infected with Fusarium Head Blight during every performed inoculation in our research. On the other hand, the tested inoculation with *Alternaria* spp. was not successful under field conditions. The inoculation with *Alternaria* spp. was also tested on the other crops (cumin or colza) (Khan et al., 2012; Özer and Bayraktar, 2015). It was tested on wheat with success – under laboratory conditions (Vergnes et al., 2006). Therefore, it was tested statistically with the natural infection together. All these results are shown in Table 8. A statistically significant difference between *F. culmorum* and *F. graminearum* DNA infestation rate was confirmed via Mann-Whitney test.

Testing and evaluation of the degree of grain contamination with pathogen produced the following results – a middle strong infestation with *F. graminearum* and a strong infestation with *F. culmorum* (Table 1). The artificial inoculation had a minimum impact on the degree of grain contamination with *Alternaria* spp. DNA. It caused neither higher contamination of grains with that pathogen (Table 3), nor a higher concentration of *Alternaria* spp. DNA in grains (Table 4). It brought about an inverse effect (Table 4). Compared to the natural conditions (0.039 μg DNA Asp/ 100mg of grain), the inoculation of grains with *Fusarium* spp. produced a slight decrease of *Alternaria* spp. DNA level in grains (0.033 μg DNA Asp/ 100 mg of grain). Such a decrease of the grain contamination with *Alternaria* spp. was provoked by a competitive interaction of *F. graminearum*. They occurred on wheat plants in the field, and therefore competed for the same resources. In the experiment done by Saß et al. (2007), *Alternaria alternata* was clearly suppressed when growing together with *F. graminearum*.

Table 6. Kruskal-Wallis ANOVA – evaluation of factor of variety of spelt (*Triticum spelta* L.)

***Fusarium culmorum* (μg DNA Fc/100mg of grain)**			
Variety	*N*	*Sum of scores*	*Mean score*
Ceralio	18	633.0000	35.16667
Spring spelt	24	648.0000	27.00000
Rubiota	18	549.0000	30.50000
H (2, N = 60) =2.249430 p=0.3247			
***Fusarium graminearum* (μg DNA Fg/100mg of grain)**			
Variety	*N*	*Sum of scores*	*Mean score*
Ceralio	18	661.0000	36.72222
Spring spelt	24	602.0000	25.08333
Rubiota	18	567.0000	31.50000
H (2, N= 60) =4.652641 p =0.0977			
***Alternaria* spp. (μg DNA Asp/ 100mg of grain)**			
Variety	*N*	*Sum of scores*	*Mean score*
Ceralio	18	605.0000	33.61111
Spring spelt	24	614.0000	25.58333
Rubiota	18	611.0000	33.94444
H (2, N= 60) =3.173588 p =0.2046			

Table 7. Mann-Whitney Test by variable Winter variety vs. Spring variety (concentration of DNA of pathogen in μg/100mg of grain)

Variable	***Rank sum***	***Valid N***	***Rank sum***	***Valid N***	***U***	***Z***	***p-value***	***Z adjusted***	***2*1 sided exact p***
	WINTER		***SPRING***						
F. culmorum	1182	36	648	24	348	1.260	0.208	1.260	0.208
F. graminearum	1228	36	602	24	302	1.954	0.050	1.954	0.051
Alternaria spp.	1216	36	614	24	314	1.773	0.076	1.773	0.076

Note: marked tests are significant at p<0.050; U = U value, Z = Z value

Table 8. Mann-Whitney Test by variable *Alternaria* natural infection vs. *Fusarium* spp. inoculation (concentration of DNA of pathogen in µg/100mg of grain)

Variable	***Rank sum***	***Valid N***	***Rank sum***	***Valid N***	***U***	***Z***	***p-value***	***Z adjusted***	***2*1 sided exact p***
	NATURAL		**INOCULATION**						
F. culmorum	792	36	1038	24	126	-4.610	0.001	-4.610	0.000
F. graminearum	864	36	966	24	198	-3.523	0.001	-3.523	0.001
Alternaria spp.	1192	36	638	24	338	1.411	0.158	1.411	0.159

Note: marked tests are significant at p<0.05000; U = U value, Z = Z value

Table 9. Mann-Whitney Test by variable DEHULLED/HULLED (concentration of of DNA of pathogen in µg/100mg of grain)

Variable	***Rank sum***	***Valid N***	***Rank sum***	***Valid N***	***U***	***Z***	***p-value***	***Z adjus-ted***	***2*1 sided exact p***
	DEHULLED		***HULLED***						
F. culmorum	565	24	611	24	265	-0.464	0.643	-0.464	0.646
F. graminearum	552	24	624	24	252	-0.732	0.464	-0.732	0.468
Alternaria spp.	423	24	753	24	123	-3.392	0.001	-3.391	0.001

Note: marked tests are significant at p<0.05000; U = U value, Z = Z value

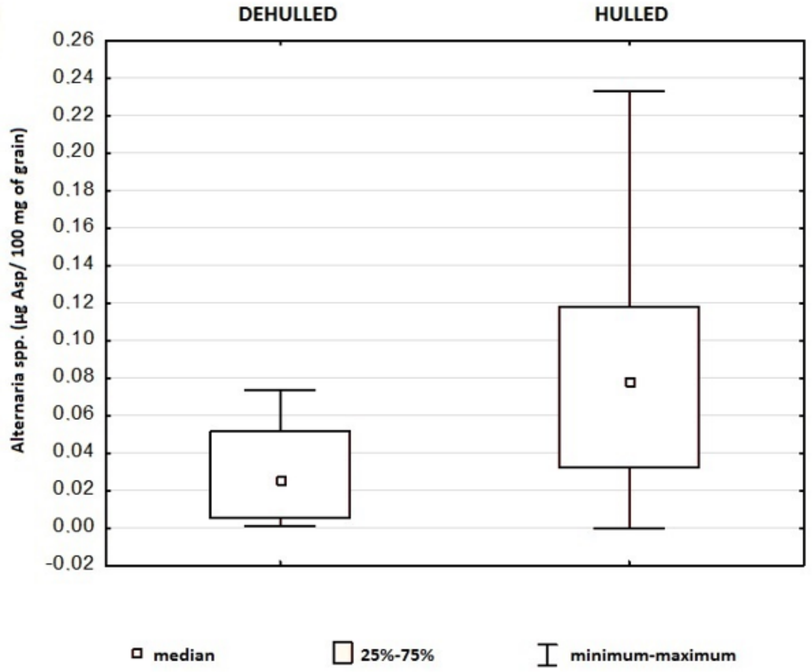


Fig. 1. Differences of grain contamination by the DNA of *Alternaria* spp. before and after dehulling (all the samples)

The factor of dehulling of the grain was studied and analysed in depth (when hulls were removed from spelt wheat spikes). It had the strongest and statistically significant effect (Mann-Whitney test – Table 9) and caused the contamination of grain with *Alternaria* spp. DNA to decrease (Table 4). Dehulled (peeled) grains were less contaminated with *Alternaria* spp. DNA (reduction from 0.09 µg DNA Asp/ 100mg of grain to 0.02 µg DNA Asp/ 100 mg of grain). Such a considerable reduction in the grain contamination with *Alternaria* spp. is shown in Fig. 1; the degree of contamination even decreased to zero in some tested samples. The dehulling of grain had a positive effect on individual years and inoculation options as well (Fig. 2). Released results of the research showed that hulls protected spelt kernels inside of *Alternaria* spp. from infection up to 50 %. The above-mentioned findings indicate that hulls efficiently protect spelt kernels from *Alternaria* spp. infestation and their toxicological metabolites (Vuckovic et al., 2013).

The technological operation of grain dehulling had a strong impact on the degree of grain contamination with *Fusarium* spp. DNA; it was, nevertheless, non-significant (Table 9), as there was a wide range and high variability of tested samples (Table 4). There was an evident lower degree of grain contamination with *Fusarium avenaceae*, *equiseti* (Table 1), *poae*, and *sporotrichioides* (Table 3). The degree of grain contamination with *Fusarium culmorum* DNA decreased from 10.72 µg DNA Fc/100 mg of grain to 2.07 µg DNA Fc/100mg of grain (Table 4).

The reduction in contamination with *Fusarium culmorum* DNA is shown in Fig. 3. Our research produced similar results with *Fusarium graminearum*;

the degree of grain contamination with *Fusarium graminearum* decreased from 1.47 µg DNA Fg/100 mg of grain to 0.29 µg DNA Fg/100 mg of grain (Table 5). The degree of grain contamination with *Fusarium graminearum* DNA decreased dramatically in 2013 (Fig. 4). Previous research had indicated that hulls acted efficiently as barriers to *Fusarium* mycotoxins in hulled *Triticum* species (Castoria et al., 2005; Suchowilska et al., 2010; Wiwart et al., 2004). Wiwart et al. (2011) compared *Fusarium* toxins in spelt and common wheat indicated that the concentrations of fungal metabolites were lower in spelt than in common wheat cultivars. There is a conclusion – dehulling can reduce contamination of fungi and toxins on spelt kernels. More surveys need to be conducted, considering the lack of knowledge on *Alternaria* toxins in food and feed in Europe and worldwide.

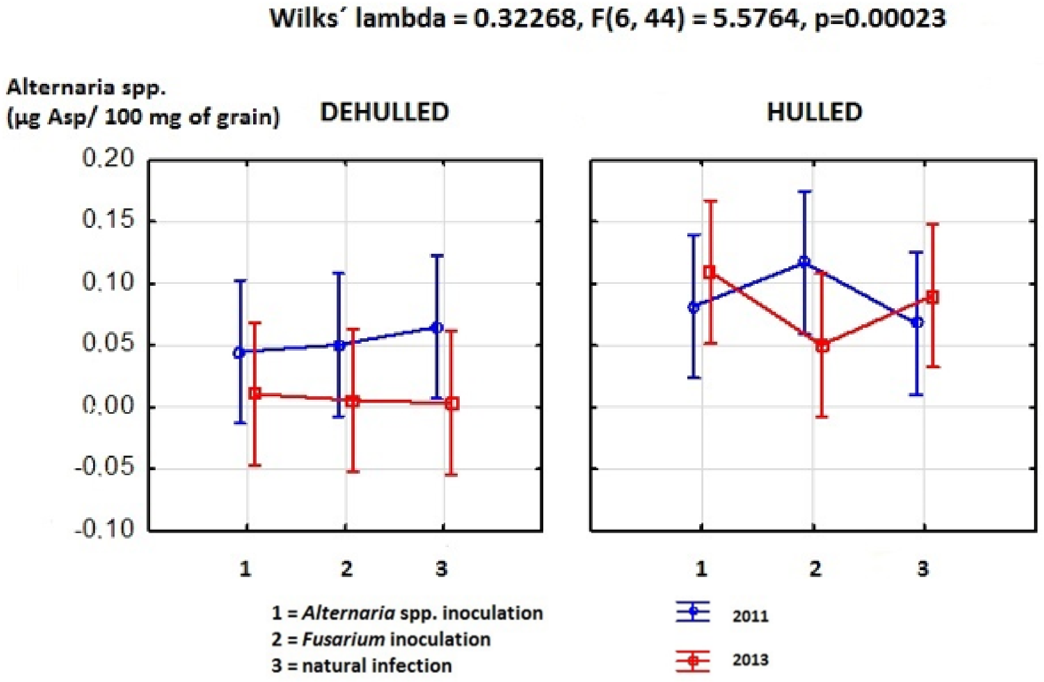


Fig. 2. Differences in grain contamination by DNA of *Alternaria* spp. in different years and grain treatment (results in 2011 were simmilar to 2012)

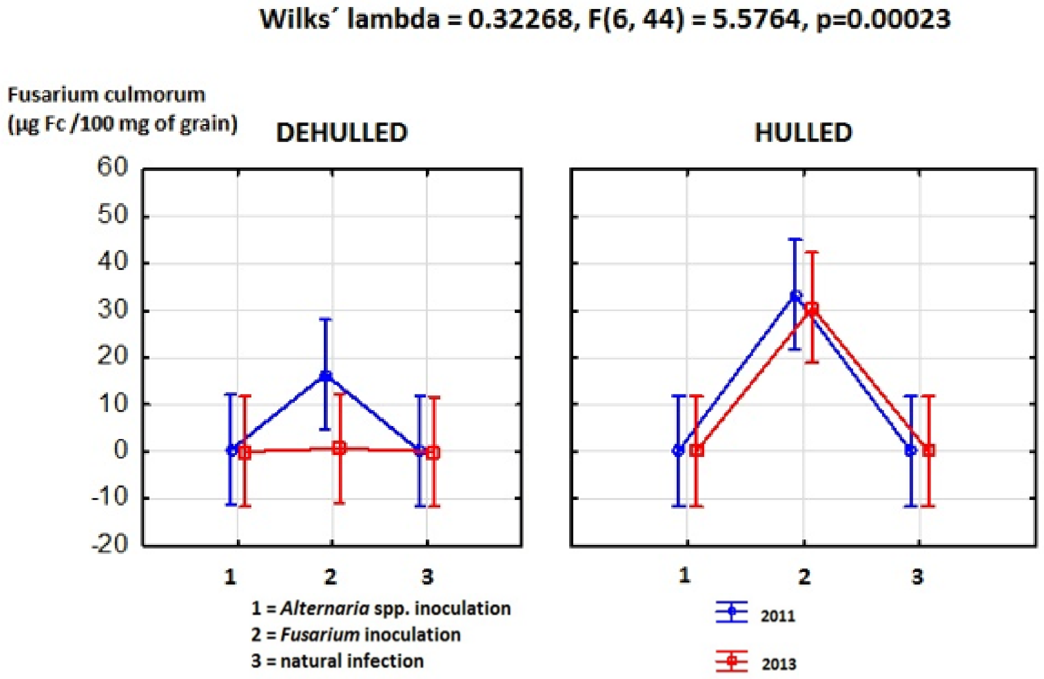


Fig. 3. Differences in grain contamination by DNA of *Fusarium culmorum* in different years and grain treatment

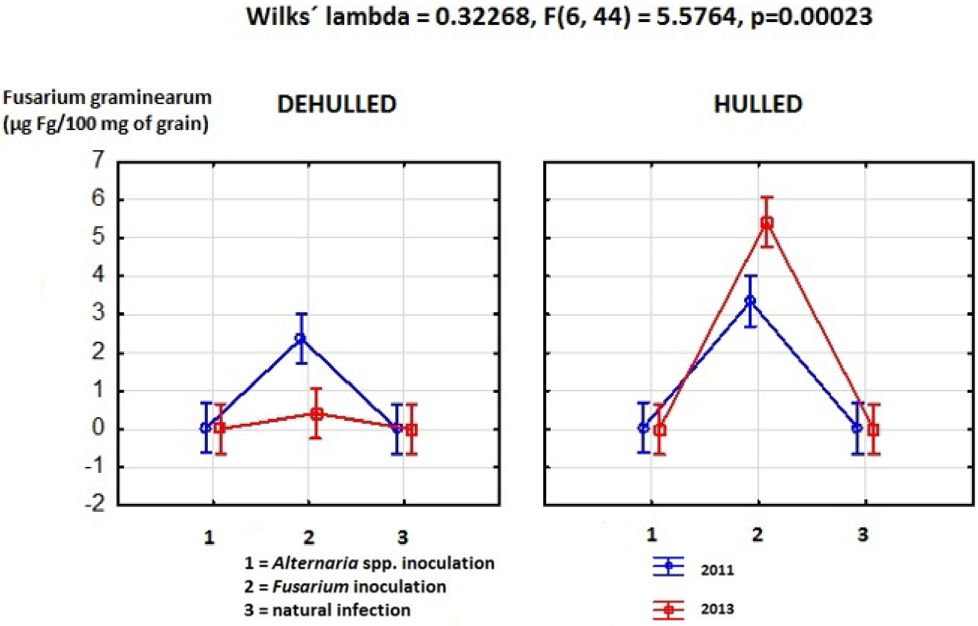


Fig. 4. Differences of grain contamination by DNA of *Fusarium graminearum* in different years and grain treatment

4. Conclusions

The degree of grain contamination with *Fusarium* spp. and *Alternaria* spp. was not influenced by the farming system. There were differences in the degree of grain contamination within individual years. Growing form (winter or spring) did not influence the degree of grain contamination either.

The artificial inoculation of grains with *Fusarium culmorum* and *graminearum* was successful. The natural infestation with *Alternaria* spp. was also studied and evaluated. The artificial inoculation with *Alternaria* spp. was not successful. Strong infestation of grains with *Fusarium* spp. led to low natural contamination of grains with *Alternaria* spp.

The technological operation of grain dehulling was performed and it was highly efficient there – the grain contamination with secondary metabolites and DNA of *Fusarium* spp. and DNA of *Alternaria* spp. decreased. The grain dehulling had a positive effect and reduced the degree of contamination of strongly infested varieties (that were infested artificially – by artificial inoculation). Therefore, it might be dangerous and risky to feed animals with whole spelt wheat spikes.

There are a lot of ambiguities about the biology of *Fusarium* ssp. – how they develop in hulls and grains. Much more experiments with *Fusarium* spp. and *Alternaria* spp. involved have to be carried out in order to make the protecting role of hulls clear – if and how they protect spelt wheat and other hulled wheat species from *Fusarium* spp. and *Alternaria* spp. and possible contamination.

Acknowledgement

This work was supported by the research project No. NAZV QJ1310072 of the National Agency for Agricultural Research of the Ministry of Agriculture of the Czech Republic and the University of South Bohemia in České Budějovice (project No. GAJU 094/2016/Z). I would like to thank Dr. Martin Šlachta for help with statistical data analysis.

References

Aoki T., O'Donnell K., (1999), Morphological and molecular characterization of *Fusarium pseudograminearum* sp. nov., formerly recognized as the Group 1 population of *F. graminearum*, *Mycologia*, **91**, 597-609.

Berthiller F., Dall'Asta C., Schumacher R., Lemmens M., Adam G., Krska R., (2005), Masked mycotoxins: determination of a deoxynivalenol glucoside in artificially and naturally contaminated wheat by liquid chromatography-tandem mass spektrometry, *Journal of Agricultural and Food Chemistry*, **53**, 3421-3425.

Binder E.M., Tan L.M., Chin L., Handl J., Richard J., (2007), Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients, *Animal Feed Science and Technology*, **137**, 265-282.

Birzele B., Prange A., Kramer J., (2000), Deoxynivalenol and ochratoxin A in German wheat and changes of level in relation to storage parameters, *Food Additives and Contaminants*, **17**, 1027-1035.

Castoria X.X., Lima G., Ferracane R., Ritieni A., (2005), Occurence of mycotoxin in farro samples from south Italy, *Journal of Food Protection*, **68**, 416-420.

Champeil A., Fourbet J.F., Dore T., Rossignol L., (2004), Influence of cropping system on *Fusarium* head blight and mycotoxin levels in winter wheat, *Crop Protection*, **23**, 531-537.

Chelkowski J., (1989), *Formation of Mycotoxins Produced by Fusarium in Heads of Wheat, Triticale and Rye*, In: *Fusarium - Mycotoxins, Taxonomy and Pathogenicity*, Chelkowski J. (Ed.), Elsevier, Amsterdam, 63-84.

Cirillo T., Ritieni A., Visone M., Cocchieri R.A., (2003), Evaluation of conventional and organic Italian foodstuffs for deoxynivalenol and fumonisins B-1 and

B-2, *Journal of Agricultural and Food Chemistry*, **51**, 8128-8131.

Demeke T., Clear R.M., Patrick S.K., Gaba D., (2005), Species-specific PCR-based assays for the detection of *Fusarium* species and comparison with whole seed agar plate method and trichothecene analysis, *International Journal of Food Microbiology*, **103**, 271-284.

D'Mello J.P.F., Placinta C.M., Macdonald A.M.C., (1999), *Fusarium* mycotoxins: a review of global implications for animal health, welfare and productivity, *Animal Feed Science and Technology*, **80**, 183-205.

Doohan F.M., Parry D.W., Jenkinson P., Nicholson P., (1998), The use of species-specific PCR based assays to analyse *Fusarium* ear blight of wheat, *Journal of Plant Pathology*, **47**, 197-205.

Janovská D., Leišová-Svobodová L., Capouchová I., Konvalina P., Káš M., (2015), Natural *Fusarium* occurrence qualitative evaluation on cereals under conventional and organic system in the Czech Republic, *Acta Fytotechnica et Zootechnica*, **18**, 19-21.

Etzerodt T., Gislum R., Laursen B.B., Heinrichson K., Gregersen P.L., Jørgensen L.N., Fomsgaard I.S., (2016), Correlation of deoxynivalenol accumulation in *Fusarium* - infected winter and spring wheat cultivars with secondary metabolites at different growth stages, *Journal of Agricultural and Food Chemistry*, **64**, 4545-4555.

FAO, (2000), Food safety and quality as affected by organic farming. FAO Regional Conference for Europe, Porto, On line at: http://www.fao.org/docrep/meeting/X4983e.htm.

Filtenborg O., Frisvad J.C., Samson R.A., (2000), *Specific Associations of Fungi to Foods and Influence of Physical Environmental Factors*, In: *Introduction to Food and Airborne Fungi*, Samson R.A., Hoekstra E.S., Frisvad J.C., Filtenborg O. (Eds.), Centraal Bureau voor Schimmelcultures, Utrecht, 306-320.

Jelínková Z., Moudrý J.jr., Bernas J., Kopecký M., Moudrý J., Konvalina P., (2016), Environmental and economic aspects of *Triticum aestivum L.* and *Avena sativa* growing, *Open Life Sciences*, **11**, 533-541.

Jestoi M., Somma M.C., Kouva M., Veijalainen P., Rizzo A., Ritieni, A., Peltonen K., (2004), Levels of mycotoxins and sample cytotoxicity of selected organic and conventional grain-based products purchased from Finnish and Italian markets, *Molecular Nutrition and Food Research*, **48**, 299-307.

Knudsen I.M.B., Elmholt S., Hockenhull J., Jensen D.F., (1995), Distribution of saprophytic fungi antagonistic to Fusarium culmorum in two differently cultivated field soils, with special emphasis on the genus *Fusarium*, *Biological Agriculture and Horticulture*, **12**, 61-79.

Konvalina P., Capouchová I., Stehno Z., Moudrý J. jr., Moudrý J., (2011), *Fusarium* identification by PCR and DON content in grain of ancient wheat, *Journal of Food, Agriculture and Environment*, **9**, 321-325.

Konvalina P., Stehno Z., Capouchová I., Zechner E., Berger S., Grausgruber H., Janovská D., Moudry J., (2014), Differences in grain/straw ratio, protein content and yieldin landraces and modern varieties of different wheat species under organic farming, *Euphytica*, **199**, 31-40.

Kuzdralinski A., Solarska E., Mazurkiewicz J., (2013), Mycotoxin content of organic and conventional oats from southeastern Poland, *Food Control*, **33**, 68-72.

Leišová L., Kučera L., Chrpová J., Sýkorová S., Šíp V., Ovesná J., (2006), Quantification of Fusarium culmorum in wheat and barley tissues using real-time PCR in comparison with DON content, *Journal of Phytopathology*, **154**, 603-611.

Lücke W., Steinbach P., Herten K., (2003), Phytosanitary observation in the organic farming of Mecklenburg-Vorpommern (in German), *Gesunde Pflanzen*, **55**, 117-120.

Maeder P., Fliessbach A., Dubois D., Gunst L., Fried P., Niggli U., (2002), Soil fertility and biodiversity in organic farming, *Science*, **296**, 1694-1697.

Magkos F., Arvantit F., Zampelas A., (2006), Organic food: buying more safety or just peace of mind? A critical review of the literature, *Critical Reviews in Food Science and Nutrition*, **46**, 23-56.

Malmauret L., Parent-Massin D., Hardy J.L., Verger P., (2002), Contaminants in organic and conventional foodstuffs in France, *Food Additives and Contaminants*, **19**, 524-532.

Miedaner T., Cumagun C.J.R., Chakraborty S., (2008), Population genetics of three important head blight pathogens *Fusarium graminearum*, F. *pseudograminearum* and F. *culmorum*, *Journal of Phytopathology*, **156**, 129-139.

Khan M.M., Khan M.R., Mohiddin F.A., (2012), The relative performance of different inoculation methods with *Alternaria brassicae* and *A. brassicicola* on Indian Mustard, *Plant Pathology Journal*, **11**, 93-98.

Ostrowska-Kołodziejczak A., Stuper Szablewska K., Kulik T., Buśko M., Rissmann I., Wiwart M., Perkowski J., (2016), Concentration of fungal metabolites, phenolic acids and metals in mixtures of cereals grown in organic and conventional farms, *Journal of Animal and Feed Sciences*, **25**, 74-81.

Özer G., Bayraktar H., (2015), Determination of fungal pathogens associated with *Cuminum cyminum* in Turkey, *Plant Protection Sciences*, **51**, 74-79.

Parry D.W., Jenkinson P., McLeod L., (1995), *Fusarium* ear blight (scab) in small grain cereals - a review, *Plant Pathology*, **44**, 207-238.

Pasanen A.L., Yli Pietilä K., Pasanen P., Kalliokoski P., Tarhanen J., (1999), Ergosterol content in various fungal species and biocontaminated building materials, *Applied Environmental Microbiology*, **65**, 138-142.

Parry D.W., Nicholson P., (1996), Development of PCR assay to detect *Fusarium poae* in wheat, *Plant Pathology*, **45**, 872-883.

Pasquali M., Migheli Q., (2014), Genetic approaches to chemotype determination in type B-trichothecene producing *Fusaria*, *International Journal of Food Microbiology*, **189**, 164-182.

Perkowski J., Pavlova A., Šrobarova A., Stachowiak J., Golinski P., (2002), Group B trichothecenes biosynthesis in wheat cultivars after heads inoculation with *Fusarium culmorum* isolates, *Biologia*, **57**, 765-771.

Prelusky D.B., Rotter B.A., Rotter R.G., (1993), *Toxicology of Mycotoxins*, In: *Mycotoxins in Grain: Compounds other than Aflatoxin*, Miller J.D., Trenholm H.L. (Eds.), Eagan Press, St. Paul, Minnesota, 359-404.

Richard J.L., (2007), Some major mycotoxins and their mycotoxicoses: an overview, *International Journal of Food Microbiology*, **119**, 3-10.

Salem N.M., Ahmad R., (2010), Mycotoxins in food from Jordan: preliminary survey, *Food Control*, **21**, 1099-1103.

Scholten O.E., Steenhuis-Broers G., Timmermans B., Osman A., (2007), *Screening for Resistance to Fusarium Head Blight in Organic Wheat Production*, Proc. COST 860 SUSVAR workshop, Velence, vol. 1, 20-23.

Skaug M.A., (1999), Analysis of Norwegian milk and infant formulas for ochratoxin A, *Food Additives and Contaminants*, **16**, 75-78.

Suchowilska E., Kandler W., Sulyok M., Wiwart M., Krska R., (2010), Mycotoxin profiles in the grain of *Triticum monococcum*, *Triticum dicoccum* and *Triticum spelta* after head infection with *Fusarium culmorum*, *Journal of the Science of Food and Agriculture*, **90**, 556-565.

Saß V., Milles J., Krämer J., Prange A., (2007), Competitive interactions of *Fusarium graminearum* and *Alternaria alternata* in vitro in relation to deoxynivalenol and zearalenone production, *Journal of Food, Agriculture & Environment*, **5**, 257-261.

Vergnes D.M., Renard M.E., Duveiller E., Maraite H., (2006), Identification of *Alternaria* spp. on wheat by pathogenicity assays and sequencing, *Plant Pathology*, **55**, 485-493.

Vuckovic J., Bodroza-Solarov M., Vujic D., Bocarov-Stancic A., Bagi F., (2013), The protective effect of hulls on the occurrence of *Alternaria* mycotoxins in spelt wheat, *Journal of the Science of Food and Agriculture*, **93**, 1996-2001.

Wiwart M., Perkowski J., Budzynski W., Suchowilska E., Busko M., Matysiak A., (2011), Concentrations of ergosterol and trichothecenes in the grains of three *Triticum* species, *Czech Journal of Food Science*, **29**, 430-440.

Wiwart M., Perkowski J., Jackowiak H., Packa D., Borusiewicz A., Buoeko M., (2004), Response of some cultivars of spring spelt (*Triticum spelta*) to *Fusarium culmorum* infection, *Die Bodenkultur*, **55**, 29-36.

Woese K., Lange D., Boess C., Bogl K.W., (1997), A comparison of organically and conventionally grown foods-results of a review of the relevant literature, *Journal of the Science of Food and Agriculture*, **74**, 281-293.

Yli-Mattila T., Rämö S., Hietaniemi V., Hussien T., Carlobos-Lopez A.L., Cumagun C.R.J., (2013), Molecular quantification and genetic diversity of toxigenic *Fusarium* species in northern Europe as compared to those in southern Europe, *Microorganisms*, **1**, 162-174.

Yörük E., Tunali B., Kansu B., Ölmez F., Uz U., Zümrüt I.M., Sarıkaya A., Meyva G., (2016), Characterization of high-level deoxynivalenol producer *Fusarium graminearum* and *F. culmorum* isolates caused head blight and crown rot diseases in Turkey, *Journal of Plant Diseases and Protection*, **123**, 177-186.

Zachariasova M., Dzuman Z., Veprikova Z., Hajkova K., Jiru M., Vaclavikova M., Zachariasova A., Pospichalova M., Florian M., Hajslova J., (2014), Occurrence of multiple mycotoxins in European feeding stuffs, assessment of dietary intake by farm animals, *Animal Feed Science and Technology*, **193**, 124-140.

Zain E.M., (2011), Impact of mycotoxins on humans and animals, *Journal of Saudi Chemical Society*, **15**, 129-144.

THE IMPORTANCE OF SOIL ORGANIC MATTER

M. Kopecky[1*†], L. Kolar[1], R. Vachalova[1], J. Bernas[1], P. Konvalina[1], J. Moudry jr.[1], D.K. Tran[1]

[1]University of South Bohemia in Ceske Budejovice, Faculty of Agriculture, Studentska 1668, Ceske Budejovice, Czech Republic, mkopecky@zf.jcu.cz

ABSTRACT

Land is an irreplaceable factor of production (resource) for the agriculture all over the world. Soil fertility rate is determined by a lot of factors; e.g. soil organic matter content and quality. Neither non-professionals, nor professionals distinguish individual constituents existing in the soil. They call and consider all the organic matters to be "hummus". However, the soil organic matter is divided into two parts - "animate" part which mostly consists of roots of taller plants and soil organisms, and "inanimate" part. There are two groups of elements existing in the inanimate part of the soil organic matter we should distinguish clearly between; they have completely different properties.These two groups of elements are the primary soil matter and the hummus. The primary soil matter is mostly comprised of dead roots of plants and soil organisms. It can be partly decomposed but it has not undergone the humification process. Concerning its resistance to biodegradability, it consists of stable fractions and less stable ones. Its cation-exchange capacity is marginal and negligible. High cation-exchange capacity, and great oxidation and hydrolyse stability are typical properties of the hummus. There are fulvic and humic acids, as well as humins (synthesized high-molecular compounds) among them.This concept of soil organic matter allows a better understanding of its role not only in the assessment of the quality and quantity of humus and primary organic matter in soils but also in the study of soil management and technologies, relations of aerobic and aerobic decomposability, insight into the reaction kinetics of organic matter decomposition in different conditions and in the assessment of the quality of digestates from biogas stations as organic fertilizers.

Keywords: Humus, primary soil organic matter, soil.

SOIL AND THE SOIL ORGANIC MATTER (SOM)

Soil forms part of the terrestrial ecosystems. A lot of complex processes take place in the soil (Sarapatka et al. 2010). The soil is defined as a mixture of various organic and mineral elements. It is a three-phase system from the physical point of view; it consists of a solid (organic and inorganic part), liquid (soil water and a solution of nutrients) and a gaseous (soil air) phase. It is an open system from the organic point of view; it consists of two elementary components. Various inorganic and organic elements form an inanimate component of the soil; minerals usually prevail there. Soil organisms form an animate component of the soil; plant and animal organisms live in the soil (Kolar et al. 2014a).

Composition of a soil-forming substrate, climate, biological factors, relief of a terrain and time are ranked among the natural factors determining creation and development of the soil (Kratina et al. 2010). The substrate is created by mechanical and chemical decomposition of rocks; it is mixed with the organic fraction then. Soil is created progressively, and this process takes a long time (Kolar et al. 2014a). Soil has to be seen as a natural dynamic formation; it is created and it develops in the surrounding environmental conditions (Tomasek 2000).

For humans, fertility is the most important soil property. It is an ability to ensure, thanks to certain indispensable conditions (water and nutrients in particular), existence and reproduction of plants, animals and humans who depend on plants. Non-production functions of the soil have become important recently; these are a stabilization function, a landscape function and a hygienic function. In order to fulfil all these functions, the soil has to be "healthy". Healthy soil is characterised by a lot of organisms supporting and fulfilling many essential functions of the soil, including a circulation of nutrients in the soil. Number of organisms and their species are different in various soil types; they always depend on absorption of the organic matter into the soil (Bot and Benites 2005).

Significance of the organic matter for the soil fertility is obvious. Farmers have discovered the soil containing more organic matter to have better water management, to be processed easily and to be warmed easily (as it is darker). However, scientists have been dealing with the soil organic matter since the 18th century (Kolar et al. 2014a). A lot of articles about positive effects of the soil organic matter have been published in many scientific and professional resources so far. The positive effects are weakened or eliminated, if there is absence of the organic matter in the soil. Soil containing little SOM is characterized by worse soil structure and lower stability of soil aggregates (Darwish et al. 1995). It has smaller hydraulic conductivity and water-holding capacity too (Leroy et al. 2008), and it sorbs less nutrients as well (Lal et al. 2015). Proportion of the soil organic carbon is significant from its sequestration and impact on the climate changes point of view (Stockmann et al. 2013). Proportion of SOM is influenced by the landscape factors, use of land and the farming system too (Kubat et al. 2008). Organic fertilizers should be used in order to increase the proportion of SOM in the farming soil (Hutchinson et al. 2007); generally said, the organic fertilizers enrich the soil with SOM (Aguilera et al. 2013; Parras-Alcantara et al. 2015). There are the organic fertilizers as follows: green fertilizer, compost, manure or sewage sludge (Tejada and Gonzales 2008; Tejada et al. 2008). The final effect of fertilizing depends on amount of the fertilizers we use, on their quality and on the environmental conditions as well (von Lützow et al. 2006). In general, we should not expect more SOM than the controlling mechanisms existing between the soil, plants and the climate indicate. For example, sand soil may contain a lot of organic matter in the warm climate, however the same proportion of organic matter may be insufficient in finely textured soil in the cold climate (Kolar et al. 2014a).

ASSESSMENT OF THE SOIL ORGANIC MATTER

Soil organic matter is an unusually complicated heterogeneous mixture of organic material mostly composed of plant and microbial residues and it contains mono- to polymeric molecules of organic substances, lignin, various proteins, various polysaccharides (cellulose, hemicelluloses, chitin, peptidoglycans), lipids and another aliphatic material (waxes, fatty acids, cutin, suberin, terpenoids). A number of semi-products originate from this basic mixture of primary soil organic matter in the exothermic decomposition process of mineralization as well as in the endothermic synthetic process of humification including the products of humification – fulvic acids, humic acids, humins and their other reaction products, salts of humus acids and organomineral compounds – complex heteropolar salts and adsorption complexes (Kolar et al. 2009).Baldock and Nelson (2000) define the SOM as a sum of all the natural and thermally modified biological elements that exist in the soil (both animal and non-animal, in any phase of decay and decomposition).

Nowadays, a proportion of organic carbon is usually determined in the soil; the organic carbon indicates the total amount of SOM, either humidified or non-humidified. SOM is usually divided into fractions (a degree of its stability during the hydrolysis in oxidation is the determining factor). A proportion of hummus (H) is set up in the soil organic matter; it is determined by a degree of soil humidification S_H, or a ratio of carbon of fulvic acids or humic acids on the total organic carbon in a soil sample, expressed in %. The hummus quality is determined by a ratio of C_{HA} to C_{FA}. It has several shortcomings – it works on the assumption that humic acids are better than fulvic acids for the agriculture. It is, however, a very simplified perspective (Kolar et al. 2014a). There is another problem – there is always a mixture of fulvic and humic acids in the soil. But properties of the humus acids depend greatly on their relative molecular weight; therefore, lower humic acids are, for example, usually closer to fulvic acids than to higher humic acids, as far as the mobility of heavy metals is concerned (Stevenson and Cole 1999). A colour quotient ($Q_{4/6}$), a rate of absorbance of solution of humus acids in two different wavelengths of 450 and 650 nm is even less reliable indicator – it only expresses a degree of condensation of their aromatic seeds. The colour quotient is determined, after humus is extracted from the soil. However, it is commonly known that such an extraction of humus modifies its properties profoundly, including a cation-exchange capacity (CEC), which is its crucial property (Vachalova et al. 2016).

PRIMARY SOIL ORGANIC MATTER AND HUMUS

A group of soil organic elements that have not been humidified yet has got completely different properties from another group of soil organic elements that have already been humidified. Therefore, it is necessary to distinguish, in spite of common and usual practice, two different groups of the soil organic matter. There is a "primary soil organic matter" (PSOM) and a "humus". They are both, nevertheless, very often confused and called "soil organic matter" or "humus"; even professional public usually considers them to be synonyms. A decrease of C_{OX} (carbon determined by wet oxidation of $K_2Cr_2O_7$ in H_2SO_4) in the soil is very often considered to be a serious reduction of humus, although it is usually a decrease of PSOM in fact. It is usually caused by stronger activity of micro-edaphone. It can also have a positive effect – it can increase a proportion of real humus in the soil (that only consists of the humidified elements of the soil organic matter – humic acids, fulvic acids and humins) (Kopecky et al. 2016).

These two terms have been confused by a historic mistake; a proportion of humus was determined by multiplication of C_{OX} by a conversion factor of 1.724. The conversion factor was based on the following assumption: if a humic acid contains 58% of carbon, how much humic acid (humus) matches to the total 100% proportion of carbon? 100:58 = 1.724. However, the calculation was not correct as scientists supposed there was just one humic acid species containing 58% of carbon (it had been defined by an alkaline infusion from garden compost mixed with an acid). Nowadays, we know a wide range of humic acid exists. Scientists made another mistake in the past research; they supposed all C_{OX} belonged to humus, but they omitted the fact a part (or the majority) of organic carbon belonged to PSOM (decomposed or undecomposed roots, root hairs, root exudates, post-harvest plant residues, fallen leaves, organic fertilizers and dead organisms) (Vachalova et al. 2016).

The major difference between H and PSOM lies in two elementary functions these two groups fulfil differently. These are CEC and a stability (resistance) against decomposing processes, or a mineralization speed.

Because of its stability, humus can become neither resource of energy (for the soil micro-edaphone), nor resource of nutrients it is comprised of. It fulfils, however, a lot of other positive functions which are different from positive functions PSOM fulfils. PSOM can have a considerable sorption capacity but, compared to humus, its ion exchange capacity is negligible. The ion exchange capacity of humus is indispensable in the soil. The effort to replace humus by any synthetic ion exchangers cannot be successful: Although they have a high value of CEC, they also show high selectivity for ions of higher valence and low ion-exchange elasticity, and their desorption of calcium and magnesium is poor (Vachalova et al. 2014).

CEC of humus can be determined with the conductometric titration of a suspension of soil sample (it is introduced in H^+ cycle via HCl). A volumetric solution of $Ba(OH)_2$ is used there (Sandhoff, 1954). First of all, the ion exchange capacity of humus and the colloid mineral fraction of the original soil suspension are determined; humus oxidizes with hydrogen peroxide (15%) in acetic acid then. Determining the ion exchange capacity by Sandhoff'sconductometric method again, we get the difference between results that corresponds to the ion exchange capacity of humus contained in our soil sample; it reflects the quality of this humus (Kopecky et al. 2016).

QUALITY OF THE PRIMARY SOIL ORGANIC MATTER

Biodegradability makes a big difference between PSOM and H. Whereas, humus elements decompose very slowly during the mineralization process (from a point of view of a human's life, we can even say they do not decompose at all), natural PSOM has lower or higher ability to decompose during exothermic decomposing processes running in the soil. PSOM is a necessary resource of energy for soil microorganisms. Therefore, it is very important to deal with the amount and quality of this matter (Kolar et al. 2009). A qualitative proportion of every stability fraction (labile, semi-labile, semi-stable and stable fraction) is crucial for the PSOM quality. A lot of authors consider higher amount of easily-decomposing SOM components to be a significant indicator of the potential soil fertility (Ghani et al. 2003, Haynes 2005; Maia et al. 2007). It is, however, a black-and-white and one-sided perspective we cannot agree with completely. The easily-decomposing fraction of the SOM reacts and modifies quite fast (Ghani et al. 2003). The amount of this easily-decomposing fraction is directly proportional to an amount of the soil microbial biomass (Lovell and Jarvis 1998). Therefore, if the weather is warm and wet enough, the soil microbial biomass reproduces very fast. The faster the soil microbial activity is, the faster the soil organic matter mineralization is (Lalande et al. 2009), and the PSOM labile fractions are used up quickly. The amount of soil microorganisms decreases quickly then. Therefore, a mixture of organic matter consisting of labile, semi-labile and semi-stable fractions is considered to be a good-quality PSOM (Vachalova et al. 2016).

We propose to apply a method by Kolar et al. (2014b) in order to detect the quality of PSOM. This method is based on a progressive oxidation of a soil sample with the solution of 0.07 M $K_2Cr_2O_7$ in 12 M H_2SO_4 under the temperature of 60 degrees Celsius. The rate constant of this reaction can be determined by the reaction kinetics of combustion of soil organic matter (carbon loss) in soil samples, which is a degree of quality of primary (non-humified) soil organic matter.

The higher the value is, the less stabile this fraction is and hence of a better quality. Humus is so stabile under these conditions that it does not participate in the oxidation reaction. The amount of primary soil organic matter is given by the total carbon content (C_1) resulting from this oxidation, which is completed by raising the temperature to 100 °C for 45 minutes, when stabile primary fractions of soil organic matter oxidize but humus does not.

The amount of humus is given by total carbon content of the soil sample, which is labelled as C_2 and determined by the oxidation of 0,27 M $K_2Cr_2O_7$ in concentrated H_2SO_4 at 135 °C for 30 minutes according to ISO DIS 14235. The amount of carbon corresponding to humus may be obtained by subtraction C_1 from C_2.

REFERENCES

Aguilera E, Lassaletta L, Gattinger A, and Gimeno BS (2013). Managing soil carbon for climate change mitigation and adaptation in Mediterranean cropping systems: a meta-analysis. Agriculture, Ecosystems and Environment.168(1). pp. 25-36.

Baldock JA and Nelson PN (2000).Soil organic matter.In: Sumner ME (ed.) Handbook of Soil Science.CRC Press.

Bot A and Benites J (2005). The importance of soil organic matter: Key to drought-resistant soil and sustained food production. Food and Agriculture Organization of the United Nations.

Darwish OH, Persaud N, and Martens DC (1995).Effect of long-term application of animal manure on physical properties of three soils.Plant and Soil.176(2). pp. 289-295.

Ghani A, Dexter M, and Perrott KW (2003). Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. Soil biology and biochemistry. 35(9) pp. 1231-1243.

Haynes RJ (2005). Labile organic matter fractions as central components of the quality of agricultural soils: an overview. Advances in agronomy. 85. pp. 221-268.

Hutchinson JJ, Campbell CA, and Desjardins RL (2007).Some perspectives on carbon sequestration in agriculture.Agricultural and Forest Meteorology.142(2). pp. 288-302.

Kolar L, Horacek J, Vachalova R, Vachal J (2014b). Method of measuring amount and quality of soil organic material. CZ Patent. B6 304 265. 5 February 2014.

Kolar L, Kuzel S., Horacek J, Cechova V, Borova-Batt J, and Peterka J (2009). Labile fractions of soil organic matter, their quantity and quality.Plant, Soil and Environment.55(6). pp. 245-251.

Kolar L, Moudry J, and Kopecky M (2014a).The Book of humus.ZERA.

Kopecky M, Kolar L, and Borova-Batt J (2016).The new method of determination of the quantity and quality of primary soil organic matter and humus.Proceedings from International Conference Soil – the non-renewable environmental resource, Brno, pp. 135-142.

Kratina J, Boruvka L, Tejnecky V, Drabek O, and Sebek O (2010). Differences in characteristics of mountain forest soils on acid and basic parent rocks. Geological research in Moravia and Silesia, 17(1-2). pp. 50-57.

Kubat J, Cerhanova D, Mikanova O, and Simon T (2008).Methodology for the assessment of the quantity and quality of soil organic matter in arable land. Crop Research Institute.

Lal R, Negassa W, and Lorenz K (2015). Carbon sequestration in soil. Current Opinion in Environmental Sustainability. 15(1). pp 79-86.

Lalande R, Gagnon B, and Royer I (2009).Impact of natural or industrial liming materials on soil properties and microbial activity.Canadian journal of soil science.89(2). pp. 209-222.

Leroy BLM, Herath HMSK, Sleutel S, De Neve S, Gabriels D, Reheul, and Moens M (2008). The quality of exogenous organic matter: short- term effects on soil physical properties and soil organic matter fractions. Soil use and management.24(2).pp. 139-147.

Lovell RD and Jarvis SC (1998).Soil microbial biomass and activity in soil from different grassland management treatments stored under controlled conditions.Soil Biology and Biochemistry.30(14). pp. 2077-2085.

Maia SMF, Xavier FAS, Oliveira TS, Mendonça ES, ndAraújoFilho JA (2007). Organic carbon pools in a Luvisol under agroforestry and conventional farming systems in the semi-arid region of Ceará, Brazil. Agroforestry systems.71(2). pp. 127-138.

Parras-Alcantara L, Diaz-Jaimes L, and Lozano-Garcia B (2015). Organic farming affects C and N in soils under olive groves in Mediterranean areas. Land Degradation and Development. 26(8). pp. 800-806.

Sandhoff H (1954). KonduktometrischeBestimmung des T- Wertes von Böden und Bodenbestandteilen.Journal of Plant Nutrition and Soil Science.67(1). pp. 24-38.

Sarapatka B, Abrahamova M, Cizkova S, Dotlacil L, Hluchy M, Kren J, ..., and Pokorny J (2010). Agroecology: the way-out for sustainable agriculture. Bioinstitut.

Stevenson FJ and Cole MA (1999). Cycles of soils: carbon, nitrogen, phosphorus, sulfur, micronutrients. John Wiley & Sons.

Stockmann U, Adams MA, Crawford JW, Field DJ, Henakaarchchi N, Jenkins M, ..., and Wheeler I (2013). The knowns, known unknowns and unknowns of sequestration of soil organic carbon. Agriculture, Ecosystems and Environment.164(1). pp. 80-99.

Tejada M and Gonzalez JL (2008). Influence of two organic amendments on the soil physical properties, soil losses, sediments and runoff water quality. Geoderma. 145(3), pp. 325-334.

Tejada M, Gonzalez JL, Garcia-Martinez AM, and Parrado J (2008). Application of a green manure and green manure composted with beet vinasse on soil restoration: effects on soil properties. Bioresource technology.99(11). pp. 4949-4957.

Tomasek M (2000). Soils of the Czech Republic.Czech geological survey.

Vachalova R, Borova-Batt J, Kolar L, and Vachal J (2014).Selectivity of Ion Exchange as a Sign of Soil Quality.Communications in soil science and plant analysis.45(20). pp. 2673-2679.

Vachalova R, Kolar L, and Muchova Z (2016).Primary soil organic matter and humus, two components of soil organic matter.Slovak University of Agriculture in Nitra.

von Lützow M, Kögel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, and Flessa H (2006). Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions – a review. European Journal of Soil Science.57(4). pp. 426-445.

LCA – TOOL FOR EVALUATION OF IMPACT OF ORGANIC AGRICULTURE ON GREENHOUSE GASES EMISSIONS

J. Moudrýjr.[1*†], J. Bernas[1], J. Moudrý[1], P. Konvalina[1], M. Kopecký[1], T. D. Khoa[1]
[1]University of South Bohemia in ČeskéBudějovice, Branišovská 31a, ČeskéBudějovice,
Czech Republic, jmoudry@zf.jcu.cz

ABSTRACT

Organic farmingis regarded as asustainableagricultural system contributing to environmental protection. Balancing the environmental and economic aspects, it fulfils the production function while minimizing environmental impacts as far as possible. In crop production, differences are clearly evidentespecially for fertilization andapplication of pesticides. This alsoimpacts the yields, which play a significant role in overall assessment of environmental impacts ofthose farming methods.For more precise assessment of differences in environmental impacts of conventional and organic farming, we need to be able to measure and quantify such impacts. LCA (Life Cycle Assessment) may serve as a tool for the evaluation of farming systems. The use of LCA is presented onthe cultivation of corn in conventional and organic farming and the evaluation of GHG emissions. The emission load was calculated by the simplified method LCA, impact category: climate. The LCA framework was set for the farming stage of growing. The inputs and outputs were related to the unit of one hectare, and the resulting value was converted to the functional unit of 1 kg of corn. The output was the yield per hectare, and the inputs were the technological operations, and the quantity of seeds, fertilizers and plant protection products. The calculation also includes a calculation of field emissions. The results based e.g. on the yields in eastern Java show cleardifferencesbetween conventional and organic growing of cornparticularly in the category fertilization where the CO_{2e} emissions per kg of corn are almost four times higherin conventional farming. Overall production is0.669 kg CO_{2e} / kg of corn in organic and 1.089 kg CO_{2e} / kg of corn in conventional farming.

Keywords: LCA, greenhousegasesemissions, corn, organicfarming

---

INTRODUCTION

Organic farming is perceived as a system with a number of positive impacts. Environmental friendliness is often considered as one of its most significant features (Robertson et al. 2000, Pretty et al. 2002). This positive impact is usually mentioned in connection with all components of the environment, i.e. soil, which in general has higher organic matter content in organic farming (Mondelaers et al. 2009;Fliessbach et al. 2007;Mäder et al. 2002), biodiversity and agrobiodiversity (Demo et al. 2004;Šarapatka et al. 2008; Hole et al. 2005), water (Lies et al. 2001; Haas et al. 2002;Niggli et al.2011) and, last but not least, climate. For example, American research comparing impacts of organic and conventional farming on a long-term basis, Rodale Institute's Farming Systems Trial, confirms that by introducing organic farming across the USA, the increased carbon sequestration in soil would reduce CO_2 emissions by up to a quarter (LaSalle and Hepperly 2008). Brandt and Svendsen (2011) also point out that organic farming has greater potential to reduce GHG emissions compared to conventional farming systems, with the greatest difference being due to the absence of synthetic fertilizers. Küstermann and Hülsbergen (2008) also came to similar conclusions, saying that organic farming systems generally generate lower amount of N_2O and CO_2 emissions due to lower inputs. This is consistent with the findings previously made by Haas et al. (1995), as well as Bos et al. (2007).

The impact of the environmental system on mitigation is usually quantified per unit of area, but it is important, from the point of view of objectivity, to recalculate it also to the unit of production. GHG emissions are typically lower in environmental systems, both per unit of area and per unit of production. However, the environmental saving per unit of area is roughly double compared to calculation per unit of production due to lower organic farming yields (Nemecek et al. 2005). Thus, the disadvantage of organic farming is lower production per unit of area, increasing the unit load of production by emissions. Average yields in Europe, for example, of organic wheat reach 80 % compared to conventional production (Lackner, 2008). Also, Mondelaers et al. (2009) report that organic farm yields are on average by 17 % lower than in the conventional farming system. Pimentel et al. (2005), on the other hand, state that even with some high-production plants, such as corn, organic farming systems can reach yields comparable to conventional systems. Thus, the yield level plays a key role also in assessing the emission load of organic farming and its comparison with the loads arising from conventional farming systems.

In order to be able to verify the argumentation on lower climate load, or lower GHG emissions by organic farming, it is necessary to evaluate and measure agricultural processes in different conditions and areas. To measure GHG emissions, the Life Cycle Assessment (LCA) method also seems to be one of the suitable methods. It is a tool for evaluating the environmental impacts of the product life cycle based on the assessment of the influence of material and energy flows that the monitored system exchanges with its surroundings (Kočí 2009). Social or economic aspects can be included in its framework, but the main focus is on the environmental component. The LCA method is also an invaluable tool for assessing GHG emissions related to product formation (Finnveden et al. 2009). Stern et al. (2005) and Brentrup et al. (2004) also consider the LCA a suitable tool for evaluating the environmental impacts of agricultural production. This is consistent with the findings of Jensen et al. (2005), who note that over the past decades, the LCA has been supplemented by methods and databases that enable it to be used also in the assessment of impacts within the agricultural sector.

Within the crop production, it is appropriate to assess first the cultivation of crops that are significant in terms of the extent of the areas on which they are grown. These include corn, which is one of the world's most widely grown crops, and together with wheat, rice and soya covers about 70 % of caloric consumption of mankind (Šarapatka et al. 2008). In many countries of the world, it is one of the most cultivated crops, for example, in Indonesia, it is the second most widely grown crop behind rice (Swastika et al. 2004) and it is the most cultivated one also in many European countries or in the USA. The right choice of the system of its cultivation can thus have a significant impact also in terms of mitigating GHG emissions.

MATERIALS AND METHODS

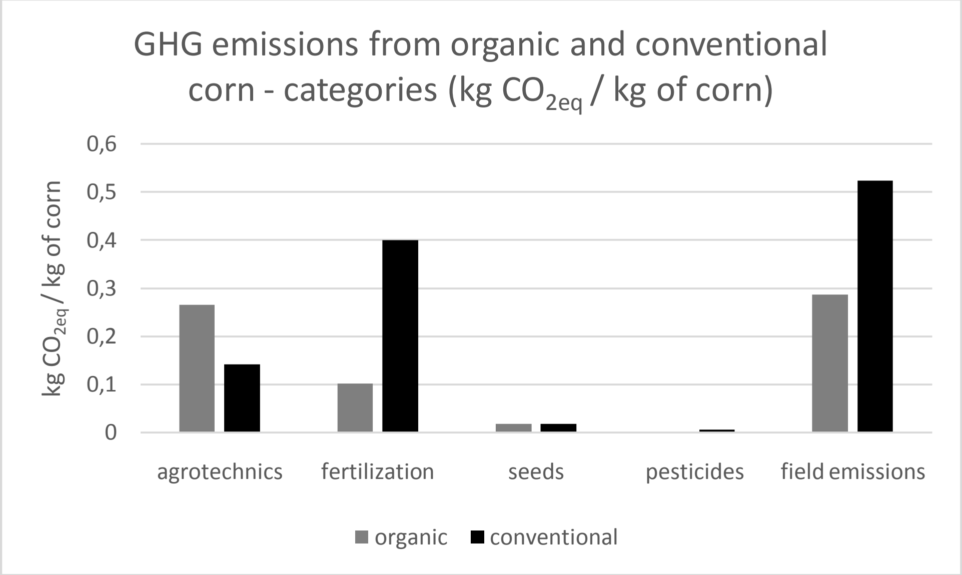
A simplified Life Cycle Assessment (LCA) method, defined by international standards ČSN EN ISO 14 040 (CNI 2006a) and ČSN EN ISO 14 044 (CNI 2006b), has been used as a tool for calculating the emission load rate. The results of the study were related to *Climatechange* impact category, expressed by carbon dioxide equivalent indicator (CO_{2eq} = 1x CO_2 or 23x CH_4 or 298x N_2O). The calculation was performed by SIMA Pro software with integrated ReCiPe Midpoint (H) method. The functional unit of the system was represented by 1 kg of the final product, i.e. 1 kg of corn grain. Technological procedure of corn cultivation was based on data from specialized literature and the Ecoinvent database. The database was partially adjusted in accordance with the conditions of the evaluated region.

The model life cycle includes the farm phase (field emissions, seed and planting, fertilizers, plant protection products, agrotechnical operations). Infrastructure loads (agricultural buildings, machinery, manufacturing infrastructure, means of transport) were not included in the life cycle and data were not evaluated. In addition to emissions from inputs in the form of fertilizers, the so-called field emissions (N_2O emissions) released after the application of nitrogen fertilizers are produced. The IPCC (*Intergovernmental Panel on ClimateChange*) methodology serves for their quantification (De Klein et al. 2006).

RESULTS AND DISCUSSION

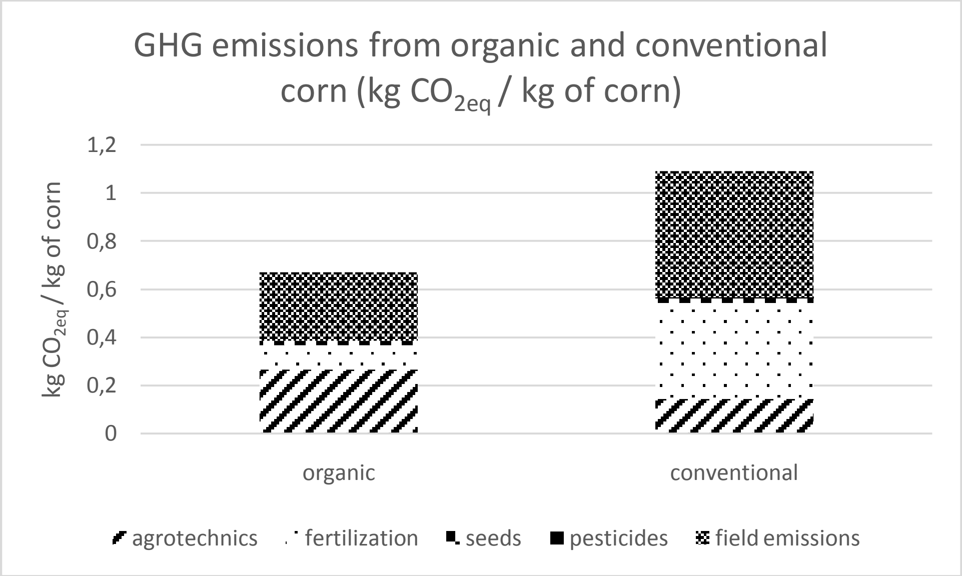
The impacts of corn cultivation in conventional and organic farming systems show different impacts in terms of GHG emissions. In terms of the LCA method, emissions are divided into five subcategories (agrotechnical operations, fertilization, seed, pesticides, and field emissions), with conventional farming showing higher load in the majority of them even after recalculation per unit of production.

Fig. 1 – Greenhouse gases emissions from organic and conventional corn – categories (kg CO_{2eq} / kg of corn)



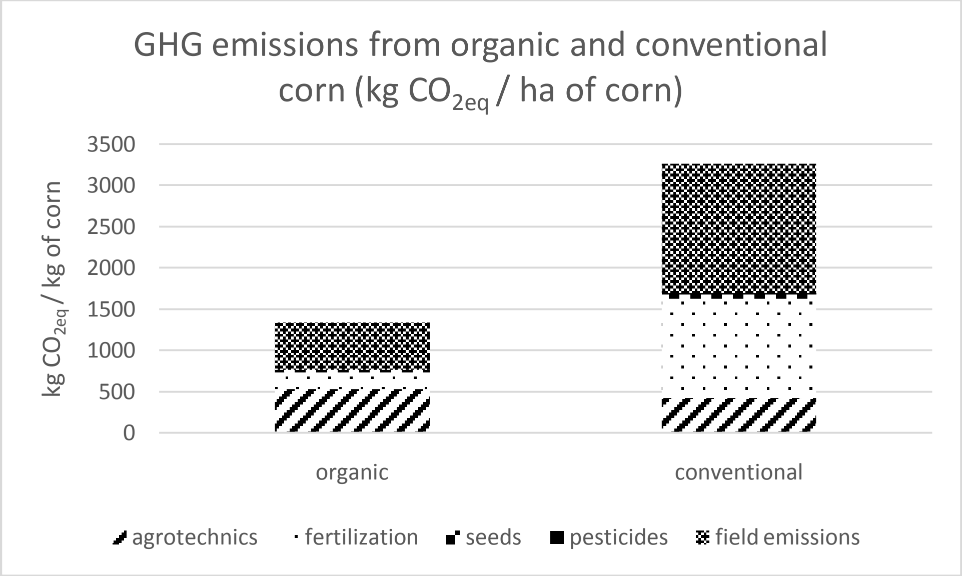
As can be seen from the Chart no. 1, only in the agrotechnical operation category, the load of organic farming is higher in relation to conventional farming (0.265 kg of CO_{2eq} / kg of corn in the organic farming system and 0.141 kg of CO_{2eq} / kg of corn in the conventional farming system). This is due to greater need for interventions in the treatment of corn without the use of pesticides and, at the same time, due to lower corn yield in the organic farming system. The category of seed has a relatively negligible impact from the point of view of GHG emissions (0.017 kg of CO_{2eq} / kg of corn in the organic farming system and 0.018 kg of CO_{2eq} / kg of corn in the conventional farming system) and also pesticides that are used only in the conventional farming system (0.005 kg of CO_{2eq} / kg of corn). However, pesticide application has a great environmental impact in other impact categories. From the point of view of GHG emissions, the main difference between conventional and organic farming system originates in particular in the fertilization phase (0.101 kg of CO_{2eq} / kg of corn in the organic farming system and 0.400 kg of CO_{2eq} / kg of corn in the conventional farming system) and in the subsequent phase of field emissions (0.286 kg of CO_{2eq} / kg of corn in the organic farming system and 0.525 kg of CO_{2eq} / kg of corn in the conventional farming system). This difference is caused mainly by the use of synthetic fertilizers in the conventional farming system. This is consistent with the findings of, for example, Tokuda and Hayatsu (2004), Mori et al. (2005) and Zou et al. (2005), who claim that with the increasing use of chemical fertilizers and manure, the proportion of N_2O released from the soil is usually also increasing. Higher load. The increase of the emission load due to the use of synthetic fertilizers is then also stated by Fott et al. (2003), or Biswas et al. (2008).

Fig. 2 – Greenhouse gases emissions from organic and conventional corn (kg CO_{2eq} / kg of corn)



The overall emission load in the environmental system, as can be seen from the Chart no. 2, is 0.669 kg of CO_{2eq} / kg of corn in the organic farming system, compared to 1.089 kg of CO_{2eq} / kg of corn in the conventional farming system. An important factor is the conversion of the load from the unit of area to the unit of production, i.e. to the kilograms of CO_{2eq} per one kilogram of corn. For example, Brandt and Svendsen (2011) note that the difference in the emission load of conventional and organic farming is very significant, when we relate this load to the unit of area, but it is partially reduced after conversion to the unit of production.

Fig. 3 – Greenhouse gases emissions from organic and conventional corn (kg CO_{2eq} / ha)



The emission load quantified per unit of area is shown in the Chart no. 3. It can be seen that almost two and a half times higher CO_{2eq} emissions (1338.7 kg of CO_{2eq} / ha in the organic farming system and 3267.8 kg of CO_{2eq} / ha in the conventional farming system) arise on one hectare from the corn grown under the conventional farming system.

CONCLUSION

Organic farming shows lower emission load when cultivating corn, both after conversion to the unit of area and to the unit of production. Since corn is one of the world's most significant cultivated crops also in terms of sown areas, a change in the system may be a tool for reducing GHG emissions. Partial savings can be achieved in particular by changes in fertilization by nitrogen fertilizers and partially also in agrotechnics.

ACKNOWLEDGEMENT

This work was supported by the University of South Bohemia in České Budějovice research project GAJU 094/2016/Z.

REFERENCES

Biswas WK., Barton L, Carter D (2008). Global Warming Potential of Wheat Production in South Western Australia. A Life Cycle Assessment. Water and Environment Journal.22.pp.206-216.

Bos JFFP, de Haan JJ, Sukkel W, Schils RLM. (2007). Comparing Energy Use and Greenhouse Gas Emissions in Organic and Conventional Farming Systems in the Netherlands. In: Niggli U, Leifert C, Alföldi T, Lück L, Willer H (Eds.), 3rd International Congress of the European Integrated Project Quality Low Input Food (QLIF): Improving Sustainability in Organic and Low Input Food Production Systems. 20.-23.3.2007, University of Hohenheim, Hohenheim, Germany, pp. 439-442.

Brandt US, Svendsen GT (2011). A Project-Based System for Including Farmers in the EU ETS.Journal of Environmental Management. 92.pp. 1121-1127.

Brentrup F, Küsters J, Kuhlmann H, Lammel J (2004). Environmental Impact Assessment of Agricultural Production Systems Using the Life Cycle Assessment Methodology - I. Theoretical concept of a LCA method tailored to crop production. European Journal of Agronomy. 20.pp. 247-264.

ČNI 2006a. ČSN EN ISO 14 040: Environmentální management Posuzování životního cyklu – Zásady a osnova. (Environmental Management Life cycle Assessment – Principles and Framework.) Český normalizační institut. Praha.

ČNI 2006b. ČSN EN ISO 14 044: Environmentální management Posuzování životního cyklu - Požadavky a směrnice. (Environmental Management Life Cycle Assessment – Requirements and Instructions.) Český normalizační institut. Praha.

De Klein C, Novoa RS, Ogle A, Smith KA, Rochette P, Wirth TC, McConkey, BG, Mosier A, Rypdal K (2006). N_2O emissions from managed soils, and CO_2 emissions managed from lime and urea aplications. In: Eggleston S et al. (eds.). 2006 IPCC Guidelines for National Greenhouse Gas Inventories., Agriculture, Forestry

Finnveden G, Hauschild MZ., Ekvall T, Guninée J, Heijungs R, Hellweg S et al (2009). Recent developments in life cycle assessment. Journal of Environmental Management 2009. 91.pp. 1-21.

Fliessbach A, Oberholzer HR, GunstL, Mäder P (2007). Soil Organic Matter and Biological Soil Quality Indicators after 21 Years of Organic and Conventional Farming. Agriculture, Ecosystems and Environment. 118.pp. 273–284.

Fott P, Pretel J, Neužil V, Bláha J (2003). Národní zpráva České republiky o inventarizaci emisí skleníkových plynů. ČHMÚ, Praha

Haas G, Berg M, Köpke U (2002). Nitrate leaching: comparing conventional, integrated and organic agricultural production systems. In: Steenvoorden J,Claessen F, Willems J (eds.)Agricultural Effects on Ground and Surface Waters: Research at the Edge of Science and Society. Oxfordshire, IAHS, 2002. pp. 131-136.

Haas G, Geier U, Schulz D, Köpke U (1995). A Comparision of Conventional and Organic Agriculture - Part 1: Climate-Relevant Carbon Dioxide Emission from the Use of Fossil Energy. Berichteüber Landwirtschaft. 73.pp. 401-415.

Jensen AA, Hunkeler D, Gaillard G, Hellweg S, Christiansen K (2005). LCM 2005 - Innovation by Life Cycle Management. International Journal of Life Cycle Assessment. 10. pp. 451-453.

Kočí V (2009). Posuzování životního cyklu – Life cycle assessment LCA. Ekomonitor spol.s.r.o., Chrudim

Küstermann B, Kainz M, Hülsbergen KJ (2008). Modeling Carbon Cycles and Estimation of Greenhouse Gas Emissions from Organic and Conventional Farming Systems. Renewable Agriculture and Food Systems. 23.pp.38–52.

Lackner M. (2008). Sozialökologische Dimensionen der österreichischen Ernährung: Eine Szenarienanalyse. Institute of Social Ecology, Klagenfurt University, Vienna, Austria

LaSalle T, Hepperly P (2008). Regenerative Organic Farming: a Solution to Global Warming. Rodale Institute, Kutztown, Pensylvania, USA

Liess M, Schulz R, Berenzen N, Nanko-Drees J, Wogram J (2001). Pflanzenschutzmittel-Belastung und Lebensgemeinschaften in Fliessgewässern mit landwirtschaftlich genutztem Umland. Technische Univerität Braunschweig, Berlin

Mäder P, Fliebbach A, Dubois D, Gunst L, Fried P, Niggli U (2002). Soil Fertility and Biodiversity in Organic Farming. Science. 296.pp. 1694-1697.

Mondelaers K, Aertsens J, Van Huylenbroeck G (2009). A Meta-Analysis of the Differences in Environmental Impacts between Organic and Conventional Farming. British Food Journal. 111. pp. 1098-1119.

Mori A, Hojito M, Kondo H, Matsunami H, Scholefield D (2005). Effects of Plant Species on CH_4 and N_2O Fluxes from a Volcanic Grassland Soil in Nasu, Japan. Soil Science & Plant Nutrition. 51.pp. 19–27.

Nemecek T, Erzinger S (2005). Modelling Representative Life Cycle Inventories for Swiss Arable Crops. International Journal of Life Cycle Assessment. 10.pp. 68-76.

Niggli U, Fliessbach A, Hepperly P, Scialabba N (2011). Zemědělství s nízkými emisemi skleníkových plynů. Bioinstitut, Olomouc

Pimentel D, Hepperly P, Hanson J, Douds D, Seidel R (2005). Environmental, Energetic, and Economic Comparisons of Organic and Conventional Farming Systems. BioScience. 55. pp. 573–582.

Pretty JN, Ball AS, Xiaoyun L, Ravindranath NH (2002). The Role of Sustainable Agriculture and Renewable-Resource Management in Reducing Greenhouse-Gas Emissions and Increasing Sinks in China and India. Philosophical Transactions of the Royal Society. 360.pp. 1741-1761.

Robertson GP, Paul AP, Harwood RR (2000). Greenhouse Gasses in Intensive Agriculture: Contributions of Individual Gases to the Radiative Forcing of the Atmosphere. Science. 289.pp. 1922-1925.

Šarapatka B, Niggli U, Čížková S, Dytrtová K, Fišer B, Hluchý M, Just T, Kučera P, Kuras T, Lyth P, Potočiarová E, Salaš P et al. (2008). Zemědělství a krajina – cesty k vzájemnému souladu. Univerzita Palackého v Olomouci, Olomouc

Stern S, Sonesson U, Gunnarsson S, Oborn I, Kumm KI, Nybrant T (2005). Sustainable Development of Food Production: A Case Study on Scenarios for Pig Production. Ambio. 34.pp. 402-407.

Swastika DKS, Kasim F, Sudana W, Hendayana R, Suhariyanto K, Gerpacio RV, Pingali PL (2004). Maize in Indonezia – Production SystemConstraints, and ResearchPriorities. CIMMYT, Mexico

TokudaS, Hayatsu M (2004). Nitrous Oxide Flux from a Tea Field Amended with a Large Amount of Nitrogen Fertilizer and Soil Environmental Factors Controlling the Flux. Soil Science & Plant Nutrition. 50. pp. 365–374.

Zou J, Huang Y, Lu Y, Zheng X, Wang Y (2005). Direct Emission Factor for N_2O from Rice-Winter Wheat Rotation Systems in Southeast China. Atmospheric Environment.39. pp. 4755-4765.

DOI:10.22616/rrd.23.2017.048

TECHNOLOGICAL AND SENSORY QUALITY OF GRAIN AND BAKING PRODUCTS FROM SPELT WHEAT

Marketa Kyptova, **Petr Konvalina**, **Tran Dang Khoa**
University of South Bohemia in Ceske Budejovice, Czech Republic
kyptova.marketa@seznam.cz

Abstract

This work deals with the baking quality of the spelt wheat grain (*Triticum spelta* L.) compared with bread wheat (*Triticum aestivum* L.). Mixed flours were made of different share of spelt wheat and bread wheat (in total 11 mixtures) in 2016 in the Česke Budejovice in the laboratories of the Faculty of Agriculture. The technological quality of these mixtures was analyzed, focusing on standard evaluation methods (protein content, characteristic of gluten or swellability of protein). The analysis was supplemented by complete rheological analysis made by Mixolab II. Bread was used as a model product. Subsequently, sensory evaluation of baked bread from the previously prepared mixtures was done. Part of the analysis was to estimate the economic basic bread recipe with different proportions of bread wheat and spelt wheat. The results were statistically analyzed via STATISTICA 9.1 (StatSoft, Inc., USA). It was proved that the flour made of spelt can give cereal products with a higher nutritional value. The results have shown that the spelt grain is much more suitable for baking. Its advantage is the higher protein content and higher resistance of kneading of the dough and starch gelatinization rate, which was statistically confirmed. The main disadvantage is the higher price of spelt. According to the results, the ideal utilization of spelt wheat based on sensory analysis and economic calculations seems to be the mixture of spelt wheat and bread wheat, which results in an undeniable decrease of the product cost, and hence effects the common customer choice and taste preferences.

Key words: spelt wheat; bread wheat; technological quality; baking test; sensory evaluation.

Introduction

Cereals are the most widespread of all the crops. They are grown worldwide. Wheat is ranked among four most significant crops from the human caloric intake point of view (Moudry *et al*., 2013a; Moudry *et al*., 2013b; Jelinkova *et al*., 2016). Apart from their nutritional, health and technological parameters, impact of cereals of the environmental sustainability was also evaluated in our research, pursuant to our attempt to evaluate farming and food products from broader point of view (Dalgaard *et al*., 2006). Food consumption patterns were counted and assessed in particular (Wallgren & Hojer, 2009). As far as alternative wheat cultivars are concerned, spelt wheat is the most widespread alternative wheat species in the organic farming; spelt wheat growing areas have been extending (Korczyk – Szabo & Lacko – Bartosova, 2013; Konvalina *et al*., 2014). In 2014, spelt wheat was grown on the area of 2,000 ha and the average yield amounted to 2.11 t ha^{-1} in the Czech Republic (Hrabalova, 2015). In Austria, for instance, the growing area is larger than in the Czech Republic nowadays; spelt wheat is grown on the area of almost 10,000 ha there. Any statistics mapping spelt wheat growing areas worldwide do not exist. However, spelt wheat is supposed to be grown on the area of hundreds of thousands of hectares in total. A wide range of products are made from spelt wheat grains – bakery products, various semi-products, pasta or coffee (Konvalina *et al*., 2011; Stehno *et al*., 2010).

Spelt wheat is often said to contain more nutrients in its grains. Its grains are supposed to be more easily digestible than bread wheat ones. There is not, nevertheless, any unequivocal evidence for that claim. High grain protein content (13 – 20%) has already been described in details. Compared to bread wheat, spelt wheat contains much more proteins. Spelt wheat composition of amino acids is very similar to bread wheat one, if related to the same grain protein content (Ranhotra *et al*., 1995). In spite of high grain protein content, spelt wheat grain is suitable for malt and beer production (Krieger, 2004). Grela, Baranowska & Krusinski (1993) noticed some differences in E-vitamin content between spelt wheat and bread wheat. Compared to bread wheat grains, spelt wheat grains contain more zinc (Ranhotra *et al*., 1995). Cubadda and Marconi (1996) described a high baking quality of spelt wheat in their study, despite spelt wheat gluten being less tough or firm and provoking worse rheological properties of dough than bread wheat one (Schmitz, 2006). Therefore, it is highly advisable to use a mixture of spelt wheat and bread wheat flour for the production of bakery products. Considering that spelt wheat is sold at a higher price than bread wheat, such a mixture of flours allows us to produce bakery products at lower prices. Gluten stability and rheological properties of dough have improved thanks to hybridizing spelt and bread wheat cultivars. Such species are not, nevertheless, accepted by consumers in Austria or Switzerland. They are highly sensitive to wheat gluten. Some grains might even contain more carotenoids that cause darker colour of spelt wheat bakery products, compared to bread wheat ones (Grausgruber *et al*., 2004; Schmitz, 2006). The Italian research showed (Marconi *et al*., 2002) that pasta could be made from spelt wheat grains containing more proteins (Lacko-Bartosova & Lacko-Bartosova, 2016). In order to make good-quality pasta, temperature has to be increased when pasta is being dried. A study showed (Ruibal-Mendieta *et al*., 2005) that wholegrain spelt wheat flour was richer in fats than bread wheat one, and unsaturated fatty acids in particular. High ash, copper, iron, zinc, magnesium or phosphorus contents were detected in some spelt wheat samples (and in the aleuronic layer of caryopsis in particular). On the other hand, there was 40% less leaf acid in spelt wheat than in bread wheat. Digestible fibre content was also lower in hulled wheat grains than in naked wheat ones (Grausgruber *et al*., 2004).

This article aims at evaluating primary parameters of the milling and baking quality. Such evaluation is accompanied with a complex rheological evaluation (carried out with Mixolab II) and sensory analysis as well. Optimizing technological and economic parameters of the mixtures of spelt and bread wheat is another partial objective.

Materials and Methods

Wheat samples: The samples were purchased from a commercial shop, products were from Bioharmonie bio baking flour. The study was done in 2016 in the Česke Budejovice in the laboratories of the Faculty of Agriculture. Evaluation of parameters of the milling and baking quality: Standardized methods were used to determine Gluten index, falling number (ICC 107/1), wet gluten content (ICC 137/1), Zeleny test (ICC 116/1) and moisture (ČSN ISO 712). The nitrogen content was determined according to the Kjeldahl method (ICC 105/2); we used conversion factors, known as N factors, 5.70 for all flours. Advanced evaluation of the baking quality was made by rheological system Mixolab II (ICC 173). Sensory analysis and economic cost analysis: The bread-making test was done using the standard procedure: 300 mLwater, 500 g flour, 16 g salt, 16 g sugar, 3 g cumin and 3 g dried yeast in the home breadmaker Moulinex Home Bread Inox on the 4 h program. The sensory analysis of various bread types was performed (those types of bread differed in the spelt wheat share). Twenty-five participants participated in the evaluation of 5 different breads. They were asked to fill in a questionnaire which included the following questions: taste, smell, visual aspects of bread crust and bread crumb, touch and hearing impressions. Statistical data analysis: The data were statistically analysed (at level $p < 0.05$) by analysis of variance to determine significant differences among samples using program STATISTICA 9.1 (StatSoft, Inc., USA).

Results and Discussion

Evaluation of primary parameters of the milling and baking quality

Table 1 shows the summary of all baking quality parameters that were monitored and evaluated in our research. Measured values corresponded to long-time figures. High falling number values were measured for both wheat species; they indicated a minor damage to grain starch as a consequence of pre-harvest lodging. We measured very high values of falling number; they exceeded the limit and were several times higher. Such high values could have a negative effect on volume and sensory properties of bakery products and bread crumb (Every *et al*., 2002). Gluten index (GI) indicates the stability and flexibility of gluten. Higher values of GI are better for mechanical dough processing. Bread wheat or stronger bread wheat mixtures attained higher values than spelt wheat or any stronger spelt wheat mixture in our research. There was a statistically significant difference between spelt and bread wheat values. On the other hand, there was a minimum difference in Zeleny test values between spelt and bread wheat. Spelt and bread wheat were similar in protein swelling capacity; pursuant to Tukey HSD test, it was a statistically significant trend.

Wet gluten content is closely linked to grain protein content (Famera *et al*., 2015). Spelt wheat usually attains higher values of grain protein content than bread wheat. It is similar to cereal mixtures. 100% pure spelt flour contained higher amount of proteins in our research. It is a general trend nowadays – spelt wheat grains contain more nutritionally valuable proteins than bread wheat ones, in the organic farming in particular. There was a statistically significant difference between 100% bread wheat sample and spelt wheat one in our research; however, there were statistically non-significant differences in every single mixture and they mingled. Dvoracek & Curn (2003) described the following trend – there are more protoplasmic protein fractions, and nutritionally valuable albumins and globulins in particular, in spelt wheat grains. Bread wheat grains usually contain more spectra of prolamins which have a positive effect on their technological quality - e. g. they attain higher values of Gluten Index (see Table 1). On the other hand, these are proteinous fractions that might cause wheat to be toxic to people suffering from celiac disease (Petr *et al*., 2003).

Advanced evaluation of the baking quality – rheological properties of dough

We analysed it with Mixolab II that allowed us to assess the complex rheology of dough during the baking process (Papouskova *et al*., 2011). In fact, we studied and evaluated the stability of dough – an ability and capacity of dough to rise and to keep leavening gas or a product of rising activity (produced by yeast cells activity) – carbon dioxide. The analysis carried out with Mixolab II also involved an evaluation of starch quality (Kahraman *et al*., 2008). The impact of starch on the baking quality was detected and

Table 1

Basic parameters of baking quality (average of two repetitions)

Sample	Spelt wheat (%)	Bread wheat (%)	Falling number (s)	GI	Wet gluten	Zeleny test (mL)	Protein content (%)
1	100	0	585^{h}	52.3^{a}	43.8^{k}	11.0^{a}	14.84^{e}
2	90	10	528^{ac}	58.1^{b}	42.2^{j}	11.5^{ab}	14.78^{e}
3	80	20	516^{b}	59.8^{c}	41.8^{i}	11.5^{ab}	14.59^{de}
4	70	30	537^{d}	60.0^{d}	40.5^{h}	11.0^{a}	14.41^{de}
5	60	40	533^{cd}	60.9^{e}	39.8^{g}	12.0^{bcd}	14.07^{ce}
6	50	50	562^{g}	66.6^{f}	37.3^{f}	11.0^{a}	13.77^{bc}
7	40	60	527^{a}	68.0^{h}	36.7^{e}	11.8^{bc}	13.53^{abc}
8	30	70	506^{f}	69.7^{i}	36.1^{d}	12.3^{cd}	13.28^{ab}
9	20	80	471^{e}	67.4^{g}	33.7^{b}	12.3^{cd}	13.20^{ab}
10	10	90	524^{a}	70.0^{j}	35.3^{c}	12.5^{d}	12.95^{a}
11	0	100	514^{b}	72.4^{k}	33.1^{a}	12.0^{bcd}	12.55^{a}

Note: Values marked with the same letter did not show statistically significant differences at a significance level of $p < 0.05$ (Tukey HSD test).

evaluated in our research. We mostly detected and evaluated starch gelatinization and degradation, which meant a stability of hot gel (Mixolab applications handbook, 2008).

As the detailed figures in Diagram 1 and Table 2, Table 3 show, spelt wheat flour is more stable, and spelt wheat dough leavens longer. It is also obvious that high values of C1 are kept if a spelt wheat share decreases to 50%. A positive effect of top-quality spelt wheat persists. From the Amplitude point of view (the Amplitude reflects and expresses the flexibility of dough), there are not any significant differences. It has been 95% confirmed by the statistics as well. Lower value of the 50% mixture was provoked by a measurement deviation in our research.

The most stable dough was made from 100% spelt wheat flour in our research. Such trend showed that spelt wheat dough needed to be worked and processed intensively. Less stable dough was made from a mixture of spelt wheat and bread wheat; the stability of mixed dough reduced considerably. Such dough should be worked and processed with care and did not need to be kneaded too much. However, Kohajdova & Karovicova (2007) showed the contrary trend. Therefore, we will have to deal with this aspect more in the future. Rheological properties of dough deteriorated (in all C2-C5 parameters) if bread wheat share increased in a flour mixture in our research. Proportion of proteins decreased and they got weaker. Starch got less gelatinized and it was less stable. Retrogradation values were also lower. From the sensory point of view, spelt wheat bread should be more attractive to consumers (spelt wheat attains higher values of all the parameters we have been measuring); it stays fresh longer and it does not crumble. We detected very small differences as both kinds of flour were quite good-quality in our research. There were also small differences from the statistical point of view.

As far as α direction (C1-C2) was concerned, minimum differences in protein weakening speed (when proteins are heated, they weaken) were noticed in our research. Such result showed that the baking technology and temperature should be similar to spelt wheat. There was a noticeable difference in starch gelatinization speed (β direction, C3-C4) between spelt wheat and bread wheat. Spelt wheat starch got gelatinized later (more slowly) than bread wheat one. Therefore, spelt wheat dough requires longer time of baking and lower temperature in general. Enzymatic degradation speed of spelt wheat is lower, as γ direction (C5-C4) shows (see Diagram 1).

Correlations shown in Table 4 demonstrate a relation between parameters. C1 parameter and amplitude had a statistically non-significant correlation in our research. No statistically significant correlation emerged between these parameters and the other ones. Zeleny test and protein content had an interesting negative correlation. A negative correlation also existed between gluten index and protein content. Both negative correlations were statistically significant (99.9%). Such a negative correlation confirmed that spelt wheat contained more proteins and attained lower values of Zeleny test and gluten index. Schober, Clarke & Kuhn (2002) state that spelt wheat dough has got worse rheological properties than bread wheat one. They also noticed that rheological properties of dough improved if the share of spelt wheat decreased in flour mixture. Stiegert and Blanc (2000) confirmed that protein weakening had a negative effect on dough and it made it less stable. Generally said, we have come to the same conclusions as the above-mentioned authors. We, nevertheless, recorded the contrary trend in the dough stability in our research. Spelt bread leavened less, and it had got lower bread volume – which complied with Kohajdova & Karovicova (2007). Bread volume is not the only and deciding factor; sensory properties of bread are also important. Therefore, the sensory analysis was made.

Sensory analysis and economic cost analysis

Results of the sensory analysis showed that pure bread wheat was found the best of all types of bread in our research (it got 2.22 points on average). It is not so difficult to interpret it – bread wheat bread resembles those sold in shops and supermarkets. Mixed bread wheat/spelt wheat bread (there is 30% of spelt wheat in it) was found the second best one (it got 2.44 points in the sensory analysis). It is also available in shops and supermarkets. Mixed bread wheat/spelt wheat bread (there is 70% of spelt wheat in it) was found the third best one (it got 2.49 points in the test). The last two mixtures were very similar to each other in

Table 2

Detailed results of the analyzes on Mixolabu II (parameters dough rheology)

Sample	Spelt wheat (%)	Bread wheat (%)	C1	Amplitude	Stability	C2
			min	Nm	min	Nm
1	100	0	2.72^{a}	0.07^{a}	6.99^{d}	0.40^{b}
2	90	10	2.71^{a}	0.06^{a}	5.59^{c}	0.39^{ab}
3	80	20	2.81^{a}	0.06^{a}	5.07^{bc}	0.37^{ab}
4	70	30	2.60^{a}	0.06^{a}	4.79^{abc}	0.36^{ab}
5	60	40	2.89^{a}	0.05^{a}	4.61^{abc}	0.37^{ab}
6	50	50	2.84^{a}	0.05^{a}	4.20^{ab}	0.38^{ab}
7	40	60	2.85^{a}	0.08^{a}	4.54^{abc}	0.36^{ab}
8	30	70	2.94^{a}	0.06^{a}	4.39^{ab}	0.37^{ab}
9	20	80	2.85^{a}	0.065^{a}	3.87^{a}	0.38^{ab}
10	10	90	2.58^{a}	0.065^{a}	3.90^{a}	0.35^{a}
11	0	100	3.07^{a}	0.055^{a}	4.05^{ab}	0.37^{ab}

Note: Note: Values marked with the same letter did not show statistically significant differences at a significance level of $p < 0.05$ (Tukey HSD test). C1 = Dough hydratation and mixing, C2 = The protein weaking.

Table 3

Detailed results of the analyzes on Mixolabu II (parameters dough rheology)

Sample	C3	C4	C5	α	β	γ
	Nm	Nm	Nm	Nm	Nm	Nm
1	1.660^{a}	1.523^{a}	2.471^{ab}	-0.076^{ab}	0.652^{b}	0.001^{bc}
2	1.633^{a}	1.501^{a}	2.4355^{ab}	-0.087^{a}	0.538^{ab}	-0.005^{abc}
3	1.628^{a}	1.518^{a}	2.5025^{b}	-0.081^{ab}	0.267^{a}	-0.01^{abc}
4	1.5635^{a}	1.4595^{a}	2.3375^{abc}	-0.09^{a}	0.431^{ab}	-0.012^{abc}
5	1.5985^{a}	1.494^{a}	2.358^{abc}	-0.073^{ab}	0.411^{ab}	0.016^{c}
6	1.597^{a}	1.492^{a}	2.355^{abc}	-0.069^{ab}	0.344^{ab}	-0.013^{abc}
7	1.571^{a}	1.441^{a}	2.274^{abc}	-0.072^{ab}	0.405^{ab}	-0.027^{ab}
8	1.56^{a}	1.457^{a}	2.316^{abc}	-0.069^{ab}	0.31^{ab}	-0.005^{abc}
9	1.587^{a}	1.477^{a}	2.3135^{abc}	-0.049^{b}	0.295^{ab}	-0.024^{abc}
10	1.554^{a}	1.4365^{a}	2.231^{ac}	-0.065^{ab}	0.317^{ab}	-0.04^{a}
11	1.5585^{a}	1.427^{a}	2.1585^{c}	-0.069^{ab}	0.3^{ab}	-0.024^{a}

Note: Values marked with the same letter did not show statistically significant differences at a significance level of $p < 0.05$ (Tukey HSD test). C3 = Starch gelatization, C4 = Starch breakdown, C5 = Starch retrogradation, α = protein weakening speed under heating effect, β = speed of starch gelatinization, γ = speed of enzyme degradation.

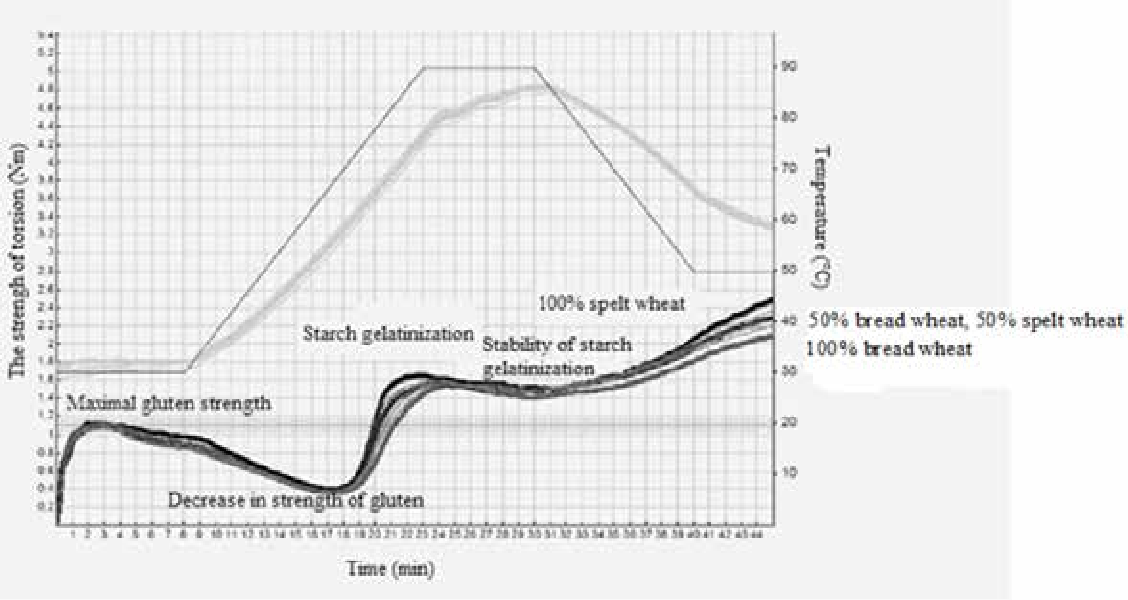


Figure 1. Comparison of the 11 mixtures with different proportions of spelled flour and flour of bread wheat.

Table 4

Results of correlation analys

	Mean±SD	S	C2	C3	C5	α	β	PC	FN	GI	WG
S	4.73 ± 0.91	1									
C2	0.37 ± 0.01	0.57**	1								
C3	1.59 ± 0.04	0.62**	0.85***	1							
C4	1.48 ± 0.04	0.43*	0.81***	0.91***							
C5	2.34 ± 0.11	0.63**	0.68***	0.81***	1						
α	-0.07 ± 0.01	-0.48*	-0.09^{ns}	-0.22^{ns}	-0.28^{ns}	1					
β	0.39 ± 0.13	0.74***	0.33^{ns}	0.24^{ns}	0.26^{ns}	-0.35^{ns}	1				
γ	-0.01 ± 0.02	0.47*	0.45*	0.47*	0.59**	-0.34^{ns}	0.34^{ns}				
PC	13.81 ± 0.77	0.78***	0.56**	0.71***	0.84***	-0.62**	0.56**	1			
FN	527 ± 28	0.65**	0.42^{ns}	0.41^{ns}	0.35^{ns}	-0.43*	0.59**	0.53*	1		
GI	64 ± 6	-0.87***	-0.58**	-0.69***	-0.76***	0.53*	-0.69***	-0.94***	-0.59**	1	
WG	38 ± 4	0.84***	0.5*	0.66***	079***	-0.65***	0.62**	0.97***	0.62**	-0.95***	1
ZT	11.70 ± 0.55	0.56**	-0.52*	-0.45*	-0.46*	0.56**	-0.47*	-0.69***	-0.7***	0.66***	-0.66***

Note:S = Stability, C1 = Dough hydratation and mixing, C2 = The protein weaking, C3 = Starch gelatization, C4 = Starch breakdown, C5 = Starch retrogradation, α = protein weakening speed under heating effect, β = speed of starch gelatinization, γ = speed of enzyme degradation, PC = protein content, FN = falling number, GI = gluten index, WG = wet gluten content, ZT = Zelny test.

the analysis. Pure spelt wheat bread got 2.61 points and was found worse in general. It is understandable as it is not widely spread or available in shops or supermarkets. Consumers are not used to it. According to the results of the sensory analysis, bread made from a mixture of bread wheat and spelt wheat is supposed to be the best and is highly recommended. It is more attractive to consumers and is sold for a reasonable price. Results of the sensory analysis are shown in Table 5. It confirmed the finding a lot of authors had revealed – bread made from a mixture of spelt wheat flour and any other kind of flour was supposed to be the best (Kohajdová & Karovičová, 2007).

Cost analysis – bread made from a flour mixture, various spelt wheat shares

Spelt wheat is mostly bought by consumers who use it in order to bake home-made bread. Nowadays, there is a lack of flour on the market. It leads to an important consequence – a high price of spelt wheat

Table 5

The results of sensory evaluation of bread prepared from various mixtures

Order	Mixture	Point evaluation
1	Bread wheat (100%)	2.22
2	70% bread wheat, 30% spelt wheat	2.44
3	30 % bread wheat, 70% spelt wheat	2.49
4	50% bread wheat, 50% spelt wheat	2.59
5	Spelt wheat (100%)	2.61

Table 6

Assessment of the costs of bread baked from a mixture of different proportions of spelt and bread wheat

Proportion of spelt wheat (%)	Proportion of bread wheat (%)	Price for 0.75 kg of bread (EUR)
100	0	1.74
90	10	1.67
80	20	1.56
70	30	1.48
60	40	1.37
50	50	1.30
40	60	1.22
30	70	1.11
20	80	1.04
10	90	0.93
0	100	0.78

flour. A simple economic cost analysis was made: costs of home-made bread were calculated. Bread wheat flour's price was 1.35 EUR per kg^{-1}, whereas spelt wheat flour's price was 3.13 EUR per kg^{-1}. Other ingredients cost 0.18 EUR per one baking cycle.

As Table 6 shows, pure (100%) spelt wheat bread costs 1.74 EUR, whereas pure (100%) bread wheat bread costs 0.78 EUR. Pure spelt wheat bread is twice as expensive. Its price is not accepted by a lot of consumers. Therefore, it is better to bake bread from a mixture of spelt wheat e.g. (50% of spelt wheat). The price of one baking cycle drops to 1.30 EUR.

Conclusions

Cereal products made from spelt wheat have got a higher added value. Bread was used as a model product in our research. The results of our research showed that spelt wheat grains were suitable for baking (from the technological point of view). It was confirmed by usual baking quality methods and a complex rheological analysis carried out with Mixolab II. Spelt wheat is also more suitable, as its grains have got much higher nutritional value than bread wheat grains. Results of the sensory analysis and economic cost analysis showed that spelt wheat should be mixed with bread wheat and used for baking. This is the optimal solution for spelt wheat. If we use such a mixture, we come to a provable baking cost reduction and the sensory properties of such bakery products are closer to consumer preferences of taste.

Acknowledgements

This work was supported by the research project No. NAZV QJ1310072 of the National Agency for Agricultural Research of the Ministry of Agriculture of the Czech Republic and the University of South Bohemia in České Budějovice (project No. GAJU 094/2016/Z). I would like to thank to Dr. Martin Šlachta for help with statistical data analysis.

References

1. Cubadda, R., & Marconi, E. (1996). Technological and nutritional aspects in emmer and spelt. In: Padulosi, S., Hammer, K., Heller, J. (Eds.), Hulled wheat, Proc. 1st Int. Workshop on hulled wheat, 21 – 22 July 1995, Castelvecchio Pascoli, Italy, pp. 203 – 211. IPGI, Rome, Italy.

2. Dalgaard, R., Halberg, N., Kristensen, I.S., & Larsen, I. (2006). Modelling representative and coherent Danish farm types based on farm accountancy data for use in environmental assessments. *Agriculture, Ecosystems & Environment*, 117(4), 223 – 237.
3. Dvoracek, V., & Curn, V. (2003). Evaluation of protein fractions as biochemical markers for identification of spelt wheat cultivars (*Triticum spelta* L.). *Plant Soil Environ*, *49*, 99 – 105.
4. Every, D., Simmons, L., Al-Hakkak, J., Hawkins, S., & Ross, M. (2002). Amylase, falling number, polysaccharide, protein and ash relationships in wheat millstreams. *Euphytica*, 126(1), 135 – 142. DOI: 10.1023/A:1019699000975.
5. Famera, O., Mayerova, M., Buresova, I., Kourimska, L., & Prasilova, M. (2015). Influence of selected factors on the content and properties of starch in the grain of non-food wheat. *Plant, Soil and Environment*.
6. Grausgruber, H., Scheiblauer, J., Schonlechner, R., Ruckenbauer, P., & Berghofer, E. (2004). *Variability in chemical composition and biologically active constituents of cereals*. Eds. J. Vollmann, H. Grausgruber, & P. Ruckenbauer (pp. 23 – 26). Genetic Variation for Plant Breeding.
7. Grela, E., Baranowska, M., & Krusinski, R. (1993). Tocopherol contents of legumes and cereals. *Przemysl Spozywczy*, 47(11), 311 – 312.
8. Hrabalova, A. Ed. (2015). Rocenka Ekologické zemedelstvi v Ceske republice. (Yearbook of Organic farming in the Czech Republic) Mze. Praha, 72 s. (in Czech).
9. Jelinkova, Z., Moudry, J., Bernas, J., Kopecky, M., & Konvalina, P. (2016). Environmental and economic aspects of *Triticum aestivum* L. and *Avena sativa* growing. Open Life Sciences, 11(1), 533 – 541. DOI: 10.1515/biol-2016-0069.
10. Kahraman, K., Sakıyan, O., Ozturk, S., Koksel, H., Sumnu, G., & Dubat, A. (2008). Utilization of Mixolab® to predict the suitability of flours in terms of cake quality. *European Food Research and Technology*, 227(2), 565 – 570. DOI: 10.1007/s00217-007-0757-y.
11. Kohajdova, Z., & Karovicova, J. (2007). Effect of incorporation of spelt flour on the dough properties and wheat bread quality. *Żywność Nauka Technologia Jakość*, *14*(4).
12. Konvalina, P., Capouchova, I., Stehno, Z., Moudry jr, J., & Moudry, J. (2011). Fusarium identification by PCR and DON content in grain of ancient wheat. *Journal of Food, Agriculture & Environment*, 9(3&4), 321 – 325.
13. Konvalina, P., Moudry, J. srov., Suchy, K., Capouchova, I., Janovska, D. (2014). Diversity of carbon isotope discrimination in genetic resources of wheat. *Cereal Research Communications*. 42(4). 687 – 699.
14. Korczyk – Szabo, J., Lacko – Bartošová, M. (2013). Crum texture of spelt bread. *Journal of Central European Agriculture*, 14(4), 1343 – 1352.
15. Korber-Grohne, U. (1989). The history of spelt (*Triticum spelta*) on the basis of archaeobotanical findings from Neolithic to Medieval times, and the data by written sources until today. *Dire, Treignes*.
16. Krieger, M. (2004). Bierspezialitaten aus Dinkel, Emmer und Einkorn. Schrift (Beer specialities from Dinkel, Emmer and Einkorn). Genetics Resources 23, 176 – 181. (in German).
17. Lacko-Bartosova, M., Lacko-Bartosova, L. (2016). Effect of farming system on colour components of wheat doodles. *Potravinarstvo*, 10(1), 413 – 417.
18. Marconi, E., Carcea, M., Schivone, M., & Cubadda, R. (2002). Spelt (*Triticum spelta* L.) pasta quality: Combined effect of flour properties and drying conditions. *Cereal Chemistry*, 79(5), 634.
19. Mixolab applications handbook. (2008). Rheological and enzymatic analysis. Chopin Applications Laboratory.
20. Moudry, J., Jelinkova, Z., Jaresová, M., Plch, R., Moudry, J., & Konvalina, P. (2013a). Assessing greenhouse gas emissions from potato production and processing in the Czech Republic. *Outlook on Agriculture*, 42(3), 179 – 183.
21. Moudry, J., Jelinkova, Z., Plch, R., Moudry, J., Konvalina, P., & Hyspler, R. (2013b). The emissions of greenhouse gases produced during growing and processing of wheat products in the Czech Republic. *Journal of Food Agriculture & Environment*, 11(1), 1133 – 1136.
22. Papouskova, L., Capouchova, I., Kostelanska, M., Skerikova, A., Prokinova, E., Hajslova, J., & Famera, O. (2011). Changes in baking quality of winter wheat with different intensity of Fusarium spp. contamination detected by means of new rheological system. *Czech Journal of Food Sciences*, 29(4), 420 – 429.
23. Petr, J., Michalik, I., Tlaskalova, H., Capouchova, I., Famera, O., Urminska, D., & Knoblochova, H. (2003). Extention of the spectra of plant products for the diet in coeliac disease. *Czech journal of food sciences*, 21(2), 59 – 70.
24. Ranhotra, G., Gelroth, J., Glaser, B., & Lorenz, K. (1995). Baking and Nutritional Qualities of a Spelt Wheat Sample. Food Sci. Technol.-Lebensm.-Wiss. Technol. 28, 118 – 122.

25. Ruibal-Mendieta, N.L., Delacroix, D.L., Mignolet, E., Pycke, J.M., Marques, C., Rozenberg, R., & Delzenne, N.M. (2005). Spelt (Triticum aestivum ssp. spelta) as a source of breadmaking flours and bran naturally enriched in oleic acid and minerals but not phytic acid. *Journal of agricultural and food chemistry*, 53(7), 2751 – 2759. DOI: 10.1021/jf048506e.
26. Schmitz, K. (2006). Dinkel ein Getreide mit Zukunft für die Bäckerei. (Spelled a grain with future for the bakery) *Getreidetechnologie*, 59(1), 48 – 51. (in German).
27. Schober, T.J., Clarke, C.I., & Kuhn, M. (2002). Characterization of functional prperties of gluten proteins in spelt cultivars using rheological and quality factor measurements. *Cereal Chemistry*, 79(3), 408.
28. Stehno, Z., Bradova, J., Dotlacil, L., & Konvalina, P. (2010). Landraces and obsolete cultivars of minor wheat species in the Czech collection of wheat genetic resources. *Czech Journal of Genetics and Plant Breeding*, 46 (Special Issue).
29. Stiegert, K., & Blanc J.P. (2000). Japanese demand for wheat protein quantity and quality. *Journal Agric. Res. Econ.* 22, 104 – 119.
30. Wallgren, C., & Hojer, M. (2009). Eating energy- identifying possibilities for reduced energy use in the future food supply system. *Energy Policy*, 37(12), 5803 – 5813.

Chapter 12

Rheological and Technological Quality of Minor Wheat Species and Common Wheat

Petr Konvalina, Heirich Grausgruber,
Khoa Tran Dang, Ondrej Vlasek, Ivana Capouchova,
Zdenek Sterba, Karel Suchy, Martina Stolickova,
Marketa Kyptova, Jaroslav Bernas and
Marek Kopecky

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67229

Abstract

Wheat is an important food grain source that nurtures millions of people around the world. Not only does wheat contain a large number of nutrients such as protein, wet gluten, etc., but also it has a lot of antioxidants such as dietary fibre, tocopherols, tocotrienols, etc. In a majority of cases, attention has been drawn to evaluate the grain yield and its components rather than its quality. The present investigation was carried out to evaluate the differences between minority wheat species and common wheat to determine the best rheological characteristics, technological quality as well as correlations between rheological and technological traits. The results revealed that hulled wheat species had a high protein content and wet gluten content. Einkorn and emmer were not suitable for 'classical' baking processing. But there is potential for other products, e.g. wheat rice (einkorn) or pasta (emmer). Spelt should be possible to be used in 'classical' baking industry, but the best solution is to use grain as a mixture with bread wheat. Also, this study showed a genotype variation in the antioxidant activity of einkorn, emmer, spelta and *Triticum aestivum*.

Keywords: wheat, quality, rheological properties, antioxidant capacity, organic farming

1. Introduction

Wheat is a plant grown on more land area than any other commercial crop. It is also one of the most important food grain sources for people all over the world because of the universal use of wheat for a wide variety of products such as bread, noodles, cakes, biscuits, etc. Wheat kernel is composed of endosperm (81–84%), bran (14–16%) and germ (2–3%) [1]. Endosperm is the inner part playing a role as storage of energy and functioning protein. Bran is the outer layer protecting the grain, and germ is the kernel's reproduction system. Whereas wheat endosperm contains mostly starch and protein, bran and germ are rich in dietary fibre, vitamins, minerals and phytochemicals playing an important role in nutrition and health benefits for humans [2]. The customers are, therefore, strongly recommended to consume whole grain foods with at least three servings per day. Recent studies have shown that regular consumption of whole-wheat grain has been found to be associated with reduced total mortality, as well as reduced risk of coronary heart disease, ischemic stroke, type 2 diabetes [3], hypertension in women and colorectal cancer [4].

Over the last few years, despite the development of organic farming throughout Europe, there are not enough varieties that have been purposely bred for organic farming [5]. Conventional bred and tested varieties which were reproduced under the organic farming conditions are grown there [6]. But there are many references from different authors [7] that reported lower baking quality of bread wheat within organic farming. On the other hand, there are many neglected wheat species which have potential to be grown in organic farming and provide high-quality grain [8].

Original cultivars and landraces (e.g. spelt wheat) are the most usual organically cultivated cereal species. Their yield rate is supposed to be lower. Therefore, they have been pushed out of the conventional farming system and replaced by common wheat species. Obsolete cultivars and landraces are also highly appreciated as valuable genetic resources because they are unique and irreplaceable genetic resources for further development of the biological and economic potential of cultural crops. The neglected cereal species have become attractive in the Czech Republic. Spelt wheat (*Triticum spelta* L.) was created by interbreeding of the Tausch's multigraft (*Aegilops tauschii* syn. *squarrosa* L.) with emmer wheat. It is a cultural hulled wheat species and has got 42 chromosomes. There are winter and spring forms of spelt wheat [9]. In 2001, a winter spelt wheat variety called Rubiota was bred in the Crop Research Institute in Prague-Ruzyne and registered. Nowadays, the largest areas of spelt wheat can be found in the Western European countries, such as Germany, Belgium, northern France and Switzerland. There are about 30,000 ha of spelt wheat areas in all of these countries and regions [10]. Spelt wheat has become more attractive in the Czech Republic too – thanks to the development of organic farming. In 2014, spelt wheat crop stands at 2058 ha in the Czech Republic, and the average yield rate attained is 2.81 t/ha. Having the origin from Turkey, *Triticum macha* Dekapr. and Menabde. has got 42 chromosomes as well. This variety ranks among hexaploid wheat species and is cultivated only in the Caucasus region and currently in Russia. It was not grown commercially in Europe or the USA either [11]. It has not been explored too much. Winter varieties are frost proof. This wheat species prefers mid-dry soil types with neutral pH. This is a late winter wheat species and plants have got long stalks. Grains stay in spikelet for a long time; they are kept there even if threshed. They are elliptical, red and mid-hard [11]. Based on foreign literature data, both hulled wheat species are attractive because of their nutritional parameters. Both species contain more proteins (13.5–19%) [12]. Wet gluten content varies from 35 to 45% (but it can be up to 48%) [13]. SDS test values are similar to common wheat values (40–60 mL). Digestible starch content in spelt wheat plants is also similar to the one in common wheat plants. Digestible saccharide content in spelt wheat plants is much lower than the one in common wheat plants. There are fairly less insoluble fibres in the spelt wheat plants than in the common wheat plants [12].

Our chapter is aimed at comparing the baking quality of grains of the different species with the baking quality of grains of modern common wheat. It is also partly aimed at assessing individual parameters of the dough rheology and comparing it with the results of usual grain quality measurement and assessment. The second aim of this chapter is to determine the contents of antioxidant activity (tocopherols) in varieties of einkorn (*Triticum monococcum* L.), emmer (*T. dicoccum* Schuebl [Schrank]), spelt (*T. spelta* L.) and *Triticum aestivum* L. and identify the richest sources for improving the nutritional value of bread, pasta and other wheat products.

2. Materials, methods and results

2.1. Materials and methods

2.1.1. Baking quality and rheological properties

The used varieties were from the Gene Bank of the Crop Research Institute in Prague-Ruzyne, including *T. macha* Dekapr. and Menabde, *T. spelta* L. and control varieties of *T. aestivum* L. – variety SW Kadrilj. Varieties were sown on the organic certified research area of the University of South Bohemia in Ceske Budejovice, Czech Republic, and the University of Natural Resources and Life Sciences, Vienna, Austria, during 2014. The seeding rate was adjusted for a density of 350 germinable grains per m^2. The crop stands were treated in compliance with the European legislation (the European Council (EC) Regulation No. 834/2007 and the European Commission (EC) Regulation No. 889/2008).

Characteristics of the conditions of the University of South Bohemia in Ceske Budejovice research area: mild warm climate, soil type – pseudo gley cambisols, kind of soil – loamy sand soil and altitude of 388 m. Characteristics of the conditions of the University of Natural Resources and Life Sciences research area: located in Raasdorf, the soil was Calcaric Phaeozems (WRB) from loess with a silty loam texture, with the altitude of 156 m.

Quality analysis: The following parameters were tested after harvesting and dehulling of the grains by the International Association for Cereal Chemistry (ICC) methods: crude protein content (ICC 105/2); index of sedimentation – SDS test (ICC 151); wet gluten content (ICC 106/2), gluten index (ICC 155) and baking experiment [14]. For the detailed evaluation of baking quality, Mixolab II. System (accepted as the ICC standard method No. 173 – ICC 2006) was used, which makes possible to evaluate physical dough properties such as dough stability or weakening, and starch characteristics in one measurement (**Table 1**).

Statistical analysis: data were analysed by the Statistica 9.0 (StatSoft Inc., USA) programme. Regression and correlation analyses provided the evaluation of interdependence. The comparison of varieties and their division into statistically different categories were provided by Tukey’s HSD test.

Time for C1 (min)	The time evolution of the dough. The stronger the flour, the longer the time evolution (time to reach C1)
Amplitude (Nm)	The elasticity of the dough. The higher the value, the greater the flexibility of flour
Stability (min)	Resistance against kneaded dough. The longer the duration, the more the flour is considered stronger
C2	Measured attenuation of protein due to mechanical work and temperature
C3	Measures the gelling starch
C4	It measures the stability of the hot gel
C5	Measured starch retrogradation in cooling phase
Guideline α (C1–C2)	Attenuation rate of protein in warming
Guideline β (C3–C4)	Speed starch gelatinisation
Guideline γ (C5–C4)	The rate of enzymatic degradation

Table 1. Description of Mixolab II. phases.

2.1.2. Quality of pasta

A mixture of tested wheat varieties – einkorn wheat, emmer wheat, spelt wheat and bread wheat (the SW Kadrilj cultivar) – was milled into semolina and flour. The semolina was used to make pasta and the selected baking properties of the flour were determined. A reference method was used for the determination of moisture content of flour; the Falling Number method according to Hagberg-Perten was used, as well. The amount of nitrogen in the flour was measured according to Kjeldahl; the sedimentation index was calculated on the basis of Zeleny's test, and the wet gluten quantity and quality were determined with the Glutomatic System.

Semolina pasta was prepared in the pasta machine MPF2.5, and subsequently, a cooking test was performed. The cooking test focused on the determination of the boiling properties, binding and swelling capacity as well as the amount of sediment. The sensory evaluation of the cooked pasta samples was carried out by a group of 10 evaluators.

2.1.3. Antioxidant capacity

The used varieties came from the Gene Bank of the Crop Research Institute in Prague-Ruzyne. In the precise, 3-year field experiments in 2010, 2011 and 2012, four varieties of wheat einkorn *T. monococcum* L., eight varieties of emmer (*T. dicoccum* Schuebl [Schrank]), seven varieties of spelt (*T. spelta* L.), four varieties of landraces of bread wheat (*T. aestivum* L.) and three varieties of spring wheat (*T. aestivum* L.) as control (SW Kadrilj, Vanek, Jara) were used.

Varieties were sown on the organic certified research area of the University of South Bohemia in Ceske Budejovice, Czech Republic. The seeding rate was adjusted for a density of 350 germinable grains per m^2. The crop stands were treated in compliance with the European legislation (the

European Council (EC) Regulation No. 834/2007 and the European Commission (EC) Regulation No. 889/2008). Characteristics of the conditions of the University of South Bohemia in Ceske Budejovice research area: mild warm climate, soil type – pseudo gley cambisols, kind of soil – loamy sand soil and altitude of 388 m.

The following methodology is based on the description in a paper by Lachman et al. [14]. Laboratory analysis of composed finely ground wheat samples (ca 5.0 g) were weighed into 100 mL volumetric flasks and dissolved in methanol. The flasks were filled up with methanol to a volume of 100 mL. For AOA determination, 100 μL aliquots of sample solutions were pipetted. Indirect method described by Roginsky and Lissi was used [15]. Sample containing antioxidants reacts with a solution of stable synthetic radical being converted to a colourless product (DPPH assay). Methanolic DPPH solution [absorbance (t0) 0.600 ± 0.01] was prepared and 100 μL of the sample were added. Reaction time was 20 min. Absorbency was measured at wavelength $\lambda = 515$ nm. AOA was calculated as the decrease of absorbency according to the equation (1): $AOA (\%) = 100-[(At20/At0) \times 100]$ (1) where At20 is the absorbency in time 20 min and At0 is the absorbency in time 0 min. Calculated AOA was expressed in mg Trolox/kg DM. At0 and At20 were determined from the standard calibration curve ($r2 \geq 0.9945$). Calibration curves were prepared using working solutions of Trolox in methanol between 5 and 25 μg Trolox/mL (LOD = 0.601 μg Trolox/mL, LOQ = 2.000 μg Trolox/mL, RSD = 1.83%). All samples were analysed in duplicates.

2.2. Results

2.2.1. Baking quality and rheological properties

Part of our work focuses on finding any differences in the baking quality between the tested varieties. It is also aimed at evaluating correlations between the baking quality parameters determined by common methods and in every single stage of Mixolab II. **Table 2** shows the tested varieties and their average values do not differ statistically from each other in the amplitude, stability, C2–C5, Gamma directive and Falling Number (the Mixolab II. stages are explained in **Table 2**). On the other side of the coin, there were statistically significant differences in C1 stage, Alpha and Beta directives, protein content, wet gluten, gluten index and SDS test. Statistically, significant differences and correlations existed between the following stages. C1 stage had a positive correlation with gluten index and dough stability. According to **Table 2**, a control variety of *T. aestivum* L. was different from the other varieties in C1 stage, which was confirmed by a high gluten index value and more stable dough as well. A positive correlation existed between protein content and C4 + C5 stages, wet gluten content and Gamma directive. *T. macha* Dekapr. and Menabde contained the highest amount of proteins. Wet gluten had a negative correlation with C1 stage. If dough contains more wet gluten, it does not need to be worked so hard mechanically [16]. A positive correlation existed between wet gluten content and protein content. On the other hand, higher gluten index value enhanced dough to develop and had a negative correlation with protein content and wet gluten content. *T. aestivum* L. attained the highest gluten index values. SDS test had a negative correlation with Alpha directive, which relates to a starch grain size and resistance –

Species	C1	Amplitude	Stability	C2	C4	C5	Alpha
T. macha	3.08^{a}	0.09^{a}	7.8^{a}	0.36^{a}	1.1^{a}	1.8^{a}	-0.08^{a}
T. spelta	4.45^{a}	0.07^{a}	8.9^{a}	0.39^{a}	1.2^{a}	1.9^{a}	-0.09^{ab}
T. aestivum	6.69^{b}	0.07^{a}	9.8^{a}	0.44^{a}	1.3^{a}	2.0^{a}	-0.10^{b}
Species	**Beta**	**Gamma**	**Protein content**	**Wet gluten**	**GI**	**SDS**	**Falling Number**
T. macha	0.45^{a}	-0.05^{a}	15.2^{b}	36.07^{b}	55.4^{a}	42.75^{a}	526^{a}
T. spelta	0.63^{b}	-0.07^{a}	14.99^{a}	36.9^{a}	56.0^{a}	43.08^{a}	425^{a}
T. aestivum	0.65^{ab}	-0.07^{a}	13.1^{a}	19.3^{a}	96.8^{b}	49.40^{b}	463^{a}

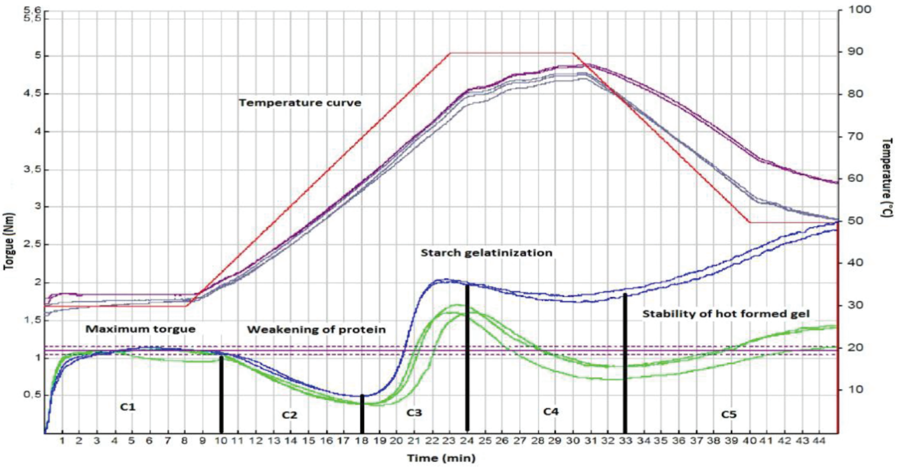
Note: Values marked with the same letter are, based on Tukey's HSD test, statically significantly different at a significance level $P \geq 0.05$.

Table 2. Average values of tracked characteristic on Mixolab II. machine and basic parameters of baking quality.

the bigger and the better quality the grains are (prime ones), the more they swell and the less resistant they are to higher temperatures [10].

Statistically non-significant differences and correlations existed in the following stages. C2–C2 stage had a positive correlation with C3–C5 stages and Beta and Gamma directive and Falling Number. This indicates that the baking technology must be adapted to its properties. C3 – the so-called amylase peak – indicates a different composition of starch and size fractions of starch grains [10]. Samples originating from Vienna research locality contained a higher amount of small starch grains (second ones) which are bound tightly to the protein matrix, and they gelatinise in higher temperatures. On the contrary, samples originating from the Ceske Budejovice research locality contained a higher amount of good-quality big starch grains (prime ones) which gelatinise in lower temperatures. A positive correlation existed with C4 and C5, Falling Number and Beta and Gamma directives. C4 – less stable dough – needs to be baked longer at lower temperature. They do not need to be worked so hard mechanically [16]. This parameter had a strong correlation with C2. In C5, starch gets cooler, starch structure changes and starch gets harder. Retrogradation had a positive correlation with Falling Number. For amplitude, stability and Gamma directive, see **Table 2**. Falling Number had a strong correlation with stability, C2–C5 stages, Beta and Gamma directives and protein content. It is one of the most significant features determining flour baking quality [10].

Triticum macha Dekapr. and Menabde: there were significant differences in the protein-weakening stage (C2) (see **Figure 1**). These were caused by an increasing temperature and mechanical processing of dough. Large differences between two of our localities existed since the starch gelatinisation stage (C3). Samples originating from Vienna research locality attained the amylase peak at the same stage as samples of the control common wheat. Values, enzymatic activity and stability were lower in Ceske Budejovice. Such differences were kept until C5 stage – retrogradation – solidification. *Triticum spelta* L: There were enormous differences since C2 stage as well. Since C3 stage, samples originating from Vienna research locality attained the amylase peak. Those samples from Vienna kept the same or similar test results in C4 stage too.

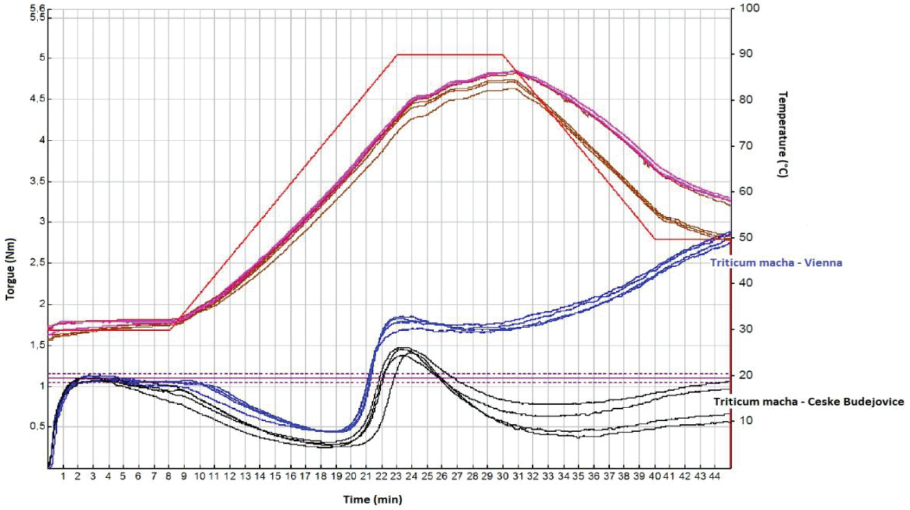


serie testy	Hyd (%)	Work basis	Vyhlazená ivka	teplota sto	teplota mička
Mendel net 2 SW Kadrilj bez F	63,0	b14	🟦	🟧	🟥
Mendel net 3 SW Kadrilj a CB 2014	61,6	b14	🟦	🟧	⬜
Mendel net 4 SW Kadrilj b CB 2014	61,6	b14	🟦	🟧	⬜
Mendel net 47 SWKadrilj a Vídeň 2014	62,8	b14	🟩	🟪	⬜
Mendel net 48 SWKadrilj b Vídeň 2014	62,3	b14	🟩	🟪	⬜

Figure 1. Rheological properties of control variety of *Triticum aestivum* L. - SW Kadrilj

There were large differences in stability between samples originating from Ceske Budejovice locality. In the last C5 stage, spelt wheat varieties attained higher average values than *T. macha* varieties. *T. aestivum* L. (control variety) – SW Kadrilj: larger differences between common wheat varieties arose during the test, since C3 stage (see **Figure 2**). These were the largest differences during the enzymatic degradation (C4 stage). It meant a very different enzymatic activity in every single sample. It was reflected in the retrogradation of starch as well (C5).

Such differences between Vienna and Ceske Budejovice research samples were caused by different conditions in every research locality (climate, weather changes, soil quality, agro-technology and quality of harvested material and post-harvest arrangements). There are



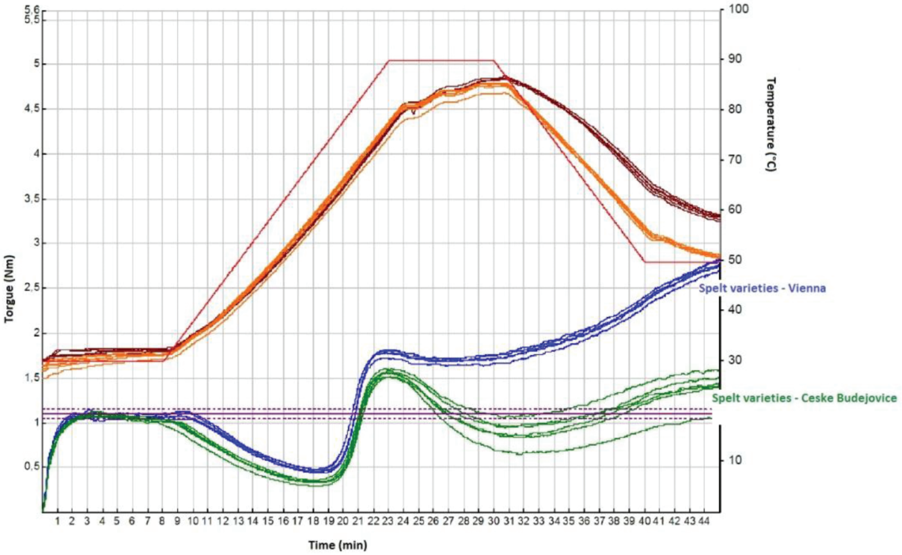
serie testy	Hyd (%)	Work basis	Vyhlazená ivka	teplota sto	teplota mkha
Mendel net 23 Macha 1a CB 2014	62,0	b14	🟫	⬛	🟥
Mendel net 24 Macha 1b CB 2014	62,0	b14	🟫	⬛	⬜
Mendel net 25 Mach 2a CB 2014	64,8	b14	🟦	🟪	⬜
Mendel net 26 Mach 2b CB 2014	65,8	b14	🟦	🟪	⬜
Mendel net 27 Mach 1 a Vídeň 2014	64,0	b14	🟩	🟧	⬜
Mendel net 29 Mach 2 a Vídeň 2014	66,5	b14	🟩	🟧	⬜
Mendel net 28 Mach1 b Vídeň 2014	66,3	b14	🟦	⬛	⬜
Mendel net 30 Mach 2 b Vídeň 2014	63,6	b14	🟦	⬛	⬜

Figure 2. Rheological properties of control variety of *Triticum macha* Dekapr and Menabde

more precipitations and irregular rains in Ceske Budejovice. Rain occurs during the harvest period too. Water percentage in dry matter is a significant factor influencing Falling Number and behaviour of proteins in grains. On the other side of the coin, there are minimum precipitations in Vienna, and almost no rain occurs during the harvest period. However, there is a good-quality soil in Vienna and the total amount of nutrients in the soil is balanced (**Figure 3**).

2.2.1.1. Quality of bread

The results of determination of baking quality are summarised in **Table 3**. It is evident that the lowest protein content was measured in bread wheat flour. This fact is also confirmed



serie testy	Hyd (%)	Work basis	Vyhlazená ivka	teplota sto	teplota mícha
Mendel net 11 SP6 a CB	62,7	b14	🟧	🟪	🟥
Mendel net 12 SP6 b CB	62,7	b14	🟧	🟪	☐
Mendel net 14 SP7 b CB	63,5	b14	🟩	🟨	☐
Mendel net 13 SP7 a CB	63,5	b14	🟩	🟨	☐
Mendel net 16 SP8 b CB	61,8	b14	🟦	🟨	☐
Mendel net 15 SP8 a CB	61,8	b14	🟦	🟨	☐
Mendel net 57 SP8_8_1 Vídeň	64,3	b14	🟦	⬛	☐
Mendel net 56 SP8_3_4 Vídeň	65,0	b14	🟦	⬛	☐
Mendel net 34 SP6_3_2 Vídeň	62,0	b14	🟪	🟥	☐
Mendel net 35 SP6_7_6 Vídeň	62,8	b14	🟪	🟥	☐
Mendel net 37SP7_2_3 Vídeň	63,7	b14	⬛	🟩	☐
Mendel net 38 SP7_6_1 Vídeň	62,2	b14	⬛	🟩	☐

Figure 3. Rheological properties of *Triticum spelta* L. varieties.

by the classification in a statistically different group ($P < 0.05$). Similarly, the lowest protein content was detected in white spelt flour. The observed result is consistent with the data published in the literature, where the protein content is normally referred above the limit

Kind of flour	Protein content (%)	Zeleny's test (mL)	Gluten index	Wet gluten content (%)	Falling Number (s)	Bread volume (cm^{3})
Spelt – whole grain flour	12.77^{a}	10.0^{a}	57^{a}	40.7^{ab}	441^{b}	1500^{a}
Spelt – white flour	14.93^{c}	11.0^{a}	55^{a}	42.4^{b}	560^{d}	1725^{c}
Bread wheat – whole grain flour	9.98^{b}	10.0^{a}	52^{a}	40.4^{ab}	406^{a}	2190^{d}
Bread wheat – white flour	12.70^{a}	14.0^{b}	83^{b}	30.8^{a}	496^{c}	1610^{b}

Note: Values marked with the same letter are, based on Tukey's HSD test, statistically significantly different at a significance level $P \geq 0.05$.

Table 3. Selected parameters of baking quality (mean of two replications).

of 15%. The amount of wet gluten in the samples was in optimum quantity excluding white bread wheat flour that showed a statistically significant difference from white spelt flour. The values resulting from the Zeleny's test were generally low. Only bread wheat reached higher values. Low values of sedimentation are general problems of hulled wheat, which are due to the genetic background to some extent. The gluten index was determined to assess the gluten quality. As expected, the highest amount of gluten was found in bread wheat flour (also confirmed by Tukey's HSD test). However, the value of gluten index in whole-wheat bread flour was surprisingly low. A partial explanation was found based on the correlation analysis (**Table 4**), because the flour had low Zeleny's values. The correlation between these values and the values of gluten index was statistically significant (r = 0.89). Flour of both wheat varieties showed high values of the Falling Number – an indicator of damage to the starch grains due to the pre-harvest sprouting. The values are very high (exceed the standard). Such a high Falling Number may have negative effects on loaf volume, as well as the sensory evaluation of bread crumb.

Parameter		Mean ± SD	1	2	3	4	5
Protein content (%)	(1)	12.6 ± 1.9					
Zeleny's test (mL)	(2)	11.3 ± 1.8	0.23^{ns}				
Gluten index	(3)	62.0 ± 14.0	0.12^{ns}	0.89^{*}			
Wet gluten content (%)	(4)	38.5 ± 5.2	0.11^{ns}	-0.88^{ns}	-0.89^{*}		
Falling Number (s)	(5)	475.8 ± 62.3	0.92^{*}	0.42^{ns}	0.24^{ns}	-0.04^{ns}	
Bread volume (cm^{3})		1756.3 ± 280.9	-0.69^{*}	-0.34^{ns}	-0.42^{ns}	0.25^{ns}	-0.44^{ns}

Note: $^{*}P < 0.05$; ^{ns}, non-significant.

Table 4. The results of correlation analysis (mean of a`ll flour kinds).

The most objective parameter of the baking quality is a determination of the loaf volume. In the present case, a modified methodology was used, and hence the values are for guidance only. Whole-wheat bread showed the highest values. Conversely, the lowest values were found in whole-wheat spelt flour. The results do not fully correspond with the values regarding the gluten index and Zeleny's test (**Table 3**). Correlation analysis results (**Table 4**) indicate a negative correlation (r = −0.69) between the bread volume and protein content. A possible explanation is the fact that spelt is generally higher in protein, but it is of lower baking quality than bread wheat (**Figure 4**).

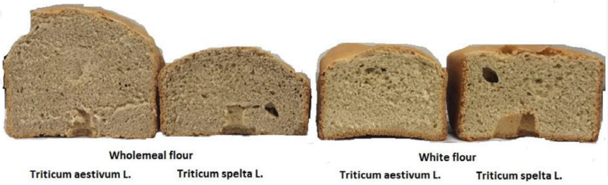


Figure 4. The result of the baking test. A cross-section of bread, in order from left to right: a bread of whole-wheat flour, finely ground flour, whole-wheat spelt flour, finely ground flour, bread wheat flour and spelt flour.

Respondents rated the bread made of whole-wheat, finely ground flour that is the best within the sensory evaluation. The main reason was its high volume. Eight out of 10 respondents described this bread visually appealing. Taste, of course, is a very important indicator of the quality of bread. For all breads, the taste was evaluated as pleasant and less intense. In overall assessment, the bread made of whole-wheat, finely ground flour and bread wheat flour received the highest rating. On the contrary, spelt bread gained only an average rating in the overall evaluation.

2.2.1.2. Quality of pasta

The resulting values of baking properties of flour, which may also affect the quality of pasta, were analysed using the correlation analysis. **Tables 5** and **6** show the results of Tukey's HSD test at a level of significance ($P \geq 0.05$). The tables also present the assessment of cooked pasta.

Species	Protein content (%)	Zeleny's test (mL)	Gluten index	Wet gluten content (%)	Falling Number (s)
Einkorn	15.59^{b}	8.50^{a}	25.8^{a}	36.45^{b}	387^{a}
Emmer	16.04^{d}	13.00^{a}	45.9^{b}	38.40^{c}	470^{c}
Spelt	15.74^{c}	21.0^{b}	76.0^{c}	42.26^{d}	403^{a}
SW Kadrilj – bread wheat	12.79^{a}	34.0^{c}	86.1^{d}	30.88^{a}	187^{b}

Note: Values marked with the same letter are, based on Tukey's HSD test, statistically significantly different at a significance level $P \geq 0.05$.

Table 5. Selected parameters of baking quality of different wheat species.

Species	Firmness	Binding capacity	Swelling capacity	Sediment	Increase of volume	Increase of weight
Einkorn	13^a	105^a	2.48^a	180^c	120.5^c	105^a
Emmer	10^b	100^c	2.28^a	110^b	102^a	100^c
Spelt	13^a	108^a	2.41^a	79.5^a	105.0^a	108^a
SW Kadrilj – bread wheat	11^c	95^b	2.20^a	80.0^a	94.5^b	95^b

Note: Values marked with the same letter are, based on Tukey's HSD test, statistically significantly different at a significance level $P \geq 0.05$.

Table 6. Selected parameters of quality of different wheat species.

According to CSN 46 1100-2 (the Czech Republic's standard quality), an amount of N-substances in wheat for food use should reach 10.8–13.7%, which corresponds with the sample of bread wheat flour. The fact that hulled wheat is higher in protein has been confirmed within this study. The tested wheat varieties contained 16% of N-substances. Generally, the sedimentation index was low in hulled wheat samples. It may be therefore stated that these varieties are not, in contrast to bread wheat, suitable for baking purpose. On the other hand, these values do not affect the quality of pasta to a great extent. By contrast, gluten index and the amount of sediment have an impact on it. In this case, negative correlation indicates that increased gluten index decreases an amount of sediment with 99.9% probability, when cooking pasta. Gluten index of einkorn wheat is very low, which consequently resulted in the relatively large amount of sediment. The interesting information shown by this statistical method is the dependence of wet gluten on the amount of water bound by pasta during boiling. The amount of water absorbed by pasta thus increases due to the higher wet gluten content together with the weight of pasta. Spelt showed the highest values of wet gluten and thereby the highest binding; conversely, the lowest amounts were found in bread wheat. The Falling Number method did not prove any evidence showing a connection with the quality of pasta.

The sensory evaluation included tasting and filling out a questionnaire. Colour, surface (smooth, rough and floury), edges (sharp and rough), texture (compact and cracked) and firmness (strong, fragile, crumbly and translucent) of uncooked pasta had been evaluated. In consequence, colour, hardness (undercooked, al dente and overcooked), shape (appropriate and deviation of shape), flavour (excellent, good, fair and poor with foreign taste), odour (pleasant and unpleasant) and surface (sticky, slightly sticky and dry) of cooked pasta had been evaluated. Based on the data gained from the questionnaires, it may be assumed that the pasta production of hulled wheat was assessed positively by the respondents on the whole (**Figure 5**).

2.2.2. Antioxidant activity of different wheat species

Whole grain phytochemicals have an antioxidant activity, the ability to scavenge free radicals that may oxidise biologically relevant molecules [17]. Due to this, whole-wheat foods could

Figure 5. Differences in pasta colour.

contribute to the health benefits of people such as reducing the risk of heart disease, diabetes type 2, cancer, etc. In the present study, there were highly significant differences ($p < 0.05$) among 26 varieties for antioxidant activity (**Figure 6** and **Table 7**).

Mean antioxidant activity among varieties ranged from 225.45 mg Trolox/kg DW to 400.83 mg Trolox/kg DW. This demonstrates a broad range of antioxidant content in wheat species. There were eight groups in which the means were not significantly different from one

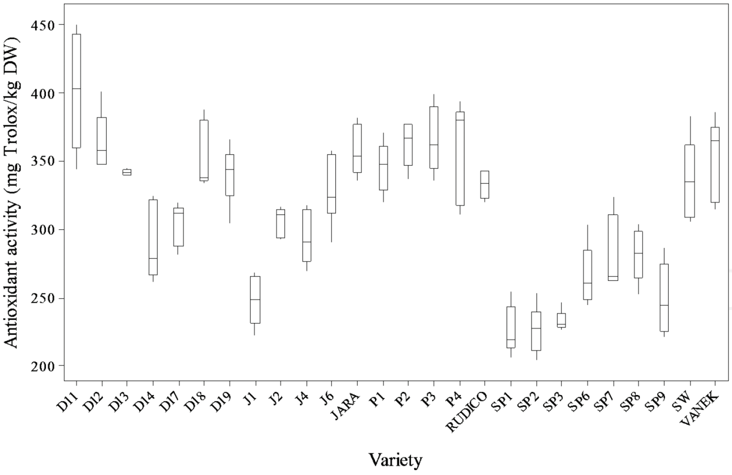


Figure 6. Antioxidant activity content of wheat varieties. Values expressed as mg Trolox/kg DM. D11 – Weisser Sommer; D12 – May-Emmer; D13 – *T. dicoccum*; D14 – *T. dicoccum*; D17 – *T. dicoccum*; D18 – *T. dicoccum*; D19 – *T. dicoccum*; J1 – *T.*; J2 – *T. monococcum*; J4 – *T. monococcum*; J6 – *T. monococcum*; P1 – *T. aestivum*; P2 – *T. aestivum*; P3 – *T. aestivum*; P4 – *T. aestivum*; SP1 – *T. spelta*; SP2 – *T. spelta*; SP3 – *T. spelta*; SP6 – *T. spelta*; SP7 – *T. spelta*; SP8 – *T. spelta*; SP9 – *T. spelta*; SW – SW Kadrilj.

Variety	**D11***	**D12***	**D13***	**D14***	**D17***	**D18***
AOA (mg Trolox/kg DM)	400.83^{a}	364.15^{ab}	341.60^{bc}	288.36$^{e–g}$	304.56$^{c–f}$	351.62^{b}
Variety	D19*	RUDICO*	J1**	J2**	J4**	J6**
AOA (mg Trolox/kg DM)	339.92^{bc}	332.90$^{b–d}$	247.42^{gh}	306.16$^{c–f}$	293.23^{df}	327.73$^{b–e}$
Variety	P1***	P2***	P3***	P4***	SP1****	SP2****
AOA (mg Trolox/kg DM)	345.88^{bc}	362.25^{ab}	365.26^{ab}	360.95^{ab}	225.45^{h}	226.55^{h}
Variety	SP3****	SP6****	SP7****	SP8****	SP9****	JARA
AOA (mg Trolox/kg DM)	232.63^{h}	265.56$^{f–h}$	280.63^{fg}	281.10^{fg}	248.82^{gh}	357.36^{ab}
Variety	SW	VÁNEK				
AOA (mg Trolox/kg DM)	336.98$^{b–d}$	353.70^{b}				

Values marked with different small letters are significantly different at $P \leq 0.05$.

*Emmer varieties.

**Einkorn varieties.

***Landrace of *T. aestivum*.

****Spelt varieties.

Table 7. Content of antioxidant activity in different wheat grains.

another. Having 400.83 mg Trolox/kg DW, D11 variety belonged to the lead group and was significantly different from all other varieties except P3, D12, P2, P4 and JARA. In contrast, the varieties containing the lowest content of antioxidant were SP6, SP9, J1, SP3, SP2 and SP1 with 266.57 mg Trolox/kg DW, 248.82 mg Trolox/kg DW, 247.42 mg Trolox/kg DW, 232.63 mg Trolox/kg DW, 226.55 mg Trolox/kg DW and 225.45 mg Trolox/kg DW, respectively.

The findings of Lachman et al. [14] showed that the antioxidant activity content of seven varieties ranged between 134.0 and 197.5 mg Trolox/kg DW. In this study, our results are approximately two times higher than these ones. This means that the varieties in our experiment are potential for breeding new wheat varieties, as well as essential sources of functional food ingredients.

It is known that antioxidant activity content can be influenced by stress factors of the weather conditions during the vegetation period and genotype effects. Comparing the data of four species collected from 2010 to 2012 (**Figure 7**) show that there is a decrease in the mean of antioxidant during the 3-year period by 23.26 mg Trolox/kg DW. These differences are, however, not statistically significant.

The cultivated diploid (einkorn), tetraploid (durum wheat), hexaploid (bread wheat) and other varieties possess antioxidant activity due to their content of hydrophilic (phenolics, selenium) and lipophilic (carotenoids, tocopherols) antioxidants (**Figure 8**) [18].

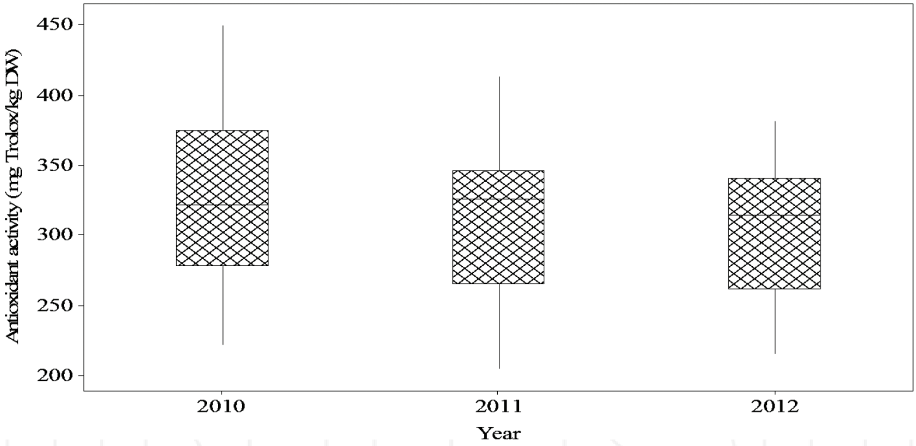


Figure 7. Antioxidant activity in 26 varieties harvested in 2010, 2011 and 2012.

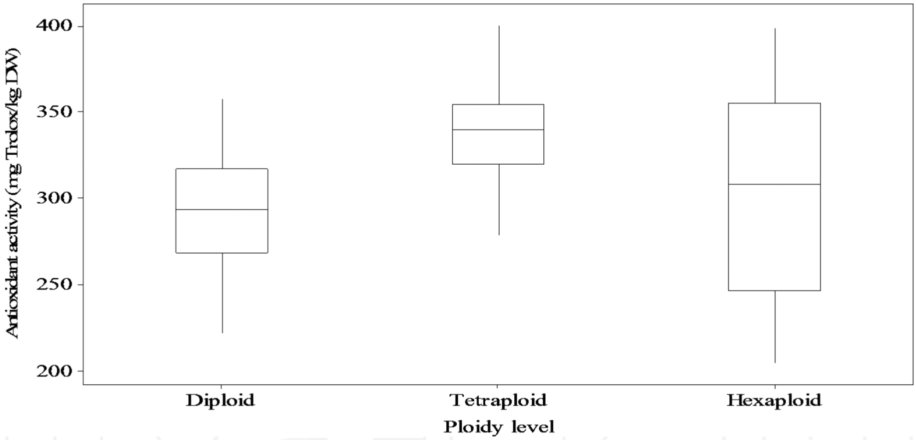


Figure 8. Content of antioxidants in wheat grains from the harvests 2010, 2011 and 2012.

Analysing ANOVA, Tukey's HSD revealed statistically significant differences between tetraploid and diploid as well as tetraploid and hexaploid. The mean antioxidant of tetraploid from 2010 to 2012 (340.49 ± 39.11 mg Trolox/kg DW) was higher than the value of diploid and hexaploid (293.64 ± 34.82 mg Trolox/kg DW) and (303.08 mg Trolox/kg DW), respectively. Our results are different from those of Lachman et al. [14]. While antioxidant values in our findings increase from diploid (einkorn) to tetraploid, the reverse is true for Lachman's results. This is because our experiment used 26 varieties in 3 years compared to seven varieties in 2 years.

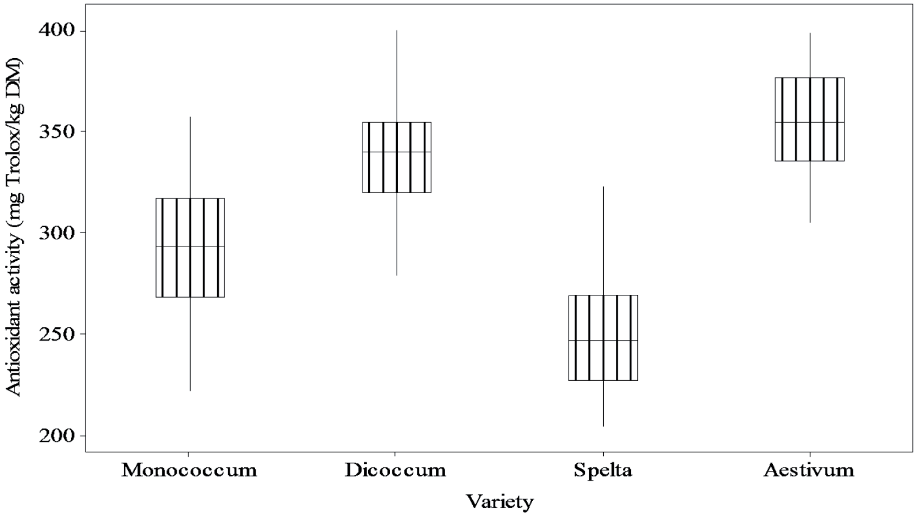


Figure 9. Antioxidant activity values of four species.

Figure 9 illustrates the differences of four varieties. *T. aestivum* and emmer wheat shared the highest value with 354.44 ± 24.97 mg Trolox/kg DW) and 340.49 ± 39.11 mg Trolox/kg DW, respectively, followed by *T. monococcum* (293.64 ± 34.82 mg Trolox/kg DW). Having 251.54 ± 29.60 mg Trolox/kg DW, *T. spelta* had the lowest value in total of four species ($P < 0.05$).

2.3. Conclusions

Compared to samples originating from Ceske Budejovice, samples originating from Vienna attained higher and more balanced values in all the stages of Mixolab II. testing. SW Kadrilj was the only common wheat variety that attained a similar protein weakening speed when heated and worked mechanically (C2). There were enormous differences between *Triticum macha* Dekapr. and Menabde and *Triticum spelta* L. Baking technology must be adapted to the requirements of these two wheat species; dough must be worked more sensitively. In spite of this fact, these species can be used for baking purposes.

The working hypothesis, i.e. the use of spelt, einkorn and emmer wheat is technically feasible within the pasta production, was confirmed based on the testing. All wheat varieties are high in protein. Boiling time is not significantly different and pasta swells to the extent close to the pasta commonly available on the market. The taste of evaluated pasta was not assessed negatively, and the consumers, who are used to consuming more whole grain products, could feel the distinctive flavour of products made of einkorn and emmer wheat and spelt, which generally fades fast during milling from pasta made of white durum wheat or bread wheat flour.

Wheat contains huge essential antioxidants such as dietary fibre, tocopherols, tocotrienols, etc. The consumption of wheat is associated with reducing risk of chronic diseases including type 2 diabetes, obesity and cardiovascular disease. In this study, the content antioxidant activity of 26 varieties of whole wheat is reported. Antioxidant activity was ranged from 225.45 mg Trolox/kg DW to 400.83 mg Trolox/kg DW. The antioxidant activity values were significantly different among varieties, ploidy level and wheat accessions.

The general conclusion is that hulled wheat species had a high protein content and wet gluten content. Einkorn and emmer were not suitable for 'classical' baking processing. But there is potential for other products such as wheat rice (einkorn) or pasta (emmer). Spelt will be possible to be used in 'classical' baking industry. The best solution will be the use of spelt wheat grain mixed with bread wheat grain. Also, this study showed a genotypical variation in the antioxidant activity of einkorn, emmer, spelta and *T. aestivum*.

Acknowledgements

The research was financially supported by the National Agency for Agricultural Research of the Ministry of Agriculture of the Czech Republic (Project No. NAZV QJ1310072) and the University of South Bohemia in Ceske Budejovice (Project No. GAJU 094/2016/Z).

Author details

Petr Konvalina[1]*, Heirich Grausgruber[2], Khoa Tran Dang[1], Ondrej Vlasek[1], Ivana Capouchova[3], Zdenek Sterba[1], Karel Suchy[1], Martina Stolickova[1], Marketa Kyptova[1], Jaroslav Bernas[1] and Marek Kopecky[1]

*Address all correspondence to: konvalina@zf.jcu.cz

1 University of South Bohemia in Ceske Budejovice, Faculty of Agriculture, Czech Republic

2 Department of Crop Sciences, BOKU – University of Natural Resources and Life Sciences, Tulln, Austria

3 Czech University of Life Sciences Prague, Czech Republic

References

[1] Pomeranz, Y. 1988. Chemical composition of kernel structures in wheat. Chemistry and Technology, Vol 1. American Association of Cereal Chemists, St. Paul Minesota: 97–158.

[2] Van Hung, P. 2016. Phenolic compounds of cereals and their antioxidant capacity. Critical Reviews in Food Science and Nutrition, 56(1): 25–35

[3] Mpofu, A., Sapirstein, H.D., Beta, T. 2006. Genotype and environmental variation in phenolic content, phenolic acid composition, and antioxidant activity of hard spring wheat. Journal Agriculture Food Chemistry, 54(4): 1265–1270..

[4] Schatzkin, A., Mouw, T., Park, Y. 2007. Dietary fiber and whole-grain consumption in relation to colorectal cancer in the NIH-AARP Diet and Health Study. The American Journal of Clinical Nutrition, 85: 1353–1360.

[5] Konvalina, P., Stehno, Z., Capouchová, I., Zechner, E., Berger, S., Grausgruber, H., Janovská, D. and Moudry, J. 2014. Differences in grain/straw ratio, protein content and yield in landraces and modern varieties of different wheat species under organic farming. Euphytica, 199: 31–40

[6] Lammerts Van Bueren, E.T., Struik, P.C. and Jacobsen, E. 2002. Ecological concepts in organic farming and their consequances for an organic crop ideotype. Netherlands Journal of Agricultural Science.

[7] Wolfe M.S., Baresel, J.P., Desclaux, D., Goldringer, I., Hoad, S., Kovacs, G., Löschenberger, F., Miedaner, T., Østergård, H. and Lammerts Van Bueren, E.T. 2008. Developments in breeding cereals for organic agriculture. Euphytica, 163: 323–346.

[8] Piergiovanni, A.R., Laghetti, G. and Perrino, P. 1996. Characteristics of meal from hulled wheats (*Triticum dicoccon* Schrank and *T. spelta* L.): an evaluation of selected accessions. Cereal Chemistry, 73: 732–735.

[9] Moudry J., Strasil Z. 1999. Cultivation of alternative crops. University of South Bohemia. České Budějovice: 11–13.

[10] Prugar, J. 2008. The quality of plant products on the threshold of 3rd millennium. Research Institute of Brewing and Malting, PLC. Prague, ISBN 9788086576282.

[11] Magness, J.R., Markle, G.M., Compton, C.C. 1971. Food and feed crops of the United States. Interregional Research Project IR-4, IR Bul. 1 (Bul. 828 New Jersey Agr. Expt. Sta.).

[12] Konvalina P., Capouchova I., Stehno Z., Kas M., Janovska D., Škerikova A., Moudry J. 2012. Growing and using of spelt in organic farming – Methodology for practice. Crop Research Institute. Prague: 40.

[13] Michalova A., Merhaut K., Hradil R., Pribis P., Hutar M., Doubravska M., Valuskova M. 2001. Czech bio cookbook, cook organic food. Fontána partnership with PRO-BIO Association. 41.

[14] Lachman, J., Orsák, M., Pivec, V., Jiru, K. 2012. Antioxidant activity of grain of einkorn (*Triticum monococcum* L.), emmer (*Triticum dicoccum* Schuebl [Schrank]) and spring wheat (*Triticum aestivum* L.) varieties. Plant Soil Environment, 58: 15–21.

[15] Vitaly Roginsky , Eduardo A. Liss. 2005. Review of methods to determine chain-breaking antioxidant activity in food. Journal Food Chemistry, 92: 235–254.

[16] Papouskova, L., Capouchova, I., Kostelanska M., Skerikova, A., Prokinova, E., Hajslova, J., Salava, J., Famera, O. 2011. Changes in baking quality of winter wheat with different intensity of Fusarium spp. contamination detected by means of new rheological system Mixolab. Czech Journal of Food Science, 29: 420–429.

[17] Liu, R. 2007. Whole grain phytochemicals and health. Journal of Cereal Science, 46: 207–219.

[18] Hidalgo, A., Brandolini, A., Gazza, L. 2008. Influence of steaming treatment on chemical and technological characteristics of einkorn (Triticum monococcum L. ssp monococcum) wholemeal flour. Food Chemistry, 111: 549–555.