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Doctoral dissertation:

Nutrition potential of new oats and spring wheat cultivars

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Prague August, 2023

ACKNOWLEDGEMENTS

First and foremost, with profound appreciation, I extend my heartfelt gratitude to my supervisor prof. Ing. Lenka Kouřimská, Ph.D. who support me throughout this study. Her support and mentorship have been a source of inspiration throughout my Ph.D.

I am deeply indebted to the Department of Microbiology, Nutrition, and Dietetics, as well as the Faculty of Agrobiology, Food, and Natural Resources at CZU, for offering me a place in this doctoral program.

My sincere thanks also go to my consultant, Dr. Ing. Pavel Horčička and Selgen for their generosity in providing me with samples and access to their laboratory.

Furthermore, I wish to express my heartfelt gratitude to Ing. Matěj Božik, Ph.D., Prof. Ing. Ivana Capouchová, Ph.D., Doc. Ing. Kateřina Pazderů, Ph.D., and Ing. Sarvenaz Khalili Tilami, Ph.D., for their support, insightful guidance, and encouragement throughout this scholarly endeavor.

I am deeply grateful and would like to extend my heartfelt appreciation to my beloved family member. I am truly grateful for everything they do and for being a special part of my life.

SUMMARY

Based on global grain production, wheat (*Triticum aestivum* L.), and oats (*Avena sativa* L.) hold prominent positions as essential sources of nutrition for both humans and animals. Interestingly, wheat ranks as the second main grain crop, serving as a staple food providing essential nutrition to billions. Similarly, oats rank as the sixth main grain crop, holding a unique position among cereals. Previously regarded primarily as horse feed, oats have now gained significant attention not only in the public and food industry but also in the pharmaceutical sector. Their nutritional potential continues to play a vital role in global diets, making them promising cereals in sustaining humanity's well-being. The overall aim of the thesis was to highlight the nutrition potential of oat and spring wheat cultivars, by investigating their fat content, fatty acid composition, carotenoid content, tocol (vitamin E) content, and phenolic compounds.

Oat is a rich source of fat, and the fatty acid composition of oats is desirable for human consumption. The lipid component of oats has nutritional, medical, and technological potential. Consequently, the fat content and the fatty acid composition of oats play a crucial role in determining the grain's energy and significantly impact overall nutritional quality. In our research, we have investigated 38 diverse genotypes of oats, including hulled and naked Canadian and European varieties. Additionally, we have also evaluated six oat varieties registered between 2011 and 2014 and eight yellow oat varieties. These oats are commonly cultivated worldwide.

The lipid of milled oat grains was extracted using petroleum ether. Fat content was determined through a gravimetric method, after drying at 103 ± 2 °C until a constant weight was achieved. After preparing fatty acid methyl esters from fresh fat, the fatty acid composition was analyzed using GC-MS. Finally, the proportions of fatty acids were calculated using the area normalization method, and the results are expressed as relative percentages of each fatty acid.

According to our results, the lipid content of Canadian and European oat genotypes was 2.9 – 6.29% and 3.52 –9.51% respectively. Notably, the French genotype, Ivore, presented the highest quantity of oleic acid and total MUFA content. Additionally, the Russian genotype Lidya contained the highest palmitic acid. This study found that the PUFA/SFA ratio was 1.39 – 2.05 in Canadian genotypes and 1.21 – 2.24 in European oat genotypes. Importantly, Canadian oat genotypes have a higher proportion of unsaturated fatty acids compared to European genotypes, suggesting they offer a healthier choice for consumers. This study's uniqueness lies in its illustration of how diverse oat genotypes can influence the fat content and

fatty acid composition even if they are grown under the same climatic condition and within a single season.

When comparing the fat content of naked and hulled oats, it was found that naked oats contained a higher amount. Moreover, husk removal led to changes in the fatty acid compositions in oats, resulting in an increase in PUFA and a decrease in MUFA. Furthermore, dehulled oats, after husk removal, contained a higher fat compared to hulled oats. Consequently, husk removal led to a reduction in the proportion of oleic acid and an increase in linoleic acid. Additionally, the result of our study has also found the major fatty acids of naked oats and hulled oats are the same but the composition is different. Additionally, our research reveals that oat lipids have low atherogenicity and thrombogenicity indices, indicating their potential health benefits. The findings of these studies highlight the dietetic significance of new oat cultivars hold for both human food and animal feed.

Due to its relatively higher fat content among cereals, oats are susceptible to oxidation reactions. This vulnerability is mainly attributed to the presence of a high proportion of MUFA and PUFA in oat oil. So, we examined the oxidative stability of crude oat lipids together by comparing the two stability tests Rancimat and the Schaal oven test. Additionally, we determined whether tocols, known for their antioxidant properties, could extend the stability of oat oils. The outcomes of the Schaal oven test were remarkable, with all samples demonstrating an induction period of more than 50 days. On the other hand, Rancimat test, the induction period of Saul was longer than others, 9.02 h at 100°C. Additionally, our study confirmed that oats indeed contain significant tocols (Sanitini with 155.2 mg/kg of oil). Our study has found that the highest tocol content in oats correlates with greater stability in Rancimat test.

On the other hand, wheat is a highly demanded cereal throughout the globe. A substantial portion of the global population relies on daily diets composed of food made from wheat. During the past few decades, various publications have suggested consuming whole grain food products due to their positive health impacts. Therefore, our study aims to analyse the nutritional composition of coloured spring wheats in comparison with winter wheats, with a particular focus on bioactive phytochemicals, including carotenoids, phenolic compounds, and tocopherols. Coloured wheats are carotenoid-rich, mainly lutein (over 80% of carotenoids). Ferulic acid dominates among analyzed colored wheat varieties. Beta-tocotrienol is the main vitamin E form. Among the six coloured wheat varieties studied, we found 30-36 mg/kg DW of tocols, 5-21 mg/kg DM of carotenoids, and 94-197 mg/kg of phenolic compounds in the samples. Spring wheat has higher tocols and carotenoids content, while winter wheat has more

phenolic compounds. The potential health advantages of consuming whole grains could stem from the phytochemicals within them.

Most of the wheat grains are consumed in the form of bread/bun, which is popular in every part of the world and constitutes a significant portion of daily diets. Sensory analysis of buns is essential as it allows to us understand how these phytochemicals in coloured wheat may influence the sensory attributes of final products. Linear unstructured graphical scale was used for sensory profile evaluation of descriptors focused on overall appearance liking, pleasantness of colour, the intensity of colour, overall taste liking, overall intensity of taste, intensity of sweet taste, intensity of salty taste, and overall sample acceptance. The results showed variations in preferences and perceptions among the assessors for different bun samples. AF Oxana (winter wheat) bun received higher scores in most attributes, while AF Jumiko had the lowest scores in several attributes. Tercie (spring wheat) bun scored highest for overall appearance liking. Last but not least, winter wheat affects colour and appearance attributes, while spring wheat influences taste attributes.

Keywords: oat, oil stability, lipid composition, tocols, wheat, carotenoids, phenolic compound

ABBIBRATIONS

AVAs: Avenanthramides

BHT: Butylated Hydroxytoluene

BMI: Body Mass Index

CVD: Cardiovascular diseases

C16:0: Palmitic Acid

C18:0: Stearic Acid

C18:1: Oleic Acid

C18:2 cis-9,12: Linoleic acid

DAD: Diode Array Detector

DM/ DW: Dry Matter/ Dry Weight

EFSA: European Food Safety Authority

FAOSTAT: Food and Agriculture Organization Statistical Division

FDA: Food Drugs Administration

FLD: Fluorescence Detector

GC: Gas Chromatography

GC-MS: Gas Chromatography-Mass Spectrometry

HCL: Hydrochloric Acid

HPLC: High-Performance Liquid Chromatography

HPLC-TOF-MS: High-Performance Liquid Chromatography-Time-of-Flight Mass Spectrometry

IP: Induction period

ISO: International Organization for Standardization

IWGSC: International Wheat Genome Sequencing Consortium

LOD: Limit of Detection

LOQ: Limit of Quantitation

LPS: Lipopolysaccharides

MPOD: Macular Pigment Optical Density

MUFA: Monounsaturated Fatty Acids

NaOH: Sodium Hydroxide

NO: Nitric Oxide

N(ω)-3: Omega-6

N(ω)-6: Omega-6

PEG: Polyethylene Glycol

PF: Protection Factor

PLs: Polar Lipids

PUFA: Polyunsaturated Fatty Acids

PVDF: Polyvinylidene Fluoride

LDL: Low-Density Lipoproteins

OBG: Oat Beta-glucan

RPM: Revolutions Per Minute

RWC: Relative Weight Change

SFA: Saturated Fatty Acids

SCFAs: Short-chain fatty acids

SMC: Smooth Muscle Cells

TAG: Triacylglycerol

tBME: Tert-Butyl Methyl Ether

TBHQ: Tert- Butyl Hydroquinone

TKW: Thousand Kernel Weight

UFA: Unsaturated Fatty Acids

UV: Ultra Violet

YPC: Yellow Pigment Content

YI: Yellow Index

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CHAPTER 1

Introduction

1 LITERATURE REVIEW

1.1.1 Oat

Oat (*Avena sativa* L.) is a cereal crop belonging to the family Gramineae (Poaceae). It has three ploidy levels: diploid, tetraploid, and hexaploid. The actual information about the origin of oat has yet to be discovered, and it probably originated in the Mediterranean basin or the Middle East (Rines et al., 2006). Oats are broadly divided into two categories, hulled oats (*Avena sativa* L.) and naked oats (Avena nuda L.). Hulless oats, which are also known as naked oats, are believed to be originated in China. Naked oats were migrated from China to Europe, and during the mid-seventeen-century, cultivation was started in Britain (Burrows, 2011).

The precise data regarding the time and location of oat domestication are lost in antiquity (Coffman, 1961). However, some records indicate oats have been cultivated since the Bronze Age (Stewart and McDougall, 2014). Oat was domesticated much later than wheat and barley (Murphy and Hoffman, 1992). Before domestication, it was considered a weed in other cereals like wheat and barley (Barker, 1985). Oat is annual grass cultivated in a temperate, cool, subtropical environment. It can be used as an essential nutritious foodstuff for human consumption as well as animal husbandry as forage, fodder, straw for bedding, hay, silage, and chaff (Mushtaq et al., 2014). Previously, oat was mainly used as animal feed, for instance, horse feed.

Oats have superior adaptability to different soil types and can perform better on acidic soils than other small-grain cereal crops. They are primarily grown in cool, moist climates, and they can be susceptible to the adverse effects of hot and dry weather, starting from head emergence and continuing until maturity. Due to these reasons, world oat production is generally focused within the latitudes of 35 to 65°N and 20 to 46°S. Most of the oat's global production comes from spring-sown cultivars, although autumn sowing is common in higher altitude regions (Ahmad et al., 2014). Oats have multiple purposes, including human consumption, forage, and fodder. Additionally, oats are grown to produce straw for bedding, hay, haylage, silage, and chaff. Oats are an important winter fodder, often fed as green, but the surplus is converted into silage or hay, which is used during periods of fodder shortage. They are preferred as feed for all animals, including horses, dairy cows, poultry, and young breeding animals, due to their soft straw and valuable grain. (Suttie and Reynold, 2004; Morey, 1961)

Oats serve various purposes, including human consumption, forage, and fodder. They are grown to produce straw for bedding, hay, haylage, silage, and chaff. Oats are an essential winter

fodder, fed green, or converted into silage or hay during fodder shortages. They are preferred as feed for all animals, including horses, dairy cows, poultry, and young breeding animals, due to their soft straw and valuable grain.

However, nowadays, oats have been considered a functional food because it provides beneficial health effects for consumers and decreases the risk of various diseases (Sterna et al., 2016). Over the last decade, public attention to oats has grown as a portion of healthy food due to their biochemical components like dietary fibres, polyphenols, proteins, and multiple functional compounds (Boukid, 2021). Oats are primarily produced in Russia, Canada, Poland, Finland, Australia, the USA, Spain, the UK, Sweden, and Germany. The global oat production was 23 million tonnes in 2019 (FAOSTAT, 2021).

Husked and naked oats are the same species. Husk oats are surrounded by lemma and palea called 'hull.' Whereas in naked oats, the lemma is lesser lignified than in hulled oats, and the lemma is thinner and less rigidly curved around the seed; thus, the kernel becomes free from the husk during harvesting. Oat husks have very low digestibility compared to the kernel, meaning husked oats have lower nutritional value than other cereals. In the process of husk removal from the husked oats, it requires a considerable energy input (Rines et al., 2006; Hackett, 2018).

Compared to other cereals, naked oats are generalized to have a more significant amount of crude fat and total protein and a smaller amount of crude fibre (Biel et al., 2009). Moreover, the protein value of naked oats cultivars is higher than other cereals, whereas lysine is a limited amino acid (Petkov et al., 2001). Husked oat contains a higher amount of fibre which decreases energy availability, especially for non-ruminants, and this is the reason that limits the value of oats as an animal feed. Naked oats can address this problem because it has high energy, lipid, protein, and low fibre content (Givens et al., 2004). Furthermore, the presence of a husk tends to reduce the bulk density of husked oats, which also increases the transportation cost compared to naked oats. However, the presence of a husk can avoid damage to the kernel. Hackett (2018) concluded that in comparison to husked oats, naked oats have a similar or higher potential to produce kernel yield.

1.1.2 Oat structure and chemical composition

The oat grain is a complex matrix containing a protective hull and the groat (caryopsis) and comprises the bran, germ, and starchy endosperm (Figure 1). The bran is a coarse outer layer

of the groat, rich in minerals, vitamins, and cell wall polysaccharides, primarily cellulose, arabinoxylan, and β-glucan (Mälkki and Virtanen, 2001 Grundy et al., 2018).

The protein content in husked oats ranged from 10.5 to 15.7%, while fat content varied from 5.1 to 9.6%. The starch content in husked oats was reported at 48% compared to 31.5% in naked oats. For vitamin E, husked oats contained 7.8 mg/kg, while naked oats had 9.5 mg/kg. Additionally, the total dietary fibre was found to be 17.6 g/100 g in husked oats and 22.9 g/100 g in naked oats. Moreover, husked oats had 14.3 g/100 g of soluble dietary fibre, whereas naked oats had 17.6 g/100 g. Finally, β–glucans were present at a level of 3.1 g/100 g in husked oats and 3.3 g/100 g in naked oats (Sterna et al., 2016). Oats are rich in abundant dietary fiber, especially soluble β-glucans, which is a distinguishing feature of oats in comparison to other cereals. Other valuable micro-components, such as phenolic compounds, are also present in oats; among them, avenanthramides have garnered significant attention (Webster, 2016). Concerning the human diet, starch is the major glycaemic carbohydrate. Moreover, the starch granules in oats are smaller than those in barley, corn, and wheat, ranging from 3 to 10 μm (Zhang et al., 2021).

Generally, oats have well-balanced amino acid composition without anti-nutritional factors. Oats' protein comprises globulin, albumins, prolamins, and glutelins (Klose and Arendt, 2012). Compared to other cereals like corn and wheat, oat protein is considered more nutritious, with adequate essential amino acid content (Mohamed et al., 2009). Prolamins are the most dominant amino acid in most cereals, such as wheat, barley, and rye, whereas the prolamin content of oats is only 4-15 % of total protein. Compared to other cereals, the consequence of low-prolamin and high-globulin in oats provides balanced amino acids essential for monogastric animals and humans (Klose and Arendt, 2012).

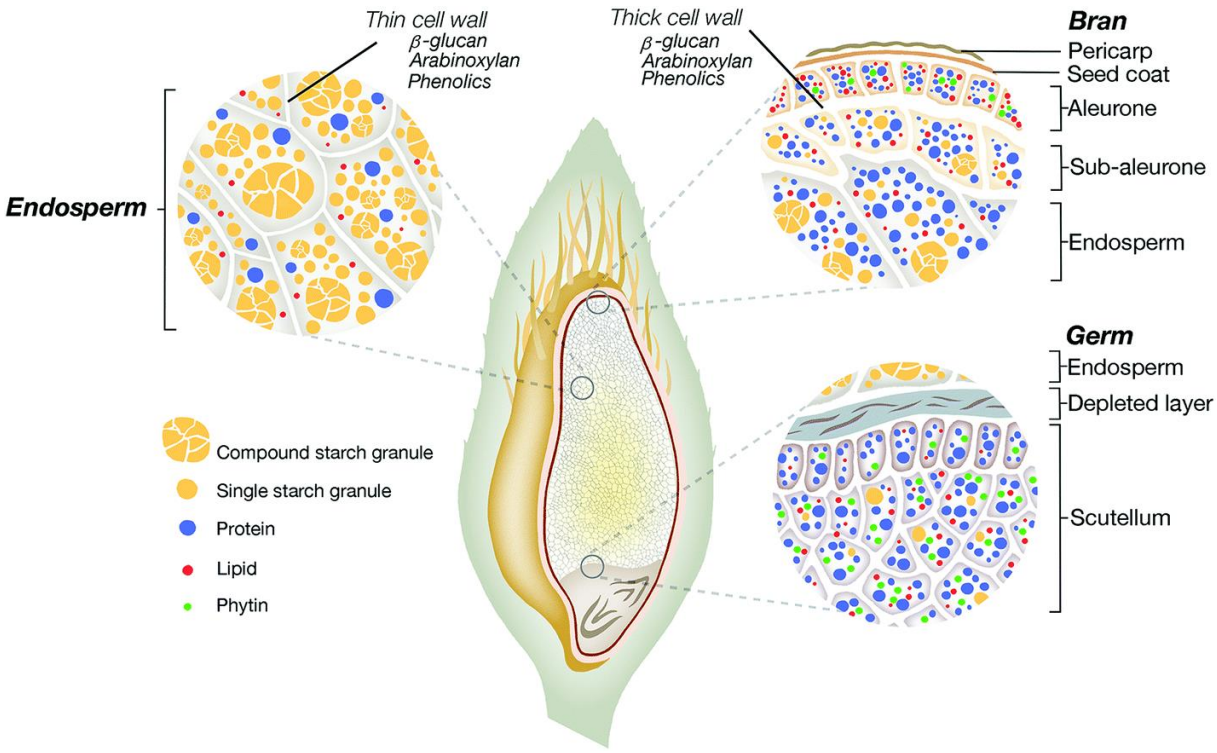


Figure 1: Structural representation of the oat grain and the nutritional distribution (Grundy et al., 2018)

Oat is one of the richest and most economical sources of soluble dietary fiber. Research has found that oat dietary fiber effectively lowers cholesterol and blood sugar and prevents various diseases (Drozdowski et al., 2010 and Tiwari and Cummins, 2011). After the decision, the Food Drugs Administration (FDA) in January 1998 issued its final rule allowing a health claim to make on the labels of foods containing soluble fiber from whole oats, the popularity of oatmeal and other oat products also increased. Whole oats products can provide up to 3 grams of soluble dietary fiber daily. Beta-D-glucan, a nondigestible polysaccharide, is the principal component of the soluble fiber in whole oats. In cereals (oat and barley), beta-glucan is composed of mixed-linkage (1,3) (1,4) -β-D-glucose units (Di Luzio et al., 1997) and (Tohamy et al., 2003). Oat is a significant source of β-glucan. Brunava et al. (2014) reported the content of β-glucans 3.15 and 3.29 g/100 g in the studied husked and naked oat varieties, respectively. The β-glucan content in oat ranges between 2.3 to 8.5/100 g (Welch et al., 2000), while oat groats or dehulled oat contain 3-5% of β-glucan (Anttila et al., 2004). Oat β-glucan is present in the oat kernel, which is a viscous polysaccharide made up of units of the monosaccharide D-glucose. The husk content of oat grains is about 25-30% of the seed. Oat kernel contains about 85% of insoluble dietary fiber in its unprocessed state. Oat hull can be further processed to extract more than 90% of insoluble dietary fiber (Butt et al., 2008 and Regand et al., 2011). In the remaining edible part, 6-9% of dietary fiber can be found, about half of which is insoluble fiber. The

principal component of soluble oat fiber is a linear polysaccharide (1,3), (1,4) -β-D-glucan, usually called β-glucan, which is located in the endosperm cell wall (Daou and Zhang, 2012). Moreover, 100 grams of oats contain abundant quantities of several minerals: calcium (53.85 mg), iron (4.73 mg), magnesium (176.92 mg), phosphorus (523.08 mg), potassium (428.85 mg), sodium (1.92 mg), zinc (3.97 mg), copper (0.62 mg), and manganese (4.92 mg). One hundred grams of oats contain the following vitamins: thiamine (0.76 mg), riboflavin (0.14 mg), niacin (0.96 mg), pantothenic acid (1.35 mg), vitamin B-6 (0.12 mg), and folate (55.77 μg) (Sangwan et al., 2014). In oats, vitamin E ranged from 4.5 to 12.3 mg/kg (Sterna et al., 2016). black, white, and yellow-coloured oats had mineral content where iron was 2.5-3.0 mg/kg DM, copper 0.2-0.4 mg/kg DM, zinc 1.6-2.0 mg/kg DM, magnesium 62.4-89.1 mg/kg DM, calcium 44-102.7 mg/kg DM, and potassium 241.7-258.3 mg/kg DM (Alemayehu et al. 2021). Oat contains 20-30 μg/100 g of folate and 10-15 μg/100 g of biotin. The mineral content of oat is 2-3% (Sami et al., 2014).

Table 1: Composition of oat grain (Sterna et al., 2016)

Parameters	Husked oats	Naked oats
Protein content, %	10.58 ± 0.67	15.71 ± 1.10
Fat content, %	5.15 ± 0.19	9.66 ± 1.17
Starch, %	48.08 ± 0.29	31.55 ± 3.72
Vitamin-E, $mg.kg^{-1}$	7.80 ± 2.36	9.50 ± 2.11
Total dietary fibre, $g.100\ g^{-1}$	17.63 ± 1.52	22.97 ± 1.89
Soluble dietary fibre, $g.100\ g^{-1}$	14.32 ± 1.89	17.63 ± 3.11
β–glucans, $g.100\ g^{-1}$	3.15 ± 0.19	3.29 ± 0.26

1.1.3 The health benefits of consuming oats

High levels of serum cholesterols and low-density lipoproteins (LDL) are known to increase cardiovascular diseases (CVD); consuming oats have shown to reduce serum total cholesterol and LDL levels, thereby reducing the risk of CVD (Whitehead et al. 2014). Beta-glucan-rich oats or oat-based products have been found to reduce blood lipid levels and blood pressure by regulating insulin metabolism in a mild hypercholesterolemia subject. Vicious oat beta-glucan slows down the absorption of macronutrients in the digestive tract, leading to reduced insulin response and postprandial blood glucose, ultimately lowering blood pressure (Anderson et al. 1990, Davidson 1991, Dreher and Dreher, 2018).

Obesity can risk health complications like CVD, musculoskeletal disorders, diabetes, and cancers. Studies show that dietary fiber and whole grain intake are crucial in weight loss. Consuming oat products rich in oat Beta-glucan (OBG) creates a sense of satiety and stomach fullness, curbing hunger. OBG's viscous and hydrating properties contribute to delayed gastric emptying, thereby reducing overall body weight and body mass index (BMI) and central adiposity (WHO, 2021; Rebello et al., 2016; Chang et al., 2013). Additionally, Type-2 diabetes has been increasing due to unhealthy diets rich in refined grains, red and processed meat, and added sugar-containing beverages. Numerous studies have shown that OBG lower postprandial glucose levels. The European Commission has approved a health claim regarding the potential of OBG to reduce postprandial glycemia. According to this claim, one should consume 4 g of OBG for every 30 g of available carbohydrates per meal (Bowman et al., 2011; EFSA, 2011).
Celiac disease (CD), also referred to as gluten intolerance, is an immune-mediated gastrointestinal disorder induced by the consumption of dietary gluten or related proteins in genetically susceptible individuals (Fasano and Catassi, 2012). Gluten is made up of glutamine and proline, which is a storage protein present in grains, which is difficult to digest in the upper gastrointestinal tract and can damage the small-intestinal mucosa. The European Commission Regulation No. 41/2009, of 20 January 2009, states that oats can be included in a diet of gluten allergies patient. A study reported that celiac patients who consumed an average of 24 g of an oat-based diet daily for 8 years found no damage to the small-bowel mucosal villous damage, inflammation, or gastrointestinal symptoms (Vassiliou 2009, Kaukinen et al., 2013).
Positive links between the biologically active compounds of oats and a reduced risk of cancer have been highlighted (Martínez-Villaluenga and Peñas, 2017). The cancer-preventive properties and anti-tumor activity of OBG were observed against epithelial lung cancer, skin cancer cells, and colon carcinoma (Choromanska et al.,2015; 2018; Shen et al., 2016). Both beta-glucan (soluble and insoluble) have been shown to reduce faecal bile acid levels, promote the production of short-chain fatty acids, and induce apoptosis in precancerous cells in mice, helping to prevent colon carcinoma (Shen et al., 2016). Avenanthramides have demonstrated attenuating effects in an in *vitro* study, suggesting that the consumption of oats and oats bran could reduce the proliferation of colon cancer cells (Guo et al., 2010).
Oats are rich in antioxidants, including vitamin E (tocols), phytic acid, phenolic compounds, flavonoids, sterols, and avenanthramides. Tocols can help prevent chronic disease, stroke, cancer, CVD, and premature aging (Peterson, 1995 and Skoglund et al., 2008). Regardless of its high or low molecular weight, the aqueous extract of OBG has demonstrated anti-inflammatory effects in enteritis induced by lipopolysaccharides (LPS) (Suchecka et al., 2015 and Wilczak

et al., 2015). OBG has reduced colon tissue damage and disease activity index. Furthermore, OBG has effectively reduced lipid peroxidation and inflammation caused by exercise (Liu et al., 2015).

The supplement of high and low molecular weight of OBG beneficially reduces oxidative stress in mice with lipopolysaccharide (LPS)-induced enteritis which indicated the antioxidant properties of oats. High molecular weight OBG creates an environment that aids in the regeneration of the mucosal membrane. Hence it lowers oxidative stress (Wilczak et al., 2015). It has been reported that more than 25 Avenanthramides (AVA) compounds are present in the oats, and 2C, 2F, and 2P are the most abundant. Among them, 2C has the highest total antioxidant capacity (Yang et al., 2014). Whole oats contain distinctive phytochemicals like high levels of beta-glucan, phenolics, and lipids, which are beneficial for gut health. In *vitro* fermentation, OBG interestingly produces short-chain fatty acids (SCFAs), primarily propionate. These SCFAs can be either reabsorbed into the circulation or utilized by other microbes (Shen et al., 2012; Hughes et al., 2007; Myhrstad et al., 2020).

Synthetic AVA dramatically can inhibit the proliferation of smooth muscle cells (SMC) and increase nitric oxide (NO) production which is crucial for the development of atherosclerosis. Additionally, consuming oat bran and atorvastatin can reduce atherosclerosis (Nie et al., 2006). Overall, oats are a promising functional food with several health benefits.

1.1.4 Lipid content of oats

Lipids are a structurally diverse group of molecules soluble in organic solvents (Ahmed et al., 2016). They consist of triacylglycerol, formed by esterification of three fatty acids with alcohol and glycerol molecules. Fatty acids are considered the 'building blocks' of lipids (Lee, 2015). Lipids can be broadly divided into "simple" (e.g., fatty acids, sterols, and acylglycerols) and "complex" groups (e.g., glycerophospholipids and glycosphingolipids) based on hydrolysis products (Fahy et al., 2005). Two primary classes of lipids are polar lipids, mainly composed of phospholipids, and neutral lipids, mainly composed of triacylglycerols, monoacylglycerols, and diacylglycerols (Henderson and Tocher, 1987). Fats and oils serve as energy reserves for plants and animals.

Typically, cereal grains contain a low amount of oil, predominantly concentrated in the embryo and scutellum (Alexander and Seif, 1963). In comparison to the other cereals, oats are more abundant in oil ranging from 3 – 11% (Frey and Holland, 1999), with notable quantities of total

protein while exhibiting a relatively lower amount of crude fiber (Biel et al., 2009). Interestingly, unlike most cereals, the maximum portion of oat grain oil has been claimed to reside in the endosperm. Generally, oats contain approximately 5-6% oil and 55-60% starch within the grain (Doehlert et al., 2001). It has been noted that a negative correlation exists between higher fat content and starch content. The microscopic investigations found a higher fat content in endosperm in the high-oil oat varieties (Banaś et al., 2007).

The oat groats oil is desirable for human consumption, which has good flavour and is comparably stable (Holland et al., 2001). The proportion of oil contained in oat (3-11%) is higher than in other cereals like wheat (1.4-1.5%) and barley (1.3-1.8%) (Ciołek et al., 2012). Some research suggests oat contain up to 18% of oil (White et al. 2006). However, the most widespread hulled oat cultivar contained around 5-6% of oil; conversely, the naked oat generally contains 9% of oil (Sterna et al., 2016). Oat oil is a rich source of essential polyunsaturated fatty acids (Biel et al., 2009). Additionally, several researchers investigated the fat content of oats 7-11% (Banaś et al., 2007), 5.9 - 7.9 % (Krasilnikov et al., 2018), and 4.1-8.3% (Leonova et al., 2008). These research suggest that oats are a better oil source than other cereals. The fat content of yellow and black-coloured hulled oats differs.

The distinguishing feature of husked and naked oats was fat content. Naked grain oats contain significantly higher fat content than other feed cereals, with an average of 7.9%, and they are rich sources of UFA (Zhou et al., 1998; Aro et al., 2007). Biel et al., (2009), found the crude fat level in naked grain around 8.4% DM, almost twice as high as that of oat with husk. Similarly, oat contained 5.91 - 7.87 % lipid, and the average was 6.9 % (Batalova et al., 2019). According to a study conducted by (Antonini et al., 2016) the average lipid content in 15 types of husked oats was recorded as 8.35 g/100 DM, while the average lipid content in an equal number of naked oats was 8.40 g/100 DM.

1.1.5 Oat lipid composition

The primary fatty acids in oats are palmitic (C16:0) ranged from 10.8% to 22.4%, oleic (C18:1) ranged from 19.6% to 37.9%, and linoleic acid (C18:2) ranged from 18.9% to 54.0%, which accounts for 90-95% of the lipid profile (Halima et al., 2015).

The capacity to accumulate the maximum proportion of oil in the endosperm of oat is high. On the other hand, maize is the only cereal with high fat content, but most of its oil is accumulated in the embryo (Leng, 1961). Moreover, the research conducted by Iowa State University showed that oat oil was found in a range of 6.9-18.1%; these high-oil oats were obtained from

the recurrent selection breeding regime. The study shows that oat fat content and tocotrienol concentration, mainly located in the endosperm, were correlated (r2=0.83), whereas the tocopherol concentration was not correlated with the fat content. The amount of protein and β-glucan concentration increased, but the starch concentration decreased with the increasing oil concentration (Peterson and Wood, 1996).

Fifteen different cultivars of *Avena sativa* and three cultivars of Avena byzantine were chosen to evaluate grain yield and nutritional quality of different climatic conditions in the years 2004 and 2005. All tested variables were influenced mainly by the environment than by genotypes (Martínez et al., 2010).

For the first time, Banaś et al. (2007) provided the characterization of the lipid deposition while the grain is developing in various parts of oat grain tissue by using two kinds of cultivars, one high oil (10% oil in Matilda cultivar) and another medium oil (6% oil in cultivar Freja). They concluded that about 86-90% of lipid was stored in the endosperm, as shown by the chemical and microscopical analyses (Heneen et al., 2009; Banas et al., 2007). Nearly 84% of oil deposits while the grain develops when the seed is still a milky endosperm. These two research shows the microscopic study of oil bodies fused in the starchy endosperm, which are less associated with proteins. Once the seed matured, it formed smears of oil distributed in the embryo, scutellum, and aleurone layers. Consequently, Heneen et al. (2009) explained that the oil is mainly stored close to the enzyme production sites related to mobilization and germination.

Oats are rich in MUFA oleic acid and PUFA linoleic acid, which account for around 40% and 36%, of the total oil respectively (Zhou et al., 1998; Ben Halima et al., 2015). Several authors reported the proportion of dominating fatty acids, which is given in Table 2.

Approximately 75% of the total fatty acids present are unsaturated, while palmitic acid constitutes the main saturated fatty acid, accounting for 14-17% of the total content (Saastamoinen et al., 1989). The proportion of saturated fatty acids in oat grains was between 15.6% and 34.4%, while the unsaturated fatty acid content ranged from 65.6% to 84.2% (Ahmet et al., 2019).

Similarly, in a study by Kan et al., 2015 the composition of oat grain oil was found to contain 16.8% palmitic acid, 40.55% oleic acid, 38.54% linoleic acid, 1.8 % steric acid and 0.9% linolenic acid. Generally, oil rich in linoleic acids is considered unsuitable for oil-food products because of the higher chances of autoxidation which increase instability and can form off flavour. Interestingly, the oil's quality and intended use are determined by the content of oleic acid and linoleic acid.

In addition, other fatty acids contribute to the composition, including stearic acid at 1% to 1.9%, erucic acid at 0.7% to 1.1%, and α-linolenic acid at 1.1% to 1.7%. Compared to other vegetable oils, oat oil contains higher levels of saturated fatty acids, specifically ranging from 16.9% to 19.6%. In contrast, sunflower oil contains 10% to 12% saturated fatty acids, rapeseed oil contains 4% to 6%, and linseed oil contains 8% to 11% and 14% to 20% saturated fatty acids (Krasilnikov et al., 2018). Furthermore, the ratio of oleic acid to linoleic acid in oat oil is almost equal, placing oat oil in the oleic-linoleic group. Oat grain possesses a diverse range of lipases and lipoxygenases, so studying the changes in lipid composition during grain storage would be a promising area of research (Youngs, 1986). Surprisingly, the human body lacks efficient systems for the chemical conversion of compounds from one essential fatty acids family to another; the nutritive properties of oat lipids were determined based on the metabolic pathways of linoleic acids n-6 family essential fatty acids. Linoleic acid, including the fatty acids of the n-6 family, is an important compound as a precursor of hormonal compounds. On the lipid profile of the blood serum of diabetes patients, linoleic acid has been shown to have a positive effect; the deficiency can risk the development of atherosclerosis. The recommended linoleic acid consumption is around 5-8% of total caloric intake (McDonald and Fitzpatrick, 1999; Krasilnikov et al., 2018). Moreover, oat oil comprises a more significant part of TAG and a lesser part of polar lipids (PLs). When 33 assessions from 10 wild species and 13 assessions of cultivated oats were analyzed, wild oat was found to be having 18:1 higher proportion of fatty acid and fat content than cultivated oats. Interestingly, they found unusual FAs in cultivated oats, including 15-hydroxy 18:2 and novel 7-hydroxy hexadecanoic acid (Leonova et al., 2008).

Table 2: Dominating fatty acids in oats

General information	Palmitic %	Oleic %	Linoleic %	Source
Australian oats	17–19.3	37.9–41.1	36–39.2	(Zhou et al., 1998)
Six spring oat cultivars	21.4–22.8	30.7–1.5	36–38.2	(Kouřimská et al., 2018)

Conventional cropping system (In average)	18.76	36.21	38.75	(Capouchová et al., 2021)
Organic cropping system (In average)	18.56	36.49	38.85	(Capouchová et al., 2021)
	15.5–17.4	37.2–42.1	38.6–42.5	(Saastamoinen et al., 1989)
Five oat genotypes	15.5-17.4	36.2–40.4	38.4–41.6	(Sterna et al., 2016)
Fifteen cultivars in two crop years	16.2-21.8	28.4-40.3	36.6-45.8	(Youngs and Püskülcü, 1976)
Five naked oats	15.3–17.8	33.5–36.7	35.9–38.7	(Batalova et al., 2019)

1.1.6 Health perceptive of oat lipids

The nutritional quality of oats, including maintaining and optimizing fatty acid composition, is a key component of oat breeders in response to the growing awareness of the health-promoting of oats (Valentine et al., 2011).

Oat oil fatty acid composition is a primary concern regarding its effect on human health, mostly cholesterol levels. Higher serum cholesterol level is contributed by saturated fatty acids like palmitic (16:0). Generally, a diet rich in unsaturated fatty acids and low in saturated fatty acids, cholesterol level, and trans-fatty acids is fit for human consumption (Holland et al., 2001). On the other hand, an increase in the proportion of polyunsaturated fatty acids increases the chances of oxidation, leading to rancidity and undesirable flavour.

There is a relation between the fatty acid composition and the dietary indices of atherogenicity and thrombogenicity. Atherogenicity and thrombogenicity show a relationship between saturated and unsaturated fatty acids (Connor, 2000). Furthermore, the leading factor of mortality is cardiovascular diseases. Generally, saturated fatty acids increase low–density

lipoprotein (LDL) cholesterol, which is a risk factor for cardiovascular disease. In contrast, monosaturated fatty acid and polyunsaturated fatty acid-rich food are subjected to hypocholesterolemic potential. The recommended saturated fat intake should be less than 10% of daily energy consumption. However, several countries consume more SFAs than the recommended value, such as Canada at 10.4%, the USA at 11%, and European at 15.5%.

Additionally, the Western diet is characterized by a high intake of n-6 compared to n-3, in which the dominant PUFA is linoleic acid (15:1-20:1) (Liput et al., 2021). Gebauera et al. (2005) suggested that a diet rich in steric/palmitic and oleic/stearic and a decrease in the ratio of n-6/n-3 would prevent several diseases. The ratio of n-6/n-3 in oats was 25:1, whereas the recommended ratio is less than 4:1, indicating that oats contain high linoleic acid (Ma Jiang et al., 2016; Kouřimská et al., 2018). Additionally, the atherogenicity index of oat was only 0.17-0.19. A typical Western diet has higher levels of n-6, with a 10-30:1 ratio of n-6/n-3 (Ma Jiang et al., 2016).

The fatty acid composition of oat lipids is gaining great interest from nutritionists as well as oat breeders due to the nutritional significance of fatty acids. Different individual fatty acids (FA) have different impacts on human health. In scientific literature, the ratio of fatty acids is generally used to compare the potential impact of fats on human health. The ratio of the sum of polyunsaturated fatty acids to the sum of saturated fatty acids (PUFA/SFA) is most commonly used, with the World Health Organization (2003) recommending a PUFA/SFA ratio higher than 0.4 for optimal health. Decreasing the ratio between n-6 and n-3 fatty acids, as well as increasing the ratio between stearic/palmitic acid (C18:0/C16:0) and oleic/stearic acid (C18:1/C18:0), is considered desirable for the prevention of various diseases (Gebauer et al., 2005 and Sterna et al., 2014).

The ratio of saturated fatty acids: monounsaturated fatty acids: polyunsaturated fatty acids is the key factor which determines the health index of the lipids; and the ratio of these fatty acids in oats is 0.5: 1: 1; which is similar to the current market standard of 0.7:1:1 (Yang et al., 2019). This similarity in the fatty acid ratio allows for the development of high-quality edible oil. Oleic acid, classified as a monounsaturated fatty acid, has a stronger oxidation stability than polyunsaturated fatty acids (Halima et al., 2015). Interestingly, it can lower the low-density lipoprotein cholesterol level without reducing the beneficial high-density lipoprotein cholesterol (Kolar et al., 2019). Oat bran is rich in linoleic acid, which benefits the elderly and middle-aged people (Dach and Schieberle., 2021). Linoleic acid is an essential fatty acid which is played an integral role in prostaglandin synthesis. Various study has documented that this

fatty acid can improve blood microcirculation, reduce blood cholesterol and triglyceride levels, and enhance memory and cognitive abilities (Heneen et al., 2009; Tong et al., 2014). Oat is rich in important phenolic compounds, are ferulic acids, caffeic acid, and avenanthramides (Tian et al., 2020). These antioxidants effectively hinder the oxidative rancidity of oat oil and scavenge free radicals, thereby contributing to delaying aging and potential anti-cancer properties (Chen et al., 2016; Tian et al., 2020). In fact, oat oil has superior antioxidant capacity compared to tert-butyl hydroquinone (TBHQ), vitamin C, and Trolox (Tian et al., 2020).

1.1.7 Oat tocols

Tocols are collective forms of tocopherols and tocotrienols, which are phenolic compounds and lipid-soluble compounds found in cereals and other sources. They are commonly referred to as vitamin E. They are monophenols with four homologues – alfa, beta, delta, and gamma. The number and the location of methyl groups differ from each other (Kamal-Eldin and Appelqvist, 1996; Shahidi et al., 2016). Tocols have antioxidant activities that significantly protect MUFAs and PUFAs against their oxidation (Shahidi et al., 1996). Tocopherol's side chain is fully saturated, whereas tocotrienols have three double bonds in the phytyl side chain. Tocols possess the ability to inhibit lipid oxidation in cells, which makes them valuable for stabilizing food products. Tocols stability may be influenced by the fatty acid composition, the storage and processing, and the cooking procedure (Rossi et al., 2007; Shin et al., 2009 and De Camargo et al., 2012).

Antioxidants play a crucial role in promoting health by countering the harmful effects of free radicals, which can risk membrane damage, heart disease, aging, and cancer in living organisms (Halliwell, 1999). The quality and concentration of lipids may entail different concentrations of specific antioxidants. Different antioxidants function through diverse mechanisms, which can result in synergistically or antagonistically, therefore influencing the overall antioxidative capacity. When it comes to food preservation and health benefits, knowledge about how plants optimize different antioxidants in tissue with varying lipid concentrations, and compositions is important. (Shahidi, 1997 and Bryngelsson et al., 2002). Because of being a lipophilic compound, tocols are closely associated with the lipid components in the sample matrix. To analyze these compounds, sample preparation should involve either solvent extraction or saponification using hydrolysis (Panfili et al., 2004). Tocols can be quantified by using HPLC or GC (Balz et al., 1993; Abidi and Mounts, 1997). In GC analysis, sample saponification is generally performed to remove the acyl lipids, which can interfere with the analysis process.

However, in HPLC, this step is not critical; separation can be achieved using reversed-phase systems with fluorescence detection. Generally, it may not always be necessary to separate all eight vitamer species in foods because they do not contain the complete range of vitamers. Meanwhile, the separation of all vitamers is desirable for cereal samples. (Radaelli et al., 2004; Kamal-Eldin et al., 2000 and Ryan et al., 2007).

α-tocotrienol was found to be predominant tocols in oats, followed by α-tocopherol and other β-homologues were found in smaller amounts. In Italian trials, the accumulation of tocols was influenced by the location of oat cultivation (Radaelli et al., 2004). The effects of food processing on tocols concentration have been largely neglected. In rolled oats, the total tocol concentration was reported as 32 mg/kg, with a similar distribution of vitamers to that of oat grains, whereas in puffed oats, the α-tocotrienol was found in a lower concentration. The concentration of tocols was observed to degrade during storage at room temperature, and the degradation rate was enhanced by exposure to air (Piironen et al., 1986).

The concentration of tocols in oats is in the range of 13.6–36.1 mg/kg (Peterson, 1995; Holasová et al., 1998 and Shewry et al., 2008); further, they are unevenly distributed in the oat kernels. Various factors influence the tools' level in the cereals like oats, such as milling, malting, baking, extrusion, cooking, and product development (Tiwari and Cummins, 2009). Oat germ is rich in tocopherols, whereas tocotrienols are absent in germ, and tocotrienols are mainly concentrated in the endosperm. Among oat tocols, the sum of α-tocopherols and α-tocotrienol account for 86–91 %. In unprocessed groats, tocols are stable for over seven months, while within one to two months, tocols are degraded in processed oats (Peterson, 2001). The tocol profile in hulls and groats is similar, but significantly higher concentrations are later observed (Bryngelsson et al., 2002). These distributions are significant since the tocols possess different biological activities. Generally, oats are consumed as a whole, so the uneven distribution of tocols within the oat kernel is important. A positive correlation was observed between the fat content and the tocotrienols across various oat varieties (Peterson and Wood, 1997). However, in some studies, a positive correlation between them was not observed (Peterson et al., 2007)

1.1.8 Factor influencing nutritional composition of oats

The nutritional value of oats presents a complex interplay of various elements, leading to a diverse chemical composition. The oat grain composition is influenced by factors such as genotype, growing environment, and their interactions, contributing to the variability in oat

composition. Additionally, differences arise between naked and husked oats. The oat composition is influenced by postharvest treatment and seasonal variations, as demonstrated by Kouřimská et al. (2018). Furthermore, research suggests that enhancing oat fatty acid composition is achievable through breeding techniques (Zhou et al., 1998). The proportions of fatty acids depend on factors such as extraction methods, cultivars, and storage conditions. The difference in the fat content and the fatty acid compositions may be attributed to the growth condition, genetic and climatic factors, harvest conditions, storage conditions, post-harvest treatment, and other treatments that can be done before final uses, and the variations in analytical procedures (Smouse, 1979; Green, 1986 Harris et al., 1980 and Singh et al., 1990). A look into tocols reveals that their concentration variance is subject to the influence of both environmental conditions and genetic factors (Peterson, 1995).

1.2 Spring wheat

1.2.1 Wheat origin and importance in the human diet

Wheat is a prehistoric crop providing energy requirements for the human diet worldwide. Wheat offers several end products at reasonable prices, so the demand for wheat is increasing gradually. Compared to other commercial crops, wheat is planted in more land areas. Approximately 8 to 10 thousand years ago, farmers of Fertile Crescent developed bread wheat from the einkorn grass and emmer (Shiferaw et al., 2013). In this region, the first "green revolution" for wheat started when people began to domesticate some species of wheat, including einkorn (*Triticum monococum*) and the emmer wheat (*Triticum turgidum* ssp. dicoccum) (Mac Key, 2005) which was later derived to modern bread and durum wheat. Bread and durum wheat cultivar have higher TKW (Thousand Kernel Weight) than emmer (Konvalina et al., 2008; Faris et al., 2014). Billions of people consume wheat daily; it is the major staple food in many diets and contributes to a major proportion of daily energy intake (Dinu et al., 2018). In the autumn season, winter wheat is sown, which develops slowly during the cold season (Thorup-Kristensen et al., 2009). On the other hand, spring wheat is sown in spring and harvested in late summer or early fall.

Additionally, wheat was originally from the Levant region (Eastern Mediterranean region of Western Asia); now, it is cultivated worldwide. Hexaploidy wheat is presumed to be originated from north-western Iran or north-eastern Turkey (Bonjean and Angus, 2001). Several scientists indicated that Iran, southwest of the Caspian Sea, was the hometown of f hexaploidy wheat. Several authors described the diffusion of wheat cultivars from Fertile Crescent to various parts of Asia, Europe, and Africa (Frankel, 1981; Kislev, 1984; Bar-Yosef, 1998). Fertile Crescent is well known for its diversified region, and the archaeological evidence suggests that the wild taxa of four species of wheat were first domesticated in this region.

Moreover, the wheat spread began in the 8th and 7th millennia BP (before the present). Among two routes from where the wheat was expanded in Europe, the first route was Anatolia to Greece (ca. 8,000 BP); later, it was expanded to Italy, southern France, and Spain (ca. 7,000 BP), which carried emmer, einkorn and a small proportion of tetraploid and hexaploidy wheat and the second route was from Transcaucasia and Caucasus, Southern Russia to Central Europe which includes mainly bread and club wheat (Bonjean and Angus, 2001). Similarly, wheat was spread in Africa in 6000 BP through several routes, including Egypt to Sudan, Ethiopia, and Libya; Greece to Crete and Libya; Italy to Tunis, Algeria, and Morocco. At the same time, the diffusion of wheat in Asia was from northern Iran. It reached western Pakistan in 6500 BP

(Jarrige and Meadow, 1980) after 1000 years in India. In China, wheat was expanded through the Silk Road (Zeven, 1980).

The majority of wheat produced in the world is *Triticum aestivum* (95%) which is a hexaploid species, also known as 'common,' 'bread,' or 'soft' wheat (IWGSC, 2014), and the remaining are generally Triticum durum also known as 'durum' which is a tetraploid species and generally used in making pasta. In 2021/22, the global wheat production was 778.6 million metric tons. China was the largest producer of wheat with 134.3 million metric tons, followed by India (107.5 million metric tons), Russia (85.9 million metric tons), the United States (49.7 million metric tons), and Canada (35.1 million metric tons).

1.2.2 Composition of wheat

Wheat (*Triticum aestivum*) kernels comprise endosperm 81-84%, bran 13-17% and 2-3% germ (Figure 3). Whereas the endosperm consists majority proportion of starch 60-75 %, protein 6-20 %, moisture around 10% and lipids 1.5-2% (Barak et al., 2015). The bran is the outer cover of the grain, composed of several layers, and it protects the inner part of the endosperm material. Bran is rich in minerals and vitamin B and it is separated from endosperm in the first stage of milling. More than half the amount of bran consists of water-insoluble fibre (53%), especially cellulose and pentosans, polymers based on arabinose and xylose; these are tightly bound to proteins. Further, the outer endosperm is called as aleurone layer. It is rich in proteins and enzymes and plays a major role in cell germination. Protein and carbohydrates each make up 16% of the bran's total dry matter. The mineral content is high, amounting to 7.2%. In comparison, the inner layer of endosperm is called mealy or starchy endosperm (Šramková et al., 2009). The mealy endosperm is dominated by carbohydrates, and other components like fats (1.5%), protein (13%): albumins, globulins, gluten complex, minerals ash (1.5%), and dietary fibre (1.5%) (Belderok et al., 2000). Moreover, the germ is composed of protein (25%) and lipid (8-13%) minerals (4.5%). Wheat germ is rich source of vitamin E (Šramková et al., 2009).

The chemical composition of wheat may affect the flour properties of dough kneading (water absorption rate); dough properties like hardness, viscosity, elasticity, extensibility, plasticity, and water retention; gluten network formation; and cooking characteristics such as shape retention, hardness, chewing viscosity and shrinkage (Park et al., 2009 Huang and Lai 2010; and Marchetti et al., 2012).

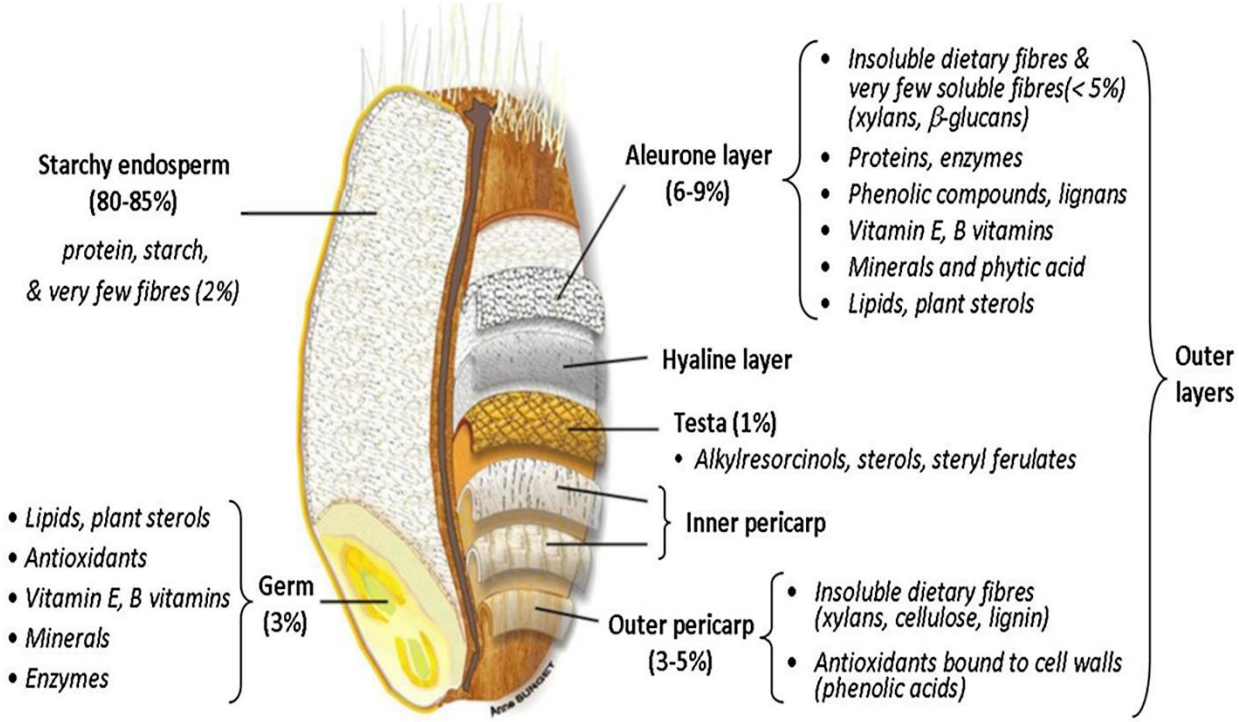


Figure 2: Histological structure of wheat grain (Barron et al., 2007)

The wheat protein can be divided into two classes: gluten and non-gluten. Non-gluten proteins contain water-soluble albumin and globulins which are insoluble in water but soluble to dilute salt solution. The proportion of non-gluten protein from the total protein is ca. 15-20% (Borght et al., 2005). The molecular weight of these proteins is mostly lower than 25,000 (Veraverbeke and Delcour, 2002); some may have up to 70,000. The rest of the proteins are gluten protein, i.e., 80-85% of total wheat protein. These proteins are insoluble in water. A strong, cohesive, viscoelastic network forms when it is hydrated and mixed, which is essential in dough making. Gliadins (molecular weight between 30,000 and 80,000) and glutenins make gluten proteins. Gliadins have single chains, and when hydrated, they become extremely sticky and are rich in glutamine and proline (Shewry, 2003).

Moreover, wheat contains 2.1% of minerals, mostly zinc, and iron, with some trace minerals like selenium and magnesium. It also contains some vitamins like thiamine (vitamin B1) and pantothenic acid (vitamin B5) (Kumar et al., 2011). The amounts of essential elements of wheat grain differ in the milling fractions. The amounts of potassium, phosphorus, and selenium are high in wheat grain and magnesium, and calcium is present in a smaller amount. Bulut (2022) investigated the concentration of minerals in 42 types of bread wheat varieties and found copper at 1.38 ppm, iron at 15.6 ppm, manganese31.1 ppm, selenium at 0.066 ppm, zinc 2.19

ppm, arsenic 0.041 ppm, cadmium 0.0064 ppm, cobalt 0.052 ppm, chrome 0.147 ppm, nickel 1.2 ppm and lead 0.185 ppm. Additionally, it has been reported that environmental and genotype factors affect the mineral content of wheat. The grain morphology of wheat influences the distribution of nutrients. The bran of wheat is the richest source of minerals. (Ciudad-Mulero et al., 2021). Shewry et al. (2011) evaluated vitamin B in 24 winter wheat and 2 spring wheat; the concentration of vitamin B1 (thiamine) 5.53-13.55 μg/g DW, vitamin B2 (riboflavin) 0.77-1.40 μg/g DW, vitamin B6 (pyridoxine) 1.27-2.97 μg/g DW and vitamin B3 (niacin) 0.16-1.74 μg/g DW. Further, the bioavailable content of vitamin B3 was about 10-15% (0.16-1.74 μg/g DW). Interestingly, the concentration of vitamin B2 was not correlated with the concentration of other vitamins. In contrast, the concentration of vitamins B1, B3, and B6 are correlated and positively correlated to the temperature from heading to harvest.

1.2.3 Wheat carotenoids

Over 600 carotenoids (tetraterpenoids) are located in the chloroplast and chromoplast of plants. Carotenoids can be divided into two groups: xanthophylls which contain one or more oxygen, for example, lutein, neoxanthin, violaxanthin, and zeaxanthin carotenes which are linear tetraterpenoid hydrocarbons without oxygen, β-carotene (Figure 4). They have three colours: orange, yellow and red (Van den Berg et al., 2000; Lachman et al., 2017). Carotenoids are found in plants and microorganisms. They have broadly distributed pigments with various structures and functions, which is why carotenoids are the most studied pigments (Britton, 1998). Mostly, carotenoids and tetraterpenoids are C40 compounds with eight isomeric units. They are linked in a symmetrical and linear structure. Hydrogenation, dehydrogenation, oxidation reactions, and cyclization can change the basic cyclic structure (Oliver, 2000). To sum up, carotenoids possess similar properties, such as they are natural antioxidants.

Factors like light, heat, acids, and enzymatic oxidation can change the structure of carotenoids from trans-isomers to cis-structure, which depends on the unsaturation degree of carotenoids. It results in a minor decrease in colour and provitamin activity (Schroeder and Johnson, 1995).

In general, lutein is the most common carotenoid. After that, zeaxanthin, followed by antheraxanthin, α-carotene, β-carotene, and that β-cryptoxanthin is present as a minor component, or sometimes it may not be detected (Lachman et al., 2017). Penfili et al. (2004) also reported that lutein, with its stereoisomer zeaxanthin and two non-pro-vitamin A xanthophylls, are predominant carotenoids.

Shewry and Hey (2015) reported the carotenoid contents of several wheat samples: on average total carotenoid in einkorn was found at 2.26 mg/kg DW, in durum 3.58 mg/kg DW, in spelt 2.16 mg/kg DW and bread wheat 2.36 mg/kg DW. The concentration of lutein was higher in einkorn wheat, then after durum, followed by emmer, spelt, and bread wheat. The order of zeaxanthin was durum > einkorn > emmer > bread > spelt wheat. Regarding the content of (α+β)-carotene, the order was einkorn > spelt > emmer > bread wheat. With this data, it can be concluded that einkorn, emmer, durum, and spelt wheat are the richest sources of carotenoids. High total carotenoids were found in T. aegilopoides 8.3 ± 0.8 mg/kg DW, T. thaoudar 8.0 ± 0.9 mg/kg DW, T. monococcum 7.3 ± 0.8 mg/kg DW and T. durum 6.2 ± 0.1 mg/kg DW (Brandolini et al., 2015). Konopka et al. (2004) investigated the carotenoid content in Polish winter wheat flour, which ranged from 156 to 228 μg/100 g. Furthermore, the lutein concentration of three wheat species was investigated by Leenhardt et al. (2006), who reported that the average lutein content was 5.75 ± 0.17 μg g−1 in einkorn DM, 3.32 ± 0.18 μg g−1 DM in durum and 1.24 ± 0.17 μg g−1 DM in bread wheat.

The main colour components in durum wheat are endosperm carotenoids. The yellow pigment content (YPC) in the whole kernel is correlated with the yellow index (YI) of semolina (correlation coefficients > 0.94 (Fratianni et al., 2005; Abdel-Aal et al., 2007; Digesù et al., 2009). Moreover, in modern varieties, the YPC is found in higher quantities than in the older varieties, and wild populations (Digesù et al., 2009), and the reason is the intense breeding activities to have higher grain pigment concentration. Some environmental factors can influence the YI of semolina. Even in adverse environmental conditions, YPC has shown an increase in durum wheat, such as wet and cool conditions (Clarke et al., 2006) and water and salt stress (Katerji et al., 2005; Borrelli et al., 2011). The increment of the plant defense machinery against stress, like antioxidant molecules, might be the reason for it.

The major representation of carotenoids in durum wheat is xanthophyll lutein, which accounts for 86 to 94% of total carotenoids (Abdel-Aal et al., 2007; Digesù et al., 2009). Other carotenoids, except for xanthophyll lutein, only represent 3 to 5%, such as carotenes, esterified lutein, zeaxanthin, z-isomers of lutein, and zeaxanthin (Panfili et al., 2004; Fratianni et al., 2005; Abdel-Aal et al., 2007; Digesù et al., 2009). Furthermore, it has been documented that the endosperm had the highest total carotenoid and lutein contents (Hentschel et al., 2002; Abdel-Aal et al., 2007; Borrelli et al., 2008). The lutein is highly preserved, but the β-carotene might be lost at a relatively low-level during milling (Borrelli et al., 2008).

1.2.4 Role of carotenoids in human nutrition

Ribaya-Mercado and Blumberg (2004) documented the provitamin A activity of carotenoids. These carotenoids show antioxidation capacity, which can minimize the risk of chronic degradation diseases and provide a protection factor from ocular diseases (Abdel-Aal et al., 2007; Nishino et al., 2009). It has been proven that carotenoids are precursors of vitamin A, with various health advantages such as antioxidant properties, decreased risk of cardiovascular ailment, anti-obesity, defense of the macula region of the retina, immune system reinforcement, etc. (Mezzomo et al., 2015). Carotenoids are transformed into vitamin A to satisfy the body's requirements (Mezzomo and Ferreira, 2006).

It has been reported that carotenoids, predominantly β-carotene, can be converted into vitamin A (retinol) (Gurmu et al., 2014). Concerning eye health and the carotenoid relationship, two carotenoids are found in the human retina: lutein, zeaxanthin, and the isomer meso-zeaxanthin (Bernstein et al., 2016). The benefits of carotenoids can be seen in ocular function and health. Additionally, lutein/zeaxanthin can increase the macular pigment optical density (MPOD). The visual performance of humans can be improved by the intake of lutein/zeaxanthin (Eggersdorfer and Wyss, 2018). Lutein and zeaxanthin react against free radicals as antioxidants which protect the retina against photo-damage and peroxidation. Although the mechanism is not clearly understood yet, several carotenoids seem to affect cognitive functioning and could be their antioxidant activities (Killer et al., 2005). A placebo-controlled study showed the beneficial cognitive effects on older women and men with the supplementation of lutein plus zeaxanthin (Hammond et al., 2017).

Additionally, some studies suggested that carotenoids may have beneficial effects on preventing cardiovascular diseases such as oxidative stress, inflammation, thrombosis, and dyslipidemia. Lutein intake may be beneficial in lowering the risk of coronary heart disease and stroke (Leermakers et al., 2016). Interestingly, β-cryptoxanthin has a stimulating effect on the formation of bone (Yamaguchi and Uchiyama, 2003). Some carotenoids (β-carotene) are associated with the activation of the immune system, and for cardiovascular disease patients, they act as inflammatory markers (Jonasson et al., 2003). Though it is controversial, it has been reported that lycopene intake can reduce the risk of lethal prostate cancer (Mucci et al., 2014). Lastly, carotenoids like lutein/zeaxanthin (present in human milk) are associated with an infant's visual and cognitive development (Henriksen and Chan, 2014).

1.2. 5 Wheat phenolic compounds

Polyphenolic compounds are secondary metabolites with one or several benzenic cycles and one or several hydroxy functions in all vascular plants. (Munin and Edwards-Lévy, 2011). Phenolic compounds are the phytochemicals commonly found in fruits, vegetables, and grains. Some studies suggest that phenolic compounds may protect against degenerative diseases (Mazza, 2000). The main beneficial characteristic of phenolic compounds is their antioxidant activity. It has been suggested that the intake of whole-grain foods protects against coronary heart disease (Liyana-Pathirana and Shahidi, 2005). Additionally, degenerative illnesses like cancer, autoimmune disease, sclerosis, and Parkinson's disease can be caused by oxidative stress. The rich diet of Phenolic compounds can minimize oxidative damage and thus avoid aging and age-related diseases. Their mechanism is scavenging the free radicals from cell metabolism. They are known to have antioxidant, antimicrobial, antiviral, antibacterial, and anti-tumor properties (Haminiuk et al., 2012). The most predominant phenolics found in cereals are benzoic acid derivatives like vanillic, gallic, and syringic acids and p-coumaric, ferulic, and caffeic acid (derivatives of cinnamic acid). Ferulic acid accounts for 70-90% of the total. In cereal grains, phenolic acids mostly remain in the insoluble bound form, linked with cellulose, protein, and lignin (cell wall structure). Thus, it has low bio-accessibility (Žilić et al., 2016). The highest antioxidant activity among the wheat fraction is in the aleurone layers, followed by the bran. It has been documented that the debranned bread and wheat had 3.7 and 2.4-fold lower antioxidant activity than the bran fractions. The milling process highly influences the content of the phenolic compound of wheat and its antioxidant activities (Žilić et al., 2012). The sum of the antioxidant capacity of phenolic fractions of whole wheat was 47.12 mmol Trolox Eq/kg, whereas the total antioxidant capacity was 19.22 mmol Trolox Eq/kg. Seventy phenolic compounds, including coumarins, anthocyanins, phenolic acids, etc., have been identified in modern and old wheat genotypes using HPLC-TOF-MS analyses (Brazier-Hicks et al., 2009).

2 HYPOTHESIS AND AIMS

New oat and coloured spring wheat cultivars are a good source of nutritionally valuable substances including polyunsaturated fatty acids, tocols, carotenoids and phenolic compounds.

Aims

1) The main aim of the research is to investigate the lipid content and fatty acid profiles of new oat cultivars. The nutritional value of the oil will be compared between naked and common oat varieties. The fatty acid composition of oat husk will be compared to that of the oat grain. Additionally, the study will investigate the stability of fat against oxidation.

2) In the case of new coloured spring wheat cultivars, the research aims to identify and quantify carotenoids in grains. The study aims to compare the content of phenolic compounds and tocols in different coloured wheat cultivars. Furthermore, the study will perform a sensory analysis of buns prepared using flour from coloured spring wheat and compare it with buns made from winter wheat flour.

HYPOTHESIS

1a) New varieties of oats are an essential source of lipids and contain more lipids than traditional cultivars.

1b) European and Canadian oat genotypes may have different lipid content and fatty acid compositions.

1c) New varieties of naked oats (Avena nuda L.) may have higher amounts of polyunsaturated fatty acids, resulting in a higher nutritional value than common hulled oats (Avena sativa L.).

1d) Fatty acids of new oat varieties are more susceptible to oxidation during grain storage and processing.

1e) The oat husk may have a different fatty acid composition than the oat grain.

2a) Selected coloured spring wheat cultivars could be a reliable source of biologically active carotenoids, with higher content compared to winter wheat cultivars.

2b) Coloured spring wheat could contain a substantial amount of phenolic compounds and tocols.

CHAPTER 2

Materials and Methods

3 EXPERIMENTAL PART

3.1 STUDY 1 - Lipid content and fatty acid profile of various European and Canadian hulled and naked oat genotypes

3.1.1 Plant material

The sample collection consisted of 38 oat genotypes, cultivated in the fields of Selgen breeding company. These genotypes were grown in a single season, under similar climatic conditions. The collections of samples included hulled and naked genotypes originated from various countries, namely Britain, Canada, Estonia, Finland, France, Ireland, Norway, Russia, and Sweden.

3.1.2 Sample preparation and fatty acid determination

For sample milling, a Scarlett Silver Line SL 1545 coffee grinder. Approximately 7g of ground oat samples were placed into an extraction timber and covered with cotton. The lipids were extracted using petroleum ether in a Soxhlet glass apparatus, following the ISO 659:2009 guidelines, for a duration of 240 minutes. The fatty acid composition was analyzed according to a method described by Kouřimská et al. (2018). Fatty acid methyl esters were prepared according to the guidelines provided by ISO 12966–2:2017. Approximately o.5 g of extracted lipids was re-esterified. Then peaks were analyzed by gas chromatography coupled with mass spectrometry (GC-MS) using an Agilent 7890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a Restek's Rt-2560 capillary column (0.25 mm ID, 100 m, 0.20 μm thickness, Restek Corporation, Bellefonte, PA, USA). Detailed information regarding the plant material and the sample preparation and fatty acid determination is provided in the original paper attached below.

3.2 STUDY 2 - Assessing the tocopherol content and oxidative stability of the crude oat oil using the Schaal oven and Rancimat tests

3.2.1 plant material and extraction of fat

Samples were collected from Selgen a.s. breeding station in Stupice, Czech Republic which contained six naked oats (Kamil, Oliver, Patrik, Marco Polo, Santini, and Saul) and two hulled (HO) (Atego, Korok). Lipid extraction was done by using Rendall hot extraction method at approximately 70°C to avoid oxidation. The remaining PET was evaporated by a rotary vacuum evaporator.

3.2.2 Schaal oven test

5 grams of extracted crude oat oil was mixed with pork lard because gravimetric detection of the oxygen-increased absorption (induction period) requires 25 g of lipids for one determination. To provide a similar condition, 5 g of rapeseed or sunflower oil was also added with 20 g of natural pork lard without any food additives. These blends of samples were added into 100mL beakers of the same diameter (50 mm). In addition, 25 g pork lard was used as a control. All samples and controls were kept inside the oven (Binder GmbH, Tuttlingen, Germany) at 60 ± 2 °C. The weight of samples was measured every 3-4 days in analytical balances (Kern analytical balance ABS-N_ABJ-NM Darmstadt, Germany) for 80 days until an induction period (IP) was clearly detectable from the graphical dependence of the relative weight change on time. Due to time- consuming extraction process and the low yield of oat oil, the Schaal oven test experiment was conducted only once for each type of oat oil. The relative weight change (RWC) was calculated using the formula:

Relative weight changes = ((w2-w) -(w1-w))/(w1-w))

Where,

w= weight of beaker without sample

w1= weight of beaker and sample with pork lard at day 0

w2= weight of beaker and sample with pork lard at a certain day

3.2.3 Rancimat test

The analysis involved testing 5 grams of crude oat oil using a Metrohm Rancimat model 892 (Herisau, Switzerland), following the recommended protocol by Metrohm AG (2019). Steam of purified air 20 L/h was passed through 0.5 g oat oil. PEG 3000 is held at a constant

temperature of 100°C or 110°C according to the guidelines provided in ISO method 6886:2016. Subsequently, the effluent air from the oil sample was directed through a vessel filled with deionized water to generate bubbles.

3.2.4 Tocopherols analysis

1 gram of crude oat oil was weighed and transferred into a 10 mL volumetric flask and then filled with heptane. For the analysis of tocopherols and tocotrienols (collectively referred to as tocols), a high-performance liquid chromatograph (HPLC) equipped with a fluorescence detector (FLD, G7121A) was employed. The FLD was set at 298 nm for excitation and 330 nm for emission. Separation of tocols was achieved using an Agilent LiChrospher DIOL column (Eschenstr. 582024; Taufkirchen, Germany) with dimensions of 4 × 250 mm and a particle size of 5 μm. The column temperature was maintained at 35°C. A mobile phase consisting of a mixture of tert-butyl methyl ether (tBME) and n-heptane in a 5:95 (v/v) ratio was used, and the flow rate was set at 1 mL/min. The analytical conditions followed previously established protocols (Agilent, 2010). The attached manuscript contains comprehensive details regarding the methodology and materials used in Study 2.

3.3 STUDY 3 - Comparative analysis of lipid content and fatty acid composition in hulled and naked oats compared with dehulled grains and husks

3.3.1 Oat samples

The eight oat samples were obtained from the Selgen a.s. breeding station (Stupice, Czech Republic). Among eight variety, two were hulled (Atego and Korok) and six were naked oats ((Kamil, Marco Polo, Oliver, Patrick, Santini, and Saul).

3.3.2 Post-harvest sample specifications, sample preparation, determination of crude fat content, and fatty acid composition

The post-harvest sample specification was determined by (FOSS Infratec 1241; FOSS Analytical A/S, Hillerød, Denmark). It determined the total protein and moisture content (n = 10, RSD ≤1%). The sample preparation, and determination of crude fat and fatty acid composition was done as described in study 1.

3.3.3 Statistical analysis

The statistical analysis comprised two- way analysis variance (ANOVA) using IBM SPSS Statistics 29.0.0.0 (Armonk, New York, USA) software and a level of significant P = 0.05. The mean ± standard deviation was evaluated using a Tukey's HSD (honestly significant difference).

3.4 STUDY 4 - Fat content and fatty acid profiles of recently registered varieties of naked and hulled oats with and without husks

3.4.1 Oat samples

Similar to previous study, samples were obtained from Selgen a.s. breeding Stupice, Czech Republic. These oat varieties were registered between 2011 and 2014. Four of the oat varieties were hulled (Cavaliere, Kertag, Selodon, and Gregor), while two were naked varieties (Kamil and Otakar). The selected oats are mainly grown in Poland, Spain, United Kingdom, Russian Federation, Canada USA and South Africa.

3.4.2 Sample preparation and determination of lipid content and composition

Sample preparation and determination of lipid content and composition is done according to the instructions provided earlier in the 3.1.3.

3.4.3 Statistical analysis

The statistical analysis involved two-way analysis of variance (ANOVA), principal compound analysis (PCA), and cluster analysis. These statistical analyses were conducted by using STATISTICA v. 12.0 (StatSoft, Inc., Tulsa, OK, USA). To identify significant differences among samples, Sheffé's test was performed at a significance level of 5%. The statistical analysis focused on major fatty acids (more than 1% representation): C16:0, C18:0, C18:1 cis-9, C18:2 cis-9,12, C18:3 cis-9,12,15, C20:0, C20:1 cis-11, C20:4 and C20:5 cis-5,8,11,14,17. Although there were also minor fatty acids present at low levels, their differences may not have been nutritionally significant.

3.5 STUDY 5 - Nutritional composition of coloured spring wheat focusing on carotenoids, tocopherols, and phenolic compounds

3.5.1 Wheat samples

Analyzed coloured wheat were obtained from the Selgen a.s. breeding station (Stupice, Czech Republic; GPS 50°313" N, 14°384" E, 287 m.a.s.l.). Samples were stored after harvest in paper bags in a box in the dark at room temperature of 25 °C for a month before being analyzed. Due to the insufficient availability of an adequate quantity of coloured spring wheat, two varieties of coloured spring wheat (Pexeso and Tercie) were compared with four varieties of winter wheat (AF Zora, AF Jumiko, AF Oxana, and Sultan) varieties.

3.5.2 Chemicals and reagents

Chemical preparation: 1% of vit C (1 gram of vitamin C was mixed with 30 ml water) and fill it till 200 ml methanol. So, the final volume is 200ml. To prevent phenolic compounds degradation during extraction process vitamin C was added.

3.5.3 Sample preparation and fat content determination

The coloured wheat samples were ground in a Scarlett Silver Line SL 1545 coffee grinder (Ariette-Scarlett, Firenze, Italy) for three minutes. Grinded wheat samples were mixed with petroleum ether and allowed to stand overnight (at temperature of 27°C). The reason was to avoid the potential oxidation during the extraction of wheat oil. After filtration, the petroleum ether was evaporated using a vacuum evaporator.

For the extraction of fat, a Soxhlet glass apparatus was used according to ISO 659: 2009. Approximately 10g of homogenized sample was carefully weighted and placed into an extraction thimble. The fat was extracted by the petroleum ether for 240 min. Fat content was determined gravimetrically after drying at 103 ± 2 °C to a constant weight. Each sample was analyzed three times (n=3).

3.5.4 Tocols determination

To perform the analysis, one gram of wheat oil extract was carefully weighed and placed into a 10mL volumetric flask. The flask was then filled with heptane to reach a final volume of 10 mL. The tocols in the sample were analyzed using high-performance liquid chromatography

(HPLC) equipped with a fluorescence detector (FLD, G7121A). The FLD was set at 298 nm and 330 nm wavelengths for excitation and emission, respectively. A mobile phase consisting of a mixture of tBME (tert-butyl methyl ether) and n-heptane in a volumetric ratio of 5:95 (v:v) was utilized for the separation of tocols. The mobile phase was delivered at a flow rate of 1mL/min. The tocols were separated using a 4 x 250 mm id, 5 µm particle size, Agilent LiChrospher DIOL column (Eschenstr. 582024, Taufkirchen, Germany), which was thermostated at 35°C. The analytical methods used in this study were briefly described in (Agilent, 2010). For peak identification and quantification, a mixture of α, β, γ, and δ isomers of tocols standards were dissolved in n-hexane. Quantification relied on six-point calibration curves for each tocol, ensuring a minimum correlation coefficient of ≥ 0.998. The limit of detection (LOD) and limit of quantitation (LOQ) values for various standard solutions are provided below:

α-tocopherol: $R^2 = 0.9982$, LOD = 6.36, LOQ = 19.29

β-tocopherol: $R^2 = 0.9980$, LOD = 2.20, LOQ = 6.67

γ-tocopherol: $R^2 = 0.9976$, LOD = 3.46, LOQ = 10.50

α-tocotrienol: $R^2 = 0.9956$, LOD = 2.35, LOQ = 7.12

β-tocotrienol: $R^2 = 0.9983$, LOD = 1.47, LOQ = 4.44

δ-tocotrienol: $R^2 = 0.9947$, LOD = 5.14, LOQ = 15.58

3.5.5 Sample preparation and chromatographic analysis of carotenoids

The method of sample extraction, chromatographic separation, identification, and quantification of carotenoids was described in a previous study by (Paznocht et al. 2021). The collected 100 grams of samples were milled in Scarlett Silver Line SL 1545 coffee grinder (Ariette-Scarlett, Firenze, Italy), and subsequently, 2g was weighted for the analysis. Finely ground samples were extracted with 12 mL ethanol/acetone/hexane mixture (1:1:2, v/v/v) into a 50 ml plastic falcon tube; which was vortexed and left for 24h in a refrigerator at 4 °C. After a day sample was vortexed for 1 minute again (Basic 3; IKA Werke GmbH & Co. KG, Staufen, Germany). The next step was sonicated the samples for 10 min in an ultrasonic bath (PS 04; Powersonic-Notus, Ltd., Vráble, Slovakia), and later, samples were centrifuged (5810R; Eppendorf, Hamburg, Germany) for 5 min at 8228 rcf (20 °C, 6000 rpm). After transferring 9 mL supernatants into another glass tube, the sediments were re-extracted with 12 mL of the extraction mentioned above mixture. Extracted supernatants were mixed in the same flask and evaporated with a vacuum at 40 °C (Rotavapor R-200; Büchi Labortechnik, AG, Flawil,

Switzerland). The dry residue was reconstituted with 1 mL ethanol/acetone (3:2, v/v) solution that contained 0.2% BHT and finally filtrated through a syringe filter (PVDF, 0.45 μm) into an HPLC vial (amber).

3.5.6 Sample preparation and chromatographic analysis of phenolic compound

The phenolic content was extracted from wheat grains using a modified method described in (Klimczak et al., 2007) (Skoczylas et al., 2020). Approximately 15 grams of milled wheat flour were homogenized with 15 grams of 1% vitamin C in a methanol solution in a 1:1 (w:w) proportion. Then, 1 gram of the prepared sample was taken, and 1 ml of NaOH (2M) was added to initiate hydrolysis. The mixture was then left undisturbed for 4 hours. Afterward, the acidity of the samples was adjusted to a pH level between 2 and 2.8. To increase acidity, 0.9 ml of HCL was added carefully, and if the pH was below the desired range, NaOH was added. Following this, the sample was centrifuged at 5000 RPM for 15 minutes. The resulting supernatant was transferred to a 5ml flask and brought to the desired volume. This solution was then centrifuged again at 18000 RPM for 15 minutes and subsequently filtered using syringe filters to ensure cleanliness.

HPLC analysis was performed using a Dionex UltiMate 3000 HPLC system equipped with a Cosmosil 5C18-MS-II column (250 x 4.6 mm ID, 5 μm) (Nacalai Tesque, INC. in Kyoto, Japan) and a DAD detector from (Thermo Scientific in Germering, Germany). Two eluents were used as the mobile phase: Eluent A was a 2% (v/v) aqueous acetic acid solution, and Eluent B was 100% methanol. The flow rate of the mobile phase was maintained at 1 mL/min throughout the 50-minute analysis. The elution program consisted of the following gradient: Eluent A was set at 95% for 0 minutes, 70% for 10 minutes, 50% for 25 minutes, 30% for 35 minutes, and 95% for 40 minutes. The injection volume was 20 μL, and the column temperature was 25 °C.

During analysis, peaks were monitored at 245 nm, 280 nm, 320 nm, and 360 nm. The identification of peaks was based on their retention times and comparison with the UV spectra of standards. Quantification of individual phenolic acids was performed using a calibration curve with an ($R2 \geq 0.998$) following retention times and DAD absorption spectra of external standards. Pure compounds (p-hydroxybenzoic acid, catechin, gallic acid, vanillic acid, p-coumaric acid, isoferulic acid, ferulic acid, syringic acid, caffeic acid, o-coumaric acid, chlorogenic acid, luteolin, and apigenin) were used as external standards. Samples were prepared and analyzed in triplicate.

3.6 STUDY 6 - Sensory analysis of buns made from coloured spring and wheat

3.6.1 Bun varieties and preparation

In this study, buns were made in a laboratory at the Selgen Breeding Institute. Same varieties of coloured wheat were evaluated as evaluated in previous study. The focus was on coloured wheat cultivars with higher levels of phytochemicals and their effect on sensory characteristics of bakery products. Initially, post-harvest characterization of wheat samples was conducted, involving the evaluation of moisture content, starch DM, Zeleny index, and hardness of the wheat grains. Prior to milling, the moisture level was adjusted by adding water to a closed container one week before the milling process. Subsequently, the wheat grains were milled to obtain flour. Approximately 40ml of water was added to 80 grams of wheat flour, which was then further mixed with specific ingredients, including 1.2 grams of salt, 0.8 grams of sucrose, 4 grams of yeast, 850 µl of lard, and 0.1 gram of ascorbic acid.

The flour and ingredients were mixed thoroughly for 30 seconds. Subsequently, the dough was placed inside an oven with a temperature of 31.9°C for 20 minutes. The dough was then allowed to rest for 15 minutes before being transferred to another oven with a temperature of 32°C for an additional 25 minutes of baking. The dough was weighed and divided into three equal pieces, which were shaped into round buns using a specific device. These buns were placed inside a baking oven with a temperature of 250°C for a final baking period of 20 minutes. During this stage, 20 ml of water was added to the oven to create a steam environment during baking. As a result of this meticulous procedure, the buns were successfully produced.

3.6.2 Sensory Analysis

The bun was sliced into equal size and shape. One serving consisted of six different slices of bun in a tray. The code number was written near each slice.

3.6.3 Sensory Evaluation

Sensory profiling in this study was conducted using a linear graphical oriented unstructured 100 mm scale, following the guidelines specified in ISO 13299:2016. The sensory panelists were from the Department of Microbiology, Nutrition, and Dietetics, comprising 10 assessors (7 females and 3 males) with ages mostly ranging between 21 and 43 years. The sensory eval-

uation took place at the Czech University of Life Sciences in Prague. Each assessor was provided a cup of water, a tray containing sliced pieces of bread, a 100 mm scale, and a form for recording their evaluation results.

The form included a list of attributes for sensory profiling, which encompassed overall appearance liking, pleasantness of colour, the intensity of colour, overall taste liking, the overall intensity of taste, intensity of sweet taste, intensity of salty taste, and overall sample acceptance. The assessors utilized the graphical scale to express their perceptions and preferences for each attribute, enabling a comprehensive sensory analysis of the bread samples. The scale utilized in this analysis has interval properties, where the intervals between its levels are assumed to be equal (continuous line-scale). Typically represented as a horizontal line, the left-hand end of the scale is assigned a low rating (commonly labeled as zero). In contrast, the right-hand end is designated with a high rating (often set as 100).

Overall sample acceptance:

Not accepted (0) Accepted (100)

3.6.4 Statistical analysis:

The data/graphs were statistically analyzed by STATISTICA v. 12.0 (StatSoft, Inc., Tulsa, OK, USA). The spider chart of attributes of buns was created by Excel. The statistical analysis comprised one-way analysis variance (ANOVA) conducted IBM SPSS Statistics 29.0.0.0 (Armonk, New York, USA) software and a level of significant $p < 0.05$. Tukey's HSD (honestly significant difference) test was performed to identify statistically significant differences, which are indicated by different superscript letters.

Figure 3: Buns made from coloured spring and winter wheats

CHAPTER 3

RESULTS

4 RESULTS

4.1 STUDY 1 - Lipid content and fatty acid profile of various European and Canadian hulled and naked oat genotypes

The Norwegian oat contained the highest lipid content (7.13 ± 0.52 g/100 g), which was significantly different from other varieties. The Swedish and Russian genotypes had a lipid content below 5 g/100 g, which was significantly distinct from the lipid content of the remaining tested varieties. Pusahybrid had the highest lipid content (9.51 g/100 g), while Navaro had the lowest (2.9 g/100 g), and both of these genotypes showed significant differences compared to the other tasted verieties ($p < 0.05$). The lipid content of Canadian and European oat genotypes is provided in the attached original paper below.

No significant difference was observed in palmitic acid content, with a relative content ranging from 17.05% to 19.77%. Whereas AC Preaknes had a significant difference in oleic acid content (39.04%) compared to other Canadian genotypes, Walderm, Navaro, OA 504-5, and Shadow. Similarly, Walderm had the highest quantity of linoleic acid with a 39.05% representation, while AC Preaknes contained the lowest amount of linoleic acid with 32.54%, which differed from Walderm and Navaro genotypes. The sum of SFA ranged from 20.29% to 24.56% showing no significant difference.

Among the European genotype oats, Lidya contained highest concentration of palmitic acid at 21.21%, showed significant different from Ivore. In contrast, Ivore contained highest amount of oleic acid (40.87%), which had significant difference ($p < 0.05$) from all other European genotypes except Katri. Similarly, Dakar had highest linoleic acid content (40.63%), marked divergence from Belinda and Ivore.

The major fatty acid composition of naked and hulled oats highlighted in Table 4. Hulled oats had a marginally higher palmitic acid content (18.68%) compared to naked oats (18.35%). There was no significant difference in the levels of stearic acid between naked oats (2.1%) and hulled oats (2.25%). Hulled oats had a higher oleic acid proportion (35.97%) than naked oats (35.35%). Additionally, naked oats contained more linoleic acid (37.92%) compared to hulled oats (36.93%). Detailed information regarding the representation of fatty acid composition in the Canadian, European, hulled, and naked oat genotypes and the comparison of fatty acid contents of Canadian and European oat genotypes is provided in the attached original paper below.

Table 3. Major fatty acid composition based on the country of origin (% of all identified fatty acids)

Sample name	C16:0	C18:0	C18:1 *cis*-9	C18:1 *cis*-13	C18:2 *cis*-9,12	C20:1 *cis*-11	C18:3 *cis*-9,12,15	SFA	MUFA	PUFA
CAN	18.64 ±1.07^{a}	2.39 ±0.55^{ab}	36.50 ±1.50^{ab}	1.06 ±0.12^{bcd}	36.76 ±2.21^{ab}	0.98 ±0.11^{abc}	1.20 ±0.15^{d}	22.41 ±1.40^{a}	39.17 ±1.41^{abc}	38.42 ±2.28^{b}
EST	18.29 ±0.66^{a}	2.29 ±0.55^{ab}	34.99 ±1.34^{cde}	1.05 ±0.12^{cd}	38.15 ±1.50^{ab}	1.02 ±0.18^{abc}	1.69 ±0.11^{a}	21.95 ±0.72^{a}	37.74 ±1.05^{cde}	40.31 ±1.65^{ab}
FIN	18.48 ±1.08^{a}	2.47 ±0.45^{a}	36.84 ±2.02^{ab}	1.02 ±0.15^{d}	36.49 ±1.48^{b}	0.95 ±0.11^{c}	1.41 ±0.20^{cd}	22.33 ±1.31^{a}	39.36 ±2.10^{ab}	38.31 ±1.61^{b}
FRA	18.32 ±1.41^{a}	2.12 ±0.68^{ab}	35.88 ±2.63^{bcd}	1.14 ±0.15^{abc}	37.28 ±2.52^{ab}	1.11 ±0.16^{ab}	1.41 ±0.19^{cd}	21.96 ±1.93^{a}	38.88 ±2.68^{cde}	39.16 ±2.58^{ab}
GBR	19.14 ±1.42^{a}	2.19 ±0.40^{ab}	36.02 ±1.32^{bc}	1.18 ±0.14^{ab}	36.23 ±1.76^{b}	1.10 ±0.11^{ab}	1.31 ±0.25^{cd}	22.86 ±1.63^{a}	39.12 ±1.36^{abcd}	38.02 ±1.88^{b}
IRL	19.66 ±0.79^{a}	2.55 ±0.78^{ab}	34.57 ±0.78^{cde}	1.24 ±0.25^{a}	37.52 ±1.33^{ab}	0.83 ±0.16^{bc}	1.40 ±0.07^{abc}	23.24 ±0.54^{a}	37.25 ±1.98^{de}	39.51 ±1.85^{ab}
NOR	17.37 ±0.52^{a}	2.12 ±0.00^{ab}	37.69 ±0.62^{a}	1.05 ±0.03^{bcd}	36.83 ±0.26^{ab}	1.10 ±0.03^{ab}	1.42 ±0.04^{cd}	20.71 ±0.40^{a}	40.49 ±0.64^{a}	38.79 ±0.25^{ab}

RUS	18.89 ±1.79[a]	1.92 ±0.40[b]	34.60 ±1.61[de]	1.17 ±0.07[abc]	37.95 ±2.07[ab]	1.15 ±0.22[a]	1.45 ±0.22[bc]	22.24 ±2.29[a]	37.76 ±1.16[cde]	40.00 ±2.27[ab]
SWE	18.19 ±0.27[a]	1.91 ±0.28[b]	34.29 ±1.42[e]	1.12 ±0.11[abc]	39.35 ±0.74[a]	1.08 ±0.23[abc]	1.65 ±0.19[ab]	21.42 ±0.36[a]	37.14 ±0.99[e]	41.44 ±1.01[a]
$HSD_{0.05}$	2.0	0.61	1.34	0.13	2.70	0.19	0.22	2.55	1.46	2.74

Values are expressed as the mean ±standard deviation. Each analysis was performed in duplicate, independently. Number of samples in each category (CAN= 7, EST= 2, FIN = 5, FRA= 8, GBR= 6, IRL= 2, NOR= 1, RUS= 5, SWE= 2).

Table 4. Major fatty acid composition of naked and hulled oats (%)

Oat type	C16:0	C18:0	C18:1 *cis*-9	C18:1 *cis*-13	C18:2 *cis*-9,12	C20:1 *cis*-11	C18:3 cis-9,12,15	SFA	MUFA	PUFA
Hulled	18.68 ±1.40[a]	2.26 ±0.60[a]	35.97 ±2.21[a]	1.16 ±0.15[a]	37.92 ±2.29[a]	1.06 ±0.16[a]	1.43 ±0.23[a]	22.37 ±1.80[a]	38.84 ±2.09[a]	39.80 ±2.44[a]
Naked	18.53 ±0.94[a]	2.11 ±0.31[a]	35.35 ±1.44[b]	1.10 ±0.11[b]	36.93 ±1.12[b]	1.05 ±0.19[a]	1.37 ±0.22 [b]	21.96 ±0.92[a]	38.24 ±1.24[b]	38.79 ±1.29[b]

Values are expressed as the mean ±standard deviation. Each analysis was performed in duplicate, independently.

4.2 STUDY 2 - Assessing the tocopherol content and oxidative stability of the crude oat oil using the Schaal oven and Rancimat tests

Schaal oven test

Inclusion of rapeseed and sunflower oil to the pork lard led to increased stability of these mixtures, as the vegetable oils contain higher levels of natural antioxidants, such as tocols, in comparison to the lard-only samples. The induction periods and protection factors of all tested samples were presented in **Table 5**. The induction periods for all oat samples in lard exceeded fifty days. The protection factors of all hulled and naked oat samples ranged from 7.8 to 8.6 days.

Table 5: Induction period (IP) and protection factor of oats, rapeseed and sunflower oil with pork lard

Oil sample (5 g) + pork lard (20 g)	Induction period (days)	Protection factor
Atego (hulled oat)	56.0	8.6
Kamil (naked oat)	54.0	8.3
Korok (hulled oat)	55.5	8.5
Marco Polo (naked oat)	51.0	7.8
Oliver (naked oat)	55.0	8.5
Patrik (naked oat)	56.0	8.6
Santini (naked oat)	56.0	8.6
Saul (naked oat)	52.0	8.0
Rapeseed oil	23.0	3.5
Sunflower oil	14.0	2.2
Control - pork lard (25 g)	6.5	1.0

Protection factor = IP sample/IP control

Rancimat test

Table 6 presents the induction periods of various fats and oils, including oat, rapeseed, rice, and sunflower oils, pork lard, and butter, when subjected to the accelerated oxidation Rancimat test at 100°C and 110°C. The results demonstrated that Kamil oil oxidized faster among the analyzed oat samples at both temperatures, with induction periods of 4.12 hours at 100°C and

1.49 hours at 110°C. In contrast, Marco Polo, Oliver, Atego, Patrik, Santini, and Korok had induction periods ranging from 5 to 7 hours at 100°C and 2 to 3 hours at 110°C. Saul exhibited the longest oxidation time, with an average induction period of 9.02 hours at 100°C and 4 hours at 110°C.

Table 6. Induction periods of samples and controls (0.5 g samples + 3 g polyethylene glycol-PEG 3000) at 100°C and 110°C in the Rancimat test.

Samples	100°C Induction period (h)	110°C Induction period (h)
Kamil	4.12 ± 0.30^{b}	1.49 ± 0.47^{b}
Saul	9.02 ± 0.27^{a}	4.00 ± 0.30^{a}
Marco Polo	6.80 ± 1.06^{ab}	2.90 ± 0.15^{b}
Oliver	7.29 ± 0.5^{ab}	3.33 ± 0.32^{ab}
Atego	5.06 ± 0.27^{b}	2.22 ± 0.09^{b}
Patrik	5.45 ± 0.47^{b}	3.50 ± 0.33^{ab}
Santini	5.96 ± 0.59^{b}	2.92 ± 0.23^{b}
Korok	6.86 ± 1.15^{ab}	2.83 ± 0.11^{b}
Pork lard	1.18 ± 0.04	0.77 ± 0.05
Butter	1.07 ± 0.06	0.66 ± 0.08
Rapeseed oil	1.34 ± 0.07	0.75 ± 0.02
Sunflower oil	1.32 ± 0.08	0.81 ± 0.06
Rice oil	2.14 ± 0.05	1.26 ± 0.19

Values are expressed as means ± standard deviation (SD) of independent analyses run in triplicate. Different lowercase letters in a column indicate significant differences ($p \leq 0.05$). Only oat samples were included in the ANOVA.

Tocopherols and tocotrienols in oats

Oat tocols analysis alongside sunflower oil and pork lard for comparison were presented in Table 7. The highest concentrations of tocols were found in the Sanitini genotype (155.3 mg/kg), followed by Korok (105.9 mg/kg). In contrast, Marco Polo contained the lowest tocols among the analyzed varieties (62.1 mg/kg). Among eight varieties of oats experimented with in this study, α-tocopherol emerged as the most abundant form of tocols, except in the cases of Patrik and Saul. Furthermore, hulled oats, i.e., Atego and Korok, contained higher tocols content than most of the analyzed oats, with the exceptions of Santini and Saul. The manuscript attached below contains comprehensive information regarding the results of the Schaal oven test, Rancimat test, and tocol concentration.

Table 7: Concentration of tocopherols and tocotrienols in oats, sunflower oil and pork lard (mg/kg)

	α-tocopherol	α-tocotrienol	β-tocopherol	β-tocotrienol	γ-tocopherol	γ-tocotrienol	Total tocols	Total tocotrienols	Total tocopherols
Saul	63.44 ± 1.52^{c}	65.65 ± 4.98^{a}	3.61 ± 0.09^{bcd}	8.76 ± 1.06^{a}	1.20 ± 0.21^{b}	1.80 ± 0.31^{a}	144.46 ± 6.18^{a}	68.25 ± 1.90^{b}	76.21 ± 7.95^{a}
Marco Polo	28.31 ± 3.77^{f}	23.53 ± 1.59^{d}	4.69 ± 0.37^{bc}	4.51 ± 0.96^{b}	0.73 ± 0.65^{c}	0.27 ± 0.06^{c}	62.05 ± 3.79^{d}	33.73 ± 2.81^{d}	28.32 ± 2.98^{e}
Patrik	32.35 ± 3.33^{f}	35.55 ± 4.48^{c}	2.42 ± 0.74^{cd}	6.01 ± 2.38^{ab}	ND	ND	76.33 ± 4.07^{cd}	34.76 ± 3.04^{d}	41.57 ± 2.34^{cd}
Sanitini	89.81 ± 7.47^{a}	48.54 ± 2.52^{b}	7.50 ± 1.37^{a}	6.91 ± 0.83^{ab}	2.05 ± 0.35^{a}	0.47 ± 0.18^{b}	155.29 ± 6.31^{a}	99.36 ± 6.75^{a}	55.93 ± 1.40^{b}
Oliver	44.07 ± 4.34^{e}	21.76 ± 2.84^{d}	1.84 ± 0.76^{d}	7.21 ± 0.40^{ab}	ND	ND	74.89 ± 7.68^{cd}	45.92 ± 4.71^{cd}	28.97 ± 3.02^{e}
Atego	56.67 ± 3.33^{cd}	35.16 ± 3.01^{c}	5.81 ± 0.72^{ab}	4.14 ± 1.63^{b}	ND	ND	101.78 ± 8.20^{b}	62.47 ± 3.73^{b}	39.3 ± 4.63^{de}
Korok	48.72 ± 4.34^{de}	46.48 ± 2.51^{b}	5.45 ± 2.0^{ab}	5.32 ± 0.84^{ab}	ND	ND	105.97 ± 7.56^{b}	54.17 ± 6.02^{bc}	51.80 ± 1.81^{bc}
Kamil	44.23 ± 3.47^{e}	40.48 ± 1.45^{bc}	2.26 ± 0.74^{cd}	6.42 ± 2.31^{ab}	ND	0.51 ± 0.08^{b}	93.90 ± 5.77^{bc}	46.49 ± 3.38^{cd}	47.41 ± 3.38^{bcd}
Sunflower oil	78.16 ± 5.09^{b}	ND	1.48 ± 0.13^{d}	ND	0.16 ± 0.03^{d}	ND	79.77^{cd}	ND	79.77^{a}
Pork lard	2.09 ± 0.36^{g}	ND	ND	ND	ND	ND	2.09^{e}	ND	2.09^{f}

ND = not detected. Different lowercase letters in a column indicate significant differences ($p \leq 0.05$). Values are expressed as means ± SD of independent analyses in triplicate ($n = 3$).

4.3 STUDY 3 - Comparative analysis of lipid content and fatty acid composition in hulled and naked oats compared with dehulled grains and husks

Lipid content

The finding demonstrates that naked oats had a higher lipid content compared to hulled oats, regardless of whether they had husks or not. Among naked oats, Kamil contained the highest lipid content with 6.03 ± 0.37 g/100 g, followed by Patrik with 5.27 ± 0.38 g/100 g. Whereas Santini had significantly lower lipid content among all tasted varieties, 3.20 ± 0.19 g/100 g. The dehulling of Korok and Atego led to an increase in lipid content comparable to certain naked oat varieties like Marco Polo and Oliver.

Fatty acid composition

The dominant fatty acid identified was C18:1 cis-9 (oleic acid), with 30-40 % among the samples, and close to it was C18:2 cis-9,12 (linoleic acid). Palmitic acid (C16:0) was also present in relatively high amounts, ranging from 19-26% of total fatty acids. Additionally, this study also demonstrated that Patrik contained the highest content of C18:1 cis-9 at 39.34%. Atego (dehulled) had the highest content of palmitic acid (C16:0) at 26%, followed by Korok husk at 24.8%. Dehulled Korok grain and Marco Polo had a high content of linoleic acid, approximately 38%. The removal of husk in both varieties resulted in an increase in linoleic acid, although the differences were not statistically significant. Last but not least, the ratio of unsaturated/saturated in naked oats ranged from 2.9 to 3.6, while the husk of Atego and Korok had only 2.2 and 2 ratios, respectively. The manuscript attached below contains comprehensive information regarding the lipid content and fatty acid composition results of hulled, naked, and dehulled oats and the husk.

4.4 STUDY 4 - Fat content and fatty acid profiles of recently registered varieties of naked and hulled oats with and without husks.

The highest fat content was found in Kamil (naked cultivar) and lowest in Kertag (hulled). Additionally, the hulled oats had significantly lower fat content than the HOBs (4.52 g/100 g; $P < 0.0001$). Although the hulled and naked varieties were similar, they still exhibited significant differences in fat content ($P = 0.0055$).

Fatty acid composition of hulled oats

In all cases, linoleic acid emerged as the predominant fatty, accounting for 38.40 % of the total composition. It was followed by oleic acid, 32.88 % and palmitic 19.8 %. Kertag contained lowest content of palmitic acid, which was significantly different from Cavaliere ($P = 0.0005$) and Seldon ($P = 0.0002$). Conversely, Kertag had the highest content of linoleic acid, significantly differing from Cavaliere ($P = 0.0248$) and Seldon ($P = 0.0002$). The three predominant fatty acid collectively accounted over 90 % of the total fatty acid content.

Fatty acid composition in naked oats

Kamil demonstrated highest content of oleic acid, with significantly differed from all hulled oats ($P < 0.01$), except for the naked oat Otakar. Regarding linoleic acid Kamil had lowest amount of it, comparing to all hulled and naked oats it had significant differences ($P < 0.05$), except Seldon.

Fatty acid composition of dehulled oats

Dehulled oats remarkably differ in their major fatty acid content. Particularly, the oleic acid content accounted 28.69 %, which was lower than hulled oats (32.88%) and naked oats (35.81%) samples. Furthermore, notable variations in fatty acid composition were observed before and after dehulling in Cavaliere ($P < 0.0001$), Kertag ($P = 0.0276$), and Seldon ($P < 0.0001$) samples.

Comparison of hulled, dehulled, and naked oat samples

Notably the most abundant fatty acids were oleic acid (35.8% (naked oats) > 32.9% (hulled oats) > 28.7% (dehulled oats)) and linoleic acid (40.2% (dehulled oats) > 38.4% (hulled oats) > 37.1% (naked oats)).

4.5 STUDY 5 - Nutritional composition of coloured spring wheat focusing on carotenoids, tocopherols, and phenolic compounds

Tocols in wheat (Tocopherols and tocotrienols)

The total concentration of tocols (vitamin E) in the different wheat samples, ranging from 30.99 to 35.91 mg/kg DM (Table 9). Among the varieties, Pexeso had the highest total concentration of tocopherols and tocotrienols combined, while AF Jumiko had the lowest. AF Zora had a significantly higher alfa-tocopherol concentration than the other wheat varieties, measuring 12.2 ± 1.32 mg/kg DM. Conversely, Pexeso wheat had the lowest amount of alfa-tocopherol, measuring only 7.36 mg/kg DM. This value was significantly lower than the other varieties, except for Tercie. Pexeso also had the highest concentration of alfa-tocotrienol (4.22 ± 0.33 mg/kg DM), which significantly differed from all other tested varieties except Tercie. AF Jumiko contained the least amount of alfa-tocotrienol. In terms of beta-tocopherol, Pexeso demonstrated the highest concentration at 4.71 ± 0.29 mg/kg DM, followed by Tercie. Similarly, Pexeso had the highest amount of beta-tocotrienol (19.62 ± 2.47 mg/kg DM), significantly differing from the other varieties. Furthermore, a higher total tocol content was observed in spring wheat (Pexeso and Tercie) compared to winter wheat.

Table 8 : Tocopherols and tocotrienols content in coloured wheat varieties

Wheat sample	alfa tocopherol	alfa-tocotrienol	beta tocopherol	beta tocotrienol	total mg/kg DM
Tercie	8.34 ± 0.45^{cd}	3.53 ± 0.27^{ab}	4.67 ± 0.11^{a}	16.63 ± 1.92^{b}	33.17
Sultan	9.18 ± 0.76^{bc}	3.21 ± 0.31^{bc}	3.92 ± 0.62^{ab}	16.37 ± 1.73^{b}	32.68
Pexeso	7.36 ± 0.56^{d}	4.22 ± 0.33^{a}	4.71 ± 0.29^{a}	19.62 ± 2.47^{a}	35.91
AF Jumiko	10.27 ± 1.02^{b}	2.19 ± 0.16^{d}	2.34 ± 0.14^{c}	16.19 ± 1.37^{b}	30.99
AF Zora	12.2 ± 1.32^{a}	2.32 ± 0.34^{cd}	2.49 ± 0.27^{c}	15.37 ± 1.92^{b}	32.38
AF Oxana	9.65 ± 0.69^{b}	2.68 ± 0.13^{bcd}	3.68 ± 0.31^{b}	17.24 ± 1.63^{ab}	33.25

Carotenoids in wheat

Carotenoid content of different coloured wheat samples and the sum of these carotenoids were presented in Table 10. Among the analyzed samples, Pexeso (spring coloured wheat) presented the most abundant presence of carotenoids, with a significant concentration of lutein, 17.0 ± 0.21 mg/kg DM. This was accompanied by a moderate amount of lutein monoester 1.77 ± 0.11 mg/kg DM and lutein isomers 2.07 ± 0.17 mg/kg DM, resulting in a total carotenoid content of 21.03 ± 0.29 mg/kg DM. These findings suggest that Pexeso is a rich source of carotenoids. AF Jumiko presented a substantial presence of lutein 7.60 ± 0.35 mg/kg DM and lutein monoesters 1.38 ± 0.71 mg/kg DM, contributing to a significant total carotenoid content of 9.88 ± 1.00 mg/kg DM. Similarly, Tercie showed a comparable level of lutein 7.20 ± 0.20 mg/kg DM but lower quantities of lutein monoesters 0.80 ± 0.25 mg/kg DM, resulting in a total carotenoid content of 9.10 ± 0.31 mg/kg DM. In contrast, Sultan presented the lowest carotenoids content overall, particularly with a significantly lower concentration of lutein 1.80 ± 0.50 mg/kg DM and lutein isomers 0.21 ± 0.07 mg/kg DM, which makes only 5.13 ± 0.80 mg/kg DM carotenoids in total. Whereas AF Oxana and AF Zora demonstrated intermediate levels of carotenoids, with AF Oxana having a slightly higher concentration of total carotenoid content, 7.24 ± 0.82 mg/kg DM, compared to AF Zora, 6.55 ± 0.19 mg/kg DM.

Table 9: Carotenoids content in coloured wheat varieties

Wheat Sample	Lutein (all-E-lutein) (mg/kg DM)	Zeaxanthin (E-isomer) (mg/kg DM)	Lutein monoesters (mg/kg DM)	Lutein isomers 13-Z-lutein 13'-Z-lutein 9-Z-lutein 9'-Z-lutein (mg/kg DM)	Sum (mg/kg DM)
AF Jumiko	7.60 ± 0.35^{b}	0.19 ± 0.01^{ab}	1.38 ± 0.71^{ab}	0.73 ± 0.02^{b}	9.88 ± 1.00^{b}
Pexeso	17.0 ± 0.21^{a}	0.13 ± 0.01^{bc}	1.77 ± 0.11^{ab}	2.07 ± 0.17^{a}	21.03 ± 0.29^{a}
Tercie	7.20 ± 0.20^{b}	0.22 ± 0.03^{a}	0.80 ± 0.25^{b}	0.83 ± 0.01^{b}	9.10 ± 0.31^{bc}
Sultan	1.80 ± 0.50^{d}	0.14 ± 0.03^{bc}	2.95 ± 1.70^{a}	0.21 ± 0.07^{c}	5.13 ± 0.80^{d}
AF Oxana	5.70 ± 0.55^{c}	0.12 ± 0.03^{c}	0.51 ± 0.12^{b}	0.86 ± 0.14^{b}	7.24 ± 0.82^{bc}
AF Zora	5.30 ± 0.13^{c}	0.11 ± 0.02^{c}	0.46 ± 0.07^{b}	0.64 ± 0.03^{b}	6.55 ± 0.19^{cd}

Phenolic compound

The concentrations of various phenolic compounds in different wheat genotypes are presented in **Table 11**. Among the phenolic compounds analyzed, ferulic acid was found to be the most abundant. Notably, AF Jumiko (winter coloured wheat) exhibited the highest overall content of phenolic compounds, with a sum of 197.09 mg/kg. Regarding ferulic acid, AF Jumiko had the highest concentration at 176.40 mg/kg, significantly differing from the other genotypes except with Pexeso. Conversely, Sultan (78.87 mg/kg) and Terecie (91.61 mg/kg) contained the lowest concentrations of ferulic acid. Regarding vanillic acid, AF Jumiko (4.11 mg/kg) and Sultan (2.67 mg/kg) had the highest levels. While Sultan (4.0 mg/kg) and AF Jumiko (3.59 mg/kg) had the highest concentrations of gallic acid, significantly differing from the other genotypes. Sultan also showed the highest concentration of syringic acid (1.5 mg/kg) and kaempferol (2.24 mg/kg), demonstrating significant differences compared to all other cultivars.

Jumiko (3.89 mg/kg) and Sultan (3.78 mg/kg) had the highest amounts of caffeic acid. AF Jumiko contained the highest overall sum of phenolic compounds (197.09 mg/kg), significantly differing from the other genotypes. On the other hand, Terecie (94.969 mg/kg) and Sultan (104.56 mg/kg) had the lowest sums of phenolic compounds.

Table 10: Phenolic compounds in spring wheat varieties

Samples name	Ferulic acid	Va-nillic acid	Gallic acid	Syrin-gic acid	Caf-feic acid	P-cou-maric	Kaempferol	Sum
AF Jumiko	176.4 ± 2.53^{a}	4.11 ± 0.12^{a}	3.59 ± 0.13^{a}	1.50 ± 0.07^{a}	3.89 ± 1.09^{a}	5.30 ± 0.53	2.24 ± 0.23^{a}	197.09^{a}
AF Oxana	112.78 ± 5.0^{bc}	1.29 ± 0.43^{bc}	3.01 ± 0.13^{b}	0.81 ± 0.03^{c}	2.59 ± 0.03^{c}	4.91 ± 0.17	0.24 ± 0.04^{c}	123.74^{bc}
Pexeso	137.1 ± 42.54^{ab}	1.91 ± 0.51^{bc}	2.99 ± 0.25^{b}	1.14 ± 0.3^{b}	3.28 ± 0.14^{b}	4.79± 1.09	0.28 ± 0.13^{c}	125.65^{b}
Sultan	78.87± 5.07^{d}	2.67 ± 1.80^{ab}	4.00 ± 0.08^{a}	0.74 ± 0.07^{c}	3.78 ± 0.59^{ab}	4.44 ± 1.6	0.45 ± 0.12^{bc}	104.56^{c}
Tercie	91.61± 9.3^{d}	0.83 ± 0.06^{c}	2.86 ± 0.30^{b}	0.98 ± 0.04^{bc}	3.63 ± 0.15^{b}	4.39 ± 0.23	0.24 ± 0.04^{c}	94.97^{c}
AF Zora	109.95± 12.13^{bc}	2.12 ± 0.93^{bc}	3.12 ± 0.15^{b}	1.03 ± 0.19^{bc}	3.21 ± 0.07^{b}	3.71 ± 0.23	0.59 ± 0.09^{b}	151.49^{bc}

STUDY 6 - Sensory analysis of buns made from coloured spring and wheat

Table11: Basic characteristics of coloured wheat flour samples

Wheat sample	AF Jumiko	AF Oxana	AF Zora	Tercie	Pexeso	Sultan
Protein in DM (%)	12.1	14.6	15.3	12.8	13.1	14.7
Starch DM	70.7	68.2	69.6	68.5	70.2	69.5
Fat content g/100g	1.31	1.19	1.31	1.32	1.32	1.16
Moisture %	13.7	13.5	13.6	12.9	14.1	13.4
Ash %	0.9	0.6	0.6	0.6	0.7	0.7
Water absorp-tion %	61.9	57.9	58.2	55.3	54.8	61.4
Zeleny sedi-mentation vol-ume [ml]	13	25	36	56	22	24
Wet gluten DM	29.4	30.4	34.5	28.2	29.4	25.9
Hardness	20	10	32	35	13	23

The average scores of sensory attributes of the wheat bun as per the assessors were indicated in Table 12. The sensory analysis of the bun samples revealed varying levels of preference and perception among the assessors. According to the rating provided by the assessors, bun made from Tercie flour had the higher scores for overall appearance liking (71.8), whereas AF Jumiko had the lowest (50.8). Similarly, AF Oxana bun scored the highest scores in every of attributes except the overall appearance of liking, pleasantness of colour (67.6), the intensity of the colour (71.8), overall taste liking (69.3), the overall intensity of taste (59.3), the intensity of sweet test (41.2), the intensity of salty test (37.8) and overall sample acceptance (66.6).

Conversely, the AF Zora bun scored lowest in several attributes, such as the intensity of the colour (34.9), the overall intensity of taste (47), and the intensity of the sweet test (31).

Table 12: The sensory attributes in coloured wheat bun (average scores)

Sample name	Overall appear-ance lik-ing	Pleasant-ness of colour	Inten-sity of colour	Over-all taste liking	Overall inten-sity of taste	Inten-sity of sweet test	Inten-sity of salty test	Overall sample ac-ceptance
AF Jumiko	50.8	48.9^{b}	49.5^{bc}	48.3	50	30.1	29.1	52.2
AF Oxana	64.3	67.7^{ab}	71.8^{a}	69.3	59.3	41.2	37.8	66.6
AF Zora	57	52.1^{b}	34.9^{d}	53.1	47	31	27.3	52.4
Tercie	71.8	76.2^{a}	56.6^{ab}	56.2	47.7	33.1	32.4	56.2
Sultan	66.1	66.7^{ab}	65.0^{ab}	55	50.3	33.9	33.4	53.7
Pexeso	51.3	49.9^{b}	45.0^{cd}	60.4	51.9	31.5	36	60.6

Statistically significant differences between variables were denoted by different superscript letters ($p < 0.05$).

Figure 4: Spider chart depicting bun attributes

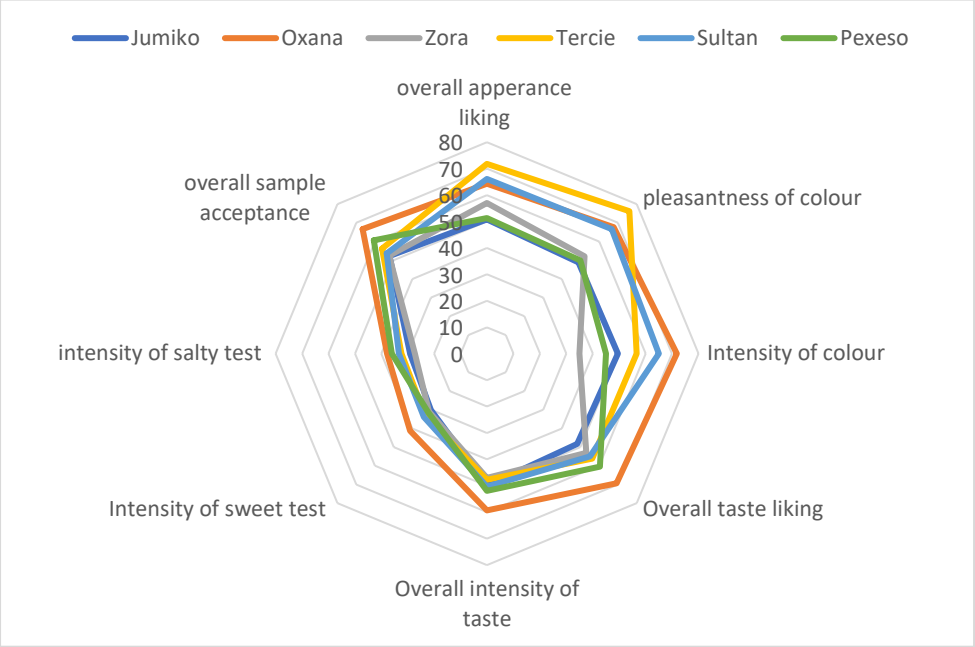


Figure 5: Overall sample acceptance compared to the age of assessors

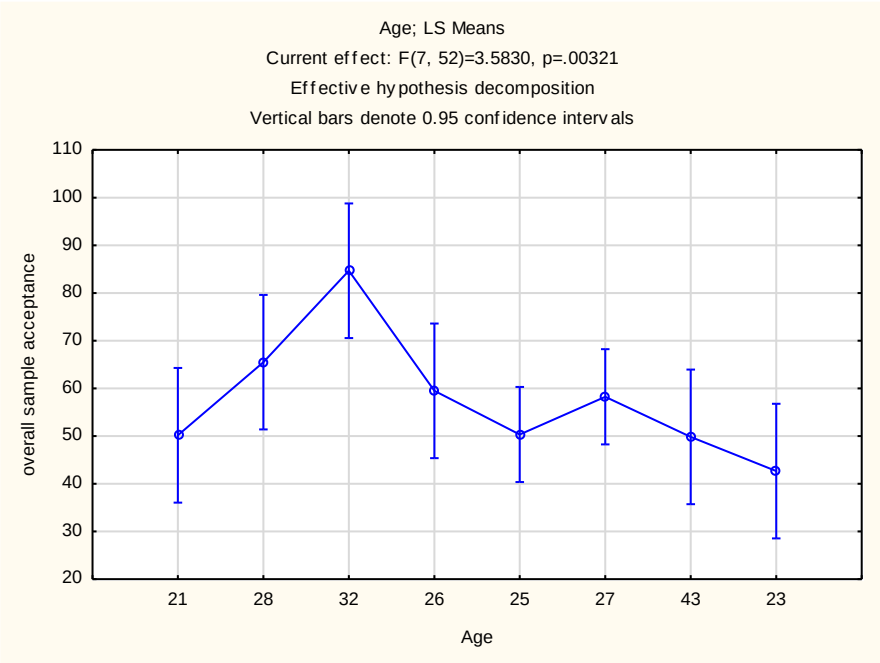


Figure 6: Intensity of buns colour

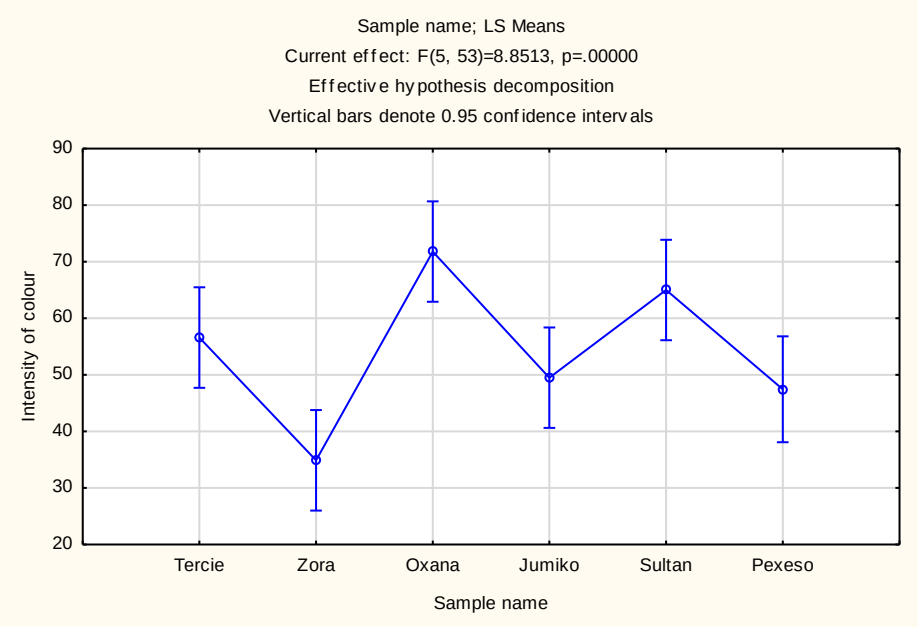


Figure 7: Intensity of salty test in different buns

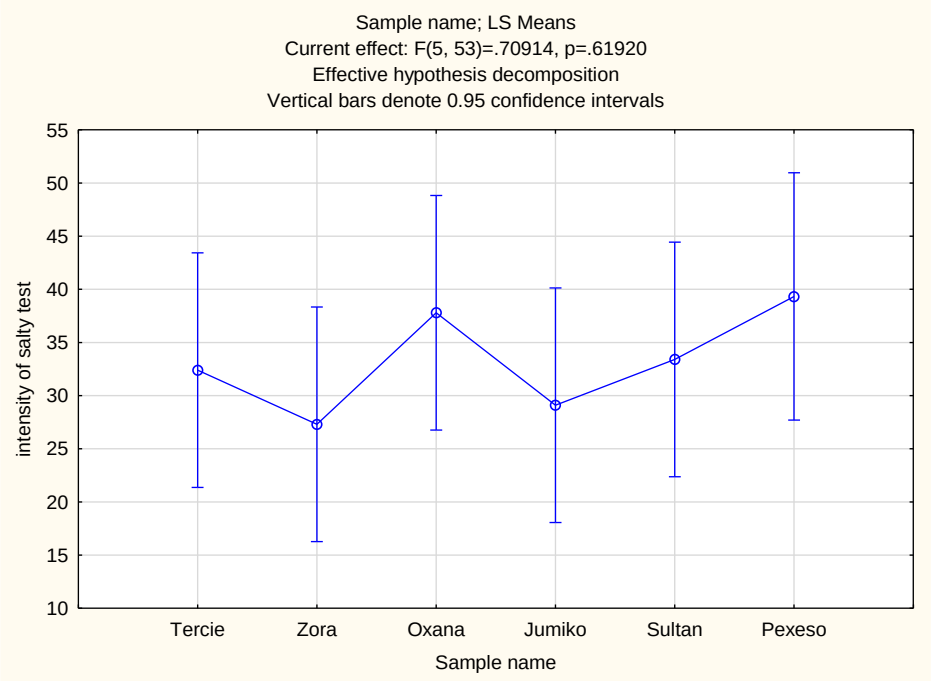


Figure 8: Intensity of sweet test in different buns

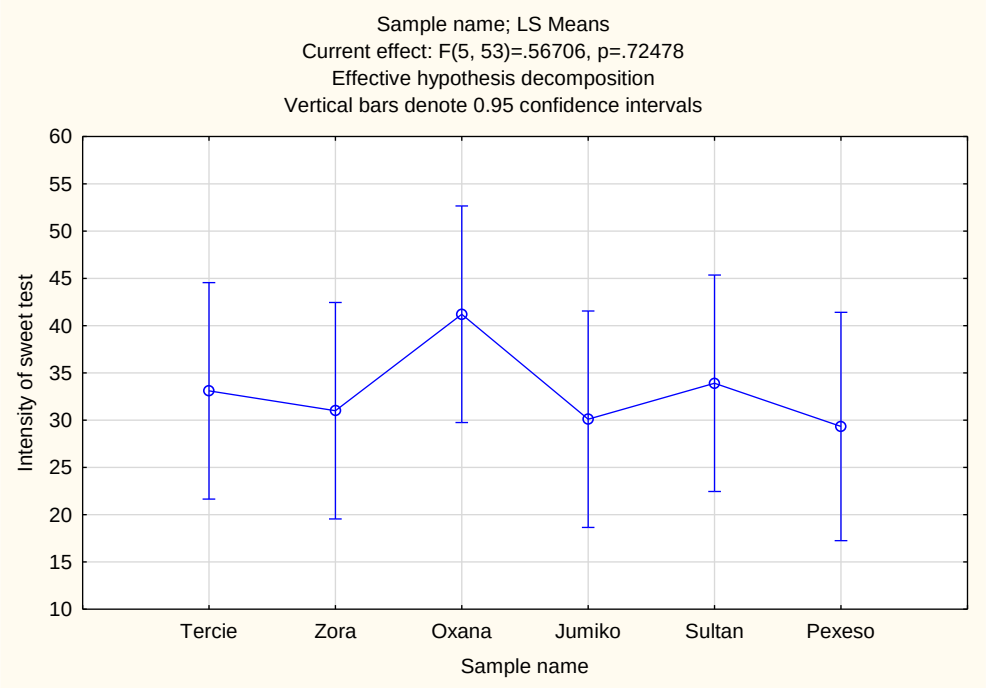


Figure 9: Intensity of overall appearance liking for different buns

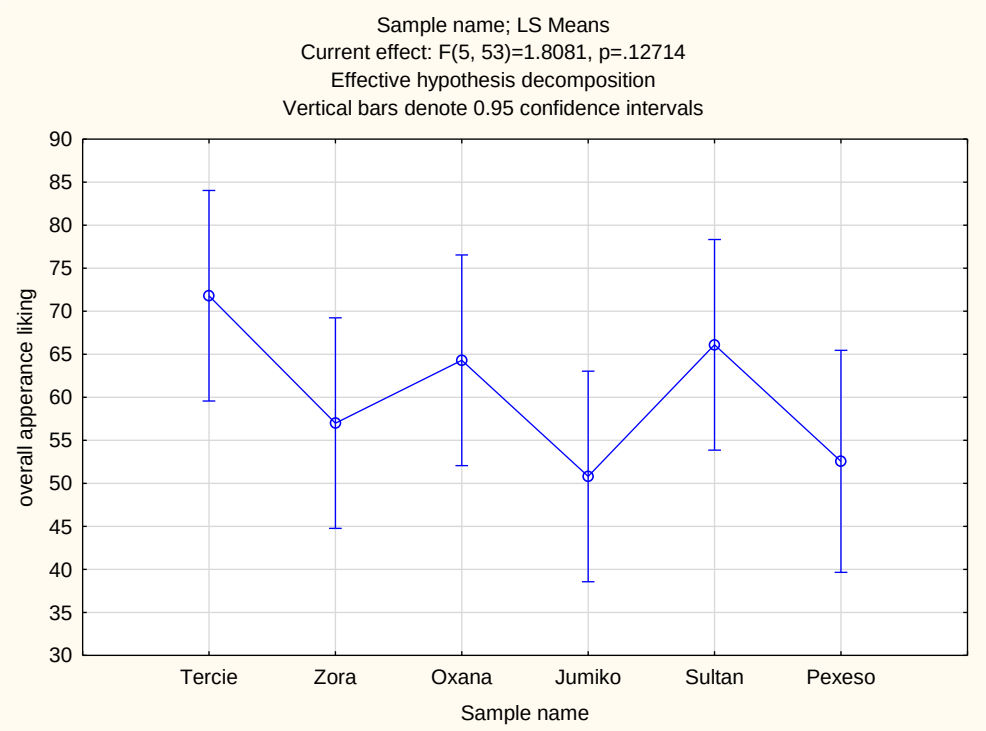


Figure 10: Intensity of overall intensity of taste in different buns

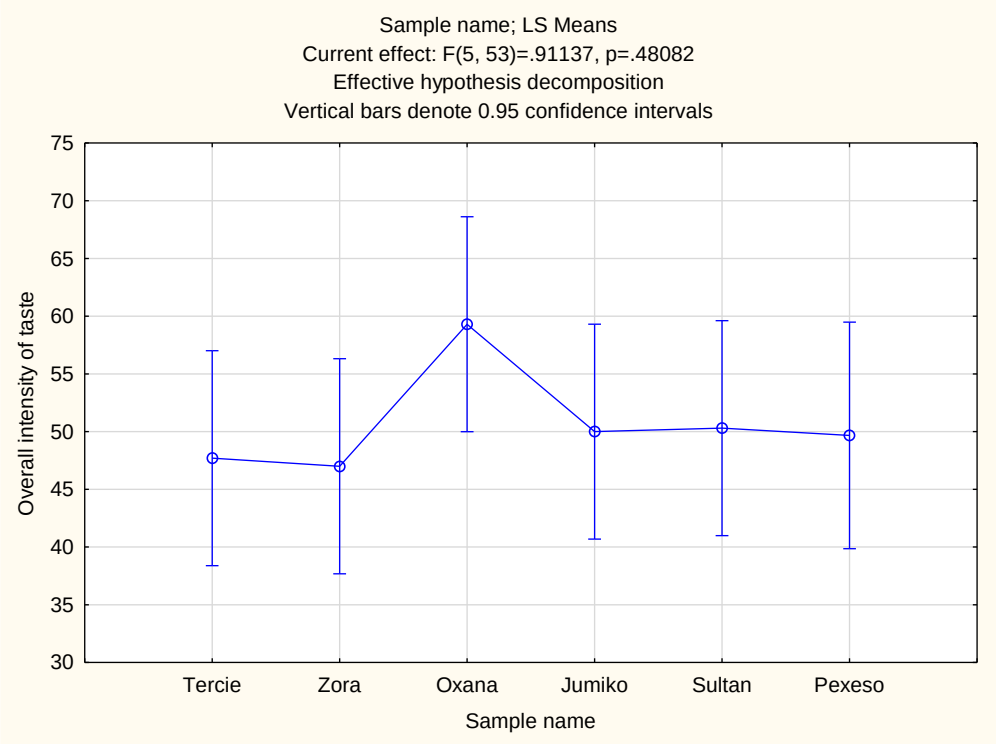


Figure 11: Intensity of overall sample acceptance in different buns

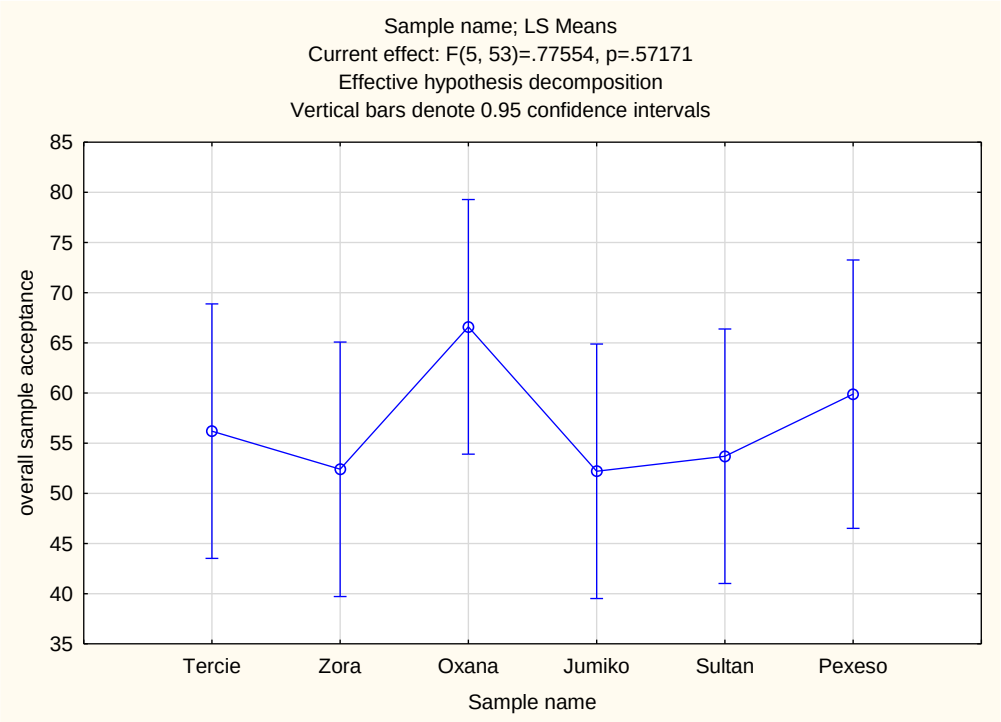


Figure 12: Intensity of overall taste liking in different buns

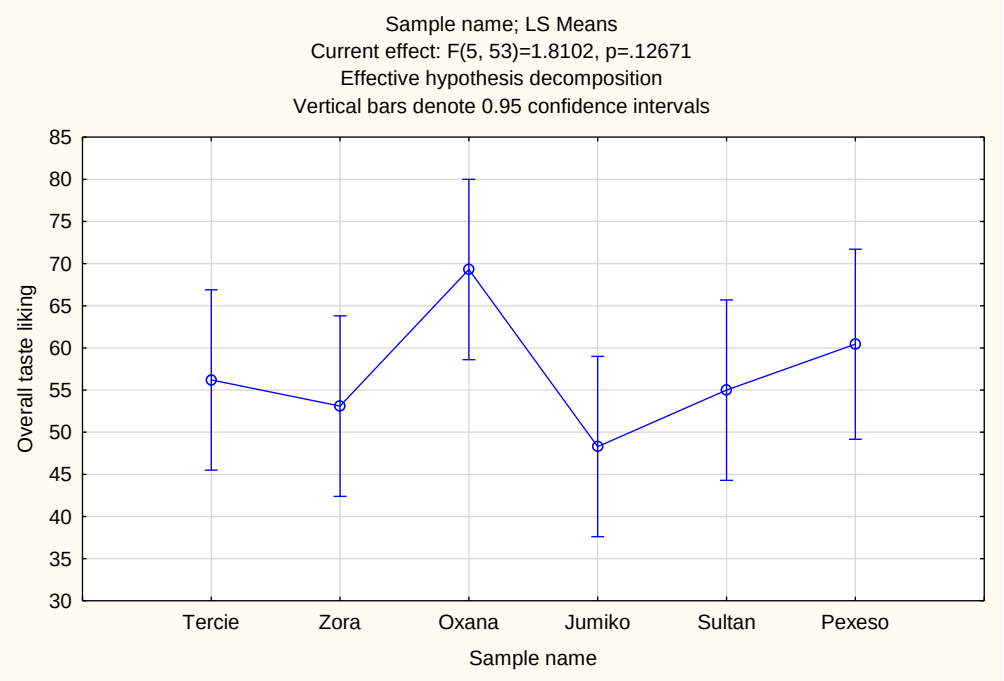


Figure 13: Intensity of pleasantness of colour in different buns

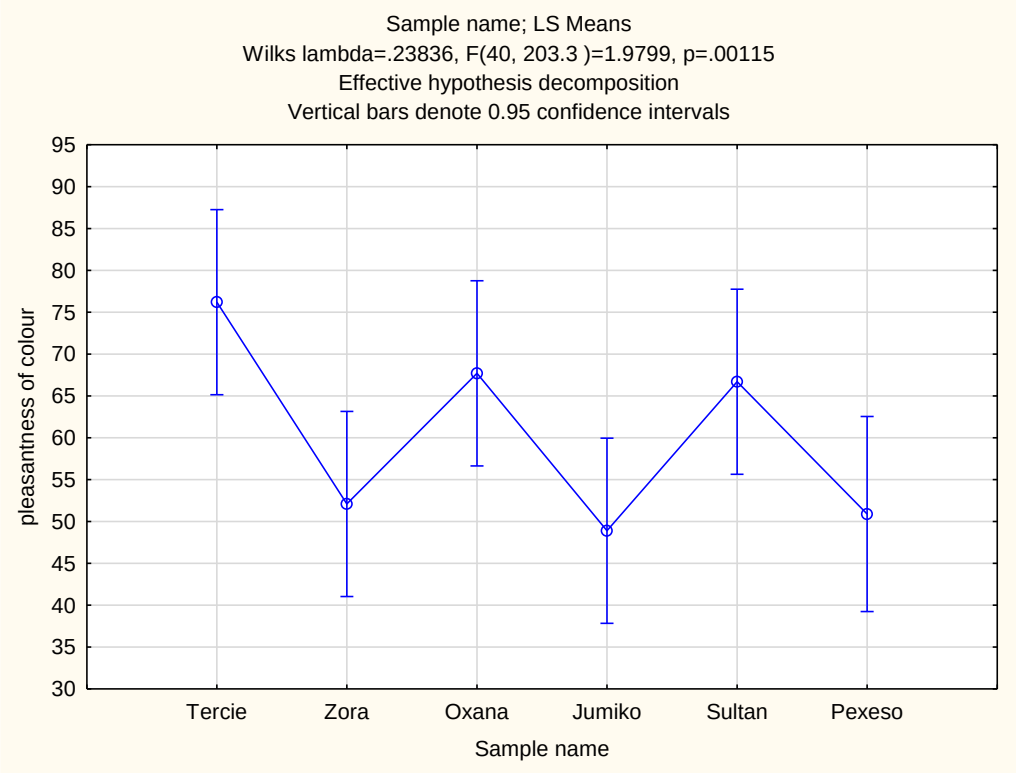
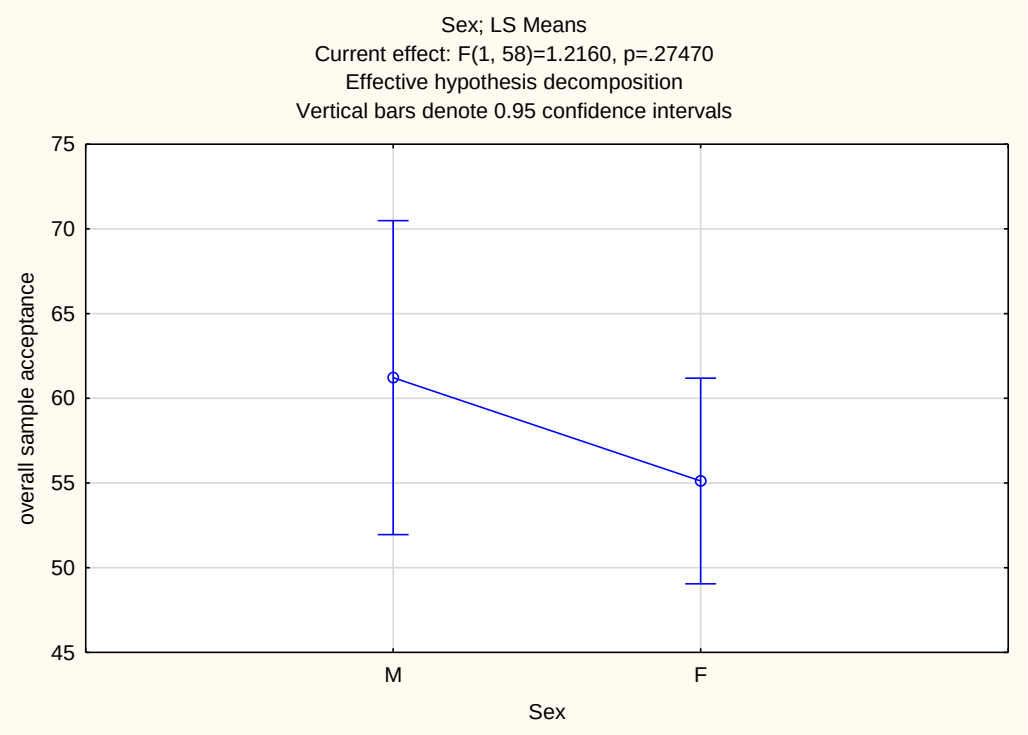


Figure 14: Intensity of overall sample acceptance compared to gender



CHAPTER 4

DISCUSSION

GENERAL DISCUSSION

STUDY 1 – Lipid content and fatty acid profile of various European and Canadian hulled and naked oat genotypes

This work summarized and discussed fatty acid profiles of oats from different continents. Unlike other studies dealing with the oil composition of oats from different regions, this study compares a wide range of varieties from different countries of origin, which were propagated under the same conditions in one growing location in the Czech Republic. Therefore, it was possibility to compare differences between oat varieties in addition to considering growing and climate conditions. The lipid content of various oats ranged from 3.0-9.5 g/100g, which aligns well with previous findings such as 5.9-7.9 g/100 g reported by (Batalova et al., 2019), 5-6 g/100 g reported by (Banaś et al., 2007), 4.8-6.0 g/100 g reported by (Capouchová et al., 2021) and 2.9-5.8 g/100 g reported by (Kouřimská et al., 2021). The temperature has been identified as a key factor that influences the lipid content of oats, as reported by several authors (Saastamoinen, 1998). A similar notable illustration of this relationship between the lipid content and the plant growing temperature can be observed in the case of Lidiya (Russian genotype), which originates from extremely cold agricultural regions and presents the highest lipid content. Besides temperature, it is worth considering that soil nutrition also strongly impacts oats' lipid content.

The combination of linoleic, oleic, and palmitic acids in Canadian and European genotypes was 91.90% and 91.56%, respectively. Our findings support the conclusions drawn in the study by Thacker et al. (2004), who stated that the content of palmitic and linoleic acids was 17.20% and 39.10%, respectively. Moreover, their study also found a higher proportion of oleic acid (42.80%) in the high-fat Canadian SA96121 oat genotype (a cross-breed of ND870425 × CDC Boyer) compared to our findings. In our study, the proportions of palmitic, oleic, and linoleic acids in CDC Boyer were 18.25%, 37.45%, and 36.14%, respectively.

The reported values for palmitic, oleic, and linoleic acids were 16.7% to 20.5%, 31.7% to 38.9%, and 37.2% to 44.4%, respectively, in the analysis of 18 different oat genotypes grown at 6 different locations (Doehlert et al., 2013), which are consistent with our findings. Furthermore, they also observed environmental and genotypical variations, similar to our study except for C14:1 and C16:0. Similarly, Batalova et al. (2019) reported palmitic acid ranging

from 15.5% to 17.8%, oleic acid from 33.5% to 36.7%, and linoleic acid from 35.9% to 38.7% in the 7 different Russian naked oats, which corresponds to our findings of 18.9%, 34.6%, and 38%, respectively. In another study, Sterna et al. (2016) analyzed five oat genotypes and found comparable values for C16:0, C18:1, and C18:2, ranging from 15.5% to 17.4%, 36.2% to 40.4%, and 38.4% to 41.6%, respectively.

The proportion of SFA, MUFA, and PUFA were 21.96 %, 38.24 %, and 39.80 % in hulled oats and 22.43%, 38.79%, and 38.78% in naked oats, respectively. Our findings align with the research conducted by (Capouchová et al., 2021), who observed 21.22% SFA, 37.79% MUFA, and 41.28% PUFA in oats cultivated using a conventional cropping system.

STUDY 2 - Assessing the tocopherol content and oxidative stability of the crude oat oil using the Schaal oven and Rancimat tests

This study is one of few studies to successfully find the correlation between tocol concentrations and the thermal stability of oats. This study represents a significant advancement in understanding their relationship. Our findings align with previous research that reported 90 ppm α-tocopherol, 13 ppm γ-tocopherol, and 6.5 ppm δ-tocopherol (Saga et al., 2013). Additionally, in a study of 12 oat cultivars, a wider range of tocopherol levels (19-31 mg/kg of dry matter) was reported by (Peterson and Qureshi, 1993). In general, various factors influence tocol concentration in oat oils, including environmental conditions, genotypes, and extraction methods.

Our study has also provided evidence of oat oils' exceptional thermal stability properties. The results of our current study indicate a significantly higher protection factor (PF) compared to a previous study conducted by Holasova et al. (2002), which reported a PF of 1.8.

In the Schaal oven test, we did not identify a correlation between tocol content and the IP of oat oils. Besides tocopherols and tocotrienols, other components like avenanthramides, phenolic compounds, phytic acids, flavonoids, and sterols also contributed to antioxidant properties. In the Rancimat test, total tocol concentration correlated with the IP at 110°C, total tocopherols correlated with IP at 100°C, and β-tocotrienol content correlated with IP at both temperatures. A previous study using the Rancimat test demonstrated that the IP of sunflower oil was 226.2 min at 110°C and 127.2 min at 120°C (Almoselhy, 2021). After the first month of storage, Rapeseed oil had an IP of 5 h, and in the twelfth month of storage, it had a 3.2 h IP. Both of these were subjected to the Rancimat test at 120°C (Maszewska et al., 2018). These values are higher than the results of our studies.

The current study's findings align with previous research indicating that oat oil and its fractions have an oxidation IP of 6.9 hours at 110°C (AG, 2019). Oat oil, at minimum concentrations of 1-5 %, significantly increased the IP of lard and tallow by 2-8-fold from non-antioxidant levels. The manuscript attached below contains comprehensive information regarding the discussion of study 2.

STUDY 3 - Comparative analysis of lipid content and fatty acid composition in hulled and naked oats compared with dehulled grains and husks

In this study, the total lipid content in various oat samples ranged from 3.2 to 6 g/100g. These findings align with previous studies that documented lipid content in oats ranging from 3% to 11%, which is comparatively higher than in other cereals such as wheat (1.4% to 1.5%) and barley (1.3% to 11%) (Ciołek et al., 2012). The current study corroborates the conclusions of previous investigations by Banaś et al. (2007), Flander et al. (2007), and Saastamoinen et al. (1990), who reported lipid content in oats ranging from 7% to 11%, 5% to 9%, and 5.6% to 7.5%, respectively.

Additionally, Capouchová et al. (2021) reported a lipid content of 6 g/100g in the Patrik variety, which slightly exceeded the measurements of our current study. Furthermore, Boeck et al. (2018) reported higher fat content values for Oliver (6.02 g/100g), Kamil (7.36 g/100g), and Saul (5.41 g/100g) compared to the fat content values obtained in our study. A study conducted by Welch et al. (1983) found that oat husks contained 1% to 2.2% of lipids, higher than the findings of our present study. The attached manuscript below contains comprehensive information regarding the discussion of Study 3.

STUDY 4 Fat content and fatty acid profiles of recently registered varieties of naked and hulled oats with and without husks.

The current study contributed valuable insights into the lipid content, composition and lipid health indices of globally produced and recently registered hulled oats with husk and naked oats. We also compared these characteristics in dehulled husked oats and naked oat varieties. The study was performed to complete and fill in the missing nutritional composition and lipid index data for new oat cultivars recently registered and harvested in the Czech Republic and other regions. The lipid content of naked oat cultivars was significantly higher (~7.9 % more) than other cereals like barley, wheat, and maize. The results align with the lipid content ranges reported by (Boeck et al., 2018) (5.3-7.4%), (Kourimska et al., 2018) (2.9-6.1%) and (Leonova et al., 2008) (4.1-8.3%). Additionally, Biel et al. (2009) stated that 8.4 % of oats grain dry matter fat is slightly higher than in the present study (2.88 – 5.82 %). The factors that affect the lipid content in grains include genetic factors, season, growth conditions, post-harvest treatments, and storage conditions (Ahmet et al., 2019).

Oat lipids, known for their richness in polyunsaturated fatty acids (PUFA), exhibit remarkable stability against oxidation due to their high content of antioxidants such as vitamin E and polyphenols (Peterson, 2001). Consequently, dehulled oat grains can be stored for extended periods without rancidity (Peltonen-Sainio et al., 2004). Oat contained higher levels of palmitic and oleic acids than various cereals, barley, wheat, and maize, as reported in a comparative study by (Batalova et al., 2019). Our results found a higher level of linoleic acid in the oat samples, which is consistence with the findings by Tong et al. (2014), who further emphasized the nutritional benefits of oat oil in reducing blood lipid, plasma cholesterol levels, and hepatic. Moreover, the majority of PUFAs identified in the analyzed oat samples were n-6, whereas n-3 was identified at a level of ≤3%. Interestingly, the naked oats had a lower level of n-3 than hulled oats varieties, and dehulling process increased the n-6 content in hulled oats. The manuscript attached below contains comprehensive information regarding the discussion of Study 4.

STUDY 5 - Nutritional composition of coloured wheat focusing on carotenoids, tocopherols, and phenolic compounds

Among the coloured wheat, tocol was detected in a range of 30.99 – 35.91 mg/kg DW. The range of α-tocopherol was 7.36 – 12.2 mg/kg DW, α-tocotrienol 2.19 – 4.22 mg/kg DW, β-tocopherol 2.34 – 4.71 mg/kg DW, and β- tocotrienol 15.37 – 19.62 mg/kg DW. The results are in line with the reported data by (Lachman et al., 2018), who documented that the total tocols in spring wheat varied between 22 – 43.67 mg/kg DW, with an average value of 34.52 mg/kg DW across all varieties in spring wheat. In contrast, winter wheat contained 24.48 ± 4.44 mg/kg DW (average value). This study also reported that only four out of eight tocols were identified (β-T3, α-T3, β-T, and α-T) in their study. The individual mean value of α-tocopherol was 14.11 mg/kg DW, α-tocotrienol 2.59 mg/kg DW, β-tocopherol 5.08 mg/kg DW, and β-tocotrienol 12.74 mg/kg DW.

The current study results align with the previous findings of Tsao (2007). Tsao reported that β-tocotrienol was the most abundant tocol in einkorn, with a concentration of 48.22 mg/kg DW. It was followed by α-tocotrienol 12.77 mg/kg DW, α-tocopherol 12.18 mg/kg DW, and β-tocopherol 4.79 mg/kg DW. The average ratio of tocotrienols to tocopherols in einkorn was estimated to be 1:3.68. In our study, the most abundant tocol is β- tocotrienol, whereas the ratio of tocotrienols to tocopherol is 1:1.10 to 1:2.24. Similarly, the HEALTHGRAIN project evaluated dietary fiber and bioactive components in a wide range of wheat varieties, including 150 bread wheat lines at a single site, 50 lines of other wheat species and cereals at the same site, and 23-26 bread wheat lines across six environments. The results revealed a range of tocol concentration in common bread wheat was 27.6–79.7 mg/kg, durum wheat ranged from 40.1 to 62.7 mg/kg, spelt showed concentrations between 40.2 and 50.6 mg/kg, einkorn contained levels ranging from 29.0 to 57.5 mg/kg, and emmer contained concentrations within the range of 29.0–57.5 mg/kg (Shewry et al., 2013). The results of our study were lower compared to previous findings, perhaps due to the difference in the genotypes, analytical methods and the growing environment.

In the evaluation of fifteen diploid, tetraploid, and hexaploid accessions from various Triticum species it was found that *T. thaoudar* contained 75.1 ± 3.95 mg/kg DM, *T. aegilopoides* contained 70.8 ± 3.35 mg/kg DM, *T. monococcum* contained 66.8 ± 3.82 mg/kg DM, and *T.*

urartu contained 63.9 ± 2.91 mg/kg DM (Brandolini et al., 2015 and Hidalgo et al., 2006). The tocols in various wheat species, where α-tocopherol ranged from 9.9 ± 0.01 to 12.9 ± 0.16, α-tocotrienol ranged from 4.2 ± 0.03 to 17.5 ± 0.85, β-tocopherol ranged from 2.9 ± 0.04 to 8.8 ± 0.07, and β-tocotrienol ranged from 23.1 ± 0.34 to 45.9 (Brandolini et al., 2015). These findings are higher compared to the results obtained in the current study, because of the differences in the genotypes, analytical methods, growing year and the environment.

Furthermore, the highest tocol contents were observed in diploid wheat, with β-tocotrienol ranging from 37.90 to 45.90 mg/kg dry weight (DW). This was followed by α-tocopherol ranging from 9.9 to 12.6 mg/kg DW, while the lowest contents were reported for β-tocopherol, ranging from 2.9 to 5.5 mg/kg DW. Additionally, the total tocol content in durum wheat was 33.75 mg/kg DW, in bread wheat, it was 36.85 mg/kg DW, and in triticale, it was 33.00 mg/kg DW (Irakli et al., 2011). These findings correspond to the results obtained in the current study.

Our study found that the total carotenoid range in wheat samples was 6.55 - 21.03 mg/kg DM. Antheraxanthin, α-carotene, β-carotene, Z-isomers of zeaxanthin, and lutein diesters were not detected in the analyzed samples. The range of carotenoid concentration in various wheat species was (α+β)-carotene ranging from 0.5 - 0.7 mg/kg DM, β-cryptoxanthin ranging from 0.05 - 0.09 mg/kg DM, lutein ranging from 1.1 - 6.9 mg/kg DM, zeaxanthin ranging from 0.3 - 0.8 mg/kg DM, and total carotenoids ranging from 1.4 - 8.3 mg/kg DM (Brandolini et al., 2015). The sum of total carotenoids in the eight Triticum subspecies ranges from 3.35 mg/kg DM to 16.79 mg/kg DM, which corresponds to the current study. Lutein is recognized as the predominant carotenoid, comprising 91 % of the total carotenoid content (Hidalgo et al., 2006). Similarly, in spring and winter wheat, lutein comprises 71.3 - 83.3% (Konopka et al., 2006).

Carotenoid levels (mainly lutein) vary across the wheat varieties. In bread wheat, the concentrations of lutein are relatively low, ranging from 0.1 – 2.5 mg/kg DM, whereas durum wheat contained 1.5 - 4.8 mg/kg DM of lutein (Hidalgo et al., 2006, Panfili et al., 2004). The highest carotenoids content among the wheat cultivar is found in einkorn, with an average of 8.5 mg/kg dm and a range of 5.3 to 13.6 mg/kg DM (Abdel-Aal et al., 2002, Brandolini et al., 2008, Hidalgo et al., 2006). Environmental factors undoubtedly influence the concentration of carotenoids and tocols in cereals, genetic compounds with heritability values also influence the carotenoids in cereals (Hidalgo et al., 2006). Furthermore, the total carotenoid content was

reported to be 8.41 µg/g (DM) in einkorn accessions, and 62.75 µg/g (DM) in T. *turgidum* and T. *aestivum* controls. Our findings correspond to the value of einkorn accessions.

The carotenoid contents of lutein, zeaxanthin, and β-carotene were determined in 5 einkorn, 5 emmer, and 5 spring wheat and reported that Lutein was the predominant carotenoid in wheat cultivars, approximately 83% on average, followed by zeaxanthin at around 10% and β-carotene at approximately 7% (Lachman et al., 2013). Moreover, lutein concentrations were found to be 1.096 mg/kg DM in spring wheat, 5.246 mg/kg DM in Einkorn, and 0.761 mg/kg DM in Emmer. Zeaxanthin levels were 0.144 mg/kg DM in spring wheat, 0.351 mg/kg DM in Einkorn, and 0.138 mg/kg DM in Emmer. β-Carotene concentrations were 0.116 mg/kg DM in spring wheat and 0.195 mg/kg DM in Einkorn. Ramachandran et al. (2010) identified lutein as the primary carotenoid constituent in durum wheat. The ratio of trans-zeaxanthin to trans-lutein was inverse-correlated with yellow pigment concentration.

In this study, ferulic acid emerged as the predominant phenolic compound, ranging from 78.87 to 176.4 mg/kg. Additionally, moderate levels of vanillic acid, gallic acid, syringic acid, caffeic acid, p-coumaric, and kaempferol were detected. Overall, the total content of phenolic compounds ranged from 94.97 to 197.09 mg/kg. The interest in phenolic compounds primarily arises from their antioxidant properties. Their capacity as potent "free radical scavengers" has garnered significant attention, leading to garnered significant attention for numerous studies present in various plant species, cereals included. Consistent with earlier research (Klepacka and Fornal, 2006), our study also confirms that ferulic acid is present in the highest concentration among wheat components.

The results of the current study are consistent with the findings reported by Hernández et al. (2011). They also observed that Ferulic acid demonstrated the highest concentrations in both *Triticum aestivum* (272 µg/g) and *Triticum turgidum* (253 µg/g). Moreover, in *T. aestivum*, vanillic acid (4.53 µg/g), syringic acid (31.4 µg/g), and p-coumaric acid (21.2 µg/g) were detected. Similarly, in *T. turgidum*, the levels of vanillic acid (4.49 µg/g), syringic acid (26.7 µg/g), and p-coumaric acid (18.7 µg/g) were also found.

The phenolic compound analysis of six hard spring wheat varieties revealed varying concentrations of vanillic acid (8.17-9.69 µg/g), caffeic acid (7.6-12.87 µg/g), syringic acid

(11.61-16.05 µg/g), p-coumaric acid (28.45-37.22 µg/g), and ferulic acid (371.04-441.02 µg/g). These findings align with the results of our current study (Mpofu et al., 2006). In that study, the influence of environmental factors on total phenolic content (TPC) and antioxidant activity (AOA), as well as vanillic acid, syringic acid, and ferulic acid, was found to be significantly higher than the impact of genotypes.

Our study observed that ferulic acid accounted for a maximum portion, ranging from 83.06 - 90.50 %, of the total phenolic content in coloured wheat. This finding is consistent with earlier research by Zuchowski et al. (2011), who reported that a range of ferulic acid represented 85.3 - 89.3% of total phenolic acid content in spring wheat. Additionally, that study reported syringic acid at 5 - 7.7 %, p-coumaric acid at 1.8 - 3. 0%, and vanillic acid (VA) at 1.9 - 2.3 %. Conversely, our results revealed vanillic acid at 0.8 - 2.81%, syringic acid at 0.65 - 0.94 %, and p-coumaric acid at 2.69 - 4.68%. Our findings partially align with those of the previous study. Regular consumption of phenolic acids has been shown to promote the anti-inflammatory capacity in humans. Thus, developing wheat varieties abundant in phenolic acids has been an important issue (Shamanin et al., 2022). Nowadays, attention is given in wheat grain phenolic compounds due to the growing preference for natural antioxidants which will help to reduce risk of chronic diseases (Tian et al., 2021).

Genotype (contributed 9 – 32 %), environment (contributed 56 – 79 %) and their interplay (contributed 8- 13 %) significantly influenced wheat grain phenolic compounds and the antioxidant activity (Moore et al., 2006; Martini et al 2015 and Zrcková et al., 2019). Conversely, it has been reported in that the impact of the environment on the phenolic compounds levels in spring wheat is unclear (Zuchowski et al., 2011).

Furthermore, the study has highlighted a contrast between spring and winter wheat: spring wheat exhibited higher levels of tocols and carotenoids, while winter wheat contained elevated levels of phenolic compounds. Further research is needed for a comprehensive analysis of phytochemicals and antioxidant activities across diverse varieties of whole-coloured wheat samples and compared with other wheat varieties.

Study 6: Sensory analysis of buns made from coloured spring and wheat

Bread and buns are popular bakery products consumed in large quantities worldwide. They provide remarkable nutrition to the human with macronutrients (carbohydrates and protein) and micro nutrition (iron, calcium, and B vitamins). Due to their extensive consumption, a considerable amount of wheat bread is wasted globally because of undesirable sensory attributes such as flavour, taste, and aroma, as well as microbial activities and physical changes (Mollakhalili-meybodi et al., 2023). While addressing microbial activities and physical changes can be approached differently while modifying sensory properties through sensory evaluation and adjusting bakery products based on customer preferences can contribute to global food security.

Several factors can influence the sensory evaluation of bakery products composed of wheat flour. The reduction in flour particle size led to a decrease in aftertaste intensity. The way of milling (roller or stone mill) also affects the sensory attributes of whole meal bread (Kihlberg et al., 2004 and Protonotariou et al., 2020). Moreover, factors including flour extraction degree, protein content, and gluten content, salt, water content, leavening agents (which will produce several components like alcohols, esters, aldehydes, and ketones), processing steps (mixing, fermentation, and baking) will affect the sensory perception of bread and bun (Salehifar et al., 2010; Boz and Karaoğlu, 2013; Avramenko et al., 2018 and Mollakhalili-meybodi et al., 2023). No data is present regarding the sensory analysis test of buns/bread produced from the same samples utilized in our experiment.

A significant difference was found in the colour of the bun. The results show variations in the pleasantness of colour and intensity of colour. There were some inconsistencies regarding the panellists, which are common in sensory analysis and the variations might be relatively minor compared to the differences observed between the samples (Annett et al., 2007). The taste is an important sensory descriptor for consumers (Hrivna et al., 2015). Significant differences were not observed in terms of taste attributes. Bun made up of AF oxana variety scored higher in most of the attributes, followed by those made from Tercie. Conversely, buns crafted from the AF zora variety which has dark seed colour, yielded lower scores in multiple attributes. As discussed by Campbell (1970) high protein content could be associated with the degree of darkness in seed colour. In addition, the quality of wheat bread is influenced by the amount

and type of storage protein within the endosperm (Li and Beta, 2011). Whereas, in our study, the protein content of AF Zora was high than other varieties, but the colour of the floor and bun was not darker than other varieties.

As reported by (Šebestíková et al., 2023), except blue AF Oxana variety, other varieties (AF Zora, KM 111-18, Vanessa, and AF Jumiko) had a notable bitter aftertaste and off-flavours in their sensory evaluation of bread made from coloured wheat. Our findings indicated that all taste-related attributes received high scores in the case of AF Oxana's bun. Hemdane et al. (2016) suggested that this could potentially happen by the attributes of enzymes present in the bran, which will create an unpleasant taste sensation. As per the review of Dhua et al., (2021) the possibility of bitter taste could be ascribed to proanthocyanidins formed through flavonoid biosynthetic pathway. Nevertheless, these off-flavours can impact the overall product acceptability.

The laboratory-based bun production process was characterized by simplicity and fundamental techniques, whereas bun and bread production in commercial bakeries and factories predominantly aligns with consumer preferences. As a result, sensory attributes tend to be more pronounced in bakery and factory settings compared to laboratory conditions. Therefore, new coloured wheat carries tremendous potential concerning bakery production. Further studies are required involving diverse coloured wheat compositions for forming buns and bread, encompassing additional attributes not covered in this study.

CHAPTER 5

CONCLUSION

The outcomes of this study provided valuable insights into how oat lipid content and fatty acid composition can differ by the genotypes even though they are grown in the same location, same condition, and same season. The study has yielded notable findings that genotypes can influence the composition of oats and the Canadian genotypes contained a higher amount of unsaturated fatty acid than the European genotypes. This study found variations in lipid content and fatty acid composition among oats, however, the precise relationship between the factors influencing the fatty acid profile and the geographical origin of oats warrants further investigation.

Moreover, the husk removal affects the lipid content by increasing the lipid content in dehulled (hull-less) oats, as well as changing the fatty acid composition of oats by changing the proportion of PUFA and MUFA. The naked oats appear to be having a higher proportion of lipid content than the husked oats. Additionally, naked oat varieties showed distinct differences from both hulled and dehulled samples in terms of lipid content and fatty acid composition. Oat varieties showed low atherogenicity and thrombogenicity indices; which can lower the risk of developing cardiovascular diseases. Further research is needed on oat hulls as animal feed and effective utilization of oat husk.

Our and various studies have confirmed oats are rich in unsaturated fatty acids and thus oat oils are more susceptible to oxidation during processing and storage. Upon the stability test against Schaal oven and Rancimat tests, oat oils showed remarkable resistance to oxidation in both tests. The exceptional oxidative stability of oat oil is attributed to its higher tocol content and potentially other bioactive compounds. Furthermore, the concentrations of tocopherols and tocotrienols showed correlations in some cases of Rancimat tests but with not induction period of Schaal oven test. Further research is needed to explore in the role of oats antioxidation compounds and their relations to the thermal stability of oat oil.

Coloured wheat is good sources of antioxidants such as tocols, carotenoids and phenolic compounds. Coloured wheat is rich in carotenoids, primarily lutein accounted more than 80% of all detected carotenoids. Ferulic acid emerged as the predominant phenolic acid among the analysed colour wheat varieties. Additionally, beta-tocotrienol took precedence as the predominant form of vitamin E across all coloured wheat varieties. The health benefits derived

from consuming whole grains might be attributed to the phytochemicals they contain. Moreover, spring wheat had more tocols and carotenoids than winter wheat. On the other hand, winter wheat had a greater quantity of phenolic compounds compared to spring wheat.

In a sensory analysis of buns made from coloured wheat, the "AF Oxana" bun scored the highest in most attributes, and other varieties also received decent scores. The production of buns in a bakery or factory will likely scores higher compared to buns made under laboratory conditions. Taking all of these factors into consideration, it can be concluded that coloured wheat, which contains significant amounts of phytochemicals, can be effectively utilized in the bakery industry. This usage will contribute to both health benefits and an enhanced taste experience.

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LIST OF PUBLICATIONS

Kouřimská, L., **Pokhrel, K**., Božik, M., Tilami, S.K. and Horčička, P., 2021. Fat content and fatty acid profiles of recently registered varieties of naked and hulled oats with and without husks. Journal of Cereal Science, 99, p.103216.

Pokhrel, K., Kouřimská, L., Pazderů, K., Capouchova, I. and Božik, M., 2022. Lipid content and fatty acid profile of various European and Canadian hulled and naked oat genotypes. Journal of Cereal Science, 108, p.103580.

Manscript under review

Pokhrel, K., Kouřimská, L., Tilami, S.K. and Rudolf, O., Oxidative Stability of Crude Oils Relative to Tocol Content from Eight Oat Cultivars: Comparing the Schaal Oven and Rancimat Tests. Available at SSRN 4517532.

Conference paper

Pokhrel Kshitiz., 2021. Fat content and fatty acid profiles of recently registered varieties of naked and hulled oats with and without husks Czech Food Chem 2021.

Manscript in progress

Comparative analysis of lipid content and fatty acid composition in hulled and naked oats: A study of dehulled grains and husks

Nutritional composition of coloured wheat focusing on carotenoids, tocopherols, and phenolic compounds

Sensory analysis of buns made from coloured spring and wheat.

Journal of Cereal Science 108 (2022) 103580

Contents lists available at ScienceDirect

Journal of Cereal Science

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

Lipid content and fatty acid profile of various European and Canadian hulled and naked oat genotypes

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ARTICLE INFO

Keywords:
Oats
European genotypes
Canadian genotypes
Lipid content
Fatty acid profile

ABSTRACT

Oat is considered the cereal of the future, presenting unique lipid composition in relation to that of other cereals. As the genotype factor could affect fatty acid composition of oat lipids this study evaluated the lipid content and fatty acid composition of 31 oat genotypes from various countries in Europe and 7 genotypes from Canada as representatives of two different continents and major oat producers. The lipid content of the Canadian oat genotype was 2.9%–6.29%, whereas that of the European one was 3.52%–9.51%. In Canadian oats, the dominant fatty acids were linoleic (36.76%), oleic (36.50%), and palmitic acids (18.57%) whereas, in European oats, the content of linoleic (37.25%), oleic (35.67%) and palmitic acid (18.64%) in average. Among the three major fatty acids, significant differences were mostly found in oleic acid rather than in palmitic and linoleic acids. Lidia (Swedish genotype), Ivore, and Dakar (French genotype) oats contained the highest quantities of palmitic, oleic, and linoleic acids, respectively. No significant differences were observed in the total quantity of saturated fatty acids. Conversely, significant differences were observed in the monounsaturated fatty acids for the majority of the cases. This study has demonstrated the effect of genotypes on lipid content and the fatty acid composition in oats, regardless they were grown within one season under same climatic conditions.

1. Introduction

The majority of the world's population depends on cereals and their derivatives for energy and nutrition. Oats belong to the *Poaceae* family and are considered an annual grass. In the past, the primary use of oats (*Avena sativa* L.) was for animal feeding, whereas today, oat grains have become a part of the human diet because of the health benefits they present.

Oats are a rich source of carbohydrates (mainly in the starch form) and lipids (of which 90% is present in the endosperm). Studies showed that the consumption of oats reduces the total cholesterol by 2%–19%, LDL-cholesterol by 4%–23%, and systolic blood pressure by 4%–6% Thies et al. (2014). Oats contain several components that decrease the risk of cardiovascular diseases, such as avenanthramides, polyphenolic compounds, water-soluble β-glucans, tocochromanols, phytic acid, melatonin, phytosterols, and inositol phosphates Banaś and Harasym (2021).

Regarding the fatty acid composition, three main fatty acids present in oats are oleic, linoleic, and palmitic acids. Moreover, unsaturated fatty acids are considered to be healthier than saturated ones and oats contain the highest quantity of unsaturated fatty acids among other conventional cereals like wheat, barley, rice, etc and therefore oats being advertised as a 'superfood' van den Broeck et al. (2015).

Essential fatty acids refer to those PUFA (polyunsaturated fatty acids) which cannot be synthesized in the body thus that should be provided by foods Kaur et al. (2014). Humans require two types of PUFA: n-3 and n-6. There is some strong evidence which suggested that essential fatty acids (particularly, eicosapentaenoic acid, docosahexaenoic acid, and α-linolenic) help in reducing cardiovascular diseases, promote mental health, and reduce the risk of type 2 diabetes mellitus Glick and Fischer (2013). In oats, α-linoleic acid was found to be the predominant fatty acid (around 40%) Kouřimská et al. (2021). The ratio of n-6 to n-3 was 25:1 Kouřimská et al. (2018), and the PUFA:SFA ratio was 1.7–2.1 Kouřimská et al. (2021). According to Ma et al. (2016) the recommendation of dietary n-3 PUFA is from 1.8 to 1.9 g per day for the optimal human health, and the ratio of n-6/n-3 PUFA should be less than

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https://doi.org/10.1016/j.jcs.2022.103580
Received 7 July 2022; Received in revised form 7 October 2022; Accepted 17 October 2022
Available online 21 October 2022

4:1. The EFSA Panel on Dietetic Products, Nutrition, and Allergies recommended that the intake of total fat should be between 20 and 35% of energy intake for adults, and saturated fatty acid intake should be as low as possible EFSA (2010). The Panel did not set any dietary reference value for total MUFA and PUFA and no specific values for the n-3/n-6 ratio. It set only an adequate intake of 4% of energy for linoleic acid, 0.5% for alpha-linolenic acid, and 250 mg/day for eicosapentaenoic acid plus docosahexaenoic acid for adults EFSA (2010).

The effect of environmental conditions on oat nutritional composition and grain yield were confirmed by Martinez et al. (2010). Similarly, the relationship between the temperature and lipid composition was investigated by Saastamoinen (1998). Low temperature positively correlated to the synthesis of fatty acids like oleic, linoleic, and eicosenoic acids whereas the synthesis of myristic, palmitic and palmitoleic acids decreased significantly by the low temperature. Additionally, the low temperature increased the oil content of oats.

Oats are widely grown in temperate zones including Northwest Europe and Canada, as a spring crop and adapt well to the wet and cool summer seasons. Moreover, oat cultivation is expending to the Mediterranean agroecological conditions whereunder oat varieties of different origins have adapted to autumn showing. Autumn showing is also increasing in other countries such as south China, south Japan, and the temperate area of North America Sánchez-Martín et al. (2014). Most oats grow between latitudes of 40° and 60° N in America, mid to north of Europe, and Asia, whereas some oats grow in the western hemisphere i.e., South America, Australia, and New Zealand. Compared with other cereals, oats can tolerate acidic soil and wet weather Givens et al. (2004). According to Buerstmayr et al. (2007), who evaluated 120 worldwide oat genotypes for their agronomic and grain quality character, the European breeds showed higher yielding entities, while the American and Canadian oats showed better agronomic traits, like plant height and groat protein percentage. Martinez et al. (2010) documented that oat genotypes depend more on environmental aspects than genetics in grain yield and nutritional attributes. Furthermore, factors that affect the fat and fatty acid profiles of oats are the hereditary characters and the soil and climatic conditions during vegetation Banaś and Harasym (2021).

As far as we know, there is still no study comparing fatty acid profiles between oats from different continents. Therefore, the aim was to evaluate the differences in the lipid content and fatty acid composition of 38 oat varieties from the collection of the breeding station (gene sources of European and Canadian origin); mainly for the purposes of further use in breeding. However, if there is interest, it would be possible to use some varieties directly for cultivation for food use. Unlike other studies dealing with the oil composition of oats from different regions, this study compares a wide range of varieties from different countries of origin, which were propagated under the same conditions in one growing location in the Czech Republic. This experimental design enabled us to compare the oat properties and differences not depending on growing and climate conditions, but reflecting only the differences between oat varieties. The samples included both naked and hulled varieties and the difference in their fatty acid profiles was also monitored.

2. Material and methods

2.1. Plant material

Sample collection consisted of 38 oat genotypes grown within one season under same climatic conditions in the fields of the Selgen breeding company in Krukanice (western part of the Czech Republic, 474 m above sea level). The collection involved both hulled and naked genotypes of British, Canadian, Estonian, Finnish, French, Irish, Norwegian, Russian, and Swedish origins. The tested genotypes are listed in Table 1. All seeds came from the Selgen's own multiplication. Oat genotypes were cultivated in the field plots using oilseed rape as a preceding crop. A total of 400 kg/ha of NPK fertilizer (15:15:15) was applied before oat sowing, and herbicidal treatment was performed. After harvest, the oat grains of the hulled genotypes were dehulled using a laboratory dehuller and used for analyses.

2.2. Sample preparation and lipid content determination

A Scarlett Silver Line SL 1545 coffee grinder (Ariette-Scarlett, Firenze, Italy) was used for milling the samples. Approximately 7 g of ground oat samples were placed into an extraction timber (Cytiva Whatman grade high performance cellulose extraction thimbles, Sweden) and were covered with cotton. The lipids were extracted in a Soxhlet glass apparatus using petroleum ether for 240 min according to the guidelines provided by ISO 659:2009. The analysis of the lipid content was carried out gravimetrically after drying at 103 ± 2 °C to a constant weight. Each analysis was performed in triplicate (n = 3).

2.3. Fatty acid composition determination

Fat from the homogenised oat samples was extracted as described in section 2.2, but the solvent was evaporated only by the rotary vacuum dryer, without the final drying at 103 °C, to avoid undesirable fatty acid oxidation. Moreover, butylated hydroxytoluene was added during the extraction. Approximately 10 g of homogenised sample was taken into the extraction timber and covered with cotton, and it was extracted using petroleum ether for 2 h. The extracted fat was dried in a rotary vacuum evaporator (Heidolph Hei-VAP Core HL G3; Heidolph Instruments GmbH, Schwabach, Germany) at 40 °C. The fatty acid composition was analyzed following a method described by Kouřimská et al. (2018). Fatty acid methyl esters were prepared according to ISO 12966–2:2017. Approximately 0.5 g of the extracted lipid was re-esterified and then analyzed by gas chromatography coupled with mass spectrometry (GC-MS) using an Agilent 7890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a Restek's Rt-2560 capillary column (0.25 mm ID, 100 m, 0.20 μm thickness, Restek Corporation, Bellefonte, PA, USA). Analysis of the fatty acid profile of each sample was conducted in duplicate. The methylated fatty acids were identified using a Restek Food Industry FAME mix (cat#35077) and by comparing their mass spectra with those reported in the National Institute of Standards and Technology Library (NIST, USA). The proportions of the fatty acids were calculated using the area normalisation method and expressed as relative percentage of all identified fatty acids. A sample chromatogram of fatty acid methyl esters in oat sample and standard are shown in Fig. 1.

2.4. Statistical analysis

The data were statistically analyzed by ANOVA (complete three-factor model with interactions and two-factor model for hulled and naked oats separately) using the SAS program (SAS Institute, Cary, NC, USA, version 9.4), and a level of significant P = 0.05. The mean ± standard deviations were evaluated using a Tukey's HSD (honestly significant difference). The evaluation was conducted only on the major fatty acids presenting more than 1% (C16:0, C18:0, C18:1 *cis*-9, C18:1 *cis, cis*-13, C18:2 *cis, cis*-9,12, C20:1 cis-11, C18:3, all *cis*-9,12,15), and for the sums of saturated, monounsaturated, and polyunsaturated fatty acids (SFA, MUFA and PUFA).

3. Results

3.1. Lipid content

The total lipidic content of the evaluated genotypes is presented in Table 2. The Norwegian oat presented the highest lipid content (7.13 ± 0.52 g/100 g), which was significantly different compared to the other varieties. The Russian and Swedish oats had a lipid content of <5 g/100

Table 1
The analyzed oat genotypes.

Genotype name	Type	Colour	Country of origin
AC Preaknes	Hulled	Yellow	Canada
CDC Boyer	Hulled	Yellow	Canada
Navaro	Naked	Yellow	Canada
OA 504-5	Naked	Yellow	Canada
Shadow	Naked	Yellow	Canada
Riel	Hulled	Yellow	Canada
Walderm	Hulled	Yellow	Canada
Jaak	Hulled	Yellow	Estonia
Kalle	Naked	Yellow	Estonia
Aarre	Hulled	Yellow	Finland
Belinda	Hulled	Yellow	Finland
Katri	Hulled	Yellow	Finland
Roope	Hulled	Yellow	Finland
Veli	Naked	Yellow	Finland
Avesta	Hulled	Black	France
Black	Hulled	Black	France
Creole	Hulled	Black	France
Dakar	Hulled	Black	France
Ivore	Hulled	Yellow	France
Longchamp	Hulled	Yellow	France
Poncho	Hulled	Yellow	France
Ebene	Hulled	Black	France
Maris Oberon	Hulled	Black	United Kingdom
CC4146	Hulled	Black	United Kingdom
Maelor	Hulled	Yellow	United Kingdom
Maldwyn	Hulled	Yellow	United Kingdom
Lennon	Naked	Yellow	United Kingdom
Zuton	Naked	Yellow	United Kingdom
Pusahybrid	Hulled	Yellow	Ireland
Kermit	Hulled	Yellow	Ireland
Martin	Hulled	Yellow	Norway
Gana	Naked	Yellow	Russia
Tjumenski golozernoi	Naked	Yellow	Russia
Apollon	Hulled	Yellow	Russia
Bison	Hulled	Yellow	Russia
Lidya	Hulled	Yellow	Russia
Salo	Hulled	Yellow	Sweden
Sang	Hulled	Yellow	Sweden

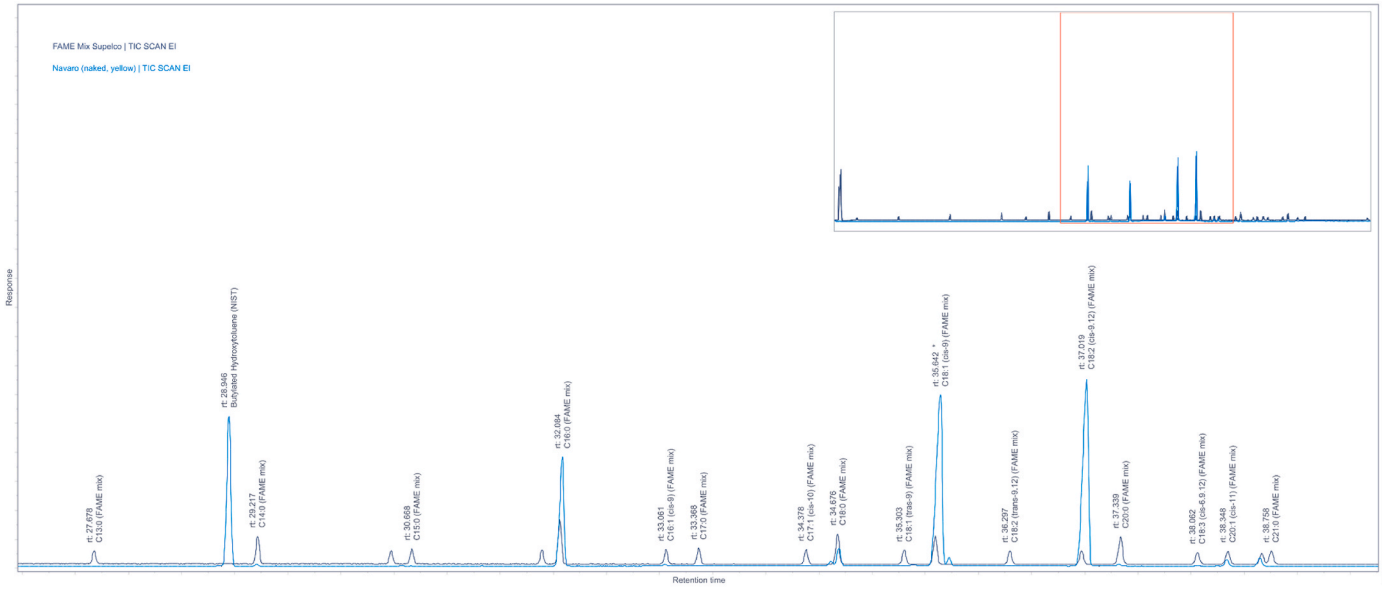

Fig. 1. Chromatogram of fatty acid methyl esters in oat sample (Navaro) and standard (FAME mix).

g, which was statistically different than that of the other tested varieties. Pusahybrid presented the highest lipid content and Navaro the lowest, 9.51 and 2.9 g/100 g, respectively. Both varieties were significantly different from the other tasted varieties ($p < 0.05$). Regarding the lipid content of hulled and naked varieties based on the continent of origin, no differences were found. Among the naked varieties, British oats contained the highest quantity of lipid, whereas among the hulled variety, oats from Norway had the highest quantity of lipid content.

3.2. Fatty acid composition of Canadian oat genotypes

The fatty acid composition of Canadian genotype oats is shown in Table 3a together with the statistically significant differences indicated by means of indices. The minor fatty acids having contents between 0.01% and 0.43% were C14:0, C20:0, C22:0, C22:1 *cis*-13, C24:0, C24:1 cis-15, C18:3 *cis*-9, *trans*, *trans*-11,13, and C26:0.

No significant difference was observed in the palmitic acid content among the Canadian genotype oats, presenting a relative content of 17.05%–19.77%. AC Preaknes contained 39.04% oleic acid which differed from Walderm, Navaro, OA 504–5, and Shadow, wherein linoleic acid was the dominant fatty acid. Waldern contained the highest quantity of linoleic acid (39.05%), whereas AC Preaknes presented a significantly lower content (32.54%) than Walderm and Navaro. No significant difference was observed in the sum of SFA, which was between 20.29% and 24.56%. Similarly, AC Preaknes contained the highest quantity of MUFA (41.32%), which was significantly more ($p < 0.05$) than in Shadow (37.19%). Walderm contained the highest quantity of PUFA (40.93%) followed by Navaro (40.3%), and both these varieties differed from AC Preaknes (34.12%). Overall, the dominant fatty acids in the Canadian oat genotypes were linoleic (36.76%), oleic (36.50%), and palmitic acids (18.64%).

3.3. Fatty acid composition of European oat genotypes

The major fatty acid composition of the European genotype oats is presented in Table 3b. Lidya contained the highest quantity of palmitic acid among the European oats (21.21%) and differed significantly from Ivore. In contrast, Ivore contained the highest quantity of oleic acid (40.87%), and was different ($p < 0.05$) from all the other European genotypes except Katri. Similarly, Dakar contained the highest quantity of linoleic acid (40.63%), which was significantly higher than that in Belinda and Ivore. Regarding SFA, the European genotypes contained 20.23%–24.75%, and no significant difference was observed in this group. Ivore contained the highest quantity of MUFA (43.64%) and differed ($p < 0.05$) from all other European genotypes except Belinda. Dakar contained the highest quantity of PUFA (42.63%) and was different from Belinda and Ivore. Overall, the dominant fatty acids in European oat genotypes were linoleic (37.25%), oleic (35.67%), and palmitic acids (18.64%).

3.4. Comparison of fatty acid contents of Canadian and European oat genotypes

The contents of major fatty acids based on the country of origin are presented in Table 4. In general, the dominant fatty acids in the tested oat genotypes were linoleic (32.54%–40.63%), oleic (32.66%–40.54%), and palmitic acids (17.0%–21.21%), respectively. It is worth mentioning that in most cases, significant differences were observed, except saturated fatty acids. Differences were mostly observed in oleic, cis-13-octadecenoic, gondoic, and linoleic acids, as well as in the sums of MUFA and PUFA. The Swedish genotype, Lidya, contained the highest quantity of palmitic acid, whereas the French genotype, Ivore, contained the lowest. Overall, no significant difference was observed in the relative contents of palmitic acid in the tested oats. The representation of oleic acid in the tested genotypes varied significantly. Ivore contained the highest quantity of oleic acid, which differed from the tested genotypes except European genotypes Belinda (Finnish) and CC4146 (British) and Canadian genotype AC Preaknes. In contrast, Kermit, an Irish genotype, contained the lowest quantity of oleic acid (32.66%). Dakar, a French genotype, contained the highest quantity of linoleic acid and differed from AC Preaknes, Belinda, and Ivore. European genotype oats contained a higher quantity of α-linolenic acid than Canadian oat genotypes. The proportion of the major fatty acids influenced the order of the sums of MUFA and PUFA, but no differences were observed in the total SFA. The highest quantity of PUFA was observed in Dakar, whereas the lowest was found in AC Preaknes, which differed significantly. The quantity of MUFA among the oat varieties differed remarkably: Ivore presented the highest MUFA content and was different from all other tested genotypes except AC Preaknes, Belinda, CC4146, and Creole. The lowest MUFA content was observed in Dakar. The PUFA/SFA ratio was 1.39–2.05 in Canadian genotypes and 1.21–2.24 in European oat genotypes.

Table 2
Lipid content (g/100 g) of oats according to their genotype, country of origin and oat type (hulled, naked).

Sample name	Lipid content g/100g
Aarre	5.3 ± 0.5^{ab}
AC Preaknes	5.2 ± 1.6^{ab}
Apollon	3.5 ± 0.8^{ab}
Avesta	4.4 ± 0.3^{ab}
Belinda	6.3 ± 1.0^{ab}
Bison	3.9 ± 0.1^{ab}
Black	6.3 ± 1.7^{ab}
CC4146	7.3 ± 2.2^{ab}
CDC Boyer	6.3 ± 0.7^{ab}
Creole	4.5 ± 1.0^{ab}
Dakar	6.2 ± 1.0^{ab}
Ebene	6.5 ± 1.0^{ab}
Gana	4.9 ± 0.6^{ab}
Ivore	5.8 ± 0.2^{ab}
Jaak	6.7 ± 0.3^{ab}
Kalle	4.6 ± 0.1^{ab}
Katri	5.3 ± 0.3^{ab}
Kermit	3.9 ± 0.5^{ab}
Lennon	7.2 ± 2.0^{ab}
Lidya	7.6 ± 1.0^{a}
Longchamp	6.8 ± 1.3^{ab}
Maelor	5.4 ± 0.1^{ab}
Maldwyn	6.2 ± 0.3^{ab}
Maris Oberon	4.2 ± 0.1^{ab}
Martin	7.1 ± 0.5^{ab}
Navaro	3.0 ± 0.2^{b}
OA 504-5	7.0 ± 0.7^{ab}
Poncho	5.6 ± 0.3^{ab}
Pusahybrid	9.5 ± 2.1^{ab}
Riel	6.2 ± 0.8^{ab}
Roope	5.4 ± 0.7^{ab}
Salo	4.6 ± 0.6^{ab}
Sang	4.4 ± 0.7^{ab}
Shadow	5.8 ± 0.2^{ab}
Tjumenski golozernoi	4.8 ± 0.3^{ab}
Veli	5.6 ± 0.7^{ab}
Walderm	4.8 ± 0.3^{ab}
Zuton	5.5 ± 0.2^{ab}
$HSD_{0.05}$	4.59
Country	
CAN	5.54 ± 1.47^{ab}
EST	6.62 ± 1.23^{ab}
FIN	5.59 ± 0.64^{ab}
FRA	5.76 ± 1.12^{ab}
GBR	5.95 ± 1.45^{ab}
IRL	6.69 ± 3.50^{ab}
NOR	7.13 ± 0.52^{a}
RUS	4.90 ± 1.59^{b}
SWE	4.54 ± 0.51^{b}
$HSD_{0.05}$	2.18
Hulled	5.71 ± 1.39^{a}
Naked	5.35 ± 1.49^{a}
$HSD_{0.05}$	0.61

Values are expressed as mean ±standard deviations (SD) and honestly significant difference (HSD). Each analysis was performed in triplicate (n = 3). Statistically significant differences between variables were denoted by different superscript letters ($p < 0.05$).

3.5. Comparison of fatty acid contents of hulled and naked oat genotypes

Table 5 presents the differences in the major fatty acid composition between naked and hulled oats. The palmitic acid content was different in hulled (18.68%) and naked (18.35%) oats. Stearic acid was present at 2.1% and 2.25% in naked and hulled oats, respectively, with no statistical significance in both cases. Different contents of oleic acid were found in hulled and naked oats, at 35.97% and 35.35%, respectively. Cis-13-octadecenoic acid was present at different levels in naked (1.16%) and hulled oats (1.10%). Similarly, more linoleic acid (37.92%) was detected in naked than in hulled oats (36.93%). In summary, the content of oleic, cis-13-octadecenoic, and linoleic acids differed

Table 3a
Major fatty acid composition of Canadian genotype oats (% of all identified fatty acids).

Sample name	C16:0	C18:0	C18:1 *cis*-9	C18:1 *cis*-13	C18:2 *cis*-9,12	C20:1 *cis*-11	C18:3 all *cis*-9,12,15	SFA	MUFA	PUFA
AC Preaknes	19.6 ± 0.4^{ab}	3.5 ± 0^{a}	39.0 ± 1.1^{abc}	0.8 ± 0.1^{gh}	32.5 ± 0.6^{d}	0.9 ± 0.1^{cdef}	1.1 ± 0.1^{defg}	23.7 ± 0.5^{a}	41.8 ± 1.1^{abc}	34.5 ± 0.6^{d}
CDC Boyer	18.2 ± 0.1^{ab}	2.2 ± 0.1^{abcdef}	37.5 ± 0.1^{cdef}	1 ± 01^{efgh}	36.1 ± 0.6^{abcd}	1.2 ± 0.1^{abcdef}	1.1 ± 0.1^{efg}	21.1 ± 0.2^{a}	40.7 ± 1.1^{bcdefg}	38.3 ± 0.5^{abcd}
Navaro	17.5 ± 0.5^{ab}	2.5 ± 0.1^{abcdef}	35.8 ± 0.1^{defghijk}	1.1 ± 0.1^{defgh}	38.5 ± 0.3^{abc}	1.0 ± 0.1^{bcdef}	1.3 ± 0.1^{bcdefg}	20.5 ± 0.6^{a}	38.7 ± 0.1^{cdefghijk}	40.7 ± 0.5^{abc}
OA 504-5	19.4 ± 0.3^{ab}	2.5 ± 0.1^{abcdef}	36.0 ± 0.6^{defghijk}	1.2 ± 0.1^{abcdef}	36.1 ± 0.8^{abcd}	1.0 ± 0.1^{bcdef}	1.5 ± 0.2^{abcdef}	22.4 ± 0.1^{a}	39.1 ± 0.6^{cdefghij}	38.5 ± 0.6^{abcd}
Riel	18.9 ± 0.5^{ab}	2.2 ± 0.2^{bcdef}	36.7 ± 1.6^{cdefghi}	1.2 ± 0.1^{abcdef}	36.9 ± 2.1^{abcd}	0.9 ± 0.1^{cdef}	1.0 ± 0.2^{gf}	21.5 ± 0.7^{a}	39.7 ± 1.6^{cdefghij}	38.8 ± 2.3^{abcd}
Shadow	19.8 ± 0.5^{ab}	2.0 ± 0.1^{bcdef}	34.6 ± 0.4^{ghijklm}	1.1 ± 0.1^{degf}	38.1 ± 0.1^{abcd}	0.9 ± 0.1^{bcdef}	1.2 ± 0.1^{cdefg}	22.3 ± 0.5^{a}	37.4 ± 0.4^{hijkl}	40.2 ± 0.1^{abcd}
Walderm	17.1 ± 0.4^{ab}	1.7 ± 0.1^{def}	36.0 ± 0.5^{defghijk}	1.1 ± 0.1^{defg}	$39.1 \pm 0.^{abc}$	1.1 ± 0.1^{bcdef}	1.2 ± 0.1^{cdefg}	19.3 ± 0.5^{a}	39.2 ± 0.6^{cdefghij}	41.5 ± 0.1^{abc}

Values are expressed as the mean ± standard deviation. Each analysis was performed in duplicate, independently.

Table 3b
Major fatty acid composition of European genotype oats (% of all identified fatty acids).

Sample name	C16:0	C18:0	C18:1 *cis*-9	C18:1 *cis*-13	C18:2 *cis*-9,12	C20:1 *cis*-11	C18:3 all *cis*-9,12,15	SFA	MUFA	PUFA
Apollon	18 ± 0.3^{ab}	1.8 ± 0.1^{cdef}	35.5 ± 0.4^{defghijkl}	1.1 ± 0.0^{cdef}	38.6 ± 0.2^{abc}	1.0 ± 0.1^{bcdef}	1.6 ± 0.1^{abcde}	20.3 ± 0.2^{a}	38.5 ± 0.5^{cdefghijk}	41.2 ± 0.3^{abc}
Avesta	17.1 ± 0.1^{ab}	1.7 ± 0.1^{def}	35.1 ± 0.5^{efghijklm}	1.1 ± 0.1^{defg}	39.6 ± 0.4^{ab}	1.2 ± 0.1^{abcdef}	1.5 ± 0.1^{abcdef}	19.3 ± 0.1^{a}	38.4 ± 0.2^{defghijk}	42.2 ± 0.3^{ab}
Belinda	17.4 ± 0.2^{ab}	3.1 ± 0.1^{abc}	40.6 ± 0.4^{ab}	0.8 ± 0.1^{h}	33.5 ± 0.1^{cd}	0.9 ± 0.1^{bcdef}	1.1 ± 0.1^{defg}	21.0 ± 0.1^{a}	43.4 ± 0.4^{ab}	35.5 ± 0.2^{d}
Bison	17.9 ± 0.6^{ab}	1.8 ± 0.7^{cdef}	33.5 ± 0.2^{jklmn}	1.2 ± 0.1^{abcdef}	39.7 ± 0.5^{ab}	1.3 ± 0.1^{abc}	1.3 ± 0.1^{bcdefg}	20.4 ± 1.0^{a}	37.2 ± 0.2^{ijkl}	42.4 ± 0.7^{ab}
Black	18.1 ± 0.2^{ab}	1.5 ± 0.1^{ef}	35.9 ± 0.2^{defghijk}	1.1 ± 0.1^{cdef}	38.1 ± 0.4^{abcd}	1.3 ± 0.1^{ab}	1.5 ± 0.1^{abcdef}	20.1 ± 0.26^{a}	39.3 ± 0.2^{cdefghij}	40.6 ± 0.5^{abc}
CC4146	18.4 ± 0.1^{ab}	2.8 ± 0.1^{abcdef}	38.1 ± 1.3^{abcd}	1.0 ± 0.1^{efgh}	35.0 ± 0.8^{abcd}	1.0 ± 0.1^{bcdef}	1.2 ± 0.2^{cdefg}	21.7 ± 0.1^{a}	41.1 ± 1.1^{abcde}	37.1 ± 1.1^{bcd}
Creole	17.7 ± 0.2^{ab}	1.6 ± 0.1^{ef}	37.86 ± 0.2^{bcde}	1.4 ± 0.1^{ab}	36.6 ± 0.2^{abcd}	1.1 ± 0.1^{abcdef}	1.5 ± 0.1^{abcde}	19.7 ± 0.4^{a}	41.3 ± 0.2^{abcd}	39.0 ± 0.2^{abcd}
Dakar	19.1 ± 3.0^{ab}	1.6 ± 0.4^{ef}	31.5 ± 0.5^{n}	1.1 ± 0.1^{defg}	40.6 ± 4.9^{a}	0.9 ± 0.1^{bcdef}	1.5 ± 0.3^{abcdef}	21.5 ± 3.6^{a}	34.8 ± 0.8^{l}	43.7 ± 4.4^{a}
Ebene	19.3 ± 0.1^{ab}	3.1 ± 0.3^{ab}	34.8 ± 0.2^{fghijklm}	1.0 ± 0.1^{efgh}	36.9 ± 0.9^{abcd}	0.9 ± 0.1^{cdef}	1.4 ± 0.1^{abcdefg}	23.0 ± 0.5^{a}	37.7 ± 0.3^{ghijkl}	39.3 ± 0.8^{abcd}
Gana	18.7 ± 0.3^{ab}	2.0 ± 0.1^{bcdef}	36.9 ± 0.3^{cdefgh}	1.1 ± 0.1^{defg}	37.1 ± 0.1^{abcd}	0.8 ± 0.1^{ef}	1.4 ± 0.1^{abcdefg}	21.1 ± 0.3a	39.6 ± 0.3^{cdefghij}	39.3 ± 0.1^{abcd}
Ivore	17.0 ± 0.4^{b}	2.4 ± 0.9^{abcdef}	40.9 ± 0.1^{a}	01.0 ± 0.3^{efgh}	34.1 ± 0.5^{bcd}	1.1 ± 0.2^{abcdef}	1.1 ± 0.1^{efg}	19.9 ± 1.3^{a}	44.1 ± 0.7^{a}	36.1 ± 0.6^{dc}
Jaak	18.8 ± 0.3^{ab}	2.4 ± 0.1^{abcdef}	36.1 ± 0.6^{defghij}	1.0 ± 0.1^{fgh}	37.1 ± 0.5^{abcd}	0.9 ± 0.1^{cdef}	1.6 ± 0.1^{abc}	21.7 ± 0.2^{a}	38.8 ± 0.7^{cdefghijk}	39.5 ± 0.5^{abcd}
Kalle	17.7 ± 0.2^{ab}	2.2 ± 0.2^{abcdef}	33.9 ± 0.7^{jklmn}	1.2 ± 0.1^{cdef}	39.3 ± 1.3^{abc}	1.2 ± 0.1^{abcdef}	1.8 ± 0.1^{a}	20.5 ± 0.1^{a}	37.3 ± 0.9^{ijkl}	42.2 ± 1.0^{ab}
Katri	20.3 ± 0.1^{ab}	2.9 ± 0.1^{abcd}	34.5 ± 0.3^{ghijklm}	1.0 ± 0.1^{fgh}	36.7 ± 0.3^{abcd}	0.8 ± 0.1^{f}	1.4 ± 0.1^{abcdefg}	23.8 ± 0.1^{a}	37.2 ± 0.3^{jkl}	39.0 ± 0.4^{abcd}
Kermit	20.3 ± 0.4^{ab}	1.9 ± 0.4^{bcdef}	32.7 ± 1.3^{mn}	1.5 ± 0.1^{a}	39.0 ± 0.57^{abc}	1.0 ± 0.1^{bcdef}	1.4 ± 0.1^{abcdefg}	22.7 ± 0.9^{a}	36.0 ± 1.5^{kl}	41.3 ± 0.7^{abc}
Lennon	17.5 ± 0.3^{ab}	2.2 ± 0.1^{bcdef}	36.4 ± 0.9^{cdefghi}	1.2 ± 0.1^{bcdef}	37.3 ± 1.1^{abcd}	1.2 ± 0.2^{abcd}	1.5 ± 0.1^{abcdef}	20.2 ± 0.3^{a}	39.9 ± 1.3^{cdefgh}	39.9 ± 1.0^{abcd}
Lidya	21.2 ± 3.7^{a}	2.5 ± 0.6^{abcdef}	34.2 ± 1.5^{hijklmn}	1.1 ± 0.1^{cdef}	35.0 ± 3.0^{abcd}	1.2 ± 0.1^{abcdef}	1.3 ± 0.1^{bcdefg}	24.6 ± 4.5^{a}	37.8 ± 1.4^{fghijk}	37.6 ± 3.2^{abcd}
Longchamp	18.4 ± 0.3^{ab}	2.8 ± 0.4^{abcde}	36.2 ± 1.1^{defghij}	1.2 ± 0.1^{abcdef}	35.8 ± 1.6^{abcd}	1.2 ± 0.1^{abcde}	1.2 ± 0.1^{bcdefg}	21.9 ± 0.3^{a}	39.9 ± 1.2^{cdefghij}	38.2 ± 1.5^{abcd}
Maelor	20.6 ± 2.3^{ab}	2.4 ± 0.6^{abcdef}	35.1 ± 0.2^{efghijklm}	1.1 ± 0.1^{cdef}	35.4 ± 3.0^{abcd}	1.1 ± 0.1^{abcdef}	1.2 ± 0.1^{bcdefg}	23.7 ± 2.9^{a}	38.5 ± 0.3^{defghijk}	37.8 ± 3.3^{abcd}
Maldwyn	19.6 ± 1.0^{ab}	1.8 ± 0.1^{def}	35.2 ± 0.8^{efghijklm}	1.3 ± 0.1^{abcd}	36.4 ± 0.5^{abcd}	1.1 ± 0.1^{abcdef}	1.7 ± 0.1^{ab}	22.0 ± 1.3^{a}	38.7 ± 0.8^{cdefghijk}	39.2 ± 0.5^{abcd}
Maris Oberon	19.1 ± 1.9^{ab}	2.2 ± 0.2^{bcdef}	36.6 ± 0.1^{cdefghi}	1.1 ± 0.1^{cdef}	35.5 ± 3.0^{abcd}	1.1 ± 0.1^{abcdef}	1.0 ± 0.2^{g}	22.0 ± 0.2^{a}	40.2 ± 1.1^{bcdefg}	37.8 ± 3.4^{abcd}
Martin	17.4 ± 0.4^{ab}	2.1 ± 0.1^{bcdef}	37.7 ± 0.6^{cde}	1.1 ± 0.1^{defgh}	36.8 ± 0.3^{abcd}	1.1 ± 0.1^{abcdef}	1.4 ± 0.1^{abcdefg}	20.0 ± 0.4^{a}	40.9 ± 0.64^{bcdef}	39.1 ± 0.3^{abcd}
Poncho	20.0 ± 2.1^{ab}	2.3 ± 0.5^{abcdef}	34.8 ± 0.3^{fghijklm}	1.2 ± 0.1^{abcde}	36.5 ± 2.5^{abcd}	1.0 ± 0.1^{bcdef}	1.6 ± 0.2^{abcd}	22.9 ± 2.9^{a}	38.0 ± 0.2^{efghijk}	39.1 ± 2.6^{abcd}
Pusahybrid	19.0 ± 0.3^{ab}	3.2 ± 0.1^{abcdef}	36.5 ± 0.3^{defghij}	1.0 ± 0.1^{cdef}	36.0 ± 0.2^{abcd}	0.70 ± 0.1^{cdef}	1.4 ± 0.1^{abcde}	22.7 ± 0.4^{a}	38.0 ± 0.2^{cdefghijk}	38.2 ± 0.2^{abcd}
Roope	18.6 ± 0.4^{ab}	2.1 ± 0.1^{abcdef}	35.6 ± 0.2^{defghij}	1.2 ± 0.1^{cdef}	37.3 ± 0.2^{abcd}	1.1 ± 0.1^{cdef}	1.7 ± 0.1^{abcde}	21.2 ± 0.1^{a}	38.8 ± 0.3^{cdefghijk}	40.0 ± 0.1^{abcd}
Salo	18.2 ± 0.47^{ab}	2.2 ± 0.1^{bcdef}	35.4 ± 1.1^{defghijklm}	1.0 ± 0.1^{defgh}	38.7 ± 0.4^{abc}	0.9 ± 0.1^{cdef}	1.5 ± 0.1^{abcdef}	20.8 ± 0.53^{a}	38.1 ± 1.1^{efghijk}	41.1 ± 0.6^{abc}
Sang	18.2 ± 0.1^{ab}	1.7 ± 0.1^{def}	33.2 ± 0.2^{klmn}	1.2 ± 0.1^{abcdef}	40.0 ± 0.1^{a}	1.3 ± 0.1^{abc}	1.8 ± 0.1^{a}	20.4 ± 0.1^{a}	36.7 ± 0.1^{jkl}	41.1 ± 0.2^{ab}
Tjumenski golozernoi	18.7 ± 0.29^{ab}	1.5 ± 0.1^{f}	32.8 ± 0.2^{lmn}	1.3 ± 0.1^{abcd}	39.3 ± 0.4^{abc}	1.5 ± 0.1 ^{a}	1.8 ± 0.1^{a}	20.8 ± 0.4^{a}	36.7 ± 0.8^{jkl}	42.4 ± 0.2^{ab}
Veli	17.8 ± 0.2^{ab}	2.1 ± 0.1^{bcdef}	37.1 ± 0.6^{cdefg}	1.0 ± 0.1^{defgh}	37.7 ± 0.8^{abcd}	0.9 ± 0.1^{cdef}	1.4 ± 0.1^{abcdefg}	20.3 ± 0.2^{a}	39.8 ± 0.7^{cdefghij}	39.9 ± 0.4^{abcd}
Zuton	19.7 ± 0.5^{ab}	1.9 ± 0.1^{bcdef}	34.7 ± 0.2^{fghijklm}	1.4 ± 0.1^{abc}	37.9 ± 0.2^{abcd}	1.0 ± 0.1^{bcdef}	1.2 ± 0.1^{bcdefg}	22.1 ± 0.5^{a}	37.93 ± 0.4^{fghijk}	40.0 ± 0.2^{abcd}
$HSD_{0.05}$	4.20	1.28	2.81	0.27	5.68	0.4	0.47	5.38	3.07	5.78

Values are expressed as the mean ± standard deviation. Each analysis was performed in duplicate, independently. Statistical evaluation was carried out together for all samples in Tables 3a and 3b

significantly between the hulled and naked oat genotypes. The average PUFA/SFA ratio in hulled oats was 1.78 and in naked oats 1.77.

4. Discussion

4.1. Lipid content of the tested oat samples

The goal of this study was to analyze and compare the lipid content and lipid profile of various European and Canadian oat varieties. Our research showed that the lipid content of oats of different genotypes ranged from 3.0 to 9.5 g/100 g. Such results are in good agreement with reported lipid contents of oats: 5–6 g/100 g was reported by Banaś et al. (2007); Batalova et al. (2019) reported 5.9–7.9 g/100 g; Capouchová et al. (2021) reported 4.8–6.0 g/100 g; Kouřimská et al. (2021) reported 2.9–5.8 g/100 g. The unique characteristic of oats is the higher lipid content than other cereals, such as wheat, rice, millet, barley, and rye,

Table 4
Major fatty acid composition based on country of origin (% of all identified fatty acids).

Sample name	C16:0	C18:0	C18:1 *cis*-9	C18:1 *cis*-13	C18:2 *cis*-9,12	C20:1 *cis*-11	C18:3 all *cis*-9,12,15	SFA	MUFA	PUFA
CAN	18.64 ± 1.07^{a}	2.39 ± 0.55^{ab}	36.50 ± 1.50^{ab}	1.06 ± 0.12^{bcd}	36.76 ± 2.21^{ab}	0.98 ± 0.11^{abc}	1.20 ± 0.15^{d}	22.41 ± 1.40^{a}	39.17 ± 1.41^{abc}	38.42 ± 2.28^{b}
EST	18.29 ± 0.66^{a}	2.29 ± 0.55^{ab}	34.99 ± 1.34^{cde}	1.05 ± 0.12^{cd}	38.15 ± 1.50^{ab}	1.02 ± 0.18^{abc}	1.69 ± 0.11^{a}	21.95 ± 0.72^{a}	37.74 ± 1.05^{cde}	40.31 ± 1.65^{ab}
FIN	18.48 ± 1.08^{a}	2.47 ± 0.45^{a}	36.84 ± 2.02^{ab}	1.02 ± 0.15^{d}	36.49 ± 1.48^{b}	0.95 ± 0.11^{c}	1.41 ± 0.20^{cd}	22.33 ± 1.31^{a}	39.36 ± 2.10^{ab}	38.31 ± 1.61^{b}
FRA	18.32 ± 1.41^{a}	2.12 ± 0.68^{ab}	35.88 ± 2.63^{bcd}	1.14 ± 0.15^{abc}	37.28 ± 2.52^{ab}	1.11 ± 0.16^{ab}	1.41 ± 0.19^{cd}	21.96 ± 1.93^{a}	38.88 ± 2.68^{cde}	39.16 ± 2.58^{ab}
GBR	19.14 ± 1.42^{a}	2.19 ± 0.40^{ab}	36.02 ± 1.32^{bc}	1.18 ± 0.14^{ab}	36.23 ± 1.76^{b}	1.10 ± 0.11^{ab}	1.31 ± 0.25^{cd}	22.86 ± 1.63^{a}	39.12 ± 1.36^{abcd}	38.02 ± 1.88^{b}
IRL	19.66 ± 0.79^{a}	2.55 ± 0.78^{ab}	34.57 ± 0.78^{cde}	1.24 ± 0.25^{a}	37.52 ± 1.33^{ab}	0.83 ± 0.16^{bc}	1.40 ± 0.07^{abc}	23.24 ± 0.54^{a}	37.25 ± 1.98^{de}	39.51 ± 1.85^{ab}
NOR	17.37 ± 0.52^{a}	2.12 ± 0.00^{ab}	37.69 ± 0.62^{a}	1.05 ± 0.03^{bcd}	36.83 ± 0.26^{ab}	1.10 ± 0.03^{ab}	1.42 ± 0.04^{cd}	20.71 ± 0.40^{a}	40.49 ± 0.64^{a}	38.79 ± 0.25^{ab}
RUS	18.89 ± 1.79^{a}	1.92 ± 0.40^{b}	34.60 ± 1.61^{de}	1.17 ± 0.07^{abc}	37.95 ± 2.07^{ab}	1.15 ± 0.22^{a}	1.45 ± 0.22^{bc}	22.24 ± 2.29^{a}	37.76 ± 1.16^{cde}	40.00 ± 2.27^{ab}
SWE	18.19 ± 0.27^{a}	1.91 ± 0.28^{b}	34.29 ± 1.42^{e}	1.12 ± 0.11^{abc}	39.35 ± 0.74^{a}	1.08 ± 0.23^{abc}	1.65 ± 0.19^{ab}	21.42 ± 0.36^{a}	37.14 ± 0.99^{e}	41.44 ± 1.01^{a}
$HSD_{0.05}$	2.0	0.61	1.34	0.13	2.70	0.19	0.22	2.55	1.46	2.74

Values are expressed as the mean ± standard deviation. Each analysis was performed in duplicate, independently. Number of samples in each category (CAN = 7, EST = 2, FIN = 5, FRA = 8, GBR = 6, IRL = 2, NOR = 1, RUS = 5, SWE = 2).

Table 5
Major fatty acid composition of naked and hulled oats (% of total identified).

Oat type	C16:0	C18:0	C18:1 *cis*-9	C18:1 *cis*-13	C18:2 *cis*-9,12	C20:1 *cis*-11	C18:3 cis-9,12,15	SFA	MUFA	PUFA
Hulled	18.68 ± 1.40^{a}	2.26 ± 0.60^{a}	35.97 ± 2.21^{a}	1.16 ± 0.15^{a}	37.92 ± 2.29^{a}	1.06 ± 0.16^{a}	1.43 ± 0.23^{a}	22.37 ± 1.80^{a}	38.84 ± 2.09^{a}	39.80 ± 2.44^{a}
Naked	18.53 ± 0.94^{a}	2.11 ± 0.31^{a}	35.35 ± 1.44^{b}	1.10 ± 0.11^{b}	36.93 ± 1.12^{b}	1.05 ± 0.19^{a}	1.37 ± 0.22 ^{b}	21.96 ± 0.92^{a}	38.24 ± 1.24^{b}	38.79 ± 1.29^{b}
$HSD_{0.05}$	0.56	0.17	0.37	0.04	0.75	0.05	0.06	0.71	0.41	0.76

Values are expressed as the mean ± standard deviation. Each analysis was performed in duplicate, independently. Number of samples in each category (hulled = 29, naked = 9).

and our findings reinforce this fact. Additionally, our analyses showed that the lipid content of Ivore (French genotype) was 5.8 g/100 g, which agrees with the values reported by Nakurte et al. (2013) (5.1 g/100 g for the same genotype).

The lipid content of Canadian oats was 3.0–7.0 g/100 g, with an average value of 5.5 g/100 g, which is lower than those reported in previous studies (4.6–11.6 g/100 g for the same type Sahasrabudhe (1979)). The European oats contained 3.5–9.5 g/100 g lipids, whereas the lipid content of Belinda and Lennon oats was 6.9 and 6.8 g/100 g, respectively, which are similar to the values reported by Chappell et al. (2017) (6.91 and 6.8 g/100 g respectively). No significant differences were observed in the fat content of European and Canadian oats.

The reported values of the lipid content of naked oats are higher than those of husked oats Sterna et al. (2016); Batalova et al. (2019); Kouřimská et al. (2021), whereas our results were not in agreement with these findings. In this study, the lipid content of hulled oats (5.7 g/100) was slightly higher than that of naked oats (5.4 g/100 g), with the difference not being significant ($p > 0.05$).

The main factor affecting the oil content of oats is the low temperature, and various authors, including Saastamoinen (1998), documented the effect of temperature on the oil content. A good example of this interaction is Russian genotype Lidiya, originating from one of the coldest agricultural lands on the planet, and presenting the highest lipid content. In addition to temperature, soil nutrition might influence the oil content.

4.2. Major fatty acids in European and Canadian oats

The analysis of the triglycerides of the tested oat genotypes indicated three major fatty acids: linoleic, oleic, and palmitic acids. The total quantity of these three fatty acids was 91.90% in the Canadian and 91.56% in European oat genotypes. Our findings were similar to those reported by Thacker et al. (2004), stating that the content of palmitic and linoleic acids was 17.20% and 39.10%, respectively. The same study reported that the proportion of oleic acid in the high-fat Canadian SA96121 oat genotype (cross-breed of ND870425 × CDC Boyer) was 42.80%, which is higher than our findings; in our study the proportions of palmitic, oleic, and linoleic acids in CDC Boyer were 18.25%, 37.45%, and 36.14%, respectively.

Doehlert et al. (2013) analyszed 18 different oat genotypes grown at 6 different locations for their palmitic, oleic, and linoleic acid contents and reported values of 16.7–20.5%, 31.7–38.9% and 37.2–44.4%, respectively, which agree with our findings. They also reported genotypic and environmental variations (apart from C14:1 and C16:0), which is in line with our findings. Moreover, Batalova et al. (2019) reported the following ranges of palmitic, oleic, and linoleic acids in 7 different Russian naked oats: 15.5–17.8%, 33.5–36.7%, 35.9–38.7%, respectively, whereas our study reports 18.9%, 34.6%, and 38% in Russian oat genotypes. Sterna et al. (2016) analyzed five oat genotypes and reported similar values for C16:0, C18:1, and C18:2: 15.5%–17.4%, 36.2%–40.4%, and 38.4%–41.6%, respectively. Ahokas and Manninen (2000) analyzed the composition of the dominant fatty acids in four Finnish oatmeal husked caryopses and reported 15.1%–16.7% of palmitic, 37.4%–40.6% of oleic, and 38.4%–39.1% of linoleic acids. Such ranges are close to the average values obtained from our research: 18.4% palmitic, 37.2% oleic, and 36.39% linoleic acids. According to Pearcy (1978), several factors can affect the fatty acid composition, such as the growth temperature affecting the lipid composition and the degree of unsaturation of lipids. Reszczyńska and Hanaka (2020) suggested that variations in environmental and physiological conditions can allow the

remodelling of the fatty acid composition. Chen and Thelen (2013) and Murata and Wada (1995) studied the changes in fatty acid unsaturation levels as a result of cold temperatures. They reported that in order to keep accurate plant membrane fluidity and a freezing tolerance, a higher degree of lipid unsaturation is required.

SFA, MUFA, and PUFA in Canadian oat genotypes were 22.41%, 39.17%, and 38.42%, respectively. The contents of SFA, MUFA, and PUFA in European oat genotypes were 20.78%, 36.06%, and 39.17%, respectively. Our findings in case of Swedish genotypes agree with those of Saga et al. (2013), who reported 17.4% SFA, 39.4% MUFA, and 42.9% PUFA. The Canadian oat genotypes contained more unsaturated fatty acids (77.59%) than the European oat genotypes (75.22%). Our results are also in line with the values reported by Sterna et al. (2016) (unsaturated acid content of 78–81.5%).

4.3. Hulled and naked European and Canadian oat genotypes

The relative differences in the contents of palmitic, stearic, and gondoic acids as well as the total SFA in European and Canadian oats were not significant. However, the differences in the contents of oleic, *cis*-13-octadecenoic, and linoleic acids, as well as the sums of MUFA and PUFA were significant, with the quantities being higher in the hulled than in naked variety. The proportions of SFA, MUFA, and PUFA in naked oats were 21.96%, 38.24%, and 39.80%, whereas in hulled oats were 22.43%, 38.79%, and 38.78% respectively.

Our results agree with those obtained by Kouřimská et al. (2021), who reported 20.97%–23.45% and 23.42%–23.51% for SFA, 33.91%–35.48% and 36.23%–38.61% for MUFA, and 41.07%–45.12% and 37.88%–40.34% for PUFA in hulled and naked oats, respectively. Our findings are also in agreement with Capouchová et al. (2021), who found 21.22% SFA, 37.79% MUFA, and 41.28% PUFA in oats cropped with a conventional cropping system. Regarding the lipidic profiles of hulled and naked oats, significant differences were observed in C18:1 *cis*-9, C18:1 *cis*-13, and C18:2 *cis*-9,12.

5. Conclusions

This study aimed at providing comparative information about the lipid content and fatty acid composition of 38 different European and Canadian oat genotypes grown in one location. The highest lipid content was found in Irish genotype Pusahybrid. French genotype Dakar presented the highest quantity of linoleic acid and was the richest source of PUFA among the analyzed genotypes. Similarly, Ivore, which is a French genotype, presented the highest quantity of oleic acid and total MUFA. Additionally, Russian genotype Lidya contained the highest palmitic acid. The PUFA/SFA ratio was 1.39–2.05 in Canadian genotypes and 1.21–2.24 in European oat genotypes. This study confirmed that Canadian oats contained more unsaturated fatty acids than European oats and are, therefore, a healthier choice for consumers. In conclusion, we have found the difference in lipid content and the fatty acid composition of oat but clear relationship between the factors influencing the fatty acid profile and the geographical origin of oats should be the subject of further research.

Author statement

Kshitiz Pokhrel: wrote the original draft, did the literature review, conducted the experiments, and evaluated the results analyses; **Lenka Kouřimská:** designed and managed the experiments, reviewed, and edited the manuscript; **Kateřina Pazderů:** did conceptualization of statistical evaluation, data curation, reviewed, and edited the manuscript; **Ivana Capouchová:** collected the samples, wrote, reviewed, and edited the manuscript; **Matěj Božik:** carried out the GC chemical analyses, wrote, reviewed, and edited the manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

Data will be made available on request.

Acknowledgements

This study was supported by the project NAZV MZe of the Czech Republic No: QK1810102 and by the METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2018100), including access to its facilities.

The authors would like to thank Mr. and Mrs. Chour from the Selgen breeding company for the cultivation of the tested oat genotypes.

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Author Contributions: KP wrote the original draft, performed the literature review, conducted the experiment on Schaal oven test and HPLC, and edited the manuscript; LK designed and managed the experiment, evaluated the analyses, and participated in manuscript modifications, revisions, and editing; OR analysed samples on Rancimat, was involved in investigation, and contributed to writing and revision. SKT validated the analytical procedure and the establishment of tocopherol analysis, writing, revision, and editing the manuscript. All authors have approved the submitted version.

Funding: This work was supported by the METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2023064) including access to its facilities.

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

Oxidative stability of crude oils relative to tocol content from eight oat cultivars: Comparing the Schaal oven and Rancimat tests

Oats are a good source of oil containing polyunsaturated fatty acids and natural antioxidants such as tocols. This study investigated tocol composition relative to oxidative stability in crude oils from eight oat cultivars. Tocol content in the cultivars was as follows: Sanitini > Saul > Korok > Atego > Kamil > Patrik > Oliver > Marco Polo. Sanitini had the highest tocol content (155.3 mg/kg of oil), whereas Marco Polo contained only 62.0 mg/kg of oil. Extracted oat oils were subjected to the Schaal oven test at 60 ± 2°C and the Rancimat test at 100°C and 110°C to determine oxidative stability. The induction period of oat samples ranged from 51 to 56 days in the Schaal test, with a protection factor of 7.8–8.6. The Rancimat test yielded induction periods ranging from 3.78 h to 9.31 h at 100°C and 1.11 h to 4.25 h at 110°C. Both tests revealed remarkable stability against oxidation. Therefore, oat oil, particularly from the Sanitini cultivar, could be a valuable and stable source of natural antioxidants for the food and pharmaceutical industries. However, a significant correlation between Schaal-derived induction period and tocopherols or tocotrienols was not identified. This outcome indicates that oil stability is due to more than tocol presence, with other antioxidants in the crude oil likely playing important roles. In contrast, the Rancimat test revealed that total tocol and total tocopherol content were correlated with induction periods at 110°C and 100°C, respectively, while β-tocotrienol content was correlated with induction period at both temperatures.

Keywords: Oxidative stability, Rancimat test, Schaal oven test, tocopherols, tocotrienols, Avena sativa

1. Introduction

Oats (Avena sativa L.) are currently receiving global attention because of their unique composition and nutritional properties, with the food industry increasing the use of oats as a raw material (Sang and Chu, 2017). Oats contain more crude oil (Chen et al., 2016) and lipids (up to 18%) (Banaś et al., 2007) than other cereal grains. While the fat content is still at lower levels than traditional oilseeds (e.g. sunflower or rapeseed), oats remain an appealing source of edible oils. However, a potential disadvantage is that their high concentration of unsaturated fatty acids increases the risk of oxidation and the formation of free radicals, which is both harmful and generates unpleasant flavours.

Oat accounts for 2.9-9.51 g/100g of lipid content (Pokhrel et al., 2022) and it contains 23% saturated fatty acids (mainly palmitic acid), 34% monounsaturated fatty acids (mainly oleic acid), and 43% polyunsaturated fatty acids (mainly linoleic acid) (Kouřimská et al., 2021) . This composition has important implications for the flavour of oat products, as it affects also food oxidation, where unsaturated fatty acids content is particularly influential. Lipid reactions in oat products can result in bitter, astringent, or rancid flavours (Peterson, 2001; Viscidi et al., 2004; Jaksics et al., 2023 and Molteberg et al., 1996). Food processing and storage can worsen this problem through lowering the effectiveness natural antioxidants in oats. For instance, peeling and grinding increases susceptibility to oxidation, while heat treatment destroys antioxidants. Unprocessed oats undergo slow hydrolysis and oxidation because of their low enzyme activity and strong antioxidants. Nevertheless, nonenzymatic oxidation can still occur. Storing oats stably requires balancing between enzyme inactivation and antioxidant preservation.

Oats are a rich source of antioxidants in free and bound forms. Natural antioxidants are primarily concentrated in the outer kernel layers and include tocols, phytic acid, phenolic compounds, and avenanthramides (Peterson, 2001). Also contributing to the crop's antioxidant properties which are flavonoids and sterols. Unlike other cereals, oat seeds contain up to 30 avenanthramides (Hernandez-Hernandez et al., 2021), secondary metabolites with antioxidant activity that contribute to fresh taste and protect against rancidity (Molteberg et al., 1996). Another key group is phenolic compounds, comprising one aromatic ring with an acidic group and one or more hydroxyl groups. Their antioxidant and anti-inflammatory effects give rise to numerous health benefits. Thus far, phenols identified and quantified in oat extracts include ferulic acid, p-coumaric acid, caffeic acid, vanillic acid, p-hydroxybenzoic acid, 4-hydroxyphenylacetic acid, vanillin, and catechol (Banaś and Harasym, 2021; Xing and White, 1997).

Total phenolic content in oats was significantly correlated with antioxidant activity (L. Emmons et al., 1999). Of particular note are tocopherols and tocotrienols, also known collectively as tocols or E-vitamers. Tocotrienols exhibit stronger antioxidant properties than tocopherols, and both have been detected in oat grains (Gangopadhyay et al., 2015). Together, α-tocopherol and α-tocotrienol account for 90% of all tocols present in oats (Bryngelsson et al., 2002). The Rancimat and Schaal oven tests are common methods used to determine oxidative stability, including anisidine, peroxide, and acid values (Maszewska et al., 2018). The Schaal oven test, also known as the accelerated oxidation method, is particularly convenient for evaluating oil stability and is frequently employed in the food industry for evaluating the lipid quality of cookies (Abasolo, 2021). The Rancimat test is widely used to evaluate bakery and cosmetic products (Abasolo, 2021).

Despite the availability of data on oat antioxidants and their properties, limited information is available regarding the stability of oils extracted from different oat varieties. Therefore, the objective of this study was to compare oxidative stability of oils from eight oat varieties using the Schaal and Rancimat tests, then correlating the results with tocol levels. This study aims to determine the stability of oat oil against two accelerated thermal tests, and to see if tocols will prolong the stability of oil.

2. Material and methods

2.1 Chemicals and reagents

Polyethylene glycol 3000 (PEG) was obtained from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Petroleum ether (PET), tert-butyl methyl ether (tBME), and n-heptane (HiPerSolv Chromanorm) of analytical purity and High-Performance Liquid Chromatography (HPLC) grade were obtained from VWR Chemicals (BDH Israel). Tocols (tocopherols and tocotrienols) were purchased from ChromaDex (Los Angeles, California, USA).

2.2 Plant material and fat extraction

Six naked (NO) (Kamil, Oliver, Patrik, Marco Polo, Santini, and Saul) and two hulled (HO) (Atego, Korok) oat varieties were obtained from the Selgen a.s. breeding station in Stupice, Czech Republic (GPS 50°313” N, 14°384” E, 287 m.a.s.l.).

Oat samples were ground using a Scarlett Silver Line SL 1545 coffee grinder (Ariette-Scarlett, Firenze, Italy) for 3 min. Approximately 10 g of sample was placed into Cytiva Whatman grade

high-performance cellulose extraction thimbles (Sweden) and covered with cotton. Crude oat lipids were obtained using the Rendall hot extraction method (E4 Behrotest, Labor-Technik, Düsseldorf, Germany) at approximately 70°C to avoid oxidation. Extraction lasted for 1 h, and for the next 40 min, most of the PET was evaporated. The remaining PET was removed using a rotary vacuum evaporator (Heidolph Hei-VAP Core HL G3, Heidolph Instruments GmbH). Approximately 100 g of raw oat oil was required to extract 5 g of purified oat oil.

2.3 Tocol analysis

To avoid potential oxidation, oat oil was extracted at room temperature (<27°C). Ground oat samples were mixed with petroleum ether and allowed to stand overnight. After filtration, petroleum ether was evaporated using a vacuum evaporator. Extracted oil (1 g) was weighed and placed into a 10 mL volumetric flask that was then filled with heptane. Tocopherols and tocotrienols (tocols) were analysed using a high-performance liquid chromatograph (HPLC) equipped with a fluorescence detector (FLD, G7121A). The detector was set at 298 nm for excitation and 330 nm for emission. Tocols were separated using a 4 × 250 mm id, 5 µm particle size, Agilent LiChrospher DIOL column (Eschenstr. 582024; Taufkirchen, Germany), maintained at 35°C. The mobile phase was a mixture of tBME and n-heptane (5:95, v/v) with a flow rate of 1 mL/min. Analytical conditions followed previous reports (Aligent, 2010). Tocol standards (mixture of α, β, γ, and δ isomers) were dissolved in n-hexane for peak identification and quantification with six-point calibration curves per tocol. Respectively, the correlation coefficients of the calibration curves (≥ 0.998), Limit of detection (LOD) (µg/mL), and Limit of quantitation (LOQ) (µg/mL) of standard solutions were as follows: α-tocopherol (0.9982, 6.36, 0.00), β-tocopherol (0.9980, 2.20, 6.67), γ-tocopherol (0.9976, 3.46, 10.50), α-tocotrienol (0.9956, 2.35, 0.00), β-tocotrienol (0.9983, 1.47, 4.44), and δ-tocotrienol (0.9947, 5.14, 15.58).

2.4 Schaal oven test

Extracted oat oil was subjected to the Schaal oven test along with refined rapeseed (K-classic, Czech Republic) and sunflower (GIANA, K-classic, Czech Republic) oils. Pork lard was used as the base for all samples because gravimetric detection of increased oxygen absorption (induction period) requires approximately 25 g of lipids per run, an unfeasible amount given the low oil yield from oats.

Crude oat oil, rapeseed oil, or sunflower oil (5 g each) plus 20 g of natural pork lard (K-classic, Czech Republic) were added to 100 mL beakers of the same diameter (50 mm). The control was 25 g of natural pork lard. All samples were kept inside the oven (Binder GmbH, Tuttlingen, Germany) at 60 ± 2°C. Sample weight was measured every 3–4 days on an analytical balance (Kern analytical balance ABS-N_ABJ-NM, Darmstadt, Germany) for 80 days until induction period (rapid increase in weight) was clearly detectable. The protection factor (PF) was the result of dividing the induction period (IP) per sample by the control IP (pork lard only). Owing to the time-consuming extraction process and low oil yield, the Schaal oven test was conducted only once per oat variety. Relative weight change (RWC) was calculated using the following formula:

$$RWC\ [g/g] = ((w2-w) - (w1-w))/(w1-w)$$

where w = weight of beaker without sample at day 0 [g], w1 = weight of beaker and sample with pork lard at day 0 [g], w2 = weight of beaker and sample with pork lard on any given day [g].

2.5 Rancimat test

The Rancimat test was applied on 5 g of crude oat oil, the samples mentioned in section 2.4, as well as rice oil (GASTON, s.r.o., Zlín, Czech Republic) and butter (Madeta a. s., České Budějovice, Czech Republic). The test was performed using a Metrohm Rancimat model 892 (Herisau, Switzerland), as recommended previously (Metrohm AG, 2019). The heat transfer medium was polyethylene glycol, suitable for non-traditional samples of oils, foodstuffs, and cosmetics (Agilent, 2010 and Metrohm AG, 2019). A stream of purified air 20 Lh−1 was passed through 0.5 g of oil or fat and 3 g of PEG 3,000 held at a constant temperature of 100°C or 110°C under conditions described in ISO method 6886:2016. Subsequently, effluent air from the oil sample was passed through a vessel filled with deionised water to create bubbles. Water conductivity was continuously monitored in StabNet 1.1 (Metrohm AG, Herisau, Switzerland), and the induction time was automatically calculated.

2.6 Statistical analysis

Data were analysed in IBM SPSS version 29 (Armonk, New York, USA) and expressed as mean ± standard deviation (SD). One-way ANOVA and Tukey's post-hoc tests were used to determine

between-group differences. Significance was set at $p < 0.05$. Pearson's Correlation coefficients were calculated to determine relationships between the Schaal oven and Rancimat tests.

3. Results

3.1 Tocopherols and tocotrienols in oats

Table 1 shows the results of tocol analysis for eight oat genotypes compared with sunflower oil and pork lard. Sunflower oil was chosen as a representative, highly unsaturated plant oil with reasonable tocopherol content. In contrast, pork lard is high in saturated fats with low to no tocol content. Among the oat cultivars, total tocol content was highest in Sanitini (155.3 mg/g of oil), followed by Saul (144.5 mg/kg of oil) and Korok (105.9 mg/kg of oil). Tocol content was lowest in Marco Polo (62.1 mg/kg of oil). Tocopherol was the most abundant form of tocol in all cultivars except Patrik and Saul, where α-tocotrienol was the predominant form. Interestingly, Saul had the highest α-tocotrienol content (8.8 mg/kg of oil), a potentially valuable trait for oat breeding programs. Notably, β-tocopherol was detected at low concentrations in all genotypes, with the highest content reaching 7.5 mg/kg of oil in Santini. Sunflower oil had comparable tocopherol content (78.1 mg/kg of oil) as most oat samples. As expected, pork lard was low in tocol content (approximately 2 mg/kg). Hulled oats, Atego, and Korok contained higher tocol content than most naked oats, except for Santini and Saul. The between-cultivar differences in tocol content were significant.

3.2 Schaal oven test

Respectively, Figures 1–3 show the oxidation kinetics of oats and controls i.e. oxidation kinetics of Oliver, Kamil, Patrik, Atego (Fig. 1); hulled (Korok) and naked (Marco Polo, Santini, Saul) oats (Fig. 2); and pork lard, rapeseed oil, and sunflower oil (Fig. 3). Adding pork lard to rapeseed and sunflower oils increased stability through elevating natural antioxidants levels compared with lard-only samples.

The IPs for all oat samples in lard exceeded 50 days (Table 2). The PFs of all hulled and naked oat samples ranged from 7.8 to 8.6 days. Atego, Patrik, and Santini exhibited the highest PFs. Oats with higher tocol levels (Saul and Santini) had slightly longer IP than other oats, but tocol content and IPs were not significantly correlated.

3.3 Rancimat test

The results of the Rancimat test showed that Kamil oxidised faster than other oat varieties, with an average IP of 4.12 h at 100°C and 1.49 h at 110°C (Table 3). In contrast, Saul oxidised the slowest,

with an average IP of 9.02 h at 100°C and 4 h at 110°C. The IPs of other oat samples ranged between 5 to 7 h at 100°C and between 2 to 3 h at 110°C. When comparing the IPs of other oils and fats with oats, rice oil had lower oxidation stability; its IP was 2.14 and 1.26 h at 100°C and 110°C, respectively. The proportion of the main fatty acid groups (SFA: MUFA: PUFA 1:2:2) is similar between rice oil and oat oil.

The results of comparing oxidation rates between crude oat oils and refined edible oils/fats (including lard and butter) at 100°C indicated that the former (IP = 4–9 h) was more stable than the latter (Figure 4). In contrast, refined edible oils and fats had IPs of approximately 1 h. Thus, the process of refinement may lower natural antioxidant content of oils.

Crude oat oils were extremely stable at both test temperatures. Oat oils and other oils differed slightly in IPs at 110°C (Figure 5). However, oat oil was more stable (IP range = 2.8–5.9 h) than other edible oils and fats. These results further suggest that refining processes decrease natural antioxidant content of edible plant oils and lower oxidation stability. However, because butter and lard have naturally low antioxidant levels, they are already fairly unstable.

4. Discussion

This study is one of few to successfully find correlation between thermal stability and tocol concentrations in oats. Total tocol concentration in our oat oil samples aligned with previous research that reported 90 ppm α-tocopherol, 13 ppm γ-tocopherol, and 6.5 ppm δ-tocopherol (Saga et al., 2013). In contrast, another study reported lower concentrations of tocols (19.0 mg/kg dry matter) than our findings (Holasova et al., 2002), whereas a third investigation on 12 oat cultivars found a wider range in tocopherols levels, from 19 to 31 mg/kg of dry matter (Peterson and Qureshi, 1993). For example, a study that examined the effect of irrigation on tocopherol concentrations found that irrigation significantly increased tocopherol concentrations from non-irrigated levels (under irrigation: α-tocopherol = 8.8 mg/kg, α-tocotrienol = 17.4 mg/kg, total tocopherols = 27.1 mg/kg; non-irrigated: α-tocopherol = 7.4 mg/kg, α-tocotrienol = 11.5 mg/kg, total tocopherols = 18.8 mg/kg) (Jackson et al., 2008).

A comparative study of oats, rice bran, and soybean oil (Tong et al., 2014) found that in their oat oil samples, the amount of α-tocopherol, β-tocopherol, γ-tocopherol, and α-tocotrienol was 157 mg/kg, 119 mg/kg, 107 mg/kg, and 346 mg/mL, respectively. Their sum was 729 mg/kg, higher than the total tocol content of rice bran (307 mg/kg) and soybean oil (710 mg/kg). Another previous study on five hulled and naked oat genotypes found significantly lower α-tocopherol concentrations (4.5–12.3 mg/kg) (Sterna et al., 2016) than current study. However, oat tocol concentrations in this

study were higher than concentrations (2.95 μg/g α-tocopherol) in a prior report (Chen et al., 2016) that extracted oat oil with petroleum ether. Overall, the data suggest that a variety of factors influence tocol concentration in oat oil, including genotype, environmental conditions, and the extraction method.

Oat oils possess remarkable thermal stability properties. The PF for oats was 1.8 in a previous study (Holasova et al., 2002), considerably lower than our results in general. This disparity can be attributed to differences in the experimental design. Our study extracted and measured crude oat oil, whereas the other study measured dried ground oat samples mixed with 10 g lard at 70°C (Holasova et al., 2002). Similarly, another report (Saga et al., 2013) indicated that adding 5% and 10% crude oat oil to fish oil increased stability by approximately two-fold, based on Schaal oven test results at 70°C. Moreover, pure oat oil did not increase in weight after 50 days at 70°C, indicating that oat oil is highly resistant to oxidation, corroborating our findings of high stability.

Similarly, a prior analysis of oat, soybean, and feta flours using the Schaal oven test (Berghofer et al., 1998) noted exceptionally potent antioxidative effects in native oat flour. Moreover, during tempeh production, oats and soybeans exhibited significantly greater changes in antioxidant activity than raw soybean flour. Specifically, thermal stress during the steaming step decreased antioxidative activity, whereas fermentation increased it.

Furthermore, a study examining the rancidity resistance of diacylglycerol-enriched soybean oil and palm olein when exposed to heat (Wang et al., 2010) found that soybean oil had an IP of 9.46 h at 110°C. In contrast, diacylglycerol-enriched soybean oil had an IP of only 4.21 h; its higher unsaturated fatty acid concentrations and lower tocopherol levels decreased its oxidative stability. At the same temperature, palm olein had an IP of 21.53 h, whereas diacylglycerol-enriched palm olein only had an IP of 5.40 h. Both soybean and palm olein oils had longer IPs than the oat samples analysed in our study.

Adding a mixture of oat oil and PUFA-rich fish oil exerts protective effects on cow's milk (Saga et al., 2013). Relatedly, the IP of lard was 5 days at 65°C (Liang and Schwarzer, 1998), similar to our result of 6.5 days. However, when lard was mixed with δ-tocopherols and rosemary extract, IP increased significantly to 36 days.

Based on its fatty acid profile, oat oil (24% saturated fatty acid [SFA], 39% monounsaturated fatty acid [MUFA], 36% polyunsaturated fatty acid [PUFA]) was expected to have a shorter IP than what we actually obtained from the Rancimat test. The fatty acid profile is in contrast to lard (35% SFA, 47% MUFA, 18% PUFA) and butter (66% SFA, 30% MUFA, 4% PUFA); the lower antioxidant

levels of saturated oils resulted in shorter IPs than the unsaturated oat oils. Next, in our study unsaturated rapeseed (8% SFA, 66% MUFA, 26% PUFA) and sunflower (11% SFA, 35% MUFA, 54% PUFA) oils had slightly longer IPs than saturated fats, but noticeably shorter IPs than oat oils. Thus, fatty acid composition appears to exert a smaller effect on oxidation than the presence of antioxidants in oat oils.

We did not identify a correlation between tocol content and the IP of oat oils when running the Schaal oven tests. While tocopherols and tocotrienols are important in enhancing oxidative stability of oat oils, other components in oats may also have protective effects. Components with antioxidant potential include avenanthramides, phenolic compounds, phytic acids, flavonoids, and sterols. In the Rancimat tests, total tocol concentration correlated with the IP at 110°C, while total tocopherols correlated with IP at 100°C and β-tocotrienol content correlated with IP at both temperatures. Hence, other compounds with antioxidant activity did not appear to be effective under the Rancimat test conditions.

Sunflower oil consists of 12.3% SFA, 34.5% MUFA, and 53.2% PUFA. Previous research using the Rancimat test demonstrated that the IP of sunflower oil was 226.2 min at 110°C and 127.2 min at 120°C (Almoselhy, 2021). Similarly, rapeseed oil contains 7.4% SFA, 63.9% MUFA, and 28.1% PUFA. Its IP was 5 h during the first month of storage and 3.2 h in the twelfth month of storage when exposed to a Rancimat test at 120°C (Maszewska et al., 2018). These values are higher than the IPs we observed for rapeseed and sunflower oil at 100°C (1.34 h and 1.32 h, respectively).

Our findings are consistent with reports that the Rancimat test yielded an IP of 6.9 h for oat oil and its fractions at 110°C (AG, 2019). At concentrations of 1–5%, oat oil increased the IP of lard and tallow two- to eight-fold from non-antioxidant levels. When the Rancimat method (100–130°C) was used to assess the stability of olive oil (semi-fine, fine, refine, and extra virgin) (Farhoosh and Hoseini-Yazdi, 2014) , the findings revealed that extra virgin olive oil was the most stable, lasting for 55.1 h at 100°C and 5 h at 130°C, while semi-fine oil only lasted 15.6 h at 100°C and 1.6 h at 130°C. Notably, however, olive oil was more stable than oat oil at both temperatures.

In our study, we demonstrated that oat oil can better slow oxidation during heating and cooking than rapeseed and sunflower oils (Figures 4 and 5). Introducing new oat cultivars with increased yield and higher antioxidant content widens their applicability in various food and industrial sectors. Furthermore, the stability of these cultivars will improve processing to obtain refined oat oil.

In summary, the result of current study indicate that oats contain more tocols than common edible vegetable oils (e.g. rapeseed, sunflower, and rice oils) and saturated fats (butter and lard),

contributing to their superior oxidative stability. Other bioactive components such as avenanthramides also play a role in the stability of oat oil against oxidation. The current study provides the better understanding of oat oil stability through correlation analysis of tocol concentrations and thermal stability. The study reveals higher tocol concentrations in oat oil compared to other edible oils and fats, indicating its superior oxidative stability.

5. Conclusions

This study provided valuable comparative data on the stability of crude oat oil using two common tests (Schaal oven and Rancimat tests). Schaal oven test results were more similar across cultivars, whereas between-cultivar differences emerged in the Rancimat test. The Saul variety had the highest IP and α-tocotrienol content. Compared with commercially available oils and fats, oat oil had greater oxidative stability. Additionally, hulled oats (except Santini and Saul) contained higher tocol content than naked oats. Tocopherol and tocotrienol concentrations in oat oils were not significantly correlated with the Schaal-derived IP. Nevertheless, total tocols were correlated with Rancimat-derived IP at 110°C, total tocopherols were correlated with Rancimat-derived IP at 100°C, and β-tocotrienol was correlated with Rancimat-derived IP at both temperatures.

Correlation between tocols and thermal stability observed in this study provides insights for the selection and optimization of processing conditions to preserve the quality and stability of oat oil during heat treatment. This knowledge can be utilized to develop healthier and more stable oat-based products. The study concluded that in addition to the tocopherols and tocotrienols, other components with antioxidant potential e.g., avenanthramides, phenolic compounds, phytic acid could contribute on the stability of oat against the oxidation. The limitations of this study are this study was not aimed to measure peroxide values of oat oil. Additionally, the study did not thoroughly investigate the antioxidant potential of other oat components and the effects of different processing conditions on oat oil stability. Future research should focus on investigating the specific antioxidant mechanisms of oat components like avenanthramides, phenolic compounds, and phytic acid, and their contributions to oat oil stability.

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Table 1: Concentration of tocopherols and tocotrienols in oats, sunflower oil and pork lard (mg/kg)

	α-tocopherol	α-tocotrienol	β-tocopherol	β-tocotrienol	γ-tocopherol	γ-tocotrienol	Total tocols	Total tocotrienols	Total tocopherols
Saul	63.44 ± 1.52^{c}	65.65 ± 4.98^{a}	3.61 ± 0.09^{bcd}	8.76 ± 1.06^{a}	1.20 ± 0.21^{b}	1.80 ± 0.31^{a}	144.46 ± 6.18^{a}	68.25 ± 1.90^{b}	76.21 ± 7.95^{a}
Marco Polo	28.31± 3.77^{f}	23.53 ± 1.59^{d}	4.69 ± 0.37^{bc}	4.51 ± 0.96^{b}	0.73 ± 0.65^{c}	0.27 ± 0.06^{c}	62.05 ± 3.79^{d}	33.73 ± 2.81^{d}	28.32 ± 2.98^{e}
Patrik	32.35± 3.33^{f}	35.55 ± 4.48^{c}	2.42 ± 0.74^{cd}	6.01 ± 2.38^{ab}	ND	ND	76.33 ± 4.07^{cd}	34.76 ± 3.04^{d}	41.57 ± 2.34^{cd}
Sanitini	89.81± 7.47^{a}	48.54 ± 2.52^{b}	7.50 ± 1.37^{a}	6.91 ± 0.83^{ab}	2.05 ± 0.35^{a}	0.47 ± 0.18^{b}	155.29 ± 6.31^{a}	99.36 ± 6.75^{a}	55.93 ± 1.40^{b}
Oliver	44.07± 4.34^{e}	21.76 ± 2.84^{d}	1.84 ± 0.76^{d}	7.21 ± 0.40^{ab}	ND	ND	74.89 ± 7.68^{cd}	45.92 ± 4.71^{cd}	28.97 ± 3.02^{e}
Atego	56.67± 3.33^{cd}	35.16 ± 3.01^{c}	5.81 ± 0.72^{ab}	4.14 ± 1.63^{b}	ND	ND	101.78 ± 8.20^{b}	62.47 ± 3.73^{b}	39.3 ± 4.63^{de}
Korok	48.72± 4.34^{de}	46.48 ± 2.51^{b}	5.45 ± 2.0^{ab}	5.32 ± 0.84^{ab}	ND	ND	105.97 ± 7.56^{b}	54.17 ± 6.02^{bc}	51.80 ± 1.81^{bc}
Kamil	44.23 ± 3.47^{e}	40.48 ± 1.45^{bc}	2.26 ± 0.74^{cd}	6.42 ± 2.31^{ab}	ND	0.51 ± 0.08^{b}	93.90 ± 5.77^{bc}	46.49 ± 3.38^{cd}	47.41 ± 3.38^{bcd}
Sunflower oil	78.16 ± 5.09^{b}	ND	1.48 ±0.13^{d}	ND	0.16 ±0.03^{d}	ND	79.77^{cd}	ND	79.77^{a}
Pork lard	2.09 ± 0.36^{g}	ND	ND	ND	ND	ND	2.09^{e}	ND	2.09^{f}

ND = not detected. Different lowercase letters in a column indicate significant differences ($p \leq 0.05$). Values are expressed as means ± SD of independent analyses in triplicate (n = 3).

Table 2. Induction period (IP) and protection factor of oats, rapeseed and sunflower oil with pork lard

Oil sample (5 g) + pork lard (20 g)	Induction period (days)	Protection factor
Atego (hulled oat)	56.0	8.6
Kamil (naked oat)	54.0	8.3
Korok (hulled oat)	55.5	8.5
Marco Polo (naked oat)	51.0	7.8
Oliver (naked oat)	55.0	8.5
Patrik (naked oat)	56.0	8.6
Santini (naked oat)	56.0	8.6
Saul (naked oat)	52.0	8.0
Rapeseed oil	23.0	3.5
Sunflower oil	14.0	2.2
Control - pork lard (25 g)	6.5	1.0

Table 3. Induction periods of samples and controls (0.5 g samples + 3 g polyethylene glycol-PEG 3000) at 100°C and 110°C in the Rancimat test.

Samples	100°C Induction period (h)	110°C Induction period (h)
Kamil	4.12 ± 0.30^{b}	1.49 ± 0.47^{b}
Saul	9.02 ± 0.27^{a}	4.00 ± 0.30^{a}
Marco Polo	6.80 ± 1.06^{ab}	2.90 ± 0.15^{b}
Oliver	7.29 ± 0.5^{ab}	3.33 ± 0.32^{ab}
Atego	5.06 ± 0.27^{b}	2.22 ± 0.09^{b}
Patrik	5.45 ± 0.47^{b}	3.50 ± 0.33^{ab}
Santini	5.96 ± 0.59^{b}	2.92 ± 0.23^{b}
Korok	6.86 ± 1.15^{ab}	2.83 ± 0.11^{b}
Pork lard	1.18 ± 0.04	0.77 ± 0.05
Butter	1.07 ± 0.06	0.66 ± 0.08
Rapeseed oil	1.34 ± 0.07	0.75 ± 0.02
Sunflower oil	1.32 ± 0.08	0.81 ± 0.06
Rice oil	2.14 ± 0.05	1.26 ± 0.19

Values are expressed as means ± standard deviation (SD) of independent analyses run in triplicate. Different lowercase letters in a column indicate significant differences ($p \leq 0.05$). Only oat samples were included in the ANOVA.

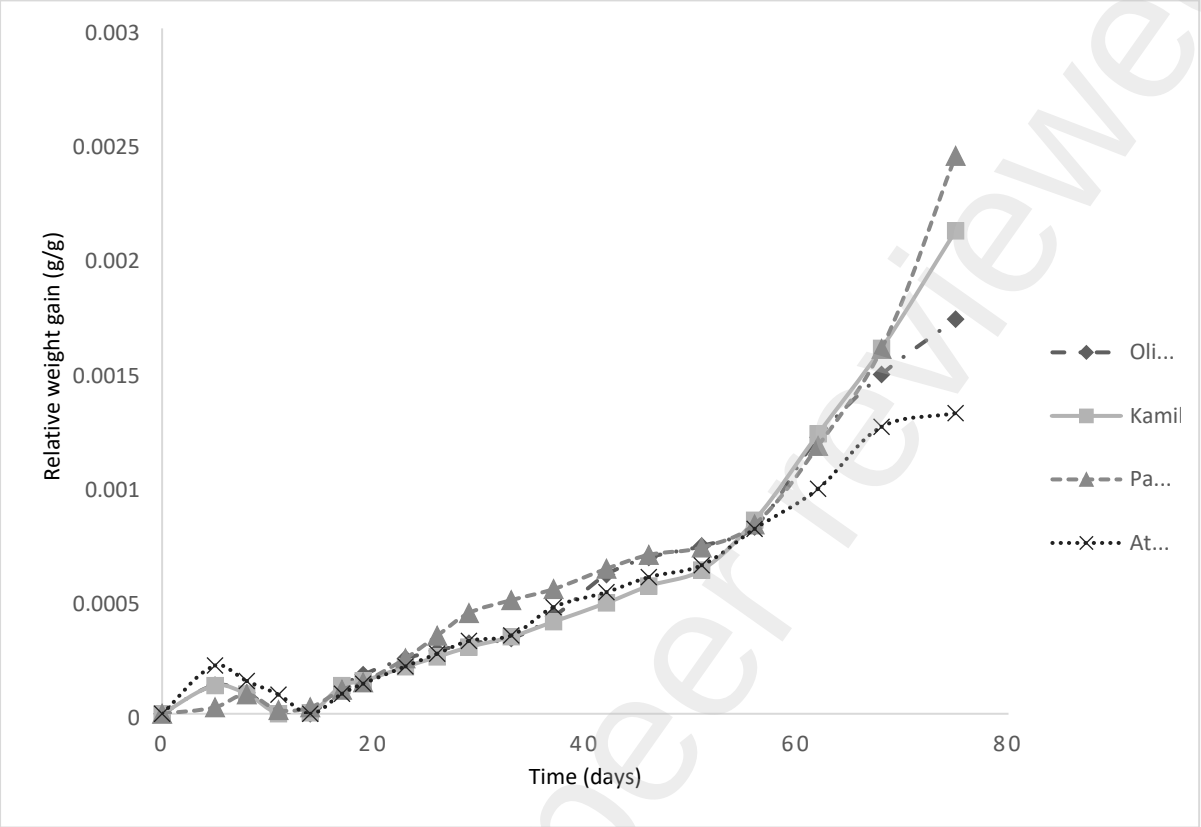

Figure 1. Oxidation time-course of yellow oat oil mixed with pork lard in the Schaal oven test

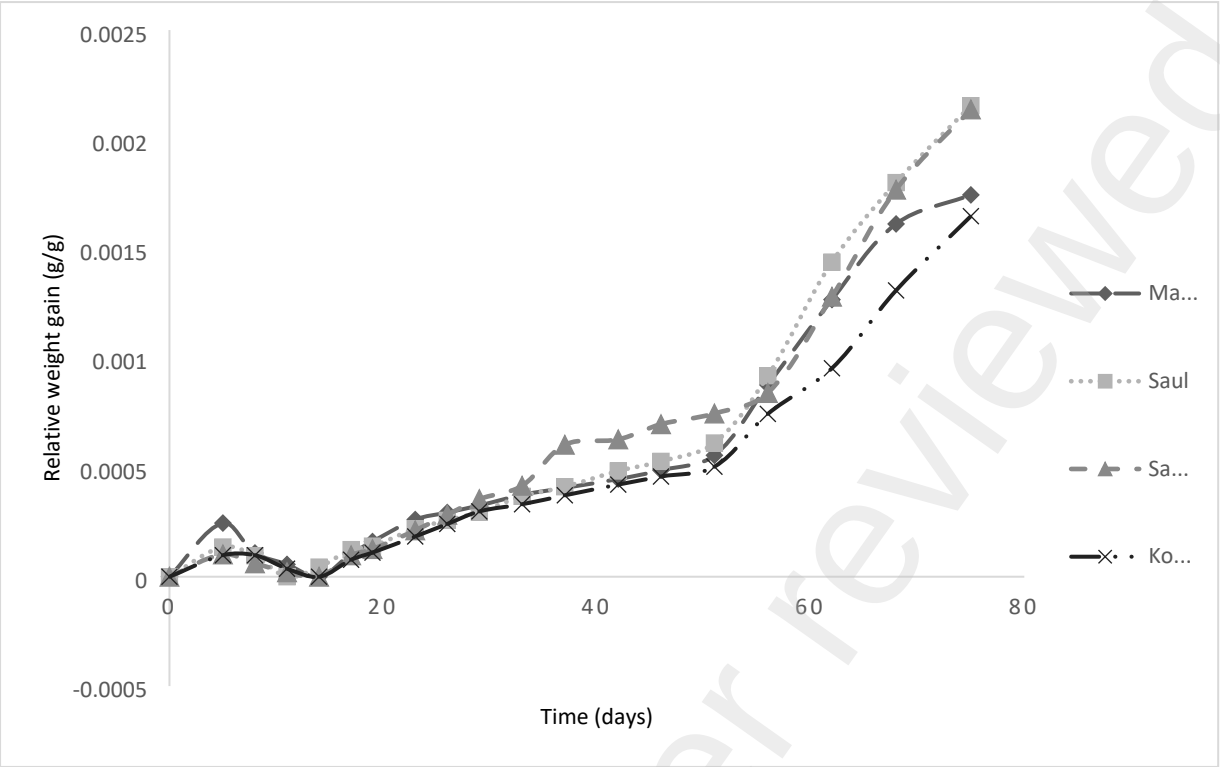

Figure 2. Oxidation time-course of hulled and naked oat oils mixed with pork lard in the Schaal oven test.

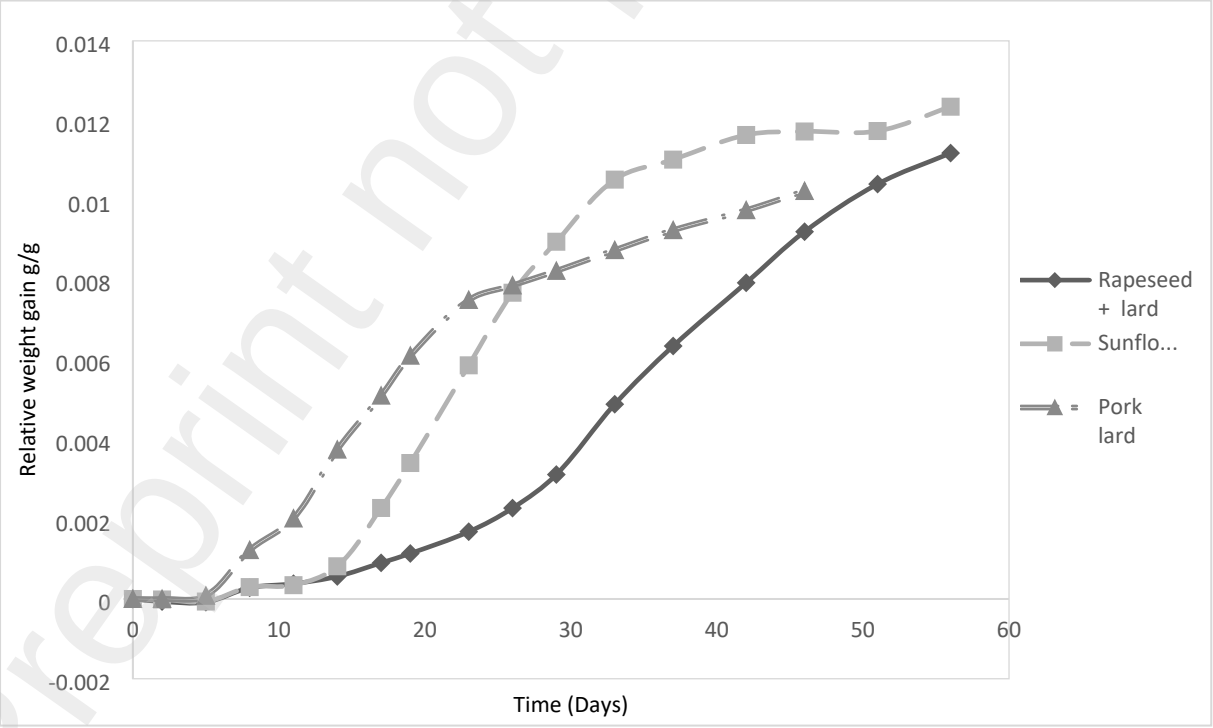

Figure 3. Oxidation time-course of pork lard alone (control), as well as rapeseed and sunflower oils mixed with pork lard in the Schaal oven test.

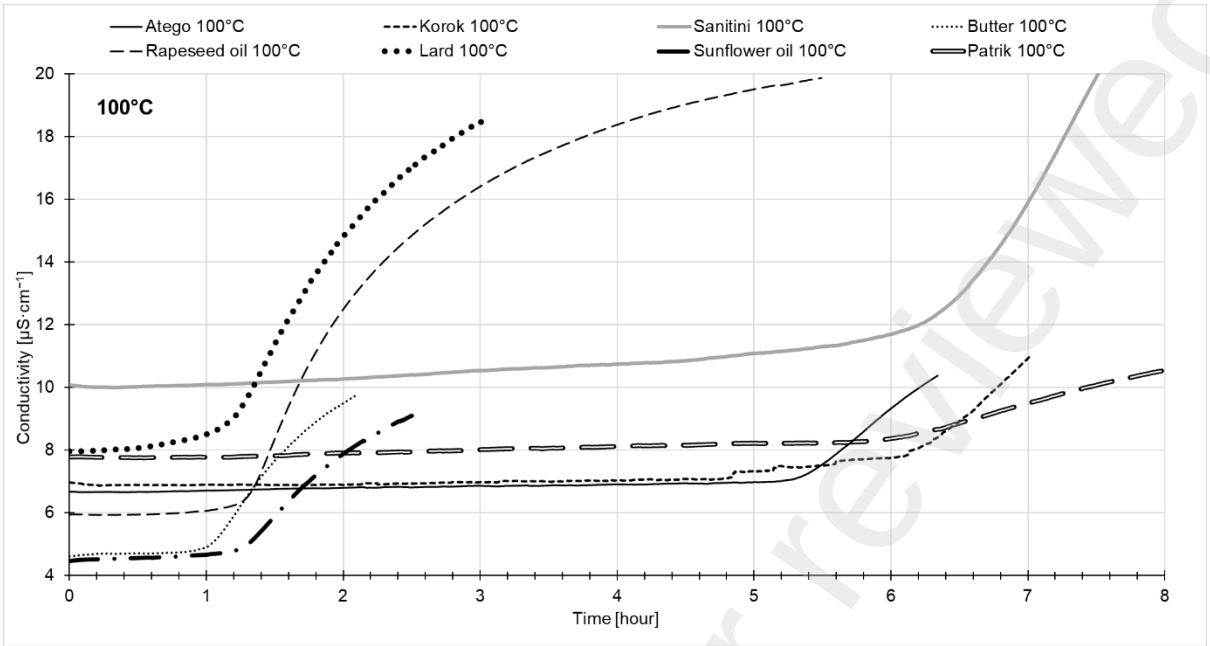

Figure 4. Stability curves for selected varieties of oat oils, butter, and lard at 100°C in the Rancimat test.

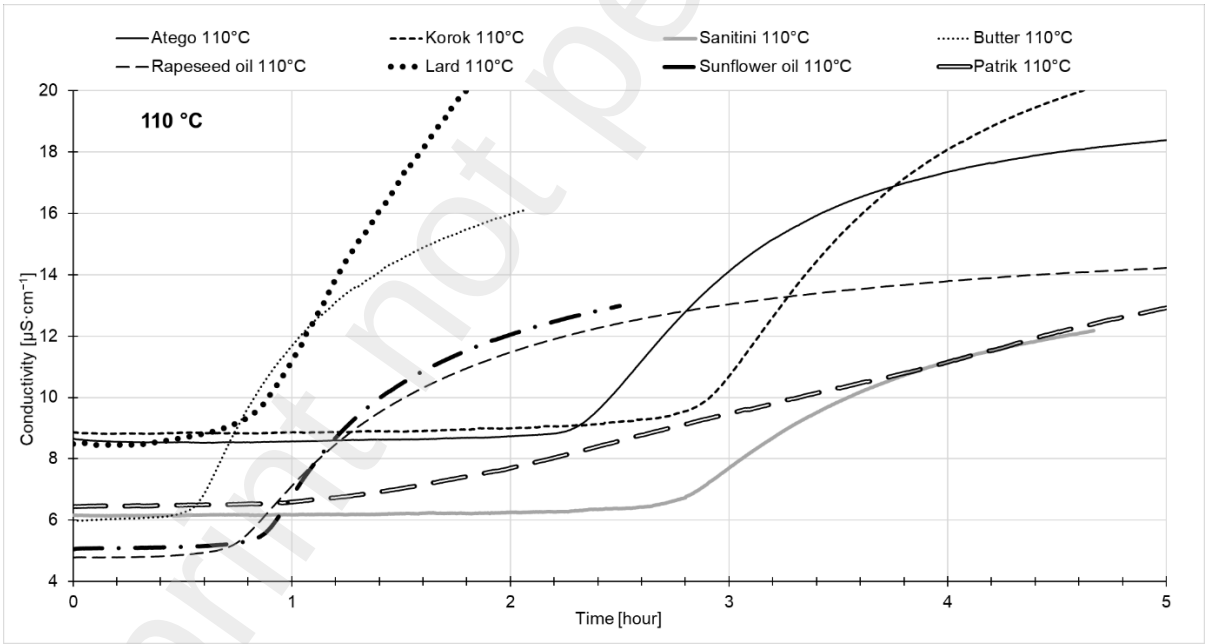

Figure 5. Stability curves for selected varieties of oat oils, edible oils and fats at 100 °C

RESEARCH ARTICLE

Comparative analysis of lipid content and fatty acid composition in hulled and naked oats: A study of dehulled grains and husks

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Abstract

Background: Oat (*Avena sativa* L.) has distinctive multifunctional characteristics and nutritional profile, and a large amount of oat-processing by-product comprises husk, which contains lipids and other nutrients. In this study, we analysed the fat content and fatty acid profiles of six naked oat varieties (Kamil, Marco Polo, Oliver, Patrik, Santini, and Saul), two hulled oat varieties (Atego and Korok), and their dehulled grains and husks. **Results:** The fat content varied from 3.73 g/100 g (Santini) to 6.03 g/100 g (Kamil) in naked oats; 3.2 g/100 g in Atego and 3.21 g/100 g in Korok with husk; 0.63 g/100 g in Atego husk and 0.68 g/100 g in Korok husk. Dehulled oats have a higher fat content than hulled oats. Linoleic and oleic acids were the predominant fatty acids. Oat husks contained maximum amounts of saturated fatty acids (32.8% in Korok and 29% in Atego). Husk removal reduced the proportion of oleic acid and increased that of linoleic acid. Oat husk contained the least amount of linoleic acid and the highest amount of eicosadienoic (C20:2 cis 11,14) and archidonic (C20:4 cis-5,8,11,14) acids. **Conclusion:** Oats are a significant source of both fats and unsaturated fatty acids. Moreover, oat husks contribute to the fat content although their fatty acid composition, with higher palmitic acid and lower linoleic acid levels, differs from that of naked, hulled, and dehulled oats.

Keywords: naked oats, hulled oats, dehulled oats, oat husks fat content, fatty acid composition.

1. Introduction

Wheat, rice, and maize are the leading cereals regarding global production, whereas oats (*Avena sativa* L.) ranks sixth. Historically, oats have mainly been utilised as animal/horse feed; however, such usage has been gradually decreasing because of the growing interest in the potential utilisation of oats as a human food [1] . Oat crops have received increasing attention because of their associated health benefits and the increasing popularity of plant-based diets. According to recent data 25.05 million metric tons of oats were produced globally in the year 2022/23 [2].

Compared with other cereals, oat grains contain larger amounts of crude fat and protein. The higher lipid content constitutes a source of energy and unsaturated fatty acids. Generally, oat groat oil concentration ranged from 3.1 to 11.6 g/100 g of oil, with a mean value of 7.0 g/100 g, which can be increased to 18.1 g/100 g by breeding for high lipid content [3]. Oat hulls cover approximately about 25–35% of the seed weight. Though they do not possess longer function as metabolic tissues at seed maturity, oat hull plays an important protective role by preventing kernel breakage during threshing and protecting against fungal pathogens. However, dehulling of oats for use in the pharmaceutical or nutritional industries costs farmers money. Therefore, naked oats may be a more suitable option for this purpose. However, oat hulls are rich in fibre and can benefit livestock, such as cattle, sheep, and horses, by offering nutritional value similar to that of cereal straw in terms of available crude protein and energy for ruminants [4]. Besides their use as animal feed, oat husks have potential as starting materials for biorefinery processes, production of bio-based films for packaging, and composites for construction and building materials. A study by Schmitz et al. (2020) shows that oat and rice husk, after being burned at 950°C to generate calcium hydroxide, can be used to improve the calcination properties of cements [5].

According to Girardet and Webster (2011) oat husks is the primary by-product of the oat-milling process [6] and industrial husks generation constitutes a global problem because of difficulties in reutilisation and disposal. Husk can be used for hydrogen production from cellulose extracted with polyvinyl alcohol [7] or chemically produced as furfural and food-grade fibre by treatment with alkali [8]. Therefore, because of their potential use, oat husks should not be considered as waste. Oat husk comprises the cell wall (>83%), which contains equal amounts of cellulose and hemicellulose besides lignin 2–10 g/100 g, ash 3.5–9 g/100 g, protein 1.6–5 g/100 g, and oil 1–2.2 g/100 g [9].

Lipid proportions of four different oat husk batches grown in similar locations, but under different climatological circumstances varied from 0.5 to 1.5 % (Schmitz et al. 2020). The ranges of SFA, 18:1, and 18:2 concentrations in seven different Swedish oat husk samples were 24–36.5% (mean 30.4%), 25.7–36.3% (mean 29.4%), and 28.8–35.8% (mean 31.7%), respectively [10]. The authors also noted that the average total lipid content of the husks was 3 g/kg DM. Kouřimská et al. (2021) investigated the fatty acid profile of naked and hulled oats with and without hulls[12]. Based on the research, this study aimed to access lipid content and the fatty acid profile of hulled oats before and after dehulling, husk of the hulled oat and compare those with naked oats. Furthermore, the impact of dehulling on these nutritional parameters, with a particular focus on fatty acid composition, was examined.

2. Materials and methods

2.1. Oat samples

The oat samples used in this study were provided by the Selgen a.s. breeding station (Stupice, Czech Republic; GPS 50°313″ N, 14°384″E, 287 m.a.s.l.). The oat varieties used in this study were registered between 2002 and 2018. Of the eight yellow varieties,

six (Kamil, Marco Polo, Oliver, Patrick, Santini, and Saul) were naked oats and two (Atego and Korok) were hulled oats; the samples have been described in Table 1.

2.2. Post-harvest sample specifications

The post-harvest characterisation of the samples was undertaken using a grain analyser (FOSS Infratec 1241; FOSS Analytical A/S, Hillerød, Denmark) that quantified the basic quality criteria for purchase (total protein and moisture contents). Each sample was measured ten times, and the relative standard deviation of the method was less than 1%. The post-harvest characteristics of the samples are given in Table 2.

2.3. Sample preparation and lipid content determination

The oats were milled for 3 min using a Scarlett Silver Line SL 1545 coffee grinder (Ariette-Scarlett, Firenze, Italy). In the case of hulled oats, grains and hulls were separated manually to prevent breakage and avoid mixing powdery oats with the hulls. From 30 g hulled oats of the Atego and Korok varieties, 7.3 and 7.6 g hulls, respectively, were obtained, with a grain-to-husk ratio of approximately 4:1. The separated hulled and dehulled oats were then milled. Ground oat samples, weighing approximately 15 g, were placed in high-performance cellulose extraction thimbles of Cytiva Whatman grade and subsequently covered with cotton. Following the procedures stipulated in the ISO 659:2009 guidelines, lipids were extracted from the samples using petroleum ether in a Soxhlet glass apparatus for 240 min. The petroleum ether–lipid solution was halved, and one portion was used to determine the fat content and the other to analyse the fatty acid profile. A rotary vacuum evaporator (Heidolph Hei-VAP Core HL G3; Heidolph Instruments GmbH, Schwabach, Germany) was used

to evaporate petroleum ether. The lipid content was determined gravimetrically after drying at 103 ± 2°C to a constant weight. Each sample was analysed in triplicate (n = 3).

2.4. Determination of fatty acid composition

The mixture of solvent and oat lipid reserved for the determination of fatty acid composition was treated with utmost care to ensure that it was not exposed to high temperatures (not more than 60 ± 2°C) throughout the entirety of the sample preparation and extraction process. A rotating vacuum evaporator was used to evaporate the petroleum ether to avoid high temperature-induced unfavourable fatty acid oxidation. The fatty acid composition of the samples was analysed by using a method described by Kouřimská et al. (2018)[13]. To analyse fatty acid profiles, approximately 0.5 g extracted fat was re-esterified. The preparation and determination of fatty acid methyl esters were undertaken according to ISO 12966-2:2011 specifications using gas chromatography coupled with mass spectrometry (GC-MS) on an Agilent 7890 gas chromatograph (Agilent Technologies). Each extract was injected three times to ensure accuracy and precision. Methylated fatty acids were identified using the Restek Food Industry FAME mix (cat#35077), and their mass spectra were compared with those reported in the National Institute of Standards and Technology Library (NIST, USA). Relative proportions of fatty acids were determined using the area normalisation method and then expressed as a percentage of all identified fatty acids.

2.6. Statistical analysis

Statistical analysis comprised one-way analysis of variance (ANOVA) using IBM SPSS Statistics 29.0.0.0 (Armonk, New York, USA), with a level of significance of *P*

= 0.05. The mean ± standard deviation was evaluated using Tukey's HSD (honest significant difference) test.

3. Results

3.1 . Fat content

Table 3 shows the results of the lipid analysis of various oat cultivars, including naked oats, hulled oats before and after their dehulling, and their husks. The findings revealed that naked oats had a higher lipid content than hulled oats, with or without husks, excluding naked Santini which had a lower lipid content than both the hulled cultivars before dehulling. Among the naked oat cultivars, Kamil demonstrated the highest lipid content (mean 6.03 ± 0.37 g/100 g), followed by Patrik (5.27 ± 0.38 g/100 g). In contrast, Santini had significantly lower lipid content as compared to Kamil, with values of 3.73 ± 0.21 g/100 g. Hulled Atego had the lowest lipid content (mean 3.20 ± 0.19 g/100 g) among all the tested oat cultivars. Dehulling of Korok and Atego hulled oats increased the lipid content of the grains as compared to that of most naked oats. Additionally, the husks of both Korok and Atego had very low lipid content (<0.7 g/100 g).

3.2. Fatty acid composition

Table 4 presents the fatty acid composition of naked oats. Significant inter-sample variations were observed. Oleic acid (C18:1 *cis*-9) emerged as the predominant fatty acid, constituting 30–40% of the total fatty acids in the analysed samples. The proportions of linoleic acid (C18:2 *cis*-9,12) and oleic acid were comparable. Additionally, palmitic acid (C16:0) was present in relatively high amounts, ranging from (19–26% of total fatty acids). Moreover, moderate amounts of other fatty acids,

such as stearic (C18:0) and linolenic (C18:3 cis-9,12,15) acid, were detected and, others, such as myristic (C14:0) and margaric (C17:0) acids, were present in minor amounts or were undetected. Additionally, the current study further demonstrated that Patrik contained the highest amount of oleic acid (39.34%).

Table 5 presents the fatty acid compositions of hulled oats with and without husks from hulled oats. The Atego grain contained the highest amount of C16:0 (26 %), followed by Korok husk (24.8 %). Dehulled Korok grains and naked Marco Polo showed a high content of C18:2 *cis*-9,12 (>38%). In the case of Korok and Atego, husk removal increased the relative proportion of linoleic acid in the grains of hulled oats, though the differences were not statistically significant. Besides the three major fatty acids, C18:0 was the fourth most abundant fatty acid, with Kamil containing the highest amount (4.6%). These findings suggest that the fatty acid composition of oats is affected by husk removal. The removal of husk from Atego oats resulted in a notable alteration in the concentration of oleic acid, indicating a significant difference in MUFA levels.

Furthermore, dehulled Atego grains contained approximately half of the MUFA compared to other samples (22%), whereas the amount of PUFA increased to 47.8%. The husks of oats had the highest amount of SFA compared to the naked, hulled, and dehulled oats. The proportion of SFA in Korok husk and Atego husks was more than 31%. The Patrik variety contained the lowest amount of SFA among all analysed samples. Finally, the unsaturated/saturated ratio in naked oats ranged from 2.9–3.6, whereas the husks of Atego and Korok had ratios of only 2.2 and 2.0, respectively.

4 Discussion

4.1 Lipid content

In this study, the total lipid content of various oat samples ranged from 3.2 to 6 g/100 g. These results are consistent with previous reports indicating total lipid contents of 3–11 g/100 g lipid content in oats, which is higher than that in other cereals, such as wheat (1.4–1.5 g/100 g) and barley (1.3–11 g/100 g)[14]. The results of the present investigation are consistent with those of previous studies[15,16] that revealed lipid contents of 7–11 g/100 g and 5–9 g/100 g, respectively, in oats. According to these authors, oats contained the highest amount of lipid (5.35 g/100 g), whereas other cereals, including durum wheat, common wheat, rye, barley, and triticale, had fat contents of 1.19 to 1.76 g/100 g.

Leonova et al. (2008) examined the fat content and fatty acid composition of 33 oat cultivars, wherein wild genotypes had fat content of 4.1 to 8.3 g/100 g [17]. The study reported that oat genotype greatly influenced the oil content of oats. The current study's results are consistent with those of Pokhrel et al. (2022), who reported a lipid content of 3.0 to 9.5 g/100 g in oats of different genotypes [18]. Furthermore, the findings of this investigation are consistent with those of Saastamoinen et al. (1990), who documented that the oil content of oats ranged from 5.6 to 7.5 g/100 g, and that the oil content increased during the growing period at low temperatures [19].

An earlier study reported that the lipid composition of Korok was 2.85 g/100 g [12]. However, upon removal of the hull, the proportion recorded was 4.31 g/100 g. In contrast, the present study revealed that Korok fat content with the husk was 3.21 g/100 g, whereas this content increased to 4.27 g per 100 g after husk removal. These findings indicated a slightly lower fat content than that reported in a previous study, wherein Patrik had a fat content of 5.59 g/100 g, which aligns closely with the findings of the present study (5.27 g/100 g)[13]. The lipid content of Patrik has been reported to be 6 g/100 g [20], which slightly surpasses the measurement recorded in the current study. It

is noteworthy that in every variety, hulled oats had lower lipid content than naked oats. Except for the Santini variety, both the dehulled Atego and Korok varieties displayed lower lipid contents than the other naked varieties. Furthermore, the fat content of Oliver (6.02 g/100 g), Kamil (7.36 g/100 g), and Saul (5.41 g/100 g) reported by (Boeck et al. in 2018) were higher than the fat content values obtained in this study [21].

A study by Welch et al. (1983) found a higher lipid content (1–2.2 g/100 g) in oat husks than what we found in the current study. The findings of this study suggest that naked oats contain a higher amount of fat compared to other cereals, while the removal of husk increases fat content [9]. Several factors influence oat fat content, including season and postharvest treatments, as indicated by Kouřimská et al. (2018) [13]; moreover, genetics, growth, and storage conditions (Kouřimská et al. 2021) [12] play a significant role. Soil nutrition is an important factor that affects oat fat content (Saastamoinen 1998) [22]. Thus, the variability in oat lipid content among studies may be attributed to the diverse influences of these factors.

4.2 Comparison of fatty acid composition

This study aimed to investigate the predominant fatty acids present in oat samples and husks. Our findings showed that the total composition of the three major fatty acids, including linoleic, oleic, and palmitic acid, ranged from 91.2–96.6% in oats and was approximately 88% in husks. Specifically, the percentage of linoleic acid (C18:2) varied from 33.9–38.3%, oleic acid (C18:1) content from 30.1–39.3 %, and palmitic acid (C16:0) content from 19.4–25.2% in oat samples, except for the husk. These findings are consistent with previously reported results [13], which included palmitic acid at approximately 22%, oleic acid at 31%, and linoleic acid at 36%, which are similar to

241 the values obtained in our study. In contrast, the proportion of palmitic acid in the
242 present study was higher than that in previous results [23] of a study that investigated
243 seven naked oat cultivars and reported 15.34–17.41% of palmitic acid. Similarly,
244 another study [24], which evaluated five different naked oats, reported concentrations of
245 palmitic acid at 17%, oleic acid at 43%, and linoleic acid at 24.3%, which were not
246 consistent with our findings.

247 Martinez et al. (2010) [25] documented the fatty acid composition of 18 distinct
248 types of whole oat grains in a semi-arid environment, revealing an average composition
249 characterized by 23.2% palmitic acid, 2.3% stearic acid, 42.8% oleic acid and 24.9%
250 linoleic acid. In comparison, lower proportions of oleic acid and higher proportions of
251 linoleic acid were observed in the present study. Similarly, an analysis of the fatty acid
252 composition revealed that palmitic acid accounted for 14.4–17.4%, whereas oleic acid
253 constituted 37.2–40.5%, and linoleic acid contributed 38.6–42.5%; here, linoleic acid
254 comprised 1.6–2% of the total composition [22] . These findings are consistent with those
255 of the present study.

256 The ratio of polyunsaturated fatty acids to saturated fatty acids (PUFA/SFA) in
257 oats, including dehulled oats, varied from 1.3–1.7, which aligns with the earlier findings
258 (Pokhrel et al. 2022) [18]. However, this study's investigation of oats with hulls
259 demonstrated a PUFA/SFA ratio of less than 1. Compared to other cereals, naked oats
260 contain considerable amounts of unsaturated fatty acids [26].

261 Furthermore, the sum of unsaturated fatty acids in oats was higher than in other
262 cereals at 80.12%, except for rye which shared a similar proportion of 81.46% [27].
263 Significant increases in the synthesis of oleic, linoleic, and eicosenoic acids were
264 observed under low temperature conditions, whereas the synthesis of myristic, palmitic,

and palmitoleic acids significant decreases [19]. It is worth noting that compared to other cereals, oats have the highest ability to accumulate a significant proportion of oil in the endosperm, whereas maize accumulates oil mostly in the embryo. In addition, the fatty acid composition of oats is more likely to be influenced by environmental conditions than by the genotype [28]. Therefore, the environment in which oats are grown can significantly affect their fatty acid composition.

This study investigated the effects of husk removal on the fatty acid composition of two hulled oat varieties, Korok and Atego. According to Biel et al. (2014), the removal of husks significantly decreases the amounts of palmitic and stearic acids, while the amount of linoleic acid increased significantly [26]. Our study found that, in Korok, the removal of husks resulted in a decrease in oleic, palmitic, and stearic acids and an increase in linoleic acid. In the case of Atego, husk removal increased the relative percentages of linoleic and palmitic acids, while significantly decreasing oleic acid. According to Liu (2011), who compared the fatty acid compositions of hulled and dehulled oats, dehulled oats had almost double the oil content and a 4% increase in oleic acid (from 32.3 to 36.5%), whereas palmitic, stearic, linoleic, and linolenic acids slightly decreased [30].

Oat hulls, a by-product of grain milling, can be used as animal feed for pigs and poultry. They contain high levels of lignin (insoluble fibre). Pigs fed the diet containing oat hulls had a higher daily gross energy intake than pigs fed the other diets. Oat husk diets also resulted in an increased intake of fat and non-starch polysaccharides. Furthermore, oat hull diets can positively affect the gastrointestinal microbiota, increase short-chain fatty acid production, and improve intestinal barrier integrity. Short-chain fatty acids have beneficial effects on gut health by affecting diet, microbiota, the intestinal barrier, and immunity. For broiler chickens, including 3% oat hulls in their

diets can potentially improve growth performance and carcase weight. Finally, perhaps most importantly, the use of oat hulls in animal husbandry can help reduce production costs [31 32 33].

The analysis of fatty acid composition in the present study revealed that the percentages of saturated fatty acids (SFA), oleic acid (C18:1), and linoleic acid (C18:2) in Atego husks were 30.9%, 35.4%, and 29.6%, respectively. In Korok husk, the percentages were 33.2% for SFA, 34.7% for oleic acid, and 28.3% for linoleic respectively. These findings contrast with those previous study [10], which reported a range of 24–36.5% (mean 30.4%) for SFA, 25.7–36.3% (mean 29.4%) for oleic acid, and 28.8–35.8% (mean 31.7%) for linoleic acid in seven different Swedish oat husk samples. Interestingly, a previous study observed a higher content of linoleic acid than oleic acid, which is contrary to the findings of the current study. Further research is necessary to explore oat hulls as functional food ingredients and to investigate their effects on texture, stability, and sensory attributes in diverse food applications. Additionally, their utilisation as animal feed requires an examination of their impact on growth, nutrient utilisation, and overall health across different animal species.

5 Conclusion

The present study investigated the lipid and fatty acid compositions of various oat cultivars, including naked oats, hulled oats, and their husks before and after dehulling. Naked oats had higher lipid content than hulled oats, and the Kamil cultivar had the highest lipid content. The removal of husks affected the fatty acid composition of oats, particularly in dehulled Atego grains, which showed a significant increase in palmitic acid and a decrease in oleic acid, resulting in a higher proportion of PUFA and a lower

proportion of MUFA. These findings suggest that oat husks contain a substantial amount of palmitic acid and stearic acid, contributing to a higher SFA content than in oats before dehulling. In contrast, the sum of PUFA was significantly lower in husks than in hulled oats. Additionally, the lipid content of naked oats was significantly higher than that of hulled oats (Atego and Korok), with notable differences observed among the Patrik, Oliver, and Kamil varieties. Furthermore, after the removal of husks, dehulled oats exhibited an increase in lipid content, approaching a proportion comparable to that of naked oats, although they still showed significant differences compared with the Kamil variety. This study showed that oat husks contain fat with a different fatty acid composition when compared to hulled oats, and this aspect should be considered in their use as animal feed.

Conflict of Interest

Author Contributions: **Kshitiz Pokhrel** conceptualisation, formal analysis writing - original draft preparation, conducted the experiment, evaluated results, statistical evaluation; **Lenka Kouřimská** methodology, supervision, funding acquisition, validation, writing - revisions and editing; **Matěj Božik** chemical analysis on GC, writing - revisions and editing; **Novel Kishor Bhujel** writing some parts in literature review and editing. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: [to the proof-reading company will be added.] This work was supported by the METROFOOD-CZ Research Infrastructure Project (MEYS Grant No: LM2023064), which provided access to the research facilities.

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Table 1: Descriptions of analysed yellow oat samples

Oat cultivar	Origin	Registration year
Kamil (naked)	Izak x (10029Cn × KR 9478)	2012
Marco Polo (naked)	Tibor x Atego	2018
Oliver (naked)	(vL8250 x D16 / 84) x (Jumbo x KR 90-40)	2012
Patrik (naked)	Avenuda x (Azur x Master)	2015
Santini (naked)	Tibor x Atego	2018
Saul (naked)	(Dragon x S 16908) x KR 5278	2006
Atego (hulled)	Gramena x Auron	2002
Korok (hulled)	Atego x KR 93682	2011

437 Table 2: Post harvest characterization of oat samples (g/100 g)

Sample	Protein	Moisture
Kamil	18.10	9.71
Oliver	17.41	9.80
Marco Polo	18.36	9.80
Patrik	15.66	9.93
Santini	17.49	9.82
Saul	17.72	9.89
Atego	14.91	11.36
Korok	14.39	10.22

438 $n = 10$, RSD $\leq 1\%$.

439

Table 3: Lipid content in naked oats, hulled oats with and without husks, and husks from hulled oats.

Sample	Lipid content (g/100g)
Kamil	6.03 ± 0.37^{a}
Oliver	4.60 ± 0.23^{bc}
Marco Polo	4.29 ± 0.17^{bcde}
Patrik	5.27 ± 0.38^{ab}
Santini	3.73 ± 0.21^{cde}
Saul	4.48 ± 0.20^{bcd}
Atego before dehulling	3.20 ± 0.19^{e}
Korok before dehulling	3.21 ± 0.09^{de}
Atego after dehulling	4.13 ± 0.32^{bcde}
Korok after dehulling	4.27 ± 0.29^{bcde}
husks from Atego	0.63 ± 0.12^{f}
husks from Korok	0.68 ± 0.06^{f}

Values are expressed as mean ± standard deviations (SD), number of replication (n=3).

Mean values with different superscript letters ($^{a, b, c}$) were significantly different ($p > 0.05$).

Table 4: Composition of fatty acids in naked oats (in % of all fatty acids)

Fatty acid (%)	Patrik	Saul	Santini	Oliver	Kamil	Marco Polo
C14:0	0.39 ± 0.10^{cd}	0.63 ± 0.11^{c}	0.51 ± 0.17^{cd}	0.58 ± 0.22^{cd}	0.28 ± 0.10^{d}	0.47 ± 0.08^{dc}
C16:0	19.44 ± 0.98^{c}	21.79 ± 0.98^{ab}	21.01 ± 0.75^{ab}	20.56 ± 0.08^{bc}	20.09 ± 0.71^{bc}	19.65 ± 0.87^{bc}
C16:1 *cis*-9	0.44 ± 0.10^{b}	0.39 ± 0.06^{b}	0.39 ± 0.01^{b}	0.48 ± 0.12^{b}	0.36 ± 0.10^{b}	0.48 ± 0.09^{b}
C17:0	0.68 ± 0.18^{a}	ND	0.10 ± 0.01^{b}	0.15 ± 0.04^{b}	0.08 ± 0.03^{b}	ND
C17:1 *cis*-10	ND	0.03 ± 0.01^{b}	1.03 ± 0.27^{a}	0.09 ± 0.01^{b}	0.05 ± 0.01^{b}	ND
C18:0	0.92 ± 0.65^{d}	2.63 ± 0.46^{bcd}	1.88 ± 0.09^{cd}	1.99 ± 0.67^{cd}	4.62 ± 0.89^{a}	1.80 ± 0.20^{cd}
C18:1 *trans*-9	0.10 ± 0.07^{b}	ND	ND	ND	ND	ND
C18:1 *cis*-9	39.34 ± 0.64^{a}	36.03 ± 1.92^{abc}	36.16 ± 1.92^{abc}	37.89 ± 0.18^{ab}	37.99 ± 0.83^{ab}	37.21 ± 0.06^{abc}
C18:2 *cis*-9,12	36.54 ± 2.29^{ab}	36.34 ± 0.37^{ab}	36.67 ± 0.59^{ab}	35.49 ± 1.39^{ab}	33.08 ± 0.50^{bc}	38.02 ± 0.21^{ab}
C20:0	0.21 ± 0.04^{b}	0.25 ± 0.02^{b}	0.21 ± 0.03^{b}	0.22 ± 0.06^{b}	0.44 ± 0.08^{b}	0.19 ± 0.02^{b}
C18:3 *cis*-9,12,15	1.32 ± 0.17^{b}	1.16 ± 0.15^{b}	1.28 ± 0.15^{b}	1.42 ± 0.21^{b}	1.37 ± 0.13^{b}	0.17 ± 0.01^{c}
C20:2 *cis*-11,14	ND	0.14 ± 0.01	0.07 ± 0.01	0.29 ± 0.04	0.18 ± 0.06	ND
C22:0	0.11 ± 0.04^{c}	0.08 ± 0.05^{c}	0.09 ± 0.05^{c}	0.25 ± 0.09^{c}	0.16 ± 0.01^{c}	0.13 ± 0.02^{c}
C22:1 *cis*-13	0.23 ± 0.03	0.16 ± 0.04	0.18 ± 0.01	0.17 ± 0.01	0.19 ± 0.08	ND
C24:0	0.17 ± 0.09^{b}	0.22 ± 0.01^{b}	0.26 ± 0.07^{b}	0.20 ± 0.03^{b}	0.26 ± 0.02^{b}	0.17 ± 0.02^{b}
C24:1 *cis*-15	0.07 ± 0.05^{abc}	0.06 ± 0.01^{c}	0.11 ± 0.03^{abc}	0.12 ± 0.01^{abc}	0.06 ± 0.01^{c}	0.10 ± 0.01^{bc}
ΣSFA	21.92 ± 1.50^{d}	25.59 ± 1.53^{bcd}	24.06 ± 1.54^{cd}	23.95 ± 0.99^{cd}	25.92 ± 1.43^{bcd}	22.41 ± 1.21^{d}
ΣMUFA	41.49 ± 0.81^{a}	37.83 ± 2.03^{abc}	39.16 ± 2.32^{a}	40.16 ± 0.70^{ab}	40.02 ± 0.73^{ab}	37.95 ± 0.10^{abc}
ΣPUFA	36.54 ± 2.29^{ab}	36.48 ± 0.37^{ab}	36.74 ± 0.59^{ab}	35.78 ± 1.17^{ab}	33.26 ± 0.44^{bc}	38.23 ± 0.33^{ab}

n = 3, ND: not detected. Values are expressed as mean ± standard deviations (SD). Mean values with different superscript letters in the same row were significantly different ($p > 0.05$).

Table 5: Composition of fatty acids in hulled oats with and without husks, and husks from hulled oats (in % of all fatty acids)

Fatty acid (%)	Korok	Atego	Korok grain	Atego grain	Korok husks	Atego husks
C14:0	0.57 ± 0.08^{cd}	0.46 ± 0.05^{cd}	0.56 ± 0.02^{dc}	0.51 ± 0.1^{cd}	1.34 ± 0.09^{a}	1.00 ± 0.18^{b}
C16:0	21.34 ± 1.69^{ab}	21.58 ± 0.81^{ab}	20.87 ± 0.31^{bc}	26.17 ± 2.23^{a}	24.81 ± 0.18^{ab}	23.23 ± 1.64^{ab}
C16:1 *cis*-9	0.46 ± 0.22^{b}	0.28 ± 0.09^{b}	0.33 ± 0.00^{b}	0.34 ± 0.07^{b}	0.90 ± 0.02^{a}	0.49 ± 0.02^{b}
C17:0	0.15 ± 0.00^{b}	0.09 ± 0.00^{b}	0.99 ± 0.00^{a}	0.10 ± 0.02^{b}	0.50 ± 0.11^{b}	0.26 ± 0.00^{b}
C17:1 *cis*-10	0.31 ± 0.07^{b}	0.04 ± 0.00^{b}	ND	0.05 ± 0.00^{b}	ND	ND
C18:0	2.68 ± 0.09^{bcd}	2.46 ± 0.20^{bcd}	1.93 ± 0.05^{cd}	2.42 ± 0.55^{bcd}	3.52 ± 0.08^{ab}	2.99 ± 0.35^{bc}
C18:1 *trans*-9	1.41 ± 0.13^{ab}	0.87 ± 0.11^{ab}	ND	ND	ND	1.58 ± 0.29^{a}
C18:1 *cis*-9	34.85 ± 0.40^{bc}	35.99 ± 1.93^{bc}	33.73 ± 1.17^{cd}	30.11 ± 1.57^{d}	34.78 ± 1.10^{bc}	35.40 ± 2.19^{bc}
C18:2 *cis*-9,12	35.43 ± 1.38^{ab}	33.91 ± 0.40^{abc}	38.32 ± 0.72^{a}	35.35 ± 3.56^{ab}	28.35 ± 0.61^{c}	29.66 ± 3.11^{c}
C20:0	0.29 ± 0.07^{b}	0.42 ± 0.07^{b}	0.22 ± 0.01^{b}	0.28 ± 0.08^{b}	1.08 ± 0.09^{a}	1.28 ± 0.26^{a}
C18:3 *cis*-9,12,15	1.75 ± 0.00^{b}	2.22 ± 0.35^{a}	2.88 ± 0.05^{a}	3.57 ± 0.71^{a}	2.70 ± 0.00^{a}	1.54 ± 0.17^{b}
C20:2 *cis*-11,14	ND	ND	0.09 ± 0.00	0.08 ± 0.01	ND	ND
C22:0	0.25 ± 0.00^{c}	0.72 ± 0.32^{bc}	0.36 ± 0.03^{c}	0.33 ± 0.11^{c}	1.32 ± 0.05^{ab}	1.69 ± 0.27^{a}
C22:1 *cis*-13	0.21 ± 0.02	0.22 ± 0.09	0.19 ± 0.00	0.27 ± 0.05	ND	ND
C24:0	0.30 ± 0.02^{b}	0.23 ± 0.07^{b}	0.27 ± 0.01^{b}	0.31 ± 0.10^{b}	0.67 ± 0.05^{a}	0.53 ± 0.05^{a}
C24:1 *cis*-15	ND	0.18 ± 0.01^{a}	0.12 ± 0.00^{abc}	0.15 ± 0.04^{ab}	ND	ND
ΣSFA	25.58 ± 1.60^{bcd}	25.96 ± 1.43^{bcd}	24.31 ± 0.27^{bcd}	30.12 ± 3.16^{abc}	33.23 ± 0.80^{a}	30.97 ± 3.37^{ab}
ΣMUFA	38.99 ± 0.53^{ab}	39.79 ± 2.06^{ab}	37.25 ± 1.07^{bc}	22.03 ± 2.24^{c}	38.38 ± 1.07^{abc}	39.00 ± 1.40^{ab}
ΣPUFA	35.43 ± 1.38^{ab}	33.91 ± 0.40^{bc}	38.41 ± 0.72^{a}	47.83 ± 2.93^{ab}	28.35 ± 0.61^{c}	29.66 ± 2.11^{c}

n = 3, ND: not detected. Values are expressed as mean ± standard deviations (SD). Mean values with different superscript letters in the same row were significantly different ($p > 0.05$).

Journal of Cereal Science 99 (2021) 103216

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: http://www.elsevier.com/locate/jcs

Fat content and fatty acid profiles of recently registered varieties of naked and hulled oats with and without husks

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ARTICLE INFO

Keywords:
Avena sativa
Avena nuda
Fatty acid profile
Atherogenicity and thrombogenicity indices

ABSTRACT

The dietary fibre and lipid content of grains is variable. Hence, it is important to estimate the nutritional quality of grains destined for human and animal consumption. We investigated the lipid content and fatty acid composition of new hulled oat cultivars before and after dehulling, and naked varieties. The fat content varied from 2.6 g/100 g (yellow hulled; Kertag) to 5.2 g/100 g (naked; Kamil). The predominant fatty acids were linoleic (36.03–41.16%) and oleic (27.8–37.1%). The naked and dehulled cultivars contained more crude fat than the hulled cultivars with husks. The hull removal also changed the fatty acid profile of hulled oat samples. These dehulled samples also differed from naked oat varieties. The naked Kamil variety had the highest content of monounsaturated fatty acids (38.61 ± 0.36). The dehulled Cavaliere variety had the highest polyunsaturated fatty acids content (47.67 ± 0.62). All cultivars displayed very low atherogenicity (0.25–0.30) and thrombogenicity (0.43–0.55) indices. The results of this study underscore the dietetic importance of new oat cultivars for human and animal consumption.

1. Introduction

Oat (*Avena sativa* L.) has multiple uses as a raw material because of its valuable physiological and nutritional properties, which can substantially contribute to food security for humans and animals. Hence, consumer demand for oat production continues to increase. The global oat market was estimated at 4.90 billion USD in 2018 and is expected to growth by 5.5% within the period 2020–2025 (https://www.marketdataforecast.com/market-reports/oats-market) together with the oat consumption which is around 23 million tons per season. The most widely cultivated and consumed hulled oat varieties have yellow, white, and black hulls. However, there are also naked oat varieties *(Avena nuda* L.). Hulled oat has relatively lower nutritional and caloric value comparing to other cereals, and is often used as feedstuff for horses as it has a high fibre content. Moreover, dehulling increases the final cost of this material (Gorash et al., 2017). Therefore, development of new, highly productive naked oat cultivars could accommodate oat production and consumption demands.

Most cereal grains have a low lipid content. In contrast, oat may contain up to 18% lipid (Banas et al., 2007). Moreover, oat has a technologically and nutritionally desirable fatty acid composition (Zhou et al., 1999). It contains the unsaturated linoleic and oleic acids and the saturated palmitic acid. Together, these fatty acids constitute around 90–95% of the total oat lipid content (Ben Halima et al., 2015). Novel oat varieties provide relatively higher yield, are more resistant to lodging and oat rust, and have a higher lipid content. Therefore, accurate fat content and composition information is required to assess the potential of hulled oat varieties for feed and oilseeds and husked or naked varieties for breakfast cereals, bars, and other processed foods.

The dietary fibre and lipid content of oat varies widely among cultivars. Thus, it is necessary to estimate the nutritional quality of oat prior to its consumption by humans and animals. Recent studies focusing on the bioactive components of cereals (Loskutov et al., 2019) demonstrated the importance of these constituents in human nutrition (Smulders et al., 2018) and animal feeding (Krochmal-Marczak et al., 2020). The fat composition and antioxidant activity of cereals should

Abbreviations: DM, dry matter; HO, hulled oat before dehulling; HOB, hulled oat after dehulling; FA, fatty acids; IA, index of atherogenicity; IT, index of thrombogenicity; MUFA, monounsaturated fatty acids; NO, naked oat; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

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https://doi.org/10.1016/j.jcs.2021.103216
Received 6 January 2021; Received in revised form 26 March 2021; Accepted 30 March 2021
Available online 4 April 2021

also be evaluated (Polonskiy et al., 2020). Certain studies focused on hull fortification in animal feed (Ratanpaul et al., 2019; Adewole et al., 2020). Assessing the nutritional health indices; index of thrombogenicity (IT) and index of atherogenicity (IA) can provide valuable information indicating the risk of formation of atherosclerosis and developing a blood clot respectively (Ulbricht and Southgate, 1991).

Our previous study (Kourimska et al., 2018) focused on determining the fat content and fatty acid profile of hulled oat varieties with husks. In the present study, our aims were to evaluate the total lipid content, fatty acid profile and lipid health indices of globally produced and recently registered naked and hulled oats with husks. We also compared these characteristics in dehulled husked oats and naked oat varieties. Another objective was to fill in missing nutritional composition and lipid index data for new oat cultivars recently registered and harvested in the Czech Republic and other regions.

2. Material and methods

2.1. Oat samples

Oat samples were provided by the Selgen a.s. breeding station (Stupice, Czech Republic; GPS 50°313" N, 14°384" E, 287 m.a.s.l.). The studied oat varieties were registered between 2011 and 2014. Six spring varieties (which do not undergone vernalization unlike winter cereals), grown throughout Europe (mainly Poland, Spain, UK) and the other non-European countries (Russian Federation, Canada USA and South Africa) were selected: four (Cavaliere, Kertag, Selodon, and Gregor) of hulled (HO) and two (Kamil, and Otakar) of naked (NO) oat (Table 1). Oat seeds were sown in April at 3 cm depth, the seeding rate was 4.5 million seeds per hectare, and rapeseed was used as pre-crop. Nitrogen (60 kg N/ha) was applied as fertilizer, and the harvest took place in August.

2.2. Post-harvest sample specifications

The approximate composition of all post-harvest oat samples was determined with a grain analyser (FOSS Infratec 1241; FOSS Analytical A/S, Hillerød, Denmark; Table 2). Each sample was measured 10 × and the relative standard deviation (RSD) was <1%.

2.3. Sample preparation

Samples were milled for 3 min in a Scarlett Silver Line SL 1545 coffee grinder (Ariette-Scarlett, Firenze, Italy). For the hulled oat samples, the grains were manually separated to obtain dehulled samples. HOs with and without hulls (HOBs) and NOs were analysed.

Table 1
Description of analysed oat cultivars.

Oat cultivar	Origin	Registration (year)[a]
Cavaliere (hulled, black)	Auron × Ebene	2013
Gregor (hulled, yellow)	Vok × Azur	2014
Kertag (hulled, yellow)	[(Lo7573 × KR TFP) × Gramena] × Atego	2012
Seldon (hulled, yellow)	(Jumbo x Diplomat) × Leo	2014
Kamil (naked, yellow)	Izak x (10029Cn × KR 9478)	2012
Otakar (naked, yellow)	Izak x [(KR-9478 × Abel) × Abel]	2011

[a] National List & Plant Variety Rights Database, Central Institute for Supervising and Testing in Agriculture, Czech Republic (http://eagri.cz/public/web/en/ukzuz/portal/).

Table 2
Crude protein and moisture content (g/100 g sample) in oat cultivars.

Variety	Crude protein	Moisture
Cavaliere	13.6	18.8
Gregor	15.8	11.2
Kertag	14.2	11.8
Seldon	14.1	11.7
Kamil	15.6	10.8
Otakar	14.3	10.6

$n = 10$, RSD ≤1%.

2.4. Determination of dry matter and fat content

Dry matter (DM) was determined with an infrared (IR) moisture balance (Precisa HA 300; Precisa, Dietikon, Switzerland). One gram of homogenised sample was dried at 105 °C for 18–20 min to a constant weight (the weight loss was <2 mg/30 s). The fat content was determined by continuous Soxhlet extraction according to ISO 659:2009 (Kourimska et al., 2018). Each sample was analysed three times ($n = 3$).

2.5. Determination of fatty acid composition

Homogenised oat samples were extracted as described in Section 2.4 <Determination of dry matter and fat content>. To avoid oxidation, the final drying step was omitted, and the solvent was driven off in a rotary vacuum evaporator (Heidolph Hei-VAP Core HL G3; Heidolph Instruments GmbH, Schwabach, Germany). Approximately 0.5 g extracted fat was re-esterified for fatty acid profile analysis. Fatty acid methyl esters were prepared according to ISO 12966–2:2011 and measured in an Agilent 7890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to a mass spectrometer (Kourimska et al., 2018). The proportion of each fatty acid was calculated by the area normalisation method and the results were expressed as % of each fatty acid. Methylated fatty acids were identified with Restek Food Industry FAME mix (Cat. No. 35077; Restek, Bellefonte, PA, USA) and by comparing their GC-MS spectra against those in the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA) library. Fat was extracted three times from each sample and each analysis on GC was performed in duplicate.

2.6. Lipid health indices

Indices of atherogenicity (IA) and thrombogenicity (IT) were calculated according to Ulbricht and Southgate (1991) using the following equations:

$$IA = \frac{C12:0 + 4 \times C14:0 + C16:0}{\Sigma MUFA + \Sigma PUFA\ n-6 + \Sigma PUFA\ n-3} \quad (1)$$

$$IT = \frac{C14:0 + C16:0 + C18:0}{0.5\ \Sigma MUFA + 0.5\ \Sigma PUFA\ n-6 + 3\ \Sigma PUFA\ n-3 + \frac{\Sigma PUFA\ n-3}{\Sigma PUFA\ n-6}} \quad (2)$$

2.7. Statistical analysis

The statistical analysis comprised two-way analysis of variance (ANOVA), principal component analysis (PCA) and cluster analysis, and was performed in STATISTICA v. 12.0 (StatSoft, Inc., Tulsa, OK, USA). Sheffé's test was performed at the 5% significance level to identify significant differences among treatment means. The statistical analysis was performed on the major fatty acids: C16:0, C18:0, C18:1 *cis*-9, C18:2 *cis*-9,12, C18:3 *cis*-9,12,15, C20:0, C20:1 *cis*-11, C20:4 and C20:5 *cis*-5,8,11,14,17. These were fatty acids with a content of more than 1% at least once. Besides, there were low levels of the minor fatty acids, but the differences among them may have been inconclusive from the nutritional point of view.

3. Results

3.1. DM and fat content

The hull:grain weight ratios for the hulled varieties after their dehulling were as follows: Cavaliere and Kertag, 27:73; Seldon, 26:74; Gregor, 25:75. DM, total fat, and fat in DM in the various oat samples are listed in Table 3. The effects of cultivar and husk removal on DM content were investigated. No statistically significant differences were found among the hulled and naked cultivars in terms of DM content. However, in case of the effects of husk removal, the HOs had significantly higher DM (89.97 g/100 g) than the HOBs (88.73 g/100 g) ($P = 0.0106$). NO cultivars showed no significant difference in terms of DM content.

The highest fat content was measured for the NO cultivar Kamil. In contrast, the yellow hulled cultivar Kertag had the lowest fat content. The various cultivars and the hulled and dehulled samples differed in terms of fat content. The NO cultivars Kamil and Otakar had significantly higher fat content (5.12 g/100 g) than the HOs (3.02 g/100 g; $P < 0.0001$). In turn, the latter had significantly lower fat content than the HOBs (4.52 g/100 g; $P < 0.0001$). The HOB and NO varieties were similar but nonetheless significantly different in terms of fat content ($P = 0.0055$). Moreover, the HOs significantly differed from the NO cultivars in terms of fat content ($P = 0.0002$). The fat in DM content significantly increased in HO samples after their husk removal ($P = 0.0004$). Overall, the naked and dehulled oat cultivars contained more crude fat than the hulled varieties.

3.2. Fatty acid composition of hulled cultivars

The fatty acid compositions of the oat cultivars are listed in Table 4. Linoleic acid predominated in all cases (38.40%) followed by oleic acid (32.88%) and palmitic acid (19.80%). Other fatty acids representing >1% of the total included stearic, arachidic, gondoic, α-linolenic, arachidonic, and eicosapentaenoic acids. All other fatty acids had minor representation. Among the HO variety, Kertag had the lowest palmitic acid content and significantly differed from Cavaliere ($P = 0.0005$) and Seldon ($P = 0.0002$). Conversely, Kertag had the highest linoleic acid content and significantly differed from Cavaliere ($P = 0.0248$) and Seldon ($P = 0.0002$). There were no statistically significant differences among the HO oat varieties in terms of oleic acid content. The three predominant fatty acids comprised >90% of the total FA content.

3.3. Fatty acid composition of naked cultivars

The NO cultivar Kamil had the highest oleic acid content and it significantly differed from all HO cultivars ($P < 0.01$) but not from the NO cultivar Otakar. Moreover, Kamil had the lowest linoleic acid content and it significantly differed ($P < 0.05$) from all HO and NO cultivars except Seldon. Unlike the NO Kamil, the major fatty acid composition of the NO Otakar resembled those of most HO cultivars. Otakar had a significantly higher palmitic acid content than Gregor or Kertag ($P < 0.05$). The three predominant fatty acids were the same in the naked and hulled cultivars. However, the order of FA composition differed between these oat classes.

Table 3
Average dry matter, fat, and fat in DM content (g/100 g) in oat cultivars.

Sample	Dry matter (DM) (g/100 g)	Fat content (g/100 g)	Fat in DM content (g/100 g)
Cavaliere	90.40 ± 0.08^{a}	2.86 ± 0.08^{d}	3.16
Cavaliere B	88.10 ± 0.49^{bc}	4.27 ± 0.15^{bc}	4.85
Gregor	89.23 ± 0.24^{a}	3.49 ± 0.10^{cd}	3.91
Gregor B	90.46 ± 0.15^{ab}	4.79 ± 0.33^{ab}	5.29
Kertag	90.07 ± 0.03^{a}	2.59 ± 0.22^{d}	2.88
Kertag B	87.44 ± 0.78^{c}	4.48 ± 0.19^{ab}	5.13
Seldon	90.18 ± 0.13^{a}	3.16 ± 0.14^{d}	3.51
Seldon B	89.35 ± 0.25^{ab}	4.53 ± 0.15^{ab}	5.07
Kamil	89.73 ± 0.50^{ab}	5.22 ± 0.37^{a}	5.82
Otakar	89.78 ± 0.21^{ab}	5.02 ± 0.05^{ab}	5.59

B: dehulled cultivars. Values are expressed as mean ± standard deviations (SD). The number of independent replicate experiment n = 3. Different superscript letters indicate statistically significant differences between variables at $p < 0.05$ within the same column. Fat in DM was calculated from the average values of fat and DM.

3.4. Fatty acid composition of dehulled oat samples

The fatty acid compositions of the hulled oat samples after their dehulling (HOB) are also listed in Table 4. The HOB significantly differed in terms of their major fatty acid content. The oleic acid content (28.69%) was lower in the HOB than the original HO (32.88%) and NO (35.81%). The fatty acid compositions significantly differed before and after dehulling in Cavaliere ($P < 0.0001$), Kertag ($P = 0.0276$), and Seldon ($P < 0.0001$). The oleic acid content in the hulled and dehulled Gregor cultivar did not significantly differ. However, the dehulled Cavaliere B ($P = 0.0321$) and Seldon B ($P = 0.0138$) samples had significantly higher linoleic acid content than the original samples with husks. The hulled and dehulled Seldon and Cavaliere samples did not differ in terms of their C18:2 content. Nevertheless, Cavaliere B and Seldon B had significantly higher arachidonic acid content than the HO ($P < 0.05$) and NO ($P < 0.0001$) cultivars. Thus, husk removal influenced the proportions of the individual fatty acids in the oat grains.

3.5. Comparison of hulled, dehulled, and naked oat samples

There were significant differences among the HO, HOB, and NO samples in terms of their major fatty acid compositions ($P < 0.05$). However, the palmitic acid content did not significantly differ between HO and HOB (19.8%) and was relatively higher in NO (20.9%). The most abundant fatty acids were oleic (35.8% (NO) > 32.9% (HO) > 28.7% (HOB)) and linoleic (40.2% (HOB) > 38.4% (HO) > 37.1% (NO)).

Total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) content in all samples is listed in Table 5. The SFA content in the HO, HOB, and NO oats was in the range of 21–23.5%. Only the HO and NO cultivars significantly differed ($P = 0.0022$). The SFA proportions were similar for most samples (Kamil, Cavaliere, Cavaliere B, Otakar, Seldon, Seldon B) and in the range of 23–23.5%. Only Kertag had significantly less SFA (21%) than all other samples ($P < 0.05$) except Gregor, Gregor B, and Kertag B.

The MUFA content was the highest in Kamil and significantly differed ($P < 0.05$) from all other cultivars except Otakar ($P = 0.2689$). Cavaliere B and Seldon B had the lowest MUFA and significantly differed ($P < 0.0001$) from all other samples except Gregor B and Kertag B. Similar results were obtained for comparisons of individual sample groups. Significant differences ($P < 0.0001$) in MUFA content were observed among all groups. The NO cultivars had the highest MUFA (37.4%) followed by HO (34.7%) and HOB (29.9%).

For most samples including Gregor, Kertag, Cavaliere B, Gregor B, Kertage B, Seldon B, the total PUFA content was in the range of 44.3–47.6%. Kamil, Otakar, Cavaliere, and Seldon had lower PUFA content (37.9-41.8%), and the PUFA content in Kamil significantly differed from all other samples ($P < 0.05$) except Otakar ($P = 0.1952$). NO had significantly less PUFA (39.1%) than HO (43.1%) and HOB (47.2%; $P < 0.0001$). The hulled samples without husks had the lowest MUFA content and the highest PUFA content. In contrast, the NO had the lowest PUFA content and the highest MUFA content. The differences between HO, HOB and NO samples were nicely seen from the PCA and hierarchical clustering graphs (Figs. 1a and b and 2). Samples of hulled oats with husks (having more linolenic, gondoic and eicosapentaenoic acids) differ from these samples after their dehulling (determined by higher levels of linoleic, arachidic and arachidonic acids), and naked varieties were significantly apart from them (having more palmitic,

Table 4
Fatty acid composition of hulled, dehulled, and naked oat varieties (% of total identified).

Fatty acid	Hulled				Dehulled				Naked	
	Cavaliere	Gregor	Kertag	Seldon	Cavaliere B	Gregor B	Kertag B	Seldon B	Kamil	Otakar
C14:0	0.45 ± 0.07	0.36 ± 0.02	0.30 ± 0.01	0.40 ± 0.06	0.39 ± 0.03	0.37 ± 0.01	0.32 ± 0.02	0.39 ± 0.02	0.32 ± 0.01	0.35 ± 0.02
C16:0	20.95 ± 0.56	18.79 ± 0.98	18.67 ± 0.30	20.76 ± 0.37	20.00 ± 0.89	19.30 ± 0.82	19.42 ± 0.21	20.38 ± 0.9	20.59 ± 0.33	21.14 ± 0.62
C16:1 *cis*-9	0.32 ± 0.04	0.22 ± 0.02	0.30 ± 0.01	0.29 ± 0.02	0.28 ± 0.04	0.23 ± 0.02	0.31 ± 0.02	0.24 ± 0.02	0.23 ± 0.00	0.23 ± 0.01
C17:0	0.05 ± 0.02	0.06 ± 0.02	0.06 ± 0.03	0.07 ± 0.01	0.04 ± 0.05	0.04 ± 0.03	0.04 ± 0.02	0.03 ± 0.03	0.07 ± 0.00	0.07 ± 0.01
C17:1 *cis*-10	0.05 ± 0.01	0.05 ± 0.03	0.02 ± 0.01	0.04 ± 0.04	ND	0.02 ± 0.01	0.01 ± 0.02	ND	0.03 ± 0.01	0.05 ± 0.01
C18:0	1.91 ± 0.33	1.47 ± 0.07	1.21 ± 0.09	1.53 ± 0.16	1.32 ± 0.08	1.31 ± 0.13	1.04 ± 0.03	1.32 ± 0.08	2.46 ± 0.10	1.86 ± 0.05
C18:1 *cis*-9	33.52 ± 1.68	32.62 ± 1.02	32.13 ± 0.13	33.26 ± 0.97	27.81 ± 0.64	29.91 ± 1.77	29.2 ± 0.96	27.85 ± 0.88	37.06 ± 0.11	34.56 ± 0.36
C18:1 *trans*-9	0.04 ± 0.04	0.14 ± 0.05	0.10 ± 0.05	0.10 ± 0.07	ND	ND	ND	ND	0.01 ± 0.02	ND
C18:2 *cis*-9,12	38.06 ± 1.06	38.77 ± 0.28	39.40 ± 0.19	37.36 ± 0.66	40.18 ± 0.91	39.92 ± 0.89	41.16 ± 0.69	39.64 ± 1.17	36.03 ± 0.24	38.19 ± 0.55
C20:0	0.07 ± 0.04	0.55 ± 0.24	0.67 ± 0.03	0.28 ± 0.19	1.31 ± 0.26	0.94 ± 0.51	1.16 ± 0.2	1.11 ± 0.45	0.08 ± 0.09	ND
C18:3 *cis*-6,9,12	0.06 ± 0.02	0.03 ± 0.02	0.07 ± 0.01	0.05 ± 0.01	0.07 ± 0.03	0.02 ± 0.03	0.03 ± 0.03	0.06 ± 0.05	0.02 ± 0.00	0.03 ± 0.01
C20:1 *cis*-11	1.32 ± 0.17	1.00 ± 0.21	1.14 ± 0.06	1.22 ± 0.28	0.86 ± 0.13	0.90 ± 0.16	0.83 ± 0.12	0.83 ± 0.18	1.04 ± 0.22	0.93 ± 0.16
C18:3 *cis*-9,12,15	1.97 ± 0.25	1.66 ± 0.09	1.83 ± 0.09	2.02 ± 0.26	1.54 ± 0.04	1.53 ± 0.15	1.68 ± 0.07	1.61 ± 0.13	1.35 ± 0.05	1.38 ± 0.05
C20:2 *cis*-11,14	0.09 ± 0.02	0.03 ± 0.03	0.05 ± 0.02	0.06 ± 0.03	0.02 ± 0.03	0.01 ± 0.02	ND	ND	0.06 ± 0.02	0.21 ± 0.27
C20:3 *cis*-8,11,14	0.21 ± 0.13	0.42 ± 0.36	0.63 ± 0.11	0.47 ± 0.19	0.26 ± 0.10	0.50 ± 0.35	0.28 ± 0.12	0.58 ± 0.15	0.05 ± 0.03	0.23 ± 0.17
C20:4 *cis*-5,8,11,14	0.29 ± 0.16	2.09 ± 0.83	2.04 ± 0.09	1.14 ± 0.85	4.68 ± 1.14	3.51 ± 1.70	3.10 ± 0.44	4.60 ± 1.79	0.09 ± 0.05	ND
C24:0	<0.01	0.21 ± 0.11	0.07 ± 0.04	0.04 ± 0.05	0.20 ± 0.10	0.38 ± 0.44	0.22 ± 0.16	0.31 ± 0.19	ND	ND
C20:5 *cis*-5,8,11,14,17	0.39 ± 0.08	0.97 ± 0.39	1.10 ± 0.29	0.48 ± 0.29	0.49 ± 0.28	0.67 ± 0.54	0.84 ± 0.28	0.71 ± 0.48	0.28 ± 0.03	0.30 ± 0.19
C24:1 *cis*-15	0.23 ± 0.06	0.21 ± 0.12	0.22 ± 0.06	0.24 ± 0.23	0.13 ± 0.11	0.13 ± 0.06	0.08 ± 0.05	0.06 ± 0.07	0.24 ± 0.04	0.47 ± 0.21
C22:6 *cis*-4,7,10,13,16,19	ND	0.36 ± 0.13	ND	0.21 ± 0.15	0.43 ± 0.10	0.31 ± 0.16	0.28 ± 0.05	0.26 ± 0.10	ND	ND

ND: not detected. Values are expressed as mean ± standard deviations (SD). The number of independent replicate experiment = 3, technical repeats of the same sample = 2; (n = 6). Significant differences were calculated for major fatty acids which were at least once more than 1%.

Table 5
Fatty acid contents (% of total identified) and atherogenicity and thrombogenicity indices of oat cultivars.

Fatty acid	Hulled				Dehulled				Naked	
	Cavaliere	Gregor	Kertag	Seldon	Cavaliere B	Gregor B	Kertag B	Seldon B	Kamil	Otakar
∑SFA	23.45 ± 0.40^{a}	21.44 ± 0.82^{b}	20.97 ± 0.27^{b}	23.08 ± 0.36^{a}	23.25 ± 0.69^{a}	22.34 ± 0.73^{ab}	22.20 ± 0.25^{ab}	23.54 ± 0.77^{a}	23.51 ± 0.36^{a}	23.42 ± 0.71^{a}
∑MUFA	35.48 ± 1.91^{b}	34.23 ± 1.11^{b}	33.91 ± 0.11^{bc}	35.15 ± 1.54^{b}	29.08 ± 0.62^{d}	31.18 ± 1.97^{cd}	30.43 ± 1.19^{d}	28.99 ± 1.14^{d}	38.61 ± 0.40^{a}	36.23 ± 0.45^{ab}
∑PUFA	41.07 ± 1.67^{d}	44.33 ± 1.54^{bc}	45.12 ± 0.31^{ab}	41.78 ± 1.75^{cd}	47.67 ± 0.68^{a}	46.48 ± 1.58^{ab}	47.37 ± 1.01^{a}	47.48 ± 1.16^{a}	37.88 ± 0.25^{e}	40.34 ± 0.56^{de}
∑n-3	2.36 ± 0.32^{ab}	2.99 ± 0.61^{a}	2.93 ± 0.35^{a}	2.71 ± 0.28^{a}	2.46 ± 0.36^{ab}	2.51 ± 0.55^{ab}	2.81 ± 0.37^{a}	2.59 ± 0.50^{ab}	1.63 ± 0.08^{b}	1.69 ± 0.21^{b}
∑n-6	38.70 ± 1.40^{ef}	41.33 ± 1.08^{cd}	42.18 ± 0.17^{bc}	39.07 ± 1.64^{de}	45.21 ± 0.52^{a}	43.97 ± 1.33^{ab}	44.57 ± 1.15^{ab}	44.89 ± 0.77^{a}	36.25 ± 0.31^{f}	38.65 ± 0.38^{ef}
n-3/n-6	0.061 ± 0.01^{ab}	0.072 ± 0.01^{a}	0.070 ± 0.01^{a}	0.069 ± 0.01^{a}	0.054 ± 0.01^{ab}	0.057 ± 0.01^{ab}	0.063 ± 0.01^{ab}	0.057 ± 0.01^{ab}	0.045 ± 0.00^{b}	0.044 ± 0.00^{b}
IA	0.30 ± 0.01^{a}	0.26 ± 0.02^{cd}	0.25 ± 0.01^{d}	0.29 ± 0.01^{ab}	0.28 ± 0.02^{abcd}	0.27 ± 0.01^{abcd}	0.27 ± 0.01^{bcd}	0.29 ± 0.02^{abc}	0.29 ± 0.01^{abc}	0.29 ± 0.01^{ab}
IT	0.53 ± 0.01^{ab}	0.44 ± 0.04^{cd}	0.43 ± 0.01^{d}	0.50 ± 0.02^{abc}	0.49 ± 0.03^{bcd}	0.47 ± 0.03^{cd}	0.45 ± 0.01^{cd}	0.49 ± 0.04^{abc}	0.55 ± 0.01^{a}	0.55 ± 0.03^{a}

IA: index of atherogenicity; IT: index of thrombogenicity; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids. Different superscript letters indicate statistically significant differences between variables at $p < 0.05$ within the same row.

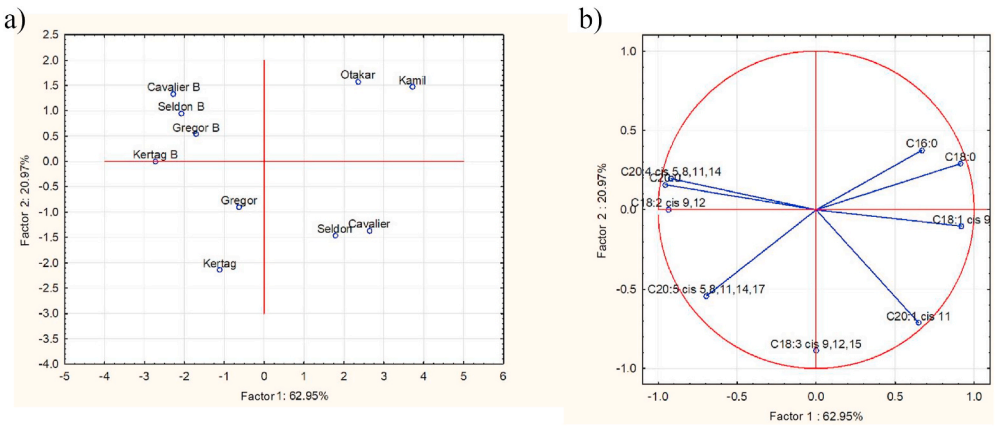

Fig. 1. a) PCA plot of individual data calculated from fatty acid profiles. b) PCA plot of variables calculated from fatty acid profiles.

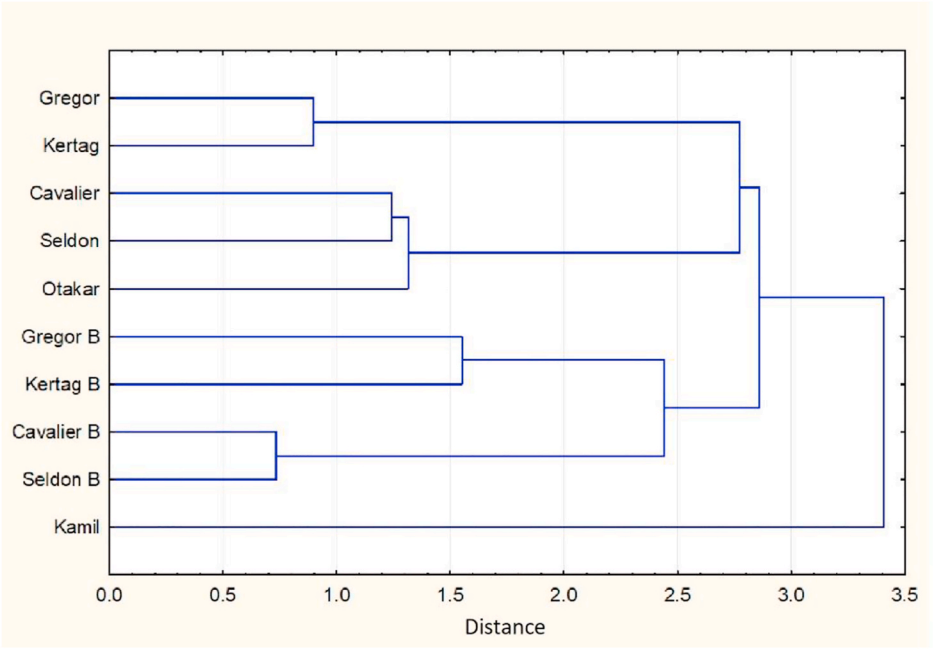

Fig. 2. Single linkage cluster dendrogram for tested oat samples.

stearic, and oleic acids). Thus, it demonstrates that naked oat varieties did not have the same fatty acid profiles as hulled oat samples after their dehulling.

Most of the PUFAs in the oats were n-6. However, n-3 fatty acids were also detected (Table 5) albeit at levels < 3% in all samples. Kamil and Otakar displayed significantly lower n-3 fatty acid content than the other NO and HO cultivars ($P < 0.0001$). Dehulling increased the n-6 content in the hulled oat samples. Hence, HOB had the highest n-6 PUFA content (44.7%; $P < 0.001$), followed by HO (40.3%; $P < 0.001$) and NO (37.4%; $P < 0.001$). Kamil had significantly ($P < 0.05$) lower n-6 content than all other samples except Cavaliere and Otakar. The n-3:n-6 fatty acid ratios were low in all samples. Nevertheless, significant differences ($P < 0.01$) were observed among oat types, namely, HO (0.068) > HOB (0.058) > NO (0.044).

3.6. Lipid health indices

For all cultivars, the calculated IA and IT were mainly within the same range (Table 5). Naked oats Kamil and Otakar had the highest IT, hulled Cavaliere and Seldon as well as naked Kamil and Otakar had the highest IA. The effect of oat breed (hulled x naked oat) was significant in case of IT ($P < 0.01$), but not in IA. Considering all hulled oats altogether, the effect of dehulling on both the IA and IT indices were insignificant, though there were differences between some individual samples of varieties (Table 5).

Overall, NO had relatively more MUFA and less PUFA than HOB. Therefore, the loose of hulls in the naked varieties that are easily removed by threshing in addition to the differences between varieties might affect the proportions of unsaturated fatty acids. But oat variety and breeding seem to have the strongest influence on these indices.

4. Discussion

The present study provided useful information about lipid composition and content in hulled oat varieties before and after dehulling and naked varieties cultivated under various climatic conditions. NO cultivars contain substantially higher fat content (~7.9% more) than other feed cereals such as barley, wheat, or maize. Oat is a rich source of unsaturated fatty acids (Zhou et al., 1998). Biel et al. (2009) reported that the crude fat content in NO is nearly double that in HO. In our study, the highest fat content was observed in the NO varieties Otakar (5.22%) and Kamil (5.02%). However, these values were about half those

reported by Sterna et al. (2014) who indicated that HO varieties contain significantly less fat (4–5.67%) than NOs (8.9–10.7%). In our study, fat content in the oat varieties were consistent with those previously reported by Leonova et al. (2008) (4.1–8.3%), Boeck et al. (2018) (5.3–7.4%), and Kourimska et al. (2018) (2.9–6.1%). Biel et al. (2009) stated that oat grain DM contains 8.4% fat which was slightly higher than the levels measured in the present study (2.88–5.82%). Naked Kamil (5.82%) and Otakar (5.59%) displayed the highest fat in DM content. Boeck et al. (2018) reported an oat DM content in the range of 91.5–96.5% which was higher than the 87.4–90.5% range determined here.

Husks contain very little fat. Moreover, they have low digestibility and can, therefore, influence the nutritional quality of the HO varieties used in animal feed (Peltonen-Sainio et al., 2004). HOB and NO cultivars are considered more valuable as nutritional components in domestic animal feed than their HO counterparts because the former contain relatively less fibre and more PUFA and have superior digestibility. Although dehulling is expensive, NO varieties are preferred for food processing while HO cultivars are used in malting and other industrial applications (Antonini et al., 2016). In the present study, the lipid content in the HO cultivars before dehulling were in the range of 2.59–3.49. In contrast, the lipid content in the NO or HOB cultivars were in the range of 4.27–5.22. HO varieties with husks had higher DM content than they did after they were dehulled. Our results suggest that the NO cultivars had a higher fat content than the HO cultivars and that hull removal significantly increased the fat content and altered the fatty acid composition of the grains.

Genetics, growth conditions, season, post-harvest treatment, and storage affect grain fat content (Ahmet et al., 2019). Despite their high PUFA content, oat lipids are relatively stable to oxidation as they also contain high levels of antioxidants such as vitamin E and polyphenols (Peterson, 2001), Thus, dehulled grains may be stored for long periods without becoming rancid (Peltonen-Sainio et al., 2004). A comparative study on various cereals (oat, barley, wheat, and maize) reported comparatively higher palmitic and oleic acid content in oat (Batalova et al., 2019). The linoleic, oleic, and palmitic acid content in seven NO varieties were in the ranges of 36.2–38.7%, 33.5–36.7%, and 15.3–17.8%, respectively. In this study, we found that in all cultivars tested, linoleic acid predominated followed by oleic and palmitic acids. Furthermore, the NO varieties exhibited higher palmitic and oleic acid content than the HO cultivars while the latter contain relatively more linoleic acid. Hence, hull removal significantly altered the major unsaturated fatty acid content in oat.

The fatty acid content in foods has a substantial impact on human health. Palmitic acid (16.87%) and myristic acid (0.31%) in oat negatively affect blood cholesterol levels. In contrast, linoleic and linolenic acids are essential for mammalian nutrition. Diets deficient in these fatty acids can increase the risk of atherosclerosis (Krasilnikov et al., 2018). Here, high linoleic acid contents were detected in the oat samples. This finding was consistent with the report of Tong et al. (2014) who stated that oat oil is nutritionally beneficial as it lowers blood lipid and hepatic and plasma cholesterol levels. In the present study, the HO and NO cultivars differed in terms of total SFA content while the MUFA content differed among all sample groups. The HO samples without husks had the lowest MUFA and the highest PUFA whereas the NO samples had the lowest PUFA and the highest MUFA. Most of the PUFAs were n-6 in the oat samples but they also contained n-3 fatty acids at levels $\leq$3%. The NO varieties had lower n-3 content than the HO varieties. Moreover, dehulling increased the n-6 content in the HO cultivars.

The fatty acid ratio in foods markedly influences human health. A low n-6:n-3 PUFA ratio is preferable (EFSA, 2010). Elevated n-6 consumption accompanied by low n-3 intake is associated with increased risks of obesity, vascular diseases, arthritis, and autoimmune and inflammatory disease (Simopoulos, 2008). In general, cereals are rich in n-6 but poor in n-3 and their n-6/n-3 PUFA ratios are high (Khalili Tilami and Samples, 2018). The World Health Organization recommends a PUFA:SFA ratio >0.4 for optimal health (FAO, 2008). Therefore, the PUFA:SFA ratios of 1.7–2.1 calculated for the oat cultivars in the present study suggest that oat may be beneficial to human health. The observed elevated stearic/palmitic acid (C18/C16) content may help reduce the incidence of coronary heart disease and other conditions (Sterna et al., 2014). Here, we found that the NO Kamil had the highest C18/C16 values (0.05–0.12). Thus, it might be one of the more healthful oat varieties.

A high IA value is correlated with an elevated risk of atherosclerosis whereas a high IT value is associated with increased risks and incidences of blood clot and cardiovascular disease (Ulbricht and Southgate, 1991; Garaffo et al., 2011). Consistent with the findings of Kourimska et al. (2018), the calculated IA and IT values were low and confirmed the findings of Pritchard et al. (2000), who reported that oat oil indices are very close to those of rice oil. Hence, the oat varieties tested in this study are associated with a low incidence of coronary heart disease. The IA and IT values in certain cultivars were very close to those reported for an Inuit diet (IA = 0.39 and IT = 0.28) which is rich in fish and marine mammals (Ulbricht and Southgate, 1991). The low IA and IT values measured for all cultivars in this study confirmed that the regular consumption of processed oat grains could reduce the risk of developing cardiovascular diseases.

In conclusion, the present study revealed that the NO and HOB oat samples contained more crude fat than the HO cultivars with husks. Furthermore, the NO Kamil variety had the highest MUFA while the HOB Cavaliere variety without husks had the highest PUFA. Linoleic and oleic acid were the predominant fatty acids in all cultivars and hull removal significantly altered their content in dehulled samples as well as proportion of other saturated and unsaturated fatty acids. Both hulled and dehulled samples also differed from naked oat varieties All oat cultivars had very low atherogenicity and thrombogenicity indices. Therefore, their regular consumption could help lowering the risk of developing cardiovascular diseases. The different proportions of unsaturated fatty acids in naked varieties comparing to HO and HOB samples was reflected in their higher IT.

Funding

This work was supported by the METROFOOD-CZ Research Infrastructure Project [Ministry of Education, Youth and Sports (MEYS), Grant No: LM2018100], including access to its facilities and from the European Regional Development Fund - Project No. CZ.02.1.01/0.0/0.0/16_019/0000845.

Author contributions

LK designed and managed the whole experiment, evaluated the results of analyses, wrote some chapters of the manuscript, and participated in its modifications and revisions. KP wrote the initial draft and did the literature review. MB carried out the instrumental analysis, wrote the corresponding analytical part of the text and revised the manuscript. SKT wrote some chapters, revised the text, and made the final corrections. PH took part in the experimental design, provided samples and information about their origin. All authors approved the submitted version.

CRediT authorship contribution statement

Lenka Kouřimská: Supervision, Methodology, Funding acquisition, Validation, Formal analysis, Writing – review & editing. **Kshitiz Pokhrel:** Writing – original draft, Investigation. **Matěj Božik:** Investigation, Visualization. **Sarvenaz Khalili Tilami:** Writing – original draft, Writing – review & editing. **Pavel Horčička:** Conceptualization, Methodology.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

The authors thank Miss Jana Floriánová for analysing the samples, Mrs. Blanka Dvořáková for technical support in the chemical analyses, and Editage for their manuscript proofreading and editing service.

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