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Cold-pressing of oil from nine varieties of technical hemp at various conditions

MASTER'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled *"Cold-pressing of oil from nine varieties of technical hemp at various conditions"* independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague 25th April 2019

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Aleš Bartoníček

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Abstract

This study analysed oil-related qualities of seeds of nine technical hemp varieties and examined the effect of different conditions during pressing on the oil yield and amount of residual oil in hemp cake. Cold-pressing was used because of the high polyunsaturated fatty acids content in the *Cannabis sativa* L. seeds.

Seeds of varieties Białobrzeskie, Fedora, Santhica, Fibrol, Finola, Futura, Tiborszállási, Kompolti hibrid and KC Virtus were pressed at different conditions (100, 200, 300 kN and 20 °, 40 °, 50 °C) to determine oil yield. Hemp cakes were tested for residual oil by solvent extraction by petroleum ether. Oil-free hemp cakes were analysed for protein content by Kjeldahl method. Fatty acid profile of extracted hemp cake residual oil was examined by means of GC-MS and GC-FID.

Higher force and higher temperature during pressing resulted in higher oil yield and simultaneously in lower residual oil in hemp cake. There were noticeable differences in oil content among varieties. Total oil content was highest in Fibrol variety (36.17 ± 0.53 %) and lowest in Santhica variety (32.29 ± 0.30 %). Varieties responded to pressing differently. Białobrzeskie and Fedora were established as the most suitable for pressing in terms of oil recovery. The protein content of dry oil-free seeds was highest in Santhica, Fibrol, Finola, Futura (39.08 ± 0.82 %; 38.91 ± 0.50 %; 38.84 ± 0.95 %; 38.65 ± 1.34 %, respectively) and lowest in Kompolti hibrid (34.88 ± 1.07 %). The highest amount of PUFAs was found in variety Finola (76.72 %). Kompolti hibrid had the highest content of essential FAs (73.99 %) and the lowest ω6/ω3 EFAs ratio (3.06) and can be considered as the best hemp variety for human nutrition.

Key words: *Cannabis sativa*, technical hemp, hemp seed, hemp cake, fatty acid

Abstrakt

Tato studie zkoumá vlastnosti semen technického konopí. Práce se zabývá výtěžností oleje ze semen devíti odrůd technického konopí; zbytkovým olejem a obsahem bílkovin v pokrutinách; profilem mastných kyselin. Kvůli vysokému obsahu polynenasycených mastných kyselin v semenech rostliny *Cannabis sativa* L. byl olej lisován za studena.

Semena odrůd Białobrzeskie, Fedora, Santhica, Fibrol, Finola, Futura, Tiborszállási, Kompolti hibrid a KC Virtus byla lisována za různých podmínek (100, 200, 300 kN a 20°, 40°, 50°C). Cílem bylo stanovení výnosu oleje. Obsah zbytkového oleje v pokrutinách (konopném „koláči“) byl zjištěn extrakcí petroletherem. Pokrutiny zbavené oleje byly podrobeny analýze obsahu bílkovin pomocí Kjeldahlovy metody. Profil mastných kyselin oleje extrahovaného z pokrutin byl určován plynovou chromatografií s hmotnostní spektrometrií (GC-MS) a plynovou chromatografií s plamenově ionizačním detektorem (GC-FID).

Vyšší síla a vyšší teplota při lisování vyústily ve vyšší výnos lisovaného oleje a zároveň v nižší obsah zbytkového oleje v pokrutinách. Při lisování byly zaznamenány znatelné rozdíly mezi výnosy oleje napříč odrůdami. Nejvyšší celkový obsah oleje byl zjištěn u odrůdy Fibrol (36,17 ± 0,53%) a nejnižší u odrůdy Santhica (32,29 ± 0,30%). Experimenty odhalily, že vyšší celkový obsah oleje není zárukou vyššího výnosu při lisování oleje. Odrůdy Białobrzeskie a Fedora se jeví jako nejvhodnější k lisování. Obsah bílkovin v sušině zbavené oleje byl nejvyšší u Santhicy, Fibrolu, Finoly a Futury (39,08 ± 0,82%; 38,91 ± 0,50%; 38,84 ± 0,95%; 38,65 ± 1,34%, ve stejném pořadí) a nejnižší v odrůdě Kompolti hibrid (34,88 ± 1,07%). Kompolti hibrid byl zároveň stanoven jako odrůda s nejvyšším zastoupením esenciálních mastných kyselin (73,99%) a nejnižším poměrem omega-6 a omega-3 mastných kyselin (3,06); a může být tedy považován za odrůdu nejvíce vyhovující účelu lidské výživy. Nejvyšší obsah polynenasycených mastných kyselin byl naměřen v odrůdě Finola (76,72%).

Práce byla napsána v anglickém jazyce.

Klíčová slova: *Cannabis sativa*, technické konopí, konopné semeno, pokrutiny, mastné kyseliny

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List of the abbreviations used in the thesis

AD	anno Domini
ANOVA	analysis of variance
BC	before Christ
CBD	cannabidiol
CBDA	cannabidiolic acid
CBGA	cannabigerolic acid
CH_3	methyl group
CO_2	carbon dioxide
COOH	carboxyl group
CS	*Cannabis sativa*
EFA	essential fatty acid
EU	European Union
FA	fatty acid
Fr.	Fries (Elias Magnus), a botanist
GC	gas chromatography
GC-FID	gas chromatography – flame ionization detector
GC-MS	gas chromatography – mass spectrometry
L.	Linnaeus (Carolus), a botanist
Lam.	Lamarck (Jean-Baptiste), a botanist
LC-PUFA	long chain polyunsaturated fatty acid
LDL	low-density lipoprotein
Lib.	Libert (Marie-Anne), a botanist
PUFA	polyunsaturated fatty acid
Sacc.	Saccardo (Pier Andrea), a botanist
SC-PUFA	short chain polyunsaturated fatty acid
ssp.	subspecies
THC	tetrahydrocannabinol
THCA	tetrahydrocannabinolic acid
Tukey’s HSD	Tukey’s honestly significant difference test

Motto:

“And therefore it cannot worth reading be,

Being compil'd by such an one as he.”

The Praise of Hemp-Seed by John Taylor, 1620

1. Introduction and Literature Review

Cannabis sativa L. (also called hemp) is a versatile plant and had been used by mankind for at least thousands of years for its fibre, oil, psychoactive and medicinal effects. For many reasons, the 20th century AD was not very lenient to cannabis, and the hemp was made illegal, especially in the Western world. This gave rise to a new kind of cannabis, so-called "technical hemp" with a limited tetrahydrocannabinol (THC) content (Miovský 2008). Seeds of some of these technical hemp varieties were subjected to experiments presented in this thesis.

1.1. *Cannabis sativa* L.

1.1.1. History

The oldest evidence of using cannabis by mankind, as suggested by Miovský (2008), is indirect. Effect of psychoactive compounds on Paleolithic and Neolithic art is suspected, and is highly probable that hemp was one of them. The archeological research on site of circa 10 thousand years old stone-age village in Taiwan discovered clay shards with imprinted coarse threads. It is suggested that this is the oldest evidence of hemp use, because of an assumption that no other material than silk and hemp was used at the time in this locality. A Chinese 1972 discovery of residues of hemp dress from the period of Zhou dynasty (1122-249 BC) is the oldest direct evidence of hemp use.

The cannabis plant is connected with many Asian myths and tales, the best known is the story of Siddhartha Gautama Buddha, who, according to legend, was surviving eating only one hemp seed per day for six years (Citti C et al. 2017).

Herodotus from Halicarnassus, who lived in the 5th century BC, is giving a testimony about hemp clothes from Thracia, which closely resemble linen. He also describes a burial ritual, in which Scythians make a booth from woollen felts, put inside a dish with hot stones and *"take some of this hemp-seed, and, creeping under the felt coverings, throw it upon the red-hot stones; immediately it smokes, and gives out such a vapour as no Grecian vapour-bath can exceed; the Scyths, delighted, shout for joy, and this vapour serves them instead of a water-bath"* (Herodotus 1910).

During the Middle Ages, cannabis was widely used in Europe, mainly for threads and clothing. The Chinese knowledge of making hemp paper was brought to Italy in the 12th century. Cannabis, probably because of an Islamic ban on alcohol, became a common intoxicant in Arabic culture in the form of hashish (Miovský 2008).

With the development of transport, the possibility to import more potent cannabis from southern countries to Europe occurred, and European medicine rediscovered its effects (Conrad 2007). Medicinal usage peaked in the 19th century and declined at the beginning of 20th century. Two factors should be noted:

- During the 19th century products made of cannabis were almost only effective tools for pain-management. However, due to lipid-binding nature of cannabis medicinal compounds and a lack of an analytical method to measure its potency, the medicinal practice later favoured water-soluble opioids, for their more precise dosing and a possibility to introduce them intravenously by syringe (Conrad 2007).
- The second factor was the development of synthetic fabrics made of crude oil[1] and usage of cheaper cotton. Hemp therefore became neglected (Conrad 2007, Miovský 2008).

In 1961 United Nations adopted a pact, which categorized marihuana as one of the most dangerous drugs (International Narcotics Control Board 2018).

1.1.1.1. Czechoslovakia and Czechia

Evidence for growing cannabis in the area of today's Czech Republic in the times bygone, author remarks, can be found in many folk songs. As an example: *"Aničkа konopí močila, žabka ji do vody skočila"*[2] or *"Za ten len, za ten len, za ty konopičky, aby nám vyrostly, nebyly maličký"*[3].

[1] See also a famous Marihuana Tax Law from 1937

[2] Translation: *"The girl Anička was wetting hemp and the frog jumped into the water"*

[3] Translation: *"For the flax, for the flax, for the hemp plants, shall they grow and not be small"*

There are also historical accounts of growing cannabis in the Middle Ages – the name of castle Konopiště, first mentioned in the year 1318; or written record from the year 1495: *"...mají konopí poskonných i hlavatých 15 záhonův vytrhati..."* (OJ 1929)[4].

Original Czechoslovak variety called Rastislavické (konopí) was cultivated in south Slovakia from 1958 till 1988 (Miovský 2008). Another variety grown in the 1980s in Czechoslovakia was Uniko B (Moudrý 2011). These varieties cannot be grown today under EU legislation, as their tetrahydrocannabinol levels are higher than allowed.

1.1.2. Taxonomy of *Cannabis*

Cannabis is both plain and cultivated, widely distributed plant, very likely originating in Central Asia (Moudrý 2011). From this region, this adaptable plant was brought to other parts of the world by humans. Cannabis use is tightly bounded with the developement of mankind (Miovský 2008).

Classified first as Urticaceae, then as Mareaceae, cannabis is now a part of order Cannabaceae, which is *Cannabis* sharing with common hop (*Humulus lupulus* L.) *Cannabis sativa* L. was identified by Carolus Linnaeus in the year 1737 in the picturesque Indian region with snowy peaks of the Himalayas looming above (Miovský 2008). Other species, *Cannabis indica* Lam. was discovered 50 years later by Frenchman Jean Baptiste de Lamarck. In 1924, a third specie *Cannabis ruderalis* Janisch. was discovered by a botanist D.E. Janischewsky in southeast Russia. It is possible, that these three species developed as a result of human cultivation (Rätsch in Miovský 2008).

Cannabis sativa is the most widely distributed species of hemp. It is divided into two subspecies (Váša in Miovský 2008):

- *Cannabis sativa* ssp. *Spontanea* - small weed plant, with rich branches, short internodes, small leaves and small seeds. It is very resistant to pests.
- *Cannabis sativa* ssp. *Culta* - taller plant with straighter stem and bigger leaves. Bigger, rounder, seeds. More demanding cultivation and lower resistance.

[4] Notice the use of word „poskonný" – meaning male plant and „hlavatý" – meaning female plant, original words connected with hemp growing, which already disappeared from Czech language.

Cannabis indica Lam. is up to 1.5 metres tall, prone to branching and creating up to twelve leaflets in a leaf. Dark seeds are of marble colour (Miovský 2008). *Cannabis indica* Lam. is grown in area from North Africa to Central Asia for the aim of obtaining its resin and producing hashish (ČSAZ 1971; Rätsch in Miovský 2008). It grows as a wild plant in Pakistan (Miovský 2008).

Cannabis ruderalis Janisch. – the taxonomy based on Žukovski is considering a third species – *Cannabis ruderalis* Janisch. It is a 0.6 m small plant with a thin stem and infrequent leaves (Špaldon in Miovský 2008).

Miovský (2008) quote Rätsch's note of various species or names of varieties, which were considered and used during botanical history: "*Cannabis sativa* Linné 1737, *Cannabis lupulus* Scopoli 1772, *Cannabis indica* Lamarck 1783, *Cannabis foetens* Gilibert 1792, *Cannabis erratica* Sievers ex Pallas 1796, *Cannabis macrosperma* Stokes 1812, *Cannabis generalis* Krause 1905, *Cannabis americana* Houghton et Hamilton 1908, *Cannabis gigantea* Crevost 1917, *Cannabis ruderalis* Janischewsky 1924, *Cannabis pedemontana* Camp 1936, *Cannabis intersita* Sojak 1960"

As Miovský (2008) states, another division of hemp is possible based on geography: *C. Borealis, C. Medioruthenica, C. Australis* and *C. Asiatica*.

Because cannabis is very flexible, adaptable and changing according to conditions, the division of hemp into classes is still a subject of botanical discussion (Booth in Miovský 2008).

The term "technical hemp" is nothing more than temporary name according to Miovský (2008), it is reflecting the jurisdiction of European and North American states, which in last decades are again exploring the possibilities of growing cannabis, but cannabis restricted to a certain limit in the content of psychoactive compounds. Varieties with THC lower than 0.3 % are allowed to grow in the EU since 1996 (Kriese et al. 2004).

However, as Citti (2017) claims: *"There exist varieties of CS selected for the lack of tetrahydrocannabinolic acid-synthase which produces only cannabidiolic acid. They are called „legal", thus they can be cultivated for fibre and seed production"*. Technical hemp varieties with low THC content usually contain a high concentration of cannabidiol (CBD) (Citti et al. 2017).

1.1.3. Botany of *Cannabis sativa* L.

Cannabis sativa is an annual flower. It is usually dioecious, meaning that the male and female reproductive organs are found on separate plants. Male plants are taller, slimmer, with lighter coloured leaves and they become ripe 4 - 6 weeks earlier. Female plants are stronger, darker and have more leaves (Moudrý 2011). Monoecious varieties also exist, predominantly as a result of breeding. They play a more important role in contemporary agriculture than the dioecious varieties, because of better pollination and uniform ripening (ČSAZ 1971; Miovský 2008).

The main tap root is usually 0.3 – 0.4 m long but can be as long as 2 metres in deep alluvial soils. Lateral roots are emerging from main roots and are abundantly developed mainly in peat soils (ČSAZ 1971). Generally, it is possible to say, that root system size corresponds to the type of the soil (Moudrý 2011). Size of a root system is small compared to above-ground parts of plant and cannabis is relatively demanding for both water and nutrients, which should be provided by high-quality, deeply loosen soil (Poliščuk & Hadinec 1952).

Stem length is usually 2 metres (Moudrý 2011) but can reach up to 6 metres (Miovský 2008). It is straight, 3 - 30 mm wide[5] (ČSAZ 1971). Colour of the stem is changing from green to yellowish green as the plant ages and an old stem can become woody and turn brown when over-ripen due to the influence of wind conditions (Miovský 2008). Its bast part contains 13.5 - 19.5% of fibre. Highest fibre content is in long and thin stems (ČSAZ 1971). The hollow stem is composed of 7 - 15 internodes. The stem is round at the bottom, six-rounded in the lower third, four-rounded at the higher part and longitudinally grooved for whole length (UNODC 2009). The deeper grooves and lower number of internodes mean higher fibre yield (ČSAZ 1971). The height of the stem is positively (and extent of branching negatively) correlated with a higher density of sowing (UNODC 2009; Moudrý 2011).

The side branches are coming in alternate or opposite direction from the main stem. The leaves stalk, petioles, are 20 - 70 mm long with a narrow groove on the upper side (UNODC 2009).

[5] Miovský (2008) states that maximum width can be up to 60 mm

Hemp has two oblong cotyledons (seed leaves). When they fall off, the first node is created (Miovský 2008). The leaves are decussate (opposite to each other) on the stem and become alternate and more dense towards the end in an inflorescence. Palmate leaves possess 3 - 13 linear-lanceolate leaflet blades, narrowed at both ends, with a coarsely serrated edge and teeth pointing towards the tip of the leaf. The leaves are dark or grey green. The bottom side of the leaf is of lighter colour and "hairy" with resinous glands (UNODC 2009). As the plant grows and ages, it sheds the most bottom, dead, leaves (Miovský 2008).

The male flowers are found in panicles on male plants. These male flowers (staminate) are made of five small hairy white-green or yellow sepals about 2.5 - 4 mm long and five pendulous stamens, consisting of a tiny filament and an anther (UNODC 2009). The anthers create a lot of pollen, which is transferred by wind up to 12 km (ČSAZ 1971). Male plants are in blossom for 20 - 25 days, after the bloom they die (Miovský 2008).

The female flowers (pistillate) are born in pairs, sessile, and they are of dark green colour. Two long and slender stigmas are coming from an ovary, which is enclosed by a small green beaked bract (UNODC 2009).

Female plants start to bloom 3 - 10 days after male plants. The pollination is possible 14 - 15 days after the pollen has ripened. It takes 30 - 40 days from pollination to mature seeds (Váša in Miovský 2008).

Hemp seed, botanically an achene, is tightly covered by a thin wall of the ovary (UNODC 2009). It has a hard shell and ellipsoid shape. It contains a small portion of endosperm and has a big embryo in a shape of horse-shoe (Miovský 2008). Size is circa 2 - 5 mm in length, 2 - 4 mm width, size depends on the variety. Colour of seed is grayish green, brown or black with marble colouring. Thousand seeds weight from 8 to 26 grams (ČSAZ 1971).

Hemp demands temperature sum between 1 800 to 2 800 °C for growing, depending on a variety (ČSAZ 1971). Central European varieties take 120 to 130 days to grow when grown for thread, 150 days when grown for a seed (Miovský 2008). The optimal temperature for seed to germinate is 30 – 35 °C. Young plants are able to survive brief frost up to -5 °C. Amount of water needed to grow hemp is about two-times the amount needed by cereals, precipitation should be 250 - 300 mm during vegetation period

(ČSAZ 1971), but Moudrý (2011) suggests precipitation of 500 mm. Cannabis plant should be grown in the area which is not very windy, with a lesser danger of hailstorms occurring. An ideal soil is a deep loamy soil, with a neutral or slightly alkaline pH of 7 - 7.6. It should not be grown on acidic or too heavy soils and soils with high water level (ČSAZ 1971). A good crop to grow on the same field year before hemp can be maize, potatoes or legumes. When grown after winter cereals, the animal dung should be added to the field.

Fertilization with 20 tonnes of animal dung combined with 60 - 80 kg N, 40 - 50 kg P_20_5 and 100 - 150 kg K_2O is recommended for 1 hectare. (ČSAZ 1971) Experiments with fertilization showed an effect on cannabinoid content (Hanuš & Dostálová 1994). The highest content of CBD was found in fertilization by NK (a combination of calcium nitrate and potassium salt). The highest content of THC was found in fertilization by PK (a combination of superphosphate and potassium salt). Based on this study of Hanuš (1994), Miovský (2008) concludes that both meteorological conditions and fertilization of soil highly affects the profile of cannabinoids. Thus, many repeated test should be carried, before the characteristics of a variety is set (Miovský 2008).

1.1.3.1. Cannabinoids

Hemp is well known to contain various cannabinoids, most prominent of them probably tetrahydrocannabinol (THC) and cannabidiol (CBD). THC is a psychoactive substance, antiemetic and is supporting appetite. However, many pages have been written about these substances and because this work is concerned about different utilization of cannabis plant, they will be spoken of only marginally. The content of Δ-9-THC is positively correlated with the amount of sunlight during growing. If the latter is higher (number of sunny days) the first is also higher (Miovský 2008).

THC is not synthetised by plant directly but is a product of decarboxylation of tetrahydrocannabinolic acid (THCA). THCA is created from cannabigerolic acid (CBGA) by the action of tetrahydrocannabinolic acid-synthase (THCA-synthase). Likewise, CBD is the decarboxylation product of cannabidiolic acid (CBDA) created by cannabidiolic acid-synthase (CBDA-synthase) from CBGA. The decarboxylation process is sped up by heat and therefore THC content in oil is increased by heating of the oil. That implies that hemp growers in southern Europe have harder times to comply with the THC limits for foods than north European farmers (Citti et al. 2017).

Cannabidiol (CBD) does not have psychotomimetic effects as THC and may have antipsychotic effects instead (Boggs et al. 2018). Its mechanism of action is not so clearly understood as that of THC. Discussion about possible antiepileptic, anti-inflammatory, neuroprotective, analgesic effects of CBD is still ongoing (Citti et al. 2017; Boggs et al. 2018). [6]

The measurement of THC content is critical to approve each variety of cannabis under current legislation. However, THC content varies highly in different parts of plants, being highest in flowers and lowest in roots and if this is not taken into account results of an analysis can be misleading (UNODC 2009). Therefore, it is important to standardize the procedure of THC content analysis. According to Miovský (2008), all the "hard parts" (roots and stem) are removed and analysis is done on leaves and flowers. UNODC (2009) states, that flowering tops should be cut at the length of about 0.2 m.

The EU legislation permits varieties with CBD to THC ratio over 2 (Callaway 2004).

1.1.3.2. Pests, diseases and environment interaction

Pests affecting cannabis are: hop flea beetle (*Psylliodes attenuata* Koch), caterpillars of Silver Y moth (*Autographa gamma* L.), cannabis aphid (*Phorodon cannabis* Passerini) or European corn-borer (*Ostrinia nubilalis* Hübner) (ČSAZ 1971; Miovský 2008).

Sclerotinia sclerotiorum (Lib.) Massee can cause white mold growth on a cannabis plant. A grey mold *Botrytis cinerea* Pers can also occur (Miovský 2008). Plant

[6] Where some authors use the word "may" (affect) (Boggs et al. 2018) other authors use the word "proven" (effects) (Citti et al. 2017). It is not in the power of the thesis's author to judge them and decide which of them is right. It is known, that this word "may" (and other words of this sort, as "perhaps" or "possibly") are susceptible to such "volatility" and "instability", that they are prone to "disappear" in some scientific works. Sometimes what is a "speculation" in an original article, becomes a "high probability" in a second article and "rock-solid certainty" in a third article as citing goes.

Sometimes it is important to admit that we just do not know, especially in the case of complicated interactions between chemical substances which do not occur in nature separately, but act together in their complexity as cannabinoids do.

pathogen *Gibberella pulicaris* (Fr.) Sacc. can attack hemp and cancer can also appear on plants (Miovský 2008, Moudrý 2011).

Among parasitic plants, European dodder (*Cuscuta europaea* L.) and hemp broomrape (*Orobanche ramosa* L.) are attacking hemp (ČSAZ 1971).

Technical hemp is usually not susceptible to diseases, however changing of growing location each year and ensuring diversity of crops grown is recommended (Miovský 2008).

Conrad (2007) suggests that cannabis is positively affecting soil, air, water and other organisms. The broad root is aerating the soil. Wide leaves suppress the growth of weeds and they prevent the ground from further drying when falling off during the dry season. Hemp should also help to remove heavy metals from soil and reduce pesticide crystal clusters. In addition to this, hemp is offering shelter to a broad spectrum of organisms, ranging from insect to medium-size mammals.

Conrad (2007) is stating that substances contained in cannabis should offer protection against molds and insects and even that moths will avoid a granary, which was used for hemp drying.

1.1.4. Hemp for fibre

When grown for fibre, hemp is sown very densely in rows, 75 to 125 mm apart (ČSAZ 1971), according to Moudrý (2011) the suggestion is up to 250 mm. For one hectare about 100 to 120 kg seeds are used. (ČSAZ 1971) The reason for dense sowing is that the plants will become taller and more straight with less branching (Sladký 2004). The depth of sowing should be 40 – 60 mm (Procházka 1991).

Generally, it is possible to say, that the more resin on the flower, the lower is the quality of fibre obtained (Miovský 2008). It should be harvested before plant energy is focused on reproduction and seed creation. The ideal time for harvest is when 75 to 90 % of male plants are flowering. Cut stems are left on the field to dry, which takes 5 to 8 days in warm, sunny and rainless conditions. Dried stems are tied to sheafs and dragged on stubble field to remove remaining leaves (ČSAZ 1971).

Because of a decline in hemp growing during the 20th century, its cultivation was not a subject of agriculture mechanization, unlike other crops. However, some special

hemp harvesting machines exist, for example, Clipper 4.3 MMH, designed to be drafted behind a tractor. It is of Czech origin and capable of harvesting 6 hectares per hour (Roslenkonoplja 2018). Other machines for hemp harvesting is a Russian ŽK-1.9 or German Balkenmäher (Moudrý 2011).

Stems should be separated based on length, width, colour and quality to different categories[7]. Hemp stems are than soaked in pools for 75 to 100 hours in warm water or 150 to 180 hours in non-heated water. Hemp stems are broken mechanically on series of cogwheels and then advanced to the scutching machine. Out of the input of 100 kg of stems without leaves, in the end, 15.5 to 19 kg of fibre is obtained, from that amount 10 - 18 % is long bast fibre and rest is hemp hurds (ČSAZ 1971). Moudrý (2011) states, that yields of fibre are between 0.5 to 1.2 tonne per hectare and yields of hemp hurds is between 1.5 to 4 tonnes per hectare. The humidity of fibre is usually between 8 and 9 % (Procházka 1991).

Hemp long bast fiber contains 53 – 74 % of celulosis and hemp hurds contain 32 - 38 % of cellulose (Van der Werf 2001). However, according to Procházka (1991), claim cellulose content of 77 % and 0.5 to 0.6 % content of wax and fats and 0.8 to 1 % of ash. Hemp fiber is very strong and resistant in a humid environment (ČSAZ 1971). Hemp fibre can be up to 4.5 metres long (in contrast with 20 mm long cotton fibre) and is up to eight times stronger and four times more durable than cotton, but is not so demanding for water and pesticide inputs (Miovský 2008). Cannabis thread is the most fine and soft among all bast fibre plants (flax, jute, ramie). It has high tensile strength and is resistant against heat. At 370 °C, it will change colour and at 1 000 °C it will carbonize, but it will not burst into flames (Miovský 2008).

Hemp fibre is used for the production of ropes, canvas[8], cords, strings, nets, hoses, sacks, textile and clothes. Bioplastics or brakes for cars, airplane casing, ski or snowboards and many other things can be made from hemp. Hemp hurds are used as an

[7] According to old Czechoslovak Norm for hemp processing from 1970s (ČSN 46 2431): First class stems are longer than 1.3 m, width less than 7 mm, yellow or yellow green colour, degree of degradation by pest and hailstorms less than 5 %, total volume of weeds less than 3 % and total volume of small underdeveloped hemp plants less than 2 %.

[8] The word “canvas” itself is derived from “cannabis”.

isolation material in construction, plastic pipelines can be made from hemp. “Hempstone” is material to make musical instruments or furniture. When one part of lime, one part of water and four parts of hemp hurds are mixed, “hempcrete” is created. Hempcrete can be used as good isolation and fire resistant material. With a 15 % density of traditional concrete, this material is suitable for areas prone to earthquake. Hemp hurds can also found their use as bedding for animals or as a substrate for gardening. Hemp textile can be recycled to create hemp paper. Hemp paper is strong, resilient and easier to bleach compared to traditional paper. This hemp paper is used for making of cigarette papers, paper money and probably most renowned as a paper for printing large volume books, such as the Holy Bible (ČSAZ 1971; Callaway 2004; Miovský 2008; Moudrý 2011).

1.1.5. Hemp for seed

When hemp is grown for its seed, it is not sown so densely. Rows are set 0.5 to 0.7 m apart and about 15 kg of seeds are used for one hectare. If planted in a square-shape manner into nests 0.7 x 0.7 or even 1 m apart, only 4 to 5 kg of seeds are needed per one hectare (ČSAZ 1971; Procházka 1991). Depth of sowing is same as for fibre production.

The field should be weeded two to three times. When one third of male plants are flowering, it is recommended to move a cord over plant tops to improve pollination (ČSAZ 1971). The seeds in the inflorescence are maturing from bottom to top. Harvest should be done when the seeds in the bottom part of the inflorescence have ripened, while the top seeds are not fully ripening and partially green. It is recommended to harvest during higher air humidity or in the morning so the loss of seeds is minimized (ČSAZ 1971).

In the past, it was common to mow hemp, bound it into sheafs and put them together into a stook to dry. Then seed was treshed by a treshing machine, for example, a soviet tresher MLK-4.5, directly on the field (ČSAZ 1971). Today, everything is done in one step, for example by CASE IH harvester (Moudrý 2011). The seed is then left to dry. The moisture content of seed after drying is between 6.5 to 9 % (Němec 1941; Callaway 2004).

The hemp seed contains between 24 to 40 % oil (Procházka 1991; Kriese et al. 2004).

Antioxidative tocopherols (which are part of a group of fat-soluble compounds called “vitamin E”) are present in hemp seed in a concentration ranging from 14 to 34 mg per 100 g of seeds according to Kriese (2004). However, research conducted by Callaway (2004) suggests much higher numbers of 90 mg of vitamin E in 100 g of seeds of Finola variety.

1.1.5.1. Hempseed protein

The protein content is usually between 20 - 25 % (Miovský 2008), but Procházka (1991) mentions protein content even as low as 15 %.

Two main proteins are albumin, a globular protein, and edestin, a legumin. Hempseed contains all of the nine essential amino acids (Callaway 2004; Miovský 2008). Amino acid profile of hempseed protein is comparable to high-quality proteins as egg white or soy bean. Hempseed protein is rich in methionine and cystine, it also possesses a fair amount of arginine and glutamic acid (Callaway 2004).

1.1.5.2. Hemp cake

Hemp cake is a residue of oil pressing. It contains usually between 20 to 30 % protein (Procházka 1991), 10 to 20 % of residual oil and high amount of dietary fibre (Miovský 2008). The residual oil can be extracted using chemical solvents (Miovský 2008).

The cake, due to its high protein content, can be used as animal fodder. Hessle (2008) concluded, that hemp cake offers the same nutrition as soybean meal and improve rumen function because of high fiber. There is a good potential hidden in feeding animals by hemp cake, as it is a waste from oil pressing.

A gluten-free flour made of hemp cake can serve as a healthy food for human as well (Miovský 2008).

1.2. Lipids

Lipids, as stated by Hoffmann (2003), are a large and diverse class of organic molecules, usually insoluble in water, but soluble in non-polar solvents. They are an important substance for both animals and plants.

The broader term “lipids” include fatty acids, glycerol derived lipids (oils, fats and phospholipids), sphingosine-derived lipids, steroids and their derivates, terpenes and their derivates, certain aromatic compounds, long chain alcohols and waxes. In conjugation with proteins or carbohydrates, they are called lipoproteins and liposaccharides. The fat-soluble vitamins can be also considered a class of lipids (Hoffmann 2003).

The lipids constitute the cell membrane and serve as an energy reserve. Among other functions, protective coatings of cells, waterproof covering of plant (Hoffmann 2003) or transfer of vitamins A, D, E and K (Novotný 2015) can be noted.

Titer point is an important attribute of lipids. It is the temperature, at which the lipid re-solidifies. The oil, fat, wax or butter is heated to melt, then stirred to cool down. When the temperature stays constant for 30 seconds or begin slightly to rise, the titer point is the highest temperature indicated by this rise (O’Lenick et al. 2008).

The terms fat, oils and waxes are commonly misused, and titer point can help us to differentiate them. Fats have titer point above 40.5 °C, oils have titer point under 40.5 °C. The butter has titer point under 40.5 but higher than 20 °C. O'Lenick et al. (2008) define oil as *“stays liquid at room temperature”*.

1.2.1. Oils

Oils are nonpolar, neutral chemical substances, which are hydrophobic and lipophilic and takes a form of viscous liquid at room temperature. Oils are characterized by a high carbon and hydrogen content, can be flammable and surface active. A broad spectrum of chemical compounds, which differs in structure, properties or use, could be included in this definition (Dijkstra 2016).

Origin of oils can be animal, plant or petrochemical. Oils can be volatile or non-volatile. Oils serve as food, fuel, lubrication, they can be applied in medicine or used as a basis for various materials, *i. e.* paint, plastics and others. Chemically, the substance called oil is a mixture of triglycerides. They consist of a glycerin backbone which is esterified by three (usually different) fatty acids (Dijkstra 2016).

The rule goes, the longer and containing less unsaturated bonds, the more waxy (or solid) the molecule is (Hoffmann 2003).

The classification of vegetable oils is arbitrary (Dijkstra 2016). They can be divided based on the origin (palm oil, sunflower oil etc.) or the content of fatty acids (which fatty acid is the most prominent, does the oil contain essential fatty acids etc.).

1.2.2. Fatty acids

A fatty acid is a carboxylic acid with long, (usually) straight, carbon chain. The number of carbons (usually) ranges from C_{10} to C_{20} (Hoffmann 2003).[9]

French chemist Michel Eugène Chevreul introduced the concept of fatty acids in the first half of 19th century (Chevreul 1823).

The fatty acid is marked by the number of carbons and the number of double bonds. These numbers are separated by a double dot. For example, stearic acid (saturated) containing 18 carbon and no double bonds will be marked as 18:0. Should the double bond occur, its position needs to be stated. Counting from a -COOH end, the fatty acid is marked by Greek letter delta Δ and number in upper index indicating the position of the double bond. Oppositely, counting from other ($-CH_3$) end, letter omega ω is used.[10] For example palmitoleic acid (unsaturated) with one double bond after seventh carbon from the $-CH_3$ end will be marked as 16:1 ω-7 or $16{:}1\Delta^9$ counted from -COOH end. They can be saturated (without carbon double bonds) or unsaturated (one or more double bond). When the carbon double bond occurs, *cis* and *trans* isomer can be distinguished (Hoffmann 2003).

1.2.3. Oil recovery

Oil is stored in so-called oil bodies, organelles inside the plant cells. These lipid containing structures in seeds function as energy storage for a new plant. The mechanical stress applied to the plant cells leads to the rupture of cell walls and the oil is obtained (Tzen & Huang 1992).

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[9] IUPAC, Compendium of Chemical Terminology (1997), states *"Natural fatty acids commonly have a chain of 4 to 28 carbons"*.

[10] omega "ω" can be replaced by Latin letter "n" (ω-3 = n-3)

Many different methods of oil recovery have been developed during history. Among the most primitive methods is the basic wet rendering process, when material containing the oil is boiled in water and oil is collected from the top of the vessel (Bredeson 1983).

Three most commonly used modern methods are:

1. Hydraulic pressing
2. Expeller pressing
3. Solvent extraction

1.2.3.1. Hydraulic pressing

Patented in 1795 by Joseph Bramah, a hydraulic press is based on Pascal's principle, that pressure in a closed system is constant. Smaller and bigger piston are connected by the hydraulic fluid. A small force applied to a small area piston will result in a large force on the large area piston (Mustakas 1980).

Hydraulic pressing is a batch-type method and requires much of hand labour (Mustakas 1980) and its use declined steeply from the second half of 20th century. Yields are also smaller than for other methods. Now considered partially obsolete for mass production, hydraulic pressing still finds its place where the low temperature of the oil is sought.

The quality of hemp oil is decreased by temperatures over 50 °C. The higher temperature reduces the content of unsaturated fatty acids. Therefore, so-called cold pressing is recommended to be used for obtaining hemp oil, when quality is preferred instead of quantity. On the other hand, hemp cakes gained by cold hydraulic pressing contain more than 10 % of residual oil and have a higher value as a fodder (Sladký 2004).

The moisture content of the pressed seeds is affecting the amount of the recovered oil and even the change of 0.1 % in the moisture can have a significant effect on the oil yield (Hickox 1953). Duration of the pressing is also affecting the oil yield. Hickox (1953) reports the residual oil in the cottonseed cake to be 5.1 % after 7.5 minutes of pressing but only 3.45% residual oil after 45 minutes and 3.25 % after 90 minutes of pressing.

1.2.3.2. Expeller pressing

Starting in the 1940s, expeller presses have quickly begun to replace the hydraulic presses. Unlike the hydraulic press, the expeller press allows the continuous pressing, marked by low residual oil in the cake, but also much higher pressing temperature. Hemp cake contains only 3 – 5 % of oil, but the temperatures during expeller pressing can raise up to 170 °C (Dunning 1956; Sladký 2004).

1.2.3.3. Solvent extraction

Solvent extraction is another relatively new method. Solvent extraction can be used as a first-line oil obtaining method for material with lower oil content (soybeans) or as a second-line method following expeller pressing for sources with high oil content (sunflower, flaxseed, cottonseed etc.) (Mustakas 1980).

A batch-type process at first, the solvent extraction evolved into a continuous process after the First World War. The most commonly used solvent is n-hexane. The disadvantage of n-hexane is its extreme flammability. Chlorinated hydrocarbons were proposed as a non-flammable hexane substitution. However, their toxicity prevented their broader use. Alcohol solvents as ethanol or isopropyl alcohol can be used for oil extraction, but the effectivity is a rather small compared to hexane, unless the temperatures near boiling are used. Problems with the oil recovery and its purification occur in the case of ethanol and ethanol is thus not used in the commercial practice (Mustakas 1980; Snyder & Wilson 2003).

Solvent extraction is characterized by fairly high efficiency, the oil yield is over 99 % (Bargale et al. 1999).

1.2.4. Vegetable oils in human nutrition

Nutrition means providing the human body with substantial nutrients, which are necessary for the proper function of the organism. The vegetable oils play an important role in nutrition, as they usually contribute to circa 30 - 40% of human energy intake (Fábry 1992). Compared to animal fats, plant fats are richer in unsaturated fatty acids (Hoffmann 2003).

As previously stated, oils play an important role not only as a fuel for the organism, as a constituent of cell membranes but also a supplement of so-called vitamin F (Hoffmann 2003). "Vitamin F" is an obsolete term for essential fatty acids (EFAs).

The human body has the capacity to produce fatty acids structures. However, because it is lacking some desaturase enzymes, it cannot synthetize unsaturated fatty acids with double bonds beyond carbon 9 and 10 (Hoffmann 2003). The two short chain polyunsaturated fatty acids (SC-PUFA), which cannot be synthetized in the human organism are linoleic and linolenic fatty acids (Da Porto et al. 2012). These two PUFA are produced only by plants and human nutrition must contain either plants capable of synthetizing them or meat of animals feeding on such plants (Hoffmann 2003).

Long chain polyunsaturated fatty acids (LC-PUFA) are created from SC-PUFA in the human body. For example, gamma-linolenic acid is formed by dehydrogenation of linoleic acid. Dijkstra (2016) states, that this process is slow in some people[11] and these may benefit from ingestion of oils containing gamma-linolenic acid.

Simopoulos (2002) argues, that the ratio between ω-6 and ω-3 essential fatty acids changed from around 1/1 at the times before the First Agricultural Revolution to 15/1-16.7/1 in contemporary Western diets. Linoleic and α-linolenic acids are competing for Δ-6-desaturase enzyme. Simopoulos (2002) states, that lower ω-6 and ω-3 EFAs ratio positively affects conditions as cancer, inflammatory and autoimmune diseases, and that *„in the secondary prevention of cardiovascular disease, a ratio of 4/1 was associated with a 70% decrease in total mortality"*.

1.2.5. Hemp oil

Cold-pressing of hemp seed results in a quality oil with chlorophyll content, which gives the oil a green colour. The oil has a nutty flavour (Miovský 2008).

The hemp seed oil contains a high portion of PUFAs, has an iodine value of 154 - 165 (Anwar et al. 2006) and is therefore considered a drying oil. Drying oil is an oil with a high number of unsaturated double bonds which, in the presence of oxygen, create polymers between fatty acids chains. When exposed to air, light and heat in a thin layer,

[11] Due to a deficiency in Δ-6-desaturase (Kriese et al. 2004)

drying oils create a solid, elastic and sticky film. Heat, light and air, in this sequence, affect the process from the most affecting to the least affecting. By absorbing oxygen, oil becomes heavier, up to 13 % in the case of hemp oil (Andés 1901).

Thanks to this characteristic, hemp oil is used as a varnish for wood finishing or as an additive for paints (Oomah et al. 2002).

It is also interesting to note, that process of drying of oil is exothermic, the heat which is created can lead to spontaneous combustion of clothes, papers or other materials soaked in drying oil, as allegedly happened in 1995 in One Meridian plaza, when rags saturated with linen oil ignited themselves (New York Times 1991).

Anecdotal evidence of faster cuts and burns healing, improving influenza, improving skin conditions, alleviating allergy symptoms and symptoms of inflammatory diseases when hemp oil is applied, is thought to have a base in the fatty acid profile of oil. Eicosanoids, which include prostaglandins, are metabolised out of essential fatty acids (EFA). Eicosanoids play a role in chronic diseases (Callaway 2004). Lack of EFAs and other PUFAs in diet can lead to disease, therefore their supplementation can improve human health. PUFAs supplementation may lower levels of LDL-cholesterol and blood pressure, it can also lower clot formation (Callaway 2004).

The oil usually contains 90 % unsaturated fatty acids. It contains linoleic acid (18:2 ω-6), alpha-linolenic acid (18:3 ω-3) and their respective biological metabolites gamma-linolenic acid (18:3 ω-6), stearidonic acid (18:4 ω-3) (Oomah et al. 2002, Callaway 2004). There is also a significant amount of unsaturated oleic fatty acid (18:1 ω-9). Palmitic acid (16:0), stearic acid (18:1), arachidic acid (20:0) and behenic acid (22:0) are contributing to 10 % of saturated fatty acids (Miovský 2008).

The hemp oil has "perfectly balanced" ratio 3/1 of linoleic and linolenic PUFAs (Oomah et al. 2002).

Moudrý (2011) states the average content of fatty acids in hemp oil is: palmitic 6.6 %, stearic 2.6 %, oleic 14.9 % linoleic 56.7 % and linolenic 19.2 %.

Da Porto (2012) examined the fatty acid composition of hemp oil of variety Felina, extracted by supercritical CO_2 extraction (40 °C, 300 bar, 45 kg CO_2/kg feed) and by Soxhlet extraction with n-hexane. Her results are shown in Table 1.

Table 1. Fatty acid composition of hempseed oil, variety Felina (Da Porto et al. 2012)

	Supercritical CO_2		Soxhlet (n-hexane)	
Fatty acid composition (%)	Mean	Std. Dev.	Mean	Std. Dev.
Palmitic acid (C16:0)	5.19	0.03	5.37	0.13
Stearic acid (C18:0)	1.57	0.03	1.56	0.05
Oleic acid (C18:1)	10.99	0.35	11.51	1.05
Linoleic acid (C18:2ω6)	59.77	0.74	59.16	0.85
γ-Linolenic acid (C18:3ω6)	3.42	0.16	3.48	0.15
α-Linolenic acid (C18:3ω3)	18.15	0.31	17.96	0.23
Eicosenoic acid (C20:1)	0.78	0.03	0.8	0.01
Behenic acid (C22:0)	0.13	0.02	0.18	0.03
EFAs sum	77.92		77.12	
ω-6/ω-3 ratio	3.29		3.29	
PUFAs sum	81.35		80.6	
Monounsaturated	11.12		11.66	
Saturated	7.54		7.74	
Polyunsaturated/saturated ratio	10.79		10.42	

β-Sitosterol, a substance known to block absorption of cholesterol, is not present in hemp oil which has been pressed cold (Miovský 2008).

The oil is good for making light body oils and creams for its penetration into the skin (Oomah et al. 2002). It can be also used for human nutrition (ČSAZ 1971, Kriese et al. 2004), but it loses its nutritional value by heating or frying (Miovský 2008). The oil has a relatively low smoke point 165 °C (Chu 2004).

According to the experiments by Anwar (2006), the hemp oil yield from the hydraulic cold-pressing at 60 °C was 26.9 - 31.5 %. The seeds were dried in a vacuum oven at 60 °C to 4 – 5 % moisture content prior to the pressing, then cooked at 60 °C for 5 minutes. The pressing was done by a manual hydraulic press and took 20 minutes. The pressure was in the range 35 - 50 MPa.

Like for other oils, biodiesel can be created by transesterification of hemp oil (Das 1997).

2. Aims of the Thesis

The aim of this work was to compare oil- and seed-related qualities of nine different technical hemp (*Cannabis sativa* L.) varieties.

Specific aims were:

- To measure oil yields of hemp seed pressing.
    1. Hypothesis was that hemp variety affected the oil yield.
    2. Hypothesis was that higher temperature during pressing led to higher oil yield.
    3. Hypothesis was that higher force applied led to higher oil yield.
- To measure residual oil in hemp cakes.
    4. Hypothesis was that higher temperature during pressing led to lower residual oil in the hemp cake.
    5. Hypothesis was that higher force applied during pressing led to lower residual oil in the hemp cake.
- To measure the protein content of oil-free hemp cake.
    6. Hypothesis was that different conditions during pressing affected the protein content of oil-free hemp cake.
- To determine the fatty acid profile of hemp cake residual oil.

3. Methods and Materials

3.1. Plant material

Nine varieties of *Cannabis sativa* L. were used in this study, namely: Białobrzeskie, Fedora, Santhica, Fibrol, Finola, Futura, Tiborszállási, Kompolti hibrid, KC Virtus.

Hemp was grown in conditions of Central Europe in Czechia, Central Bohemia, in Masojedy village, 26 kilometres southeast of Prague. The hemp was sown in May 2016 and harvested between October and November 2016.

According to the data from meteorological station Praha-Libuš, 23 km to the west of Masojedy, temperature sum for a period between 1st May 2016 and 31st October 2016 was 3 100 °C (CHMI 2019).[12] Sum of monthly precipitation for the region of Central Bohemia between May and October 2016 was 358 mm (CHMI 2019).

3.1.1. Hemp varieties

3.1.1.1. Białobrzeskie

Białobrzeskie is a Polish monoecious, semi-early ripening variety of hemp. Plants are circa 2.5 m tall (Jankauskienė & Gruzdevienė 2012; iHempFarms Ltd 2017).

It is used mainly for fibre production, for cordage or military fabrics, but also for technical oil products. Białobrzeskie is a result of cross-breeding of mono- and diecious varieties and was registered in the year 1968 (Jankauskienė & Gruzdevienė 2010).

According to sources cited by Jankauskienė and Gruzdevienė (2010), it has a seed yield of 800 to 1 000 kg per hectare, stem yield of 10 to 12 tonnes per hectare and content of good quality fibre is around 28 %.

[12] This number can differ insignificantly for Masojedy, *i. e.* is expected to be a little bit lower, since Praha-Libuš meteorological station is found in a city.

It has high crop density and very good dry mass yield of 11.6 tonnes per hectare according to research by Jankauskienė and Gruzdevienė (2012), but their previous article (2010) mentions dry biomass yield even 18 tonnes per hectare.

CBD content is between 1 and 1.5 % and THC content is lower than 0.12 %.

Oil content in seeds should be 30 – 32 % (iHempFarms Ltd 2017).

3.1.1.2. Fedora

Fedora is a French monoecious variety, medium-early ripening (Konopko 2015; Baldini et al. 2018).

The plant is over 2 m tall (iHempFarms Ltd 2017; Baldini et al. 2018). Leaves are medium green with a medium length of the petiole (CPVO 2018).

Seed yield was experimentally determined to be 790 kg of seeds per hectare and stem yield around 7 tonnes per hectare. Dry biomass yield is relatively low at 9.27 tonne per hectare (Baldini et al. 2018).

CBD content is between 1.5 to 2 % and THC content lower than 0.06 % (Konopko 2015).

Reports about oil content in seeds vary, from 24 % (Baldini et al. 2018), 27 % (Siano et al. 2019) up to 33 % (Kriese et al. 2004).

3.1.1.3. Santhica

Santhica is a French monoecious, early ripening variety (Konopko 2015).

Plants are over 2 m tall (iHempFarms Ltd 2017). Leaf petiole is short and central leaflet is short and narrow. The seed testa is of yellowish brown colour. The pith of main stem in cross-section is of medium width (CPVO 2018).

Dry mass yield is between 10 to 12 tonnes per hectare (Jankauskienė & Gruzdevienė 2010 and 2015). It is a variety grown for fibre, the stems contain more than 35 % of fibre (Konopko 2015).

CBD content is between 1 to 1.5 % and THC content is negligible, lower than 0.02 % (Konopko 2015).

Oil content in seeds should be lower than 26 % (Konopko 2015).

3.1.1.4. Fibrol

Fibrol is a Hungarian, medium ripening variety. Although Fibrol is registered as a monoecious variety, there can be a significant presence of male plants (Baldini et al. 2018).

Plants are almost 2.5 m high (Baldini et al. 2018). The seed testa is of light grey colour. Petioles of leaves lack anthocyanin coloration (CPVO 2018). It was registered in the year 2006 (Salentijn et al. 2015).

Stem yield is about 7 tonnes per hectare. Seed yield per hectare is very low, just 360 kg per hectare (Baldini et al. 2018).

Oil content in seeds is around 27.5 % (Baldini et al. 2018).

3.1.1.5. Finola

Finola is a Finnish, dioecious, very early maturing variety. It is grown mainly for oil (Chandra et al. 2017).

Plants are short stature, 1.5 m tall. Due to the low height, it is possible to harvest Finola with modern agricultural machines. Plants are tolerant to frost (down to -5 °C) at all growing stages (Callaway 2004).

Petioles of leaves have strong anthocyanin coloration. The main stem is thin. Grooves on the main stem are shallow. Seeds have weak marbling and testa is of medium grey colour (CPVO 2018). It was registered in the year 2003 (Salentijn et al. 2015).

Seed yield can be more than 2 000 kg per hectare (Callaway 2004).

The THC content is ranging from 0.05 to 0.32 % (Chandra et al. 2017).

Oil content in seeds is 35.5 % (Callaway 2004).

3.1.1.6. Futura

Futura is a French monoecious, late maturing variety (Konopko 2015; Chandra et al. 2017).

It is between 2.5 to 3 m high (iHempFarms Ltd 2017). Seeds have strong marbling and testa is of grey brown colour (CPVO 2018).

Dry mass yield is around 12 tonnes per hectare (Jankauskienė & Gruzdevienė 2012). Stem yield is 8.3 tonnes per hectare. Seed yield is 400 kg per hectare (Baldini et al. 2018). Stems contain between 30 to 35% fibre (Konopko 2015).

CBD content is between 1.5 to 2 % (Konopko 2015), according to iHempFarms Ltd (2017) up to 3 %. The THC content is lower than 0.12 %.

Accounts of the oil content in seeds vary depending on the source from 25 % (Baldini et al. 2018), 29 % (Konopko 2015) up to 31.75 % (Kriese et al. 2004).

3.1.1.7. Tiborszállási

Tiborszállási is a Hungarian, dioecious late maturing variety (Konopko 2015). It is grown for both fibre and seed (Chandra et al. 2017).

The plants are between 2.5 to 3.5 m tall (iHempFarms Ltd 2017). Tiborszállási was registered in the year 2004 (Salentijn et al. 2015).

Dry matter yield is 9.5 tonnes per hectare according to Amaducci (2008). However, other sources state more than 12 tonnes per hectare (Konopko 2015).

Seed yield is between 500 to 800 kg per hectare (iHempFarms 2017). The fibre content in stem is between 26 to 30 % (Konopko 2015).

CBD content is between 2 to 3 % and THC content is lower than 0.2 % (Konopko 2015).

The oil content of seeds is around 27 % (Konopko 2015).

3.1.1.8. Kompolti hibrid TC

Kompolti hibrid[13] TC is a Hungarian, hybrid, very late variety (Sankari 2000). Kompolti hibrid TC is a *"three-way-cross hybrid where two selections of Chinese origin 'Kinai Kétlaki' (dioecious) and 'Kinai Egylaki' (monoecious), and 'Kompolti' were combined"* (Štiasna et al. 2015).

Plants are between 3.5 and 4.5 m tall (iHempFarms Ltd 2017). It was registered in year 1983 (Salentijn et al. 2015).

[13] Sometimes written as "Kompolti hybrid"

Seed yield is between 500 to 800 kg per hectare (iHempFarms Ltd 2017). Stem yield was experimentally established by Sankari (2000) to be 7.3 tonnes per hectare and fibre content ranged from 19 to 26 %.

CBD content is between 2 to 3 % and THC content is lower than 0.12 % (iHempFarms Ltd 2017).

Accounts of oil content in seeds vary depending on source from 25 % (iHempFarms Ltd 2017), 30.4 % (Höppner & Menge-Hartmann 1994) up to 33 % (Kriese et al. 2004).

3.1.1.9. KC Virtus

KC Virtus is a Hungarian late variety (iHempFarms Ltd 2017). It is a single-crossing hybrid, unisexual in F1, dioecious in F2 (Gabrielová 2017).

Plants are 2.5 to 3.5 m tall (iHempFarms Ltd 2017). The seeds are grey with medium marbling (Agromag 2018). KC Virtus was registered in the year 2013 (Salentijn et al. 2015).

It is a fibre variety, the fibre content is between 26 to 30 %. Seed yield is circa 800 to 1 000 kg per hectare (iHempFarms Ltd 2017). Dry mass yield is between 10 to 12 tonnes per hectare (Agromag 2018).

CBD content is 5 % and THC content is 0.12 % (Ferrante et al. 2019).

Oil content is around 29 % (iHempFarms Ltd 2017).

3.1.2. Handling of seeds

Hemp seeds were stored in sacks in dark cold room until April 2018. The storing temperature was 5 °C. After the storing period seeds were transported to the laboratory and cleaned because they contained dust, small branches and other impurities. A blower which was a part of Farmet® seed preparation station was used for this purpose.

Seeds were blown through by air, which caused the lighter material to flow and concentrate in the middle of the apparatus. Twigs and dust; underdeveloped, small or damaged seeds were removed by this action and this waste contributed to circa 10 % of the weight of the original material. About four kilograms of seeds of each variety were

purified and stored in the laboratory in transparent plastic bags with closing strips for later use.

3.2. Cold-pressing

The testing was performed at the Czech University of Life Sciences Prague (CULS), Faculty of Engineering, Department of Mechanical Engineering. The tests were carried out by a hydraulic press Tempos ZDM 50 with a maximum power of 500 kN, produced by the company TEMPOS®, Czech Republic.

First trial pressing was carried on hemp seeds of unknown variety, kindly provided by Ing. Iva Kučerová, Ph.D. By this trial pressing the optimal pressing conditions were set at the forces of 100, 200 and 300 kN and temperatures 20 °, 40 ° and 50 °C.[14]

All the combinations of forces and temperatures (9 combinations) were carried out on each variety two times. The third pressing was planned to be conducted later, however, the seeds stored in the laboratory were meanwhile attacked and infested by moths. In total 162 pressings were done and the same number of hemp cakes was obtained. All pressings were carried out between April and August 2018.

The metallic cylinder used for pressing was consisting of two parts, an inner (smaller) cylinder and an outer (larger) cylinder. The bore of the outer cylinder was 60 mm, the same as the diameter of the inner cylinder, so the smaller cylinder fitted into the bigger cylinder. The seeds were differing in a size among varieties. Thus, the seeds were put inside the outer cylinder and closed by the inner cylinder, in a manner to ensure the height of the chamber created is 80 mm and the volume of the pressed seeds remains the same for each variety (circa 130 g of seeds were used, depending on a variety). The sieve with an aperture of circa 0.5 mm was placed at the bottom of the outer cylinder. The outer cylinder was closed at the bottom by a bottom lid with holes of circa 2 mm. The bottom

[14] There were no means available to ensure the exact temperature of 20 °C. Therefore "20 °C" rather means a room temperature in a laboratory or "an absence of heating". During the hot days, air-conditioning was activated in the laboratory.

lid was held in its place by two pegs. A scheme of this pressing vessel can be seen in Figure 1.

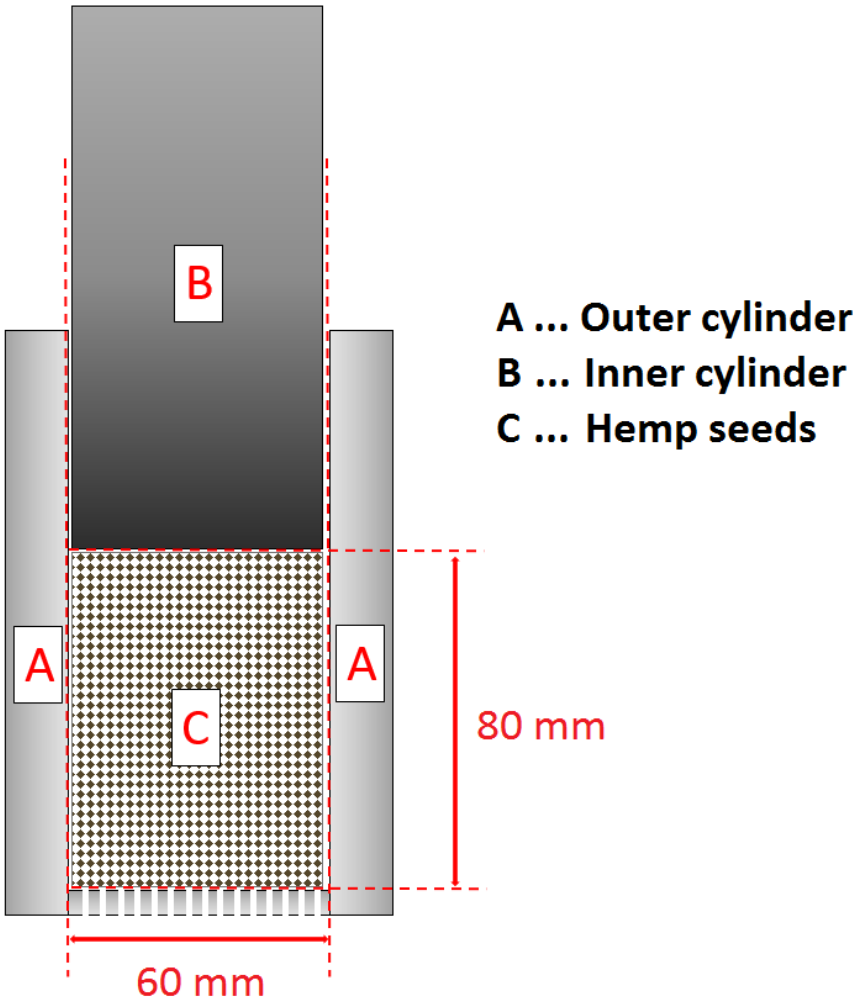

Figure 1. Scheme of the pressing vessel

The temperature of 40 ° and 50 °C was ensured firstly by pre-heating the seeds in an oven (types UN110m and HCP 108, both by Memmert®, Germany) at the desired temperature and secondly by putting a heating sleeve on the metallic pressing cylinder. The cylinder had to be heated in some advance due to a certain thermal inertia of the metal.

Seeds were taken out of the bag or out of the oven and measured in a plastic container on the electronic balance (KB 2000-2N, KERN & Sohn GmbH, Germany) with a precision of d = 10 mg. They were then poured into the pressing cylinder. The cylinder was placed into a plastic container, which served as a collector of pressed oil. The cylinder in the plastic container was positioned under the hydraulic press. As the lower platform

of the hydraulic press was rising, the inner cylinder was pushed into the outer cylinder until the desired force was reached. This took more than 5 minutes. When the desired force was reached, the cylinder was left in its place for circa 60 seconds to let the oil leak out. After that, the platform was retracted and the metallic pressing cylinder was placed into the wide metallic pan, which was there to ensure no seed is lost during manipulation. The pressed seeds, so-called "cake"[15], were removed from the cylinder into a plastic container and weighted. The oil yield was calculated from the weight of seeds before and after pressing.

Oil was removed from the plastic container by a spatula into a plastic storage container with a lid, marked and kept at the refrigerator.

The hemp cakes were vacuum-packed in plastic bags, marked and stored in the refrigerator.

The pressure was calculated from the pressing force divided by the area of the inner cylinder (2.827×10^{-3} m). The work was calculated as the sum of multiples of the distance travelled by the platform of the hydraulic press in 0.02 second and the current force in that moment as shown in the Equation 1. These values were recorded by a computer operating the hydraulic press.

$$W = F_1 \Delta s + F_2 \Delta s + \ ... + F_n \Delta s$$

Equation 1. The work conducted by the hydraulic press

3.3. Solvent extraction

The extraction by petroleum ether was used to remove the remaining oil from the pressed seeds and to determine the amount of residual oil in hemp cake. The testing took place in a laboratory of the Faculty of Environmental Sciences, CULS, Prague.

Automatic solvent extractor SER148 (VELP® Scientifica, Italy), capable of measuring six samples at once, was used for extraction in this study.

[15] It was not possible to talk about a "cake" under these pressing conditions. Hemp seeds have a very hard coating and the pressed seeds were falling apart and were not compact by any means.

The hemp cake was ground to a fine powder. Around 6 grams were taken and the exact amount was measured accurately on the electronic balance (FR-200 MK II, AND, Japan) with a precision of d = 0.1 mg. The grounded cake was put into a cotton extraction thimble and enclosed by cotton padding. The boiling stones were placed into an extraction beaker and the beaker was weighted. The extraction thimble with a magnetic ring was attached to the apparatus. The beakers were filled with about 100 ml of petroleum ether and placed into their positions on the extractor's hotplate. The cold water flowing in the top of apparatus assured the condensation of vaporized petroleum ether and its return to the cycle. When the solvent began to boil, the thimbles with powdered seeds were emerged into it for 120 minutes. All of the oil remaining in the plant material was dissolved in the petroleum ether. After this time the thimbles were lifted into a position "washing" and left for 60 minutes. Then the valve was closed for the recovery of the solvent, which lasted about an hour.

The beakers with oil were put into the oven (KBC G100/250, Premed, Poland) for about 30 minutes to let the remaining petroleum ether evaporate, then put into a desiccator to cool and weighted. The content of residual oil in hemp cake was calculated as shown in the Equation 2.

$$\text{Hemp cake residual oil [\%]} = \frac{m_2 - m_1}{m_{PM}} * 100$$

m_1 ... weight of beaker before extraction

m_2 ... weight of beaker (with oil) after extraction

m_{PM} ... weight of the plant material used

Equation 2. Residual oil in hemp cake

Each hemp cake obtained by the previous pressing was tested one time, 162 samples were tested in total.

3.4. Protein analysis

The protein content of the oil-free hemp cake was measured by the Kjeldahl method. The testing took place in a laboratory of the Faculty of Environmental Sciences, CULS, Prague.

The procedure was done in accordance with the norm ČSN 46 1011–18 (2003). One gram of oil-free hemp cake was weighted with the precision of 1 mg and added to the mineralizing tube. Two tablets (3.5 g K_2SO_4 + 3.5 mg Se) were added, followed by addition of 20 ml of concentrated H_2SO_4 and everything was mixed. Tubes were then inserted into a mineralizing block, a device which provided constant heating to 420 °C. Two tubes without plant material were also put as a "blind sample". The mineralization went on for approximately 105 minutes, until the liquid became transparent. Tubes were then left to cool down. Each tube was inserted into automatic distillation unit. The apparatus added 50 ml of distilled water. An automatic distillation by water vapour with an addition of 80 ml of 40 % NaOH commenced. Emerging ammonia was captured into 30 ml of 1 % H_3BO_3 with Tashiro's indicator. The amount of ammonia was measured by titration by 0.2 N H_2SO_4.

The moisture content of the oil-free hemp cake was measured by moisture analyser MAC 110 (Radwag®, Czech Republic).

A standard conversion factor 6.25 was used to convert nitrogen content to protein content in a calculation, complying with the research of Mattila (2018). The protein content of oil-free hemp cake was calculated as shown in the Equation 3.

$$\text{Protein content [\%]} = \frac{0.28 * 6.25 * \text{consumption of } H_2SO_4 * \text{factor } H_2SO_4}{100 - \text{moisture content in \%}} * 100$$

Equation 3. Protein content in dry oil-free hemp cake

Variety Białobrzeskie was tested for pressing temperatures 20 °, 40 ° and 50 °C, since no significant effect of pressing temperature or force on protein content was found, other varieties were further tested only for pressing temperatures 20 ° and 50 °C.

Each variety was tested at least twelve times, 114 samples were tested in total.

3.4.1. Calculated protein content in a raw seed

Additionally, the approximate protein content in a raw seed was calculated as shown in the Equation 4. See the section Limitations.

$$\text{Protein content in a raw seed [\%]} = \frac{100 - \text{TOC [\%]}}{100} \times \text{PCiDOFS [\%]}$$

TOC ... Total oil content

PCiDOFS ... Protein content in dry oil-free seed

Equation 4. Calculated protein content in a raw seed

3.5. Fatty acid profile

Oil extracted from hemp cake was subjected to fatty acid profile analysis by chromatography method. Each combination of pressing conditions (in total 9 per variety) was examined in quadruplicate. The testing took place in a laboratory of Faculty of Environmental Sciences.

3.5.1. Sample preparation

Oil extracted from each hemp cake by petroleum ether was stored in an Eppendorf® tube in a freezer.

100 µl of hemp oil was transferred to 10 or 25 ml volumetric flask. 0.5 ml of petroleum ether (Penta, CZ) and 0.5 ml of benzene (Chemapol, CZ) were added. 1 ml of derivatization agent (0.4 M methanolic NaH, both delivered by Sigma Aldrich, CZ) was added. Samples were shaken and let stand for 20 minutes at room temperature. Approximately 10 ml of distilled water were added into the volumetric flask, shaken and let stand overnight to separate the organic layer. Then 50 µl of the organic layer was transferred into a vial, diluted with 950 µl of hexane and stored in a fridge before further analysis.

3.5.2. Identification of fatty acid composition

The fatty acid composition was determined by the analysis of samples by GC-MS (GC – Agilent Technologies 7890A, MS – Agilent Technologies 5975C, USA).

Separation was executed on Restek 13199 RT-2560 (99.5 m x 250 μm x 0.2 μm) column. Helium was used as carrier gas at constant flow of 1.2 ml/min. A 1 μl sample was injected with a split ratio 50:1 at 225 °C. The oven temperature was at 70 °C for 2 min, then raised by 5 °C/min to 225 °C and held for 9 min, then raised by 10 °C/min to 240 °C and held 2 min. MSD Transfer Line temperature was set up to 150 °C. Scan parameters of MS were set to low mass 40.0 and high mass 400.0. The temperature was set to 230 °C for MS Source and 150 °C for MS Quad. The run time was 45.5 min. For the identification of FA the measured spectra and retention times were compared with available standard Supelco® 37 FAME mix (Sigma Aldrich, CZ), spectra were also compared with data in NIST database, SW version 2.0.

3.5.3. Relative quantification of fatty acids

Quantification of FA was performed on GC-FID (Agilent 7890A). The separation was done on the same column as described above. Helium was used as carrier gas at constant flow 1.5 ml/min. A 0.2 μl sample was injected with a split ratio 50:1 at 225 °C. The oven temperature was at 100 °C for 2 min, then raised by 5 °C/min to 240 °C and held for 3 min. The run time was 33 min.

3.6. Statistical analysis

Data obtained by experiments were analysed by three-way ANOVA. Post-hoc Tukey's HSD test was used to evaluate differences between hemp varieties. Analyses were conducted at 95 % confidence level ($p < 0.05$). Software programs IBM® SPSS® Statistics 25 for analyses and MS Excel® 2016 for graphs and calculations were used.

4. Results and discussion

4.1. Oil pressing

The pressing by forces 100 kN, 200 kN and 300 kN resulted in the pressure of circa 35.4 MPa, 70.7 MPa and 106.1 MPa, respectively. The total amount of work done by pressing at forces 100 kN, 200 kN and 300 kN equalled to circa 770 J, 1 050 J and 1 320 J, respectively.

The oil yield has been rising with increasing temperature and force, as can be seen in Figure 2. See appendix *(pages II – VI)* for figures of oil yield of all varieties.

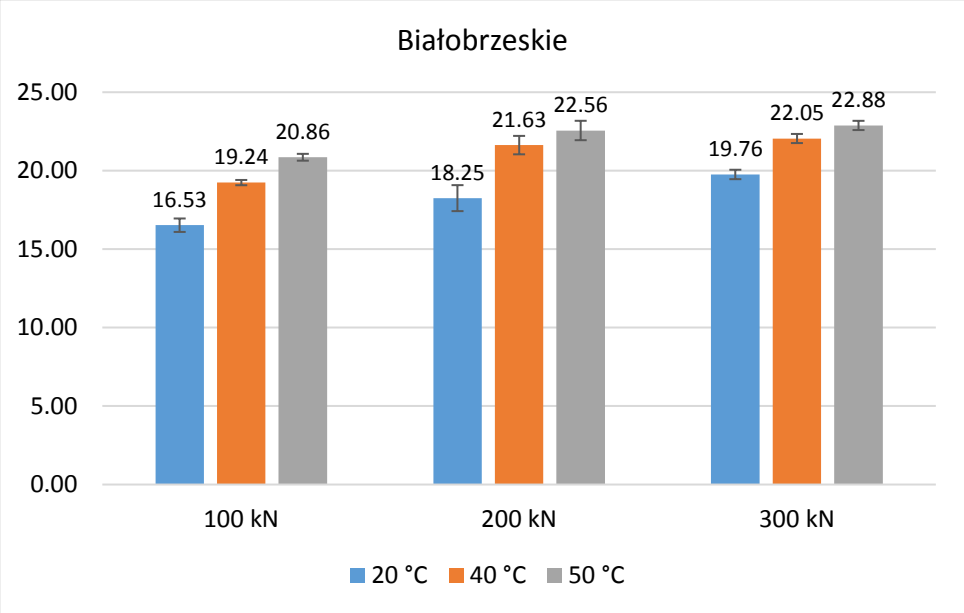

Figure 2. Oil yield (%) of variety Białobrzeskie

The difference between oil yields among varieties is shown in Table 2 which illustrates the average oil yield from all 18 pressings.

Table 2. Average oil yield

	Oil yield (%)	
	Mean	Std. Dev.
Białobrzeskie	20.42	2.13 a
Fedora	19.66	2.40 b
Santhica	15.86	2.00 f
Fibrol	19.83	2.60 b
Finola	18.85	2.31 c
Futura	18.40	2.24 d
Tiborszállási	18.41	2.32 d
Kompolti hibrid	17.68	2.13 e
KC Virtus	17.61	2.05 e

Yields of varieties followed by the same letter were not significantly different according to Tukey's HSD test at α=0.05

The influence of hemp variety, the temperature during pressing and force of pressing on oil yield was confirmed by statistical software. Tukey's HSD test at 95 % confidence level confirmed that higher temperature resulted in higher oil yield. Higher pressure resulted in higher oil yield as well. Therefore the first three hypotheses formulated in the Aims section are confirmed and accepted as true.

The mean yield of all varieties was lowest at 100 kN and 20 °C (14.34 ± 1.23 %) and highest at 300 kN and 50 °C (21.55 ± 1.46 %).

The lowest average oil yield from all pressings was in variety Santhica (15.86 ± 2.00 %). The highest average yield from all pressings was from variety Białobrzeskie (20.42 ± 2.13 %).

The results of the cold-pressing in this study are lower than the oil yield figures (26.9 - 31.5 %) reported by Anwar (2006), even though he reports the range of pressure 35 – 50 MPa only. That is probably due to three factors. The first is the higher temperature (60 °C) used in his study. Second is the time, as his pressing took 20 minutes. In this study, the hydraulic press was retracted 60 seconds after the desired pressure was reached. The last factor, possibly influencing the oil yield, can be the different moisture content of the seeds. Both the duration of the pressing and the moisture content of the seeds are known to be the factors affecting the oil yield (Hickox 1953).

4.2. Residual oil in hemp cake

Unsurprisingly the residual oil was negatively correlating with the amount of oil obtained by pressing. The higher temperature and higher force applied during pressing, the less oil remained in hemp cake, as can be seen in Figure 3. See appendix *(pages VII – IX)* for figures of residual oil of all varieties.

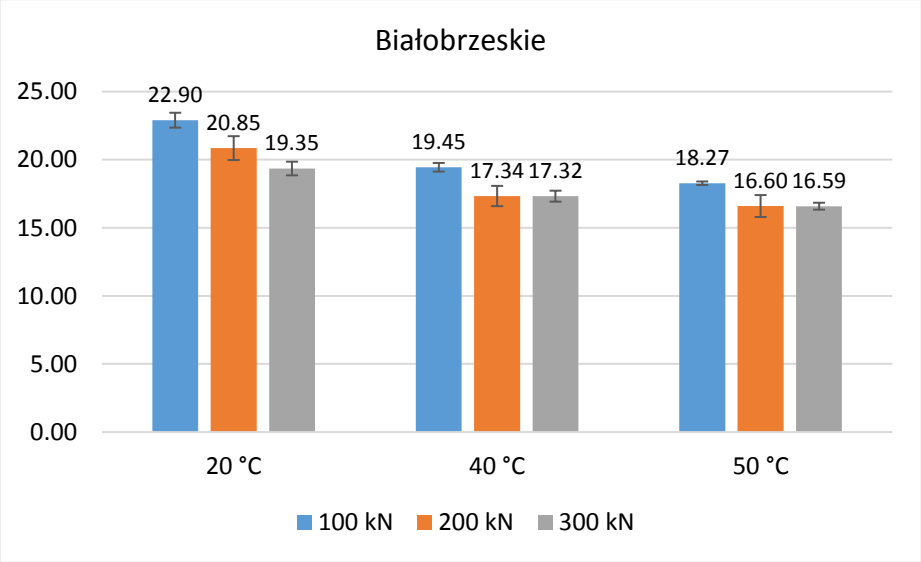

Figure 3. Residual oil (%) in hemp cakes of variety Białobrzeskie

Influence of variety, temperature and force were statistically significant. Tukey's HSD test at 95 % confidence level confirmed that higher temperature during pressing led to a lower amount of residual oil. Also, the higher the force applied during pressing, the lower was residual oil in hemp cake. Fourth and fifth hypotheses formulated in Aims section are also accepted as true.

Table 3. Average residual oil in hemp cakes

	Residual oil (%)	
	Mean	Std. Dev.
Białobrzeskie	18.74	2.14 c
Fedora	17.48	2.37 d
Santhica	19.48	1.99 b
Fibrol	20.31	2.41 a
Finola	19.43	2.27 b
Futura	19.12	2.14 bc
Tiborszállási	19.00	2.02 bc
Kompolti hibrid	18.55	1.96 c
KC Virtus	18.81	1.87 c

Residual oil of varieties followed by same letter were not significantly different according to Tukey's HSD test at α=0.05

The lowest mean residual oil remained in hemp cake after pressing conditions 300 kN and 50 °C (17.75 ± 1.69 %) and highest mean residual oil was after pressing by force 100 kN at 20 °C (22.81 ± 0.72 %).

The lowest mean residual oil was found in variety Fedora (17.48 ± 2.37 %) and the highest amount of oil remained in hemp cake of variety Fibrol (20.31 ± 2.41 %), as can be seen in Table 3.

These findings agree with a literature review only partially. Sladký (2004) states that hemp cake contains more than 10 % residual oil. While this is de facto true, the lowest residual oil (14.00 %) recorded in variety Fedora after pressing at temperature 50 °C is still 4 % more than the lowest number mentioned by Sladký. According to Miovský (2008), hemp cake contains 10 to 20 % of residual oil. A lot of samples exceeded 20 % of residual oil, the highest residual oil was found in variety Fibrol after pressing at temperature 20 °C and force 100 kN (24.35 %). It can be speculated, that the use of higher pressing forces and temperatures usually occurs in agricultural practice and therefore literature findings do not exceed 20 % of residual oil in hemp cake.

4.3. Total oil content

Total oil content stated in Table 4 was calculated as the sum of oil yielded by pressing and residual oil in hemp cake recalculated to content in a raw seed. This allows a better comparison with literature figures.

Table 4. Oil content of seeds

	Total oil (%)	
	Mean	Std. Dev.
Białobrzeskie	35.37	0.35 b
Fedora	33.76	0.23 d
Santhica	32.29	0.30 f
Fibrol	36.17	0.53 a
Finola	34.66	0.29 c
Futura	34.04	0.61 d
Tiborszállási	33.95	0.42 d
Kompolti hibrid	32.99	0.52 e
KC Virtus	33.14	0.43 e

The oil content of varieties followed by the same letter was not significantly different according to Tukey's HSD test at α=0.05

Lowest oil was found in Santhica, which is not very surprising because it is a variety grown for fibre. Still the measured 32 % oil content fairly exceeds 26 % mentioned by website Konopko (2015), the only figure about Santhica's oil content that had been found during literature research.

The highest oil was in Fibrol variety, which again exceeds the figure 27.5 % stated by Baldini (2018). Notice that although Białobrzeskie variety surpassed Fibrol in oil yield from pressing, the Fibrol variety had almost one percent higher total oil content. Compare also figures of average oil yield for varieties Fedora (19.66 ± 2.40 %) and Finola (18.85 ± 2.31) from Table 2 with figures of total oil content in Table 4 (33.76 ± 0.23 % and 34.66 ± 0.29 %, respectively). This indicates, that seeds of different varieties respond to pressing differently and that higher total oil content is not a guarantee of higher oil yield from pressing.

Białobrzeskie and Fedora emerged from the experimental testing as the two most promising varieties in relation to their pressing characteristics and oil content.

Most of the results were (sometimes significantly) higher than the figures found in the literature. The only exception is Finola variety. Overall, obtained results concurred the most with experimental findings of Kriese (2004).

4.4. Protein content

4.4.1. Protein content in oil-free dry seed matter

The results of the analysis of protein content in oil-free dry seed matter are presented in Table 5.

Table 5. Protein content of the oil-free dry seed

	Protein content (%)	
	Mean	Std. Dev.
Białobrzeskie	37.27	1.23 c
Fedora	36.20	0.84 d
Santhica	39.08	0.82 a
Fibrol	38.91	0.50 ab
Finola	38.84	0.95 ab
Futura	38.65	1.34 ab
Tiborszállási	38.35	0.79 b
Kompolti hibrid	34.88	1.07 e
KC Virtus	37.20	0.73 c

The protein content of varieties followed by the same letter was not significantly different according to Tukey's HSD test at α=0.05

The highest protein content was found in the variety Santhica and the lowest in Kompolti hibrid.

Variety Białobrzeskie was tested by means of Tukey's HSD test at confidence level 95 %. There were no significant effects of varying temperature or force during pressing on the protein content in the hemp cake. Statistical analysis did not discover any significant effect of pressing conditions on protein content. The sixth hypothesis was rejected.

4.4.2. Calculated protein content in a raw seed

For the purpose of comparison with literature research, protein content in dry oil-free seed matter was recalculated to approximate content in raw seeds. Figures are shown in Table 6. See the section Limitations.

Table 6. Calculated protein content in a raw seed

	Calculated protein (%)	
	Mean	Std. Dev.
Białobrzeskie	21.83	0.65
Fedora	22.27	0.51
Santhica	24.44	0.54
Fibrol	22.86	0.33
Finola	23.48	0.64
Futura	23.49	0.69
Tiborszállási	23.42	0.49
Kompolti hibrid	21.25	0.78
KC Virtus	23.05	0.48

Calculated values correspond with figures of protein content 20 – 25 % mentioned by Miovský (2008). Protein content 15 – 16 % in seeds stated by Procházka (1991) seems at least questionable.

4.5. Fatty acids

Through the means of gas chromatography analysis, ten fatty acids have been found out in hemp cake residual oil in total. Nine were successfully identified, one remains unknown. A comparison with findings of Da Porto (2012) can lead to speculation that this unknown fatty acid was possibly an eicosenoic acid (C20:1).

Moudrý's (2011) list of fatty acids had proved to be very limited.

Results of fatty acids composition analysis for different pressing conditions of variety Białobrzeskie can be seen in Table 7. Total procentual content of polyunsaturated fatty acids, monounsaturated fatty acids, saturated fatty acids, the ratio between polyunsaturated/saturated FA, the content of essential FA and the ratio between omega 6 and omega 3 were calculated. It can be seen from this results, that the lowest (thus the best) ω-6 / ω-3 essential fatty acids ratio for this variety (3.61) was found to be at pressing conditions 40 °C and 300 kN or 50 °C and 200 kN. If compared to results of the analysis

of variety Felina by Da Porto, Felina variety had ω-6 / ω-3 EFA ratio (3.29) lower than variety Białobrzeskie and therefore should be more beneficial for human consumption (Simopoulos 2002) than variety Białobrzeskie. See appendix *(pages XI - XV)* for figures of fatty acids composition of all varieties.

Average fatty acids composition is presented in Table 8.

Residual oil from hemp cakes of variety Kompolti hibrid had the lowest ω-6 / ω-3 EFA ratio (on average 3.06) and therefore Kompolti hibrid seems like the most suitable variety for consumption in terms of health effect (Simopoulos 2002). Kompolti hibrid was also the best in terms of essential fatty acids content (on average 73.99 %). Variety Finola had also fairly low average ω-6 / ω-3 EFA ratio (3.13) and had the highest average content of polyunsaturated fatty acids (76.72 %). Owing to this quality, variety Finola could become the most promising source of oil used as a drying oil in varnishes and paints (Oomah et al. 2002).

As a curiosity, the FA profile of the dried oil had been measured. Some samples (pressed at temperature 20 °C, variety Futura) had dried out due to mishandling after the solvent extraction and the experiment had to be repeated. Profile of fatty acids was nevertheless determined and can be found in the Table 9 in the appendix (*page X*). Changes in the favour of saturated fatty acids and parallel decline of polyunsaturated fatty acids content can be noticed.

Table 7. FA profile of hemp cakes residual oil of variety Białobrzeskie

Variety	Białobrzeskie																	
Temperature	20 °C						40 °C						50 °C					
Force	100 kN		200 kN		300 kN		100 kN		200 kN		300 kN		100 kN		200 kN		300 kN	
Fatty acid composition (%)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Palmitic acid (C16:0)	6.40	0.01	6.47	0.05	6.57	0.01	6.36	0.02	6.43	0.03	6.46	0.01	6.50	0.00	6.50	0.01	6.48	0.03
Stearic acid (C18:0)	3.03	0.00	3.00	0.01	3.09	0.01	3.06	0.01	3.00	0.02	2.98	0.00	3.06	0.01	3.04	0.00	3.02	0.01
Oleic acid (C18:1)	12.56	0.01	12.38	0.09	12.55	0.08	12.59	0.01	12.43	0.10	12.29	0.02	12.47	0.04	12.36	0.00	12.37	0.02
Elaidic acid (trans-C18:1)	0.83	0.00	0.85	0.01	0.86	0.00	0.84	0.01	0.86	0.01	0.87	0.00	0.87	0.00	0.88	0.00	0.88	0.00
Linoleic acid (C18:2ω6)	55.54	0.03	55.69	0.02	55.75	0.01	55.62	0.05	55.68	0.03	55.69	0.07	55.54	0.02	55.56	0.02	55.68	0.06
Arachidic (C20:0)	0.91	0.00	0.91	0.00	0.92	0.00	0.90	0.00	0.89	0.00	0.90	0.00	0.91	0.00	0.91	0.01	0.90	0.00
γ-Linolenic acid (C18:3ω6)	3.05	0.03	3.05	0.02	3.06	0.03	3.07	0.02	3.04	0.01	3.06	0.01	3.03	0.01	3.02	0.01	3.02	0.01
α-Linolenic acid (C18:3ω3)	15.31	0.04	15.31	0.04	14.87	0.05	15.25	0.07	15.36	0.09	15.43	0.06	15.30	0.03	15.40	0.01	15.34	0.06
Unknown acid	0.93	0.00	0.92	0.00	0.91	0.01	0.93	0.00	0.92	0.00	0.93	0.00	0.92	0.00	0.92	0.00	0.92	0.00
Behenic acid (C22:0)	0.35	0.00	0.35	0.00	0.35	0.00	0.34	0.00	0.34	0.00	0.35	0.00	0.35	0.00	0.35	0.00	0.35	0.00
EFAs sum	70.85		71.00		70.63		70.87		71.04		71.12		70.84		70.96		71.02	
ω-6/ω-3 ratio	3.63		3.64		3.75		3.65		3.63		3.61		3.63		3.61		3.63	
PUFAs sum	73.90		74.05		73.69		73.93		74.08		74.17		73.87		73.99		74.04	
Monounsaturated	13.39		13.23		13.41		13.43		13.29		13.16		13.34		13.23		13.25	
Saturated	10.68		10.74		10.93		10.66		10.66		10.68		10.82		10.80		10.75	
Polyunsaturated/saturated ratio	6.92		6.89		6.74		6.94		6.95		6.94		6.83		6.85		6.89	

Table 8. Average FA profile of hemp cakes residual oil of all varieties

	Fatty acid profile of hemp cake residual oil of different varieties (average from all pressing conditions)																	
Variety	Białobrzeskie		Fedora		Santhica		Fibrol		Finola		Futura		Tiborszállási		Kompolti hibrid		KC Virtus	
Fatty acid composition (%)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Palmitic acid (C16:0)	6.46	0.06 d	6.76	0.15 c	7.13	0.45 b	7.23	0.39 c	6.77	0.42 c	7.28	0.86 c	7.28	0.31 c	6.05	0.14 e	6.45	0.19 d
Stearic acid (C18:0)	3.03	0.03 c	2.97	0.18 d	3.00	0.24 cd	3.12	0.16 b	2.74	0.17 f	3.46	0.41 a	3.14	0.13 b	2.59	0.06 g	2.83	0.08 e
Oleic acid (C18:1)	12.44	0.10 d	11.41	0.11 e	13.23	1.42 c	14.34	0.30 a	9.52	0.35 f	14.01	1.05 b	14.02	0.41 b	13.36	0.16 c	13.32	0.18 c
Elaidic acid (trans-C18:1)	0.86	0.02 c	0.90	0.03 b	0.96	0.02 a	0.96	0.04 a	0.91	0.04 b	0.93	0.08 ab	0.84	0.09 cd	0.80	0.01 e	0.82	0.01 de
Linoleic acid (C18:2ω6)	55.64	0.07 ab	55.62	0.56 ab	55.46	0.75 b	55.42	0.50 b	54.84	0.28 cd	54.77	0.79 d	55.09	0.25 c	55.78	0.12 a	55.85	0.17 a
Arachidic (C20:0)	0.91	0.01 d	0.94	0.08 c	0.94	0.09 c	0.99	0.06 b	1.04	0.07 a	0.97	0.11 b	1.00	0.04 b	0.78	0.02 f	0.87	0.03 e
γ-Linolenic acid (C18:3ω6)	3.04	0.02 b	2.94	0.46 c	1.81	0.33 d	1.59	0.05 e	4.35	0.16 a	1.85	0.20 d	1.83	0.09 d	1.16	0.02 f	1.49	0.03 e
α-Linolenic acid (C18:3ω3)	15.29	0.16 e	16.04	0.48 d	15.38	1.02 e	15.27	0.61 e	17.53	0.51 b	15.01	1.28 f	15.31	0.50 e	18.22	0.26 a	17.12	0.29 c
Unknown acid	0.92	0.01 a	0.94	0.12 a	0.60	0.13 b	0.18	0.15 e	0.29	0.18 de	0.35	0.05 cd	0.32	0.14 cde	0.44	0.10 c	0.33	0.07 cd
Behenic acid (C22:0)	0.35	0.00 e	0.38	0.02 de	0.39	0.03 de	0.40	0.16 d	1.36	0.09 a	0.52	0.14 bc	0.54	0.05 b	0.42	0.01 d	0.49	0.02 c
EFAs sum	70.93		71.65		70.84		70.69		72.37		69.77		70.40		73.99		72.97	
ω-6/ω-3 ratio	3.64		3.47		3.61		3.63		3.13		3.65		3.60		3.06		3.26	
PUFAs sum	73.97		74.59		72.64		72.28		76.72		71.62		72.23		75.15		74.46	
Monounsaturated	13.30		12.31		14.19		15.30		10.44		14.94		14.85		14.16		14.14	
Saturated	10.75		11.04		11.47		11.73		11.92		12.23		11.94		9.85		10.64	
Polyunsaturated/ saturated ratio	6.88		6.75		6.33		6.16		6.44		5.85		6.05		7.63		7.00	

Means of a FA followed by the same letter were not significantly different among varieties according to Tukey's HSD test at α=0.05

4.6. Limitations known to the author

The calculated protein content of whole seeds is of an approximate value and functions just as an orientation value for better comparison with literature. The exact moisture of seeds was not measured before pressing but during the protein analysis. The moisture content changed (increased) during the storage in the fridge. According to moisture measurement by master's student Rebeka Hadlová, the moisture content of seeds ranged between 3.4 – 3.7 % in raw seeds. Author's measurements of moisture after storage found out moisture usually exceeded 7 %. Therefore the author suggests to readers to rather look at the protein content of oil-free dry seed matter to gather reliable figures.

The temperature 20 °C during pressing should be rather looked upon as a "room temperature". As it has been already stated, there were no means available to ensure that the temperature was always exactly 20 °C and it is possible that this temperature raised up to 25 °C.

5. Conclusions

The thesis proved the influence of pressing temperature and pressing force on oil yield.

Oil yield from pressing at different conditions, residual oil in hemp cakes and protein in dry oil-free hemp cake were measured for nine technical hemp varieties.

Fatty acid profiles of these varieties were also analysed. Ten fatty acids were found and nine of them were successfully identified.

The gas chromatography analysis of fatty acid profile brought out variety Finola as the best for use as a drying oil. Variety Kompolti hibrid was established as the best for human consumption for its highest essential fatty acids content and lowest omega 6 / omega 3 essential fatty acids ratio. Kompolti hibrid's high quality oil is however balanced by a rather small amount of oil in the seed and the lowest measured protein content of the seed. The Englishmen say: "*You can't have your cake and eat it too".* This holds true also for the qualities of seeds - there is no variety which can excel in all measured traits simultaneously. The consideration of the purpose of use is crucial for choosing the right variety to grow.

Overall oil content was found to be highest in seeds of variety Fibrol (36.17 ± 0.53 %). The results have shown that the varieties do not respond to the pressing equally. It was proven, that higher total oil content *per se* does not mean higher oil yield from pressing.

Out of these nine varieties, variety Białobrzeskie and Fedora are the most suitable for pressing in terms of oil yield and they can be recommended to practice where the maximization of yields is an aim.

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Appendices

List of the Appendices:

Appendix 1: Figures of oil yield (%) from pressing

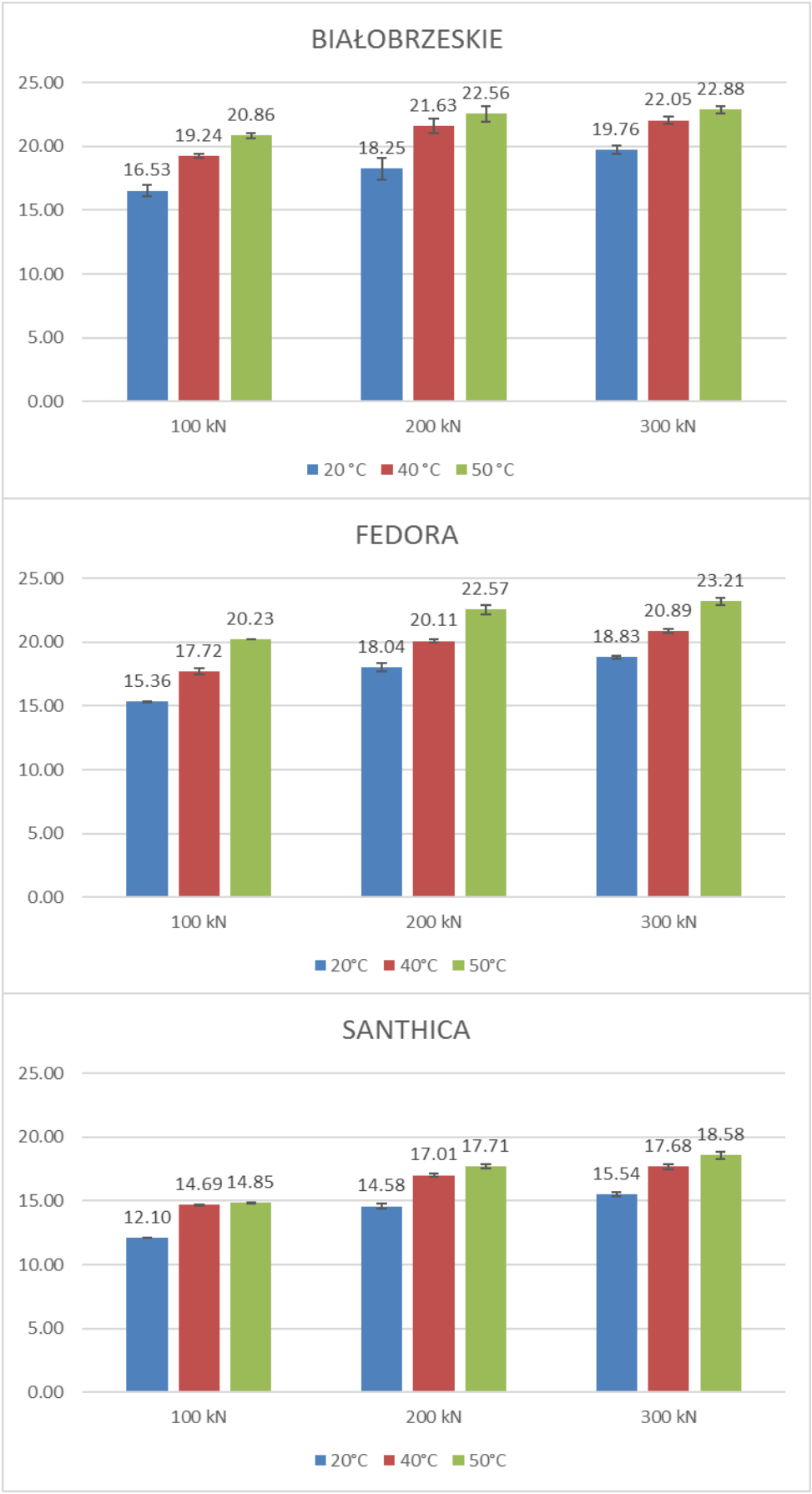

Figure 4. Oil yield (%) of variety a) Białobrzeskie b) Fedora c) Santhica

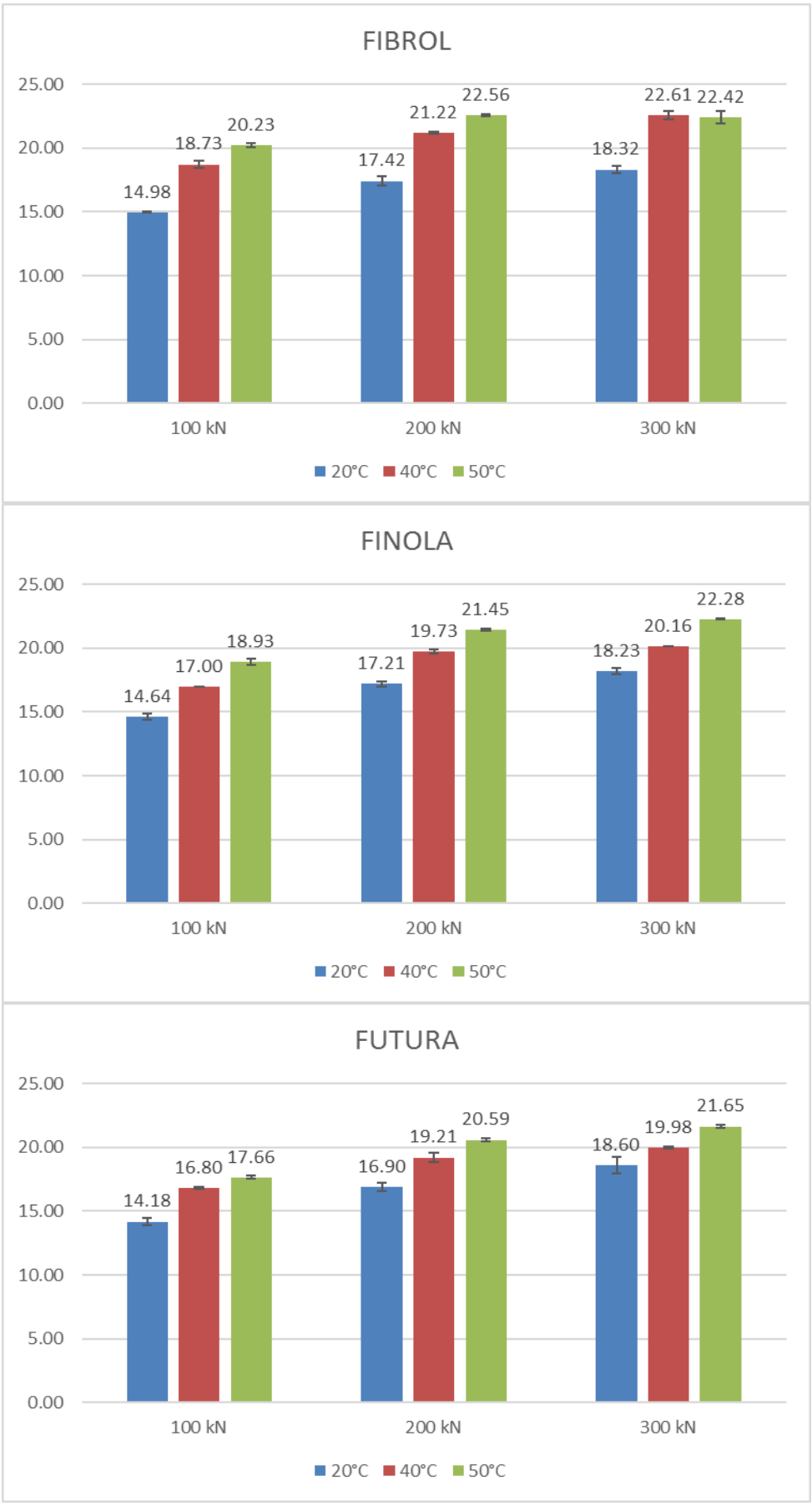

Figure 5. Oil yield (%) of variety a) Fibrol b) Finola c) Futura

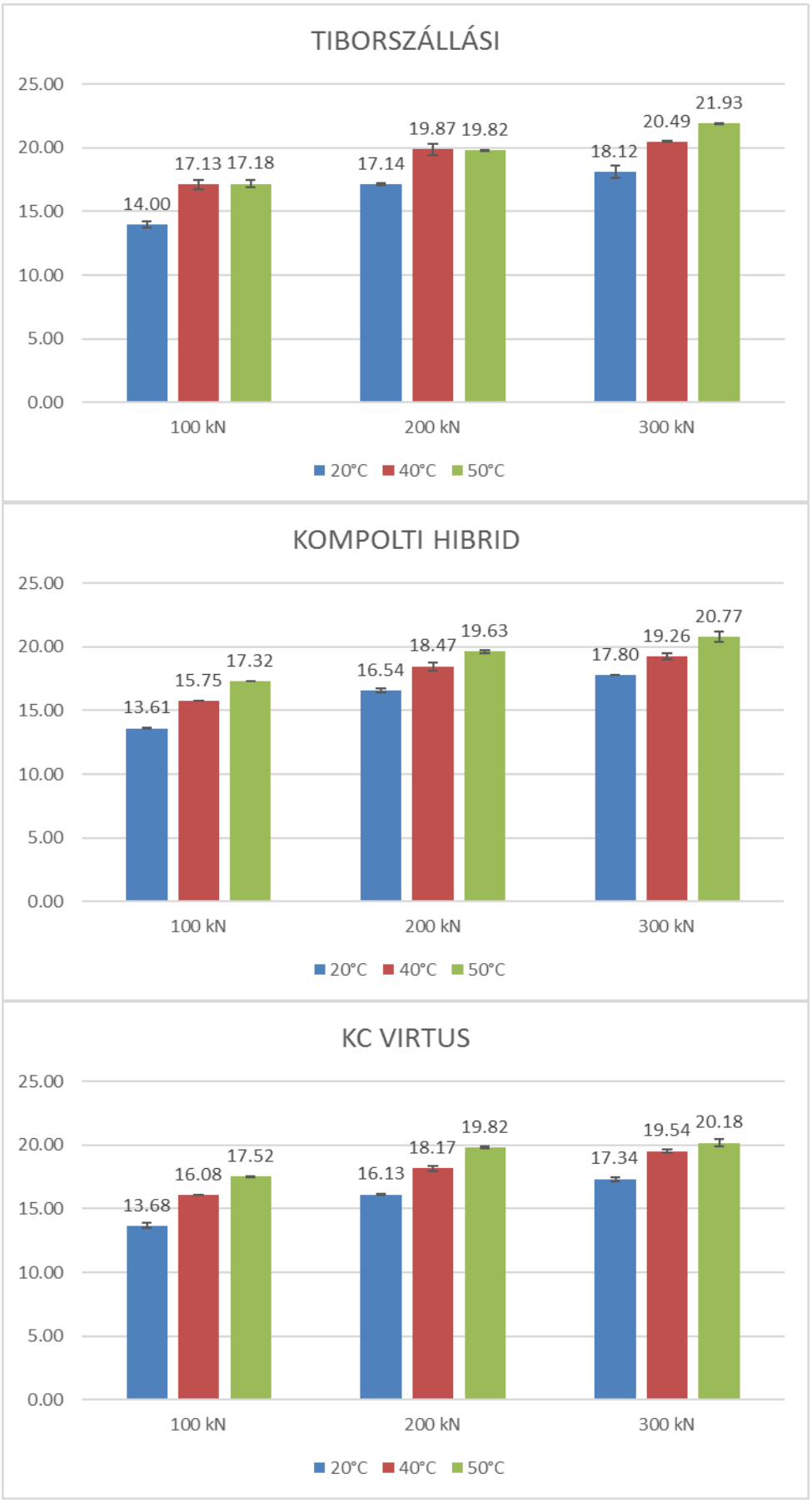

Figure 6. Oil yield (%) of variety a) Tiborszállási b) Kompolti hibrid c) KC Virtus

Figure 7. Oil yield (%) of all varieties at force a) 100 kN v) 200 kN c) 300 kN

v

Figure 8. Oil yield (%) of all varieties at temperature a) 20 °C b) 40 °C c) 50 °C

Appendix 2: Figures of hemp cake residual oil (%) (by variety)

Figure 9. Residual oil (%) in hemp cakes of variety a) Białobrzeskie b) Fedora c) Santhica

Figure 10. Residual oil (%) in hemp cakes of variety a) Fibrol b) Finola c) Futura

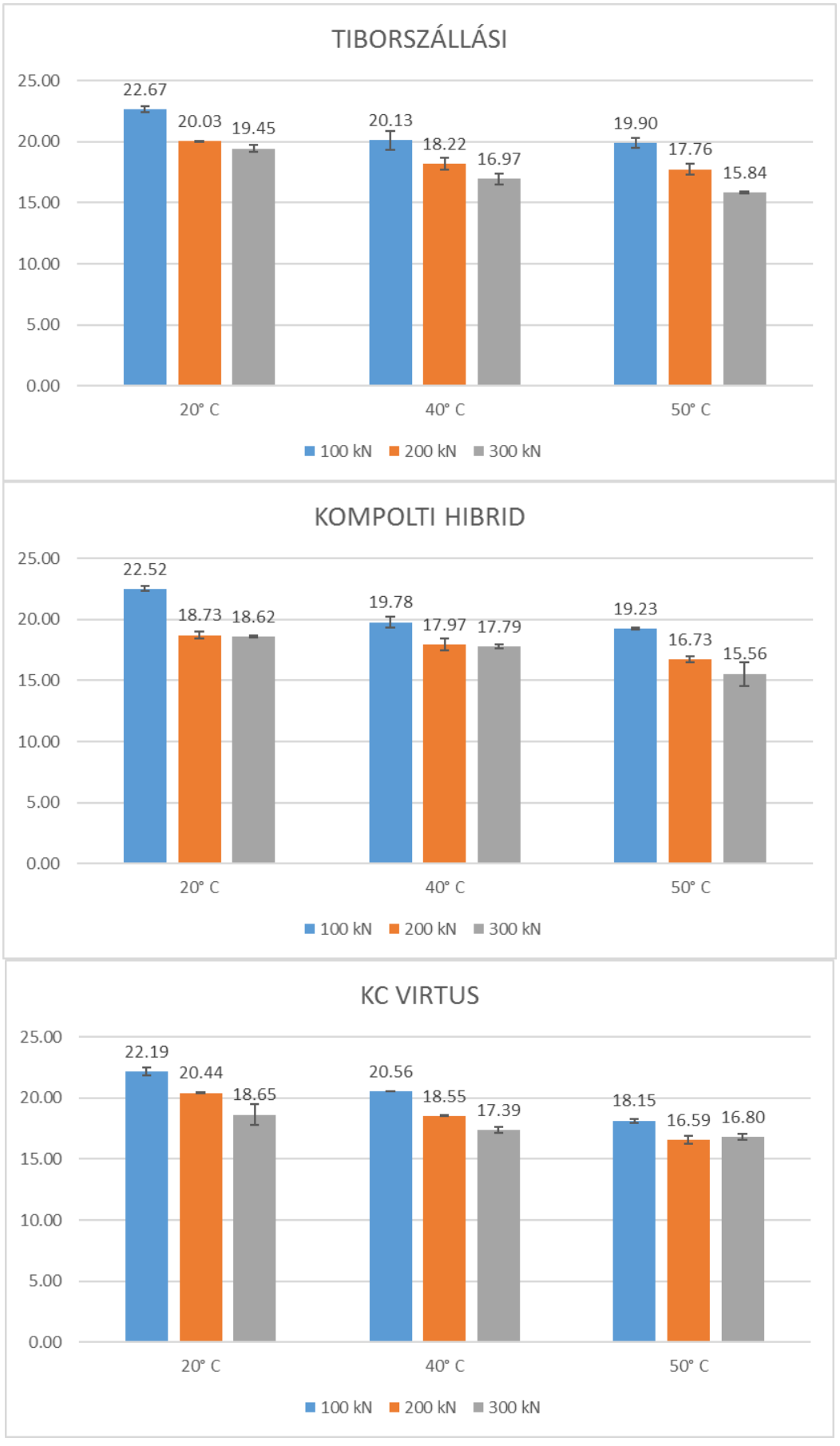

Figure 11. Residual oil (%) in hemp cakes of variety a) Tiborszállási b) Kompolti hibrid c) KC Virtus

Appendix 3: Fatty acid profile of hemp cake residual oil (by variety)

Table 9. FA profile of the dried oil, variety Futura

Variety	Dried oil, variety Futura					
Temperature	20 °C					
Force	100 kN		200 kN		300 kN	
Fatty acid composition (%)	Mean	SD	Mean	SD	Mean	SD
Palmitic acid (C16:0)	15.92	2.43	16.43	0.62	15.35	1.63
Stearic acid (C18:0)	7.36	1.30	7.55	0.24	7.11	0.84
Oleic acid (C18:1)	24.57	3.25	24.82	0.68	24.42	2.05
Elaidic acid (trans-C18:1)	1.63	0.22	1.66	0.02	1.63	0.15
Linoleic acid (C18:2ω6)	32.09	2.56	30.28	2.18	33.43	3.79
Arachidic (C20:0)	2.11	0.38	2.29	0.21	2.12	0.18
γ-Linolenic acid (C18:3ω6)	0.15	0.27	0.43	0.04	0.48	0.11
α-Linolenic acid (C18:3ω3)	4.26	0.81	3.80	0.50	4.30	0.82
Unknown acid	0.21	0.36	0.13	0.22	0.42	0.29
Behenic acid (C22:0)	0.00	0.00	0.00	0.00	0.00	0.00
EFAs sum	36.34		34.08		37.72	
ω-6/ω-3 ratio	7.54		7.97		7.78	
PUFAs sum	36.50		34.50		38.20	
Monounsaturated	26.20		26.48		26.05	
Saturated	25.39		26.27		24.58	
Polyunsaturated/saturated ratio	1.44		1.31		1.55	

Table 10. FA profile of hemp cakes residual oil of variety a) Białobrzeskie b) Fedora

Variety	Białobrzeskie															
Temperature	20 °C						40 °C						50 °C			
Force	100 kN		200 kN		300 kN		100 kN		200 kN		300 kN		100 kN		200 kN		300 kN	
Fatty acid composition (%)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Palmitic acid (C16:0)	6.40	0.01	6.47	0.05	6.57	0.01	6.36	0.02	6.43	0.03	6.46	0.01	6.50	0.00	6.50	0.01	6.48	0.03
Stearic acid (C18:0)	3.03	0.00	3.00	0.01	3.09	0.01	3.06	0.01	3.00	0.02	2.98	0.00	3.06	0.01	3.04	0.00	3.02	0.01
Oleic acid (C18:1)	12.56	0.01	12.38	0.09	12.55	0.08	12.59	0.01	12.43	0.10	12.29	0.02	12.47	0.04	12.36	0.00	12.37	0.02
Elaidic acid (trans-C18:1)	0.83	0.00	0.85	0.01	0.86	0.00	0.84	0.01	0.86	0.01	0.87	0.00	0.87	0.00	0.88	0.00	0.88	0.00
Linoleic acid (C18:2ω6)	55.54	0.03	55.69	0.02	55.75	0.01	55.62	0.05	55.68	0.03	55.69	0.07	55.54	0.02	55.56	0.02	55.68	0.06
Arachidic (C20:0)	0.91	0.00	0.91	0.00	0.92	0.00	0.90	0.00	0.89	0.00	0.90	0.00	0.91	0.00	0.91	0.01	0.90	0.00
γ-Linolenic acid (C18:3ω6)	3.05	0.03	3.05	0.02	3.06	0.03	3.07	0.02	3.04	0.01	3.06	0.01	3.03	0.01	3.02	0.01	3.02	0.01
α-Linolenic acid (C18:3ω3)	15.31	0.04	15.31	0.04	14.87	0.05	15.25	0.07	15.36	0.09	15.43	0.06	15.30	0.03	15.40	0.01	15.34	0.06
Unknown acid	0.93	0.00	0.92	0.00	0.91	0.01	0.93	0.00	0.92	0.00	0.93	0.00	0.92	0.00	0.92	0.00	0.92	0.00
Behenic acid (C22:0)	0.35	0.00	0.35	0.00	0.35	0.00	0.34	0.00	0.34	0.00	0.35	0.00	0.35	0.00	0.35	0.00	0.35	0.00
EFAs sum	70.85		71.00		70.63		70.87		71.04		71.12		70.84		70.96		71.02	
ω-6/ω-3 ratio	3.63		3.64		3.75		3.65		3.63		3.61		3.63		3.61		3.63	
PUFAs sum	73.90		74.05		73.69		73.93		74.08		74.17		73.87		73.99		74.04	
Monounsaturated	13.39		13.23		13.41		13.43		13.29		13.16		13.34		13.23		13.25	
Saturated	10.68		10.74		10.93		10.66		10.66		10.68		10.82		10.80		10.75	
Polyunsaturated/saturated ratio	6.92		6.89		6.74		6.94		6.95		6.94		6.83		6.85		6.89	
Variety	Fedora															
Temperature	20 °C						40 °C						50 °C			
Force	100 kN		200 kN		300 kN		100 kN		200 kN		300 kN		100 kN		200 kN		300 kN	
Fatty acid composition (%)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Palmitic acid (C16:0)	6.90	0.01	6.89	0.06	7.02	0.07	6.63	0.02	6.52	0.06	6.74	0.06	6.69	0.00	6.64	0.01	6.77	0.01
Stearic acid (C18:0)	3.14	0.01	3.11	0.05	3.18	0.03	3.07	0.02	3.01	0.02	3.07	0.05	2.73	0.01	2.69	0.01	2.73	0.00
Oleic acid (C18:1)	11.44	0.04	11.36	0.05	11.55	0.03	11.58	0.04	11.40	0.06	11.50	0.10	11.36	0.04	11.20	0.06	11.30	0.01
Elaidic acid (trans-C18:1)	0.88	0.00	0.89	0.00	0.89	0.01	0.87	0.00	0.87	0.01	0.89	0.00	0.94	0.00	0.94	0.01	0.96	0.01
Linoleic acid (C18:2ω6)	55.07	0.01	55.19	0.10	55.20	0.04	55.21	0.05	55.39	0.05	55.33	0.04	56.38	0.07	56.37	0.01	56.43	0.01
Arachidic (C20:0)	1.01	0.00	1.01	0.01	1.03	0.01	0.97	0.01	0.96	0.01	0.98	0.01	0.83	0.01	0.82	0.00	0.83	0.00
γ-Linolenic acid (C18:3ω6)	3.25	0.00	3.27	0.04	3.21	0.02	3.25	0.01	3.31	0.03	3.26	0.01	2.31	0.01	2.27	0.02	2.28	0.01
α-Linolenic acid (C18:3ω3)	15.75	0.03	15.72	0.04	15.34	0.10	15.85	0.05	15.98	0.14	15.68	0.19	16.58	0.02	16.90	0.00	16.52	0.01
Unknown acid	1.03	0.01	1.03	0.01	0.99	0.01	1.03	0.00	1.06	0.02	1.02	0.01	0.78	0.00	0.79	0.01	0.77	0.00
Behenic acid (C22:0)	0.39	0.00	0.40	0.00	0.41	0.00	0.38	0.00	0.38	0.00	0.39	0.00	0.36	0.00	0.36	0.00	0.37	0.00
EFAs sum	70.82		70.90		70.54		71.06		71.37		71.01		72.96		73.27		72.96	
ω-6/ω-3 ratio	3.50		3.51		3.60		3.48		3.47		3.53		3.40		3.34		3.41	
PUFAs sum	74.07		74.17		73.76		74.32		74.68		74.27		75.27		75.55		75.23	
Monounsaturated	12.32		12.24		12.44		12.45		12.27		12.38		12.30		12.13		12.26	
Saturated	11.44		11.40		11.63		11.06		10.86		11.17		10.62		10.51		10.70	
Polyunsaturated/saturated ratio	6.47		6.50		6.34		6.72		6.87		6.65		7.09		7.19		7.03	

Table 11. FA profile of hemp cakes residual oil of variety a) Santhica b) Fibrol

Variety	Santhica																	
Temperature	20 °C						40 °C						50 °C					
Force	100 kN		200 kN		300 kN		100 kN		200 kN		300 kN		100 kN		200 kN		300 kN	
Fatty acid composition (%)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Palmitic acid (C16:0)	6.74	0.03	6.77	0.02	6.76	0.01	7.74	0.09	7.72	0.06	7.83	0.04	6.82	0.01	6.88	0.05	6.92	0.04
Stearic acid (C18:0)	2.71	0.03	2.73	0.01	2.72	0.02	3.30	0.03	3.29	0.05	3.34	0.02	2.99	0.01	2.98	0.01	2.98	0.00
Oleic acid (C18:1)	11.26	0.08	11.32	0.10	11.18	0.13	14.56	0.06	14.42	0.14	14.56	0.06	14.02	0.03	13.89	0.14	13.87	0.02
Elaidic acid (trans-C18:1)	0.95	0.00	0.96	0.00	0.96	0.00	0.99	0.01	0.98	0.01	0.99	0.00	0.93	0.01	0.93	0.01	0.95	0.01
Linoleic acid (C18:2ω6)	56.46	0.04	56.39	0.06	56.46	0.08	54.63	0.04	54.64	0.11	54.59	0.02	55.27	0.07	55.33	0.07	55.32	0.02
Arachidic (C20:0)	0.83	0.01	0.84	0.01	0.84	0.00	1.04	0.01	1.05	0.01	1.06	0.01	0.93	0.00	0.94	0.01	0.94	0.01
γ-Linolenic acid (C18:3ω6)	2.29	0.02	2.27	0.01	2.26	0.01	1.56	0.06	1.56	0.01	1.49	0.01	1.62	0.01	1.61	0.01	1.60	0.02
α-Linolenic acid (C18:3ω3)	16.57	0.09	16.53	0.08	16.64	0.11	14.07	0.13	14.22	0.16	13.99	0.10	15.47	0.06	15.48	0.14	15.46	0.00
Unknown acid	0.78	0.01	0.77	0.01	0.77	0.00	0.48	0.02	0.48	0.01	0.46	0.00	0.54	0.00	0.54	0.00	0.54	0.00
Behenic acid (C22:0)	0.36	0.00	0.37	0.00	0.37	0.00	0.43	0.00	0.43	0.00	0.43	0.00	0.37	0.00	0.38	0.00	0.38	0.00
EFAs sum	73.03		72.92		73.10		68.70		68.86		68.58		70.74		70.81		70.78	
ω-6/ω-3 ratio	3.41		3.41		3.39		3.88		3.84		3.90		3.57		3.57		3.58	
PUFAs sum	75.33		75.19		75.36		70.26		70.41		70.07		72.37		72.42		72.38	
Monounsaturated	12.21		12.28		12.14		15.55		15.40		15.55		14.94		14.82		14.81	
Saturated	10.65		10.71		10.68		12.51		12.48		12.67		11.11		11.18		11.22	
Polyunsaturated/saturated ratio	7.07		7.02		7.05		5.62		5.64		5.53		6.51		6.48		6.45	
Variety	Fibrol																	
Temperature	20 °C						40 °C						50 °C					
Force	100 kN		200 kN		300 kN		100 kN		200 kN		300 kN		100 kN		200 kN		300 kN	
Fatty acid composition (%)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Palmitic acid (C16:0)	7.70	0.14	7.76	0.12	7.84	0.04	7.00	0.01	7.03	0.06	7.15	0.04	6.80	0.02	6.86	0.05	6.91	0.05
Stearic acid (C18:0)	3.35	0.18	3.31	0.06	3.35	0.04	3.07	0.03	3.07	0.04	3.07	0.03	2.97	0.03	2.97	0.02	2.96	0.04
Oleic acid (C18:1)	14.44	0.47	14.67	0.20	14.74	0.09	14.59	0.06	14.49	0.15	14.25	0.06	14.06	0.08	13.95	0.14	13.91	0.05
Elaidic acid (trans-C18:1)	0.97	0.05	1.00	0.02	1.03	0.03	0.95	0.03	0.95	0.01	0.93	0.02	0.92	0.02	0.93	0.02	0.92	0.02
Linoleic acid (C18:2ω6)	54.09	1.78	55.43	0.32	55.13	0.31	55.68	0.26	55.61	0.16	55.72	0.20	55.61	0.21	55.69	0.17	55.78	0.12
Arachidic (C20:0)	1.06	0.05	1.06	0.03	1.09	0.02	0.94	0.01	0.96	0.02	0.95	0.01	0.93	0.01	0.96	0.05	0.93	0.02
γ-Linolenic acid (C18:3ω6)	1.51	0.06	1.60	0.07	1.49	0.03	1.67	0.07	1.63	0.03	1.58	0.01	1.62	0.03	1.62	0.02	1.60	0.02
α-Linolenic acid (C18:3ω3)	14.34	0.45	14.82	0.29	14.70	0.21	14.97	0.09	15.25	0.05	15.20	0.04	16.02	0.08	16.09	0.15	16.08	0.02
Unknown acid	0.21	0.36	0.00	0.00	0.44	0.28	0.36	0.22	0.17	0.30	0.27	0.27	0.15	0.25	0.00	0.00	0.00	0.00
Behenic acid (C22:0)	0.34	0.19	0.12	0.21	0.10	0.18	0.48	0.01	0.48	0.01	0.48	0.01	0.52	0.02	0.52	0.02	0.52	0.03
EFAs sum	68.44		70.25		69.83		70.64		70.86		70.92		71.63		71.78		71.86	
ω-6/ω-3 ratio	3.77		3.74		3.75		3.72		3.65		3.66		3.47		3.46		3.47	
PUFAs sum	69.95		71.84		71.32		72.32		72.49		72.50		73.26		73.40		73.46	
Monounsaturated	15.41		15.67		15.77		15.54		15.44		15.18		14.98		14.88		14.83	
Saturated	12.44		12.25		12.37		11.49		11.55		11.65		11.22		11.31		11.33	
Polyunsaturated/saturated ratio	5.62		5.86		5.76		6.30		6.28		6.22		6.53		6.49		6.49	

Table 12. FA profile of hemp cakes residual oil of variety a) Finola b) Futura

Variety	Finola																	
Temperature	20 °C						40 °C						50 °C					
Force	100 kN		200 kN		300 kN		100 kN		200 kN		300 kN		100 kN		200 kN		300 kN	
Fatty acid composition (%)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Palmitic acid (C16:0)	7.10	0.11	7.19	0.05	7.65	0.40	6.52	0.07	6.61	0.11	6.67	0.05	6.43	0.04	6.43	0.03	6.34	0.13
Stearic acid (C18:0)	2.92	0.06	2.90	0.02	3.08	0.16	2.65	0.03	2.68	0.04	2.68	0.03	2.61	0.02	2.61	0.01	2.56	0.08
Oleic acid (C18:1)	9.79	0.14	9.78	0.03	10.29	0.33	9.37	0.02	9.38	0.10	9.42	0.04	9.29	0.03	9.30	0.04	9.09	0.27
Elaidic acid (trans-C18:1)	0.93	0.05	0.94	0.02	1.01	0.05	0.89	0.02	0.90	0.01	0.92	0.01	0.88	0.01	0.89	0.01	0.87	0.01
Linoleic acid (C18:2ω6)	54.84	0.07	54.86	0.11	54.68	0.28	54.98	0.31	55.03	0.23	54.93	0.10	55.09	0.11	55.03	0.11	54.11	1.43
Arachidic (C20:0)	1.10	0.01	1.11	0.02	1.18	0.06	1.01	0.02	1.02	0.02	1.03	0.02	0.98	0.02	0.99	0.02	0.97	0.04
γ-Linolenic acid (C18:3ω6)	4.27	0.06	4.20	0.07	3.99	0.20	4.47	0.05	4.45	0.05	4.41	0.03	4.48	0.05	4.46	0.04	4.45	0.10
α-Linolenic acid (C18:3ω3)	17.28	0.26	17.26	0.04	16.29	0.64	17.87	0.05	17.73	0.20	17.60	0.03	17.89	0.06	18.04	0.13	17.84	0.44
Unknown acid	0.00	0.00	0.00	0.00	0.18	0.30	0.35	0.22	0.34	0.21	0.49	0.03	0.49	0.07	0.44	0.05	0.34	0.21
Behenic acid (C22:0)	1.31	0.05	1.29	0.03	1.14	0.08	1.43	0.03	1.41	0.03	1.39	0.03	1.43	0.03	1.41	0.02	1.41	0.06
EFAs sum	72.12		72.11		70.97		72.85		72.76		72.53		72.98		73.07		71.95	
ω-6/ω-3 ratio	3.17		3.18		3.36		3.08		3.10		3.12		3.08		3.05		3.03	
PUFAs sum	76.38		76.31		74.96		77.32		77.21		76.95		77.46		77.53		76.40	
Monounsaturated	10.72		10.72		11.31		10.26		10.28		10.34		10.17		10.18		9.96	
Saturated	12.42		12.49		13.05		11.61		11.72		11.77		11.46		11.44		11.28	
Polyunsaturated/saturated ratio	6.15		6.11		5.74		6.66		6.59		6.54		6.76		6.78		6.77	
Variety	Futura																	
Temperature	20 °C						40 °C						50 °C					
Force	100 kN		200 kN		300 kN		100 kN		200 kN		300 kN		100 kN		200 kN		300 kN	
Fatty acid composition (%)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Palmitic acid (C16:0)	6.46	0.05	6.57	0.07	6.44	0.06	6.72	0.01	7.29	0.45	6.88	0.06	7.76	0.09	8.60	0.30	8.80	0.32
Stearic acid (C18:0)	3.08	0.03	3.11	0.03	3.04	0.05	3.22	0.02	3.50	0.21	3.26	0.06	3.70	0.06	4.09	0.15	4.20	0.15
Oleic acid (C18:1)	13.02	0.09	13.16	0.03	12.76	0.24	13.41	0.19	14.41	0.76	13.47	0.17	14.49	0.22	15.47	0.44	15.89	0.49
Elaidic acid (trans-C18:1)	0.86	0.02	0.86	0.00	0.86	0.01	0.88	0.04	0.96	0.06	0.89	0.02	0.97	0.04	1.04	0.05	1.07	0.06
Linoleic acid (C18:2ω6)	55.14	0.36	55.68	0.17	55.18	0.62	55.05	0.88	54.88	0.44	55.50	0.21	54.73	0.14	53.24	0.61	53.51	0.13
Arachidic (C20:0)	0.89	0.05	0.84	0.00	0.83	0.01	0.97	0.09	0.98	0.06	0.92	0.03	1.03	0.01	1.13	0.05	1.17	0.05
γ-Linolenic acid (C18:3ω6)	2.18	0.22	2.01	0.03	1.98	0.03	1.91	0.03	1.76	0.13	1.93	0.02	1.77	0.04	1.59	0.06	1.52	0.04
α-Linolenic acid (C18:3ω3)	16.03	0.07	16.27	0.18	16.44	0.22	15.69	0.28	14.75	0.96	15.66	0.25	14.41	0.31	13.04	0.50	12.78	0.60
Unknown acid	0.32	0.26	0.38	0.10	0.43	0.20	0.30	0.19	0.36	0.26	0.38	0.22	0.25	0.25	0.34	0.21	0.37	0.22
Behenic acid (C22:0)	0.63	0.02	0.63	0.01	0.66	0.08	0.58	0.01	0.50	0.08	0.57	0.02	0.48	0.02	0.41	0.03	0.20	0.20
EFAs sum	71.17		71.94		71.61		70.74		69.63		71.16		69.14		66.28		66.29	
ω-6/ω-3 ratio	3.44		3.42		3.36		3.51		3.72		3.55		3.80		4.08		4.19	
PUFAs sum	73.35		73.95		73.59		72.65		71.38		73.09		70.91		67.86		67.81	
Monounsaturated	13.88		14.02		13.62		14.29		15.37		14.36		15.46		16.51		16.95	
Saturated	11.06		11.15		10.98		11.48		12.27		11.62		12.96		14.22		14.37	
Polyunsaturated/saturated ratio	6.63		6.63		6.70		6.33		5.82		6.29		5.47		4.77		4.72	

Table 13. FA profile of hemp cakes residual oil of variety a) Tiborszállási b) Kompolti hibrid

Variety	Tiborszállási																	
Temperature	20 °C						40 °C						50 °C					
Force	100 kN		200 kN		300 kN		100 kN		200 kN		300 kN		100 kN		200 kN		300 kN	
Fatty acid composition (%)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Palmitic acid (C16:0)	7.07	0.01	7.31	0.08	7.21	0.15	7.48	0.05	7.63	0.01	7.85	0.29	6.83	0.12	6.92	0.14	7.18	0.10
Stearic acid (C18:0)	3.06	0.02	3.12	0.03	3.10	0.08	3.24	0.01	3.30	0.02	3.35	0.10	2.97	0.04	2.98	0.06	3.10	0.04
Oleic acid (C18:1)	13.88	0.06	13.88	0.03	13.92	0.21	14.15	0.04	14.46	0.05	14.91	0.51	13.55	0.22	13.55	0.16	13.85	0.06
Elaidic acid (trans-C18:1)	0.84	0.02	0.88	0.02	0.87	0.02	0.88	0.02	0.90	0.01	0.60	0.42	0.84	0.02	0.85	0.02	0.87	0.01
Linoleic acid (C18:2ω6)	55.47	0.07	55.29	0.21	55.19	0.23	55.03	0.05	54.78	0.20	54.63	0.33	54.94	0.83	55.27	0.19	55.21	0.21
Arachidic (C20:0)	0.96	0.01	0.98	0.02	0.98	0.03	1.02	0.02	1.04	0.02	1.09	0.06	0.96	0.02	0.95	0.03	0.99	0.03
γ-Linolenic acid (C18:3ω6)	1.88	0.01	1.86	0.02	1.81	0.03	1.79	0.01	1.74	0.02	1.66	0.05	1.98	0.20	1.91	0.09	1.83	0.05
α-Linolenic acid (C18:3ω3)	15.79	0.11	15.60	0.14	15.43	0.20	15.07	0.10	14.81	0.07	14.22	0.71	15.63	0.26	15.91	0.36	15.35	0.12
Unknown acid	0.07	0.13	0.13	0.22	0.38	0.26	0.41	0.04	0.31	0.22	0.25	0.18	0.36	0.21	0.46	0.10	0.49	0.06
Behenic acid (C22:0)	0.57	0.01	0.54	0.01	0.54	0.01	0.51	0.01	0.50	0.01	0.43	0.06	0.63	0.11	0.57	0.01	0.53	0.01
EFAs sum	71.26		70.89		70.62		70.10		69.59		68.86		70.57		71.18		70.56	
ω-6/ω-3 ratio	3.51		3.55		3.58		3.65		3.70		3.84		3.52		3.47		3.60	
PUFAs sum	73.14		72.74		72.43		71.88		71.33		70.52		72.54		73.08		72.40	
Monounsaturated	14.72		14.76		14.79		15.03		15.37		15.51		14.39		14.39		14.72	
Saturated	11.66		11.95		11.84		12.25		12.47		12.72		11.39		11.41		11.80	
Polyunsaturated/saturated ratio	6.27		6.09		6.12		5.87		5.72		5.54		6.37		6.40		6.13	
Variety	Kompolti hibrid																	
Temperature	20 °C						40 °C						50 °C					
Force	100 kN		200 kN		300 kN		100 kN		200 kN		300 kN		100 kN		200 kN		300 kN	
Fatty acid composition (%)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Palmitic acid (C16:0)	5.99	0.05	6.06	0.03	6.08	0.03	6.11	0.03	6.27	0.07	6.24	0.07	5.88	0.02	5.89	0.05	5.91	0.02
Stearic acid (C18:0)	2.53	0.03	2.59	0.01	2.58	0.03	2.64	0.01	2.70	0.02	2.69	0.02	2.54	0.02	2.55	0.01	2.54	0.01
Oleic acid (C18:1)	13.30	0.09	13.29	0.13	13.32	0.08	13.65	0.18	13.56	0.04	13.49	0.12	13.17	0.07	13.27	0.07	13.19	0.05
Elaidic acid (trans-C18:1)	0.79	0.01	0.79	0.01	0.80	0.01	0.80	0.01	0.81	0.01	0.81	0.01	0.79	0.02	0.80	0.01	0.79	0.02
Linoleic acid (C18:2ω6)	55.60	0.48	55.81	0.09	55.72	0.19	55.69	0.15	55.64	0.16	55.81	0.10	55.84	0.15	55.98	0.08	55.91	0.11
Arachidic (C20:0)	0.77	0.01	0.79	0.01	0.79	0.01	0.80	0.01	0.81	0.02	0.81	0.01	0.76	0.00	0.77	0.01	0.77	0.01
γ-Linolenic acid (C18:3ω6)	1.16	0.03	1.19	0.01	1.17	0.00	1.13	0.01	1.15	0.01	1.13	0.02	1.17	0.01	1.16	0.01	1.17	0.01
α-Linolenic acid (C18:3ω3)	18.28	0.19	18.38	0.12	18.18	0.06	17.90	0.07	17.88	0.09	17.87	0.19	18.50	0.20	18.51	0.18	18.46	0.12
Unknown acid	0.65	0.62	0.33	0.08	0.47	0.14	0.45	0.17	0.35	0.21	0.38	0.20	0.53	0.17	0.31	0.06	0.49	0.10
Behenic acid (C22:0)	0.42	0.02	0.43	0.01	0.42	0.01	0.41	0.01	0.41	0.01	0.41	0.01	0.43	0.01	0.42	0.01	0.43	0.01
EFAs sum	73.87		74.19		73.89		73.59		73.52		73.68		74.34		74.49		74.37	
ω-6/ω-3 ratio	3.04		3.04		3.07		3.11		3.11		3.12		3.02		3.02		3.03	
PUFAs sum	75.03		75.38		75.07		74.72		74.66		74.81		75.51		75.65		75.54	
Monounsaturated	14.09		14.08		14.12		14.45		14.36		14.30		13.96		14.07		13.99	
Saturated	9.71		9.86		9.87		9.96		10.19		10.14		9.61		9.63		9.65	
Polyunsaturated/saturated ratio	7.73		7.64		7.61		7.50		7.33		7.38		7.85		7.86		7.83	

Table 14. FA profile of hemp cakes residual oil of variety KC Virtus

Variety	KC Virtus															
Temperature	20 °C						40 °C						50 °C			
Force	100 kN		200 kN		300 kN		100 kN		200 kN		300 kN		100 kN		200 kN		300 kN	
Fatty acid composition (%)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Palmitic acid (C16:0)	6.32	0.02	6.29	0.01	6.33	0.00	6.64	0.04	6.73	0.05	6.77	0.04	6.31	0.02	6.32	0.05	6.37	0.05
Stearic acid (C18:0)	2.76	0.01	2.75	0.01	2.78	0.02	2.91	0.01	2.95	0.04	2.98	0.01	2.79	0.01	2.79	0.02	2.78	0.02
Oleic acid (C18:1)	13.38	0.06	12.99	0.17	13.22	0.03	13.47	0.17	13.51	0.11	13.62	0.05	13.25	0.07	13.19	0.09	13.24	0.11
Elaidic acid (trans-C18:1)	0.81	0.02	0.81	0.01	0.80	0.00	0.83	0.02	0.83	0.02	0.84	0.02	0.82	0.01	0.81	0.01	0.81	0.02
Linoleic acid (C18:2ω6)	56.03	0.13	55.95	0.13	56.08	0.02	55.72	0.10	55.56	0.21	55.65	0.22	55.97	0.15	55.82	0.35	55.86	0.25
Arachidic (C20:0)	0.87	0.01	0.85	0.02	0.86	0.00	0.90	0.00	0.90	0.01	0.92	0.01	0.86	0.01	0.85	0.01	0.86	0.01
γ-Linolenic acid (C18:3ω6)	1.53	0.01	1.52	0.01	1.50	0.02	1.47	0.01	1.45	0.01	1.45	0.02	1.52	0.03	1.50	0.01	1.48	0.02
α-Linolenic acid (C18:3ω3)	17.21	0.07	17.65	0.16	17.21	0.08	16.80	0.16	16.79	0.09	16.67	0.05	17.27	0.12	17.33	0.10	17.13	0.16
Unknown acid	0.22	0.23	0.27	0.16	0.36	0.02	0.42	0.17	0.36	0.23	0.25	0.27	0.30	0.20	0.42	0.35	0.37	0.21
Behenic acid (C22:0)	0.49	0.00	0.51	0.01	0.48	0.00	0.47	0.02	0.46	0.01	0.46	0.01	0.50	0.01	0.49	0.01	0.49	0.01
EFAs sum	73.24		73.60		73.29		72.52		72.35		72.32		73.24		73.15		72.99	
ω-6/ω-3 ratio	3.25		3.17		3.26		3.32		3.31		3.34		3.24		3.22		3.26	
PUFAs sum	74.77		75.12		74.79		73.99		73.81		73.77		74.76		74.65		74.47	
Monounsaturated	14.19		13.80		14.02		14.30		14.34		14.47		14.07		14.00		14.06	
Saturated	10.44		10.40		10.46		10.92		11.04		11.13		10.46		10.45		10.50	
Polyunsaturated/saturated ratio	7.16		7.22		7.15		6.78		6.68		6.63		7.15		7.14		7.09	

Appendix 4: Photodocumentation

Figure 12. Cleaning of the seeds by Farmet® air blower (Source: Author)

Figure 13. Disassembled metallic cylinder used for pressing (Notice the heating sleeve with the red wire) (Source: Author)

Figure 14. TEMPOS® ZDM 50 hydraulic press capable of creating a force of 500 kN

(Source: Author)

Figure 15. Assembled metallic cylinder (Source: Author)

Figure 16. Hemp seeds did not proved to be as resistant to moths as stated by Conrad (2007). The seeds stored in laboratory for further pressing were attacked by moth larvae (Source: Author)

Figure 17. Hemp cakes were stored in a fridge before extraction (Source: Author)

Figure 18. SER148 Solvent Extraction Unit (Source: Author)

Figure 19. Agilent 7890A (Source: Author)

Figure 20. Samples in vials prepared for GC (Source: Author)