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Faculty of Tropical AgriSciences

Quality evaluation of cold-pressed hemp seed oil of industrial hemp varieties (*Cannabis sativa* L.)

MASTER'S THESIS

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CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

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DIPLOMA THESIS ASSIGNMENT

Bc. Rebeka Hadlová

Tropical Crop Management and Ecology

Thesis title

Quality evaluation of cold-pressed hemp seed oil of industrial hemp varieties (Cannabis sativa L.)

Objectives of thesis

The thesis is focusing on sensoric qualities of oil obtained from different varieties of technical hemp. It deals with technological processing and analysing freshly pressed hemp oil and comparing it with already stored samples. Among the quality analysis belong sensorics, chemical, physical analysis and oxidative stability.

Methodology

Analysing the sensoric qualities of hemp oil based on determination of peroxide value, iodine value, acidity value, saponification value and ester value, comparing the results in different time intervals and storage conditions.

The proposed extent of the thesis

60 str

Keywords

hemp oil, sensorics analyses, peroxide value, acidity value, iodine value, ester value, saponification value, cannabis sativa, technical hemp

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Lawless HT, Heymann H. 2010. Sensory Evaluation of Food: Principles and Practices.
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Declaration

I hereby declare that I have done this thesis entitled “Quality evaluation of cold-pressed hemp seed oil of industrial hemp varieties (*Cannabis sativa* L.)” 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 Faculty of Tropical AgriSciences, CULS.

In Prague 26th April

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Rebeka Hadlová

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Abstract

Hemp oil is becoming more and more valuable thanks to its content of polyunsaturated fatty acids, α-linolenic acid and linoleic acid. And also, the fact of relatively low content of saturated fatty acids. Thanks to its high amount of unsaturated fatty acid and chlorophyll, the hemp oil prone to rancidity. The process of devaluation of oil is fasten up by heat and light, therefore it should be stored in dark cold place.

The diploma thesis focused on determination of changes occurring in hemp oil obtained from 9 different varieties of *Cannabis sativa* L., namely: Sathica, Futura, Fedora, Fibrol, Kompolti hybrid, KC Virtus, Bialobrzeskie, Tiborsallasi and Finola. Oil samples were stored under different storage conditions: under the room temperature and in the fridge.

Chemical properties of the samples were investigated several times over 24 weeks by peroxide value, iodine value, saponification value, acidity value, ester value, when degustation panel assessed organoleptic properties of freshly prepared oil samples.

The experiments pointed out that the refrigerated samples were more stable, and their shelf life was prolong. The overall most stable variety was proven to be Bialobrzeskie. It has the lowest values or was at the top of the lowest value. That goes for both type of storage. The highest values occur in case of iodine value, but still was not that far from general mean. On the other side of the scale, with the most repetition of high values was variety Fibrol for room conditions and Finola for cool conditions. But still was not that far on the scale from other varieties. In case of sensory evaluation as the most tasteful variety was also evaluated Bialobrzeskie.

After all, the experiments showed that longer shelf life, overall oil quality and decreasing inside processes (oxidation, changing colour, rancidity) is provided by correct storage at lower temperatures.

Key words: hemp seed oil, sensory analyses, peroxide value, acidity value, iodine value, ester value, saponification value, cannabis sativa, technical hemp.

Abstrakt

Popularita konopného oleje roste díky množství polynenasycených mastných kyselin, kyseliny linolové a kyseliny α-linolenové, ale i díky relativně nízkému obsahu nasycených mastných kyselin. Pro vysoký obsah nenasycených mastných kyselin a chlorofylu dochází u konopného oleje k častému žluknutí. Tento nechtění proces degradace je urychlen teplem a světlem a ptoto je dobré olej skladovat na temných a chladných místech.

Diplomová práce se zaměřila na sledování změn konopného oleje získaného z 9 odrůd Cannabis sativa L., slovně: Sathica, Futura, Fedora, Fibrol, Kompolti hybrid, KC Virtus, Bialobrzeskie, Tiborsallasi a Finola. Olejové vzorky byly skladovány v jiných podmínkách: v pokojových a lednicových teplotních podmínkách.

Chemické vlastnosti získaných vzorků byly zkoumány několikrát po dobu 24 týdnů. Mezi zkoumané vlastnosti patřilo peroxidové číslo, jódové číslo, číslo zmýdelnění, číslo kyselosti a esterové číslo. Degustační panel se na druhé straně věnoval organoleptickým vlastnostem čerstvě vylisovaných olejových vzorků.

Studie prokázala, že lednicové vzorky jsou více stabilní a jejich životnost je delší. Celkově nejvhodnější odrůda s nejvyšší stabilitou byla Bialobrzeskie. Její hodnoty byly nejnižší, nebo se pohybovaly mezi nejnižšími u obou způsobů skladování. Na druhé straně, s nejvyššími hodnotami pro pokojové podmínky byla odrůda Fibrol a pro lednicové Finola. V rámci degustace se nejlépe umístila, jako nejvíce chutná, Bilobrzeskie a jako nejméně chutná Finola.

Obecně bylo pokusy prokázáno, že delší životnost, celková kvalita oleje a zpomalení vnitřních procesů (oxidace, změna barvy, žluknutí) je způsobeno vhodným skladováním pri nižších teplotách.

Klíčová slova: konopný olej, senzorické analýzy, peroxidové číslo, číslo kyselosti, jódové číslo, esterové číslo, číslo zmýdelnění, cannabis sativa, technické konopí

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List of the abbreviations used in the thesis

AV- Acidity Value

EV- Ester Value

IV- Iodine Value

PV- Peroxide Value

SD- Standard Deviation

SV- Saponification Value

1. Introduction and Literature Review

Cannabis sativa serves as a plant with many different types of usage for many years. It has been part culture and history as a traditional fiber and oil plant. Nowadays it is also being classified as an energy plant. The plant can be found all over the world, from tropical areas to the most northern parts of the world, thanks to its ability to adapt.

The spectrum of use is wide from food industry mostly for oil production, pasta making, bread making and other products, to chemical industry for colours, brines, cements, soaps, shampoos, creams etc. Lot of ecological, high-quality, eco-friendly products are being made from the fiber and leaves. The seeds contain between 22-25% of digestible proteins, all of the essential amino acids and high amount of ω- 3 a ω- 6 acids.

The technical hemp is cultivated to contain as little as possible of psychoactive agents. The amount is lower than 0.3%.

1.1. Role of vegetable oils in human nutrition

Vegetable oils can be obtained either from oilseeds (rapeseed, sunflower seed, cocoa butter) or from legumes (peanut, soybean), fruits (olives, palm oil, rice bran) and nuts (walnuts, almond). After obtaining the oil by pressing the plants or their parts it is processed and cultivated to become high-quality oil suitable for human consumption. The increasing worldwide consumption can be seen in Table 1. (Foster et al. 2009a).

Table 1. Production of four major vegetable oils in % in selected years (Gunstone 2011a).

Oil	1995/96	2000/01	2005/06	2008/09
Palm	22.8	27.1	30.2	32.2
Soybean	28.5	29.7	29.1	27.1
Rapeseed	15.6	14.8	14.6	15.5
Sunflower seed	12.8	9.1	8.9	9.0
Total	79.7	80.7	82.8	83.8

Thanks to rising interest in the biofuels and growing health concerns, the popularity of vegetable oils has rapidly increased over the last 30 years. The main compound of the oils are triacyglycerides, the main nutrient is fat. Another nutrient present in large amounts is vitamin E (tocopherols and tocotrienols). One of the biggest advantages is that vegetable oils are also main source of natural plant sterols and contain minor components (squalene and sphingolipids) known for providing a wide range of health benefits (Foster et al. 2009b).

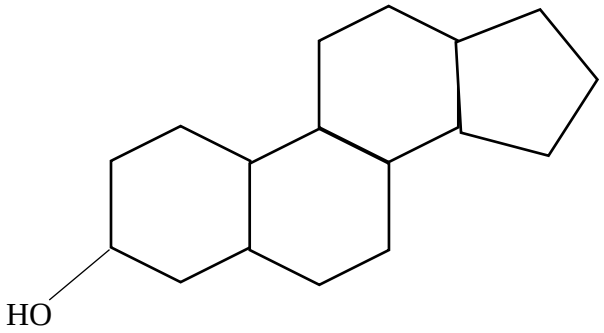

Figure 1. Sterol chemical structure (Author 2019).

Except for palm kernel and coconut oils, which are high in saturates, other oils tend to be high in monounsaturated or polyunsaturated fats. The overall amount of fatty acids in plant oils varies. All of the oils are a mixture of different fatty acids in different proportions (Petrovic 2008).

The content of fatty acids in oils is responsible for the functionality of each type of oil, hand in hand with the minor components. However the properties of the oils can be changed by modification through technical processes including hydrogenation (leads to increased hardness and stabilization of fats without having to increase the amount of saturates to a great extent), fractionation, seed breeding and interesterification (so the oil has functional properties and the desired organoleptic qualities, without the formation of trans fatty acids) (Gunstone 2011a).

As time passes, more and more studies are being conducted delivering increasing evidence that fatty acids are vital component in human nutrition because of their therapeutic and prophylactic function in the approach to many severe diseases such as cardiovascular, inflammation etc, in growth and development of human embryo, brain function. A number of the fatty acids are known to have anticancer potential. The more

studies are done on the importance of fatty acids in human nutrition the more they are gaining attention. It is necessary to mention that fatty acids do not serve just as an essential component of human diet but also have a high potential for industrial use such as production of soaps (can be seen in Figure 2.) and detergents, cosmetics, lubricants, ink, varnish, paints etc. (Kumar et al. 2016).

Figure 2. Bar of olive oil soap (Nummelin 2013).

Having said that, both markets (nutritional and industrial) of oilseed crops are rapidly developing. Thanks to different structures of fatty acid the exceptionality of physico-chemical properties make plants useful and wanted (Kumar et al. 2016).

But there is no evidence suggesting that one vegetable oil is better and more beneficial than any other in terms of additional health benefits. There have been a few high-quality scientific trials comparing the health benefits and outcomes of individuals consuming different oils. It must be said that due to manufacturing procedures using vegetable-based

oils in food industry, most people consume wide range of vegetable oils each day without even realizing it. Usually the oil preferences depend on the functionality of the oil for specific food applications, requirements for taste, personal preference or cost. The crucial thing to remembered is that oil no matter if it contains good or bad ones, is almost 100% fat. Therefore, oil itself and products made from it should only be included in human diet in reasonable, moderate quantities (Foster et al. 2009b).

1.2. Oil extraction

Vegetable oils have been used in different cultures for centuries. The extraction of oil is based on removal of oil components, mostly seeds. There are two main methods; mechanical one using oil mill or chemical using a solvent. The obtained oil is usually purified or, if necessary, can be refined or chemically altered.

Mechanical extraction is also known as “crushing” or “pressing.” This method is usually used to produce the traditional oils (olive, coconut etc.). There are several types of this kind of extraction. Expeller-pressing, with the help of screw press, ram press, and ghani (powered mortar and pestle). Figure 3. and 4. express example of mechanical crusher.

Figure 3. Mechanical crusher use for oil extraction (Author 2018).

Figure 4. Mechanical crusher used for oil extraction (Author 2018).

The solvent extraction is mostly applied in commercial sector using such dissolving agents to produce higher yields. It is faster and cheaper. One of the most known solvents is petroleum-derived hexane. Carbon dioxide can serve as its non-toxic alternative. The newer industrial oils (e.g. soybean, corn oil) are obtained this way.

1.3. Vegetable oils

Combination of vegetable and animal sources, leads to production of 117 million tonnes of world oil and fat production per year. About 80% of the total oil and fat production is used for food purposes, the other 14% is used as a basis of the oleochemical industry and the rest 6% serves as an animal fodder. Figure 5 serves as an illustration of the vegetable oil variety (Olaoluwa R. & Sanni Muideen 2017).

Figure 5. Types of vegetable oils (Sabbah 2016).

There are three main sources of vegetable oil:

- Byproducts. For example, cotton and corn, both plants are primarily cultivated either for cotton processed for fiber or corn processed for cereal. In both examples, the oil is a byproduct. It is similar n case of soybean. Its yield has mostly two purposes: oil and meal. Oil represents 18% and meal 79% of the dried beans. The demand for the soybean sometimes depends on one of these aspects sometimes on the other. Peanuts can also be included, since half of the yield is crushed and processed for oil and meal and the rest is consumed as a nut (Olaoluwa R. & Sanni Muideen 2017)
- Tree crops. The biggest disadvantage is that these crops cannot be annually adjusted based on the current demand. The trees need to be planted and mature before they give useful crop. Afterwards the trees can provide crops for 25-30 years. Sometimes the usefulness can be even longer (in case of olive trees) (Olaoluwa R. & Sanni Muideen 2017)
- Annual crops. Rape, sunflower and linseed belong in this category. The decision which one of the crops to plant fully depends on the farmer's/planter's decision. Usually the choice is either oilseed crop or cereals and depends on agricultural and economic factors (Olaoluwa R. & Sanni Muideen 2017)

Table 2. Production, exports and imports (millions tonnes) of 10 oilseeds and of 17 oils and fats in selected countries in 2000/01 (Gunstone 2011b).

		Oilseed			Oils and fats		
	Population (millions)	Production	Exports	Imports	Production	Exports	Imports
World	6133	306.9	64.7	64.7	117.1	37.7	37.7
Malaysia	23	3.4	-	0.7	13.6	11.5	0.4
Argentina	37	29.6	5.9	-	5.3	4.5	-
Canada	31	10.7	6.3	0.8	2.2	1.1	0.4
Australia	19	2.9	1.8	-	0.9	0.5	0.2
US	286	85.1	28.2	0.8	2.2	1.1	0.4
Brazil	173	38.7	12.8	0.6	5.5	1.4	0.3
Indonesia	215	5.1	-	1.5	8.9	5.6	-
China	1263	47.8	0.9	14.4	15.8	0.2	2.9
India	1025	20.8	0.2	-	6.7	0.2	5.9
EU-15	377	14.6	0.7	20.5	15.2	2.6	5.2

1.3.1. Palm oil

Palm oil is the second most produced oil right after soybean oil. It is in first place in terms of trade, making up to 44% of all oil and fat exports and still growing. The whole production and exports are dominated by Malaysia responsible for 51% of palm oil production and 63% of exports. The second largest producer (31%) and exporter (26%) is Indonesia. In both countries, the production is still growing. Palm oil is consumed worldwide. It can be found in retail food and snack produce, personal care and cosmetics, biofuel and other energy production, animal feed, pharmaceutical, industrial and foodservice industries (Dijkstra 2015).

1.3.2. Rapeseed/canola oil

Canola oil is the third most produced oil. China, India, Canada and Japan are among the biggest producers. Only 12% of the oil serves for export, 48% of all of the production is from Canada. The strong seed trade is important. Rapeseed oil, same as the palm oil, has many practical uses, such as food industry, cooking, frying, cosmetics, as a biodiesel etc. The picture below represent rapeseed oil with the seeds (Taylor et al. 2013). Illustration of canola oil can be seen in Figure 6.

Figure 6. Sample of canola oil with seeds (Tadimalla Teja 2018).

1.3.3. Sunflowerseed oil

Sunflower oil is the last representative of the four major oils and fats. Of the total oil production, it takes up about 9% share. The oil is typical for its composition of different fatty acids. The major producers are the countries from region of former USSR, Argentina and Europe in general. 27% of production is exported, mostly from Argentina. The refined form is used for cooking at a wide range requiring low to extremely high temperatures. The unrefined version mostly serves as a salad dressing. The crushed seeds left after obtaining the oil serve as an animal feed, fertilizer or fuel and are commonly called seed meal (Gunstone 2011b).

1.3.4. Peanut oil

About 53% of all grown peanuts are crushed in order to obtain oil. The market for peanut oil is very small. The biggest producers are China and India, which together represent 7% of the entire market. Other smaller producers are African countries. Peanut oil is suitable for frying and sautéing. Usually the flavor is neutral, but some varieties can taste nutty. Also, one of the biggest advantages is its unique quality to not absorb flavors from different foods, which has been fried in it (Gunstone 2011b)

1.3.5. Cottonseed oil

The cottonseed trade market is only small scale. The biggest producer is China with its 29% of total production, followed by India, the US, the former USSR, Pakistan, Brazil and Turkey. The oil is frequently used for frying, deep-frying and baking. Its neutral taste intensifies the natural taste of food. It is also commonly used in processed foods, serves as ingredient in margarines, icings and whipped toppings. Other than that it can be used in cosmetics. Soaps with addition of cottonseed oil are well suited for washing wool and in general are added to laundry detergents. Other products range from rubber to insecticides and explosives (Liu et al. 2009). The basic steps of processing cotton seed to oil can be seen in Figure 7.

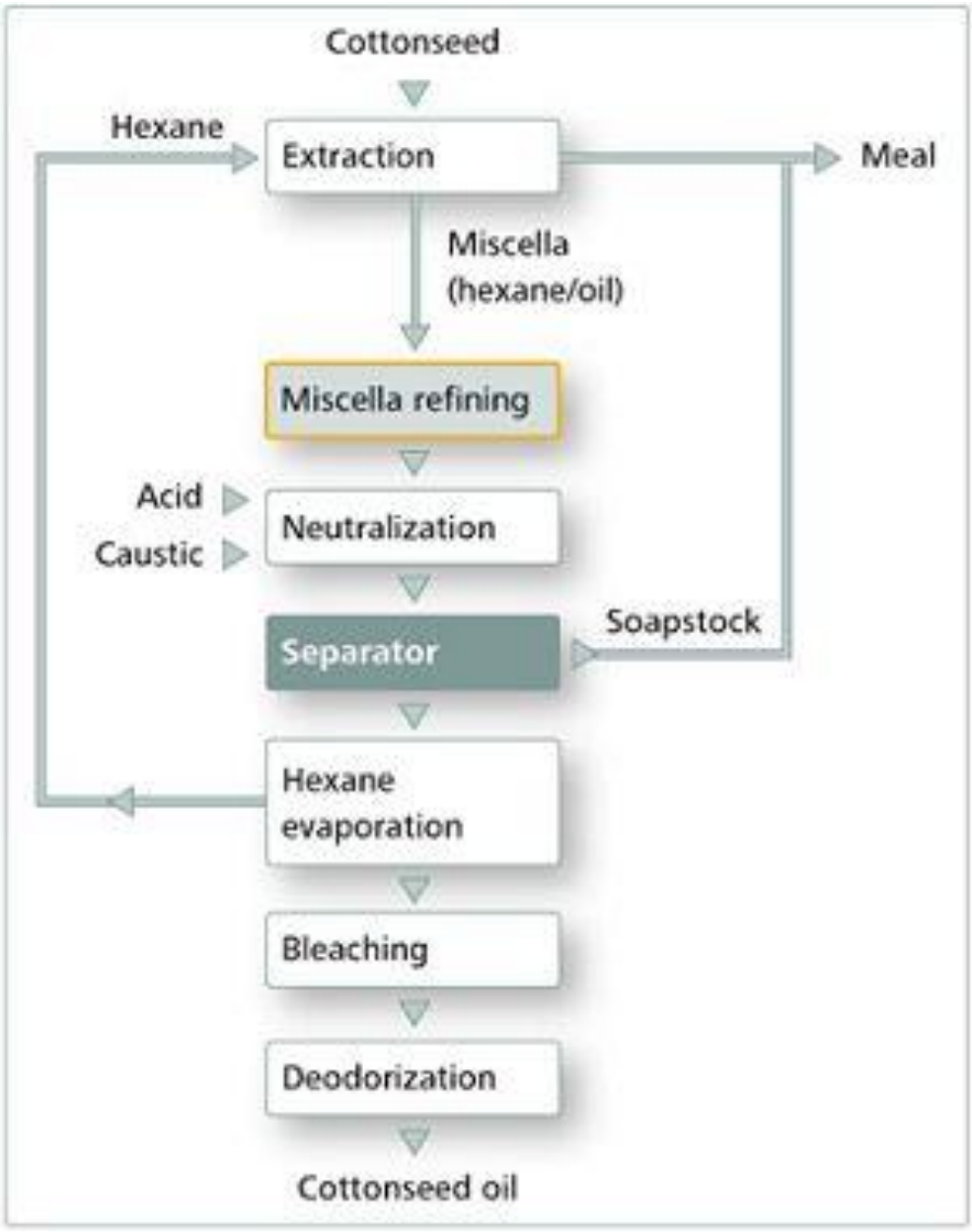

Figure 7. Steps of processing cotton seeds to oil (Agico Group 2019).

1.3.6. Coconut oil

Major exporters of coconut oil are the Philippines and Indonesia (Figure 8), on the other hand the US and EU are the biggest importers. However, the statistical records about coconut oil are imbalanced and unreliable because of the climatic and politic instability of the countries where it is produced. It has significant food and non-food uses such in all kinds of cosmetics (soaps, body lotions, toothpastes etc.), food industry and others. The biggest disadvantage is its strong flavor, which can influence the final taste (Marina et al. 2009).

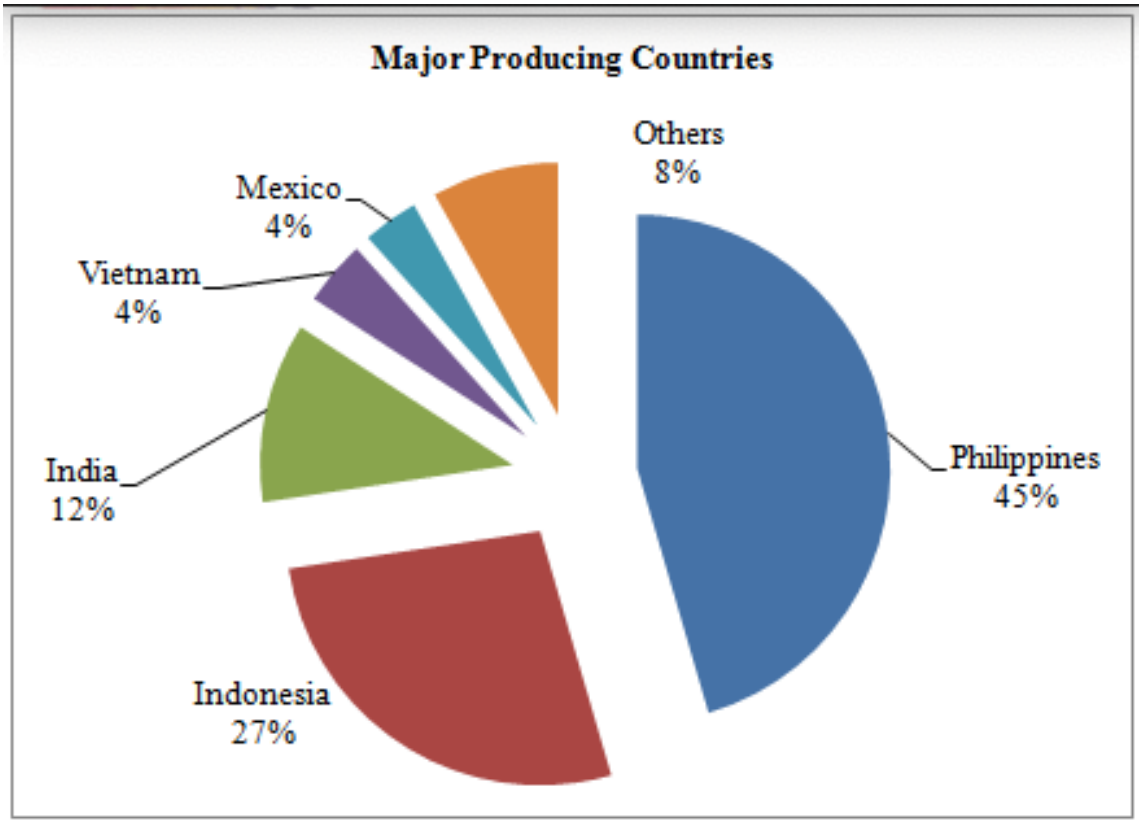

Figure 8. Producers of coconut oil (Coconut Development Board 2018).

1.3.7. Palmkernel oil

Palmkernel oil is the second major lauic oil. Its availability is a bit lower as the coconut oil popularity grows day by day. Malaysia and Indonesia are the dominant producers and exporters. Thanks to its content of myristic and lauric acids, the oil is well-suited for the manufacturing of soaps, washing powders and personal cosmetic products (Young 1995). The differences between palmkernel oil and palm oil can be seen in Figure 9.

Figure 9. Reddish palm oil made from the pulp of oil palm fruit; clear palm kernel made from kernels (Naliaka 2019)

1.3.8. Olive oil

Olive oil has a long history of use, which goes way back to pre-biblical times. This oil is typical for Mediterranean region, nevertheless the demand from Northern Europe countries and the US is rapidly increasing. Olive oil plays crucial role in a healthy Mediterranean diet. This oil is not recommended for cooking thanks to its amount of unsaturated fats which makes it susceptible to oxidative damage when cooked. Olive oil is mostly use cold, drizzled on prepared food. But not to forget, olive is also widely used in cosmetics (creams, soaps, on its own, body lotion etc.) (Owen et al. 2000).

1.3.9. Corn oil

Corn oil belongs among the less expensive vegetable oils. The biggest producer, consumer and even exporter is the US. The oil is extracted from the germ of corn. As a result of high smoke point, the refined version of the oil serves as a valuable frying oil. It is also used as a key component in some margarines. Other industrial use of corn oil include biodiesel, soap, salve, paint, inks, textiles etc. (Barrera-Arellano et al. 2019).

1.3.10. Sesame oil

China, India and Burma are the major producers of sesame oil. The consumption of it largely takes place also in those countries thanks to its enormous popularity. The taste and aroma are described as nutty. The light type is used as a frying oil and the darker one as a flavoring agent. It has tendencies to become rancid if it is kept open for longer period of time. It can also be used in industry for manufacturing cosmetics, illuminants, insecticides etc. (Lyon 1972).

1.3.11. Linseed oil

Linseed oil is one of the rarest ones. Its main use is oil-based industry (oil paint, putty and wood finish) thanks to its high unsaturation. Recently it has seen a rise in popularity as a food oil. The use has inclined since the viability of synthetic alkyd resins has increased (Gunstone 2011b).

1.3.12. Special oils

As for most food fats, plant oils help intensify the taste of food but also can be use in cosmetics, as aromatherapy and other types of industry. The option from which is possible to obtain the oils has expanded in the past few decades. For example, avocado seed oil, almond oil, grapeseed oil, walnut oil, pumpkin seed oil and many others. Among those unusual oils also belongs hemp oil.

1.4. Cannabis characteristics

Cannabis sativa L. is an herbaceous annual plant from family Cannabinaceae. It is considered to be the only species in the genus *Cannabis.* After maturation, it develops into a rigid, woody structure and reaches height of 1-5 metres. If the plants are not crowded, branches have a tendency to spread freely and the central stalk can grow 3-6 centimetres in diameter. If the plants are in thick stands, the stems are unbranched, without foliage with the exception of the top and the stem reaches a diameter from 6 to 20 mm (Ehrensing 1998).

Most varieties have hollow stems. Leaves are palmately compound with 5 to 11 pointed, serrate leaflets 50 to 150 millimetres long and 10 to 20 millimetres wide. If the soil is

light and well drained hemp roots can be 2 to 5 meters deep and secondary roots branches can grow up to 0.8 m below the surface (Bocsa & Karus 1998).

The plant is usually dioecious, having staminate and pistillate; each having its typical growth characteristics. Male (staminate) plants are tall and slender with only few leaves around the flowers, meanwhile female (pistillate) plants are short and stocky with a large number of leaves at each terminal inflorescence and in contrast to male plants, stay alive until the seeds are matured. Female plant is illustrated in Figure 10. and male in Figure 11. Because of the growing interest in hemp, monoecious varieties have been developed with the help of breeding and selection (Amaducci & Gusovius 2010).

Figure 10. Female flowering plant (Alchimia 2014).

Figure 11. Male *Cannabis sativa* plant (Alchimia 2014).

The seeds can be coloured from light brown to dark grey. Usually they are smooth, nearly spherical achenes and are sometimes mottled. The amount of fiber in the seeds depends on whether the plant is monoecious that means around 16 grams per thousand seeds and or dioecious, 21 grams. The amount of oil reaches somewhere between 29-34% (Sacilik et al. 2003).

The stalks have hollow woody core surrounded by the vascular cambium and the rest is presented by phloem, cortex and epidermal tissue. The fiber can be either long, coarse which spreads almost at the entire length of the stalk or short, fine ones which tent to stick to the woody core. The bark content in the fiber differs from 14-48% depends on the genotype and plant height (Bocsa & Karus 1998).

1.4.1. Cannabis sativa

Cannabis sativa is the most spread variety of *Cannabis.* It is annual, dioecious plant which stem reaches height from 0.8m to 4m (Kubánek 2009). Female plants are bigger with more leaves of dark green colour. On the other hand, male plants are taller, slimmer and leaves are light green (Kubánek 2009).

The stem is totally free from THC (delta-9-trans-tetrahydrocannabinol). In comparison with cotton the fiber is longer, stronger and isolation function is better. Main root is upright, globular, soft and fleshy. Afterwards from the bottom it becomes woody. The leaves are alternating (on the bottom the pairs are in opposite leaf arrangement), serrated and have peculiar and diagnostic venation. The seeds can be big up to 0.5cm and are brownish, greenish ashy colour (Kubánek 2009). In Figure 12. we can see example of hemp seeds.

Figure 12. Sample of *Cannabis* seeds (Author 2019)

Cannabis sativa includes three main types; north, south and temporary. The north type has vegetation period between 50-80 days, is less than 1m tall and is spread in the north area of the former Soviet Union and partially in Finland. The south type, also known as late or partially late, has height ratio between 2.5m-4m (in tropical areas can reach even 7m). It ripens after 120-165 days. It also has great yield of very soft fiber but yield of seeds is quite small. The stems are branched and well covered with leaves (Kubánek 2009). The temporary type has evolved by crossbreeding of the north and south types. Its height is around 1.7m- 2.5m. Vegetation period is between 90- 120 days. Because of the breeding it disposes with good yield of seeds and fiber (Anwar et al. 2006).

1.4.2. Cannabis indica

Cannabis indica is dioecious, annual plant that can reach height from 1-1.8 metres. The biggest difference between *Cannabis indica* and *Cannabis sativa* is that the *C. sativa* species stalk is more fibrous than that of *C. indica*. The plant is cultivated in India, Afghanistan, Iran, Turkey, Northern Africa and Syria for the resin. Tepals and foliage are

used as a material for psychoactive effects (Miovský 2008). The differences between leaves of *Cannabis sativa* and *Cannabis indica* are illustrated in Figure 13. and 14.

Figure 13. *Cannabis sativa* leaf (Procházková & Bečka 2014).

Figure 14. *Cannabis indica* leaf (Procházková & Bečka 2014).

1.4.3. Cannabis ruderalis

The maximum height of the plant reaches 0.6-1.5 metres. Stalk is almost branchless and slightly grooved. The foliage is not so dense. The seeds are small with irregular black

spots. This cultivar is resistant to pests and diseases. The plant is originally from southeast Russia, precisely western Siberia, the Caucasus Mountains, the Ural Mountains and area around Volga. Later it expanded into Middle (Moravia and Slovakia) and North-east Europe (Poland, Lithuania, Estonia) (Kubánek 2009).

1.4.4. Advantages of hemp cultivation

The main processes involved in the growing and processing of hemp are mostly environmentally friendly. Growing hemp is ecological itself. Additionally, hemp is also good as a rotational crop and has the ability to absorb pollutants (heavy metals) from soil. The plant eliminates weeds by suppressing them through its rapid growth and high leaf coverage. This way it eliminates the necessity to apply weed control chemicals. Hemp does not require chemical fertilizers and if it does, only on a small scale. The leaves of the plant have multiple applications- as a natural fertiliser- left on the site, animal litter, manufacture of paper (hemp hurds), fiberboard in construction and furniture making. Overall nothing or just little of the plant is wasted (Sponner et al. 2005).

The seeds, thanks to their content of oil can be used for fuel and fatty acids for the production of plastics and other industrial products. The meal obtained from oil extraction serves as feed for livestock or as a fertilizer. The stalks, after harvesting, are used for their high-quality bast fibers. Fast technical progress allowed for optimization and effectiveness of production of all of the applications mentioned above. As a result, each end product can now be obtained at lower costs, with environmentally friendly and safer technologies (Fortenbery & Bennett 2004b).

1.4.5. Hempseed oil

Hempseed offers both, positive health benefits, such as lowering of cholesterol and high blood pressure, and high nutritional value (Kenneth Jones 1995). For centuries it has been consumed in food, has had crucial role in folk medicinal preparations and has served as a feed. The seeds contain 20-25% protein, 20-30% carbohydrates, 10-15% of insoluble fiber and wide range of minerals (Leizer et al. 2000).

The oil itself has been used for manufacturing of printer's ink, wood preservative, detergents and soaps. After the ratio (3:1) of the two essential polyunsaturated fatty acids was discovered, it was recommended for human nutrition. Thanks to presence of γ-

linolenic acid and the optimal ratio of linoleic and linolenic acids, the oil is also optimal for light body oils and lipid-enriched creams which allows their high penetration into the skin (Oomah et al. 2002).

Since Hemp is still a novel, nonconventional crop, the methodology for oil quality determination is under investigation to improve the economical and environmental performances of the components and byproducts (Oomah et al. 2002).

1.4.6. Cultivation

Hemp is believed to be one of the first cultivated crops, even before flax and cotton. The first cultivation dates back 4.000-60000 years ago in China (Kim & Mark 2018). By the 16th century, hemp became vital cash crop in Europe (Fortenbery & Bennett 2004b).

The cultivation itself depends on the purpose for the final purpose, as for all the other crops. There are different varieties for seed and fiber production. The fiber varieties reach around 3-4 m of height just in three to four months and are typical for having minimal foliage. The harvest for fiber usually takes place right before the seeds are fully mature, which is seventy-ninety days after seedings. To prevent branching the seeds are planted in narrow rows. This way of plantation also increases stalk height and the percentage of the best fibers- the primary ones and the shorter secondary ones (Meijer et al. 1995).

Harvest for the seed production usually occurs four to six weeks later than that for the fiber. In contrast to fiber plantation, hemp for the seed production is planted farther apart to endorse branching and allow for better seed development. The seeding rate is much lower than for the fiber production; more specifically one-fifth (Kraenzel et al. 1998).

The industrial variety of hemp is adaptable to wide range of environmental conditions. Nevertheless, for higher yields, the plant has a high demand for supply of nutrients and plentiful moisture throughout the growing season. Best conditions are well-drained loam soils with great amount of organic matter. Optimal temperature is between 13 and 22°C (Ehrensing 1998).

In the beginning of the growth, hemp requires around 30 cm of rainfall. After getting well rooted, it can bear drier conditions. With extreme drought seeds are premature and produce dwarfed plants (Fortenbery & Bennett 2004a).

The plant does not need biocides for the most part. Damage caused by insects or diseases are rare but can occur. The biggest threat are some bird species that feed on the plant's seeds. The fiber varieties are very competitive, mostly with weeds and therefore need only little herbicides. Weeds can also be totally cut off by proper planting timing. But when hemp is cultivated for seed or as dual seed and fiber crop, the canopy is not intense enough and herbicides must be used (Fortenbery & Bennett 2004a).

1.4.7. Utilization

The market potential for both hemp fiber and seed depends on its ability to compete with already well-established sources in terms of characteristics, quality and price. Current fiber market includes special textiles, paper and composites. Till 2000 hemp fiber represented only 8% of jute production and 27% of flax production. Flax fiber is very similar to hemp regarding fiber quality and processing requirements (Fortenbery & Bennett 2004a).

Hemp has multiple uses, for example weed control, pest and disease resistance, elimination of pesticides and when being part of crop rotation, improving the soil. Therefore, hemp is suitable candidate both for conventional and for organic farming, providing high biomass production with low inputs (Ranalli & Venturi 2004).

Hemp fiber was the main reason for its planting from the beginning. Fibers are used for basic raw materials in order to produce ropes, canvas or clothing. The peak of the production for fiber was the three past centuries but after World War II production declined because of the introduction of synthetic fibers, high-labour cost, associations with illegal narcotics, high cotton production etc. But with the growing interest in renewable resources hemp is slowly coming back in style (Ranalli & Venturi 2004).

Other opportunities on the market for hemp fiber include molded car parts, fiberglass substitutes and composites. The use of the fiber in car industry comes from the idea that it is lighter and easier to recycle than other materials (Thompson et al. 1998). To replace fiberglass with non-fibers is applicable only in such places where moisture is no problem. The success of hemp fiber in such industries depends on its ability to compete with other sources of non-wood origin and on its supply satisfactory amount throughout the entire year (Fortenbery & Bennett 2004a).

The market for hemp seed centers around two main areas; whole seed as a food ingredient or crushed for oil and meal. Thanks to its composition, hempseed is highly nutritious (around 20% of high-quality digestible proteins). The oil can be used in multiple ways as stated above. It needs to be said that it is fairly unstable and tends to go bad quickly. The overall market remains limited. The factors for such are need for mechanical crushing together with solvent extraction to maximize the oil yields. Because of not undergoing degumming and bleaching many consumers do not find the oil appealing due to taste and appearance. As stated above, the oil oxides quickly thus is necessary to keep it in dark-colored bottles, has short shelf life and is not suitable for frying (Fortenbery & Bennett 2004a).

1.5. Oil quality

Throughout the entire process (from manufacturing and packaging to distribution and retail sale) in the food supply chain it is crucial to test the quality of oil (Ahmad 2014). With multiple cases of food fraud and dilution, the analyses become more acute over the past few years. Researchers at the U.S. Pharmacopeial Convention found that vegetable oils, particularly olive oil, is the most vulnerable to adulteration and represents the best documented cases of food fraud. Even though no official regulation is set for the evaluation of the quality of edible oils, the amount and content of free fatty acids, peroxide value and p-anisidine value are normally used to establish the quality of edible oils (Kerr & Dunford 2018). In recent past, thanks to significant advances in the analytical methods, many types of adulterated food are now detectable. The analysis of results from testing the authenticity and chemical properties of vegetable oil has been researched in more detail. Among others, gas chromatography and high-performance liquid chromatography have been recently used to classify triglycerine content. The basic scheme of gas chromatography can be seen in Figure 17. The other properties of the oil are always connected to the type and relative amount of each constituent triglyceride in the sample (Kerr & Dunford 2018).

1.5.1. Spectrophotometry

Spectrophotometry is a scientific method which measures how much light is absorbed by chemical solution by measuring the intensity of light as a beam of light passed through sample solution. The principle is that every compound absorbs, reflects or transmits light over different but defined wavelength. This method is also widely used to measure accurate amount of a known chemical substance in a solution. Overall this quantitative analysis is used in diverse fields such as chemistry, physics, biochemistry (determination of enzyme-catalysed reactions), material and chemical engineering and clinical applications (examination of blood or tissue for diagnosis) (Augustyn et al. 2009).

Thanks to spectrophotometer device the intensity of light absorbed after it passes through sample solution is measured. By measuring the intensity of light, the quantity of a known chemical can be determined (Vo 2018). There are two types of classification depending on the range of wavelength.

UV- visible spectrophotometer: base is light over the ultraviolet range (185-400nm) with visible range (400-700 nm) of electromagnetic radiation spectrum.

IR spectrophotometer: base is light over infrared range (700-1500nm) of electromagnetic spectrum (Vo 2018).

The absorption or the transmission in visible spectrophotometry can be established by the detected colour. To be specific, solution absorbing light over all visible ranges (transmit none of visible wavelengths) appears to be black. In contrast, if all visible wavelengths are transmitted (absorbs nothing) the solution appears to be white. Or if the solution is absorbing red light, it appears to be green. Because the colour green is complementary to colour red. The visible spectrophotometry is usually use to set down the wavelengths so the specific beam of light passing through a solution sample (Augustyn et al. 2009). The basic structure of spectrophotometer is illustrated in Figure 15.

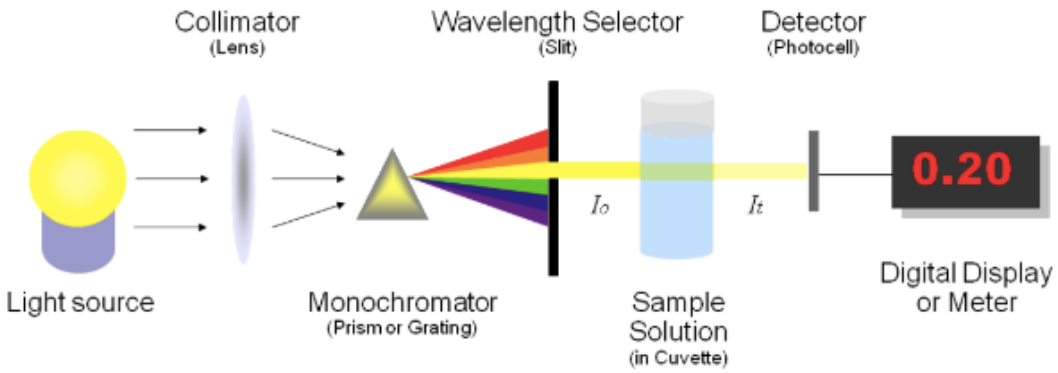

Figure 15. Basic structure of spectrophotometer (Vo 2018).

Usually spectrophotometer is assembled of two devices; a spectrometer and photometer. Spectrometer serves as a producer of wanted wavelength of light. The lens transmits a straight beam of light which then passes through the prism to split into a spectrum. The split also passes only the wavelengths that are wanted. Photometer identifies the amount of photons that are being absorbed and send the information to a digital display (Augustyn et al. 2009).

It is necessary to have spectrometer producing wide range of wavelengths because various compounds absorb different wavelengths. The place where the absorbance of two or more species is the same is called isosbestic point (Figure 16.) (Vo 2018).

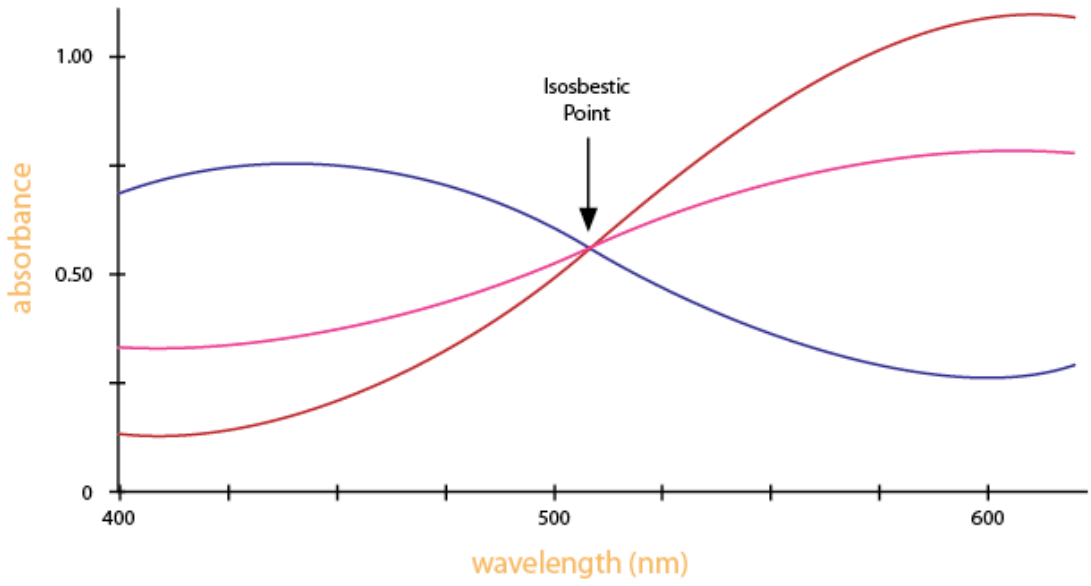

Figure 16. An example of isosbestic point (Sanjeev et al. 2012).

1.5.2. Sensory analyses (SA)

During the second half of the twentieth century sensory evaluation grew expeditiously. One of the reasons was expansion of the processed food and consumers products industries (Lawless & Heymann 2010). The analytical techniques are about accurate measurement of human responses to food with minimized distracting effects such as brands and other information influencing the perception of the consumers. Having said that, the foundation of the analyses is to separate the sensory qualities of food itself and allow product developers, food scientists and managers to use the obtained information for other food industry purposes (Meilgaard et al. 2007).

Using sight, touch, taste, and hearing, sensory evaluation allows to measure, analyse and interpret consumers' responses (Stone & Sidel 2004). Sensory analyses provide guidelines on how to prepare and serve the samples under controlled conditions so the whole process can be reproducible. There are certain ways how to minimalize the biasing factors such as individual booths for the connoisseurs, different order of tested samples, labels etc. Precise temperature, volume, and spacing time must be established (Lawless & Heymann 2010).

The measuring process is based on numerical data collected to set rules and relationships between product characteristics and human perception. One example would be the generalized numerical evaluation performed by individuals to reflect how strongly each product smells or tastes. Thanks to guidelines provided by behavioral and experimental psychology, the measurements can be evaluated also with their potential pitfalls and liability (Lawless & Heymann 2010).

After obtaining the data the next crucial part is to analyze them. Thanks to variability of human responses it's vital to use appropriate statistical analyses and experimental design which allows to draw a realistic conclusion. This needs to be done to prevent faults such as motivation of the participants, their past history with similar product, their sensitivity or their mood. (Meilgaard et al. 2007)

The last process of SA is to interpret the results. For precise conclusion it is necessary to consider the method of experiment, the limitations of the experiment, context of the hypothesis, background knowledge and the context of the study (Stone & Sidel 2004).

1.5.3. Storing conditions

Studies have been conducted on analysis of seed oils quality by multiple researchers because of increasing demands for both human consumption and for industrial applications (Kyari 2008). Light and storing conditions are often forgotten or underestimated factors in studies of oil quality. The activity of oil can increase with the increasing amount of light and with higher temperatures (Amer & Mehlhorn 2006).

The storing conditions of each oil differ. Most of them oxidize over time and eventually become rancid no matter how they are stored. The rancid oil can be identified by changes in aroma and taste. The aroma becomes sharp, bitter and unpleasant and can be easily distinguished from the fresh oil one. But some oils still can have sharp aroma at the fresh state, therefore it is recommended to test the taste and smell of the oil in the beginning (Amer & Mehlhorn 2006).

In general oils that are highly saturated (coconut oil) have stable shelf life. Oils rich in essential fatty acids and polyunsaturated fats are those with shorter shelf life. Shelf life of vegetable oils may be extended by adding an anti-oxidant. Example of such anti-oxidant are mixed tocopherols such as T-50 and T-80 vitamin E. Those need to be used at a rate of 0.04-1.0% in order to protect the oils (Amer & Mehlhorn 2006).

The oils included in the experiments were always stored in one of two ways. One sample of each cultivar was refrigerated, and the other was kept outside at room temperature. All of the samples were kept in dark glass bottle and deoxidized.

1.5.3.1. Colour

The colour of hemp oil is usually described as a grassy green with golden shades. The shade of the green depends on amount of chlorophyll in oil. The amount of chlorophyll can also be influenced by the way of oil extraction. In general, it is known that extracted oils have lighter colour than oils obtained by cold pressing. The shade also becomes lighter when the oil is purified (Rezková & Kouřimská 2013).

1.5.3.2. Taste

The taste depends mostly on the particular cultivar and method of harvesting. In most cases the taste is characterized as nutty. In comparison with different oils for example sunflower oil, hemp oil tastes stronger and sourer. This is caused by the amount of fatty

acids. Simultaneously the taste is less oily. The aftertastes can be defined as earthy, grassy and even constricting. Too rancid, bitter nutty taste is undesirable because they indicate aging of oil (Rezková & Kouřimská 2013).

1.5.3.3. Smell

Like any other type of fat, when it comes to smell of oil, it is a vital indicator of changes in composition and of oil- rancidity. The same rule applies to hemp oil. The description of the smell is grassy or weedy reminding the consumers of freshly cut grass or crushed hemp leaf. If the seed are drier, the taste of the final oil is more herbaceous (Rezková & Kouřimská 2013).

1.5.4. Peroxide value

Peroxide value serves to tell the quantity of hydroperoxides present in fats and oils. Hydroperoxides toxic to humans are those that oxidize first during the primary stages of oxidation. It is not recommended to evaluate the quality based only on peroxide value. This is because low peroxide value does not necessarily mean low level of oxidation. That could also be caused by progressive oxidation which converted the primary oxidation products into secondary products. That is why other indicators are recommended (Fiebig 2003).

Activity of air oxide on fatty acids causes oxidation of fats. This process is undesirable and decreases sensory and nutritious values of oils. Unpleasant smell and taste are caused by presence of aldehydes and ketones. Peroxides and monomers accumulate in fats as by-products. Those processes cause oxidation, lead to loss of nutritional value and crucial unsaturated fatty acids. Peroxide value is one of the quantitative measures.

Peroxide value determines the content of arising fatty peroxides and hydroperoxides. It reflects the amount of oxide able to oxidize iodide to iodine. It is expressed in μg active oxide in 1 g of fat.

1.5.5. Acidity value

Acidity value is used to express the amount of potassium hydroxide in milligrams which is mandatory to neutralize free fatty acids in 1 g of oil (Vitz et al. 2017). It is an indicator of the state of the oil, which directly influences quality of material and products.

It is essential to dissolve the sample in polar solvent system, usually ethanol or ethanol-ether mixture, and then titrate while hot with solution of KOH with phenolphthalein indicator.

1.5.6. Saponification value

Saponification value reports how much mg of KOH is needed for saponification of 1 g of fat or 1 g of isolated lipid. The sample is cooked together with ethanolic solution KOH. After it is titrated with standard volumetric solution of hydrochloric acid.

1.5.7. Ester value

The ester value is the amount of mg of KOH needed for saponification of esters in 1 g of fat. It is used for approximate calculation of the amount of present glycerin.

1.5.8. Anisidine value

p-Anisidine value is a method used to determine amount of secondary oxidation products formed by disruption of the primary oxidation products during broader oxidation. The secondary compounds are mostly aldehydes such as 2,4-dienals and 2-alkenals (Tompkins & Perkins 1999).

The anisidine value and peroxide value are two qualitative characteristics of oils. Anisidine value measures the amount of aldehydes. Rancidity of oil correlates with the increase of amount of unsaturated fatty acids which also includes aldehydes. Long-term storing and repeated reheating also lead to increase of the anisidine value.

The value is centuple of growth of absorbance measured solution of wavelength 350 nm in 10 mm cuvette after reaction with p-anisidine. It is counted for 1 g of sample dissolved in 100 ml of solvent.

1.5.9. Iodine value

Iodine value is defined as an amount (in %) of halogens re-count to iodine. It is the measurement of the content of unsaturated fatty acids. Therefore, it is used to tell the origin and quality of oil. Oils with iodine value higher than 150 are ranked drying. Oils with iodine value between 100-150 are semi-drying and oils under 100 are non-drying.

This method can be performed either with Hanus (iodine monobromide) or Wijsov solution (iodine monochloride).

2. Aims of the Thesis

The main objective of the thesis was to investigate the influence of nine different varieties of *Cannabis sativa* L. on the chemical and sensory properties of cold pressed oil from hemp seed.

Specific aims were to process literature review focusing on hemp, its cultivation, utilization and oil quality properties. As well as investigation of two different storage condition over the period of 24 weeks: a) under the room temperature b) in the fridge. Further, evaluation of nine different varieties namely Santhica, Bialobrzeskie, Futura, Fedora, Tiborsallasi, Kompolti hybrid, Finola, KC Virtus and Fibrol on the chemical and sensory properties with respect to different storage conditions and interval of storage. Finally, comparison of nine different varieties on peroxide value, iodine value, saponification value, acidity value, ester value and sensory properties such as color, odor, flavor and taste.

3. Material and Methods

3.1. Plant material

The seeds of nine varieties of *Cannabis sativa* L. (Santhica, Bialobrzeskie, Futura, Fedora, Tiborsallasi, Kompolti hybrid, Finola, KC Virtus and Fibrol) where obtained for the study from plants grown in Masojedy. The location can be found in Central Bohemian Region and lies on east side of Prague. Its geographical coordinates are 50° 2' 0" North, 14° 47' 0" East. The seeds were sowed in May 2016. The harvest took place on turn of September and November of 2016. According to Czech Hydrometeorological Institute the mean temperature for each month from May till October was 14.2°C, 17.8°C, 19.3°C, 17.9°C, 16.8°C. The mean precipitation during those five months was 58 mm, 77 mm, 95 mm, 32 mm, 39 mm and 57 mm. The soil typical for this area is luvisol widespread in temperate climates and are labelled as generally fertile. After harvesting, the seeds were stored under stabile conditions: in the dark, dry place, at 5°C.

3.1.1. Finola

Finola is variety originally from Finland. It is a type of industrial hemp cultivated for seed oil. The plant is short, quickly maturing, dioecious and auto-flowering. The foliage is chopped and worked into the soil as a fertilizer. The cultivation is similar to the other oilseed crops, for example rapeseed. Average weight of 1000 seeds are 12-15 grams. The seeds are smaller at northern latitudes (>50°N) because of cooler climate. The bigger seeds can be found at southern latitudes (<50°N) which are warmer. The average yield differs and depends on the climate. In maritime climate near latitude 60°N it is 1000kg/ha and in continental climates (50°N) it is around 2000 kg/ha. With increasing latitude, the height of the plant also increases (Callaway 2010).

The seeds need warm, moist, well-drained soils rich in organic matter. It is not recommended to use corn, oats, rye, wheat and other oilseed crop as rotation crops because of their tendency to catch diseases. Thus, it is recommended to use soybean, legumes, potatoes or grass breaking crops (clover) (Gallaway 2016).

The harvest period is approximately between 100-130 day after sowing. At this time, only top third is being harvested and combined for grain. This minimizes cutting and wrapping problems. Harvest begins after maturing. The grain moisture is between 10-15% (Gallaway 2016).

The seeds contain 30-35% oil. The oil is rich in vegetable protein (25%), carbohydrates (30%), vitamins, minerals, phytosterols and polyphenols. The oil is used as an animal feed, human feed, as a drying oil in the production of specialty inks, paint and small percentage is used in conventional diesel fuels. The fiber consists of 50-70% cellulose and 5-7% lignin. It is used for non-woven insulations, bio-mats, geotextiles, nets, tea bags, cigarette paper and other special papers. Together with biomass it is also used as animal bedding, geotextiles and in compost substrates. The fiber quality and its use for woven application depends on the sex of the plant from which it has been obtained (Callaway 2010).

3.1.2. Fedora

Variety Fedora also belongs among the monoecious types. Its origin is in France. The optimal climate is Atlantic. This climate is typical for its low temperature changes throughout the year. The precipitation is also equal the whole year. This variety reaches maturity early, <125 days. The stand is high between 200-250 cm, which may be limiting for combine harvesting. Those can reach 250 cm with difficulties (Marrot et al. 2013).

The yield differs depending on the climactic conditions, soil nutrient availability, sowing date, seeding rate and time of the harvest. But in general, this variety is one of those with bigger yield >1.2 t/ha. The content of the oil in the seeds is 30-32%. The size of it is right in the middle of the size charts with 16-18 grams/1000 seeds. Fedora variety is mostly cultivated for its fiber quality. The amount of fiber in the seed is 30-35%. (Marrot et al. 2013).

3.1.3. Bialobrzeskie

The variety Bialobrzeskie comes from Poland. It was bred as a monoecious variety with high economic value. Its popularity is steadily growing to this day not just in Poland, but all over the whole European Union. The plant is adapted to Polish climate and soil

conditions. But when the conditions are appropriate elsewhere it provides high and stable yields (Grabowska et al. 2008).

The water requirements depend on the growth. That means the highest need for water is in June and July. The roots can reach down 2-3 m deep. Its deep roots enable the plant to reach and collect water from deeper layers of soil and survive times of drought. Regarding temperature, it is necessary to provide sufficient heat during June and July. The seed germination occurs at 8-10°C within 10-12 days. Thanks to the ability of young plants to survive frost, the early sowing is possible (Grabowska et al. 2009).

Due to its oil content of 30-32% and fiber 26-30%, the use of this variety is universal. That means that it can be used for human or animal feed, in oil industry, in fiber industry etc (Grabowska et al. 2009).

3.1.4. Fibrol

Fibrol variety also belongs among the monoecious and it is from Hungary. This variety is cultivated for its extreme content of oil <35%. It was registered in 2006 so it is a rather new variety. Fibrol was also selected from hybrid parental variety Fibrimom 21-63. (Finta-Korpeľová 2010)

3.1.5. Tiborsallasi

The variety Tiborszallasi is originally from Hungary too and is the typical hemp because it is dioecious. The male and female flowers are not on the same plant. It is free flowering, southern type hemp. The stem of the plant is lighter green and grooved with dark green leaves. Each leaf has nine mini-leaves. Optimal climate for it is continental. It is representative of late maturing varieties (<145 days). That favors gathering cannabinoids. The plant is more on the higher scale (250-350 cm), therefore it is cultivated for the fiber. The fiber yield is approximately 12-15 t/ha and fiber content in stem is 26-30%. The amount of oil in the seed, which are the bigger ones 18-20 g/1000 seeds, is below average; 26-28% (Cosentino et al. 2012).

3.1.6. Futura

The Futura variety is originally from France. It is also one of the monoicous plants. The cultivation is one of the easier ones. The seeds are planted in the usual way (by splashing

in water, between two napkins or directly into soil. The optimal climate for it is Atlantic, which means just small temperature changes between day and night (Svennerstedt & Sevenson 2006)

The vegetative cycle is quite long, and maturity takes place after <145 days so it is a late one. But thanks to it, this cultivar has higher cannabinoid profile 1.5-2.0%. Therofore, this variety is universal and can be used not just for fiber (fiber content 30-35%) but also for oil, proportion of extractable oil is 28-30%. The stand is around 250-350 cm high. The grain yield is 0.8-1.0 t/ha. The seed usually weights 16-18 grams/1000 seeds (de Bruijn et al. 2009a)

3.1.7. Kc Virtus

Kc Virtus is a variety from Hungary. This variety is dioecious. It has been cultivated mostly for fiber. The yield of the stem for fiber is very high. And the fiber percentage in the stem is 27.5% (Ferrante et al. 2019).

The vegetative period is around 150 days. The plant can reach up to 3.5 m and is known for its bigger seeds (up to 20g/1000 seeds). Overall the grain yield is between 800-1000 kg/ha. This variety was cultivated to produce biomass (Ferrante et al. 2019).

3.1.8. Kompolti hybrid

Kompolti hybrid is also a variety from Hungary. It belongs among the dioecious varieties. The vegetative period is rather long (160 days). At the maturity, the plant can reach height of almost 3.5m. The content of cannabinoids is around 2.00-3.00% (Ehrensing 1998).

This variety serves mostly as source of fiber. The fiber content is the highest in Europe. Usually fiber content in the stem is around 30 %. The amount of oil is on the lower scale with maximum reaching only 26%. The overall biomass yield is 12.15 t/ha (van der Werf 2008)

3.1.9. Santhica

Santhica is originally from France. It is a monoecious plant; whose optimal climate adaptation is Atlantic one. Its vegetative cycle is neither short nor long. The maturity takes place around <135 days. The plant is also mediocre in the meaning of maximum

height reaching 2.5 metres. The seed yield is normally around 0.8-1.0t/ha with oil content smaller than 26% (de Bruijn et al. 2009b)

The variety is mostly cultivated for fiber. The biomass yield is from 8-10t/ha and the fiber content in the stem is >35%. The seed size is 18 grams/1000 seeds. The plant is usually harvested by hand and not mechanically (Fournier et al. 2004).

3.2. Cold pressing of oils

The cold pressing of oil was carried out in the laboratory of the Department of Mechanical Engineering, Faculty of Engineering Czech University of Life Sciences Prague (CULS) between 6th to 9th August 2018 for the storage experiment and between 16th to 18th January 2019 for the sensory panel.

3.2.1. Preparation of the seeds

According to the preliminary pressing, when was not possible to run the press machine because of low moisture content of the seeds (approx. 3.4%-3.7%), it was necessary to moisten the seeds before the pressing. Without the moisturizing, it was not possible to process the seeds. Therefore, the entire procedure of obtaining the oil took three days. On the humidity chamber Memmert HCP 108 (Memmert GmbH.,Gemany) was set humidity to 95% and the temperature was set to 35°C. Each cultivar was moistened for 24 hours. Afterwards the humidity of the seeds was 8.6%-9.2%.

3.2.2. Extraction

The oil was extracted using the press machine Farmet DUO 3F (Farmet a. s.,Czech Republic). The Farmet machine (Figure 17) was set on 80°C, jet (10mm) was loosen up for three revolution and the revolutions of the press were set on 100%.

Moisturized seed mixed with dry seeds in the ratio 2.5:1 were used for the pressing. To obtain approx. 1 litre of oil it was necessary to use 2.5 kg of wet seeds and 1kg of dry ones. Total 3.5 kg of seed of each variety was used for pressing. The weights used for weighting were Kern KB 2000-2N (Kern & Sohn, Germany)

Figure 17. The crusher.

3.3. Storing the oil

The oil of nine varieties was after pressing poured separately in bigger dark glassed bottles and kept resting for one day to allow the sediments to settle. Afterwards oil of each variety was divided into 9 (8+ 1) smaller, dark glassed bottles (Figure 18.). In each bottle was about 90 mL of oil. All bottles were labelled with date and variety. There were 2 sets of 4 bottles of each variety: one set was stored in refrigerator (4°C) and second set at room temperature (22°C). Oil was analyzed after storing for 1 day, 2 weeks, 4 weeks, 12 weeks and 24 weeks. One bottle with oil (the ninth one) was used for analyses without storing, immediately the next day.

Figure 18. Dark glassed smaller bottles with oil.

3.4. Chemical analysis

Chemical analyses of oil was carried out in the laboratory of the Department of Food Science, Faculty of Agrobiology, Food and Natural Resources, CULS between 6th August 2018 to 1st February 2019. Chemical characteristics such as peroxide value, acidity value, saponification value, ester value and iodine value were analyzed in five intervals over the 24 weeks period (0 day, 2 weeks, 4 weeks, 12 weeks and 24 weeks after the pressing). The photographic documentation of chemical processes happening during each analyzes can be found in Appendix I.

3.4.1. Peroxide value (PV)

The experiment was done according to ČSN EN ISO 3960.

3.4.1.1. Chemicals

To obtain the peroxide value of the samples the following chemicals were required: chloroform ($CHCl_3$ and CH_3COOH in ration of 2:3), solution of KI (must be prepared fresh; 5 of KI was dissolved in boiled distilled water, freshly prepared starch (1g of starch was boiled with 25mL of distilled water and then another 25mL of distilled water were added) last solution was sodium thiosulfate, 0.01M, which served as a titration solution. And of course, the oil samples.

3.4.1.2. Laboratory equipment

As for the laboratory equipment it was necessary to have four Erlenmeyer flask (250mL) with stoppers, cylinder (50mL and 5mL), 1mL pipette, spoon, funnel, 100mL beaker with stopper, 10mL flask, cooker and thermometer.

3.4.1.3. Procedure

The first step to start the experiment was to weight out 4grams, with the precision of 0.0001g, of the oil sample in the Erlenmeyer flasks (work in triplicates to get proper results plus a blind sample, meaning one without the oil). Second step was to mix the weighed-out oil in 30-50mL mix of $CHCl_3$ and CH_3COOH, until it dissolves. In the third step, 1mL of freshly prepared solution of KI was added and blended together. Later the Erlenmeyer flasks with a mixture of everything were placed in the dark for 20min. After

20min, 30mL of distilled water was added. Then with the help of titration solution $Na_2S_2O_3$ the content of the flasks was titrated until the yellowish colouring disappeared. Afterwards 1g of starch was added and the mixture is titrated for the second time until the purple colouring disappeared even from the chloroform layer. The blind sample was processed the same way except for the addition of 4g of oil.

3.4.1.4. Calculation

The following equation was used to calculate the peroxide value:

$$\mathbf{PV}=\frac{(\boldsymbol{a}-\boldsymbol{b})}{\boldsymbol{n}} * \boldsymbol{c} * \boldsymbol{f} * \mathbf{8} * \mathbf{1000};\ [\text{mmol/L}] \qquad \mathbf{(1)}$$

where a= amount of $Na_2S_2O_3$ consumed by titration, b= amount of $Na_2S_2O_3$ consumed by titration of blind sample, c= concertation of the $Na_2S_2O_3$ solution, f = factor of the $Na_2S_2O_3$ solution, n= weight of the oil sample.

The final number was arithmetical mean of the three samples. Because peroxide value was mostly stated in mEq, there is also alternative equitation with mEq units.

$$PV=\frac{(\boldsymbol{a}-\boldsymbol{b})*\boldsymbol{c}*\mathbf{8}*\mathbf{1000}}{\boldsymbol{n}};\ [\text{mEq/kg}] \qquad \mathbf{(2)}$$

3.4.2. Acidity value (AV)

The experiment was done according to ČSN EN ISO 660.

3.4.2.1. Chemicals

For determination of acidity value 3 chemicals were needed. First one is 96% ethanol (which must be neutralized by adding a few drops of phenolphthalein and 0.1 M KOH till the solution is lightly pink for at least 15-30s) then ethanolic solution of KOH was also demanded, c= 0.5 mol*L^{-1}. 1% phenolphthalein was the last solution.

3.4.2.2. Laboratory equipment

To be able to do this experiment it was crucial to have cooker, cylinder (50mL), 150mL flask, 250mL Erlenmeyer flask (4x), spoon, funnel and scoopula.

3.4.2.3. Procedure

All the steps were performed in triplicates with the oil sample and one time without the oil as a blind sample. As for the first step, 4g of the oil sample was weighed out in the Erlenmeyer flask with the precision of 0.0001g. In the second step, 100mL of the neutralized ethanol which had been preheated to 60-65ºC and 2mL of phenolphthalein was added. All the chemicals were carefully mixed together until they dissolve. After it was essential to immediately titrate it with solution of KOH until the solution was pink-purple for at least 30 s. Consumption of KOH during the titration should be somewhere between 5-50mL.

3.4.2.4. Calculation

To get the precise number it was required to calculate the amount of used chemicals.

$$AV = \frac{a*c*M}{n}; \quad [mg/g] \qquad (3)$$

where a= consumption of KOH solution, c= concentration of KOH solution, M= molar weight of KOH ($g*mol^{-1}$) and n= weight of the oil sample.

AV is arithmetical value of the three samples.

3.4.3. Saponification value (SV)

The experiment was done in according to ČSN EN ISO 3657.

3.4.3.1. Chemicals

For the experiment to obtain Saponification value it was necessary to have ethanolic solution of KOH, $0.5mol*l^{-1}$, solution of HCl, $0.5mol*L^{-1}$ and 1% solution of phenolphthalein.

3.4.3.2. Laboratory equipment

For this experiment cooker, Erlenmeyer flask (250 mL, 4x), cylinder (50mL), pipette (25mL), spoon, funnel, scoopula, boiling stones and reverse cooler were used.

3.4.3.3. Procedure

All the steps were performed in triplicates with oil and one without the oil for blind experiment. First step was to weigh out 2g of the oil, into the Erlenmeyer flask, with the precision of 0.0001g. 25mL of KOH solution was added with a pipette and a few boiling stones was added too. The Erlenmeyer flasks were then attached to reverse cooler and the solution was boiled for one hour. After one hour 3-5 drops of phenolphthalein were added. Titration of the solution was the last step. It was performed using HCl, 0.5 $g*mol^{-1}$. The experiment was finished when the solution was clear.

3.4.3.4. Calculation

To get SV it was necessary to count the amount of used chemicals.

$$SV = \frac{(V0 - V1) * c * f * 56{,}1}{m}; \text{ [mg/g]} \quad \textbf{(4)}$$

where V_0 = the amount of HCl used for titration of blind sample, V_1= the amount of HCl used for titration of samples with oil, c= concentration of used HCl ($mol*L^{-1}$), f= factor of HCl solution and m= weight of the oil.

To get the most accurate number, arithmetical mean from the three samples was calculated.

3.4.4. Ester value (EV)

The ester value was counted just from Saponification values and Acidity values.

EV= SV-AV; [mg/g of fat] **(5)**

3.4.5. Iodine value (IV)

The experiment was done according to ČSN EN ISO 3961.

3.4.5.1. Chemicals

These chemicals were needed for obtaining the iodine value: chloroform, solution of iodine monobromide (also known as Hanus solution) of 0.1 mol.L^{-1} concentration, 10% solution of KI, indicator starch glue and solution of $Na_2S_2O_3$ c= 0.1N.

3.4.5.2. Laboratory equipment

For this experiment cooker, 25mL and 100mL graduated cylinders, 150mL bask, 300mL Erlenmayer flask with stopper (4x), spatula and pipette were needed.

3.4.5.3. Procedure

The first step of the experiment was to weigh 0.25g oil with 0.0001g precision into the Erlenmayer flask. Second step was to add 25ml of Hanus solution. Then the flask was sealed off with the stopper which needs to be dipped in the solution of 10% KI to stop evaporation of iodine. Then the flask was shook and placed in dark for one hour. After one hour the stopper was washed with distilled water and 25ml of 10% KI was added. Within 1-2 minutes of 10% KI addition, 100mL of distilled water was added. Next step was titration with 0.1 M solution of $Na_2S_2O_3$ solution. The $Na_2S_2O_3$ solution was yellow. After adding 3-5mL of starch glue it was titrated once again until the liquid section lost colour.

The whole experiment was done 3 times with the oil samples plus once without the oil as a blank one.

3.4.5.4. Calculation

Iodine value was defined by the following formula.

$$IV = \frac{(a-b)*c*f*12{,}692}{n};\ [g\ I_2*100g^{-1}] \qquad \textbf{(6)}$$

where a is the amount of $Na_2S_2O_3$ needed for titration of blank test, b is the amount of $Na_2S_2O_3$ needed for titration of the test with oil, f stands for factor of $Na_2S_2O_3$ solution, c is for concentration of $Na_2S_2O_3$ solution and n is the precise amount of oil.

The final result was arithmetic mean of results obtained from each of the three tests.

3.5. Sensory analysis

Sensory analyses was performed in the laboratory of the Department of Food Science, Faculty of Agrobiology, Food and Natural Resources, CULS. It took place from 16th to 18th January 2019. Organoleptic properties of 9 different varieties of freshly pressed oil were assessed by 22 independent assessors. Assessors were selected and trained. Because of high amount of the samples, panel was divided in to two sets in two days. The samples were coded by 3-digit code to avoid that any information related to the product influences the judges' answers. Each of the participants obtained 20 mL of each sample, water, apple and bread to clear the taste pallet in-between each sample. For evaluation was used the profile method and it has been used 90 mm unstructured graphic scale. Figure 20a and Figure 20b express the original sensory analysis form. Table 3 is showing the parameters and orientation of each criteria of evaluation.

Table 3. Parameters and orientation of sensory evaluation.

Parameter and orientation	0	9
General look	Dislike	Like
General colour	Dislike	Like
General odour	Dislike	Like
General taste	Dislike	Like
Fusty/mudy sediment	Slightly intensive	Extremely intensive
Musty-humid-earthy	Slightly intensive	Extremely intensive
Acid-sour	Slightly intensive	Extremely intensive
Metallis	Slightly intensive	Extremely intensive
Rancid	Slightly intensive	Extremely intensive
Fruity	Slightly intensive	Extremely intensive
Bitter	Slightly intensive	Extremely intensive
Pungent	Slightly intensive	Extremely intensive

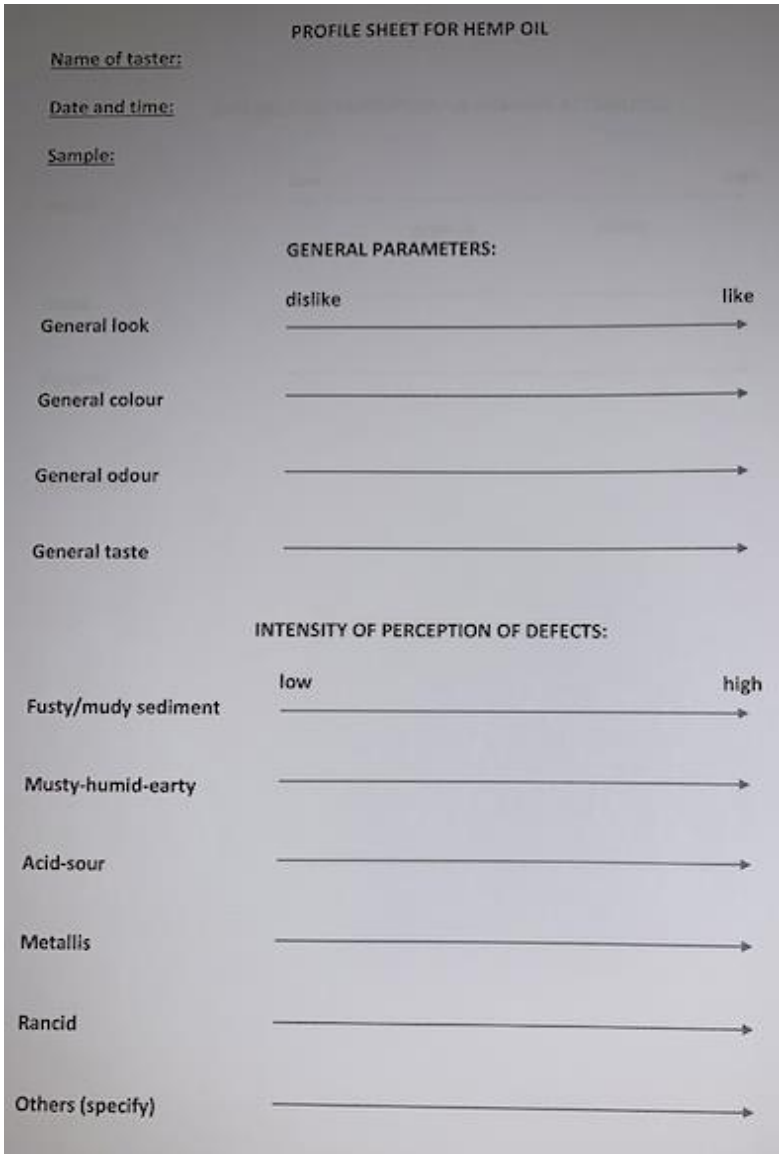

Figure 19a. Example of taste data sheets use for sensory evaluation.

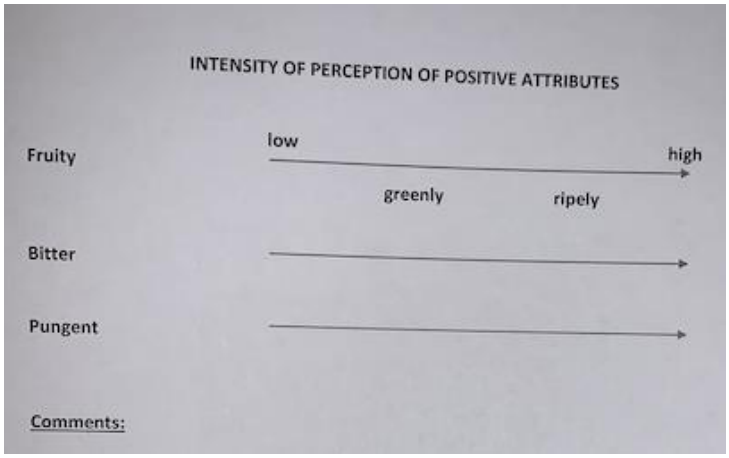

Figure 19b. Example of taste data sheets use for sensory evaluation.

3.6. Statistical analysis

Data were analyzed with the IBM SPSS Statistics software version 25 (IBM, US).

For the establishment that the results were right, two-way repeated measures Anova was done. The reason why this statistical test was chosen is because there are two within-subjects factors (storage type, time of the experiment) where each of it consists of two or more categorical levels (room condition, cool condition and right after, 2 weeks, 4 weeks, 12 weeks, 24 weeks after).

The next step was the test for determination if the results have any outliers based on residuals. The resume was that there were no outliers, as assessed by examination of studentized residuals for values bigger than ± 3. This was true for all of the analyses.

Afterwards it was necessary to test the data for normality, because the two-way repeated measures. For that was used Shapiro-Wilk's test on the residuals. All the gained values should be more than 0.05. To establish the post hoc values it was used the Tukey test.

The next step was to conclude whether there are any statistically significant simple main effects. So storage means were tested for differences in CRP concentration between experiments set at each time intervals and vice versa. The results showed significant differences between the type of storage.

For other experiments was the development almost similar, the values had the tendency to increase and room condition for storage had them higher.

4. Results and discussion

4.1. Type of storage

The type of storage directly influences the quality of the oil. Changes and increasing tendency of every tested values can be seen for every variety.

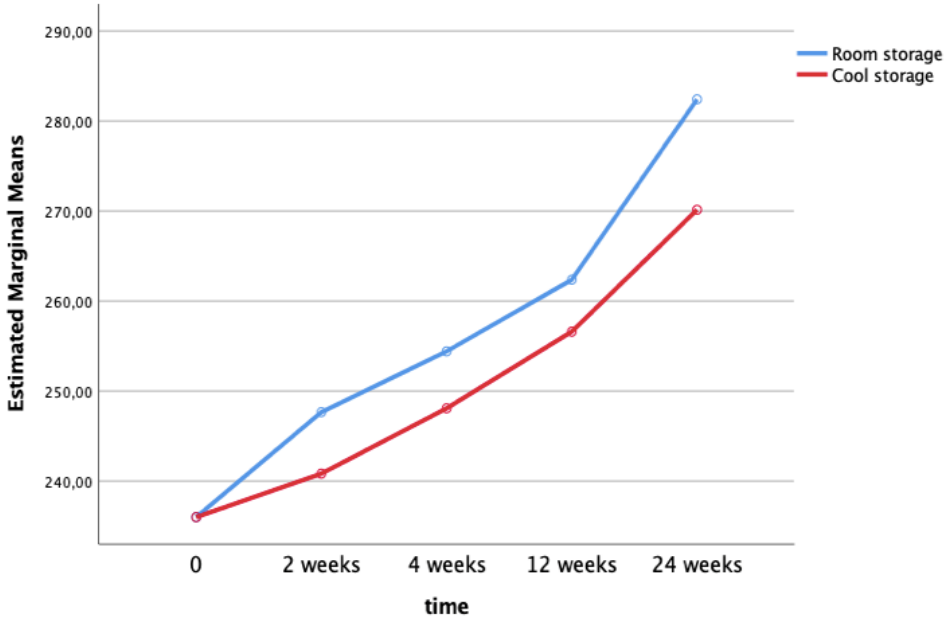

Figure 20. The development of the means (of all varieties) of the saponification value under different storing conditions.

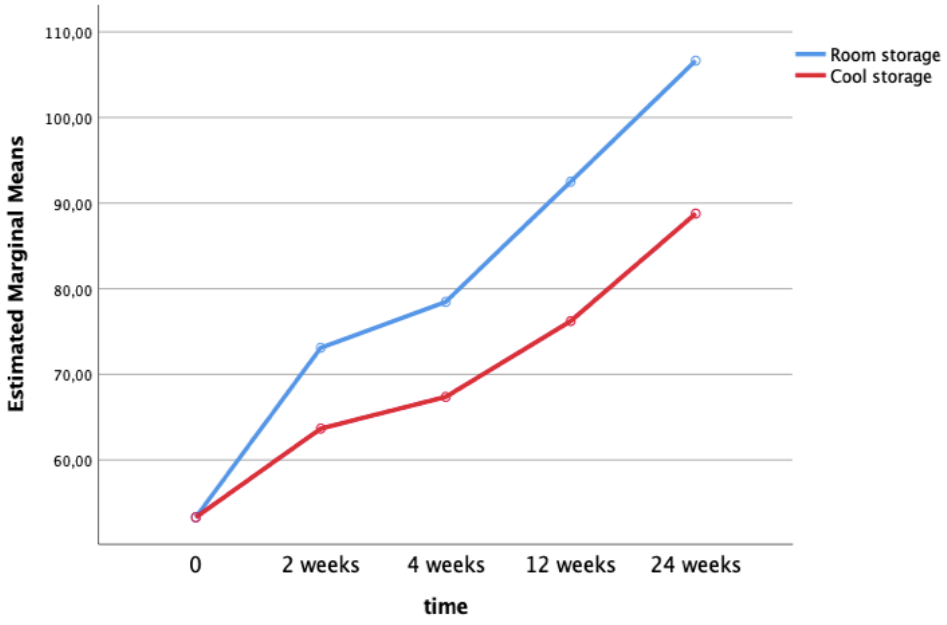

Figure 21. The development of the means (of all varieties) of the peroxide value under different storing conditions.

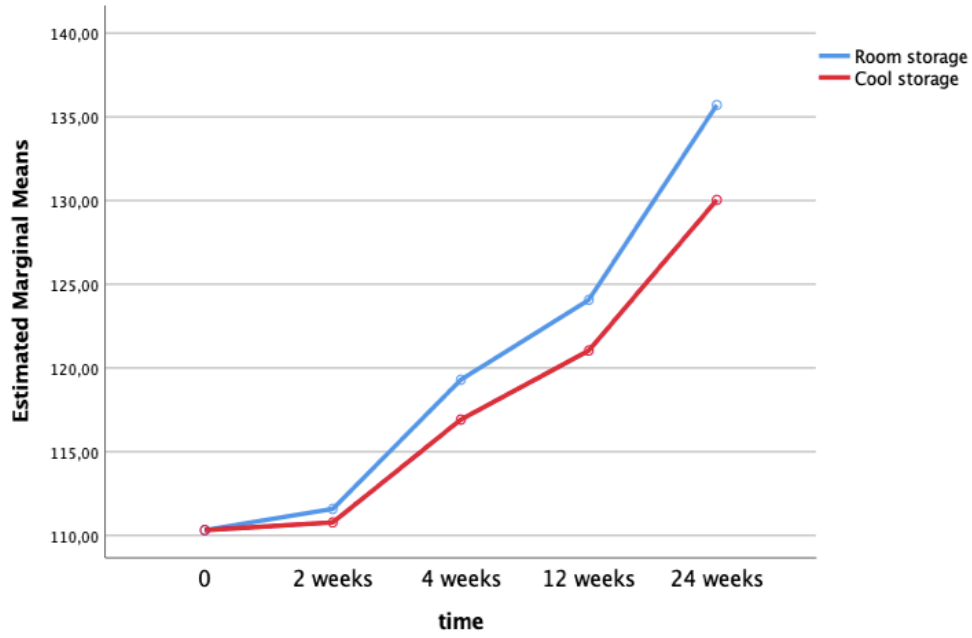

Figure 22. The development of the means (of all varieties) of the iodine value under different storing conditions.

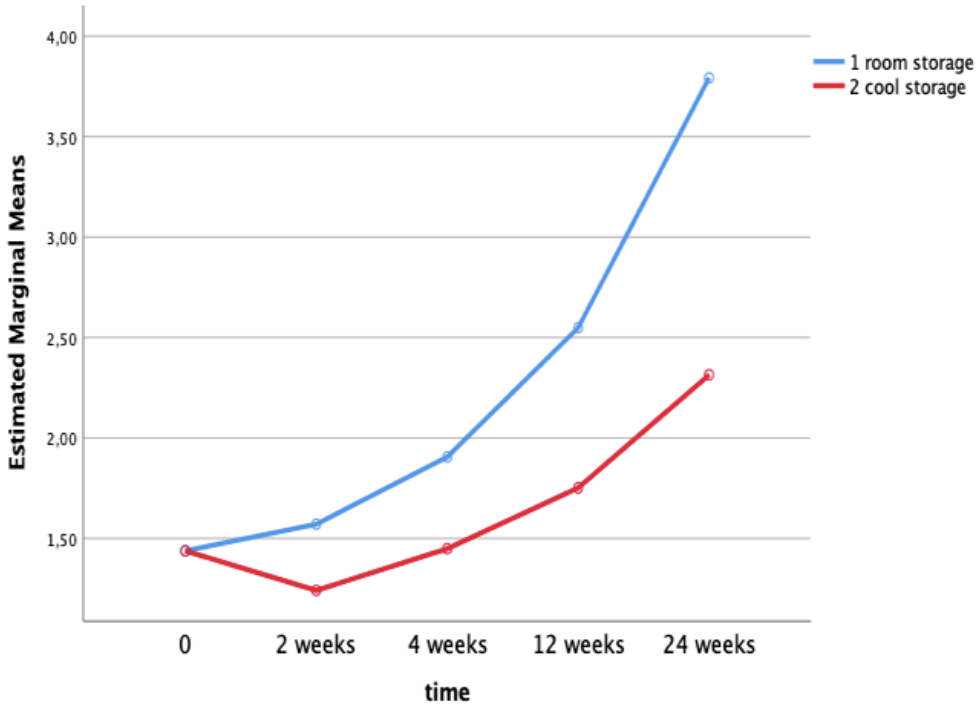

Figure 23. The development of the means (of all varieties) of the acidity value under different storing conditions.

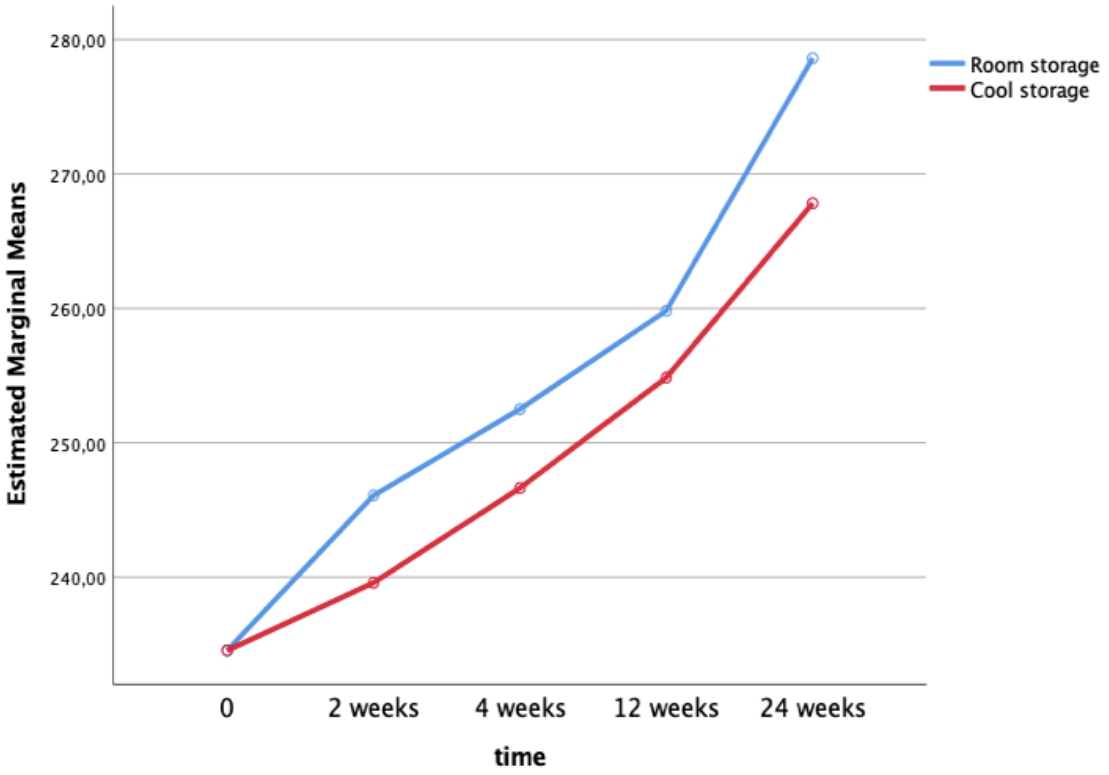

Figure 24. The development of the means (of all varieties) of the ester value under different storing conditions.

The results showed (Figures 21-24.) that the type of storage changed the values enormously in-between the varieties and experiments. The increasing values are shown on the variation range and mean.

There was statistically significant interaction between type of storage and time on the values of the quality analyzes. The statistical difference between the type of storage was proven by Pairwise comparison with $p = 0.5$.

The results of all the tested values illustrate that the type of storage has the most significant influence on all monitored values. Values were repeatedly measured at previously determined time points and they were compared to the values measured right after the initial extraction. Those samples, which were stored in cool conditions did not deteriorate as fast as samples stored in room conditions. I reached this conclusion based on evaluation of all measurements, which showed a clear trend of increase in all recorded values in samples stored at room temperature, indicating worsening of the quality of oil. The difference in respective values based on their storage reached one third. From the results it is obvious that samples stored in cool conditions, where temperature was around had lower values occur. Therefore, I conclude that this kind of storage is more convenient.

Based on published sources, storing hemp oil at 4°C and without access of light is possible for up to 4 months (Procházková & Bečka 2014). Where the samples which were kept in room condition, where the temperature runs around 22°C, show higher stage of rancidity. Under those conditions, the storage period is guaranteed only for 2.5 months. According to Callaway (2002) and Ruman & Včeláková (2008) hemp oil should be consumed till 6 months after initial opening. To prevent the oxidation process, it is possible to add some antioxidant such as resin of oregano or rosemary or phylloquinone. Those antioxidants are able to prolong the expiration date by several days.

4.2. Storage during time

4.3. Peroxide value

The growth of the values throughout the experiments is visible for all the varieties and for both types of storage as can be seen in Figure 21a. and Figure 21b.

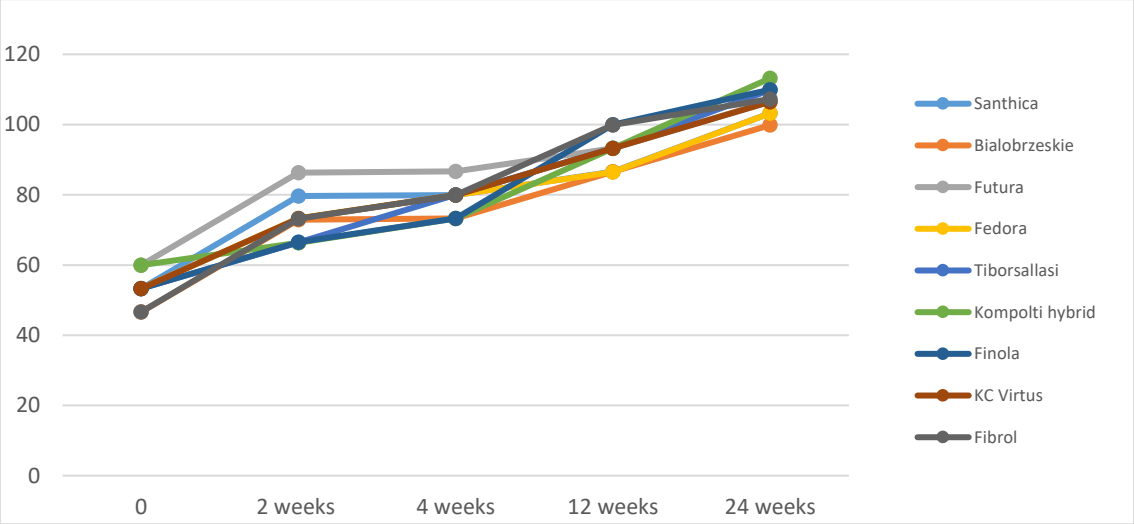

Figure 25a. Graph of development of peroxide value- room condition for every variety.

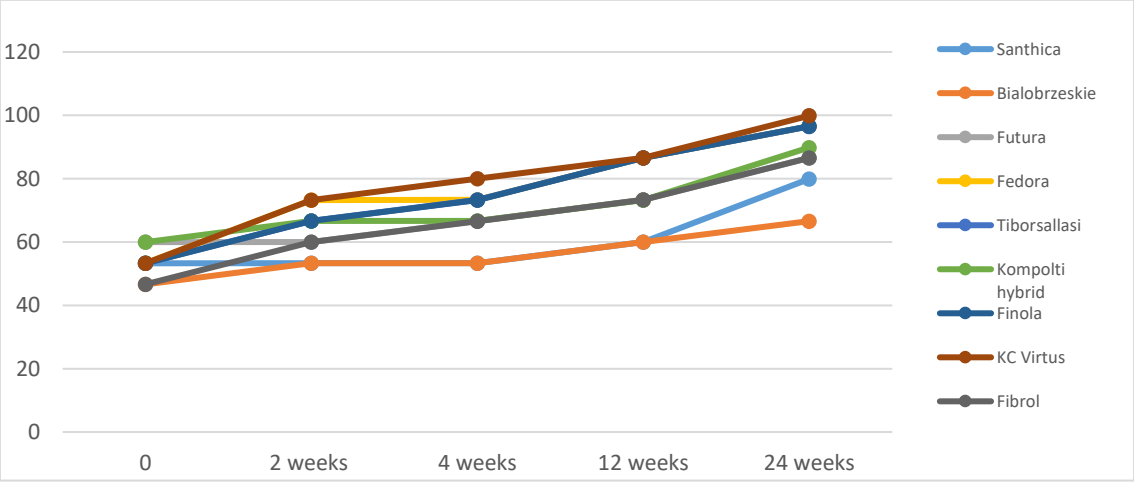

Figure 21b. Graph of development of peroxide value- cool conditions for every variety.

From the results in the graphs (Figure 21a and 21b) it is obvious that the variety with the lowest values no matter which type of storage was applicated is Bialobrzeskie. As far as the variety with the highest values goes the results are split between Kompolti hybrid (for room conditions) and KC Virtus (fridge conditions). That being said the value of the varieties is not statistically different.

The study proved that for obtaining better numbers for peroxide value analysis, the cool storage conditions are better. The best results had Bialobrzeskie variety stored in 4°C temperature with 66.59 μg active oxide in 1 g of fat. The worst results were obtained form room conditions storing in variety Kompolti hybrid (113.21 μg active oxide in 1 g of fat). According to Poustkova et al. 2010 the range where the peroxide value is considered as higher is 10 - 20 mekv O2.kg-1 (the value of Kompolti hybrid was 8 mekv O2.kg-1). None of studied variety exceeded this range.

4.4. Acidity value

With acidity value the values were during the testing consistent. The final 6th measurement was crucial for deciding which variety has the increasing tendency at the lowest. For room storing, the variety with best results was Fedora, which was not the case in fridge storing where the best results had Kompolti hybrid. On the other hand, the worst values, under room condition, has Fibrol and for cool conditions it was Bialobrzeskie. The development can be seen in Figure 26a. and Figure 26b.

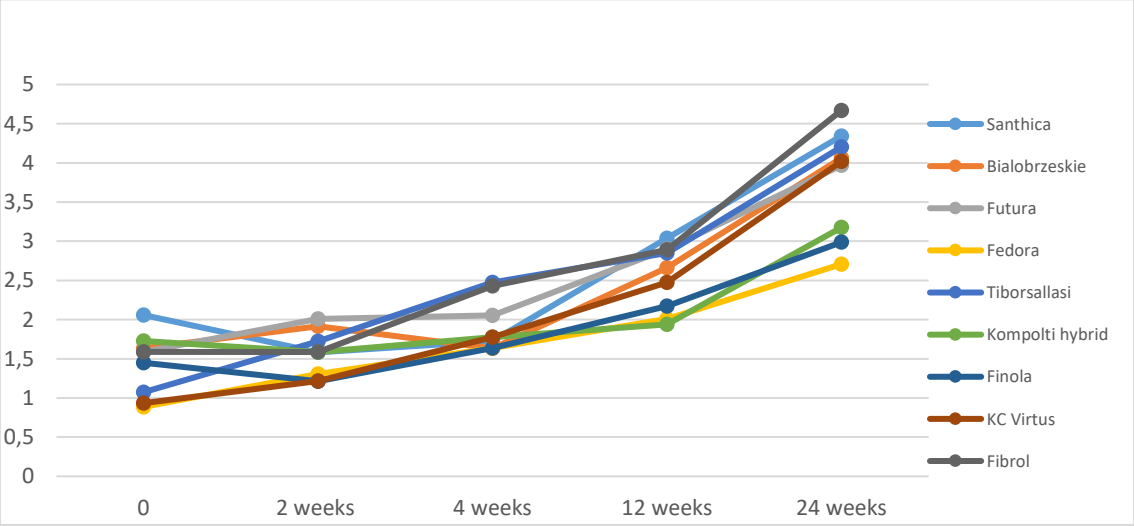

Figure 26a. Graph of development of acidity value- room conditions for every variety.

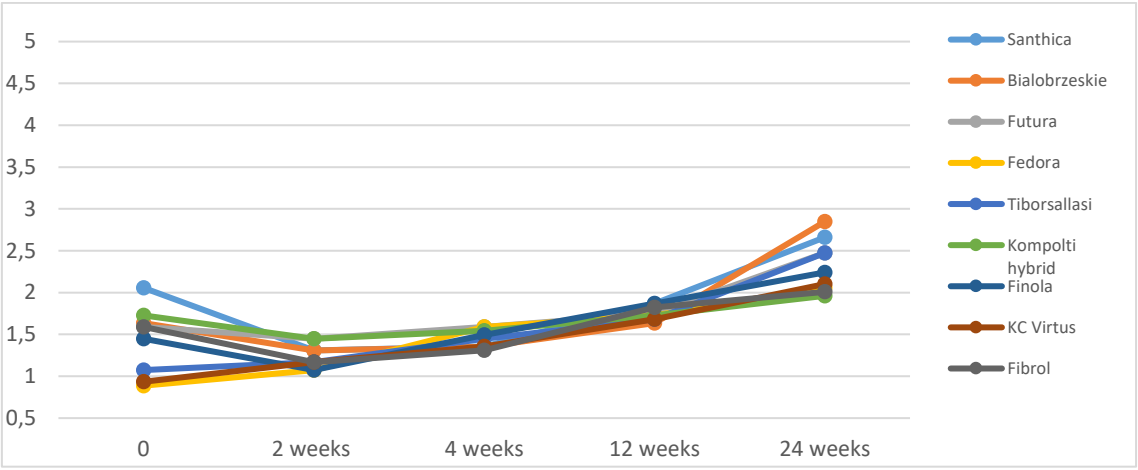

Figure 26b. Graph of development of acidity value- cool conditions for every variety.

The acidity value expresses the state of hydrolytic splitting. During oil aging, such splitting is no exception and it is accompanied by release of fatty acids. According to Codex Alimentarius (2009) the top limit is max. 4.00 mg KOH/g of fat. The most stable development was observed in variety Kompolti hybrid under cool storing conditions with the mean 1.682 mg KOH/g of fat. On the contrary the variety, with the highest content of free fatty acids, was Santhica stored in room conditions. The mean amount was 2.549 mg KOH/g of fat. So, the obtained values corresponds with these specifications.

4.5. Saponification value

As for increasing tendencies of the values, saponification analyses is no different. For all the varieties, no matter the type of storage, the results are increasing as can be seen in Figure 27a. and Figure 27b.

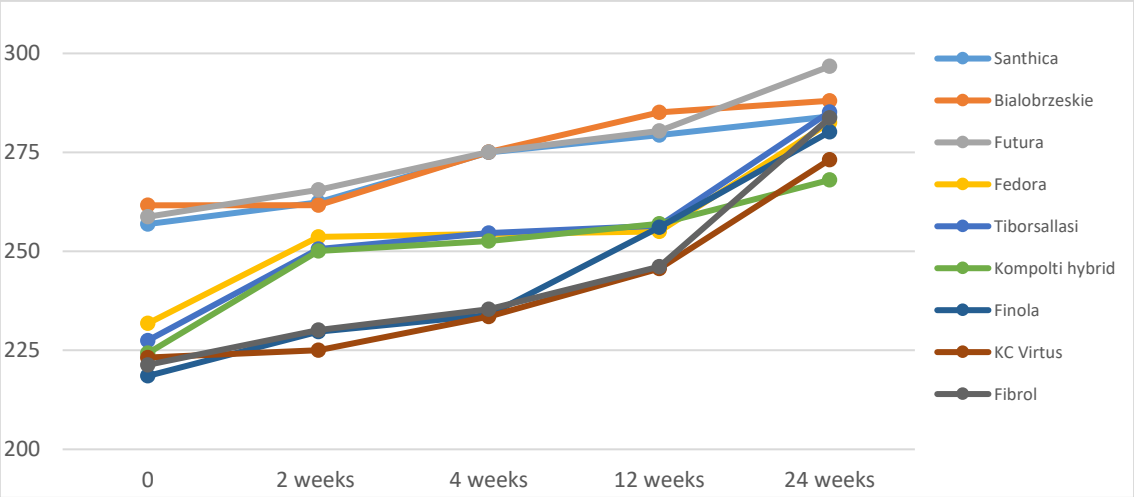

Figure 27a. Graph of development of saponification value- room conditions for every variety.

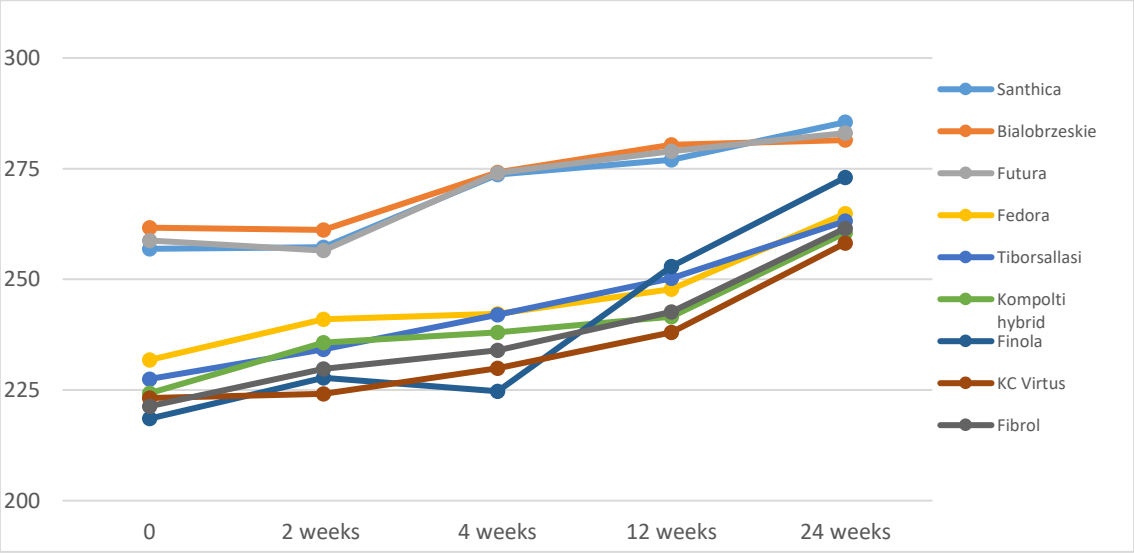

Figure 27b. Graph of development of saponification value- cool conditions for every variety.

The saponification results for room conditions were, the lowest values Kompolti hybrid and the highest variety Futura. For the cool conditions the best variety was KC Virtus and the last variety was Santhica.

The official value set by Ministry of Agriculture (2009) is 195 mg KOH/g of fat. From establishing the means we can see that variety with lowest mean was KC Virtus with 234.695 mg KOH/g of fat stored under cool conditions. On the opposite side of the scale is variety Futura with mean 275.303 mg KOH/g of fat stored in room conditions. In study made by Mikulcová et al. (2017), the result where (206 mg KOH/g of fat) also higher and considered as acceptable. Therefore, I conclude that these values, despite being borderline, would be acceptable.

4.6. Iodine value

The development of the varieties was same as for the previous analyses. The tendency of the values was to increase as it is visible in Figure 28a and Figure 28b.

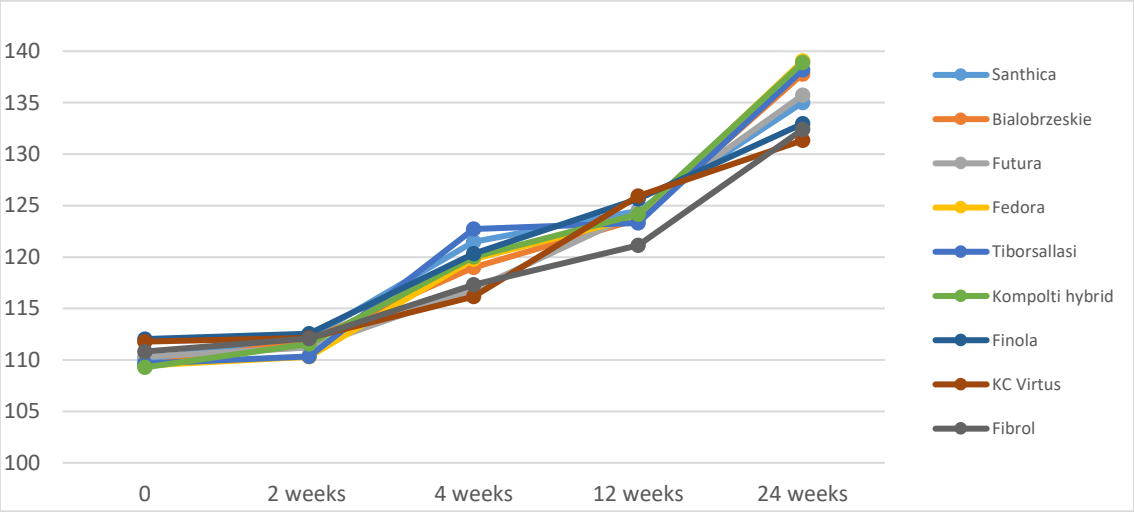

Figure 28a. Graph of development of iodine value- room conditions for every variety.

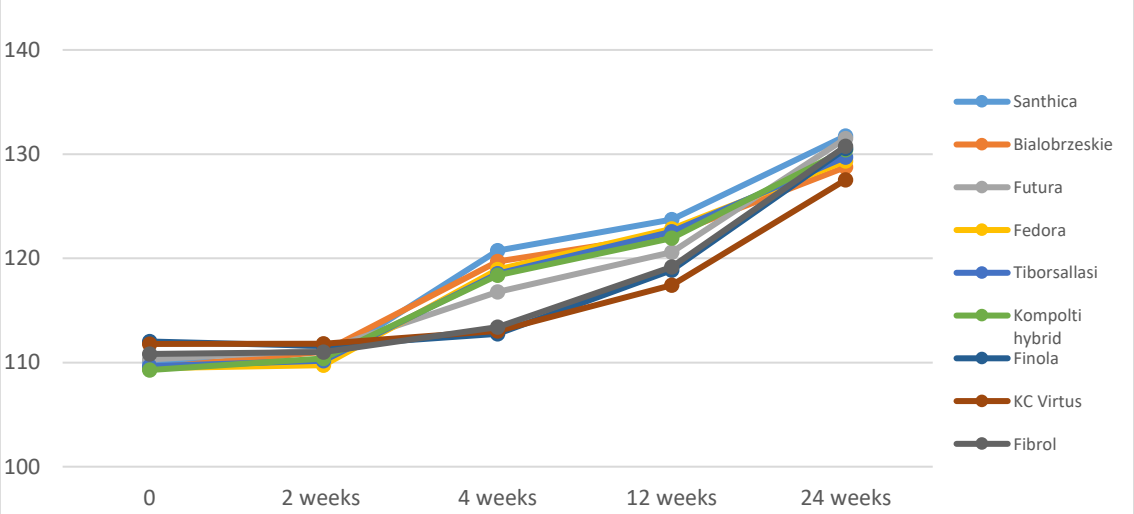

Figure 28b. Graph of development of iodine value- cool condition for every variety.

The results of iodine values showed that for room storing conditions is KC Virtus the one with the lowest number. And on the other side of scale is Fedora variety. For cool conditions results the variety with the lowest values the same- KC Virtus and the highest number had Santhica.

For iodine value all of the varieties have increasing tendency over time. During the iodine value analysis, the differences between the type of storage were not that significant. As Krivá & Věžníková (2010) proved the maximum value of iodine analysis is 170 [g I_2*100g^{-1}]. The results acquired by this project correspond with this range. To be precise this limit has not been surpassed by any of the tested varieties under any type of storage conditions. Yet the cool conditions storage obtained better results as predicted.

4.7. Ester value

The results (Figure 29a and Figure 29b) were the same as for saponification value.

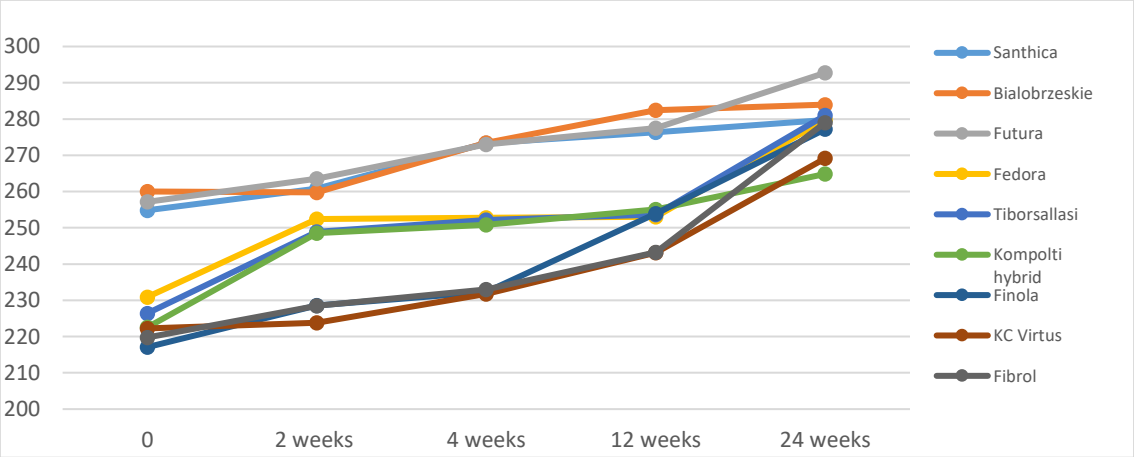

Figure 29a. Graph of development of ester value- room condition for every variety.

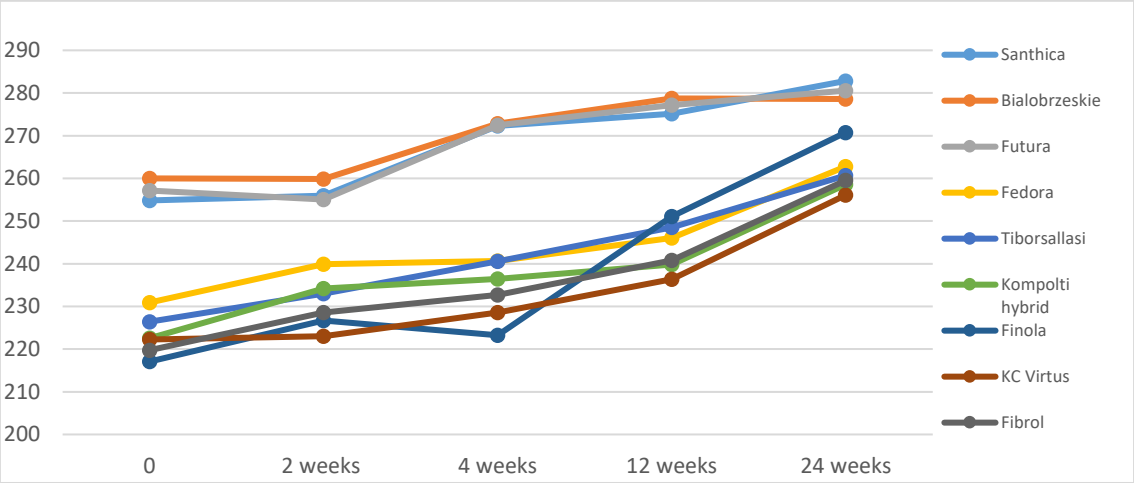

Figure 29b. Graph of development of ester value- cool condition for every variety.

The variety with lowest values for room conditions was Kompolti hybrid, the opposite position went to Futura.

As far as cool conditions go the best variety was KC Virtus and the one with the highest number was Santhica.

Because the value is set from distinction of saponification value and acidity value, the results are same as for saponification analysis. For obtaining the amount of glycerol, the result needs to be multiplied by 0.547.

4.8. Variety

For establishment the best variety was used descriptive statistics. For all the varieties was during each analyses set mean and variation range which was then compared with results for every tested value. The variation range is obtain by calculating the difference between the value of the first measurement and the last one. The results for each experiment can be seen in Table 4a and 4b.

Table 4a. Overview of variation range for cool conditions.

	Variation Range				
	Peroxide value	Acidity value	Saponification value	Iodine value	Ester value
Santhica	26.63	1.35	28.61	21.67	28.00
Bialobrzeskie	**19.97**	1.54	**20.29**	19.29	**18.87**
Futura	26.61	1.03	26.54	21.21	25.52
Fedora	43.25	1.17	33.04	19.88	31.87
Tiborsallasi	43.21	1.40	35.67	20.08	34.27
Kompolti hybrid	29.87	**0.51**	36.25	21.14	36.02
Finola	43.23	1.17	54.47	18.94	53.63
KC Virtus	46.57	1.17	35.01	**15.76**	33.85
Fibrol	39.91	0.84	40.19	19.94	39.78

Table 4b. Overview of variation range for room conditions.

	Variation Range				
	Peroxide value	Acidity value	Saponification value	Iodine value	Ester value
Santhica	49.98	2.76	27.18	24.95	24.89
Bialobrzeskie	53.23	2.43	**26.41**	28.33	**24.21**
Futura	**46.64**	2.38	37.97	25.43	35.59
Fedora	49.93	1.82	50.74	29.59	48.92
Tiborsallasi	56.56	3.13	57.73	28.53	54.60
Kompolti hybrid	53.24	**1.59**	43.75	29.61	42.30
Finola	56.64	1.77	61.65	20.93	60.11
KC Virtus	53.19	3.08	49.98	**19.56**	46.90
Fibrol	60.64	3.08	62.36	21.58	59.28

The results, for both types of storing, showed that the variety with the lowest changes in the values and the lowest numbers is Bialobrzeskie. On contrary the least convenient and suitable variety for long time storing under cool conditions is Finola and for room conditions it is Fibrol.

4.9. Sensory evaluation

The results of the sensory analysis are shown in the Table 6.

Table 5. Overview of mean and SD values for every variety and every criteria with the best score highlighted.

	Santhica	SD	Bialobrzeskie	SD	Fedora	SD	Tiborsalli	SD	Kompolti hybrid	SD	Futura	SD	Finola	SD	Fibrol	SD	KC Virtus	SD
general look	4.13 $_{a}$	2.13	**6.56** $_{c}$	1.55	6.05 b,c	1.83	4.81 $_{a,b,c}$	2.04	4.59 $_{a,b}$	2.53	5.50 $_{a,b,c}$	1.79	5.79 $_{a,b,c}$	1.72	6.02 $_{a,b,c}$	1.96	4.32 $_{a,b}$	2.49
general colour	4.33 $_{a}$	2.56	**6.84** $_{b}$	1.94	6.21 a.b	2.02	5.32 $_{a,b}$	2.23	4.57 $_{a,b}$	2.68	5.98 $_{a,b}$	1.93	6.06 $_{a,b}$	1.96	6.30 $_{a,b}$	1.82	4.49 $_{a,b}$	2.89
general odour	4.70 $_{a,b}$	2.38	**5.47** $_{b}$	2.26	5.71 a,b	2.37	4.60 $_{a,b}$	2.51	4.99 $_{a,b}$	2.47	4.77 $_{a,b}$	2.34	4.22 $_{a}$	2.49	5.56 $_{a,b}$	1.94	4.34 $_{a}$	2.78
general taste	4.28 $_{a}$	2.75	5.73 $_{a}$	2.07	4.69 $_{a}$	2.71	4.01 $_{a}$	2.64	4.29 $_{a}$	2.84	4.47 $_{a}$	2.36	3.91 $_{a}$	2.66	**5.77** $_{a}$	1.96	4.28 $_{a}$	2.58
fusty/mudy sediments	3.08 $_{a}$	0.89	1.79 $_{b}$	2.01	2.57 $_{a}$	2.32	2.90 $_{a}$	2.65	2.97 $_{a}$	2.77	2.54 $_{a}$	2.39	2.53 $_{a}$	2.44	**1.59** $_{a}$	1.84	3.03 $_{a}$	2.91
musty/humid/earthy	3.03 $_{a}$	3.18	2.06 $_{a}$	2.73	1.89 $_{a}$	2.53	2.64 $_{a}$	2.93	2.56 $_{a}$	3.16	2.07 $_{a}$	2.36	2.23 $_{a}$	2.60	**1.77** $_{a}$	2.39	3.04 $_{a}$	3.25
acid/sour taste	1.24 $_{a}$	1.95	1.03 $_{a}$	1.39	0.89 $_{a}$	1.69	0.91 $_{a}$	1.45	0.76 $_{a}$	1.21	**0.69** $_{a}$	1.00	1.08 $_{a}$	1.51	1.02 $_{a}$	1.72	1.23 $_{a}$	1.82
metallis	0.76 $_{a}$	0.88	1.06 $_{a}$	1.51	1.22 $_{a}$	1.72	2.06 $_{a}$	2.34	**0.68** $_{a}$	0.94	0.97 $_{a}$	1.26	1.91 $_{a}$	2.41	1.07 $_{a}$	1.84	1.06 $_{a}$	1.35
rancid	1.26 $_{a}$	1.89	0.77 $_{a}$	1.37	0.79 $_{a}$	1.54	1.26 $_{a}$	1.82	0.94 $_{a}$	1.64	1.01 $_{a}$	1.91	1.22 $_{a}$	1.68	**0.68** $_{a}$	1.14	0.85 $_{a}$	1.13
fruity	2.59 $_{a}$	2.52	2.61 $_{a}$	2.18	2.85 $_{a}$	2.14	2.46 $_{a}$	2.27	2.04 $_{a}$	2.02	2.26 $_{a}$	1.99	2.14 $_{a}$	1.95	**2.94** $_{a}$	2.35	2.71 $_{a}$	2.38
bitter	1.66 $_{a}$	1.78	1.14 $_{a}$	1.55	1.59 $_{a}$	1.89	1.53 $_{a}$	1.95	1.16 $_{a}$	1.63	1.74 $_{a}$	2.22	1.70 $_{a}$	1.88	1.18 $_{a}$	1.59	**1.23** $_{a}$	1.64
pungeancy	1.54 $_{a}$	2.19	1.51 $_{a}$	2.02	1.79 $_{a}$	2.73	1.59 $_{a}$	2.16	1.02 $_{a}$	1.72	1.36 $_{a}$	2.33	1.08 $_{a}$	1.18	**1.01** $_{a}$	1.79	1.62 $_{a}$	2.47

$_{a_c}$Mean values with different superscripts within the same row are signifcantly diferent ($p < 0.05$).

Overall, it is visible that the one variety, which was the most visually attractive ($p < 0.05$), was Bialobrzeskie which was also evaluated as the one with best colour ($p < 0.05$). The best ranking for category general odour was determined as well variety Bialobrzeskie ($p < 0.05$). The variety with the best taste, with smallest amount of sediments, with the lowest after taste and acid/sour taste, with the fruitiest taste and the least rancid and pungeant scored Fibrol, unfortunately only in parameter fusty/muddy sediments was statistical different ($p < 0.05$). As well as the evaluation of the rest of parameter were statistically insignificant: the lowest metallis taste (Futura), the lowest rancid taste (Kompolti hybrid) and the lowest bitter (KC Virtus). To sums it up, as the most tasteful, eye catching, and good-looking variety was evaluated Bialobrzeskie.

As far as the sensory evaluation goes there were not any enormous differences among the varieties. The greatest problem of the analysis is its subjectivity. Each taster has different preferences and could be influenced by personal mood, hunger etc. For future evaluation and taste testing I recommend including more subjects in order to obtain more precise results.

The results of all the tested values illustrate that the type of storage has the most significant influence on all monitored values. Values were repeatedly measured at previously determined time points and they were compared to the values measured right after the initial extraction. Those samples, which were stored in cool conditions did not deteriorate as fast as samples stored in room conditions. I reached this conclusion based on evaluation of all measurements, which showed a clear trend of increase in all recorded values in samples stored at room temperature, indicating worsening of the quality of oil. The difference in respective values based on their storage reached one third. From the results it is obvious that samples stored in cool conditions, where temperature was around had lower values occur. Therefore, I conclude that this kind of storage is more convenient.

5. Conclusion

Cold pressed seed oil from nine different varieties of *Cannabis sativa* L. namely Santhica, Bialobrzeskie, Futura, Fedora, Tiborsallasi, Kompolti hybrid, Finola, KC Virtus and Fibrol was evaluated in this study. Chemical characteristics such as peroxide value, iodine value, saponification value, acidity value and ester value served as indicators of quality of the hemp oil stored for 24 weeks in two different conditions, under the room temperature and in the fridge. Increase in the measured values indicates decrease in quality of the oil. The decrease is characteristic by appearance of unwanted changes not only in chemical composition but also in sensory properties.

According to the results, it proved crucial to keep the hemp oil in dark and cold, 4°C. The values obtained from the experiment, prove that no matter which variety at which time I tested, every time the values where lower for refrigerator storage. Under cool conditions, the variation within range of individual values was indisputably smaller in contrast to room temperature. The change was observed for each tested variety. Room storing varieties had higher means than cool storing ones.

Bialobrzeskie was analysed to be the most stable variety for long-term storage. This variety was also evaluated, by the 22-member sensory panel, as a variety with the most eye pleasing colour and overal general look and odour.

Hemp oil has its own characteristic aroma, colour, taste and nutritional values, which are changing with time and storage conditions. After 24 weeks the changes are visible in all aspects. The most significant changes were observed in the oxidation stability.

To slow such undesirable changes down, it is necessary to choose the most suitable storage conditions, mainly temperature but also other aspects play role. Among those belong access of light as well as the right packaging. If those terms are met, the longevity of the oil can be extended at least by days.

Overall, the study can be further used to examine the maximum capacity for storing each variety, to establish the most convenient variety for long-term storage periods, extending the 24 weeks mark. Such analysis will be useful for evaluation of the quality factors of products made from the oil etc. To monitor the differences among varieties and influence of the storage conditions on them, further testing is scheduled at the 36 week- and 48 weeks – mark.

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Appendices

List of the Appendices:

Appendix 1: Chemical analyses - photo documentation

Figure 30. Prepared samples for obtaining peroxide value. State before first titration by solution $Na_2S_2O_3$.

Figure 31. Peroxide value sample after first titration by solution $Na_2S_2O_3$.

Figure 32. Peroxide value sample after adding colour indicator- starch.

Figure 33. Final look of the peroxide value sample after second titration by solution $Na_2S_2O_3$.

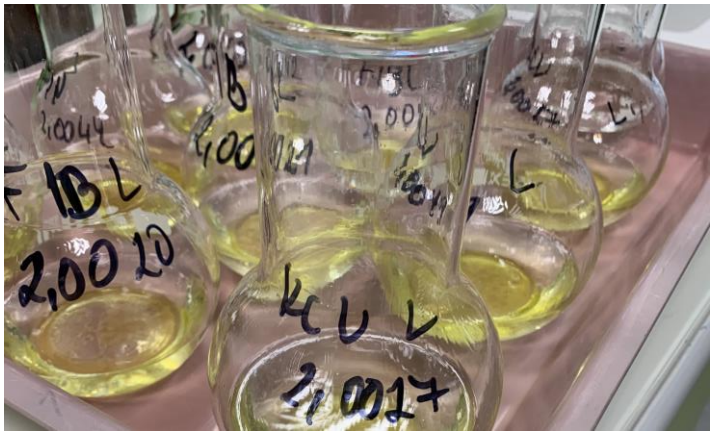

Figure 34. Acidity value oil samples with added neutralized ethanol.

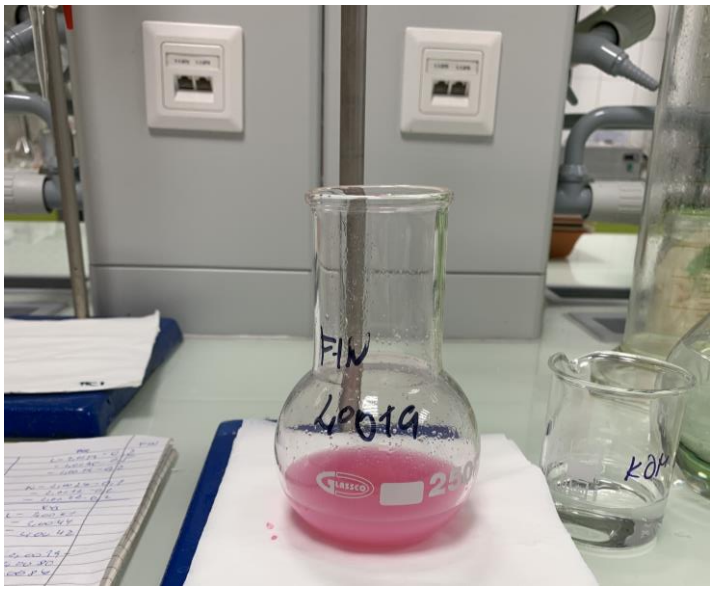

Figure 35. Acidity value sample after titration by KOH.

Figure 36. Set of reverse coolers for saponification value analysis.

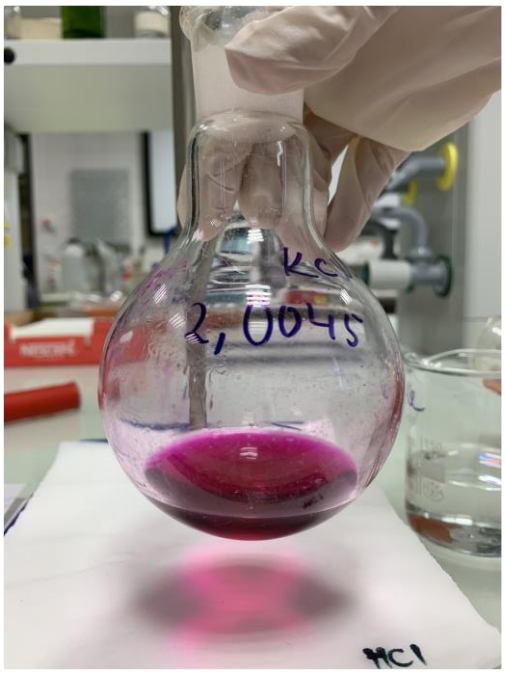

Figure 37. Saponification value sample with added colour indicator 1% solution of phenolphthalein.

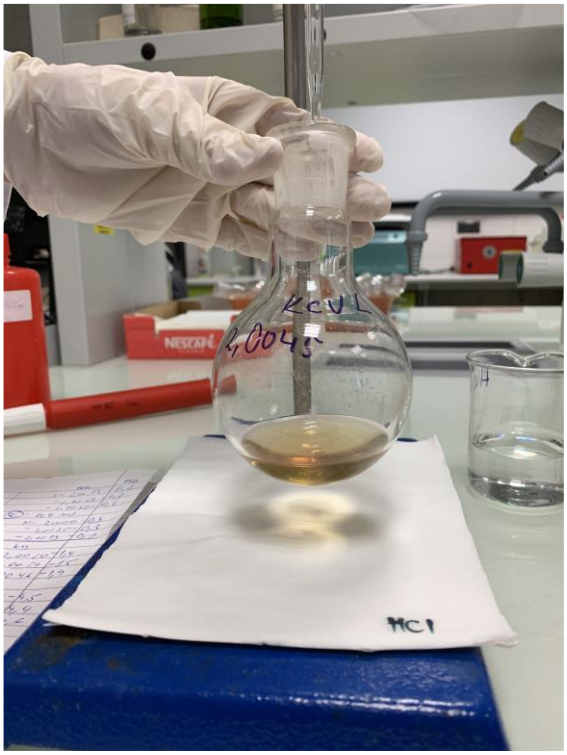

Figure 38. Saponification value sample after titration by solution HCl.

Figure 39. Iodine value sample with added colour indicator- starch.

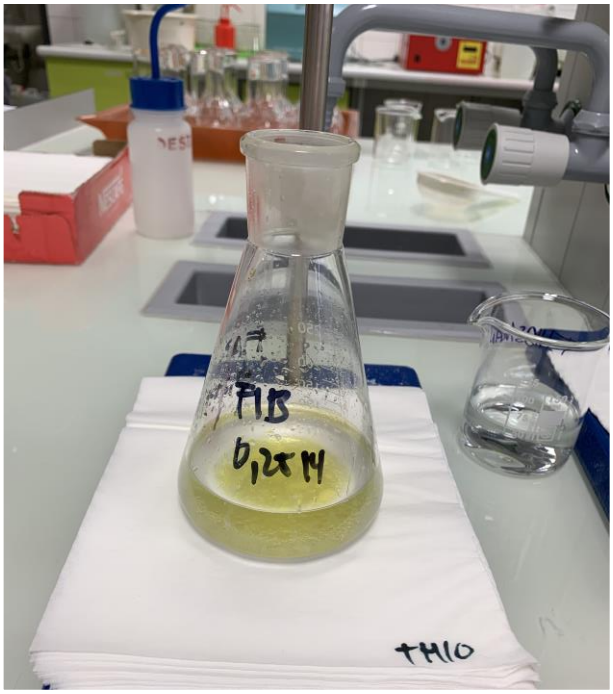

Figure 40. Iodine value sample after second titration by solution $Na_2S_2O_3$.