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Quality of olive oil during storage in different packaging materials

MASTER'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled Quality of olive oil during storage in different packaging materials independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague, 22nd April 2022

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Abstract

Extra virgin olive oil is an excellent source of natural fat, originated in the Mediterranean basin. Nowadays, olive oil is expanded and appreciated worldwide. Besides its positive content, olive oil carries substances that are not stable, and its durability depends on the storage conditions such as packaging, temperature, light, and oxygen. This thesis aimed to compare the influence of two packaging materials (glass and Tetra Pak®) on extra virgin olive oil quality parameters. To simulate household conditions, the headspace was created by removing half of the content. Each sample was stored under two different storage conditions (dark & cold, light & warm). Quality parameters and chemical properties of the samples were investigated four times over four months by analytical titrations; oxidation stability was identified by oxitest reactor and volatile compounds causing rancidity were determined chromatographically (GC-MS). The results of titrations did not show any significant differences in the quality of stored olive oil, nevertheless; the induction periods measured by the oxitest reactor were the shortest for olive oils with headspace indicating their susceptibility to rancidity. To conclude, no difference was found between packaging materials and their stability. The quality of extra virgin olive oil is unstable primary due to a large amount of the headspace in the bottle, particularly, when the olive oil is stored in light & warm conditions regardless of the packaging material.

Keywords: *Olea europaea*, shelf-life, headspace, stability, vegetable oil, edible oil

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List of the abbreviations used in the thesis

AV	acid value
EVOO	extra virgin olive oil
FAO	The Food and Agriculture Organization
FF	final full
FFA	free fatty acids
G	glass - before applied conditions
GC-MS	Gas chromatography - mass spectrometry
G-D	glass - dark
G-D-FF	glass - dark - final full
G-D-H	glass - dark - headspace
G-L	glass - light
G-L-FF	glass - light - final full
G-L-H	glass - light - headspace
H	headspace
IOC	International Olive Council
IP	induction period
mEq	milliequivalents
MUFA	monosaturated fatty acids
PET	polyethylene terephthalate
PV	peroxide value
RT	room temperature
SPME	Solid - phase microextraction technique

SV	saponification value
T	Tetra Pak® - before applied conditions
T-D	Tetra Pak® - dark
T-D-FF	Tetra Pak® - dark - final full
T-D-H	Tetra Pak® - dark - headspace
TGA	triacylglycerols
T-L	Tetra Pak® - light
T-L-FF	Tetra Pak® - light - final full
T-L-H	Tetra Pak® - light - headspace
WHO	World Health Organization

1. Literature Review

1.1. Botanical description

The olive, *Olea europaea* L. is a characteristic fruit tree from the family Oleaceae. *O. europaea* is a small evergreen tree up to 20 meters high or grey-green branched shrub growing to a height of 5 meters. Its root system is extensive with main roots which could grow up to 200 centimetres in diameter. The leaves are opposite, simple, and without stipules. The upper part of the leaf is dark grey-green and globous. The lower part of the leaf is silvery scaly, and pinnately veined (PROTA 2021). To survive the hot and dry climates of the subtropical region, the olive tree has a protective coat in the form of trichomes on the underside of the leave (Laurentiu 2022). The inflorescence is an axillary panicle with small white bisexual fragrant flowers (PROTA 2021). Most olive varieties are self-pollinating. At the beginning of the spring, during 12-15 weeks of temperature fluctuation, when the temperature occurs below 10 °C, there is a jarovisation associated with the production of flowers. If the temperature drops under -7 °C, fruit production could be inhibited. On the other hand, higher production is being supported when the tree is strongly pruned. After the process of pollination, the flower is fertilised and later, the fruit is created (Laurentiu 2022). The fruit of the olive tree is a globose to ellipsoid drupe, which is found in a size of 0.5 – 4 cm x 0.5 – 2.5 cm (PROTA 2021). It consists of three layers: a lignified endocarp usually containing one seed, in the middle there is a fleshy mesocarp containing the oil, and a top layer is a slender epicarp. The olive could weigh up to 20 grams (Guo et al. 2018).

1.2. Harvesting and Processing of virgin olive oil

Olive oil production begins with the harvest. Harvesting is the last step in the field production of an olive crop (Ferguson 2006). However, the type of harvesting method has a significant effect on the oil quality and for that reason, olives are traditionally harvested by hand (Saglam et al. 2014). As well as the choice of harvesting method, the choice of

harvesting time is essential for obtaining the highest quality and quantity of olive oil (Camposeo et al. 2013).

After the harvest, the olives are washed to separate impurities, twigs, and leaves. To achieve the highest oil quality, it is required to process olives within 24 hours of harvest (Mchugh 2015). There are several extraction methods: pressing, percolation, and centrifugation (Kapellakis et al. 2008).

As a pressing method, the traditional press is the oldest oil extraction procedure. Traditional olive oil processing begins with the crushing of olives with 2 – 6 stones in the mill to obtain an olive paste (Kalogianni et al. 2019). The olive paste is slowly mixed to improve the oil extraction and it allows the fruit enzymes to produce desirable flavours and aromas. It is mixed for 20 – 45 minutes. Longer mixing time could increase yield but it could also decrease the shelf-life and quality. Subsequently, the paste is spread onto disks and placed into the press to separate water and oil. After pressing, liquids are separated by centrifugation or decantation (Mchugh 2015). There are some advantages of this method such as low moisture content in the oil, cheap equipment, and low waste of water. However, this process is not persistent, has insufficient capacity, and has high labour costs. For this reason, this traditional method has been replaced by modern ones such as metallic crushers, centrifugation separation systems, and malaxers (Kalogianni et al. 2019). After the processing, new virgin olive oils still contain some undesirable particles. These pieces are removed by filtration or sedimentation to the bottom of the containers (Ciafardini & Zullo 2018).

Olives and olive oil are basically part of every European household, especially in the Mediterranean region. In Table 1, there are the top greatest olive oil producers in the world. Spain is the biggest producer of olive oil in the world with a year production of 1,129,233 tonnes. Italy is in second place and the third place belongs to Greece with 290,476 tonnes per year. Thus, olive oil is primarily produced in European countries in the Mediterranean basin (FAO 2022). Nevertheless, olive trees are currently planted in California, Chile, and Argentina, too (Vossen 2007).

Table 1 The biggest producers of olive oil, data from 2019 (FAO 2022)

Area	Production [t]
Spain	1,129,233
Italy	336,581
Greece	290,476
Tunisia	239,500
Turkey	217,800
Morocco	204,200
Portugal	154,063

1.3. Storage of olive oil

The olive fruit and its oil are key elements in the cuisine of the Mediterranean region. Nowadays, olive oil is worldwide expanded and eaten for its taste, aroma, and effects on the human body. Nevertheless, the quality of olive oil depends on different factors such as olive cultivar, olive tree cultivations, type of harvest, processing method, and storage (Di Giovacchino et al. 2002). Also, olive oil contains substances that are not stable and its durability depends on the storage conditions such as temperature, light, and oxygen (Serrano et al. 2016; Sanmartin et al. 2018). Degradation of olive oil compounds could lead to classifying them to a different specific quality grade when bottled. Consequently, olive oil may be of inferior quality when purchased and consumed (Lolis et al. 2019). Attributes of packaging material affect its chemical and sensorial qualities (Sanmartin et al. 2018). Materials used for the packaging of olive oil are dark-coloured glass, aluminium, tinplates, polyethylene, plastic-coated paperboard (known as Tetra-Brick®), and multilayer pouches (Lolis et al. 2019). According to Abbadi et al. (2014), the best container to maintain the extra virgin olive oil (EVOO) quality was glass. Glass represents a sound barrier against moisture and gases. On the other hand, glass is a transparent bottle that could lead to photo-oxidation (Sanmartin et al. 2018). For this reason, it is essential to protect the oil against oxidative deterioration (Sanmartin et al. 2018).

1.4. Health benefits

The consumption of olive oil, particularly EVOO, has an increasing trend due to its high dietetic and nutritional value. These health effects are principally correlated to the mixture of bioactive compounds such as triacylglycerols (TGA), polyphenols, tocopherols, and carotenoids (Gavahian et al. 2019). In addition, olives contain an abundant amount of mineral compounds, including iron, potassium, calcium, and sodium. Olives are rich in vitamins too, especially vitamin E and K (Guo et al. 2018).

The olive oil consists of 98 % of TGA. TGA are a group of glycerol esters with various fatty acids. The most dominant fatty acids present in olive oil are monosaturated fatty acids (MUFA), mainly oleic acid (Kuban-Jankowska et al. 2018). The high content of MUFA seems to have anti-hypertensive, anti-inflammatory, and anti-thrombotic effects (Donat-Vargas et al. 2022).

In the case of phenolic compounds, there are two predominant polyphenols: hydroxytyrosol and oleuropein. Both polyphenols are responsible for antioxidant activity. Polyphenols are considered as natural compounds that reduce the development of cardiovascular and neurodegenerative diseases (Kuban-Jankowska et al. 2018).

For instance, hydroxytyrosol captures free radicals and reactive oxygen or nitrogen in the human body. Oleuropein is effective against bacteria, viruses, moulds, and fungi. Moreover, the antioxidant properties of oleuropein protect cells from genetic damage which could lead to oncogenesis. Another positive effect of oleuropein on the human body is an anti-angiogenic impact which prevents or slightly slows down tumour development (Kuban-Jankowska et al. 2018).

All these characteristic bioactive compounds make olive oil one of the healthiest edible oil worldwide (Gavahian et al. 2019). It is well known that the consumption of olive oil has a positive effect on human health (Ciafardini & Zullo 2018). It prevents breast cancer (Foscolou et al. 2018), modifications of inflammatory responses (Ciafardini & Zullo 2018), obesity, and diabetes mellitus type 2 (Gavahian et al. 2019). According to Donat-Vargas et al. (2022), the recommended consumption of EVOO is 20 to 30 grams per day to obtain the maximum benefit against cardiovascular illnesses.

1.5. Quality and Standards of olive oil

The olive oil has many rules and standards to guarantee the product's authenticity. Such regulations are issued by the European Union, the International Olive Council (IOC), and the Codex Alimentarius. The European Union is a member of the IOC, the only intergovernmental organisation globally to assemble olive oil-producing and consuming stakeholders (International Olive Council 2021). The Codex Alimentarius is an organisation for international food standards supported by The Food and Agriculture Organization (FAO) and World Health Organization (WHO) (Codex Alimentarius Council 2017). Codex Alimentarius and IOC cooperate, and they have similar standards which are described below. Standards define the physical, chemical, and organoleptic characteristics of olive oil. The quality and the purity of olive oil are measured by the content of chemical compounds. Also, these regulations established methods for their analysis. Moreover, olive oils must comply with the standards depending on where they are traded (Conte et al. 2019). In this chapter, selected chemical parameters of EVOO are summarised in detail according to the Codex Alimentarius and IOC.

EVOO is a type of highest-rated olive oil obtained from the fruit of olive trees exclusively mechanically under thermal conditions that do not lead to alterations in the oil (International Olive Council 2021). Every oil has to pass the test of Free Fatty Acid, Peroxide Value, UV Absorbency, Volatile Compounds, Insoluble Impurities, Flash Point, Metal Traces, and so on, to be defined according to regulations (Vossen 2005). In this thesis only some of the quality characteristics were applied, hence only a few criteria are listed below.

Table 2 gives the main quality parameters and the regulations of EVOO. Free acidity (FFA) cannot exceed the level of 0.8 grams of oleic acid per 100 grams (not more than 0.8 %. Peroxide value (PV) could not be higher than 20 milliequivalents (mEq)[1] of active oxygen per kilogram of oil. Saponification value (SV) must be between 184 and 196 mg KOH per gram of oil (Codex Alimentarius Council 2017; International Olive Council 2021). In the case of acid value (AV), regulations are used in accordance with

[1] The equivalent is the amount of substance that can react with one mole of counter-ion carrying and unit charge. The mEq is 1 / 1,000 equivalent. Because of the amounts found in chemical terms, the unit mEq is more common (Nelson 2018).

Czech legislation. The official value set by the Ministry of Agriculture of the Czech Republic (2022) is lower than 4.0 milligrams KOH per gram.

Table 2 Regulations of qualitative parameters

Parameter	Amount	Unit	Source
PV	≤ 20	mEq O_2 / kg	(Codex Alimentarius Council 2017)
FFA	≤ 0.8	g oleic acid / 100g	(Codex Alimentarius Council 2017)
SV	184 - 196	mg KOH / g	(Codex Alimentarius Council 2017)
AV	≤ 4.0	mg KOH / g	(The Ministry of Agriculture of the Czech Republic 2022)

The European Union has established regulations on marketing standards for olive oil and its characteristics (European Commission 2012). Nevertheless, these standards are not for household conditions. In this research, household conditions are simulated by creating the headspace in bottles.

1.6. Packaging materials

Food packaging plays an important role in product accessibility, advertising, protection, and storage. The olive oil quality is affected mainly by storage conditions, especially by the type of packaging material. Packaging material can provide a barrier to oxygen and light transmission. Packaging has been a key factor in the worldwide dissemination of olive oil, especially owing to its contribution toward the retention of the oil quality. Although the design of olive oil bottles is valuable for advertising. On the other hand, it is necessary to ensure an adequate shelf-life of the product (Esposto et al. 2021).

During the time, various containers were used and studied for qualitative aspects. As the most commonly used packaging materials are considered: glass, metal, Tetra Pak®, and various types of plastic materials (Esposto et al. 2021).

The most widely used material for packaging olive oils is glass. That is not only due to marketing requirements but also because glass prevents the permeation of oxygen into the bottle. On second thought, transparent glass leads to photo-oxidation of olive oil and reduction of its shelf-life. But most of the oils are packed into coloured glass bottles,

which prevents or slows down the oxidation process. For example, green bottles protect the oil from wavelengths of 300 – 500 nm (Piergiovanni & Limbo 2009). One group of scientists studied the olive oil in green glass material and ultraviolet grade absorbing glass. Based on the data obtained in their study, the green glass container and a certain extent of ultraviolet grade absorbing glass containers allow light transmission and therefore induce oxidative rancidity. Although most of the EVOOs are commercially distributed in bottles made of glass, as an indicator of high quality, this material does not maintain those properties for which the product is highly appreciated by consumers (Esposto et al. 2021).

Metal containers are manufactured using tinplate and aluminium. Tinplate containers have been used for a long time for oil packaging and are still appreciated because of many advantages, such as protection against access to oxygen, light, microorganisms, and water vapour. Moreover, the inside of the container is protected with food-approved enamels that protect the metal from the corrosiveness of the product (Piergiovanni & Limbo 2009).

Plastic containers are a relatively new type of edible packaging due to their comparatively low price and low weight. Among plastics, polyethylene terephthalate (PET) material has many advantages, including clarity, chemical inertness, low oxygen permeability, and great mechanical properties and that is why PET became a substantial piece of the olive oil retail market (Piergiovanni & Limbo 2009). Pristouri et al. (2010) researched the effect of container oxygen permeability. They used clear glass, clear PET, and clear polypropylene bottles. They concluded that containers with high oxygen transmission rates, such as polypropylene, are not suitable for the packaging of olive oil. Also, packaging olive oil in low oxygen transmission rate bottles, such as PET, does not effectively protect the olive oil beyond 3 months in the presence of light (Pristouri et al. 2010).

New packaging formats have been introduced in the market including bag-in-box systems, lined cartons, and paperboard laminate cartons (Piergiovanni & Limbo 2009). Tetra-Brick® is a carton package better known as Tetra Pak which is the official name of the company that makes Tetra-Brick®. Tetra-Brick® packaging is inexpensive and is considered more suitable because it contains a metal lining, thereby protecting the oil from penetrating light and oxygen (Kiritsakis et al. 2002; Samaniego-Sánchez et al. 2012). Samaniego-Sánchez et al. (2012) studied EVOO stored at glass, PET,

and Tetra-Brick®. Their results showed that Tetra-Brick® packages appear to be the most appropriate containers for maintaining the quality of EVOOs since they protect the oil from both light and oxygen, which are directly related to nutritive attributes, and quality parameters of EVOO, at least for a limited time.

1.7. Peroxide value

Every edible oil is prone to oxidation during storage. As an essential indicator of the initial stages of oxidation, the peroxide value is determined. Peroxide value measures the total hydroperoxides content and monitors lipid oxidation during oil preservation (Zhang et al. 2021). Hydroperoxides are known as primary oxidation products which influence the intensity of the flavour and odour. During secondary oxidation, hydroperoxides are broken down into aldehydes and ketones, which are responsible for off-flavours (de la Torre-Robles et al. 2019). They are influenced by many factors, such as oxygen, and temperature, but also a large amount of oxygen could be dissolved in oil when the oxygen partial pressure in the headspace is high (Zhang et al. 2021). An increased level of peroxide indicates oxidised and poor quality oils (Li & Wang 2018).

PV is usually determined by titration analysis. Titration is the most common method. There are also other methods which are described below in the chapter Oxidative stability. The titration method is based on the hydroperoxides contained in oil that react with potassium iodide to form molecular iodine, which is titrated using a thiosulfate solution (Zhang et al. 2021).

According to IOC regulations, the peroxide value cannot exceed the level of 20 mEq O_2 per kilogram of oil (International Olive Council 2021).

Samaniego-Sánchez et al. (2012) from Spain studied EVOOs under different storage conditions for 9 months. As a packaging material, they used polyethylene terephthalate (PET), glass, and Tetra-Brik®. All containers were closed for the whole experiment. The initial PV for the oil in glass was 4.76 mEq O_2/ kg. Whereas the oil in Tetra-Brik® has the initial PV lower with the amount of 2.26 mEq O_2/ kg. The worst PV had PET material with 5.33 mEq O_2/ kg. After three months, the PV at room temperature (RT) in glass raised to 9.83 mEq O_2/ kg, in Tetra-Brik® to 8.86 mEq O_2/ kg, and in PET to 14.8 mEq O_2/ kg. In the case of oils in the refrigerator, the oil in glass has PV 8.40 mEq

O_2/ kg, the oil in Tetra-Brik® has PV 8.13 mEq O_2/ kg, and the oil in PET has the PV 9.80 mEq O_2/ kg. As a result, they found out that higher PV is in the oils stored at RT. The photo-oxidation reactions in the oils are initiated more slowly at lower temperatures. Also, none of the oil exceeded the maximum peroxide limit for EVOO after 9 months. The most significant increase was in oil stored in PET containers because it has the highest permeability of all these packaging materials (Samaniego-Sánchez et al. 2012).

Di Serio et al. (2018) from Italy conducted research on seven EVOOs in dark green glass bottles in diffuse light in RT. An initial PV was 9.4 mEq O_2/ kg as the mean of 7 varieties of EVOO. After 4 months, the PV increased to 10.2 mEq O_2/ kg. Their results showed that the primary oxidation already started, but it does not influence the quality as the second phase of oxidation leads to the rancid organoleptic defect. One variety out of seven exceeded the limit after only 10 months with a value of 21.5 mEq O_2 per kg. Four other varieties exceeded the limit after 12 months. Only two of all varieties did not exceed the standard after 12 months (Di Serio et al. 2018).

Another research team, Lolis et al. (2020) from Greece, studied EVOO under different storage temperatures (15 °C, 22 °C, and 37 °C). EVOO was stored in a dark-coloured glass bottle for up to 18 months. The initial PV was 12.31 mEq O_2/ kg. After three months, the PV of oil stored at 15 °C was 13.41 mEq O_2/ kg. The oil stored at 22 °C had the PV 13.95 mEq O_2/ kg. The PV of oil stored at an abuse temperature of 37 °C was 15.09 mEq O_2/ kg. Overall, none of the samples exceeded the limit value after 18 months of storage indicating that even at the abuse temperature of 37 °C, oxidative changes in the EVOO were limited (Lolis et al. 2020).

Italian scientists studied just one type of olive oil. Oil was packaged into a one-litre bottle of dark-green glass. The bottles were maintained at RT under artificial light and away from heat sources. It was being monitored for 12 months. The PV has risen to a maximum value of 19 mEq O_2/ kg in the third month of storage. And then, the PV started to decrease to 12.8 mEq O_2/ kg after 12 months. This behaviour could be explained by the initial increase in hydroperoxides, which are compounds formed during the primary step of the oxidation process. Afterwards, these compounds bring about substances responsible for off-flavours. After one year of storage, the occurrence of the rancid defects was observed so that is why they recommended an optimum time of storage of the oil up to 9 months (Lanza et al. 2015).

Pristouri et al. (2010) from the University of Ioannina, Greece, studied the packaging material (PET, polypropylene, and clear glass), oxygen transmission, and the effect of storage temperature, but also the influence of headspace in the container. Bottles were stored in the dark at 22 °C. PV was firstly exceeded in the polypropylene sample in the ninth month. In the PET sample, there was a large headspace (about 0.5 litres) that resulted in a drastic increase of PV values after 12 months of storage. Due to an extremely high concentration of oxygen, the olive oil quality was drastically regressed. At such high oxygen headspace concentrations, the effect of this parameter was the most critical (Pristouri et al. 2010).

1.8. Free fatty acids

Virgin olive oil contains about 98 % of lipids, mostly triglycerides, followed by a small quantity of diglycerides and a variable quantity of FFA which are used as a marker of oil quality (Jabeur et al. 2015). FFA are products of hydrolysis of triglycerides in edible oils (Di Pietro et al. 2020). Their formulation occurs primarily during ripening, processing, and storage. An elevated level of FFA indicated hydrolysed fruit or poor-quality olive oil made from defective fruit, improperly processed, or incorrectly stored oil (Li & Wang 2018). The high content of FFA results in poor flavour quality and stability of the oil (Di Pietro et al. 2020).

The international standard for FFA in EVOO is established as not more than 0.8 grams of oleic acid per 100 grams (Codex Alimentarius Council 2017; International Olive Council 2021).

An applied method to measure the content of FAA is the so-called acid value (AV). The AV indicates how many milligrams of potassium hydroxide are needed to neutralise the acidic fraction found in one gram of oil. This method is based on the titration of the sample with a standardised ethanolic solution of potassium hydroxide using phenolphthalein as an indicator (Di Pietro et al. 2020).

Samaniego-Sánchez et al. (2012), in addition to PV, also described the FFA as a percentage of oleic acid. The initial value of FFA for the oil in the glass was 0.14 %, the oil in PET has 0.23 %, and the oil in Tetra-Brik® has 0.12 %. After three months, FFA increased in every container. The oil in glass at RT has 0.27 %, oil in PET at RT has 0.34 %, and in Tetra-Brik® at RT has 0.34 %. In refrigeration, the oil in glass has 0.33 %,

in PET 0.40 %, and in Tetra-Brik® the value was 0.31 %. In this experiment, the lowest increment was found in the oils stored in Tetra-Brik® because the Tetra-Brik® contains a metal lining, thereby protecting the oil from penetrating light and oxygen. For these oils, the AV was practically equal at RT and at a refrigerated temperature which means that acidity is not affected by temperatures. Furthermore, even after 9 months, the values did not exceed standards (Samaniego-Sánchez et al. 2012).

According to Lolis et al. (2020), the percentage of acidity increased with storage time and temperature. The initial AV was 0.70 % of oleic acid. After three months, the changes were following: stored at 15 °C AV was 0.72 %. At 22 °C AV was 0.79 %. The results indicated that the upper limit value for acidity was reached after 9 months at 22 °C. In the case of storage at 15 °C, the respective limit was reached after 12 months. At the abnormal temperature of 37 °C, the limit was reached after only 3 months of storage (Lolis et al. 2020).

Pristouri et al. (2010) also studied the acidity of the EVOO. The initial value of EVOO in glass was 0.63 % of oleic acid. The oil was stored in the dark at 22 °C. After three months the value increases to 0.68 %. The increase in temperature in the dark and increase in the headspace in the dark resulted in the highest acidity values after 12 months of storage (Pristouri et al. 2010).

1.9. Saponification value

The saponification value (SV) is related to all fatty acids present in the sample, free acids as well as esterified acids. The SV is defined as an amount of potassium hydroxide in milligrams needed to neutralise the free fatty acids and saponify the esters contained in one gram of oil (Barret 2018). In other words, SV is an indicator of the molecular weight of triglycerides in oil (Cobzaru et al. 2016). SV is one of the most common parameters used to characterise fats and oils with the former being an indication of the degree of unsaturation, which is essential in monitoring hydrogenation processes (Xu et al. 2018). SV is mainly used to establish oils for biodiesels (Azam et al. 2010).

According to Codex Alimentarius Council, the SV for virgin olive oils should be between 184 milligrams to 196 milligrams of potassium hydroxide per gram of oil (Codex Alimentarius Council 2017).

SV is determined by many methods, the most known is the conventional method which is performed by analytical titration. The sample is heated for at least 30 minutes with potassium hydroxide in ethanolic solution to complete saponification of the oil. Subsequently, the sample is titrated with a hydrochloric acid solution using phenolphthalein as an indicator (Dalla Nora et al. 2018).

1.10. Volatile compounds

Extra virgin olive oil flavour is usually characterised by pleasant sensory impressions that are appreciated by consumers. High-quality olive oils have a profile of volatile compounds that generated balanced flavour of green and fruity sensory characteristics. These compounds include aldehydes, ketones, esters, and alcohols (Morales et al. 2005).

Volatile compounds are retained by EVOO during their mechanical extraction process from olive fruits (Angerosa et al. 2004). On the other side, they could be influenced by several factors, such as cultivar, geographic region, ripeness, harvest and processing methods (da Silva et al. 2012).

After the oil is extracted from the fruit and stored, the oxidation of fatty acids begins. During storage, oxidation reactions reduce the high nutritional value of EVOO and modify its characteristic flavour through the development of off-flavours from hydroperoxide decomposition products. The volatile compounds, which are responsible for the pleasant sensory impression became less dominant and at the same time, those which are responsible for the negative aspects arise. The main sensory defect, which develops during olive oil storage, is correlated with the oxidation process in the rancid off-flavour (Kotsiou & Tasioula-Margari 2015).

The unsaturated aldehydes 2-heptenal, 2-octenal, and 2-decenal can be considered as the main contributors to the rancid defect due to their very low odour thresholds followed by the saturated aldehydes such as pentanal, hexanal, heptanal, nonanal, and octanal. Additionally, acetic, hexanoic, and butanoic acids contribute to the rancid sensory profile. All these compounds are related to the perceptions of rancid, fatty and oily (Kotsiou & Tasioula-Margari 2015).

In order to find the volatile compounds that are responsible for the odour quality, sensory assessment methods are used. Nevertheless, they are not simple, inexpensive and a permanent staff of trained sensory analysts is required. Moreover, the subjective opinion of the sensory analyst influences the final overall evaluation too, and some flaws have been pointed out. Thus, analytical methods based on the identification and quantification of volatiles are needed to achieve the correct classification of EVOO in an efficient way. In this regard, solid-phase microextraction is the most used system in the isolation and preconcentration of volatiles, prior to gas-chromatographic analysis (Romero et al. 2015).

1.11. Oxidative stability

Lipid oxidation has been recognised as a major problem affecting edible oils, as it is the cause of critical deteriorative changes in their chemical, sensory and nutritional properties. As already mentioned in the peroxide value chapter, oxidation normally proceeds slowly at the initial stage, and this is measured by PV. Then a sudden rise occurs in the oxidation rate (Velasco & Dobarganes 2002). The oxidative stability is expressed as an induction period (IP) which means the period of time before a dramatic increase in the oxidation rate begins (Tsao et al. 2021). EVOO has a high resistance to oxidative deterioration mainly due to its fatty acid composition, and also because contains minor compounds with antioxidant activity among which polyphenols stand out (Velasco & Dobarganes 2002).

The most important external variables influencing olive oil stability towards oxidation are temperature, oxygen concentration and light (Velasco & Dobarganes 2002). The temperature is the most critical factor. According to Riciputi & Caboni (2017), the oil oxidation rate is usually slow at room temperature. The influence of light on photosensitised oxidation is also important. Minor compounds (such as chlorophyll) could be excited electronically due to the absorption of light. Therefore, preventing photooxidation during shelf-life is very important to ensure high oxidative stability (Velasco & Dobarganes 2002).

There are several methods for evaluating the oxidative stability of oils. These are mainly methods for the prediction of the olive oil resistance to oxidation under storage conditions. These methods are applied for the measurement of oxidative stability at low

and high temperatures. Firstly, the Schaal oven test measures the rancidity and PV, and it is the most straightforward accelerated test. Secondly, the Active Oxygen Method measures the time to reach a predetermined PV under specific test conditions. Thirdly, Oxydograph is a method used to measure the induction time as the point of maximum change in the rate of oxygen uptake (Velasco & Dobarganes 2002). Another useful method is the Rancimat method, also called Oxidative Stability Index; it measures the oil oxidation resistance under accelerated conditions. Rancimat provides valuable data on the susceptibility to oxidation under the same operating conditions in oils of high stability (Ceci & Carelli 2010). Lastly, the method, which is used in this research, is an OXITEST® reactor which is an innovative instrument to measure the oxidative stability of fat foods based on the monitoring of pressure over a period of time in analytical chambers, where the sample is submitted at high oxygen pressure and high temperature (Caruso et al. 2017).

Most of these methods are suitable only for oils or fat extracted from foods but are not applicable to the whole food. Among them, the OXITEST® method also allows to measure the oxidation in the whole food and could directly detect changes in oxygen pressure inside a chamber and automatically determine the IP. Moreover, OXITEST® provides an ideal environment where light and oxygen exposures are controlled (Tsao et al. 2021).

2. Aims of the Thesis

The main objective of this thesis was to compare the influence of packaging material on qualitative parameters of extra virgin olive oil. The secondary subject of interest of this thesis was to observe light conditions, temperature, and the amount of the headspace in the bottles. Twelve bottles of olive oil were used, six for each type of packaging material: dark glass and Tetra Pak®.

The specific aims of the study were to analyse qualitative parameters, such as peroxide value, acid value, and saponification value by titration methods.

Furthermore, the autooxidation of olive oil employing an oxidation reactor under the action of pressure and temperature was detected. This method makes it possible to know the oxidative stability of olive oil as soon as possible.

Finally, volatile compounds were detected by gas chromatography-mass spectrometry. This method made it possible to determine the amount of the most volatile substances in olive oil.

3. Materials and Methods

The experimental part was performed in the laboratories of the Faculty of Tropical Agriculture at the Czech University of Life Sciences Prague.

3.1. Olive Oil

For analysis, two types of storage containers (two different packaging materials) of extra virgin olive oil (EVOO) were used. Firstly, EVOO stored in dark green glass was a mixture of four Spanish varieties: Arbequina, Cornicabra, Hojiblanca and Picual. It was produced by Aceites García de la Cruz, S.L. Secondly, EVOO stored in Tetra Pak® container was a mixture of four Spanish varieties: Arbequina, Picual, Picuda, and Hojiblanca. Both EVOOs were mechanically extracted only from olives.

Twelve bottles of olive oil were used, six for each type of packaging material: dark glass and Tetra Pak®. As room temperature (RT) and refrigerator represent the most common storage conditions of olive oil in a normal household, half of the bottles were placed in one and the other half in other conditions creating 4 groups (Table 3).

Table 3 Sorting of storage

dark & cold[1]	**light & warm**[2]
3 glass bottles (G-D)	3 glass bottles (G-L)
3 Tetra Pak® containers (T-D)	3 Tetra Pak® containers (T-L)

[1] a refrigerator with a temperature of 6 °C and no light exposure
[2] laboratory table at the room temperature of 21 °C (simulating worktop of a kitchen unit) with a light regime of 8 – 12 hours of daylight in December – March in the Prague, Czech Republic (coordinates: 50°1′N 14°4′E)

Initially, samples from four bottles (two bottles from storage conditions and packaging material) were taken to conduct the analyses of: G-D, G-L, T-D, T-L. At the same time, four other bottles (two bottles from each storage condition and packaging material) were opened, half of the content was removed, and the other half was left for further measurements of quality and headspace composition. This method

was applied to create a headspace (H) in the bottles, recreating a regular household use and storage of olive oil. These bottles with the headspace were stored in two different conditions: cold darkness (fridge) and warm light (room temperature, exposure to light during the day). The remaining four bottles were left unopened in two different storage conditions (dark & cold and light & warm) for later analyses. These last bottles were marked as final full (FF). In Table 4, there is a description of the abbreviation of each sample with a full meaning.

Table 4 Explanation of the applied conditions

Abbreviation	Full meaning
G	glass - before applied conditions
T	Tetra Pak® - before applied conditions
G-D	glass - dark
G-L	glass - light
G-D-H	glass - dark - headspace
G-L-H	glass - light - headspace
G-D-FF	glass - dark - final full
G-L-FF	glass - light - final full
T-D	Tetra Pak® - dark
T-L	Tetra Pak® - light
T-D-H	Tetra Pak® - dark - headspace
T-L-H	Tetra Pak® - light - headspace
T-D-FF	Tetra Pak® - dark - final full
T-L-FF	Tetra Pak® - light - final full

Figure 1 shows the design of the bottles with the corresponding environment and storage type. Tetra Pak® is represented by square rectangles, while glass is represented by rectangles with rounded corners. The headspace is depicted using unhatched spots. The diagram shows all twelve bottles used in this experiment.

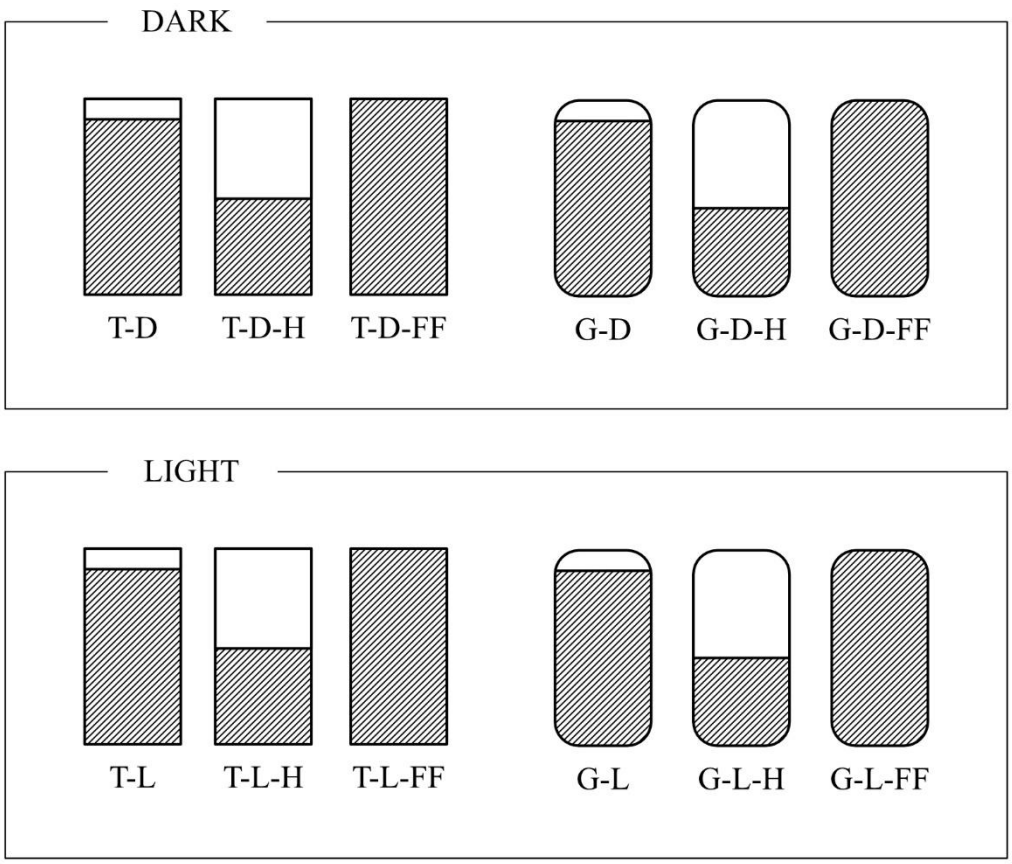

Figure 1. Scheme of research design

3.2. Chemicals

Used chemicals:

Acetic acid

Distilled water

Ethanol

Phenolphthalein

Potassium hydroxide

Potassium iodide

Sodium thiosulfate

Starch

Trichloromethane

Chemical Preparations:

Preparation of phenolphthalein indicator 1 %

i. 1 g of phenolphthalein
ii. 50 % ethanol solution consisting of 50 mL ethanol and 50 mL distilled water
iii. Dissolve the phenolphthalein thoroughly in the 50 % ethanol solution

Ethanolic solution of KOH, c(KOH) = 0.1 mol. l^{-1}

i. Normalan Potassium hydroxide Pentanal® – exact quantity for the preparation of 1000 mL of ethanol
ii. Mix the ampoule of KOH with 1000mL of ethanol

Neutralised ethanol

i. Warm the ethanol up to 65 °C in a water bath
ii. Put 3 drops of phenolphthalein indicator and add 0.5 mL of 0.1 M KOH to the pink colour

Water solution of Potassium iodide KI

i. 5 grams of KI was mixed with 5 mL of boiled water

Volumetric solution of $Na_2S_2O_3$, c($Na_2S_2O_3$) = 0.01 mol. l^{-1}

i. Normalan Sodium thiosulfate Pentanal® – exact quantity for the preparation of 1000 mL of water
ii. Mix the ampoule of KOH with 1000mL of water

Volumetric solution of HCl, c(HCl) = 0.5 mol. l^{-1}

i. Normalan Hydrochloric acid ROTH® – exact quantity for the preparation of 1000 mL of water
ii. Mix the ampoule of HCl with 1000 mL of water

Starch solution (indicator)

i. Heat 200 mL of water at 80 °C
ii. Measure 2 g of starch and mix with 20 mL of water
iii. Mix hot water with dissolve starch

Ethanolic solution of KOH, c(KOH) = 0.5 mol. l^{-1}

i. Measure 28.055 g of Potassium hydroxide
ii. Mix with 986.274 mL of ethanol

3.3. Methods

Four analyses were made during the whole experiment that lasted four months (from December to March). The first one was executed at the beginning of the experiment (the containers have not been placed yet in their corresponding environments). The rest of the analyses were done on monthly basis.

Two samples from each bottle and one blank sample were taken and analysed in each measurement. In case the difference between them was greater than 10 %, a third sample was analysed.

3.3.1. Analytical titrations

- **Peroxide value**

The experiment was performed according to ČSN EN ISO 3960.

Five grams of oil sample was weighted into an Erlenmeyer flask. 50 mL of trichloromethane and acetic acid (the ratio of them was 2:3) was added to the flask with oil. 1 mL of water solution of potassium iodide was added by pipette. After that, the flask was closed and left in the dark for 20 minutes. Later, 50 mL of water was added to the flask and titrated with the volumetric solution of sodium thiosulfate till the yellow colour disappeared. 1 mL of starch solution was then added, which turned purple. It is shown in Figure 13 in Appendix 3. The sample was titrated again with the volumetric solution of sodium thiosulfate so that the purple colour got out of the chloroform layer.

OPTIMIZATION – In this research, the flask with the solution was left in the dark overnight. By this optimization, the solution got darker colour and the change during the titration was more visible.

The calculation was according to The International Fragrance Association (2019)

$$PV = \frac{(a-b)}{n} \; c * f * 1000$$

Where is a.. consumption of a volumetric solution of $Na_2S_2O_3$ (c = 0.01 mol. l^{-1}) of own determination in mL,
b.. consumption of a volumetric solution of $Na_2S_2O_3$ (c = 0.01 mol. l^{-1}) of blank sample in mL,
c.. concentration of a volumetric solution of $Na_2S_2O_3$ in mol. l^{-1},
f.. factor of a volumetric solution,
n.. sample weight in grams.

- **Acid value**

The experiment was performed according to ČSN EN ISO 660.

Five grams of oil sample was weighted into Florence flask. 100 mL of neutralised ethanol with a temperature about 65 °C was added by a graduated cylinder. Also, 2 mL of phenolphthalein was added by pipette. All these solutions were mixed with the oil. By the burette, each sample was titrated with 0.1 M KOH till the pink-purple colour appeared. The colour change is shown in the Figure 14 in Appendix 3.

Calculation

$$AV = \frac{a\,c\,M}{n}$$

Where is a.. consumption of an ethanolic solution of KOH (c = 0.1 mol. l^{-1} in mL),
c.. concentration of an ethanolic solution of KOH (c = 0.1 mol. l^{-1} in mL),
M.. Molar Mass of KOH in g. mol^{-1},
n.. sample weight in grams.

- **Saponification value**

The experiment was performed according to ČSN EN ISO 3657.

Three grams of oil sample was weighted into a boiling flask. Then, 50 mL of ethanolic solution of KOH was poured into the sample flask. Samples were placed on a heating mantle. Condensers were attached to each sample flask, and it was boiled for one hour. After saponification, the flask was removed, and the phenolphthalein indicator was added by five drops. Immediately the sample was titrated with 0.5 mol. l^{-1} hydrochloric acid solution till the pink colour completely disappeared.

The blank sample was prepared the same way, but the sample was only heated to boiling point and subsequently titrated.

Calculation:

$$SV = \frac{(V_0 - V_1) * C * f * 56.1}{m}$$

Where V_0.. consumption of a volumetric solution of HCl (c = 0.5 mol. l^{-1}) of a blank sample in mL,
V_1.. consumption of a volumetric solution of HCl (c = 0.5 mol. l^{-1}) of own determination in mL,
c.. concentration of a volumetric solution of HCl in mol. L^{-1},
f.. factor of a volumetric solution HCl,
m.. sample weight in grams.

3.3.2. Oxitest Method

The Velp Scientifica Oxidation Test Reactor, OXITEST® reactor (Velp Scientifica, Usmate, Milan, Italy) was used in this experiment.

Approximately, 8 grams of EVOO were measured into each sample-holder container. The OXITEST® has two separate oxidation chambers that could be used simultaneously. Therefore, into each chamber base, two spacers were placed and then

the sample-holder containers were loaded into the OXITEST® chambers. The O-rings were put in the dedicated grooves. Then the covers were placed, and the screws were tightened. Then the analyse has been started. All of the analyses were carried out under the same conditions of temperature (90°C) and oxygen pressure (6 bar). During the sample oxidation, the instrument recorded the oxygen pressure drop inside the oxidation chambers due to the chemical reactions. At the end of the test, the software automatically calculated the Induction Period using the Least Squares Method.

3.3.3. GC-MS Analysis

The volatile compounds were extracted in duplicate by using the Solid-phase microextraction technique (SPME). 75 µm phase thickness fiber (Carboxen®/ Polydimethylsiloxane Fiber) was used. One gram of EVOO sample was weighed into a 10 mL vial and sealed. After that, the vial was warmed to 50 °C and mixed for 10 minutes. SPME fiber was exposed for 30 minutes to the headspace of the vial and the volatiles were then adsorbed on the fiber by this process, the vial was still heated and mixed during this process. This process is shown in the Figure 15 in Appendix 3. Afterwards, the fiber was introduced into the injection port and kept for 10 minutes for the desorption of volatiles.

An Agilent Technologies 7890 B GC System gas chromatograph with an HP-5MS column measuring 30 m x 0.25 mm x 0.25 µm was used for the measurement. The carrier gas was helium with a flow rate of 1 millilitre per minute. An Agilent Technologies 5977 A mass spectrometer was used to identify the substances. The initial GC oven temperature program was 40 °C. This temperature was held for 4 minutes. Subsequently, the temperature was increased at a rate of 5 °C per minute to 120 °C. Upon reaching this temperature, the rate was immediately increased to 20 °C per minute until a final temperature of 280 °C was reached.

3.3.4. Statistical analysis

Statistical analysis was performed by the Software TIBCO Statistica™. For graphs and calculations, MS Excel® was used.

The effects of the material and time on the measured parameters were evaluated statistically. Initially, it was necessary to test the data for normality. Shapiro-Wilk test was used. Then the data were tested for homogeneity by Levene's test. As the data did not follow the normality and homogeneity assumptions, the non-parametric test Kruskal-Wallis was used. The differences between the individual means were considered significant at $p < 0.05$.

4. Results

Data of each measurement of chemical processes happening during each analysis can be found in Appendix 1.

4.1. Peroxide value

The results are divided into two tables by their specific environment: dark & cold and light & warm. Each table (Table 5 and Table 6) shows the results of the measurement of peroxide value from the initial value to three months. In

Table 13, there are full measurements of each sample.

Table 5 Peroxide value, dark & cold

T	G-D	G-D-H	G-D-FF	T-D	T-D-H	T-D-FF
0	4.70 ± 3.23	4.70 ± 3.23	4.70 ± 3.23	1.70 ± 3.24	1.70 ± 3.24	1.70 ± 3.24
1	2.40 ± 0.40	-	-	4.20 ± 4.20	-	-
2	15.60 ± 0.20	19.60 ± 0.20	-	1.70 ± 0.33	1.90 ± 0.52	-
3	2.60 ± 0.00	1.60 ± 0.20	1.60 ± 0.20	2.00 ± 0.20	1.60 ± 0.20	1.60 ± 0.20

T - storage time in months

Results are expressed as mEq per kg^{-1}

See Table 4 to understand the abbreviations

Table 6 Peroxide value, light & warm

T	G-L	G-L-H	G-L-FF	T-L	T-L-H	T-L-FF
0	4.70 ± 3.23	4.70 ± 3.23	4.70 ± 3.23	1.70 ± 3.24	1.70 ± 3.24	1.70 ± 3.24
1	1.00 ± 0.20	-	-	5.20 ± 2.00	-	-
2	9.80 ± 7.07	13.67 ± 7.01	-	1.60 ± 0.20	2.70 ± 1.34	-
3	3.00 ± 0.00	3.40 ± 0.40	1.80 ± 0.00	2.40 ± 0.20	2.00 ± 0.20	1.20 ± 0.20

T - storage time in months

Results are expressed as mEq per kg^{-1}

See Table 4 to understand the abbreviations

In Table 4, there is a description of all abbreviations used in this experiment. The most important factor of both tables is that none of the results of the peroxide value exceeded the specified standard of 20 mEq per kg^{-1}. The initial value of samples in the glass was 4.70 mEq per kg^{-1}. The Tetra Pak® values were 1.70 mEq per kg^{-1}. After three months, the values for G-D-H, G-D-FF, T-D-H, and T-D-FF unified at 1.60 mEq per kg^{-1}. In the case of oils on light and warm, the values were more diverse. The highest peroxide value was measured for G-D-H after two months with a value of 19.60 mEq per kg^{-1}.

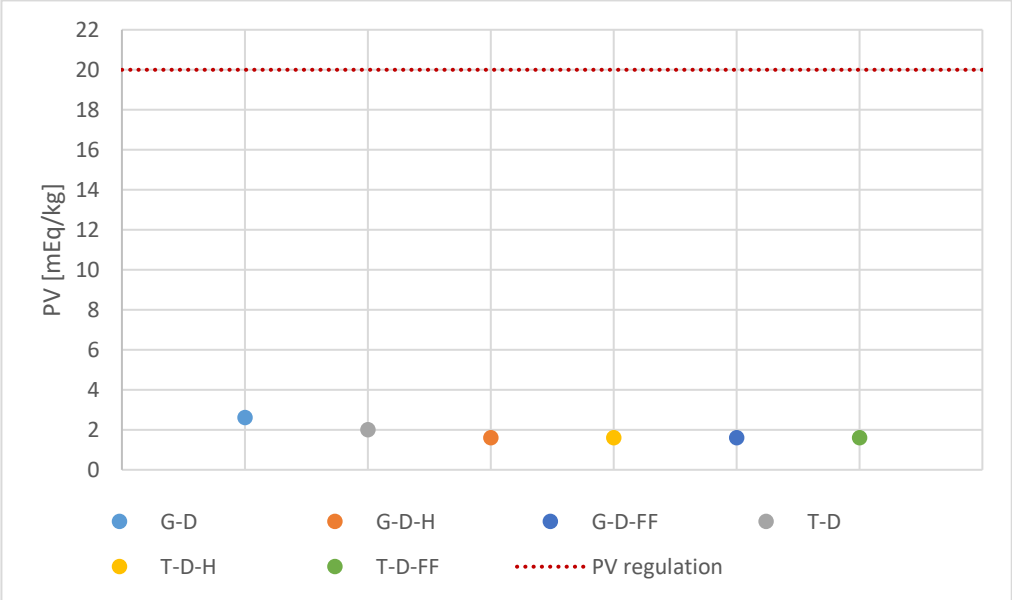

Figure 2 PV results after three months, dark & cold

The regulation is 20 mEq per kg^{-1}; see dotted red line in a graph

See Table 4 to understand the abbreviations

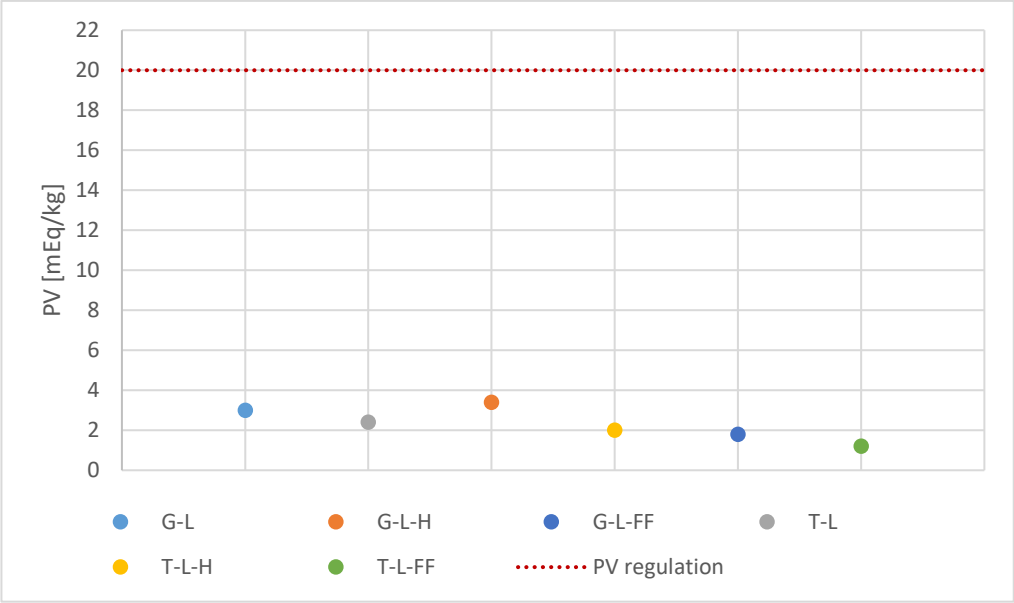

Figure 3 PV results after three months, light & cold

The regulation is 20 mEq per kg^{-1}; see dotted red line in a graph

See Table 4 to understand the abbreviations

From the results of PV after three months (Figure 2 and Figure 3) it is obvious that values in both conditions continue to be similar. Not a single sample deviated from the others. All values are approximately ten times smaller than the specified standard.

According to statistical analysis, there was no significant difference between the means of the PV results within time and material.

4.2. Acid value

The results are divided into two tables by their specific environment: dark & cold and light & warm. Each table (Table 7 and Table 8) shows the results of the measurement of acid value from the initial value to three months. In Table 14, there are full measurements of each sample.

Table 7 Acid value, dark & cold

T	G-D	G-D-H	G-D-FF	T-D	T-D-H	T-D-FF
0	0.86 ± 0.11	0.86 ± 0.11	0.86 ± 0.11	0.82 ± 0.14	0.82 ± 0.14	0.82 ± 0.14
1	0.50 ± 0.06	-	-	0.67 ± 0.00	-	-
2	0.45 ± 0.22	0.67 ± 0.00	-	0.67 ± 0.00	0.79 ± 0.11	-
3	0.67 ± 0.00	0.45 ± 0.00	0.56 ± 0.11	0.45 ± 0.00	0.45 ± 0.00	0.67 ± 0.00

T - storage time in months

Results are expressed as mg KOH per g of oil

See Table 4 to understand the abbreviations

Table 8 Acid value, light & warm

T	G-L	G-L-H	G-L-FF	T-L	T-L-H	T-L-FF
0	0.86 ± 0.11	0.86 ± 0.11	0.86 ± 0.11	0.82 ± 0.14	0.82 ± 0.14	0.82 ± 0.14
1	0.34 ± 0.11	-	-	0.34 ± 0.11	-	-
2	0.45 ± 0.00	0.56 ± 0.11	-	0.67 ± 0.00	0.67 ± 0.22	-
3	0.45 ± 0.00	0.45 ± 0.00	0.56 ± 0.11	0.45 ± 0.00	0.67 ± 0.00	0.79 ± 0.11

T - storage time in months

Results are expressed as mg KOH per g of oil

See Table 4 to understand the abbreviations

In Table 4, there is a description of all abbreviations used in this experiment. None of the values exceeded the limit of the Czech standard, which is set at 4.0 mg KOH per g of oil. The initial value of samples in glass bottles was 0.86 mg KOH per g of oil. In the case, when the sample was in Tetra Pak® containers, the initial value was 0.82 mg KOH per g of oil. The highest final value in the third month had the T-L-FF sample with the amount of 0.79 mg KOH per g of oil.

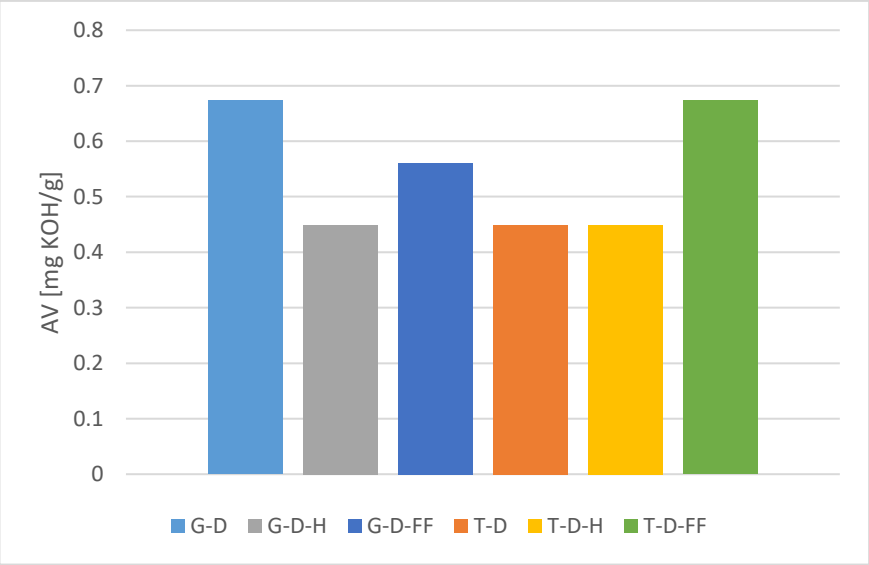

Figure 4 AV results after three months, dark & cold

The regulation is 4 mg KOH per g of oil

See Table 4 to understand the abbreviations

Figure 5 AV results after three months, light & warm

The regulation is 4 mg KOH per g of oil

See Table 4 to understand the abbreviations

With the AV results after three months in both conditions were consistent. From the results in the graphs (Figure 4 and Figure 5), it is evident that not a single sample deviated from the others. All values are approximately three times smaller than the specified standard.

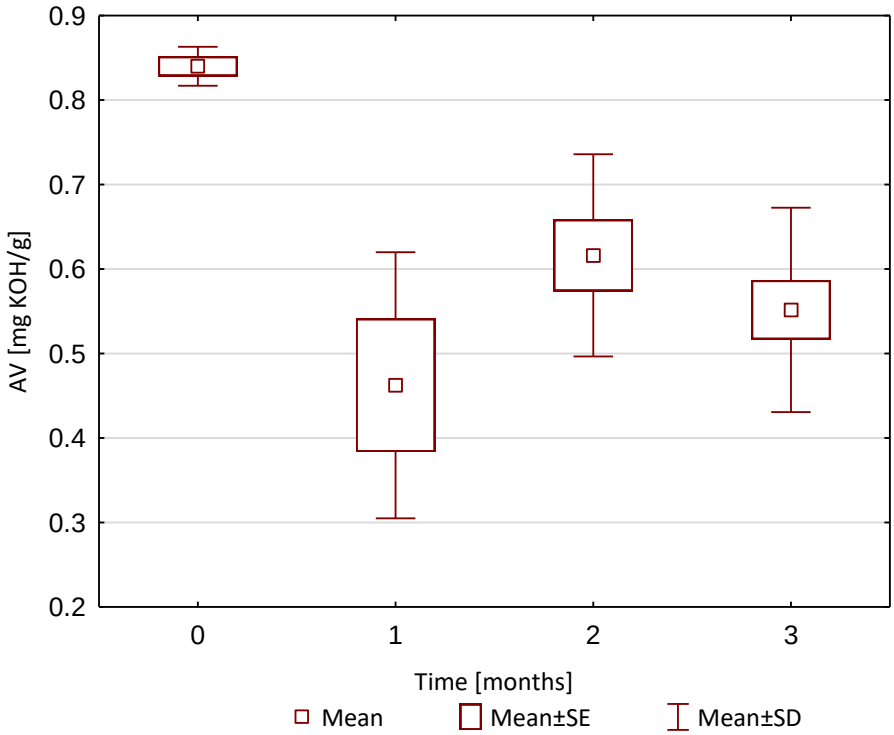

Figure 6 Means of the AV results within time

In accordance with statistical analysis, there was a significant difference between the means of the AV results within time. Figure 6 shows that there is no difference between the first, second, and third month. In contrast, the initial month of AV result was higher in comparison with other months. In comparison, there was no significant difference between the means of the AV results within material.

4.3. Saponification value

The results are divided into two tables by their specific environment: dark & cold and light & warm. Each table (Table 9 and Table 10) shows the results of the measurement of peroxide value from the initial value to three months. In Table 15, there are full measurements of each sample.

Table 9 Saponification value, dark & cold

T	G-D	G-D-H	G-D-FF	T-D	T-D-H	T-D-FF
0	195.10 ± 2.64	195.10 ± 2.64	195.10 ± 2.64	192.61 ± 1.76	192.61 ± 1.76	192.61 ± 1.76
1	185.13 ± 0.94	-	-	177.65 ± 2.80	-	-
2	181.39 ± 5.61	187.00 ± 1.87	-	186.07 ± 0.94	179.52 ± 1.87	-
3	197.29 ± 0.00	198.22 ± 0.94	196.35 ± 0.94	195.42 ± 0.00	193.55 ± 1.87	195.42 ± 0.00

T - storage time in months

Results are expressed as mg KOH per g of oil

See Table 4 to understand the abbreviations

Table 10 Saponification value, light & warm

T	G-L	G-L-H	G-L-FF	T-L	T-L-H	T-L-FF
0	195.10 ± 2.64	195.10 ± 2.64	195.10 ± 2.64	192.61 ± 1.76	192.61 ± 1.76	192.61 ± 1.76
1	180.46 ± 0.00	-	-	175.78 ± 2.80	-	-
2	181.39 ± 1.87	183.26 ± 3.74	-	183.26 ± 3.74	184.20 ± 0.94	-
3	197.29 ± 1.87	196.35 ± 0.94	192.61 ± 0.94	193.55 ± 3.74	198.22 ± 2.81	190.74 ± 0.93

T - storage time in months

Results are expressed as mg KOH per g of oil

See Table 4 to understand the abbreviations

In Table 4, there is a description of all abbreviations used in this experiment. The initial values were 195.10 mg KOH per g of oil for glass samples and 192.61 mg KOH per g of oil for Tetra Pak® samples. In the third month, which was also the final month of measurement, G-D, G-D-H, G-D-FF, G-L, G-L-H, and T-L-H exceeded the standard which is from 184 to 196 mg KOH per g of oil.

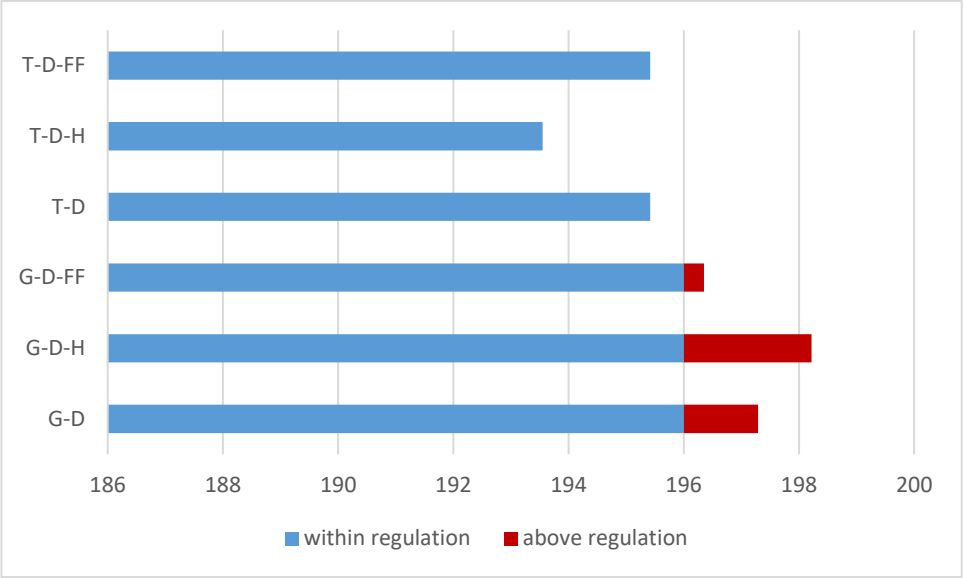

Figure 7 SV results after three months, dark & cold

The regulation is 196 mg KOH per g of oil

See Table 4 to understand the abbreviations

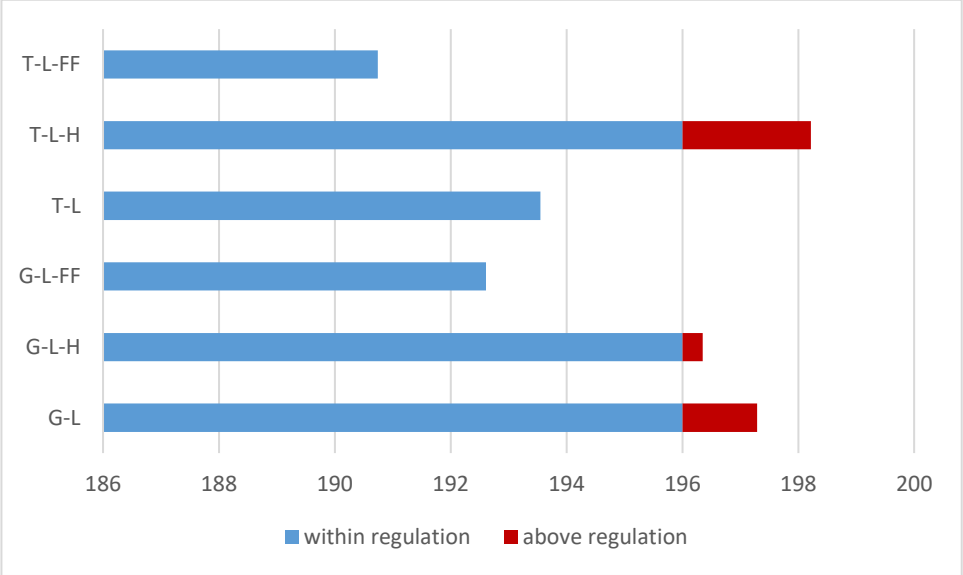

Figure 8 SV results after three months, light & warm

The regulation is 196 mg KOH per g of oil

See Table 4 to understand the abbreviations

With the SV results after three months in both conditions, limits were exceeded in six samples. From the results in Figure 7, it is evident that G-D-FF, G-D-H, and G-D exceeded 196 mg KOH per g of oil after three months. G-D-H sample exceeded the regulation the most from the sample in dark and cold conditions. It is also visible

from Figure 8, that the limits were also exceeded in T-L-H, G-L-H, and G-L. The SV in light and warm conditions was the highest in the T-L-H sample.

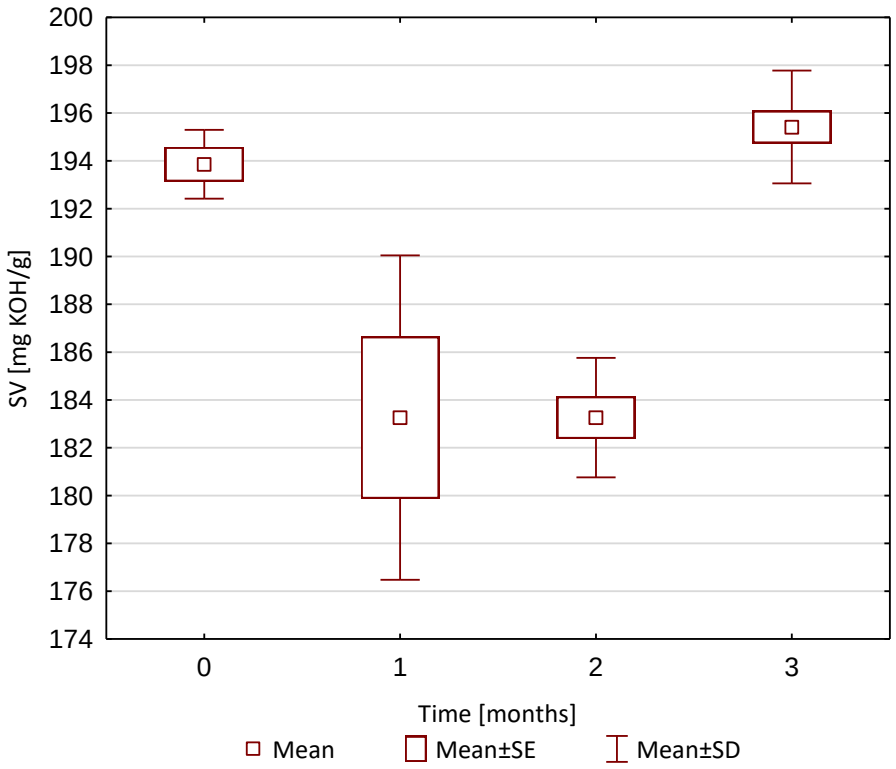

Figure 9 Means of the SV results within time

In accordance with statistical analysis, there was a significant difference between the means of the SV results within time. From Figure 9 could be seen that there is no difference between the initial time and the second month. In the case of the third month, the SV result was significantly higher in comparison with the first and second month. On the other hand, there was no significant difference between the means of the SV results within material.

4.4. OXITEST

The results are divided into two tables by their specific packaging. Both graphs (Figure 10 and Figure 11) show the results of measuring the induction period (IP) at a given pressure. IP is the time required to reach the starting point of oxidation. Table 16 contains all IP results measured by the OXITEST® reactor in this research. In Table 4, there is a description of all abbreviations used in this experiment.

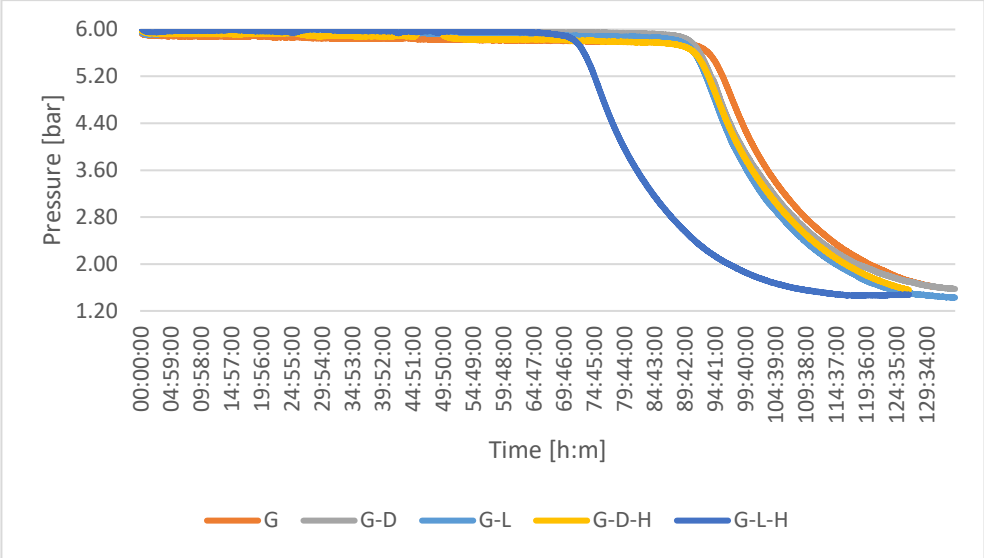

Figure 10 Induction period curve, Glass

See Table 4 to understand the abbreviations

Figure 10 shows the IP in time for the measured oils in the glass packaging. The longest IP had sample G, which is not so different from G-D, G-L, and G-D-H. In contrast, G-L-H had a significantly shorter period than other samples in glass packaging.

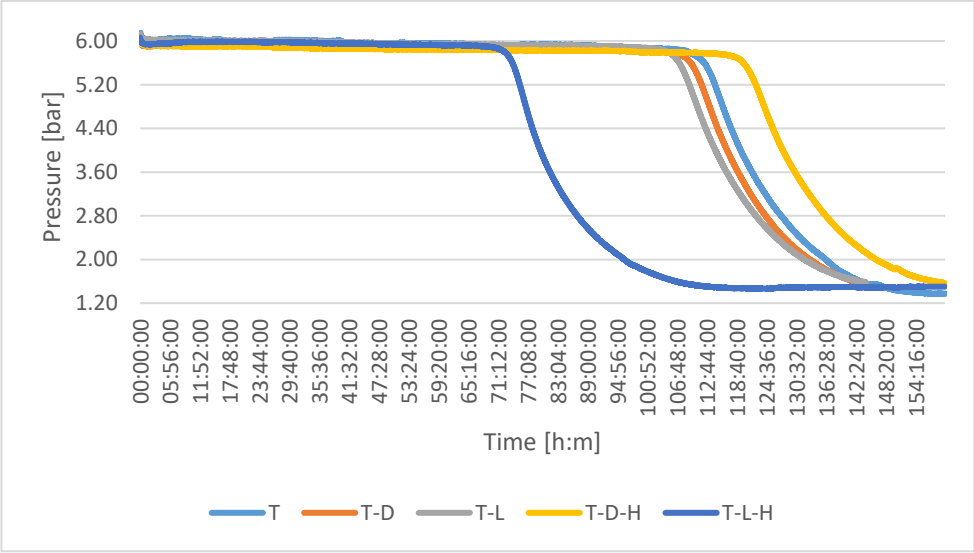

Figure 11 Induction period curve, Tetra Pak®

See Table 4 to understand the abbreviations

Figure 11 shows the IP in time for the measured oils in the Tetra Pak® packaging. The longest IP had sample T-D-H. The results of T, T-D, and T-L are not significantly

different. On the other hand, the T-L-H sample is significantly shorter than all other samples.

4.5. GC-MS Analysis

The results are divided into two tables by their given packaging material: glass and Tetra Pak®. Each table (Table 11 and Table 12) shows ten volatile compounds from the analysis, which occurred in all samples most often, and made reference to literature sources, such as: Morales et al. (2005), Kotsiou & Tasioula-Margari (2015), Cherfaoui et al. (2019).

Table 11 Evolution of the peak area of selected volatile compounds, glass

	initial	1 month		2 month			
Volatile compounds	**G**	**G-D**	**G-L**	**G-D**	**G-L**	**G-D-H**	**G-L-H**
Hydrazinecarboxamide	44.43	40.99	16.89	24.68	29.96	38.84	35.73
Ethanol	129.23	133.20	-	129.91	-	152.47	135.41
Ethyl Acetate	37.14	11.45	8.78	27.40	13.09	16.39	17.07
1-Penten-3-one	40.85	16.43	14.13	32.89	-	22.01	18.69
1-Butanol, 3-methyl-	2.93	6.23	5.24	9.20	4.57	4.80	4.07
Hexanal	73.92	53.12	48.69	62.46	47.30	65.84	38.32
2-Hexenal	103.84	80.31	94.88	111.01	107.59	139.63	59.26
3-Hexen-1-ol	65.19	54.28	100.52	107.75	97.31	114.88	20.79
Nonanal	3.29	4.84	4.30	2.72	5.01	6.60	4.09
Acetic acid	2.92	11.35	-	14.28	20.67	23.60	21.87

Areas are expressed x 10^5

See Table 4 to understand the abbreviations

Table 12 Evolution of the peak area of selected volatile compounds, Tetra Pak®

	initial	1 month		2 month			
Volatile compounds	**T**	**T-D**	**T-L**	**T-D**	**T-L**	**T-D-H**	**T-L-H**
Hydrazinecarboxamide	41.15	4.71	-	29.89	39.39	34.77	38.61
Ethanol	333.33	124.72	188.78	266.76	273.20	268.94	167.13
Ethyl Acetate	55.02	19.79	26.61	24.87	29.70	29.02	29.15
1-Penten-3-one	27.68	13.73	15.04	23.00	19.16	16.34	-
1-Butanol, 3-methyl-	-	5.36	7.44	4.42	5.30	4.84	-
Hexanal	80.09	45.29	52.52	68.88	63.43	60.59	92.39
2-Hexenal	88.65	30.44	66.24	81.00	68.65	74.28	1.79
3-Hexen-1-ol	136.13	39.27	41.33	74.12	62.76	65.01	38.10
Nonanal	3.84	5.32	5.43	7.02	5.96	5.96	5.67
Acetic acid	-	9.40	9.92	14.35	-	16.30	69.19

Areas are expressed x 10^5

See Table 4 to understand the abbreviations

Selected volatile compounds had changed according to their specific packaging material and over time either. The peak area for ethanol in glass bottles was around 136 x 10^5. While Tetra Pak® samples had an average peak area for ethanol of 232 x 10^5. The peak area in ethyl acetate had a declining trend in both packaging materials. As with ethyl acetate, the peak area of 1-penten-3-one was also declining for both packaging materials. The peak areas of 1-butanol, 3 methyl- and nonanal were small but crucial for oil stability. The peak area of hexanal was slightly larger at the olive oils in the Tetra Pak®. The peak area of acetic acid in both packaging materials increased over time.

5. Discussion

The main objective of this thesis was to compare the influence of packaging material on qualitative parameters. Several studies (Pristouri et al. 2010; Samaniego-Sánchez et al. 2012; Di Serio et al. 2018; Ghanbari Shendi et al. 2018) showed the influence that storage material, temperature, and time could affect the quality parameter values of the olive oil.

In this research, peroxide value results after three months did not exceed the maximum limit of the European regulation. As in this work, Samaniego-Sánchez et al. (2012) studied olive oil in glass and Tetra Pak® and none of the analysed oils exceeded the maximum peroxide limit for EVOO even after nine months. In the case of a study from Turkey (Ghanbari Shendi et al. 2018), they studied PV for twelve months. In the seventh month of their monitoring, there was a reversal, and the values began to decrease again after reaching 26.6 mEq per kg^{-1}. This phenomenon also manifested itself in an experiment by Lanza et al. (2015) which also monitored the PV for 12 months. However, in this case, there was a sudden decrease in the third month. This behaviour can be explained by an initial increase in hydroperoxides, which are flavourless compounds produced during the primary step of oxidation. Afterwards, these compounds give rise to substances responsible for off-flavours - secondary oxidation (Lanza et al. 2015). This behaviour probably manifested itself in this research, specifically in the sample in the glass bottles. The turning point occurred in the second month in both conditions (dark & cold, light & warm). Then the values started to fall again. Figure 12 shows the breaking point of PV of G-L-H and T-D in comparison with already mentioned articles (Lanza et al. 2015; Ghanbari Shendi et al. 2018). As well as in the article of Lanza et al. (2015), the European regulation was not exceeded in this study.

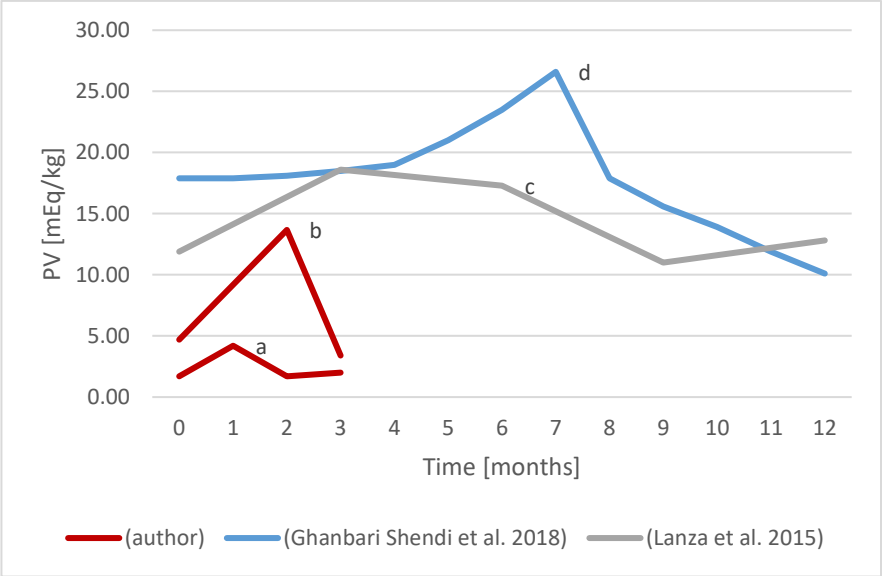

Figure 12 Comparison of the breaking points of PV (Lanza et al. 2015; Ghanbari Shendi et al. 2018)

[a] Tetra Pak® in dark & cold

[b] Glass in light & warm with headspace

[c] Glass, light & warm

[d] Amber glass, warm, headspace with nitrogen gas

As reported by Samaniego-Sánchez et al. (2012), the acidity value of all the oils under observation was found to increase throughout their experiment, with the lowest increment corresponding to the oils in Tetra Pak®. Considering the environment, RT and refrigerator are practically equal, as well as glass and Tetra Pak® packaging. As in this research, the values in both environments and packaging are comparable during the entire experiment.

The results of saponification value after three months showed that all samples in the glass in dark & cold exceeded the standard. Furthermore, both packaging materials under light & warm with a crafted headspace exceeded the standard, as well as light & warm glass. To the best of our knowledge, there is no publication describing the effect on quality parameters, chemical composition and not even organoleptic properties and health effects when the standard is exceeded.

Oxidative stability was mainly affected by the amount of the headspace in the bottle and by given conditions. The IP had a decreasing tendency which is visible in Figure 10 and Figure 11. Both dark glass bottle and Tetra Pak® container appear to be suitable packaging materials. Their effect on oil stability was not conclusive. In contrast,

by creating the headspace in combination with light and warm, we proved that the starting point of oxidation occurred significantly earlier. Pristouri et al. (2010) also monitored the function of the headspace, but in a different packaging material in a clear PET bottle. However, they concluded either that the large headspace volumes should be avoided indicating the need for consumption of olive oil within a given container as soon as possible (Pristouri et al. 2010).

The olive oil volatile compounds changed during storage. In both packaging materials (glass and Tetra Pak®), the largest peak area of selected volatile compounds had ethanol. Ethanol has also been reported in other studies (Lanza et al. 2015; Cherfaoui et al. 2019). This occurrence could be potentially produced by microbial activity, based on Cherfaoui et al. (2019) study. On the other hand, this compound does not have to be a product of oxidation. According to Gómez-Coca et al. (2016), ethanol will always be present in newly extracted oils because ethanol is not only a fermentation by-product but also is formed in the fruit during aroma development (Gómez-Coca et al. 2016). Nonanal and 1-butanol, 3 methyl- occurred in very small areas during the time in this research. In accordance with Kotsiou & Tasioula-Margari (2015), they found these compounds also in very low amounts in the fresh sample. So we are suggesting that the selected oil samples were of high quality. Acetic acid had also appeared in both packaging materials. And its levels were increasing throughout storage time. Acetic acid contributes to the rancid sensory profile (Kotsiou & Tasioula-Margari 2015). In addition, it was observed that the area of peak of hexanal increased during the storage period of Tetra Pak® olive oil. According to Lanza et al. (2015), hexanal could be a useful marker of oxidation, since it comes only from the secondary oxidation of the linoleic hydroperoxide radical.

In most publications dealing with the storage conditions or storage materials of extra virgin olive oils, it is possible to observe trends in individual parameters. These changes, increase or decrease with changes in the oil, depending on which parameter is involved. Certain trends can be observed as the quality of the oil decreases during storage. In our study, all methodological procedures were carefully followed to ensure that there was as little variation as possible (ideally none at all) in the individual measurements. Nevertheless, it is not possible to derive a clear trend for all parameters. This fact could not be identified until almost before the end of the storage experiment. Due to the length of the experiment, it was also not possible to repeat the whole experiment. It is essentially

impossible to identify retrospectively the reason why trends in changes in individual parameters cannot be identified from the experiment. The only option is to continue the research and re-run the experiment in order to obtain any conclusive trends.

6. Conclusions

Extra virgin olive oils from two packaging materials (glass and Tetra Pak®) were evaluated in this thesis. According to the results, none of the monitored parameters was critical to the effect on quality parameters determined in this study even after four months of storage experiment. Both monitored packaging materials appear to be adequate.

The peroxide value may not only increase during storage but may also decrease back due to the secondary oxidation.

Thanks to the oxidation reactor, it was found that the oxidation stability decreased in time due to the open bottles, especially in the case when the headspace had been created in the bottles and when the bottles had been stored in light & warm conditions. The effect of packaging materials on oxidation was negligible.

Analysis of volatile compounds showed that during storage increased peak areas of substances caused the rancidity of extra virgin olive oils in both packaging materials.

To conclude, to maintain the quality of extra virgin olive oils as long as possible, it is necessary to limit the headspace in the container and reduce exposure to light conditions. It is desired to consider how much olive oil one household could consume and accordingly buy a smaller package to prevent the formation of an ample headspace and thus prevent faster oxidation.

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8. Appendices

List of the Appendices:

Appendix 1: Tables with measurement of analytical titration

Table 13 Measured value of PV

Time	Material	a	b
Initial			
	G	4.75 ± 1.44	2.4
	T	3.25 ± 1.45	2.4
1 month			
	G-D	4.20 ± 0.16	3
	G-L	3.50 ± 0.08	3
	T-D	5.10 ± 1.71	3
	T-L	5.60 ± 0.82	3
2 months			
	G-D	8.30 ± 0.08	0.5
	G-L	5.40 ± 3.06	0.5
	G-D-H	10.30 ± 0.08	0.5
	G-L-H	7.33 ± 3.03	0.5
	T-D	1.35 ± 0.15	0.5
	T-L	1.30 ± 0.09	0.5
	T-D-H	1.45 ± 0.23	0.5
	T-L-H	1.85 ± 0.60	0.5
3 months			
	G-D	1.80 ± 0.00	0.5
	G-L	2.00 ± 0.00	0.5
	G-D-H	1.30 ± 0.08	0.5
	G-L-H	2.20 ± 0.16	0.5
	G-D-FF	1.30 ± 0.08	0.5
	G-L-FF	1.40 ± 0.00	0.5
	T-D	1.50 ± 0.08	0.5
	T-L	1.70 ± 0.08	0.5
	T-D-H	1.30 ± 0.08	0.5
	T-L-H	1.50 ± 0.08	0.5
	T-D-FF	1.30 ± 0.08	0.5
	T-L-FF	1.10 ± 0.08	0.5

a – measurement of titration with standard deviation [mL]

b – blank sample [mL]

Table 14 Measured value of AV

Time	Material	a
Initial		
	G	0.77 ± 0.08
	T	0.73 ± 0.11
1 month		
	G-D	0.45 ± 0.04
	G-L	0.30 ± 0.08
	T-D	0.60 ± 0.00
	T-L	0.30 ± 0.08
2 months		
	G-D	0.40 ± 0.16
	G-L	0.40 ± 0.00
	G-D-H	0.60 ± 0.00
	G-L-H	0.50 ± 0.08
	T-D	0.60 ± 0.00
	T-L	0.60 ± 0.00
	T-D-H	0.70 ± 0.08
	T-L-H	0.60 ± 0.16
3 months		
	G-D	0.60 ± 0.00
	G-L	0.40 ± 0.00
	G-D-H	0.40 ± 0.00
	G-L-H	0.40 ± 0.00
	G-D-FF	0.50 ± 0.08
	G-L-FF	0.50 ± 0.08
	T-D	0.40 ± 0.00
	T-L	0.40 ± 0.00
	T-D-H	0.40 ± 0.00
	T-L-H	0.60 ± 0.00
	T-D-FF	0.60 ± 0.00
	T-L-FF	0.70 ± 0.08

a – measurement of titration with standard deviation [mL]

Table 15 Measured values of SV

Time	Material	V_0	V_1
Initial			
	G	39.87	19.00 ± 0.24
	T	39.87	19.27 ± 0.16
1 month			
	G-D	37.90	18.10 ± 0.08
	G-L	37.90	18.60 ± 0.00
	T-D	37.90	18.90 ± 0.24
	T-L	37.90	19.10 ± 0.24
2 months			
	G-D	39.20	19.80 ± 0.49
	G-L	39.20	19.80 ± 0.16
	G-D-H	39.20	19.20 ± 0.16
	G-L-H	39.20	19.60 ± 0.33
	T-D	39.20	19.30 ± 0.08
	T-L	39.20	19.60 ± 0.33
	T-D-H	39.20	20.00 ± 0.16
	T-L-H	39.20	19.50 ± 0.08
3 months			
	G-D	40.50	19.40 ± 0.00
	G-L	40.50	19.40 ± 0.16
	G-D-H	40.50	19.30 ± 0.08
	G-L-H	40.50	19.50 ± 0.08
	G-D-FF	40.50	19.50 ± 0.08
	G-L-FF	40.50	19.90 ± 0.08
	T-D	40.50	19.60 ± 0.00
	T-L	40.50	19.80 ± 0.33
	T-D-H	40.50	19.80 ± 0.16
	T-L-H	40.50	19.30 ± 0.24
	T-D-FF	40.50	19.60 ± 0.00
	T-L-FF	40.50	20.10 ± 0.08

V_0 – blank sample [mL]

V_1 – measurement of titration with standard deviation [mL]

Appendix 2: Induction period of each analysis

Table 16 Oxitest, IP results

Material	Date 1	IP1	Date2	IP2
G	15/12/21	93:52	06/01/22	93:16
G-D	20/01/22	91:49	02/02/22	92:18
G-L	20/01/22	90:47	21/02/22	88:33
G-D-H	01/03/22	91:31	16/03/22	94:44
G-L-H	01/03/22	72:10	07/03/22	72:21
T	06/01/22	101:53	12/01/22	110:03
T-D	26/01/22	109:14	02/02/22	113:34
T-L	26/01/22	106:38	21/02/22	122:36
T-D-H	10/02/22	120:11	16/03/22	120:02
T-L-H	10/02/22	73:20	07/03/22	71:08

Appendix 3: Photo documentation

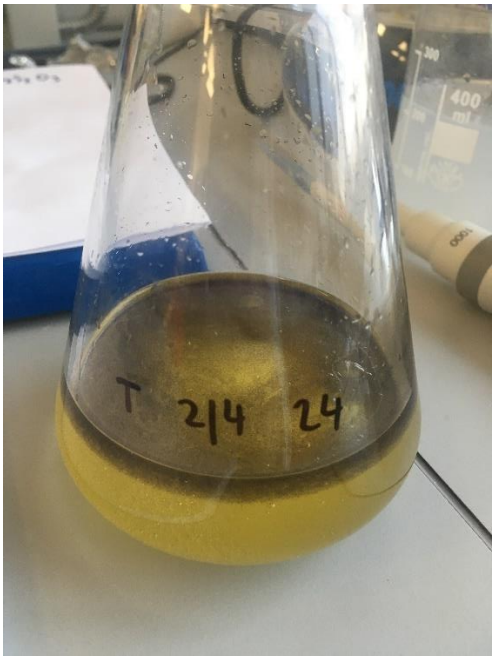

Figure 13 Peroxide value sample before the second titration

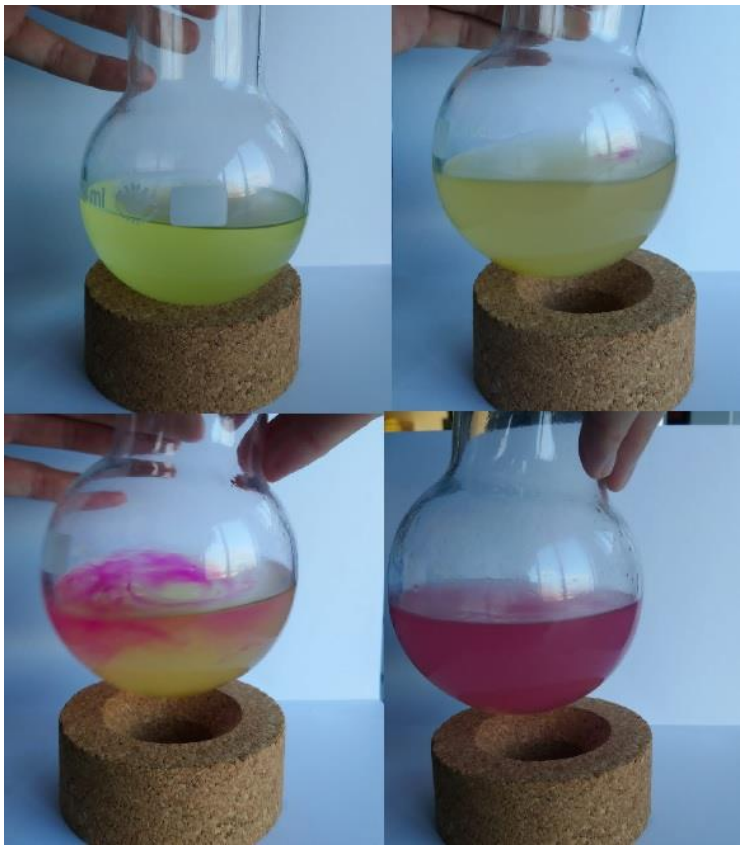

Figure 14 Acid value sample during titration

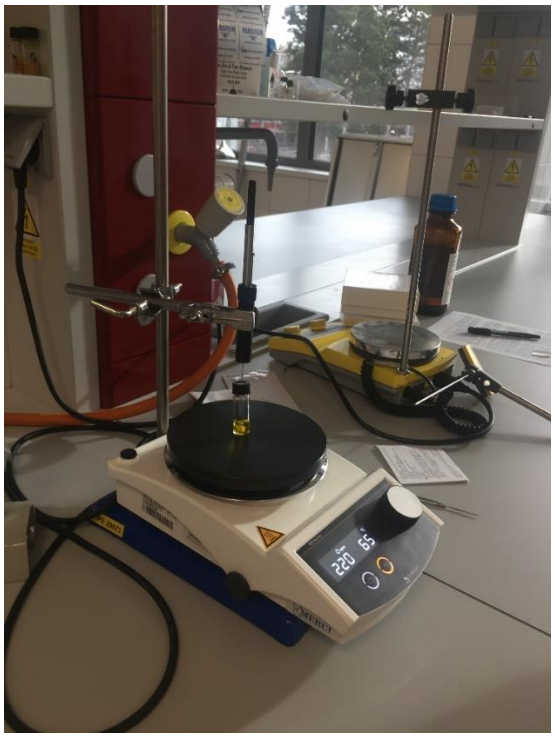

Figure 15 Exposed fiber in the headspace of the vial