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MONITORING THE STABILITY OF FORTIFIED COLD-PRESSED SUNFLOWER OIL UNDER DIFFERENT STORAGE CONDITIONS

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ABSTRACT

The aim of the study was to evaluate the stability of cold-pressed sunflower oil mixed with different seeds and herb. The seeds and herb were added at 1% and 5% concentrations; samples were divided into 2 groups: stored in the dark and light. The primary products of oxidation and chlorophyll content were monitored during 3 months of storage. The results showed very low oxidation stability of experimentally produced cold-pressed oil mixtures/dressings, especially during storage on the light. The samples with 5% of hemp herb addition showed the best stability since they have peroxide value under 20 mekv $O_2.kg^{-1}$, both in the dark and on the light. Other samples, both stored in the dark and on light, were declared as not for human consumption due to high oxidative product development. The research represents an important storability evaluation of products that can be found on the market and that can be found very attractive for consumers.

Keywords: sesame; chia; hemp; oil dressings; storability

INTRODUCTION

The production of cold-pressed edible plant oils includes only pressing and occasional filtering of plant material rich in oil. Many consumers found this production attractive and this food commodity is becoming more popular. Especially because in this kind of edible oil production, organic solvents are prohibited. The advantage of cold-pressed oil is that natural beneficial components, that are present in the raw material, are preserved **(Febrianto and Yang, 2011; Parker et al., 2003)**.

Sunflower has good tolerance to drought and different soil typology. These properties have made sunflowers to be one of the most important oilseeds in the world, both in developed and developing countries. The production of cold-pressed oil has been increasing due to the chance for smaller farmers to produce it, while the demand for it is increasing too **(Foppa Pedretti et al., 2019)**. On the other hand, edible plant cold-pressed oil has low oxidative stability. The fortification of this food commodity should lead to longer shelf life and consequently the better oxidative stability **(Mazaheri et al., 2019)**.

Cold-pressed oils, including sunflower cold-pressed oil, are a good source of polyphenols, chlorophylls, and carotenoids. These compounds have beneficial properties for humans since they have anti-inflammatory, anticancer properties, same as antioxidant and antibacterial activities **(Song et al., 2019; Wu et al., 2019)**. The total area in the Czech Republic used for the planting of oilseeds is 412 060 ha **(Zehnalek, 2019)**.

Food fortification has been gaining in popularity recently. Chia seeds are often called "superfoods" due to the high nutritional value for humans. Chia, hemp, and sesame seeds can be used for the fortification of cold-pressed plant oils since they have a beneficial nutritional composition **(Urbizo-Reyes et al., 2019; Bartkiene et al., 2019; Kermani et al., 2019)**.

On the other side, the quality and stability of these seeds fortified cold-pressed oils are questionable. The study aimed to monitor oxidative-hydrolytic stability and nutritional profile of cold-pressed sunflower oil fortified with o chia, sesame, and hemp seeds, as well as hemp herb.

Scientific hypothesis

The fortification influence on the stability of cold-pressed sunflower oil. Since chlorophylls can act as antioxidants and prooxidants independence on storage conditions we are expecting different stability properties of experimentally produced oil mixtures/dressings.

MATERIAL AND METHODOLOGY

The Velox cultivar of sunflower (*Helianthus annuus*) was cultivated in the Czech Republic and was obtained from the company Olejářství z Horňácka, Uherské Hradiště. Sunflower seeds were harvested and stored in a dry cellar until pressing. After milling the oil was stored in the dark until mixing with seeds and herb (15 days).

Table 1 The samples description.

Samples	Sample description
1% SS1	1% sesame seeds
5% SS5	5% sesame seeds
1% SKS1	1% hemp seeds
5% SKS5	5% hemp seeds
1% SCH1	1% chia seeds
5% SCH5	5% chia seeds
1% SKC1	1% hemp tea
5% SKC5	5% hemp tea
SK	without addition of seeds/tea

Table 2 The measured parameters in sunflower samples at the beginning of the experiment.

	Peroxide value (mekv $O_2.kg^{-1}$)	Free fatty acids mg $KOH.g^{-1}$	Total phenol content $mg.mL^{-1}$	Chlorophyll content ppm
Sunflower oil	8.20 ±0.87	0.07 ±0.01	0.23 ±0.01	2.78 ±0.19

The sesame seeds (*Sesamum indicum* L.), hemp seeds (*Cannabis sativa* L.), and chia seeds (*Salvia hispanica* L.) were obtained from the retail shop "Zdraví z přírody" in Brno, the Czech Republic. The hemp herb was obtained from the retail shop "Sativa-Medical" in Brno, the Czech Republic.

The sesame/chia/hemp seed and hemp herb were added to the sunflower cold-pressed oil in the following concentrations: 1% and 5%. The samples were stored in glass bottles and divided into two groups, one group was covered with aluminum foil and stored in the dark; another group was exposed to the daylight, without aluminum covering. Samples were stored at 25 °C at the Department of Vegetable Foodstuffs Hygiene and Technology, Faculty of Veterinary Hygiene and Ecology, the University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic (GPS: 49°12'59.2"N 16°35'41.0"E). The control samples were without seed/herb addition. The samples were analyzed at the beginning of the experiment and after 1 and 3 months of storage. The description of the samples is given in Table 1.

The peroxide value (PV) was determined according to **ISO 3960 (2017)** standard. 5 g of the sample was mixed with 30 ml of a mixture of chloroform and glacial acetic acid (2:3 ratio). The mixture was shaken for 1 minute, then 30 mL of distilled water and 5 mL of 1% starch solution were added. The sample was titrated with 0.01M $Na_2S_2O_3$. The blank sample was prepared in the same way with the addition of water instead of the sample.

The determination of chlorophylls was performed by the spectrophotometric method according to **Kraljić et al. (2013)**. The absorbance of chlorophylls in oil was measured at 670, 630, and 710 nm against cyclohexane (blank sample) by spectrophotometer (CE7210 DIET-QUEST, Cambridge, England).

Statistical analysis

Statistical significance at $p < 0.05$ was evaluated by one-way ANOVA analysis of variance, and parametric Tukey post hoc test (in the case when Levene's test showed equal variances $p > 0.05$) and nonparametric Games–Howel post hoc test (in the case when Levene's test showed unequal variances $p < 0.05$) for finding differences within groups. Overall differences among samples were checked by principal component analysis (PCA) using SPSS 20 statistical software (IBM Corporation, Armonk, USA).

RESULTS AND DISCUSSION

Table 2 is showing the results of the samples (cold-pressed sunflower oil samples without seeds and herb) at the beginning of the experiment.

The amount of primary products of oxidation, represented as peroxide values (mekv $O_2.kg^{-1}$), in the samples of sunflower oil (mixtures, same as without seeds/herb addition) after one month of storage can be seen from Table 3. The results are clearly indicating the high influence of storage conditions on the formation of hydroperoxides. After one month of storage, both on light and in the dark, only SKC5 (5% of hemp herb addition) samples showed better oxidation stability than control samples (SK).

3 months of storage showed an even higher oxidation ratio. Significant ($p < 0.05$) changes occurred in almost all oil mixture. The significant differences between samples stored in the dark and in light can be also seen from the principal component analysis (Figure 1). Out of all investigated samples only sample SKC5, stored in the dark, had a lower peroxide value than 20 mekv $O_2.kg^{-1}$. All other samples had peroxide value over 20 mekv $O_2.kg^{-1}$. This finding is making these samples be declared as not for human consumption.

The peroxide value, as the method for the determination of primary oxidation products, can be significantly influenced by the presence of antioxidants, such as phenols and chlorophylls. Chlorophylls have been proven to be good antioxidants in cold-pressed olive oil, but only during the storage in the dark. When cold pressed oil is stored on light, chlorophylls were found to act as prooxidants and they even make food matrix to oxidize more rapidly.

Table 3 Determination of peroxide value (mekv $O_2.kg^{-1}$).

Samples	Storage in dark		Storage on light	
	After 1 month	After 3 months	After 1 month	After 3 months
1% SS1*	12.06 ±1.32^{a**}	41.13 ±0.38^{b}	67.08 ±1.84^{c}	186.76 ±0.62^{d}
5% SS5	10.18 ±0.58^{a}	35.49 ±0.01^{b}	37.77 ±2.72^{ac}	123.78 ±0.32^{b}
1% SKS1	10.48 ±0.45^{a}	42.86 ±0.69^{b}	32.40 ±0.53^{c}	134.92 ±0.90^{d}
5% SKS5	12.56 ±0.87^{a}	60.29 ±0.09^{b}	52.96 ±0.26^{c}	169.29 ±1.23^{d}
1% SCH1	20.77 ±0.84^{a}	62.47 ±0.40^{b}	24.78 ±0.84^{a}	108.93 ±0.99^{c}
5% SCH5	21.08 ±0.46^{a}	62.54 ±0.55^{b}	26.60 ±0.72^{c}	125.88 ±0.30^{d}
1% SKC1	13.24 ±1.4^{a}	50.34 ±0.90^{b}	51.46 ±6.03^{ab}	209.24 ±0.11^{c}
5% SKC5	5.99 ±0.09^{a}	10.33 ±0.86^{a}	3.47 ±3.51^{a}	73.04 ±2.28^{b}
SK	8.96 ±0.29^{a}	62.98 ±0.15^{b}	17.98 ±1.42^{a}	80.42 ±0.29^{c}

Note: *Samples description is shown in Table 1; **lowercase letters shown statistically significant difference (p <0.05) between columns.

Table 4 Determination of chlorophyll content (ppm).

Mycotoxins	Storage in dark		Storage on light	
	After 1 month	After 3 months	After 1 month	After 3 months
1% SS1*	2.35 ±0.10^{a**}	2.19 ±0.04^{b}	0.28 ±0.00^{c}	0.24 ±0.00^{d}
5% SS5	0.13 ±0.09^{ac}	3.27 ±0.28^{c}	0.37 ±0.00^{b}	0.32 ±0.00^{da}
1% SKS1	2.35 ±0.04^{a}	1.49 ±0.02^{b}	0.27 ±0.00^{c}	0.22 ±0.00^{d}
5% SKS5	2.34 ±0.05^{a}	1.28 ±0.01^{c}	0.26 ±0.00^{d}	0.22 ±0.00^{c}
1% SCH1	3.15 ±0.04^{a}	0.70 ±0.03^{b}	0.27 ±0.00^{c}	0.26 ±0.00^{d}
5% SCH5	3.82 ±0.12^{a}	1.49 ±0.03^{d}	0.25 ±0.00^{c}	0.21 ±0.00^{c}
1% SKC1	2.50 ±0.06^{a}	1.72 ±0.09^{b}	0.26 ±0.00^{c}	0.21 ±0.00^{d}
5% SKC5	3.00 ±0.05^{a}	1.97 ±0.08^{a}	0.28 ±0.00^{b}	0.21 ±0.00^{c}
SK	2.47 ±0.12^{a}	1.57 ±0.05^{b}	0.28 ±0.00^{c}	0.24 ±0.00^{d}

Note: *Samples description is shown in Table 1; **lowercase letters shown statistically significant difference (p <0.05) between columns.

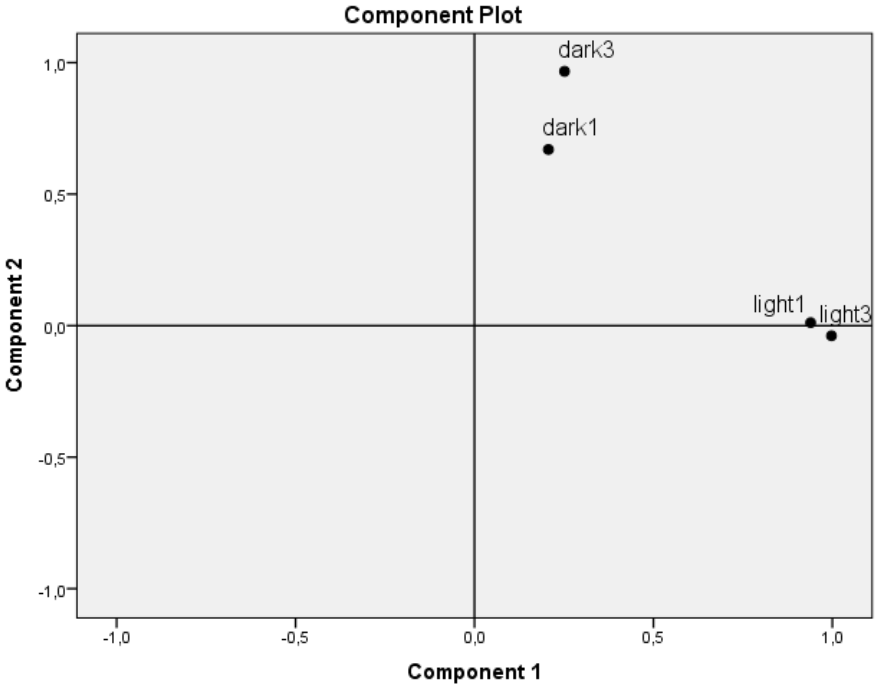

Figure 1 Principal component analysis (PCA) of samples‘ peroxide values. Note: dark1 – the samples stored 1 month in the dark; dark3 – the samples stored 3 months in the dark; light1 – the samples stored 1 month on light; light3 – the samples stored 3 months on light.

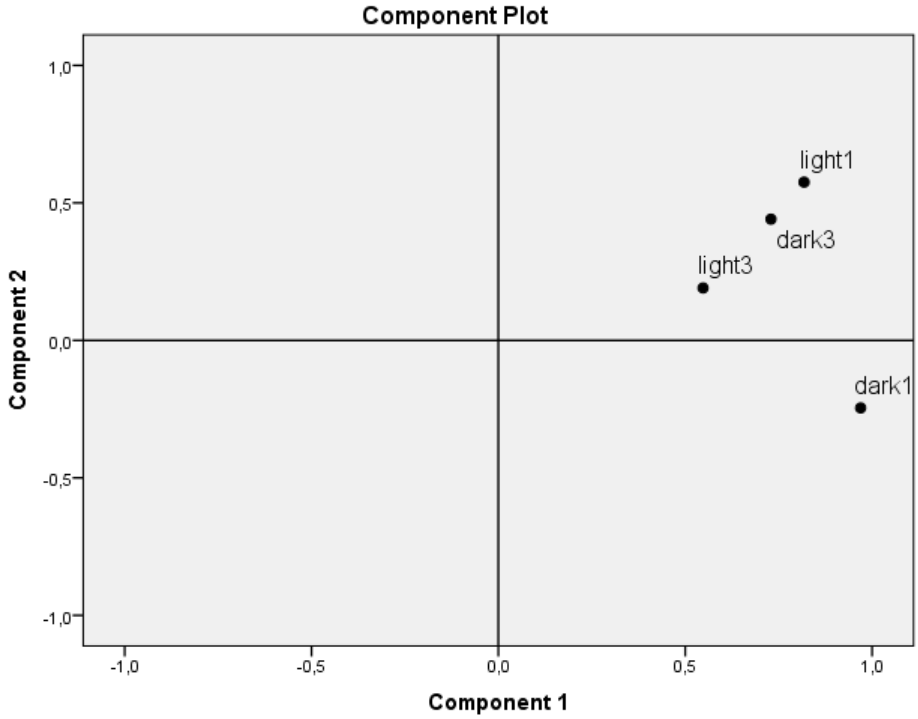

Figure 2 Principal component analysis (PCA) of chlorophyll contents in samples stored in the dark and light. Note: dark1 – the samples stored 1 month in the dark; dark3 – the samples stored 3 months in the dark; light1 – the samples stored 1 month on light; light3 – the samples stored 3 months on light.

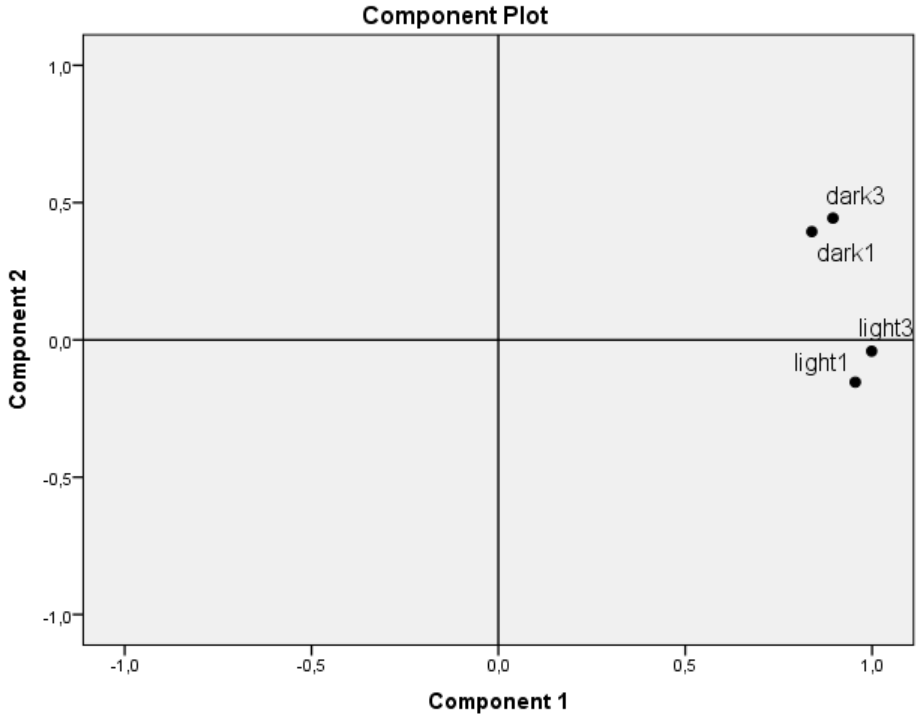

Figure 3 Principal component analysis (PCA) of overal differences between samples stored in the dark and on light dark1 – the samples stored 1 month in the dark; dark3 – the samples stored 3 months in the dark; light1 – the samples stored 1 month on light; light3 – the samples stored 3 months on light.

That is the main reason for the better oxidation stability of refined edible plant oils, since polyphenol, carotenoid and chlorophyll compounds are removed during the production **(Choe and Min, 2006)**. The results are emphasizing that cold-pressed sunflower oil has a very short shelf life since the majority of the investigated samples developed rancid taste. Rancidity is developed when peroxide value is over 20 mekv $O_2.kg^{-1}$ **(Ekwenye, 2006)**. Oxidation stability is the main property of edible plant oil and our results indicated very low stability of cold-pressed sunflower oil. The fortification/addition of different seeds and herb did not affect positively oxidation stability. Oppositely, the fortification resulted in higher oxidation degradation, especially after 3 months of storage on light (Table 3). Photooxidation, which influences the oxidation processes of samples stored on light, is producing singlet oxygen that is more reactive than triple oxygen (produced by auto-oxidation) and leads to faster oxidation. Sunflower can be declared worldwide as the most consumable edible oil. Though, the disadvantage of sunflower oil, especially cold-pressed, is its low oxidative stability **(Caponio et al., 2005)**.

Table 4 is showing the ratio of chlorophyll content in sunflower oil, both fortified and not fortified, during 3 months of storage on light and in the dark. It can be seen the amount of chlorophyll degradation during the storage period. The samples stored on light had significantly (p <0.05) lower chlorophyll content than samples stored in the dark. The degree of chlorophyll degradation is also clearly visible from the principal component analysis, where the sample stored one month in the dark formed one group and differ from the rest samples (Figure 2). The distinguish between samples stored in the dark and on the light during 3 months of storage are also visually observable from Figure 3. The results of the samples stored in the dark and in light, analyzed by principal component analysis, formed separate groups. A certain amount of chlorophylls occurs in cold-pressed vegetable oil, while these contents are removed in refined oils by a refining process **(Szydłowska-Czerniak et al., 2019)**. Chlorophyll contents decrease during longer storage periods and with higher storage temperatures **(Rasul and İnanc, 2014; Islam et al., 2019)**. Under light chlorophylls degrade more rapidly than in the dark **(Lee et al., 2014)**. Chlorophylls are affecting the beneficially nutritional profile of oils and also oil color. The auto-oxidation is triggered when pigments are exposed to light or heat. Chlorophylls are capable to transform electromagnetic radiation energy to triplet oxygen and produce highly oxidative singlet oxygen. Consequently, unsaturated fatty acids react with these free radicals. In this way, the quality of the oil is degraded **(Diaz et al., 2019)**. During auto-oxidation primary products of oxidation, hydroperoxides are triggering free radical formation reaction and off-flavor products. These off-flavor products are formed by the release of short-chain fatty acids during oxidation **(Kiritsakis and Markakis 1984; Kiritsakis, 1995)**.

Chlorophylls are strong antioxidants, but when oil is stored in the dark since on light they have acted as prooxidants **(Choe and Min, 2006)**. That is the reason for the following requirement: edible plant refined oil should have chlorophyll content under 1 $mg.kg^{-1}$ **(Szydłowska-Czerniak et al., 2019)**. The same trend of lowering chlorophyll content during 2 months of storage, under light, was noticed with cold-pressed olive oil **(Caponio et al., 2005)**.

CONCLUSION

The study emphasized cold-pressed edible plant oils storage issues, such as sunflower oil. The study unambiguously indicates that the storability/stability of cold-pressed sunflower oil is highly questionable. Fortification with seeds and herb did not affect significantly (p <0.05) experimentally produced oil mixtures/dressings since oxidation even in fortified samples were over the consumption limit. Certain, slightly better oxidation stability was noticed in fortified samples during 3 months storage in the dark. The only samples with peroxide value below 20 mekv $O_2.kg^{-1}$ (the palatable limit) were samples prepared with 5% of the hemp herb. The study showed very low oxidation stability of cold-pressed sunflower oil; the fortification was reasonable only in the samples with the addition of 5% hemp tea, only these samples stayed significantly more stable in comparison with other experimentally produced samples. The study emphasized the rapid degradation nature of chlorophylls during storage under the light. Since these kinds of products can be already found on the market and can be found attractive for consumers, our study represents valuable information for producers and consumers.

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