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RESEARCH ARTICLE

Influence of the ethanol distillation process on the quality of post-fermentation corn oil with respect to corn germ oil

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Abstract

Post-fermented corn oil has a specific quality that differs from germ corn oils. In the fermentation process of corn starch, the fat fraction remains in the wort and is distilled with ethanol. The studies compared the fraction of corn fat isolated by hexane extraction differs from the fraction of fat isolated from the distillate broth. It has been observed that the alcohol distillation process itself does not negatively affect the basic quality parameters of the post-fermented oil. The oil after ethanol distillation was found to have much higher oxidation stability. The obtained results further indicate that post-fermentation oils differ in quality from germ corn oils in terms of acid number, hydroxyl number and oxidative stability, moreover they have a significant amount of carotenoids.

KEYWORDS
corn oil, ethanol distillation, fermentation broth, post-fermentation corn oil

Introduction

Maize (Zea mays) is a cereal grain with a low fat content (2–4%), with a significant proportion of triacylglycerols located in the germ of the grain. The majority component is starch, with a content of 67–72% [Jyoti et al., 2022] and this determines the use of maize as a food and feed product.

Corn oil is not very popular in Poland, unlike in the USA and India [Jyoti et al., 2022; Klopfenstein et al., 2013]. In Poland, the leading oilseed crop is rapeseed [Kapusta, 2015], while corn is grown for other food purposes and animal feed. The main food products from this crop are flour, starch, corn syrup, and cooking oil obtained from corn germ [Jarosławski et al., 2017]. Corn plays a significant role in animal nutrition due to it being a rich source of carotenoids and other valuable biologically active substances [Skwarek, 2020]. Common industrial methods for obtaining corn oil from germ are pressing and solvent extraction, mainly with hexane [Susik, 2021]. There are methods for fat extraction using ethanol as a solvent, where the extraction efficiency can be as high as 93% [Kwiatkowski and Cheryan, 2002]. In order to improve quality, crude corn oil is also subjected to physico-chemical refining.

In recent years, corn grain has gained popularity as a feedstock in the fermentation process that results in bioethanol production, which is used as an additive for fuels [Mohanty, Swain, 2019]. In Poland, 1.2 million hectares were allocated for the cultivation of corn for grain in 2021/2022, which was used as an energy resource for bioethanol production, among other uses [CSO, 2023]. As part of the development of technology to produce bioethanol from whole corn grain, the possibility of recovering corn oil after corn starch fermentation and alcohol distillation (post-fermentation corn oil after ethanol distillation) has arisen. The oil is obtained from the sub-distillation residue (whole or thin stillage) by centrifugation and concentration [Susik, 2021].

Ethanol distillation is a process requiring specific technological conditions, where elevated temperature (above the boiling point of ethanol) is a key parameter. In addition, distillation requires rectification (fractional distillation). Depending on the capacity and size of the equipment, the corn oil, which is highly emulsified in the corn wort, is kept in unfavourable conditions, which can result in a deterioration of its quality parameters. Oils with a high content of polyunsaturated fatty acids are particularly

prone to oxidation, resulting in the loss of valuable fatty acids and the formation of primary and secondary oxidation products. The stability of vegetable oils is strongly dependent on the process conditions used to extract and refine the oil, such as high temperature and the presence of enzymes and oxygen. Under these conditions, compounds such as peroxides, aldehydes, ketones, hydroxyl compounds, epoxy compounds and many others can form and negatively affect the quality of the oil [Pignitter, 2012].

There is a lack of scientific reports on the effect of distillation on the quality of post-fermentation corn oil, which has different quality characteristics compared to germ corn oils. The aim of the study undertaken was to test the possibility of isolating the fat fraction from the wort before it undergoes ethanol distillation (post-fermentation corn oil before ethanol distillation) and to evaluate the effect of the ethanol distillation process on the quality of post-fermentation corn oil. Particular attention was paid to comparing the quality of post-fermentation corn oils and those obtained from corn germ.

MATERIALS AND METHODS

Research material

Post-fermentation corn oil after ethanol distillation and wort before distillation were obtained from a local bioethanol producer located in southern Poland. The research material was stored under refrigerated conditions in dark glass packaging. Corn oils from germs came from: pressed from Hungary (Olvita), refined from Italy (Pedrisol).

Reagents and solutions

The following reagents were used in the research: hexane (Lach-Ner s.r.o., Neratovice, Czech Republic), potassium hydroxide in ethanol (Chemland, Stargard, Poland), acetic anhydride (Chempur, Piekary Śląskie, Poland), p-toluenesulfonic acid (Sigma-Aldrich, ST. Louis, USA), pyridine (Chempur, Piekary Śląski, Poland).

Recapture post-fermentation corn oil before ethanol distillation

The post-fermentation broth was extracted with hexane to recover oil strongly emulsified with the aqueous phase. The operation was repeated 3 times. Then, the water and solid phases were separated from the miscella (a mixture of hexane and oil). Using an IKA RW10 rotary evaporator (IKA-Werke GmbH & Co. KG, Staufen, Germany), hexane was evaporated from the miscella, controlling the temperature in the bath in the range of 50-70˚C. A fraction post-fermentation corn oil was obtained from the wort (fermentation corn oil before ethanol distillation).

Standardized analytical methods

The basic parameters specify the quality of the oil were determined using standardized methods: the acid number (LK) of the tested oil was determined according to the PN-ISO 660 standard, the peroxide value (LN) according to PN-EN ISO 3960, the iodine value (LJ) according to PN-ISO 3961, anisidine (LA) acc. PN-EN ISO 6885, oxidation stability according to PN-EN ISO 6886.

Hydroxyl number determination

The determination of the hydroxyl number (LOH) involved performing a quantitative acetylation reaction with acetic anhydride using pyridine as a solvent and titration of excess acetic anhydride with ethanolic potassium hydroxide solution (c = 0.5 mol/dm3) according to BN-68 6110-13. The hydroxyl number was expressed as milligram of potassium hydroxide in 1 g of oil, using correction for acid groups.

Determination of fatty acid composition

The quantitative composition of the fatty acids was determined using gas chromatography (GC). A 50 mg oil sample was placed in a flask for transesterification of fatty acids to methyl esters using sodium methanolate (8g NaOH in 1000 ml methanol). The sample was heated for 5 min under a reflux condenser. Phenolphthalein (0.2% solution in methanol) and sulphuric acid VI (3 ml 96% sulphuric acid in 100 ml methanol) were added to the cooled solution for decolourisation. The sample was again brought to the boil (5 min). Sodium chloride and isooctane were then added to the cooled solution to transfer the resulting esters to the organic phase. After shaking and phase separation, the organic phase was collected for analysis on a chromatograph. An Agilent Technologies chromatograph with an FID detector (7890B GC chromatograph model G3440B, California, USA) was used, with a 30 m × 0.25 mm × 0.25 µm J&W DB-23 column (Agilent Technologies, California, USA). Process conditions: helium as the carrier gas at a constant pressure of 14 psi, FID at 280°C with a hydrogen flow rate of 40 ml/min and a helium flow rate of 25 ml/min. 1 µl was injected with a 50:1 split ratio at a dispenser temperature of 250°C. Chromatographic analysis was carried out according to the temperature programme recommended by the column manufacturer. Initially, a temperature of 50˚C was maintained for 1 min, then the oven temperature was increased to 250˚C at a rate of 25°C/min to 175°C and 4°C/min to 230°C for 5 minutes. The column was calibrated using the FAME standard (Sigma Aldrich, Laramie, USA) containing fatty acid esters from C4:0 to C24:0, with a total of 37 compounds, which were assigned appropriate retention times. Isooctane (Chempur, Piekary Śląskie, Poland) was used as a solvent. Results were expressed in grams per 100 g of oil relative to the total amount of fatty acids.

Carotenoids determination

The carotene content was determined using the PORIM method (PORIM, 1990). The sample was dissolved in hexane. The solution was placed in a 1 cm wide measurement cuvette and the absorbance was measured at A446 wavelengths using an AquaMate Plus UV-Vis spectrophotometer (Thermo Fisher Scientific Inc. UK). Carotenoid content, expressed as β-carotene, in milligrams per kilogram of oil.

Statistical analysis

Statistical analysis was performed using Statistica software (TIBCO Statware Inc. 2017, version 13). To assess the significance of differences between the results, one-way ANOVA and the Tukey HSD test were used, at the level of statistical significance $p \leq 0.05$.

RESULT AND DISCUSSION

Table 1 shows the results of the corn oil samples taken from production, two of which were post-fermentation oils before and after ethanol distillation, and another two of which were corn germ oils purchased from the market. The acid value of the post-fermentation oils was significantly higher than that of the oils obtained by reference methods. Scientific reports confirm that its value does not exceed 15% expressed as free fatty acids [Kerr et al., 2016]. It was observed that ethanol distillation did not

Table 1. Analysis results: acid number (AV), peroxide number (PV), anisidine number (pAV), iodine value (IV), hydroxyl number (OHV) and oxidative stability of post-fermentation corn obtained before and after the distillation of ethanol and commercial corn germ oils.

Corn oil	AV	PV	pAV	IV	OH V	Oxidative stability
	mgKOH/g	*meq O_2/kg*		*gI_2/100g*	*mgKOH/g*	*h*
Post-fermented corn oil after ethanol destilation	22,0 ± 1,32^{a}	0,01 ± 0,0^{a}	1,25 ± 0,5^{a}	121 ± 3^{a}	0,43 ± 0,05^{a}	18,2 ± 0,6^{a}
Post-fermented corn oil after ethanol destilation	21,0 ± 1,32^{a}	0,01 ± 0,0^{a}	3,23 ± 0,8^{b}	124 ± 3^{a}	2,85 ± 0,15^{b}	6,4 ± 0,2^{b}
Pressed corn oil	4,0 ± 0,24^{b}	4,43 ± 1,0^{b}	1,01 ± 0,5^{a}	122 ± 3^{a}	3,09 ± 0,16^{b}	7,2 ± 0,2^{b}
Refined corn oil	0,5 ± 0,03^{c}	0,18 ± 0,04^{c}	2,75 ± 0,7^{b}	123 ± 3^{a}	19,59 ± 0,98^{c}	10,5 ± 0,3^{c}

Values are presented as mean ± SD for triplicates. The mean values marked with different descriptors differ significantly in the column at the significance level of $p \leq 0.05$ (Tukey's HSD test).

alter this parameter in the post-fermentation corn oils tested. The difference in mass number between post-fermentation oils and corn germ oils is due to the use of whole corn grains in the starch fermentation without prior germ removal. Yeast, enzymes and water are added during the fermentation process, which may facilitate the hydrolysis of the fat contained in the corn wort and, as a result, the post-fermentation oils had an increased acid value.

The peroxide value of the oils tested was relatively low, below 5 meq O_2/kg, with the exception of refined corn oil. Post-fermentation corn oils before and after distillation showed trace amounts of peroxides. Research by Hanson and colleagues confirms the low peroxide value, which was less than 1.3 meq O_2/kg fat [Hanson et al., 2015]. Ethanol distillation had no significant effect on the peroxide value in the post-fermentation oils. In the case of corn germ oils, the higher peroxide value level may have resulted from a longer storage period [Moigradean et al., 2012]. Significant differences were observed in the anisidine value, which was higher in the post-fermentation corn oil before ethanol distillation than in the post-fermentation corn oil after distillation. It is presumed that the reason for the increase in anisidine value was the increased temperature during hexane extraction (67˚C). Similar conclusions were reached by researchers Junyusen and colleagues [Junyusen et al., 2022]. So far, the effect of hexane extraction of post-fermentation oils from wort has not been studied. The mechanism for the formation of secondary oxidation products is closely linked to oil oxidation and peroxide value. However, the presented results show that the correlation between the increase in anisidine value and peroxide value was not confirmed. This can only be explained by the presence of polar antioxidant substances that were not washed out of the wort with the oil by hexane. Statistical analysis showed that there were no significant differences in the iodine value of the corn oils analysed. In addition, from the data collected, it cannot be established that the unsaturated triacylglycerols are adversely affected by the distillation process.

During processes accompanied by high temperature, light, air or moisture, spontaneous oxidation and fat degradation may occur. Under such conditions, compounds containing epoxy and hydroxyl groups in their structure can be formed [Pignitter et al., 2012]. The formation of hydroxyl groups in oils before and after ethanol distillation was tested using simple titration methods. The lowest content of hydroxyl groups was observed in the post-fermentation oil obtained after ethanol distillation. Statistically similar values for hydroxyl number were found in the post-fermentation corn oil obtained by hexane extraction from wort (before ethanol distillation) and corn oil pressed from corn germ. A significant presence of hydroxyl groups was observed in corn germ oil that had been subjected to standard physico-chemical refining methods. From the results, it can be concluded that ethanol distillation had less effect on the formation of additional hydroxyl groups in the oil through oxidation than standard oil production. We can distinguish two groups of compounds in oils that contain a hydroxyl group. The first are natural compounds found in vegetable oils, e.g. phenolic compounds. Phenolic compounds, e.g. cinnamic acid and benzoic acid derivatives, are considered to be biologically active substances that improve the oxidative stability of oils [Rabiej-Kozioł et al., 2020]. The second group of hydroxyl compounds are those formed by the oxidation of fatty acids and may even result in a combination of compounds containing hydroxyl and epoxy groups. An example of such a case may be the formation of the 9,10-epoxy-11-hydroxy-12-octadecenoate methyl ester from oleic acid [Xia and Budge, 2017]. In order to determine more accurately which hydroxyl compounds and in what quantity we are dealing with, a broader range of studies should be conducted.

The oxidative stability of corn oils varies depending on the oil extraction method [Junyusen et al., 2022] and the refining processes used [Almeoda et al., 2018]. It was observed that post-fermentation corn oil after ethanol distillation had the highest oxidative stability measured at 110˚C in the Rancimat accelerated oxidation test. The fermentation process, during which the corn fat fraction is present, results in the release of a significant amount of antioxidant substances, also found outside the corn germ, which affect the stability of the oil. High levels of these substances result in the protection of this oil in the subsequent process, i.e. ethanol distillation. In contrast, post-fermentation corn oil obtained prior to ethanol distillation had lower oxidative stability. It is suggested that this may have been due to the use of hexane as a solvent, which, due to its non-polar properties, resulted in much less extraction of polar antioxidant substances with the oil [Jedidi et al., 2020]. The other corn oils also showed lower oxidative stability.

Table 2 shows the fatty acid composition of the tested corn oil samples. The data presented in the table show that there were no statistically significant differences between the oils tested, either the post-fermentation oils or those obtained from corn germ. The fatty acid composition was not altered by ethanol distillation.

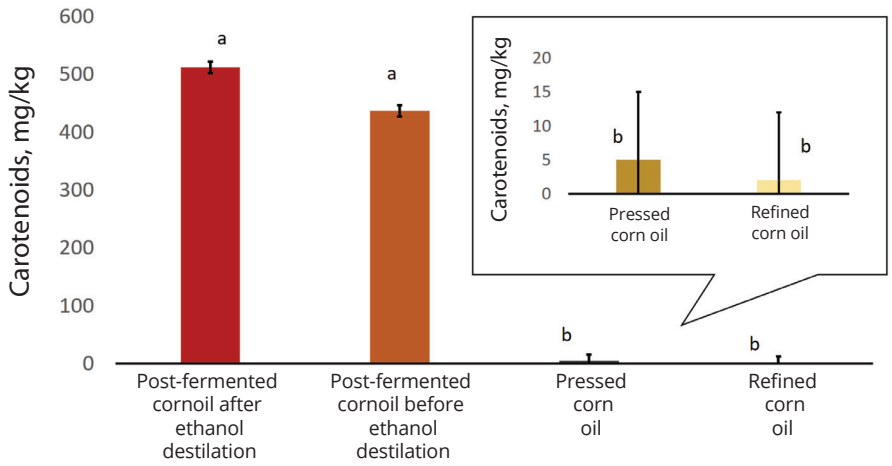

Figure 1. Carotenoid content of post-fermentation corn oil after and before distillation of ethanol and commercial corn germ oils.

Another parameter was the level of carotenoid content. When analysing the data presented in Figure 1, it is apparent that the post-fermentation corn oils were characterised by a much higher total carotenoid content than corn oils obtained by reference methods (pressing, extraction, refining). No significant differences were found between oil obtained before and after ethanol distillation. A large disproportion in carotenoid content was observed between post-fermentation oils and those obtained from the germ, indicating that a significant proportion of the carotenoid compounds occur outside the corn germ. This is supported by studies showing that more than 90% of the total carotenoid content is found in corn endosperm [Wong et al., 2004]. The whole

Table 2. Fatty acid composition of post-fermentation corn oil obtained before and after distillation of ethanol and commercial corn germ oils.germ oils.

Fatty acids	Corn oil			
	Post-fermented corn oil after ethanol destilation	Post-fermented corn oil before ethanol destilation	Refined corn oil	Pressed corn oil
	g/100g	*g/100g*	*g/100g*	*g/100g*
C12:0	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
C14:0	0.3 ± 0.1^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
C16:0	11.4 ± 0.3^{ab}	11.3 ± 0.3^{a}	11.3 ± 0.3^{a}	12.2 ± 0.3^{b}
C16:1	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2 ± 0.1^{a}	0.1 ± 0.0^{a}
C17:0	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.0 ± 0.0^{a}
C17:1	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
C18:0	1.9 ± 0.1^{ab}	1.9 ± 0.1^{ab}	2.0 ± 0.1^{b}	1.7 ± 0.1^{a}
C18:1	30.4 ± 0.8^{a}	31.0 ± 0.8^{a}	31.4 ± 0.8^{a}	30.2 ± 0.8^{a}
C18:1 trans	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
C18:2 cis	53.6 ± 1.4^{a}	$53,2 \pm 1.4^{a}$	52.5 ± 1.4^{a}	52.9 ± 1.4^{a}
C18:2 trans	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
C18:3n3	0.9 ± 0.1^{a}	0.9 ± 0.1^{a}	0.9 ± 0.1^{a}	1.1 ± 0.1^{a}
C20:0	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}
C20:1	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.3 ± 0.1^{a}	0.4 ± 0.1^{a}
C22:0	0.1 ± 0.0^{a}	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}	0.2 ± 0.0^{a}
C22:1	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
C24:0	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}
∑ NKT	14.4 ± 0.4^{a}	14.1 ± 0.4^{a}	14.2 ± 0.4^{a}	14.7 ± 0.4^{a}
∑ NNKT	30.9 ± 0.8^{a}	$31,5 \pm 0.8^{a}$	32.0 ± 0.8^{a}	30.8 ± 0.8^{a}
∑ WNKT	54.5 ± 1.4^{a}	54.1 ± 1.4^{a}	53.4 ± 1.4^{a}	54.0 ± 1.4^{a}
Others	0.2 ± 0.1^{a}	0.3 ± 0.1^{a}	0.4 ± 0.1^{a}	0.5 ± 0.1^{a}

corn kernels subjected to the fermentation process resulted in a fat fraction enriched with a significant amount of carotenoids. In contrast, the ethanol distillation process did not reduce the overall content of this group of compounds in the post-fermentation oils. Corn oils obtained from pressed and refined germs are characterised by a low content of carotenoids.

CONCLUSION

Post-fermentation corn oil is an innovative oil that differs from corn oils present on the market. The main difference is the method of obtaining post-fermentation oils from those obtained directly from pressed as well as refined corn germ. The primary objective of the study was to determine if it is possible to obtain post-fermentation corn oil before ethanol is distilled from the corn wort, and to verify whether ethanol distillation affects the quality of the post-fermentation oil. As a result of the data obtained, it was found that ethanol distillation had no effect on the increase of values characteristic of oils such as acid, peroxide or anisidine value. Ethanol distillation did not cause changes in the fatty acid composition or carotenoid content. It was observed that post-fermentation corn oil after ethanol distillation has a low hydroxyl value and high oxidative stability. The quality of the post-fermentation oils was also compared with corn germ oils. There was a significantly higher degree of hydrolysis of the post-fermentation oils, indicating the need for a refining process. The above-mentioned studies are preliminary studies and define the direction of further work on the effects of fermentation and alcohol distillation processes on the quality of post-fermentation corn oil.

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