

Wheat germ oil extracted by supercritical carbon dioxide with ethanol: Fatty acid composition

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SUMMARY: In this work, supercritical fluid extraction (SFE) using CO₂ with ethanol as entrainer was performed at a temperature of 40 °C under a pressure of 21 MPa. For comparison, a similar extraction without the entrainer was carried out. The extraction yield of wheat germ using supercritical CO₂ with ethanol was slightly higher (10.7 wt%) than that of extraction without the entrainer (9.9 wt%). Fractions of SFE extracts were collected separately during the experiments and the composition of fatty acids in each fraction was analyzed. The SFE extracted oils were rich (63.4-71.3%) in the most valuable polyunsaturated fatty acids (PUFA) and their content in all collected fractions was approximately constant. Similar PUFA contents were found in the reference samples of oils extracted by *n*-hexane (66.2-67.0%), while the commercial cold-pressed oil contained significantly less PUFA (60.2%). These results show a higher nutritional value of the oil obtained by extraction with supercritical CO₂ than cold pressed oil which is generally considered to be very valuable.

KEYWORDS: *Entrainer; Extraction; Polyunsaturated fatty acids; Supercritical CO₂; Wheat germ oil*

RESUMEN: *Aceite de germen de trigo obtenido mediante extracción con dióxido de carbono supercrítico con etanol: Composición en ácidos grasos.* En este trabajo, la extracción con fluidos supercríticos (SFE) usando CO₂ con etanol como agente de arrastre se realizó a 40 °C bajo una presión de 21 MPa. Se ha llevado a cabo la comparación con una extracción similar sin agente de arrastre. El rendimiento de la extracción de germen de trigo usando CO₂ supercrítico con etanol fue ligeramente mayor (10,7% en peso) que la de extracción sin agente de arrastre (9,9% en peso). Se recogieron por separado fracciones de extractos SFE durante los experimentos y se analizó la composición de ácidos grasos en cada fracción. Los aceites extraídos mediante SFE eran ricos en los ácidos grasos poliinsaturados más valiosos (63,4-71,3%), (PUFA) y su contenido en todas las fracciones recogidas fue aproximadamente constante. Un contenido similar de PUFA fueron encontrados en muestras de referencia de los aceites extraídos con *n*-hexano (66,2-67,0%), mientras que el aceite prensado en frío comercial contenía significativamente menos PUFA (60,2%). Estos resultados muestran un mayor valor nutritivo del aceite obtenido por extracción con CO₂ supercrítico que el aceite prensado en frío que generalmente se considera que es muy valioso.

PALABRAS CLAVE: *Aceite de germen de trigo; CO₂ supercrítico; Entrainer; Extracción de ácidos grasos poliinsaturados*

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1. INTRODUCTION

Wheat is one of the most common cereal crops. However, it is generally consumed as white flour obtained from the endosperm which constitutes about 85% of the grain. The endosperm does not contain the highest nutritional value and consists mainly of starch and storage proteins. A relatively small portion of the grain, called the germ, which accounts for about 2-3% of its volume, contains 26% protein, 17% sugar and about 10% oil rich in valuable unsaturated fatty acids (Wang and Johnson, 2001; Durante *et al.*, 2012). Wheat germ is one of the richest natural sources of vitamin E. It also contains vitamin B and many micronutrients. This valuable part of the grain is mostly treated as a by-product in the production of flour and is used as animal feed. Only a portion of it is sold as an ingredient for human diets in health food stores. A small amount is subjected to cold-pressing or extraction in order to recover oils rich in nutrient components. This high-quality oil is used for example as a component of food, pharmaceutical or cosmetic preparations.

Cold-pressing is a very old, but still common method of producing high quality oil. An advantage of pressed oil is the lack of chemical contaminants, which is a prerequisite for its application as a component of healthy food. Unfortunately, the efficiency of this method is low in comparison with others and garners about 50% of total oil content in wheat germ (Panfili *et al.*, 2003). Solvent extraction using a Soxhlet apparatus is much more effective than pressing and enables producers to recover about 90% of the total oil. Its efficiency depends on the type of solvent used. The most commonly applied solvent is *n*-hexane, but the use of petroleum ether, chloroform and ethanol have also been investigated (Dunford *et al.*, 2003; Piras *et al.*, 2009). Besides extraction under normal pressure, a pressurized solvent extraction has also been studied (Dunford *et al.*, 2003). Another method that extracts the wheat germ oil of similar quality is aqueous enzymatic extraction (Li *et al.*, 2011; Xie *et al.*, 2011).

Supercritical extraction with CO₂ is, perhaps, the most promising method which combines the advantages of both methods mentioned above. Supercritical extraction of wheat germ has been intensively studied in recent years. The effect of factors such as pressure, temperature, CO₂ flow, particle size and moisture content in the feed on the extraction efficiency was studied. Durante *et al.* (2012) found that the optimal conditions for the best oil yield were: pressure 30-35 MPa, temperature 60-70 °C, moisture content 2.6%, particle size 30 mesh (particles smaller than 0.595 mm) and a CO₂ flow rate of 4 dm³·min⁻¹. Panfili *et al.* (2003) estimated that the best performance used a pressure of 38 MPa, temperature 55 °C, the particle size of

0.35 mm and a CO₂ flow equal to 1.5 dm³·min⁻¹. In contrast, the optimal conditions determined by Gomez *et al.* (2000) differed from the previous ones and were as follows: 15 MPa, 40 °C and 1.5 dm³·min⁻¹ of CO₂. According to Jiang *et al.* (2011) a maximum oil yield of 10.46% was achieved when the particle size was 60-80 mesh (0.170-0.250 mm), water content 4.37%, pressure 30 MPa, temperature 40 °C and CO₂ flow rate 20 kg·h⁻¹. Optimizing the efficiency of extraction at a flow rate of 2 g·min⁻¹, Gelmez *et al.* (2009) determined the following conditions: 44.2 MPa, 40 °C. However, in order to obtain a higher content of tocopherols and greater antioxidant capacity, it is better to use the following conditions: 14.8-16.5 MPa, 40-60 °C. According to Ge *et al.* (2002) the most favourable conditions for the maximum efficiency of extraction of vitamin E were 26.2 MPa and 40 or 45 °C. Similar temperature and pressure (40°C and 25 MPa) were used by Özcan *et al.* (2013).

Summarising the results of the cited works, the temperature does not significantly affect the efficiency of extraction and a temperature of about 40°C is the most often recommended. The increase in pressure improves the yield, but lower pressures of 14.8-26.2 MPa should be used to increase the content of valuable components in the extract.

Supercritical extraction is often used for extracting oil from seeds. The most common non-polar solvent is CO₂, but sometimes small amounts of a polar entrainer are added to enrich the extracted oil in polar nutrients (King *et al.*, 2001; Salgın, 2007; Salgın *et al.*, 2011; Jozwiak *et al.*, 2013). However, we did not find reports describing wheat germ supercritical extraction using CO₂ with the addition of an entrainer in the literature.

The aim of this work was to study the extraction of wheat germ oil using supercritical CO₂ with addition of ethanol, as a safe polar entrainer and to compare the result with a similar extraction without entrainer and with a conventional solvent extraction. Extract fractions were collected separately during the course of the experiments and their composition was analyzed to determine the content of various fatty acids. Whether the use of an entrainer affects the fatty acid profile in the total extract and subsequent fractions was investigated.

2. MATERIALS AND METHODS

2.1. Materials

Two kinds of germ produced from wheat grown in Poland were used for extraction. The first was the wheat germ offered by the Kupiec company (Poland), commercially available in stores and intended for direct consumption. The second raw material was the wheat germ received directly from a local mill in Ciechanowiec (Poland).

The moisture in wheat germ feeds was determined using Moisture Analyzers (Radwag MAC 50/NH) and amounted to 8.06% and 8.26%, respectively.

The comparative sample was the cold-pressed wheat germ oil, purchased from the company LOGIS-TECH (Mirków, Poland) and the sample was denoted as CPO.

2.2. Extraction methods

The wheat germ samples from the Kupiec company and from the Ciechanowiec mill were tested in extraction with supercritical CO₂ with the addition of ethanol as entrainer. For comparison wheat germ from the Kupiec company was extracted without entrainer. Each extraction was carried out using the wheat germ without grinding.

The scheme of the test unit for supercritical extraction is shown in Figure 1. The volume of the extractor was 150 cm³, and that of separators about 50 cm³. Details of the apparatus have been described elsewhere (Jozwiak *et al.*, 2013, Bujnowski *et al.*, 2011). A pressure of 21 MPa and temperature of 40 °C were selected as standard conditions of SFE experiments. The average flow rate of CO₂ was about 60 dm³·h⁻¹ (at ambient pressure).

Prior to the extractions with entrainer, 10 cm³ of ethanol was added directly to the germ, and then 2 cm³·h⁻¹ of ethanol were fed simultaneously with CO₂ to the extractor. Fractions of the extract were collected every 6 hours (3 hours for extraction without ethanol) or if the amount of the extract was too small, time for collection was prolonged to ensure an adequate amount of oil for analysis purposes. In the case of extraction of wheat germ from the Kupiec company, the temperature of the last fraction was increased to 60 °C in order to monitor whether such a temperature increase improved the yield. Samples of extracts obtained by supercritical extraction of

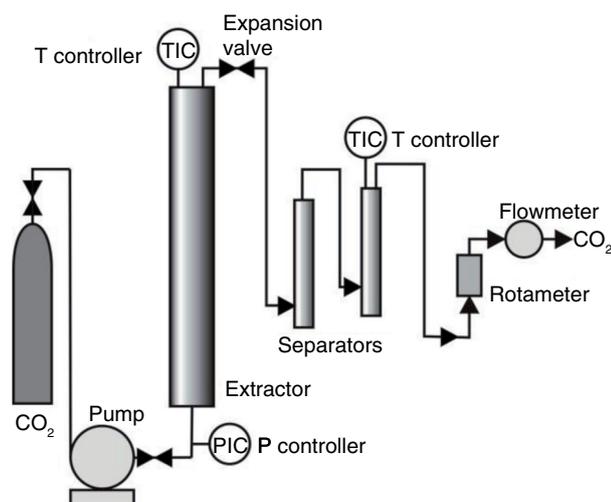


FIGURE 1. Scheme of apparatus for supercritical extraction tests.

wheat germ from the Kupiec company with and without ethanol entrainer were denoted as SFE-KE and SFE-K, respectively, and extract samples obtained by the supercritical CO₂ extraction with ethanol of the feed from the mill were denoted as SFE-ME.

For comparison with the SFE extracts, extractions of both types of wheat germ were also performed using a Soxhlet apparatus with *n*-hexane (POCh, 95%, for HPLC) as solvent and the samples were denoted as SE-KH and SE-MH, respectively. Extractions for each of the two types of germ were carried out twice and both extracts were combined. For a single extraction, about 70 g of wheat germ and 450 cm³ of *n*-hexane were used. The extraction was carried out for 8 h.

2.3. Analytical method

Fatty acids composition analysis was performed after derivatization to form the methyl esters using the AOCS Ce 1-62 method (American Oil Chemists' Society, 1989). Methyl esters of long-chain fatty acids were prepared according to AOCS Ce 2-66 method (American Oil Chemists' Society, 1989). A GC7890A gas chromatograph with a flame ionization detector (FID) (Agilent Technologies) was used. The chromatograph was equipped with a SUPELCOWAX 10 capillary column 30 m length, 0.32 mm internal diameter, 0.25 μm film thickness. The chromatographic conditions were: detector temperature of 260 °C, injector temperature of 250 °C, volume injected of 1 μL, the carrier gas was helium with a flow rate of 1 ml/min and split flow ratio 50:1. The oven temperature programme was set as follows: 200-260 °C (4 °C·min⁻¹), 260 °C (10 min). Uncertainty of fatty acid content determination, expressed by relative standard deviation (RSD) depends on fatty acid content:

- RSD = 1.3% for contents higher than 40%,
- RSD = 3.0% for contents higher than 10 and lower than 40%,
- RSD = 5.9% for contents higher than 1 and lower than 10%,
- RSD = 11.3% for contents lower than 1%.

Fatty acids were identified using a GC/MS analysis with the help of Wiley 9th Edition / NIST 2009 Mass Spectral Library and by comparing the GC retention times of the peaks with the retention times of standards.

3. RESULTS AND DISCUSSION

3.1. Extraction yield

The conditions of supercritical extraction and amounts of obtained fractions of oil extracts are listed in Table 1. In each of the three SFE extractions,

TABLE 1. The results of wheat germ extraction using supercritical CO₂ without (SFE-K) and with ethanol (SFE-KE and SFE-ME) carried out at 40 °C under pressure of 21 MPa

	Fraction number	Extraction time of subsequent fraction (h)	solvent/feed amount ratio (dm ³ ·g ⁻¹)	Mass of oil fraction (g)
SFE-K (wheat germ produced by Kupiec, feed amount 90.98 g, average flow rate of CO ₂ at ambient pressure 63.0 dm ³ ·h ⁻¹)	1	3	2.1	2.4004
	2	3	4.1	1.95225
	3	3	6.2	1.4024
	4	3	8.3	1.2671
	5	3	10.4	0.97075
	6	3	12.4	0.49205
	7	3	14.5	0.18505
	8	5.5	18.3	0.19015
	9	3	20.4	0.0894
	10*	3	22.5	0.0577
SFE-KE (wheat germ produced by Kupiec, feed amount 94.8 g, average flow rate of CO ₂ at ambient pressure 58.4 dm ³ ·h ⁻¹)	1	6	3.7	6.2024
	2	6	7.4	2.9740
	3	6	11.1	0.8093
	4	12	18.5	0.1260
	5*	6	22.2	0.0619
SFE-ME (wheat germ produced at the Ciechanowiec mill, feed amount 109.6 g, average flow rate of CO ₂ at ambient pressure 63.1 dm ³ ·h ⁻¹)	1	6	3.5	5.7353
	2	6	6.9	2.7456
	3	6	10.4	1.8323
	4	6	13.8	0.5109
	5	18	24.2	0.2414

*The temperature of collecting this fraction was increased to 60 °C in order to check whether such a temperature increase improved the yield.

the first fractions were the largest but subsequently decreased with time. In order to check whether the increase in temperature increased the efficiency of extraction, the last fractions in both extractions of wheat germ from Kupiec were collected at a temperature raised to 60 °C. However, there was no visible change.

Figure 2 shows the dependence of the total yield of the three SFE extractions on solvent/feed amount ratio. As seen in the graph, the addition of ethanol to CO₂ slightly improved the efficiency of extraction. In the extraction without entrainer (SFE-K), a yield of 9.9 wt% was achieved, and smaller amounts of extracts were collected, therefore a long time, i.e. over 32 h was necessary to extract all the extractable components. In the supercritical extraction of wheat germ from the Kupiec company with the addition of entrainer (SFE-KE) the yield reached 10.7 wt%, which was a little higher than the yield of 9.9 wt% in the extraction without ethanol (SFE-K). In the case of SFE-ME extraction, the yield was about 10.1 wt%, which was a little lower than in a similar extraction of wheat germ from Kupiec. The difference in yields may be due to differences in the

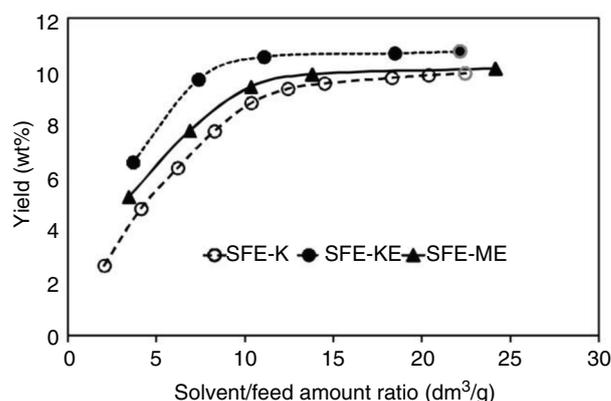


FIGURE 2. The effect of solvent/feed amount ratio on the yield of supercritical extraction of wheat germ using CO₂ (SFE-K) and CO₂ with ethanol (SFE-KE and SFE-ME).

amount of extractable components or moisture contents of the raw materials.

Comparative samples of wheat germ from the Kupiec company and from the Ciechnowiec mill, extracted using *n*-hexane, achieved a yield of

TABLE 2. The percentage contents of PUFA, MUFA and SFA groups of fatty acids in wheat germ oils obtained by different methods of extraction (SFE-K, SFE-KE, SFE-ME, SE-KH and SE-MH) and cold-pressing method (CPO)

Group of fatty acids	Type of wheat germ and extraction method								
	SFE-K		SFE-KE		SFE-ME		SE-KH	SE-MH	CPO
	Content range in fractions	Content in overall extract	Content range in fractions	Content in overall extract	Content range in fractions	Content in overall extract			
PUFA	63.6-68.6	66.1	65.4-72.1	66.8	64.4-65.9	65.5	67.2	66.3	61.6
MUFA	13.1-17.7	14.2	9.3-20.0	14.0	13.7-20.9	15.4	14.0	15.1	21.5
SFA	14.5-22.7	19.2	13.8-20.5	18.8	12.9-20.5	18.4	18.8	18.4	16.8

11.3 wt% and 10.7 wt%, respectively, and the tendency was similar to the supercritical extraction. Total yields of extracts observed in both the solution and supercritical extraction were slightly higher or similar to those reported in the literature (Gomez *et al.*, 2000; Jiang *et al.*, 2011).

3.2. Fatty acid composition

For all oil fractions obtained by supercritical extractions, *n*-hexane extractions and cold-pressed oils, the fatty acid compositions were determined. This allowed us to check whether the fatty acid composition changed during the extraction and whether use of ethanol as an entrainer affected their composition. The nutritional value of fats is greater if the content of polyunsaturated fatty acids (PUFA) is higher. The presence of monounsaturated fatty acids (MUFA) is also profitable, while saturated fatty acids (SFA) have the lowest value. The concentrations of the above mentioned groups of acids in wheat germ oil obtained by different methods of extraction and the cold-pressing method are summarized in Table 2. The results show that the addition of ethanol to the supercritical CO₂ did not affect the fatty acid composition of the whole collected extract (SFE-K compared with SFE-KE). It can also be noted that the total concentrations of the three groups of fatty acids in the extracted oil were similar to those obtained by other investigators (Gomez *et al.*, 2000; Jiang *et al.*, 2011; Özcan *et al.*, 2013; Eisenmenger and Dunford, 2008).

The collected wheat germ oils are characterized by a high content of PUFA in all fractions, varying from 63.6 to 72.1%. Similar values of 66.3-67.2% were observed for oils extracted by *n*-hexane, but a little lower value of 61.6% for cold-pressed oil. The contents of various PUFA in subsequent fractions of oils corresponding to the values of solvent volume to feed mass ratios are shown in Figure 3. The major polyunsaturated acid was linoleic acid (C18:2). Its content was very similar in all fractions of oils obtained using supercritical extraction methods, regardless of whether CO₂ was used with the addition of ethanol or alone. This content ranged

from 54.2 to 60.6%. Similar values were found in the oils extracted with *n*-hexane from the germ produced by Kupiec, the germ from the Ciechanowiec mill and cold-pressed oil, which were 57.8, 58.0 and 55.0%, respectively.

The second polyunsaturated acid with the highest content in the oil was linolenic acid (C18:3) and as in the case of linoleic acid, its content was practically the same in subsequent oil fractions obtained from the supercritical extraction. There were some differences between the three extractions, but they were smaller than the estimated detection error. In the case of SFE-K extraction, these contents in all fractions were 8.7-9.3%, and for SFE-KE extraction they were 8.4-11.3%. However, in the SFE-ME extraction the contents of linolenic acid were lower varying in the range of 7.9-8.4%. Comparable linolenic acid contents were found in the oils extracted with *n*-hexane, i.e. 9.2 and 8.2% for germ from the Kupiec company and from the Ciechanowiec mill, respectively. A slightly lower content of this acid, i.e. 6.5%, was determined for the cold-pressed oil.

Eicosadienoic acid (C20:2) and heptadienoic acid (C17:2) were also identified in the tested oils, but they were present in small amounts. The contents of the first acid in all fractions were about 0.2%, and the second acid about 0.05%. Similar values were observed for the oils extracted using *n*-hexane and the cold-pressed oil.

The contents of various MUFA in all the extracted fractions are presented in Figure 4. The main acid of this group in the wheat germ oil was oleic acid (C18:1). In the case of the SFE-K extraction, concentrations of this acid gradually increased in the subsequent fractions from 10.5% in the first fraction to 13.6% in the middle fraction, and then remained essentially constant. During SFE-KE extraction, the oleic acid content increased in subsequent fractions from 10.8% to 14.1% in the middle fraction, and then decreased to 7.7% in the last one. Similarly, in the SFE-ME the concentration increased from 11.6 to 14.5% in the fourth fraction and dropped to 13.5% in the last one. The contents of oleic acid in the *n*-hexane-extracted oils obtained from germs from the Kupiec company and from

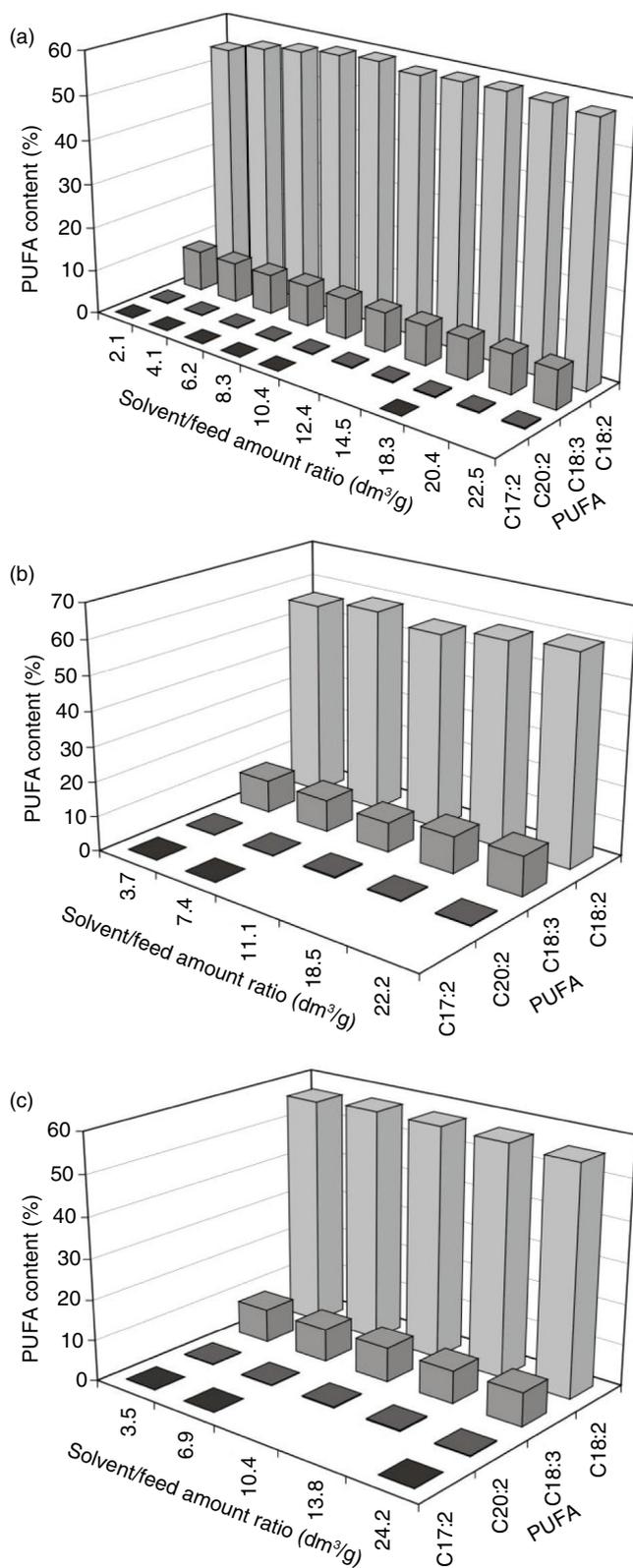


FIGURE 3. Content of various PUFA in subsequent fractions (collected after reaching specified value of solvent/feed amount ratio) of: a) SFE-K extraction, b) SFE-KE extraction, c) SFE-ME extraction.

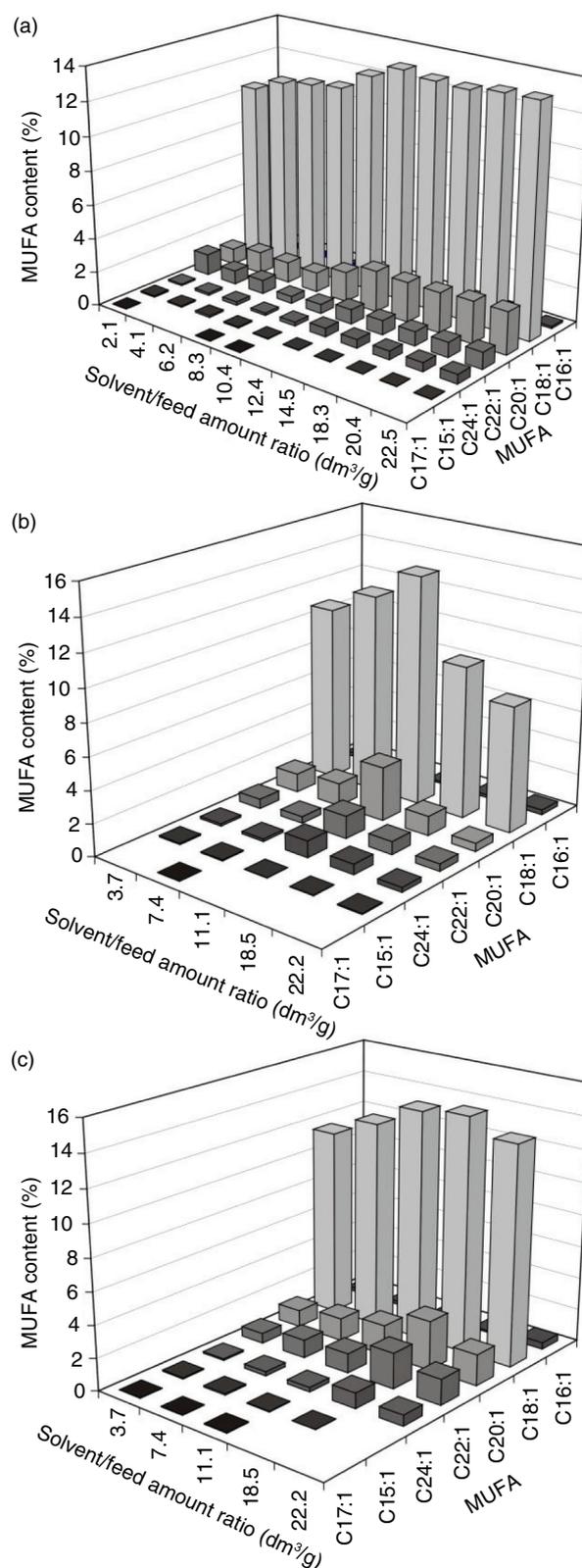


FIGURE 4. Content of various MUFA in subsequent fractions (collected after reaching specified value of solvent/feed amount ratio) of: a) SFE-K extraction, b) SFE-KE extraction, c) SFE-ME extraction.

the Ciechanowicz mill were 11.5 and 12.5%, respectively, and they were similar to those observed for the SFE extracts. The highest content of that acid, i.e. 19.7%, was found in the cold-pressed oil.

A similar variation in the concentrations as that for oleic acid can be observed in the subsequent fractions of extracts in the case of eicosenoic acid (C20:1). However, in this case, the concentrations were significantly lower. Maximum levels were 2.5, 3.3 and 3.0% for the SFE-K, SFE-KE and SFE-ME extraction, respectively. The values found in the oils extracted with *n*-hexane were lower and amounted to 1.4 and 1.3% for the germ from the Kupiec company and Ciechanowicz mill, respectively. The cold-pressed oil contained 1.1% of eicosenoic acid.

Other MUFA, such as C16:1, C22:1, C24:1, C15:1 and C17:1, were also identified in the tested oils. However, the contents of these acids were much lower than those discussed above. The total content of MUFA was the highest in the cold-pressed oil and amounted to 21.5% (Table 2), whereas it was 14.0 and 15.1% in the oils extracted by *n*-hexane for germ from the Kupiec company and Ciechanowicz mill, respectively. The content of MUFA in the SFE fractions showed similar variability to the main acids of this group. In the fractions obtained by the SFE-K, SFE-KE and SFE-ME extractions, maximum MUFA contents were observed at 17.7, 20.0 and 20.9%, respectively.

SFA are mainly a source of energy, but unfortunately also cause an increase in the level of low-density cholesterol, and therefore a low content of SFA in oil is preferred. Wheat germ oil has a relatively low total concentration of SFA. The examined cold-pressed oil contained 16.8% SFA. The Oils extracted by *n*-hexane from germ derived from the Kupiec company and Ciechanowicz mill included a little more SFA, 18.8% and 18.4%, respectively.

The results of the concentration analysis of various SFA in the fractions of oils obtained by SFE extraction are shown in Figure 5. The main SFA in the oil investigated was palmitic acid (C16:0). In cold-pressed oil, its content was the lowest and amounted to 14.3%. Slightly higher contents of the acid were found in oils extracted with *n*-hexane: 17.7% (Kupiec) and 17.4% (Ciechanowicz). The concentration of palmitic acid in the fractions of SFE extracted oils varied and depended on the time at which a particular fraction was collected and on the presence of the entrainer. In the case of SFE-K extraction, 21.9% of the acid was found in the first fraction, but this content systematically decreased in subsequent fractions to the level of about 13.5%. A slightly different variation in the concentration of C16:0 acid was observed in the case of CO₂ extraction with ethanol addition. A high content (19.5%) of this acid was found in the first fractions obtained from both SFE-KE and SFE-ME and in subsequent fractions it decreased to 11.8% (SFE-KE) and

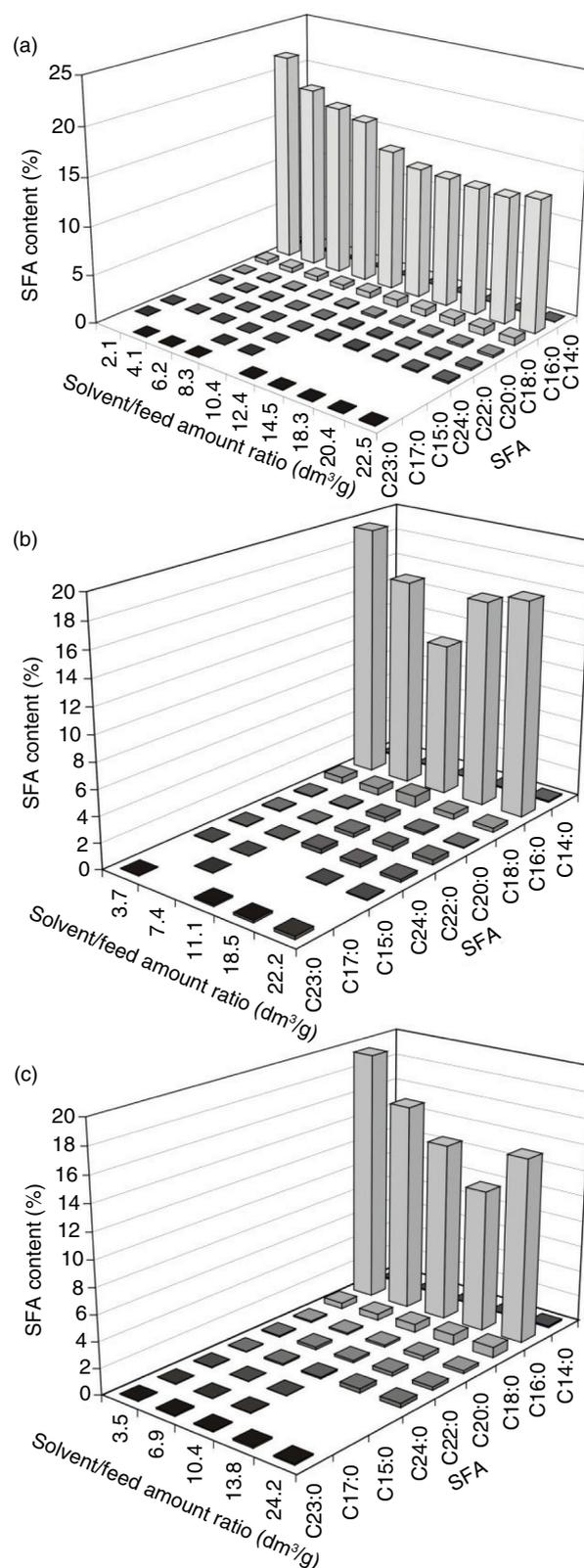


FIGURE 5. Content of various SFA in subsequent fractions (collected after reaching specified value of solvent/feed amount ratio) of: a) SFE-K extraction, b) SFE-KE extraction, c) SFE-ME extraction.

11.0% (SFE-ME) and then increased again. Such a relationship was mainly due to a change in the composition of the extracted MUFA and SFA and this trend was opposite to that observed for MUFA concentrations (Figure 4).

Comparing the results from Figures 4b, c and Figures 5b, c, one can notice that the selection of some fractions with a relatively high MUFA and relatively low SFA content (third for SFE-KE and fourth for SFE-ME) provide the possibility of obtaining a product with especially high nutritional value.

4. CONCLUSIONS

The use of ethanol as an entrainer to the supercritical CO₂ extraction slightly improved the efficiency of the extraction and had practically no effect on the PUFA content in the extract. The PUFA contents in the extracts were very close to their contents in the oil obtained by *n*-hexane extraction. It was also stated that the PUFA contents were similar in all the oil fractions obtained by SFE.

The contents of SFA and MUFA in all the oils were significantly less than the PUFA contents. It was stated that average contents of MUFA and SFA in fractions of SFE extracts and their contents in the solvent extracts were similar. However, the contents of MUFA and SFA changed in subsequent fractions of oils obtained by SFE. In the middle fractions of extracts obtained with use of entrainer the maximum MUFA content and minimum PUFA content was observed.

Such variability in the fatty acid profile of consecutive fractions permits the use of fractionation in supercritical extraction to obtain more valuable oil fractions with high contents of PUFA, and higher MUFA contents than in the oil obtained without fractionation.

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