

OPTIMIZATION OF CO-SOLVENT ADDITION IN SUPERCRITICAL FLUID EXTRACTION OF FAT WITH CARBON DIOXIDE

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This investigation is concerned with supercritical fluid extraction (SFE) using CO₂, as an analytical technique for total fat extraction from food and feed samples. Its most significant advantages are safety, cleanness, and shorter extraction time. The main limitation of this technique includes the difficulty of extracting polar lipids due to the non-polar character of the solvent (CO₂) used for the extraction. The influence of ethanol as a co-solvent on the SFE of mash pig feed was investigated in this paper. Total fat content was determined by SFE and Soxhlet method for ten commercially available mesh pig feeds. Yields of the fat extracted by both methods were plotted one against the other and compared. Statistically significant difference ($p \leq 0.05$) has been found only between the total fat obtained by the Soxhlet extraction and SFE by pure CO₂. Based on the mathematical model, maximum yield of the extracted fat is achieved at an ethanol addition of 0.67 ml/g of sample, when the other parameters are the same as recommended by the producer's procedure.

KEY WORDS: supercritical fluid extraction, co-solvent, Soxhlet extraction, mash feed

INTRODUCTION

The majority of lipids in all natural fats and oils are triglycerides, which can be completely removed by simple organic extraction techniques, such as Soxhlet extraction. However, structural lipids are predominantly phospholipids, which can contain a high percentage of polyunsaturated fatty acids (PUFA) and their inclusion is essential when determining total fat content, or in the example of complete fatty acid profile. Acid hydrolysis extraction easily releases the mentioned fatty acid group, but this type of extraction is extremely aggressive and leads to chemical degradation of the extracts, making them unsuitable for further analysis. Chloroform/methanol extraction methods, such as Folch extraction, can completely extract the lipids from biological tissues, with no or minimal damage, but these techniques require both long time of extraction and the use of expensive and dangerous chemicals, which can lead to health risk of laboratory personnel (1).

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Supercritical fluid extraction (SFE) with CO₂ has been used as an analytical method for the determination of total fat content in food, due to a lower impact on lipid components, and thus, minimal chemical damage (2). This technique is less hazardous and has no negative effects on the environment. CO₂ is inexpensive, nontoxic, and non-flammable, easily removed from the extract, and has a high interpenetration in solid matrices (3-6). In the early 1980s, numerous researchers began their studies on SFE and evaluated the impact of extraction variables on the yield, recovery, and composition of the extracted oils from various sources (7, 8). The most significant variables are temperature, particle size, and moisture content of the examined material, pressure, extraction time, CO₂ flow rate, and ratio of added solvent, all of which need to be optimized for upgrading the efficiency of the process (9, 10).

The extraction that uses only pure supercritical CO₂ usually yields good recoveries of nonpolar lipids. Polar lipids may remain unextracted, because of their lower solubility in the supercritical CO₂. Therefore, samples containing a certain amount of these types of lipid may present difficulties in the extraction (11). The polarity of supercritical CO₂ can be varied by using a co-solvent (modifier) such as methanol, ethanol, or in some cases even water, in order to improve the extraction of polar lipids (12). Modifiers can be introduced as mixed fluids in the pumping system, or with the aid of a second pump, or by simply injecting the modifier as a liquid onto the sample before the extraction (9, 13). A variety of alcohols, acetone, and hexane can be used for the extraction of oilseed (14).

The aim of this investigation was to develop an SFE method suitable for the determination of total fat content in mash pig feed, and other feed or food materials with similar physical and chemical characteristics, and to predict optimal volume of pure ethanol as a co-solvent in the extraction process. The SFE results with adopted volume of ethanol were compared with those obtained using the Soxhlet extraction method.

EXPERIMENTAL

Pig feed samples

Ten commercially available mash feeds for fattening pigs were used in this study. All samples belonged to the same group of products, produced by different manufacturers from Serbia. Samples were marked with numbers from 1 to 10. Pig feed samples were stored at room temperature in dark, for not more than five days before the extraction. The nominal contents of main nutrients were: protein (approx. 15%), cellulose (approx. 5%), ash (approx. 8%), and moisture (8-12%).

Determination of moisture content

Gravimetric method, also known as "oven-dry", was used to determine moisture content in accordance with applicable national regulation and AOAC Method 950.46 (15). Approximately, 5 g of sample was placed into small iron beaker with appropriate cap, and mixed with sand. The filled beaker was placed in an oven at the temperature of 103±2°C, and dried to the constant mass (± 0.005 g). Moisture content was expressed as the percentage (% w/w).

Supercritical fluid extractions

A LECO TFE-2000 fat analyzer was used for SFE, using CO₂ with a purity of 99.995%. Temperature, extraction flow rates and pressure were adopted from the existing LECO procedures. Extracting pressure was 62.05 MPa (9000 psi), and CO₂ flow rate was 1.3 l/min. Cell temperature and heated variable restrictor (HVR) temperature was set at 100 °C. The collection vials on the instrument remained near room temperature of 30°C (16). “Leco Dry” infusorial soil (LECO, St. Joseph, MI, USA) was used as absorbent to remove traces of water from samples. Ethanol (p.a. ≥ 97.5%), when used, was added as co-solvent to the mix, prior to transferring sample into the CO₂ flow. Static extraction time (hold time) was set at 0 minute, and dynamic extraction time was set at 45 minutes. Infusorial soil was added in the amount of 2.2g. Considering the mass of the examined sample and estimated fat content based on previous experience, different volumes of co-solvent were added (0 ml, 0.3 ml, 0.6 ml, and 1 ml).

Amount of 1.0 g of homogenized mash feed was weighed into a glass beaker with the accuracy of ±0.001 g. Targeted mass of absorbent was added into the beaker and the sample was vigorously dispersed with a glass rod. After dispersing the sample, co-solvent was added in previously determined volume to the mix, blended, and thus prepared mixture was transferred into a metal extraction thimble (12 cm length and 10 mm diameter). Filled extraction thimbles were closed with approximately 0.5 g of glass wool on the top and with an appropriate cap. Glass scintillation vials (Wheaton, Millville, NJ, USA) were used as vessels for collecting extracted fat. The prepared thimbles and collection vials were placed in the instrument. After finishing the extraction step, the instrument was depressurized, and the collection vials were removed from the instrument. The next step was de-gassing of extracted fat in the collection vials for ten minutes, and achieving a constant weight of the extract. Fat content was expressed as the percent by weight, (%; w/w).

Soxhlet extractions

Soxhlet extractions were performed on a Büchi 810 Soxhlet fat extraction apparatus (Soxtec system HT, 1043 Extraction Unit, Foss Tecator AB, Höganäs, Sweden) in accordance with the manufacturer’s procedure and AOCS Method Ba 3-38 (17). Extractions were performed with petroleum ether (40 - 60°) as a solvent. Sample weight was 3g, and the extraction time was 1.5 h, at 80°C. After removal from the apparatus, the extracted lipids were allowed to dry at room temperature while passing air over the samples for approximately 60 minutes, until they reached a constant weight. Fat content was expressed as the percentage, %; w/w (gravimetric method).

Statistical analysis

STATISTICA software version 10 (Statsoft, Tulsa, OK, USA) was used for analyzing variations (analysis of variance – ANOVA) and for Tukey’s HSD comparison of the means of samples extracted with various volumes of added co-solvent. The program was also used for the optimization of co-solvent amount and determination of equitation’s

parameters. Differences among means with the probability $p \leq 0.05$ were accepted as indicators of statistically significant differences, and the differences among means with $0.05 \leq p \leq 0.10$ were accepted as indicators of differentiating tendencies.

RESULTS AND DISCUSSION

Since water influences on the supercritical extraction process (9), moisture contents of all samples were determined before the extraction of lipids from the samples. The values of moisture content were found to be in the range from 10.34% to 11.13%.

To investigate the effect of addition of co-solvent, each of the ten samples was extracted in a series. The procedures recommended by manufacturer were used as starting points and initial measurements were performed without co-solvent addition (16). Furthermore, different volumes of co-solvent were added (0.3, 0.6, and 1 ml). The obtained contents of fat extracted from the investigated mash pig feeds are shown in Table 1.

Table 1. Total fat yield (% w/w) of mash pig feed determined by SFE

Sample	Amount of added ethanol as a co-solvent(ml)			
	0	0.3	0.6	1
1	3.81 ± 1.01 ^a	3.93 ± 0.60 ^{ab}	4.4 ± 0.02 ^b	4.02 ± 0.61 ^{ab}
2	3.15 ± 0.50 ^a	3.66 ± 0.50 ^a	4.12 ± 0.91 ^a	3.96 ± 0.48 ^a
3	3.97 ± 0.01 ^a	3.85 ± 0.51 ^a	4.12 ± 0.11 ^a	3.94 ± 0.42 ^a
4	1.91 ± 0.13 ^a	1.98 ± 0.23 ^a	2.03 ± 0.18 ^a	2.00 ± 0.18 ^a
5	1.74 ± 0.06 ^a	1.98 ± 0.04 ^b	1.91 ± 0.09 ^{ab}	1.96 ± 0.07 ^{ab}
6	2.04 ± 0.5 ^a	2.06 ± 0.32 ^a	2.32 ± 0.21 ^a	2.21 ± 0.27 ^a
7	3.45 ± 0.29 ^a	3.92 ± 0.59 ^{ab}	4.36 ± 0.06 ^b	4.05 ± 0.38 ^{ab}
8	3.79 ± 0.09 ^a	3.91 ± 0.41 ^a	4.21 ± 0.13 ^a	4.01 ± 0.03 ^a
9	3.32 ± 0.13 ^a	3.85 ± 0.16 ^{ab}	4.02 ± 0.18 ^b	3.74 ± 0.24 ^{ab}
10	3.46 ± 0.08 ^a	3.62 ± 0.07 ^a	3.81 ± 0.2 ^a	3.74 ± 0.11 ^a
Mean value	3.06	3.28	3.53	3.36

The values are represented as mean ± SD, n = 3

a, b, c, different superscripts within the same row indicate significant differences ($p \leq 0.05$)

The increase in the yield of extracted fat with increasing volume of the co-solvent added is expected and it is in accordance with the literature data. The use of a co-solvent, especially ethanol, has been studied extensively due to its impact on the solubility of lipids. Solubility enhancement is a result of an increase in the density of the supercritical CO₂ + co-solvent mixture, or intermolecular interactions between the co-solvent and a particular solute (9). A great increase of phospholipids solubility in supercritical CO₂ by adding ethanol was reported in many recently published papers. It was noticed that the increased solubility of phospholipids-rich fraction was directly proportional to the amount of added ethanol (1, 13). Several sources reported the solubility enhancement for

some fatty acids using ethanol as a co-solvent mainly due to hydrogen bonding interactions (1, 10).

Fat content was also determined in all ten samples by Soxhlet extraction method in duplicate. As it is shown in Figure 1, the obtained results of both extraction methods were plotted one against the other, and the linear function $Y = X$ was obtained. Soxhlet extraction gave slightly higher values for the extraction yield of fat than SFE (all marks are positioned on the right hand side of the $Y = X$ function in Figure 1). Statistically significant difference ($p \leq 0.05$) in total fat content has been determined by Soxhlet extraction and in the SFE it was noticed only in experiments without ethanol.

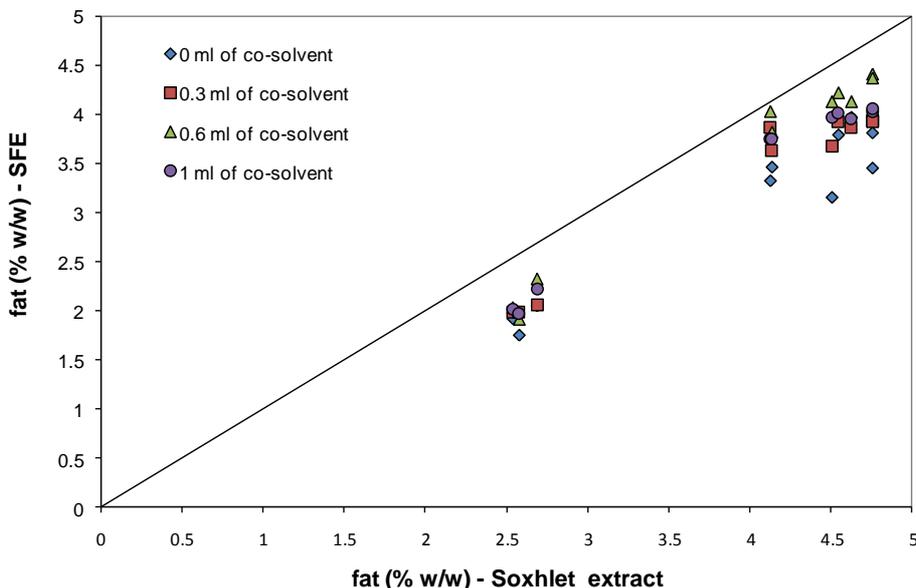


Figure 1. Comparison of Soxhlet extraction and SFE employing various volumes of added ethanol

The experimental data were used to determine a mathematical equation which would describe the dependence of the extracted fat yield on the volume of added ethanol. The mean values of data of ten analyzed samples groups were used for the equation fitting. The effect of co-solvent amount on the fat extraction was well described by a second order polynomial equation [1]:

$$Y = A + BX + CX^2 \quad [1]$$

where Y is the yield of extracted total fat, and the independent variable X is the volume of added co-solvent (ml). The high determination coefficient ($R^2 = 0.93$) confirmed this conclusion (Table 2). Values of the polynomial equation parameters are given in Table 2.

Table 2. Regression equation coefficients for total fat yield (% w/w) obtained by SFE

Coefficient	Type	Value
A	<i>const.</i>	3.038
B	<i>linear</i>	1.314
C	<i>quadratic</i>	-0.976
R^2		0.93

Figure 2 shows the influence of the added co-solvent on the amount of extracted total fat from investigated samples. The maximum yield of extracted fat calculated from the experimental results was found by using partial derivatives, and it has been achieved at a volume of co-solvent of 0.67 ml. By including this value of co-solvent in the estimated equation, mean value of extracted fat content for ten examined samples has been found at a level of 3.48 %. The same values can be read from the graph (Figure 2).

Johnson et al. found that SFE with ethanol as a co-solvent appears to be a good alternative to the Soxhlet method with petroleum ether for extraction of total fat (3). Shih et al. reported that there were no significant differences in fat contents determined by the Soxhlet method and SFE for the extraction of fat from encapsulated oil products (18). In comparison with Soxhlet extraction, the SFE was slightly more effective in the case of their experiment. Other sources also indicated that the amount and composition of total fat in salmon meat by SFE agreed well with the results from the Soxhlet procedure. Quantitative recoveries in the SFE were obtained by adding of 1ml ethanol to 1 g sample in the extraction cell before the extraction (19).

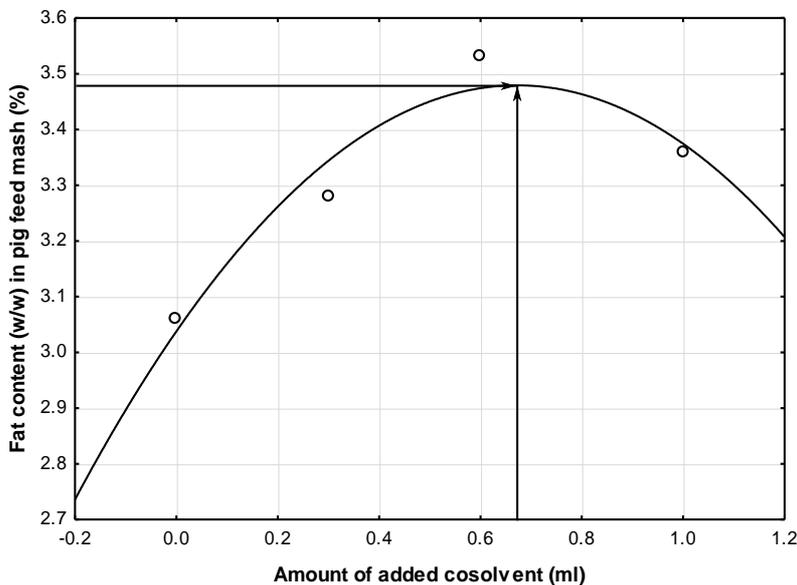


Figure 2. Influence of the amount of co-solvent on the yield of extracted fat from pig feed mash

CONCLUSION

According to the results shown in this paper, the process of SFE can easily be optimized for food or feed materials by regression analysis. Since the co-solvent influences the yield of extracted fat, its appropriate amount has to be determined before the analysis of any specific material.

Comparison between the SFE and Soxhlet extraction showed insignificant differences in the yield of extracted fat ($p > 0.05$), except for the case when no co-solvent was used in the SFE. Therefore, SFE is recommended for the determination of total fat content instead of Soxhlet method, as equally precise, but less toxic and hazardous. Based on experimental results, the effect of the co-solvent amount on the fat content was described by a fitted polynomial equation. The maximum yield of extracted fat (mean value of ten examined samples = 3.48 %) has been achieved at a volume of the co-solvent of 0.67 ml.

It can be concluded that SFE has a considerable potential as an environmentally friendly and reliable technique for total fat determination, which can replace conventional extraction methods.

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ОПТИМИЗАЦИЈА ДОДАТКА КО-РАСТВОРАЧА У СУПЕРКРИТИЧНОЈ ЕКСТРАКЦИЈИ МАСТИ ПОМОЋУ УГЉЕНДИОКСИДА

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Суперкритична екстракција (СЕ) помоћу CO₂ добро је позната техника која се користи за екстракцију укупних масти из узорака хране за људе и животиње. Најзначајније предности ове технике над осталима су смањен ризик од озледа приликом манипулације, смањена штетност по здравље људи и околину и краће време екстракције. Широку употребу технике ограничавају потешкоће које се јављају

приликом екстракције поларних липида, услед неполарног карактера растварача (CO_2). У овом раду испитана је употреба етанола као ко-растварача ради побољшања екстракције поларних липида у процесу СЕ масти из узорка потпуне смеше за исхрану свиња. Укупан саджај масти одређен је у десет комерцијалних узорака потпуне смеше за тов свиња различитих произвођача из Србије, доступних на тржишту, применом две методе: СЕ и екстракције по Soxhlet-у, након чега су добијени резултати упоређени. Статистички значајна разлика у приносима екстраката ($p \leq 0,05$) примећена је једино између резултата екстракције по Soxhlet-у и СЕ када ко-растварач (етанол) није додат у узорак. Максималан принос екстракције, израчунат на основу добијеног математичког модела, остварује се при додатку етанола од 0,67 мл на 1г узорка, док су остали параметри подешени како је препоручено произвођачком процедуром. На основу добијених резултата закључује се да се СЕ може поуздано употребљавати као алтернатива традиционалним методама екстракције, уз претходну оптимизацију процеса за испитивани аналит.

Кључне речи: суперкритична екстракција, ко-растварач, екстракција по Soxhlet-у, потпуна смеша

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