

Effect of Extrusion Cooking on Extractable Lipids and Fatty Acid Composition in Sifted Oat Flour

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ABSTRACT

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Sifted oat flour was processed at 25.0, 27.5, and 30.0% moisture content in a twin-screw extruder at screw speed 300 rpm. The preset temperatures of the extruder barrel were 120, 150, or 180°C. Raw material and extrudates were analyzed for the content of diethyl ether-extractable lipids, with and without hydrolysis, and the content of chloroform-

methanol-water saturated butanol (C/M/WSB) extractable lipids. The lipid extracts were analyzed for fatty acid (FA) composition. Percentage distribution of palmitic, oleic, and linoleic acids were significantly different in the different lipid extracts. Extrusion processing influenced the amounts of extractable lipids, while FA composition was not affected.

Interest in the health benefits of oats (Anderson and Bridges 1993) has stimulated much research in recent years. However, the effects of processing conditions on oat products have not been as extensively studied. Quantity and composition of protein, starch, dietary fiber, and lipids will affect processing conditions and contribute to the properties of the end product.

Most of the oat lipids are located in the bran and the starchy endosperm (Youngs et al 1977). These kernel fractions comprise the greatest part of the total kernel weight (Youngs 1972). An important difference between oats and other small cereal grains is the high lipid concentration in the oat groat. About 80% of the groat lipids are in the endosperm, while for other cereals the percentage in this fraction is usually ~50% (Morrison 1978). In an ideal separation of oat bran from the endosperm, the layer of aleurone cells, which is very rich in lipid, is removed with the bran (Youngs 1986). The lipid content of oats varies considerably; Brown and Craddock (1972) reported a range of 3.1–11.6% free lipids in oat groat from more than 4,000 entries in the world collection.

Youngs and Forsberg (1987) stated that the scutellum and embryonic axis contain a much higher concentration of lipids extractable by polar solvent systems, such as butanol, ethanol, methanol, or chloroform (polar lipids, PL). Usually, 80–90% of the oat lipids can be extracted by diethyl ether, petroleum ether, or hexane (free lipids, FL) (Youngs et al 1977). Starch lipid extraction requires a long time and use of polar solvents at ambient or elevated temperature. Nonstarch lipids can be quantitatively extracted using polar solvents at ambient temperature for just 10 min in an ethanol-ether-water (EEW) system, a water-saturated *n*-butanol (WSB) system, or benzene-ethanol-water (BEW) system. Once the nonstarch lipids are removed, the starch lipids can be extracted in ~3 hr with WSB at 90–100°C. The nonstarch and starch lipids each have a characteristic composition (Morrison et al 1975).

Results from different studies have shown considerable variation in fatty acid (FA) composition of whole oat or groat lipids in different cultivars. In previous studies, the FA distribution was: 14–26% palmitic acid (16:0), 1–4% stearic acid (18:0), 25–48% oleic acid (18:1), 24–48% linoleic acid (18:2), 1–5% linolenic acid (18:3), and only trace amounts of myristic acid (14:0), arachidic acid (20:0), and behenic acid (22:0) (Frey and Hammond 1975, Welch 1975, Youngs and Püskülcü 1976, de la Roche et al

1977). FA composition of various lipid classes might be altered during processing due to lipolytic activity or to interactions between lipids and other constituents.

Decrease in hexane or ether extractable lipids after extrusion cooking and baking have been reported in several studies (Schweizer et al 1986, Addo and Pomeranz 1991, Guzman et al 1992). Decrease in lipid recovery might be expected to depend for example, on complex formation between lipid and starch during processing, where lipids might be entrapped inside the amylose helix. Schweizer et al (1986) found that additional acid hydrolysis was required to recover the lipids of extruded wheat flour. Colonna and Mercier (1983) found almost equal recovery of lipids in extruded manioc starch and the raw material when acid hydrolysis was included before extraction with hexane and boiling methanol-water. The quality of the lipid influenced the amount of complexed starch as addition of linoleic acid resulted in more complexed starch than addition of soy oil. Thermally distinct forms of complex, depending on crystallization temperature, were identified by Biliaderis and Galloway (1989).

The aim of this study was to investigate effects of different temperatures and moisture levels during extrusion processing on extractability of lipids and changes in FA composition. Such data could be useful to optimize processing conditions, to predict the shelf life of a product, and to evaluate the need for addition of antioxidants or components to bind lipids and prevent oxidation of unsaturated FA.

MATERIALS AND METHODS

Flour Samples

Commercially obtained sifted oat flour (80% extraction) from Regal Mølle, Moss, Norway, was used. The oat flour was stored at 4°C and 50% rh until extrusion processing.

Sample Preparations

Samples from the extruded material were ground on a laboratory mill (Ultra-Centrifugal Mill, Type ZM-1, Kurt Retsch GmbH & Co, Haan, Germany) to a particle size <0.5 mm before chemical analyses. The oat flours were analyzed without further grinding. All analyses were performed in duplicate.

Analyses of Flour Protein, Total Dietary Fiber, Starch and Ash

Content of protein, total dietary fiber (TDF), starch, and ash of the flours and extrudates were analyzed in duplicate. Protein content was determined according to AACC Method 56-10, N × 6.25 (AACC 1995). Total dietary fiber (TDF) was determined according to AACC Method 32-05; starch content was determined according to the method described by Holm et al (1986); and ash content was determined according to AACC Method 08-01.

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Lipid Analysis of the Flour and Extrudates

Diethyl ether extractable lipids. Oat flour (5 g) was extracted with diethyl ether by Soxtec extraction (Soxtec System HT 1043/1046, Tecator AB, Höganäs, Sweden), using 40 mL of diethyl ether, boiling for 15 min, and rinsing for 60 min (Tecator AN 301, ASN 3414).

Lipids extracted by diethyl ether after hydrolysis. Total lipid content of the flours was determined by an additional hydrolyzing step before diethyl ether extraction. Acid hydrolysis was performed by heating the samples with 50 mL of 3M HCl for 1 hr before filtration. The residue was rinsed with distilled water, dried and extracted as described above.

Chloroform-methanol-WSB extractable lipids at 20°C. Samples of ~0.3 g of flour or extruded materials were mixed with 6 mL of chloroform, methanol, and WSB (C/M/WSB at 3:2:1) and 100 µL C-19 trinonadecanoin (10 mg/mL of chloroform) as internal standard for subsequent FA analysis. The mixture was mechanically rotated for 3 hr at room temperature. Solvent (5 mL) was transferred to another tube and dried by nitrogen flushing at 40°C.

WSB extractable lipids (100°C). Flour (~3 g) or extruded materials were mixed with 5 mL of WSB and 100 µL of C-19 trinonadecanoin (10 mg/mL of chloroform) added as an internal standard. Samples were held in boiling water for 2 hr and centrifuged at 2,500 rpm for 10 min. After centrifugation, the supernatant was transferred to fresh tubes, and the extraction was repeated twice, but the last boiling step was 1 hr. The extracts were pooled, cooled, and dried at 80°C by flushing with nitrogen.

FA Composition by Gas Chromatography Analysis

C/M/WSB and WSB-dried extracts were dissolved in 1 mL of benzene; 1 mL of methanolic 3N HCl and 200 µL of 2,2-dimethoxypropane were added, and the mixture was left at ambient temperature for at least 6 hr (usually overnight). Hexane (4 mL) and 1 mL of 5% NaCl were then added, and the samples were shaken and centrifuged at 2,500 rpm for 10 min. The organic phase was transferred to a fresh tube and 1 mL of 2% NaHCO₃ was added; the mixture was shaken and centrifuged at 2,500 rpm for 10 min. The organic phase was transferred to a fresh tube, Na₂SO₄ was added, and the sample was transferred to a vial and sealed before gas chromatography (GC) analysis.

Lipids (~25 mg) extracted by diethyl ether and diethyl ether after hydrolysis were dissolved in methanol with 2% H₂SO₄, and heated for 2 hr at 80°C for methylation. After cooling, water and 4 mL of hexane were added to give a final volume 100 mL. The organic phase was then transferred to vials and sealed before GC-

TABLE I
Screw Configuration^a Used for Extrusion of Oat Flour

Section Length (from Die to Hopper)	Type of Element
170 mm	Transition elements
120 mm	Six reversed and five forward elements
550 mm	Transition elements
150 mm	Feed section

^a Screw diameter, 37 mm. Screw element dimensions (for all elements, except feed section): flight height, 7.5 mm; helix angle, 30°; pitch, 40 mm; and axial flight tip thickness, 2 mm.

TABLE II
Content of Starch, Lipids, Fiber, Protein, and Ash in the Oat Flour Used in the Extrusion Experiment

Component	Percent (as is)
Starch	68.2
Lipid	6.9
Total dietary fiber	4.4
Protein	10.3
Ash	1.2
Moisture	11.0

analysis. The methylated FA were analyzed on a gas chromatograph (GC 8000 series, Carlo Erba, Milan, Italy) equipped with a DB 23-capillary column (30 m × 0.25 mm, i.d.) (J&W Scientific) and flame-ionization detector. The carrier gas was hydrogen, and the flow rate was 2 mL/min with a split vent flow at 100 mL/min. The injector temperature was 250°C and the detector temperature was 300°C. Initial column temperature was maintained for 2 min at 50°C. The temperature was increased by 10°C/min, kept at 140°C for 10 min, at 160°C for 5 min, and at 180°C for 20 min. Identification of individual FAs was based on the elution profiles of standards of C6, C8, C10, C12, C13, C14, C16, C18:0, C18:1, C18:2, C18:3 (Sigma Chemical Co., St. Louis, MO), and C20:0 (Alltech Associates, Inc., Deerfield, IL). Integration was performed by a chromatopac integrator (C-R3A, Shimadzu Corp., Kyoto, Japan).

Statistical Analyses

Data were analyzed by ANOVA using the general linear model (GLM) of SAS Version 6.0 (SAS Institute, Cary, NC 1989). Relationships among variables were also studied by correlation analysis (SAS Version 6.0).

Extrusion Conditions

The extrusion cooking was done on a co-rotating twin screw extruder (Continua 37/27D, Werner & Pfleiderer, Stuttgart, Germany). The power of the motor at maximum speed (400 rpm) is 7.6 kW. A full-factorial design (two factors each at three levels and three replicates) was employed. Screw speed was kept constant (300 rpm) during the experiment. The screw configuration is presented in Table I. Extrusion was performed at preset temperatures of 120, 150, and 180°C. Processing temperature was measured by a thermocouple immediately before the products exited the die (*results not shown*). Moisture contents of the extrusion feeds were 25.0, 27.5, and 30.0%, respectively. Water feed was 5 L/hr, and the different moisture contents were obtained by varying the flour feed rate of the volumetric feeder equipped with twin screws from 22 to 34 kg/hr. The selected moisture levels and temperatures were chosen from preliminary experiments. The feed

TABLE III
Extractable Lipids in Raw Materials and Extrudates^a

Processing Temperature, °C	C/M/WSB ^b at 20°C (%)	Diethyl Ether (%)	Diethyl Ether After Hydrolysis (%)	Free Lipids ^c (%)
Unprocessed	6.1b ^d	6.8b	7.7b	88.8b
120	3.7b	3.3d	7.7b	42.4d
150	2.8b	2.2c	7.7b	28.5c
180	2.6b	2.3c	7.7b	29.9c

^a Means from three replicates.

^b C/M/WSB = chloroform, methanol and water saturated butanol.

^c Free lipids calculated as proportion of lipids extracted by diethyl ether with and without acid hydrolysis.

^d Numbers in same column with different letters are significantly different.

TABLE IV
Fatty Acid Distribution of Lipids in Raw Materials and Extrudates Extracted by Diethyl Ether, Diethyl Ether after Hydrolysis, C/M/WSB^a Extraction at 20°C, and WSB Extraction at 100°C

Extraction Method	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0
Diethyl ether without hydrolysis	18.9a ^b	1.7a	41.2a	36.9a	1.3a	0.8a
Diethyl ether with hydrolysis	24.0c	2.2b	40.0b	31.4b	0.9a	0.8b
C/M/WSB	19.4a	1.9a	38.1c	39.3c	1.7c	0.8a
WSB	21.3b	1.6a	36.5d	38.2a	1.4b	0.7a

^a C/M/WSB = chloroform, methanol and water saturated butanol.

^b Numbers in same column with different letters are significantly different.

section was water-cooled and the temperature at the other zones was kept constant at either 120, 150, or 180°C.

The shape of the die opening was a slot 7.9 mm wide and 1.1 mm high. The speed of the rotating knives was adjusted to give squares of the extrudate. The extrudates were dried to ~90% dry matter for 24 hr at ambient temperature. The results from the analyses of the protein, total lipids, dietary fiber, starch, and ash content of the oat flour are shown in Table II.

RESULTS AND DISCUSSION

Influence of Processing Conditions on Lipid Extraction

Results from lipid extractions are presented in Table III. The amount of diethyl ether extractable lipids was significantly lower in extruded samples than in raw material. With increasing extrusion temperature, decreasing amounts of diethyl ether extractable lipids were obtained. However, no significant difference in diethyl ether extractable lipids was found between samples at 150 and 180°C. Equal amounts of lipids could be extracted from raw material and extrudates by diethyl ether extraction after hydrolysis. The polar solvent system (C/M/WSB at 20°C) extracted less lipids than the amounts obtained from diethyl ether extraction after hydrolysis. This indicated that some lipids bound to other components, for example, lipids complexed with amylose were not extracted by the polar solvent system at ambient temperature. Increasing extrusion temperature tended to decrease the amount of C/M/WSB-extractable lipids, but the effect was not statistically significant (Table III). Colonna and Mercier (1983) found that boiling water-methanol mixture extracted amounts of lipids close to those obtained by acid hydrolysis followed by hexane extraction from extruded manioc starch. Morrison and Coventry (1989) found that lipid was extracted at 100°C, and that even polar solvents containing alcohol-water mixtures such as WSB extract mainly nonstarch lipids at room temperature.

In an earlier study, Morrison and Coventry (1985) had reported that two 2-hr and one 1-hr extraction using propanol-water mixture at 100°C gave yields of lipids equal to or slightly higher than that by acid hydrolysis. Our results suggested that extrusion processing resulted in binding of lipids, as indicated by a decrease in diethyl ether extractable lipids after processing. This is consistent with results obtained by Nierle et al (1980), who also found variation in extractability of the lipids from extruded wheat and corn, using petroleum ether, C-M and butanol-water mixtures.

There was also a lower proportion of lipids extractable by diethyl ether in extruded corn than in extruded wheat. In the present study, the nonpolar fraction of the lipids extracted by diethyl ether after hydrolysis seemed to increase in the extruded oat flour when compared to the raw material (*results not shown*), indicating binding of nonpolar lipids increased during processing.

The amount of diethyl ether extractable lipids was not influenced by moisture content at the medium and the highest temperature levels, but at the lowest extrusion temperature, the lowest moisture level resulted in more diethyl ether extractable lipids (54.5%) than higher moisture levels at that temperature (29.9%). (However, the effect of moisture level was not statistically significant). Binding of lipids in complexes is a likely explanation for reduced extractability. According to Meuser et al (1987), complex formation of lipid and wheat starch starts above a threshold specific mechanical energy (SME) value and processing temperature. In this study, SME was significantly affected by the extrusion temperature and varied from 263.5 kJ/kg to 364.8 kJ/kg, with an average of 350 kJ/kg at 120°C, 337 kJ/kg at 150°C, and 272 at 180°C (*results not shown*). According to the findings reported by Meuser et al (1987), the level of starch-lipid complexing is expected to be relatively high within the range of SME.

In this study, low temperature and high throughput might have restricted the complex formation in the material and complex form-

ation thus seemed more sensitive to moisture content at low temperatures. Diethyl ether extractable lipids in the raw material constituted up to 88.8% of lipids extracted by diethyl ether after hydrolysis, which is in good agreement with results of Youngs et al (1977), who reported 80–90% of diethyl ether extractable lipids (FL) in oats. Guzman et al (1992) studied binding of lipids before and after extrusion of cornmeal at three different temperatures. They reported free lipids (FL) before extrusion to be 81.34%, while after extrusion, at temperatures of 50–60°C and 120–125°C, the proportion of FL was 23.41–29.65%. These results correspond to that obtained by extrusion of oat flour at 150 and 180°C in our study.

FA Distribution

Only trace amounts of the FA shorter than C16:0 and longer than C20:0 were detected by GC analysis. Since the majority of the FA in oats are C16:0, C18:1, and C18:2, only FA of chain lengths between C16:0 and C20:0 will be discussed.

The percentage distribution of C16:0, C18:0, C18:1, C18:2, C18:3, and C20:0 are presented in Table IV. Since no significant effect of extrusion temperature or moisture level were found, mean values are presented. FA composition varied with extraction methods used. These results correspond well with results reported by Guzman et al (1992). They found that FA distribution of the lipid fraction extracted by petroleum ether at room temperature in corn meal samples were similar before and after extrusion. Results from ANOVA showed that the percentage of C16:0 in ether-extractable lipids was not significantly different from the amount found in extracts from C/M/WSB. Otherwise, the distribution of C16:0, C18:1, and C18:2 in the lipids from the different extraction methods were significantly different (Table IV). Amount of FA from lipids extracted by C/M/WSB at 20°C were higher in C18:2 and lower in C18:1 content than diethyl ether-extractable lipids. FA from WSB extracts were higher in C16:0 and lower in C18:1 and C18:2 than C/M/WSB extracts. Diethyl ether extractable lipids were lower in C16:0 and higher in C18:1 and C18:2 than lipids extracted by diethyl ether after hydrolysis.

CONCLUSIONS

Different temperatures during processing significantly influenced the recovery of diethyl ether-extractable lipids in the extrudates. At 150 and 180°C, the selected moisture levels did not influence the amount of diethyl ether-extractable lipids, while at 120°C, the amount was highest at the lowest moisture level. Using the polar solvent system C/M/WSB at 20°C, lipid yield was almost equal to yield obtained by ether extraction. With the additional step of acid hydrolysis before diethyl ether extraction, equal amounts of lipids in processed and unprocessed oat flour were obtained. FA distribution varied with lipid extraction method. The proportion of C16:0 was higher in the total lipid extract than in the other lipid fractions. However, the FA distribution in the different lipid extracts were similar for raw material and processed oat flour.

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