Improvement of Lipid Stability in Oat Products by Alkaline Wet-Processing Conditions

Kirsi Liukkonen,* Anu Kaukovirta-Norja, and Simo Laakso

Laboratory of Biochemistry and Microbiology, Department of Chemical Engineering, Helsinki University of Technology, SF-02150 Espoo, Finland

Oats were wet-fractionated into fiber, starch, and protein fractions under different pH conditions, and lipid contents and compositions were determined in each fraction. In a process utilizing water without a pH adjustment, the accumulation of free fatty acids (FFA) was a characteristic feature so that they constituted up to 6%, 18%, and 27% of total lipids in fiber, starch, and protein fractions, respectively. In contrast, in an alkaline process initially adjusted to pH 9 or above, the FFA levels were close to that found in dry oat meal. When the fractions from the alkaline process were dried and resoaked in nonadjusted water, no lipid hydrolysis was found, while in fractions from the corresponding process in nonadjusted water FFA contents rose to 14%, 44%, and 77% in the fiber, starch, and protein fractions, respectively. It is therefore concluded that lipid deterioration associated with oat processing and storage of the products so obtained can be favorably affected by slightly alkaline fractionation conditions.

INTRODUCTION

Among the cereals, oats are recognized for their good nutritional as well as functional properties (Kahlon, 1989; Kühnau, 1985; Paton, 1986; Schrickel, 1986; Wood et al., 1989). However, oat lipids are very liable to undergo deteriorative changes during processing (Youngs, 1986), which complicates the utilization of, e.g., oat fiber, protein, starch, and other valuable products for human and animal nutrition. In intact grains oat lipids show little change (Welch, 1977), but when grains are ground and soaked in water, the release of free fatty acids (FFA) from triglycerides (TG) is a rapid response as recently reported (Liukkonen et al., 1992). Excessive FFA adversely affect the flavor and keeping qualities of final oat products (Kent, 1983). So, new techniques which are applicable to industrial processes to minimize the possibility of rancidity development during wet fractionation and subsequent storage and further processing might be welcome.

In several known wet fractionation processes for cereals, pH adjustments are used to solubilize, or precipitate, cereal components or to help soften the grains (Burrows et al., 1984; Cluskey et al., 1973; Hohner and Hyldon, 1977; Phillips and Sallans, 1966; Wood et al., 1978). However, processes including pH adjustments with the purpose of affecting the quality of cereal lipids have not been published. Our previous study (K. Liukkonen, A. Kaukovirta-Norja, and S. Laakso, 1992, unpublished results) revealed that oats' tendency to lipid hydrolysis in aqueous milieu can be very efficiently affected by pH adjustments. In this work we have studied the applicability of pH adjustments to eliminate the development of hydrolytic rancidity during oat wet fractionation into good quality fiber, starch, and protein products.

MATERIALS AND METHODS

Materials. Oat grains were the Finnish cultivar Veli harvested in 1989. Dipentadecanoylphosphatidylcholine, heptadecanoic acid, dipentadecanoin, triheptadecanoin, and heptadecanoic acid methyl ester were used as standards in the TLC and GLC analyses and were purchased from Sigma. Silica gel plates 60 were purchased from Merck. HPLC grade solvents were used for all fatty acid analyses. All other chemicals were of reagent grade or better.

Oat Fractionation. Sixty-five grams of ground, dehulled oats was slurried in water, 50 mM sodium acetate buffer (pH 5), 50 mM sodium borate buffer (pH 9), 50 mM sodium hydroxide, or 50 mM ammonia to form an approximately 24% slurry (dry substance content). The slurry was mixed with a pestle for 15 min at 15 °C and homogenized with an Ultra Turrax for 1 min. The fiber fraction was separated from protein–starch mixture in a continuous saftcentrifuge (AEG, Model ESF 102, Germany) and washed with the same fractionation medium. The protein–starch mixture was screened (mesh size 88 μm) to remove residual fiber and separated by centrifuging at 16000*g* for 20 min. Portions of the fractions were directly freeze-dried, stored in vacuum at room temperature, and used for lipid extraction and water soaking. The remainder of the fractions from the alkaline process was washed with water to pH 7 before the freeze-drying step.

Water Soaking. A 1–2-g sample representing dry fiber, protein, or starch was soaked for 15 h in 5–10 mL of distilled water in a 50-mL shaking flask (200 rpm, 20 °C). The suspension was used as such for lipid extraction.

Lipid Extraction. The water-soaked suspensions were extracted by shaking (250 rpm, 28 °C) for 8 h in 19 volumes of chloroform–methanol (2:1 v/v) according to the method of Folch et al. (1957). The freeze-dried samples (1–2 g) were suspended in 5–10 mL of water and extracted as above. After 8 h of extraction, the mixtures were centrifuged (1460*g*) for 10 min to remove insoluble material. The extraction was repeated for 2 h with the same amount of chloroform–methanol. The extracts were combined and evaporated to dryness in a rotary evaporator. Lipids were dissolved in 20 mL of chloroform–methanol (100:1 v/v), divided in 1-mL portions in test tubes, evaporated to dryness under N_2, and stored at −20 °C under N_2 before analysis. The samples were used to determine the total fatty acids and major lipid classes.

Separation of Major Lipid Classes by TLC. The samples were redissolved in 0.5 mL of chloroform–methanol (100:1 v/v) and supplemented with 50 μg of PL, FFA, DG, and TG standards (specified under Materials). The mixtures were applied on silica plates, and the plates were developed with petroleum ether–diethyl ether–acetic acid (80:30:1 v/v). Lipid classes were visualized under UV light after the plates were sprayed with 0.01% Rhodamine 6G, scraped off, and used for fatty acid determinations.

Preparation and Analysis of Fatty Acid Methyl Esters. Fatty acid methyl esters were prepared by saponification and methylation essentially as described by Suutari et al. (1990). The methyl esters were analyzed by GLC. The major fatty acids were identified by comparing their retention times with that of a reference mixture (Sigma) containing different fatty acid methyl esters [12:0ω, 14:0ω, 16:0ω, 16:1(*n*−7), 18:0ω, 18:1(*n*−7), 18:1(*n*−9), 18:2(*n*−6,9), 18:3(*n*−6,9,12), 20:0ω, and 22:0ω]. To determine

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Table I. Effect of Fractionation Medium on Yields of Oat Fractions

fractionation medium	yield,[a,b] % fiber[c]	starch[c]	protein[c]	total
water	16.7	46.1	25.5	88.3
50 mM sodium acetate buffer, pH 5	20.5	49.6	16.2	86.3
50 mM sodium borate buffer, pH 9	27.3	48.1	6.4	81.8
50 mM sodium hydroxide	20.3	43.8	5.6	69.7
50 mM ammonia	19.7	45.5	8.6	73.8

[a] From untreated flour. [b] Average of two experiments with SD less than 15%. [c] The terms refer to fiber, starch, and protein fractions.

the extractable total fatty acids, 30 μg of heptadecanoic acid methyl ester standard was added prior to saponification.

Determination of Lipid Classes. To determine the total amounts of different lipid classes, a mixture of PL, FFA, DG, and TG standards with uncommon fatty acids was added prior to lipid class separation by TLC as described above. The identified spots were scraped off and saponified, and the fatty acid methyl esters were prepared as described. Individual lipid classes were quantitated according to the peak area of the standard fatty acid.

Gas Chromatography. A Hewlett-Packard Model 5890A gas chromatograph equipped with a flame ionization detector, a capillary inlet system, a HP-FFAP (25 m × 0.2 mm × 0.3 μm) column, and a Model 7673A high-speed automatic liquid sampler with a 10-μL syringe was employed. The column temperature was programmed from 70 to 200 °C at the rate of 25 °C/min. The injector and detector were maintained at 250 °C. The column inlet pressure was 150 kPa. The flow rate for the makeup gas was 30 mL/min He, and for the detector gases the rates were 40 mL/min H_2 and 400 mL/min air. The column flow rate was 1.0 mL/min and the septum purge flow rate 1–2 mL/min. Split injection at a split ratio 1:20 was employed. Peak areas were measured by using a Hewlett-Packard Model 3365A integrator.

Calculations. The relative amounts of fatty acids in total lipids and in different lipid classes were determined as a percentage of total peak area. The absolute amounts of the fatty acids in total lipids were calculated per 1 g of sample dry weight by comparison of the peak areas to that of the methyl ester standard without using any conversion factors. The amounts of major lipid classes per 1 g of sample dry weight were determined by comparing the area of the fatty acids from a lipid class to that of the corresponding standard. The percentage distribution of different lipid classes was determined as a percentage of total amounts of lipid classes. As an approximation, it was assumed that the polar lipids consist of phosphatidylcholine only. The errors in the experiments (SD, $n = 2$) were <15% for yields of oat fractions, <10% for total fatty acid contents and lipid class compositions, and <5% for fatty acid compositions.

RESULTS AND DISCUSSION

Effect of Fractionation pH on Yields of Oat Fractions. The fractionation method used in this study was varied only with respect to the initial pH of the process water. The methods are correspondingly termed as acidic, water, or alkaline processes. The yields of fiber, starch, and protein fractions from the various fractionation methods are presented in Table I. The yields of total dry matter varied between the processes, mainly reflecting increased protein solubility in the alkaline process waters. Otherwise, in light of the reproducibility of the fractionation method (Table I), the distribution of material among the fractions was not significantly affected by the pH of the process water.

Effect of pH on Total Fatty Acids. In all of the processes lipids were carried into each fraction irrespective of the pH used (Table II). Alkaline processes increased total fatty acid contents in fiber, especially in processes based on dilute sodium hydroxide or ammonia. Instead, fatty acid contents of protein fractions obtained by the alkaline processes were reduced remarkably when compared to the respective fractions in the acidic and water processes. Starch fractions from the alkaline processes, especially from those based on sodium hydroxide, had lower fatty acid contents, possibly because saponified lipid was removed with the soluble protein (Paton, 1986). Additional water washings of the fractions from the alkaline processes to about pH 7 further removed lipids. Lipid stability in fiber, starch, and protein fractions from acidic, water, and alkaline processes was further studied by soaking the freeze-dried fractions in water for 15-h periods and by determining lipid compositions in the slurries. After the water soakings, the total fatty acid contents of any of the fractions varied less than 10% of the mean value obtained before the soaking, independent of the process used for their isolation.

Fatty acid compositions of the fractions (Table III) were also quite similar to those of respective oat grains (Liukkonen et al., 1992), palmitic, oleic, and linoleic acids being the major fatty acids. Furthermore, 15-h soakings had no detectable effect on the fatty acid compositions irrespective of the pH of the process used for their isolation,

Table II. Fatty Acid Contents of Oat Fractions[a] after Fractionation in Different Media

fractionation medium	fatty acid content,[b,c] mg/g fiber	starch	protein	water-washed fiber	water-washed starch	water-washed protein
water	49.9	14.3	137.3	*d*	*d*	*d*
50 mM sodium acetate buffer, pH 5	55.3	24.0	142.9	*d*	*d*	*d*
50 mM sodium borate buffer, pH 9	57.4	11.3	80.9	34.8	5.9	40.0
50 mM sodium hydroxide	68.1	2.0	55.3	53.5	*d*	*d*
50 mM ammonia	69.0	9.4	31.6	64.0	*d*	*d*

[a] Fatty acid content of oat grains was 5.2% (dry weight basis). [b] mg/g, mg of fatty acid/sample dry wt. [c] Average of two experiments with SD less than 10%. [d] Not determined.

Table III. Fatty Acid Compositions[a] (Percent) of Oat Fractions after Fractionation in Different Media

fractionation medium	16:0ω fiber	starch	protein	18:1n–9 fiber	starch	protein	18:2n–6,9 fiber	starch	protein	others[b] fiber	starch	protein
water	16.3	17.6	16.6	39.4	39.3	39.6	38.5	36.2	38.9	5.8	6.9	4.9
50 mM sodium acetate buffer, pH 5	15.8	17.0	16.7	40.7	40.3	40.2	38.1	37.0	38.2	5.4	5.7	4.9
50 mM sodium borate buffer, pH 9	15.2	18.2	16.3	41.0	36.5	38.4	38.1	38.0	39.8	5.7	7.3	5.5
50 mM sodium hydroxide	14.8	21.9	17.8	42.2	33.4	39.7	38.5	38.2	36.8	4.5	6.5	5.7
50 mM ammonia	15.1	17.7	20.7	40.9	37.8	36.0	39.2	37.6	36.9	4.8	6.9	6.4

[a] Average of two experiments with SD less than 5%. [b] Includes 12:0ω, 14:0, 16:1(n–7), 18:0ω, 18:1(n–7), 18:3(n–6,9,12), and unidentified fatty acids.

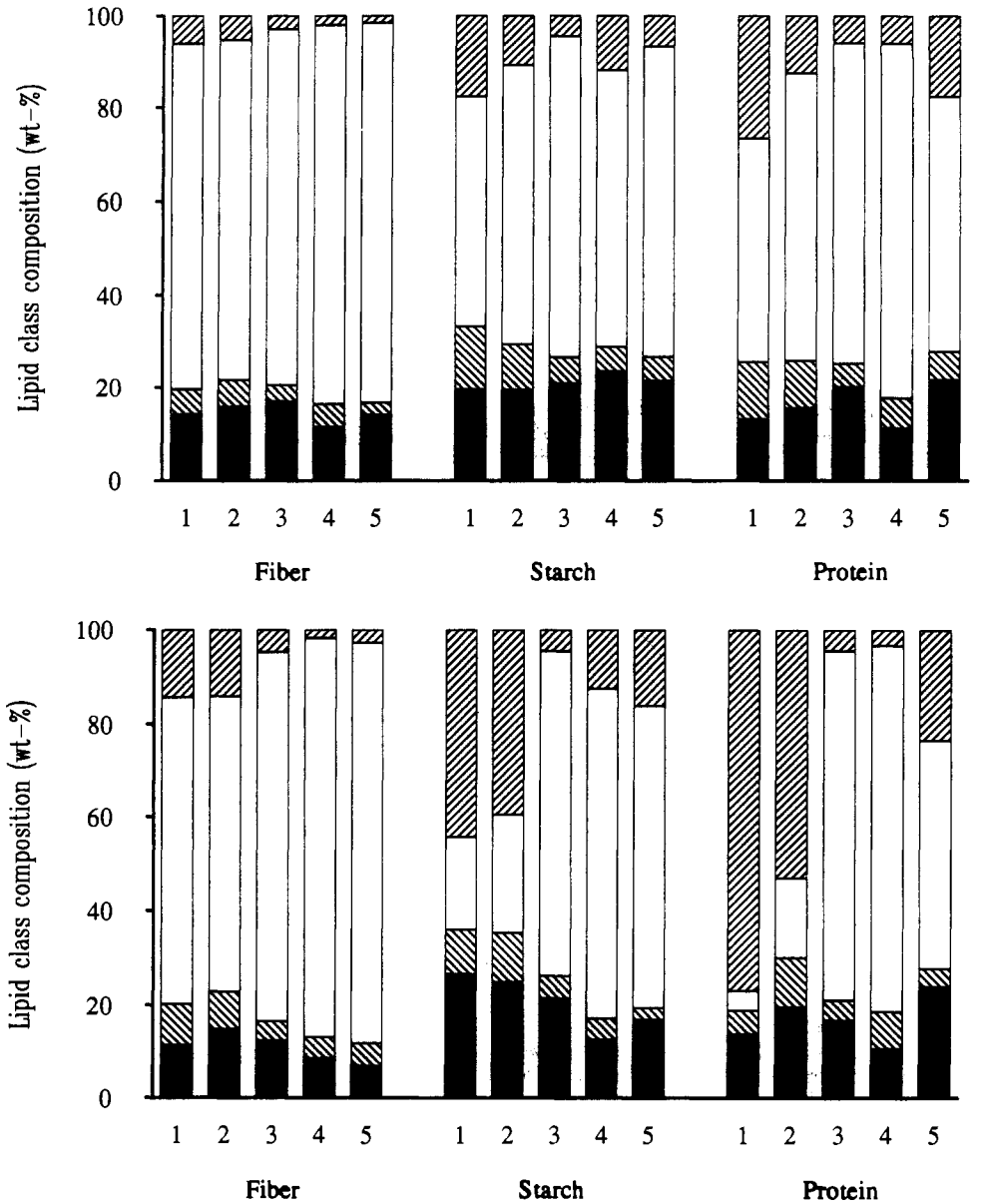

Figure 1. (a, top) Lipid compositions of oat fractions after fractionation in different media. (b, bottom) Effect of subsequent water soakings (15 h) on the compositions. Fractionation media: (1) water; (2) 50 mM sodium acetate buffer, pH 5; (3) 50 mM sodium borate buffer, pH 9; (4) 50 mM sodium hydroxide; (5) 50 mM ammonia. (Left-slashed bar) Free fatty acids; (open bar) triglycerides; (right-slashed bar) diglycerides; (black bar) polar lipids.

indicating that at least significant oxidation of unsaturated acids did not occur.

Effect of pH on Lipid Class Compositions. When oats were wet-fractionated by the water process, FFA contents rose to 6%, 18%, and 27%, in fiber, starch, and protein fractions, respectively (Figure 1a); these levels in starch and protein fractions are significantly higher than those reported for undamaged oat grains (3–10%) (Sahasrabudhe, 1979; Welch, 1977). During the 15-h water soaking of the fractions from the water process, accumulation of FFA proceeded further so that their content rose to 14%, 44%, and 77% in fiber, starch, and protein fractions, respectively. As a result, TG contents were reduced (Figure 1b).

When the fractionation process was initiated at pH 9 or above with the aid of borate, sodium hydroxide, or ammonia, FFA contents of fiber, starch, and protein fractions remained at lower levels than in the respective fractions obtained by the water process (Figure 1a). During subsequent water soaking of the fractions from the alkaline processes, TG hydrolysis was still very efficiently arrested (Figure 1b). The additional water washings of fiber fractions prepared in borate, sodium hydroxide, or ammonia and starch and protein fractions prepared in borate to pH 7 did not result in FFA accumulation (Figure 2a), not even during the subsequent water soaking (Figure 2b). This indicates that alkaline pH is essential in the beginning of the wet process. The hydrolytic tendency during the process (Figure 1a) and subsequent water soaking of the fractions (Figure 1b) was also reduced when the acidic process was used, but the same efficiency of inhibition as in the alkaline processes was not reached.

In present study the consideration of starch lipids applies mainly to those which are located on the surfaces of the starch granules. The internal lipids are poorly extracted from undamaged starch granules in chloroform–methanol at ambient temperature (Morrison, 1981). The composition of internal lipids differs from that of surface lipids (Morrison, 1978), and in native starch granules the latter are not affected under the conditions of any of the processes used (Liukkonen and Laakso, 1992). Instead, hydrolytic lipid changes in starch surface are positively affected by

Table IV. Fatty Acid Compositions[a] (Percent) of Triglycerides in Oat Fractions after Fractionation in Different Media

	16:0ω			18:1n-9			18:2n-6,9			others[b]		
fractionation medium	fiber	starch	protein	fiber	starch	protein	fiber	starch	protein	fiber	starch	protein
water	15.5	16.9	16.3	39.1	40.3	41.0	39.6	37.5	37.7	5.8	5.3	5.0
50 mM sodium acetate buffer, pH 5	14.7	16.2	15.5	40.0	40.3	40.2	39.7	37.9	38.9	5.6	5.6	5.4
50 mM sodium borate buffer, pH 9	14.4	15.2	15.2	39.9	39.0	39.4	41.2	39.7	40.6	4.5	6.1	4.8
50 mM sodium hydroxide	14.9	16.9	15.8	41.6	39.8	40.4	38.9	37.3	39.3	4.6	6.0	4.5
50 mM ammonia	14.9	16.7	16.2	40.3	40.7	39.7	40.5	37.0	38.3	4.3	5.6	5.8

[a] Average of two experiments with SD less than 5%. [b] Includes 12:0ω, 14:0ω, 16:1(n-7), 18:0ω, 18:1(n-7), 18:3(n-6,9,12), and unidentified fatty acids.

Table V. Fatty Acid Compositions[a] (Percent) of Polar Lipids in Oat Fractions after Fractionation in Different Media

	16:0ω			18:1n-9			18:2n-6,9			others[b]		
fractionation medium	fiber	starch	protein	fiber	starch	protein	fiber	starch	protein	fiber	starch	protein
water	22.8	26.0	22.9	21.8	22.2	23.6	48.9	44.8	47.9	6.5	7.0	5.6
50 mM sodium acetate buffer, pH 5	22.2	25.3	22.8	24.4	22.0	22.5	46.9	47.1	48.4	6.5	5.6	6.3
50 mM sodium borate buffer, pH 9	21.8	28.0	22.1	23.5	19.6	21.0	49.5	45.0	51.3	5.2	7.4	5.6
50 mM sodium hydroxide	18.5	32.1	22.2	30.2	19.1	25.0	45.4	41.0	44.9	5.9	7.8	7.9
50 mM ammonia	22.9	29.6	27.6	26.4	17.9	20.3	44.8	44.5	44.7	5.9	8.0	7.4

[a] Average of two experiments with SD less than 5%. [b] Includes 12:0ω, 14:0ω, 16:1(n-7), 18:0ω, 18:1(n-7), 18:3(n-6,9,12), and unidentified fatty acids.

Table VI. Fatty Acid Compositions[a] (Percent) of Free Fatty Acids in Oat Fractions after Fractionation in Different Media

	16:0ω			18:1n-9			18:2n-6,9			others[b]		
fractionation medium	fiber	starch	protein	fiber	starch	protein	fiber	starch	protein	fiber	starch	protein
water	22.0	15.4	14.8	35.4	43.6	41.5	35.7	34.9	38.8	6.9	6.1	4.9
50 mM sodium acetate buffer, pH 5	18.7	17.6	16.9	39.0	44.0	41.6	34.0	32.3	35.8	8.3	6.1	5.7
50 mM sodium borate buffer, pH 9	21.6	17.3	18.6	35.0	38.6	36.4	37.4	39.4	38.7	6.0	4.7	6.3
50 mM sodium hydroxide	19.3	27.7	28.9	33.9	22.8	25.7	39.8	42.5	38.1	7.0	7.0	7.3
50 mM ammonia	33.1	17.5	34.2	32.7	32.7	29.0	27.8	42.3	31.8	6.4	7.5	5.0

[a] Average of two experiments with SD less than 5%. [b] Includes 12:0ω, 14:0ω, 16:1(n-7), 18:0ω, 18:1(n-7), 18:3(n-6,9,12), and unidentified fatty acids.

the alkaline process conditions as seen in this study. In conclusion, the alkaline processes give oat fractions with lipid class composition very similar to that of undamaged oat grains, where the TG component dominates and FFA exist naturally only in small amounts (De La Roche et al., 1977). Furthermore, this lipid class composition is well maintained even though the fractions are thoroughly washed with nonadjusted water and/or further soaked in nonadjusted water for extended periods of time.

Effect of pH on Fatty Acid Composition of Lipid Classes. When fatty acid compositions of TG, PL, and FFA were determined separately in fiber, starch, and protein fractions representing the acidic, water, and alkaline processes (Tables IV–VI) palmitic, oleic, and linoleic acids were always prominent fatty acids, contributing over 90% of all fatty acids as reported earlier (Youngs, 1986). The fractionation process had little effect on the fatty acid composition of TG (Table IV), which remained practically similar to that of native oat grains (Liukkonen et al., 1992) in each of the fractions considered. In contrast, the fatty acid compositions of PL (Table V) varied slightly and that of FFA (Table VI) most notably from one process to another, possibly reflecting some dependence on the fractionation conditions used.

The water soaking (15 h) was not found to affect the fatty acid compositions of fiber TG in spite of the TG hydrolysis occurring in the fiber fractions from the acidic and water processes. During the water soaking of the starch fractions the percentage of linoleic acid in TG decreased slightly and that of palmitic acid increased, respectively. The change was most notable (4.6 percentage points) in starch from the water process, where the hydrolysis was highest, and zero in starch from the alkaline process utilizing borate. During the water soaking of protein fractions representing the alkaline processes, the fatty acid compositions of TG remained unchanged, but in protein fractions representing the water and acidic processes, the percentage of linoleic acid in TG decreased while the percentage of palmitic acid increased, respectively. However, the change in linoleic acid was only 3.6 percentage points at maximum.

In PL the water soaking also decreased the percentage of linoleic acid (less than 10 percentage points) when the fractions were from acidic and water processes. However, the alkaline processes utilizing borate or sodium hydroxide yielded fractions where such changes were not detected. On the basis of the data on FFA (Figures 1 and 2; Tables II and VI) the applicability of the alkaline processes seems evident. The content of FFA remains low, and then changes possibly occurring in FFA composition would be of minor contribution to the quality of total fat.

Conclusions. This study suggests that oats can be wet-fractionated into fiber, starch, and protein fractions without concomitant FFA formation if the process water is adjusted slightly alkaline. The beneficial effect of the slightly alkaline conditions will probably be emphasized in industrial-scale processes where lag times are longer than in the laboratory-scale process described. Further, the fractions from the alkaline processes resist lipid hydrolysis even though they are resoaked in water without any pH adjustments, in contrast to fractions originating from either the water or acidic processes. The same phenomenon is reproduced even though fractions from the alkaline processes are washed with water to pH of about 7 and subsequently soaked in nonadjusted water. So, it seems that pH adjustment is essential in the beginning of the fractionation, possibly by causing irreversible changes in the oat slurries. The fact that only slight pH elevation was needed (pH 8–9) is in poor accordance with the reported pH profile of oat lipase with

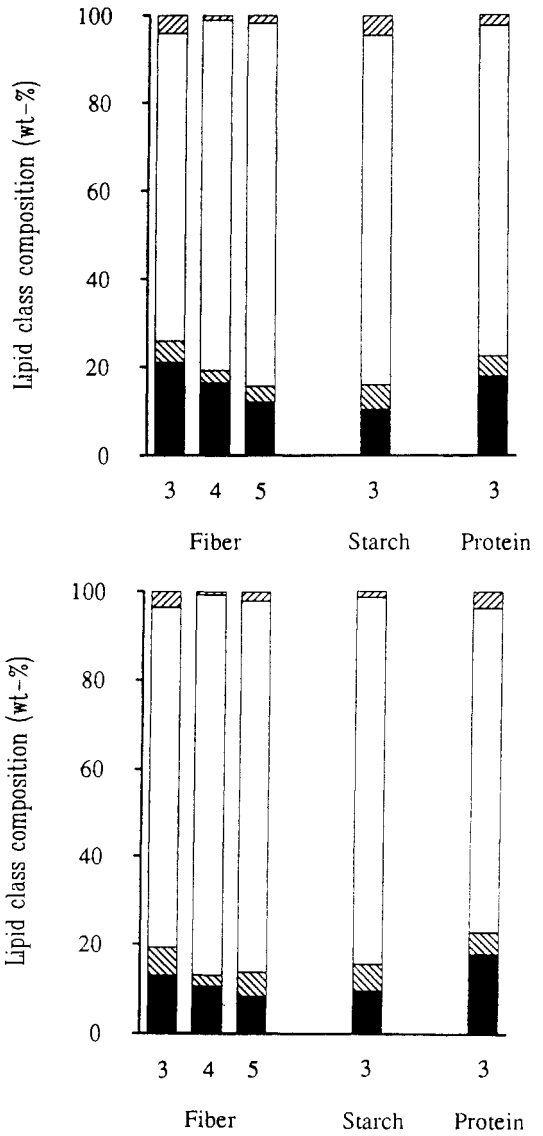

Figure 2. (a, top) Lipid compositions of water-washed oat fractions after fractionation in different media. (b, bottom) Effect of subsequent water soakings (15 h) on the compositions. Fractionation media: (3) 50 mM sodium borate buffer, pH 9; (4) 50 mM sodium hydroxide; (5) 50 mM ammonia. (Left-slashed bar) Free fatty acids; (open bar) triglycerides; (right-slashed bar) diglycerides; (black bar) polar lipids.

a pH optimum at 7.5 (Matlashewski et al., 1982). The alkaline isolation conditions are known to increase protein solubility. Therefore, cleaner starch and fiber fractions and higher protein contents in the waste stream can be expected. The described pH adjustment is easily applicable to existing industrial wet processes, and only low amounts of alkali are needed to obtain the effective pH. The alkaline wet processes probably change the microflora of the wet process and diminish the danger of explosion associated with dry fractionation processes. With the alkaline process preliminary procedures such as heat treatment of groats to destroy lipolytic enzymes become unnecessary.

ABBREVIATIONS USED

GLC, gas–liquid chromatography; TLC, thin-layer chromatography; TG, triglycerides; DG, diglycerides; PL, polar lipids; FFA, free fatty acids. Fatty acids are denoted by the number of carbon atoms followed after a colon by the number of double bonds.

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