Influence of Water Processing on the Composition, Behavior, and Oxidizability of Barley and Malt Lipids

Anu R. Kaukovirta-Norja,*,† Pia K. Kotiranta,† Anne-Mari Aurola,† Pekka O. Reinikainen,‡ Juhani E. Olkku,‡ and Simo V. Laakso†

Laboratory of Biochemistry and Microbiology, Helsinki University of Technology, P.O. Box 6100, FIN-02015 HUT, Finland, and Oy Lahden Polttimo Ab, P.O. Box 22, FIN-15141 Lahti, Finland

The fate of lipids during mashing of malt is not an unambiguously resolved question. To gain insight into the behavior of lipids during mashing, lipid reactions were studied in aqueous slurries of barley, malt, and samples taken during malting and in different mashing processes. An ability for lipid hydrolysis increased as the malting process progressed, being most significant in samples taken during kilning and from malt. However, although lipolysis occurred in the aqueous slurries, a corresponding accumulation of free fatty acids (FFA) could not be detected when assayed by conventional extraction methods. As a consequence, an apparent reduction of total lipids occurred. On the other hand, the use of more destructive methods for analysis revealed that upon hydrolysis the liberated fatty acids enter a nonextractable, protective complex phase. The results of the present study suggest that the FFA liberated during mashing are complexed and carried out of the process with spent grains without extensive oxidation.

Keywords: *Barley; malt; malting; lipid oxidation; lipid protection; mashing*

INTRODUCTION

Oxidation of cereal lipids and activities of oxidizing enzymes have received much attention with reference to the quality of cereal foods and the development of rancidity during food processing. On the other hand, the hydrolysis of cereal lipids has often been neglected. Lipolysis should, however, be considered of prime importance as it is usually the first step in the reaction chain of lipid deterioration and therefore profoundly influences the overall quality of foods. Free fatty acids (FFA) can be directly responsible for altering the properties of food, but more importantly, they are also susceptible to oxidation, complexation, and reacylation, which gives rise to numerous compounds that in turn can influence the taste, odor, structure, and physical properties of the product.

The lipolytic activity of different cereals has been measured by various methods, which have been reviewed by several authors (Morrison, 1978; Galliard, 1983; Stauffer, 1987). Oat is reported to have a remarkably higher lipase activity than, for example, wheat, barley, or rye (Matlashewski et al., 1982; Galliard, 1983; O'Connor et al., 1992). In the ungerminated cereal grain, the lipase activity is mainly located in the outer layers of the kernel (Hutchinson et al., 1951; Galliard, 1983), whereas endosperm has little or no activity (Urquhart et al., 1983). In mature, clean, and ungerminated grain, the presence of lipase in the germ of some cereals is questionable (Galliard, 1983). In a germinating cereal grain lipase activity has been reported to increase in starchy endosperm, aleurone tissue, scutellum, and embryo (Taverner and Laidman, 1972). Moreover, Jensen and Heltved (1982) showed in their studies that the initial site of formation of lipolytic enzymes is in the scutellum, from where the enzymes are later gradually diffused into the entire endosperm.

In whole grain, ungerminated or germinated, the action of lipolytic enzymes is difficult to estimate because the localization of lipids and lipid-degrading enzymes is poorly understood. Anness and Baxter (1983) reported a 20% reduction of neutral and polar lipids due to lipolytic activity during germination of barley. During germination the majority of liberated fatty acids are thought to act as a fuel resource for growth, but the distribution and movements of such FFA are not well-known.

Processing of cereals usually begins with milling and soaking of groats or flour with water, giving enzymes and their substrates an opportunity to react with each other. Under these circumstances it is of importance to evaluate the kernel properties. These properties include the potential for lipolytic activity, the restriction of this lipolytic action, and the capability of the material to protect liberated fatty acids and thus prevent further harmful reactions, for example, oxidation. The lipolytic activity of cereals with reference to pure substrates is well-known (Sahasrabudhe, 1982; Urquhart et al., 1983; O'Connor, 1988), and information is thus available on the potential of lipolytic action of cereals. However, such data are not adequate for the evaluation of the inherent lipolysis that occurs during processing. Therefore, the changes in native cereal lipids due to lipolytic activity in the kernel (originating either from cereal or from microbes) give more relevant information on lipolytic reactions that occur during processing. During

* Address correspondence to this author at the Helsinki University of Technology, P.O. Box 6100, FIN-02015 HUT, Finland (telephone 358-9-451 2555; fax 358-9-462 373; e-mail Anu.Kaukovirta-Norja@hut.fi).

† Helsinki University of Technology.

‡ Oy Lahden Polttimo Ab.

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this study the problem of lipid hydrolysis, lipid oxidation, and lipid metabolism in general during water processing has been approached by studying changes in native cereal lipids.

MATERIALS AND METHODS

Barley. Finnish malting barley varieties Kymppi (crop years 1991, 1993, 1994, and 1995) and Kustaa (crop year 1991) were used.

Other Materials. The standards for thin-layer chromatography (TLC) and gas−liquid chromatography (GC) were purchased from Sigma Chemical Co., St. Louis, MO, and were as follows: dipentadecanyolphosphatidylcholine (P-7285), heptadecanoic acid (H-3500), triheptadecanoin (T-2151), dipentadecanoin (D-8508), and heptadecanoic acid methyl ester (H-4515). Silica gel plates (5721) were purchased from Merck, Darmstadt, Germany. HPLC grade solvents were used for all fatty acid analyses. All other chemicals were of reagent grade or of a higher grade.

Malting. All experiments were carried out using malts that were micromalted by employing 44 h of steeping with various wet and dry periods (6 h wet, 13 h dry, 5 h wet, 19 h dry, and 1 h wet) at 12−14 °C, 5 to 6 days of germination at 14−16 °C, and 21−22 h of kilning. Samples were taken after each step during steeping and after every germination day. Malting samples, except malt and malt with rootlets, were freeze-dried and stored below 8 °C. Rootlets were removed from freeze-dried samples and from malt.

Mashing. Mashings were performed in laboratory scale according to the method of Sjöholm et al. (1994). The temperature profile of mashings was as followed: 48 °C for 30 min, 63 °C for 30 min, 72 °C for 30 min, and 80 °C for 10 min. The rate of temperature increase was 2 °C/min. Wort was separated, cooled, and freeze-dried. Spent grains were washed with cold (4 °C) water, cooled, and freeze-dried. The milling conditions, the separation of wort and spent grains, and the use of additives varied from mashing to mashing.

Water Treatment. A 0.5 g flour sample of barley, malt, or a malting sample was soaked for 15 h (200 rpm, 23 °C) in 2.5 mL of distilled water. Prior to lipid extraction of the untreated samples, 2.5 mL of distilled water was added after the chloroform/methanol (2:1 v/v) addition.

Lipid Extraction. The Folch method (Folch et al., 1957) and the Morrison method (Morrison, 1985a) were used for extraction of lipids from barley, malt, malting samples, and spent grains.

Folch Method. A 0.5 g untreated flour sample or a 0.5 g water-treated sample containing 2.5 mL water was used for Folch extraction. Before extraction, 2.5 mL of water was added in untreated, dry samples to get similar water contents in all samples. Samples were extracted by shaking (240 rpm, 26 °C) in 19 volumes chloroform/methanol (2:1 v/v) according to the procedure of Folch et al. (1957). Extraction was repeated twice (1 × 6 h, 1 × 2 h). The extracts were combined and evaporated to dryness under N_2 in a rotary evaporator. Lipids were dissolved in 10 mL of chloroform/methanol (1:1 v/v), divided into 500−1000 μL aliquots 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 total fatty acid and lipid class contents.

Morrison Method. Samples were extracted according to the procedure of Morrison (1985a) using an *n*-propanol/water mixture (3:1 v/v) at 90−100 °C with a ratio of 20 mL of solvent/g of sample. The extraction was repeated three times (2 × 2 h, 1 × 1 h). The extracts were combined and treated as above.

Separation of Lipid Classes. The dried extracts were redissolved in 200 μL of chloroform/methanol (1:1 v/v), followed by the addition of 50 μg each of the polar lipid (PL), diglyceride (DG), FFA, and triglyceride (TG) standards. The samples were then applied onto silica plates. PL, TG, DG, and FFA were separated by developing the plates with petroleum ether/diethyl ether/acetic acid (80:30:1 v/v). Lipid classes were visualized by spraying with 0.01% Rhodamine 6G and detected under UV light, scraped off, and used for fatty acid determination. The sum of separated lipid classes is defined as *total lipids*.

Determination of Total Fatty Acids. The amount of total fatty acids was determined by saponificating the Folch extracts without separating the lipid classes.

Preparation and Analysis of Fatty Acid Methyl Esters. Fatty acids were saponified and converted to methyl esters as described by Suutari et al. (1990). The methyl esters were analyzed using GC, and major fatty acids were identified by comparing their retention times with those of known standards (Sigma). The total extractable fatty acids were determined by adding 30 μg of an internal standard, heptadecanoic acid methyl ester, to each sample prior to saponification and methylation.

Direct Saponification. Total fatty acids of water-treated, freeze-dried flour of barley, malt, or malting sample or of untreated flour of corresponding samples were treated as above without prior extraction of lipids. A 50 mg sample was used for analysis.

Gas Chromatography. A Hewlett-Packard Model 5890A gas chromatograph equipped with a flame ionization detector, a capillary inlet system, an 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 (Hewlett-Packard, Avondale, PA). The column temperature was programmed from 70 to 200 °C at a rate of 25 °C/min. The column inlet pressure was 150 kPa. The flow rate for the makeup gas, He, was 30 mL/min, and the flow rates for the detector gases 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. The split ratio was set at 1:20. 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 the total peak area. Absolute amounts of the individual fatty acids in total lipids were calculated per gram of dry weight of the sample by comparison of the peak area to that of the internal methyl ester standard without any conversion factors. The total amount of fatty acids was determined as the sum of all individual fatty acids. Amounts of major lipid classes per gram of dry weight of the sample were determined by comparing the area of the fatty acids from a lipid class to that of the corresponding standard. The distribution of different lipid classes was determined as a percentage of total weights of lipid classes.

The degree of fatty acid unsaturation (DUS) in the lipid fraction was calculated as $\Delta\text{mol} - 1 = [\Sigma(\%\ \text{monoene}) + 2(\%\ \text{diene}) + 3(\%\ \text{triene})]/100$.

Accuracy of the Results. All of the analyses were done at least in duplicate. The standard deviations for analyses were determined, and CVs were calculated to be <7% for total lipids and for different lipid classes at the maximum 10% for PL, 7% for TG, 4% for DG, and 5% for FFA fraction.

RESULTS AND DISCUSSION

Barley and Malt Lipids. The lipid content of barley cultivar Kymppi-91 was 28 mg/g. The amount of lipids decreased 14% during malting, being 24 mg/g in lager malt. The reduction of lipids may be considered as an expected change attributable to respiration and energy usage of kernel during the process (Anness and Baxter, 1983). Barley lipids contained 69% TG, 20% PL, 10% DG, and 2% FFA. The respective values for lager malt were 66, 25, 4, and 5%, indicating only minor changes in the composition of kernel lipids during malting. In samples taken during the malting process the lipid class composition was very similar to those of barley and malt as reported earlier (Kaukovirta-Norja et al., 1993). Anther barley cultivar (Kustaa) and different crops of Kymppi used in the present experiments contained 28−

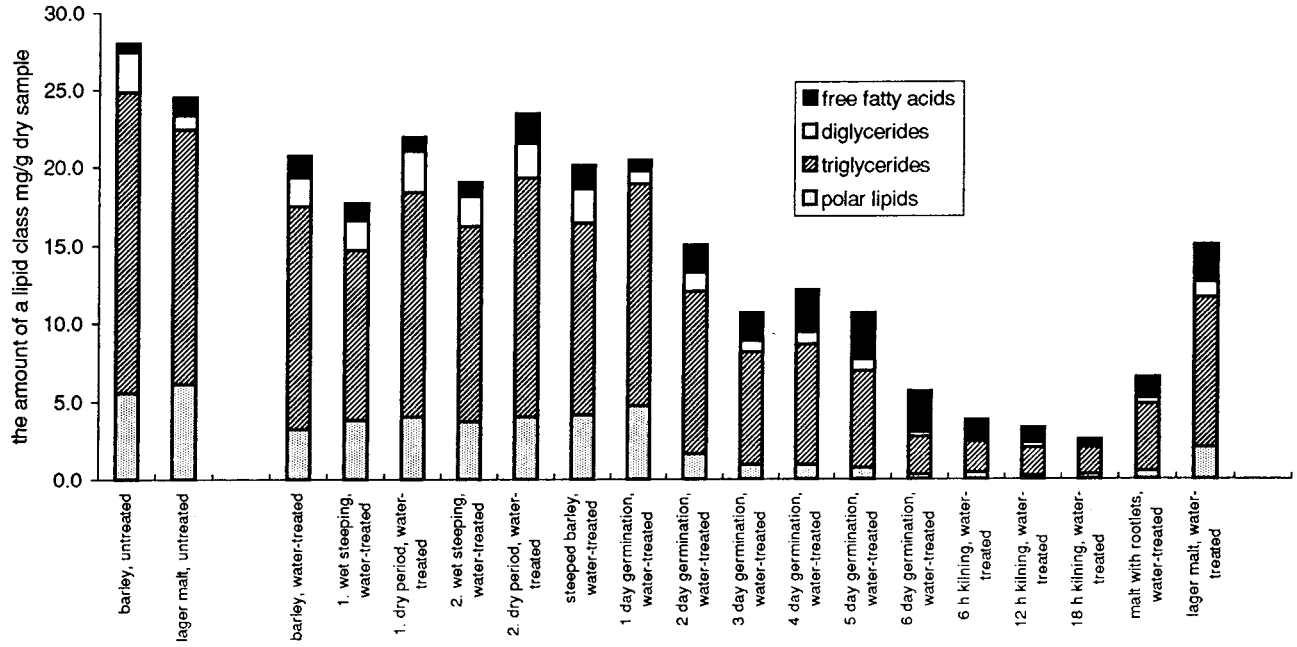

Figure 1. Lipid content (milligrams per gram) and composition of barley, malt, and malting samples after water treatment analyzed according to the Folch method followed by separation of lipid classes. The lipid composition of untreated, native barley and malt is presented for comparison. (1, wet steeping—sample taken after first wet period of steeping, etc.)

34 mg/g lipids with a composition very similar to that of Kymppi-91. Lipid contents are in accordance with the lipid content previously reported for barley by Morrison (1978).

Development of Lipolytic Activity during Malting. The development of lipolytic ability during malting of barley was studied by preparing flour—water suspensions and by measuring changes in total lipid content and lipid class composition after a fixed period of water incubation. The incubation time was chosen according to the study of Liukkonen et al. (1992). Using the Folch method for lipid extraction, the total amount of lipids as well as various lipid classes left in suspensions after water treatment varied markedly depending on the stage of malting from which the sample originated (Figure 1). In samples from the steeping stage of malting, the lipid content was slightly increased in comparison to the water-treated barley sample (Figure 1). On the other hand, in samples taken from the first day of germination, a reduction in lipids was seen after water treatment and the reduction in lipids was most prominent in samples from the kilning phase, where only 10—20% of the barley lipids were present after water treatment (Figure 1). The amount of malt lipids after water treatment was higher than in water-treated kilning samples because during the latest hours of kilning high temperatures had partially inactivated lipolytic enzymes of malt. Therefore, lipolysis and further loss of lipids was not so great in malt as in kilning samples. The amounts of total fatty acids in the same Folch extracts of barley and malting samples had a similar trend as obtained by lipid class separation followed by fatty acid analysis in Figure 1. As seen in Figure 2, the reduction in total lipids (*total* in Figure 2) and in total fatty acids (*totFA* in Figure 2) correlated well with the reduction in PL and TG. This suggests that during the water treatment of the malting samples extensive hydrolysis of TG and PL had occurred, especially in samples taken during the last days of germination and during kilning. Moreover, the reduction in total lipids correlated also with this lipolytic ability.

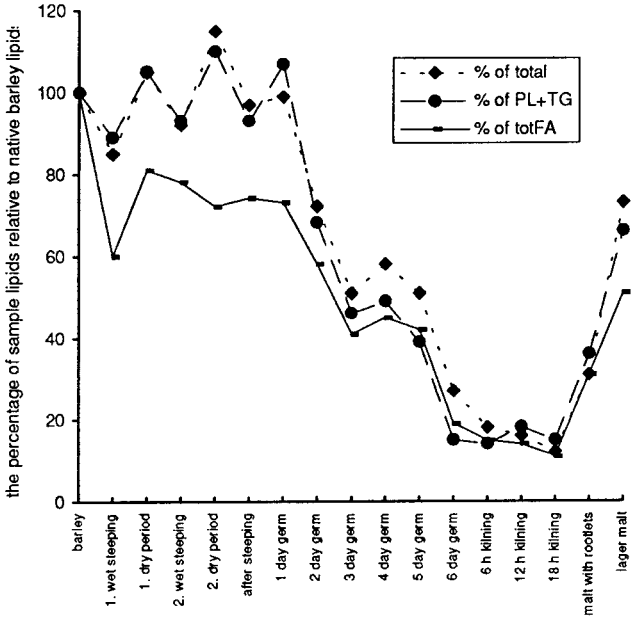

Figure 2. Comparison of lipids of malting samples with lipids of barley after water treatment (the amount of lipids in water-treated barley is set as 100%). Comparison is made for the total amount of lipids, for the sum of PL and TG, and for the total fatty acid content (totFA). All of the extractions were made by the Folch method. (1, wet steeping—sample taken after first wet period of steeping, etc.)

On the other hand, lipolysis should lead to corresponding increments in the amount of FFA in water-treated samples. This did not, however, occur. The proportion of FFA from total lipids varied from 7% in barley to 30% in samples from kilning. It is evident that these proportions cannot account for the loss of TG and PL fractions. It may thus be suggested that the relatively low amount of FFA is due to lipid oxidation, which would follow lipolysis, especially as the proportion of easily oxidizable 1,4-pentadiene fatty acids is high among the fatty acids of barley.

Table 1. DUS in Lipid Classes (TG, PL, and FFA) and Total Fatty Acids of Barley and Malting Samples before and after Water Treatment

	TG (DUS)[a]		PL (DUS)		fFFA (DUS)		total fatty acids (DUS)	
sample	untreated	water-treated	untreated	water-treated	untreated	water-treated	untreated	water-treated
barley	1.54	1.50	1.32	1.28	0.90	0.61	1.42	1.33
1st wet steeping	1.49	1.44	1.39	1.24	0.85	0.77	1.35	1.26
1st dry period	1.52	1.47	1.41	1.29	0.85	0.68	1.36	1.28
2nd wet steeping	1.50	1.48	1.40	1.23	0.84	0.66	1.35	1.26
2nd dry period	1.48	1.46	1.40	1.33	0.94	1.08	1.44	1.29
steeped barley	1.49	1.45	1.32	1.33	0.89	0.83	1.34	1.32
1 day germn	1.44	1.48	1.46	1.42	0.88	1.26	1.38	1.37
2 day germn	1.50	1.47	1.49	1.29	0.96	1.07	1.41	1.36
3 day germn	1.51	1.51	1.42	n.d.	1.22	1.16	1.38	1.36
4 day germn	1.53	1.50	1.51	0.97	0.98	1.23	1.42	1.35
5 day germn	1.53	1.49	1.46	1.02	1.15	1.19	1.44	1.35
6 day germn	1.57	1.49	1.53	1.04	1.11	1.21	1.45	1.35
6 h kilning	1.54	1.51	1.31	0.97	1.14	1.09	1.42	1.40
12 h kilning	1.50	1.54	1.48	1.03	1.15	1.28	1.41	1.30
18 h kilning	1.60	1.57	1.46	0.96	0.95	0.68	1.44	1.29
22 h kilning	1.59	1.56	1.54	1.06	0.92	1.02	1.52	1.36
lager malt	1.60	1.49	1.40	1.18	1.12	1.15	1.44	1.34

[a] The analysis was carried out at least two times, and SD for the DUS values was <0.05.

DUS of Different Lipid Classes. The DUS of different lipid classes was determined to elucidate the possible occurrence of lipid oxidation during the water treatment. The determination of the DUS of lipid classes prior to water treatment (untreated samples in Table 1) showed that the malting process itself did not have a marked effect on the DUS of lipid classes. On the other hand, following water treatment of samples, changes in the DUS of lipid classes were seen in all other fractions except for the TG fraction (Table 1). In all water-treated samples the DUS of the PL fraction was lower than in the PL fraction of untreated samples, indicating that unsaturated fatty acids were somewhat more susceptible to hydrolysis by phospholipase than were saturated fatty acids.

The most remarkable differences in the DUS between the samples was seen in the FFA fraction after water treatment (Table 1). In malting samples, for which the reduction of lipids was found to be the largest during water treatment (samples from the third day of germination until lager malt, Figure 1), a specific reduction in unsaturated FFA was not seen. On the contrary, results in Table 1 show that the stronger the liberation of fatty acids from TG and PL during water treatment, the higher was the DUS of the FFA fraction. It appeared that liberated 1,4-pentadiene fatty acids were not lost more easily than other FFAs. On the other hand, in barley and steeping samples, the unsaturated FFA were lost more easily than their saturated counterparts. It appears that lipid oxidation occurred predominantly in malting samples with low lipolytic potential and, consequently, with a small proportion of FFA. Therefore, it can be concluded that the increment in the ability of the samples to hydrolyze TG and PL as the malting process progresses does not lead to extensive oxidation of unsaturated FFA in an aqueous process. This means that the extent of lipolysis and the oxidation of linoleic acid do not correlate, but the oxidation of lipids during water processing depends on the activity of oxidative enzymes in the sample. An example of this is the 18 h kilned sample, for which the composition of FFA after water treatment differed from that of other kilning samples (Table 1). The reason for the behavior of the lipids in the 18 h kilned sample might have been an extraordinarily high oxidative activity as reported earlier (Kaukovirta-Norja et al., 1993) connected with a high lipolytic activity (Figure 1).

Table 2. Effect of Water Treatment on Total Fatty Acid Content and DUS of Fatty Acids of Barley and Malting Samples Analyzed by Direct Saponification without Extraction

	mg of lipids/g of sample[a]		DUS[b]	
sample	untreated	water-treated	untreated	water-treated
barley	30.1	30.9	1.43	1.33
steeped barley	29.4	35.9	1.43	1.28
1 day germn	30.8	37.6	1.45	1.36
2 day germn	30.4	37.2	1.46	1.35
3 day germn	30.9	37.2	1.47	1.34
4 day germn	28.8	35.5	1.47	1.34
5 day germn	28.2	36.8	1.49	1.34
6 h kilning	29.5	35.4	1.49	1.32
12 h kilning	24.6	34.9	1.51	1.33
18 h kilning	29.0	37.2	1.49	1.33
22 h kilning	28.5	35.9	1.50	1.37
lager malt	26.7	37.3	1.45	1.38

[a] The analysis was carried out at least two times with CV <7%. [b] The analysis was carried out at least two times, and SD for the DUS values was <0.05.

Analysis of Nonextractable Lipids. The fate of the lipids, lost especially as FFA, was further studied by direct saponification of freeze-dried water-treated samples. For comparison, this direct method was used also for the corresponding samples lacking the water treatment. Table 2 shows that the water treatment did not reduce the lipid content of any samples. In addition, the reduction of lipids that was found to occur during the water treatment when the Folch method was used proved to be apparent. The DUS values of all water-treated samples were very similar but continued to be highest in lager malt and in a late kilning sample. Furthermore, the DUS of water-treated samples was only moderately lower than that of untreated samples (Table 2). Therefore, during water treatment neither oxidation nor degradation of FFA occurred to any significant extent. The FFAs produced under conditions of extensive lipolysis seemed to be rendered nonextractable by the Folch method.

However, the apparently missing FFAs proved to be difficult to trace. Table 3 shows two different maltings for which the lipid amounts of the Folch extract and the

Table 3. Influence of Water Treatment on Extractability of Lipids from Cereal Materials: Lipid Content of Folch Extracts and Folch Residues after Extraction Compared to Lipid Content of Samples without Extraction [Two Malts with Different Lipolytic Activities (Malt A/Malt B)]

	mg of lipid/g of sample[a]						
water-treated sample	barley[b]	steeped barley	1 day germn[c]	5 day germn	12 h kilning[d]	18 h kilning	malt
direct saponification	31.9/34.5	37.4/39.1	34.8	34.0/38.3	24.1	34.4/32.1	29.4/30.4
Folch extract	17.1/16.1	16.8/15.2	19.8	17.2/9.1	6.2	15.7/11.5	15.3/19.4
Folch residue	5.3/10.3	4.4/11.3	4.9	1.2/4.2	4.4	1.3/4.6	0.8/4.0
extract + residue	22.4/26.4	21.2/26.5	24.7	18.4/13.3	10.6	17.0/16.1	16.1/23.4
lipids recovered (%)	70/76	57/68	71	54/35	44	49/50	55/77

[a] The analysis was carried out at least two times with CV <7%. [b] Malt A/malt B. [c] Only malt A. [d] Only malt B.

Table 4. Extractability of Lipids from Untreated Barley and Malting Samples: Lipid Content of Folch Extracts and Folch Residues Compared to Lipid Content of Samples without Extraction

	mg of lipids/g of sample[a]				
untreated sample	barley	steeped barley	5 day germn	18 h kilning	malt
direct saponification	28.9	31.4	25.2	27.5	28.9
Folch extract	17.3	19.4	23.6	24.8	21.9
Folch residue	3.9	3.5	0.5	1.3	1.2
extract + residue	21.2	22.9	24.1	26.1	23.1
lipids recovered (%)	73	73	96	95	80

[a] The analysis was carried out at least two times with CV <7%.

Folch residue have been added together and compared to the lipid amount obtained from the direct saponification of samples. The sum of the lipids of the Folch residue and the lipids of the Folch extract did not give fatty acid concentrations that would correspond to lipid amounts that were apparently lost in the Folch method after water treatment (Table 3). Instead, fatty acids were found to be scattered partly in the particular extraction residues and in the aqueous gelatinous phase formed by the solvent used in the Folch method (data not shown). A comparison of the results in Table 3 to those of Table 4 shows that in untreated samples almost all of the lipids were found in the Folch extract and the residue. The only exceptions were the barley and early steeping samples in which some oxidation of lipids might have occurred.

According to the above results the lipolytic capacity develops during malting of barley in the kernels. In aqueous suspensions it causes marked changes in the lipid class composition, but only minor changes occur in the total quantity of lipids. Despite the hydrolysis of TG and PL, readily extractable FFA did not accumulate even though FFAs are polar compounds that are usually expected to be more soluble in water than other lipids, for example, glycerides. Furthermore, the fatty acid composition of total lipids indicated that oxidation of unsaturated lipids did not occur. On the basis of these observations it appears that in an aqueous suspension of malt, FFA may enter a protective phase concomitant with their formation. In applications of malt, this phenomenon may have an important effect on product quality and further use.

Loss of Lipids during Mashing. Mashing of barley represents a typical process in which a loss of lipids has been reported (Anness and Reed, 1985a; Kaukovirta-Norja, 1993) and has been suggested to be due to lipid oxidation (Anness and Reed, 1985b). The lipid balances in eight mashings with various conditions were studied (Table 5). The Folch method and its modified version for lipid analysis of wort (Anness and Reed, 1985a) indicated that the total reduction of lipids during mashing varied between 12 and 43% depending on the mashing conditions. Results were in accordance both with the report of Anness and Reed (1985a,b) and with the above experiments on water treatment of malting samples. Worts contained only 0.2−0.3% of malt lipids, whereas the majority of lipids was found in spent grains. The analysis of fatty acid composition of worts and spent grains showed again no specific reduction in polyunsaturated fatty acids.

Lipids in Spent Grain. The fate of lipids during mashing was further studied by analyzing lipids of two malts and their corresponding spent grains by the Folch and Morrison methods (Morrison, 1985a). As the latter method includes a destructive extraction with hot *n*-propanol/water, it is applicable, for example, for the analysis of tightly complexed lipids. When subjected to lipid analysis by the Folch method, malts contained 19−27 mg/g of lipids and the respective spent grains 12−17 mg/g. The relative proportions of the lipid classes in malt and spent grains shown in Table 6 suggest that lipolysis had occurred during mashing, although when the Folch method was used, it was not possible to elucidate the fate of the missing FFA portion. A change from the Folch method to the Morrison method increased the lipid content of malt to 28−37 mg/g (complexed lipids were now also included) and that of spent grains to 39−46 mg/g. However, despite the increased lipid yield in malt, the content of FFA increased only by 2%, whereas in spent grains the FFA proportion increased from 10 to >20%. Thus, it is evident that, by using the destructive Morrison method, the tightly complexed FFA could be detected. The results also indicate that the loss of lipids during mashing is significantly smaller than previously estimated (Anness and Reed, 1985a,b). It is possible that in the lipolytically active aqueous suspensions, the liberated fatty acids neither disappear nor are oxidized but are protected from further reactions, including even solvent extraction, by complexation to the water-insoluble material. In processes such as mashing, during which the insoluble material is removed from the process prior to its degradation, such a complexation phenomenon serves as a means of lipid removal. In light of the results of lipid balances in Table 5, this lipid removal seems not to be sensitive to variations in malt quality or mashing conditions. Overall, the results show that this lipid protective characteristic is developed during malting and demonstrates that the use of high-quality malt in mashing instead of, for example, barley would ensure a more efficient means to reduce the reactivity of FFA. The FFA complexes formed during the lipolytic water treatments, such as mashing, may

Table 5. Lipid Balances in Eight Laboratory Scale Mashings When 60 g of Malt Was Used [Extraction of Spent Grain Lipids by the Folch Method; Extraction of Wort Lipids by the Method of Anness and Reed (1985)]

	no. of mashing experiment[a]							
	1	2	3	4	5	6	7	8
total lipids into mashing (mg)	1374	1374	1374	1374	1258	971	1286	1132
lipids in spent grains (mg)	885	896	1215	1026	719	670	970	930
lipids in wort (mg)	4	4	3	3	3	3	3	2
lipid loss in mashing (mg)	480	474	156	345	536	298	313	200
lipid loss in mashing (%)	36	35	12	25	43	31	25	18

[a] The analysis was carried out at least two times with CV <7%.

Table 6. Amount of Lipids of Two Malts and the Respectative Spent Grains with Two Extraction Methods [F, Method by Folch (1957); M, Method by Morrison (1985)]

	mg of lipid/g of dry sample[a]				
	PL	TG	DG	FFA	sum of lipids
malt 1; F	6.8	18.2	1.0	1.3	27.3
spent grains 1; F	2.6	8.2	0.6	1.2	12.6
malt 1; M	13.6	20.3	0.9	2.5	37.2
spent grains 1; M	11.7	21.8	2.7	10.0	46.2
malt 2; F	5.0	12.9	0.5	0.9	19.2
spent grains 2; F	3.3	10.5	1.0	2.1	16.9
malt 2; M	10.9	14.5	0.6	1.7	27.8
spent grains 2; M	11.0	17.1	1.7	8.1	37.9

[a] The analysis was carried out at least two times with CV <10%.

be multiple and difficult to analyze at a molecular level, especially if heat treatments are involved. As long as the stability of these complexes is poorly understood, unexpected loads of FFA may occur in aqueous processes of malt during which extensive amylolytic or proteolytic action can occur or insoluble material is not removed at all.

CONCLUSIONS

The present study has shown that during the malting process the lipolytic potential of barley increases markedly. When malting samples were milled and mixed with water, >80% of PL and TG could be hydrolyzed. It was seen that the stronger the hydrolysis of PL and TG during water treatment, the larger was the reduction of the total amount of lipids. However, when lipids were analyzed directly from the samples without extraction, no reduction in the amount of lipids was seen. The extractability of lipids into specific solvents was different for the samples taken at the different stages of malting, probably because of differences in the kernel composition. The malting processes themselves were responsible for biochemical and structural changes in the kernel. In an aqueous environment, these changes could lead to selective complexation of lipolysis products (FFA), which would consequently become unextractable in chloroform/methanol solvents. The binding of lipids with other compounds of grain has been reported in various connections. Starch−lipid complexes [reviewed, for example, by Morrison (1985b)] and lipid binding proteins (Frazier, 1981; Ponz et al., 1984; Zawistowska, 1986) with reference to dough making are the most well-known lipid interactions. The selectivity of complexation for FFA and the tightness of the complexes in aqueous flour mixtures observed in the present study were highly unexpected.

The fact that the liberated fatty acids were also protected from oxidation supports the view that actual complexation occurs between FFA and other flour components. Iwami et al. (1988) reported that, for example, gliadin-encapsulated unsaturated fatty acids were well-protected against oxidation, showing that cereal proteins themselves can form oxidation resistant complexes with lipids. However, in the present study the protective effect was not seen in barley or in samples taken after a short steeping in water. In these samples the small fractions of FFA showed a remarkable reduction of polyunsaturated fatty acids during the water processing. Essentially the capability to protect lipids from oxidation was apparently formed during the germination and kilning stages of malting.

In summary, this work presents a new aspect of the quantitative role of total lipids during mashing. Moreover, this view on physical protection of lipids gives an additional view on lipid oxidation in cereal processes.

LITERATURE CITED

Anness, B.; Baxter, E. The control of lipids and their oxidation products during malting and brewing. *EBC Proceedings of the 19th Congress; European Brewery Convention*; London, 1983; pp 193−200.

Anness, B.; Reed, R. Lipids in the brewery—A material balance. *J. Inst. Brew*. **1985a**, *91*, 82−87.

Anness, B.; Reed, R. Lipids in wort. *J. Inst. Brew*. **1985b**, *91*, 313−317.

Folch, J.; Lees, M.; Sloane Stanley, G. A simple method for isolation and purification of the total lipids from animal tissues. *J. Biol. Chem*. **1957**, *226*, 497−509.

Frazier, P.; Daniels, N.; Russell Eggitt, P. Lipid−protein interactions during dough development. *J. Sci. Food Agric*. **1981**, *32*, 877.

Galliard, T. Enzymatic degradation of cereal lipids. In *Lipids in Cereal Technology*; Barnes, P. J., Ed.; Academic Press: London, 1983; pp 111−147.

Hutchinson, J.; Martin, H.; Moran, T. Location and destruction of lipase in oats. *Nature* **1951**, *167*, 758−759.

Iwami, K.; Hattori, M.; Yasumi, T.; Ibuki, F. Stability of gliadin-encapsulated fatty acids against autoxidation. *J. Agric. Food Chem*. **1988**, *36*, 160−164.

Jensen, S.; Heltved, F. Visualization of enzyme activity in germinating cereal seeds using a lipase sensitive fluorochrome. *Carlsberg Res. Commun*. **1982**, *47*, 297−303.

Kaukovirta-Norja, A.; Reinikainen, P.; Olkku, J.; Laakso, S. Lipolytic and oxidative changes of barley lipids during malting and mashing. *J. Inst. Brew*. **1993**, *99*, 395−403.

Liukkonen, K.; Montfoort, A.; Laakso, S. Water-induced lipid changes in oat processing. *J. Agric. Food Chem*. **1992**, *40*, 126−130.

Matlashewski, G.; Urquhart, A.; Sahasrabudhe, M.; Altosaar, I. Lipase activity in oat flour suspensions and soluble extracts. *Cereal Chem*. **1982**, *59*, 418−422.

Morrison, W. The stability of wheat starch lipids in untreated and chlorine-treated cake flakes. *J. Sci. Food Agric*. **1978**, *29*, 365−371.

Morrison, W. Extraction of lipids from cereal starches with hot aqueous alcohols. *Starch/Staerke* **1985a**, *37*, 83−87.

Morrison, W. Lipids in cereal starches. In *New Approaches to Research on Cereal Carbohydrates*; Hill, R., Munck, L., Eds.; Elsevier Science Publishers: Amsterdam, 1985b; pp 61−70.

O'Connor, J.; Perry, H.; Harwood, J. Solubilization and studies of cereal lipases. *Biochem. Soc. Trans.* **1988**, *17*, 687−688.

O'Connor, J.; Perry, H.; Harwood, J. A comparison of lipase activity in various cereal grains. *J. Cereal Sci.* **1992**, *16*, 153−163.

Ponz, F.; Hernandéz-Lucas, C.; Carbonero, P.; García-Olmedo, F. Lipid binding proteins from the endosperms of wheat and oats. *Phytochemistry* **1984**, *23*, 2179−2181.

Sahasrabudhe, M. Measurement of lipase activity in single grain of oat (*Avena sativa* L.). *J. Am. Oil Chem. Soc.* **1982**, *59*, 354−355.

Sjöholm, K.; Pietilä, K.; Home, S.; Aarts, R.; Jaakkola, N. Evaluation of lautering performance of malt. *Monatsschr. Brauwiss.* **1994**, *47*, 165−171.

Stauffer, C. Ester hydrolases. In *Enzymes and Their Role in Cereal Technology*; Kruger, J., Lineback, D., Stauffer, C., Eds.; American Association of Cereal Chemists: St. Paul, MN, 1987; pp 265−280.

Suutari, M.; Liukkonen, K.; Laakso, S. Temperature adaptation in yeasts: the role of fatty acids. *J. Gen. Microbiol.* **1990**, *136*, 1469−1474.

Taverner, R.; Laidman, D. The induction of lipase activity in the germinating wheat grain. *Phytochemistry* **1972**, *11*, 989−997.

Urquhart, A.; Altossaar, I.; Matlashewski, G.; Sahasrabudhe, M. Localization of lipase activity in oat grains and milled oat fractions. *Cereal Chem.* **1983**, *60*, 181−183.

Zawistowska, U.; Bietz, J.; Bushuk, W. Characterization of low-molecular-weight protein with high affinity for flour lipid from two wheat classes. *Cereal Chem.* **1986**, *63*, 414−419.

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