

Influence of Hydrothermal Treatment on Lipid Oxidation in Barley

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ABSTRACT

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A steeping process of barley grains was evaluated regarding lipid oxidation. The steeping process was evaluated with respect to the temperature during the first steep and second steep, and the lactic acid concentration of the steep solutions. The study was conducted using a central composition circumscribed design, and response surface models were estimated with the use of partial least square. The change in the concentration of hexanal was used to monitor the oxidation during process-

ing and subsequent storage at 30°C. In all samples there was hexanal development during processing and the hexanal concentration increased considerably during storage. The results show that it is possible to optimize the process to get a lower oxidation during the subsequent storage. The temperature during the second steep in the hydrothermal process and the level of lactic acid addition were the most important factors. Both of them should be kept low to favor the oxidative stability.

Hydrothermal treatment of cereals is a process whereby the moisture content of the cereals is raised and the cereals are kept at an elevated temperature for some period of time. Earlier experience has shown that this type of process has a favorable influence on sensory properties (Meyer and Zwengelberg 1979). According to Batscheider and Bermasek (1980), hydrothermal treatment of cereals may lead to concentrating of vitamin B₁, B₂, B₆, E, and carotene. Also, the process could enhance the nutritional value by enzymatic degradation of phytate which reduces the mineral availability in cereals. It has been shown, for example, that malting of cereals followed by soaking at optimal conditions could be used to obtain flour with improved mineral availability. Such flour could be a significant source of minerals when used in production of food for infants and breakfast cereals (Larsson and Sandberg 1992). However, little is known about the effect of hydrothermal treatment on the storage stability of the product.

One important factor for the storage stability of cereals is lipid oxidation. Lipid oxidation in food is influenced by several factors such as temperature, oxygen availability, water activity, exposure to light, the concentration of antioxidants and prooxidants, fat content, and distribution, etc. The processing of the food may affect oxidation during the subsequent storage by influencing all these factors. Lipid oxidation also occurs readily during food processing, and the initial oxidation may promote further oxidation during subsequent storage (Lingnert 1992). To produce oxidatively stable products in an hydrothermal process, both process and storage conditions must be considered.

The oxidation of lipids has a great influence on the deterioration of foods, even if the lipid content is small. The content of lipids in barley is 1.9–4.6% (Morrison 1993). The major fatty acids present are unsaturated (Palmer 1989). Linoleic acid (18:2) constitutes ≈57% and linolenic acid (18:3) constitutes ≈5% of the fatty acid composition (Morrison 1978). These polyunsaturated fatty acids are particularly susceptible to oxidation and give rise to primary and secondary oxidation products such as aldehydes. Hexanal content has been correlated highly to the sensory score obtained during evaluation of oxidation in oat cereals (Fritsch and Gale 1977). Hexanal is formed mainly from the 13-hydroperoxide of linoleic acid, which is the most abundant essential fatty acid in barley.

Barley also contains eight naturally occurring tocopherols (Barnes 1983), which may act as antioxidants. α -Tocotrienol is the predominant isomer in barley. Also, there are substantial quantities of α -tocopherol, β -tocotrienol, and γ -tocotrienol (Peterson 1994).

Lipoxygenase (LOX) is important due to its prooxidative activity. LOX-1 is localized exclusively in the germ (Yang et al 1993), while a second isoenzyme LOX-2 (Baxter 1982) appears to develop only after germination. The isoenzyme LOX-1 converts linoleic acid mainly into the 9-hydroperoxide; the ratio 9- to 13-hydroperoxide is 80:20 (Yang et al 1993).

The aim of this study was to investigate the influence of hydrothermal processing on the oxidative stability of barley. A multivariate experimental design was used and response surface models were made to statistically evaluate the results.

MATERIALS AND METHODS

Raw Material

Whole barley kernels (cultivar Blenheim) of 1994 crops were provided by Skånska Lantmännen, Sweden, and used as the raw material.

Hydrothermal Process

The processing was performed in a laboratory plant at Oy Lahden Polttimo AB, Lahti, Finland.

Step 1 – Cold water wash: 11.2 kg of barley was distributed into eight steeping boxes, resulting in batches of 1.4 kg. All eight batches were washed simultaneously with ≈130 L of water (15°C) for 5 min. The washing water was drained before step 2.

Step 2 – Wet steep 1: The barley kernels (1.4 kg) were soaked in 2.1 L of steep solution (water or lactic acid solution of 0.2–0.8%) for 1 hr at a controlled temperature of 45–65°C.

Step 3 – Dry steep 1: The steep solution was removed and the kernels were dry-steeped at the same temperature as wet steep 1 for 5 hr.

Step 4 – Wet steep 2: The kernels were soaked once more in 2.1 L of steep solution for 1 hr at a controlled temperature of 55–75°C.

Step 5 – Dry steep 2: The solution was removed and the kernels were then dry-steeped at the same temperature as wet steep 2 for 15 hr.

Step 6 – Drying: Hot air was blown through the barley bed. The kernels were dried with the temperature program of 8 hr at 50°C followed by a continuous temperature increase up to 80°C for 2 hr, and finally 6 hr at 80°C. After drying, the kernels of the eight batches were combined.

Samples were taken after each of the steps 2–6 in the process. These samples were frozen and stored at –40°C until analyzed or until the start of the storage experiment.

Experimental Design

The effects of three process variables were studied: the temperature during the first steep (T1, °C), the temperature during the second steep (T2, °C), and the lactic acid concentration of the steep solutions (LA, %). A central composition circumscribed (CCC) design was used with four replicates at the center point, giving a total of 18 treatments. The experimental conditions at the

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TABLE I
Experimental Layout in Coded Values and Real Values of the Central Composite Circumscribed (CCC) Design^a

Sample	Coded Values			Real Values		
	X1	X2	X3	T1 (°C)	T2 (°C)	LA (%)
1	0	0	0	55	65	0.4
2	1	-1	1	60	60	0.6
3	-2	0	0	45	65	0.4
4	-1	1	-1	50	70	0.2
5	-1	-1	1	50	60	0.6
6	0	2	0	55	75	0.4
7	2	0	0	65	65	0.4
8	0	0	0	55	65	0.4
9	1	-1	-1	60	60	0.2
10	1	1	-1	60	70	0.2
11	0	0	-2	55	65	0
12	0	0	0	55	65	0.4
13	-1	1	1	50	70	0.6
14	1	1	1	60	70	0.6
15	0	-2	0	55	55	0.4
16	0	0	2	55	65	0.8
17	-1	-1	-1	50	60	0.2
18	0	0	0	55	65	0.4
19	-2	-2	2	45	55	0.8
20	1.4	-0.6	1.5	62	62	0.3
21	-0.4	2.2	0.5	53	76	0.1

^a Three variables with four replicates of the central point (samples 1–18); verification experiments performed in the experimental domain (samples 19–21); random experimental run order. T1 and T2 = temperatures during steep 1 and 2; LA= lactic acid concentration.

TABLE II
Influence of Lactic Acid (LA) on pH Level During Processing

Steps ^a	LA Addition				
	0.0%	0.2%	0.4%	0.6%	0.8%
AIw	6.0	5.4 ± 0.09	5.2 ± 0.09	4.9 ± 0.13	4.8
AId	5.9	5.5 ± 0.05	5.3 ± 0.07	5.0 ± 0.13	5.0
AIWw	6.0	5.2 ± 0.05	4.8 ± 0.06	4.6 ± 0.10	4.4
AIId	5.9	5.3 ± 0.01	5.0 ± 0.06	4.7 ± 0.08	4.4

^a pH level measured in suspensions of samples at different steps: AIw, after wet steep 1; AId, after dry steep 1; AIWw, after wet steep 2; AIId, after dry steep 2. Results at 0.2, 0.4, and 0.6%, respectively, are mean values of 4, 8, and 4 samples ± standard deviation. Results at 0.0 and 0.8% are single measured values.

center point were T1 = 55°C, T2 = 65°C, and LA = 0.4%. The experimental number, scaled values, and real values are given in Table I where the scaled values are X1 = (T1–55)/5, where T1 is 45–65°C; X2 = (T2–65)/5, where T2 is 55–75°C; X3 = (LA–0.4)/0.2, where LA is 0.0–0.8%. Three verification experiments were performed in the experimental domain (samples 19–21).

Evaluation of Storage Stability

Storage experiments were performed to study the oxidative stability of the processed barley grains. Processed barley grain (30 g) was weighed into 500-mL glass bottles closed with screw caps (Pyrex, France). The bottles were stored in darkness at +30°C. After 0, 6, and 12 weeks of storage, four bottles were withdrawn and stored at –40°C until analyzed. The samples were randomly analyzed within six weeks.

Methods

Oxidation during storage was monitored by analyzing the formation of volatiles. A dynamic headspace method as described by Hall et al (1985) was used with minor modifications. The kernels (30 g) were thawed and then ground in a coffee grinder (Braun 4041, Kronberg/Ts., Germany) for 15 sec. The flour was transferred into another 500-mL glass bottle tightened with a headspace-sampling adapter. The sample was allowed to equilibrate overnight (16 hr) at room temperature. Headspace volatiles were collected on an adsorbent polymer cartridge (Chromosorb 102) by leading a stream of helium through the sampling bottle and the adsorbent cartridge (40 mL/min, 75 min of sampling time). The adsorbed volatiles were thermally desorbed, condensed in a cold

TABLE III
Copper Content in Processed Samples

Sample	Cu
	(mg/100 g of dry barley grains)
1	2.6
2	2.6
3	2.5
4	2.6
5	2.6
6	2.6
7	3.2
8	3.0
9	3.2
10	3.2
11	2.0
12	3.9
13	3.8
14	4.6
15	3.8
16	5.0
17	4.6
18	4.9
19	5.0
20	4.5
21	3.0
Raw material	1.3

trap, and injected to a gas chromatograph (HP5890A). The instrumental parameters were flame ionization detector; fused silica megabore capillary column (30 m × 0.52 mm i.d.) with chemically bonded methyl-polysiloxan, DB1, film thickness, 1.5 µm; temperature program, 30–200°C, 4°C/min; carrier gas, helium, 4 mL/min. A data collection system (HP 3367) was used. Hexanal was identified by mass spectrometry, quantified with an external standard, and used as a lipid oxidation indicator. All determinations were run in duplicate.

Tocols were determined by an HPLC method described by Piironen et al (1984) with minor modifications. The barley grains were ground in a coffee grinder for 15 sec, and 5 g of ground barley was weighed into an Erlenmeyer flask. The sample was saponified and extracted by iso-hexane (HPLC-grade, Fisons, Loughborough, England). The iso-hexane extract was evaporated to dryness on a rotary evaporator at ≈40°C. The residue was redissolved in 10 mL of iso-hexane and dried with water-free sodium sulfate (Pro Analysis,

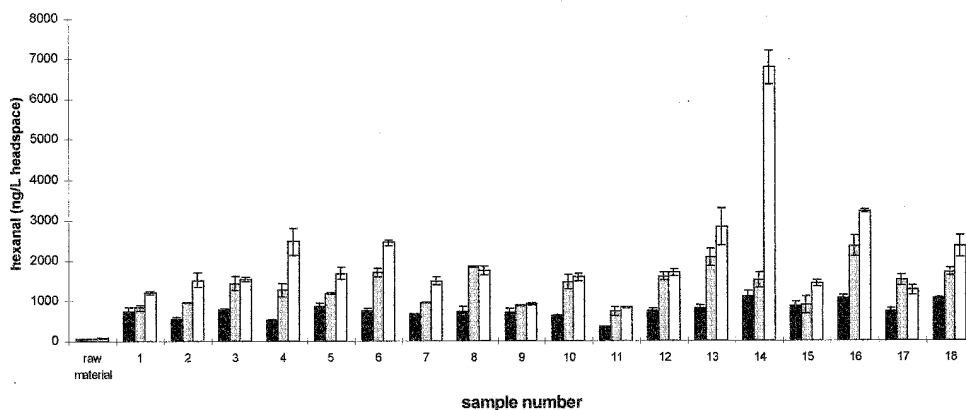


Fig. 1. Hexanal content in raw material and 18 samples measured after 0, 6, and 12 weeks of storage. Results are mean of duplicate samples. Error bars represent minimum and maximum values.

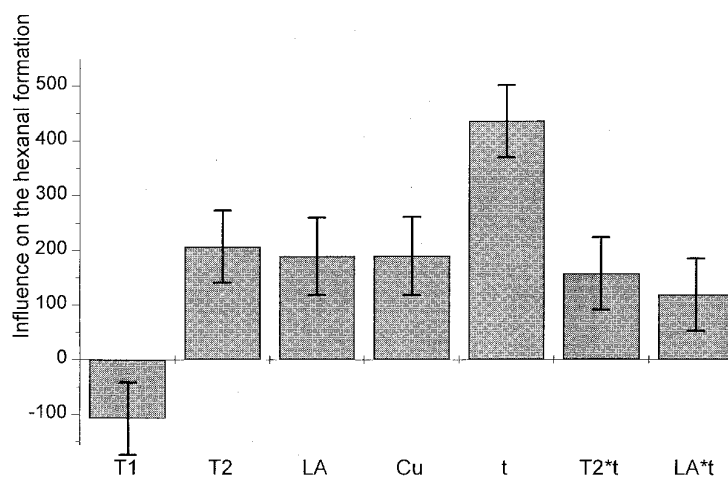


Fig. 2. Coefficients (scaled and centered) for each variable showing the influence of the variable on hexanal formation during storage. Coefficients estimated by partial least squares (PLS) analysis. T1 and T2 = temperatures for steep 1 and 2; LA = lactic acid concentration; Cu = copper content; t = storage time. Error bars represent minimum and maximum values.

E. Merck, Darmstadt, Germany). The tocopherols were separated by normal-phase chromatography on a LiChrosorb 5 Silica column (Phenomenex, Torrance, CA) 250×2.10 mm i.d., and analyzed by a fluorescence detector. The injection volume was 20 μ L. The equipment consisted of a Beckman model 110A pump, a Beckman injector, a Hewlett Packard fluorescence detector HP 1046A (excitation wavelength 292 nm slits at 2×2 mm, and emission wavelength 324 nm slits at 4×4 mm), and a Softron PC integration program version 1.0. The mobile phase was iso-hexane and 2-propanol (LiChrosolv, E. Merck) (99.8:0.2) at a flow rate of 0.5 mL/min. Tocopherols were identified by the injection of standards. Standard curves for α -tocopherol (>95%, E. Merck) and α -tocotrienol (>95%, E. Merck) were made to quantify these tocopherols. All extractions and analyses were run in duplicate.

The transition metals copper and iron were determined by atomic absorption spectroscopy (Varian model 575) at Arla FoU, Stockholm, Sweden. Ground barley (5.0 g) and 50 g of water was mixed in a cup. A 2.0 g portion of this slurry was weighed into a new cup. Nitric acid (5 mL) and hydrogen peroxide (1 mL) was added. The sample was heat-treated in a microwave oven while monitoring pressure and temperature. The final temperature was 180°C and final pressure was 200 psi. The remaining sample was diluted with 52 g of water. Before measurement, the sample was further diluted to fit within the range of the standard curve. The wavelengths used were copper 324.8 nm and iron 248.3 nm.

The water content of the samples at different steps in the process was determined by a thermo-controlled infrared dryer (Sartorius GmbH, Goettingen, Germany). The final dried products were deter-

mined by conventional oven (ISO standard 712) at Oy Lahden Poltimo AB, Lahti, Finland. The pH level was measured in a suspension of ground grains in water (1:0.5, v/v).

Lipoxygenase Activity

Barley kernels (≈ 30 g) were thawed and then ground for 15 sec in a coffee grinder (Braun AG type 4041). A 1.0 g (wet weight) sample of the ground barley was suspended in 10 mL of a 0.1M potassium phosphate buffer containing 0.01M KCl, pH 6.7, in a beaker on an ice bath, and magnetically stirred for 30 min. The suspension was then centrifuged for 15 sec (Eppendorf 5415, Hamburg, Germany) at $14,000 \text{ r} \times \text{min}^{-1}$ at room temperature, and the supernatant was collected and stored on ice (for a maximum of 30 min) before analysis. The extraction was performed in duplicate.

The lipoxygenase activity was measured at 25°C using a polarographic method described by Eriksson and Svensson (1970). The lipoxygenase activity was determined as the rate of O_2 consumption after addition of the barley extract to a linoleic acid substrate. This was performed using an oxygen analyzer (Medelco AB, Hagersten, Sweden) equipped with a YSI 4004 Clark electrode (Yellow Springs Instrument Co., Yellow Springs, OH). The substrate was 10 mM linoleic acid (>99% Nu-Chek-Prep, Elysian, MN) in a 0.1M potassium phosphate buffer, pH 6.7, and Tween 20 (0.3%) was used as the emulsifier. Supernatant (100 μ L) was injected into a 1-mL reaction chamber containing the linoleic acid substrate. The maximum rate of oxygen consumption (shortly after the addition of the extract) was used as the measure of the enzymatic activity. The results are presented as a percentage of

the activity in the raw material extract on a dry weight basis. The analyses were performed in duplicate.

Data Analysis

Response surface models were estimated with the use of partial least square (PLS). The calculations were made with the computer program MODDE version 3.0 (Umetri AB, Umeå, Sweden). The reliability of the models was evaluated by calculating the values R^2 , Q^2 , and the condition number. R^2 is the percentage of the variation of the response explained by the model. Q^2 is the fraction of the variation of the response that can be predicted by the model, determined by cross validation. The condition number is the ratio between the variation accounted for by the component with the largest variance and the variation of the component with the smallest variance, which represents a measure of the orthogonality of the design (User's Guide to MODDE, Umetri AB). Q^2 should be >0.5 if conclusions are to be drawn from the model. The model is better the closer R^2 and Q^2 are to 100%. Generally, a model is excellent if R^2 and $Q^2 > 90\%$. The condition number of the response surface model designs varies with the number of factors but should be <10 . The model is better the closer the condition number is to 1.

RESULTS AND DISCUSSION

The water content of the original barley kernels was $\approx 13\%$. After wet steep 1, AIw, it had increased to $34 \pm 2\%$, (mean of all

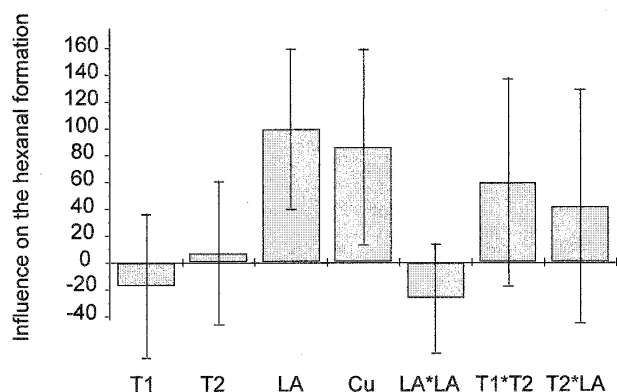


Fig. 3. Coefficients (scaled and centered) for each variable showing the influence of the variable on hexanal formation after 0 weeks of storage (model 1). Coefficients estimated by partial least squares (PLS) analysis. T1 and T2 = temperatures for steep 1 and 2; LA = lactic acid; Cu = copper; t = storage time. Error bars represent minimum and maximum values.

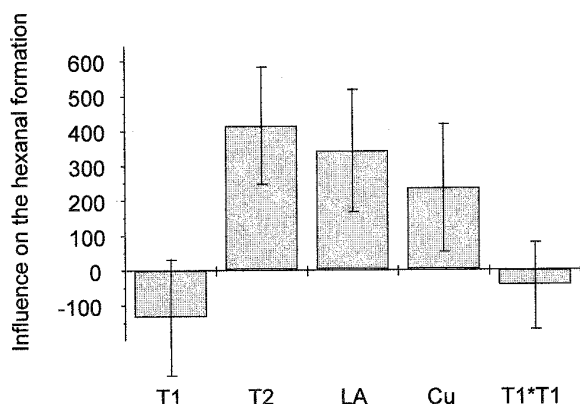


Fig. 4. Coefficients (scaled and centered) for each variable showing the influence of the variable on hexanal formation after 12 weeks of storage (model 3). Coefficients estimated by partial least squares (PLS) analysis. T1 and T2 = temperatures for steep 1 and 2; LA = lactic acid; Cu = copper; t = storage time. Error bars represent minimum and maximum values.

18 samples \pm standard deviation), after dry steep 1, AId, it was $37 \pm 2\%$, after wet steep 2, AIIw, it was $46 \pm 2\%$, after dry steep 2, AIIId, it was $51 \pm 4\%$; in the dried product, DP, it was $7 \pm 1\%$. The pH values at different stages in the process are given in Table II. With no addition of lactic acid, the pH value remained at ≈ 6 throughout the process; the addition of lactic acid could lower the pH by 1.6 units at the most.

Transition Metals

The content of transition metals, copper and iron, in the raw material and the processed barley grains was analyzed. The total copper content was 2.0–5.0 mg of Cu/100 g of dry barley grains (Table III). The raw material contained 1.3 mg of Cu/100 g, dry weight. Thus, in some samples, the copper content had increased almost four times during processing. The copper content of barley varies at 0.5–1.1 mg/100 g (Kent 1975, Låg and Steinnes 1978, Varo et al 1980). The copper content in the raw material used in these experiments was slightly higher than the reported values, but, above all, there was a remarkable increase during this specific process. Thus, the copper content was proven to be an uncontrolled variable that could have great influence on the lipid oxidation. The total iron content was also analyzed but showed only small variations among the samples, 4.8–5.2 mg/100 g of dry barley grains. The raw material contained 5.8 mg/100 g of dry barley grains. Thus, the iron decreased slightly during this process.

Volatile Determinations

The results from the hexanal measurements after 0, 6, and 12 weeks of storage at 30°C are presented in Fig. 1. A significant formation of hexanal during processing was found, and there were considerable differences between the samples at 0 weeks. Differences in the development of hexanal during storage are also seen. Considerable amounts of hexanal were formed already during processing, although the major lipoxygenase in ungerminated barley, LOX-1, oxidizes linoleic acid mainly into the 9-hydroperoxide. Still, lipoxygenase presumably plays an important role by initiating further oxidation resulting in hexanal formation

To evaluate the process variables important to oxidation, a mathematical model was fitted to the measured hexanal contents. The mathematical model was based on the scaled variables and estimated with the use of partial least square, PLS. Storage time (t) was included as an independent variable. The mathematical model used is valid only within this experimental domain.

Sample 14 was excluded from the experiment because the mathematical analyses (normal probability plot) showed it to be an outlier after 12 weeks of storage. A complete response surface model (RSM) with the outlier excluded showed an R^2 of 82%, a Q^2 of 57%, and a condition number of 6.4. This could not be considered an adequate model. In response surface models, the response (y_{calc}) is expressed as a second-order polynomial function of the examined variables (Lindgren et al 1995), in this case giving:

$$y_{\text{calc}}(\text{ng of hexanal/L of headspace}) = b_0 + b_1T1 + b_2T2 + b_3LA + b_4t + b_{11}T1^2 + b_{22}T2^2 + b_{33}LA^2 + b_{44}t^2 + b_{12}T1T2 + b_{13}T1LA + b_{14}T1t + b_{23}T2LA + b_{24}T2t + b_{34}LA t$$

The b -coefficient b_0 is a constant; b_1 , b_2 and b_3 express the main effects of each process variable; b_{11} , b_{22} and b_{33} reveal whether any of the variables give a maximum or minimum in the response; and b_{12} , b_{13} and b_{23} show the interaction effects between the variables. The prediction of the model was improved by pruning with the Q^2 value and the condition number as control variables. The pruned model gave a new polynomial function:

$$y_{\text{calc}}(\text{ng of hexanal/L of headspace}) = b_0 + b_1T1 + b_2T2 + b_3LA + b_4t + b_{24}T2t + b_{34}LA t$$

All further discussions concern this pruned model.

The pruned response surface model gave an R^2 of 79%, a Q^2 of 71%, and a condition number of 1.0, which was a better model,

but still not adequate. When the copper content was included in the model as an uncontrolled variable, the R^2 increased to 88%, the Q^2 increased to 79%, and the condition number increased to 1.5. These improved values show that the copper content variation was of great importance and should be included in the model. Without including copper as an uncontrolled variable, the model showed a significant lack-of-fit, which also supports the theory that the copper variation should be included.

The estimated PLS model can be used to show the b -coefficients (scaled and centered) for each of the variables and how the variables influence the hexanal formation during storage (Fig. 2). The figure shows that all factors significantly influence the hexanal formation. The storage time (t) was the most important variable. This was not surprising, but shows that t , using these storage conditions, had been long enough to influence the lipid oxidation more than the other model variables. There was no dominant factor among the other factors, but the results show that a low temperature (T1) and a high temperature (T2) promoted the oxidation. A high concentration of lactic acid and a high copper content also increased the oxidation. However, the results also show two interaction coefficients are significant: $T2 \times t$ and $LA \times t$.

The influence of T2 on the hexanal formation increased with t . The level of T2 (high or low) did not influence oxidation at 0 weeks, but was important after 12 weeks of storage. Similarly the influence of the added amount of LA on the oxidation increased with t . The difference between high LA (low pH value) and low LA (high pH value), regarding lipid oxidation, increased during storage.

Mathematical models in scaled variables were also fitted to the mean values of hexanal measured 1) at 0 weeks of storage, 2) after 6 weeks of storage, and 3) after 12 weeks of storage at 30°C. These three models all included the copper content as an uncontrolled variable. These models were also pruned. The Q^2 and the condition number were used as control variables. The pruned RSM for each model was estimated using PLS. Sample 14 was again shown to be an outlier after 12 weeks of storage and was not included in model 3. The influence of the variables on the hexanal formation at 0 and 12 weeks are presented in Figs. 3 and 4 showing the b -coefficients for each of the process variables in the mathematical model.

When comparing the results presented in Fig. 3 with those in Fig. 4, T2 became a significant and dominant variable in model 3, while it turned out to be a negligible variable in model 1. This is in accordance with the findings regarding interactions between T2 and t . Regarding the added amount of LA, the corresponding coefficient was significant both at 0 and 12 weeks of storage (Figs. 3 and 4). There was a difference between high and low LA already at 0 weeks, but the difference between high and low LA regarding hexanal formation became more important with t . This is not contradictory to the fact that LA was significant in both models 1 and 3.

While model 1 represents information about the influence on oxidation occurring during processing, models 2 and 3, based on data after 6 and 12 weeks of storage, respectively, include information about lipid oxidation caused both by the process and by the storage. Models 2 and 3 are almost similar. Model 2 contains two square terms, and model 3 contains one square term, in addition to the four variables (T1, T2, LA, and Cu). Model 2

contains the square terms $T1 \times T1$ and $T2 \times T2$, while model 3 only contains the square term $T1 \times T1$. Model 2 shows a Q^2 of 59%, an R^2 of 79%, and a condition number of 3.5, while model 3 shows a Q^2 of 72%, an R^2 of 86%, and a condition number of 2.5. This means that model 3 was the most reliable model and the model to use for the main conclusions about the importance of the variables influencing the oxidative stability after storage.

Figure 4 shows that factors T2, LA, and Cu had significant ($P < 0.02$) influence on oxidation. The temperature during the second steep, T2, was the most important variable containing the principal explanation of the mathematical model, followed by the added amount of lactic acid, LA, and finally the copper content, Cu. An increase in any of these three variables increased the formation of hexanal. The temperature during the first steep, T1, was not significant on a 5% level ($P = 0.101$), but the results indicated that increasing T1 decreased the formation of hexanal.

A low temperature during the second steep was beneficial to the oxidative stability. This would be expected because high temperatures would favor both oxidation during the process and the thermal decomposition of hydroperoxides already formed during the early stages of the process.

A low LA content was also beneficial to the oxidative stability. This could be due to the interactional effects of the copper content. In a purely aqueous system, copper II would exist as free ions at $pH < 6.5$ (Quinn et al 1980). Free copper ions would be expected to increase the lipid oxidation (Pokorny 1987). However, in these experiments, all samples had a $pH < 6.5$ during processing (Table II). But in a complex mixture like this, pH dependent interactions

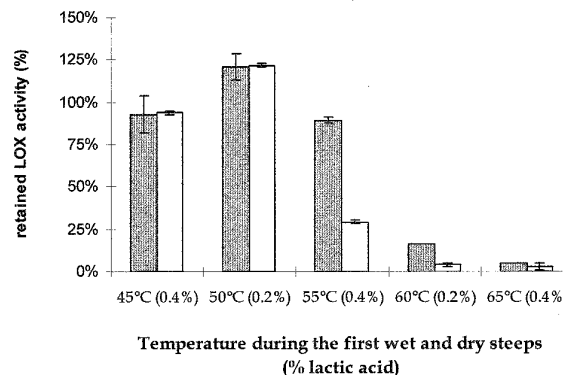


Fig. 5. Retained lipoxygenase (LOX) activity after wet steep 1 and dry steep 1 as a function of processing temperature. Samples 3, 4, 12, 10, and 7 were analyzed in process temperature order (% lactic acid in parentheses). Results are % of LOX activity of raw material. Mean of duplicate samples. Error bars represent minimum and maximum values.

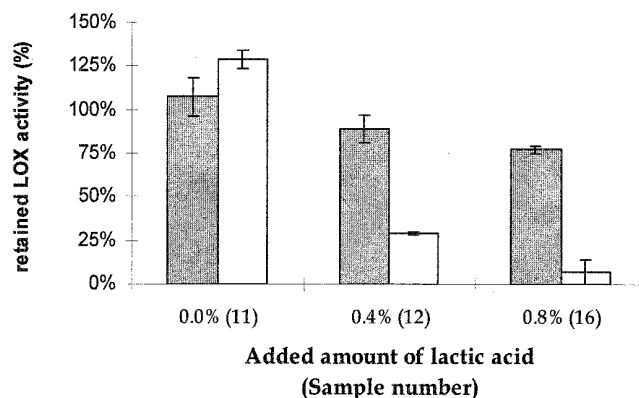


Fig. 6. Retained lipoxygenase (LOX) activity as a function of added amount of lactic acid at 55°C. LOX activity for samples 11, 12, and 16 after wet steep 1 and dry steep 1. Results are % of LOX activity of raw material. Mean of duplicate samples. Error bars represent minimum and maximum values.

TABLE IV

Hexanal Content After 12 Weeks in Samples 19–21 Using Model 3^a

Sample	Predicted Value	Confidence Levels		Measured Value
		Lower	Upper	
19	2,300	1,500	3,100	2,100
20	1,500	1,000	2,000	1,900
21	2,300	1,800	2,800	2,300

^a Values are ng of hexanal/L of headspace.

between the copper content and the variety of ligands would be anticipated. For instance, interaction with amino acids, particularly histidine and cysteine with imidazole and -SH side chains, would be expected (Quinn et al 1980).

The fact that the copper content was a significant factor in this experiment showed that these uncontrolled variations between the samples were important to the oxidation in the product. It is well known that copper may promote oxidation at trace levels, and a reduced copper content is always beneficial to oxidative stability (Irwing et al 1991). The reasons for the variations in the copper content of the processed barley kernels was later found to be a leakage of copper from the steeping equipment.

A high temperature during the first steep seems to be beneficial to oxidative stability. The temperature during the first wet steep and during the first dry steep was 45–65°C. The beneficial effect of a high temperature in this steep regarding lipid oxidation may indicate higher temperature is needed to inactivate the prooxidative enzyme, lipoxygenase. To investigate this, the lipoxygenase activity was assayed in samples taken at various points in the process.

To verify the mathematical model 3, three more experiments were performed in the experimental domain (samples 19–21, Table I). The prediction of these samples for hexanal formation after 12 weeks of storage using model 3 was compared with the measured values (Table IV). All three predicted values were reasonably in accordance with the measured values, falling within the respective confidence intervals. These results support the reliability of the model.

Lipoxygenase Activity

The results from model 3, based on the hexanal formation after 12 weeks of storage, indicated that a high temperature during the first steep, T1, correlated to storage stability. These results could possibly be explained by the fact that a high temperature is needed to inactivate the enzyme lipoxygenase. Lipoxygenase activity was therefore measured after wet steep 1, AIw, and after dry steep 1, AI_d, in the process. Seven samples were chosen for the lipoxygenase assay. Samples 3, 4, 7, 10, and 12 were chosen to cover the range of variation of the process variable T1 from 45 to 65°C (Table I). Because these samples differed regarding added LA, samples 11, 12, and 16 were chosen to specifically cover the variation of the process variable LA from 0.0 to 0.8% (Table I). The temperature T1 was 55°C for these three samples. The influence of the temperature during the first steep, T1, on the retained LOX activity in percentage of LOX activity in the raw material is presented in Fig. 5.

Figure 5 shows that the thermal inactivation of lipoxygenase started at ≈55°C. At 65°C, the enzyme was almost totally inactivated at the conditions in the process. This is in accordance with results of Lulai and Baker (1976), who reported that barley lipoxygenase was totally inactivated at 65°C. Baxter (1982) reported that at

60°C, 50–80% of the enzyme was inactivated after 30 min of heat treatment and that inactivation then proceeded more slowly. Yang et al (1993) separated the two isoenzymes of lipoxygenase in barley LOX-1 and LOX-2 and reported that LOX-1 was totally inactivated after 5 min at 55°C and that LOX-2 was totally inactivated after 5 min at 65°C. Lulai and Baker (1976) reported that the optimum temperature of barley lipoxygenase was 47°C.

The influence of the added amount of LA on the LOX retention at 55°C is presented in Fig. 6, which shows that the more LA added to the steep water (lower pH value), the more unstable was the enzyme at 55°C. The pH values in the suspensions of water and ground barley grains are given in Table II. Svensson and Eriksson (1972) similarly found lipoxygenase from peas to be more unstable against heat at lower pH levels. They studied a pH range of 4.0–6.0 in the temperature interval of 55–80°C. The results are also in accordance with results from barley samples showing lipoxygenase (LOX-1) to be more unstable against heat at lower pH within a pH range of 4.65–7.0 in the temperature interval of 50–65°C (Hugues et al 1994). Mathematical models were also made for these measurements, one after wet steep 1 and one after dry steep 1. The interactional effect between T1 and LA showed no significance ($P > 0.30$) in the models. None of the samples analyzed showed any remaining LOX activity after dry steep 2.

Tocols

α -Tocotrienol was the most abundant tocol in the barley samples, followed by α -tocopherol, β -tocotrienol, and γ -tocotrienol. This is according to the literature (Peterson 1994). The tocol content (α -tocotrienol and α -tocopherol, which contribute most to the total tocol content) in the raw material and in the 18 samples is given in Fig. 7. The tocol content of the raw material was in accordance with values reported in the literature. Barnes (1983) reported a range of 19–50 mg/kg of barley and Peterson and Querchi (1993) reported tocol concentration varying at 42–80 mg/kg. The hydrothermal processing caused substantial tocol losses.

Mathematical models were fitted to the mean values of α -tocopherol (model A) and to α -tocotrienol (model B), respectively, in samples 1–18 and were statistically evaluated by the use of PLS. The measured copper contents were also included in these mathematical models as an uncontrolled variable. Sample 18 was excluded in both model A and model B because it was an outlier, and the response surface models were pruned. The outcome from the mathematical model A showed $R^2 = 79\%$ and $Q^2 = 49\%$. The outcome from the mathematical model B showed $R^2 = 83\%$ and $Q^2 = 37\%$. The low values of Q^2 show that neither of the models was reliable. However, the results from both mathematical models gave indications that the copper content was an important variable. A high copper content decreased the content of α -tocopherol and α -tocotrienol.

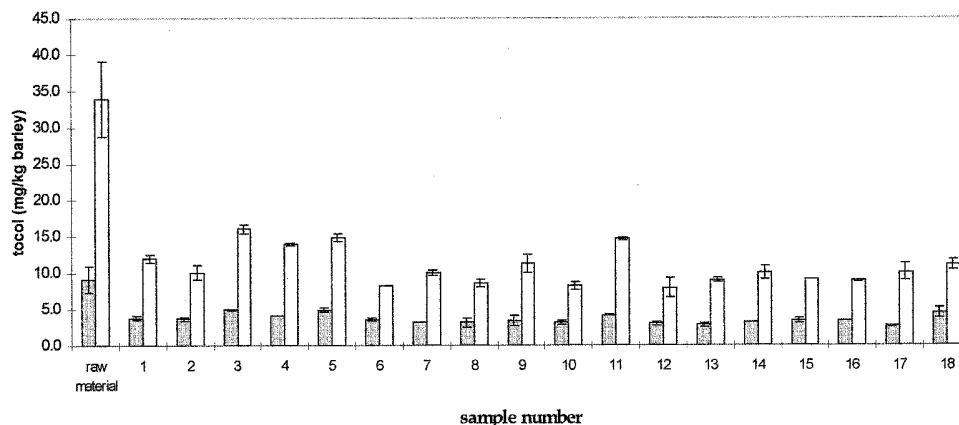


Fig. 7. Content of α -tocopherol and α -tocotrienol in mg/kg of barley grains for raw material and 18 samples, respectively. Mean of duplicate samples. Error bars represent minimum and maximum values.

Comparing model 3 with models A and B indicates that the tocol content after processing did not correlate with the hexanal content after storage. A regression analysis showed the correlation coefficient between hexanal (measurement after 12 weeks) and α -tocopherol, and between hexanal (measurement after 12 weeks) and α -tocotrienol to be 0.02 and 0.01, respectively (samples 14 and 18 were excluded). Thus, tocol measurements after processing do not seem to be a way to predict lipid oxidation during the subsequent storage. The same is true for hexanal measured directly after processing (compare Figs. 3 and 4). A regression analysis showed a correlation coefficient between hexanal measurement after 12 weeks and after 0 weeks to be 0.27 (sample 14 was excluded).

CONCLUSIONS

The experimental design, combined with the modeling and statistical evaluation, was a valuable tool in the identification of critical process parameters regarding lipid oxidation and storage stability. The temperature during the second step in the hydrothermal process and the level of LA addition were the most important factors. Both of them should be kept low to favor the oxidative stability. The copper content also proved to be a significant factor, although it was an uncontrolled variable in these experiments. A high temperature during the first steep was beneficial, which could be explained by the importance of inactivating the barley lipoxygenase. A temperature of at least 55°C was needed to inactivate the lipoxygenase. The inactivation was also influenced by the pH level, the enzyme being more unstable at a lower pH.

Considerable oxidation was taking place already during the processing, and it could be established by measuring hexanal formation as well as tocol losses during processing. However, there was no significant relation between the oxidation during processing and further oxidation during the subsequent storage. Neither the initial hexanal concentration nor the tocol content after processing could therefore be used to predict the storage stability.

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