Storage Stability of Milled Flaxseed

L.J. Malcolmson[a,]*, R. Przybylski[a], and J.K. Daun[b]

[a]Department of Foods and Nutrition, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada, and [b]Canadian Grain Commission, Grain Research Laboratory, Winnipeg, Manitoba R3C 3G8, Canada

ABSTRACT: Two samples of flaxseed, Linott and a mixture of several varieties, were milled and stored at 23 ± 2°C for 128 d in paper bags with plastic liners. Samples were evaluated at 0, 33, 66, 96, and 128 d for chemical, sensory, and volatile indicators of quality. Neither the mixed variety nor Linott samples showed a significant increase in peroxide values or conjugated double bonds throughout the 128-d storage period. Only the Linott sample showed a significant increase in free fatty acids, which was likely due to the presence of immature seed in the sample. Total volatiles increased with storage in the mixed variety sample but showed minimal change in the Linott sample. Overall, the levels of total volatiles in the milled flaxseed samples were much lower than levels reported in stored vegetable oils containing significantly lower levels of linolenic acid. Dienals, formed during polyunsaturated fatty acid oxidation, and hexanal, a compound used as an indicator of oxidative deterioration, were found at very low levels in both samples and did not reach high levels throughout the 128-d storage period. A trained sensory panel could not detect any differences in the odor properties of fresh or stored milled samples. No differences in flavor could be detected between bread made with 0- and 128-d milled flaxseed. This study showed that milled flaxseed can be stored up to 4 mon at ambient temperatures without noticeable changes in quality. The presence of endogenous antioxidants in the milled flaxseed may account for the stability observed.

Paper no. J9186 in *JAOCS 77,* 235–238 (March 2000).

KEY WORDS: Bread, ground flaxseed, milled flaxseed, storage stability.

The use of milled flaxseed in bakery products has become increasingly popular. Mixtures, with up to 15% by weight of milled flaxseed to flour, are used, mostly in whole wheat or multigrain bread formulations. Since not all bakeries are able to mill their own flaxseed, milled flaxseed is sold, usually in triple-wrapped paper bags with plastic liners (60 lb). These bags are stored at ambient temperatures until required for use. Owing to the high content of linolenic acid in flaxseed, it is generally believed that milled flaxseed has a limited shelf life. Few studies have been undertaken to examine the stability of milled flaxseed. Chen *et al.* (1) found that whole flaxseed was more stable than milled seed, but the study was carried out using extreme storage conditions (178°C with oxygen deficiency). Hettiarachchy and Barr (2) showed that citric acid and L-ascorbic-6-palmitate, alone or in combination, reduced free fatty acid formation when milled flaxseed was stored at 26°C for 4 mon. No other measures of oxidative stability were determined. Thus, the objectives of this study were to determine the stability of milled flaxseed stored at ambient temperatures characteristic of bakery operations and to measure oxidative stability using a number of techniques including volatile component analyses and chemical and sensory methods.

*To whom correspondence should be addressed at Canadian International Grains Institute, 1000-303 Main Street, Winnipeg, MB Canada R3C 3G7. E-mail: lmalcolmson@cigi.ca

EXPERIMENTAL PROCEDURES

Storage of seeds. A sample constituting mixture of currently grown varieties of flaxseed and a sample of the variety Linott were provided by the Flax Council of Canada (Winnipeg, Canada). These samples were selected for study since a mixed variety sample is the most common form available commercially, and Linott has been suggested by flaxseed users to have better storage stability. Both samples were visually determined to be similar in cleanliness and quality and were considered suitable for food use. The seeds were milled on a Retsch mill (Brinkmann Instruments Canada Ltd., Mississauga, Canada) using the pin mill attachment. Milled seed (1 kg) was packed into a triple-layer medium-weight paper bag with a 1.5-ply plastic liner similar to what is used commercially. The bags were stored at 23 ± 2°C for 33, 66, 96, and 128 d. A simultaneous storage design was selected which permitted all stored samples to be ready for analysis at the same time. With this approach, the sample to be stored the longest (128 d) was milled at the beginning of the study and placed in storage. Thirty-two days later the sample to be stored for 96 d was milled and placed in storage, etc. To ensure this was a valid approach the whole seed was analyzed at the beginning and end of the study period to ensure there were no changes in whole seed quality. As shown in Table 1 no change in whole seed quality was evident.

Chemical analysis of milled seed. Oil was extracted from the milled seed according to American Oil Chemists' Society (AOCS) method Am 2-93 (3). Peroxide values were determined on the extracted oil according to AOCS method Ca 5a-40 (3), and free fatty acids were determined by titration using the method described by Ke and Woyewoda (4). Conjugated double bonds were determined by ultraviolet absorption and expressed as the extinction of a 1% solution at 268 nm (5,6). Fatty acid composition of the extracted oil was determined

TABLE 1
Initial Quality of Whole Flaxseed

	Mixed		Linott	
Parameter	0 d	128 d	0 d	128 d
Moisture (%)	6.5		6.6	
Oil content (%)	43.4		47.3	
Peroxide value (meq/kg)	0.4	0.2	0.6	0.3
Free fatty acids (%)	0.4	0.2	0.5	0.3

using AOCS Method Ca 5a-40 (3). All analyses were performed in duplicate.

Volatile component analysis. Volatile components were determined in duplicate using a method developed for vegetable oils with modification for solid samples (7). Briefly, 500 mg milled flaxseed was placed into the gas chromatograph injector insert tube, and both ends were closed with glass wool. The tubes and glass wool were purified by heating at 380°C for at least 4 h prior to use. The tube containing the sample was placed into the injector, and volatiles were transferred onto the trapping column using helium as the carrier gas. The trapping column was immersed into liquid nitrogen. Purging was continued for 15 min at 110°C. The sample was removed from the injector and the pressure equilibrated to working conditions to initiate the run. Volatiles were separated on a fused-silicia capillary column, 60 m × 0.32 mm, coated with 0.5 µm DB-5 (J&W Scientific, Folsom, CA). Column temperature was programmed from 40 to 210°C at a rate of 2.5°C per minute. Lower and upper temperatures were held for 4 and 15 min, respectively. Separate volatile components were quantified using the internal standard, decane.

Sensory evaluation of milled seed. A nine-member trained panel evaluated the stored milled flaxseed for its odor characteristics. Seven 30-min training sessions were conducted to familiarize panelists with the odor properties of fresh and stored flaxseed, the ballot, and the procedure for handling the samples. During training, panelists were exposed to a range of stored samples including samples that had undergone prolonged storage such that oxidation had occurred. Panelists described the fresh milled flax (0 day) as having a cereal-like, slightly sour odor and the sample that had undergone prolonged storage as having a painty, rancid odor. Panelists evaluated the samples in a computerized sensory evaluation facilities equipped with individual booths. Samples were evaluated under red lights to mask possible color differences among samples. Compusense version 4.1 (Compusense Inc., Guelph, Canada), a sensory evaluation software package, provided panelists with on-screen instructions, sample presentations, and recording of judgments. Samples were prepared by placing 5 g of milled flaxseed and 2 mL of water in a 125-mL glass jar and capping with a Teflon-lined lid. Milled seed was placed in water because the odor intensity of the sample was thus enhanced, making the task easier for the panelists. The jars were placed in a waterbath held at 50°C, the temperature recommended for evaluating edible oil samples (AOCS method Cg 2-83) (3). Samples were coded with three-digit random numbers and were presented in random orders to panelists. Panelists rated the intensity of the fresh odor on a five-point category scale where 1 = none and 5 = intense. A fresh (0 d) milled flaxseed sample was provided as a reference sample at each test session. Two test sessions were required to evaluate all the samples and two replications were completed for a total of four test sessions.

Sensory evaluation of bread. Bread was made from the milled flaxseed that had been stored for 0 and 128 d according to the following formulation (all quantities in grams except where noted): flour, 855; wheat bran, 45; milled flaxseed, 100; water, 630; sugar, 40; salt, 22.5; canola oil, 10; compressed yeast, 42.5; vital wheat gluten, 40; ascorbic acid, 100 ppm; and sodium stearoyl lactylate, 5.

Six loaves of bread were prepared. After baking, the bread was allowed to cool at room temperature, sliced, placed in plastic bags, and frozen until required for analysis (2 d). Bread was removed from the freezer 2 h before evaluation. A panel of 36 participants who volunteered to serve on the panel evaluated the bread for flavor using the constant reference duo-trio method (8). Panelists received a reference sample (0 d) and two coded samples (0 and 128 d). Their task was to choose the sample that had the same flavor as the reference. Bread was prepared for evaluation by removing the crusts, cutting the slices into 3 × 3 × 1.5 cm cubes, and placing the cubes in 60-mL plastic cups with lids. Panelists evaluated the samples in the computerized sensory facility under red lights as described previously.

Statistical analyses. Analysis of variance using the general linear model procedure (9) was used to analyze data from chemical and sensory tests performed on the seed. The model included the main effects of sample and storage days and its interaction. Sensory data from the constant reference duo-trio test (bread evaluation) were analyzed according to the method described by Meilgaard *et al.* (8).

RESULTS

Initial quality of whole flaxseed. Both samples of flaxseed were considered to be of good quality as indicated by low levels of free fatty acids and peroxide values (Table 1). Linolenic acid ($C_{18:3}$) was the predominant fatty acid followed by linoleic ($C_{18:2}$) and oleic ($C_{18:1}$) acids (Table 2).

Chemical determinations. No significant ($P \leq 0.05$) changes in peroxides or conjugated double bonds were found over the 128-d storage period for either the mixed variety or Linott samples nor were differences found between the two

TABLE 2
Fatty Acid Composition (%) of Stored Flaxseed

	Mixed		Linott	
Fatty Acid	0 d	128 d	0 d	128 d
$C_{16:0}$	5.8	5.8	4.9	4.9
$C_{18:0}$	3.3	3.3	2.6	2.5
$C_{18:1}$	15.0	15.0	18.2	18.3
$C_{18:2}$	15.5	15.5	14.5	14.4
$C_{18:3}$	59.6	59.6	58.9	58.9

TABLE 3
Peroxide, Free Fatty Acid, Conjugated Double Bonds, and Odor Intensity Values for Stored Milled Flaxseed

	Linott: storage day					Mixed: storage day				
Parameter	0	33	66	96	128	0	33	66	96	128
Peroxide value (meq/kg)	0.20	0.20	0.19	0.21	0.30	0.20	0.21	0.12	0.16	0.12
Free fatty acid (%)	0.30	0.65	0.67	0.67	1.58	0.34	0.48	0.31	0.35	0.31
Conjugated double bonds (absorbance at 268 nm)	0.31	0.30	0.30	0.26	0.21	0.20	0.19	0.19	0.19	0.19
Odor intensity[a]	3.3	3.6	3.4	3.0	3.1	3.1	3.6	3.2	3.2	3.5

[a]1 = none, 5 = intense.

samples (Table 3). A significant ($P \leq 0.05$) sample by storage day interaction was found for free fatty acids. As storage increased, the level of free fatty acids in the mixed variety sample did not change, but the level in the Linott sample increased (Table 3). The increase in free fatty acids observed for the Linott sample may be attributed to enzymatic activity possibly owing to the presence of immature seed in the sample (10). Approximately 5% of the seeds were slightly discolored indicating lack of full maturity of the seed.

Volatile components. The amount of total volatiles did not increase with storage for the Linott sample (Fig. 1), whereas levels did increase for the mixed variety sample (Fig. 2) resulting in higher levels of volatiles in the mixed variety sample than in the Linott sample. Overall, the level of total volatiles that formed in the stored flaxseed samples was 10–25 times lower than values reported in stored vegetable oils (11) that contain significantly lower levels of linolenic acid. Formation of the volatile subclasses, saturated carbonyls and monounsaturated carbonyls, was also higher in the mixed variety sample than in Linott (Figs. 1 and 2). With increasing storage, the levels of saturated carbonyls increased in both samples, whereas monounsaturated carbonyls increased only in the mixed variety sample. Dienals, one of the major subclasses formed during polyunsaturated fatty acid oxidation, were found in both samples at relatively low levels (Figs. 1 and 2). Similar trends were observed among individual off-flavor components (data not shown). These components, including hexanal, which is often used as a quality indictor, were found at the ppb level, close to or below reported threshold values.

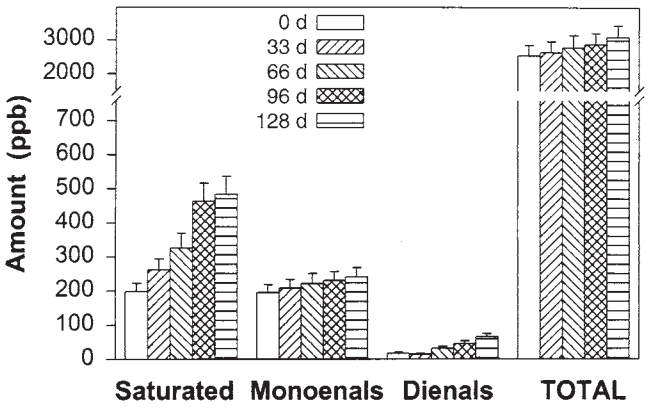

FIG. 1. Accumulation over time of total volatiles and volatile subclasses in stored milled Linott flaxseed.

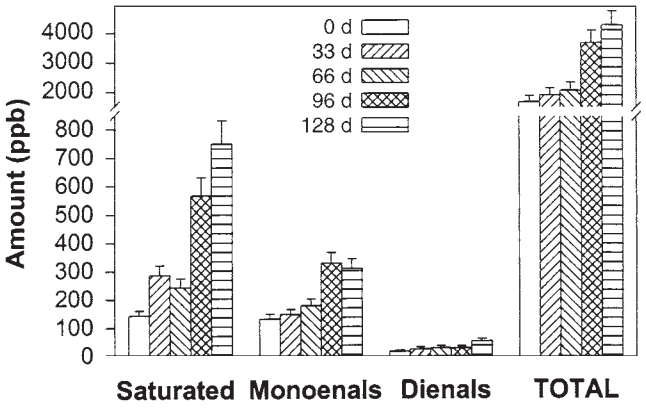

FIG. 2. Accumulation over time of total volatiles and volatile subclasses in stored milled mixed variety flaxseed.

Sensory evaluation of milled flaxseed. Panelists were not able to detect a difference in odor characteristics among the fresh or stored flaxseed samples for either the mixed variety or the Linott samples (Table 3). This finding is consistent with the results obtained for the chemical and volatile determinations.

Sensory evaluation of bread. The number of panelists who correctly selected the sample that was the same as the reference was 22 out of 36 for the bread contained the mixed variety sample and 20 out of 36 for the bread containing Linott. Neither result was significant ($P \leq 0.05$), indicating that panelists were unable to detect a flavor difference between bread made with 0-d stored milled flaxseed and 128-d stored milled flaxseed.

DISCUSSION

Both Linott and the mixed variety flaxseed were stable over 128 d of storage at 23 ± 2°C as measured by peroxide values, free fatty acids, conjugated double bonds, volatile components, and sensory evaluation. These findings suggest the presence of endogenous antioxidants in the milled flaxseed that prevented oxidation of the unsaturated fatty acids and the corresponding development of off-flavors. Additional studies should be undertaken to determine the maximal shelf life of milled flaxseed and to determine what components in the milled seed impart lipid stability.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the contribution of Donna Ryland, University of Manitoba and Ken Howard, Bert Siemens, Barry Misener, Michelle Kisilowsky, Garth Paulley, Grain Research Laboratory. The financial support of the Flax Council of Canada is acknowledged.

REFERENCES

1. Chen, Z.-Y., Ratnayake, W.M.N., and S.C. Cunnane, Oxidative Stability of Flaxseed Lipids During Baking. *J. Am. Oil Chem. Soc. 71*:629–632 (1994).
2. Hettiarachchy, N.S., and J. Barr, Effect of Additives on the Shelf-Life Stability of Lipids in Ground Flax, in *Program and Exhibit Directory*, 52nd Annual Meeting of the Institute of Food Technologists, Chicago (1991).
3. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., edited by D. Firestone, American Oil Chemists' Society, Champaign, 1990.
4. Ke, P.J., and A.D. Woyewoda, A Titrimetric Method for Determination of Free Fatty Acids in Tissues and Lipids with Ternary Solvents and *m*-Cresol Purple Indicator, *Anal. Chim. Acta 99*:387–391 (1978).
5. Ong, V.T.L., Die Notwendigkeit, neue Qualitaetsanforderungen fuer rohes Sojaoel einzufuehren, *Feite Seifen Anstrichm. 82*:169–171 (1980).
6. International Organization for Standardization, 3656 Animal and Vegetable Fats and Oils—Determination of Ultraviolet Absorbance, 1989.
7. Przybylski, R. 1991. Efficient Trapping System for Volatile Components Evaluation in Oils and Fats, in *Eighth International Rapeseed Congress Proceedings*, edited by D.I. McGregor, Saskatoon, Canada, July 9–11, 1991, Vol. 3, pp. 861–866.
8. Meilgaard, M., G.V. Civille, and B.T. Carr, *Sensory Evaluation Techniques*, 2nd edn., CRC Press Inc., Boca Raton, 1991.
9. SAS, Release 6.04 Edition, SAS Institute Inc., Cary, 1992.
10. Daun, J.K., Oilseeds—Processing, in *Grains and Oilseeds Handling, Marketing and Processing*, Canadian International Grains Institute, Winnipeg, Canada, 1993, pp. 833–935.
11. Rui, R.C., The Role of Endogenous Lipid Components in Vegetable Oil Stability, Ph.D. Thesis, University of Manitoba, Winnipeg, 1997.

[Received March 26, 1999; accepted October 26, 1999]