Oxidative Stability of Flaxseed Lipids During Baking

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This study examined the stability of whole and ground flaxseed, either alone or as an ingredient in a muffin mix, by measuring oxygen consumption and changes in α-linolenic acid under various conditions. When ground flaxseed was heated at 178°C in a sealed tube, headspace oxygen decreased from 21 to 2% within 30 min, while that of whole flaxseed decreased only slightly up to 90 min at 178°C. Under the same conditions, the oxygen consumption of lipids extracted from an equivalent amount of flaxseed was between the whole flaxseed and the ground flaxseed. After heating to 178°C for 1.5 h, α-linolenic acid decreased from 55.1 to 51.3% in ground flaxseed, and to 51.7% in lipid extracts, but it remained unchanged in the whole flaxseed. Ground flaxseed with large (<20 mesh) or small (>35 mesh) particle size absorbed more oxygen than samples with medium particle size when heated at 122°C for 8 h. Long-term storage of whole or ground flaxseed or lipid extracts showed that all three preparations were stable at room temperature for 280 d with 12 h light/dark cycles. A muffin mix, containing 28.5 wt% flaxseed flour, consumed oxygen more rapidly than a control muffin without flaxseed flour at a baking temperature of 178°C for 2 h, but the α-linolenic acid remained unchanged in both muffin mixes. Polymers derived from triglyceride oxidation and new *trans* isomers of α-linolenic acid were not detected under the present experimental conditions. Under typical baking conditions, there is minimal loss of α-linolenic acid from flaxseed, although the manner of incorporation of flaxseed in food products should be considered to minimize oxidation of α-linolenic acids.

KEY WORDS: α-Linolenic acid, flaxseed, flaxseed muffin, oxidation, oxygen absorption.

Flaxseed has been used traditionally in breakfast cereals. Nutritional research on flaxseed has increased its potential as a new ingredient for breads, buns, muffins and other bakery products (1). The interest in flaxseed consumption is related to its high content of α-linolenic acid (ALA; 18:3n-3; 50–55% of total fatty acids), dietary fiber mucilage, lignans and phenolic compounds, all of which are probably beneficial in reducing the risk factors for both coronary vascular disease and cancer (2–7).

One of the potential safety concerns about consumption of flaxseed is its content of cyanogenic glycosides (8). Potential production of oxidized compounds and reduced shelf life, due to its high ALA content, is also of concern. The objective of the present study was to examine thermal and oxidative stability of whole and ground flaxseed and of extracted flaxseed oil, either alone or as an ingredient in muffin mix, at a baking temperature of 178°C under various conditions.

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METHODS AND MATERIALS

Preparation of ground flaxseed and lipid extracts. Flaxseed (*Linum usitatissimum*) was supplied by Flax Growers Western Canada (Saskatoon, Saskatchewan, Canada). Flaxseed used in this study was the *Linott* variety unless otherwise indicated. Flaxseed was ground in a coffee grinder and screened according to various particle sizes with the Canadian Standard Sieve Series (W.S. Tyler Company of Canada, St. Catherine, Ontario, Canada). Particle size composition of the ground flaxseed ranged from >35 mesh (<500 μM), 10.7%; 25–35 mesh (500–710 μM), 56.7%; 20–25 mesh (710–850 μM), 24.2%; to < 20 mesh (>950 μM), 8.4%. Total lipids in the ground flaxseed were extracted with 20 vol of chloroform/methanol (2:1, vol/vol). After drying under nitrogen, the flaxseed total lipids were redissolved in chloroform.

Preparation of flaxseed muffin mix. The flaxseed muffin mix was prepared by mixing thoroughly the following ingredients: 960 g wheat flour, 600 g flaxseed flour, 400 g honey, 30 g canola oil, 96 g baking powder and 17 g salt. For the control muffin mix, the flaxseed flour was replaced by wheat flour, and 226 g canola oil was added instead, while the other ingredients were identical to those in the flaxseed muffin mix.

Measurement of oxygen consumption. The method described by Bunick (9) and modified by Chen and Nawar (10) was used to monitor oxygen consumption. In brief, one gram of the whole or the ground flaxseed was sealed in a glass tube (150 × 16 mm, o.d.) with a rubber stopper obtained from an evacuated blood collection tube (100 × 16 mm, o.d.; Becton-Dickinson, Rutherford, NJ), which usually maintains a vacuum for 2–3 yr. The sealed tube was leak-free. This was verified by filling the tube with nitrogen gas and monitoring by gas chromatography if headspace nitrogen concentration decreases. In case of the muffin mix, two grams were used. Two mL of chloroform containing the lipid extracts equivalent to 1 g flaxseed were pipetted into a reaction vessel, and the chloroform was removed under a gentle stream of nitrogen. The reaction tube was then flushed with air, sealed tightly and heated to either 178 or 122°C, or stored at room temperature for 280 d with 12-h dark/light cycles. The headspace oxygen was sampled only once per tube with a gas-tight syringe and analyzed in a Varian 3300 gas chromatograph, fitted with a 1/8" × 6' stainless-steel column packed with Molecular Sieve 5A (60/80 mesh) and a thermal conductivity detector (Varian, Palo Alto, CA). The percent oxygen in the headspace was calculated from the ratio of the oxygen to nitrogen. After the headspace oxygen analysis, the lipids were extracted with 20 vol of chloroform/methanol (2:1, vol/vol) and saved for fatty acid analysis.

Fatty acid analysis. Fatty acids were converted to the corresponding methyl esters with a mixture of 14% boron triflouride in methanol (Sigma Chemical Co., St. Louis, MO) and toluene (1:1, vol/vol) under nitrogen at 90°C for 45 min. Fatty acid methyl esters were analyzed on a flexible silica capillary column (SP 2560, 100 m × 0.25 mm,

i.d.; Supelco, Inc., Bellefonte, PA) in a Hewlett-Packard 5890 Series II gas-liquid chromatograph equipped with a flame-ionization detector (Palo Alto, CA).

Polymer analysis. To detect possible triglyceride polymers formed during lipid oxidation, gel permeation chromatography was carried out on two styragel columns connected in series (Waters, Marlborough, MA; 300 × 7.8 mm, i.d., 500 A) in a Waters HPLC 510 equipped with a refractive index detector. Tetrahydrofuran was used as solvent at a flow rate of 1 mL/min (11).

RESULTS AND DISCUSSION

Typical gas chromatograms of headspace air in the whole flaxseed, the ground flaxseed and the lipid extracts heated at 178°C for 1.5 h are shown in Figure 1. With ground flaxseed from two varieties, *Linott* and *Noralta*, headspace oxygen was depleted by 91% within 30 min (Fig. 2). In contrast, the whole flaxseed was stable throughout the period examined (17–18% headspace oxygen depletion). The oxygen consumption rate in the lipid extracts fell between that in the whole flaxseed and the ground flaxseed (Fig. 2).

Fatty acid data were consistent with the oxygen consumption test (Table 1). ALA decreased from 55.1 to 51.3% in the ground flaxseed, and to 51.8% in the lipid extracts after 90 min heating at 178°C. This was accompanied by a small but proportional increase in oleic acid in both the ground flaxseed and the lipid extracts (Table 1). The oxygen consumption data clearly need to be compared with the fatty acid profiles before conclusions can be made about the stability of flaxseed under these experimental conditions.

After heating the ground flaxseed at 122°C for 8 h, that with the largest particle size (mesh <20 or >950 μM) absorbed the most oxygen, followed by the ground flaxseed with the smallest particle size (mesh >35 or <500 μM; Fig. 3). The headspace oxygen concentration of the ground flaxseed with a medium particle size (mesh 20–35) decreased slower under the same experimental conditions.

It has been known that Maillard reaction products generated by thermal processes have antioxidative properties (12–15). Roasting may generate some Maillard reaction products that could make ground, roasted flaxseed resistant to lipid oxidation. To test this hypothesis, flaxseed was roasted at 110°C for 1 h and then ground prior to the oxygen consumption test. However, no difference was observed between the ground roasted flaxseed and ground raw flaxseed in terms of oxygen consumption (Fig. 4).

The oxygen consumption test showed that whole flaxseed and ground flaxseed were stable for at least 280 d of storage at room temperature. Although the stability of lipid extracts was more variable (Fig. 5), the fatty acid composition of the three samples remained unchanged from starting values (data not shown).

The flaxseed muffin mix contained five times more ALA than the control muffin mix. As expected, the flaxseed

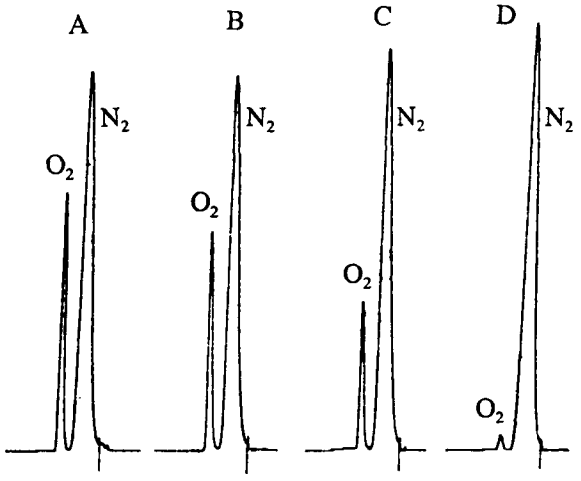

FIG. 1. Gas-chromatographic traces of headspace oxygen (O_2) and nitrogen (N_2) in reaction tubes containing: A) Control (headspace air before heating), B) whole flaxseed, C) flaxseed lipid extracts and D) ground flaxseed after 90 min at 178°C.

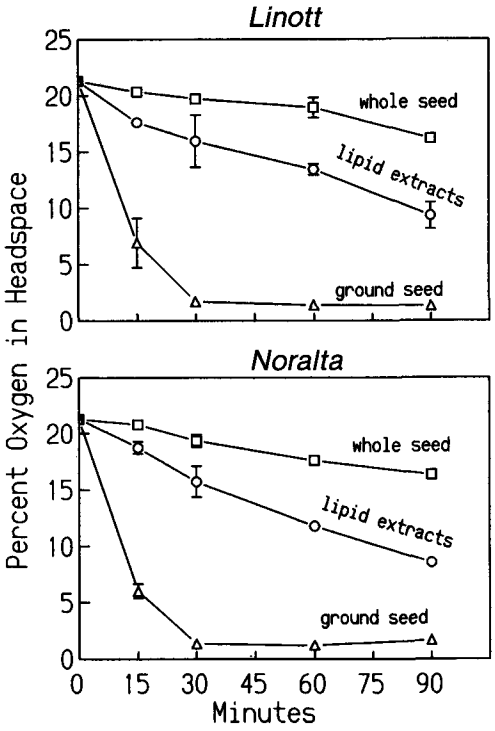

FIG. 2. Oxygen consumption profiles of two varieties (*Linott* and *Noralta*) of whole flaxseed (□), flaxseed lipid extracts (○) and ground flaxseed (△) at 178°C. Values were mean ± SD, n = 4 reaction tubes/time point.

TABLE 1

Effect of Heat Treatment (at 178°C for 90 min) on the Fatty Acid Composition (% of total fatty acids) in Whole Flaxseed, Ground Flaxseed and Lipid Extracts (mean ± SD/n = 4)

		After		
Fatty acid	Before	Whole seed	Lipid extracts	Ground
Palmitic	7.1 ± 0.6	7.5 ± 0.4	7.5 ± 0.5	8.1 ± 0.1
Stearic	2.2 ± 0.1	2.5 ± 0.2	2.8 ± 0.1	2.6 ± 0.1
Oleic	21.5 ± 0.2	22.7 ± 1.5	23.8 ± 0.7[a]	23.4 ± 0.5[a]
Linoleic	13.8 ± 0.1	13.9 ± 0.1	14.1 ± 0.1	14.0 ± 0.1
α-Linolenic	55.1 ± 0.3	53.2 ± 1.3	51.8 ± 0.9[a]	51.3 ± 0.5[a]
18:3 *trans*	trace	trace	trace	trace

[a]$P < 0.01$ in contrast to the value before heating.