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Processing and Cooking Effects on Lipid Content and Stability of α-Linolenic Acid in Spaghetti Containing Ground Flaxseed

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Research was conducted to determine the effect of processing and cooking on the content of hexane-extractable lipid and the stability of α-linolenic acid (ALA) in spaghetti fortified with ground flaxseed. Lipid content, ALA, and free fatty acids (FFA) were lower in dried spaghetti samples than in the original semolina-flaxseed mixture. The data indicate that the decline in lipid, ALA, and FFA contents occurred during the extrusion process. In contrast, conjugated diene levels were greater in dried spaghetti than in the corresponding premix. Conjugated diene level was similar for spaghetti samples dried using low- or high-temperature drying cycles, and was lower in cooked than in uncooked spaghetti. The low levels of FFA and conjugated diene indicate that ALA remained stable during processing and cooking of spaghetti fortified with ground flaxseed.

KEYWORDS: Flaxseed; α-linolenic acid; pasta; processing; semolina; stability

INTRODUCTION

Flaxseed has been reported to contain several healthful components: secoisolariciresinol diglycoside, dietary fiber mucilage, and α-linolenic acid (ALA) (*1*−*3*). ALA is an omega-3 fatty acid and has been reported to be useful in prevention and treatment of coronary artery disease, hypertension, and type 2 diabetes (*4*, *5*). ALA is the precursor fatty acid for the synthesis of eicosapentaenoic acid and docosahexaenoic acid which are associated with control of cardiovascular diseases (*6*). ALA reduces the risk of cardiovascular disease by lowering serum triglycerides and reducing the development of thrombosis and arteriosclerosis (*7*). However, oxidation of ALA is a concern because unsaturated lipids exposed to air can oxidize into compounds associated with rancidity (*8*).

ALA is associated with vegetable oil instability (*9*). The presence of a food matrix may improve ALA oxidative stability. Malcolmson et al. (*10*) reported that milled flaxseed could be stored up to 4 months at ambient temperatures without noticeable changes in quality. Chen et al. (*11*) reported ground flaxseed was stable for 280 d when stored at room temperature and a 12-h light/dark cycle (*11*). Bread (*10*), muffins (*11*), and spaghetti (*12*) have been fortified with flaxseed. Chen et al. (*11*) reported minimal oxidation of ALA from flaxseed flour during baking.

Information is lacking concerning the stability of ALA in spaghetti during processing and cooking. Elevated temperatures associated with processing and subsequent cooking of pasta could reduce ALA stability. This research was conducted to determine the effect of processing and cooking on the stability of ALA in spaghetti fortified with ground flaxseed.

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MATERIALS AND METHODS

Sample Preparation. Commercial semolina was obtained from the North Dakota State Mill and Elevator (Grand Forks, ND). Omega cultivar flaxseed was obtained from Reimers Seed Company (Carrington, ND). Flaxseed was ground on an Urschel Commitrol mill (Urschel Laboratories, Valparaiso, IN). A portion of the whole-ground flaxseed was sieved for 2 min using a rebolt machine fitted with a number 20 rebolt sieve and a number 34 XXGG sieve (Swiss silk grit gauze). The ground flaxseed that passed through the number 34 XXGG sieve (531 μm) was collected and considered fine ground. The whole-ground and fine, sieved flaxseed were mixed with semolina to concentrations of 5, 10, and 15 wt%/wt. Semolina and ground flaxseed were mixed 5 min using a cross-flow blender.

Extrusion. Semolina-flaxseed mixtures were hydrated to 32% moisture and extruded as spaghetti using a semicommercial laboratory extruder (DEMACO, Melbourne, FL). Extrusion occurred under the following conditions: extrusion temperature, 45 °C; mixing chamber vacuum, 46 cm of Hg; and auger extrusion speed, 25 rpm. Spaghetti were dried in a laboratory pasta dryer using a low-temperature (40 °C) or a high-temperature (70 °C) drying cycle (*13*).

Cooking Parameters. Spaghetti (10 g) was cooked in boiling water (300 mL) for 12 min. Spaghetti was drained into a Büchner funnel, rinsed with a stream of distilled water (∼50 mL), and then freeze-dried.

Oil Recovery and Analysis. The freeze-dried cooked spaghetti (10 g) and the dried uncooked spaghetti (50 g) samples were ground using a laboratory falling number mill (model 3100, Perten Instruments North America, Inc., Springfield, IL). Lipid oil content in ground spaghetti samples was determined using a 16-h Soxhlet extraction with hexane (*14*).

Fatty acid composition of extracted oil was determined by a modified method described by Welch (*15*) and Schwarz et al. (*16*). The fatty acids were hydrolyzed and determined as their methyl esters. Oil (40 mg) and 5 mL of 2% H_2SO_4 in methanol (v/v), containing methyl heptadecanoic acid (17:0; 200 mg/L) as an internal standard, were mixed

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Table 1. Total Lipid Content (mg/g, db[a]) of Semolina-Flaxseed Premix and Uncooked and Cooked Spaghetti

	processing[b]			cooking[b]	
		uncooked		cooked	
mixture	premix[c]	LTDC[d]	HTDC[d]	LTDC	HTDC
semolina	10.9b	5.6a	4.2a	5.6a	6.2a
semolina + WGF[e] 5%	29.2d	22.7c	23.5c		
semolina + SF[e] 5%	31.8d	22.0c	23.5c		
semolina + WGF 10%	48.4f	44.3e	45.0e		
semolina + SF 10%	51.6g	44.1e	47.2e,f		
semolina + WGF 15%	66.9h	65.4h	67.5h	68.3b	66.5b
semolina + SF 15%	71.0i	66.0h	68.2h,i	68.1b	65.8b

[a] db = dry basis. [b] Values not sharing a common letter are significantly different ($P \leq 0.01$). [c] Premix = dry mixture of semolina and ground flaxseed prior to pasta processing. [d] LTDC = low-temperature (40 °C) drying cycle; HTDC = high-temperature (70 °C) drying cycle. [e] WGF = whole-ground flaxseed; SF = sieved flaxseed.

together and heated at 100 °C for 1 h. The samples were mixed using a vortex mixer every 15 min. The samples were then cooled to room temperature. Hexane (3 mL) was added to each sample and the samples were mixed using a vortex mixer for 5 s, then distilled water (3 mL) was added, and the samples were mixed for 5 s. The solvent mixture was allowed to separate, and the top layer (hexane) was transferred to auto-sampler vials. A Hewlett-Packard (HP) 5890 gas chromatograph (Hewlett-Packard, Inc., Palo Alto, CA) fitted with a flame ionization detector and equipped with a SP-2330 fused silica capillary column (30 m × 0.25 mm i.d., and 0.20 μm film thickness) (Supelco, Bellefonte, PA) was used for analysis under the following conditions: column flow rate of 1 mL/min; initial column temperature 150 °C (held for 5 min), raised 10 °C/min to a final temperature of 180 °C; and injector and detector temperatures at 200 °C. Individual fatty acids were confirmed by retention times and quantified against peak area standard plots of known fatty acid concentrations. Each sample was analyzed in duplicate.

ALA degradation was determined by using a modified conjugated dienoic acid method (*14*). Extracted oil (80 mg) was mixed with isooctane (50 mL). A sample (1 mL) was withdrawn, and absorbance at 268 nm was measured using a HP 8452A diode array spectrophotometer (Hewlett-Packard, Inc., Palo Alto, CA).

Free fatty acid (FFA) content in the oil was determined by using a modified FFA method (*14*). Heated (60 °C) isopropyl alcohol (0.75 mL) was added to 75 mg of oil in a 25-mL Erlenmeyer flask. Then, phenolphthalein (0.5% in 95% ethanol) was added to the solution. Fatty acid content was determined by titrating with 0.00025 N KOH.

Statistical Analysis. The experiment was a randomized complete block design. Each treatment, semolina-flax mixture, was replicated three times. Data were subjected to analysis of variance. Means were separated by Fisher's Protected LSD at the $p \leq 0.01$ probability level.

RESULTS AND DISCUSSION

Content of hexane-extractable lipid was 387 mg/g of whole-ground flaxseed, 425 mg/g of sieved flaxseed, and 11 mg/g of semolina. These lipid contents are typical for flaxseed and semolina (*10*, *11*, *17*). Fifty percent of the whole-ground flaxseed passed through the 531-μm sieve. The higher lipid content of the sieved flaxseed, compared to that of the whole-ground flaxseed, indicates that sieving may have inadvertently removed more hull than cotyledon from the sample. Removing hull particles would increase lipid content because oil concentration in flaxseed cotyledon is greater than that in the hull (*18*).

Lipid content of semolina-flaxseed premixes (original semolina-flaxseed mixture prior to processing) reflects both the flaxseed concentration and the differences in lipid content between ground and sieved flaxseed (**Table 1**). However, lipid content of spaghetti containing similar amounts of whole-ground and sieved flaxseed were not different. Lipid content was statistically lower ($p \leq 0.01$) in dried spaghetti samples containing semolina alone or semolina fortified with 5 and 10% ground flaxseed than in their corresponding premixes (**Table 1**). The apparent decline in lipid content probably occurred during dough development in the extrusion process. Fabriani et al. (*19*) reported that extractable fat content decreased during the transformation of semolina into pasta. Lipids have been reported to bind to gluten (*20*, *21*). Kobrehel and Sauvaire (*21*) reported a strong interaction between lipids and durum wheat sulfur-rich glutenins. Drying temperature did not affect the lipid content of spaghetti containing whole-ground or sieved flaxseed (**Table 1**). These data support the concept that the decline in lipid content occurred during the extrusion process.

Lipid content of cooked spaghetti did not differ ($p \leq 0.01$) greatly between spaghetti containing 15% whole-ground or sieved flaxseed (**Table 1**). Although not statistically different ($p \leq 0.01$), the lipid content of cooked spaghetti containing whole-ground or sieved flaxseed tended to be lower in spaghetti dried at high temperature than that in spaghetti dried at low temperature.

Table 2. Fatty Acid Distribution (%, db[a]) of Lipids Extracted from Ground Flaxseed and Premix and Spaghetti Made from Semolina, and Semolina Fortified with 15% Ground Flaxseed[b]

sample	drying cycle[c]	palmitic	stearic	oleic	linoleic	linolenic
ground flaxseed (GF)	none	5.4	3.7	22.7	17.1	51.1
semolina	premix	19.9	0.6	21.1	54.9	3.4
	LTDC	17.0	1.7	21.8	44.7	14.9
	HTDC	20.4	1.0	21.9	53.3	3.3
semolina + GF 5%	premix	10.3	2.8	22.1	29.4	35.3
	LTDC	10.0	2.9	22.5	27.6	37.0
	HTDC	10.1	2.9	22.5	27.8	37.0
semolina +GF 10%	premix	8.3	3.2	22.3	24.4	41.9
	LTDC	8.3	3.2	22.5	23.4	42.7
	HTDC	8.3	3.2	22.4	23.6	42.5
semolina + GF 15%	premix	7.4	3.3	22.3	22.1	45.0
	LTDC	7.4	3.3	22.4	21.3	45.8
	HTDC	7.5	3.3	22.6	21.6	45.4

[a] db = dry basis. [b] Values are the average of sieved and whole-ground flaxseed. [c] Premix = dry mixture of semolina and ground flaxseed prior to pasta processing; LTDC = low-temperature (40 °C) drying cycle; HTDC = high-temperature (70 °C) drying cycle.

Fatty acid distributions of lipid extracted from premix and spaghetti made from ground flaxseed, semolina, and semolina fortified with ground flaxseed are presented in **Table 2**. The fatty acid distribution was similar for whole-ground and sieved flaxseed (data not presented). Lipids from ground flaxseed and semolina differed in their fatty acid distribution and are typical for flaxseed and semolina (*17*, *18*, *21*). ALA was the predominant fatty acid in lipid extracted from ground flaxseed (51.1%), whereas linoleic acid was the predominant fatty acid in lipid extracted from semolina (54.9%). Semolina lipids also contained a higher amount of palmitic acid (20%) than did lipid from ground flaxseed (5.4%). Premix and dried spaghetti had similar fatty acid distributions (**Table 2**). This indicates that processing and drying temperature did not affect fatty acid distribution of lipids extracted from spaghetti made from semolina or semolina-flaxseed blends.

Spaghetti made from semolina and dried at low temperature had a high percentage ALA (14.9%) relative to that of the premix (3.4%) or the spaghetti dried at high temperature (3.3%) (**Table 2**). Analysis of spaghetti made from three different sources of semolina but dried at LT indicated a wide range in percentage ALA (1.8−23.5%). This variation in percent ALA

Table 3. α-Linolenic Acid Content (mg/g, db[a]) of Semolina-Flaxseed Premix and Uncooked and Cooked Spaghetti

	processing[b]			cooking[b]	
		uncooked		cooked	
mixture	premix[b]	LTDC[d]	HTDC[d]	LTDC	HTDC
semolina	0.4a	0.8a	0.1a	0.2a	0.2a
semolina + WGF[e] 5%	10.2c	8.6b	8.8b		
semolina + SF[e] 5%	11.4c	8.0b	8.6b		
semolina + WGF 10%	20.1e	19.2d,e	19.3d,e		
semolina + SF 10%	21.7e	18.6d	19.8d,e		
semolina + WGF 15%	29.8g	29.7g	30.5g	31.5b	31.4b
semolina + SF 15%	32.3h	30.3g	30.4g	31.6b	29.8b

[a] db = dry basis. [b] Values not sharing a common letter are significantly different ($P \leq 0.01$). [c] Premix = dry mixture of semolina and ground flaxseed prior to pasta processing. [d] LTDC = low-temperature (40 °C) drying cycle; HTDC = high-temperature (70 °C) drying cycle. [e] WGF = whole-ground flaxseed; SF = sieved flaxseed.

Table 4. Free Fatty Acid Content (%, db[a]) of Lipid Extracted from Semolina-Flaxseed Premix and Uncooked and Cooked Spaghetti

	processing[b]			cooking[b]	
		uncooked		cooked	
mixture	premix[c]	LTDC[d]	HTDC[d]	LTDC	HTDC
semolina	21.6k	2.6i	3.9j	2.4b	4.3c
semolina + WGF[e] 5%	1.8g,h	1.2b–e	1.5e–g		
semolina + SF[e] 5%	2.0h	1.8g,h	1.6f,g		
semolina + WGF 10%	1.3c–f	0.8a	0.8a		
semolina + SF 10%	1.2b–e	1.0a–c	0.9a,b		
semolina + WGF 15%	1.4d–f	0.8a	0.9a.b	0.6a	0.9a
semolina + SF 15%	1.1a–d	0.8a	0.8a	0.7a	0.8a

[a] db = dry basis. [b] Values not sharing a common letter are significantly different ($P \leq 0.01$). [c] Premix = dry mixture of semolina and ground flaxseed prior to pasta processing. [d] LTDC = low-temperature (40 °C) drying cycle; HTDC = high-temperature (70 °C) drying cycle. [e] WGF = whole-ground flaxseed; SF = sieved flaxseed.

was not seen with premix or HT dried spaghetti. Despite differences in percentage ALA, the ALA content (mg/g spaghetti) was similar for premix and spaghetti made from semolina and dried at low or high temperature (**Table 3**).

ALA content in the premix and spaghetti made from semolina fortified with ground flaxseed increased with increased ground flaxseed concentration (**Table 3**). ALA content was 219 mg/g and 199 mg/g for sieved and whole-ground flaxseed, respectively. Thus, the ALA content in ground flaxseed reflects the greater lipid content of sieved than whole-ground flaxseed.

The effect of processing on ALA content was inconsistent. At a given concentration, ALA content of the premix and spaghetti was similar for semolina containing 0, 10, and 15% whole-ground flaxseed; but it was lower with spaghetti with 5% whole-ground or sieved flaxseed and 15% sieved flaxseed (**Table 3**). The data indicate that ALA content was not affected by drying temperature but might be affected during extrusion. ALA content of lipids extracted from cooked spaghetti did not differ between spaghetti dried at low and high temperatures or between spaghetti containing 15% whole-ground or sieved ground flaxseed (**Table 3**).

FFA content was 0.4% for both whole-ground and sieved flaxseed. FFA content of premix semolina was 21.6% (**Table 4**). FFA content in durum flour has been reported to range from 9 to 9.9% (*22*, *23*). The high FFA value of premix semolina oil (21.6%) could be due to lipase activity in bran, or to high concentrations of phenolic acids (*24*). Lipase found primarily in the bran is active at low moisture levels and can catalyze the hydrolysis of triacylglycerides into FFA. Lipase activity during grain storage or bran contamination in semolina would result in elevated FFA content in semolina (*25*, *26*). Additionally, phenolic acids could cause acidic conditions in the premix semolina oil and premix semolina-flaxseed oil which could affect FFA determination. FFAs are problematic as they are substrate for lipoxygenase. Lipoxygenase oxidizes unsaturated FFA resulting in rancidity.

FFA content of lipids extracted from dried spaghetti made from semolina was much lower than that found in lipids extracted from premix semolina (**Table 4**). Similarly, FFA content of lipids extracted from spaghetti containing whole-ground and sieved flaxseed tended to be lower than that in the corresponding premix. The apparent reduction in FFA content is probably due to FFA binding to starch and protein during gluten formation (*20*) which occurs during pasta extrusion. FFA content of cooked and uncooked spaghetti made from semolina was greater with spaghetti dried at high temperature than that of spaghetti dried at low temperature. However, drying temperature did not affect FFA content in cooked or uncooked spaghetti containing whole-ground and sieved flaxseed.

Conjugated dienes (0.05%) were detected in the semolina premix (**Table 5**). The presence of conjugated dienes indicates that some lipid oxidation had occurred in the semolina before processing. Conjugated dienes detected in the semolina-flaxseed premix probably originated from the semolina because conjugated dienes were not detected in the freshly ground flaxseed (data not presented).

Conjugated diene content was greater in dried spaghetti than in the corresponding premix (**Table 5**). This increase in conjugated diene was not due to drying temperature, as the conjugated diene content was similar for spaghetti dried at low- or high-temperature drying cycle. Lipid oxidation could occur during the hydration/mixing stage prior to extrusion. Oxidation is less likely to have occurred during extrusion because extrusion occurred under vacuum. Conjugated diene values for cooked spaghetti were not greater than those for uncooked spaghetti. Thus, cooking did not have a negative effect on the stability of the oil in flaxseed-spaghetti or in traditional spaghetti. In cooked spaghetti, conjugated diene values were lower in spaghetti fortified with flaxseed than in traditional spaghetti. These differences reflect the conjugated diene values found in the uncooked spaghetti samples.

These results indicate that the amount of hexane-extractable lipids and detectable FFA were reduced during pasta processing.

Table 5. Conjugated Diene Content (%, db[a]) of Lipid Extracted from Semolina-Flaxseed Premix and Uncooked and Cooked Spaghetti

	processing[b]			cooking[b]	
		uncooked		cooked	
mixture	premix[c]	LTDC[d]	HTDC[d]	LTDC	HTDC
semolina	0.05a–c	0.22f	0.22f	0.18b	0.18b
semolina + WGF[e] 5%	0.02a,b	0.13d,e	0.09c,d		
semolina + SF[e] 5%	0.02a,b	0.16e	0.13d,e		
semolina + WGF 10%	0.01a	0.10c,d	0.07b,c		
semolina + SF 10%	0.01a	0.13d,e	0.06a–c		
semolina + WGF 15%	0.03a,b	0.07b,c	0.07b,c	0.01a	0.00a
semolina + SF 15%	0.03a,b	0.10c,d	0.13d,e	0.01a	0.03a

[a] db = dry basis. [b] Values not sharing a common letter are significantly different ($P \leq 0.01$). [c] Premix = dry mixture of semolina and ground flaxseed prior to pasta processing. [d] LTDC = low-temperature (40 °C) drying cycle; HTDC = high-temperature (70 °C) drying cycle. [e] WGF = whole-ground flaxseed; SF = sieved flaxseed.

The low levels of FFA and conjugated diene indicate that the triacylglycerols and ALA remained stable during processing and cooking of spaghetti fortified with ground flaxseed. Previous research indicated that spaghetti containing ground flaxseed could be produced that had acceptable cooking quality (*12*). Thus, spaghetti fortified with ground flaxseed could be used to increase consumption of ALA in our daily diet.

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