FLAXSEED

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I. INTRODUCTION

Flaxseed or linseed (*Linum usitatissimum*) is an ancient crop that has been used for food and fiber. In North America, flaxseed is the preferred term for flax used in human consumption whereas Europeans use the term linseed for edible flax (Vaisey-Genser and Morris, 2003). Historical records indicate that flaxseed dates back to around 9000–8000 B.C. in Turkey (van Zeiste, 1972), Iran (Helbaek, 1969), Jordon (Hopf, 1983; Rollefson *et al.*, 1985), and Syria (Hillman, 1975; Hillman *et al.*, 1989). Although the evidence does not clearly show that flaxseed was cultivated, the seeds have been found alongside domesticated wheat and barley (Zohary and Hopf, 2000). Domestication of flaxseed is clearly evident around 7000–4500 B.C. (reviewed in Vaisey-Genser and Morris, 2003; Zohary and Hopf, 2000). The first probable use of flaxseed as food may have been as an ingredient in breads (Stitt, 1994) and as a laxative (Judd, 1995).

Flaxseed is grown in approximately 50 countries most of which are in theNorthern Hemisphere. In 2002, Canada was the largest producer of flaxseed accounting for approximately 33%, of the 2 million metric tons produced, followed by China (20%), the United States (16%), and India (11%) (Berglund, 2002). In general, the world supply of flaxseed has remained constant. Flaxseed acreage in the United States reached 516,000 harvested acres in 2004. Ninety-four percent of the flaxseed was grown in North Dakota followed by Montana (4%), and South Dakota (2%) (National Agricultural Statistics Service, 2005). Production in North Dakota totaled 10.5 million bushels and the yield per acre was 20.3 bushels (National Agricultural Statistics Service, 2005). The estimated harvest acreage for 2005 in North Dakota was approximately 955,000 acres (National Agricultural Statistics Service, 2006). Flaxseed acreage in the Canada totaled 1.9 million with a production of approximately 42 million bushels in 2004 (Agriculture and Agri-Food Canada, 2006).

History shows that flaxseed has been used as an ingredient in breakfast cereals and breads; however, since the 1990s, a number of products containing flaxseed have been developed primarily for the health food market. The renewed interest in flaxseed as a food source is due to findings that suggest that flaxseed can provide a variety of health benefits (Thompson and Cunnane, 2003). The components that contribute the health benefits include lignans (secoisolariciresinol diglucoside [SDG] being the predominant form), α-linolenic acid (ALA), and nonstarch polysaccharides (i.e., gum or fiber).

Flaxseed is an oilseed that contains roughly 38–45% oil. ALA, a polyunsaturated lipid, accounts for 52% of the fatty acids in the oil. Flaxseed is also a rich source of plant lignans (up to 13 mg/g flaxseed). The interest in ALA and

lignans as food ingredients has opened opportunities for the utilization of flaxseed in foods. In contrast, the same level of interest has not been observed for other flaxseed components, such as protein and dietary fiber, which account for 20% and 28% of the flaxseed, respectively (Carter, 1993). This chapter will provide a general overview of flaxseed research completed over the past 50 years with the major focus being on data from 1990 to 2006. It will highlight the basic composition, health benefits, and finally the processing and application of flaxseed.

II. FLAXSEED COMPONENTS

A. FLAXSEED OIL

Flaxseed oil content falls in the range 38–45% oil depending on location, cultivar, and environmental conditions (Daun *et al.*, 2003; Oomah and Mazza, 1997). Kozlowska (1989) reported an average of 41.4% oil content for Polish cultivars. North Dakota flaxseed cultivars ranged from 31.9% to 37.8% oil (Hettiarachchy *et al.*, 1990). Wakjira *et al.* (2004) reported oil contents between 29.1% and 35.9% among flaxseed cultivars grown in Ethiopia.

In addition to oil content, fatty acid distribution in flaxseed can be affected by environmental conditions (Taylor and Morrice 1991). Growing conditions and variety can influence the unsaturated fatty acid content in flaxseed (Daun *et al.*, 2003). In contrast, the environment may also have an undesired impact on flaxseed composition. Early and late frosts, heat damage, and drought could have detrimental effects on flaxseed quality (Daun *et al.*, 2003). Significantly lower oil contents and a darken oil from frost-damaged immature seeds was reported by Gubbels *et al.* (1994). In addition, higher concentrations of palmitic (P), linoleic (La), and linolenic (Ln) acids and lower oleic (O) acid were observed in damaged seed compared to normal seeds.

Wanasundara *et al.* (1999) reported that neutral lipids (acylglycerols and fatty acids) constitute 96% of the total lipid in flaxseed, whereas polar lipids (glycolipids and phospholipids) account for 1.4%. Stenberg *et al.* (2005) observed similar findings except that less phospholipid was detected. Froment *et al.* (1999) discussed the effects of cultivar, location, and late harvest on phospholipid content. Neutral lipid fraction of flaxseed meal was 95–98% triacylglycerols (TAG) and thus accounts for the predominant lipid in flaxseed (Oomah *et al.*, 1996).

Ayorinde (2000) reported trilinolenate (sn-LnLnLn; 35%) as the most predominant TAG in flaxseed oil. In a study, Holcapek *et al.* (2003) observed comparable results (Table I). TAG and diacylglycerol (DAG) composition of

TABLE I
TRIACYLGLYCEROL DISTRIBUTION OF FLAXSEED[a,b]

Triacylglycerols (TG)	%	Triacylglycerols (TG)	%
LnLnLn	30.4	OLnP	3.1
LaLnLn	18.7	LnLaP	3.0
OLnLn	13.5	SLaLa	1.1
LnLnP	6.9	OLaLa	1.0
OLaLn	5.9	LaLaLa	0.9
LaLaLn	5.3	OLaO	0.8
OLnO	4.2	LaOP	0.6
SLnLn	4.1	PLnP	0.5

[a]Adapted from Holcapek *et al.* (2004).
[b]P, palmitic acid; S, stearic acid; O, oleic acid; La, linoleic acid; Ln, linolenic acid.

flaxseed oil was analyzed by high-performance liquid chromatography-mass spectrometry (HPLC-MS) with atmospheric pressure chemical ionization. The most abundant TAG was again sn-LnLnLn (30.4%) but was slightly lower than the observations of Ayorinde (2000) who used matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) as a means to detect the TAG species. Other flaxseed TAG included sn-LaLnLn, sn-OLnLn, sn-LnLnP, sn-LaLaLn, and sn-OLaLn. In total, 16 TAGs were detected, which is lower than the TAG totals of soybean, sunflower, almond, pistachio, hazelnut, poppy seed, palm, Brazil nut, macadamia, and rapeseed oil samples. The composition of the DAG was mainly LnLn in flaxseed oil, which exhibited the lowest equivalent carbon number (24) among oil samples.

Flaxseed has a high ALA content, generally constituting 50–62% of total fatty acids (Daun *et al.*, 2003). Dorrell (1970) reported that fatty acid distribution in flaxseed varied depending on the anatomical fractions. The hull is the main source of palmitic acid, but it has a relatively low oil content. Lower oleic and ALA and higher linoleic contents are present in the embryo compared to whole seed. Oomah and Mazza (1997) also observed higher levels of palmitic acid in the hull. However, the hull and whole seed gave similar ALA values, compared to dehulled seed.

Fatty acid composition of flaxseed was affected by cultivar (Froment *et al.*, 1998; Oomah and Mazza, 1997). DeClercq (2005) reported that average ALA content of Canadian flaxseed grown in 2004 was 61.9%. Similar high (56.5–61%) ALA levels were reported by Bozan and Temelli (2002) for Turkish flaxseed. Results were higher than the average values of

flaxseed grown in Poland (57.1%) (Kozlowska, 1989), Ethiopia (51.9%) (Wakjira *et al.*, 2004), and North Dakota (48.4%) (Hettiarachchy *et al.*, 1990). In our laboratory, we observe ALA contents in organic flaxseed of approximately 50–52%. Linola, a low-ALA cultivar, developed for commercial vegetable oil market was reported to contain 3–4% ALA and 75% linoleic acid (LA) (Lukaszewicz *et al.*, 2004). Stenberg *et al.* (2005) reported that high-ALA flaxseed from Sweden contained 60.4% ALA, whereas high-LA flaxseed exhibited only 2.7% ALA. Furthermore, differences in oil content and fatty acid profile could be related to methods used to measure these components. Type of solvent, extraction time, and sample preparation could have a major impact on analytical results. Supercritical fluid CO_2 extraction gave a higher average ALA content (60.5%) compared to the soxhlet extraction method (56.7%) (Bozan and Temelli, 2002). Sikorska *et al.* (2005) discussed scanning fluorescence spectroscopy method as a means to detect adulteration in various oils including flaxseed oil.

B. PROTEIN

The protein content in flaxseed has been reported to be between 10.5% and 31% (Oomah and Mazza, 1993). The protein content of the most important flaxseed cultivars grown in different countries are presented in Table II. Protein values of flaxseed from Poland were generally well above 24%, whereas those from Canada were lower than 20% except for the cultivar AC Linora. The protein content of 24.1% for No. 1 Canadian Western flaxseed, from the 2001 harvest survey, was 1.7% higher than in 2000 and 1.6% higher than the 10-year mean of 22.5% (Canadian Grain Commission, 2001). Khategaon cultivar grown in India had a protein content of 21.9% (Madhusudhan and Singh, 1983). The protein contents of 11 flaxseed cultivars grown in North Dakota ranged from 26.9% to 31.6% (Hettiarachchy *et al.*, 1990). The USDA reports the protein content of flaxseed as 19.5% (USDA, 2005). Differences in protein can be attributed to both genetics and environment. However, a conversion factor of 6.5 (Oomah and Mazza, 1993) from nitrogen to protein was used in the calculation of protein content in Canada varieties, whereas a factor of 5.41 (Oomah and Mazza, 1998a) was also used in the calculation of flaxseed protein content. Thus, protein differences may be due to the conversion factor used in the determination of protein (Table II).

The protein contents of flaxseed fractionation from research work in different countries is presented in Table III. The proximate protein contents of dehulled and/or defatted flaxseed varied considerably, depending on cultivar, growth location, and seed processing. Oomah and Mazza (1997) reported that the hull fraction contains lower protein levels, and

TABLE II
PROTEIN CONTENT OF DIFFERENT FLAXSEED CULTIVARS[a]

Poland		Canada		USA	
Kozlowska, 1989		Oomah and Mazza, 1993		Hettiarachchy *et al.*, 1990	
Variety	Protein %	Variety	Protein[b] %	Variety	Protein %
Avangard	24.7	Linott	19.81	Clark	27.3
Reina	25.2	Noralta	19.82	Culbert	30.0
Viking	25.6	Dufferin	18.42	Dufferin	31.6
Ottawa	25.8	McGregor	19.07	Flor	31.0
Hera	27.4	NorLin	19.49	Linott	26.9
Zielona	27.9	NorMan	19.45	Linton	29.4
Bionda	28.0	Vimy	19.03	McGegor	27.1
LCSD200	28.2	Andro	19.89	Neche	29.5
Svapo	28.6	Somme	19.67	Norlin	28.1
–	–	Flanders	19.33	Norman	27.0
–	–	AC Linora	20.18	Verne	29.5

[a]Protein contents are calculated on dry basis.
[b]N × 6.5.

that dehulling increases protein content of flaxseed from 19.2% to 21.8%. Bhatty and Cherdklatgumchal (1990) reported a protein content of about 20% in flaxseed hull, which was identical to that reported in an earlier study by Peterson (1958). Bhatty and Cherdklatgumchal (1990) also reported that the mean protein contents for laboratory-prepared meals and commercial meals were statistically different. Researchers from Germany (Krause *et al.*, 2002) reported that flaxseed meal from dehulled seed had a protein content of 50%, which was close to the data reported by Indian researchers (Madhusudhan and Singh, 1983).

The protein content in various extracts obtained from different extraction methods is given in Table IV. Krause *et al.* (2002) extracted protein by isoelectric precipitation and micellization. The protein contents for isoelectric-precipitated protein isolate and micelle protein isolate were the same (Table IV). According to Dev and Quensel (1988), the protein contents of high-mucilage protein concentrate from seed (HMPC-S) and high-mucilage protein concentrate from expeller cake (HMPC-EC) were comparable (63.4% and 65.5%), while low-mucilage protein isolate (LMPI) contained much higher protein (86.6%). These results were consistent as Zhang (1994) also observed protein contents of 66.3% and 86.5% in the high-mucilage protein isolates HMPI and LMPI, respectively.

TABLE III
PROTEIN CONTENT OF FLAXSEED FRACTION[a]

Fractionation	Protein %	Cultivar	Reference	Country
Seed	19.2	NorMan, Linola™947, McGregor, NorLin, Omega, Flanders, and Vimy	Oomah and Mazza, 1997	Canada
Dehulled seed	21.8			
Hull	17.3			
Dehulled seed	23.9	NorMan	Lei *et al.*, 2003	
Meal	22.9			
Hull	20.3	–	Bhatty and Cherdklatgumchal, 1990	
Laboratory-prepared meal	43.9	Norlin, NorMan, and McGregor		
Commercial meal	34.7	–		
Dehulled meal	50.0	–	Krause *et al.*, 2002	Germany
Dehulled meal	48.9	Khategaon	Madhusudhan and Singh, 1983	India
Meal	49.0	Viking	Sammour, 1999	France

[a]Protein data are based on dry basis.

TABLE IV
PROTEIN CONTENT OF PROTEIN EXTRACT

Protein extract	Protein %	Reference	Country
Micelle protein isolate	93.0	Krause *et al.*, 2002	Germany
Isoelectric-precipitated protein isolate	89.0		
LMF[a]	56.4[b]	Dev and Quensel, 1988	
LMPC[a]	59.7[b]		
HMPC-S[a]	63.4[b]		
HMPC-EC[a]	65.5[b]		
LMPI[a]	86.6[b]		
HMPI	66.3	Zhang, 1994	China
LMPI	86.5		

[a]LMF, low mucilage flour; LMPC, low-mucilage protein concentrate; HMPC-S, high-mucilage protein concentrate from seed; HMPC-EC, high-mucilage protein concentrate from expeller cake; LMPI, low-mucilage protein isolate.
[b]N × 6.25.

Albumin- and globulin-type proteins are the major proteins in flaxseed. According to Madhusudhan and Singh (1983), flaxseed albumin comprised 20% of total meal protein. Marcone *et al.* (1998) reported that the globulin fraction makes up 73.4% of total protein, and the albumin constitutes about 26.6% of total protein. In contrast, Youle and Huang (1981) reported that 2S proteins accounted for 42% of the total seed proteins. Sammour (1999) also reported albumin accounted for 40.2% of the total protein content.

The amino acid profiles of flaxseed cultivars from different countries are given in Table V. Although there were variations among varieties, all flaxseeds had similar amino acid profiles. Flaxseed proteins are relatively high in arginine, aspartic acid, and glutamic acid, whereas lysine, methionine, and cystine were the limiting amino acid. Madhusudhan and Singh (1985a) investigated the amino acid composition of water-boiled flaxseed meal. Boiling appeared not to affect amino acids as the amino acid composition between the boiled and raw flaxseed meal were not significantly different. In contrast, germination of the seed significantly changed the amino acid content in the flaxseed. Wanasundara *et al.* (1999) reported that the total amino acid content of the flaxseed, after an 8-day germination, increased by about 15 times, with the greatest increase (i.e., 200 times) being observed in glutamine and leucine compared to the original seed. Wanasundara and Shahidi (1993) reported the amino acid profiles of solvent-extracted flaxseed meal and compared those to commercial meal. Commercial meal had slightly lower values for all amino acids than the laboratory-prepared meal (Table VI). They also noted that there were only minor changes in the amino acid content of flaxseed meal

TABLE V
AMINO ACID CONTENT (G/100 G PROTEIN) OF DIFFERENT FLAXSEED CULTIVARS

	Poland	Canada[a]			India
	Kozlowska, 1989	Oomah and Mazza, 1993			Madhusudhan and Singh, 1985a,b
Amino acid	LCSD 200	Norlin	Foster	Omega	Khategaon
Ala	5.40	4.4	4.7	4.5	4.3
Arg	9.75	9.2	10.0	9.4	11.5
Asp	10.40	9.3	10.0	9.7	11.2
Cys	–	1.1	1.8	1.1	–
Ser	–	4.5	4.7	4.6	5.1
Glu	22.50	19.6	20.0	19.7	19.8
Gly	6.41	5.8	5.9	5.8	4.8
His	1.42	2.2	2.1	2.3	2.5
Leu	–	5.8	6.0	5.9	5.8
Ile	3.53	4.0	4.1	4.0	4.6
Lys	1.80	4.0	4.0	3.9	4.1
Met	1.44	1.5	1.4	1.4	1.7
Phe	4.94	4.6	4.8	4.7	5.9
Pro	3.16	3.5	3.8	3.5	4.6
Thr	–	3.6	3.8	3.7	3.9
Tyr	1.53	2.3	2.4	2.3	3.3
Val	5.69	4.6	5.1	4.7	5.6

[a]Adapted from Oomah and Mazza (1993).

when changing from a nonpolar solvent to a solvent with increased polarity. Bhatty and Cherdklatgumchal (1990) also reported higher amino acid contents in laboratory-prepared meal than commercial meals, which is comparable to amino acid data of linseed meal reported previously by Sosulski and Sarwar (1973) and Madhusudhan and Singh (1985a).

The amino acid profiles were consistent among researchers with abundances in arginine, aspartic acid, and glutamic acid but deficient in sulfur-containing amino acids. Amino acid profiles in different flaxseed protein fractions are listed in Table VII. Sammour *et al.* (1994) and Madhusudhan and Singh (1985a) found the contents of glutamic acid and lysine were higher in albumin than in globulin, whereas, methionine was higher in globulin. Chung *et al.* (2004) also found similar amino acid profiles (Table VII) of the major protein fractions to previous researchers (Madhusudhan and Singh, 1985b; Marcone *et al.*, 1998). The data suggests that the amino acid composition of the protein fractions is less variable than total protein content of the

TABLE VI
AMINO ACID COMPOSITION OF LABORATORY-PREPARED AND COMMERCIAL FLAXSEED MEALS (G/100 G PROTEIN)

	Wanasundrara and Shahidi, 1994			Bhatty and Cherdklatgumchal, 1990	
Amino acid	Hexane extracted	MAW-HE[a]	Commercial meal	Laboratory prepared	Commercial meal
Ala	4.81	4.64	4.61	5.4	5.5
Arg	11.50	11.20	9.78	11.8	11.1
Asp	9.18	9.16	8.03	12.5	12.4
Cys	3.29	3.39	3.16	3.8	4.3
Glu	16.70	16.36	14.45	26.3	26.4
Gly	6.44	6.26	5.64	7.0	7.1
His	2.69	2.46	2.36	2.9	3.1
Ile	4.78	4.54	4.19	5.2	5.0
Leu	6.70	6.39	5.96	6.8	7.1
Lys	4.38	4.14	3.92	4.1	4.3
Met	1.45	1.41	1.24	2.2	2.5
Phe	5.13	4.91	4.63	5.3	5.3
Pro	3.64	3.65	3.32	5.2	5.5
Ser	4.94	4.99	4.48	5.8	5.9
Thr	3.40	3.33	3.00	4.9	5.1
Try	0.46	0.46	0.25	1.8	1.7
Tyr	2.21	2.12	1.98	2.9	3.1
Val	5.75	5.64	5.02	5.6	5.6

[a]MAW-HE, meal extracted with a multiple solvents (methanol-ammonia-water/hexane-extracted meal).

flaxseed. Furthermore, the country of origin has minimal impact on amino acid composition even if total protein did change.

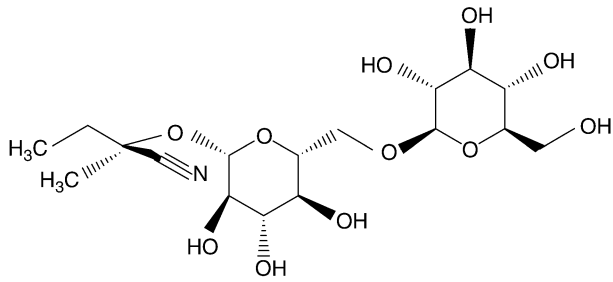
C. CYANOGENIC GLYCOSIDES

Flaxseed contains cyanogenic glycosides, such as linamarin, linustatin, lotasutralin, and neolinustatin (Figure 1), which release toxic hydrogen cyanide upon hydrolysis. Cyanogenic glycosides content differs depending on location in the plant and stage of development (Niedzwiedz-Siegien, 1998).

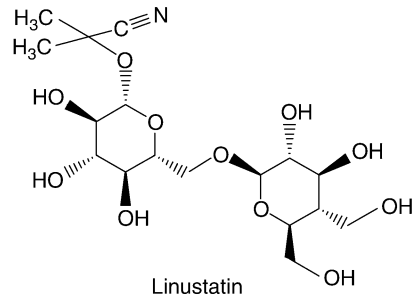
Ten Canadian flaxseed cultivars were analyzed for total cyanide content (Chadha, 1995) and contents of individual cyanogenic glycosides (Oomah *et al.*, 1992). Chadha *et al.* (1995) determined cyanide content in 10 cultivars of flaxseed using an autohydrolysis method that required up to 5 hours of hydrolysis time. The maximum cyanide values were typically obtained

TABLE VII
AMINO ACID CONTENT (G/100 G PROTEIN) OF FLAXSEED PROTEIN FRACTIONS

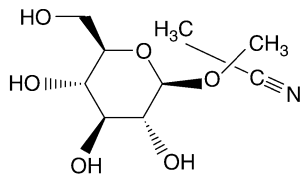
	France		India		USA	Canada	
	Sammour *et al.*, 1994		Madhusudhan and Singh, 1985b		Marocone *et al.*, 1998	Chung *et al.*, 2004	
Amino acid	Globulin	Albumin	12 S protein	Albumin	11 S protein	Major fraction	Whole protein extract
Ala	3.8	3.4	4.8	1.9	5.5	5.7	6.9
Arg	11.5	11.1	12.5	13.1	12.6	11.9	8.4
Asp	10.1	8.7	11.3	5.5	12.4	12.3	10.3
Cys	1.1	0.36	–	3.5	0.9	0.6	1.4
Glu	17.1	27.3	19.8	35	24.3	21.8	21.5
Gly	9.8	8.1	4.8	8.3	5.4	5.6	10.9
His	1.9	2.1	2.5	1.6	2.6	2.5	1.8
Ile	4.1	3.6	4.6	2.8	5.6	4.6	4.2
Leu	6.6	5.9	5.8	5.4	5.9	5.8	5.9
Lys	4.0	5.0	3.1	4.9	3.1	3.2	3.4
Met	2.0	1.1	1.7	0.8	1.3	1.3	1.3
Phe	4.0	3.1	5.9	2.4	6.3	5.8	4.1
Pro	2.1	2.2	4.5	3.0	–	4.2	3.9
Ser	8.0	5.7	5.1	3.9	6.5	4.6	6.4
Thr	6.1	4.1	3.9	2.1	3.6	3.1	3.9
Try	–	–	1.3	2.0	–	–	–
Tyr	2.4	2.2	2.3	1.4	2.4	2.4	1.7
Val	4.1	4.1	5.6	2.6	5.1	4.7	4.7



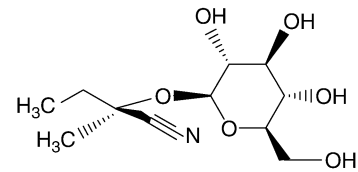
Neolinustatin



Linustatin



Linamarin



Lotasutralin

FIG. 1 Cyanogenic glycosides from flaxseed.

by 2–3 hours of hydrolysis (Table VIII). Oomah *et al.* (1992) reported that the amount of three cyanogenic glycosides present in 10 Canadian cultivars were significantly different, with linustatin and neolinustatin being the most abundant cyanide-containing compounds. Linamarin was reported to be present at very low levels (<32 mg/100 g seed) in 8 of the 10 cultivars (Table VIII). Oomah *et al.* (1992) concluded that the content of these three cyanogenic glycosides was dependent on cultivar, location, and the year of production of the seed, with cultivar being the most important factor.

TABLE VIII
CYANOGEN CONTENT (MG/100 G SEED) IN DIFFERENT FLAXSEED CULTIVARS

	Chadha *et al.*, 1995	Oomah *et al.*, 1992		
Cultivar	Cyanide	Linamarin	Linustatin	Neolinustatin
Ac Linora	14.5	19.8	269	122
Andro	19.6	16.7	342	203
Flanders	12.5	13.8	282	147
Linott	14.4	22.3	213	161
McDuff	15.4	–	–	–
McGregor	13.8	25.5	352	91
Noralta	–	20.3	271	163
Norlin	15.1	ND[a]	295	201
NorMan	12.4	ND[a]	231	135
Somme	16.6	27.5	322	149
Vimy	13.7	31.9	262	115

[a]ND, not detected.

Various procedures have been investigated to detect the cyanogenic glycoside via the release of hydrogen cyanide (HCN) (Amarowicz *et al.*, 1993; Bhatty, 1993; Kobaisy *et al.*, 1996; Kolodziejczyk and Fedec, 1995; Oomah *et al.*, 1992). Kobaisy *et al.* (1996) compared the accuracy of three methods, barbituric acid-pyridine, pyridine-pyrazolone, and HPLC, for the determination of cyanogens in flaxseed. They found that the total hydrogen cyanide values obtained by all three methods were not statistically different, although those obtained by the HPLC method were higher than those from the colorimetric methods. Their finding disagreed with those of Schilcher and Wilkens-Sauter (1986), who found that the HCN concentrations determined using HPLC were lower than those determined by a photometric method. Amarowicz *et al.* (1993) reported the chromatographic techniques for the preparation of linustatin and neolinustatin from flaxseed. The compounds isolated this way had a high purity (>99%) and could be used as standards for the analysis of cyanogenic glycosides by HPLC (Amarowicz *et al.*, 1993).

Various methods could be used to reduce the cyanogenic glycosides content in the seed. Wanasundara *et al.* (1993) investigated the use of solvents extraction as a means to reduce cyanogenic glycosides content of flaxseed meal. They found that extraction of flaxseed with alkanol-ammonia-water/hexane resulted in enhanced removal of cyanogenic compounds from 41 to

TABLE IX
HYDROGEN CYANIDE CONTENT (MG/100 G SEED) AFFECTED BY PROCESSING OF THE FLAXSEED

	Feng *et al.*, 2003		Yang *et al.*, 2004	
Processing method	HCN	Reduction (%)	HCN	Reduction (%)
Flaxseed raw	37.7		15.8	
Autoclaved	26.5	29.7	11.5	27
Microwave	6.35	83.2	2.8	82
Pelleted	9.88	73.8	–	–
Oven heated at 130°C, 10 minutes	3.16	16.2	–	–
Oven heated at 130°C, 20 minutes	2.91	22.8	–	–
Solvent extracted	–	–	1.7	89
Water boiled	–	–	ND[a]	100

[a]ND, not detected.

16 mg/100 g (total cyanogenic glycosides as HCN equivalents) compared to using hexane alone. Cyanogenic glycosides could be further removed by increasing solvent volumes, duration of the extraction period, and number of extraction stages. Yang *et al.* (2004) reported hydrogen cyanide reductions of 89%, 27%, 82%, and 100% using solvent extraction, autoclaving, microwave roasting, and water boiling, respectively (Table IX). These results were very close to those reported by Feng *et al.* (2003), who reported that autoclaving and microwave heating reduced hydrogen cyanide by 29.7% and 83.2%, respectively (Table IX). In their study, they also reported a 73.8% hydrogen cyanide reduction using a California pellet mill. In addition, hot air oven heating at 130°C for 10 and 20 minutes resulted in 16.2% and 22.8% reduction of hydrogen cyanide.

Research has also shown that commercial processing of flaxseed could affect the cyanogenic glycosides content in flaxseed (Oomah and Mazza, 1997, 1998a). These authors noted that dehulling increased linamarin content in the cotyledon or embryo fraction in all cultivars except Omega, where linamarin decreased by 50% to 23 mg/100 g seed (Oomah and Mazza, 1997). A reduction was also observed in linustatin and neolinustatin content. The mean values of seven cultivars show that linamarin increased from 19 mg/100 g seed to 67 mg/100 g seed, and linustatin and neolinustatin contents increased by 150%, after dehulling. Thus, total cyanogenic glycosides increased from 311 mg/100 g to 515 mg/100 g after dehulling (Oomah and Mazza, 1997). Commercial processing of flaxseed increased the cyanogenic glycosides from 309 to 476 mg/100 g (Oomah and Mazza, 1998a).

D. DIETARY FIBER (MUCILAGE OR GUM)

Flaxseed mucilage, associated with hull of flaxseed, is a gum-like material and composed of acidic and neutral polysaccharides. The neutral fraction of flaxseed mainly contains xylose (62.8%), whereas the acidic fraction of flaxseed is comprised mainly of rhamnose (54.5%), followed by galactose (23.4%) (Cui *et al.*, 1994a). A study by Warrand *et al.* (2005) found that the neutral monosaccharides were a mixture of three major families of polymers, arabinoxylans with a constant A/X ratio of 0.24, and various amount of galactose and fucose residues in the side chains. Acidic hydrolysis yields xylose, galactose, arabinose, rhamnose, galacturonic acid, fucose, and glucose (BeMiller, 1973; Erskine and Jones, 1957).

Extraction of flaxseed gum has been extensively investigated (Cui *et al.*, 1994b; Garden, 1993; Luo *et al.*, 2003; Oomah and Mazza, 2001). Luo *et al.* (2003) extracted the flaxseed gum by hot water (90–95°C) for 50 to 60 minutes and achieved the gum yield of 13–14%. Among the extract methods used to separate flaxseed gum, including centrifugation, steam heating, and vacuum filtration, the steam heating method was chosen for its efficient separation of gum from the rest of the seed (Luo *et al.*, 2003). Normal-pressure drying, reduced-pressure drying, freeze drying, microwave drying, and spray drying methods have all been tested to obtain a dry flaxseed gum. Spray drying was found to be the ideal method and was scaled up for industrial production (Luo *et al.*, 2003). Using response surface methodology, Cui *et al.* (1994b) determined that the optimum conditions for the gum extraction were a temperature of 85–90°C, a pH of 6.5 to 7.0 and water: seed ratio of 13:1. Response surface methodology was also used to optimize the spray drying to achieve maximum yield and functionality (rheological properties) of flaxseed gum (Oomah and Mazza, 2001). In another study, Garden (1993) extracted flaxseed gum from the outer coating of Neche flaxseed. In her research, a nonspecific protease was used to purify the flaxseed gum. Ultrafiltration with a hollow membrane cartridge was used to concentrate the mixture. Garden (1993) reported that the yield of crude flaxseed gum from flaxseed was 5.0% and that of purified gum was 4.5%.

Flaxseed mucilage composition varies due to the growing environment and cultivars. Oomah *et al.* (1995a) reported the variation (3.6–8%) in the content of water-soluble polysaccharides in flaxseeds from different geographical regions and cultivars. Glucose was reported to be the most abundant main monosaccharide in flaxseed gum with a mean value of 28.9% followed by xylose, galactose, rhamnose, and arabinose. However, xylose was reported to be present at the highest level (up to 40%) in the polysaccharides of seven Canadian cultivars extracted at 40°C (Chornick *et al.*, 2002). The xylose content was affected by ethanol, in that a 40% ethanol

solution yielded higher mucilage than the 75% ethanol using precipitation as a means to concentrate the gum. In another study, Cui *et al.* (1996) studied yield and composition of flaxseed gums from six brown and six yellow cultivars. They noted that within yellow seeds, the minimum yield (5.2%) of gum was obtained from the APF 9006 cultivar and the maximum yield (6.5%) was from the Foster cultivar. They also noted that NorMan brown seed cultivar had the highest yield of 7.9%, whereas Royal has the lowest yield of 5.5%. Compared to gum yields (6.35%) from brown seeds, the yellow seed had a lower mean yield (5.85%) of gum among the 12 cultivars tested (i.e., six cultivars of each seed color). Xylose, galactose, rhamnose, and arabinose were the main components of both yellow seed and brown seed gums, with the content of 37.5, 19.2, 17.8, and 14.7% in yellow seeds, respectively, and 32.9, 21.6, 20.2, and 12.9% in brown seeds, respectively (Cui *et al.*, 1996). These results were consistent with data reported by Oomah *et al.* (1995a). However, the glucose contents (5.6% for yellow seed and 6.8% for brown seed) were much lower when compared to that of Oomah *et al.* (1995a), in which they reported the glucose (28.9%) to be the leading monosaccharide in flaxseed gum. A larger yield of mucilage was reported by Fedeniuk and Biliaderis (1994). They used higher extraction temperatures and Vega clay to purify the crude mucilage extract by reducing up to 80% of the protein that coextracted with the gum. More protein is typically extracted at higher temperature (80°C) than at lower 40–60°C temperatures.

Flaxseed gums extracted from different cultivars or by different methods exhibited various physiochemical properties (Cui and Mazza, 1996; Cui *et al.*, 1994a). Cui and Mazza (1996) tested for moisture, ash, mineral and nitrogen contents, amino acid composition, and intrinsic viscosity in both lab-prepared and commercial gums. Further characterization of the flaxseed gum was achieved by analysis of monosaccharide composition, galacturonic acid content, and ^{13}C NMR spectra. They found that intrinsic viscosity of flaxseed gum ranged from 434 to 658 ml/g, which was very different from gum Arabic (14.4 ml/g), guar gum (1135 ml/g), and xanthan gum (1355 ml/g). They concluded that among the gum extracted from the four cultivars, there were large variations in physicochemical properties. In another study, Cui *et al.* (1994a) noted that dialyzed flaxseed gums contain lower protein and higher carbohydrates and slightly lower mineral contents, with a similar monosaccharide ratio as crude flaxseed gums. These variations in flaxseed gums may be potentially suited for specific applications (Cui and Mazza, 1996). For additional review regarding applications of flaxseed gums, see the review of Chen *et al.* (2002).

Shan *et al.* (2000) reported that flaxseed gum had good foamability, stability, emulsibility, and salt resistance, and that flaxseed gum has the

same rheology as non-Newtonian flow. The gum viscosity remains stabile over a broad pH 6 to12 (Shan *et al.*, 2000). In contrast, Mazza and Biliaderis (1989) reported that the viscosity of 0.05–0.5% (w/v) solutions exhibited Newtonian-like behavior at concentrations below 0.2% and shear thinning at concentrations above 0.2% (w/v) (Mazza and Biliaderis, 1989). Oomah and Mazza (1998b) found that the soluble carbohydrate content of flaxseed after commercial processing increased from 98.6 to 177.9 g/kg on a dry basis. Thus, variations in carbohydrate may account for different flow properties.

In addition to hot water extraction, chemical and enzymatic treatments were also investigated to remove the flaxseed mucilage. Wanasundara and Shahidi (1997) reported that soaking of seeds in sodium bicarbonate solution improved the removal of mucilage from seeds as compared with using water alone. Treatment with Celluclast® 1.5 L (45 mg protein/100 g for 3 hours or 22.5 mg protein/100 g for 6 hours) had a similar effect in reducing the mucilage content of the seed as soaking the seed in sodium bicarbonate solution (0.05 M, pH 8.16) for 12 hours. The effect of two enzymes, Pectinex Ultra SP and Viscozyme® had a similar effect in removal of mucilage as Celluclast® 1.5 L (Wanasundara and Shahidi, 1997).

E. POLYPHENOLS AND LIGNANS

Phenolic compounds are widely distributed in plants. In oilseeds, phenolic compounds occur as the hydroxylated derivatives of benzoic and cinnamic acids, coumarins, flavonoid compounds, and lignans (Ribereau-Gayon, 1972). Oomah *et al.* (1995b) reported the total phenolic acids (PA) in eight Canadian cultivars ranged from 790 to 1030 mg/100 g with esterified PA accounting for 48–66% of the total PAs, which was comparable to the 54% found by Varga and Diosady (1994). Dabrowski and Sosulski (1984) reported that flaxseed contained 811 mg/100 g phenolic compounds in defatted flour. In another study, Velioglu *et al.* (1998) reported the total phenolics contents of flaxseed and flaxseed gum prepared by different methods. They noted that gum prepared by different methods could retain total phenolics as high as 1422 mg/100 g and as low as 328 mg/100 g from flaxseeds with original total phenolics of 473 and 509 mg/100 g, respectively. A study in our laboratory (Hall and Shultz, 2001) measured the SDG and PAs, including ferulic, coumaric, caffeic, chlorogenic, gallic, protocatechuic, p-hydroxybenzoic, sinapic, and vanillic acids in defatted flaxseed and non-defatted flaxseed extracts (Table X). The total phenolic content in the defatted flaxseed extract and non-defatted flaxseed extract were 13,233, and 5420 mg/100 g extract, respectively.

The main lignan in flaxseed is SDG (Figure 2). Also present are a number of other lignans, that is, matairesinol (MAT), lariciresinol, hinokinin,

TABLE X
PHENOLIC COMPOUNDS CONTENTS (MG/100 G)[a]

Phenolic compounds	NDFE[b]	DFE[b]
Ferulic acid	161	313
Coumaric acid	87	130
Caffeic acid	4	15
Chlorogenic acid	720	1435
Gallic acid	29	17
Protocatechuic acid	7	7
p-Hydroxybenzoic acid	1719	6454
Sinapic acid	18	27
Vanillin	22	42
Total	2767	8440
SDG	2653	4793

[a]Adapted from Hall and Shultz (2001).
[b]NDFE, non-defatted flaxseed extract; DFE, defatted flaxseed extract.

arctigenin, divanillyl tetrahydrofuran nordihydroguaiaretic acid, isolariciresinol, and pinoresinol (Muir *et al.*, 2000). The main interest in these compounds is as precursor to mammalian lignans, which have been shown to have health-promoting activity (see Section III later).

Analytical methods of extraction and hydrolysis for quantifying lignan content in flaxseed has been extensively investigated (Charlet *et al.*, 2002; Degenhardt *et al.*, 2002; Eliasson *et al.*, 2003; Harris and Haggerty, 1993; Liggins *et al.*, 2000; Mazur *et al.*, 1996; Obermeyer *et al.*, 1995; Rickard *et al.*, 1996; Westcott and Muir, 1996). Liggins *et al.* (2000) reported lignan content of MAT, secoisolariciresinol (SECO), and shonanin in Cambridge and Argentinian linseed. The former had a higher SECO and shonanin content (1262 mg/100 g dried seed) than the latter (880 mg/100 g dried seed), and a lower MAT content (5.9 mg/100 g dried seed) compared to that of the latter (9.1 mg/100 g dried seed). However, lignan contents in flaxseed and its meal reported by several other researchers were slightly lower, ranging from 81 to 371 mg/100 g dried wt (Mazur *et al.*, 1996; Obermeyer *et al.*, 1995; Setchell *et al.*, 1999; Thompson *et al.*, 1991, 1997). Johnsson *et al.* (2000) reported SDG contents of defatted flaxseed flour and whole flaxseeds for cultivars grown in Sweden and Denmark. They noted that among the 14 cultivars from Sweden, cultivars Flanders and Mikael had the lowest and highest SDG contents in defatted flaxseed flour of 1170 and 2270 mg/100 g dry matter, respectively. The SDG content of defatted flaxseed flour in 15 cultivars grown in Denmark ranged from 1440

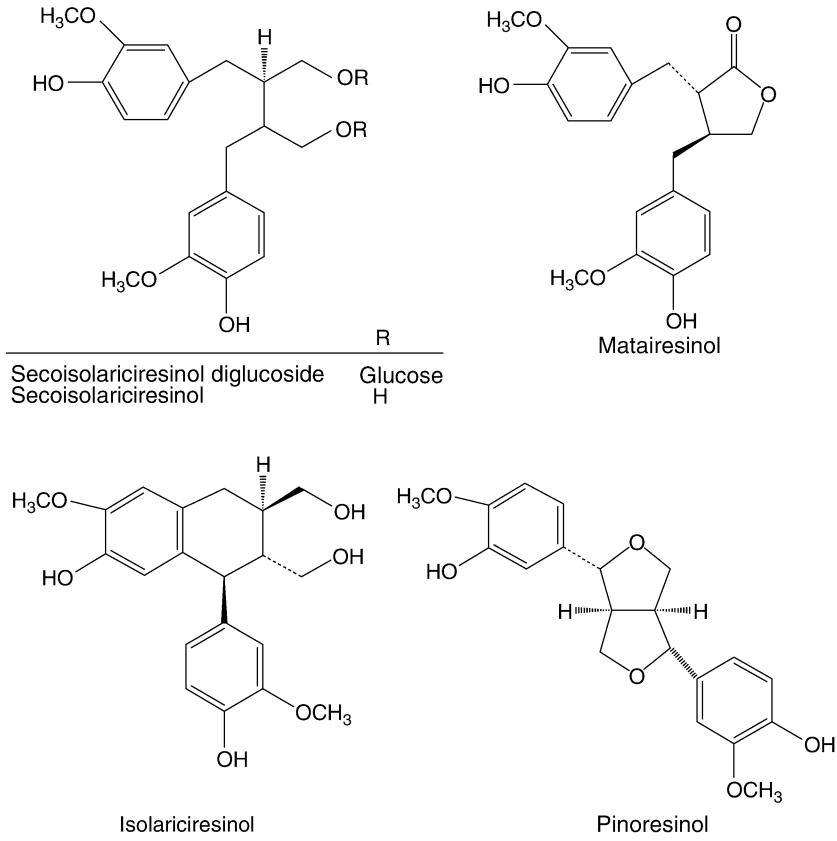


FIG. 2 Major lignans found in flaxseed.

to 2080 mg/100 g dry matter (Johnsson *et al.*, 2000). SDG contents in 27 Swedish cultivars were 1410 to 2590 mg/g dried seed (Eliasson *et al.*, 2003), which was slightly higher than the findings of Johnsson *et al.* (2000). Zimmermann *et al.* (2006) reported that flaxseed obtained from sites grown in Germany and Spain had lignan contents that were strongly correlated to cultivar and to a lesser extent the growing environment.

In addition to solvent extraction of lignans, a dry mechanical method for concentrating the lignan SDG was developed by Madhusudhan *et al.* (2000). As a result, the content of SDG increased from 1290 and 1430 mg/100 g in whole Neche and Omega seed, respectively, to 2760 and 2380 mg/100 g in the hull-rich fractions (Madhusudhan *et al.*, 2000).

F. OTHER COMPONENTS

Tocopherols consist of α, β, γ, and δ isomers and are effective antioxidants. Oomah *et al.* (1997a) observed that γ-tocopherol (9.04 mg/100 g seed) was the predominant isomer of Canadian flaxseed cultivars. Total tocopherol ($r = 0.42$) and γ-tocopherol ($r = 0.41$) values were correlated with seed oil content. Kamm *et al.* (2001) reported the distribution of tocopherols and tocotrienols in high and low linolenic flaxseed. Results were similar to the findings of Oomah *et al.* (1997a) in which γ-tocopherol content was greater (430–575 mg/kg oil) in high ALA flaxseeds, whereas low linolenic flaxseed exhibited lower (170 mg/kg oil) values. Bozan and Temelli (2002) compared tocopherol levels in oil extracted by supercritical CO_2 fluid and soxhlet (Table XI). Soxhlet-extracted oil had greater tocopherol levels (76.4 mg/100 g oil). These authors speculated that the temperature–pressure combination may have influenced the tocopherol extraction by supercritical CO_2 fluid.

Major flaxseed sterols are stigmasterol, camp sterol, and δ-5 avenasterol (Daun *et al.*, 2003). Obtusifoliol, gramisterol, and citrostadienol constituted 45%, 22%, and 12%, respectively, of the total 4α-monomethylsterol in flaxseed (Kamm *et al.*, 2001). Squalene content of flaxseed oil was reported as 4 mg/100 g oil, which was significantly lower than olive, corn, and rice bran oils. Squalene content is an intermediate compound of biosynthesis of plant sterols, which may have protective effects on lipid quality. Squalene could act as a peroxy radical scavengers in high polyunsaturated fatty acid oil (Dessi *et al.*, 2002).

Pretova and Vojtekova (1985) reported the presence of lutein, β-carotene, and violaxanthin in flaxseed. Carotenoids may serve as secondary antioxidants and scavenge singlet oxygen. In addition, carotenoids can function as chain-breaking antioxidants by trapping lipid-free radicals, in the absence of singlet oxygen (Belitz *et al.*, 2004). Daun *et al.* (2003) reported that Canadian flaxseed exhibited a range of 0–2 mg/kg chlorophyll which mostly disappeared during maturation.

TABLE XI
DISTRIBUTION OF TOCOPHEROLS AND TOCOTRIENOLS IN FLAXSEED (MG/100 MG OIL)[a]

Extraction method	α-Tocopherol	α-Tocotrienol	β- + γ-Tocopherol	δ-Tocopherol
Supercritical CO_2	0.21	0.67	53.74	0.95
Soxhlet	0.46	0.42	73.90	1.64

[a]Adapted from Bozan and Temelli (2002).

III. HEALTH BENEFITS

A. INTRODUCTION

1. α-Linolenic acid (ALA) and n-6 to n-3 fatty acid ratio

Flaxseed is one of the leading sources of ALA (Hauman, 1998), an omega-3 (n-3) fatty acid, which is essential for maintaining human health. The US Institute of Medicine, Food and Nutrition Board (2002) and Health Canada (Morris, 2003a) recommend ALA intakes of 1.1 and 1.6 g/day for women and men, respectively. Further recommendations of 1.3 and 1.4 g/day have been extended to lactating and pregnant women. Healthy individuals who have no signs of ALA deficiency are the basis for these recommendations. In infants, a 500 mg/day intake of long chain omega-3 has been recommended for proper neurological and cognitive development (Carver, 2003; Institute of Medicine, 2002). Other recommendations have been summarized by Nettleton (2003).

Nutrient deficiencies are difficult to research in humans because the test subject would have to be removed from that nutrient for an extended period of time, possibly to leading a state of starvation. Thus, clinical deficiencies of n-3 fatty acids have been reported by only two researchers. Both of these studies involved one patient each and different clinical symptoms were observed in the patient prior to ALA supplementation (Bjerve *et al.*, 1988; Holman *et al.*, 1982). Thus, our knowledge of symptoms of ALA deficiency is not complete. Furthermore, a complete removal of ALA may not be required for the manifestation of clinical symptoms related to an improper balance between ALA and other fatty acids in the diet. Many of these symptoms can be related to the overproduction of the proinflammatory eicosanoids, synthesized from fatty acids other than ALA, resulting in heart disease and cancer (Morris, 2003b).

Within the last decade, the health benefits of ALA have been documented in numerous studies and may be related to an improved n-6 to n-3 fatty acid intake. Nettleton (2003) summarized the recommendations of leading health organizations regarding the proper ratio of n-6 to n-3 fatty acid intake. Most organizations agree that a 5:1 to 10:1 n-6 to n-3 fatty acid ratio is preferred (Institute of Medicine, 2002; WHO/FAO, 2003). However, a typical western diet has an n-6 to n-3 fatty acid ratio well beyond 10:1; thus, flaxseed can be a valuable lipid source to improve the n-6 to n-3 fatty acid ratio due to the high n-3 content of flaxseed oil.

The recommendation for the n-6 to n-3 ratio stems from studies dating back to 1950 (Greenberg *et al.*, 1950). Studies show that ALA and LA (L, n-6) function synergistically at low concentration, but become competitive

at higher intakes (Bourre *et al.*, 1990; Greenberg *et al.*, 1950). Furthermore, excessive ALA intake may not necessarily be better since high ALA can act as a substrate inhibitor in the conversion to long chain n-3 fatty acids (Vermunt *et al.*, 2000). Arguments against the necessity of ALA in the diet have focused on the lack of ALA conversion to long chain n-3. The conversion of ALA to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in humans varies from 0.2% to 6% (Emken *et al.*, 1994; Pawlosky *et al.*, 2001); suggesting dietary factors may ultimately influence conversion. This was supported by several studies showing that dietary lipid influenced the conversion of ALA to EPA and DHA (Allman *et al.*, 1995; Emken *et al.*, 1999; Kestin *et al.*, 1990; Valsta *et al.*, 1996). Simopoulos (1999) recommends a LA to ALA ratio of 4:1 (e.g., 15 g LA: 3.7 g ALA) based on optimal conversion of ALA (11 g) to EPA (1 g). Thus, the proper balance between the n-6 and n-3 fatty acids in studies assessing conversion of ALA to long chain n-3 fatty acids EPA and DHA must be considered. Furthermore, iron, zinc, and possibly magnesium and vitamin B6 deficiencies could influence the conversion of ALA to long chain n-3 fatty acids (Cunnane and Yang, 1995; Cunnane *et al.*, 1987).

2. *Lignans*

Flaxseed is the richest source of plant lignans (Thompson *et al.*, 1991). SDG is the predominant lignan in flaxseed with minor amounts of pinoresinol and MAT (Meagher *et al.*, 1999; Thompson *et al.*, 1991). The lignans of flaxseed are phytoestrogens and serve as precursors in the production of mammalian lignans. Flaxseed lignans convert to the mammalian lignans enterolactone and enterodiol by intestinal flora (Adlercreutz *et al.*, 1982; Axelson and Setchell, 1981; Axelson *et al.*, 1982; Borriello *et al.*, 1985; Wang *et al.*, 2000).

A dose-dependent formation of mammalian lignans has been reported (Nesbitt *et al.*, 1999; Rickard *et al.*, 1996). In humans, a dose-dependent response was based on daily dietary intake of flaxseed between 5 and 25 grams as flaxseed or in the form of a baked product (Nesbitt *et al.*, 1999). Higher flaxseed intakes may not produce additional mammalian lignan concentrations based on the observation in rats that showed 4.4 μmol SDG/d did not produce higher mammalian lignan concentrations than the 2.2 μmol SDG/d treatment (Rickard *et al.*, 1996).

The concentration of the plasma lignans was also time dependent. The first day of the study showed that there was a continued increase in lignan concentrations in the plasma over a 24-hour period after the consumption of 25 grams of flaxseed. Whereas by the eighth day of the study, a high-level mammalian lignans was observed at the time of the flaxseed intake and that no significant change in plasma lignan was observed (Nesbitt *et al.*, 1999). The dose and

time dependences are critical parameters that must be considered in clinical investigations and may account for differences observed by researchers.

The metabolic products of the mammalian lignans have not been fully elucidated. Jacobs and Metzler (1999) found that enterolactone and enterodiol converted into 12 and 6 metabolites, respectively, using an *in vitro* hepatic microsome assay. This research group (Jacobs *et al.*, 1999; Niemeyer *et al.*, 2000) also found these metabolites in the urine of humans fed flaxseed and in the urine and bile of rats given the pure mammalian lignans intraduodenally. The metabolites were oxidation products of the mammalian lignans. The most interesting metabolite was the addition of hydroxyl units to the aromatic rings to make dihydroxy benzene ring structures (Figure 3). Heinonen *et al.* (2001) also found similar metabolites from syringaresinol using a human fecal inoculum assay.

The presence of the oxidized metabolites is unique and may provide additional reasons for the health benefits of lignans. Classical antioxidant mechanisms show that the addition of an ortho hydroxyl group to a monophenol enhances the antioxidant activity of the original monophenol. Thus, some of the mammalian lignan metabolites may actually have greater or different activity than the parent lignan. Kitts *et al.* (1999) reported that enterolactone and enterodiol had greater antioxidant activity than the parent

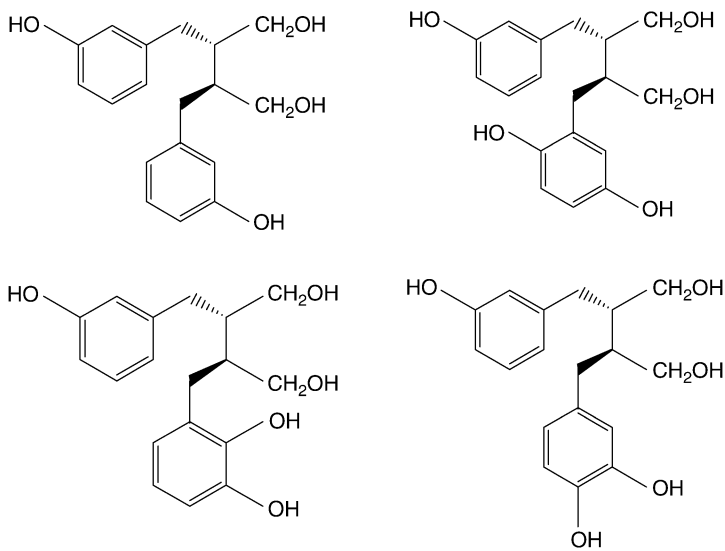


FIG. 3 Oxidized mammalian lignans observed by Jacobs *et al.* (1999) and Niemeyer *et al.* (2000) in rat and human urine.

lignan (SDG), suggesting that the metabolites might be the reason for the health benefits of the plant lignans.

The health benefits of flaxseed lignans is thought to be due to antioxidant activity, primarily as hydroxyl radical scavengers (Kitts *et al.*, 1999; Prasad, 1997a), and as estrogenic and antiestrogenic compounds due in part to the structural similarity to 17-β-estradiol (Adlercreutz *et al.*, 1992; Waters and Knowler, 1982). The behavior of the lignans depends on the biological levels of estradiol. At normal estradiol levels, the lignans act as estrogen antagonists, but in postmenopausal women (i.e., low estradiol levels) can act as weak estrogens (Hutchins and Slavin; 2003; Rickard and Thompson., 1997). Other activities related to estrogen include the *in vivo* synthesis of 2-hydroxy estrogen, a compound that may protect against cancer (Haggans *et al.*, 1999), and inhibit the binding of estrogen and testosterone to receptors on sex-binding globulin (Martin *et al.*, 1996). For further information regarding the estrogenic and antiestrogenic activity of flaxseed, see the review of Hutchins and Slavin (2003).

3. *Other components*

As previously mentioned, flaxseed is a rich source of dietary fiber (28%). Dietary fiber has been widely viewed as a component essential to lowering the risk of colon cancer. The flaxseed protein is similar to soy thus may be beneficial to health. Bhathena *et al.* (2002) first reported that flaxseed protein was effective in lowering plasma cholesterol and triacylglycerides (TAG) compared to soy and casein protein in male F344 and obese SHR/N-cp rats. The role of protein in disease prevention warrants further investigation. Components, such as PAs and flavonoids, may also contribute to the health benefits of flaxseed.

B. ROLE OF FLAXSEED IN CARDIOVASCULAR DISEASE PREVENTION

1. *Affect on biological lipid status*

a. Affect of ALA and flaxseed oil on lipid status. Kelley *et al.* (1993) reported that serum TAG, cholesterol, high-density lipoproteins (HDL), apoproteins A-I and B (markers for cardiovascular disease) of men fed a flaxseed oil (6.3% ALA) diet were not significantly different from the basal diet. However, an increase in ALA in serum and peripheral blood mononuclear cells (PBMNC) was found, as were EPA and DHA in the PBMNC. Men given a high ALA/low LA diet had significantly higher ALA in their plasma

phospholipid, cholesteryl esters, and TAG and neutrophil phospholipids (Mantzioris *et al.*, 1994). The men on the diet containing flaxseed oil had higher (2.5 fold) EPA levels in their plasma lipids and neutrophil phospholipids (Mantzioris *et al.*, 1994). These results support the suggestion of Chan *et al.* (1993) that the low LA:ALA (n-6 to n-3) ratio in the diet was important for incorporation of long chain n-3 into platelets and plasma phospholipids. Ranhotra *et al.* (1992) noted that flaxseed oil or blends of flaxseed oil and sunflower oil promoted cholesterol reduction in hypercholesterolemic rats compared to diets formulated with hard fats. These authors suggested that a diet with the appropriate balance of n-6 and n-3 fatty acids was preferred over diets high in n-6 fatty acids.

Wilkenson *et al.* (2005) also found that dietary fatty acid intake could affect biological lipid status. ALA and EPA levels in the plasma erythrocytes increased by 225% and 150%, respectively, in subjects fed a high ALA diet (i.e., 0.5 to 1 LA:ALA ratio). A reduction in arachadonic acid was also observed in the patients fed the high ALA diet compared to the high LA (i.e., 27.9 to 1 LA:ALA ratio). A 12.4% reduction in total plasma cholesterol and a 10% reduction in HDL cholesterol were also observed in patients on the flaxseed oil diet (Wilkenson *et al.*, 2005). Hussein *et al.* (2005) also reported similar findings in 57 male subject fed high ALA diets (17 g/day). ALA and EPA contents increased by three- and twofold in erythrocyte phospholipids, respectively. A 50% increase in docosopentanoic acid (DPA) levels in erythrocyte phospholipids was also observed but increases in LA, AA, or DHA contents were not found. Furthermore, ^{13}C tracer studies supported the observations in that the AA formation was directly related to LA intake and inversely related to ALA intakes (Hussein *et al.*, 2005). Gerster (1998) found radioisotope-labeled ALA conversion to EPA and DPA was affected by diet. In diets high in saturated fats, the ALA conversion to EPA and DHA was 6% and 3.8%, respectively. A 40–50% reduction in ALA conversion was found for diets high in n-6 polyunsaturated fats compared to diets high in saturated lipids.

Clandinin *et al.* (1997) found that fish oil significantly lowered plasma TAG compared to flaxseed and olive oils. However, total cholesterol and low density lipoprotein (LDL) levels in humans were slightly lower, but not significantly, in flaxseed and olive oils diets compared to fish oil. In this study, a lack of a difference between the control diet (i.e., olive oil) and flaxseed diet on plasma lipids may have been due to the small number of subjects (i.e., 26). In a placebo-controlled, parallel study involving 150 hyperlipidemic subjects, significant reduction in fasting plasma TAG after 2 months in subjects given 1.7 g EPA+DHA per day was observed (Finnegan and Minihane, 2003). However, after 6 months on this diet, the significance in the reduction diminished. The 9.5 g ALA/day significantly increased the EPA concentrations in

plasma phospholipids without significantly altering plasma TAG (Finnegan and Minihane, 2003).

b. Affect of flaxseed on lipid status. Weanling rats fed diets containing 20–40% flaxseed for 90 days had significantly lower total serum cholesterol and TAG levels than rats on flaxseed-free diets (Ratnayake *et al.*, 1992). Kritchevsky (1995) noted that rats fed a 20% flaxseed diet had a serum and liver cholesterol level that was 25% lower than rats fed a diet containing 10% flaxseed. The combination of defatted flaxseed meal and flaxseed oil caused a significant reduction in cholesterol levels in rats whereas the full fat flaxseed and flaxseed oil alone were not as effective (Ranhotra *et al.*, 1993).

Incorporating full fat flaxseed meal into the diet eliminated the adverse effect of hydrogenated soybean oil on serum cholesterol levels in hypercholesterolemic rats (Ranhotra *et al.*, 1993). In addition, full fat flaxseed meal enhanced the cholesterol-lowering effect of diets containing flaxseed oil. Cunnane *et al.* (1993) reported a 9% reduction in cholesterol (18% for LDL) in females fed 50 g of flaxseed per day. In addition, the n-3 fatty acid level increased in plasma and erythrocytes. No differences in plasma ALA were found in subjects fed 50 g flaxseed or 20 g flaxseed oil. Thus, when evaluating plasma ALA, the form in which ALA is consumed may not be as important as the level because 20 g flaxseed oil and 50 g flaxseed have equivalent ALA concentrations (12 g). However, a decreased postprandial glucose response was found in subjects fed 50 g flaxseed diet suggesting that soluble fiber has a positive impact on health and supports the consumption of flaxseed as a source of n-3 fatty acids. Jenkins *et al.* (1999) also noted a 5% and 8% reduction, respectively, in serum total and LDL levels in subjects fed partially defatted flaxseed. These researchers attributed the LDL reduction to the gum (i.e., soluble fiber) component of the flaxseed.

Arjmandi *et al.* (1998) reported a 6.9% reduction in cholesterol of postmenopausal women fed 38 g flaxseed. A 14.7% reduction in serum LDL was noted whereas serum HDL cholesterol and TAG were not affected. A 7.4% reduction in apoprotein A levels was also noted in the subjects. Identical reduction (7.5%) in apoprotein A levels was also noted in postmenopausal women on a 40-g/day flaxseed diet (Lucas *et al.*, 2002). Dodin *et al.* (2005) reported that significant reductions in serum total and HDL cholesterol of postmenopausal women on a 40-g/day flaxseed diet were observed in comparison to a wheat germ control diet. However, the authors considered these changes to be of little clinical importance. A significant observation was that flaxseed and wheat germ could reduce the severity of menopausal symptoms. Lemay *et al.*, (2002) also noted that a diet containing 40 g flaxseed/day improved menopausal symptoms in 25 hypercholesterolemic menopausal women. Glucose and insulin levels were lowered by the

flaxseed intake; however, only small nonsignificant changes in cholesterol levels were observed.

An extensive study was completed on rats to determine the effects of flaxseed intake during pregnancy and throughout the lives of their offspring (Wiesenfeld *et al.*, 2003). The general finding of the study support others in that an increase in ALA and EPA and decrease in AA were observed in the rats and their offspring fed flaxseed. Pregnant rats fed 20% and 40% flaxseed diets had significantly lower plasma LA compared to the rats on the control diet. A dose-dependent increase in plasma ALA were observed in all flaxseed treatments (20% and 40% flaxseed, 13% and 26% low-fat flaxseed meal) compared to the plasma of rats fed a nonflaxseed diet. Plasma AA contents also were significantly lower than the control for rats on all flaxseed diets except 20% flaxseed (Wiesenfeld *et al.*, 2003). Only the 20% and 40% diets were sufficient to promote a significant increase in plasma EPA. Similar trends in the results were observed in the offspring (i.e., F_1 rats). The F_1 rats on the 40% flaxseed diets had significantly lower total cholesterol after 90 days from weaning. As in other studies, HDL cholesterol accounted for most of the reduction. Based on their finding, Wiesenfeld *et al.* (2003) concluded that high flaxseed (40%) and flaxseed meal (26%) diets were safe based on the lack of observed deleterious effects in pregnant and F_1 rats.

2. *Affect on inflammatory markers and atherosclerosis*

Atherosclerosis is a disease that is characterized by deposition and accumulation of cholesterol and other blood lipids in blood vessel walls. Factors affecting the development atherosclerosis include high cholesterol (i.e., hypercholesterolemia), inflammatory compounds, such as eicosanoids and cytokines, and reactive oxygen species (Prasad, 1997b; Ross, 1999). Tumor necrosis factor (TNF), interleukin 1-β (IL-1 β), and platelet-activating factor (PAF) have been shown to promote the production of oxygen-free radicals by polymorphonuclear leucocytes (PMNLs) (Braquet *et al.*, 1989; Paubert-Braquet *et al.*, 1988; Stewart *et al.*, 1990). Reactive oxygen species can be synthesized during the metabolism of arachidonic acid to prostaglandins (Panganamala *et al.*, 1976) and leukotrienes (Murota *et al.*, 1990). Thus, control of these factors would result in a reduction of atherosclerosis and cardiovascular disease.

Caughey *et al.* (1996) reported that flaxseed oil inclusion into the diet of healthy volunteers resulted in 30% reduction in the production of the cytokines TNF-α and IL-1 β. These cytokines were inversely related to EPA levels in mononuclear cells. An intake of 1.8 g EPA and DHA per day and 9.0 g ALA per day over 4 weeks resulted in a 20%, 26%, and 36% reduction in IL-1 β, prostaglandin E_2 (PGE_2), and Thromboxane B_2 (TXB_2),

respectively (Mantzioris *et al.*, 2000). As in the previous study, EPA levels in mononuclear cells were inversely related to the inflammatory markers. Kew *et al.* (2003) reported a reduction of PGE_2 production in spleen mononuclear cells when EPA and DHA were located at the *sn*-1(3) position of the TAG. In contrast, EPA and DHA incorporation into the spleen mononuclear cells phospholipids was not dependent on the location of the fatty acid on the dietary TAG.

Allman *et al.* (1995) noted that platelet EPA levels were more than double for individuals fed flaxseed oil compared to sunflower oil group. Platelet EPA:arachidonic acid ratio (i.e., marker for thromboxane production and platelet aggregation potential) increased in the flaxseed group, thus a protective effect against cardiovascular disease, over LA-rich oils, would be expected. Their findings support the decreased platelet aggregation observed in hyperlipidemic subjects fed flaxseed (Bierenbaum *et al.*, 1993).

Zhao *et al.* (2004) reported that a high-LA (12.6% and 3.6% energy from LA and ALA, respectively) or -ALA (10.5% and 5.6% energy from LA and ALA, respectively) diet was significantly better than an average American (7.7% and 0.8% energy from LA and ALA, respectively) diet in controlling inflammatory markers in 23 subjects. Subjects on the high-ALA diet had lower serum LA and AA but higher serum ALA, EPA, and DPA than the high-LA diet. Serum C-reactive protein was also improved in subjects on the high-LA and -ALA diets compared to the average American diet. However, the reduction in C-reactive protein was only significant in the high-ALA diet (Zhao *et al.*, 2004). Vascular cell adhesion molecule-1 and E-selectin were lower in subjects on the ALA diet when compared to the LA diet. These parameters were inversely related to serum EPA and to a lesser extent DPA. The conclusion of the study was that the reduction of CVD by ALA occurs through several mechanisms based on the findings that the high ALA affected both lipids/lipoproteins and C-reactive protein/cell adhesion molecular parameters (Zhao *et al.*, 2004).

Prasad (1997b) found that a 46% reduction in hypercholesterolemic atherosclerosis (HCA) resulted from a daily flaxseed intake of 7.5 g/kg body weight in New Zealand white rabbits. This reduction was remarkable considering the fact that a lowering of serum cholesterol was not achieved during the 8-week feeding study. No atherosclerotic plaques developed in the rabbits on the cholesterol-free diets. Furthermore, flaxseed diets were able to decrease the oxygen-free radicals production by PMNLs. Subsequent work by this author assessed the role of ALA and lignan in the control of HCA (Prasad, 2005; Prasad *et al.*, 1998).

Rabbits fed a low-ALA flaxseed diet (7.5 g/kg body weight/day) plus 1% cholesterol had higher serum TAG and very low density lipoprotein (VLDL) cholesterol than the control group on a 1% cholesterol diet. However, total

cholesterol and LDL cholesterol at week 4 were lower by 14% and 17%, respectively, in the flaxseed plus 1% cholesterol diet compared to the 1% cholesterol diet. Further lowering (31% and 32%, respectively) of total cholesterol and LDL cholesterol were observed by week 8; addition of flaxseed to the diet was favorable (Prasad *et al.*, 1998). A 69% reduction in atherosclerotic plaques formation was observed in rabbits fed flaxseed plus 1% cholesterol diet compared to the 1% cholesterol diet. These authors suggested that the ALA was not responsible for the prevention of atherosclerosis. Prasad (2005) suggested that the lignan may act to prevent oxygen radical production by PMNLs, thus effectively reducing atherosclerosis. Data to support this hypothesis was based on several observations.

Rabbits fed a 0.5% cholesterol diet had atherosclerotic plaques on over 50% of the aorta surface whereas the 0.5% cholesterol plus 40 mg lignan complex/kg body/per day reduced atherosclerosis by 34.4% (Prasad, 2005). Furthermore, the added lignan complex lowered the total cholesterol, LDL cholesterol, serum, and aortic malondialdehyde by 20%, 14%, 35%, and 58%, respectively. Unlike previous studies, the lignan enhanced HDL cholesterol by 25% and 30% in normocholesterolemic and hypercholesterolemic rabbits, respectively. Slightly higher (33% and 35%) reductions in total cholesterol and LDL cholesterol, respectively, were observed in rabbits on a 1% cholesterol diet containing 15 mg SDG/kg/day (Prasad, 1999). A 73% reduction in atherosclerosis was observed in the rabbits given the SDG diets compared to the 1% cholesterol diets.

Similar trends were observed in ovariectomized Syrian hamsters (Lucas *et al.*, 2004). Flaxseed diets of 7.5%, 15%, or 22.5% for 120 days were able to prevent the rise in plasma cholesterol that was observed in the ovariectomized hamsters. Nearly 61% of the aorta contained fatty streaks in the ovariectomized hamsters, whereas the ovariectomized hamsters on the 7.5% and 22.5% flaxseed diets had aortic fatty streaks over 7.5% and 7.2% of the aorta, respectively (Lucas *et al.*, 2004). This difference was nearly an 88% reduction in atherosclerosis compared to ovariectomized hamsters.

3. *Significant clinical and epidemiological studies*

Although a large number of clinical studies on small groups of patients have been completed, only a small number of studies have evaluated large populations regarding potential benefits of flaxseed. Mozaffarian *et al.* (2005) completed a 14-year follow-up involving 45,722 men in the Health Professionals Follow-up Study. The first major conclusion from the study was that both plant and seafood n-3 fatty acids could reduce the risk of coronary heart disease. Furthermore, the association between ALA and reduced risk of coronary heart disease was enhanced in subjects consuming low levels of

EPA and DHA. In fact, these researchers observed a 47% and 58% lower risk of coronary heart disease and nonfatal myocardial infarctions, respectively, for each 1 g increase in ALA in subjects consuming low levels of EPA and DHA (Mozaffarian *et al.*, 2005). In contrast to low-EPA and -DHA consumption, ALA intake was not associated with reduced coronary heart disease in men with high- ($\geq$250 mg/d) EPA and DHA intake. This observation may be due to the way in which ALA is metabolized and that the already high EPA and DHA diminished the conversion of ALA to other anti-inflammatory lipids.

A second conclusion was that n-6 content in the diet did not appear to influence the benefit of ALA (Mozaffarian *et al.*, 2005), which also was supported from earlier studies (Djoussé *et al.*, 2001; Hu *et al.*, 1999). That the n-6 levels did not significantly alter ALA efficacy may be attributed to a number of factors such as LA intakes by the test subjects may not reflect the general population (Mozaffarian *et al.*, 2005). However, increasing concentrations of ALA reduced the risk of coronary heart disease (Djoussé *et al.*, 2001; Hu *et al.*, 1999). In the Family Heart Study (Djoussé *et al.*, 2001), higher ALA intakes were always associated with reduced prevalence odds ratio of coronary heart disease, even at high-LA intakes. These researchers also concluded that the ALA and LA acted synergistically. In contrast, a higher ALA and lower LA (i.e., approximately 5:1 LA:ALA) intake resulted in decreased platelet reactivity in Moselle farmers, suggesting a lower risk of myocardial infarctions (Renaud *et al.*, 1986). Zhao *et al.* (2004) also reported higher total n-3 fatty acids as serum LA:ALA ratios decreased. Thus, further research is needed to clarify discrepancies in experimental and clinical data.

In the Lyon Diet Heart Study, survivors of myocardial infarctions on a Mediterranean style diet rich in ALA were less likey to experience a second episode (de Lorgeril *et al.*, 1994). These researchers reported that nonfatal myocardial infarctions and total death in subjects on the experimental and control diets were reduced by 75% and 70%, respectively. Fewer nonfatal myocardial infarctions also were reported in subjects consuming an Indo-Mediterranean diet rich in ALA (Singh *et al.*, 2002). The Nurse's Health Study (Albert *et al.*, 2004) also showed that diets rich in ALA reduce the risk of dying from coronary heart disease. The study was a 16-year follow-up involving approximately 76,000 women. Women on the highest ALA (1.5 g/day) diet had a 21% and 46%, respectively, lower risk of dying from coronary heart disease or sudden cardiac death compared to women on a 0.7 g ALA/day diet.

The concentration of ALA in adipose tissue was also associated with a reduction in nonfatal acute myocardial infarctions (Baylin *et al.*, 2003; Guallar *et al.*, 1999). However, only the relationship in the study by Baylin *et al.* (2003) was sufficiently strong. In this study, 964 survivors of myocardial infarction in

Costa Rica were the test subjects. The results showed that the subjects with the highest adipose tissue ALA had a lower risk of additional myocardial infarctions.

The National Heart, Lung, and Blood Institute Family Heart Study showed that higher dietary ALA intake was associated with lower prevalence of carotid plaques (Djoussé *et al.*, 2003a). One interesting observation in the Djoussé *et al.* (2003a) investigation was that ALA was inversely related to the thickening of internal and bifurcation segments of the carotid arteries whereas LA and long chain n-3 fatty acids were not related to carotid artery disease. In a cross-sectional designed study involving approximately 4400 men and women between the ages of 24 and 93, ALA consumption was inversely related to plasma TAG concentrations (Djoussé *et al.*, 2003b). The men and women consuming the highest ALA had 26% and 14.6%, respectively, lower plasma TAG than the subject with the lowest ALA intake. This observation was in contrast to other reports that suggested ALA consumption increases plasma TAG (Bemelmans *et al.*, 2002; Layne *et al.*, 1996; McManus *et al.*, 1996). Djoussé *et al.* (2003a) suggested that higher ALA intakes might have been the reason for the discrepancies between studies.

The Multiple Risk Factor Intervention Trial (Dolecek, 1992) included over 12,000 men over an 8-year period. The results showed that higher ALA intakes were associated with lower risks of death due to coronary heart disease and cardiovascular disease. Furthermore, a 28% reduction in risk of stroke was associated with a 0.06% increase in the ALA content of serum phospholipids (Simon *et al.*, 1995). Other studies have since supported the association between ALA and reduction in stroke risk (Leng *et al.*, 1999; Vartiainen *et al.*, 1994). Vartiainen *et al.* (1994) followed a Finnish population of approximately 28,000 men and women over 20 years and found that a 60% reduction in mortality from stroke was associated with increased ALA consumption. In a study involving approximately 1,100 subjects, individuals suffering a stroke had significantly lower ALA concentrations in the red blood cell (Leng *et al.*, 1999).

Inflammation is an important factor in the development of cardiovascular disease. Most clinical studies involving inflammation parameters have been relatively small. The Nurses Health Study involving 727 women was the largest study designed to determine the effects of n-3 fatty acids on biomarkers of inflammation and endothelium activation (Lopez-Garcia *et al.*, 2004). They found an inverse association between ALA intake and plasma concentrations of C-reactive protein (a marker for inflammation), Interlukin-6, and E-selectin. Bemelmans *et al.* (2004) also found an inverse association between C-reactive protein and ALA intake in a randomized, double-blind placebo-controlled study involving 103 hypercholesterolemic subjects.

In general, ALA intake was associated with reduced risks of cardiovascular disease in many of the large population studies despite inconsistencies in serum lipid data. Only one study (Zutphen Elderly Study) did not report a benefit of ALA in reducing cardiovascular disease (Oomen *et al.*, 2001). However, this study relied on dietary intake of ALA from margarine and meat thus raising questions regarding the outcome of the study.

The benefits of flaxseed and flaxseed oil have been demonstrated in a number of animal and human studies. The resulting biological benefits may be related to the potential of ALA to block the formation and release of proinflammatory eicosanoids and cytokines, reducing apolipoprotein B formation, blocking platelet activation factor, and improving blood vessel flexibility (Morris, 2003b).

C. ROLE OF FLAXSEED IN CANCER PREVENTION

1. Introduction

Cancer is a complex disease characterized by several stages: initiation, promotion, proliferation (i.e., rapid growth), and metastasis (spread). Because of the complexity of this disease, developing treatments to control or eliminate the disease is difficult. Furthermore, not all cancers are alike, thus treatments may work against one type of cancer but not others. Treatment is complicated because many factors, such as inflammatory compounds, hormones, nutrients, and exposure to toxins, all contribute to the growth and spread of the disease. This section of the chapter will highlight only a few studies involving the role of flaxseed in cancer prevention. For more in-depth information, please see the reviews of Bougnoux and Chajés (2003), Saarinen *et al.* (2003), and Thompson (2003)

2. Breast cancer

a. General studies using flaxseed. Early cancer studies used Sprague-Dawley (SD) rats injected with the carcinogen dimethylbenzanthracene (DMBA) as a means to assess the anticarcinogenic activity of flaxseed. These rats were generally fed 5% or 10% flaxseed or defatted flaxseed prior to preinitiation (i.e., before injection of DMBA) or during the early promotion stage of carcinogenesis. Reduction in tumor cell proliferation (Serraino and Thompson, 1991, 1992), reduction in mammalian tumor size and number (Thompson *et al.*, 1996a,b), and having a positive affect in controlling the initiation and promotional stages of mammalian cancers (Serraino and Thompson, 1992) were observed in these early investigations.

The susceptibility of a women to breast cancer is believed to start very early in life (Russo *et al.*, 2001). Maternal and prepuberty dietary intakes and hormonal exposure play a significant role in the development to breast cancer (Hilakivi-Clarke *et al.*, 2001; Russo and Russo, 1995). Terminal end buds (TEBs) formation is a critical stage in the development of the mammary glands. During puberty or exposure to estrogen, the TEBs mature into alveolar buds and lobules (i.e., differentiated TEBs). Prior to this time, TEBs remain undifferentiated. Due to the high number of proliferative epithelial cells, undifferentiated TEBs are very susceptible to carcinogens (Russo and Russo, 1978). Furthermore, a correlation exists between the number of undifferentiated TEBs and cancer risk (Russo and Russo, 1978). Thus, interventions early in life may reduce cancer risks.

Tou and Thompson (1999) observed endocrine changes in virgin female rat offspring of dams fed either 5% or 10% flaxseed or the equivalent amount of SDG in 5% flaxseed (SDG-5f) diets. Mammary structure changes, primarily the density of TEBs, were observed in 50-day-old rats exposed to the 5% flaxseed diet during gestation and lactation and over a lifetime exposure. However, no changes in TEBs were observed in these rats fed a 5% flaxseed diet after weaning. Alveolar bud density increased in the 50-day-old rats fed the 10% flaxseed diet whereas TEB density decreased. The 50-day-old rats exposed to the SDG-5f diet during gestation and lactation had fewer TEBs and Alveolar buds. The major conclusion from this study was that the lower TEB formation in rats exposed to low flaxseed levels was due to delayed puberty and reduced number of estrous cycles (Tou and Thompson, 1999). In contrast, the lower TEB formation and higher alveolar bud density in rats exposed to 10% flaxseed levels was due to an earlier onset of puberty and increased number of estrous cycles. The final conclusion from this study was that mammalian cancers could be due to the reduction in TEBs (Tou and Thompson, 1999).

Further support for early exposure to flaxseed has recently been reported (Chen *et al.*, 2003). In this study, dams were fed either a basal diet or one containing 10% flaxseed or the equivalent amount of SDG in 10% flaxseed (SDG-10f) during the lactation period (i.e., up to 21 days). At no time was the rat offspring (i.e., pups) exposed directly to flaxseed during the suckling/lactation period. The pups were then given DMBA and 21 weeks later sacrificed. The tumor incidence, tumor load, mean tumor size, and tumor number were all significantly lower by approximately 31%, 51%, 44%, and 47%, respectively, in the 10% flaxseed group and 42%, 63%, 68%, and 45%, respectively, in the SDG-10f group compared to the control (Chen *et al.*, 2003). The observed improvements in tumorigenesis were due to mammary gland differentiation for rats exposed to flaxseed or SDG (Tan *et al.*, 2004). Near the end of the study (i.e., day 49–51 postnatal), lower numbers of

TEBs were observed and expression of epidermal growth receptor and estrogen receptors (ER) decreased in rats exposed to both the flaxseed- and SDG-fortified diets.

Downregulation of expression of epidermal growth receptor, insulin-like growth factor I, and vascular endothelial growth factor in mice injected with human breast cancer has been reported (Chen *et al.*, 2002; Dabrosin *et al.*, 2002). In both these reports, MDA MB 435 (cells that are known to metastasize) human breast cancer cells were injected into athymic mice and subjected to the basal diet or 10% flaxseed diet at week 8. Tumor growth rates and total incidence of metastasis were reduced significantly during the 7-week feeding (Chen *et al.*, 2002). The suppression of angiogenesis may account for the slow tumor growth rate and lower incidence of metastasis in mice on the 10% flaxseed diet (Dabrosin *et al.*, 2002).

In contrast to MDA MB 435 cells, MCF-7 human breast cancer cells lines are ER-positive (i.e., estrogen dependent). Chen *et al.* (2004) assessed the role of flaxseed on the inhibition of ER-positive cancers using MCF-7 human breast cancer cells lines injected into mice. In this study, a 10% flaxseed diet was fed to mice with low- (0.035 nmol/L) and high- (0.3 nmol/L) blood estrogen levels. A 74% reduction in pretreatment tumor size was observed in the low-estrogen mice. In contrast, the tamoxifen group initially reduced tumor size but in the end, no tumor size reduction occurred (Chen *et al.*, 2004). The combination of flaxseed and tamoxifen produced a 53% reduction in tumor size in mice as compared to the tamoxifen-treated mice. In mice with high blood estrogen, 22%, 41%, and 50% reductions in tumor size were observed in mice on the flaxseed, tamoxifen, and flaxseed plus tamoxifen diets, respectively, compared to the estrogen positive control. Increased apoptosis and reduced tumor proliferation were the reasons for the tumor size reductions (Chen *et al.*, 2004).

In human studies, flaxseed consumption has had positive impacts on carcinogenesis and parallel observations from animal studies. A double-blind, placebo-controlled, prospective clinical trial involving 39 women, newly diagnosed with breast tumors, was completed to evaluate the effect of flaxseed consumption on tumor growth (Thompson *et al.*, 2000). Subjects given flaxseed (25 g/day) diets had reduced tumor cell proliferation and c-erB-2 expression, and an increased apoptosis index compared with women who ate whole-wheat muffins.

Haggans *et al.* (1999) reported that 28 postmenopausal women on diets supplemented with 10 g/day of ground flaxseed had significantly higher urinary 2-hydroxyestrone (2-OHE1) excretion and ratio of 2-OHE1:16α-OHE1 (16α-hydroxyestrone). A high level of 16α-OHE1is associated with increased cell proliferation and thus is a measure of possible cancer risk (Gupta *et al.*, 1998). Brooks *et al.* (2004) also reported significant higher

concentrations of 2-OHE1 in urine of women fed a diet containing 25 g flaxseed/day than in the control and soy flour–fortified diets. In this study, 2-OHE1 and the ratio of 2-OHE1:16α-OHE1 increased by 103% and 98%, respectively, compared to the levels found by Haggans *et al.* (1999) supporting a dose-dependent response between the levels tested. Higher 2-OHE1 excretion and ratio of 2-OHE1:16α-OHE1 were also reported by Slavin *et al.* (2002). Furthermore, 10 g flaxseed/day decreased estradiol levels and estrone-sulfate concentrations.

Thompson *et al.* (2005) reported that tumor cell proliferation and c-erbB2 expression decreased by 34% and 71%, respectively, whereas cell apoptosis increased (31%) in menopausal women fed a diet containing 25 g of flaxseed. Changes in c-erbB2 score and apoptosis index were correlated with total flaxseed intake whereas cell proliferation, as measured by Ki-67 labeling index, was not. A main conclusion from these studies was that the lignans and, to a lesser extent, ALA were responsible for the anticarcinogenic activity.

b. Studies involving lignans. Lignans could be a significant part of a treatment regimen for cancer based on the large number of small-scale studies. Thompson *et al.* (1996a) reported a 46% reduction in the number of tumors per rat fed a diet that contained purified SDG at 1.5 mg/day (equivalent to a 5% flaxseed/day intake) compared to the basal diet. The feeding started 1 week after DMBA induction. A second study involving the same level of SDG showed that established tumor volumes were reduced by 54% and that new tumor numbers were 50% lower than the control and other treatments (Thompson *et al.*, 1996b). In this particular study, the 2.5% and 5% flaxseed diets also had positive effects on tumor reduction and growth. In contrast, 0.7 or 1.5 mg SDG/day dietary intakes did not significantly reduce tumor size in SD rats injected with N-methyl-N-nitrosourea (Rickard *et al.*, 1999). However, tumor size was lowest in rats fed the 5% diet and all treatments reduced tumor multiplicity and grade. The anticancer effect of flaxseed against N-methyl-N-nitrosourea-promoted cancers was thought to be due partly to the reduction of insulin-like growth factor I (Rickard *et al.*, 1999).

Mousavi and Adlercreutz (1992) found that enterolactone stimulated the growth of MCF-7 cells *in vitro* at low concentrations (0.5 and 10 μM), but at concentrations above 10 μM an inhibitory activity was observed. Estrogen stimulated the growth of MCF-7 cells, similar to 1 μM enterolactone, at concentrations of 1 nM. However, the combination of estrogen (1 nM) and enterolactone (1 μM) inhibited cell growth. These authors suggested that enterolactone might have inhibited aromatase and 17 β-hydroxysteroid dehydrogenase, enzymes important in estrogen production. Brooks and Thompson (2005) reported that 10 μM concentrations of

enterolactone and enterodiol significantly inhibited aromatase activity in MCF-7 cells by 37% and 81%, respectively. Enterolactone concentration of 20 μM also significantly inhibited (25%) aromatase activity; however, 1 and 50 μM had no inhibitory activity. In contrast, all concentrations of enterodiol inhibited the aromatase activity (Brooks and Thompson, 2005). The greatest 17 β-hydroxysteroid dehydrogenase inhibitions (84 and 59%) were found at the 50 μM concentrations of enterolactone and genistein, respectively. A significant reduction in androstenedione-stimulated cell proliferation was also observed in enterolactone-treated MCF-7 cells. The enterodiol treatment did not reduce androstenedione-stimulated cell proliferation and in some instances (10 and 50 μM) increased cell proliferation (Brooks and Thompson, 2005). Saarinen *et al.* (2002) reported that enterolactone inhibited the growth of DMBA-inducted cancers in rats. To achieve this inhibition, a dose of 10 mg/kg body weight was required, which resulted in a plasma level of 0.4 μM. Although weak, aromatase activity inhibition was observed.

In a study by Chen and Thompson (2003), the effects of enterolactone, enterodiol, tamoxifen, or combinations of these were evaluated in estrogen receptor negative human breast cancer cells MDA-MB-435 and MDA-MB-231. At physiologically relevant concentrations, all three chemicals were effective against steps (i.e., cancer cell adhesion, invasion, migration) in the metastasis process. However, the effects were concentration dependent. Enterolactone inhibited MDA-MB-435 cell adhesion at 1 μM concentration but not at 5 μM, whereas cell adhesion was not inhibited at 1 μM concentration of enterodiol. However, the 5 μM concentration of enterodiol was effective against cell adhesion. In the MDA-MB-231 cells, enterolactone (5 μM) but not enterodiol inhibited cell adhesion. Tamoxifen prevented cell adhesion in both cell lines (Chen and Thompson, 2003). Combinations of enterolactone, enterodiol, and tamoxifen, at 1 μM concentration each, had the greatest reduction (36%) in cell adhesion. Cell invasion in general was inhibited by the lignans and tamoxifen; however, the 5 μM concentration was most effective in both cell lines (Chen and Thompson, 2003). In contrast, Magee *et al.* (2004) found that enterolactone was not effective against cell migrations at concentrations of 2.5, 10, or 50 μM. Furthermore, SDG and enterodiol were only effective against cell migrations at the 50 μM concentration. Differences in the results may be due to the methodology used by the investigators. Enterolactone and enterodiol both inhibited cell migrations at all concentrations (0.1, 1, and 10 μM) tested whereas tamoxifen was ineffective against cell migration (Chen and Thompson, 2003). The authors concluded that combination of these three chemicals could be effective against breast cancer.

A number of factors may contribute to the various anticancer activity of flaxseed (Thompson *et al.*, 2005). The behavior of the lignans depends on

the biological levels of estradiol. At normal estradiol levels, the lignans act as estrogen antagonists but in postmenopausal women (i.e., low estradiol levels) can act as weak estrogens (Hutchins and Slavin, 2003; Rickard and Thompson., 1997). Tumor size reductions were observed in MCF-7 breast tumors in ovariectomized mice fed a diet containing flaxseed or flaxseed lignan; however, tumors were larger in rats fed diets containing soy products (Powers *et al.*, 2004). These authors suggested that the tumor growth and MCF-7 cell proliferation in mice fed soy-based diets was due to an estrogenic effect.

The presence of flaxseed lignans in MCF-7 tumors and the observed lignan binding to ER suggests that lignan function may be ER mediated (Adlercreutz *et al.*, 1992; Saarinen *et al.*, 2000). Although the lignans have been shown to be protective against breast cancer, minor structural alterations may influence overall activity (Saarinen *et al.*, 2005). Thus, many of the aforementioned benefits might be the results of specific structural features needed for lignans to bind to ER.

Flaxseed was among the best food sources in the prevention of *in vivo* spontaneous chromosomal damage in mice (Trentin *et al.*, 2004). The exact reason for the chromosomal damage prevention was not identified; however, the mechanism may be related to the antioxidant function of flaxseed components. Lignans have antioxidant activity and thus may contribute to the anticancer activity of flaxseed (Kangas *et al.*, 2002; Kitts *et al.*, 1999; Prasad 1997a, Yuan *et al.*, 1999).

c. Studies involving ALA. Few studies involving the role of ALA in breast cancer have been reported. Most of these studies used flaxseed oil and not ALA directly. Thompson (2003) reported a summary of these studies and in general a positive benefit has been associated with flaxseed oil intake and tumor prevention. A 10% flaxseed oil diet reduced tumor growth and metastasis incidents. The presence of ALA in breast adipose tissue was inversely related to breast cancer risk (Klein *et al.*, 2000; Maillard *et al.*, 2002). However, ALA content in other biomarkers did not provide the same results (Bougnoux and Chajès, 2003). Rao *et al.* (2000) reported that the number of tumors and tumor growth in general was influenced by the ratio of n-6 to n-3 fatty acids. The closer to a ratio of one gave the greatest benefit. This suggests the role of ALA in breast cancer has not been completely elucidated.

3. *Other cancer models*

a. Prostate cancer. Tumor cell proliferation decreased and apoptosis increased in TRAMP (i.e., transgenic adenocarcinoma mouse prostate) mice fed a 5% flaxseed diet for 30 weeks (Lin *et al.*, 2002). These authors reported that no differences in cell proliferation or apoptosis were observed

at the 20-week evaluation. Byland *et al.* (2000) also reported that tumor numbers and growth decreased and an increase in apoptosis in nude mice model. These authors concluded that enterolactone was not related to the antitumor effect.

In contrast, enterodiol and enterolactone were believed to be partly responsible for the growth inhibition of three human prostate cancer cell lines (Lin *et al.*, 2001). Morton *et al.* (1997) reported that higher enterolactone levels in prostatic fluid were associated with populations with a low risk of prostate cancer. In a small clinical study, prostate cancer cell proliferation decreased and apoptosis increased in men fed 30 g of flaxseed per day (Demark-Wahnefried *et al.*, 2001). A significant factor which may have influenced this study was that the subjects were on a low-fat diet. A subsequent study by the authors further supported the role of flaxseed in combination with a low-fat diet as a means to control prostate growth (Demark-Wahnefried *et al.*, 2004). In this study, prostate-specific antigen level and cell proliferation both decreased from baseline after only 6 months on the dietary regime.

Cameron *et al.* (1989) reported that flax oil prevented tumor formation in mice whereas mice on the corn oil and safflower oil diets had the greatest number of tumors. A diet consisting of 10% flax oil was sufficient to reduce tumor growth and metastasis in mice compared with corn or fish oil diets (Fritsche and Johnston, 1990). Although flaxseed and flaxseed oil and lignans have been shown to be beneficial, additional studies are needed to identify the mechanisms by which flaxseed or its components function to reduce prostate cancer.

b. Colon and skin cancer. Although not extensively evaluated, flaxseed has been shown to inhibit colon and skin cancers in cell cultures and in animal studies as reviewed by Thompson (2003) and Morris (2003a). Oikarinen *et al.* (2005) reported that flaxseed oil may be responsible for preventing colon carcinogenesis in multiple intestinal neoplasia (Min) mice. Dwivedi *et al.* (2005) also supported this finding that flaxseed oil prevented colon tumor development in rats. These authors used an azoxymethane-induced colon tumor model and reported that corn oil, high in omega-6, did not prevent tumor development. Danbara *et al.* (2005) reported that a 10 mg/kg dose of enterolactone, by subcutaneous injection three times per week, reduced the expression of colo 201 human colon cancer cell in athymic mice. Using various testing protocols, Danbara *et al.* (2005) concluded that the tumor suppression was due to apoptosis and decreased cell proliferation. In general, flaxseed may be a valuable tool in the fight against various cancers. Further research is needed in clinical settings to support the role of flaxseed in cancer prevention in human populations.

4. *Epidemiological studies*

a. Breast cancer. In general, the consumption of flaxseed or lignans has been inversely associated with breast cancer. Ingram *et al.* (1997) reported a significant reduction in breast cancer risk among women consuming high levels of the enterolactone. The reduction in breast cancer risk was further supported by the increased excretion of lignan (Dai *et al.*, 2003). Linseisen *et al.* (2004) reported that enterodiol and enterolactone were inversely related to breast cancer risks in premenopausal German women. Women with palpable cysts and high-serum enterolactone concentrations had lower risks of developing breast cancer than women with palpable cysts with a low enterolactone intake (Boccardo *et al.*, 2004).

Low plasma concentration of enterolactone was associated with an increased breast cancer risk in 248 breast cancer cases selected from three population-based cohorts in Sweden (Hulten *et al.*, 2002). Tonkelaar *et al.* (2001) reported that high urinary enterolactone was weakly associated with increased breast cancer risks in postmenopausal women. Kilkkinen *et al.* (2004) concluded that high serum enterolactone was not associated with a reduced risk of breast cancer, as serum enterolactone did not differ between the case and control groups. The results may be due to the population of women tested in the study. Using data collected from a case-controlled study nested within the New York University Women's Health prospective cohort study, Zeleniuch-Jacquotte *et al.* (2004) compared serum enterolactone and breast cancer risk. They found higher enterolactone concentrations in the case group compared to the control group for the premenopausal women. Howevere, no significant differences in serum enterolactone concentrations were found between the control and case group for the postmenopausal women. These findings led the authors (Zeleniuch-Jacquotte *et al.*, 2004) to conclude that circulating enterolactone did not provide protection against breast cancers.

McCann *et al.* (2004) reported that enterolactone intakes were not associated with breast cancer risk in postmenopausal women. However, the highest quartile of dietary lignan intake was strongly associated with reduced cancer risks in premenopausal women. The different observations (McCann *et al.*, 2004; Zeleniuch-Jacquotte *et al.*, 2004) regarding the premenopausal women might suggest that biomarkers beyond serum enterolactone concentrations may be required to assess the relationship between enterolactone and breast cancer risks. Furthermore, Olsen *et al.* (2004) reported that breast cancer risk increased as plasma enterolactone decreased. However, this relationship was statistically stronger for estrogen receptor negative breast cancer and only a weak association between plasma enterolactone and lower breast cancer risk in estrogen receptor positive cases. Thus, the contradictory

reports may be due to differences in the type of breast cancer (i.e., estrogen receptor positive or negative). An inverse association between high serum enterolactone and lower risk of breast cancer was observed in Finnish women (Pietinen *et al.*, 2001). This effect was observed in both pre- and postmenopausal women and contradicts other reports. Again, this result shows the high variability in epidemiological studies and that additional research may be needed to clarify the role of enterolactone and breast cancer risk.

In contrast to lignans, few studies have been reported regarding the relationship between ALA and breast cancer. In a meta-analysis, Saadatian-Elahi *et al.* (2004) reported a significant protective effect of total n-3 fatty acids and breast cancer risk. In this assessment, three cohort and seven case-control studies were reviewed. The case-control studies revealed an inverse association between ALA and breast cancer risk. High dietary intakes of ALA were correlated with a reduced breast cancer risk (Franceschi *et al.*, 1996). This study involved 2569 women with breast cancer and the result was supported by a cohort study conducted in the Netherlands (Voorrips *et al.*, 2002). A significant inverse association was found between ALA content in breast adipose tissue and breast cancer risk (Klein *et al.*, 2000; Maillard *et al.*, 2002). Furthermore, ALA to LA ratio close to one was also significantly associated with lower breast cancer risk (Maillard *et al.*, 2002).

Minimal to no association between breast cancer risk and subcutaneous ALA has been reported (London *et al.*, 1993; Simonsen *et al.*, 1998). In contrast, an increased risk of breast cancer has been associated with high ALA intakes (De Stéfani *et al.*, 1998). The link between ALA and breast cancer is weak in this study because most of the ALA was obtained from red meat. Thus, differentiating the effects from red meat and ALA intakes on breast cancer is difficult.

b. Prostate cancer. In the majority of the epidemiological studies, the authors concluded that prostate cancer was associated with ALA intake. However, inconsistencies in the reported data suggest that ALA may not be the component responsible for the observed risk. The influence of total dietary fat intake and caloric intake has been associated with increased risk of prostate cancer (Denis *et al.*, 1999; Giles and Ireland, 1997). Thus, many of the studies that draw conclusions on the association between ALA and prostate cancer may not be real.

Giovannacci *et al.* (1993) concluded from prospective data in the Health Professionals follow-up study that prostate cancer risk was associated with ALA intake. In contrast, Schuurman *et al.* (1999) reported a nonsignificant inverse relationship between ALA intake and prostate risk from data obtained in a cohort study completed in the Netherlands. In the Physicians'

Health Study (Gann *et al.*, 1994) and the Health Professionals Follow-up Study (Giovannacci *et al.*, 1993) eating red meat also emerged as a risk factor for prostate cancer. Leitzmann *et al.* (2004) reported that the ALA from both plant and animal sources was "suggestively" associated with an increased risk of advanced prostate cancer. However, total prostate cancer risk or early stage prostate cancer was not associated with ALA intake.

To complicate further the relationship between ALA and prostate cancer risk, Mannisto *et al.* (2003) reported that prostate cancer risk and ALA decrease when smokers were included in the model. This suggests that factors other than diet need to be considered in the models for determining causative relationships. Case-controlled studies also provide conflicting conclusions regarding the role of ALA in prostate cancer. For a complete review of these studies, see Bougnoux and Chajès (2003).

Kilkkinen *et al.* (2003a,b) reported that serum enterolactone was not associated with an increased prostate cancer risk. However, they also concluded that serum enterolactone concentration did not provide a protective effect against prostate cancer in male smokers. Statin *et al.* (2002) also concluded that high serum enterolactone was not associated with a lower risk of prostate cancer in a nested case-controlled study. The conflicting results between clinical and epidemiological studies suggest that the role of ALA and lignans in prostate cancer are far from being conclusive and that additional research in this arena is needed.

D. ROLE OF FLAXSEED IN DIABETES PREVENTION

Low glycemic index foods containing soluble fiber not only prevent certain metabolic ramifications of insulin resistance, but also reduce insulin resistance (Reaven *et al.*, 1993). Soluble fiber and other components of flaxseed fractions could potentially affect insulin secretion and its mechanisms of action in maintaining plasma glucose homeostasis. Flaxseed was shown to reduce the postprandial blood glucose response in humans (Cunnane *et al.*, 1993; Jenkins *et al.*, 1999). A consumption of 50 g/day ground flaxseed by young females over a 4-week period caused a reduction in blood glucose levels (Cunnane *et al.*, 1993). Similar findings were observed in postmenopausal women fed a 40 g/day flaxseed fortification diet (Lemay *et al.*, 2002). Bread containing 25% flaxseed gave a glycemic response that was 28% lower than the control (no flaxseed) bread (Jenkins *et al.*, 1999).

Prasad *et al.* (2000) reported that rats fed 22 mg SDG/kg and treated with the diabetes-promoting chemical streptozotocin had 75% lower incidence of type-1 diabetes than the streptozotocin-treated control group. However, the serum glucose of the SDG plus streptozotocin-treated rats had significantly higher serum glucose levels than streptozotocin-treated control group.

In contrast, the rats treated with SDG and streptozotocin that did not develop diabetes had lower serum glucose than the streptozotocin-treated control group. Rats fed no streptozotocin (control) or 22 mg SDG/kg body weight without streptozotocin did not develop type-1 diabetes and had serum glucose that where not significantly different. Similar results were observed in the formation of malondialdehyde, an oxidative stress indicator (Prasad *et al.*, 2000). In another type-1 model using Bio-Breed diabetic-prone rats, 71% of the rats fed 22 mg SDG/kg body weight did not show signs of diabetes whereas only 27% of the Bio-Breed diabetic prone rats had no signs of diabetes (Prasad, 2000a). Furthermore, pancreatic and serum malondialdehyde contents were lower in the rats treated with SDG whereas pancreatic antioxidant reserves were higher in these same rats.

The addition of 40 mg SDG/kg body weight reduced the incidence of type-2 diabetes in Zucker diabetic fatty (ZDF) rats by 80% (Prasad, 2001). In addition, blood glucose, glycated hemoglobin, and serum malondialdehyde were all lower in the rats treated with SDG at 72 days of age. In contrast, blood glucose levels were not significantly different between the SDG-treated rats and the control at 42 days. Oxidative stress plays a significant role in the etiology of type-1 and type-2 diabetes (Seghrouchni *et al.*, 2002; Shinomiya *et al.*, 2002). Kaneto *et al.* (1999) reported that oxidative stress can promote the overexpression of cyclin-dependent kinase inhibitor p21 mRNA resulting in reduced insulin production. Thus, SDG may indirectly affect this inhibitor by acting as an antioxidant (Prasad, 1997a, 2000b). Furthermore, diabetics have higher phosphoenolpyruvate carboxykinase, a liver enzyme important for gluconeogenesis, levels that result in higher glucose production (Consoli *et al.*, 1989). Prasad (2002) found that SDG inhibited phosphoenolpyruvate carboxykinase thus indicating another means by which SDG can act as an antidiabetic agent.

In contrast to ground flaxseed and SDG, ALA did not regulate glucose levels in elderly (Ezaki *et al.*, 1992) and type-2 diabetic (McManus *et al.*, 1996) populations fed 3 g/day and 35 mg/kg body weight, respectively. Kleeman *et al.* (1998) also reported that flaxseed oil had no effect on insulitis in diabetic-prone rats. In contrast to the antioxidant functions of SDG, *in vivo* oxidation of ALA may be in part one reason for the lack of activity of ALA in diabetes.

E. SAFETY OF FLAXSEED

1. Antinutrients

Flaxseed has several compounds that may negatively influence health and well-being. In some cases, the negative impact might simply be an assumption based on literature reports of similar compounds from other foods. The two

components that have been questioned most frequently are the cyanogenic glycosides and linatine, an antipyridoxine factor.

Cyanogenic glycosides are not exclusive to flaxseed. These compounds can be found in a number of plants including apples, cherries, and cassava. Many of the health concerns regarding cyanogenic glycosides stem from studies showing that cassava was toxic to animals and humans (McMahon *et al.*, 1995). However, cassava contains significantly more cyanogenic glycosides than flaxseed. Furthermore, the release of hydrogen cyanide from flaxseed would be minimal and below the toxic, lethal dose. At the recommend daily intake of about 1–2 tablespoons, approximately 5–10 mg of hydrogen cyanide is released from flaxseed, which is well below the estimated acute toxic dose for an adult of 50–60 mg inorganic cyanide and below the 30–100 mg/day humans can detoxify (Roseling 1994). Daun *et al.* (2003) reported that a person would have to consume 8 cups (i.e., 1 kg) of ground flaxseed to achieve acute cyanide toxicity.

In addition to cyanogenic glycosides, trypsin inhibitor, linatine and phytic acid are other antinutrients contained in flaxseed. Trypsin inhibitor activity (TIA) in flaxseed was low when compared to those in soybean and canola seeds. Bhatty (1993) reported laboratory-prepared flaxseed meals containing 42–51 units of TIA, which was slightly higher than 10–30 units observed by Madhusudhan and Singh (1983) and commercially obtained flaxseed meal (14–37 units). The contents of phytic acid were significantly different among cultivars. AC Linora has a lowest phytic acid content of 2280 mg/100 g and low ALA yellow-seeded cultivar Linola 947 has the highest content (3250 mg/100 g seed) among the eight cultivars reported (Oomah *et al.*, 1996).

Kratzer (1946) reported that pyridoxine supplementation in chicks on diets containing a linseed meal was necessary to counteract the vitamin B_6 deficiency. Klosterman *et al.* (1967) identified the antipyridoxine factor linatine. Although linatine is a problem in chicks, flaxseed has not been associated with a vitamin B_6 deficiency in humans. In fact, no affect on serum pyridoxine levels in subjects consuming 45 grams of flaxseed per day over 5 weeks was observed (Dieken, 1992).

2. *Role of flaxseed in reproduction*

Extensive investigations into the effects of flaxseed consumption, by mothers during gestation and lactation, on the offspring have been reported. Various effects in the offspring of rats fed flaxseed at levels between 5% and 40% have been observed (Collins *et al.*, 2003; Flynn *et al.*, 2003; Orcheson *et al.*, 1998; Tou and Thompson, 1999; Tou *et al.*, 1998, 1999; Ward *et al.*, 2000, 2001a).

Flynn *et al.* (2003) reported that flaxseed (20% or 40%) or flaxseed meal (13% or 26%) diets did not alter the toxicological properties of rat serum in a way that would disrupt organogenesis. No significant embryotoxicity was observed in rat embryos cultured in serum obtained from pregnant rats fed flaxseed diets. Although the growth of the embryo was affected, no dose-dependent response was observed thus the findings might be a chance occurrence unrelated to flaxseed treatment (Flynn *et al.*, 2003). These authors concluded that the exposure serum obtained from pregnant rats fed flaxseed diets was not teratogenic to embryos.

Flaxseed consumption did not affect the pregnancy of the rats (Collins *et al.*, 2003; Tou *et al.*, 1998). Flaxseed (20% and 40%) and flaxseed meal (13% and 26%) diets did not affect fertility, litter size, or survival of offspring (Collins *et al.*, 2003). However, length of gestation, anogenital distance and index, onset of puberty, and estrous cycles were affected by flaxseed consumption.

Lower offspring birth weights were observed in rats fed a 10% flaxseed diet (Tou *et al.*, 1998). In contrast, the higher flaxseed (20% and 40%) and flaxseed meal (13% and 26%) diets did not significantly affect birth weight (Collins *et al.*, 2003). With the exception of the 40% flaxseed diet, female rats gain more weight on the diets containing 20% flaxseed and 13% and 26% flaxseed meal than the control rats from weaning to maturity (Collins *et al.*, 2003).

Female rats on a 5% flaxseed diet had delayed onset of puberty whereas earlier puberty onset was observed in female rats on a 10% flaxseed diet (Tou *et al.*, 1998, 1999). In both studies, the average weight of the female rats at puberty was lower than the control group. Higher flaxseed (20% and 40%) and flaxseed meal (13% and 26%) diets did not affect the onset of puberty or weight of the female rats at puberty (Collins *et al.*, 2003). Lengthened estrous cycle of the female rats was reported in all studies and supports the anti-estrogenic activity of flaxseed (Collins *et al.*, 2003; Orcheson *et al.*, 1998; Tou *et al.*, 1998, 1999). Orcheson *et al.* (1998) reported that a 10% flaxseed diet produced irregular estrous cycles similar to the addition of 3 mg SDG/kg fortified diet.

Ward *et al.* (2000) reported that terminal mammary end buds decreased and alveolar buds increased in rat offspring exposed to 10% flaxseed or the equivalent SDG diet during pregnancy and lactation. However, exposure to the same flaxseed or SDG levels only during lactation did not affect reproductive organs (Ward *et al.*, 2001a). Lina *et al.* (2005) reported a decrease in ovary weights in rats fed the lignan 7-hydroxymatairesinol. These authors suggested a 160 mg/kg body weight/day no adverse effect level, which is similar to the 177.2 mg SDG/kg body weight/day reported by Ward *et al.* (2000). Furthermore, no adverse effect level is approximately 160-fold greater than the proposed daily lignan intake in humans (Lina *et al.*, 2005).

In male offspring, reduced postnatal weight gains were observed in the lower flaxseed diets (Tou *et al.*, 1998) whereas high flaxseed diets did not affect postnatal weight gain, although the 40% flaxseed diet did have lower weight gain than the control (Collins *et al.*, 2003). At puberty, the rats on the flaxseed or flaxseed meal were heavier than the rats on the control diets. The onset of puberty was delayed in rats fed the 20% flaxseed and 13% and 26% flaxseed meal diets, but not in the 40% flaxseed diet. Collins *et al.* (2003) suggested that hormonal effects on the male reproductive system might be the cause of the observed delay in puberty.

Tou *et al.* (1999) reported that serum testosterone and estradiol levels were higher in rats fed a 10% flaxseed diet compared to the control. In addition, this diet produced higher relative accessory sex gland and prostate weight (Tou *et al.*, 1999). In contrast, lifetime exposure to 5% flaxseed diets reduced prostate weights. Sprando *et al.* (2000a) also found significant reductions in prostate weight in offspring from dams fed flaxseed (20%) or flaxseed meal (13% or 26%) diets. Testes weight was not significantly altered in offspring of dams fed flaxseed (20% or 40%) or flaxseed meal (13% or 26%) diets (Sprando *et al.*, 2000b) during suckling. Ward *et al.* (2001a) also reported no adverse effects on testes and prostate weights of offspring from dams fed a flaxseed (10%) diet during lactation. The absolute volume in the seminiferous tubules was significantly lower in offspring exposed to 20% or 40% flaxseed diets. However, this difference was not considered biologically relevant because the seminiferous tubule diameter, an indicator of spermatogenic activity, was not affected (Sprando *et al.*, 2000b). This combined with the observation that homogenization-resistant spermatid counts and daily sperm production rates were not affected indicated further that spermatogenesis was not affected by the high flaxseed or meal diets (Sprando *et al.*, 2000a,b). Furthermore, testis structure was not significantly affected by exposure to high flaxseed or meal diets (Sprando *et al.*, 2000b).

The development bone is a hormone dependent process. Thus, flaxseed and in particular lignans could influence bone development. Ward *et al.* (2001b) found that rats exposed to 88 or 177.3 mg SDG/kg body weight/day had higher bone strength than the basal diet at 50 days postnatal. However, the significant higher bone strengths were lost at day 132 postnatal. The bone mineral content were generally not significantly different between the basal and treatment groups except at postnatal day 132 where the higher lignan treatment had lower bone mineral content than the basal diet. In contrast, bone strength at postnatal day 50 were significantly lower in male rats fed 10% flaxseed diets compared to the basal diet (Ward *et al.*, 2001c). However, by postnatal day 132 no differences in bone strength, bone mineral density, or bone mineral content were observed. The authors concluded that

the exposure to SDG did not have a negative effect on bone strength (Ward *et al.*, 2001b,c).

Babu *et al.* (2003) reported that a significant reduction in concanavalin A spleen lymphocytes proliferation in pregnant rats fed a 40% flaxseed diet. Spleen lymphocytes proliferation was significantly lower in a 90-day-old offspring on the 40% flaxseed diet and exposed to phytohemagglutinin. In both cases, interleukin-2 formation was not affected by flaxseed intake. The levels of LA and AA in serum and tissues combined with relatively constant interleukin-2 concentrations lead to the conclusion that flaxseed could be used in treating autoimmune diseases (Babu *et al.*, 2003).

3. *Metabolism of flaxseed lignan*

Rickard and Thompson (1998) followed the metabolism of ^{3}H-SDG in rats. They found that a chronic (10 day, 1.5 mg/day) exposure to SDG could increase lignan levels up to threefold compared to acute (1 day) exposure. In a follow-up study involving feeding of ^{3}H-SDG to rats, Rickard and Thompson (2000) reported that blood radioactivity peaked at 9 hours for both the chronic and acute groups. Furthermore, only slight reductions in blood radioactivity were noted after 24 hours in both treatment groups. Regardless of the treatment group, 75–80% of urine radioactivity was due to enterodiol, enterolactone and SECO. This combined with blood radioactivity profiles suggests that lignans are metabolized in a similar manner between the chronic and acute treatment groups.

Humans appear to be able to effectively handle single dose of lignans. Nesbitt *et al.* (1999) observed that mammalian lignan contents in urine started to increase 9 hours after ingesting 5, 15, or 25 grams of ground flaxseed. The excreted lignan content was dose dependent but remained higher than baseline values for up to 24 hours. The pharmacokinetics of ingested SDG has been reported in humans (Kuijsten *et al.*, 2005). The maximum plasma levels of enterodiol and enterolactone were reached 14.8 and 19.7 hours after ingestion of SDG, respectively. The mean elimination half-life was 4.4 and 12.6 hours for enterodiol and enterolactone, respectively. Mean residence times of 20.6 and 35.8 hours for enterodiol and enterolactone were observed, respectively (Kuijsten *et al.*, 2005). In all pharmacokinetic measurements, the time to reach maximum mammalian lignan concentration in plasma and urine was longer in men. Given the vast number of research reports on health benefits combined with positive safety results, flaxseed may be an effective food ingredient that could enhance health status in humans.

IV. FLAXSEED QUALITY AND END USE FUNCTIONALITY

A. INTRODUCTION

Flaxseed is utilized as a main food ingredient in order to enhance functional foods (Oomah and Mazza 1999). Whole or ground flaxseed can be used in various food products, such as bread (Carter, 1993; Garden, 1993), pasta (Lee *et al.*, 2003; 2004a; Manthey *et al.*, 2000, 2002a), candy, chocolate bar, chocolate (Kozlowska, 1989), muffin, bagel, bun, cereals, salad toppings (Carter, 1993), corn snack (Ahmed, 1999), cake (Lee *et al.*, 2004b), tortilla (Ghosh *et al.*, 2004), ice cream (Hall and Schwarz, 2002), yogurt (Hall *et al.*, 2004), or can be consumed as a roasted snack (Kozlowska, 1989; Schorno *et al.*, 2003; Tulbek *et al.*, 2004). Flaxseed is also processed for oil, which is a major product for the organic food industry (Wiesenborn *et al.*, 2004). Oomah (2001) and Hall (2002) reported flaxseed as a functional food source. In this section, end product use of flaxseed will be discussed.

B. SENSORY PROPERTIES OF FLAXSEED

In general, flaxseed has a pleasant nutty flavor (Carter, 1996). However, sensory characteristics have not been fully evaluated and consumer preference of flaxseed is largely dependent on seed quality. Appearance, color, and flavor attributes could be variable depending on cultivar and growing conditions. Carter (1996) indicated that ground flaxseed samples from different cultivars had acceptable quality. However, the Omega cultivar exhibited the highest sensory score whereas McGregor was statistically rated the lowest (Figure 4). A composite sample was tasted first and accepted as a reference

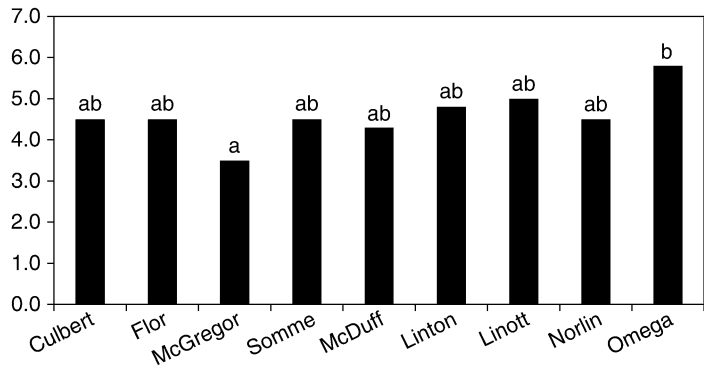


FIG. 4 Sensory scores of flaxseed cultivars observed by Carter (1996).

TABLE XII
PHENOLIC ACID CONTENTS (G/KG) OF WHOLE AND DEHULLED FLAXSEED[a,b]

	Total phenolic acid	
	Whole	Dehulled
Flanders	16.85b	21.03a
Linola™947	5.37e	9.75d
McGregor	6.99d	19.78ab
NorLin	8.05c	16.53c
NorMan	16.85b	16.71c
Omega	16.91b	18.61b
Vimy	18.15a	18.85b

[a]Adapted from Oomah and Mazza (1997).
[b]Values not sharing a common letter are significantly different ($p \leq 0.05$).

with a rating of 5. Linott and Linton exhibited similar flavor profile, slightly lower than Omega. The panelists found McGregor to have a slightly higher bitterness than other cultivars, whereas Omega was slightly milder.

Grinding can generate flavor volatiles and enhance the accessibility of the cyanogenic glycosides and phenolic components, which may then be more easily detected by sensory panelists. In addition, components, such as PAs and cyanogenic glycoside, may contribute to bitterness (Chiwona-Karltun *et al.*, 2004). Oomah and Mazza (1997) analyzed the phenolic and cyanogenic compounds of flaxseed cultivars. Results indicated that dehulled McGregor cultivar yielded the highest linamarin and linustatin content (Table VIII). In contrast, Flanders and Omega cultivars had the lowest linamarin content and total cyanogenic glycoside levels. Cultivars showed statistical differences ($p \leq 0.05$) in terms of PA contents in McGregor, NorLin and Linola™947 cultivars were the lowest, whereas Omega, Flanders, NorMan, and Vimy had higher values (Table XII). Linola™947 had the lowest PA content among the dehulled samples, whereas Flanders the highest. Based on this data, the sensory scores reported by Carter (1996) may be due to the lower cyanogenic glycoside present in the Omega cultivar and not as much on the PA content.

C. FLAXSEED PROCESSING

1. Milling and fractionation

Schorno *et al.* (2004) compared milled flaxseed production using several mills which included roller, burr, hammer, and centrifugal cutting mills. The burr mill was found to be the least efficient for flaxseed milling. The roller mill

caused particle adherence to rolls due to the high-surface lipid content. High-surface lipid content, which could trigger lipid oxidation, increased due to the higher feed values. The centrifugal cutting mill and hammer mill showed greater potential for flaxseed milling.

Flaxseed has two flattened cotyledons, which constitute the greater portion of the embryo. The embryo is surrounded by a seed coat that consists of a hull and an adherent layer of endosperm. Hull and cotyledon can be utilized separately as a functional ingredient (Oomah and Mazza 1997; Wiesenborn *et al.* 2002). Hence dehulling and milling studies have been carried out for flaxseed utilization purposes.

Oomah and Mazza (1997) reported that the dehulling process significantly decreased water absorption capacity and viscosity of flaxseed. Dehulled Omega showed the lowest viscosity among the cultivars, whereas Linola™847 and NorMan had the highest. NorMan gave the least reduction in terms of viscosity. Oomah and Mazza (1998b) noted that microwave drying was a valuable pretreatment for abrasive dehulling applications. Higher hull yields resulted from the microwave pretreated seeds compared to untreated seeds.

Tostenson *et al.* (2000) reported the use of a pearler for fractionation of the flaxseed. The data indicated higher hull fraction yields compared to abrasive dehulling. Madhusudhan *et al.* (2000) found that centrifugal cutting mill could be successfully applied to fractionate hull and germ fractions. Results suggested a negative correlation between the oil and SDG contents. Hull fractions could be utilized as a good source of SDG, whereas dehulled seed could be processed for flaxseed oil. Wiesenborn *et al.* (2003) discussed continuous abrasive milling and results showed that the process efficiency was affected by cultivar, moisture content, and feed rate. SDG content of fractions was negatively related to oil content, which was consistent with the results of Madhusudhan *et al.* (2000). Wiesenborn *et al.* (2003) noted that the SDG content was an indicator of purity of the embryo and hull fractions, which was related to cultivar and growing location and conditions.

2. *Storage stability of milled flaxseed*

White and Jayas (1991) observed a twofold increase in free fatty acids (FFA), which was correlated to discolored seed content. This observation also triggered a rapid loss of germination capacity. Significant color changes (from red–brown to dark brown) in flaxseed were observed at various storage times when the seed was stored at 40–50°C at a relative humidity level of 35% and above. Seed storage conditions can affect seed color, which might affect flaxseed end use. Dark flaxseed percentage in a seed lot was related to poor lipid stability in milled flaxseed (Pizzey and Luba, 2002). The authors investigated lipid oxidation in milled flaxseed samples containing

2.7% and 25% dark flaxseed. The milled flaxseed sample with 25% dark seed produced higher FFA and peroxide values, suggesting that presence of dark seed could cause undesirable quality of the milled flaxseed if not removed prior to milling. Results were consistent with the conclusions of White and Jayas (1991) in terms of seed color. Pizzey and Luba (2002) demonstrated the fact that that Canadian and US number 1 grade flaxseed could contain high levels of dark seed depending on the season. The role of lipase and other enzymes in flaxseed stability is not well understood.

Wanasundara *et al.* (1999) showed that flaxseed lipase had an activity of 160 units/g prior to germination and that after germination activity increased to as high as 354 units/g. High FFA content in immature seed reported by Malcolmson *et al.* (2000) indicates that lipase activity might be greater in immature seed than in mature seed. Information concerning lipase activity in immature flaxseed was not found. However, Daun (1993) presented data showing that frost-damaged immature canola had higher FFA content than normal canola. Though this information is not for flaxseed, the same trend may be observed for flaxseed.

Whole flaxseed remains oxidatively stable for many years; however, high moisture conditions during storage can trigger enzymatic-promoted oxidation. Enzymes, such as lipoxygenase (LOX), might promote sufficient oxidation to affect flaxseed quality. Thus, knowing the enzyme level might provide flaxseed handlers with a strategy to prevent enzymatic-promoted oxidation. LOX is soluble in the cytoplasm of cells and has been found in chloroplasts (Vick and Zimmerman, 1987) and lipid bodies of oilseeds (Feussner *et al.*, 1995). LOX requires substrate with the pentadiene system; thus only the unsaturated fatty acids, linoleic, and linolenic acids, in flaxseed can function as LOX substrate. LOX has a preference for FFA substrates more than TAG (Hamilton, 1994). Flaxseed LOX produces hydroperoxides at the C-13 position (80%) and C-9 position (20%) in linoleic substrates (Zimmerman and Vick, 1970). With linolenic acid, 88% of the C-13 hydroperoxide and 12% of the C-9 hydroperoxide is formed (Zimmerman and Vick, 1970).

LOX in young and developing plant tissues generally have greater activity than in mature tissues (Siedow 1991; Zhuang *et al.* 1992). Thus, flaxseed with high LOX activity may be indicative of either germinated and or immature seeds. LOX content is affected by cultivar × location × year interaction but quantitative differences in LOX content and LOX activity are primarily due to cultivar (Oomah *et al.*, 1997b). Flaxseed was reported to contain from 1.63 to 5.98 g/kg of LOX (Oomah *et al.*, 1997b) with Linola cultivar having the highest content. Linola is very high in LA and low in linolenic acid, and that LA is the preferred flaxseed LOX substrate (Zimmerman and Vick, 1970). This suggests that linolenic acid content is less important than LA content in regards to LOX content in flaxseed. Investigations by Oomah

et al. (1997b) showed that LOX content varied significantly with cultivar; however, the relationship between LOX and flaxseed stability was not determined. The data suggests that the fatty acids in the TAG are an important factor in lipoxygenase-promoted oxidation of flaxseed. Additional research to correlated lipoxygenase and oxidative stability is needed.

Thermal and oxidative stability of whole and milled flaxseed and extracted flaxseed oil at 178°C has been reported (Chen *et al.*, 1994). Researchers showed that milled flaxseed exhibited the highest level of oxygen consumption followed by flaxseed lipid extract, whereas whole seed showed little change over a period of 90 minutes at 178°C. The ALA content decreased significantly from 3.8% to 3.3% for milled flaxseed and flaxseed lipid extract, respectively. Results indicated that oxidative susceptibility of milled flaxseed was related to particle size. Chen *et al.* (1994) reported that oxygen consumption was highest in the coarse fraction (>950 micron), followed by the fine fraction (<500 micron), and the granular (intermediate) size fractions (500–710 and 710–850 micron) were the most stable as measured by the lower oxygen consumption. High oxygen consumption by large-sized particles was suggested to be due to ample air space between flaxseed particles. Thus, greater oxygen diffusion into the pile of coarse-milled flaxseed may be more probable than for the fine-milled flaxseed. Surface area and tight packing of fine-milled flaxseed particles might have contributed to the lower oxidation rate observed in contrast to coarse-milled flaxseed (Chen *et al.*, 1994). We have observed similar observations in pasta made from different sized flaxseed particles, whereby pasta made with large flaxseed particles oxidized more readily than pasta made with small flaxseed particles.

Malcolmson *et al.* (2000) reported the storage stability of milled flaxseed stored at ambient temperatures. The result showed that peroxide value and conjugated diene did not significantly change during storage (128 day). Linott cultivar exhibited a significant increase in terms of FFA (0.3% to 1.58%). About 5% of the Linott seed was slightly discolored indicating the seed lacked full maturity. Malcolmson *et al.* (2000) speculated that high FFA content in milled flaxseed sample was due to the presence of immature seed in the sample. Chlorophyll and moisture content could also be significant parameters, which are likely to be high in immature seed and may trigger lipid oxidation in milled flaxseed.

In the follow-up report, Przybylski and Daun (2001) tested different lots of milled flaxseed for oxidation. These samples were stored in loosely closed plastic bags protected from light at ambient temperatures for up to 20 months. The authors observed that one sample stored for 11 months had higher FFA content (9.70%) and had higher off-flavor characteristic than samples that were stored for 0 and 20 months. Peroxide values for samples varied between 2.4–3.4 meq/kg and flaxseed stored for 11 months actually

had the lowest value in contrast to FFA. Researchers suggested the presence of sufficient moisture or damaged seed might have promoted lipolytic activity during storage.

In a study conducted in the author's research lab, storage stability of flaxseed stored under various conditions was tested. Ground flaxseed packed in plastic or aluminum bags were stored for 4 weeks at 30°C, then for 3 weeks at 60°C in a temperature-controlled chamber under light. Peroxide values of all samples increased from 0.8 to 1.3 meq/kg oil in flaxseed stored in aluminum foil bags at 60°C at the end of 7 weeks of storage, which showed protective effect on flaxseed oxidation against light exposure and high temperature. Peroxide values of the flaxseed stored in plastic bags averaged 1.5 meq/kg oil at the end of 4 weeks of storage, but then increased to 5.0 meq/kg oil after three additional weeks of storage at 60°C. In terms of GC-MS analysis, flaxseed stored in plastic bags exhibited significantly higher propanol, pentanol, and hexanol secondary oxidation products, whereas these same volatiles were observed to be at lower concentrations in flaxseed stored in aluminum bags. In contrast, butanol, the primary volatile, was higher in the samples stored in aluminum bags compared to the samples stored in the plastic bags. Alkoxy radicals can react with unsaturated lipid to form stable and innocuous alcohols (Frankel, 2005). However in this case, hydroperoxides produced by lipoxygenase might go through an isomerization and rearrangement to produce aldehydes, short chain alcohols, vinyl ethers, or oxo acids (Frankel, 2005). We mainly observed the oxidation end product volatiles of LA during storage. We speculate that lipoxygenase, which might promote enzymatic oxidation (Frankel, 2005) may have been triggered during the milling process thus promoting the formation of LA primary oxidation and eventually to the secondary products. One particular compound, 2-pentyl furan (butter, green bean flavor), was detected in flaxseed stored in plastic bags, which is a main secondary end product volatile of LA (Frankel, 2005). High temperature storage (60°C) increased significantly the headspace volatile concentration in flaxseed; however, peroxide values did not increase to a great extent at the end of 7-week storage. This indicated that possible decomposition of the hydroperoxides into secondary oxidation products occurred. Overall the aluminum bags provided better shelf life stability for ground flaxseed during elevated storage.

D. FLAXSEED OIL

1. Extraction of flaxseed

Edible flaxseed oil is largely sold in organic food markets, thus mechanical pressing is preferred by the food industry due to the absence of alternative

and convenient solvents (Wiesenborn *et al.*, 2004; Zheng *et al.*, 2003). Zheng *et al.* (2002) reported that pressing dehulled flaxseed yielded twice the oil output compared to whole flaxseed. Wiesenborn *et al.* (2004) compared the processing of whole flaxseed and dehulled seed. Oil yield was significantly higher in whole seed, possibly indicating that higher oil temperature (66°C) promoted the oil extraction compared to lower temperature (33°C) expelling. Results were consistent with the previous results indicating the aid of fiber during pressing (Zheng *et al.*, 2003). Nevertheless higher oil temperatures of whole flaxseed during cold pressing could be a concern for the industry, because autooxidation may be triggered. Zheng *et al.* (2005) discussed the specific mechanical energy during screw pressing of whole and dehulled seed. Results indicated that a reduction in moisture content of flaxseed (from 12.6% to 6.3%) triggered friction among the seeds in turn, causing a significant increase in specific mechanical energy and heat. Data were consistent with the previous study and suggested that pretreatment of the flaxseed prior to pressing help to reduce exposure of the oil to high temperatures and preventing possible off flavors.

In order to improve oxidative stability, supercritical CO_2 fluid extraction could be used for flaxseed oil extraction (Bozan and Temelli, 2002). This method demonstrated higher ALA content compared to soxhlet extraction. In contrast, tocopherol content was lower (Table XI). Temperature and pressure profiles did not alter the fatty acid profile and exhibited similar results at 50 and 70°C and pressures of 35 and 55Mpa. Stability of oil was not reported, and since limited knowledge is available further research is required with regards to shelf life and comparisons to cold-pressing operation.

2. *Flaxseed oil stability*

Hadley (1996) reported the use of flaxseed oil in frying applications. At higher temperatures (177–191°C) lipid oxidation rate was rapid and ALA content was significantly reduced. Furthermore, a fishy flavor in the oil developed during frying. Although not evaluated, 1-penten-3-one may have formed during this process causing the fishy flavor. Van Ruth *et al.* (2001) noted the use of soybean extracts in flaxseed oil as an antioxidant source. Extracts of soybean seeds reduced the formation of primary oxidation products up to 30%, and secondary lipid oxidation products up to 99%. Various antioxidants could be applied in flaxseed oil (Rudnik *et al.*, 2001). Ascorbyl palmitate, citric acid, ascorbic acid, ethoxylated glycol, and α-tocopherol blend exhibited stronger antioxidant capacity compared to butylated hydroxyanisole.

Lukaszewicz *et al.* (2004) reported the oxidative properties of oil extracted from various flax cultivars. Linola cultivar (high LA), with the lowest content of linolenic acid, exhibited the highest conjugated diene values when heated

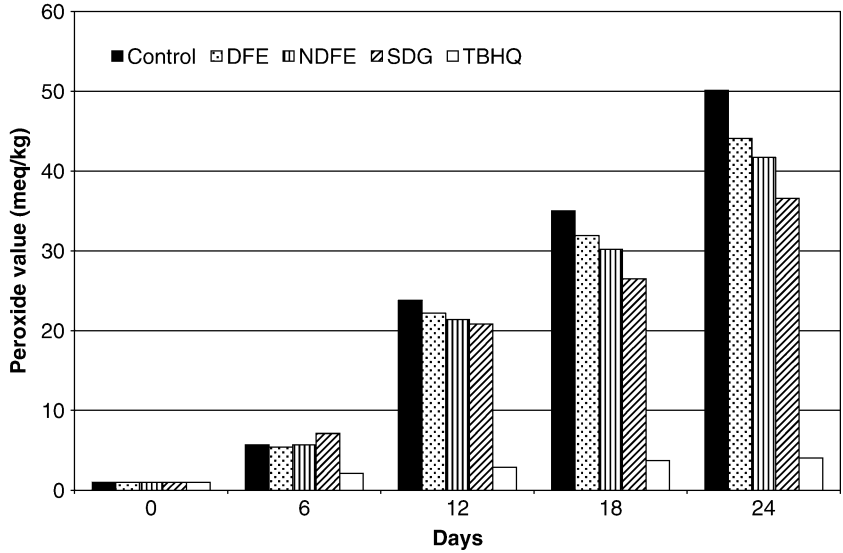


FIG. 5 The antioxidant activity of non-defatted flaxseed extract (NDFE), defatted flaxseed extract (DFE), SDG, and tertiary-butylhydroquinone (TBHQ) in stripped corn oil observed by Hall and Shultz (2001).

for 40 minutes at 140°C. Abby (high linolenic acid), which is a British flaxseed cultivar, gave the lowest conjugated diene and thiobarbutiric acid values. No relationship was demonstrated between oxidation attributes of cultivars and ALA content whereas ALA was negatively correlated with other fatty acids. The observations by Lukaszewicz *et al.* (2004) suggest factors other than oil may be responsible for the observed oxidation behavior of the different cultivars. In the author's laboratory (Hall and Shultz, 2001), antioxidant and prooxidant activities of flaxseed phenolics and SDG were tested. SDG significantly ($p < 0.05$) decreased lipid oxidation rate in corn oil, compared to ground defatted and nondefatted flaxseed extracts (Figure 5). At the 18- and 24-day evaluations, the SDG-treated oil samples had significantly ($p < 0.05$) lower peroxide values than the control oil. However, no differences in peroxide values of the oil treated with the flaxseed extracts and SDG were observed (Figure 5). In the second part of the study, SDG was compared to tocopherol and phenolic compounds. The corn oil treated with SDG had significantly ($p < 0.05$) lower peroxide values at the 11-day storage evaluation than the control (Figure 6). Although not significant, the peroxide values of the combined PA and SDG were lower than the control. The tocopherol treatment (220 ppm) was prooxidant, but

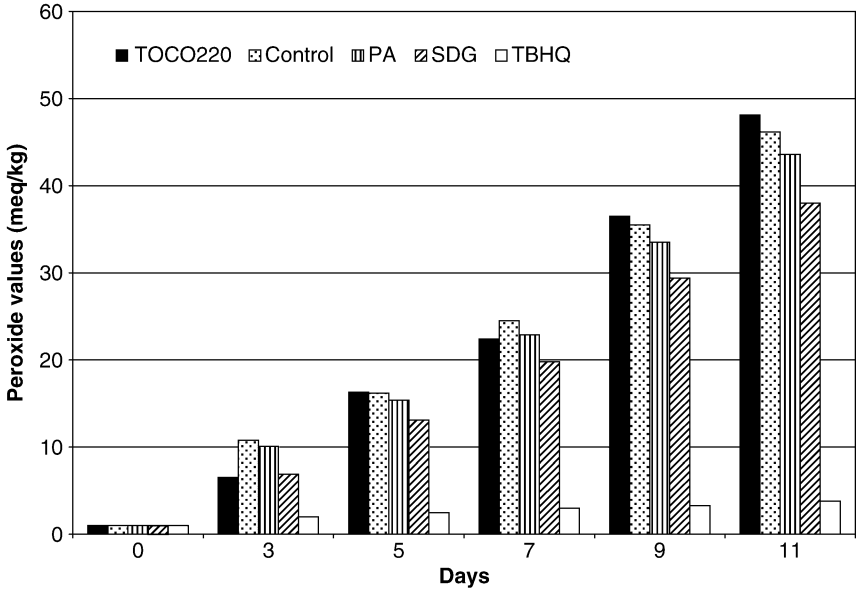


FIG. 6 The antioxidant activity of SDG, phenolic acid (PA), tocopherol (TOCO = 220 ppm), and tertiary-butylhydroquinone (TBHQ) in stripped corn oil observed by Hall and Shultz (2001).

combined with SDG and PA the prooxidant activity of tocopherol was eliminated (Figure 7). The results from our work are consistent with previous findings of Shahidi *et al.* (1995) who also observed antioxidant activity of flaxseed extracts. Wiesenborn *et al.* (2005) reported a relationship between headspace volatile analysis and sensory properties of cold-pressed flaxseed oil. Results indicated that significant differences between samples for painty-bitter flavors and overall quality. Thus, solid phase microextraction analysis could be a means of determining flaxseed oil quality since head space volatiles correlated with descriptive sensory profile.

E. FLAXSEED GUM

Mason and Hall (1948) noted the potential use of flaxseed gum in soft drinks, candy, processed cheese, jellies, and fruit juice. Garden (1993) reported that flaxseed gum significantly improved bread quality and shelf life, and suggested the use of the gum fraction as a food ingredient in food products. Chemical, physical, and functional properties of flaxseed gum have been documented (Chornick *et al.*, 2002; Cui *et al.*, 1994b, 1994c; Mazza and

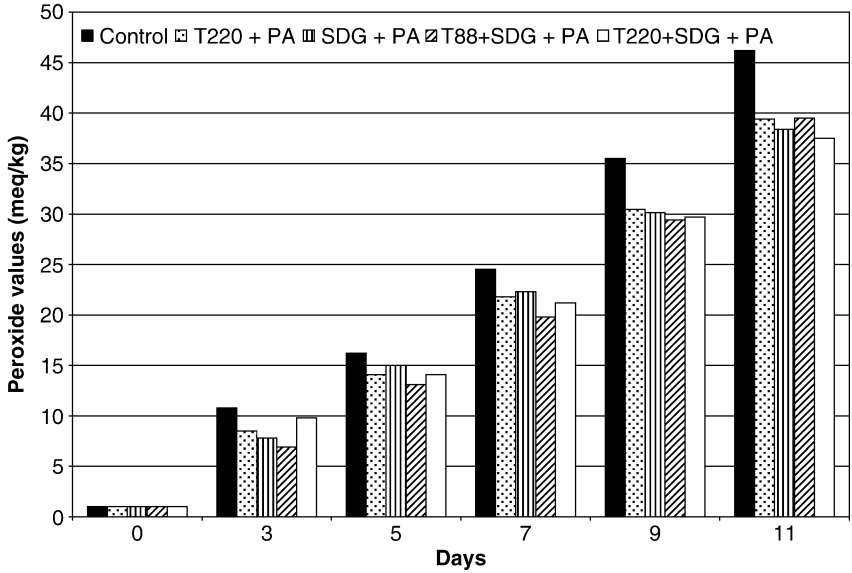


FIG. 7 The antioxidant activity of SDG, phenolic acid (PA), and tocopherol (TOCO = 88 or 220 ppm), alone or in combinations, in stripped corn oil observed by Hall and Shultz (2001).

Biliaderis, 1989; Oomah and Mazza, 1998b). Flaxseed gum exhibited good foam stability at a level of 1% and maximum viscosity at pH 6.0–8.0 (Mazza and Biliaderis, 1989). Oomah and Mazza (1998b) reported that lipid removal significantly increased apparent viscosity values of flaxseed gum. Furthermore, viscosity of seed, cake, and flake samples was significantly related to protein ($r = 0.97$) and carbohydrate ($r = 0.91$) fractions, which were related to mucilage fraction of the seed.

Cui *et al.* (1994c) reported genotypic differences of flaxseed gum in terms of chemical and rheological properties. Yellow flaxseed cultivars showed stronger dynamic rheological properties in aqueous solutions, compared to brown seeds. It was observed that neutral polysaccharide fraction of flaxseed gum caused weak gel forming. NorMan cultivar exhibited the highest properties among the brown cultivars. Moore *et al.* (1996) reported that apparent viscosity of flaxseed gum was stable at a wide pH range (4.5–7.0).

Cui and Mazza (1996) reported that neutral polysaccharides have a larger molecular size than acidic polysaccharides and flaxseed gum showed superior moisture retention properties compared to carboxymethylcellulose, Arabic, guar, and xanthan gums. Acidic polysaccharides show more shear

thinning properties than neutral polysaccharides. The pH of a 0.5% flaxseed gum dispersion was observed to be 6.4 (Huang *et al.*, 2001). The emulsion made from 0.5% flaxseed gum lacked storage stability as microbial spoilage occurred before 30 days (Huang *et al.*, 2001). We have observed similar results with aqueous extracts of flaxseed gums where the gum solution becomes moldy within 30 days. Thus, the gum should be dried as quickly as possible once extracted from the flaxseed to prevent molding of the extract.

Flaxseed had the highest protein content of various gums tested by Huang *et al.* (2001); however, the stabilizing effect of the flaxseed gum on the emulsion was less than fenugreek, yellow mustard, gum Arabic, and methylcellulose. Apparent viscosity value of flaxseed gum was the third highest following xanthan and locust bean gum. Huang *et al.* (2001) attributed the results mainly to protein content, since protein content is significantly related to emulsion and foam stability of colloidal solutions.

Chornick *et al.* (2002) tested the mucilage content among the Canadian flaxseed cultivars. Results showed diversity in terms of molecular weight, structural conformation, and proportion of neutral to acidic polysaccharides. Xylose content exhibited significant correlation with viscosity values. Mucilage viscosity significantly increased with higher levels of neutral polysaccharide content, which was consistent with the results of Cui and Mazza (1996). In contrast, Warrand *et al.* (2005a) reported a reduction in flaxseed cake mucilage viscosity compared to whole seed mucilage. Steady shear rheological flow properties indicated a very low viscosity level which might have been due to oil extraction conditions (high temperature and pressure). Thus, oil extraction technique could be a major factor that impacts the mucilage and gum properties. In a follow-up study, Warrand *et al.* (2005b) investigated the neutral polysaccharides of flaxseed mucilage after fractionation by size-exclusion chromatography. The presence of arabinoxylans (1,4 linked β-D-xylans) with a constant Arabinose/Xylose ratio of 0.24 was observed with variable galactose and fucose residues connected to the side chains. These authors speculated that the association between polymers could be the reason for the observed rheological properties.

There has been an interest in utilizing flaxseed pectin as an encapsulation agent for shark liver oil (Diaz-Rojas *et al.*, 2004). Flaxseed pectin did not exhibit acceptable results; however, researchers suggested the use of the pectin fraction be combined with alginate coated by chitosan. In a study reported by Qin *et al.* (2005), flaxseed gum stabilized the cloudy appearance of carrot juice and reduced creaming. Thus, suggesting that the soluble gum could be used as dispersing and stabilizing agents in beverages. Limited knowledge is available regarding flaxseed gum and mucilage in food model systems, thus further research is required to understand the basic interactions between flaxseed gum and food components.

F. ROASTED FLAXSEED

Kozlowska (1989) discussed the use of roasted flaxseed in bread and confectionery products. Schorno *et al.* (2003) and Tulbek *et al.* (2004) reported roasted flaxseed quality. Higher roasting temperature and longer roasting time conditions indicated greater moisture loss and flaxseed exhibited significantly lower moisture contents (0.1–0.3%), after roasting samples for 24 minutes at 160 and 180°C. The recorded moisture loss might include volatiles that are lost under the conditions of the test; however, the major loss should be considered to be moisture. When the roasting temperature was increased to 180°C, significant changes were observed in color properties. Roasted flaxseed brightness significantly ($p \leq 0.05$) decreased at 160 and 180°C; however, no color differences were observed at 140°C. Samples roasted at 160 and 180°C were higher in redness and lower in yellowness than control and samples roasted at 140°C. Extractable lipid increased with roasting temperature but not with duration. Observations were consistent with the research of Yoshida and Takagi (1997), who found that roasting time and temperature increased lipid extraction in sesame seed.

Jung *et al.* (1999) extracted pyrazines from the oil extracted from roasted red pepper seeds and reported that the oil had a pleasant nutty and peanut butter-like aroma. Similar aroma was noted in roasted flaxseed oils after soxhlet extraction. GC-MS analysis indicated methyl pyrazine, 2,5-dimethyl pyrazine, trimethyl pyrazine, and 3-ethyl-2,5-dimethyl pyrazine as the most abundant Maillard end products in roasted flaxseed products (Figure 8). Pyrazines contributed to the roasted flavor of roasted products. The threshold levels of pyrazines are very low, thus low pyrazine contents could significantly affect the flavor profile of roasted products (Belitz *et al.*, 2004). According to the sensory ranking test, the top three roasted flaxseed treatments were 160°C for 16 and 24 minutes, and 180°C for 8 minutes. These treatments were screened for further sensory evaluation. Consumer preference tests indicated that roasted flaxseed had better flavor, texture, and overall acceptability than the nonroasted control sample. Roasting significantly improved the aroma and flavor properties of flaxseed. We believe that the presence of pyrazines in the roasted flaxseed affected consumer preference. Kozlowska (1989) reported similar results and the pleasant taste of roasted flaxseed. Principal component analysis of sensory results was used to show that overall acceptability was significantly related to flavor and texture, whereas appearance was not determined to be a consumer preference factor in the roasted flaxseed. The cracking or splitting of some of the flaxseed during roasting may be the cause for the lack of correlation between preference and appearance. Although minimal, the scanning electron micrograph (SEM) illustrates a split seed that occurs during roasting (Figure 9).

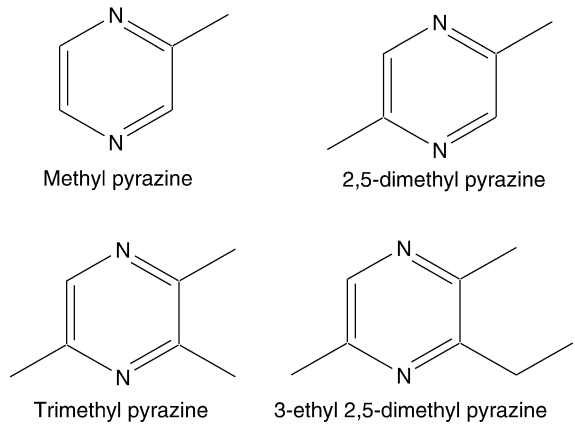


FIG. 8 Flavor compounds in roasted flaxseed observed by Meyers *et al.* (2004).

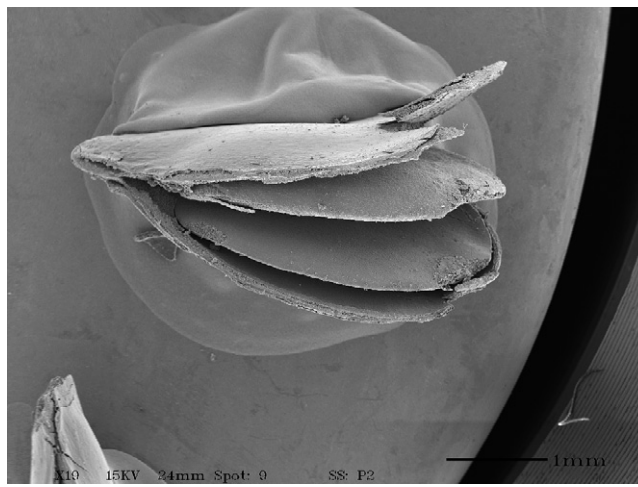


FIG. 9 Scanning electron micrograph of roasted flaxseed (160°C, 4 minutes, ×19) (Tulbek *et al.*, 2004).

Pearson correlation coefficients showed a relationship between color and extractable lipid. Lipid extraction was negatively correlated with brightness ($r = –0.68$, $p \leq 0.01$), and yellowness ($r = –0.67$, $p \leq 0.01$), whereas positively correlated with redness ($r = 0.40$, $p \leq 0.01$) color values. Thus, the extraction

of Maillard reaction byproducts during oil extraction may account for the color of the roasted flaxseed oil, and thereby explain the increase in extractable lipid observed. Peroxide values of flaxseed decreased significantly with roasting. Roasted flaxseed showed significantly ($p \leq 0.05$) lower peroxide values (0–0.2 meq/kg) when compared to the unroasted sample (1.8 meq/kg). In contrast, Yoshida and Takagi (1997) reported that peroxide values of roasted sesame seed increased with roasting temperature and time. FFA content of roasted flaxseed decreased with roasting applications; however, flaxseed roasted for a shorter time (4–8 minutes) had lower FFA contents than samples roasted longer (16–24 minutes). Shimoda *et al.* (1997) reported that the consumption of FFA during flavor compound formation can occur, but at high temperature and long-roasting times FFA formation occurs faster than the consumption of FFA in flavor formation. Thus, this may explain the increased FFA we observed in the flaxseed as roasting time increased. FFA content was significantly correlated ($r = 0.54$, $p \leq 0.01$) with peroxide value, indicating the corresponding decrease observed in both parameters. However, as FFA content increased at longer roasting times, peroxide values did not respond similarly. This might be due to a breakdown of the hydroperoxides and also the protective effects of endogenous antioxidants such as SDG, tocopherols, or various pyrazines.

Fatty acid compositions of roasted flaxseed samples are presented in Table XIII. The distributions of fatty acid showed statistical variations among the treatments within similar ranges. α-Linolenic, oleic, and LA were the predominant fatty acids in roasted flaxseed respectively. Hettiarachchy *et al.* (1990) reported the fatty acid composition of 11 flaxseed cultivars grown in North Dakota in 1989 to be 4.6–6.3 palmitic, 3.3–6.1 stearic, 19.3–29.4 oleic, 14.0–18.2 linoleic, and 44.6–51.5% ALA. The fatty acid composition of all samples reported in this study fall within the ranges reported by Hettiarachchy *et al.* (1990). Results were furthermore consistent with the literature findings, as fatty acid profile of roasted flaxseed was stable during and after roasting process (Kozlowska, 1989). ALA slightly decreased at longer roasting time, whereas no significant differences were detected in relation to roasting temperature. Chen *et al.* (1994) heated flaxseed samples at 178°C for 90 minutes and found that no significant difference was observed in fatty acid composition when compared to the control. When the same flaxseed was ground prior to heating, a significant decrease was observed in ALA and a significant increase observed in oleic acid (Chen *et al.*, 1994). Oleic acid content was negatively correlated with brightness ($r = -0.49$, $p \leq 0.01$) and yellowness ($r = -0.44$, $p \leq 0.01$) color values, whereas ALA was positively correlated ($r = 0.40$, $p \leq 0.05$; $r = 0.40$, $p \leq 0.05$ respectively). These findings might indicate the role of triacylglycerols or diglycerols on the development of color and flavor compounds

TABLE XIII
FATTY ACID DISTRIBUTION OF LIPIDS EXTRACTED FROM ROASTED FLAXSEED[a,b]

Roasting temperature (°C)	Roasting time (minutes)	16:0 (%)	18:0 (%)	18:1 (%)	18:2 (%)	18:3 (%)
	Unroasted	5.49bc	4.55c	27.15e	16.23d	46.28ab
140	4	5.54abc	4.51c	27.00e	16.33bcd	46.24abc
	8	5.49bc	4.32d	27.16cde	16.56a	46.05bc
	16	5.46bc	4.27de	27.05e	16.48ab	46.20abc
	24	5.58abc	4.54c	27.20cde	16.56a	45.74cd
160	4	5.27c	4.66b	26.98e	16.25cd	46.06bc
	8	5.44bc	4.49c	27.05e	16.28cd	46.27ab
	16	5.85a	4.77a	27.89a	16.24cd	44.79e
	24	5.65ab	4.48c	27.16cde	16.28cd	46.19abc
180	4	5.45bc	4.20e	26.93e	16.40abc	46.67a
	8	5.69ab	4.31d	27.36bcd	16.40abcd	46.05bc
	16	5.56abc	4.50c	27.44bc	16.46ab	45.45d
	24	5.69ab	4.58bc	27.48b	16.38bcd	45.51d
P		0.05	0.0001	0.0001	0.0001	0.0001

[a]Adapted from Tulbek *et al.*, 2004.
[b]Values not sharing a common letter are significantly different ($p \leq 0.05$).

during flaxseed roasting. In addition, microbial load on flaxseed was significantly reduced during the high temperature processing. The impact was greatest at high temperature long time combinations. Kozlowska (1989) observed that the roasting process promoted the decomposition of cyanogenic glycosides from 16.32 to 0.82 mg/kg. Thus roasting process could be means of improving the microbial quality of flaxseed and removal of undesired compounds, such as cyanogenic glycosides and vitamin antagonists, in flaxseed. However, only the extreme conditions were detrimental to the SDG content (Figure 10).

Meyers *et al.* (2004) and Tulbek *et al.* (2004) reported the storage stability of roasted flaxseed. Roasted flaxseed was stored for 16 weeks in paper bags at 25 and 30°C. Roasting process significantly decreased water activity (a_w) of flaxseed ($a_w < 0.1$). Very low water activity has been associated with rapid lipid oxidation (Fennema, 1996). Furthermore, cracked seeds expose cotyledon to air and consequently we anticipated that oxidation could be a setback during ambient storage. The storage of samples exhibited an increase in peroxide values (Figure 11) and a decrease in FFA. Elevation in oxidation rate supported our theory that, lower a_w levels enhanced oxidation. Week 16 samples gave painty and fishy aroma and flavor at 30°C. Peroxide values,

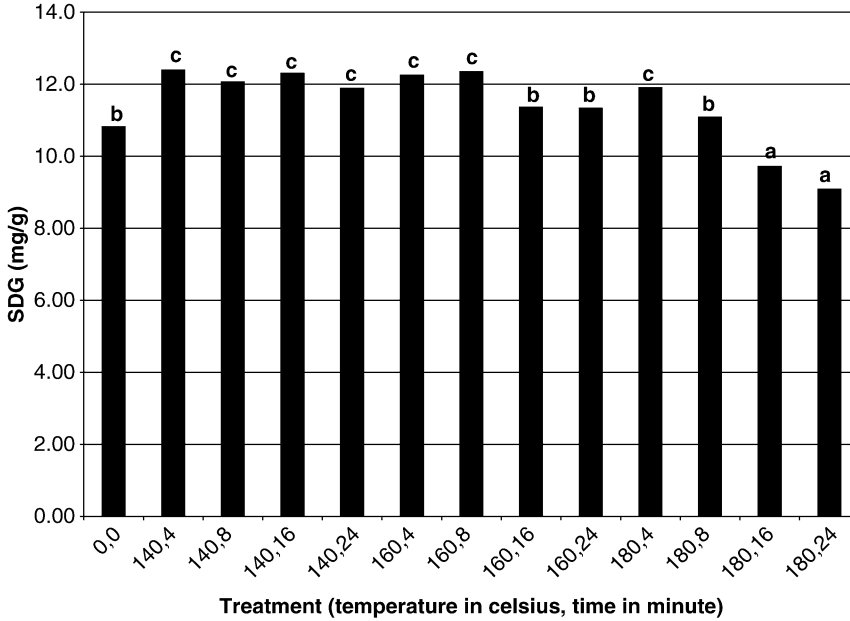


FIG. 10 Concentration of SDG (mg/g) in roasted flaxseed observed by Tulbek *et al.* (2004). Letters above bars represent significant ($p < 0.05$) differences.

propanal and hexanal contents significantly ($p < 0.05$) increased with storage time, indicating a decrease in stability of roasted flaxseed with paper bags. Data was not observed to be similar to Kozlowska (1989); however, water activity and harsh storage conditions were in a range that would accelerate the oxidation. Results indicated that storage in a paper bag and ambient temperature could not be appropriate for roasted flaxseed storage. Additional research is in progress to identify proper storage conditions.

G. BAKING APPLICATIONS

Garden (1993) reported the physical properties of wheat flour with added ground flaxseed. Dough strength was stable up to 5% ground flaxseed (Table XIV). Farinograph absorption significantly ($p \leq 0.05$) increased and dough strength remained stable up to 5% ground flaxseed addition. Peak time, an indirect indicator of dough mixing time, increased with flaxseed addition, which could lengthen baking time. However, a general weakening of farinogram curve was observed due to the decrease in stability over 5% level. Baking experiments indicated a reduction in terms of specific

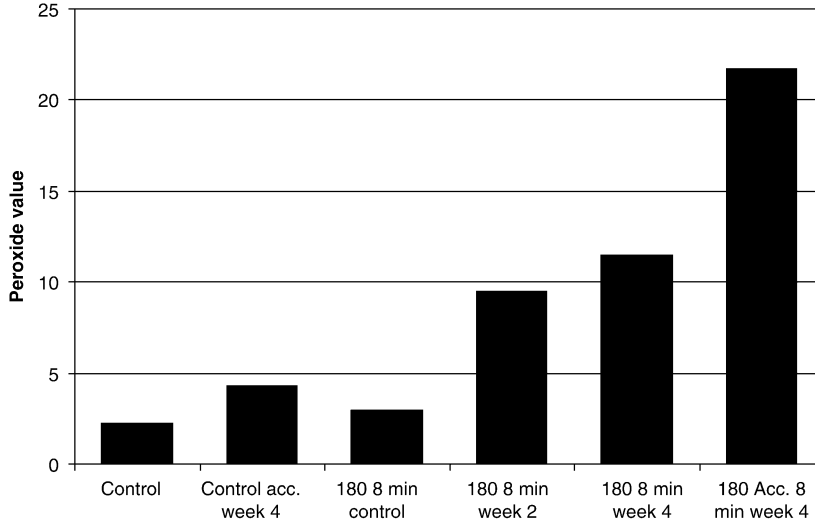


FIG. 11 Peroxide values (meq/kg) of roasted flaxseed (180°C, 8 minutes) stored at room temperature or 30°C (Accelerated = Acc) as observed by Tulbek *et al.* (2004).

volume and oven spring values with 5% ground flaxseed. The presence of TAG and flaxseed protein was suggested to cause the detrimental effects on bread properties. Ground flaxseed significantly increased bread firmness and reduced crust and crumb color quality due to the Maillards reactions possibly related to flaxseed protein and phenolic compounds (Garden, 1993). In contrast, the author observed superior results with flaxseed gum. Every 0.5% flaxseed gum addition increased water absorption of a hard spring wheat flour by 3%. Farinograph peak time slightly increased with flaxseed gum addition, whereas stability values decreased. Extensigraph analysis showed that extensibility, maximum resistance, and area values were significantly reduced with flaxseed gum, which was consistent with farinograph analysis. Nevertheless, flaxseed gum addition increased loaf volume and enhanced shelf stability of bread. Oven spring and specific volume values significantly increased at 1% flaxseed gum addition. Furthermore, crumb and crust color quality was similar to control, indicating no additional Maillard reactions. Carbohydrate–protein interactions in dough system could improve bread quality and explain some of the observations upon addition of flaxseed gum to dough.

Muir and Westcott (2000) investigated the stability of SDG in baked products. SDG content of flaxseed bread was stable during baking process

TABLE XIV
EFFECTS OF GROUND FLAXSEED ON FARINOGRAPH PROPERTIES OF LEN HARD RED SPRING WHEAT FLOUR[a,b]

Sample	Absorption (%)	Peak time (minutes)	Stability (minutes)
Control	60.5e	8.3d	21.8a
Control + 0.5% GF	61.5d	10.8c	22.0a
Control + 2% GF	62.0c	12.4b	22.0a
Control + 5% GF	63.0b	13.4a	20.8a
Control + 10% GF	65.0a	13.8a	15.4b

[a]Adapted from Garden (1993).
[b]Values not sharing a common letter are significantly different ($p \leq 0.05$).

and consistent with the observed range of SDG levels of flaxseed cultivars (Westcott and Muir, 1996). Muir and Westcott (2000) noted that recovery from the bread was only 73–75% of the theoretical yield of SDG. In their finding, the SDG in flaxseed bread was in a range of 34 to 136 mg/100 g, and that of other baked products including bagel, cookie, and muffin ranged from 60 to 120 mg/100 g. Nesbitt and Thompson (1997) also investigated lignan contents of homemade and commercial products containing flaxseed, which included bread, pancake, muffin, pizza dough, and breakfast cereal. The lignan content ranged from 1.1 to 32.4 μmol/100 g (0.33 to 9.72 mg/100 g by a conversion factor of 0.3, calculated from an average molecular weight of 300 of metabolites enterodiol and enterolactone) in the products due to the different flaxseed levels in the formulas and the different retaining ratio in the processing of different types of product (Nesbitt and Thompson, 1997). Gluten formation in bread or other bakery items could entrap flaxseed and interfere with SDG recovery, which might possibly lead to a low SDG theoretical yield.

Flaxseed powder and oat bran were utilized as a fat replacer in cake without any detrimental effects (Lee *et al.*, 2004b). Flaxseed significantly decreased viscosity at 20%; however, an increase in cake volume was observed, potentially due to the high lipid content which served as a shortening in the presence of nonstarch polysaccharides. However, more data are required to explain the observed phenomenon. Flaxseed powder addition significantly ($p \leq 0.05$) darkened crumb color and increased yellowness values. Results indicated that flaxseed was an acceptable additive when used with Nutrim oat bran.

Ghosh *et al.* (2004) reported the effects of ground flaxseed on wheat flour tortilla quality. Ground flaxseed addition significantly increased water absorption and decreased dough strength. However, the presence of nonstarch

polysaccharides in flaxseed significantly increased ($p \leq 0.05$) rapid viscoanalyzer values. Cold paste viscosity values were observed to be higher than the control. Flaxseed addition significantly decreased brightness values due to the presence of specks. Tortilla tensile properties of fresh tortillas containing 15% or more ground flaxseed were significantly different than the control and lower flaxseed treatments (5% and 10%). However, 10-day storage results did not show statistically significant differences in tensile properties. Headspace volatile analysis indicated that flaxseed in the tortilla significantly increased propanal, the major secondary volatile of ALA. Tortillas are typically baked for 1–2 minutes in an air impingement oven. Surface area of the product and lower a_w on the surface of tortilla could be the reason for the increased lipid oxidation. However, proper storage and packaging greatly diminished oxidation (unpublished data).

H. EXTRUDED PRODUCTS

Ahmed (1999) reported that flaxseed flour addition significantly reduced expansion ratio, and increased bulk density and breaking strength results, indicating a denser product. Sensory evaluation scores of the extruded flaxseed-corn snack product were lower than control. The water absorption index and water solubility index of extruded samples significantly decreased in extruded samples, but increased in raw samples. Flaxseed significantly ($p \leq 0.05$) reduced brightness and increased redness. Results were consistent with the observations of Garden (1993). Fiber fraction of flaxseed could be effective in extrusion properties due to the extensive hydrogen bonding and linear structures of flaxseed gum.

Manthey *et al.* (2000) investigated the effects of ground flaxseed in spaghetti. Dough strength significantly decreased, with small particles having the most detrimental effect on dough strength. However, medium and coarse fractions resulted in spaghetti that was too brittle whereas the fine particle size flaxseed gave acceptable spaghetti quality. Manthey *et al.* (2002a) noted that flaxseed macaroni was stable during processing and storage. FFA content was reduced due to hydration and extrusion processes. Hydrated premix showed higher conjugated diene values compared to dry premix. Manthey *et al.* (2002b) reported results consistent with the previous study. Extractable lipid and FFA content decreased due to processing. Conjugated diene results indicated that TAG and ALA were stable during processing and cooking steps (Table XV).

Lee *et al.* (2003) tested the effects of boiling, refrigeration, and microwave heating on ground flaxseed macaroni quality. Boiled macaroni and boiled-refrigerated-microwave-heated macaroni exhibited similar appearance attributes. Cooked firmness values were highest with boiled macaroni,

TABLE XV
CONJUGATED DIENE CONTENT OF LIPID (%, DB[a]) EXTRACTED FROM SEMOLINA-FLAXSEED, UNCOOKED AND COOKED SPAGHETTI[b,c]

		Uncooked		Cooked	
Mixture	Premix[d]	LTDC[d]	HTDC[d]	LTDC	HTDC
Semolina	0.05a	0.22c	0.22c	0.18b	0.18b
Semolina + 15% WGF[e]	0.03a	0.07a	0.07a	0.01a	0.00a
Semolina + 15% SF[e]	0.03a	0.10ab	0.13b	0.01a	0.03a

[a]DB, dry basis.
[b]Adapted from Manthey *et al.* (2002b).
[c]Values not sharing a common letter are significantly different ($p \leq 0.05$).
[d]Premix, dry mixture of semolina and ground flaxseed prior to pasta processing; LTDC, low temperature-drying cycle; HTDC, high temperature-drying cycle.
[e]WGF, whole ground flaxseed; SF, sieved flaxseed.

intermediate with refrigerated macaroni, and lowest with microwave-heated macaroni. Yoshida *et al.* (1990) noted a significant increase in conjugated diene values (from 0.38% to 0.78%) in microwave-heated (20 minutes) flaxseed oil. However, flaxseed macaroni was observed to be stable and ALA remained unchanged. In terms of lipid stability, results were consistent with previous results and lipid oxidation was not observed with boiling (6 minutes), refrigeration (72 hours), and microwave (20 s) treatments. Lee *et al.* (2004a) reported the stability of ground whole flaxseed and flaxseed hull in pasta. Hydration step exhibited the greatest detrimental impact on content and stability of extractable lipid in macaroni supplemented with flaxseed. Hydration decreased the amount of ALA and FFA, whereas an increase in the conjugated diene content in lipid fraction was observed. Semolina-flaxseed premix was hydrated for 10 minutes to 30% moisture prior to extrusion. The extrusion and drying processes did not affect lipid extraction or stability (Lee *et al.*, 2004a).

Hall *et al.* (2005) reported the shelf life of flaxseed macaroni in terms of SDG and lipid stability. Processing and drying methods did not affect lipid oxidation as much as the pretreatment of the flaxseed with steam or addition of the hull component. Pasta made with hull and steam-treated flaxseed had higher oxidation than pasta made with ground flaxseed. Propanal, pentane, hexanal, 2t- and 3c-hexenal, heptadienal, octanal, and nonanal volatiles were detected. These volatiles observed are similar to those reported by Malcolmson *et al.* (2000) and Jelen *et al.* (2000). Ultrahigh and high temperature-drying cycles showed slightly better oxidative stability than

low temperature-drying process. However, similar results were observed in the oxidation data for all drying applications. Pentane was observed to be the most abundant volatile of all treatments. Propanal significantly increased by week 32 in hull and steam-treated flaxseed, indicating a potential degradation of ALA. Thus, these pretreatments were detrimental to the oxidative stability of the flaxseed macaroni and were not recommended as possible alternatives to whole ground flaxseed (Hall *et al.*, 2005). Low temperature-dried pasta generally had higher levels of volatiles compared to high temperature- and ultrahigh temperature-dried macaroni. Samples did show early signs of oxidation but no detectable off-aroma was found by week 32. Nevertheless shelf life of pasta is limited as observed by high hexanal levels in pasta stored over 1 year. Volatile concentrations were similar to those reported by Malcolmson *et al.* (2000) hence macaroni containing ground flaxseed would be expected to have similar sensory properties. Minimal lipid oxidation could be due to the possible protection by native proteins of the flaxseed via an encapsulation of the oil body, formation of gluten during dough extrusion entrapping the ground flaxseed, the presence of a vacuum during extrusion, and the presence of antioxidants in durum wheat and flaxseed. In terms of SDG stability, low SDG recovery without a protease pretreatment of the samples supported the flaxseed-gluten entrapment hypothesized by Muir and Westcott (2000) in breads. No degradation in SDG was observed. Results indicated that macaroni containing ground flaxseed and dried at high temperature had very good lipid and SGD stability, which could be used as a means to enhance dietary ALA and SDG consumption.

Sinha *et al.* (2004) reported the effects of various levels of flaxseed (0–20%) on extrusion properties and cooking quality of fresh pasta. Appearance and cooking quality of fresh pasta made with flaxseed was superior at lower absorption level (29%), but brightness and yellowness scores were lower whereas redness score increased in fresh pasta. Flaxseed flour decreased energy requirement to extrude dough, by decreasing gluten strength. Cooked firmness values varied significantly ($p \leq 0.05$) (Figure 12). Control sample yielded the highest firmness and statistically differed from 5% and 10% flaxseed fortified fresh pasta ($p \leq 0.05$), whereas 15% and 20% flaxseed fortified fresh pasta gave the lowest firmness scores.

I. DAIRY PRODUCTS

Flaxseed oil has been proposed to be a valuable ingredient for ice cream products (Hall and Schwarz, 2002). Flaxseed oil replaced between 10% and 25% of the milk fat in ice cream formulas has been investigated. The 25% flaxseed product exhibited an oil-like mouth feel; however, the presence of

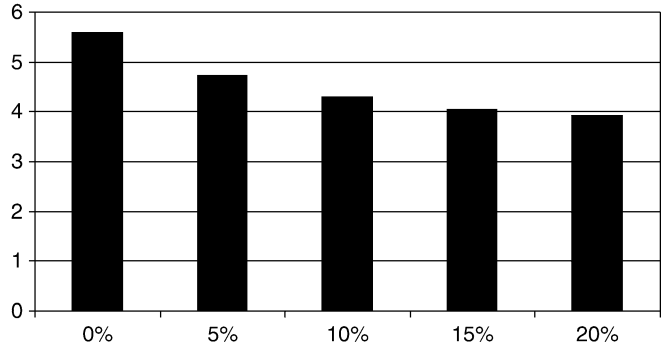


FIG. 12 Effect of flaxseed concentration on fresh pasta cooked firmness (determined by Texture Analyzer TA-XT2, g-cm) observed by Sinha *et al.* (2004).

the oil in product could not be detected by 60% of the panelists using an informal sensory evaluation. A trained sensory panel showed that 15% of the milk fat could be replaced in a vanilla ice cream without being detected. The melt time of flaxseed ice cream was not significantly different compared to control. The 25% flaxseed ice cream product gave a thin consistency compared to 10% product, whereas 10% flaxseed ice cream showed similar properties with control. Flaxseed oil addition significantly improved the fatty acid profile of frozen dessert (Table XVI). No ALA was detected in conventional chocolate ice cream, whereas ALA constituted 20.4% of the fatty acids in the lipid fraction of the flaxseed oil ice cream. Moreover, the ratio of n-3 to n-6 fatty acids was approximately 2.5 to 1 for flaxseed oil ice cream. Overall saturated fatty acid composition decreased from 64.6% to 42%. The ratio of saturated to unsaturated fatty acids was roughly 2.3 to 1 and 0.77 to 1 for the chocolate- and flaxseed oil-containing products, respectively.

Hall *et al.* (2004) reported the stability of lignan in yogurt. In addition, flaxseed extract addition did not have a negative impact on the fermentation. At 700 ppm of the flaxseed extract, lactic acid was observed to be higher than control, whereas the 7000 ppm flaxseed extract addition resulted in lactic acid levels similar to the control yogurt. In contrast, lactic acid bacteria counts in yogurts were lower than the control for the product containing 700 ppm but higher in the product containing 7000 ppm. Acetaldehyde content, the characteristic volatile of yogurt was not significantly influenced by flaxseed extract addition. Lactic acid content, pH, and acetaldehyde content were not significantly affected with regards to end product quality, whereas a reduction in SDG content was observed due to the possible interactions between SDG and

TABLE XVI
FATTY ACID PROFILE OF A COMMERCIAL CHOCOLATE ICE CREAM AND A CHOCOLATE FROZEN DESSERT CONTAINING 25% FLAXSEED OIL[a]

	% Fatty acids[b]	
	Flaxseed oil-fortified ice cream	Standard ice cream
C4–C12 fatty acids	5.2	8.8
Myristic 14:0	6.6	10.9
Palmitic 16:0	20.7	31.5
Stearic 18:0	9.5	13.4
Oleic 18:1	25.7	25.0
Linoleic 18:2	8.3	3.5
Linolenic 18:3	20.4	0.0

[a]Adapted from Hall and Schwarz (2002).
[b]Sums of the fatty acids do not equal 100% due to the fact that odd and branched chain fatty acids present in dairy are not included in the table.

yogurt proteins. SDG recovery results were consistent with the findings of Muir and Westcott (2000) and Hall *et al.* (2005).

J. ANTIFUNGAL PROPERTIES

Antifungal properties of flaxseed were tested in the author's research lab. Milled hull, embryo, or whole flaxseeds of Omega were milled and incorporated into fresh pasta at up to15% and stored in plastic bags under room temperature. Mold growth was monitored on a daily basis. Fresh pasta with 15% Omega flaxseed or fraction was mold-free after 20 days, whereas mold growth was present after 4 days in the control. Defatted flaxseed meal was also tested for their effect on the mold growth of fresh pasta. At 9% of defatted meal level, mold appeared on the same day as compared with control. In contrast, at the 15% level, there was no mold growth after 20 days. We first thought that the variance in mold growth on different fresh pasta samples was due to difference in water activity in different samples. Results from water activity showed that each sample had a water activity of approximately 0.97. These results suggested that components in flaxseed caused the observed phenomenon.

In a subsequent evaluation, a noodle formula was tested with 15% ground flaxseed addition. Noodle sheets were cut into circles and put into sterilized petri dish. The spot inoculation screening method using *Penicillium chrysogenum, Fusarium graminearum*, and *Aspergillus flavus* was conducted to test the antifungal activity of the whole ground flaxseeds. Three spots for each

microorganism were inoculated on the noodle sheets and incubated for 5 days. After which time the colony size of the microganism growth was measured. Noodle sheets incorporated with both yellow and brown seeds had less or no mold growth after 6 days of incubation, which suggested that flaxseed exhibited antifungal activity in fresh noodle. Several lots of flaxseed purchased from different suppliers were used in the study. No significant ($p > 0.05$) impact on antifungal activity was observed between flaxseed obtained from various suppliers. The antifungal activities of the yellow and brown flaxseed also were not significantly different. In contrast, microorganisms showed significantly different responses to the flaxseed treatments. Further investigation has shown that the protein and polyphenolic fractions have antimicrobial activity (Xu *et al.*, 2006).

K. VALUE-ADDED ANIMAL PRODUCTS

Flaxseed as an animal feed has been limited until recently, although the benefits of feeding flaxseed to animals have been observed for nearly 100 years. Many of these observations have become folklore such as shinner coats and improved animal health. Quantifying the benefit to animal health is needed. However, some studies have shown general health improvement along with enhancement to animal production and end product quality. For a review of the health and production issues see the extension publications of Maddock *et al.* (2005), Novak and Scheideler (1998), and Puthpongsiriporn and Scheideler (2001).

1. Poultry

The most recent interest in flaxseed as a feed has focused on enhancing the ALA and other long chain fatty acid contents in eggs, meat, and milk. Scheideler and Froning (1996) found that ALA was the major omega-3 in egg yolks. However, significant amounts of longer-chain omega-3 fatty acids, such as EPA, DPA, and DHA, were incorporated into the egg yolk phospholipid fraction. Furthermore, a linear increase in ALA content in the yolk was observed with increasing (i.e., 5%, 10%, and 15%) dietary flaxseed (Scheideler and Froning, 1996). Ahn *et al.* (1995) reported that incorporation of ALA (3%) and tocopherol (120 IU/kg) into chicken diets enhanced omega-3 content in eggs by 6.5%. Over 70% of the omega-3 lipid in the egg was ALA. The remaining omega-3 lipids were DHA and EPA, which accounted for approximately 23% and 7%, respectively. Leeson and Caston (2004) reported that dehulling of flaxseed improved ALA deposition in eggs. However, a nonsignificant reduction in EPA and DHA was observed in eggs from hens fed a dehulled flaxseed diet compared to the whole flaxseed diet.

Although minor differences were reported, sensory scores of eggs from the chickens fed the control (tallow) diet were more favorable than the eggs from the ALA diet. Scheideler *et al.* (1997) reported that the overall acceptance of fresh eggs from hens fed a flaxseed diet were not significantly different from control eggs. However, slightly lower scores in appearance and flavor were observed in eggs obtained from hens fed 10% to 15% ground flaxseed compared to whole flaxseed. Parpinello *et al.* (2006) also reported that overall acceptance of the eggs from hens fed a 2% flaxseed diet was not significantly different from the control. In boiled egg evaluations, the overall acceptability of eggs from flaxseed fed hens was lower than the acceptability of control eggs (Leeson *et al.*, 1998). However, no difference in acceptability was detected between eggs from hens fed a 10% or 20% flaxseed. Leeson *et al.* (1998) also reported that vitamin E fortification in the hen's diet did not improve the sensory characteristics of the eggs. In fact, the combined high flaxseed and high vitamin E diet produced the lowest quality eggs. The appearance of the eggs from hens fed the golden (Omega) flaxseed variety was preferred by panelists over eggs of hens fed brown (Neche) flaxseed (Scheideler *et al.*, 1997). From these reports, a number of factors, such as flaxseed variety and level and egg preparation method, may be responsible for reported differences in sensory quality.

In addition of changes in egg composition, feeding flaxseed to chickens alters the meat omega-3 content. Ajuyah *et al.* (1993) reported significantly higher omega-3 lipids in both dark and white meat from broiler chickens fed flaxseed or flaxseed combined with an antioxidant. The white meat from the chicken fed the flaxseed diets with mixed tocopherol and mixed tocopherol plus canthaxanthin had significantly more omega-3 fatty acids compared to the flaxseed alone or the control diet. In contrast to the white meat, the dark meat had higher ALA levels and less EPA and DHA. However, the overall omega-3 content was higher in the dark meat due to the higher ALA content (Ajuyah *et al.*, 1993). Gonzalez-Esquerra and Leeson (2000) also reported that ALA was deposited in the dark meat broiler chickens feed flaxseed for 1 week prior to slaughter. They also found that more long chain omega-3 fatty acids were deposited in the white or breast meat. These authors reported that the sensory characteristics of the breast meat were not affected by chicken diet. However, only diets containing menhaden oil (7.5 g/kg) or flaxseed (100 g/kg) plus 0.75 g/kg menhaden oil negatively impact the sensory quality of the thigh meat (Gonzalez-Esquerra and Leeson, 2000). Loopez-Ferrer *et al.* (1999) reported that replacement of fish oil with flaxseed oil (8.2% in the diet) in broiler chicken diets increased meat ALA content. However, a decrease in long chain omega-3 content was also observed. Feeding of the flaxseed diet throughout a 5-week period resulted in unacceptable sensory quality. In contrast, feeding the 8.2% flaxseed diet for 1 or 2 weeks prior to

slaughter resulted in meat with higher sensory scores compared to the fish oil diet (Loopez-Ferrer *et al.*, 1999).

In several studies, the deposition of the omega-3 in the TAG or phospholipids has been identified. ALA tends to deposit in the TAG fraction whereas long chain omega-3 fatty acids deposit in the phospholipids (Ajuyah *et al.*, 1993; Jiang *et al.*, 1991; Scheideler and Froning, 1996). The data reporting the deposition of omega-3 fatty acid in poultry eggs and meat is clear; however, sensory evaluation of the products give mixed results. For an extensive review of omega-3-enriched poultry meat and eggs see Elswyk (1997), González-Esquerra and Leeson (2001), and Scheideler (2003).

The enhancement of omega-3 in eggs has been used to create a new egg market. The "Omega eggs" contain 350 mg omega-3 fatty acids per egg and have lower amounts of saturated fatty acids and cholesterol (Scheideler and Lewis, 1997). The "Omega Egg" was developed at the University of Nebraska by feeding chickens a special diet containing flaxseed (Scheideler, 1998, 1999). In a small feeding trial, enriched omega-3 eggs, from hens fed flaxseed, were reported to enhance omega-3 fatty acids in blood platelet phospholipid male participants (Ferrier *et al.*, 1995). The data suggests that eggs from flaxseed fed hens could be a way to reduce the dietary omega-6 to omega-3 ratio in humans.

2. *Beef cattle*

Several researchers have looked at the use of flaxseed in beef cattle diets as a means to enhance omega-3 composition in meat. The conversion or deposition of omega-3 into milk and meat of cattle is far less efficient compared to poultry. Ruminal biohydrogenation of the unsaturated fatty acids is thought to be the main reason for the poor deposition omega-3 fatty acids in the meat. However, feeding flaxseed did enhance ALA content in beef when compared to barley- and corn-based diets (Maddock *et al.*, 2003) and Holstein steers (Drouillard *et al.* 2002, 2004). The level of long-chained omega-3 also increased in meat of cattle fed a flaxseed diet. Maddock *et al.* (2005) reported that ALA, EPA, DPA, and DHA levels were higher in the phospholipid fraction of cattle fed 8% flaxseed diet compared to the control. Drouillard *et al.* (2002, 2004) also reported enhanced EPA and DHA in cattle fed a 5% flaxseed diet. In contrast to the observed omega-3 enhancement, conflicting sensory characteristics of omega-3-enhanced beef have been reported (Drouillard *et al.* 2004; Maddock *et al.*, 2003, 2004).

Maddock *et al.* (2003) reported that marbling score were improved for when flaxseed was incorporated into the cattle diet. This observation was in agreement with that of Drouillard *et al.* (2002). The tenderness was lower for

boneless strip loins from steers fed 6% flaxseed compared to those fed 3% flaxseed. Juiciness was rated higher for boneless strip loins from cattle fed a corn diets. All diets that included flaxseed resulted in statistically similar juiciness score. In contrast, flavor of the boneless strip loins was not affected by the addition of flaxseed to cattle diet (Maddock *et al.*, 2003). Drouillard *et al.* (2004) did not find significant differences in the tenderness, juiciness, or flavor of steaks from animals fed flaxseed or a control diets.

Further investigation by Maddock *et al.* (2004) showed that tenderness scores were best, but not significant, for steaks obtained from heifers that were on a diet that contained 8% rolled flaxseed. In contrast, the Warner-Bratzler shear force values for tenderness did show significantly improved tenderness values compared to the control corn diet. Furthermore, steaks obtained from heifers fed an 8% rolled or ground flaxseed diet were significantly more tender than those steaks obtained from heifers fed an 8% whole flaxseed diet (Maddock *et al.*, 2004). As in their previous study, juciness scores of the steaks from the flaxseed fed heifers were rated lower than the steaks from cattle fed the control diet. However, the differences in juiciness of steaks obtained from heifer on the rolled flaxseed diet and the control diet did not appear to be significant (Maddock *et al.*, 2004). Thus, the data suggests that preparation of the flaxseed did have some benefit to finished product quality.

3. Dairy cattle

The information obtained from studies with dairy cattle is mixed with regards to the influence of flaxseed in milk production and composition. However, the general trend is that flaxseed in the cattle diet does influence the composition of milk. The method of feeding flaxseed may be responsible for the sometime contradictory observations regarding milk production. Kennelly and Khorasani, (1992) reported that milk production and fat content were not affected by the incorporation of rolled flaxseed up to 15% in the diets of dairy cattle. Goodridge *et al.* (2001) and Ward *et al.* (2002) also reported that milk production and fat content were not affected by flaxseed diets. Mustafa *et al.* (2003) and Gonthier *et al.* (2005) also reported that milk yield was not significantly different between cattle fed the control diet or diets containing microionized or untreated flaxseed. However, these authors did report lower milk fat content in a diet containing ground flaxseed. Petit *et al.* (2001) also reported a lower fat content in milk and lower milk production from cattle fed formaldehyde-treated flaxseed. In contrast, Petit (2002) found that a whole flaxseed-containing diet resulted in higher milk production than the control diet containing Megalac® (rumin

inert fat). However, the milk fat content was lower in the milk obtained from the cattle on the flaxseed diet compared to the Megalac® diet. When considering the fat production on a kilogram per day basis, no significant differences were observed in the milk fat production (Petit, 2002; Ward *et al.*, 2002). All research agreed that omega-3 content of milk was enhanced by the addition of flaxseed in the diet of cattle.

Kennelly and Khorasani (1992), Khorasani and Kennelly (1994), and Ward *et al.*, (2002) reported that protein content was lower in milk from cattle fed a diet containing flaxseed. In contrast, Goodridge *et al.* (2001), Petit (2002), and Petit *et al.* (2001) reported an increased protein composition of milk obtained from cattle fed flaxseed-fortified diets. Mustafa *et al.* (2003) reported similar protein contents for milk obtained from cattle on the control or flaxseed diets. Studies have been completed to evaluate the methods to reduce the rate of biohydrogenation of ALA and crude protein degradability in dairy cattle and model *in vitro* systems (Gonthier *et al.*, 2004a,b; Loor *et al.*, 2005; Petit *et al.*, 2002). Thus, the conflicting observations regarding production, and the fat and protein contents is likely due to the method of flaxseed incorporation into cattle diets and how the cattle assimilate the flaxseed. In any case, omega-3-enhanced milk can be achieved by incorporating flaxseed into the diets of dairy cattle.

4. Swine

Romans *et al.* (1995a,b) reported that incorporation of up to15% flaxseed into the diets of swine did not affect carcass traits. However, the amount of omega-3 incorporated into pork lipids varied depending on the carcass tissue. The ALA and EPA contents in the outer backfat were affected by flaxseed incorporation into the swine diet whereas no change in DHA was observed. No significant differences were observed in the ALA or EPA content of the outer backfat of swine fed a 10% or 15% flaxseed diet (Romans *et al.*, 1995a). In contrast, significantly more ALA and EPA were found in the middle/inner backfat for swine fed 15% flaxseed. The addition of 5% and 10% flaxseed in the swine diet also resulted in higher ALA and EPA contents in the backfat compared to the control. Thacker *et al.* (2004) also observed increased ALA content in backfat obtained from swine fed a 30% Linpro (i.e., extruded flaxseed and pea product) compared to the control diet containg soy meal. Significant increases in backfat ALA and EPA contents were also observed as the length of exposure to the flaxseed diets increased to 28 days (Romans *et al.*, 1995b). However, longer feeding trials showed reduced omega-3 contents in adipose tissue (Fontanillas *et al.*, 1998; Riley *et al.*, 2000).

Signficant increases in ALA and EPA were also observed in the belly and bacon of the swine. Belly and bacon from swine fed the 15% flaxseed diets had the highest ALA and EPA contents (Romans *et al.*, 1995a). Signficant increases in belly and bacon ALA and EPA contents were also observed as the length of exposure to the flaxseed diets increased to 28 days (Romans *et al.*, 1995b). Frying of the bacon resulted in a significant increase in EPA and DHA contents whereas ALA was not affected compared to uncooked bacon. The content of ALA was higher in the neutral lipid fraction compared to the polar lipid fraction. In contrast, the polar lipids contained more EPA (Romans *et al.*, 1995b). The increased EPA and DHA in the fried bacon may have been due to the incorporation of these fatty acids into the phospholipid (i.e., polar lipids) fraction and thus less impacted by the heating process. Microwaving proved to be detrimental to the omega-3 fatty acids, as signficiant reductions in their contents were observed (Romans *et al.*, 1995a,b).

The incorporation of omega-3 fatty acids into the loin was not as efficient compared to the other tissues. Feeding a diet greater than 10% flaxseed did not enhance loin ALA or EPA contents and only minimal changes were observed after 21 days of feeding (Romans *et al.*, 1995a,b). Kouba *et al.* (2003) also found increased ALA and EPA contents in the loin of the pig fed the flaxseed diet compared to the control. However, only minor changes were observed in fatty acid contents in lipids in swines fed flaxseed up to 60 days. A significant reduction in ALA and EPA contents were observed in pigs fed flaxseed for 100 days compared to the 60-day feeding.

Sensory panelists were able to identifiy the bacon from the animals fed 10% and 15% flaxseed. Romans *et al.* (1995b) reported that flavor intensity of bacon was greater for bacon obtained from animals fed flaxseed diets between 14 and 28 days compared to the control diet. As the length of the feeding trial increased, so did the fishy defect as reported by the sensory panel. In contrast, panelists were not able to identify the loin from the swine fed flaxseed up to 15% (Romans *et al.*, 1995a) or differentiate the eating quality of loin in swine fed up to 10% flaxseed (Matthews *et al.*, 2000). Riley *et al.* (2000) also observed that feeding flaxseed for 24 days resulted in loin steaks that were juicer and had greater tenderness than the loin steaks from the swine fed the control diet. No differences in taste, tenderness, and juiciness were observed between the loins from pigs fed diets containing 0.4, 0.7, or 1.0% ALA (van Oeckel *et al.*, 1996).

Feeding of animals in general can enhance the omega-3 levels in lipids and is well documented. In contrast, few studies have documented the stability of the lipids in stored meats or in processed meat products. Additional researcher is needed to evaluate the sensory characteristics of stored meats and of processed meat products containing enhanced omega-3 lipids.

V. CONCLUSION

Flaxseed is an oilseed that has had a long history dating back nearly 12,000 years. However, many people are still unaware of the potential health benefits of flaxseed and potential food applications. One cereal company (US Mills) has had flaxseed as a component in their Uncle Sams cereal for nearly 100 years. More recently, other cereal, baking, and pasta companies have incorporated flaxseed into their formulation. Many of these products are promoted as high fiber, but also have a nutrient content claim for the omega-3 fatty acid. Currently, flaxseed does not have a health claim or qualified health claim. The intent of this chapter was to provide a foundation for individuals not aware of the benefits of flaxseed and potential food applications. Although the body of evidence is growing in support of flaxseed consumption, many more studies are needed to resolve the conflicting reports regarding the health benefits, in particular the role of ALA in prostate cancer and cancer in general. Also, a daily recommendation for flaxseed has to be made by authoritative organizations. The general recommendation has been 1–3 tablespoons per day for ground flaxseed or 1 tablespoon for flaxseed oil (Morris, 2003a). These recommendations are based on data reported in the health benefits section of this chapter.

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