EFFECTS OF PASTEURIZATION (HEATING) ON MILLED FLAXSEED QUALITY

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State

University's regulations and meets the accepted standards for the degree of

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ABSTRACT

Current food industry practices include the use of pasteurization (heat treatment) as a means to reduce the microbial counts of flaxseed (*Linum usitatissimum*). Flaxseed was pasteurized using a dry air heat treatment of 148 °C for 16.25 minutes and the oxidative stability was observed over a 20 week storage period. Flaxseed quality was measured using peroxide value, percent free fatty acid (as oleic), and propanal content. Peroxide values of the pasteurized milled flaxseed were found to significantly increase ($P \leq 0.05$) by week 2. Propanal formation coincided with peroxide value development over 20 weeks of storage. Pasteurized flaxseed milled under refrigerated temperatures (10 – 18 °C) did not exhibit any significant ($P > 0.05$) improvement in oxidative stability. Flaxseed pasteurization at 148 °C for 16.25 minutes using dry heat was found to be detrimental to the oxidative stability flaxseed once milled.

ACKNOWLEDGEMENTS

To my children, Nathaniel and Connor, your love, hugs, and smiles have given me the strength, motivation, and drive to excel and do my best each and every day. Your special love has provided daily support and joy which has been a driving force in my life and work.

And lastly, heartfelt and sincere thanks to my amazing wife, Ashley, for her unconditional love, support, patience, sacrifice, and inspiration which has made achieving my goals and dreams possible. Thank you for everything that you do! To the moon and back, I love you!

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GENERAL INTRODUCTION

The use of flaxseed as a functional food ingredient has been increasing due the health benefits associated with its consumption (Oomah 2001). Flaxseed has been used as a functional ingredient to fortify several different food products such as bakery products, breakfast cereals, snack foods, and soups (Xu et al 2008). Many of the physiological benefits attributed to flax are derived from the high levels of α-linolenic acid, lignans, and the flaxseed fiber or gum (Oomah et al 2006). Several potential health benefits associated with flaxseed or flaxseed meal consumption include anticancer effects, antiviral and bactericidal activity, anti-inflammatory effect, ion reduction, laxative effect, reduction of atherogenic risks, reduction in cardiovascular diseases, hypoglycemic effect, and prevention of menopausal symptoms and osteoporosis (Babu and Weisenfeld 2003; Collins et al 2003; Dalprane et al 2010; Aliani et al 2011).

Food manufacturers are increasingly beginning to require microbial counts from food ingredients in order to achieve strict microbial criteria for their finished products. Flaxseed handlers are therefore being required to produce or provide flaxseed with low microbial counts. Flaxseed has microbial counts that are similar to commodities such as wheat (Manthey et al 2004). In addition, the microbial counts vary depending on environment and storage conditions.

Current industry practices include the use of pasteurization (heat treatment) as a means to reduce the microbial counts of flaxseed to levels acceptable to food manufacturers. However, heat treatment increases the possibility of damaging the seed and thus causing a reduction in the quality of the seed. The combination of heat treatment and improper milling can further reduce the quality. However, the combination of low temperature pasteurization followed by low temperature milling and packaging may aid in maintaining quality.

Properly milled flaxseed stored under conditions with limited access to oxygen and light had a shelf life of over 20 months (Przbylski and Daun 2001). However, food companies are

interested in microbial loads first and quality second. The new Food Safety Modernization Act may further increase the focus of food companies on the microbial issue. Thus, the purpose of this study is two-fold. First, the effects of pasteurization on milled flaxseed quality and oxidative stability were evaluated. Second, the effects of low temperature milling were evaluated to determine if the combination of pasteurization followed by cold temperature milling will affect milled flaxseed quality as compared to milling under ambient milling conditions.

This project has direct and indirect benefit to flaxseed producers. The value is in providing food companies with relevant information regarding stabilized flaxseed. There is confusion by food companies about why some flaxseed is stabilized and why some is not. One aim of this study is to determine the impact of heat treatments commonly used on flaxseed by handlers for stabilization. This study will determine if these treatments are causing undue damage such as increased susceptibility to oxidation. An output from this work will be a recommendation to the industry regarding the stabilization process and if it should be done. Currently, several companies are promoting stabilized flaxseed and based on communications with food companies, (we have experienced), that these companies have a negative perception of stabilized flaxseed (Communication with Dr. Mehmet Tulbek of Alliance Grain). Thus, this affects producers because food companies are not using flaxseed due to their experiences with poor quality stabilized flaxseed.

LITERATURE REVIEW

Flaxseed

Flaxseed, also known as linseed (*Linum usitatissimum*), is obtained from the flax plant which is a member of the Linaceae family. Flaxseed is an ancient crop which has been used both for food and for linen fiber. Flaxseed is mainly cultivated in America, Argentina, Canada, China and India today (Zhang et al 2008). The most common forms of flaxseed are brown, yellow, and golden yellow (Vadukapuram 2009). Today, flaxseed is mainly grown for its oil, which is used in the manufacture of paints, varnishes, and linoleum (Coşkuner and Karababa 2007). Flaxseed also is used as a functional ingredient due to the suggested health benefits associated with its consumption (Bravi et al 2011). Flaxseed is available as a whole seed or in one of its derivative forms: ground flaxseed, flaxseed oil, defatted flaxseed, flaxseed fiber, or lignan extract.

Flaxseed characteristics

Flaxseed dimensions are approximately 3.0–6.4 mm in length, 1.8–3.4 mm in width, and 0.5–1.6 mm in thickness. The amount of pigment in the flaxseed coat determines the color of the seed (Coşkuner and Karababa 2007). Flaxseed is commonly identified as either brown flaxseed or golden flaxseed. The composition of flaxseed varies by seed genetics, growing environment, seed processing, and method of analysis, but is typically 30–40% fat, 20–25% protein, 20–28% total dietary fiber, 4–8% moisture and 3–4% ash (Coşkuner and Karababa 2007; Zhang et al 2008).

Flaxseed is considered an oilseed as it is composed of about 40% oil, of which more than 50% is α-linolenic acid (Choo et al 2007). The main source of oil in flaxseed is found in the flaxseed cotyledon (75%) and the remaining oil is found in the seed coat/hull (22%) and the embryo/germ (3%). Triacylglycerol is the most common lipid type found in flaxseed. The

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predominant triacylglycerols in flaxseed oil are trilinolenoylglycerol (LnLnLn) at 30%, dilinolenoyllinoleoylglycerol (LnLnL) at 19%, and dilinolenoylpalmitoylglycerol (LnLnP) at 7%. The fatty acid distribution of flaxseed is primarily composed of unsaturated fatty acids: oleic, linoleic, and linolenic acids. These three unsaturated fatty acids account for more than 70% of the total fatty acid distribution (Schorno 2006). The most predominant fatty acid found in flaxseed is α-linolenic acid (ALA), which typically accounts for greater than 50% of the total fatty acids (Choo et al 2007).

Lipid oxidation

Lipid oxidation is a primary concern when it comes to the quality of food products as it can reduce the overall quality and acceptability of foods. The oxidation of flaxseed lipids, primarily ALA, can lead to the development of objectionable odors and flavors resulting in product rancidity. Additionally, oxidation can adversely affect the nutritional value of flaxseed through the degradation of vitamins, such as vitamin E (Schorno 2006).

Catalysts of oxidation include light, temperature, enzymes, metals, metalloproteins, pigments, and microorganisms (Yildirim 2009). The extent of oxidation is typically influenced by the nature of a food (fatty acid composition, number, position, geometry, and conjugation of double bonds, prooxidants, chelators, and antioxidants) and the physicochemical environment (oxygen, moisture, light, and heat) during processing, packaging and storage (Colakoglu 2007).

Lipid oxidation is considered to be enzymatic, nonenzymatic, or a combination thereof. All lipid oxidation pathways lead to the formation of lipid hydroperoxides through the replacement of a hydrogen atom on the lipid hydrocarbon chain with a hydroperoxy group (OOH) (Schorno 2006). Hydroperoxides, once formed, are susceptible to further oxidation leading to the formation of secondary reaction products such as aldehydes, ketones, acids, and

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alcohols. Many of the secondary reaction products negatively impact flavor, aroma, taste, nutritional value, and overall food quality (Yildirim 2009).

Autoxidation

Autoxidation is a non-enzymatic oxidation process that proceeds via a free radical chain reaction. The reactants of autoxidation are unsaturated fatty acids and oxygen, where the unsaturated fatty acids can be either in a bound or free form. Several different factors influence the rate of autoxidation including: fatty acid composition, degree of unsaturation, presence and activity of pro- and antioxidants, partial pressure of oxygen, nature of the surface exposed to oxygen, and the storage conditions (temperature, light exposure, moisture content, etc.) of fat/oil containing foods (Belitz et al 2009).

Foods containing high levels of polyunsaturated acids are increasingly predisposed to autoxidation. The rate of autoxidation has been found to increase with increasing degrees of lipid unsaturation whereas the number of allyl groups present increases, so too does the oxidation rate and the length of the induction period (Belitz et al 2009). Relative to stearic acid, a saturated fatty acid, the oxidation rate of the fatty acids oleic, linoleic, and linolenic are exponentially higher as the degree of unsaturation increases (Table 1). Flaxseed lipids are predominantly composed of ALA, which is the most unsaturated fatty acid found in flaxseed (Schorno et al 2010). The high content of ALA in flaxseed oil therefore predisposes it to a high susceptibility to autoxidation.

Autoxidation takes place in three distinct stages: initiation, propagation, and termination (Figure 1) (Kubow 1992). The initiation phase of autoxidation begins with the abstraction of a hydrogen atom next to a double bond of an unsaturated fatty acyl chain (LH) by a free radical initiator (X•) to produce a free lipid radical (L•) group (Kubow 1990; Ladikos and Lougovois

1990). The labile hydrogen is cleaved from the α-carbon next to a double bond of the unsaturated

fatty acid as this site results in the formation of a stable allylic radical whereby the electrons are

delocalized over three carbon atoms (Kubow 1992; Sun et al 2011). At the endpoint of the

initiation phase, the rate of oxidation is accelerated, the consumption of oxygen by the system

dramatically increases, and the concentration of peroxides is significantly increased (Laguerre et

al 2007).

Table 1. Relative oxidation rate and induction period for oxidation of fatty acids at 25 °C
(Adapted from Belitz et al 2009).

Fatty acid	Lipid number*	Induction period (h)	Oxidation rate (relative)
Stearic acid	18:0		1
Oleic acid	18:1 (9)	82	100
Linoleic acid	18:2 (9, 12)	19	1200
Linolenic acid	18:3 (9, 12, 15)	1.34	2500

* First number indicates carbon chain length; second number indicates degree of lipid
unsaturation; numbers within parentheses indicate locations of unsaturation on carbon chain.

In the propagation phase (Figure 1) of autoxidation, the lipid radical formed during the

initiation phase readily reacts with oxygen to form a lipid peroxyl radical (LOO•). The newly

formed peroxyl radical then reacts with a second polyunsaturated fatty acid chain resulting in the

formation of a lipid hydroperoxide (LOOH) and a lipid radical (L•), thus resulting in the

conservation of the lipid radical in the reaction equation (Kubow 1992). The reaction is

propagated by the abstraction of a hydrogen atom from a different fatty acid by the newly formed

lipid peroxyl radical. The lipid autoxidation rate is enhanced by the decomposition of

hydroperoxide molecules to alkoxyl and hydroxyl free radicals leading to the branching of the

autoxidation process (Schorno 2006; López-Duarte and Vidal-Quintanar 2009). The peak of

hydroperoxide formation is indicative of the onset of the termination phase (Laguerre et al 2007).

Initiation:	X• + LH	-------->	L• + XH

Propagation:	L• + O_2	-------->	LOO•
	LOO• + LH	-------->	L• + LOOH

Termination:

LO• + LO• -------->
LOO• + LOO• --------> } Non-radical products
L• + L• -------->
LOO• + L• -------->
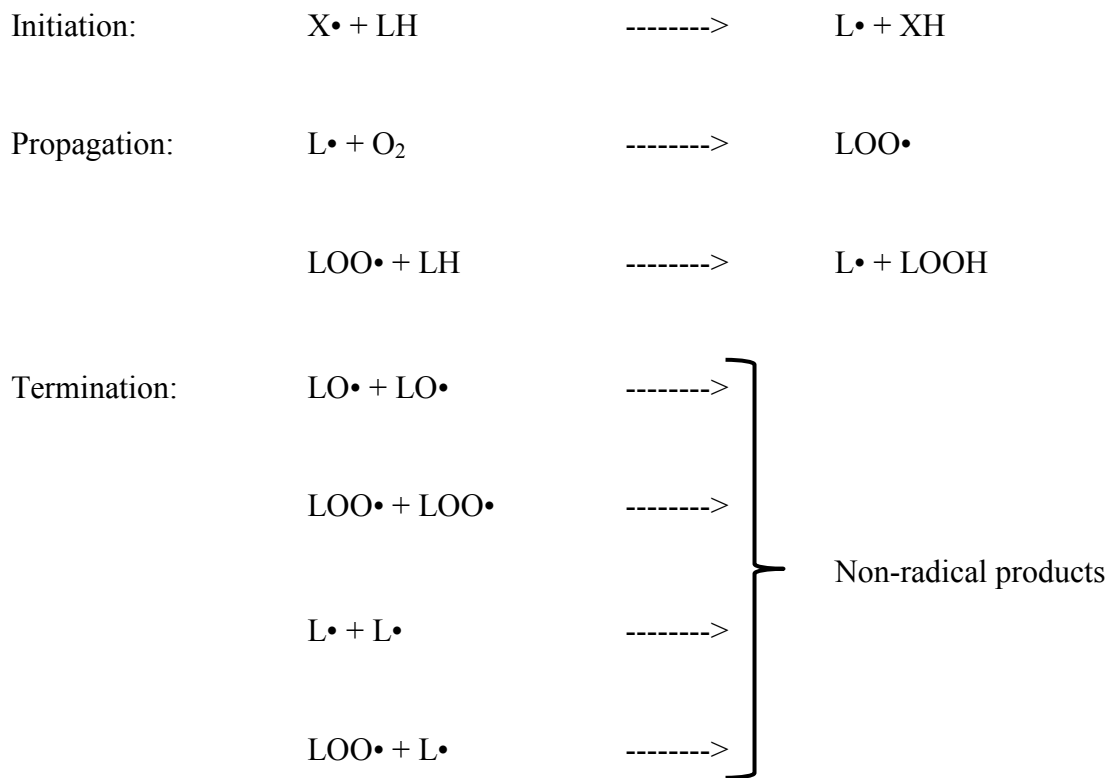
Figure 1. Autoxidation mechanism of lipids (Adapted from Kubow 1992).

The termination phase is the period where free radicals are removed from the system (Figure 1). This is accomplished by the reaction of two radical species to form a non-radical product or where the presence of a hydrogen or electron donor acts to terminate propagation (Kubow 1992). During the termination phase, the breakdown of reactive hydroperoxides (primary oxidation compounds) into secondary non-radical oxidation compounds such as hydrocarbons, aldehydes, alcohols, and volatile ketones occurs (Laguerre et al 2007). Several volatile secondary oxidation byproducts are considered responsible for the oxidized odors and flavors found in rancid foods (Schorno 2006).

ALA autoxidation is based on the autoxidation process of lipid radicals. Hydroperoxides are formed at carbon 9, 12, 13, and 16 during the autoxidation of ALA (Figure 2). The 9- and 16-

hydroperoxide isomers are the most predominant hydroperoxides formed from ALA autoxidation, accounting for 31 and 49% of the hydroperoxides, respectively (Kubow 1990; Frankel 1998). Formation of the 9-hydroperoxide occurs in the following manner (Figure 3). Hydrogen is abstracted from the methylene group on carbon-11, resulting in the formation of a pentadienyl radical. Oxygen then reacts with the pentadienyl radical at carbon-9 to form a lipid peroxyl radical at the carbon-9 location. Hydrogen is then abstracted from a different fatty acid by the peroxyl radical to form a new lipid radical and the 9-hydroperoxide. Hydroperoxides formed during autoxidation are susceptible to further oxidation and lead to the formation of several secondary products, which negatively impacts product quality due to their off-odor and flavor profiles.

9, 12, 15- octadecanoic acid
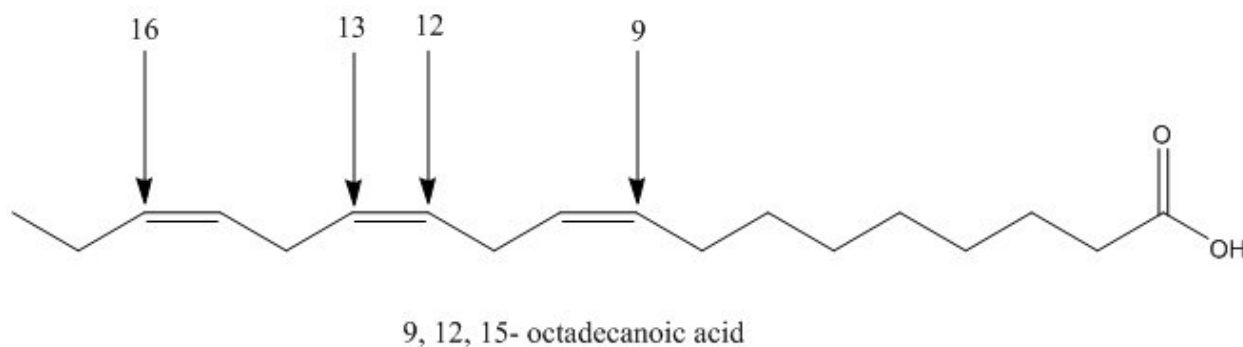
Figure 2. Alpha-linolenic acid structure and hydroperoxide formation at carbon 9, 12, 13, and 16.
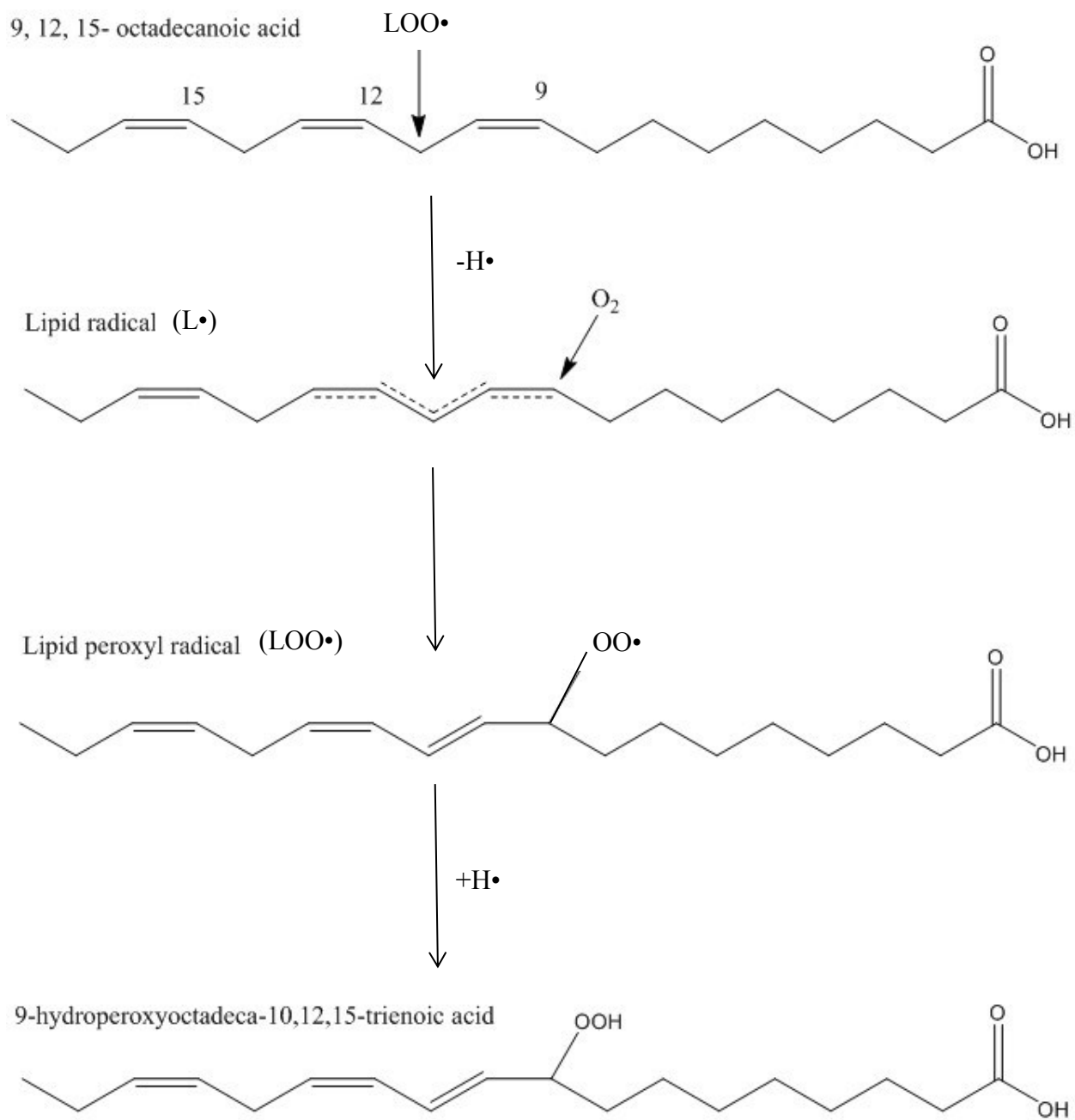
Figure 3. Autoxidation of alpha-linolenic acid to form the 9-hydroperoxide isomer.

Secondary oxidation

The decomposition of hydroperoxides formed during oxidation, leads to the formation of aldehydes, ketones, and other volatile compounds. The volatile secondary oxidation compounds produced are a concern in food products due to their low sensory detection threshold (Vadukapuram 2009).

The major volatile compound produced from the decomposition of ALA hydroperoxides is propanal, which is used as an indicator of omega-3 oxidation (Hall et al 2005). Additional secondary oxidation products produced from ALA hydroperoxide decomposition include ethane, 2,4,7-decatrienal, 2,4-heptadienal, 3-hexenal, 2-pentenal, 3,6-nonadienal, and 2-butenal (Figure 4; Frankel 1998).

Flaxseed, due to its high ALA content, is susceptible to oxidation if improperly processed (Schorno et al 2010). The use of proper milling techniques and storage conditions may aid in the reduction of the formation of secondary oxidation compounds, which negatively affects flaxseed quality.

CH₃——CH₃ Ethane

Propanal

CHO 2-butenal

2-pentenal

CHO 3-hexenal

2,4-heptadienal

3,6-nonadienal

CHO 2,4,7-decatrienal
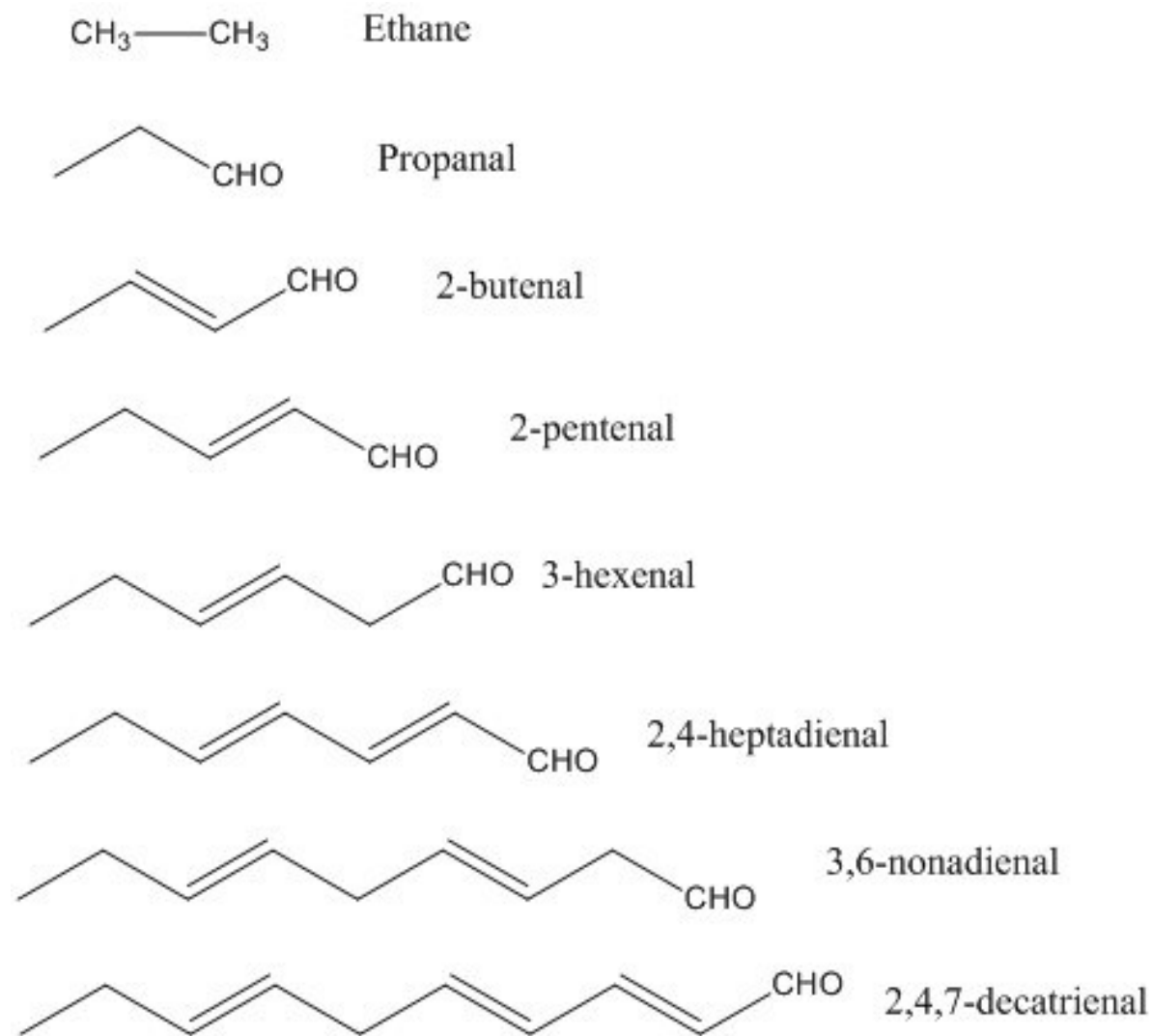
Figure 4. Secondary oxidation products from autoxidation of alpha-linolenic acid.

Milled flaxseed

Schorno et al (2004) compared the use of roller mill, burr mill, hammer mill and centrifugal cutting mill for milled flaxseed production. Burr mill was found to be the least efficient in flaxseed milling. Roller mill application caused particle adherence to the rolls due to the high surface lipid content. High surface lipid content, which can trigger lipid oxidation, increased with high feed rate. The centrifugal cutting mill and hammer mill showed greater potential for flaxseed milling, than either the roller or burr mills.

Chen et al (1994) indicated that oxidative susceptibility of milled flaxseed was related to particle size. Oxygen consumptions of various particle sizes 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). High oxygen consumption by large particles was suggested to be due to ample air space between flaxseed particles. Thus, 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).

In contrast, Schorno et al (2010) indicated that lipids in coarse flaxseed particles oxidized to a lesser extent than did lipid in fine particles. In addition, he noted that the surface lipid was greater in the finer particle and thus providing a reason for the increased oxidation in finer particles. Weisenborn et al (2005) similarly found that larger flaxseed cotyledon particles had greater stability than smaller milled flaxseed particles stored for 30 weeks at 40 °C. The results reported by Chen et al (1994) were determined using headspace oxygen consumption, which does not directly measure lipid oxidation. In contrast, the studies of Schorno et al (2010) and Weisenborn et al (2005) reported large size milled flaxseed particles were more oxidatively

stable than smaller sized particles. The studies undertaken by Schorno et al (2010) and Weisenborn et al (2005) both utilized peroxide values as the primary measurement to indicate lipid oxidation. Peroxide values are considered a direct measurement of lipid oxidation as they measure the formation of intermediate hydroperoxide compounds formed during lipid oxidation (Frankel 1998; Sahin and Sumnu 2009).

Schorno et al (2010) reported that milled flaxseed predominantly oxidized at the pile surface and very little oxidation occurred on the bottom layer when flaxseed was stored at 50 °C for 84 days of storage. The development of a surface crust layer due to the oxidation and polymerization of surface lipids, likely acted to reduce the movement of oxygen into the sample and thus, limiting the oxidation of sub-surface milled flaxseed particles. Since oxidation is promoted by elevated temperatures, it may be possible that an alteration in milling conditions, such as lower temperature milling, may act to reduce surface lipid and thereby reduce the susceptibility of flaxseed to oxidation.

The oxidative stability of stored milled flaxseed based on sample size was greatest with a small sample size (100 g), intermediate with a medium sample size (200 g), and lowest with a large sample size (300 g). The large and intermediate sample sizes acted to dilute the amount of oxidized surface lipids compared to the small sample size thus reducing the amount of sample lipid oxidation based on peroxide value (Schorno et al 2010).

The induction period is a measurement of the time required before a detectable change in the rate of oxidation takes place (Frankel 1993). Schorno (2006) reported that the induction period of milled flaxseed, as measured by peroxide value, was less at an elevated storage temperature. Milled flaxseed stored at 25 °C had an induction period of 96 days, whereas milled flaxseed stored at 50 °C had an induction period of 16 days.

Properly milled flaxseed stored under conditions with limited access to oxygen and light has been shown to be stable for over 20 months (Przbylski and Daun 2001). The oxidative stability of milled flaxseed can be negatively affected by factors including mill conditions, particle size, and storage temperature leading to the formation of off-flavor components due to lipid oxidation.

Flaxseed roasting

Koslowska (1989) discussed the use of roasted flaxseed in bread and confectionery products. Schorno et al (2004) and Tulbek et al (2004) reported that significant changes were observed in color properties at roasting temperatures of 180 °C. Roasted flaxseed brightness significantly decreased at 160 and 180°C; however, no color differences were observed at 140°C when compared to the unroasted flaxseed (Tulbek et al 2004). Samples roasted at 160 and 180°C were also less oxidatively stable compared to the control and samples heated at 140°C. The roasting experiments demonstrated that roasting dramatically altered the seed chemistry. In addition, the microbial counts were dramatically reduced under roasting conditions and to a lesser extent the non-roasting condition of 140°C.

The application of heat to flaxseed has the potential to damage the seed, leading to an increased susceptibility to oxidation. The use of a heat treatment to control microbial contamination must be used in conjunction with proper milling and storage techniques to minimize the potential for lipid oxidation and maintain product quality.

Although oxidation and the formation of secondary oxidation products are the main reasons for off-flavors in flaxseed, other reasons for off-flavors have been identified. The presence of microorganisms can contribute to the generation of off-flavors in flaxseed (Magan

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and Evans 2000). Several companies are applying heat treatments as a means to reduce microbial loads; however, surface lipids and oxidative stability have not been evaluated.

Microbial contamination in flaxseed

Research conducted in 2005 on flaxseed harvested under wet conditions showed the importance of microbial contamination in flaxseed. The suspect flaxseed had an initially malt-like flavor note that later gave way to fruit-like and weak geranium flavors. Octadienone gives a geranium-like flavor, and was present in the suspect sample, but not in a freshly ground sample from good flaxseed. The presence of alcohols, in particular 3-methyl-1-butanol, can indicate contamination by spoilage microorganisms (Tuma et al 1989; Magan and Evans 2000). *Alternaria* species have been known to affect flaxseed and could possibly be responsible for the production of 3-methyl-1-butanol (Tulbek, Personal communication). Thus, one likely explanation for the presence of 3-methyl-1-butanol in the samples was due to microbial contamination during harvest or storage, particularly under wet conditions. A milled sample of this flaxseed had an intense fruit flavor and the compound 2-butanol was identified as the most likely volatile responsible for this flavor (Tulbek, Personal communication). Thus, milling intensified the flavor characteristics of the flaxseed with high microbial loads by intensifying the release of flavors. The use of pasteurization for flaxseed can improve food safety through the elimination of pathogenic microorganisms, as well as aid in the reduction of off-flavor compounds produced from flaxseed microorganisms.

Flaxseed pasteurization

Kuchynski et al (2012) used response surface methodology to predict the pasteurization time and temperature necessary to reduce flaxseed microbial counts to acceptable levels without roasting the flaxseed during the heating process. Flaxseed was processed at 70, 120, and 150 °C

for 2, 12, and 24 minutes. The microbial load reduction was measured using aerobic plate (APC), yeast count (YC), and mold count (MC). The optimal pasteurization conditions were based on the level of microbial reduction for each respective microbial analysis.

Alterations in the pasteurization temperature and time conditions resulted in significant changes to the average APC for the heat treated flaxseed. As the temperature was increased from 70 °C to 150 °C, the average percent reduction in APC increased dramatically (Figure 5). The treatments at 70 °C exhibited a minimal effect on the APC. An increased APC was observed in the flaxseed treated at 70 °C for 24 minutes, most likely as a result of the extended pasteurization time, which allowed for the increased growth of thermophilic bacteria. As the processing time was increased to temperatures of 120 °C and 150 °C, a noticeable reduction in APC was observed, especially at 12 and 24 minutes of processing. At 150 °C, almost a complete reduction in APC was observed. The RSM analysis predicted that a pasteurization treatment carried out at 133 °C for 14.25 minutes would be required to effectively reduce the APC.

The average percent reduction of the YC was found to be significant only at a pasteurization of 150 °C for 12 and 24 minutes (Figure 6). Yeast cells were more tolerant to elevated temperatures on average compared to APC, but less tolerant than molds. The RSM predicted that a temperature of 148 °C and a time of 16 minutes would be required to sufficiently eliminate yeast.

Mold growth was found to be more tolerant on average to elevated temperatures than aerobic microorganisms and yeast. Similar to yeast, only the treatments carried out at 150 °C for 12 and 24 minutes was sufficient to significantly decrease the mold counts (Figure 7). The RSM predicted that a temperature of 148 °C and a time of 16.25 minutes would be required to sufficiently eliminate mold.

The heat tolerance of mold cells resulted in the highest predicted temperature and time requirements for flaxseed pasteurization. Thus, the optimal predicted pasteurization conditions of 148 °C for 16.25 minutes were the only conditions predicted to sufficiently reduce the APC, YC, and MC.
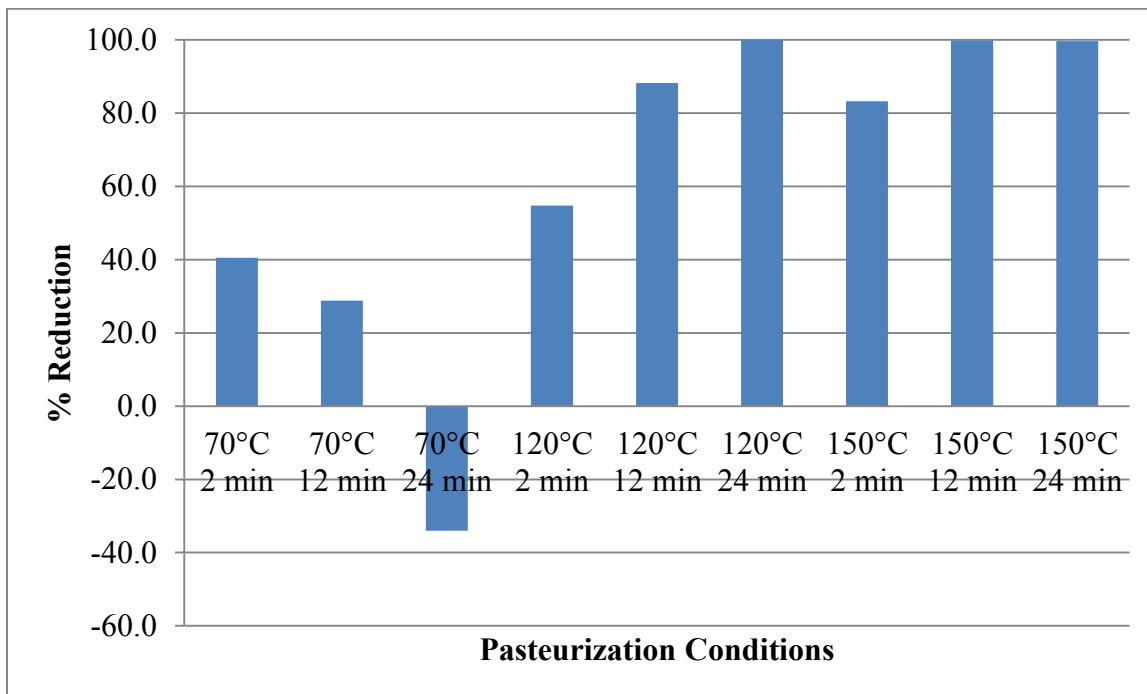
Figure 5. Average percent reduction in aerobic plate count at various pasteurization time and temperature combinations (Adapted from Kuchynski et al 2012).
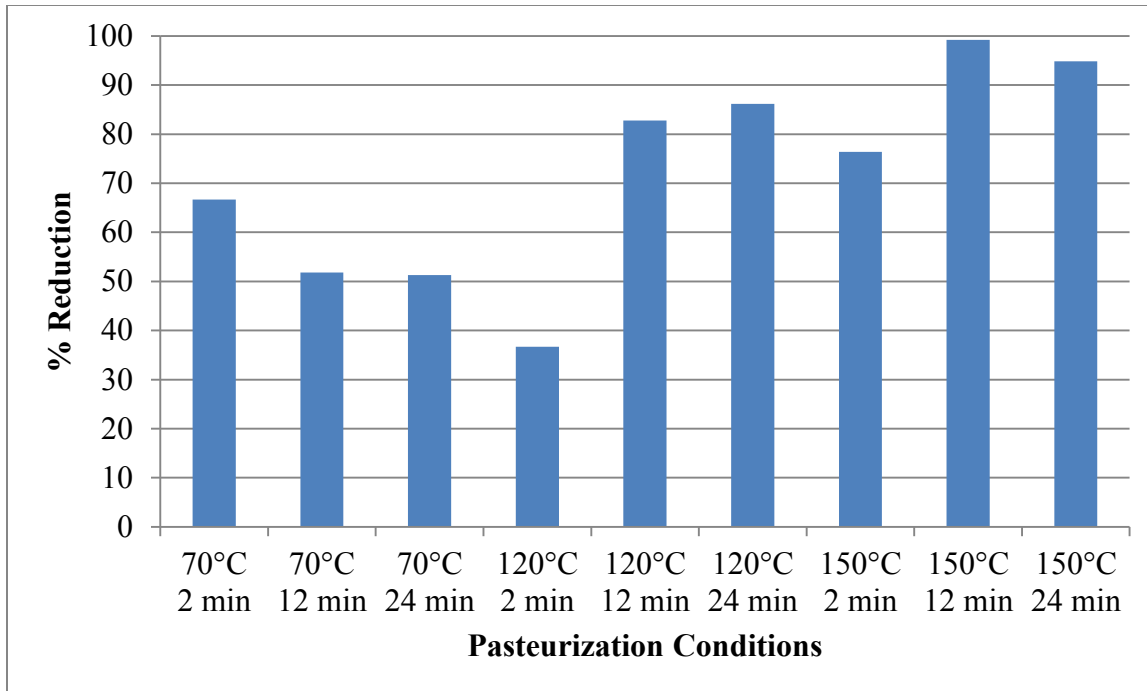
Figure 6. Average percent reduction in yeast count at various pasteurization time and temperature combinations (Adapted from Kuchynski et al 2012).
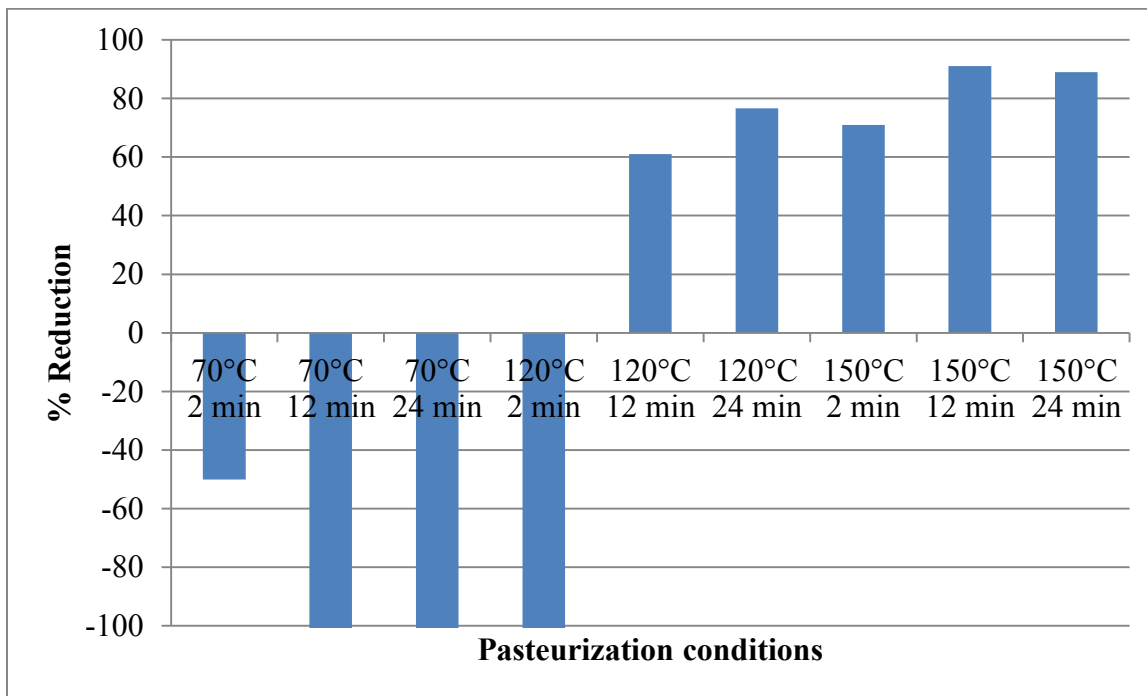
Figure 7. Average percent reduction in mold count at various pasteurization time and temperature combinations (Adapted from Kuchynski et al 2012).

Kuchynski et al (2012) processed flaxseed using the optimal pasteurization time and temperature of 16.25 minutes at 148 °C to validate the predicted pasteurization processing condition identified through response surface methodology. A 3 log average reduction in the APC was observed after pasteurization to a final average level of 2 log cfu/g (Figure 8). This was contrary to the response surface methodology predicted pasteurization process of 133°C for 14.25 minutes, which was predicted to sufficiently reduce the APC to zero or near zero. The higher final APC observed in the validation study may have resulted from the use of flaxseed containing a higher number of spore forming microorganisms, which were stressed under the heat treatment and germinated prior to taking the APC. The optimal processing condition significantly reduced the yeast (Figure 9) and mold (Figure 10) counts to zero or near zero, as was predicted by the response surface methodology. Food manufacturers seeking low APC requirements would likely consider a reduction in microbial counts to 2 log after pasteurization sufficient to meet low APC specifications for products such as flaxseed (Kuchynski et al 2012).
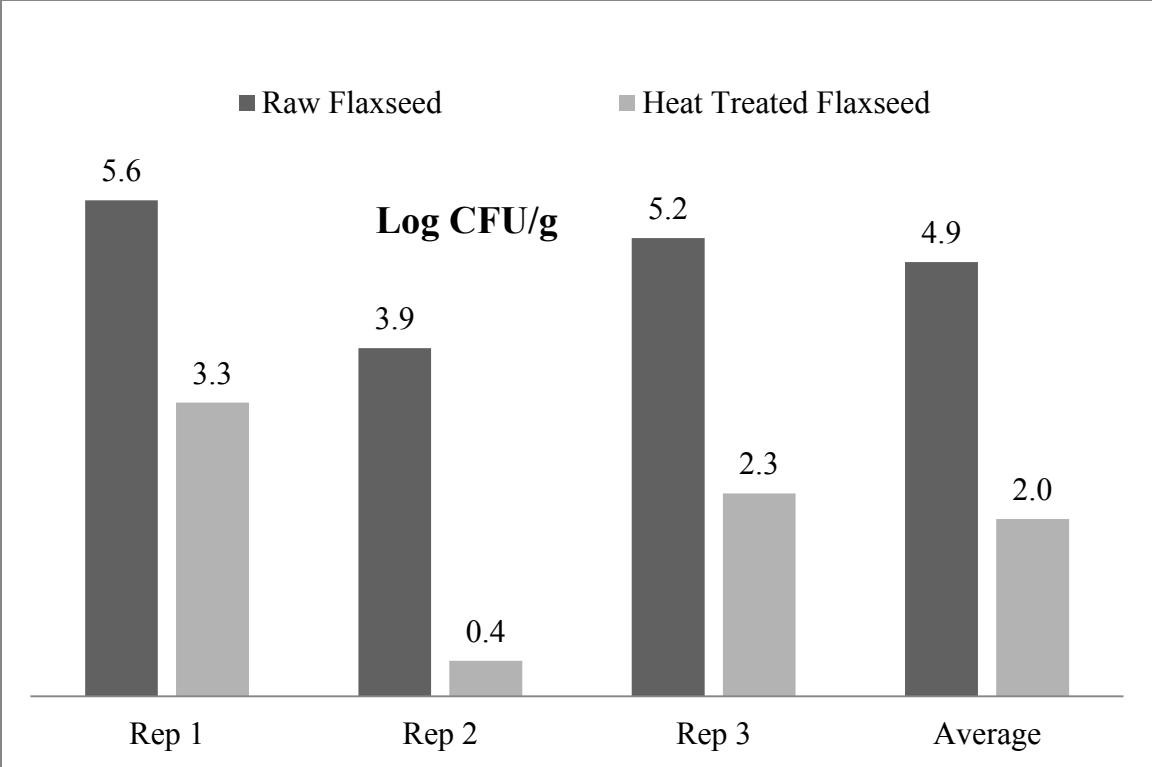
Figure 8. Aerobic plate count (log cfu/g) in flaxseed before and after heat treatment.
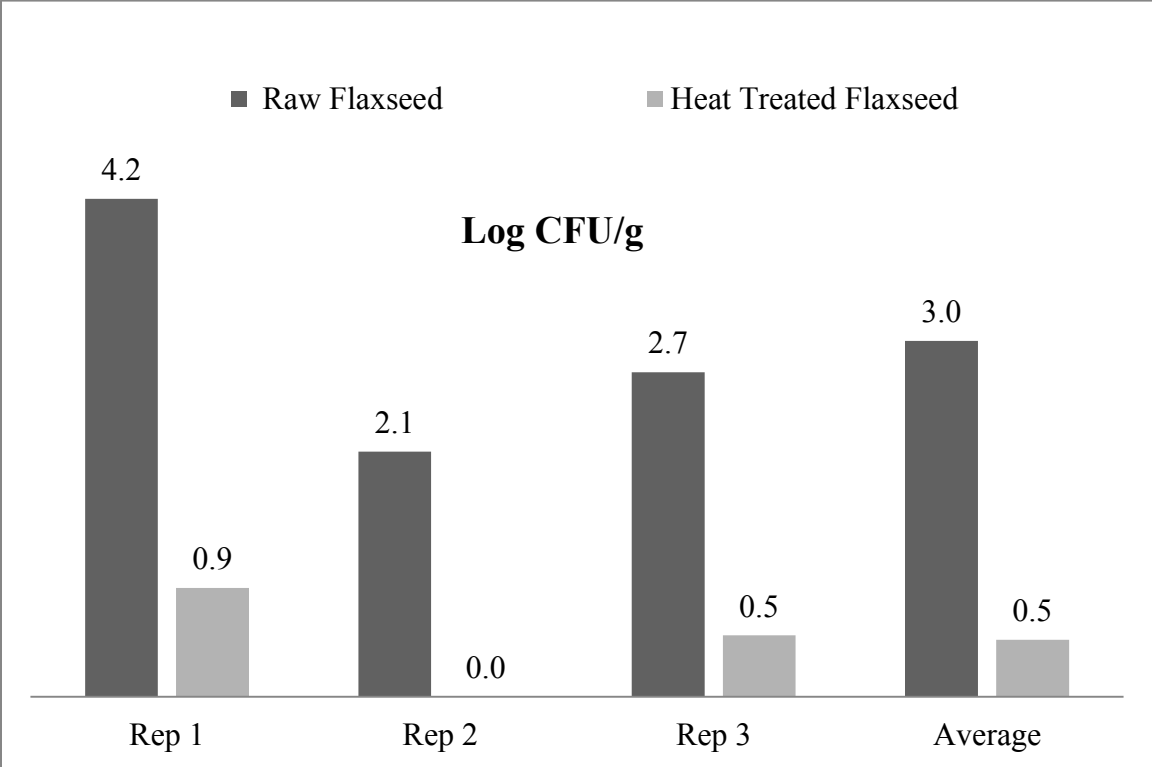
Figure 9. Yeast count (log cfu/g) in flaxseed before and after heat treatment.
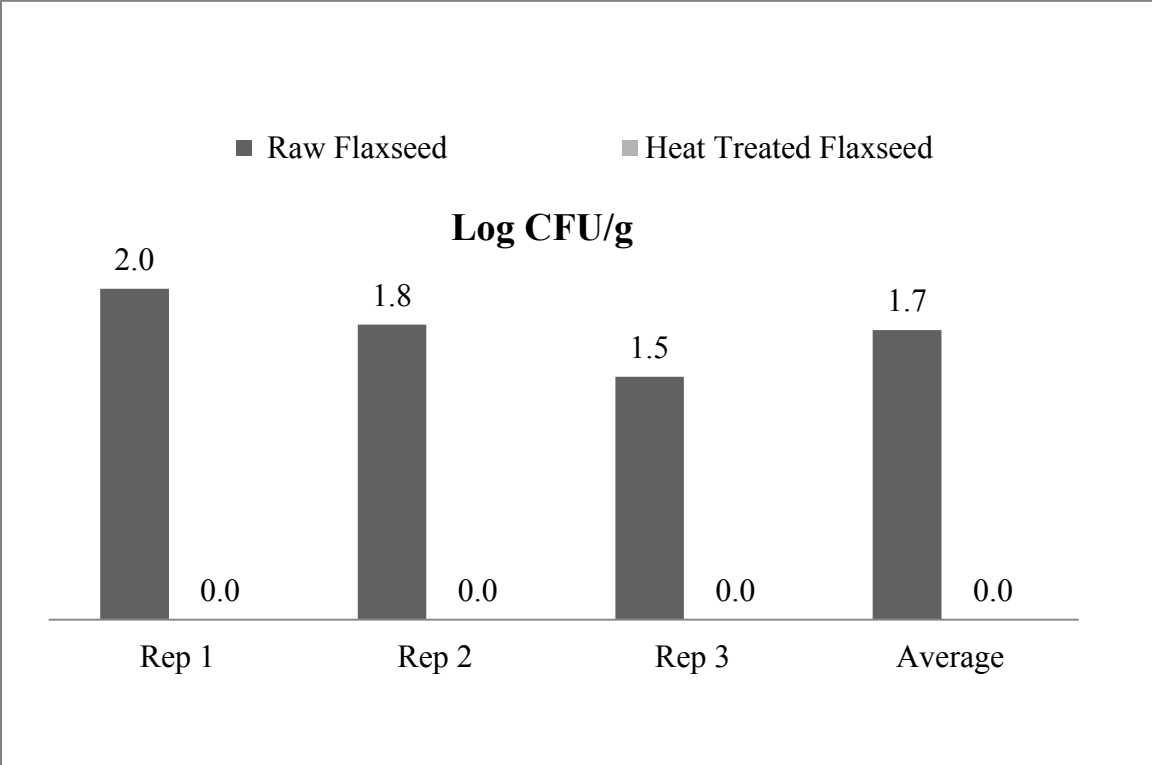
Figure 10. Mold count (log cfu/g) in flaxseed before and after heat treatment.

RESEARCH JUSTIFICATION AND OBJECTIVES

The preliminary study by Kuchynski et al (2012) provided evidence that the pasteurization of flaxseed resulted in a reduction of aerobic plate count, yeast cell count, and mold count. Currently, there is no information regarding the stability of pasteurized flaxseed milled under ambient or cold temperature conditions. This study will benefit flaxseed producers and food companies by providing relevant information regarding the impact of stabilization on the oxidative stability of milled flaxseed.

The objective of this research was two-fold: 1) to identify what the effects of pasteurization are on milled flaxseed quality and oxidative stability and 2) to determine if the combination of pasteurization followed by cold temperature milling will affect milled flaxseed quality differently from pasteurized flaxseed milled under ambient conditions.

MATERIALS AND METHODS

Approach

Heat treated (pasteurized) and raw (non-pasteurized) flaxseed were subjected to milling under either room temperature or refrigerated conditions. The oxidative stability of milled flaxseed was compared in a twenty-week storage study. The extent of oxidation was measured using peroxide value, percent free fatty acid, and the concentration of the secondary oxidation byproduct propanal.

Raw materials

Brown flaxseed was sourced from Heartland Flax (Valley City, ND), Premium Gold Flax (Denhoff, ND), and Keystone Grain (Winkler, Manitoba).

Flaxseed pasteurization

Flaxseed was heat treated using the optimal pasteurization conditions of 148 °C for 16.25 minutes in a Lincoln (Fort Wayne, IN) air impingement oven. Flaxseed from each of the listed sources was individually processed in a 2 kg batch evenly distributed onto a 26 ¾ in. x 29 ½ in. x 1 in. perforated tray. Each treatment was allowed to cool to room temperature before milling.

Flaxseed milling

The pasteurized and raw flaxseed was milled under ambient and refrigerated (10 - 18°C) conditions in a hammermill, Fitzmill Model DAS-06 (Fitzpatrick Company, Elmhurst IL) using a blunt face hammer configuration. The hammer mill was operated with a tip speed of 1341 m^{-1} (4200 rpm), a 1.71 kg min^{-1} feed rate, and a 2.0 mm screen opening size.

Sample storage and collection

The milled flaxseed was packaged in an unsealed plastic bag placed inside a Kraft paper bag. Samples were stored at room temperature for a period of twenty weeks. At each sample

procurement interval (every other week), the milled flaxseed was thoroughly mixed and a 100 g sub-sample was taken for analysis. Samples were placed in a Kraft paper bag and stored at -14 °C prior to analysis.

Surface lipid

Surface lipid was determined using a modified method of Schorno et al (2010). Milled flaxseed (10 g) was weighed onto a preformed #415 VWR filter paper. Filter papers were preformed by pressing them into a Büchner funnel with a plastic bottle having a smaller diameter than the funnel. The sample was placed into the Büchner funnel on top of a #4 Whatman filter paper pre-wetted with hexane. Hexane (50 mL) was added and the sample was vacuum filtered into a 250-mL round bottom flask. Hexane was allowed to completely evaporate from the sample before it was weighed and the percent surface lipid was calculated.

Particle size

Particle size distribution was determined using a Ro-Tap mechanical shaker with U.S. standard sieves #20, #30, and #40 (850, 600, and 425 microns, respectively). Samples (50 g) were shaken for 5 minutes. Prior to analysis of each sample, sieve screens were cleaned by shaking > 100 g iodized salt per individual sieve screen for 5 minutes. Screens were then brushed clean to remove any material present. Samples were measured in duplicate and averaged. Particle size distribution was reported by weight % of coarse (> 600 μm) and fine (<600 μm) flaxseed particle fractions.

Total lipid content

Total lipid content was determined using a 16-hour Soxhlet extraction with hexane according to AOCS Method Ba 3-38 (1998). Samples of ground flaxseed (4 g) were analyzed in duplicate and averaged.

Lipid extraction and stability

The milled flaxseed was packaged in an unsealed plastic bag placed inside a Kraft paper bag. Samples were stored at room temperature for a period of twenty weeks to identify trends in quality changes. Lipids were extracted from the milled flaxseed by combining 10 g of milled flaxseed and 100 mL of chloroform:methanol (2:1 v/v) in an Erlenmeyer flask covered in aluminum foil. Samples were stirred using a magnet bar for 30 minutes and allowed to sit for 10 minutes. Samples were filtered through a #415 VWR filter paper preformed into a cone containing a teaspoon of sodium sulfate filter aid. The chloroform:methanol (2:1 v/v) solvent was removed under vacuum using a rotary evaporator (Buchi, Uzwil, Switzerland) and a heated water bath at 40 °C. Extracted oil was purged with nitrogen gas and stored at -14 °C prior to analysis. The lipid stability of the stored flaxseed was measured using peroxide values, free fatty acid (FFA) content, and headspace volatile analysis.

Peroxide value

Peroxide values of the extracted oil were measured using AOCS Method Cd 8-53 (AOCS 1998) as modified by Crowe and White (2001). Extracted oil (0.5 g) was weighed into a 25 mL Erlenmeyer flask and 3 mL of acetic acid:chloroform (3:2 v/v) and 50 µL of potassium iodide were added and mixed for 1 min. Next, 3 mL of distilled water were dispensed into the mixture, followed by the addition of 300 µL of 1% starch indicator solution. The oil samples were titrated using either 0.001 N sodium thiosulfate or 0.01 N sodium thiosulfate until a clear color was obtained. Samples with expected peroxide values of greater than 10 meq/kg were titrated using the 0.01 N sodium thiosulfate. Samples with expected peroxide values of less than 10 meq/kg were titrated using 0.001 N sodium thiosulfate. Peroxide values were calculated using the equation: peroxide value = (S-B) * N * 1000 / W, where S = volume of titrant for sample, B =

volume of titrant for blank, N = normality of sodium thiosulfate (meq/ml), 1000 = unit conversion (g/kg), and W = sample mass (g). The peroxide values of each treatment were measured in duplicate and averaged.

Free fatty acid (as oleic)

Free fatty acid content of the extracted oil was determined using a modified AOCS Method Ca 5a-40 (AOCS 1998). Heated (60 °C) isopropyl alcohol (0.75 mL) and 0.6 mL of phenolphthalein (1% in 95% ethanol v/v) was added to 0.75 g of oil in a 25-mL Erlenmeyer flask. The lipid FFA content (as oleic acid) was determined by titrating with 0.0025 N NaOH. The percent FFA, as oleic, was calculated using the equation: % FFA (as oleic) = V * N * 282 *100 / W where V = volume of NaOH titrant (ml), N = Normality of NaOH titrant (mol/1000 ml), 282 = MW of oleic acid (g/mol), and W = sample mass (g). The FFA content of each treatment was measured in duplicate and averaged.

Headspace volatile analysis

Propanal, the major secondary oxidation product from the oxidation of ALA, was estimated using modified headspace solid phase microextraction (Hall et al 2005). Milled flaxseed (0.5 g) was placed in 4 mL vials and sealed using Teflon–faced silicone septa, which were previously heated at 100 °C for 24 h before use. Samples were initially heated in a boiling water bath for 10 min and subsequently heated in 60°C sonicating water bath for 10 min with the SPME filament (divinyl benzene / carboxene / polydimethylsiloxane) (Supelco, Bellefonte, PA) of 30/50 μm thickness inserted into the vial headspace. The SPME filament remained in the headspace for 10 min and was then transferred to the injection port of the GC, where the volatiles were allowed to desorb from the filament for 10 min. The GC system included an HP5890 gas chromatograph with a flame ionization detector and was equipped with a Zebron (Phenomenex,

USA) capillary GC column (60 m length, 0.25 mm ID and 0.25 mm film thickness). The volatile analysis was completed under the following conditions: helium flow rate of 33.7 mL/min; initial oven temperature of 40 °C ramped to 60 °C at 1 °C/min, followed by a ramp from 60 to 160 °C at 5 °C/min, and a final ramp from 160 to 250 °C at a rate of 20 °C/min with the final temperature of 250 °C held for 5 min. Final run time was set at 59.50 min. Retention time was based on a propanal standard obtained from Aldrich Chemical Co. (Milwaukee, WI). A standard curve was prepared to quantify the volatile compound propanal using a standard solution ranging in concentration from 0.8 to 80 ppm. Each standard level was prepared by adding the respective level of propanal to 10 g of finely ground saltine cracker milled using a Z-mill (Retech ZM1, Haan, Germany) and a 1.0 mm perforated screen.

Statistical analysis

The experimental design was a randomized complete block with a split plot in time, with non-time factors being flaxseed heat treatment (2 levels) and milling temperature (2 levels). Each treatment combination was replicated three times. A replicate was seed sourced from one of three vendors. The data was subjected to analysis of variance, with the treatment means being separated by Fisher's protected LSD at $p = 0.05$ (SAS 2011).

RESULTS AND DISCUSSION

Milled flaxseed characteristics

Lipid content of milled flaxseed averaged 43.0%, db (Appendix A-1). The lipid content ranged from 41.9 (Raw, cold milled) to 44.1% (Heat treated, cold milled). Surface lipid values were not significantly different ($P > 0.05$) between raw and heat treated samples at week zero (Appendix A-2), thus indicating the heat treatment applied to flaxseed prior to milling did not significantly alter the expression of surface lipid in samples compared to the raw flaxseed. A more extensive analysis of the surface lipids present during the initial weeks of oxidation would be useful to support the oxidation process. Schorno (2006) identified a negative correlation ($r=-0.903$, $P=0.01$) between surface lipid content and peroxide value, presumably due to the formation of lipid polymers. Thus, as oxidation progresses, a decrease in surface lipid content and an increase in peroxide value would be expected. Schorno (2006) found the surface lipid content of a milled flaxseed stored at 50 °C for 84 days sampled from the top (surface) layer was approximately 4.5 times lower than the surface lipid content of the lower (interior) layer of flaxseed, whereas the peroxide value of the surface layer was approximately 40 times greater than the peroxide value obtained from the interior layer.

The fine fraction (< 600 μm) of milled flaxseed was greater among all treatments compared to the coarse fraction (> 600 μm), averaging 41.9% coarse particles and 58.1% fine particles (Appendix A-3). Schorno et al (2010) and Weisenborn et al (2005) reported milled flaxseed of larger particle size was more oxidatively stable than smaller sized particles. Schorno (2006) found that the surface lipid content of flaxseed milled using a hammermill was positively correlated with the fine particle yield of flaxseed ($r=0.822$, $P=0.01$). Increased surface lipid content has been attributed to increased oxidation susceptibility in flaxseed (Schorno 2006).

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Peroxide value

The peroxide values of extracted oil from the milled flaxseed samples indicated increased susceptibility to oxidation in heat treated flaxseed compared to raw flaxseed, over the 20 week storage period (Figure 11). All four treatments initially had peroxide values less than 5 meq/kg at time zero. The oxidation level of soybean oil has been described as low, medium, and high at 1-5, 5-10, and >10 meq/kg, respectively (O'Brien 2009). Peroxide values of less than 10 meq/kg are generally considered acceptable, while peroxide values greater than 20 meq/kg correspond to very poor quality oils, which would normally have significant off flavors (Vadukapuram 2009; Nielsen 2010).

Over the course of the storage study, the raw flaxseed treatments exhibited consistently low peroxide values at levels less than 5 meq/kg. This is in accordance with the findings of Przybylski and Daun (2001). They reported that the peroxide values of milled flaxseed, when properly stored, remained stable for a period of 20 months. The peroxide values of the pasteurized flaxseed treatments were observed to diverge from the peroxide values of the raw flaxseed treatments at week 2. At this point, a significant elevation was observed in the pasteurized flaxseed peroxide values to levels greater than 20 meq/kg, which is indicative of poor oil quality. At week 10, a peak in the peroxide value of the pasteurized flaxseed treatments was observed, indicating the end of propagation and the onset of termination in the lipid oxidation process.

The early onset of lipid oxidation observed in the pasteurized flaxseed treatments showed the pasteurization process negatively impacted the stability and storability of milled flaxseed. The heat treatment of 148 °C for 16.25 minutes utilized to pasteurize milled flaxseed likely resulted in damage to the flaxseed, thereby causing an increase in the availability of surface lipid,

which was more readily oxidized. Schorno et al (2010) reported that peroxide values from the surface layer of milled flaxseed stored for 84 days at 50 °C were nearly 40 times greater than peroxide values obtained from the interior layer of the milled flaxseed. The high ALA content of flaxseed would act to further enhance lipid oxidation of the surface lipid (Vadukapuram 2009).

A flaxseed pasteurization temperature of 148 °C was intermediate to the roasting temperature (160 °C) and non-roasting temperature (140 °C) examined by Tulbek et al (2004), but was likely high enough to induce instability within the flaxseed, resulting in an increased susceptibility to oxidation. Tulbek et al (2004) reported that roasting temperatures of 160 and 180 °C resulted in reduced oxidative stability of flaxseed and indicated an alteration of flaxseed chemistry occurred at these temperatures, whereas flaxseed which was processed a 140 °C was comparatively stable to oxidation against a non-heated flaxseed sample.

Increased variability of the peroxide values were observed in all weeks, with the exception of week 0, among the pasteurized flaxseed treatments (Table 2). In contrast, all raw flaxseed samples exhibited minimal variation among recorded peroxide values. As an increase in variability was only identified in the pasteurized treatments and not the raw treatments, it is likely that the source of variation was not the flaxseed source, but rather the variation that existed due to inconsistencies in the application of the heat treatment. A reduction in the batch size during pasteurization may act to provide more uniform heating of the flaxseed.
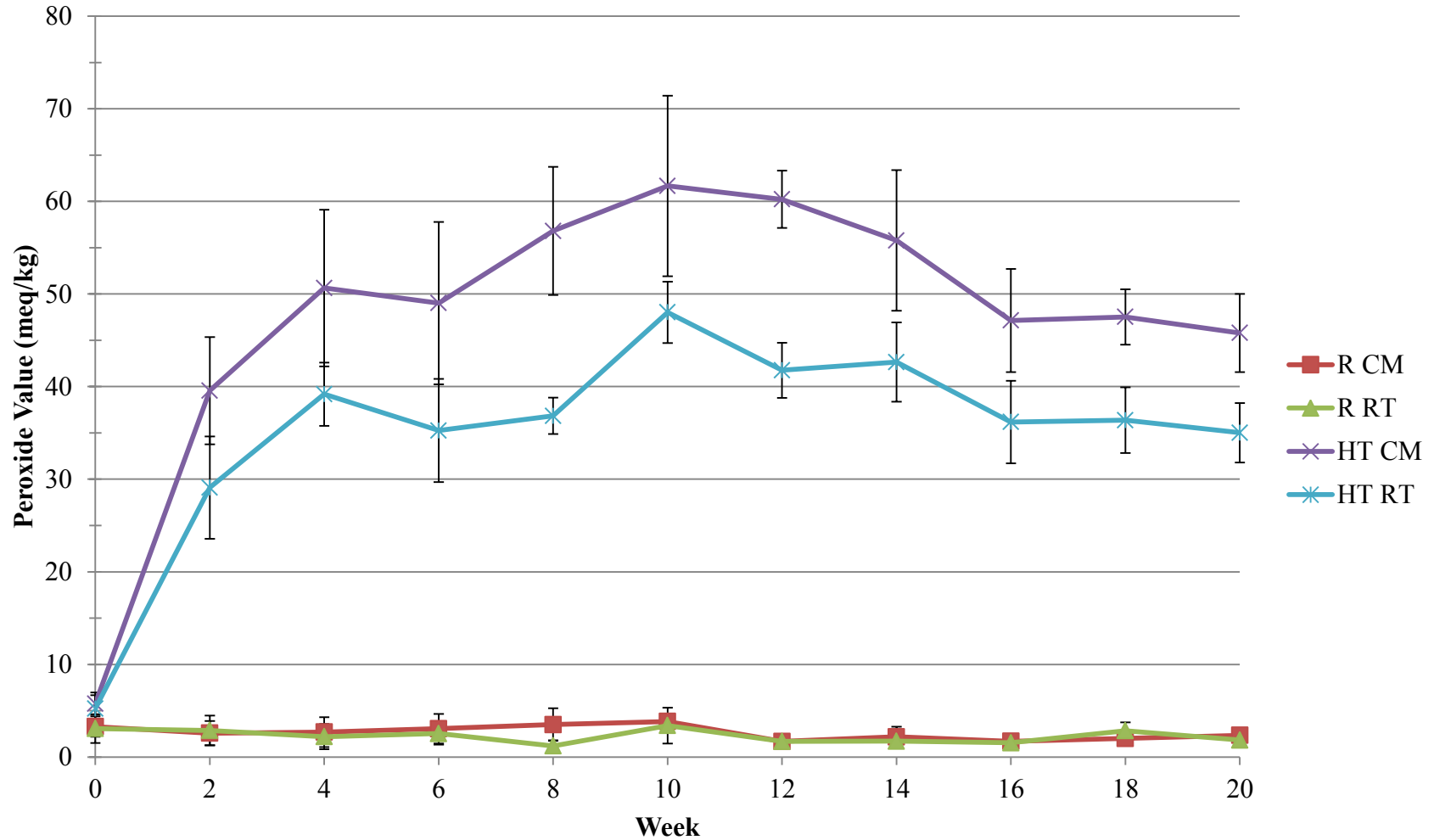
Figure 11. Peroxide values of milled flaxseed stored over 20 weeks. Treatments are: R CM – raw flaxseed, cold milled; R RT – raw flaxseed, room temperature milled; HT CM – heat treated flaxseed, cold milled; HT RT – heat treated flaxseed, room temperature milled

Table 2. Standard deviation of milled flaxseed peroxide values.

Week	Treatment*			
	R CM	R RT	HT CM	HT RT
0	1.905	2.690	2.083	2.501
2	2.300	2.770	10.019	9.569
4	2.772	2.352	14.647	5.924
6	2.775	2.081	15.175	9.654
8	3.052	1.059	11.991	3.397
10	1.249	3.330	16.896	5.744
12	0.755	0.664	5.344	5.167
14	1.913	1.202	13.158	7.421
16	1.218	1.036	9.640	7.731
18	1.168	1.529	5.173	6.156
20	1.367	1.295	7.317	5.549
Mean SD**	1.861	1.819	10.131	6.256

* Treatments are: R CM – raw flaxseed, cold milled; R RT – raw flaxseed, room temperature milled; HT CM – heat treated flaxseed, cold milled; HT RT – heat treated flaxseed, room temperature milled.
** SD – Standard deviation

No significant differences ($P > 0.05$) were observed between flaxseed milled under ambient conditions or refrigerated conditions, based on statistical analysis of the peroxide values over the 20 week storage period (Appendix A-4). However, statistical analysis by individual week indicated significant differences based on milling conditions between treatments in weeks 8 and 12 (Table 3). The differences in peroxide value due to milling conditions observed in week 8 and 12 were likely due to differences in the magnitude of the peroxide values from the pasteurized samples near the peak of oxidation as a result from increased variation of the peroxide values in the pasteurized treatments (Table 2). The overall lack of significance in peroxide values across the twenty week storage period suggests milling flaxseed under refrigerated conditions did not minimize the susceptibility of flaxseed to oxidation compared to room temperature milling. The use of refrigerated milling did not result in a reduction of surface

lipid formation during milling as was anticipated (Appendix A-2); as a result the oxidative

stability over time of the pasteurized flaxseed was similar to that of flaxseed milled using

ambient temperature conditions. Surface lipid has been identified as having a high susceptibility

to oxidation. Schorno et al (2010) found peroxide value obtained at the sample surface of milled

flaxseed stored for 84 days at 50 °C was nearly 40 times greater than the peroxide value obtained

from the lower layer of the milled flaxseed.

Table 3. Means for peroxide values, by week, of flaxseed milled under refrigerated or room
temperature conditions.

Week	Milling condition*	
	Refrigerated	Room Temperature
0	4.520 a	4.155 a
2	21.068 a	15.983 a
4	26.667 a	20.690 a
6	26.037 a	18.893 a
8	30.158 a	19.015 b
10	32.748 a	25.708 a
12	30.957 a	21.722 b
14	28.980 a	22.175 a
16	24.417 a	18.860 a
18	24.758 a	19.610 a
20	24.067 a	18.423 a

* Means within each row followed by the same letter are not significantly different at p=0.05.

Percent free fatty acid (as oleic)

The FFA content of all treatments was generally observed to be less than one percent,

although points in the study were observed above this threshold (Figure 12). The FFA content of

milled flaxseed was reported by Malcolmson et al (2000) to be stable over 128 days, with a peak

FFA level of 1.58 % reported as oleic. High levels of FFA in milled flaxseed have been

attributed to the presence of immature seeds or improper storage conditions (Przybylski and Daun 2001).

Free fatty acid content (FFA) of extracted oil from the milled flaxseed samples was statistically significant ($P \leq 0.05$) for storage week by time (Appendix A-4). As storage time increased, the level of free fatty acids in the raw flaxseed samples did not change, but the level in the pasteurized samples increased. Elevated FFA levels in the heat treated flaxseed samples were not expected to increase during the 20 week storage period because heat inactivates lipase. This in turn should prevent the formation of free fatty acids. Elevated FFA levels observed over the 20 week storage period are more likely due to a lack of sensitivity in the assay itself. The variability in FFA levels observed from week to week may be due to difficulties in identifying the end point of titration. The use of phenolphthalein indicator as a visual indicator for the endpoint of titration is a subjective measure. Phenolphthalein indicator experiences a color change over a range of pH values, beginning with a slight color change to pink at pH 8.2, and a distinct color change to dark pink at pH 10. Identification of the initial color change indicating the end of titration can be variable as it is a visual observation which is subject to differences in interpretation by the analyst who may be influenced by factors such as time of day or the type or amount of room lighting. A quantitative measurement of pH may be a better indicator for the titration endpoint of phenolphthalein indicator compared to a visual observation for the color change.
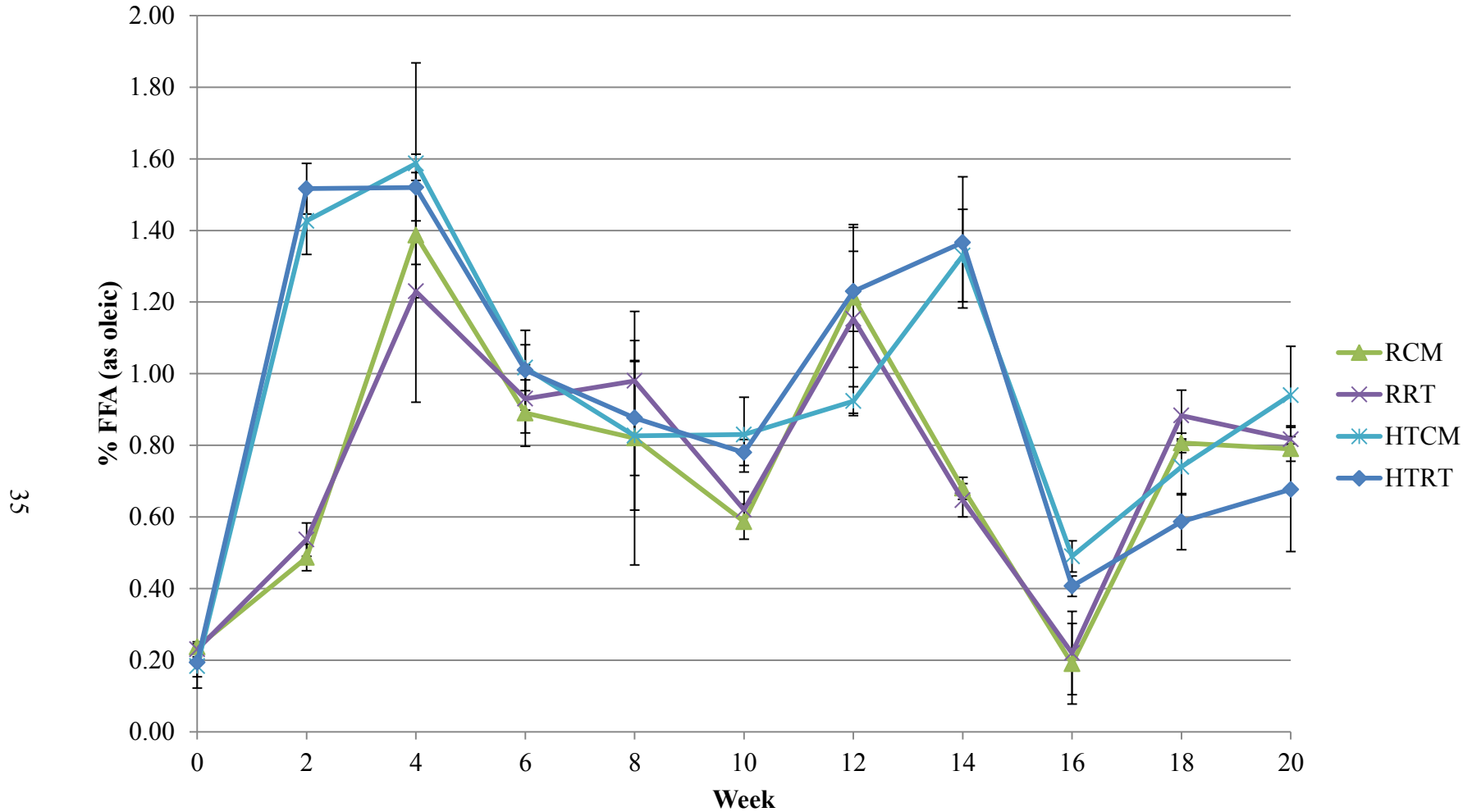
Figure 12. Percent free fatty acid (as oleic) of milled flaxseed stored over 20 weeks. Treatments are: R CM – raw flaxseed, cold milled; R RT – raw flaxseed, room temperature milled; HT CM – heat treated flaxseed, cold milled; HT RT – heat treated flaxseed, room temperature milled.

Headspace results

Propanal is one of the major aldehydes formed during the degradation of ALA and is considered a key indicator for lipid oxidation (Hall et al 2005). Properly stored milled flaxseed was reported by Malcolmson et al (2000) to be stable for up to 128 days with regards to the presence of volatile components. The propanal values (Figure 13) for raw milled flaxseed were zero or near zero over a 16 week period, and coincided with the findings reported by Malcolmson et al (2000).

The formation of propanal was observed in both of the heat treated samples and appeared to coincide with the development of peroxides over storage time. The increased propanal content by week indicated that oxidation of the heat treated samples was taking place. The application of heat likely resulted in damage to the seed, thus altering flaxseed susceptibility to oxidation similar to the findings of Tulbek et al (2004), who found that flaxseed roasted at 160 and 180 °C was significantly more susceptible to oxidation over a 4 week storage period. Tulbek et al (2004) proposed that a decrease in the roasted flaxseed water activity acted to enhance lipid oxidation. The pasteurization temperature of 148 °C was lower than the roasting temperature of 160 °C described by Tulbek et al (2004), but may have been high enough to roast the flaxseed causing heat induced damage resulting in the promotion of lipid oxidation.
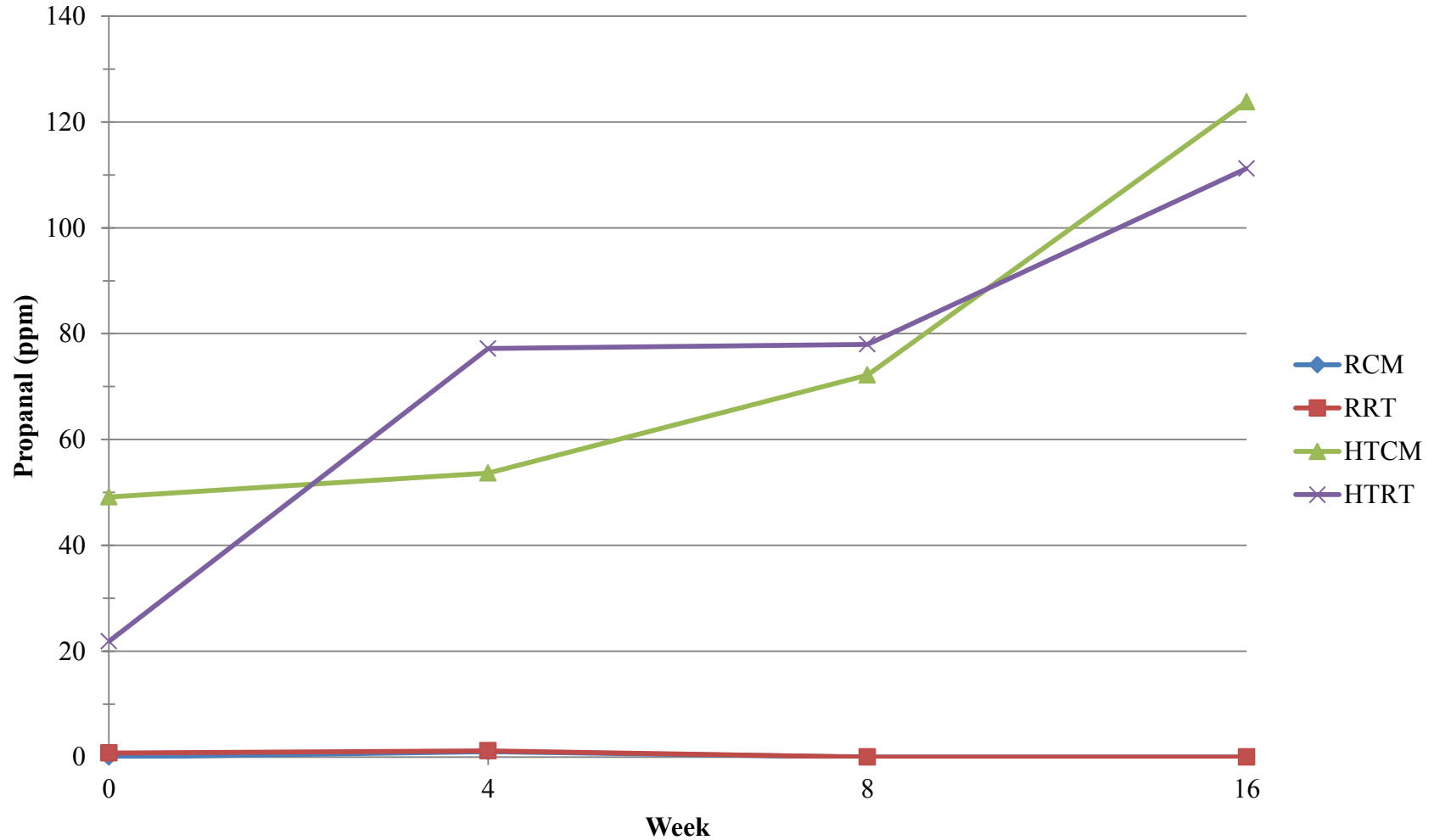
Figure 13. Propanal concentration (ppm) of milled flaxseed stored over 20 weeks. Treatments are: R CM – raw flaxseed, cold milled; R RT – raw flaxseed, room temperature milled; HT CM – heat treated flaxseed, cold milled; HT RT – heat treated flaxseed, room temperature milled.

CONCLUSION

Flaxseed pasteurization at 148 °C for 16.25 minutes was found to be detrimental to the oxidative stability of flaxseed once milled. Significant ($P \leq 0.05$) elevation of peroxide values in the pasteurized samples was found after two weeks of storage at room temperature compared to the raw flaxseed treatments. The progression of oxidation was observed through the elevation of peroxide values and propanal content over the course of the storage study. The application of heat to pasteurize flaxseed likely resulted in damage to the seed, resulting in flaxseed instability and an increased predisposition to lipid oxidation. Milling pasteurized flaxseed under refrigerated conditions did not significantly ($P > 0.05$) increase the oxidative stability compared to pasteurized flaxseed milled under ambient temperatures over the 20 week storage period. Pasteurization of flaxseed at 148 °C for 16.25 minutes using a dry heat with minimal packaging is not recommended, as it can significantly reduce the shelf life of milled flaxseed.

FUTURE RESEARCH

The oxidative stability of milled flaxseed pasteurized using the application of dry heat (148 °C) was negatively impacted by the heating process. Future research should focus on whether the pasteurization of flaxseed using dry heat (< 148 °C) can sufficiently reduce the microbial load of flaxseed without inducing instability in the seed. The identification of the temperature at which flaxseed destabilization occurs, and damage to the seed begins, would aid in identifying a target temperature for pasteurization.

Additional measures to consider include the use of modified atmospheric packaging or active packaging as a means to improve the oxidative stability of pasteurized milled flaxseed. Additionally, alternative pasteurization methods to dry heat, such as ultra-high pressure pasteurization, the use of steam pasteurization along with vacuum packaging, or moist heat may be explored to determine whether they can sufficiently reduce the microbial load without affecting flaxseed stability.

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APPENDIX

Table A-1. Percent total lipid in milled flaxseed.

Treatment	Raw	Pasteurized
	% Total lipid	
RT milling*	42.5	43.5
Cold milling	41.9	44.1

* RT = Room temperature

Table A-2. Percent surface lipid in milled flaxseed.

Treatment	Raw	Pasteurized
	% Surface lipid	
RT milling*	15.82	17.57
Cold milling	14.68	13.29

* RT = Room temperature

Table A-3. Particle size distribution of milled flaxseed at week zero.

Sample	Sieve			
	#20	#30	#40	Pan
	850 µm	600 µm	425 µm	< 425 µm
RCM	23.9	23	18.4	34.7
RRT	22.3	19.2	30.5	28
HTCM	20.8	20.1	22.8	36.3
HTRT	14.8	23.3	26.5	35.4

Sample	Coarse	Fine
	> 600 µm	< 600 µm
RCM	46.9	53.1
RRT	41.5	58.5
HTCM	40.9	59.1
HTRT	38.1	61.9
Mean PS*	41.9	58.1

*PS = Particle size

Table A-4. Analysis of variance for peroxide value and percent free fatty acid of milled flaxseed.

Source of Variation	DF	Peroxide value	% FFA
		MS	MS
Replicate (Rep)	r-1 = 2	585.3004	0.3327
Heat treatment (HT)	a-1 = 1	49463.6390 *	1.1592 *
Milling condition (MC)	b-1 = 1	1303.7918	0.0001
HT x MC	(a-1)(b-1) = 1	1158.4856	0.0057
Rep x HT x MC	(r-1)(ab-1) = 6	323.6103	0.0161
Week (W)	(c-1) = 10	501.5164 *	1.4231 *
Rep x W	(r-1)(c-1) = 20	10.9995	0.0868
HT x W	(a-1)(c-1) = 10	530.3103 *	0.3671 *
MC x W	(b-1)(c-1) = 10	21.8096	0.0180
HT x MC x W	(a-1)(b-1)(c-1) = 20	16.5287	0.0235
Error	(r-1)(ab-1)(c-1) = 60	8.5510	0.0352

* Indicates significant at p=0.05; DF=degrees of freedom; and MS=mean square