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Increased growing temperature reduces content of polyunsaturated fatty acids in four oilseed crops

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1 Increased Growing Temperature Reduces Content of Polyunsaturated Fatty Acids in Four
2 Oilseed Crops

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List of Abbreviations: Oleic acid = C18:1; Linoleic acid = C18:2; Linolenic acid =
C18:3; Gas Chromatography or Gas Chromatograph = GC; Flame Ionization Detector =
FID

22 Abstract

23 Environmental temperature directly influences the lipid profile produced by oilseeds. If
24 growing temperatures increase, as is predicted by current models, the precise profile of
25 lipids produced are likely to change. This paper develops models to predict lipid profiles
26 as a function of growing temperature. Data relating to lipid profiles of soybean (*Glycine*
27 *max*), spring canola (*Brassica napus*), spring camelina (*Camelina sativa*), and sunflower
28 (*Helianthus annuus*) were gathered from the literature and evaluated to examine the
29 influence of temperature on relative production of oleic, linoleic, and linolenic acid. For
30 each crop, a set of linear regressions was used to correlate temperature during the grain
31 fill, defined as 30 days before harvest, with the molar percentages of oleic, linoleic, and
32 linolenic acid present. An increase in temperature from 10 to 40°C resulted in an increase
33 in the production of oleic acid and a decrease in the production of linoleic and linolenic
34 acid in soybeans, canola, and sunflowers. Over the range of data available, the lipid
35 profile of camelina was temperature insensitive. To test the validity of the correlations,
36 the four crops were grown in a field study in Manhattan, Kansas simultaneously, in the
37 same environment, in 2011. The correlations accurately predicted the field data for
38 soybean, canola, and camelina but not for sunflower. The correlation for sunflower
39 under-predicted the molar amount of oleic acid and over-predicted the molar amount of
40 linoleic acid. This study indicates increasing growing temperatures from 10 to 40°C will
41 result in more monounsaturated oils and less polyunsaturated oils in soybean, canola, and
42 sunflower.

43 Keywords: Fatty acid profile; unsaturated oils; *Glycine max*; *Brassica napus*; *Camelina*
44 *sativa*; *Helianthus annuus*

45 **1. Introduction**

46 Plant lipids are important because of their use as food, fuel, and chemicals. Lipids
47 also have uses as starting materials for surfactants, lubricants, epoxides, coatings, inks,
48 polymers, and other products in the chemical industry (Metzger and Bornscheuer, 2006).
49 The lipid profile of a seed can affect its end use. In oils for human consumption, linoleic
50 acid is valued for its health benefits but linolenic acid results in oil having a poor
51 oxidative stability and shortened shelf life (Singh et al., 2010). For biodiesel production,
52 it is desirable to have a lipid profile that is highly saturated to minimize oxidation of
53 double bonds because oxidized methyl esters can form polymers that plug fuel filters and
54 damage engine performance (Monyem and Gerpen, 2001). Specific lipid profiles also
55 influences reactivity. Multiply unsaturated lipids have been shown to have a higher
56 reactivity than monounsaturated species (Singh et al., 2009, 2011).

57 Fatty acid profiles are influenced by plant type, genotype, temperature,
58 environmental conditions, and agricultural practices (Harris et al., 1978). Several studies
59 have examined the effect of temperature on fatty acid composition of the grain (Canvin,
60 1965; Aksouh et al., 2001; Ren et al., 2009). Many studies examining how temperature
61 influenced the resultant seed lipid profile were performed in greenhouses but greenhouses
62 can only approximate growing conditions in the field and cannot give a complete picture
63 of how crops will respond to different temperatures (Canvin, 1965; Aksouh et al., 2001;
64 Ren et al., 2009). Conversely, data from field studies only encompass a relatively narrow
65 temperature range (Nagao and Yamazaki, 1983; Putnam et al., 1991; Gugel and Falk,
66 2006; Gao et al., 2009). Most studies found that as temperatures rise, the percentage of
67 polyunsaturated lipids, linoleic (C18:2) and linolenic (C18:3) in particular, decreases

68 while the percentage of oleic acid (C18:1) increases. Yet each of the prior studies is
69 limited in scope, typically including only one crop grown in a handful of locations,
70 resulting in growing conditions across a limited temperature range. The general
71 consensus from these studies is that growing temperature and genotype are the main
72 factors contributing to the large variation within a crop's lipid profile (Lajara et al.,
73 1990).

74 In the current work, the literature was reviewed to determine if temperature is the
75 single dominant factor influencing lipid composition. In this paper, 25 studies of oil
76 profiles for crops grown in fields and greenhouses were compiled with temperatures
77 ranging between 10 and 40°C to provide a more complete understanding of how lipid
78 profiles are affected by temperature. Temperature during the grain fill, defined as 30 days
79 before harvest, was correlated with the percentage of major lipids contained in soybean,
80 canola, camelina and sunflower. Then the oilseed crops were grown and the lipid profile
81 of their seeds was determined and compared to the literature to demonstrate the validity
82 of the determined correlations.

83 Oilseed crops were chosen for their ability to grow in the Midwest and their
84 potential for use as a feedstock for production of biodiesel or other biochemicals.
85 Soybean (*Glycine max*) is the most valuable oilseed crop in the United States in terms of
86 production and economic value as it accounts for over 90 percent of U.S. production of
87 biodiesel and is a dominant food product (Gao et al., 2009). Over 50 million metric tons
88 of canola (*Brassica napus*) is produced annually, making it the world's third most
89 important oilseed crop behind palm and soybean (Downey, 1990). Camelina (*Camelina*
90 *sativa*) is a relatively new oilseed crop that because of its low agricultural inputs and

91 ability to grow on marginal lands, could play an important role in food and fuel
92 production in the future (Budin et al., 1995). Sunflower (*Helianthus annuus*) is one of the
93 five largest oilseed crops in the world with over 1.5 million acres of sunflower planted in
94 the US in 2011 (“Economic Research Service, USDA. ‘Table 20: Sunflowerseed:
95 Acreage planted, harvested, yield, production and value, U.S., 1980-2011’,” n.d.).
96 Compared to previous multi-crop studies on seed oil compositions, the current study is
97 distinct in that it includes camelina with the traditional crops (Werteker et al., 2010).

98 Our objectives were to gather literature data on lipid profiles over a large range of
99 growing temperatures and correlate the temperature during the grain fill to the molar
100 amount of lipid contained in the seeds. The correlations were compared to field studies to
101 demonstrate their validity.

102 **2. Materials and methods**

103 **2.1. Collecting lipid profiles from literature**

104 For field studies, literature was included in this review if the study included the
105 location where the crops were grown and harvest date (or sufficient data with which to
106 make a reasonable estimate of the date of harvest). If only the planting date was given,
107 the harvesting date was assumed to be the average of the recommended days to allow the
108 plant to grow in the field. For soybean the assumed harvest date was 100 days after
109 planting while for the short season crops, canola, camelina, and sunflower, the assumed
110 harvest date was 92 days after planting. The mean monthly maximum temperature for
111 each location was found in the National Oceanic and Atmospheric Administration’s
112 National Climatic Data Center. If the grain filling days spanned two months, the average
113 mean maximum temperature of that period was calculated, accounting for the days of

114 grain filling in each month. For greenhouse studies, literature was included in this review
115 if temperature data was given. A list of the literature included in this review can be found
116 in Table 1.

117 Genotype has been documented to have an effect on the oilseed profile, so an
118 attempt was made to control for genotype in the collected literature. Only literature
119 studies with genotypes that matched the oilseed crops grown as validation studies were
120 used. Since few studies specifically articulated the genotype of the seeds, categorization
121 strategies were employed. Soybean cultivars have been considerably modified due to
122 genetic engineering and can have a wide variety of lipid profiles. Studies with more oleic
123 acid than linoleic acid were neglected because soybean in the field studies had twice as
124 much linoleic acid than oleic. The commercial canola evaluated is significantly different
125 from the wild *Brassica napus* varieties. By definition, canola is a *Brassica napus* hybrid
126 or variety with less than 5% erucic acid, therefore *Brassica napus* oils with more than 5%
127 erucic acid were neglected. Because it has not yet reached use maturity, camelina has
128 experienced limited genetic modifications, thus all data from the literature was included.
129 Commercially available sunflower hybrids have a wide variety of lipid profiles and are
130 classified by percentage of oleic acid. The sunflower hybrid used in this study was
131 classified as a mid-oleic line with oleic acid percentages between 55-75% and linoleic
132 acid percentages between 20-42% (Grompone, 2005). Therefore, only literature studies
133 with these properties were included.

134 A linear regression was used to correlate the molar amount of each fatty acid as a
135 function of the mean maximum temperature during the grain fill. SAS software (“SAS
136 Version 8. SAS Institute Inc.,” 2006) was used to determine the parameter estimates for

137 the linear regression. Residuals for each regression were plotted to determine that errors
138 were normally distributed and that the mean of the errors was zero.

139 **2.2. Agricultural practices**

140 A randomized complete block with four replications was used to plant maturity
141 4.7 ('KS 4702' Kansas St. Univ. Manhattan, KS) soybean, canola '1651h Clearfield'
142 (Cropland Genetics St. Paul, MN), camelina (Cheyenne, Blue Sun Biodiesel Golden,
143 CO), and sunflowers '559 CL, DMR, NS' (Cropland Genetics St. Paul, MN), in
144 Manhattan, KS (98.3°W, 39.14°N) in 2011. The soybean line was chosen because its
145 maturity matched the growing season. Cropland Genetics' canola and sunflower lines
146 were chosen for their herbicide resistance. The sunflower line is resistant to drought,
147 making it suitable for planting in central and western Kansas. The camelina line was
148 chosen because it is one of the few that is tailored for dryland farming and commercially
149 available. These crops were grown simultaneously in the same location to minimize
150 variations in weather patterns and soil types. Canola and camelina were planted 11 Mar.
151 2011 and harvested 9 July 2011 and 29 June 2011 respectively. The soybean and a 95-
152 day relative maturity sunflower were planted as full season crops on 17 May 2011. The
153 sunflower crop was harvested at the end of August while the soybean crop was harvested
154 at the end of September. The non-legume crops received $112 \text{ kg} \cdot \text{ha}^{-1}$ of (N) as urea
155 $((\text{NH}_2)_2\text{CO})$ 15 - 20 days after planting. The brassica crops received $22.4 \text{ kg} \cdot \text{ha}^{-1}$ of
156 sulfur (S) from gypsum $(\text{CaSO}_4(\text{H}_2\text{O})_2)$ simultaneously with the N broadcast application.
157 Following harvest, all crops were dried to 3% moisture prior to oil extraction.

158 **2.3. Extraction of lipids**

159 Each extraction started with 100 mg of grain. An overview of the procedure can
160 be found in Figure 1. The extraction and fatty acid synthesis procedure is a modification
161 from previous work by the same lab (Kim et al., 2013). The seeds were heated at 75°C
162 for 15 min in 0.01 wt% BHT in isopropanol to inactivate lipolytic enzymes. The mixture
163 was transferred to a homogenizer to crush the seeds. To separate the triglycerides from
164 the protein solids, 1.0 mL chloroform, 1.0 mL methanol and 0.8 mL of water were added.
165 The mixture was shaken for 30 seconds and centrifuged for 10 minutes at 10,000 rpm to
166 facilitate phase separation. The chloroform layer, containing the triglycerides, was
167 transferred to a separate vial and saved. The extraction was repeated three times, each
168 time adding more chloroform to the aqueous phase, with the triglyceride fraction
169 collected in a common vial. To remove any water that might have been carried over from
170 the extraction, 0.5 mL of 1 M KCl was added as a desiccant to the triglyceride solution
171 and the mixture was shaken and centrifuged. The upper aqueous layer was removed and
172 discarded. To remove any remaining proteins, 1.0 mL of water was added and the
173 mixture was shaken and centrifuged. The aqueous layer was discarded and the
174 triglyceride solution was then dried under nitrogen and redissolved in 1000 µL of
175 chloroform.

176 For the synthesis of methyl esters, 25 µL of the triglycerides solution and 50 µL
177 of internal standard, pentadecanoic acid in chloroform, were mixed in a screw-cap tube.
178 The chloroform was evaporated and 1 mL of 3 M methanolic hydrochloric acid was
179 added to each tube. The mixture was bubbled with nitrogen to remove oxygen. The tubes
180 were heated at 78°C for 30 minutes to synthesize the methyl esters. To isolate the methyl
181 esters from water soluble compounds, 2 mL of water and 2 mL of hexane:chloroform

182 (4:1, v/v) were added to the tubes and then shaken for 30 seconds and centrifuged for 2
183 minutes. The upper layer, containing methyl esters in hexane:chloroform, was pipetted to
184 a separate vial. This separation was repeated three times, each time adding more
185 hexane:chloroform to the remaining aqueous layer, with the methyl ester fraction
186 collected in a common vial. The organic layer was dried under nitrogen. The sample was
187 then dissolved in 100 μ L of hexane and transferred to gas chromatograph (GC) vials.

188 **2.4. Analysis of lipids**

189 The GC-FID (Flame Ionization Detector) analysis was performed at the Kansas
190 Lipidomics Research Center with a 6890N GC (Agilent Technologies, Santa Clara, CA)
191 coupled to an FID. The GC was fitted with a HP-88 capillary column with a bis
192 (cyanopropyl) polysiloxane stationary phase (column length: 100 m, internal diameter:
193 250 μ m, film thickness: 0.25 μ m). Helium was used as the carrier gas at a flow rate of 1.2
194 mL min⁻¹. The injector port was maintained at 275°C. An Agilent 7683 autosampler was
195 used to inject 1 μ L of the sample in the split mode with a split ratio of 10:1. The GC
196 temperature ramp was operated as follows, initial temperature of 70 °C, ramp 1 at 15 °C
197 min⁻¹ to 175°C, ramp 2 at 1 °C min⁻¹ to a final temperature of 235°C. The FID was
198 operated at 260°C. The hydrogen flow to the detector was 30 mL min⁻¹ and air flow was
199 400 mL min⁻¹. The sampling rate of the FID was 20 Hz. The data were processed using
200 Chemstation software.

201 **3. Results and discussion**

202 **3.1. Results from literature review**

203 The molar percentage of oleic, linoleic and linolenic acids were plotted versus the
204 temperature during the grain fill for each of the four crops are presented in Figures 2, 3,

205 4, and 5, respectively. The dotted line represents the best linear fit (minimized residuals),
206 while the solid lines represent the 95% confidence intervals based on the estimation of
207 the standard deviation. The slope and y-intercepts for each of the linear regressions and
208 their respective standard deviations were determined using SAS. SAS was also used to
209 confirm that the residuals were approximately normal and the use of a linear regression
210 was appropriate for the literature values collected.

211 In soybean, canola, and sunflower, as the temperature increased, the percentage of
212 oleic acid increases while the percentage of linoleic and linolenic acids decreased. These
213 results agree with other studies that were conducted in greenhouses over broad
214 temperature ranges with canola and sunflower (12 to 27 °C) (Tremolieres et al., 1982)
215 and with studies performed in fields with soybean, canola, and sunflower over smaller
216 temperature ranges (12 to 17 °C) (Werteker et al., 2010). Camelina was unique in that its
217 lipid profile was nearly independent of growing temperature over the range investigated.
218 Other authors have found that the effect of temperature on fatty acid composition was
219 small for nine varieties of camelina, although they noted that during a particularly warm
220 year the different varieties produced 2% less linolenic acid than the same varieties during
221 a normal year (Crowley and Frohlich, 1998). Soybean and sunflower exhibited the
222 strongest trends towards more monounsaturated and less polyunsaturated fatty acids with
223 increasing temperature while canola and camelina changed minimally with increasing
224 temperature. Camelina has not been extensively studied and had fewer data points over a
225 smaller temperature range than the other crops. The data collected had temperatures
226 during the grain fill between 19°C and 28°C. This is a relatively small range compared
227 with the other three crops studied which had data from approximately 10°C to 40°C.

228 More work needs to be completed, with camelina grown in both cooler and warmer
229 temperatures to gain a more complete understanding of the effect of temperature during
230 the grain fill on the molar amounts of lipids present.

231 **3.2. Field studies**

232 For oilseeds grown near Manhattan, KS, the oil profiles varied considerably
233 between crops (Table 2). The oil profiles varied considerably between crops. Oleic acid
234 was the primary fatty acid in canola and sunflower seeds. The soybean varieties had far
235 more linoleic acid than oleic or linolenic acid. Camelina was highly unsaturated, having
236 the most linolenic acid of any of the oil seeds grown. Camelina was the only crop with
237 significant amounts of fatty acids with 20 carbons.

238 To compare percentage of fatty acid predicted by the regression to the
239 experimentally determined percentage of fatty acid contained in the seed, the temperature
240 during the grain fill must be known. The temperature during the grain fill for the
241 Manhattan, KS crops were calculated based on the growing season temperatures (Table
242 3). The experimentally determined molar percentages of the crops were compared to the
243 predicted value of the molar percentage of lipids from the regressions (Figure 6). Points
244 closest to the diagonal line represent an agreement between the lipid profile determined
245 from the plants grown in Manhattan, KS and the value predicted by the correlation from
246 the literature values. For soybean, canola and camelina, the developed correlations
247 accurately predicted the molar percentage of lipids within the confidence intervals. The
248 sunflower regression under predicted the amount of oleic acid and over predicted the
249 amount of linoleic acid. Sunflower was the only plant that did not contain linolenic acid.
250 The planted sunflower hybrid was classified as mid-oleic or having between 55 and 75%

251 oleic acid, but the grain from this trial, grown under high temperatures, had 77% oleic
252 acid. This was perhaps due to high temperatures increasing the oleic seed content above
253 typical levels. The discrepancy between the experimentally determined amount of lipids
254 and the values predicted by the regressions might have resulted because the literature
255 review purposefully excluded studies with oleic acid outside the mid-oleic range in an
256 attempt to control for genetic differences. Differences could also be explained by other
257 factors which are known to affect the fatty acid profile such as precipitation or genotype
258 (Rao et al., 1998; Gao et al., 2009). Some literature suggests that agricultural practices
259 can also affect oil profiles (Vera et al., 2007).

260 Enzymes that promote the formation of lipids are similar in all higher plants but
261 temperature affects lipid profiles to different degrees. Previous research documents that
262 the lipid profiles of all four studied crops are affected by temperature and the amount of
263 oleic acid increases while the amount of linoleic and linolenic acids decrease with
264 increasing temperature (Tremolieres et al., 1982; Wolf et al., 1982; Lajara et al., 1990;
265 Zubr and Mattha, 2002). There are two accepted explanations for how temperature causes
266 changes in the lipid profile. The earliest literature suggests that oilseeds produce more
267 linoleic acid at lower temperatures because oxygen is a necessary reactant for desaturase
268 enzyme activity and oxygen is more soluble in water at lower temperatures (Harris and
269 James, 1969). Later literature confirmed that the activity of oleoyl-phosphatidylcholine
270 desaturase, an important enzyme in the desaturation of oleic acid into linoleic acid, is
271 highly dependent on the amount of available oxygen in sunflowers (Rolletschek et al.,
272 2007). It has also been suggested that higher temperatures directly affect the lipid profile
273 by destabilizing the enzyme (Martinez-Rivas et al., 2003).

274 **4. Conclusions**

275 The lipid profile of a crop determines its ability to be used in industrial and
276 nutritional applications. The linear regressions from previously published results suggest
277 that the molar percentage of oleic, linoleic and linolenic acids contained in soybean,
278 canola, and sunflower depend on the temperature during grain fill. The molar amounts of
279 oleic, linoleic and linolenic acids in the soybean, canola, and camelina crops grown in
280 Manhattan, KS were within the 95% confidence interval of each of their respective
281 regressions. Higher temperatures will result in lower amounts of polyunsaturated lipids
282 and higher amounts of monounsaturated lipids in soybean, canola, and sunflower. As
283 average temperatures across the planet rise, oilseed crops are going to produce more
284 monounsaturated fats and less polyunsaturated fats.

285

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412 Figure 1: The procedure for extraction and separation of triglycerides from seed proteins,
413 synthesis of methyl esters, and subsequent separation from water-soluble organics from
414 oilseed crops grown for analysis by GC.

415

416 Figure 2: Linear regressions of the molar amount of the three most common lipids in
417 soybean gathered the literature plotted versus the mean high temperature during the grain
418 fill. See Table 1 for references. a) oleic acid b) linoleic acid c) linolenic acid

419

420 Figure 3: Linear regressions of the molar amount of the three most common lipids in
421 canola gathered the literature plotted versus the mean high temperature during the grain
422 fill. See Table 1 for references. a) oleic acid b) linoleic acid c) linolenic acid

423

424 Figure 4: Linear regressions of the molar amount of the three most common lipids in
425 camelina gathered the literature plotted versus the mean high temperature during the
426 grain fill. See Table 1 for references. a) oleic acid b) linoleic acid c) linolenic acid

427

428 Figure 5: Linear regressions of the molar amount of the two most common lipids in
429 sunflower gathered the literature plotted versus the mean high temperature during the
430 grain fill. See Table 1 for references. a) oleic acid b) linoleic acid

431

432 Figure 6: Comparison of predicted values from linear regression of literature values
433 versus data from crops grown in Manhattan, KS. The error bars in the x and y-direction
434 are the 95% confidence intervals for the 20 samples collected for each lipid for each crop

435 grown in Manhattan, KS and for the values collected from the literature, respectively. a)

436 oleic acid b) linoleic acid c) linolenic acid.

437

438 Table 1: Published data included in this study.

Citation (Author, Year)	Location	Plants Grown
(Aksouh et al., 2001)	Greenhouse	Canola
(Aksouh-Harradj et al., 2006)	Greenhouse	Canola
(Angelini et al., 1997)	Central Italy	Camelina
(Bhardwaj and Hamama, 2008)	Virginia	Canola
(Budin et al., 1995)	Minnesota	Camelina
(Canvin, 1965)	Greenhouse	Sunflower
(Gao et al., 2009)	Michigan	Soybean
(Gugel and Falk, 2006)	Saskatoon and Scott, Saskatchewan and Beaverlodge, Alberta	Camelina
(Iqbal et al., 2011)	Greenhouse	Canola
(Larson et al., 2002)	Gnhouse	Canola
(Lu and Kang, 2008)	Greenhouse	Camelina
(Maestri et al., 1998)	Cordoba, Argentina	Soybean
(Martinez-Force et al., 1998)	Greenhouse	Sunflower
(Nagao and Yamazaki, 1983)	Okayama, Japan	Sunflower
(Putnam et al., 1991)	Rosemount, MN	Soybean, Canola, Camelina
(Rao et al., 1998)	Fort Valley, GA	Soybean
(Ren et al., 2009)	Greenhouse	Soybean
(Rennie and Tanner, 1989)	Greenhouse	Soybean
(Robertson et al., 1971)	Tifton, GA, Baton Rouge, LA, College Station, TX	Sunflower
(Shafiullah et al., 1994)	Islamabad, Pakistan	Sunflower
(Tremolieres et al., 1982)	Greenhouse	Canola and Sunflower
(Unger and Thompson, 1982)	Bushland, TX	Sunflower
(Vantoi et al., 2012)	Columbia and Portageville, MO	Soybean
(Wolf et al., 1982)	Greenhouse	Soybean
(Zubr and Mattha, 2002)	Mullhein, Paderborn, Carlow, Germany and Uppsala, Sweden	Camelina

439 Table 2: Lipids contained in four oilseed crops grown in Manhattan, KS in 2011 listed as average of twenty samples.

Crop	Lipid (mol of lipid/total mol of identified lipids)								Sum	Harvest Date
	C16:0 Palmitic	C18:0 Stearic	C18:1 Oleic	C18:2 Linoleic	C18:3 Linolenic	C20:1 Eicosenoic	C20:2 Eicosadienoic	C20:3 Eicosatrienoic		
Soybean	11 ± 0.5	4 ± 0.5	22 ± 1.1	55 ± 1.8	8 ± 0.5	<1	ND	ND	100	Sept 20
Canola	6 ± 0.7	2 ± 0.2	62 ± 1.0	22 ± 0.5	6 ± 0.2	1 ± 0.1	<1	ND	100	July 9
Camelina	7 ± 0.9	2 ± 0.4	17 ± 1.9	21 ± 2.4	31 ± 3.4	13 ± 0.6	2 ± 0.7	1 ± 0.1	94†	June 29
Sunflower	6 ± 0.5	3 ± 0.3	77 ± 4.8	14 ± 4.8	ND	<1	ND	ND	100	Aug 25

440 ND: Not detected

441 † The molar percentages for camelina do not sum to 100 because camelina also produced C18:3n6 and C20:0 fatty acids (~3%) and
 442 detectable amounts of C22:0, C20:3n6, C22:2, C24:0 and C24:1 fatty acids.

443

444 Table 3: Monthly temperature for Manhattan, Kansas

Month	Monthly average high temperature (°C)	
	2011	30 year average
March	12.3	14.5
April	19.6	20.3
May	23.6	25.1
June	31.2	30.6
July	36.7	33.3
August	33.6	31.6
September	26.8	26.4

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