Effects of Thermal Oxidation on the Constitution of Butterfat, Butterfat Fractions and Certain Vegetable Oils[1]

D.B. Kupranycz, M.A. Amer* and B.E. Baker
Department of Food Science and Agricultural Chemistry, Macdonald College of McGill University, Ste. Anne de Bellevue, Quebec, Canada

To evaluate the thermal oxidative behavior of butterfat in comparison to selected vegetable oils, samples of winter and summer butterfat, liquid and solid butterfat fractions, and selected vegetable oils were heated at 185 C in the presence of air (30 ml/min) for 8 and 16 hr. The resultant heated fats and oils, as well as the methyl esters of their fatty acids, were examined by gel permeation chromatography. The results indicated that butterfat is much more stable to thermal oxidation than canola, sunflower and soybean oils. This was evidenced by a substantially higher content of both dimeric and higher oligomeric triglycerides in the vegetable oils than in any of the butterfat samples after both 8 and 16 hr of heating. The corn oil also exhibited a high degree of stability to thermal oxidation after 8 hr of heating. The 16 hr corn oil data, however, was less certain due to the presence of a very viscous and dark colored material which could not be removed from the oxidation flask; this was believed to contain highly polymerized oil and was not observed with any of the other samples. There were some differences in the inter- and intramolecular polymerization of the butterfat fractions compared with each other and with whole butterfat. With the winter butterfat samples, after 8 hr of thermal oxidation, both the solid and liquid butterfat fractions exhibited more stability toward intermolecular polymerization than did the whole butterfat. After 16 hr of heating the ratio of trimeric and higher oligomeric triglycerides to dimeric triglycerides increased with increasing degree of unsaturation of the butterfat and with increased time of heating. Similar trends were observed with regard to the degree of intramolecular polymerization.

Previous work (1) has shown that the thermal oxidation of a fat (heating at 180 C–200 C in the presence of air) results in a variety of complex chemical changes in the fat, both oxidative and thermolytic. This leads to the formation of numerous volatile and nonvolatile degradation products, many of which are important from the standpoints of flavor, odor and nutrition.

Of particular interest are the nonvolatile degradation products which accumulate in the thermally oxidized fats and oils and subsequently are ingested with the food. A relationship between the nonvolatile, non-urea-adduct-forming fraction of heated vegetable oils and various toxic responses has been suggested by the early work of Crampton et al. (2,3). The work of subsequent researchers (4–7) supports these findings and suggests that the nonadductable monomers and oxidative dimers are the main source of toxicity.

Numerous investigations have dealt with the characterization of various components of thermally oxidized vegetable fats and oils; corresponding data for butterfat, however, is limited. There is some evidence in the early literature that butterfat may have possible nutritional advantages over vegetable oils as a cooking fat (8–10). Knowledge of the differences in composition of thermally oxidized butterfat and of vegetable oils may suggest possible factor(s) which could be responsible for the observed nutritional advantages of butterfat. Such information is also desirable in the view of current interest within the dairy industry in the fractionation of butterfat to yield products which might have specific uses in food formulation and processing (11). To the authors' knowledge, there is no information in the literature on the thermal oxidative behavior of such butterfat fractions.

The present study deals with the characterization of nonvolatile degradation products which are formed during thermal oxidation of butterfat and butterfat fractions as well as certain vegetable oils. In particular, this study deals with the higher molecular weight compounds that form as a result of thermal oxidation of fats and oils.

EXPERIMENTAL

Fat and oil samples. Samples of winter (January) and summer (September) butterfat were prepared from fresh butter (Cooperative Agricole de la Cote Sud, Quebec) by melting the butter at 60 C, removing the top oil layer, filtering the oil through glass wool and drying the resultant product over anhydrous sodium sulfate. The oil was then refiltered (vacuum, Whatman 41 paper), flushed with nitrogen and stored at −20 C. The anhydrous butterfat was fractionated at 29 and 19 C by crystallization from molten fat to yield solid (S) and liquid (L) fractions at each temperature. The fractionation procedure has been described previously (11).

Sunflowerseed oil (Safflo, CSP Foods Ltd., Saskatchewan) and corn oil (Mazola, Best Foods, Montreal) were purchased from a local supermarket. Canola oil and soybean oil were obtained from Canada Packers Ltd. (Montreal). None of the vegetable oils contained any preservatives.

Thermal oxidation procedure. A sample (100 g) of fat or oil was placed in a three-neck round-bottom flask (500 ml) and then heated (heating mantle) at 185 C ± 2 C for 8 or 16 hr. A constant flow (30 ml/min, double stage regulator and flowmeter) of extra dry air (Linde, St. Laurent, Quebec) was passed into the oil during the heating period. The same cylinder of air was used throughout the study. The samples were stored at −20 C under an atmosphere of nitrogen until they were analyzed.

Gel permeation chromatography (GPC). The treated and untreated samples were analyzed using a High Performance Liquid Chromatograph (HPLC) supplied by Waters Associates (Milford, Massachusetts); the instrument consisted of a Model 510 pump, a U6K Universal Injector, an R401 Differential Refractometer (attenuation 8×8) and a series of three columns (7.8 mm ID × 30 cm

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*To whom correspondence should be addressed at Department of Food Science and Agricultural Chemistry, Box 187, Macdonald College of McGill University, 21,111 Lakeshore Rd., Ste. Anne de Bellevue, Quebec, Canada H9X 1C0.

length; 10^3 Å, 500 Å, and 100 Å Ultrastyragel) operated at room temperature. The column packing material was highly crosslinked styrenedivinylbenzene copolymer (<10 microns). The total permeation volume was 36 ml (or 12 ml/column), and the total void volume was 18 ml. Tetrahydrofuran (THF) stabilized with 250 ppm BHT (Anachemia, Lachine, Quebec), was used as the solvent at a flow rate of 1.0 ml/min. Peak integration was performed using a Spectra-Physics (San Jose, California) SP-4270 Integrator.

Preparation of samples for GPC. For the analysis of intact triglycerides, the samples (30 to 50 mg) were dissolved in 1 ml of THF.

Methyl esters of the unheated and heated fats and oils were prepared by IUPAC Method 2.301 (4.2) for acid oils and fats (12) with minor modifications. The methyl esters were extracted with hexane and the resultant solution dried over anhydrous sodium sulfate. The hexane was evaporated using a stream of nitrogen, and the methyl esters were redissolved in THF (30 to 50 mg methyl esters per 1 ml of solvent). High purity dimer and trimer acids were obtained from Emery Industries (Cincinnati, Ohio); they were converted to methyl esters by the procedure given previously. Standard mixtures of triglycerides, diglycerides, monoglycerides and free fatty acids were purchased from Sigma Chemical Co. (St. Louis, Missouri).

Fatty acid analyses. The preparation of methyl esters and the determination of fatty acid composition by capillary-column gas liquid chromatography was described in a previous paper (11).

RESULTS AND DISCUSSION

Figure 1 shows typical gel permeation chromatograms of butterfat and vegetable oil samples which were heated at 185 C for 16 hr; chromatograms from both the intact samples and their methyl esters are shown. The dimeric and higher molecular weight peaks which increase as a result of polymerization reactions during thermal oxidation give an indication of oil deterioration.

It should be recalled that GPC separations are based strictly on differences in molecular size. Thus, the chromatographic peaks may include both nonpolar and polar components. The relative concentrations of nonpolar and polar components will depend on the presence of polar functional groups and, in the case of polymerized fatty acids or triglycerides, on the type of linkage between fatty acid or triglyceride units (i.e., carbon to carbon, ether, hydroperoxide, or epoxide linkage) (13). It should also be noted that GPC of the intact samples gives an indication of the degree of intermolecular polymerization (i.e., reactions between fatty acids on two different triglyceride molecules) and not intramolecular polymerization (i.e., reactions between fatty acids on the same molecule). Thus, the heated oils were transformed to methyl esters to obtain additional data on the total amounts of inter- and intramolecular fatty acid dimers and higher oligomers which were formed during the thermal oxidation treatment.

Components are eluted from gel permeation columns in the order of decreasing molecular size. The series of columns which was used in this investigation resulted in the separation of normal weight butterfat triglycerides to give two peaks; these represent two broad groups of

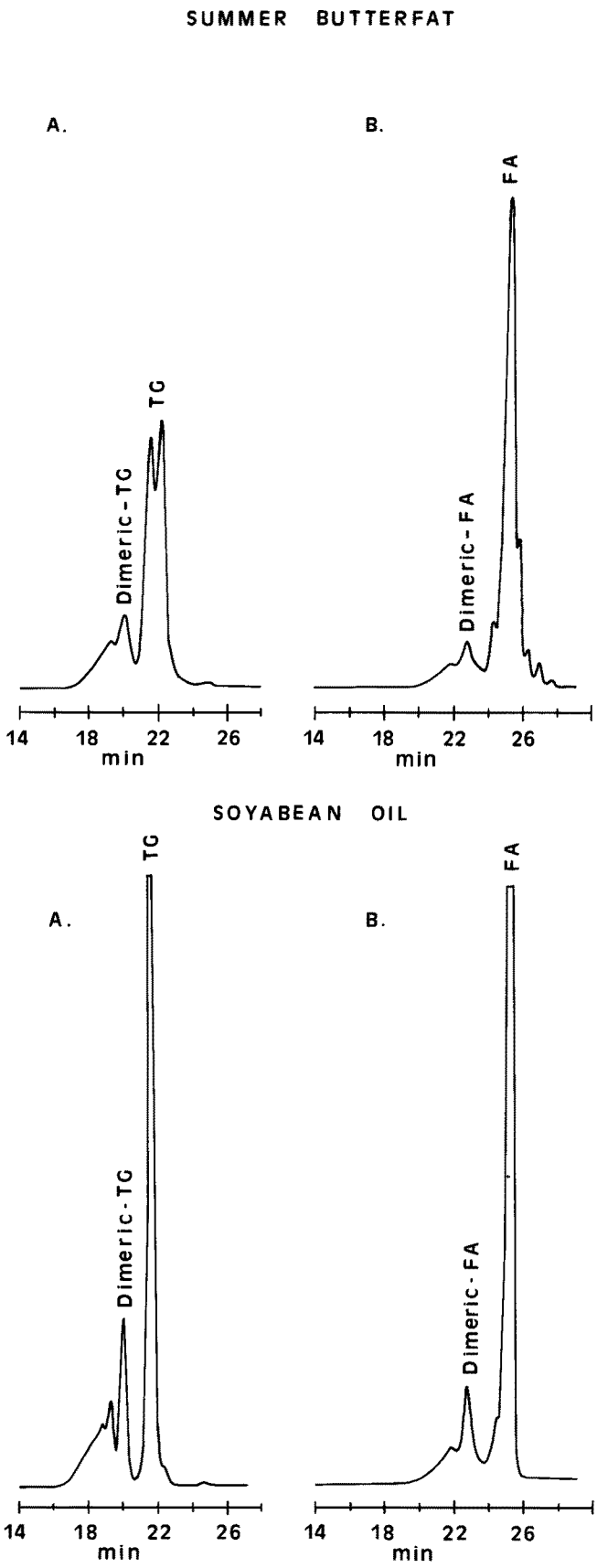

FIG. 1. Gel permeation chromatograms of thermally oxidized (185 C; 16 hr) summer butterfat and soybean oil (A, intact oil; B, methyl esters).

triglycerides which differed in molecular size (Fig. 1). The second peak represented the low molecular weight triglycerides; they are eluted with diglycerides as indicated by the retention time of standard diglycerides. The vegetable oils, on the other hand, gave a single triglyceride peak. This indicates the narrower range of composition of vegetable oil triglycerides compared to butterfat triglycerides which contain from C22 to C54 (total acyl carbon number) species (11).

The results reported in Table 1 show that all of the unheated vegetable oils contained dimeric triglycerides ranging in amounts from 0.21 to 0.59%; the unheated butterfats did not contain any detected polymeric triglycerides. The occurrence of high molecular weight compounds in fresh vegetable oils is most likely the result of processes that were used in their refinement (i.e., degumming, alkali refining, bleaching, deodorization).

The sunflowerseed, soybean and canola oils, after both 8 and 16 hr of thermal oxidation, contained substantially higher amounts of both dimeric and higher oligomeric triglycerides than did either the corn oil or butterfat samples (Table 1). It should be noted that with the corn oil samples, after 16 hr of thermal oxidation a very viscous and dark colored material remained on the inner walls of the flask after the sample was removed; this was not observed with any of the other samples. This material could contain high molecular weight components which were not accounted for in the GPC results from the corn oil samples which were heated for 16 hr. The results obtained with all of the fat and oil samples indicate that the rates of dimeric triglyceride formation, during the first 8 hr of heating, exceeded the rates of trimeric and higher oligomeric triglyceride formation. The fastest rates of formation of all polymers occurred in the sunflowerseed, soybean and canola oils. The rate of dimer formation in the sunflowerseed, soybean and canola oils decreased during the second 8 hr of heating, while the amounts of higher oligomeric triglycerides continued to increase at a steady rate throughout the 16-hr heating period. The rates of formation of dimers as compared with the higher oligomers in the sunflowerseed, soybean and canola oils suggest that dimers are formed preferentially and that the higher oligomers were formed from the dimers (addition polymerization).

The corn oil behaved more like the butterfat samples with respect to intermolecular polymerization than did the other three vegetable oils. This is somewhat surprising in view of the considerable differences in their fatty acid compositions (Table 5). It seems reasonable to assume that the probability of intermolecular polymerization increases as the degree of unsaturation of an oil increases. The results reported here, however, suggest that (at least during the first 8 hr of heating) there are factors other than degree of unsaturation which have a marked influence on intermolecular polymerization. One such factor could be the presence of naturally occurring antioxidants. It is known that untreated corn oil contains a certain amount of tocopherol (14) which could explain its slower rate of polymerization during 8 hr of thermal oxidation compared to the other vegetable oils.

TABLE 1

Monomeric, Dimeric and Higher Oligomeric Triglycerides Constitution of Unheated and Thermally Oxidized Butterfats and Vegetable Oils

Heating period (hr)	GPC fraction	% of intact sample (peak area/total area of all peaks)					
		Canola oil[a]	Sunflowerseed oil[a]	Corn oil[a]	Soybean oil[a]	Winter butterfat[b]	Summer butterfat[a]
0	Trimeric and higher oligomeric	nd[c]	nd	nd	nd	nd	nd
	Dimeric	0.21	0.59	0.21	0.25	nd	nd
	Monomeric (TG)	97.06	97.06	95.83	98.10	50.01	52.26
	Monomeric (TG, DG)	2.45	2.04	3.51	1.36	49.54	47.24
	Monomeric (FFA)	0.29	0.31	0.45	0.30	0.45	0.50
8	Trimeric and higher oligomeric	7.70	10.33	2.86	8.82	4.93	5.51
	Dimeric	12.17	15.85	8.90	13.51	9.74	9.34
	Monomeric (TG)	77.58	72.14	84.45	77.43	39.63	41.63
	Monomeric (TG, DG)	2.40	1.44	3.44	tr[d]	45.70	43.23
	Monomeric (FFA)	0.16	0.23	0.35	0.24	tr	0.28
16	Trimeric and higher oligomeric	19.65	23.10	9.60	21.14	9.12	11.48
	Dimeric	14.67	17.49	13.76	15.05	12.81	13.16
	Monomeric (TG)	62.99	59.23	72.90	63.55	34.23	34.27
	Monomeric (TG, DG)	2.69	tr	3.50	tr	43.84	41.03
	Monomeric (FFA)	tr	0.18	0.23	0.27	tr	0.05

[a]Each value for 8- and 16-hr heating represents the mean of 3 replicate samples, analyzed in duplicate.

[b]Each value represents the mean of one sample, analyzed in duplicate.

[c]Not detected.

[d]Trace (not integrated).

Tables 2 and 3 show that there are some differences in the intermolecular polymerization of the butterfat fractions in comparison to whole butterfat. The chemical and physical properties of the whole butterfat and butterfat fractions which were used in the present study were described in another paper (11).

The liquid fraction (L-19; Table 2) obtained from the winter butterfat showed some stability toward thermal oxidation (8 hr heat treatment) as compared to the other samples; this sample, however, had the highest degree of unsaturation (11). After 16 hr of heating, the solid fraction (S-29; Table 2) exhibited the greatest resistance to intermolecular polymerization. With the summer butterfat samples (Table 3), the S-29 fraction contained the lowest level of total polymeric triglycerides after both 8 and 16 hr of thermal oxidation; the L-19 fraction contained the highest levels of polymeric triglycerides. A comparison of the results obtained with winter and summer butterfats shows that the levels of dimeric triglycerides in the samples heated for 8 and 16 hr were very similar. The main differences were in the levels of high molecular weight polymers with the summer butterfat and fractions containing larger amounts of trimeric and higher oligomeric triglycerides than the corresponding winter samples. This reflected the higher proportion of unsaturated fatty acids in the summer butterfat as compared to the winter butterfat (Table 5) (11).

Table 4 summarizes the GPC data from heated fat and oil samples (16 hr) which were transformed to the corresponding methyl esters prior to GPC. These results give an indication of the total amount of polymerization (both inter- and intramolecular) which has occurred. It is reasonable to expect that the probability of having more than one unsaturated fatty acid in a triglyceride molecule will be greater with samples having high levels of unsaturated fatty acids. This, in turn, should result in an increased probability of intramolecular polymerization (15). It was observed that the sunflowerseed oil had undergone the highest degree of both inter- and intramolecular polymerization among all the fats and oils that were studied. On the other hand, the corn oil sample was relatively resistant to polymerization compared to the other vegetable oils. It is interesting to note that, despite the fatty acid composition of the corn oil, the heated oil contained primarily intermolecular polymers as indicated by the relatively low value for trimeric fatty acid methyl esters (Table 4). Trimeric fatty acids can arise only if intramolecular polymerization reactions have occurred. As indicated previously, the results for corn oil (16 hr sample) may be lower than the actual values due to losses of polymeric material on the inner walls of the oxidation flask. The gel permeation chromatograms from the fatty acid methyl esters showed that none of the fats and oils studied contained polymeric fatty acids with more than three component fatty acids.

The results obtained with the butterfat samples (both winter and summer) indicate that, in general, the total amount of polymerization increased as the level of unsaturated fatty acids in the unheated fats increased (Table 4). Pokorny et al. (15) stated that it is mainly the polyenoic fatty acids which participate in polymerization reactions of lipids. The present study indicates that this is not necessarily true, because the levels of polyenoic fatty acids in the butterfat samples alone do not account for the observed levels of polymerization. It would appear that, under conditions of thermal oxidation, both monoenoic and polyenoic fatty acids, and perhaps saturated fatty acids, once oxidized, have the potential

TABLE 2

Monomeric, Dimeric and Higher Oligomeric Triglycerides Constitution of Unheated and Thermally Oxidized Winter Butterfat and Butterfat Fractions

Heating period (hr)	GPC fraction	% of intact sample (peak area/total area of all peaks)[a]				
		Fraction[b] S-29	Fraction S-19	Whole butterfat	Fraction L-29	Fraction L-19
0	Trimeric and higher oligomeric	nd[c]	nd	nd	nd	nd
	Dimeric	nd	nd	nd	nd	nd
	Monomeric (TG)	65.86	56.05	50.01	44.83	40.55
	Monomeric (TG, DG)	33.74	43.54	49.54	54.55	58.82
	Monomeric (FFA)	0.39	0.41	0.45	0.62	0.64
8	Trimeric and higher oligomeric	4.38	4.62	4.93	5.24	5.00
	Dimeric	8.86	9.36	9.74	8.45	7.82
	Monomeric (TG)	55.38	45.93	39.63	35.16	30.73
	Monomeric (TG, DG)	31.30	40.10	45.70	50.86	56.25
	Monomeric (FFA)	0.11	tr[d]	tr	0.29	0.20
16	Trimeric and higher oligomeric	7.95	9.04	9.12	13.33	12.22
	Dimeric	12.48	12.24	12.81	11.74	11.71
	Monomeric (TG)	50.39	40.42	34.23	27.14	22.76
	Monomeric (TG, DG)	29.18	38.29	43.84	47.78	53.32
	Monomeric (FFA)	tr	tr	tr	tr	tr

[a]Each value represents the mean of one sample, analyzed in duplicate.

[b]Fraction designation indicates physical state (solid, liquid) and fractionation temperature (C).

[c]Not detected.

[d]Trace (not integrated).

TABLE 3

Monomeric, Dimeric and Higher Oligomeric Triglycerides Constitution of Unheated and Thermally Oxidized Summer Butterfat and Butterfat Fractions

Heating period (hr)	GPC fraction	% of intact sample (peak area/total area of all peaks)[a]				
		Fraction[b] S-29	Fraction S-19	Whole butterfat	Fraction L-29	Fraction L-19
0	Trimeric and higher oligomeric	nd[c]	nd	nd	nd	nd
	Dimeric	nd	nd	nd	nd	nd
	Monomeric (TG)	68.69	59.22	52.26	49.38	45.12
	Monomeric (TG, DG)	31.08	40.45	47.24	50.22	54.40
	Monomeric (FFA)	0.23	0.30	0.50	0.40	0.48
8	Trimeric and higher oligomeric	4.75	5.48	5.51	6.33	6.62
	Dimeric	8.83	9.40	9.34	8.48	8.51
	Monomeric (TG)	59.46	48.34	41.63	39.01	34.52
	Monomeric (TG, DG)	26.84	36.57	43.23	46.01	50.08
	Monomeric (FFA)	0.12	0.21	0.28	0.18	0.27
16	Trimeric and higher oligomeric	9.90	10.77	11.48	13.75	15.34
	Dimeric	12.48	12.99	13.16	11.75	11.39
	Monomeric (TG)	52.15	40.90	34.27	31.54	26.71
	Monomeric (TG, DG)	25.26	35.09	41.03	42.83	46.32
	Monomeric (FFA)	0.20	0.26	0.05	0.13	0.23

[a]Each value for 8- and 16-hr heating represents the mean of 3 replicate samples, analyzed in duplicate.
[b]Fraction designation indicates physical state (solid, liquid) and fractionation temperature (C).
[c]Not detected.

TABLE 4

Monomeric, Dimeric and Higher Oligomeric Fatty Acid Constitution of Thermally Oxidized (185 C, 16 hr) Butterfats, Butterfat Fractions and Selected Vegetable Oils

GPC fraction	Fatty acid methyl esters, % (peak area/total area)			
	Canola oil[a]	Sunflowerseed oil[a]	Corn oil[a]	Soybean oil[a]
Trimeric and higher oligomeric	5.98	5.64	2.96	6.47
Dimeric	11.04	14.89	9.24	12.50
Monomeric (fatty acids)	82.98	79.47	87.79	81.03

	Fractions from winter butterfat				
	S-29[b]	S-19[b]	Whole butterfat[b]	L-29[b]	L-19[b]
Trimeric and higher oligomeric	3.97	4.31	4.21	8.18	7.07
Dimeric	9.24	8.94	9.18	7.20	7.82
Monomeric (fatty acids)	86.79	86.75	86.61	84.62	85.11

	Fractions from summer butterfat				
	S-29[a]	S-19[a]	Whole butterfat[a]	L-29[a]	L-19[a]
Trimeric and higher oligomeric	3.59	4.50	4.96	6.44	7.92
Dimeric	7.70	8.63	8.97	8.51	8.06
Monomeric (fatty acids)	88.71	86.88	86.07	85.03	84.02

[a]Each value represents the mean of 3 replicate samples, analyzed in duplicate.
[b]Each value represents the mean of one sample, analyzed in duplicate.

TABLE 5

Fatty Acid Composition of Winter and Summer Butterfat and Selected Vegetable Oils

Fatty acid group	Methyl esters (% by weight)[a]					
	Canola oil	Sunflowerseed oil	Corn oil	Soybean oil	Winter butterfat	Summer butterfat
Saturated	7.43	10.74	12.99	15.07	70.48	66.73
Monounsaturated	58.16	18.00	26.83	22.66	24.57	27.36
Polyunsaturated	34.17	70.90	60.17	61.86	1.90	2.36
Other	0.24	0.35	—	0.42	3.04	3.55

[a]Each sample was analyzed in duplicate.

to participate in polymerization reactions.

The results reported in the present paper indicate that butterfat and the fractions of butterfat are much more stable to thermal oxidation than are certain vegetable oils (canola, sunflowerseed and soybean). The corn oil also exhibited a high degree of stability after 8 hr of heating. However, the 16-hr corn oil data was less certain due to the presence of a very viscous and dark colored material which could not be removed from the oxidation flask; this was believed to contain highly polymerized oil and was not observed with any of the other samples. The data also suggests that the degree of unsaturation of a fat or oil alone does not control the extent or rate of polymerization reactions during thermal oxidation. It is certain that unsaturation is involved in polymerization of fats; other factors, however, also could be important as would be indicated from the results with corn oil (8-hr heat treatment) and with the L-19 fraction from winter butterfat (8-hr heat treatment). It should be pointed out that, although the formation of polymeric compounds in heated fats and oils is considered to be an indication of the extent of degradation, the monomeric triglycerides or fatty acids may also be degraded by thermal or oxidative changes. Further work is being conducted in this laboratory to provide more detailed information on the constitution of the heated fats and oils.

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