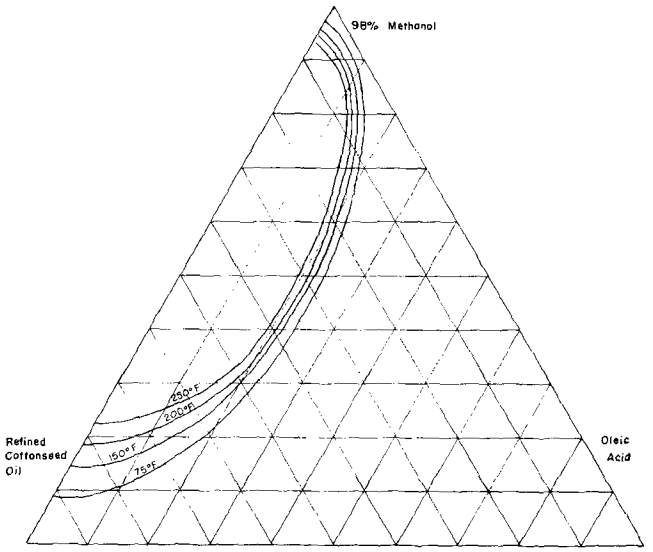

FIG. 1. Phase diagram for an oleic acid, cottonseed oil, 98% methanol system.

remains low, changing from 0.75 at 86F to 1.0 at 260F.

When the above information was available, it became apparent that the proposed process was not commercially attractive. Although the selectivity was excellent, the distribution ratio never exceeded 1.0. Systems where this ratio is 1.0 or less usually require excessive amounts of solvent. This is definitely true for the methanol system studied.

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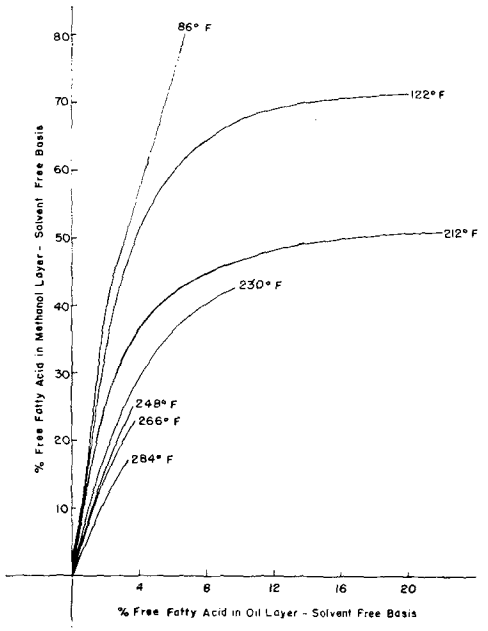

FIG. 2. Selectivity diagram for extraction of fatty acids in cottonseed oil using 98% methanol.

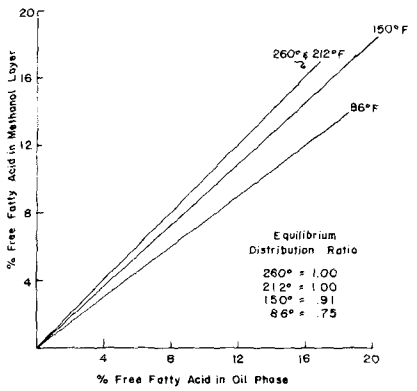

FIG. 3. Equilibrium distribution of fatty acids in 98% methanol-fatty acid-cottonseed oil system.

The Influence of Temperature, Heating Time, and Aeration upon the Nutritive Value of Fats

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Abstract

In order to determine the biological significance of the changes which occur when fats are heated to high temperatures in air, cottonseed oils were heated and aerated under several controlled conditions. In general, the data indicate that the changes induced are proportional to the severity of the conditions and that treatments more severe than those usually encountered in processing or cooking are necessary to produce detectable damage.

Oils which had been subjected to prolonged aeration at 60C (16 days or more) or exposed to air in thin layers maintained at 180–220C supplied less available energy and caused development of larger livers than untreated samples when compared in rat feeding tests. Heating in deep layers caused less damage than heating to the same temperatures in thin films, indicating that exposure to oxygen accelerates nutritional impairment.

The cooking of food in fats changes condition so greatly that direct extrapolation of data obtained in tests using fat alone is not justified. Fat extracted from foods has not been found to contain harmful substances by the tests used.

Introduction

NUMEROUS INVESTIGATORS have indicated that excessive laboratory heating and/or oxidation impairs the nutritive value of fats and may result in the formation of substances which give adverse physiological reactions when fed (1–14). While this type of research is of utmost importance in demonstrating the tendencies of fats to undergo changes under various conditions, most of it has not been designed to

determine changes which may occur during conventional processing or cooking.

Generally speaking, laboratory treatments of fat have been more severe than conventional food processing with respect to temperature, heating time, degree of aeration, or combinations of these factors. Moreover, laboratory treatments represent relatively constant, "pure" systems; whereas, during cooking the volume and temperature of the fat varies, and this system contains food particles, water, steam, and added make-up fat. Studies of samples obtained from food processors or experimental kitchens have demonstrated that marked changes in nutritional value do not occur in food fats (2,15).

The experiments reported in this paper are part of a general program to determine the nutritive value of fats obtained from commercial and home cooking operations. They were designed to study the influence of temperature, heating time, and aeration on the biological effects of fats when fed to rats.

Materials and Methods

Biological Testing Procedure. The biological methods used throughout these experiments have been previously reported (2,19). In practice, male weanling rats [1] weighing 40–50 g were fed 5.0 g of basal ration daily for a 9 day adjustment period. At the end of this period the rats were weighed, those falling within a 10 g weight range, usually 60–70 g, were divided into the test groups. Then, for a 7 day test period, each rat received 5.0 g of basal ration supplemented with a fixed quantity of test material each day. Under the conditions of the experiment the only known limiting factor is energy, and growth is proportional to the energy supplied by supplements. The total available energy of a test material may be determined by comparing the gains of rats fed the test material with the gains made by control groups fed varying amounts of prime steam lard as a source of energy. Based on data from 333 rats, assuming lard to contain 9.0 cal of available energy, the equation relating weight gain to available calories (C=kcal in the equation) has been characterized by the method of least squares, as follows:

$$\text{g gained} = 6.25 + 2.49C - 0.0426C^2$$

The energy content of a test substance may be calculated from the equation using the weight gains of rats fed the test materials and solving for C, or from a reference curve representing this equation.

At the end of the 7 day test period, the animals were weighed, chloroformed, and autopsied within two minutes after death. Experience had indicated that liver weights may vary depending upon the length of period between the last meal and autopsy. For example, when rats which had been fed at the same time one day were autopsied the following morning and afternoon, the liver as per cent of body weight averaged 5.16% for the morning series (38 animals) and 4.48% for the afternoon series (38 animals). Therefore, in the tests reported hereafter, a staggered feeding technique was used to assure that every rat was autopsied 24 ± 1 hours after the last meal.

Calculations of Liver Size. Based on data accumulated over a number of years involving 381 rats fed fresh fats and other nutritious materials of unquestioned wholesomeness, a normal liver-body weight relationship has been established for weanling rats fed under these conditions. This relationship is essentially a straight line function for body sizes of 60–110 g. The equation of the line as determined by the method of least squares is:

$$\text{g of liver} = 0.95 + 0.0538 \times (\text{g of body weight})$$

This normal curve represents data from 16 experiments in which weanling rats were fed according to the technique described by Rice et al. (19). Data from the following four experiments were included as part of the normal curve. Inspection of the equation makes it apparent that the liver as per cent of body weight is not constant but that it increases with increasing body size under these dietary conditions.

Since it has been established that rats fed fresh fat will grow more rapidly than those fed equal amounts of abused fats, a method was needed for comparing liver weights of animals differing in body size. In order to make such comparisons, it has been assumed that the above curve will indicate the "normal" or expected liver weight for a rat of any weight within the accepted range and fed under the conditions of the test. The per cent liver enlargement may then be calculated as follows:

$$\%\ \text{Enlargement} = \frac{\text{exptl liver wt} - \text{normal liver wt from curve}}{\text{normal liver wt}} \times 100$$

This method of comparing liver size is essentially the same as that of Kaunitz (4) except that the normal curve is a straight line over the range of body sizes used. The actual amount of liver enlargement produced by fat depends on the level fed. Thus, by calculating the per cent liver enlargement per g of fat, the results of two or more experiments can be compared with some degree of validity, even though the levels fed are not exactly the same.

Experimental

Experiment No. 1. Winterized cottonseed salad oil was heated in a 28 lb commercial deep fat fryer for 120 hours at 182C. The surface of the oil was fully exposed to the air, but the oil was not stirred. This treatment differs greatly from the condition used in restaurants where make-up fat is added and where the fat is stirred by the addition of food. Small samples of heated oils were collected daily. The results of the feeding of these samples appear in Table I.

The weight gains, which reflect available energy of the fats fed, decreased constantly with increased duration of heat treatment. Co-variance analyses of liver and body weights likewise revealed highly significant increases in adjusted liver sizes with increased time of heating. Oil from the same lot was used in a comparable setup for the production of 5 lb quantities of potato chips twice daily as de-

TABLE I

Influence of Prolonged Heat Treatment upon the Available Energy of Cottonseed Oil and upon Its Ability to Cause Heavier Livers

Heating time	Avg. weight gain [a]	Available energy	Liver % body weight	% Liver enlargement per g of fat
		No frying		
hr	*g*	*kcal/g*		
0	32.8	9.36	4.33	0.0
24	31.1	8.52	5.60	19.3
48	30.5	8.22	6.19	29.0
72	29.1	7.62	6.45	33.5
96	25.6	6.12	6.66	37.3
120	26.3	6.42	6.97	42.0
		Intermittent frying		
120	30.4	8.16	5.98	25.6

[a] All animals were fed 5.0 g of basal + 1.5 g of fat per rat per day.

[1] Holtzman Company.

TABLE II
Influence of Sample Size on Changes in Various Oils Heated 6 Hours at 200C.

	Saybolt viscosity 210F	Iodine No.	No. of rats	Avg. weight gain	Available energy	Liver % body wt	% Liver enlargement per g of fat
	sec			*g*	*kcal/g of fat*		
Corn Oil No. 1							
Unheated	....	121.0	8	30.5	8.22	4.57	2.7
3 Kg	....	121.1	4	32.5	9.18	4.84	6.5
400 g	....	113.8	8	29.8	7.92	5.46	15.5
200 g	156	97.2	4	23.5	5.34	6.26	31.3
Corn Oil No. 2							
Unheated	57	122.7	8	32.1	9.00	4.35	—1.2
3 Kg	59	123.7	4	33.0	9.48	4.69	4.0
400 g	71	112.0	8	31.0	8.46	5.27	13.1
200 g	128	100.0	4	28.8	7.50	6.75	37.7
Cottonseed Oil							
Unheated	64	111.4	4	30.5	8.22	4.43	1.1
3 Kg	62	108.9	4	33.3	9.60	4.33	—0.8
400 g	77	98.2	4	29.0	7.56	5.61	18.2
200 g	166	82.4	4	17.3	3.24	7.29	50.1
Lard							
Unheated	65	60.8	8	32.9	9.42	4.69	3.8
3 Kg	67	60.6	4	30.3	8.10	4.33	0.0
400 g	64	53.2	4	30.0	7.98	5.60	19.0
200 g	124	42.5	4	22.8	5.10	6.33	32.7
Hydrogenated Vegetable Oil							
Unheated	....	72.2	4	32.0	8.94	4.55	1.9
3 Kg	67	70.7	4	30.3	8.40	4.59	3.3
400 g	86	66.9	4	30.0	7.98	4.98	9.9
200 g	118	57.2	4	27.0	6.72	6.67	36.9
Tallow							
Unheated	64	41.1	3	31.3	8.58	4.48	1.1
3 Kg	67	43.4	4	29.0	7.56	4.27	—1.2
400 g	75	36.7	4	29.8	7.92	4.91	8.0
200 g	111	29.2	4	24.5	5.58	6.05	27.5

scribed by Rice et al. (20). The oil used intermittently produced significantly smaller changes than the oil with no food added (see last line, Table I).

While this experiment demonstrates that oil heated in a restaurant-type fryer at practical heating temperatures can be abused if it is heated long enough, it should be emphasized that fats obtained from actual restaurants do not show this damage even though the temperature and times are similar (2). Apparently, the rate of fat turnover, the effect of steam distillation due to the addition of water, and other factors alter the reaction. Hence, data obtained with the one system cannot be depended upon to reflect changes in the other. Moreover, it should be pointed out that even under the conditions of this laboratory experiment, the change occurred slowly. "Days" were required to produce appreciable damage rather than "hours" or "minutes."

Experiment No. 2. In this experiment, 3 kg, 400 g, and 200 g portions of different types of fats were heated for six hr at 200C. The 3 kg and 400 g samples were heated in a 4 liter stainless steel beaker placed directly on a hot plate. A motor-driven stirrer, regulated to produce a gentle agitation, was used to insure uniform temperature conditions throughout the oil without appreciable inclusion of air bubbles. The 200 g samples were heated in a 10 in. aluminum electric skillet of a type used in home cooking. Temperatures were measured by thermocouples. In this skillet, stirring was not feasible but the sample had a large exposed surface.

There were no significant differences between rats fed unheated fats and those fed fats heated in 3 kg quantities (Table II). There were highly significant differences in weight gains and adjusted liver sizes between animals fed fats from the 400 g and the 200 g treatments and both of these groups were significantly different from both the controls and from animals fed oils heated in 3 kg quantities. There were no significant differences between the various types of fats.

The batch sizes and the temperatures used in these experiments are in a range which might be found in home cooking. However, it should again be emphasized that no foods were cooked in the fats. No evidence of damage was noted (15,20) when fat was extracted from meats which are fried, roasted, or broiled under practical home conditions, or from commercial potato chips and fed to rats. The data does show a striking increase in the rate of deterioration with increased exposure to air.

Experiment No. 3. In this experiment, a balanced 3 x 3 x 3 design was used to study the effect of sample size (which indirectly controls the air exposure per g of fat), temperature, and heating time on the biological value of a winterized cottonseed oil. Samples weighing 50, 100, and 200 g were heated to temperatures of 180, 200, 220C for 30, 120, or 360 min. All samples were heated in a flat bottom aluminum cake pan 1½ in. deep by 8½ in. diameter. The cake pan was positioned in an oil bath which was heated to 192, 212, or 232 C on a hot plate. A stirrer was used in the bath to insure uniform conditions but the test samples were not stirred. At the start of each run, the cake pan was preheated in the bath before the sample was added. Zero time was recorded as the time at which a sample attained the designated temperature. Oils in the cake pan attained temperatures of approximately 180, 200, or 220C (about 12 degrees lower than that of the bath) in about 3 min. The pan and samples were cooled rapidly after the experimental period had elapsed.

Even gross inspection of the samples indicated differences in color and viscosity. Since it is well established that viscosity increases and iodine values decrease with increased heating time, no attempt was made to determine these changes. However, the 50 g samples which had been heated for 2 hr. or more became so viscous that they would have been completely unsuitable as frying agents. All samples which had been heated for the longer periods of time and at the higher temperatures became viscous and some of them were converted into gum-like materials. The 50 g and 200 g samples heated for six hr at 200C were converted to their methyl esters and the volatile portion analyzed by gas chromatography. Results of this analysis (Table III) indicate that linoleic acid is the component showing the greatest change during this heating period.

Very few, if any, biological differences were apparent in samples that had been heated for 30 min or less (Table IV). In general, however, the data indi-

TABLE III

Changes in Fatty Acid Composition of Cottonseed Oil Heated 6 hr at 200C

Fatty ester detected	Composition of volatile portion			Corrected to same palmitate basis as unused oil[b]	
	Typical unheated oil[a]	50 g heated oil	200 g heated oil	50 g heated	200 g heated
	%	%	%	%	%
Myristate	1.1	1.2	1.2	0.68	0.87
Palmitate	20.7	36.5	28.4	20.7	20.7
Stearate	2.4	3.1	1.3	1.8	1.0
Oleate	18.7	29.4	23.6	16.7	17.2
Linoleate	56.4	29.9	45.4	17.0	33.1

[a] Also 0.7% palmitoleate detected. This cottonseed oil sample was different from the starting material used to prepare heated samples, but was from a similar lot.

[b] Since only the volatile fraction can be analyzed by gas chromatographic methods, polymers produced by heating were not represented in the analyses, and values for the volatile fatty esters are high. To correct for this, it was assumed that palmitate was unaffected by the heating process, and other values were corrected by the same factor needed to reduce the palmitate value of the heated oil to the initial value.

cate that all three variables affect deterioration of the oil. Increasing the temperature, length of treatment, or exposure to air (by decreasing sample size), decreased the available energy contents of the treated oils and increased their liver enlarging capacities.

It should be noted in the 50 g series that the liver enlarging capacity leveled off at the two higher temperatures as the heating time progressed from 2–6 hr. However, the available energy content continued to decrease during this same time interval. Since the drop in available energy and the increase in liver enlarging capacity do not occur together, the two effects are apparently not caused by the same material. A similar effect has been reported by Rice et al. (20). Crampton et al. (13) postulated the presence of a relatively harmless indigestible polymeric material in heated fat along with a digestible but relatively more harmful substance.

Experiment No. 4. To determine the influence of oxidation upon nutritive value, a 3 kg quantity of winterized cottonseed oil was maintained at 60C in a 4 liter stainless steel beaker which had been positioned in an oil bath heated by a hot plate. Motor driven stirrers were used in both bath and beaker. Initially, air was pumped at a rate of 450 ml/min through a gas bubbler placed near the bottom of the beaker and close to the blades of the stirrer. Fine bubbles were dispersed in the oil by the stirrer blades. At the end of 10 days, the flow rate of air was doubled with no appreciable change in the rate of peroxide formation. Peroxide values were measured at least once every day.

On the 16th day a change in odor was detected, so a 200 g sample was removed for feeding. On the 17th day there was a large increase in peroxide value. A 200 g sample was removed that day and each day until a maximum peroxide value was reached on the

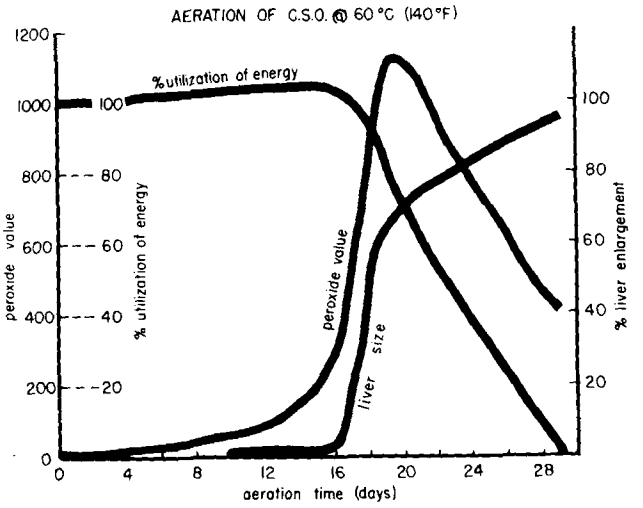

FIG. 1.

19th day. Aeration of the oil was continued until the 29th day, at which time the viscosity was so great that stirring was difficult. All samples were stored in the freezer until fed. The peroxide curve (Figure 1) shows the characteristic induction period followed by a period of rapid oxidation, then gradual decline. Feeding tests indicated that there had not been appreciable change in the available energy or the liver enlarging capacity of the fat at the end of the induction period, although peroxide values had increased to 300 me/kg.

It is significant that the greatest increase in liver enlargement and the greatest drop in available energy occurred in the period during which the peroxide values were increasing rapidly. It should also be noted that liver enlargement values increased and available energy values decreased even after peroxide values had reached a maximum and began to decline.

Discussion

In line with the purpose of this research, temperatures were selected to be similar to those used by food processors. Several investigations have demonstrated harmful effects when fats were treated at temperatures above the smoke point. Such conditions were considered beyond the scope of this paper. However, even if the temperature and heating time used to prepare a laboratory sample are the same as those used to prepare foods, the effects upon the fats should not be expected to be similar. Many other important factors such as degree of aeration and amount of make-up fat added have to be considered.

Experiment No. 1 shows that a fat heated at 182C in a commercial type deep fat fryer required "days" for development of appreciable damage. The pre-

TABLE IV

Relationship of Sample Size, Temperature, and Length of Heating Period to the Nutritive Value of Cottonseed Oil

Heating time	% Liver enlargement per g of fat[a]								
	180C			200C			220C		
	50g	100g	200g	50g	100g	200g	50g	100g	200g
min									
0	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
30	4.9	2.6	−7.8	2.2	−3.8	−1.0	2.7	2.8	1.7
120	21.3	18.3	2.3	35.4	12.8	1.3	42.3	20.2	9.0
360	51.5	38.2	20.6	35.8	37.6	27.1	38.1	47.8	29.2
	Energy utilization, kcal/g of fat[a]								
0	8.93	8.93	8.93	8.93	8.93	8.93	8.93	8.93	8.93
30	9.34	9.52	9.67	8.48	9.37	8.85	8.92	10.05	9.52
120	8.85	8.10	8.47	8.10	8.47	8.62	7.13	8.25	9.52
360	4.50	7.28	8.35	5.48	6.90	8.85	4.35	6.38	7.35

[a] Each value in the table represents the average for four rats.

sumed reason for this slow rate of deterioration is that the depth of the fat allows only a relatively small area to be exposed to the air per unit of fat. If the air exposure had been reduced or eliminated altogether, one would expect the time required to produce nutritional damage to be even longer.

In Experiments No. 2 and 3 the air exposure per unit of fat was increased by regulating the depth of the fat. Striking increases were noted in a matter of "hours" rather than "days." Because of the relatively small amounts of fat heated in these experiments it is only natural that a comparison be made with small cooking operations encountered in the home and small restaurants. However, some important differences should be noted. First of all, frying of food is completed in a matter of minutes; whereas, in the above experiments only moderate changes were noted after two hours. Secondly, the evaporation of water present in most foods lowers the temperature of the surrounding fat. The temperature of meat in a hot oven, even near the surface, never reaches the boiling point of water (15). Finally, the per cent fat exposed to the air is relatively small since most of the fat is beneath the surface of the food.

The two characteristics measured in this research, liver size and growth, are not necessarily affected by the same substance. The possibility that more than one substance is involved was particularly emphasized in Experiments No. 3 and 4, and is in line with the observations of Crampton (13) and Rice (20). The decrease in growth seems to be due to lower digestibility (1) and/or metabolic disturbances while the increase in liver size is presumably caused by a digestible substance.

Peroxide value *per se* does not necessarily correlate with either increased liver size or decreased caloric value. In the first three experiments the temperatures were too high for a buildup of peroxides in the fats; yet, after a sufficient period of time, changes in the two physiological measurements were noted. During the induction period, in Experiment No. 4 (aeration at 60C) no changes in liver size or caloric value were noted, although the peroxide value reached 300 me/kg. Also, after 29 days of aeration, when the peroxide value of the fat had dropped to ⅓ of max, liver sizes remained maximal. Kaunitz also observed increased toxicity in samples aerated past the point of maximum peroxide (21). Andrews (23) fed fractions of aerated soybean oil obtained by extraction with solvents of increasing polarity and noted a correlation of peroxide value with growth. However, Rice (20) fed fractions presumably low in peroxides (i.e., derived from cottonseed oil which had been heated to 360F for 120 hr) and noted lowered growth and increased liver size in the more polar fractions.

In Experiment No. 4, the only period during which the liver enlarging capacity and the peroxide value increased simultaneously was the period of rapid autoxidation. However, even during this oxidative phase it is not clear whether the changes are due to peroxides, breakdown products, polymers, or some other material which was formed simultaneously. While peroxide value does not necessarily correlate with either increased liver size or decreased caloric utilization, the data do not rule out the possibility that peroxides are precursors of the effective substance(s). Also, the evidence tends to emphasize the importance of the overall oxidative process in producing changes of biological importance.

There is no doubt that fats can be abused to the point of being toxic. However, Melnick, Rice and others (2,15,16,18,20) have shown that fats obtained under practical conditions from large food manufacturers, restaurants, bakeries, and home cooking operations show virtually none of the damage so characteristic of severe laboratory treatments. Also, it should be pointed out that histopathological examination of animals fed excessively aerated or heated fats have been negative (2,6,11,22). While it has been adequately demonstrated that heated fats will produce elevated organ weights, it is not clear whether or not this condition is harmful.

TABLE V

Aeration of Cottonseed Salad Oil at 60C

Level fed	Aeration time	Peroxide value	Average weight gain	Available energy per g/fat	Livers	
					% Body weight	Enlargement per g/fat
	days	*me/kg*	*g*	*kcal*		%
0.6 g	0	2	.....	.....	.....	.....
	16	305	17.7	8.25	3.93	−13.8
	17	828	17.3	7.95	4.56	10.3
	18	1030	15.7	6.75	4.97	27.2
	19	1135	14.0	5.40	5.31	41.5
	29[a]	400	5.0	.....	5.81	64.2
1.2 g	0	2	27.0	8.40	3.96	− 4.9
	16	305	26.7	8.25	4.42	0.8
	17	828	25.0	7.35	5.34	18.8
	18	1030	19.3	4.88	6.23	37.3
	19[b]	1135	4.7	.....	6.97	59.3
	29[c]	400	−14	.....	6.71	68.8

[a] One rat died; average of two rats (one rat ate 96% of food; other ate 87%).
[b] No deaths; ate average of 76% food.
[c] Two rats died; remaining rat ate 73% of food.

The appearance of materials in abused fats which do not have full energy value and which produce changes in organ size obviously suggests that the extreme handling conditions conducive to such changes should be avoided in the preparation of food until such time as the significance of these changes can be more fully evaluated. Since at present there is no indication that appreciable quantities of deleterious materials appear in commercial or home processed foods, the data on hand relating to the damage caused by abused fats is more a precaution against the use of unnecessarily severe cooking conditions than an indication of damage occurring under conventional procedures.

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Fatty Acids, Fatty Alcohols, and Wax Esters from *Limnanthes douglasii* (Meadowfoam) Seed Oil

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Abstract

Limanathes douglasii seed oil glycerides contain fatty acids which predominantly (97%) have 20 or more carbon atoms. Fatty acids were prepared by saponification; fatty alcohols, by sodium reduction of the glycerides; and liquid wax esters, by *p*-toluenesulfonic acid-catalyzed reaction of the fatty acids with the fatty alcohols. Solid waxes were prepared by hydrogenation of the glyceride oil and of the wax esters. Chemical and physical constants were determined for *Limnanthes douglasii* seed oil and its derivatives. The liquid wax esters had properties very similar to those of jojoba *(Simmondsia chinensis)* seed oil. The solid hydrogenated wax ester was identical in physical appearance and melting point to hydrogenated jojoba seed oil.

Introduction

Limnanthes douglasii (meadowfoam) (3) is a hardy annual herb native to California and adjoining Pacific Coast States. It is grown as a garden ornamental and has fragrant flowers, about 1 in. across, with white or roseate petals, which are yellowish toward the base. Its seeds are 2–2.5 mm in diameter and 3–5 mm long. The first chemical study (5) of the seeds showed that the extracted oil contained a large amount of long-chain fatty acids, 94% of which are longer than C_{18}. The isolation and chemical characterization of the major C_{20} and C_{22} acids also have been reported earlier (2,13), as well as the amino acid composition of the seed meal (14). These investigations were made as part of a research program to discover new crops that have agricultural and industrial potentials (16).

The present study reports selected chemical and physical properties of oil, fatty acids, fatty alcohols, and wax esters derived from seeds of *Limnanthes douglasii*. Because of their similarity a comparison of chemical composition and physical properties between the wax esters and jojoba oil (4,9) is also presented. Jojoba oil is a liquid ester wax of long-chain fatty acids and fatty alcohols rather than a glyceride fat and is potentially a useful raw material for the chemical and allied industries in such fields as plastics, lubricants, pharmaceuticals, and cosmetics (6,8).

Materials

Limnanthes douglasii seed was obtained from Harry Saier of Diamondale, Michigan. Botanical identity was verified by botanists of the Crops Research Division, USDA, Washington, D.C. The jojoba oil was extracted from *Simmondsia chinensis* nuts acquired from Boyce Thompson Institute, Superior, Arizona.

The bp range of the petroleum ether solvent was 33–57C. Purified ethanol was prepared by refluxing bulk, 95% ethanol with ACS-grade potassium hydroxide, 5 g per liter, for 4 hr, and distilled through a column of glass helices; bp 78C at 760 mm. Ethyl ether, toluene, and xylene were ACS grade. Methyl isobutyl carbinol (4-methyl-3-pentanol) was distilled through a column of glass helices; bp 130–132C at 760 mm.

Preparation of Samples

Oil. Oil was obtained by 60 hr Soxhlet extraction with petroleum ether of 0.5 and 1.0 kg. seed samples, which had been ground in a 6 in. hammer mill. The solvent was removed by bubbling through the solution a stream of nitrogen under reduced pressure (ca. 20 mm) and elevated temperature (heating mantle at ca. 80C) until no change in weight was observed.

Fatty Acids. The triglycerides were saponified by calculating the average molecular weight of the fatty acids from the saponification value of the oil (5) and adding 2 meq of potassium hydroxide and 1 meq of water for every meq of fatty acid. Fifty g of oil was saponified in 300 ml of purified ethanol. After refluxing for 2 hr, 1 l. of water was added, and the unsaponifiables were extracted with ethyl ether. The ether extract was washed with 1*N* K_2CO_3 and distilled water, then dried and weighed. The acids were recovered from the combined washings and soap solution by HCl acidification and ethyl ether extraction. The extract was demineralized by washing with water, and the fatty acids were obtained by removal of ether at reduced pressure and elevated temperature under an atmosphere of nitrogen.

Fatty Acid Methyl Esters. Six g of mixed methyl esters were prepared by reacting the fatty acids with diazomethane (1) for use in gas-liquid chromatography and for physical property measurements.

Fatty Alcohols. Sodium reduction of the triglycerides to fatty alcohols was carried out by following the procedure of Hansley (7). A slight modification was introduced for the hydrolysis of the sodium alkoxide and recovery of the fatty alcohol. The alkoxides (0.63 mole) were hydrolyzed in the original reaction flask by refluxing first with 100 ml of water and then with 1% excess of HCl. The acidulated system was cooled and washed with ethyl ether to extract all the organic compounds. The extract was washed with water and concentrated at ca. 20-mm pressure and ca. 150C pot temperature. The fatty acids present in the concentrate were removed by (a) extraction from ethyl ether with dilute sodium hydroxide (0.5*N*) and (b) adsorption on an activated alumina column with ethyl ether as eluting solvent. The fatty alcohols thus purified were washed with water and concentrated to constant weight at ca 20 mm and 150C.

Wax Esters. Xylene as solvent and *p*-toluenesulfonic acid monohydrate (mp 104–106C) as catalyst were used for the esterification reaction. The weight and mole ratios of fatty acids, fatty alcohols, solvent,

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