residue was saponified, and the fatty acids were recovered by extraction. The less unsaturated acids were removed by crystallization from acetone at —40°C. At this stage the filtrate contained essentially all the arachidonic acid originally present in the glands and also unsaponifiable matter. After the unsaponifiable material was removed, the arachidonic acid content of the concentrate was about 25%. These unsaturated acids were converted to their methyl esters and fractionated on a silicic acid adsorption column. The progress of the adsorption fractionation was followed by spectrophotometric examination and determination of iodine values of the eluted fractions. Methyl arachidonate of 90% purity was obtained by this means. It was further purified by fractional distillation in vacuo. The final product had an iodine value of 316.1; theory, 318.8. The purity of this preparation was further established by spectrophotometric examination, by saponification equivalents, mean molecular weight, and by x-ray diffraction patterns and melting points of a completely hydrogenated portion.

Evidence of acids of greater unsaturation than arachidonic acid in suprarenal lipids was also clearly established by spectrophotometric examination. A fraction was obtained which was estimated to contain about 80-85% of a C_{20} acid with five double bonds.

Acknowledgment

The authors are grateful to Lee Witnauer for making the x-ray diffraction examinations.

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[Received June 27, 1950]

Effects of Heat Treatment on the Stability of Lard[1]

H. J. LIPS, Division of Applied Biology, National Research Laboratories, Ottawa, Canada

ALTHOUGH high temperature treatment of fat usually changes the color of the product (3, p. 291), tends to destroy natural antioxidants (9, p. 163), induces polymerization (3, p. 290), and may produce toxicity (6, p. 43), various observations indicate the possibility of stabilizing fat by heating it with other materials. A number of patents cover the stabilization of glyceride oils by heating them at atmospheric or reduced pressure with a sugar plus one of the following: proteins, amino acids, phosphatides, phosphates, polyhydroxybenzenes, aromatic and aliphatic acids (12). Increased keeping quality has been claimed when certain fats are heated under an inert gas; or heated with spice residues, with finely divided peanuts or soybean flour, with phosphates or phosphatides in the presence of aliphatic acids, or with molasses and inactivated yeast (12). The temperature usually specified is "above 120°C."

A marked improvement in the keeping quality of butter oil resulted from heating whole butter for a short time at 205°C. and from heating the oil with skim milk powder at 205°C. (7). Spray dried milk powders (containing about 26% fat) prepared from milk preheated at 88°C. remained free from talliness about three times as long as powders prepared from milk preheated at 74°C.(13).

It was noted in these laboratories that fats recovered from fried or roasted meats had good resistance to oxidation although they had increased color and strong odor and flavor. "Butcher's" or "country" lard, a dark, high-flavored product with good keeping quality, is made by re-cooking prime steam lard with the cracklings obtained from the manufacture of kettle rendered lard (1, p. 175). Lard rendered in open, directly heated vessels often has excellent stability.

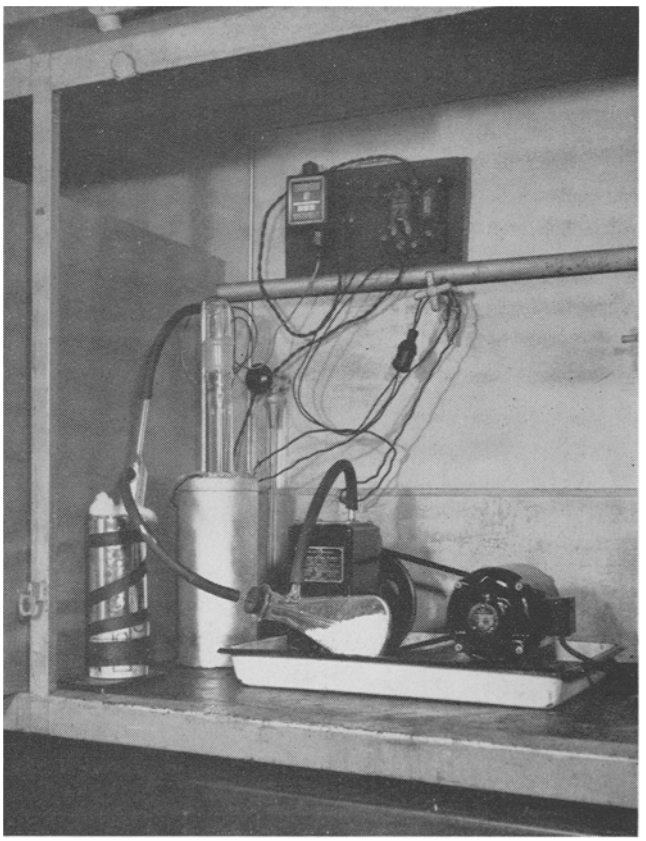

FIG. 1. Apparatus for heating test samples under vacuum.

It is apparent that practical application of this type of stabilization would involve considerations of consumer acceptance and nutritive values. However it was considered necessary first to determine the in-

[1] Issued as Paper No. 254 of the Canadian Committee on Food Preservation and as N. R. C. No. 2299.

crease in stability of fat heated with specific food substances and some of their components, and the degree of stability retained by the heated fat after bleaching and deodorizing. Lard was selected for stabilization experiments as its keeping quality without antioxidant treatment is usually poor (11).

Materials and Methods

The test materials were composites of Canadian wet and dry rendered lards. Substances added to lard in heating trials represented two types: a) products that might be heated with lard in food preparation or processing, b) chemical compounds, the action of which might clarify the nature of the heat stabilizing reaction. Fat hog backs were used in laboratory rendering experiments.

To minimize color changes, polymerization of unsaturated acids, and oxidation, the lard was heated rapidly to a high temperature and maintained at this temperature only long enough to obtain the desired stabilizing effect.

In preliminary trials it was found that the use of oil baths or of dielectric heating was impractical. The heating apparatus finally used consisted of a brass block (drilled to hold a test tube 1.5 in. in diameter), wound with resistance wire and insulated with asbestos and porcelain cement (Fig. 1). To obtain a conveniently rapid rate of heating of samples, the temperature of the block was maintained at 370°C. (±2°C.) by a vapor capsule thermoregulator. The test tubes, fitted with ground glass joints greased with lard, rested at a depth of 5.5 in. in the block, with the ground glass joint 7 in. above the block so that the joint would not become overheated. Lard samples (100 gm.) were dehydrated 30 min. in a boiling water bath, transferred at once to the block, heated for the desired length of time, and cooled to about 75°C. for filtration. The entire treatment was done under vacuum. In control experiments the lard was dehydrated and then filtered without further heating. The filtration removed any added materials that were not fat-soluble. The temperatures attained in this apparatus by 100 gm. of dehydrated lard were as follows: initial, 99°C.; 5 min., 235°C.; 10 min., 280°C.; 15 min., 296°C.

All heating trials were done in duplicate, and the heated fats were stored at 60°C., in 1-oz. bottles (6 ml. in each bottle, one bottle removed at each sampling). Keeping time was determined by peroxide oxygen measurements (5). Measurements of storage life, defined as time in days for the lard to attain a peroxide equivalent of 10 ml. of 0.002N thiosulphate per gm., agreed for duplicate heating trials on the same lard within ±1 day for times of 15 days or less, and within ±2 days for times greater than 15 days.

Nitrogen was determined by the Kjeldahl procedure, and color determinations were made photoelectrically (10) and with F. A. C. standards (2). Measurements of Swift stability (10) and refractive index (2) were made on some samples. Bleaching, deodorizing, and rendering experiments were done in all-glass, laboratory scale apparatus.

Tests on heated lards with the Emmerie-Engel reagent (4) were positive, indicating the presence of reducing substances. However, when four different heat treated lards, each of zero peroxide value, were separately mixed in known proportions with untreated, oxidized lards, the averaged peroxide values remained unchanged. This indicated that, although reducing materials present in heat stabilized lards might retard the formation of peroxides, they would not interfere with the peroxide determination once the fat had begun to oxidize.

Experimental

Whey Powder. In the first trials lard mixed with 1% whey powder was heated in air for 10 min. in an oil bath held at 230°C. Frothing subsided toward the end of the heating period when the material suspended in the fat became charred. The filtered products were dark and had a strong odor. Considerable increase in stability was obtained; Swift stability times up to 50 hr. at 97.8°C. were noted for the heated samples.

When heating trials under vacuum with the brass block apparatus were begun, times of 5, 10, and 15 min. from the moment of insertion of dehydrated samples were chosen, with 1% whey powder as the only addition. Effects of heating alone and of contact between lard and whey powder without high temperature were also determined.

The products heated under vacuum showed a marked increase in color and odor but were better in both respects than those heated in air. Keeping quality results (Table I) showed that heat treatment of lard alone was ineffective. Similarly, no improvement was noted in lard dehydrated with whey powder and filtered without high temperature application. Keeping time of lard heated with 1% whey powder increased with the duration of the high temperature process. The storage life of lard heated 15 min. with whey powder (80 days at 60°C.) is equivalent to approximately two years at 32°C. or about 12 times the average shelf life of Canadian lard at the latter temperature (11).

TABLE I

The Effect of Heat Treatments on the Stability of Lard

Treatment	Storage life, days at 60°C.
None	1
Heated to 235°C.	1
Heated to 280°C.	1
Heated to 296°C.	1
1% whey powder, dehydrated at 100°C. and filtered only	1
1% whey powder, heated to 235°C.	2
1% whey powder, heated to 280°C.	11
1% whey powder, heated to 296°C.	80

The refractive indices for all samples varied only between 1.4517 and 1.4520 at 50°C., an indication that no gross changes in fat composition were caused by the heat treatment.

Other Addends. In view of the stabilizing effect of whey powder, tests were made with whey components: casein, lactose, lecithin, and combinations of these. In addition, dried pork was tested to simulate the effect of heating lard with pork tissue during rendering. Glycine and several protein and carbohydrate materials were also added. A heating period of 12 min. (to 288°C.) and concentrations of 0.1-0.5% were used.

Storage results (Table II) indicated that, in the absence of heating, the only material that appreciably stabilized lard was lecithin, which, being fat-soluble, was not removed from the lard by filtration in the

TABLE II

The Stabilizing Effect of Various Added Materials in Lard Heated to 288°C.

(Average of results obtained with two composite lards)

Material added	Storage life, days at 60°C.	
	Unheated	Heated to 288°C.
Food Products		
0.1% whey powder	1	4
0.2% whey powder	1	4
0.5% whey powder	1	55
0.1% skim milk powder	1	3
0.25% skim milk powder	1	6
0.5% skim milk powder	1	9
0.1% white flour	1	2
0.5% white flour	1	25
0.1% barley malt	1	1
0.5% barley malt	1	11
0.5% dried pork	2	17
0.1% dried egg	1	2
0.5% lecithin	15	30
Nitrogen Compounds		
0.5% acetamide	1	1
0.1% glycine	1	2
0.5% glycine	1	10
0.5% casein	1	18
0.1% gluten	1	3
0.5% Vegamine*	1	3
Carbohydrates		
0.5% mucic acid	1	5
0.1% arabinose	1	2
0.5% arabinose	1	6
0.5% rhamnose	1	8
0.1% dextrose	1	2
0.5% dextrose	1	7
0.5% levulose	1	4
0.1% sucrose	1	1
0.5% lactose	1	22
0.5% starch	1	3
0.5% cellulose	1	2
Mixtures		
0.1%glycine + 0.1% dextrose	1	2
0.25% glycine + 0.25% dextrose	1	9
0.1% casein + 0.1% dextrose	1	3
0.25% casein + 0.25% lactose	1	12
0.17% casein + 0.17% lactose + 0.17%lecithin	7	29

* Commercial mixture of amino acids.

control experiment. With heat treatment, whey powder was the most effective addend. There was no apparent relation between stabilizing activity and nitrogen content; keeping time was increased by heating lard with carbohydrate alone. The stabilization obtained with the more complex materials, and with combinations of simpler substances, suggest that synergistic action was responsible for the more striking results obtained. It is also possible that some of the heat treatments may have removed pro-oxidant materials from the lard by adsorption or by inactivation in other ways. The stabilizing effect generally increased with concentration of the addend, but the sharp increase in storage life from 4 to 55 days with an increase in concentration from 0.2 to 0.5% whey powder may constitute an anomaly.

Table III shows examples of the increase in color (decrease in light transmission) for some of the heated lards.

Furfural and Nitrogen Ring Compounds. In the heated lards, formation of furfural compounds by dehydration of sugars and other substances, or of nitrogen ring compounds by cyclization of protein material, was possible (8, pp. 224-225, 693-969).

Some increase in nonfilterable nitrogen was found in lard heated with nitrogenous materials. Six Canadian lards showed an approximate nitrogen content of less than 0.002%. Lard rendered at 225°C. however had a nitrogen content of 0.013%, and lard heated in the block apparatus with casein for 12 min. contained 0.016% nitrogen.

The results of tests on one of the experimental lards with 0.1% of several furfural and nitrogen ring compounds added without heating are given in Table IV. Two commercial antioxidants, nordihydroguaiaretic acid and G-4, were included for comparison. Although the compounds which may dehydrate to furfurals (mucic acid and the pentoses, Table II) were effective on heating, the furfural derivatives themselves had no antioxidant activity. The nitrogen compounds were likewise ineffective.

Rendering. It was considered that a stable lard might result from the use of high rendering temperatures. A number of fat hog backs, with the skin on, were ground with about 1% of raw lean and bone. The material was mixed, weighed (2 kg.) into 5,000 ml. round-bottomed flasks with ground glass joints,

TABLE III

Effect on the Color of Lard of Heating to 288°C. Under Vacuum With Various Materials

Material added to lard	Light transmission of lard, % at 60°C., relative to mineral oil	
	at 440 mμ.	at 660 mμ.
None	68	98
Food Products		
0.1% whey powder	45	94
0.2% whey powder	37	92
0.5% whey powder	22	93
0.1% skim milk powder	58	97
0.25% skim milk powder	47	96
0.5% skim milk powder	17	85
0.1% white flour	37	94
0.5% white flour	25	87
0.1% barley malt	41	92
0.5% barley malt	46	100
0.1% dried egg	30	94
Nitrogen Compounds		
0.5% acetamide	35	91
0.1% glycine	36	94
0.5% glycine	35	96
Carbohydrates		
0.1% arabinose	21	83
0.1% dextrose	25	88
0.5% levulose	12	73
0.1% sucrose	22	84
0.5% starch	47	92
0.5% cellulose	58	90
Mixtures		
0.1% glycine + 0.1% dextrose	26	89
0.25% glycine + 0.25% dextrose	20	89
0.1% casein + 0.1% dextrose	15	84

TABLE IV

The Effect of Various Added Materials on Lard Stability

Material added	Storage life, days at 60°C.
None	1
0.01% nordihydroguaiaretic acid	56
0.1% G-4*	17
0.1% furfuryl alcohol	1
0.1% furoin	1
0.1% tetra hydro furfuryl alcohol	2
0.1% tetra hydro furfuryl lactate	1
0.1% pyrrole	1
0.1% indole butyric acid	2
0.1% o-phenanthroline	1

* Contains corn oil, lecithin, and propyl gallate.

TABLE V

Effect of Rendering Conditions on the Stability of Lard

Treatment	Storage life, days at 60°C.
Control, rendered at 100°C	2
Rendered to final temperature, 115°C	2
Rendered to 150°C., with 1% whey powder	5
Rendered to 170°C	5
Rendered to 225°C	6
Rendered to 225°C., then simultaneously bleached and deodorized one hour at 200°C	2

and heated under vacuum with a Glas-Col mantle until the contents of the flasks reached the desired temperatures (1.5-2 hr.). Rendering in a boiling water bath for 2 hr. was used as a control condition. All mixtures were filtered under nitrogen. Added stability was obtained by the use of high rendering temperatures (Table V), but increased color and odor of the lard were apparent.

Bleaching and Deodorizing. It was found that heated samples with strong odor and color between F. A. C. standards 2 and 3 were bland and light-colored (F. A. C. less than 1) after one hour's deodorization at 200°C. in contact with 1% bleaching clay, and subsequent filtration with the addition of 1% diatomaceous earth. Heat stabilized lard retained some degree of stabilization after this bleaching and deodorizing procedure (Table VI). Simultaneous heat treatment and deodorization gave a bland lard of fair stability but dark color.

Discussion

The foregoing results raise doubts as to the usefulness of some of the patented processes for stabilizing fats by heat treatments. The keeping quality of the most stable lard (heat treated with whey powder) after necessary bleaching and deodorizing, although considerably greater than that of the control material, was appreciably less than that obtained with the commercial antioxidants tested (cf. Tables IV and VI).

Although the direct application of heat treatment to lard stabilization appeared to be impractical, the observations are of wider interest since edible fats are heated extensively with other materials during extraction or rendering and when used in the preparation of foods. The temperatures attained in such processes are generally not as high as the ones reached in these experiments, and the materials usually contain considerable moisture. However the tendency for the natural antioxidants in the heated fats to be destroyed may be offset to some extent by stabilization resulting from heating in contact with protein and (or) carbohydrate material. When heating conditions are severe this effect could assume greater importance, but it might be accompanied by undesirable changes in palatability and nutritive value.

TABLE VI

The Effect of Bleaching and Deodorizing on the Stability of Heat Treated Lard

Treatment	Storage life, days at 60°C.
No heat treatment, no deodorization	1
No heat treatment, deodorized one hour at 200°C	1
1% whey powder, heated to 296°C., no deodorization	80
1% whey powder, heated to 296°C., then simultaneously bleached and deodorized one hour at 200°C	8
0.5% whey powder, simultaneously heated and deodorized one hour at 225°C	1
0.5% whey powder, simultaneously heated and deodorized one hour at 250°C	6
0.5% whey powder, simultaneously heated and deodorized one hour at 296°C	11

Summary

The storage life of lard was increased by heating it under vacuum to 288-296°C. with 0.1 to 0.5% of various protein and carbohydrate materials (dried whey was the most effective substance used), but undesirable color and odor were produced in the lard by the process, and bleaching and deodorizing to a bland, light colored end-product resulted in a loss of most of the added stability.

Acknowledgments

Appreciation is expressed to A. C. Bell for technical assistance and to the N. R. C. Radio and Electrical Engineering Laboratories for dielectric heating trials.

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[Received June 30, 1950]

Evaluation of the Twitchell Isooleic Method: Comparison With the Infrared Trans-Isooleic Method[1]

FRANK L. JACKSON and JOSEPH E. CALLEN, Procter and Gamble Company, Ivorydale, Ohio

TWITCHELL (1), in 1921, proposed a lead salt-alcohol method for the separation of solid and liquid acids. His method was an important improvement over lead salt-ether method first suggested by Gusserow (2) in 1828 and subsequently examined by numerous other investigators. Twitchell clearly pointed out that the iodine value of the twice crystallized solid fatty acid (SFA) fraction represented "isooleic acids," a term which broadly includes all the unsaturated acids appearing in the solid fatty acid fraction and which is most often applied to the unsaturated solid acids of hydrogenated fats (3). Cooperative investigation of the Twitchell method by a group of A.O.C.S. collaborators (4) led to its adoption as the A.O.C.S. official method (5) for determining solid and liquid unsaturated acids and incidentally isooleic acids.

The Twitchell method has been found satisfactory for determining saturated solid and total unsaturated fatty acids and partially satisfactory for determining the unsaturated solid acids in fats and oils. However in order to understand clearly the chemical nature

[1] Presented at the spring meeting of the American Oil Chemists' Society, Atlanta, Ga., May 1-3, 1950.