eight locations was 662, 344, and 163 pounds per acre, respectively.

The chemical composition of the seed was found to be well within the acceptable range but was markedly influenced both by the varieties and by the locations where they were grown. The average oil and protein contents on a moisture-free basis were 53.53% and 26.25%, respectively. The average iodine value of the oil was 110.0. Average protein content on the moisture- and oil-free basis was 57.28%.

The genetic and environmental influences that affect protein content of the seed also inversely affected the oil content. It was suggested that in sesame protein synthesis is favored over oil synthesis as the nitrogen supply to the seed increases.

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Behavior of Antioxidants During the Baking and Storage of Pie Crust

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A previous study on the behavior of propyl gallate and butylated hydroxyanisole in lard stored at 61°C. (6) has been extended to include a study of these and other antioxidants in pie crust stored at 61°C. Previous work in this field has been conducted by means of static experiments, that is, the time, under given conditions, for the baked material to develop organoleptic rancidity or for the fat fraction to attain a given peroxide value.

The present study involved the addition of a number of antioxidant combinations to lard employed in the preparation of pie crust. The keeping quality of the pie crust and the content of added antioxidants were determined periodically by extracting the fat from a portion of the pie crust and analyzing it for the added antioxidants and for peroxide value.

Preparation and Storage of Pie Crust

Mixing of Dough. A dough consisting of 100 parts flour, 60 parts lard, and 50 parts water was employed. Commercial pastry flour (not enriched), fresh steam-rendered lard and distilled water were used. No sodium chloride was added because it has been demonstrated that this material may function as a prooxidant due to the traces of other metals it may contain (1).

The antioxidants and/or acidic synergists were dissolved in a few drops of propylene glycol and thoroughly mixed into the lard at 45°C. The lard was cooled rapidly to 18°C. and employed in the preparation of dough.

The flour and lard were cooled to approximately 18°C., and the lard was creamed with half of the flour. The remainder of the flour was then mixed with the creamed portion, followed by the water, also at 18°C., which was mixed in lightly.

Baking of Pie Crust. The dough was spread, ¼ in. thick in rectangular aluminum trays (11 in. x 10 in. x ⅜ in.) and baked in a 205°C. oven until lightly browned. To avoid scorching of the pie crust near the edge of the trays, the turned-up edge was only ⅜ in. high because this edge served as an additional surface for heat absorption. Also the layer of dough was slightly thicker near the edge of the tray in order to utilize this additional heat input. In order to obtain reliable keeping times, uniformly baked pie crust is of the utmost importance.

Storage of Pie Crust. The pie crust was stored in a 61°C. oven. At intervals the fat from a portion of the pie crust was extracted and analyzed for added antioxidants and peroxide value.

Analytical Methods

Reagents. Light Petroleum (34-38°C.). Shake Skellysolve A (28-38°C.) with $\frac{1}{10}$ of its volume of concentrated sulphuric acid for 2 to 3 minutes. Run off the acid layer and wash the light petroleum with water and dilute alkali until free of acid. Distill through an all-glass fractionating column and collect the 34 to 38°C. fraction.

Light Petroleum (60-100°C.). Distill Skellysolve H in all-glass apparatus.

Acetone. Distill in all-glass apparatus.

Sodium Carbonate. Prepare a 1% solution of anhydrous sodium carbonate in water.

Extraction of Fat from Dough or Pie Crust. Place 55 g. of dough or 45 g. of pie crust into a Waring Blendor. Add 150 ml. of 34 to 38°C. light petroleum, cover, and blend for 30 seconds. Transfer the contents of the Blendor to a 250-ml. centrifuge bottle, stopper, and centrifuge at 1,000 r.p.m. for 5 minutes. Decant the slightly turbid light petroleum phase into a double 15-cm. Whatman No. 54 filter and collect the filtrate in a 250-ml. flask. Remove the light petroleum under reduced pressure, employing a water pump while warming the flask in a 40°C. water bath. Remove the last traces of light petroleum with a vacuum pump. This extraction procedure recovered approximately 90% of the fat in dough and 85% of that in pie crust.

Analysis of Antioxidants. The extracted fat was analyzed for one or more of the following antioxidants, propyl gallate, total butylated hydroxyanisole (BHA), 2-tert-butyl-4-hydroxyanisole (2-BHA), 3-

tert-butyl-4-hydroxyanisole (3-BHA), and nordihydroguaiaretic acid (NDGA) as previously described (3, 4). The reagents for these analytical procedures are not included in this paper. Lauryl gallate was estimated by the procedure outlined hereafter. Peroxide values were determined employing Lea's "hot method" (2).

Quantitative Analysis of Lauryl Gallate in Fat. The following procedure was employed for the determination of lauryl gallate. Dissolve 10 g. of extracted fat in 55 ml. of light petroleum (60-100°C.) and 55 ml. of acetone. Place three aliquots of this solution ranging from 3 to 30 ml. into 20 x 150 mm. test tubes and dilute to 30 ml. with a a similar lard solution containing no lauryl gallate. Add 10 ml. of 1.0% aqueous sodium carbonate, stopper, and invert the test tube twice. After one minute remove the upper light petroleum layer with a suction tube. Filter the aqueous acetone through a 9-cm. Whatman No. 42 filter and collect the filtrate in a colorimeter tube. Six minutes after adding the sodium carbonate reagent, measure the absorbency with an Evelyn colorimeter fitted with a No. 515 filter, relative to a "blank" employing 30 ml. of a solution of lard in light petroleum in place of the fat-extract solution. Under these conditions the observed absorbency at 515 mμ divided by a "K-Value" of 0.00154 yielded the concentration of lauryl gallate expressed as micrograms per aliquot employed.

Recovery of Antioxidants from Dough and Pie Crust

Propyl Gallate. It was found impossible to recover any of the propyl gallate added to dough. However lard containing 0.006% of propyl gallate, upon being blended with light petroleum, centrifuged, filtered, and the light petroleum evaporated, yielded as much as 98% of the added propyl gallate. Therefore the procedure for extracting the fat from the sample occasioned no serious loss of propyl gallate.

The use of autoclaved flour also resulted in zero recoveries of propyl gallate from dough, thus enzymatic activity cannot be responsible for the destruction of propyl gallate. The use of flours not subjected to any oxidizing "improvers," such as chlorine dioxide, also resulted in the complete loss of added propyl gallate.

Since propyl gallate reacts with metallic ions, such as iron, it was suspected that the antioxidant was being oxidized or complexed by the metallic ions present in flour. In an attempt to prove this point, flour was fractionated into four crude fractions and each fraction was employed in the preparation of dough, using lard containing 0.006% of propyl gallate. The partial analysis of these flour fractions and the resulting recoveries of propyl gallate from the doughs are given in Table I.

TABLE I

Recovery of Propyl Gallate Added to Dough Made With Flour and Four Flour Fractions

Type of flour fraction	Starch, %	Protein, %	Ash, %	Propyl gallate, % recovered
Starchy fraction 1	93.5	1.7	0.1	94
Starchy fraction 2	93.0	4.5	0.2	80
Original flour	72.0	10.8	0.4	0
Residual fraction	27.3	42.5	16.6	0
Proteinaceous fraction	2.8	92.7	2.0	0

Data in Table I indicate that high recoveries of propyl gallate were possible when predominately starchy flour fractions were employed. However as the proportion of protein and ash in the flour fractions increased, the recovery of propyl gallate rapidly decreased to zero. These results indicated that propyl gallate was destroyed or complexed by some component of flour. In this connection Sair and Hall (6) observed that some factor in flour plays an important role in the carry-through of antioxidants. These authors considered that the metallic content of flour might be responsible but were unable to confirm this assumption since the addition of metallic deactivators did not increase the keeping quality of the pie crust.

In the case of pie crust made from dough containing added citric acid or disodium dihydrogen ethylenediamine tetraacetate ("Versene"), the addition of propyl gallate as a spray in a lard solution resulted in recoveries of 3 to 10% of added propyl gallate. However after a few hours at room temperature no propyl gallate could be recovered from the pie crust. These results are in agreement with the views of Sair and Hall (6). Nevertheless the role of metallic ions in flour should not be minimized because in most cases the metallic deactivators employed are inadequate to complex all the metallic ions present. Also the small amount of propyl gallate added (approximately 0.003% of the flour) could be complexed by a small fraction of the metallic content of flour.

Lauryl Gallate. Lauryl gallate recoveries of 30% from dough and 20% from freshly baked pie crust have been obtained. The recovery of lauryl gallate sprayed onto pie crust containing "Versene" was 64% after approximately four hours at room temperature. In this case "Versene" was employed at 0.28% of the flour by weight. These results indicated that if a sufficient amount of a powerful metal deactivator was employed, the loss of added antioxidant could be reduced and the keeping quality of the pie crust increased (see Table II).

TABLE II

Effect of Different Antioxidant and Synergistic Combinations Upon the Time Required for the Fat of Pie Crust Held at 61°C. to Attain a Peroxide Value of 60

Material added to lard		Time at 61°C. to attain a peroxide value of 60
		days
Control		4
CA	Acidic synergists	5
H_3PO_4+Glycine	Acidic synergists	5
"Versene"	Acidic synergists	7
High CA	Acidic synergists	7
NDGA+CA		7
PG+CA		7
CA (Baked)+PG (sprayed on pie crust)		7
High CA (Baked)+PG (sprayed on pie crust)		7
LG+CA		13
"Versene" (baked)+PG and CA (sprayed on pie crust)		15
"Versene" (baked)+LG and CA (sprayed on pie crust)		18
BHA+NDGA+CA		21
BHA+High CA		22
BHA+PG+CA		23
BHA+LG+CA		24
BHA+CA		25

Key to table

Additive	Additives expressed as percentage of the lard employed
CA	0.004 % anhydrous citric acid
High CA	0.460 % anhydrous citric acid
H_3PO_4	0.460 % phosphoric acid
Glycine	0.040 % α-amino acetic acid
"Versene"	0.460 % disodium dihydrogen ethylenediamine tetraacetate
PG	0.006 % propyl gallate
LG	0.0095% lauryl gallate
NDGA	0.010 % Nordihydroguaiaretic acid
BHA	0.008 % 2-tertiarybutyl-4-hydroxyanisole; 0.012 % 3-tertiarybutyl-4-hydroxyanisole

The greater stability of lauryl gallate (0.0095% of the lard) in dough as compared to propyl gallate employed at the same molecular concentration was explained on the basis that lauryl gallate is insoluble in water while being soluble in fat. These properties should restrict the lauryl gallate to the lipid portion of the dough, thus avoiding such direct contact with the metallic ions of flour which should be concentrated in the aqueous phase. Propyl gallate, on the other hand, is slightly water-soluble and would diffuse into the aqueous phase and be complexed by the metallic ions.

Nordihydroguaiaretic Acid (NDGA). The apparent recovery of NDGA from dough was of the order of 2 to 6% of that added while no NDGA was recovered from pie crust. The loss of NDGA added to dough was attributed to the same factors responsible for the loss of propyl gallate.

Butylated Hydroxyanisole (BHA). Recoveries of BHA from dough have been as high as 98% though in most instances recoveries 80 to 92% were obtained. In the case of pie crust the time of baking normally employed resulted in a loss of 15 to 20% of the BHA present. The effect of time of baking at 205°C. on the loss of BHA was investigated with the results given in Figure 1.

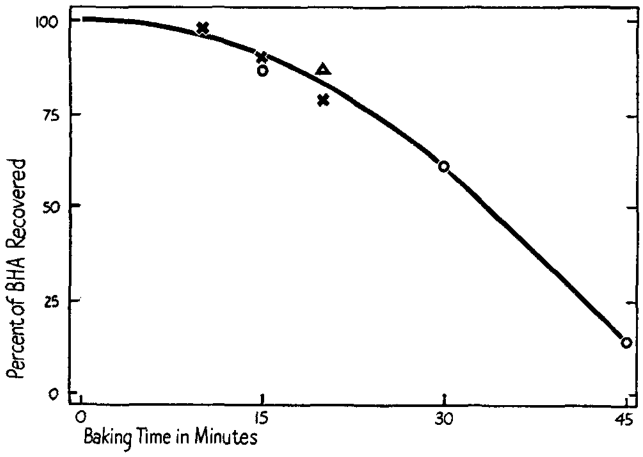

FIG. 1. Effect of baking time at 205°C. on recovery of BHA from pie crust.

Data in Figure 1 indicate that the loss of BHA proceeds at an ever-increasing rate during the baking process. The loss of BHA was due at least in part to the steam-distillation of BHA from the dough.

Recovery of BHA from Oven Vapors

A series of three experiments were conducted with the object of recovering the BHA vaporized during the baking of pie crust. Air was drawn through a 205°C. oven containing 700 g. of dough, made with lard containing 0.02% BHA, at a rate of approximately 620 ml. per second. On leaving the oven, the air and oven vapors were passed through a series of two straight condensers and one spiral condenser, cooled to 4°C. by a rapid stream of water. The condensate amounted to 80 to 86% of the weight loss during the baking of the dough. Of the BHA lost during the baking, 55 to 80% was recovered in the condensate. If the amount of BHA actually present in the condensate is calculated on the basis of the total weight lost during the baking of the pie crust, then from 64 to 85% of the BHA lost during baking was in the oven vapors. Therefore it can safely be said that the greater portion of the BHA lost during the baking of pie crust is due to vaporization.

Recovery of BHA Vaporized from Pie Crust Stored at 61°C. In order to estimate the vaporization of BHA during the storage of pie crust, lard containing 0.02% BHA was used to make pie crust, and 640 g. of this pie crust was placed in a large jar held at 61°C. A slow stream of nitrogen (10 ml. per min.) was passed through this jar and then through a series of six traps filled with absolute ethyl alcohol. After a period of 91 hours the remaining alcohol in the traps was analyzed, employing the 2,6-dichloroquinonechloroimide-borax reagents, and found to contain 0.7 mg. of BHA or approximately 2% of the BHA added to the pie crust. Since this reagent couples with BHA to produce a blue complex, errors due to other reducing substances are avoided. Due to the large volume of nitrogen employed and the partial evaporization of the alcohol in the traps it is considered significant that so much BHA was recovered from the traps.

It is important to note that whereas the loss of BHA during the baking of pie crust is normal and unavoidable, the loss of BHA by vaporization from pie crust stored at 61°C. would not be encountered under normal storage conditions at room temperature or under refrigeration.

Pie Crust Storage Experiments

A number of doughs were prepared, employing lard to which various phenolic antioxidants and/or acidic synergists had been added. The phenolic antioxidants employed included propyl gallate, lauryl gallate, NDGA, and BHA while the acidic synergists included citric acid, phosphoric acid, α-amino acetic acid (glycine) and disodium, dihydrogen ethylenediamine tetraacetate ("Versene"). When phenolic antioxidants were added, the fat in a portion of the dough was extracted and analyzed for the added antioxidants, prior to the baking processes. The dough was then baked as previously described, the fat from a portion of the fresh pie crust was extracted and analyzed for antioxidant content and peroxide value. The remainder of the pie crust was stored at 61°C. for further analyses. Samples containing acidic synergists but no phenolic antioxidants were analyzed for peroxide value only.

Results of Storage Experiments. For the purpose of comparison the data on peroxide values were summarized and expressed in terms of the time required at 61°C. for the fat in the pie crust to attain a peroxide value of 60 milliequivalents per kilogram of fat. These results are given in Table II.

Data in Table II indicate that the presence of acidic synergists alone resulted in little improvement in the keeping quality of pie crust stored at 61°C. The addition of propyl gallate or NDGA in the presence of citric acid exerted no additional effect, thereby indicating that these antioxidants were rendered chemically inactive upon addition to the dough. Lauryl gallate, on the other hand, produced a significant improvement in the keeping time of pie crust. This is attributed to the fact that lauryl gallate is insoluble in water while being fat-soluble. These properties should tend to keep the lauryl gallate in the fat phase, thereby avoiding direct contact with the metallic ions which are presumably concentrated in the aqueous phase. For these reasons lauryl gallate might avoid being oxidized or complexed by the metallic ions. The addition of "Versene" to the dough followed by the spraying of the resulting pie crust with propyl gallate and citric acid or lauryl gallate and citric acid resulted in a significant improvement in the stability of pie crust. This fact indicates that the relatively large amount of "Versene" employed was able to complex a large portion of the metallic ions present, thus avoiding their destructive action on the propyl gallate or lauryl gallate added subsequently.

The most stable pie crusts were those containing BHA and citric acid. In all cases the BHA employed was a mixture of 60% pure 3-BHA and 40% of pure 2-BHA. The use of propyl gallate plus BHA plus citric acid, NDGA plus BHA plus citric acid, lauryl gallate plus BHA plus citric acid, or the use of additional citric acid in the presence of BHA resulted in no further increase in stability. Since the addition of BHA plus citric acid or lauryl gallate plus citric acid resulted in greatly increased stability in the resulting pie crust it is surprising to find that the addition of lauryl gallate plus BHA plus citric acid did not produce greater stability than the addition of BHA plus citric acid.

Figures 2, 3, 4, 5, and 6 illustrate the loss of BHA in the presence of citric acid and other antioxidants. In all cases there was approximately 15% loss of BHA during the baking process. Thereafter the loss of total BHA, 2-BHA, and 3-BHA approximated a straight line function of the time of storage at 61°C. In the case of doughs containing propyl gallate plus BHA plus citric acid, or NDGA plus BHA plus citric acid, no propyl gallate or NDGA was found in the pie crust, and therefore no curves for the loss of these antioxidants are given in Figures 4 and 5. It is important to note that in all cases the peroxide value began to increase rapidly while a significant amount of the added BHA still remained in the pie crust. Peroxide values as high as 280 milliequivalents per kg. of fat were recorded in the presence of detectable quantities of BHA.

The curves for the loss of lauryl gallate plus BHA in the presence of citric acid are given in Figure 6. The loss of both lauryl gallate and BHA followed straight-line functions of time. In this case it was

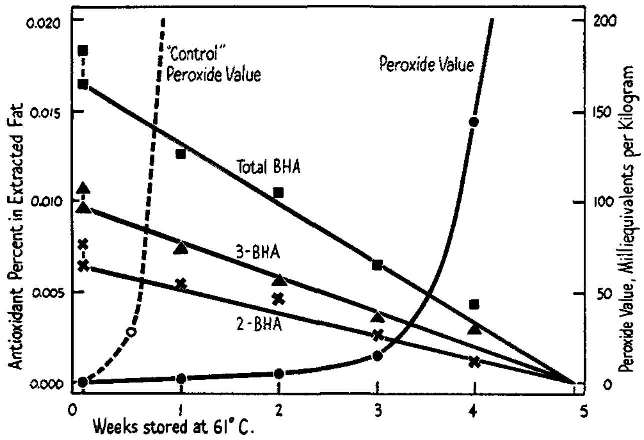

FIG. 2. Loss of BHA from pie crust containing BHA and citric acid.

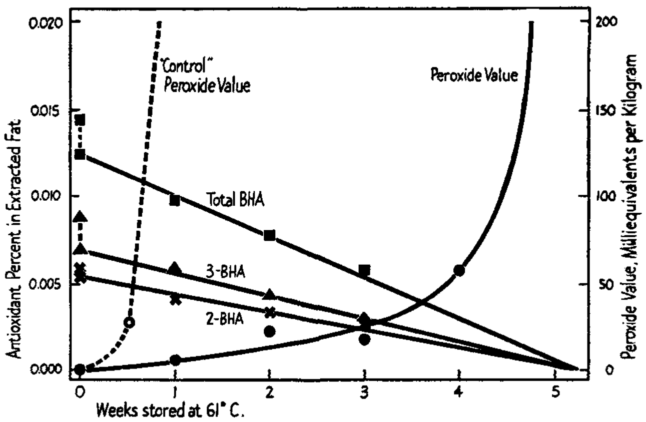

FIG. 3. Loss of BHA from pie crust containing BHA and high citric acid.

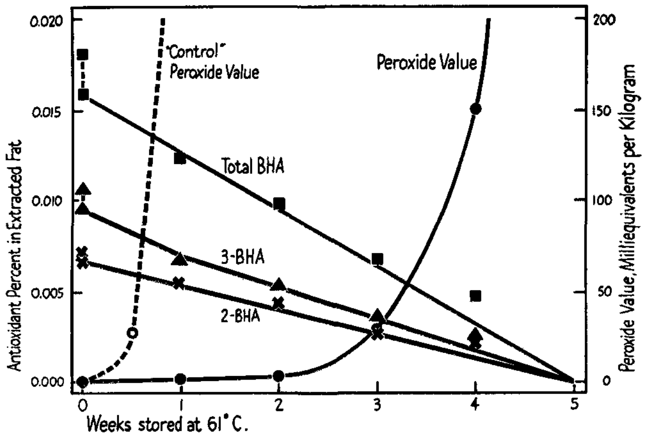

FIG. 4. Loss of BHA from pie crust containing propyl gallate, BHA and citric acid.

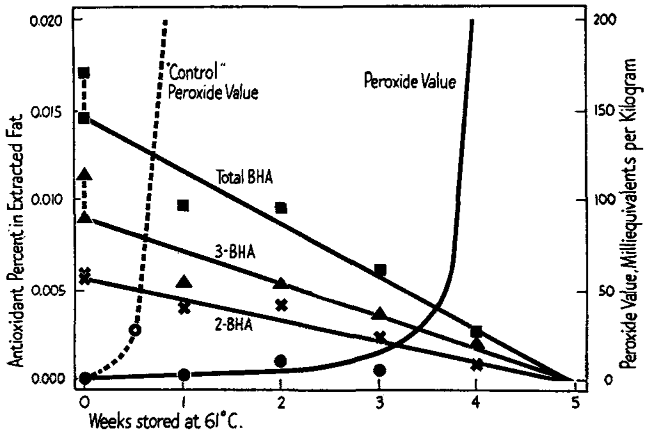

FIG. 5. Loss of BHA from pie crust containing NDGA, BHA and citric acid.

impossible to estimate the amount of 2-BHA and 3-BHA present since the analytical method is not applicable in the presence of lauryl gallate. Here again appreciable peroxide values were obtained before all the BHA was lost.

The curve for the loss of lauryl gallate in the presence of citric acid is given in Figure 7 and is approximately a straight-line function of time. A high peroxide value was attained before all the lauryl gallate was lost. In the case of pie crust made from

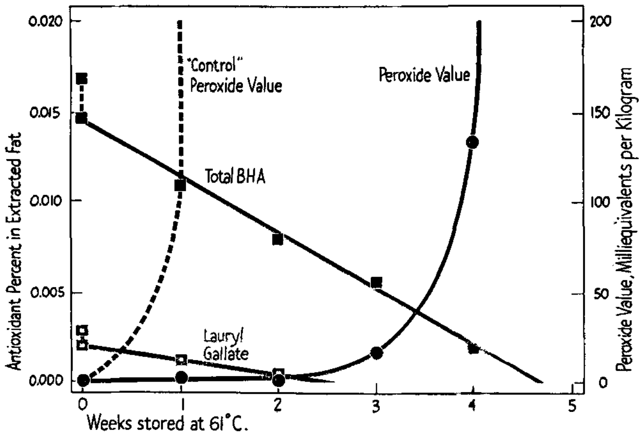

FIG. 6. Loss of BHA and lauryl gallate from pie crust containing lauryl gallate, BHA and citric acid.

dough containing "Versene" and then sprayed with lauryl gallate plus citric acid, the loss of lauryl gallate approximated a straight-line function of time as shown in Figure 8.

Pie crust containing "Versene" and then sprayed with propyl gallate plus citric acid contained 10% of the added propyl gallate after a few hours at room temperature. However after 7 days of storage at 61° C. no propyl gallate was found, therefore no curve for the loss of propyl gallate was obtained.

Summary

1. Acidic synergists such as citric acid, phosphoric acid, α-amino acetic acid (glycine), or disodium dihydrogen ethylenediamine tetraacetate ("Versene") produced no significant improvement in the keeping quality of pie crust.

2. Propyl gallate plus citric acid, or NDGA plus citric acid, produced no significant effect on the keeping quality of pie crust owing to the fact that propyl gallate and NDGA are apparently destroyed in dough made with flour.

3. Lauryl gallate plus citric acid produced a significant improvement in the keeping quality of pie crust.

4. The addition of "Versene" to dough, followed by spraying the pie crust with a solution of lauryl gallate plus citric acid or propyl gallate plus citric acid, resulted in an increase in stability.

5. The addition of BHA plus citric acid to dough resulted in the greatest stability of the pie crust. However the addition of propyl gallate, lauryl gallate or NDGA to this dough produced no further increase in the stability of the pie crust.

6. BHA is partly vaporized from dough during the baking process and also during the storage of pie crust at 61°C.

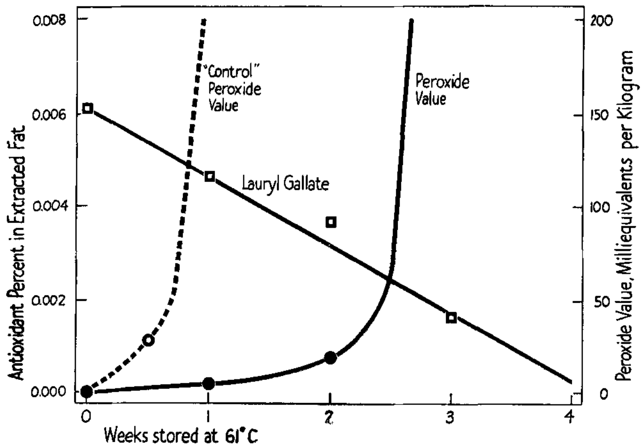

FIG. 7. Loss of lauryl gallate from pie crust. Pie crust baked with added Versene and then sprayed with lauryl gallate and citric acid.

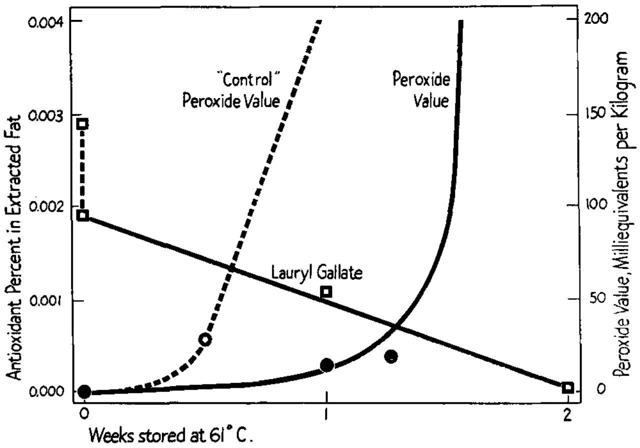

FIG. 8. Loss of lauryl gallate from pie crust containing lauryl gallate and citric acid.

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