THE ANTIOXIDANT EFFECT OF EDIBLE
FLORES DERIVED FROM OIL PRESS
CAKES IN CERTAIN FAT-CONTAINING
FOOD MIXTURES

by

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The problem of rancidity in fats and in stored foods containing fat is of major importance in the food industry and in the home.

In the investigation of factors that may influence the development of rancidity, much work has been done on the use of antioxidant materials. These studies have dealt most frequently with the effect of antioxidant substances when added to the fat itself.

Such materials act to inhibit oxidation of the fat. In this group of substances are found some naturally occurring materials, as gum guaiac or wheat germ concentrate, and many chemical compounds.

Though much attention has been given to the problem of fat rancidity and many materials have been studied as to their antioxidant effect, very little work has been done on the inhibition of rancidity in prepared food mixes and in cooked food products.

Baked foods present special problems since rancidity develops very rapidly in the fat present in such foods.
The present study was primarily concerned with the effect on fat rancidity of several oil press cakes which were incorporated in food mixes and in baked food products. The press cakes studied were peanut, soy and cotton. If it could be demonstrated that oil press cakes, usually regarded as simple by-products of the oil manufacturing industry, are effective inhibitors of fat rancidity, both in prepared mixes and in baked foods, then a new and valuable use would be suggested for these materials.

A second purpose of this study was to collect information regarding the method to be used for accelerating the development of rancidity in the food mixtures.

**HISTORICAL BACKGROUND**

Although the Soybean has been used by Oriental races as food for centuries, its introduction to Americans did not occur until 1804. At first it was used only as a food for cattle. During the first World War the oil extracted from soybeans was used by homemakers as a source of fat. In the second World War soybeans found widespread use as human food. Soy flour was used for fortifying various cereal products, cakes, breads, muffins, soups, and other products used by the army. (Lager, 1945) (26).
The first attempts to produce a satisfactory flour from soybeans met with failure. These methods either yielded a product high in fat which became rancid very rapidly, or the quality of the bean had been destroyed during processing. The first successful soybean flour was produced by Dr. Laszlo Bercze1lar of Vienna. This flour was pleasant in flavor, the nutritive value and good qualities were preserved and it was found to have excellent keeping qualities. Since this time Germany has done more than any other country in developing methods of manufacturing and using soybean flour. (Ferree, 1929; Lager, 1945) (11; 28).

The introduction of Cottonseed meal as human food was in 1876. It was not widely used except in special diets where a low starch content was needed. The presence of the toxic substance, gossypol, made it necessary to develop a method of processing in which the press cake could be used safely for stock feeding and as flour for human food. (McMath, 1940) (31).

Peanuts have been grown in America since colonial days, but only became of commercial importance about 60 years ago. From the beginning of the peanut crushing industry the press cake has been accepted as an excellent protein supplement in the feeding of farm animals.
Only in recent years has the excellence of the peanut press cake been established as human food. Early attempts to produce an acceptable flour product met with failure due to the lack of proper equipment and techniques. In 1939 the cottonseed oil-milling company which had successfully overcome the same difficulties in the production of cottonseed flour undertook the development of an acceptable peanut flour. (Payne, 1942; Ascham, 1939) (38; 1). Peanut flour is used principally in combination with wheat flour in muffins, breads, and cookies, and in diabetic diets as a means of reducing the carbohydrate value and improving the protein content. (Ascham, 1936) (1).

Soybean and peanut flours have been found to confer desirable characteristics upon certain cereal products. Johns and Finks (1921) (21); Stellar and Bailey (1938) (40); Bohn and Favor (1945) (6) found that the use of 5 per cent to 20 per cent soybean flour in bread dough increased the absorption quality of the wheat flour and produced a much slower rate of staling of the bread.

Johns and Finks (1920) (20) added peanut flour to bread and produced a palatable loaf rich in water soluble vitamins, and containing a protein mixture adequate for normal growth in rats. Bread made with a 40 per cent proportion of peanut flour yielded a loaf with a slight
decrease in whiteness of the crumb and small decrease in volume. Cookies, biscuits, muffins, griddle cakes, and waffles gave satisfactory products using 50 per cent peanut flour. (Grewe, 1945; National Peanut Council, 1942) (16; 35).

**METHOD OF MANUFACTURING FLOURS**

Soy, peanut, and cottonseed flours are by-products of oil manufacture. Certain precautions are essential for the production of edible flours that would be unnecessary if oil manufacture were the only consideration.

The methods used for the manufacture of edible press cakes agree in general for the different seeds, but may differ slightly in details. The method of manufacturing cottonseed flour may be easily adapted to soybean and peanut. (McMath, 1940; Burlison, 1936) (31; 7). The steps in the process are:

1. Clean, especially selected seeds are used.
2. Hulls and kernels are separated.
3. Kernels are rolled, then cooked at temperature approximately that of boiling water. Water may or may not be added depending on the type of cottonseeds used.
4. Oil is expressed by the hydraulic or expeller process. The meal which is left contains
only a small percentage of oil, and is the press cake.

5. Press cake is cooled and stored for 30 days before grinding.

6. The press cake is ground and the finer meal is separated from the coarser ground portion by mechanical means.

7. From a ton of cottonseeds, 300 pounds of edible flour may be produced.

Certain changes take place during the cooking process. When cottonseed is cooked, the protein separates and coagulates and binds gossypol, causing loss of its toxic properties. (Lyman, Holland, and Halé, 1944; Olcott, 1941) (29; 36).

During cooking of soybeans, the enzymes, lipoxidase and lipase are inactivated. (Balls, Axelrod and Kreis, 1943; Sumner and Tressler, 1943) (3; 41).

Cooking of peanuts results in the peptization of the nitrogenous constituents while care must be taken to prevent the denaturation of protein. (Fontaine, Samuels and Irving, 1944; Payne, 1942) (13; 36).

Some recent discoveries suggest new uses for these various flours, such as: the addition of soybean or peanut flours to increase protein value of foodstuffs;
use in bread to decrease rate of staling; and addition
to milk products to inhibit rancidity of butterfat.
(Kinsman, 1944; Payne, 1942; Payne and Stuart, 1944;
Bohn and Favor, 1945; Williamson, 1944) (25; 38; 39; 6;
42).

In addition to the special properties that have
made these flours desirable for food manufacture the
press cakes have been shown to possess antioxidant acti-
vity when added to fats. Eichberg (1942) (9) found that
the addition of small quantities of an antioxidant frac-
tion (lecithin) from soybeans improved the stability of
oils and fats.

Husher (1935; 1942) (33; 34) suggests that rancidity
in lard may be inhibited by the addition of soy or cereal
flours.

These observations indicate that the flours may
have an antioxidant effect when incorporated in food
mixes.

RANCIDITY OF FATS

Rancidity is a condition developing in fats and
baked goods containing fat in which definite off flavors
and odors are detected. Bailey (1945) (2) declares that
this phenomenon does not involve gross quantities of
rancid fat, but that only about 0.1 per cent of the fat may actually decompose to form the compounds responsible for its rancid flavor and odor.

Lea (1939) says, "The most important, and from a scientific point of view the most interesting form of rancidity is that produced by the action of oxygen of the air on the fat." (27--p. 79).

The initial step in the oxidation of fats is the addition of oxygen at or near the double bond of a fatty acid chain to form unstable compounds known as peroxides. (Bailey, 1945) (2).

\[
\begin{align*}
\text{H} & \quad \text{H} \\
\text{C} & \quad \text{C} + \text{O}_2 \\
\text{O} & \quad \text{O} \\
\end{align*}
\]

Fats such as lard, which are relatively high in oleic acid content and low in linoleic acid, will become rancid after the absorption of less oxygen than fats in which the amount of these acids are reversed. The rate at which oxygen is absorbed by fat is markedly accelerated by heat, exposure to light, particularly ultraviolet, and the presence of certain metals. (King, Roschen and Irwin, 1933; Boehm and Williams, 1943) (25; 5). The course of oxidation follows two phases:
1. Initial phase or induction period. Oxida-
tion during this phase proceeds relatively
slowly and at a more or less uniform rate.

2. Second phase. When the induction period is
ended, oxidation takes place at a greatly
accelerated rate.

The point at which the sample smells and tastes
rancid coincides with the beginning of the second phase.
(Bailey, 1945; Filer, Mattil and Longenecker, 1945)
(2; 12).

"The more highly saturated animal fats and hydro-
genated oils whose unsaturated acids consist largely of
oleic acid are relatively little altered in flavor and
odor during the early phase. Onset of rancidity in such
fats is both sudden and definite." (Bailey, 1945...p.
45) (2).

**METHODS USED IN THE DETERMINATION OF RANCIDITY**

Various methods have been developed for testing
rancidity in fats and in products made with fat. Rancid
odors and flavor may be determined organoleptically,
or rancidity may be determined by chemical methods. Of
the chemical methods for determining the degree of
oxidation the test for fat peroxides is most sensitive.

Fat peroxides are intermediate products formed
During oxidation of the fat, these peroxides decompose rapidly to form other products which are responsible for flavors and odors. They are estimated by their ability to release iodine from potassium iodide (KI) in glacial acetic acid (CH₃COOH). The peroxide value of a fat is a measure of its content of reactive oxygen in millimols of peroxide or milliequivalents of oxygen per 1000 grams of fat. (Lea, 1939; Bailey, 1945) (27; 2).

\[
\text{CH}_3 - \text{C} \quad \text{+ KI} \quad \rightarrow \quad \text{CH}_3 - \text{C} \quad \text{+ HI}
\]

\[
\text{O} - \quad \text{O} \\
\text{R} \cdot \text{HC} - \text{CH} : \text{R} \quad \text{+ 2HI} \quad \rightarrow \quad \text{R} \cdot \text{HC} - \text{CH} : \text{R} \quad \text{+ I}_2
\]

\[
\text{I}_2 \quad \text{+ 2Na_2S_2O_3} \quad \rightarrow \quad 2\text{NaI} \quad \text{+ Na}_2\text{S}_4\text{O}_6
\]

The length of time required for many fats to become rancid under normal conditions is comparatively great. For this reason accelerated tests have been introduced and are now frequently used. These tests make use of relatively high temperatures to increase the rate of oxidation. (Beadle, 1946) (4).

The Schaal oven test is a method making use of
simple incubation of the sample in an oven at 60°C. (Bailey, 1945) (2). The test was developed to provide a means of rating the relative stability of various shortenings and as an index to their keeping qualities. In the test developed by Schaal 50 gram samples were placed in beakers covered with watch glasses. The samples were smelled daily, in the morning, for rancid odors. The end of the induction period was marked by the development of a rancid odor and darkening of the sample. Peroxide formation was used in connection with the organoleptic tests to follow the development of rancidity.

Certain limitations exist in the use of a method for determining rancidity by organoleptic means:

1. Personal evaluation of the point at which rancid odors occur. Rancidity in fats with short induction periods is easier to detect. The longer induction periods introduce more difficulties since the sample changes but little from day to day. The end of the induction period is hard to determine with accuracy.

2. Color changes to indicate end point may be confusing. Some fat darkens toward the end of its induction period, but in some cases the color of the fat becomes lighter.
The Swift's stability test is one in which the sample is aerated by bubbling oxygen through the liquid fat while it is held at a constant temperature of 98°C on a water bath. (King, Roschen and Irwin, 1933) (24). This test is mainly used for determining stability of fats and oils, and has not been adapted for use with baked or prepared foods. Most of the data reported in the following papers were obtained by means of this test.

FACTORS INFLUENCING THE DEVELOPMENT OF RANCIDITY

The appearance of rancidity may be accelerated or inhibited. The presence of certain metallic salts or minute traces of certain metals have a catalytic effect upon the oxidation of fats. Copper has been found to be the most active. Because of this, copper should not be used in any of the utensils in which fats are processed.

In addition to the accelerating effect of metals, it is well established that two other factors are of prime importance in affecting the development of rancidity. The exposure of fats to light, particularly ultraviolet rays, as well as holding fats at high temperatures will accelerate the appearance of rancidity. (King, Roschen and Irwin, 1933; Boehm and Williams, 1934) (23; 5).
The development of rancidity may be inhibited by storing a fat at low temperatures and protecting it from light. The keeping quality may be greatly improved by the addition of antioxidant substances.

The manufacturers of commercial fats and oils were the first to become interested in materials which if added to their products would delay the appearance of rancidity. Since animal fat contains only small amounts of natural antioxidants a number of materials have been studied as to their effect in prolonging the keeping quality of lard and other animal fats. An antioxidant selected for this purpose should:

1. Exhibit effective inhibitory action.
2. Be easily soluble in fat.
3. Impart no foreign flavor, odor, or color on long storage.
4. Exhibit no harmful physiological effect.
5. Exhibit no changes when heated.
6. Possess ability to retard rancidity in baked goods prepared from the fats treated with it.
7. Be available in quantity and be economical.

(Higgins and Black, 1944) (18).
An efficient antioxidant must have a hydroxyl group uncombined and attached directly to an aromatic ring. The antioxidant compounds found in vegetable oils are phenolic in nature and depend for their activity on free hydroxyl groups. (Olcott, 1941) (37). Lard contains only small quantities of the phenolic type of antioxidants.

Certain synthetic antioxidants have been used in lard but their use has been discontinued because of the undesirable flavor and odor, and because they are too unstable or toxic for use in edible products. (Bailey, 1945) (2).

Some acids have been found to delay the appearance of rancidity. Most of the effective acids act only in the presence of phenolic type compounds. They are designated as synergists.

Materials which occur naturally in connection with foods have received most of the attention for stabilizing fatty foods, since such substances are unlikely to have toxic effects. Some phosphatides behave as synergists. Purified lecithin has been shown to contain no antioxidant properties. Cephalin, though similar in chemical structure to the inactive lecithin exerts an inhibiting effect on the development of rancidity. Its
inhibiting action is probably due to the phosphoric acid. The same group is present but is bound in lecithin.

\[
\begin{align*}
H & \\
\text{H-C-} & \text{OOCC}_{R_1} \nonumber \\
\text{H-C-} & \text{OOCC}_{R_2} \nonumber \\
\text{H-C-} & \text{-P-} \text{O} \text{-CH}\text{CH}_2\text{CH}_2\text{N(CH}_2\text{)}_3 \nonumber \\
\text{H} & \\
\text{Lecithin} \nonumber \\
\end{align*}
\]

\[
\begin{align*}
\begin{align*}
\text{H} & \\
\text{H-C-} & \text{OOCC}_{R_1} \nonumber \\
\text{H-C-} & \text{OOCC}_{R_2} \nonumber \\
\text{H-C-} & \text{-P-} \text{O} \text{-CH}_2\text{CH}_2\text{N(CH}_2\text{)}_2 \nonumber \\
\text{H} & \\
\end{align*}
\text{Cephalin} \nonumber \\
\end{align*}
\]

Mitchell and Black (1934) (32) and Hilditch and Paul (1939) (19) found the phospholipids of cottonseed and corn oil to be more potent in their antioxidant effect than were those of soybean.

One important class of naturally occurring antioxidants consists of the tocopherols. Their presence has been demonstrated in some vegetable oils where they undoubtedly act as the chief antioxidants. Three different tocopherols have been isolated and are desig-
nated as α, β, and γ tocopherol.

\[ \text{α-Tocopherol} \]

Although tocopherols are thought to be the chief antioxidants of vegetable oils, others exist, some of which have been identified. Chroman-5,6-quinone, an oxidation product of tocopherol, and gossypol are antioxidant materials which have been identified. Sesame and rice bran oils are unusually stable although the tocopherol content of these oils is not particularly high. It is believed that they contain antioxidants more potent than tocopherol, probably sesamol in the sesame oil. (Bailey, 1945; Columbia, 1942; Hove, 1944) (2; 15; 17).

Small quantities of soybean or oat flour or fractions of soybean have been added to lard as a means of retarding rancidity. (Musher, 1935; Dahle and Nelson, 1941) (32; 8).
Gabel and Sunderlin (1945) (14) compared the tenderness of pastries made with five different types of soy flour. They found that soy flour containing only 5 to 15 per cent of fat produced more tender pastry than did full-fat flours. The authors felt that the increase of tenderness of the pastry made from extracted flours containing 5 per cent and 15 per cent fat was due to the fat present in the flours. They found "no significant differences in the mean breaking strengths of pastries made with the extracted soy flours containing 5 per cent or 15 per cent fat and the pastries in the third series made with the defatted flour and soybean oil equivalents when the total fat in the pastries was constant. In comparing a soy flour of the expeller type containing 5 per cent fat with extracted type soy flours, it was found that the pastries made with the expeller flour were much more tender than with the extracted flour of either the 5 per cent or the 15 per cent fat content or even than pastries made with the full fat soy flour containing 22 per cent fat." From these results the authors concluded that "this difference in tenderness must have been caused by some factor other than the fat in the different types of flour." (14...p. 271, 272)
CHAPTER II

PURPOSE OF THE EXPERIMENT

The work of Hilditch and Paul (1939) (19), Dahle and Nelson (1941) (8) and others has demonstrated the effectiveness of soy, cotton and peanut flours, or certain of their fractions, as inhibitors of fat oxidation. These findings suggested the possibility that the flours might have antioxidant effect in prepared foods.

This experiment was designed:

1. To compare flours from three oil press cakes as to:
   (a) Their effect on the development of rancidity in baked pastry and pastry mixes.
   (b) Their effect on the tenderness of the pastry.

2. To obtain information regarding the method for acceleration of rancidity.
   (a) To determine whether division of samples for incubation between a few large or many small containers would influence consistency of results.
(b) To determine whether an incubator temperature of 50°C (122°F) would be sufficiently high to accelerate the appearance of rancidity within a reasonable period of time, yet not high enough to obscure the possible differences between the experimental flours.
CHAPTER III

EXPERIMENTAL PROCEDURE

Baked pastry was prepared, using a standardized formula and technique. The pastry was then crushed and distributed between the jars in which it was to be stored. These jars of pastry, as well as jars of raw pastry mix were stored at 50°C.

Samples of both pastry and mix were removed at intervals. The fat was extracted, and peroxide numbers of the fat were determined.

PROCEDURE FOR PASTRY

Ingredients

The ingredients used were all-purpose patent wheat flour, salt, lard, soy, cottonseed, and peanut flours. All ingredients were secured in quantities sufficient to serve for the entire experiment. Distilled water was used in making the pastry.

The wheat flour and lard were stored at 0°F. until needed. On the day preceding the one on which the pastry or mix was to be prepared, the weighed ingredients were placed in an incubator which maintained a temperature of 25°C. During this holding period the
temperature of all ingredients became adjusted to 25°C.

**Formula**

The basic pastry formula used was that developed by Lowe, Nelson, and Buchanan (1939) (28).

**Basic formula:**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat Flour</td>
<td>276.0 grams</td>
</tr>
<tr>
<td>Lard</td>
<td>110.0 grams</td>
</tr>
<tr>
<td>Salt</td>
<td>6.0 grams</td>
</tr>
<tr>
<td>Water</td>
<td>70.0 ml</td>
</tr>
</tbody>
</table>

For the soy, cottonseed and peanut series, 10 per cent by weight of each of these flours was substituted for wheat flour in the basic formula. No other changes were made.

The formulas used for the raw mixes were identical to those used for the baked pastries, with the exception that water was omitted from the mixes.

**Mixing**

The dough was mixed using a modification of the method described by Lowe, Nelson and Buchanan (1938) (28). A machine method for mixing was employed. The equipment consisted of a Hobart KitchenAid, Model G, its 3 quart mixing bowl and pastry knife.

The standardized technique used for combining ingredients followed this pattern: The flour and salt
were sifted once and then sifted into the mixing bowl. The lard was then added. These ingredients were mixed for three minutes at slow speed. Without stopping the machine, the mixture was scraped down with a spatula at 1½, 2, and 2½ minutes. At the end of three minutes the machine was stopped, the mixture again scraped down and the water added. Mixing at slow speed was then continued for 20 seconds.

After mixing, the dough was turned out onto a board. It was then kneaded into a ball with 10 motions of the hand. The ball of dough was cut into six strips. The two outer strips were discarded leaving the four center strips to be rolled.

Rolling

Immediately after the dough was mixed and cut into strips, it was rolled between steel cleats, 1/8 of an inch in height. To prevent the dough sticking to the rolling pin and board it was rolled between two pieces of wax paper.

The upper piece of paper was then removed. A rectangular steel cutter measuring 1½ by 2½ inches was used to cut the pastry into wafers of uniform size. The superfluous dough was trimmed away around the edges of the wafers and the trimmings discarded. The pastry
wafers were then transferred with a minimum of handling to a tin baking sheet. This was accomplished by inverting over the baking sheet the lower strip of wax paper to which the wafers adhered. By brushing a hand lightly over the paper enough pressure was exerted to cause the wafers to cling to the baking sheet. The wax paper was then peeled off.

The wafers were pricked to minimize blistering. The instrument used for pricking was a small block of wood carrying a number of evenly spaced sharp steel points.

Baking

A Despatch electric oven was used to bake the pastry wafers. The heat was thermostatically controlled and checked by thermometer. The baking sheet was placed as nearly as possible in the center of the oven to help insure uniform baking conditions. The pastry was baked for 20 minutes at 180°C. (356°F.). A stopwatch was used for timing the baking period, and the oven temperature was accurately controlled.

After removal from the oven, the pastry wafers were transferred to wire cake racks where they were allowed to cool for one hour. The breaking strength was then determined by means of the Bailey Shortometer.
Division of samples

All pastry wafers were rolled to crumbs and thoroughly mixed. For one part of the experiment the pastry crumbs were divided equally among many small jars. In a second part of the experiment, the pastry crumbs were divided equally among four large jars.

Incubation of samples

The jars of pastry crumbs were placed in an incubator which maintained a temperature of 50°C ± 0.25°C, as measured by a thermometer placed in the center of the top shelf. The temperature variations at different points in the oven were: from the bottom shelf to the top shelf, 1.7°C, and from the front to back of each shelf, 0.3°C, to 0.8°C.

In the second part of the experiment when each combined lot of pastry or mix was divided among four large jars, the jars were placed from front to back on the center shelves of the incubator.

Selection of samples for extraction

At suitable intervals, samples of pastry or mix were removed from the incubator for extraction of the fat.

In the first part of the experiment, when the pastry was held in many small containers, two samples of pastry
and two of mix were picked at random. These two supposedly were representative of the whole.

In the second part of the experiment when each lot of pastry or mix was divided equally among four large jars, samples were removed from all four jars at each incubation period studied. These four samples were representative of the whole.

Procedure for raw pastry mixes

The procedure for combining the raw pastry mixes was identical to that used for the baked pastry except that water was omitted.

The different lots of raw mix were distributed among jars in the same way as the baked pastry. Incubation and selection of samples for extraction were done in the same way as for the baked pastry.

**METHOD FOR EXTRACTING FAT**

The fat was extracted from the pastry samples with petroleum ether using Soxhlet extractors. The extraction was stopped at the end of eight hours. Excess petroleum ether was removed by distillation from the fat sample. Drying of the extracted fat was completed under a 25-30 inch vacuum at 50°C for six to eight hours.
PEROXIDE NUMBER DETERMINATION

Method

Peroxide numbers were determined using a modification of the method developed by Lea (1939) (27). The steps in the determination were as follows:

1. Sample was weighed (1.000 gram plus or minus 0.05 gram) into amber glass test tube. Duplicate determinations were made on each sample of fat.
2. Approximately 1 gram of powdered potassium iodide was added.
3. 20 ml glacial acetic acid and chloroform mixture, (2:1 by volume) were added.
4. The test tube containing the above mixture was placed immediately in a beaker of boiling water, and heated exactly 1 minute. A stopwatch was used. Care was taken so mixture did not foam over.
5. Mixture was then poured into 50 ml water in 100 ml Erlenmeyer flask.
6. The free iodine was then titrated with standardized sodium thiosulfate 0.02 N until near end point.
7. Approximately 2 ml indicator starch solution was added.
8. The mixture was titrated until colorless.
9. Peroxide number, formula:

\[
\text{me peroxide per 1000 g fat} = \\
\frac{(\text{ml Na}_2\text{S}_2\text{O}_3)(\text{Normality})}{\text{weight of fat in grams}} \times 1000
\]

Peroxide number is expressed as milliequivalents of peroxide per 1000 grams of fat
Reagents

Potassium iodate reference standard, 0.02 N.  
0.7134 g potassium iodate per liter.

Sodium thiosulphate 0.02 N. (approximately)  
5.6 g sodium thiosulphate per liter.

Acetic acid and chloroform mixture 2 parts  
glacial acetic acid, 1 part chloroform by  
volume.

Starch solution  
1 g soluble starch per 100 ml water.

Hydrochloric acid, concentrated  
30 per cent potassium iodide solution  
30 g potassium iodide in 100 ml water.

Potassium iodide in powdered or crystal form.

Standardization of sodium thiosulphate

1. 25.00 ml potassium iodate solution, 0.02 N, 
was run into a 100 ml Erlenmeyer flask

2. 10 ml potassium iodide solution was added.

3. 3 ml concentrated hydrochloric acid was added.

4. Free iodine was titrated with approximately  
0.02 N sodium thiosulphate until a light 
straw color, then 3 ml starch solution was  
added. Starch solution was freshly prepared.

5. The mixture was then titrated until colorless.

6. Formula:  \((\text{ml of potassium iodate}) \times (N \text{ of potassium iodate}) = (\text{ml of sodium thiosulphate}) \times (N \text{ of sodium thiosulphate})\).

7. Triplicate determinations were made, and each 
calculated separately. The results were then 
averaged.
CHAPTER IV

RESULTS AND DISCUSSION

Information was obtained as to the most desirable method for sampling the incubated pastry. As mentioned under "Experimental Procedure" one lot of pastry was distributed among many small jars. After incubation, samples were selected at random for extraction of the fat and determination of the peroxide numbers. Another lot of pastry was divided equally among four large jars. Each time that pastry was removed for analysis of the fat, a sample was taken from each of these jars. In the following discussion the first method is designated as Test I, the second method is designated as Test II.

Data obtained by sampling at random from numerous small containers (Test I) are compared with those obtained by sampling from four large containers (Test II) in Table I.

Differences between duplicate samples from Test I are no greater than variations among quadruplicate samples in Test II. On the other hand, a comparison of the day to day results shows considerable fluctuation among the averages from Test I. No trend is apparent, and no curve could be plotted from these data.
A curve for development of rancidity in lard would be expected to show a short induction period followed by a rapid increase in peroxide number. This type of curve was obtained when averages from Test II were plotted, as shown by the curve for plain pastry in Figure I. Since these averages represent a cross section of the changes taking place in the entire lot of pastry, the method of sampling followed in Test II should give the more accurate picture.

No definite explanation has yet been found as to the reason why all samples do not become rancid at the same time when all conditions are apparently uniform.

Erbank and Gould, (1942) (10) working with butter samples, found that when samples were spaced far apart in the oven they varied more as to the rate of oxidation than if closely grouped in the center of the oven. "66.5 per cent of the samples on the bottom shelf oxidized more rapidly than those on the top shelf." (10---p. 206). "Decreasing the size of sample or increasing surface area markedly affected the rate of oxidation." (10---p. 207). Standardization of the size of sample and size of container as well as the arrangement in the oven was found to be necessary for reliable results.

In the present study, differences in peroxide
numbers of duplicate or quadruplicate samples may have been due to temperature variations within the incubator.

A temperature of 50°C. (122°F.) was found to be satisfactory for accelerating the development of rancidity in pastry samples. It was high enough to speed up the appearance of rancidity, yet low enough to allow sampling at convenient intervals. The testing of nearly all samples was complete in approximately two months. Though some samples did not become rancid up to nine months of storage, this was not found to be an inconveniently long holding period.

A temperature of 50°C. (122°F.) is but slightly higher than that of a hot summer day. Summer temperatures around 100°F. in the shade are common in many parts of the country. Temperatures lower than 122°F. would be preferable for studying the development of rancidity, since these would approach more closely normal holding conditions. However, a lower incubation temperature would not be feasible for laboratory use.

The effect of soy, peanut and cottonseed flours on the development of rancidity in baked pastry and pastry mixes is shown in Tables II through IX and by Figures I and II.

It is evident that oxidation of the fat in plain
and peanut baked pastry followed a very similar course. Both the plain baked pastry and the baked pastry containing peanut flour became rancid within 17 days. The peanut flour present in the baked pastry exerted very little if any antioxidant effect. The last samples of peanut mix to be analyzed were removed from the incubator after 46 days. They showed no evidence of becoming rancid at that time. The plain mix showed no increase in peroxide number up to the sixtieth day of incubation, but rancidity developed between the sixtieth and seventy-fifth day.

On the other hand, soybean flour was shown to be a good antioxidant, and cottonseed flour an excellent antioxidant both in the baked pastry and in the raw mixes. The baked pastry containing soy flour did not become rancid up to 39 days of incubation. The raw soy mix became rancid after nine months. The baked pastry containing cottonseed flour showed no sign of becoming rancid up to 86 days of storage, and the raw mix showed no increase in peroxide number up to nine months.

The effectiveness of soy and cottonseed flours in delaying the appearance of rancidity is doubtless due to their content of antioxidants of different kinds. It is known that cottonseed flour contains gossypol and tocopherols as well as other antioxidant substances as
yet not identified. Gossypol, whether in its free or bound form, has been shown to be a very powerful antioxidant. (Hilditch and Paul, 1939) (19). Olcutt (1941) (37) reported that a water soluble antioxidant also is present in cottonseed. Soybean flour is known to contain tocopherols and lecithin.

Peanut flour may contain tocopherols. Inasmuch as the peanut flour did not delay rancidity in baked pastry, it may be assumed that antioxidant substances were present in very small amounts or were readily oxidized.

The lard used for preparing the pastries and mixes had a peroxide number of 8.7. In every case, the fat extracted from the freshly baked pastry had peroxide numbers lower than that of the unused lard. The same observation was made in regard to fat extracted from the dry mixes. Peroxide numbers were found to be lower than 8.7 in every fat sample from the plain mix through 60 days of incubation, from the peanut mix through 46 days, from the soy bean mix through 114 days and from the cottonseed mix through 270 days.

These lower peroxide values may have been due to reducing substances in the food mixtures which acted upon peroxides in the fat, or they may have been due to substances extracted with the fat, which were capable of reducing iodine in the iodometric method of analysis used.
The effect of soy, peanut and cottonseed flours on tenderness of baked pastry is shown in Table X. Averages of breaking strength determinations show that plain pastry was somewhat more tender than the others. The pastries in which soy, peanut or cottonseed flour had been used differed but little from each other.

The pastries in which soy, peanut and cottonseed flours were used were acceptable in color, flavor and texture. The pastries made with soy or peanut flour were slightly creamy or yellow in color. The flours added a slightly nutty flavor to the pastry. The cottonseed pastry was slightly darker in color than the others. A smaller percentage of cottonseed flour would have given a lighter colored product. The flavor was not unpleasant, but in the opinion of the author, was not as pleasing as that from soy or peanut flour.

As rancidity developed in the pastry and mixes, definite color changes were noted in the extracted fat. The fat extracted from the fresh pastry had a light yellow tint. This color was due to the carotene from the unbleached flour. As rancidity progressed the yellow tint of the fat gradually faded to colorless.

A definite color change was noted in the extracted fat from the sample of cottonseed mix that had been
incubated for nine months. When incubation was first started a faint yellow tint was characteristic of the fat, but a distinct orange-red color was present in the fat extracted from the sample that had been held for this long period of time. This may have been due to the formation of tocoquinones, caused by the oxidation of the tocopherols that are present in the cottonseed flour. The tocoquinones exhibit a red color. (Columbic, 1945; Bailey, 1945) (15; 2).
CHAPTER V

SUMMARY AND CONCLUSIONS

The effectiveness of soy, peanut and cottonseed flour in delaying rancidity in baked pastry and pastry mixes has been studied.

Baked pastry and dry mixes were made using a standardized formula, and standardized technique. The soy, peanut and cottonseed flours were substituted for 10 per cent by weight of wheat flour in the formula. The pastry and mixes were stored in glass jars at a constant temperature of 50°C. Peroxide numbers were determined at intervals on fat extracted from samples of each kind to test for the development of rancidity.

Peroxide numbers of fat extracted from samples which had been incubated for the same length of time were found to be more uniform when the pastry was in a few jars of the same size than when stored in many jars of small size.

A temperature of 50°C. was found to be satisfactory for a study of this type.

Cottonseed flour exerted the greatest antioxidant effect, and soybean was good, but peanut flour appeared to have no antioxidant value. Pastry in which peanut
flour had been incorporated was almost identical to plain pastry in the speed with which rancidity developed.

In every case the dry mixes kept better than the corresponding baked pastry.

Pastries in which soy, peanut or cottonseed flour had been used differed very little from each other in tenderness, but in each case were less tender than the plain baked pastry.
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37. Olcott, H. S. Antioxidants for edible fats and oils. Oil and Soap 18: 77-80, 1941.


APPENDIX
Table I. ---Effect on uniformity of different methods of sampling pastry as shown by peroxide numbers* of extracted fat --- Plain, baked pastry.

<table>
<thead>
<tr>
<th>Days of Incubation</th>
<th>Sample number</th>
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<th>Days of Incubation</th>
<th>Container number</th>
<th>Average</th>
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<td>I</td>
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<td>4.0</td>
<td>5</td>
<td>4.2</td>
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<td>16.6</td>
<td>8</td>
<td>6.2</td>
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<td>6.1</td>
<td>13.6</td>
<td>12</td>
<td>6.6</td>
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<td>15</td>
<td>10.8</td>
</tr>
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<td>21.2</td>
<td>12.6</td>
<td>17</td>
<td>22.4</td>
</tr>
</tbody>
</table>

* Milliequivalents $\text{H}_2\text{O}_2$ per 1000 grams fat
Table II. PLAIN PASTRY, BAKED -- Peroxide numbers\(^\circ\) of fat extracted from pastry stored at 50\(^\circ\)C.

<table>
<thead>
<tr>
<th>Days of Incubation</th>
<th>Container number</th>
<th>Average</th>
</tr>
</thead>
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<td>17</td>
<td>22.4</td>
<td>36.8</td>
</tr>
</tbody>
</table>

\(^\circ\) Milliequivalents \(H_2O_2\) per 1000 grams fat
Table III. ----PLAIN PASTRY, MIX ---- Peroxide numbers* of fat
extracted from mix stored at 50° C.

<table>
<thead>
<tr>
<th>Days of Incubation</th>
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<th>Average</th>
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<td>75</td>
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</table>

* Milliequivalents H₂O₂ per 1000 grams fat
Table IV. **PEANUT PASTRY**, BAKED **--- Peroxide numbers**°° of fat extracted from pastry stored at 50°C.

<table>
<thead>
<tr>
<th>Days of Incubation</th>
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<th>Average</th>
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</thead>
<tbody>
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</tr>
<tr>
<td>19</td>
<td>79.8</td>
<td>115.6</td>
</tr>
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</table>

° 10 per cent Peanut flour

°° Milliequivalents $H_2O_2$ per 1000 grams fat
Table V. --PEANUT PASTRY°, MIX -- Peroxide numbers" of fat extracted from mix stored at 50° C.

<table>
<thead>
<tr>
<th>Days of Incubation</th>
<th>Container number</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>46</td>
<td>3.3</td>
<td>2.8</td>
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</table>

° 10 per cent Peanut flour

" Milliequivalents $\text{H}_2\text{O}_2$ per 1000 grams fat
Table VI. ---SOYBEAN PASTRY*, BAKED --- Peroxide numbers** of fat
extracted from pastry stored at 50 C.

<table>
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<tr>
<th>Days of Incubation</th>
<th>Container number</th>
<th>Average</th>
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</thead>
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<tr>
<td>39</td>
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<td>5.1</td>
</tr>
</tbody>
</table>

* 10 per cent Soy flour

** Milliequivalents H₂O₂ per 1000 grams fat
Table VII. ---SOYBEAN PASTRY°, MIX --- Peroxide numbers** of fat
extracted from mix stored at 50° C.

<table>
<thead>
<tr>
<th>Days of Incubation</th>
<th>Container number</th>
<th>Average</th>
</tr>
</thead>
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<td>I</td>
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<td>77</td>
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<td>95</td>
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<td>114</td>
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<tr>
<td>270</td>
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</tbody>
</table>

° 10 per cent Soy flour

** Milliequivalents H₂O₂ per 1000 grams fat
Table VIII. --COTTONSEED PASTRY*, BAKED -- Peroxide numbers**
of fat extracted from pastry stored at 50°C.

<table>
<thead>
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<th>Container number I</th>
<th>Container number II</th>
<th>Average</th>
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<td>86</td>
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</table>

* 10 per cent Cottonseed flour
** Milliequivalents $H_2O_2$ per 1000 grams fat
Table IX. \textit{---COTTONSEED PASTEY\textsuperscript{o}, MIX --- Peroxide numbers\textsuperscript{oo} of fat extracted from mix stored at 50\degree C.}

<table>
<thead>
<tr>
<th>Days of Incubation</th>
<th>Container number</th>
<th>Average</th>
</tr>
</thead>
<tbody>
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<td>3.7</td>
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</table>

\textsuperscript{o} 10 per cent Cottonseed flour

\textsuperscript{oo} Milliequivalents $H_2O_2$ per 1000 grams fat
Table X. -- Influence of soy, peanut and cottonseed flours<sup>**</sup> on the tenderness of baked pastry.

<table>
<thead>
<tr>
<th>Lot&lt;sup&gt;2&lt;/sup&gt; number</th>
<th>Plain pastry</th>
<th>Soy pastry</th>
<th>Peanut pastry</th>
<th>Cottonseed pastry</th>
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<tbody>
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<td>14.9</td>
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<td>9.5</td>
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<td>Average</td>
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<td>10.8</td>
</tr>
</tbody>
</table>

<sup>*</sup> Averages of 14 to 15 wafers per lot

<sup>**</sup> 10 per cent of soy, peanut or cottonseed flour substituted for wheat flour
FIGURE I

The effect of soy, peanut, and cottonseed flours on development of rancidity in baked pastry stored at 50°C.
The effect of soy, peanut, and cottonseed flours on development of rancidity in raw pastry mixes stored at 50° C.