

# Germicidal Properties of Paraffin

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**B**ECAUSE of the widespread use of waxed papers and containers for the storage and distribution of foodstuffs and the increasing use of waxed containers for this purpose, it seems desirable to assemble the various information available in the literature and in unpublished records which deals with the bactericidal effect of the paraffination process, both on the coated paper or board and on the paraffin surface of the finished container. The following paper reviews this work and contributes further information with regard to the problems which would appear to be of interest in view of the sanitary problems involved in the distribution of foods in containers of this type.

## Historical Review

Work on this problem started as early as 1898 when hot paraffin or paraffin oil was advocated as a medium of sterilization. Forgue and Reclus (1898) for instance believed that oil over 100° C. (212° F.) was a good sterilizing agent. However, Anderson (1907) and Kieffer (1907) reported that the temperatures had to be much higher to insure sterility, an observation confirmed two years later by Conradi (1909). Anderson (1907) investigated two temperatures, 120° C. (248° F.) and 150° C. (302° F.), using spores of *Bacillus subtilis* as the test organism. Exposure of the spores of this organism in hot paraffin at the above temperatures for ten minutes was insufficient. They were killed only after 30 minutes at 150° C. (302° F.). Kieffer's experiments (1907) were more extensive. The test organisms were 2- to 8-day growth of spores of *Bacillus anthracis*, a 24-hour growth of *Staphylococcus*, and an 8-day growth of *Bacillus vulgatus*. The test organisms were carried on a small brass wire which was held in the oil for the time indicated. After removal, they were placed in sterile broth to allow the viable bacteria to develop. Exposure to hot paraffin for ten minutes at 150° C. did not destroy all of the organisms. Conradi (1909), realizing the ineffectiveness of anhydrous liquids, used much higher temperatures. At 200° C. (392° F.) spore formers were killed in one minute. Amako's results (1909) were quite similar when using *Serratia marcescens*, *Echerichia coli*, *Bacillus anthracis*, and *Salmonella schottmülleri*. Results of Dreyer and Walker (1912) and Bartlett and Kinne (1914) are in agreement with those just reported.

The most extensive investigation into the problem of thermal death times of bacteria in anhydrous menstrua was carried out by Bullock (1914). Unfortunately he did not use paraffin; the nearest approach to it might be glycerine with which he secured results not unlike those reported above for paraffin. Davis and Rosen (1917), using *Staphylococcus aureus* as a test organism, found that high temperatures were required to destroy the organism in hot paraffin. Layman's results (1917) from an investigation of hot-oil sterilization of surgical instruments were more encouraging. Results with *Staphylococcus aureus* and *albus* were so encouraging that he recommended that syringes be sterilized in oil at 150° C. (302° F.); no

data were given to support the recommendation. The sterilizing effects of hot paraffin were also studied by Dirska (1922). He used spores of *Mesentericus fuscus*, an aerobic spore former which is widely distributed in nature. The organisms were suspended on threads which were placed in the hot paraffin. Liquid paraffin at 120° C. (248° F.) did not kill the organisms in forty minutes. Thirty minutes' heating, however, at 150° C. (302° F.) destroyed the organisms. At 180° C. (356° F.) the organisms were able to survive only ten minutes' heating. Believing that the widespread use of hot liquid paraffin for sterilization of surgical instruments was ill founded, Rodenbeck carried out an extensive investigation, the results of which were reported in detail. The test organism was an aerobic spore former isolated from garden earth. This organism was heated in various anhydrous menstrua such as cumol, glycerol, fat, oil, and paraffin. Starting with 150° C. (302° F.) with a death time of some six hours, the rates progressively shortened as the temperature was raised. At 200° C. (392° F.) there was almost complete destruction of the living cells. The death rate was quite uniform to 170° C. (338° F.) where the organisms were destroyed in 40 minutes.

That many of these experiments were uncontrolled should not be overlooked. Practically no attention was given to heat resistance of the organisms used or to the numbers of organisms used. Little information is available with respect to rate of death. It is obvious from more recent work that hot paraffin wax does exert an appreciable destructive effect on bacteria.

Prucha's work (1938) may be reviewed at greater length because it bears directly on the subject of this paper. While his work was prompted largely by interest in the paper milk container, his results are probably of broader significance. In laboratory experiments Prucha used small strips of paper 1/2 by 2 1/2 inches. After the strips were inoculated, they were paraffined at different temperatures. They were then dropped into a culture tube containing 15 per cent milk and 85 per cent water as a culture fluid. Appearance of a red color was taken as evidence of viability and growth of the test organism, *Serratia marcescens* (*Bacillus prodigiosus*). Results secured with such technique are, of course, qualitative, i.e., nothing is revealed as to the extent and rate of death and should be interpreted with this fact in mind. At 71° C. (160° F.) viable cells survived for 60 seconds, at 77° C. (170° F.) for 30 seconds; at 82° C. (180° F.) only one tube out of six showed the presence of viable cells after exposure of 20 seconds, indicating that this temperature was quite lethal. The strips paraffined at 88° C. (190° F.), 93° C. (200° F.), and 100° C. (212° F.) also showed one tube with growth after 20 seconds exposure. Prucha also determined the facts for hot water using the same technique. Hot water, as was known, is more germicidal. All cells were killed after ten seconds when exposed on strips of paper at 66° C. (150° F.); in 45 seconds at 63° C. (145° F.), but not after 60 seconds at 54° C. (130° F.) and 60° C. (140° F.).

Sanborn (1939) stated that efficient paraffining of

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high quality paperboard suppresses or "seals in" the few bacteria which are in paperboard. He stressed the value of adequate coverage of board with paraffin. His tests indicate that as satisfactory coatings of wax can be obtained at 74-77° C. (165 to 170° F.) as at temperatures of 82-85° C. (180 to 185° F.). Ninety-six per cent of 730 milk containers paraffined at 165° F. yielded less than 5 colonies per container, while only 95 per cent of 1370 containers paraffined at 180° F. yielded less than 5 colonies per container. Whether this difference is significant is open to question, but the results indicate that above a temperature of 74° C. (165° F.) the bacteriological results are not materially different.

Prucha also paraffined paper milk bottles which had been heavily inoculated with the test bacterium *Serratia marcescens*. The containers were dipped into a suspension of this organism having 200,000,000 cells per cc. Such a method of inoculation would give many more bacteria in a container than would ever be found in practice. As soon as the containers were dry, they were paraffined and examined by adding 30 cc. of nutrient agar, shaking, and incubating. Many hundreds of containers were paraffined at temperatures of 5° intervals between 71° C. (160° F.) and 88° C. (190° F.). While most of the containers were free from living cells, a few showed living cells at all temperatures of paraffining. It should be emphasized that a very heavy inoculation and a very rigorous method of testing for viable cells was used.

To show the effect of strength of inoculation, another of Prucha's experiments may be quoted. When he used a suspension containing only two million bacteria per cc. and paraffined the bottles at 77° C. (170° F.), 82° C. (180° F.), and 88° C. (190° F.), not one bottle showed the presence of viable cells. A suspension of two million cells would infect a container with many more bacteria than would ever be found on a paper milk bottle. Prucha realized this fact, for he gave up this method of inoculation, finally resorting to inoculation of the container by dipping his hand into a suspension and then rubbing the inside of the container.

In the light of the above experiments, the temperatures used in the application of paraffin to food containers, namely from 175 to 185° F., would appear to provide adequate sterilization of these containers. Additional experiments as described later have been conducted which still further support that view.

#### Paraffin Characteristics

Paraffin wax which may be obtained either as crude scale or as fully refined paraffin has many characteristics which make it a suitable covering for food containers. These have been mentioned by Tanner (1938).

(1) Paraffin contributes to rigidity and strength.

The presence of paraffin on paper contributes greatly to the stiffness and shape of a container made from it. The higher the melting point of the wax, the greater the rigidity of the container. These characteristics are related not only to the melting point of the wax but also to the amount which is put on the paper. This is controlled in part at least by the temperature of paraffination; higher temperatures of paraffination permit greater "run-off" with less paraffin remaining on the paperboard. It is desirable that some middle ground be established with respect to the temperature of paraffination so as to achieve a satisfactory sterilization and at the same time allow

the container to undergo satisfactory waxing, both for rigidity and moisture protection.

(2) Paraffin improves appearance and gives a brilliant finish.

Waxed paper and paperboard has an appearance and finish not possessed by unwaxed paper. Paraffin wax brings out the colors and gives the entire package an appeal which is not possessed by unwaxed packages.

(3) Paraffin makes an hermetical seal possible.

Food packages may be sealed when paraffined or wrapped in paraffined paper.

(4) Fully refined paraffin is free from odor, color, and taste.

Fully refined paraffin wax is chemically inert. It is odorless, colorless, and tasteless itself but may pick up odors unless it is properly stored.

Below 190° F., the various grades of paraffin are stable toward both alkalis and acids. Chlorine combines slowly at room temperatures — more rapidly at higher temperatures. At ordinary temperatures paraffin is relatively stable toward oxidation, whereas at higher temperatures, oxidation may occur to yield products which may be detected in the wax by their characteristic odor. Below the waxing temperature, the rate of oxidation is slow; above the waxing temperature, the rate increases. Paraffined containers coated with oxidized wax may possess an undesirable odor and impart a taste to the contents. Proper "housekeeping" and temperature control will keep this at a minimum; in this connection, consideration should be given to the elimination of copper from the materials used in constructing a paraffining plant, for copper has a marked catalytic effect on the paraffin oxidates. Various forms of dirt and impurities in the paraffin may have the same catalytic effect.

(5) Paraffin is almost inert to bacterial attack.

Paraffin wax is very resistant to attack by micro-organisms. Those which are able to decompose it are uncommon soil types which would not be found ordinarily in foods or in the papermill. Furthermore, they act very slowly and could not be of any significance in the food industry.

(6) Paraffin is free from micro-organisms.

Paraffin is such an inert substance that it will not in itself support bacterial growth. It is subjected to the action of such heat both during preparation and while being applied to the paper that micro-organisms would be killed. It is usually molded into large cakes in the refinery by being run while hot into molds. These cakes are then wrapped in muslin-lined burlap bags or packed in sealed cardboard cartons when intended for use in the food industry. This keeps the paraffin clean. In one case known to the authors, the paraffin is transported from refinery to the factory, where it is applied to the paper, in insulated tank trucks; it is stored in heated tanks until it is used. Such a practice permits use of a paraffin which is clean and free from foreign matter. In addition to this, fully refined paraffin is treated with sulfuric acid which assists in destroying micro-organisms.

Many attempts have been made to find living bacteria in paraffin. They have resulted uniformly in

A number of specific references have been cited in which are discussed in detail the physical characteristics of paraffin: U. S. Bureau of Mines Bulletin 368, 1925, Paraffin Wax and its Properties; Bureau of Mines Bulletin 388, 1935, Manufacture of Paraffin Wax from Petroleum; Journal of the Institute of Petroleum Technology, Volume 12, 1926, J. A. Carpenter, The Physical and Chemical Properties of Paraffin Wax; Composition and Crystal Form of Petroleum Waxes, Ferris, Cowles & Henderson, Industrial and Engineering Chemistry, Volume 23, No. 6, 1931; Petroleum Waxes, Their Properties and Uses, F. W. Paget, Oil and Gas Journal, February 3, 1938.

negative results. While these results may be partially attributed to lack of a satisfactory technique, it is inconceivable that bacteria could live in solid paraffin wax.

(7) Paraffin is available with various melting points.

Waterproofing agents are used in the food packaging industry under many conditions and on various materials. Consequently they must be applied in different ways. Paraffin is available with different melting points, making it possible to vary its application with the season and other factors. Addition of higher melting point waxes is also possible.

(8) Paraffin is a waterproofing material.

The moistureproofing properties of paraffin are well known. When added to paper, it gives a product which prevents the passage of water in a relatively satisfactory manner.

(9) Paraffin is an important agent in producing a sterile food container.

Paraffination under conditions which can obtain in making paper milk containers involves two separate actions on bacteria. First, it covers and imprisons the bacteria if any are present on and in the paper. Second, when paraffining is done at 73.9° C. (165° F.) to 82.2° C. (180° F.), which is probably the best temperature range for paraffining, it does destroy a very high percentage of the miscellaneous kinds of bacteria, corresponding to the process of pasteurization and sterilization as defined in dairy industry. Paraffination as now accomplished almost completely eliminates all kinds of bacteria. This has been shown by the examination of many thousands of paraffined containers in several laboratories, as N. Y. Department of Health; Agricultural Experiment Station, Geneva, New York; Department of Bacteriology, University of Illinois; American Can Company at Maywood, Illinois and Jersey City, New Jersey. Roughly stated, over 90 per cent of the containers are sterile. The severest tests reveal no bacteria in them. The few bacteria that are found in the few containers are almost entirely spore-bearing bacteria which cannot be killed by the accepted bactericidal treatment recognized as satisfactory in sanitary controls.

Paraffin is not as effective as water at the same temperature, but it has bactericidal action if the temperature is high enough. In Prucha's experiments strips of paperboard were dipped into a suspension of *Bacillus prodigiosus* of 200,000,000 cells per ml. Each strip absorbed 0.1 ml., thus being inoculated with 20,000,000 cells. These heavily-inoculated strips of paperboard were freed of all the organisms in

- 20 seconds at 100° C. (212° F.)
- 30 seconds at 93.3° C. (200° F.)
- 30 seconds at 87.8° C. (190° F.)
- 45 seconds at 82.2° C. (180° F.)
- 45 seconds at 87.8° C. (190° F.)

In another experiment 1200 containers were handled in the milk plant before paraffining by an operator whose hands were heavily infected with *B. prodigiosus*. The bacterial suspension had about 150,000,000 cells per ml. Four hundred containers were paraffined at each of three temperatures, namely, at 76.7° C. (170° F.), 82.2° C. (180° F.), and 87.8° C. (190° F.). Only one container showed the presence of the inoculating organisms. In another experiment, the containers before paraffining were inoculated by dipping one's hand in a bacteria suspension and rubbing with it the inside of the unparaffined containers. In the

experiment 100 containers were paraffined at 71.1° C. (160° F.), 100 at 76.7° C. (170° F.), and 100 at 82.2° C. (180° F.). The bacterial suspension had 50,000,000 cells per ml. At 71.1° C. (160° F.), 91 containers were free from the bacteria and in 9 containers they survived. At 76.7° C. (170° F.), 99 containers were free from the bacteria and one was positive. At 82.2° C. (180° F.) all 100 containers were free from the inoculating bacteria.

#### The Significance of the Term "Sterilization" in Connection with Food Containers

Food containers should be clean and carry a reasonably low number of bacteria. There is no need for them to be sterile in the surgical sense, i.e., absolutely free from all living micro-organisms, because few foods are sterile. Other conceptions of the term "sterile" have grown up in the food industry. For instance, a joint committee of the American Public Health Association in 1933 (Holmquist, Chairman, 1933) defined the term for the dairy industry as follows:

"The word 'sterilization' as used here and as it has been used for years in dairy work means bactericidal treatment resulting in the devitalization of milk-borne pathogens and the further material reduction of all other bacteria."

This is a reasonable definition and may be readily used in other parts of the food preservation industry. Hot paraffin wax is in this sense a sterilizing agent. Paper and paperboard are "sterilized" by coating with hot paraffin.

It is well to point out here that paperboard is itself "sterile" in that it contains no pathogenic bacteria but a low number of harmless species which have survived the processes of papermaking or have been picked up from the air.

Hot paraffin wax shows such germicidal properties when applied even to inoculated paperboard, that reliance should be placed on it to help produce a sterile container.

Where paraffin is applied to regular stock with the few bacteria which it contains, a paraffined paperboard is secured which is practically devoid of micro-organisms. Only harmless spore-forming bacteria survive and these in very low numbers. Containers made of paraffined paper and board carry practically no bacteria while foodstuffs placed therein may contain many thousands. For instance the milk ordinances of some cities permit 30,000 bacteria per ml. in milk. A quart of such milk may then have over 27,700,000 bacteria. The container has usually only one or two which are available to the milk.

#### Bacteria in Paraffin Baths

When the bactericidal properties of hot paraffin wax became of interest, attention was turned to the presence of living micro-organisms in paraffin baths. It was suggested that if these baths became infected with bacteria, these would be picked up by the paper passing through the bath subsequently. Consequently, bacteriological examinations have been made of several paraffin baths on machines in commercial operation. In one instance over 100 samples were collected every 15 minutes from the paraffin bath on a machine on which paper milk bottles were being waxed. The temperature of the bath was 78° C. (172° F.). In only one culture tube did growth appear, and this may reasonably be attributed to contamination, for all of the inoculations had to be made beside the machine under conditions which were unfavorable for bacterio-

logical work. These experiments have also been repeated under other conditions with different machines. All of the cultures remained sterile, indicating the absence of living bacteria in paraffin baths which are operating at temperatures of 71° C. (160° F.) and above.

Additional information on this topic has been made available by Dr. Wheaton of the American Can Company.\* In extensive experiments on the germicidal effect of hot paraffin wax when used for coating paper milk containers, Dr. Wheaton passed containers which had been heavily inoculated with *Escherichia coli* and those which were not so inoculated alternately through a paraffin bath. He desired to determine whether a paraffin bath operating at 79.5° C. (175° F.) could be seeded with bacteria and whether containers could be contaminated with such bacteria. He found that the uninoculated containers could not be contaminated in this manner and that even the inoculated containers were almost completely sterilized.

#### Experimental Work on the Germicidal Effect of Hot Paraffin

To return again for a moment to the work described in the historical section, much thought was given to the possible effect of hot paraffin wax on micro-organisms. Under the conditions which obtained in these experiments results were secured which seemed to indicate that hot paraffin could not be depended upon to show marked germicidal power. Later it was learned that these experiments created conditions which did not simulate practical conditions. The inoculum was much larger than should have been used, and the results were always qualitative. When a smaller inoculum was used, even then much larger than would be expected in practice, the hot paraffin wax did show a decided germicidal action. Attempts to follow the lethal action of hot paraffin quantitatively showed a very rapid decline in viable bacteria with complete destruction in most cases in a few seconds.

In addition to the actual lethal properties of hot paraffin wax, there are those which are physical and mechanical in nature. Paraffin is known to "imbed" or "cover up" bacteria so that, should they be present on the base over which it is spread, they cannot be released. The inability of bacteria to pass through thin paraffin films has been demonstrated in the laboratory. Pure cultures of seventeen bacteria were used. In the first experiments a water suspension of the organisms was sprayed into the bottom of a sterile Petri dish; this was then dried in a vacuum desiccator. After complete desiccation had been attained, sterilized paraffin at 74° C. (165° F.) was carefully poured into the dish and allowed to harden. Sterile swabs were then rubbed at intervals over the surface of the hardened paraffin to determine whether the bacteria penetrated the paraffin films. In no case could presence of viable bacteria on the surface of the paraffin be proved.\*

In other experiments, lightly vaselined glass rods were dipped into molten paraffin held at 74° C. (165° F.). After hardening, the thin paraffin sack was slipped off and placed in 70 per cent alcohol to harden. It was then placed in 5 per cent phenol for sterilization and rinsed with sterile water. This sterile paraffin sack was then slipped over the end of a sterile glass

tube and sealed with more paraffin. It was finally filled with sterile broth and suspended through a cotton stopper in a flask of broth. The broth in the sterile paraffin sack was next inoculated with a pure culture of the seventeen bacteria used in the experiments mentioned above. After 48 hours, the broth in the flask was examined bacteriologically for the presence of cells of the test organism. None were found which would indicate that bacterial cells cannot penetrate paraffin films.

Many experiments have also been carried out on the effect of hot paraffin wax on bacteria on both strips of paperboard and on formed paper milk containers. The paperboard strips were heavily inoculated with various test organisms and, after drying, heated in water and paraffin at different temperatures.

Use of strips has several objections which greatly affect the value of results. The ratio of cut edge to surface area is too large. When such strips are dipped into the suspension of the test organism, the interior of the board may soak up large numbers of cells, thus giving an inoculation within the paper which is not realized under practical conditions. When such strips are paraffined, air bubbles are forced from the interior of the paper. Bacteria are forced out in the same manner. This is indicated by the fact that when such strips are cultured in agar medium, growth may be profuse around the edge of the strip but may be entirely absent on the board. Rice (1930, 1932) reported the same observations in his work with milk bottle caps.

It has been our desire to study the effect of hot paraffin wax on bacteria under conditions which existed in actual practice. In view of this fact, most later work has been done with formed paper containers made for milk. These were made of paperboard with a very low bacterial content and consequently had to be inoculated with test bacteria. The results of one extensive experiment carried out early in 1939 will be briefly reported here.

Half-pint milk containers were heavily inoculated with *Escherichia coli* and an aerobic spore-forming species. The containers were inoculated by filling with a heavy suspension of pure cultures and then emptying. By weighing, it was estimated that each container had received over 200,000 of the test bacteria. The containers were then dried for two and one-half hours at 90° F. to remove excess moisture. The bottles were then carefully paraffined at 74° C. (165° F.) and 79.5° C. (175° F.). The time involved was 20 seconds preheating, six seconds filling, 3, 5, and 7 seconds immersion time, 17 and 28 seconds draining time. The draining temperature was the same as the preheat period. Everything was as carefully controlled as possible. The cooling temperature was 10-15.5° C. (50-60° F.). The bottles were examined in different ways. Some were dissected under aseptic conditions, portions of the paperboard being disintegrated for analysis. Others were filled with milk and plain broth and stored at room temperature and at 4.5° C. (40° F.).

The disintegration tests showed that *Escherichia coli* did not survive the treatment briefly outlined above. No cells were viable when paraffination was done at either 74° C. (165° F.) or 79.5° C. (175° F.). Even with a short immersion time of only 3 seconds, *Escherichia coli* did not survive. Milk and plain broth from bottles (70) which had been inoculated with *Escherichia coli*, paraffined at 74° C. (165° F.), and stored at 4.5° C. (40° F.) and 21° C. (70° F.) did

\*Private communication

\*We are indebted to Mr. R. A. Kaberg for these results.

not yield this organism in a single instance. Milk from bottles paraffined at 76.6°C. (170° F.) yielded *Escherichia coli* in but few instances. The bottles which were filled with plain broth were entirely free from the organism. Bearing in mind that in such work much heavier inoculations are used than would be encountered in actual practice, the results indicate that paraffination of paper is distinctly germicidal and a practice with real sanitary advantages.

The aerobic spore-forming bacterium was used because it should have survived the treatment. It was used as a control. However, it was destroyed with almost the same regularity as *Escherichia coli*, showing again that paraffination is a germicidal process.

The authors wish to stress the importance of good technique and especially good logic in experimental work on the effect of hot paraffin on micro-organisms. To use a heavy suspension of cells much heavier than the number which would be found in practice, renders the experiments of little practical value. Quantitative results should be sought, for qualitative results might only show that one cell had survived to start growth in the culture tube.

Another warning should be given. To sterilize paperboard by high pressure steam and then subject it to paraffination, gives results of questionable value. Steam sterilization changes the nature of the board and causes it to accept paraffin in a much different manner than regular untreated board. If results of experiments in our laboratories are to be of help in practice, they must be secured under conditions which obtain in practice.

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## Licanic Acid of Oiticica Fat and a Study of Its Nutritive Value and Efficiency

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### Introduction

Within the past few years considerable attention has been devoted to a newly discovered oil bearing the native name "oiticica" or "oilzika" which is being extracted on a commercial scale from the nuts of a Northeast Brazilian tree, commonly referred to as *Licania rigida*, Bentham, of the Rosaceae family (13, 20). For botanical information concerning the tree and for a discussion of the commercial production of the oil, its properties and utilization in the manufacture of paint and varnish, the writings of Gardner (13) should be consulted. This oil is of special chemical interest because of an exceptionally high content of the glycerides of an unsaturated ketone fatty acid recently named "licanic acid."

As generally used the designation "oiticica fat" refers to the material obtained by pressing or by solvent extraction of the kernels in contra-distinction to the term "oiticica oil" which refers to the so-called polymerized product of commerce obtained by heat treatment of the oiticica fat. The fat, as Brown and Farmer (8, 9) have recently shown, normally contains the alpha-licanic acid while the oil, or the extracted material from old nuts (36), contains pre-

dominantly the stereoisomeric beta-licanic acid. Oiticica fat has already been subjected to some chemical investigation and it is proposed to continue research on one of its principal components, alpha-licanic acid.

The problem of the nutritive value of licanic acid was suggested by Alfred Rheineck of Devoe and Reynolds Company who kindly supplied for investigation enough of the acid, isolated and carefully protected from oxidation by the Brown and Farmer method (8). A neutralization equivalent of 295.0 obtained in our laboratory was practically identical with the theoretical molecular weight of licanic acid, 295.2.

### Review of Earlier Work on Oiticica and Licanic Acid

The earliest chemical investigation of oiticica fat appears to have been made during the first months of 1917 by Bolton and Revis (5), whose work showed that the fat was highly unsaturated. Additional characteristics were not reported until 1929 (2, 37, 59).

Van Loon and Steger (56, 57, 58) apparently were the first to study the fatty acid composition of oiticica fat. They claimed that the high refractive index and high iodine number were due to the presence of a geometrical isomer of elaeostearic acid belonging to a double conjugated system of double bonds. This acid they named "couepic" from *Couepia grandiflora* with

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