PHOTOCHEMICAL STUDIES OF RANCIDITY: THE MECHANISM OF RANCIDIFICATION*

By MAYNE R. COE
FOOD RESEARCH DIVISION, BUREAU OF CHEMISTRY AND SOILS, U. S. DEPARTMENT OF AGRICULTURE, WASHINGTON, D. C.

Abstract

A theory is set forth for the mechanism of rancidification which is based on a disrupted photosynthesis in the case of vegetable oils and on the photosensitizing action of haemoglobin or other animal pigment that may be present in small amounts in animal fats such as lard. Nascent hydrogen is believed to be liberated from the photosensitizer (chlorophyll or animal pigment) which unites with molecular oxygen to form loosely combined or nascent hydrogen peroxide. This unstable peroxide unites with the unsaturated bond of the tri-glyceride to form a glyceride peroxide which in turn splits into an aldehyde and forms the rancid compound.

INTRODUCTION

RANCIDITY in foods, notwithstanding the enormous amount of valuable research devoted to the subject, is still the cause of losses amounting to millions of dollars annually. Only recently has it been demonstrated that a great portion of this loss may be avoided through special packaging that excludes certain wave-lengths of light.[5 to 15] While it may be possible to save considerable sums by the use of proper kinds of wrappers for oil-bearing foods, the complete mechanism of rancidification is still unsolved and a matter of speculation.

The term "rancidity" as used in the trade and by the consumers generally refers to any off-odor noted in fats and oils. In this paper, however, the term "rancidity" is used to denote a certain type of change or spoilage in oils and fats due to molecular oxidation. Rancidity is characterized by a distinctive disagreeable odor and taste. From investigations already conducted it has been concluded that rancidity arises from the oxidation of fats by atmospheric oxygen in the presence of light or of certain metals which act as catalysts. While there are some steps in the mechanism which are still unexplainable, our experiments thus far have yielded results that bring us nearer than heretofore to an understanding of what rancidity really is.

Theory

It is the purpose of this paper to show that rancidity, in vegetable oils at least, may be correlated with the presence of chlorophyll and a disrupted photosynthesis. Protochlorophyll which is found in the seed is the precursor of chlorophyll and is naturally protected from the influence of light by the seed coating. When the seed begins to sprout or germinate, sunlight converts the protochlorophyll into the well-known green chlorophyll. The oil, which is expressed from the seed, is believed to contain part of the protochlorophyll that, in the presence of sunlight, is transformed into myriads of tiny particles of chlorophyll that act as photosensitizers.

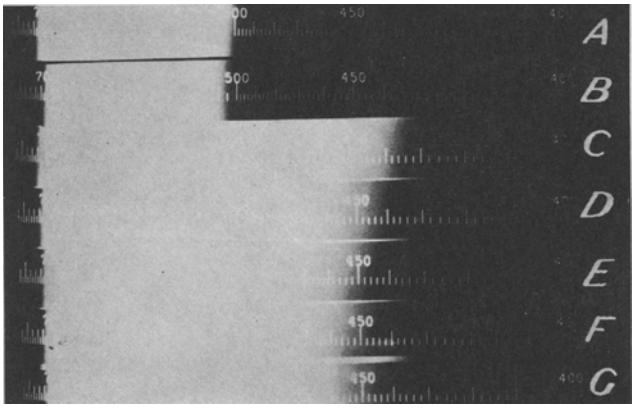

FIGURE 2

The theory relating to the development of rancidity in vegetable oils which is herein explained applies to oils that contain chlorophyll. However, it is not insisted that this explanation should apply to oils that do not contain chlorophyll. (Figure 1.)

The presence of chlorophyll in an oil is indicated by the appearance of the chlorophyll[30] band in the neighborhood of 6500 Å units and by deep absorption at the blue end of the spectrum. This may be seen when the oil is examined with a spectroscope. As the oil becomes rancid this chlorophyll absorption band disappears. (Fig. 2.)

In the course of plant metabolism—which starts with the germination of the seed—certain peroxides are known to form. Nature provides catalase in the plant which converts these poisonous peroxides into $2H_2O + O_2$. However, peroxides (including hydrogen peroxide) also

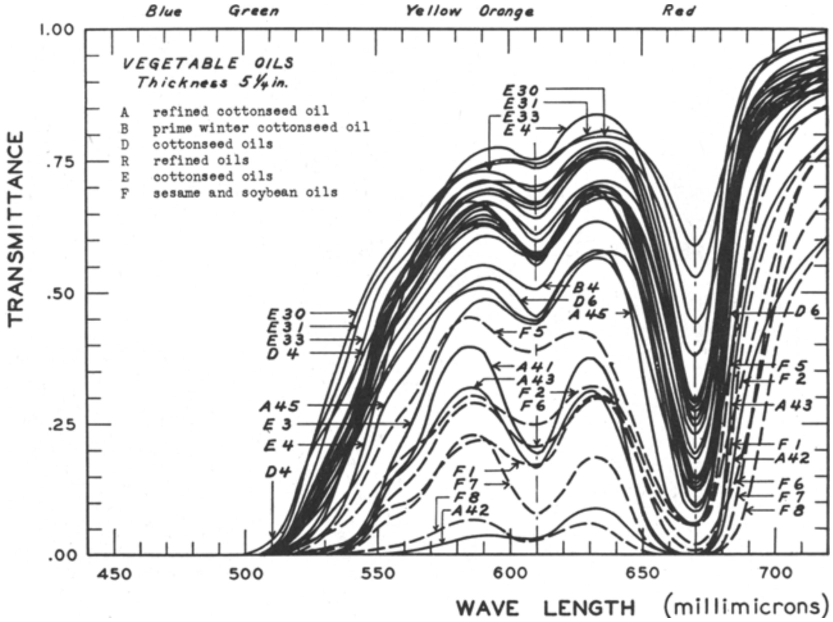

FIGURE 1.

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*Food Research Division, Contribution No. 386.

develop in an oil after it has been expressed from the seed and is exposed to sunlight but, due to the fact that little if any catalase is found in the oil, hydrogen peroxide thus formed in the oil can not be decomposed into harmless constituents and hence the oil becomes rancid. This mechanism is, for the most part an extension of previously advanced mechanisms, but departs from them in postulating HO.OH as the active oxidant in place of molecular oxygen.

Finely divided metals such as iron, copper, zinc, nickel, etc., also are considered to have the property of forming hydrogen peroxide and thus produce rancidity even in the absence of light.

Such a theory hinges the cause of the development of rancidity on the presence of nascent hydrogen peroxide in the loosely combined form HO.OH and not molecular oxygen. The organic peroxides, which may or may not be formed, do not seem to play a part until some time later when they are thought to hydrolyze[23] slowly in the presence of moisture to HO.OH. The subsequent stages then occur independently of light.

It may not be conclusively proven by the work which follows, that loosely combined OH groups in hydrogen peroxide are the primary cause of rancidity, nevertheless, this theory has been suggested by the present investigations. Rancidification may be due, first, to photochemical action closely related to photosynthesis in which nascent hydrogen peroxide is the activating compound formed by the photochemical action upon chlorophyll, second, to the presence of finely divided metals or metallic compounds, and, third, to the presence of other photosensitizers such, e.g., as may be found in lard, butter, etc.

—1—Light—

In the normal functioning of photosynthesis where light is absorbed by chlorophyll there is first an induction period: the reaction which takes place during this absorption of light may be, as suggested by Franck[19, 20, 22, 34] at one time, expressed as follows:

$$\underset{\text{(fully hydrogenated chlorophyll)}}{\text{HH chph}} + \underset{\text{(light energy)}}{h\nu} \longrightarrow \underset{\text{monodehydro-chlorophyll}}{\text{H chph}} + \text{H} \qquad (1)$$

With molecular oxygen present:

$$\text{H} + O_2 \longrightarrow HO_2 \qquad (2)$$

$$HO_2 + HO_2 \longrightarrow H_2O_2 + O_2 \qquad (3)$$

Under ordinary conditions of photosynthesis as, e.g., when a seed germinates catalase is present in the plant material to dissipate the hydrogen peroxide which is poisonous to plant metabolism, thus:

$$2H_2O_2 + \text{catalase} \longrightarrow 2H_2O + O_2 \qquad (4)$$

But when the oil is expressed from the seed the greater part of the seed constituents is left in the residue. Part of the photosensitizer, however, is extracted with the oil; this photosensitizer, viz., protochlorophyll, turns to chlorophyll upon exposure to light. Thus, when the oil is exposed to light photochemical absorption takes place in the presence of a disrupted photosynthesis mechanism. After a certain induction period, loosely combined hydrogen is liberated from the chlorophyll as the result of the absorption, and this hydrogen is believed to unite with molecular oxygen, according to equation 5, i. e., in a somewhat different manner than that illustrated by Franck in equation 3:

$$HO_2 + HO_2 \longrightarrow \underset{\text{loosely combined}}{HO.OH} + O_2 \qquad (5)$$

Experiments seem to show that the oil, separated from its natural carrier, is free of catalase; consequently the loosely combined hydroxyl groups attach themseves onto the unsaturated glyceride, thus giving rise to the rancid compound, as illustrated in 9.

In these photosensitized reactions the sensitizing substance, which is chlorophyll, acts as the light absorber and may bring about reactions of other components of the system which of themselves are not affected by light. According to the "Grotthus-Draper absorption law," only light which is absorbed can produce chemical change; in that case the active light is limited to those wave lengths which are absorbed by the chlorophyll. The absorption curves of various vegetable oils are shown in Figure I and indicate the presence of chlorophyll.

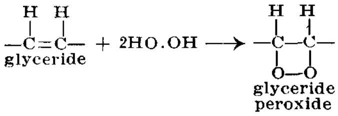
The present - day photochemical theory accepts the "Stark-Einstein equivalence law" for the explanation of the primary photochemical process; according to this law molecules absorb radiant energy in discrete quanta, the magnitude of the quanta depending upon the frequency of the absorbed light. The energy is usually expressed by hv, where "h" is Planck's constant and "v" is the frequency of the absorbed radiation. According to Blum[1] the first act in the process involves the activation of the sensitized molecule S. to give the activated molecule S':

$$S + h\nu \longrightarrow S'$$

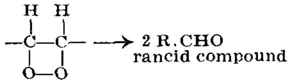
In the equations which follow this same reaction occurs in the presence of O, (Blum and Spealman[2]):

$$\text{HH chlorophyll} + h\nu \longrightarrow \text{nascent H} + \text{H chlorophyll} \qquad (6)$$

$$\text{nascent H} + O_2 \longrightarrow HO.OH \qquad (7)$$



$$\underset{\text{glyceride}}{-\overset{H}{\overset{|}{C}}=\overset{H}{\overset{|}{C}}-} + 2HO.OH \longrightarrow \underset{\text{glyceride peroxide}}{-\overset{H}{\overset{|}{C}}-\overset{H}{\overset{|}{C}}-} \qquad (8)$$



$$-\overset{H}{\overset{|}{C}}-\overset{H}{\overset{|}{C}}- \longrightarrow \underset{\text{rancid compound}}{2\,R.CHO} \qquad (9)$$

In bringing about the rancid compound the activated molecule, which is chlorophyll, appears to transfer its activation through free hydrogen to molecular oxygen which (giving rise to hydrogen peroxide in the loosely combined HO.OH state) is able to act chemically with the unsaturated glyceride. Therefore, the number of the activated chlorophyll molecules which actually take part in this chemical reaction subsequent to both the primary and the final reaction is determined by the concentration of the chlorophyll present. According to Beer's law light absorbency is proportional, within limits, to the concentration of the absorbing substance, chlorophyll, and it should be expected that this reaction would proceed the faster the greater the concentration of the sensitizer up to the point of complete absorption, since a greater number of chlorophyll molecules would be excited. However, according to Lambert's law, the intensity of light transmitted by successive layers of oil diminishes exponentially, and consequently the greatest absorption must be in the layer of oil nearest the source of radiation. Hence, in oils having an excessive amount of chlorophyll the greater part of the absorption will take place in the surface layer only.

On the other hand, refined, bleached and deodorized oils are known to become rancid much quicker than crude oils and even unbleached refined oils, and this may be attributed to the fact that in these refined oils the concentration of yellow or brown coloring pigments has been so reduced as to permit penetration of the actinic light to greater depths in the oil, thus exciting a greater number of chlorophyll molecules and therefore bringing about rancidity more quickly. One of the limiting factors in the development of rancidity in deep layers would be the amount of oxygen occluded in the oil since, according to Blum[1], considerable evidence is at hand to attribute photodynamic action to the presence of molecular oxygen.

Dognon[16] points out that there is

a linear relationship between photodynamic activity and intensity of radiation at a given concentration of chlorophyll which may explain the variations in rancidity susceptibility. Since the present theory of rancidification hinges on the formation of hydrogen peroxide in the loosely combined state, hydroxyls are then able to react with unsaturated glycerides to form the "rancid compound."

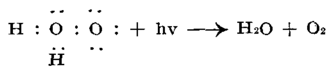
Gaffron[24] suggests that the sensitizer may act as an oxygen transporter to the acceptor, the latter being, in the case of oils, the unsaturated glyceride. He has[25] also quite definitely shown that peroxides are formed when chlorophyll in solution in isoamyl-amine is irradiated. The oxygen absorption during irradiation was measured by means of Warburg's oxygen absorption method. Gaffron found that the quantity of peroxide (hydrogen peroxide and organic peroxides) formed might be many times the amount equivalent to the quantity of chlorophyll present; this means that only a small amount of chlorophyll is necessary to bring about the reaction and that seems to be true with the development of rancidity in the case of a refined and deodorized oil.

It is very possible that three kinds of peroxides are formed when an oil is exposed to sunlight, namely, HO.OH active, H_2O_2 and organic peroxides both inactive. Blum and Spealman[2] have shown that H_2O_2 is formed when fluorescent dyes are irradiated alone in aqueous solution, which is additional evidence that hydrogen peroxide is one of the early steps in the process of autoxidation. Milas[32] states that hydrogen peroxide is known to be formed by the addition of hydrogen to molecular oxygen. The electronic structure of hydrogen peroxide which appears to favor this mechanism of rancidification may be illustrated as follows:

$$\mathrm{H:\overset{..}{\underset{..}{O}}:\overset{..}{\underset{..}{O}}:H}$$

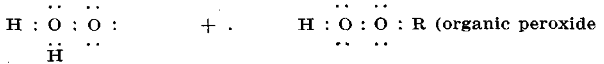
(active)

Here the OH groups are loosely combined as the peroxide is formed and due to their affinity for the unsaturated bond they separate and unite with the glyceride and produce the rancid compound. It is conceivable that not all of the peroxide will have that configuration because it is known that hydrogen peroxide under exposure to light decomposes to water and oxygen which reaction may be illustrated thus, after Milas[32]:



$$\mathrm{H:\overset{..}{\underset{\ddot{H}}{O}}:\overset{..}{\underset{..}{O}}: + hv \longrightarrow H_2O + O_2}$$

The electronic structure of both peroxides developed under a green filter may be illustrated as follows:

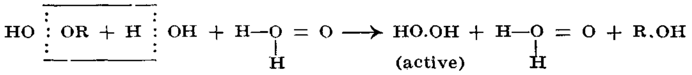


$$\mathrm{H:\overset{..}{\underset{\ddot{H}}{O}}:\overset{..}{\underset{..}{O}}: \qquad + . \qquad H:\overset{..}{\underset{..}{O}}:\overset{..}{\underset{..}{O}}:R}\ \text{(organic peroxide}$$

(in negligible amounts (inactive)

in large amounts (inactive)

In this inactive state the oil does not form the rancid compound and appears fresh. However, upon long standing under green or under total exclusion of light rancidity may develop due to the instability of the organic peroxide[23] as illustrated:



$$\mathrm{HO\,\boxed{\vdots\;OR + H\;\vdots}\;OH + H{-}\underset{\overset{|}{H}}{O} = O \longrightarrow \underset{(active)}{HO.OH} + H{-}\underset{\overset{|}{H}}{O} = O + R.OH}$$

Thus, in rancidification, a portion of the active HO.OH goes to form the rancid compound and the rest forms stable H_2O_2 which usually decomposes to form $2H_2O + O_2$. This happens under full exposure to light. Under green filter protection mostly organic peroxides develop which may break down to active loosely combined HO.OH and H_2O_2 the latter being inactive.

—2—Metals—

Continuing along the same idea relative to the activity of loosely combined hydrogen peroxide in the development of rancidity, attention is directed to the role of metals in bringing about this form of spoilage.

King, Roschen[27] and Irwin, among many others, have noted the catalytic influence of certain metals on the development of rancidity and Emery[18] and Henley found that light in the absence of metals produced rancidity in about the same time as metals did in the absence of light. As the proposed theory implies, hydrogen peroxide (HO.OH) in the active state must be produced before rancidification can take place. The work of Kufferath and Merckens[28] in 1904 and of Merckens alone in 1905[31] supports this theory. These authors were able to obtain photographic images with magnesium, aluminum, zinc, cadmium, nickel, cobalt and lead, when these metals were placed in contact with a photographic plate in the dark. They explained the darkening of the plate by these metals on the basis that the oxidation of the active metal produced hydrogen peroxide which acted on the plate. Russell[33] attributed the action to metallic oxidation in moist air and also found that on supplying to the metallic surfaces more moisture than it would obtain from the atmosphere on the photographic plate, the activity was much increased. Dombrowsky's[17] experiments also seemed to show that metals alone were not active in the production of H_2O_2 except in the presence of moisture and oxygen. Lengyl[29] believed that hydrogen formed in the presence of moisture on the metallic surface might be considered the active agent. His idea follows very closely the photochemical action of light on chlorophyll in an oil in which hydrogen first is liberated with the subsequent formation of loosely combined hydrogen peroxide which in turn is followed by stable hydrogen peroxide. Therefore, rancidity produced in oils in the presence of metals seems to be due to the same mechanism, except that one is due to photochemical action induced by chlorophyll as a photosensitizer and the other is due to the action of metals, the end products being the same.

—3—Rancidity in Animal Fats—

In the case of churned fats, such as butter, rancidity might be developed through photochemical action due, first, to the presence of natural pigments that, being closely related to chlorophyll, act as photosensitizers and, second, to the action of metals such as copper derived from the churn. Either of these two processes will develop rancidity. The presence of traces of metals in the butter may account often times for its becoming rancid even in an opaque container.

Such fats as lard become rancid readily due to the possible presence of hemoglobin, which acts either as a photosensitizer or because it contains iron from hemoglobin in the reduced state. One important reason why lard usually becomes rancid more rapidly than do vegetable oils may be because during the cooling of the lard to a solid mass oxygen is occluded in the mass and assists light in bringing about the reaction.

Catalase[3 to 14] is believed to play an important role in preventing rancidity in such fats as fat-back and bacon, both of which contain catalase in large amounts. A certain amount of the catalase is, however, destroyed in the process of smoking. While the smoking of meat has a partial preserving effect, yet bacon, because it has lost much of the catalase present in the untreated pork, is known under suitable conditions to become rancid in a comparatively short time.

Experimental

Previous work has shown that a green filter having a light transmission delimited by 4900 to 5800 Ångström units, appreciably delays the development of rancidity. This observation suggested the further idea that green plant pigments might be present in the oil and that they might be acting as photosensitizers when the oil is exposed to light. When light was excluded entirely or by the use of a green filter these green photosensitizers ceased to function.

In an article entitled "The Color and Spectral Transmittance of Vegetable Oils"[30], McNicholas gives the light transmission curves of a number of edible vegetable oils of commerce. The regions of light absorption (Fig. 1) clearly show the presence of chlorophyll and other pigments in the oils.

In order to determine the effect of various amounts of chlorophyll in oil on the development of rancidity, experiments were carried out under CX lamps as the light-source.

To 25 cc. samples of refined and deodorized vegetable oil was added varying amounts of Mg-chlorophyll from 0.0 to 10.0 mg. The results show that rancidity may be developed with an unusually low peroxide value and that the latter cannot be correlated with the degree of rancidity. The striking features of these experiements were, first, the rapidity with which rancidity developed, and, second, the fact that the peroxide value remained practically the same even though there was an increase in the degree of rancidity.

Experiments were conducted with corn oil and lard which showed that H_2O_2 is formed during the development of rancidity. Metallic copper strips were placed in both of these fats together with titanium sulphate solution, the latter to serve as an indicater for the presence of H_2O_2 when the oil or fat became rancid. It was found that as soon as these fats became rancid even in the dark, H_2O_2 was formed in sufficient amounts to give the characteristic yellow color to the titanium sulphate solution. However the test was not obtained when corn oil without metal strip was protected by a green filter even though organic peroxides were present. These experiments seem to indicate that hydrogen peroxide is formed during autoxidation of an oil and according to our present conception, active HO.OH must have been formed also since the oil developed rancidity.

Again a sample of corn oil free of peroxides was placed in a solution of titanium sulphate and irradiated with ultraviolet light until a rancid odor could be detected. It was observed at the same time that the characteristic yellow color had developed, showing, again, the presence of hydrogen peroxide as a secondary product in the development of rancidity.

In order to further support the theory regarding the presence of hydrogen peroxide in rancid oils, the Russell effect experiment was conducted (1) with rancid oil, (2) with oil protected by sextant green filter, and (3) with fresh oil of the same lot but which had been kept in a refrigerator. It was found that the photographic film placed over the rancid oil showed a pronounced image due presumably to the presence of hydrogen peroxide, while the oil which had been protected by the green filter and the perfectly fresh oil which had been kept in the refrigerator developed little or no image on the photographic film. When a photographic film was placed over benzoyl peroxide no image was obtained, indicating that the Russell effect is not produced by organic peroxides. This accounts for the fact that an oil which has been kept in the dark and which has been subjected to the photographic film gives no Russell effect. And this is true even though the oil has developed a high peroxide value.

Experiments have shown that if hydrogen peroxide is added to an oil, there is no accelerated production of rancidity indicating that hydrogen peroxide as such is not in the reactive OH state. Furthermore, the peroxides developed under protection of a green filter are not of the reactive type; hence, they must be largely organic peroxides because they do not fog the photographic film appreciably.

Our observations show that so long as there is active catalase in an oil, rancidity does not develop, as any hydrogen peroxide that is formed is decomposed by the catalase. Only when the catalase itself has lost its activity does active hydrogen peroxide function to bring about rancidity.

The inhibiting effect of catalase toward the development of rancidity was compared with that of the well-known antioxidants and it was shown that catalase delayed the formation of peroxides more efficiently than did any of the common antioxidants except pyrogallol.*

Naturally the question arises: do oils and fats contain catalase? Numerous determinations have been made of various commercial oils and fats with the expectation of finding catalase, but no catalase has been found either in the crude oil or in lard from fat-back. However, the press-cake from which the oil was obtained or from the residue from the pressed fat did contain large amounts of catalase.

Fresh oils and fats when subjecteded to ultraviolet light fluoresce with a light bluish color while rancid oils or fats under the same conditions lose this characteristic property almost entirely and acquire a dull gray color.

In order to intensify the difference between a rancid and a fresh oil under the fluorescing screen, a solution of magnesium chlorophyll in a non-fluorescing mineral oil was added to the oil. It was found that an appreciable amount of chlorophyll solution could be added to a fresh oil before the appearance of a red glow due to chlorophyll was obtained, while only a small amount was needed to obtain the same effect with a rancid oil. Experiments were then conducted with corn oil, peanut oil and lard protected and nonprotected from light, all of which oils reacted in a similar manner.

A fresh oil containing as little as 5 per cent of a rancid oil may thus be detected by means of the fluorescing screen attached to the ultra-violet lamp.

The results obtained with the chlorophyll test are significant but no attempt is being made at this time to draw any conclusions relative to the merits of the test as a means to detect rancidity of an oil.

The amount of chlorophyll that may be added to an oil before a pink fluorescence can be obtained may be a means of indicating the degree of incipient rancidity far in advance of the actual deterioration of an oil, i.e., before rancidity can be detected organoleptically.

Discussion

The theory of rancidity of an oil or fat, as heretofore understood, acquires a new angle of interpretation in view of the theories already men-

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*Unpublished data.

tioned and the experiments here recorded. It has already been revealed that a certain green filter affords a better protection against the development of rancidity than any other filter. This is because oils and fats contain photosensitizers that absorb light in the ultraviolet, violet, blue, orange and red regions and that they contribute to the development of rancidity. A sextant green filter interposed between these light-absorbing elements and the source of light retards photochemical action. That rancidity is developed by the presence of a photosensitizer in the oil or fat, is borne out by the experiments in which magnesium chlorophyll was added in varying amounts to samples of oil.

The striking features about these experiments with magnesium chlorophyll are the rapidity with which rancidity develops when even small amounts of chlorophyll have been added and the extremely low peroxide value of the oil at the moment the oils become rancid.

The experiments which indicate that hydrogen peroxide is formed during the development of rancidity appear to support the theory that active hydrogen peroxide as HO.OH must be produced in order for an oil to become rancid. The Russell effect obtained by exposing a rancid oil to a photographic film in the dark shows further evidence that active HO.OH may be formed during rancidification.

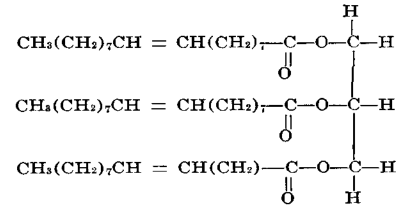
Still more evidence is embodied in the behavior of metals in oils and fats. Both the titanium sulphate test and the Russell effect give positive tests for the presence of hydrogen peroxide when an oil or fat becomes rancid.

Perhaps the most striking evidence of the presence of hydrogen peroxide in the form of HO.OH and H_2O_2 in an oil developing rancidity is the fact that catalase (added to an oil in the form of cheese, oats, wheat germ, yeast and in a concentrated solution, etc.), suppresses the peroxide value of the oil and appreciably delays the development of rancidity in a way similar to that of an antioxidant. Substances that carry catalase (such as rice bran, rice polish, pecans, soybeans, peanuts, etc.) seem to become rancid when the natural controlling factor, viz., the catalase, is destroyed either by heat or by conditions which accompany rancidification.

All efforts to determine catalase in oils and lard failed to show that catalase is present in an oil even in a hand-pressed crude oil. However, catalase was found in the press cake and animal tissue in large amounts.

All of the experiments mentioned above seem to support the theory that rancidity is induced by liberated hydrogen uniting with molecular oxygen to form both loosely combined hydrogen peroxide and stable hydrogen peroxide. The former, being active, attaches itself to the unsaturated bonds of the glyceride and produces rancidity. The free hydrogen may be produced in two ways, either by the photochemical action of light, which follows the general idea of photosynthesis, or by the presence of certain metals. In each case the mechanism of rancidification appears to be the same.

The following equations illustrate graphically the reactions which are believed to take place during the development of rancidity:

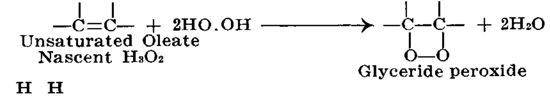


= Unsaturated tri-glyceride, a component of all vegetable oils. (10)

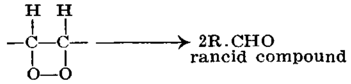
Protochlorophyll + Sunlight ⟶ Protochlorophyll changes to chlorophyll HH Chlph (11)

Chlorophyll + Sunlight + O_2 ⟶ Nascent H + H Chph monohydrogenated chlorophyll (12)

Nascent H + O_2 + Sunlight ⟶ HO.OH + H (13)

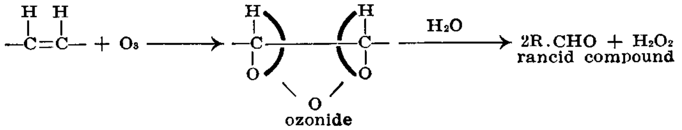


$$\underset{\text{Unsaturated Oleate}}{-\overset{\text{H}}{\underset{}{\text{C}}}=\overset{\text{H}}{\text{C}}-} + \underset{\text{Nascent H}_3\text{O}_2}{2\text{HO.OH}} \longrightarrow \underset{\text{Glyceride peroxide}}{\underset{\text{O}-\text{O}}{-\overset{\text{H}}{\text{C}}-\overset{\text{H}}{\text{C}}-}} + 2\text{H}_2\text{O} \qquad (14)$$



$$\underset{\text{O}-\text{O}}{-\overset{\text{H}}{\text{C}}-\overset{\text{H}}{\text{C}}-} \longrightarrow \underset{\text{rancid compound}}{2\text{R.CHO}} \qquad (15)$$

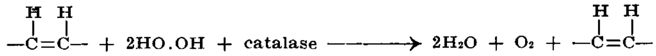
It is possible that under the action of sunlight $+O_2$, ozone O_3 is formed; the action of the ozone is illustrated as follows:



(16)

This reaction does not seem to be the usual step in the development of rancidity.

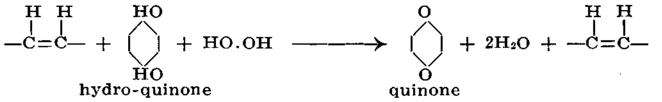
The equations above illustrate the behavior of a refined and deodorized oil; the following equations show our conception of the behavior of these same oils in the presence of antioxidants:



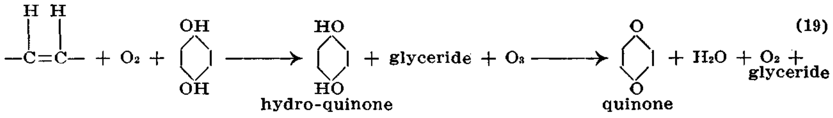
$$-\overset{\text{H}}{\text{C}}=\overset{\text{H}}{\text{C}}- + 2\text{HO.OH} + \underset{\text{(antioxidant)}}{\text{catalase}} \longrightarrow 2\text{H}_2\text{O} + \text{O}_2 + -\overset{\text{H}}{\text{C}}=\overset{\text{H}}{\text{C}}- \qquad (17)$$

No rancid compound is formed until the potency of the catalase is gone, after which rancidity develops in accordance with equations 14 and 15 above.

The action of antioxidants in stabilizing an oil is further illustrated as follows:



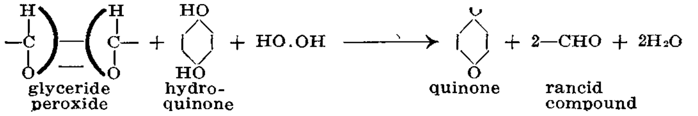
(18)



(19)

The antioxidant prevents the formation of the so-called "rancid compound" even in the presence of ozone, which latter in the absence of the antioxidant would have caused rancidity (see No. 16).

If the hydroquinone be added to an oil before rancidity has started, the development of rancidity will be definitely checked. But if rancidity has already started no such protection will be afforded (see No. 20).



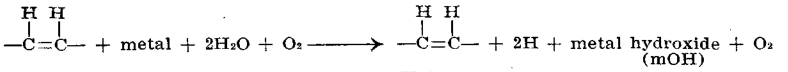
(20)

Therefore, the following is a general mechanism for the development of rancidity due to light:

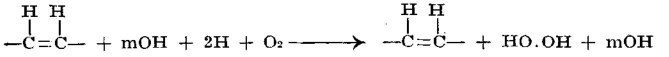
$$\text{Protochlorophyll} \xrightarrow{\text{light}} \text{Chlorophyll} \xrightarrow{\text{light}} \text{reduced Chlorophyll} + \text{free H} \longrightarrow \text{Free 2H} + \text{Molecular } O_2 \longrightarrow \text{HO.OH} \longrightarrow \underset{\text{rancid compound}}{\text{R.CHO}} \quad (21)$$

The foregoing is the theory as applied to chlorophyll which considers chlorophyll as the agent responsible for the production of HO.OH which becomes the active oxidant. It is conceived that metals also might function like chlorophyll in producing free HO.OH in which case the following equations would apply:

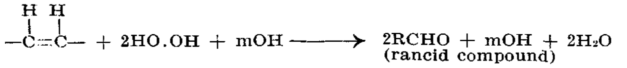
The mechanism of rancidity due to metals:



$$\overset{H}{\underset{|}{}}\!\overset{H}{\underset{|}{}}\quad -C{=}C- + \text{metal} + 2H_2O + O_2 \longrightarrow \overset{H\ H}{-C{=}C-} + 2H + \underset{(\text{mOH})}{\text{metal hydroxide}} + O_2 \quad (22)$$



$$\overset{H\ H}{-C{=}C-} + \text{mOH} + 2H + O_2 \longrightarrow \overset{H\ H}{-C{=}C-} + \text{HO.OH} + \text{mOH} \quad (23)$$



$$\overset{H\ H}{-C{=}C-} + 2\text{HO.OH} + \text{mOH} \longrightarrow \underset{(\text{rancid compound})}{2\text{RCHO}} + \text{mOH} + 2H_2O \quad (24)$$

According to what precedes, the so-called "induction period" of an oil acquires a new meaning. Formerly, it was believed that certain natural antioxidants were present which delayed the development of rancidity. According to the new theory the induction period must be attributed to the time it takes for the metal-catalyzer or photosensitizer to generate the reactive substances to a point where they react and produce organoleptic rancidity.

No attempt has been made to identify the rancid compound or compounds. A theory has been offered that seems to explain much that heretofore was difficult to account for.

Conclusions

1. A theory is offered for the development of rancidity based on the liberation of free hydrogen which unites with molecular oxygen to form loosely-combined hydrogen peroxide in the HO.OH state; this form of hydrogen peroxide unites with the unsaturated bond of the glyceride and produces the rancid compound.

2. Chlorophyll acts as a photosensitizer in vegetable oils and is believed to liberate free hydrogen under the photochemical action of light.

3. Metals are known to produce hydrogen peroxide in the presence of moisture and oxygen, and because finely divided metals in oils catalyze rancidity it is logical to believe that they produce active hydrogen peroxide of the same nature as that brought about by light in the presence of a photosensitizer.

4. The presence of catalase in an oil-bearing commodity seems to be related to the keeping qualities of that commodity and to the development of rancidity.

5. The induction period observed in oils and fats must be attributed to the time it takes for the photosensitizer or metal-catalyzer to develop the reactive substances to a point where organoleptic rancidity is formed.

6. The sextant green light filter prevents or delays the development of rancidity in oils or fats because the photosensitizer, normally present (which generates the active substances that cause the development of rancidity), is prevented from functioning by the exclusion of photo-chemically active light.

7. When substances containing catalase are enclosed in a sextant green wrapper or container, the activity of the catalase is not impaired. Under these conditions catalase prolongs the induction period of the oil or fat.

8. Animal fats become rancid because of the presence of photosensitizers which induce photochemical activity.

9. The ability of an oil to absorb added chlorophyll combined with the appearance of its fluorescence may be used to indicate the susceptibility of an oil to become rancid.

10. Organic peroxides and stable H_2O_2 do not seem to enter into the reaction during the development of rancidity and neither can they be used as a measure of the degree of rancidification.

The author wishes to express his appreciation to Mr. Wilmer C. Powick, Senior Biochemist of the Biochemical Division, Bureau of Animal Industry, and to Dr. J. A. LeClerc, Chief of the Cereal Section, Bureau of Chemistry and Soils, for their interest and helpful suggestions in preparing and reviewing the manuscript.

LITERATURE CITED

(1) Blum, H. F. 1932. Photodynamic action. Physiological Rev., vol. 12, pp. 23-55.

(2) Blum, H. F., and Spealman, C. R. 1933. Photochemistry of fluorescein dyes. J. Phys. Chem., vol. 37, p. 1123.

(3) Bodnâr, J., and Bârtfai (Bauback), J. 1936. Hitzeaktivierung der Fettkatalase. Z. Physiol. Chem., vol. 244, pp. 225-28.

(4) Clover, A. M., and Richmond, G. F. 1903. The hydrolysis of organic peroxides and peracids. Amer. Chem. Jour., vol. 29, pp. 179-203.

(5) Coe, Mayne R. 1932. Light, a factor in rancidity. Science, vol. 75, no. 1953, p. 585. June 3.

(6) Coe, Mayne R., and LeClerc, J. A. 1932. Photochemical action, a cause of rancidity. Cereal Chem., vol. 9, no. 4, p. 519, Sept.

(7) Press release, U. S. Department of Agriculture. 1934, May 24. Find blue light rays speed spoilage of foods.

(8) Coe, Mayne R., and LeClerc, J. A. 1934. Photochemical studies of rancidity. Peroxide values of oils as affected by selective light. Ind. Eng. Chem., vol. 26, no. 3, p. 245, March.

(9) Coe, Mayne R. 1934. The rancidity problem and new developments, with particular reference to the effect of light. Cereal Chem., vol. 11, no. 3, p. 241, May.

(10) Coe, Mayne R., and LeClerc, J. A. 1934. Photochemical studies of rancidity. Oil and Soap, vol. 11, no. 9, p. 189, September.

(11) Coe, Mayne R. 1934. Rancidity in foods delayed by excluding certain wave lengths of light. U. S. Department of Agriculture Year Book Separate No. 1450.

(12) Coe, Mayne R., and LeClerc, J. A. 1935. Photochemical studies of rancidity: Antioxidants vs. green light protection. Oil and Soap, vol. 12, no. 10, p. 231, October.

(13) Coe, Mayne R. 1936. Photochemical studies of rancidity: Induction period of protected and non-protected oils. Oil and Soap, vol. 13, no. 8, p. 197, August.

(14) Coe, Mayne R. 1937. Photochemical studies of rancidity: Rate of peroxide development under constant intensity of light. Oil and Soap, vol. 14, no. 7, p. 171, July.

(15) Coe, Mayne R. 1938. Uses of catalase for the preservation or delaying of rancidity. (Mimeographed sheet, U. S. Department of Agriculture.)

(16) Dognon, A. 1928. La photo-sensibilisation biologique. Influence de la concentration du sensibilisateur et el'intensite luminence. Compt. rend. soc. biol., vol. 98, p. 283.

(17) Dombrowsky, Constantin. 1908. Uber die Einwirkung der verschiedenen Stoffe inbesonders des Wasserstoffsuperoxyds auf die photographische Platte. Inaugural Dissert. Leipzig, pp. 1-68.

(18) Emery, James A., and Henley, R. R. 1922. Studies on rancidity. 1. The influence of air, light, and metals on the

development of rancidity. Ind. Eng. Chem., vol. 14, pp. 937-40.

(19) Fischer, H. 1934. Chlorophyll-A. Part 1, page 245.

(20) Franck, J. 1935. Remarks on photosynthesis. Chem. Reviews, vol. 17, no. 3, pp. 433-42.

(21) Franck, J., and Wood, R. W. 1936. Fluorescence of chlorophyll in its relation to photochemical processes in plants and organic solutions. Jour. Chem. Physics, vol. 4, pp. 551-60.

(22) Franck, J., and Herzfeld, K. F. 1937. An attempted theory of photosynthesis. Jour. Chem. Physics, vol. 5, pp. 237-51.

(23) Freer, Paul C., and Novy, Frederick G. 1902. On the formation, decomposition and germicidal action of benzoyl acetyl and diacetyl peroxides. Amer. Chem. Jour., vol. 27, pp. 161-192.

(24) Gaffron, H. 1926. Über photooxydation mittels fluorezeierender Farbstoffe. Biochem. Zeitschr., vol. 174, p. 157.

(25) ——— 1927. Die photochemische Bildung von Peroxyd bei der Sauerstoff-Übertragung durch Chlorophyll. Ber. d. deutsch. Chem. Gesell, vol. 60, p. 2229.

(26) Keenan, George L. 1926. Substances which affect photographic plates in the dark. Chemical Reviews, vol. 3, pp. 95-111.

(27) King, A. E., Roschen, H. L., and Irwin, W. H. 1933. The accelerating effect of metals on the development of peroxides in oils and fats. Oil and Soap, vol. 10, no. 6, pp. 204-7.

(28) Kufferath, A., and Merckens, W. 1904. Neue Strahlen in Harzen. Zeitschrift f. angewandte Chemie, vol. 17, pp. 1095-7.

(29) Lengyel, Bela V. 1898. Ueber die Wirkung einiger Gase und Metal auf die photographische Platte. Annalen der Physik u. Chemie, vol. 66, pp. 1162-70.

(30) McNicholas, H. J. 1935. The color and spectral transmittance of vegetable oils. Oil and Soap, vol. 12, pp. 167-78.

(31) Merckens, W. 1905. Über strahlenartige Einwirkungen auf die photographische Bromsilbergelatine. Ann. Physik., vol. 16, pp. 667-83 (4th ser.).

(32) Milas, Nicholas A. 1934. Studies in auto-oxidation reactions, VII: The dative or coordination peroxide theory of auto-oxidation. J. Phys. Chem., vol. 38, pp. 411-18.

(33) Russell, W. J. 1899. On hydrogen peroxide as the active agent in producing pictures on a photographic plate in the dark. Proc. Royal Soc., London, vol. 60, pp. 409-19.

(34) Stoll, A. 1932. Über den chemischen Verlauf der Photosynthese. Naturwiss, vol. 20, p. 955.

THE RAPID DETERMINATION OF MOISTURE

With Especial Reference to Oil Seeds and Their Products

EGBERT FREYER

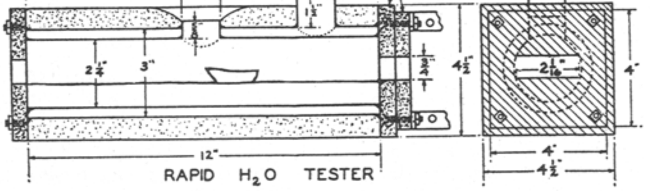
SPENCER KELLOGG AND SONS, INC., BUFFALO, N. Y.

Abstract

The principles underlying the evaporation of moisture from oil bearing materials are discussed with especial reference to rapid testing. Two forms of rapid moisture testing apparatus employing relatively high temperatures and strong induced draft, are described. Data are given to show how the results compare with determinations made in a conventional oven at the usual drying temperature, as well as a tabulation comparing the results obtained by an untrained plant operator using the rapid tester with checks on the same samples made in the laboratory. The advantages inherent in rapid moisture testing from the control standpoint are discussed. Finally, the application of the apparatus as a controlled-heat hotplate is suggested.

THE American Oil Chemists' Society's concern in moisture testing, officially, is to have a method that will yield uniform results in different laboratories, and require only a reasonable time for the determination. It is also desirable that the results approximate as closely as possible the actual moisture content of the material tested. If our requirements in this latter respect should be too exacting; that is, should we insist that the method show absolute moisture content, then the question of uniformity of results would be completely satisfied, but at the sacrifice of the second requirement —reasonably short testing time. Absolute moisture content of organic materials like seed and seed meals may be indicated only by slow desiccation at low temperatures or moderate temperatures under vacuum. So for practical reasons we vote to content ourselves with empirical results and adopt testing conditions that minimize the loss of volatile matter not water and the decomposition of the dry material. When we admit a certain amount of empiricism into our results, as we so often must do, it becomes necessary to know what conditions cause any deviation of the results from the absolute and to so state the method and its alternates that uniformity of results is preserved, with particular respect to the allowance of alternate methods. Thus, in view of the effect on the rate of drying of the vapor pressure of the air over a material being dried, we are careful to consider maximum oven load and oven ventilation. Of especial importance in this connection is the forced draft principle. Through its employment the layer of air directly over the test material is continually removed, facilitating and accelerating the evaporation of moisture; so that at a given temperature a sample tested in a forced draft oven will lose its moisture in a shorter time than in a conventional type of oven. Moreover, on the reasonable assumption that decomposition of solid matter is slower than and lags behind the evaporation of moisture, we would expect that the shorter test for the same temperature would be more nearly in agreement with the true moisture content. In other words, the forced circulation has somewhat the effect of a moderate vacuum.

As to how much of the weight loss during drying is due to the loss of matter not water, we may form a qualitative idea from the shapes of the drying curves; and some of these curves for reasonably low temperatures indicate that while moisture is still present little or no loss of other matter occurs, but that after the material has completely dried, further weight loss begins and continues, the rate of loss being greater of course at more elevated



END PLATES OF BRASS (18 GAGE) AND ½ INCH TRANSITE HAVE OPENINGS CUT AS SHOWN. BRACKETS ARE BOLTED ON ONE END FOR FASTENING ON SIDE OF DESICCATOR BOX (SHOWN) WHICH IS TO BE MADE FROM ¼-INCH MASONITE-PRESSBOARD. BOTTOM TO BE GLUED ON AND TOP TO BE REMOVABLE ¼-INCH PLATE GLASS WHICH IS TO BE FLUSH WITH LARGER TRANSITE END BLOCK. 2¼-INCH AND 3 INCH (18 GAGE) BRASS TUBING ARE STOCK SIZES. FOR BRAZING USE SILFLOSS 1/16-INCH RODS AND FLUX.

FIGURE 1