Carotene As a Natural Anti-Oxidant

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IT is not possible to obtain accurate data showing the losses incurred annually from the oxidation of fats and oils. If we may judge from the frequency of observed cases of this deterioration, however, we are led at once to the conclusion that the problem of rancidity is of tremendous scope. There are used in the United States every year, more than six billion pounds of fats and oils for edible purposes, and several billion pounds of other fats and oils for the soap industry, etc. These figures are in addition to the tremendous quantities of fats occurring naturally in meats, nuts, milk, and other foods. Every pound of this fat is subject to the deterioration caused by oxidation at some stage of its collection, storage, refining treatment, and distribution.

The chief manifestations of deterioration in fats are the development of off flavors and odors. These off flavors are often referred to as "flavor reversions" in products which have been previously refined and deodorized. Flavor reversions, other than rancidity, I believe are due particularly to impurities remaining in the fats, although they may be due, to a lesser extent, to reversible polymerization reactions of the fatty acids and the fats themselves. The impurities which cause these flavor reversions are, perhaps, gums, resins, and volatile constituents of the oils. It is logical to consider all substances present, except the fatty glycerides, as impurities.

Of all the different types of natural fats which have been subjected to experimental work in our laboratory, there are very few, if any,[1] of them entirely free from some kind of flavor or odor reversion after the usual refining treatment. It happens that some of these flavor reversions are particularly obnoxious, and in such cases, such oils or fats take an inferior position on the market.

Another cause of deterioration in fats, which is perhaps much less common than the characteristic flavor reversions, is that of rancidity from oxidation of the unsaturated fats and fatty acids. It is this type of fat spoilage which is to be considered in this paper.

Although the cause and mechanism of typical rancidity may be different than that of flavor reversion, the two types of deterioration are equally serious from the losses incurred when the fat is rejected from public flavor. The cause and prevention of oxidation of rubber, soap, mineral oils, gasoline, etc., as well as of fats and oils, have been given considerable study during the past ten years. Although much earlier work is of record, the real incentive for the intense studies during these later years comes from the classical work of Moreau and Dufraisse, which indicated the possible advantages to be obtained from the use of proper anti-oxidants.[2]

THERE are many theories of the mechanism of the oxidation reaction which brings about this typical, rancid odor.

The theory suggested by C. Engler and J. Weissberg in their *"Kritische Stutiem über die Vorgänen der Auto-oxidation," Braunschweig* (1904), and independently by Bach (*Compt. Rend.*), is supported by some evidence which shows that oxygen binds itself to the auto-oxidizable substance, as molecular oxygen,

[1]Properly refined, hydrogenated and deodorized lard apparently shows no reversion other than true oxidative rancidity. This is probably due to the fact that the fat is relatively free from extraneous matter after rendering and, therefore, the few remaining impurities are easily removed by the usual refining methods.

[2]It should be stated here that Moreau and Dufraisse preferred to use the term "anti-oxygen." They based their preference on the belief that the mechanism of the reaction which these materials retarded concerned atmospheric oxygen rather than some later stage of the oxidation reaction. It is fairly well established now that there are several stages in the oxidation of fats and oils involving, first, the linkage between atmospheric oxygen and their double bonds, followed by some sort of rearrangements of the oxygen carbon linkages, and then further followed by a split of the carbon chain. Inasmuch as no adequate proof has been yet presented to show which of these reaction stages is affected by the anti-catalyst, we consider it better to use the more general term, "anti-oxidant."

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yielding a peroxide. They postulate some sort of rearrangement afterward into atomic oxygen as a subsequent stage of oxidation.

N. A. Milas (*J. Phys. Chem., Vol.* 33, *p.* 1204, 1929; *J. O. C. S., Vol.* 53, *p.* 221, 1931; and *Chem. Rev., Vol.* 10, *p.* 295, 1932) suggests an electronic interpretation of auto-oxidation which correlates somewhat the ideas of the Engler-Bach theory with that of dehydrogenation proposed by Wieland (*Ber., Vol.* 47, *p.* 2085, 1914).

P. N. Raikow (*Z. Anorg. Allgen. Chem., Vol.* 189, *pp.* 36-52, 1930) proposes a new theory of auto-oxidation which involves the formation of oxonium intermediate compounds catalyzed by moisture.

Several theories have also been proposed for the action of anti-oxidants. These are probably best summarized by the following two references:

Titoff (*Zeig. Phys. Chem., Vol.* 45, *p.* 641, 1903) *suggests that anti-oxidants bind up or precipitate the pro-oxygen substances and thereby effect improved resistance to oxidation.*

Moreau and Dufraisse (*Compt. Rend., Vol.* 174, *p.* 258, 1922, *Chem. Rev., Vol.* 3, *p.* 113, 1926) *prefer the idea that the chemical anti-oxidants reverse the process of oxidation at one of its stages. They account, in this manner, for the tremendous activity of the extremely minute quantities of the anti-oxidant.*

The work of Moreau and Dufraisse established the phenols as anti-oxidants in fats, as well as in acrolein, with which they were initially interested. Much of the later work has confirmed the earlier results in showing the effectiveness of phenols, especially the ortho- and para-di-hydric phenols as stabilizers of certain fats under certain conditions.

IN spite of all this evidence, and the great need for a suitable anti-oxidant, these chemical substances have not come into use as stabilizers in edible products. I believe this is due to two difficulties, one of which serves to frighten workers from the idea; and the other, to limit its usefulness. In the first case, it is known that phenols, when taken in large quantities, are toxic, and because no adequate study[3] has ever been made to determine the physiological properties of minute quantities, no one has felt justified in suggesting the addition of these soluble phenols to edible products. In the second case, the simple di- or tri-hydric phenols, such as hydroquinone, pyrocatechin, and pyrogallol, are ineffective as anti-oxidants in a fat after mixing and heating the fat with any other ingredient containing moisture and protein, or mild alkalies. This latter limitation excludes positively the utilization of this means of fat stabilization for any bakery goods, by any of the simple phenols thus far listed as having anti-oxidant properties.

There are, however, several types of stabilizers which do not belong to the simple chemicals just mentioned and which carry over this stabilizing action into the finished cracker or other bakery product in which the fat may be used. It is this class of stabilizers that has been receiving special study in our laboratory[4] for the last four or five years.

It had been observed, during a long series of "keeping quality" comparisons, that some of our beef fats having constants and past histories of very similar character, possessed definite differences in their ability to withstand incubation without the development of oxidative rancidity (see Series 1). In all carefully controlled comparisons made in our earlier work, it was noted that the higher colored beef fats were superior in their resistance to oxidation. This suggested the possibility that the carotene contained in these oils might possess some anti-oxidant property. Further experiments designed to test this hypothesis gave almost complete confirmation. Carotinoids from various sources were then extracted with carefully rendered lard, and the samples incubated with controls to compare the rate of development of rancid odors and flavors.

IN the course of our experience with this method of stabilization, we have used three different accelerated tests as criteria for comparing the keeping qualities of the treated and untreated samples. These tests have been correlated as far as possible with room temperature storage tests of the various fat samples and also of bakery products made from these

[3] Olcovich and Mattill (J. Bio. Chem., Vol. 91, p. 105, 1931) mention unpublished observation that small quantities of hydroquinone have no physiological effect on experimental animals.

[4] Newton and Richardson, U. S. Patent Office, Serial 439,847, March, 1930.

fats. The first accelerated test used in this study was the simple incubation at 70° C. of a 50 gram sample contained in a half pint jar with a loose fitting screw cap. The criterion for the inception of rancidity was by smelling and tasting the sample at regular intervals. In all cases, the samples were allowed to remain in the incubator for one or two days after the development of faint rancid odors. In this manner, the rate of increase in the development of the rancid odor served as a check against the possible error of organoleptic detection of the true point of inception of rancidity. As another precaution, we used new jars only, in these tests. It is, of course, possible to wash a jar or beaker sufficiently clean to prevent any carry-over of oxidation products to catalyze oxidation in the next sample, but we have proven conclusively that such a procedure is a constant source of error even when unusual care is taken. In another series of experiments, not reported in connection with this work, it was shown that washing the glass vessel with acid dichromate solution, followed by washing several times with clear, distilled water, did not clean the vessel sufficiently to prevent its causing accelerated oxidation in the next sample.

The second test applied in our study of carotinoid stabilization was a modification of the method of Issoglio (*Ann. chim. applicata,* 1916, 1-18), as further developed by Bailey (*Cotton Oil Press,* 7, *No.* 8, 35, 1923), and adapted by workers in our laboratory. (The improvements in this method were reported before this group two years ago.)

The active oxygen test used by Lea (*Proceeding, Royal Soc., London, B* 108, 175-89, 1931, *and Dept. Sci. Ind. Research, Report of Food Investigation Board,* 193, 30), and later slightly modified by Wheeler (*Oil & Soap,* 4/32, *pp.* 89-97), was used as a criterion of the rate of oxidation during the period of incubation of our samples. This latter check of our results came, however, after this stabilizing process had been put on an operating basis and had received practical confirmation.

In these experiments, no attempts were made to isolate the carotene from the other fat-soluble materials which might occur with it in these natural sources, except to effect the usual refining treatments applied to edible shortening products. We feel justified, however, in the assumption that the stabilizing action is due to the carotinoids, since these pigments were obtained by fat extraction from so many widely varying sources, and in each case, with positive results.

In the course of our work, we observed that moderate amounts of these pigments can be bleached in the oil by subjecting to an elevated temperature. If the oil is richly colored, several hours at a temperature of 200/250° C. are required for effective bleaching. The pigments are, of course, readily bleached by oxidation if blown with air at even slightly elevated temperatures. The hydrogenation of any oil containing these pigments effects an almost complete decolorization. The course of these three reactions which brings about bleaching is not well understood. It seems, however, to be fairly well established that several groupings in the structure of carotene are necessary for the production of its color. High temperature apparently effects some sort of polymerization or molecular rearrangement, whereas oxidation and hydrogenation both bleach by elimination of some of the unsaturated groups. The action of bleaching at high temperature, or by hydrogenation, is considered practicable on a commercial scale, whereas deterioration by blowing oils with air makes the third method impracticable to use for edible products.

It was further developed, as illustrated by the data given in the following table, that bleaching of the pigments by high temperature, or by hydrogenation, augments the stabilizing action. Another interesting point illustrated in Series 6, 9, and 12 of the data given, indicates it is necessary, in order to obtain maximum stabilization of the oil, to carry out the bleaching action either by heat or hydrogenation after the addition of the pigment to the oil. The necessity for the incorporation of the pigment in the fat before treatment with heat or hydrogenation for stabilizing action, might lead one to believe that the carotinoid pigment acts either as a catalyst, effecting some change in the fat during this course of treatment, or serves to precipitate and remove, or render inert, some pro-catalyst naturally occurring in the fat. I do not consider the weight of our

Experiments

Comparison of Treated and Control Samples Incubated at 70° C. Using Odor at Various Intervals as Criterion for the Onset of Rancidity

	Comparative Keeping Quality on Basis of Control Sample Equals 100†
Series 1—	
(a) No. 2 oleo oil rendered from fresh fats containing slightly less color than the oil used in d, e, f, g (control)	100
(b) Same as "a," but deodorized by blowing with steam at 400° F. under 6 m.m. pressure	100‡
(c) Same as "a"*	200
(d) /O/ Oleo oil rendered from high colored beef fats (control)	100
(e) Same as "d"*	333
(f) /A/ Oleo stock from fresh rendered high colored beef fats, including some bone marrow fat (control)	117
(g) Same as "f"*	195
Series 2—	
(a) Prime steam lard rendered in full size plant equipment from fresh killing and cutting fats (control)	100
(b) Lard extract of paprika (5 parts paprika extracted with 100 parts same lard used for "a")*	300
Series 3—	
(a) Prime steam lard rendered from fresh killing and cutting fats (control)	100
(b) Same as "a," plus small percentage of lard extract of dried carrots	133
(c) Same as "b"*	375
Series 4—	
(a) Prime steam lard rendered from fresh killing and cutting fats (control)	100
(b) Same as "a"*	100
(c) Lard extract of alfalfa (10 parts alfalfa leaves extracted with same lard used in "a")*	150
Series 5—	
(a) Prime steam lard (control)	100
(b) Same as "a," hydrogenated to a refr. index change of 1°*	155
(c) Same as "a," hydrogenated to a refr. index change of 1.5°*	233
Series 6—	
(a) Prime steam lard (control)	100
(b) Same as "a," plus 5% of high colored palm oil, mixture refined with caustic soda and hardened to a change in refr. index of 1.6°*	325
(c) Same as "a," plus 10% of high colored palm oil, refined with caustic soda and hardened to a change in refr. index of 2.5°*	1483
(d) Mixture of equal parts of the finished products from "a" and "c"	275
Series 7—	
(a) Prime steam lard (control)	100
(b) Same as "a"*	129
(c) Same as "a," plus 5% of high colored palm oil, mix. ref. with caustic, etc.*	157
Series 8—	
(a) Prime steam lard (control)	100
(b) 98% P. S. lard, same as "a," plus 2% of high colored palm oil, mixture refined with caustic, etc.*	169
(c) 80% P. S. lard, same as "a," plus 20% of a mixture of 75% P. S. lard, the same as "a," with 25% of high color palm oil added; this 75-25% mixture refined with caustic, and hydrogenated to about 2° change in refractive index*	113
Series 9. Incubation Test—	
(a) Pie crust made from untreated prime steam lard (control)	100
(b) Pie crust made from shortening as follows: 98% P. S. lard and 2% palm oil, this shortening mixture caustic refined and hydrogenated to about 2° change in refractive index*	240
(c) Pie crust made from shortening as follows: 95% P. S. lard and 5% palm oil, this shortening mixture caustic refined, and hydrogenated to about 2° change in refractive index*	440
(d) Pie crust made from shortening as follows: 80% untreated P. S. lard, mixed with a treated product consisting of 75% P. S. lard and 25% palm oil, this latter mixture refined, and hydrogenated to about 2° change in refractive index*	200
	Index of Keeping Quality§
Series 10—	
(a) Prime steam lard rendered from fresh killing and cutting fats (control)	.6
(b) Same as "a," plus 5% of palm oil refined by treatment with caustic soda to remove free fatty acid and hydrogenated to a change in refractive index of 2° ZB	3.6
Series 11—	
(a) Prime steam lard rendered from fresh killing and cutting fats (control)	.6
(b) Same as "a," plus 5% of high colored palm oil refined with caustic to remove free fatty acid and hardened to a change in refractive index of 1.5° ZB	4.2

*Deodorized by blowing with steam at 400° F., under 6 m.m. pressure, in the presence of sufficient dry sodium bicarbonate to neutralize the free fatty acid, afterward filtered to remove trace of soap. (Attention is called to 4a and 4b, also 7a and 7b, for the effect of this treatment on control sample.)

†In our incubation studies, the results are reported in terms of the control sample, which was carefully selected in each case to represent identically the quality of lard product treated in that series. For example, series 2 and 5 were made at different times, using different raw materials, so in each case the control is reported arbitrarily as having keeping quality of 100, and the other samples rated relative to the control sample of its particular series.

‡This sample showed slightly better than the control in that a positive Kreis test did not develop as soon, although the development of rancid odor appeared to be identical with the control.

§An illustration of the results of carotene treatment, as measured by the rate of formation of volatile oxidative decomposition products (Grettie and Newton, Indus. & Eng. Chem., Analytical Edn., Vol 3, p. 171, Apr. 15, 1931) is shown in these results expressed in terms of 1% of the number of minutes required to reduce 1 c.c. of .01 N. potassium permanganate solution when the volatile decomposition products are passed through the permanganate solution.

Series 12—

results to date sufficient to establish either of these theories as a satisfactory explanation.

In considering the results of chemical antioxidants, as stated before, it was found that the presence of moisture nullified the protective action of most of them. In checking this point,

Active Oxygen

Mille-equivalents per Kilo of Fat.

Time of Incubation at 70° C.	1	2	3	4
65 hours	3.6	3.6	3.2	4.0
120 hours	6.0	4.0	5.0	7.0
187 hours	118.0 rancid	5.0	8.0	33.0
281 hours		9.5	10.0	100 rancid
304 hours		9.0	11.0	
354 hours		10.0	11.5	
388 hours		12.0	13.0	
473 hours		19.5 rancid	16.0	
523 hours		60.0 sample used up	20.0	
576 hours			24.0	
648 hours			50.0 off odor	

1. Prime steam lard refined and hydrogenated from 50.55 to 48.5° ZB.
2. A mixture of 95% P. S. lard and 5% of palm oil hydrog. from 50.3 to 48.25° ZB.
3. A mixture of 80% P. S. lard and 20% of palm oil hydrog. from 50.15 to 48.05° ZB.
4. A mixture of 75% No. 1, plus 25% No. 3 (total palm oil 5%).

the keeping qualities of crackers or piecrusts made with the fat containing the anti-oxidant were found to be a suitable criterion. The ninth series of data serves to illustrate that portion of our results which we believe shows that the stabilizing action is carried over into the finished baked product.

The work of Monahan and Schmitt (*J. Biol. Chem., 96,* 387, 1932) has confirmed, independently, our results (*Newton and Richardson U. S. Pat. Ofc. Serial No.* 439,847, *March,* 1930) obtained on carotene as a stabilizing agent. These workers have concluded that vitamin A. is also an anti-oxidant for fats.

The work of Olcovich and Mattill (*J. Biol. Chem., 91,* 105, 1931) produced contradictory results with respect to the catalytic action of carotenes on the oxidation of fats. It was their conclusion that carotene was a pro-catalyst rather than an anti-catalyst for this reaction.

The difference in the results noted in the work of these workers has been partly explained by Monahan and Schmitt (*J. Biol. Chem., 96,* 387, 1932) on the basis that carotene retards the oxidation even after the induction period has run its course, whereas the work of Mattill was concerned only with the induction period. This graph of some of our results confirms this explanation.

It should be noted further in this connection that all anti-oxidants are easily auto-oxidizable (*Moreau and Dufraisse, Chem. Rev.*). Wilstätter and Meig (*Ann. 355,* 1-28, 1907) are credited with the discovery that carotinoids help to regulate oxygen pressure in plant cells.

Carotene, with a formula of $C_{40}H_{56}$, is a highly unsaturated hydrocarbon and absorbs oxygen readily from the air under some conditions.

We are led to conclude from the increased stabilizing action of carotene after reduction by hydrogenation, together with the results of Mattill, that the oxidized derivatives of carotene may in fact have pro-oxidant properties which are over-balanced in effect by the presence of the unoxidized carotene as they are found mixed in nature.

Bunnosuke Yamaguchi (*Rept. Aeronautical Research Inst., Tokyo Imp. Univ., Vol.* 5, *pp.* 195-229, 1930) studying the anti-oxidant effect of unsymmetrical diphenylhydrazine on olive oil found "Once the reaction is started, the reaction velocity is the same as when no inhibitor is present." This is contrary to our findings with carotinoids on other fats and oils. Our results show a distinct increase in the length of the induction period, and also in the slowing up of the oxidation reaction, after it has once started, when carotinoid pigments are present.

E. Couture (*Compt. Rend., Vol.* 190, *pp.* 532-3, 1930) finds the sterols to be specific catalysts for the oxidation of the oils in which they occur naturally.

Mattill (*J. Biol. Chem., Vol.* 90, *p.* 141, 1931) states "A number of sterols of animal origin, and sitosterol from wheat, corn, and lettuce were all ineffective."

Workers in our laboratory have found some evidence of the specificity of anti-oxidants occurring naturally in oils. These have been disclosed in the patent office and will be further reported in the literature when more of them have been investigated.

During the early course of our work, it was observed that rather small quantities of free fatty acids in a mixture of fats containing carotinoid pigments prevented, to a certain extent, the bleaching action during the course of hydrogenation. Correlated with this was the observation that these samples which were difficult to bleach by hydrogenation were not stabilized to as great an extent as those in which the bleaching action took place easily. For this reason, in many of our laboratory experiments, some sodium bicarbonate was added to the fats during the deodorization period to lower the free fatty acid in the final product as far as possible. Holm, Greenbank, and Deysher (*J. Indus. Engr. Chem., Vol.* 19, *p.* 156, 1927) point out the effect of small amounts of acid on the susceptibility of fats to oxidation.

Summarizing:

The results of our work support the following conclusions:

1. Carotinoid pigments, or some substance closely associated in nature with carotinoid pigments, in five widely separated sources act as anti-oxidants in some fats.
2. The treatment of fat containing carotinoid pigment at high temperature to effect bleaching also augments the stabilizing action of the pigment.
3. The treatment of oil containing carotinoid pigment to effect hydrogenation, bleaches the pigment and augments its stabilizing action over that of hydrogenation of the oil alone, or that of the hydrogenated oil plus carotinoid pigment.
4. Anti-oxidant properties associated with carotene are carried over into the finished bakery products, such as piecrust, crackers, etc.
5. The maximum stabilizing action, accomplished by bleaching the pigment at high temperatures, or by hydrogenation, is obtained only when the pigment is mixed with the entire body of the oil before treatment.