

acid content. The proportion of saturated acids remains practically the same. The American sample (11) contains the maximum amount of linoleic acid, which is 10% higher than the present sample. The contents of oleic and saturated acids are lower in proportion. Besides the differences expected because of the methods of investigation followed, these results suggest the tendency of the composition of *Citrullus Vulgaris* seed fat to vary with environmental conditions.

The seed fat composition of *Benincasa Cerifera* has been reported from India by Phaduis *et al.* (12), who did not use spectrophotometric technique. The difference between the two results is not wide. The variation is confined to slightly higher content of linoleic acid (ca. 6%) and lower contents of saturated acid (ca. 3%) and oleic acid (ca. 2%), which in this case was probably due to adoption of older techniques.

It is evident from the results obtained that the component acids of these four allied members of the *Cucurbitaceae* family are of a more or less simple nature and do not contain triene acids like trichosanic or elaeostearic acid, which are found in some other members of this family.

The tendency toward variation in composition according to environmental conditions is also apparent. It should be found possible therefore to obtain lin-

oleic-rich oils from the members, *Benincasa Cerifera*, *Lagenaria Vulgaris*, and *Citrullus Vulgaris*, by providing proper environments. All the seeds are rich in fat content, and, if available in commercial quantities, the oils would be quite suitable for edible and soap-making purposes. The composition of the seed fat of *Cucurbita Maxima*, as reported here, approaches that of Indian sesame oil and so this can be utilized as a substitute to fulfill certain purposes.

REFERENCES

1. Shahani, H. S., Dollear, F. G., and Markley, K. S., *J. Am. Oil Chemists' Soc.*, **28**, 90 (1951).
2. Aggarawal, J. S., and Mathur, H. H., *J. Sci. Ind. Res.*, **12**, 60 (1953).
3. Hilditch, T. P., Morton, R. A., and Riley, J. P., *Analyst*, **70**, 68 (1945).
4. Hilditch, T. P., Patel, C. B., and Riley, J. P., *Analyst*, **76**, 81 (1951).
5. Official and Tentative Methods of the American Oil Chemists' Society, *Ca 6a-40* (1946).
6. Hilditch, T. P., and Shrivastava, R. K., *Analyst*, **72**, 527 (1947).
7. Jamieson, G. S., and Baughmann, W. F., *J. Am. Chem. Soc.*, **42**, 153 (1920).
8. Agarwall, R. R., and Dutt, S., *Proc. Acad. Sci. Agra and Oudh*, **5**, 227 (1934-35).
9. Pieraerts, J., *Bull. Soc. Pharmacol.*, **24**, 204 (1917).
10. Rankov, G., and Popov, A., *Fette und Seifen*, **48**, 489 (1941).
11. Ibid.
12. Dhangra, D. R., and Biswas, A. K., *J. Ind. Chem. Soc.*, **22**, 119 (1945).
13. Nolte, A. G., and Loesecke, H. W. Von, *J. Am. Chem. Soc.*, **61**, 889 (1939).
14. Phaduis, K. D., Rege, A. V., Rishawikar, D. G., and Shah, S. V., *J. Univ. Bombay*, **17A**, No. 24, 62-71 (1948).
15. Hilditch, T. P., "The Chemical Constitution of Natural Fats" (Chapman and Hall, London, 1947).

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A Comparison of Commercially Used Phenolic Antioxidants in Edible Animal Fats

W. M. GEARHART and B. N. STUCKEY, Eastman Chemical Products Inc., Kingsport, Tennessee

BUTYLATED hydroxyanisole (BHA) and propyl gallate, alone and in various combinations, are the most widely used food antioxidants today. Another phenolic antioxidant, butylated hydroxytoluene (BHT), was recently approved for food use by the Food and Drug Administration. BHT was first patented by Stevens and Gruse (6) in 1940 as a petroleum oil antioxidant and has since been used widely in the gasoline field. Seubert and Andrews (5) have shown that under accelerated conditions BHT at .01% concentration increased the stability of paraffin wax and polyethylene-paraffin wax mixtures tenfold.

Polister and Mead (4) report that when linoleic acid is exposed to ionizing radiation, a chain reaction similar to oxidation is introduced. They found however that when BHT was added in concentrations of .0003% and higher, oxidation was reduced to practically zero. Kraybill and Dugan (3) in reporting on new antioxidants stated that BHT was effective as an antioxidant for animal fats. They stated that, when used in lard, BHT was slightly more effective than BHA in increasing AOM stability but not as effective as BHA in carry-through stability as judged by the Schaal Oven Method. They also reported marked synergism between BHT and BHA in lard.

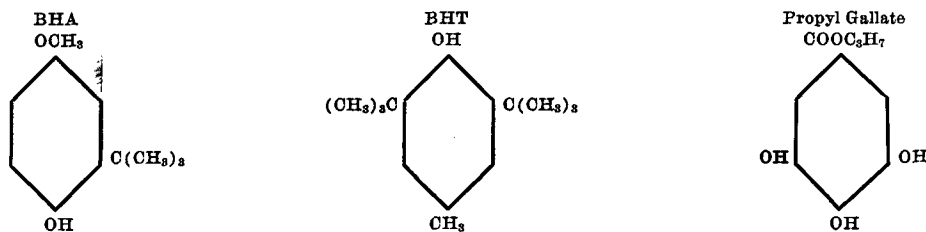
A purified form of BHT has recently been approved for food use by the Food and Drug Administration. Tappel (7) gives a very good comparison

of the properties and general characteristics of BHA, propyl gallate, and nordihydroguaiaretic acid. Moore and Bickford (1) give an excellent study on the evaluation of 13 different antioxidants on the AOM stability of lard, cottonseed oil, and cottonseed shortening. They did not however include the carry-through stability effect of the different antioxidants or the effect of various combinations of these antioxidants. It was felt therefore that a basic study of BHA, BHT, and propyl gallate was needed so that a more intelligent approach could be made in utilizing the best characteristics of each of these three food antioxidants in preparing commercial formulations. This study should include a comparison of their physical characteristics, their effect on AOM stability in lard, and the effect of their carry-through stability in lard, as judged by the Schaal Oven Method. It should include the basic antioxidants as well as synergistic mixtures of the basic antioxidants with each other and with citric acid.

Physical Properties

Figure 1 shows a comparison of the structure and the physical properties of BHA, BHT, and propyl gallate. We have shown the structural formula for only the three isomer of BHA. All the tests reported herewith for BHA were made, using BHA which contained 95% or more of the three-isomeric form. Several investigators have reported that the three isomer

Structural Formula



| | | | |
|-------------------------------|--------------------|------------------------|-------------------|
| Molecular Weight..... | 180 | 220 | 212 |
| Physical State..... | White Waxy Crystal | White Granular Crystal | White Crystal |
| Melting Point - °C..... | 48 - 55 | 69 - 70 | 146 - 148 |
| Odor..... | Phenolic | Slightly Phenolic | Slightly Phenolic |
| Percentage Solubility - 25°C. | | | |
| Water..... | 0 | 0 | 0.35 |
| Ethanol..... | 25 | 25 | 100 |
| Propylene Glycol..... | 50 | 0 | 65 |
| Cottonseed Oil..... | 30 | 20 | 1 |
| Peanut Oil..... | 40 | 25 | 0.5 |
| Lard..... | 50 | 40 | 1 |
| Yellow Grease..... | 50 | 40 | 1 |
| Mineral Oil..... | 5 | 5 | 0.5 |

Fig. 1. A comparison of the structure of physical properties of some phenolic antioxidants.

is more potent as an antioxidant than the two isomer. Our experience has been that under carefully controlled laboratory conditions the three isomer is a better antioxidant for lard than the two isomer. The difference is so slight however that under practical commercial conditions a ratio containing at least 60% of the three isomer is equal in antioxidant value to pure three-isomer BHA. From the structural formula it may be seen that BHA and BHT are very similar and should have similar properties. Propyl gallate however is quite different and has different chemical properties, as would be expected.

Another interesting fact which is shown here is the difference in odor. BHA in the pure form has a rather strong phenolic odor. BHT and propyl gallate however have practically no odor even in the concentrated state. We have found however that none of these antioxidants affect either odor or flavor when used in concentrations necessary for stabilization of fats and oil.

The chief difference in solubility among these antioxidants is the fact that BHA and BHT are highly oil-soluble and completely water-insoluble whereas propyl gallate is very slightly soluble in water and only slightly soluble in animal and vegetable oils. It may also be pointed out that BHA and propyl gallate are quite soluble in propylene glycol while BHT is completely insoluble in this food-approved solvent.

BHA is slightly more oil-soluble than BHT in both animal and vegetable fats. The solubility of both these antioxidants is so high in most fats and oils however that for all practical purposes they can be considered equal.

Experimental

The lard used in these laboratory tests was a top quality commercial lard purchased from Kingan and Company. Determinations of stability were made, using standard techniques and equipment for both the Active Oxygen Method and Schaal Oven Method. All preparation and cooking of potato chips, pastry, and crackers followed a standard procedure which has been worked out by the Foods Laboratories at Kingsport. This procedure is basically the preparation of potato chips, test crackers, and test pastry, using standard times, temperatures, and formulas.

The test foods are then stored in a 145°F. oven called a Schaal Oven. It is not within the scope of this paper to go into the details of this testing method. A panel of three experienced technicians carried out all tests for the organoleptic evaluations in the Schaal Oven Method for determining rancidity.

It should be pointed out that, in the following experiments, antioxidant concentrations were used which greatly exceed those allowed in lard by the Meat Inspection Branch. It was necessary however to use these high concentrations in order to show effectively the over-all behavior of the various antioxidants when used to stabilize lard.

Experiment I. Samples of lard (800 g.) were stabilized with BHA, BHT, and propyl gallate under carefully controlled conditions. It has been our experience that much of the poor results obtained with antioxidants is due to incomplete solubility of the antioxidant in the fat, and special care must be taken in the laboratory to stabilize all samples in exactly the same way. These samples were stabilized so that the finished samples of lard would contain .005, .01, .02, .03, .04, .05, .075, and 0.10% antioxidant. The stability of each sample was determined by the Active Oxygen Method (2). Potato chips, test pastry, and test crackers were prepared, using the various

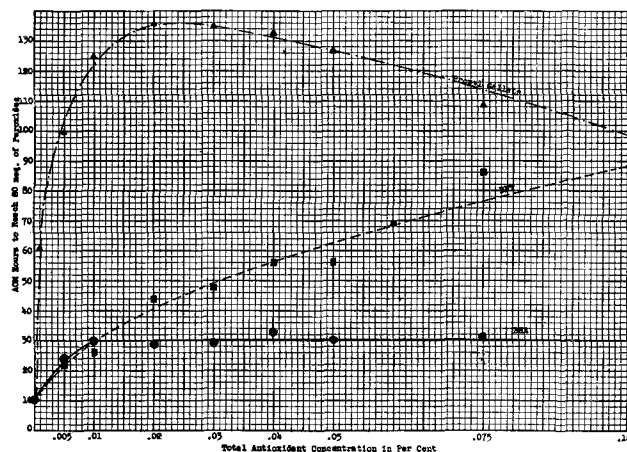


Fig. 2. The effect of basic antioxidant concentration on the AOM stability of lard.

samples of lard and placed in a Schaal oven at 145°F. Each sample was tested daily organoleptically for evidence of rancidity.

Figure 2 shows the effect of varying the concentration of BHA, BHT, and propyl gallate on the AOM stability of lard. In this graph the hours for each sample to reach a peroxide value of 20 milliequivalents are plotted against the total antioxidant concentration. It may be seen from this graph that BHA reaches its peak value at approximately .01%. BHT however has an initial antioxidant value below that of BHA but increases in antioxidant value almost directly proportional to its concentration. Propyl gallate reaches its peak value at approximately .02% and decreases in value thereafter. It may also be seen that propyl gallate is an excellent antioxidant from an AOM standpoint at very low concentrations.

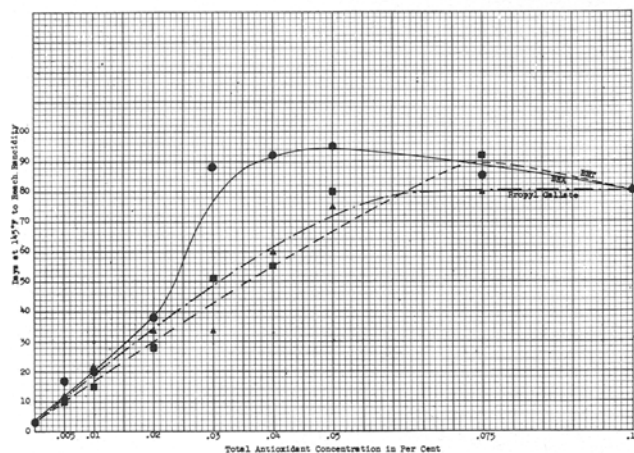


FIG. 3. The effect of basic antioxidant concentration on the Schaal oven stability of potato chips.

Figure 3 shows the carry-through effect of various concentrations of BHA, BHT, and propyl gallate when potato chips are used as the test material. In this figure days to reach rancidity at 145°F. are plotted against total antioxidant concentration. It may be seen that in potato chips BHA has the best carry-through properties. It reaches its maximum antioxidant value at about .04%. BHT and propyl gallate are quite similar in effect and reach their maximum efficiency at about .06%.

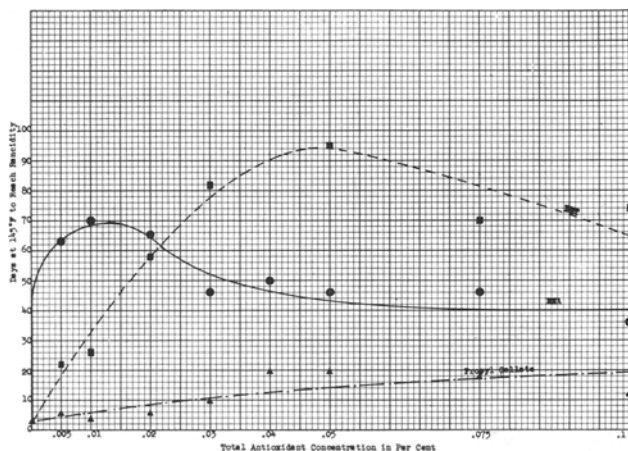


FIG. 4. The effect of basic antioxidant concentration on the Schaal oven stability of pastry.

Figure 4, showing the effect of the antioxidant concentration on pastry, again plots days to reach rancidity at 145°F. against antioxidant concentration. It may be seen that at low concentrations BHA has much better carry-through than either of the other antioxidants but reaches its peak at about .01%. BHT, while having lower initial antioxidant value, surpasses BHA at approximately .02% and reaches its peak at approximately .04%. Propyl gallate shows very little carry-through in pastry regardless of concentration.

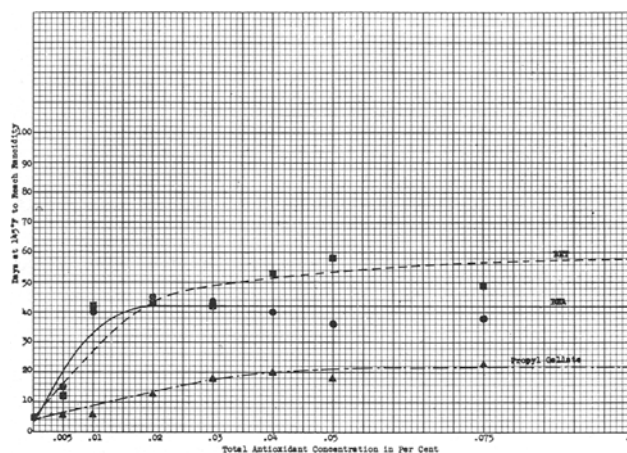


FIG. 5. The effect of basic antioxidant concentration on the Schaal oven stability of crackers.

Figure 5 plots the days at 145°F. for crackers to reach rancidity against the total antioxidant concentration. BHA and BHT are practically equal in concentrations up to .03%. At this point however BHT continues to increase stability while BHA produces no further increase in stabilizing effect. Propyl gallate again shows poor carry-through properties in crackers.

Experiment II. The fat and oil industry for a number of years has successfully used a synergistic mixture of antioxidants containing BHA, propyl gallate, and citric acid. The citric acid has been incorporated as a metal scavenger to prevent the formation of dark-colored metallic gallates. It also has some antioxidant effect, probably due to its chelating action on metallic catalyst in the fat itself. In order to determine the synergistic action of BHA with propyl gallate, BHT with propyl gallate, and BHA with BHT, samples of the Kingan lard were stabilized with the following mixtures:

1. 20% BHA, 6% propyl gallate, 4% citric acid, and 70% propylene glycol.
2. 20% BHT, 6% propyl gallate, 4% citric acid, and 70% monoglycerides.
3. 20% BHA, 20% BHT, and 60% cottonseed oil.

Lard was stabilized with varying percentages of the above mixtures so that the final antioxidant concentration would be .005, .01, .02, .03, .04, and .05% of the total antioxidant, exclusive of the citric acid. Additional lots of the same lard were also stabilized with .075 and .10% of the BHA-BHT combination to see if the high concentration of BHT would cause the stability curve to continue its rise. The AOM and Schaal oven stabilities of each lot were determined as described in Experiment I.

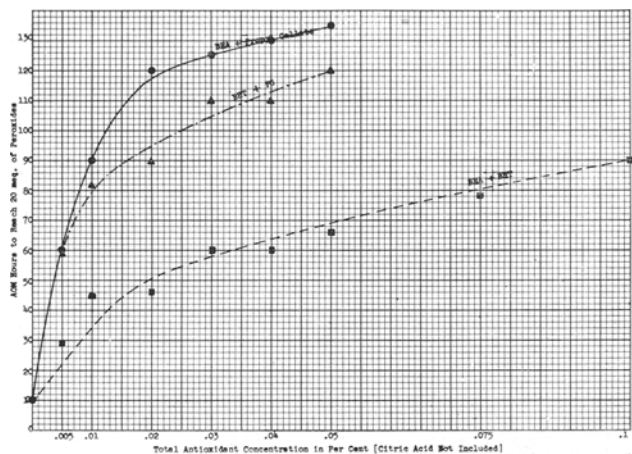


FIG. 6. The effect of synergistic antioxidant combinations on the AOM stability of lard.

Figure 6 shows the effect of varying concentrations of the above synergistic mixtures on the AOM stability of lard. As would be expected from Experiment I, mixtures containing propyl gallate produce a very high AOM stability. BHA and propyl gallate synergize to produce higher AOM stability than either of the other two mixtures. BHT apparently does not synergize with propyl gallate to any great extent. BHT and BHA apparently synergize to a very slight degree. When compared with BHA or BHT alone, as shown in Figure 2, the combination produces slightly higher AOM stability in lard at concentrations of .05% and below than equal concentrations of either antioxidant alone. At concentrations above .05% BHT alone is apparently as effective as the combination of BHA and BHT.

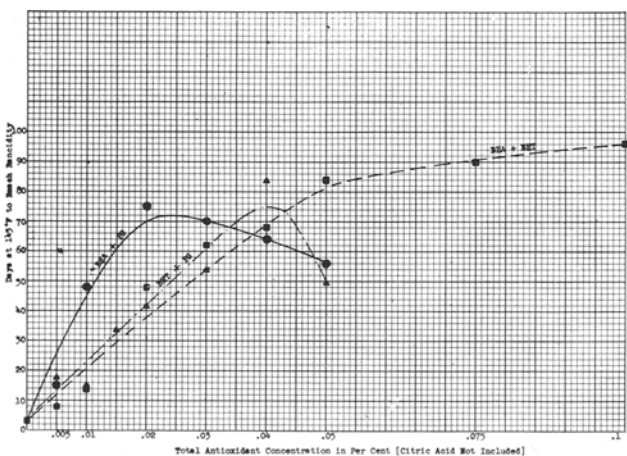


FIG. 7. The effect of synergistic antioxidant combinations on the Schaal oven stability of potato chips.

The data shown in Figure 7 indicate that in potato chips the BHA-propyl gallate mixture at concentrations of .03% and lower has better carry-through properties than either the BHT-propyl gallate or BHT-BHA mixture. The BHT-BHA mixture continues to increase the stability of the potato chips with an increase in concentration however and surpasses the other two at approximately .05%. One interesting point in this chart as well as the follow-

ing charts is that mixtures containing propyl gallate reach a peak concentration and the stability drops sharply thereafter.

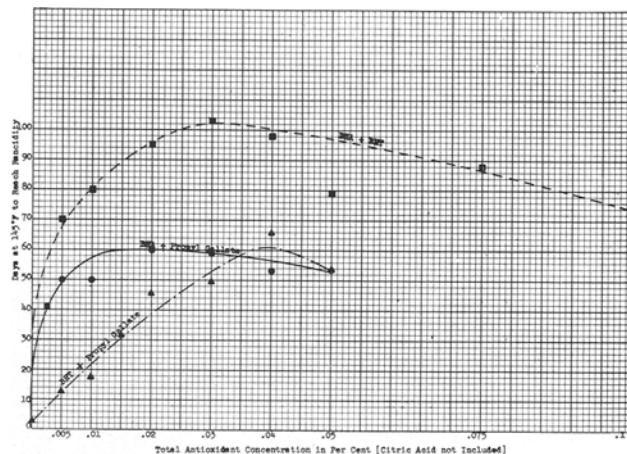


FIG. 8. The effect of synergistic antioxidant combinations on the Schaal oven stability of pastry.

Figure 8 denotes the effect of the above synergistic mixtures on the Schaal oven stability of pastry. In pastry the BHA-BHT mixture shows synergism producing stabilities in excess of either the BHA-propyl gallate or the BHT-propyl gallate mixtures. The BHA-propyl gallate mixture again produces better stability at lower concentration than the BHT-propyl gallate mixture but only equal stability at concentrations of approximately .035% and above. The BHA-BHT mixture also shows a peak efficiency at about .03% and falls off thereafter.

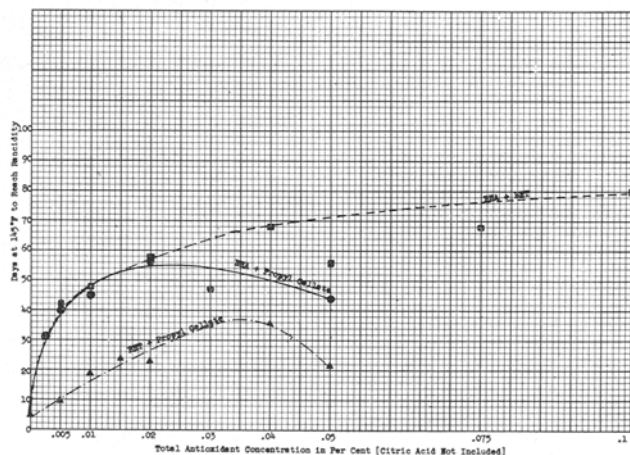


FIG. 9. The effect of synergistic antioxidant combinations on the Schaal oven stability of crackers.

The data on the effect of the synergistic mixtures on the Schaal oven stability of crackers, as may be seen in Figure 9, again show that the BHA-BHT mixture has the best carry-through properties. The BHA-propyl gallate mixture is equal to it at lower concentrations but falls off at higher concentrations. The BHT-propyl gallate mixture ranks a poor third at all concentrations.

Discussion

In general, these data show that from the standpoint of AOM stability propyl gallate, either alone

or in combination with BHA or BHT, produces the highest stability in lard. When used alone, it reaches its maximum efficiency at about .02% and decreases thereafter. BHT produces fairly high AOM values at high concentrations but would not be comparable to propyl gallate at food-approved concentrations of .01% and less. BHA at low concentrations is about equal to BHT but reaches its peak effectiveness at about .010%. Higher BHA concentrations apparently have little effect on the AOM stability of lard. The combination of BHA and propyl gallate produces higher AOM's than the combination of BHT-propyl gallate. This indicates that there is synergism between the BHA and propyl gallate at all concentrations tried but little or no synergism between BHT and propyl gallate. The combination of BHA and BHT shows a slight synergism from the AOM standpoint, but more work should be done before definite conclusions are made.

BHA alone produces the best carry-through stability at food approved concentrations. BHT, while not as good at lower concentrations, produces higher stability at concentrations above .02%. Propyl gallate is approximately equal to BHT in carry-through as tested in potato chips but apparently will not stand the high baking temperatures of either pastry or crackers. The efficiency of the synergistic mixtures containing propyl gallate is good in the case of potato chips, but when higher temperatures are used, as in the case of crackers and pastry, the BHA-BHT combination is better than either of the other two. It is believed that the marked difference in the carry-through effect of the various antioxidants in pastry as compared to crackers is due to the higher fat content used in pastry. These data as well as other work at

this laboratory have shown that carry-through data in pastry is usually more concise and clear-cut than the same data in crackers.

These data indicate that combinations of BHA and propyl gallate produce the best stability in lard if used at limits authorized by the Meat Inspection Branch. If only AOM stability is considered, propyl gallate alone is the best antioxidant. If carry-through stability alone, especially at higher temperatures, is the chief qualification, then the best antioxidant is a combination of BHA plus BHT.

Summary

Butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate were compared for antioxidant efficiency alone and in combination with each other in lard. A comparison of the physical properties of these three antioxidants was also made.

BHA was found to synergize with propyl gallate to produce the best all-around antioxidant. The mixture of BHA and BHT produced the best carry-through stability in lard, particularly when higher temperatures were used. Propyl gallate alone produces the best AOM stability in lard.

REFERENCES

1. Moore, R. N., and Bickford, W. G., *J. Am. Oil Chemists' Soc.*, **29**, 1-4 (1952).
2. King, A. E., Roschen, H. L., and Irwin, W. H., *Oil and Soap*, **10**, 105-109 (1933).
3. Kraybill, H. R., and Dugan, J. R. Jr., *Agr. and Food Chem.*, **No. 2**, 81-4 (1954).
4. Polister, Barbara Hinderer, and Mead, James F., *Agr. and Food Chem.*, **72**, No. 4, 199 (1954).
5. Seubert, R. F., and Andrews, Ed., *Modern Packaging*, **26**, No. 4, 153-54 (1952).
6. Stevens, Donald R., and Gruse, William A. (Gulf Oil), *U. S.* **2,202,877** (1940).
7. Tappel, A. L., *Food Eng.*, **26**, No. 6, 73-4 (1954).

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Investigations on the Bitter and Beany Components of Soybeans¹

H. M. TEETER, L. E. GAST, E. W. BELL, W. J. SCHNEIDER, and J. C. COWAN, Northern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois

SOYBEAN FLOUR contains substances that impart undesirable bitter and beany flavors and odors to soybean food products. Although many minor constituents of the soybean have been identified, none has been shown to be responsible for these bitter and beany flavors and odors. In fact, no investigations directed toward this specific purpose appear to have been carried out.

Horvath (4), referring to work by Schmalfluss and Treu (10), made the statement that methyl *n*-nonyl ketone is responsible for the beany flavor. This substance was however isolated from a sample of soybean oil rather than from the flour. No experimental evidence is presented to show that this ketone contributes to the flavor or odor of soybean flour.

A large number of patents covering processes for "debitting" soybeans are in existence. These processes are empirical, and there appears to be no available evidence that they are based on knowledge of the

chemical nature of the beany or bitter principles. It is believed that if this knowledge were at hand, it would be possible to devise more satisfactory debittering processes and thus to obtain edible soybean products having no objectionable flavor. This paper is a report on the progress made in studying this complex problem.

In any study of the minor constituents of a natural entity, such as the soybean, one of the more perplexing problems is that of obtaining an extract or concentrate of the minor constituents that will be suitable for detailed organic chemical investigation and analysis. Beckel (2) had observed that when ethanol was used to extract oil from soybeans, the resulting flour contained less bitter and beany flavor than did flours prepared by other means. It therefore appeared that if the oil were extracted from the beans with hexane, in which the odor and flavor substances were not very soluble, and if the flakes were then extracted further with ethanol, the resulting alcoholic solution should contain many of the minor constituents that are responsible for flavor and odor.

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