ether–petroleum ether in colorless prisms, m. p. 73.5–75°. Neutral equivalent calculated, 219.2; found, 220.6.

*Anal.* Calcd. for $C_8H_{13}NO_6$: N, 6.38. Found: N, 6.26.

**Alkaline Hydrolysis of Dialkyl 3-Methyl-3-nitro-1,2-butanedicarboxylates VII and XVI.**—Dimethyl 3-methyl-3-nitro-1,2-butanedicarboxylate (10 g.) was refluxed for thirty minutes with 10 g. of potassium hydroxide and 40 cc. of water. When the cooled solution was acidified with 20% hydrochloric acid, there was a vigorous evolution of oxides of nitrogen and 3.2 g. (47%) of teraconic acid (IX) was precipitated, m. p. 161–163° dec. This acid was identified as previously described. An attempt to obtain a further quantity of teraconic acid from the acidified mother liquor was unsuccessful.

In a similar manner, hydrolysis of 10 g. of diethyl 3-methyl-3-nitro-1,2-butanedicarboxylate (two and one-half hours) with 9 g. of potassium hydroxide yielded 3 g. (50%) of teraconic acid.

A mixture of 10 g. of dimethyl 3-methyl-3-nitro-1,2-butanedicarboxylate, 14 g. of barium hydroxide octahydrate and 50 cc. of water was refluxed for forty-five minutes. The mixture of solid barium salts which had precipitated was suspended in a small quantity of water and decomposed with 36% hydrochloric acid. Several fractional crystallizations from ether–petroleum ether were required to separate the precipitated mixture of acids (3.5 g.) into 0.6 g. of teraconic acid and 1.6 g. of 3-methyl-3-nitro-1,2-butanedicarboxylic acid.

**Reaction of Dimethyl 3-Methyl-3-nitro-1,2-butanedicarboxylate with Diethylamine.**—A solution of 23.3 g. of nitro ester VII and 7.3 g. (1 mole) of diethylamine in 20 cc. of 2-nitropropane (or benzene) was allowed to stand at 30° for fourteen days. Fractionation of the reaction mixture yielded N-nitrosodiethylamine, 15.5–16.4 g. (83–88%) of dimethyl teraconate and 1.7 g. of unchanged nitro ester which crystallized upon cooling.

## Summary

1. Triethylamine has been found to be an effective catalyst for the Michael-type condensation of methyl acrylate with nitroparaffins.

2. Alkylidenesuccinic esters are produced in good yield when dimethyl or diethyl fumarate reacts at 30° with three moles of nitroparaffin and one mole of diethylamine

3. In the presence of a smaller concentration (0.2 mole) of diethylamine, dimethyl and diethyl fumarate condense with 2-nitropropane to yield dimethyl 3-methyl-3-nitro-1,2-butanedicarboxylate and diethyl 3-methyl-3-nitro-1,2-butanedicarboxylate, respectively. Some reactions of these new nitro esters are described.

LOS ANGELES, CALIFORNIA RECEIVED APRIL 26, 1948

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[CONTRIBUTION FROM DIVISION OF FOOD TECHNOLOGY, UNIVERSITY OF CALIFORNIA]

# Deterioration of Dried Fruits. I. The Effect of Sugars and of Furfurals[1]

BY VICTORIA A. HAAS, E. R. STADTMAN, F. H. STADTMAN AND G. MACKINNEY

A wide variety of darkened foods, including dried apricots, orange and lemon concentrates, sulfited dried cabbage and some trade caramels show a characteristic absorption band in aqueous media, *ca.* 285 mμ (Fig. 1.) Studies with dried apricots have shown that the band at 285 mμ is absent in undarkened fruit and that its development is usually concurrent with browning. Since a similar band was observed in some though not in all commercial caramels, it was a logical inference that sugars were involved in the formation of the compounds responsible for this absorption. Moreover, it seemed possible that these compounds were involved in browning. It is the purpose of this paper to test these hypotheses.

## Experimental

Apricot concentrates were used in preference to dried fruit. Qualitatively, concentrates behave in the same manner as dried fruit and they possess the following advantages: they are homogeneous and smaller samples may be used without introducing large sampling errors; their composition may be readily modified by the addition or removal of substances and chemical changes can be followed readily.

**Preparation of Concentrates.**—To test the possible role of sugar in the deterioration, it was desirable to have for comparison a natural concentrate and one from which the sugar had been removed. A natural concentrate was prepared from 10 kg. of fresh apricots which were steam-treated for ten minutes to inactivate enzymes, cooled and blended in a Waring Blendor for five minutes and filtered through a thin layer of celite. The filtered juice was then concentrated at 2–3 cm. pressure, below 35° to a thick sirup, *ca.* 50% solids. To obtain sugar-free concentrate part of the filtered juice was fermented with Peerless bakers' yeast prior to concentration. It required twenty-four hours at 30° to effect a maximum decrease in reducing value, 98% of the original being destroyed. The yeast was then filtered out, and the fermented samples were concentrated to the same extent by volume as the unfermented sample. The composition of the unfermented and fermented concentrate, in grams per 100 g. concentrate, was

| | Unfermented | Fermented |
|---|---|---|
| Total solid[a] | 48.4 | 17.7 |
| Kjeldahl nitrogen | 0.0041 | 0.0025 |
| Reducing value[b] | 33.0 | 0.65 |

[a] By vacuum oven. [b] Computed as glucose after acid inversion.

It is of course not to be supposed that the elimination of sugar by fermentation is the sole change undergone by the fermented sample, nor can it be expected that the two concentrates will be exactly comparable except for the sugar.

**Measurement of Deterioration.**—Deterioration was determined by the increase in darkening, as a measure of which the optical density[1a] was determined on samples

(1) The subject matter of this series of papers (I-IV) has been undertaken in coöperation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces under contract with the University. The opinions and conclusions contained in these reports are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the War Department.

(1a) Measurements of optical densities were made on two different instruments in these papers. To avoid confusion, measurements on the photoelectric colorimeter are designated by the term 2 − log

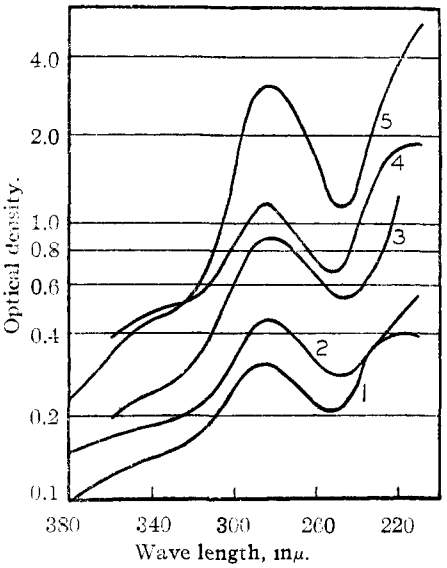


Fig. 1.—Absorption curves for different products: 1, trade caramel; 2, dark apricot blend; 3, shredded sulfited cabbage; 4, orange concentrate; 5, lemon concentrate.

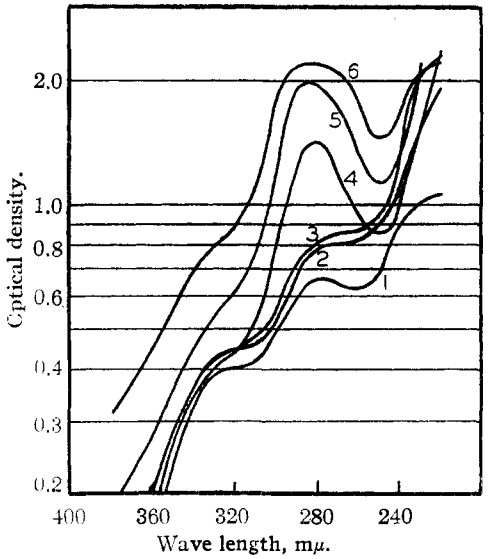


Fig. 2.—Absorption curves for natural and fermented apricot concentrates: 1, unfermented, undarkened; 2, fermented, undarkened; 3, fermented, darkened; 4, fermented plus glucose, darkened; 5, fermented plus fructose, darkened; 6, unfermented, darkened.

(diluted 1:25) with an Evelyn photoelectric colorimeter, 440 mμ filter. Where a rate measurement was desired, the reciprocal of the time in days was calculated from the time required for a sample to reach an arbitrarily selected degree of darkening, an optical density of 0.4 for the diluted sample.

For a study of the development of the band at 285 mμ and for qualitative pictures of the darkening in the visible, the Beckman DU spectrophotometer was used, with the appropriate lamp.[2]

In the succeeding experiments 5 ml. of concentrate was placed in cork-stoppered test-tubes (15 × 1.5 cm.) and stored. The samples were therefore under identical conditions with respect to oxygen.

**The Effect of Sugar.**—The following series of concentrates were incubated at 50° for two weeks: unfermented control, fermented control, fermented sample to which 30% by weight of C.P. glucose had been added, a similar sample with 30% C.P. fructose. (The unfermented control was diluted until it contained 30% sugar, and a comparable dilution was made in the fermented control.)

All samples darkened, the order being plainly visible. The unfermented control darkened most rapidly, followed in order of decreasing rate by the fermented plus fructose, the fermented plus glucose and the fermented control. The absorption of comparably diluted samples is shown in Fig. 2. It is noteworthy that the unfermented control showed no band at 285 mμ initially, nor did the fermented control, even after incubation. The band appears most prominently in the unfermented control after incubation, followed by the fermented plus fructose and then by the fermented plus glucose. The experiment clearly shows that the sugars make a major contribution to the development of the characteristic absorption at 285 mμ and to the browning.

**The Effect of Furfurals.**—Sugars are known to decompose in acid media with the formation of furfurals. Since the furfurals show prominent maxima *ca.* 285 mμ, their presence in dried apricot concentrate was suspected.[3] They are readily extractable from aqueous solution with ether and other organic solvents. Darkened apricot extracts were therefore extracted with ether. To avoid pos-

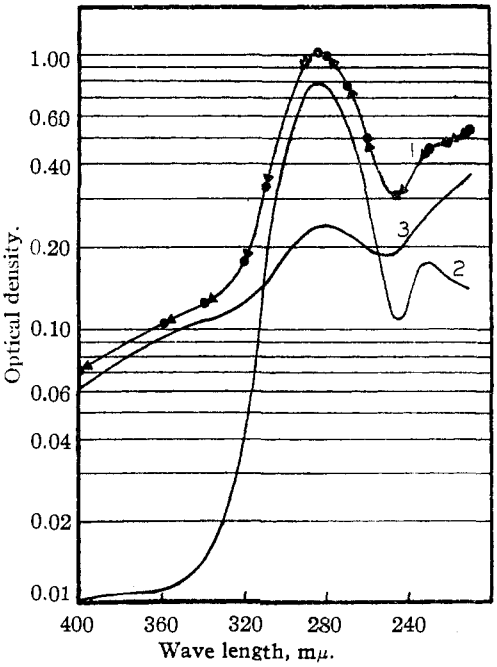


Fig. 3.—Absorption of original, ether extract and residue: 1, dark circles, original dark concentrate, dark triangles, sum of (2) and (3); 2, ether extract; 3, residue.

*G* (where *G* is the galvanometer throw), and on the Beckman DU spectrophotometer by the conventional log $1/T$ for a depth of 1 cm. for which, where concentrations are known, the absorption coefficients ($\epsilon$, with $c$ in moles per liter) can be calculated. When comparison is made of solutions with very different absorptions, the densities must be calculated on a common concentration basis, hence calculated densities of 2 to 4 may appear, *e. g.*, in Fig. 1.

(2) Paper No. IV of this series, THIS JOURNAL, 70, 3586 (1948).

(3) We wish to express our appreciation to Dr. S. M. Cantor who made available to us at this juncture results from his laboratory which indicated that the absorption in sugar solutions was due to hydroxymethylfurfural.

sible furfural formation during extraction the following precaution was taken. Extracts (in 50% alcohol) of darkened fruits were passed over ion-exchange resins (Duolites A-3 and C-3) to remove nitrogenous and acidic constituents.[4] The furfurals, if present, would not be removed, but would appear in the neutral fraction. A sample of this neutral fraction was extracted continuously for twenty hours with ether. The absorption spectra of the original, the residue and the ether-extractable material (transferred back to water) were compared (Fig. 3). The compounds responsible for the band at 285 mμ are almost completely extracted with ether and show the two banded spectrum typical of the furfurals.[2] Constituents of the ether extract reacted rapidly with 2,4-dinitrophenylhydrazine, indicating the presence of carbonyl groups. Positive furfural tests were obtained with xylidine and aniline acetate. Conclusive identification of both furfural and hydroxymethyl furfural has been accomplished.[5]

If furfurals are involved in browning, then the removal of furfurals as rapidly as they are formed should retard browning, and the addition of furfural should accelerate browning.

To test the effect of removal of furfurals, an apricot concentrate was stored at 57° and continuously extracted with ethyl acetate. (The side arm of the liquid extractor containing the concentrate was immersed in a hot water-bath at this temperature.)

In Figure 4 is plotted the optical density at 440 mμ as a function of time for four samples, three continuously extracted (in two of which extraction was stopped after 110 and 215 hours, respectively) and one not extracted at all. The results clearly show that so long as the extraction is continued darkening is negligible, but that on stopping the extraction darkening is rapid.

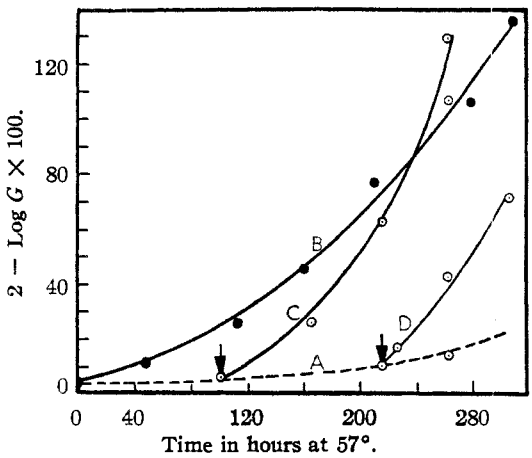


Fig. 4.—Effect of continuous extraction: A, continuous extraction; B, non-extracted concentrate; C and D, extraction discontinued at points indicated by arrows.

This, of course, shows only that the ethyl acetate extracted certain critical intermediates in browning. Furfural and hydroxymethylfurfural have been identified in the ethyl acetate fraction. There are also a number of as yet unidentified carbonyl compounds in the extract.[6]

To test the effect of furfural addition, redistilled colorless furfural (Quaker Oats Co.) was added to samples of unfermented and fermented concentrates. A fermented sample was included with sugar added as a 1:1 glucose-fructose mixture. Furfural concentrations were 0, 0.14, 0.28 and 0.54%. Measurements of the optical density at 440 mμ were made as before and rates were calculated as indicated earlier. As will be seen in Fig. 5, the rate increases with increasing concentrations over the range studied. With 0% added furfural, the fermented control has only 37% the rate of the fermented sample to which sugar has been added. The rates for these two samples are closely parallel, but the unfermented sample shows a greater response to added furfural.

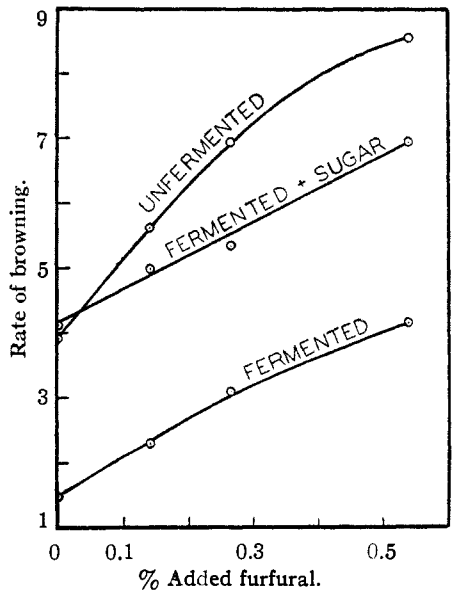


Fig. 5.—Effect of added furfural on darkening: The rate is the reciprocal of the time in days × 1000 required for the optical density to equal 0.400.

This suggests that fermentation has removed components not restored by addition of the glucose-fructose mixture. However, this explanation does not account for the fact that the fermented concentrate plus sugar and the unfermented sample have almost the same rate, in the absence of added furfural. In spite of these discrepancies, the addition of furfural always caused an increase in browning. This consideration, coupled with proof that furfurals are produced during storage, shows conclusively that furfural is a normal intermediate in some browning reaction.

**The Darkening of Furfurals.**—Furfurals are known to undergo reactions which give rise to dark colored polymers, but the mechanism in the pure substance appears to be oxidative.[7] Qualitatively, the picture in the dried fruit is quite different as is shown by the absorption characteristics of aqueous solutions of incubated concentrates, darkened with and without added furfural, and of darkened furfural exposed to air. The addition of furfural to the concentrate increases the rate of darkening, but does not change qualitatively the nature of the absorption spectrum. The absorption in the visible range for the three is shown in Fig. 6. For ease of comparison the concentrate with furfural has been superimposed on the concentrate alone, which is clearly not possible in the case of the darkened furfural.

These results show that browning caused by the addition of furfural is indistinguishable from that of natural concentrates, but differs from the browning of pure furfural. It has been observed that furfurals react with proteins and amino acids to form humin-like substances.[8] This type of reaction may be analogous to that occurring in dried fruits, but this has not yet been determined.

**Effect of Formaldehyde.**—It has been observed previously that formaldehyde in very low concentrations will inhibit browning of citrus juice concentrates.[9] Experi-

(4) Haas and Stadtman, unpublished data.
(5) Paper No. II of this series, *ibid.*, **70**, 3580 (1948).
(6) Paper No. III of this series, *ibid.*, **70**, 3583 (1948).
(7) Dunlop, Stout and Swandish, *Ind. Eng. Chem.*, **38**, 705 (1946).
(8) Dowell and Menaue, *J. Biol. Chem.*, **40**, 131 (1919).
(9) (a) Matlack and Sando, *Fruit Products J.*, **13**, 81 (1933); (b) Joslyn, *Ind. Eng. Chem.*, **33**, 308 (1941).

ments with apricot concentrates have shown the formaldehyde in concentrations of 0.15–0.30% of the total solids will greatly retard browning. When furfural and formaldehyde were added in equimolar amounts to apricot concentrates the accelerated rate of browning to be expected by the addition of furfural alone was not observed. While it cannot be assumed that formaldehyde is a specific inhibitor for furfural browning, the fact that it inhibits the browning of the natural concentrate, with and without added furfural, is entirely consistent with the conclusion that furfural is an intermediate to browning.

## Discussion

The suggestion that furfurals are involved in browning is not new.[9] However, no direct experimental evidence has been available to prove the validity of this suggestion. The above experiments show that furfurals accumulate during storage. Among the substances that might form furfurals in dried fruit are sugars, ascorbic acid, uronic acid, reductic acid and related compounds.[10] When sugars are removed from apricot concentrates by fermentation with bakers' yeast, the rate of browning is reduced one-third to one-half the normal rate, and little, if any, furfural accumulates during storage. This shows that sugars contribute materially to darkening and are a major source of furfural. It is of interest that the removal of sugars does not completely prevent browning. Therefore, if furfurals are obligatory intermediates in browning, substances other than sugars must also give rise to furfurals; otherwise more than one mechanism is involved in browning, at least one of which does not require furfural as an intermediate. Noteworthy in the latter connection is the observation that carbonyl compounds other than furfurals are produced during the storage of dried fruit. These, as yet unidentified, may also play an important role in browning.

It should be emphasized that these results refer to apricot concentrates stored under aerobic conditions. Previous results show that oxygen is not essential for browning of dried apricots but that the rate of browning is accelerated by the presence of oxygen.[11] The influence of oxygen on the furfural-type of browning in fruit has still to be determined.

(10) Isbell, *J. Res. U. S. Bureau Std.*, **33**, 45-60 (1944).
(11) *Ind. Eng. Chem.*, **38**, 324 (1946).

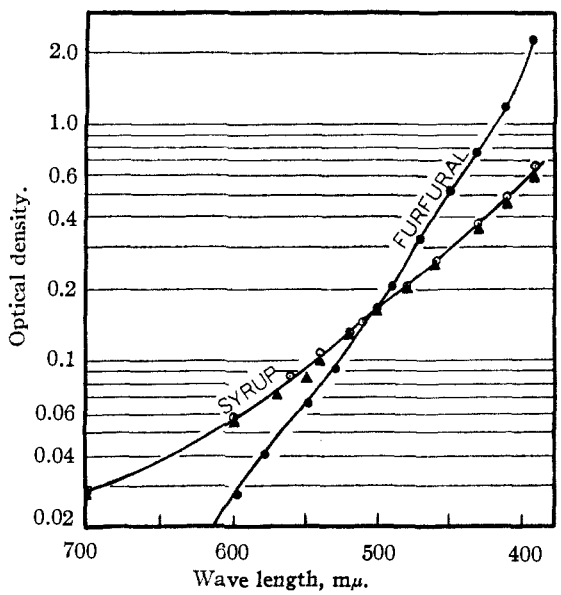


Fig. 6.—Optical densities of aqueous solutions of: 1, darkened apricot concentrate, no added furfural (open circles); 2, same with added furfural (dark triangles); 3, pure furfural, darkened in air (dark circles).

## Summary

The following evidence has been obtained to show that furfurals are involved as intermediates in the browning of apricots:

1. During the browning of dried apricots furfurals are produced.
2. Continuous ethyl acetate extraction of apricot concentrates to remove furfurals as rapidly as they are formed inhibits browning.
3. The addition of furfural to apricot concentrates increases the rate of browning.
4. The pigments produced by the addition of furfural to apricot concentrates are spectroscopically indistinguishable from the pigments produced in natural browning.
5. The formaldehyde inhibits both furfural browning and natural browning.

It has been shown that sugars are involved in the formation of furfurals and in browning.

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