a pair of overlapping triplets in the range 0.9–1.4 ppm.

The measurement of citrate salts rests on the NMR spectrum of the citrate ion. This is seen in the potassium citrate spectrum, a case where the salt is water soluble. In the case of calcium citrate it was necessary to dissolve the salt in an acidic medium and the spectrum is essentially that of citric acid, the species to be expected under these solution conditions. Attention is called to Figure 11 where low intensity spinning side bands are situated symmetrically about 0.52 ppm from the solvent peak.

The differences in the spectra recorded for the citrates and citric acid are useful. The NMR spectrum for citric acid shows signals from both methylene groups as two superimposed AB systems in the 2–3-ppm region due to the nonequivalence of the two protons of each methylene group. These are the signals available for integration and quantitative measurement. It is important to note that when the citrate spectrum is compared with citric acid, the methylene signal center is shifted downfield by about 20 Hz in the case of the acid. This difference provides a way for differentiation between samples of citric acid and its salts, but not in a mixture.

Table I presents a summary of the analytical results compiled in the study of all 17 compounds. It is noted that the internal standard and solvent are indicated for each compound. The assay results indicate that the maximum uncertainty experienced is less than 0.8%. The average values are all as might be expected for pure chemicals.

Some comment seems appropriate with regard to the process by which a particular resonance peak or cluster of resonance peaks is chosen for quantitative measurement. First, the analytically significant moiety giving rise to the resonance peak or peaks should be stable under analytical conditions and not be disturbed by proton exchange processes during the time of analysis. Second, in general the strongest resonance peak or multiplet is chosen provided that it is an independent signal standing at least 0.5 ppm if possible from any other signal. In many cases, a molecule may provide more than one analytically useful resonance signal. Use of the strongest signal clearly results in the most sensitive measurement since the ^1H NMR equivalent weight is smallest. In those instances where the strongest resonance is interfered with, an alternative choice is made. Third, the internal standard should be a compound that possesses a strong resonance signal, preferably a singlet, in the proximity of the chosen resonance signal of the specimen compound undergoing analysis.

ACKNOWLEDGMENT

The authors would like to thank the following individuals for their generous donations of FCC chemicals: A. Kimizuka, Ajinomoto USA Inc.; D. M. Winer, Hoffmann-La Roche Inc.; C. Joyce, Metachem, Inc.; A. J. Schmitz, Jr., Pfizer, Inc.; G. Gallagher, Fritzsche-Dodge & Olcott; and K. Mochizuki, Kyowa Hakko USA, Inc. Thanks also are due to William M. Richey of Case-Western Reserve University for his very helpful comments.

LITERATURE CITED

Becker, E. D., "High Resolution NMR—Theory and Chemical Applications", Academic Press, New York, N.Y., 1969.

Food Chemicals Codex, 1st ed, National Academy of Sciences–National Research Council, Washington, D.C., 1966.

Food Chemicals Codex, 2d ed, National Academy of Sciences–National Research Council, Washington, D.C., 1972.

Gutowsky, H. S., *J. Am. Chem. Soc.* **75**, 4567 (1953).

"High Resolution NMR Spectra Catalog", Vol. 1 and 2, Varian Associates, Palo Alto, Calif., 1963.

National Formulary, 14th ed, Mack Publishing Co., Easton, Pa., 1974.

Turczan, J. W., Medwick, T., *J. Pharm. Sci.* **65**, 235 (1976).

United States Pharmacopeia, 19th Revision, Mack Publishing Co., Easton, Pa., 1974.

Received for review March 8, 1976. Accepted November 11, 1976. This paper was presented at the 89th Annual Meeting of the Association of Official Analytical Chemists, Washington, D.C., Oct 16, 1975.

Browning Determination in Citrus Products

Shaul Meydav, Israel Saguy, and Isaiah J. Kopelman*

Previous methods for browning determination in citrus products suffer from serious accuracy drawbacks, especially while measuring minor differences, resulting from various processing treatment of juices and concentrates. The main inaccuracy and scattering of results were caused by carotenoid interference. A modified clarification procedure is proposed: pulp removal by centrifugation, succeeded by 1:1 dilution with ethyl alcohol for floculation of remnant cloud particles and filtration through Whatman No. 42 filter paper. By this procedure carotenoid interference was eliminated and good repeatability was obtained.

Various processed food products are susceptible to browning deterioration occurring during processing and storage. Due to browning reactions miscellaneous chemical changes develop, resulting in undesirable flavors (Hodge, 1967), nutrient losses (Labuza, 1972), and formation of brown color pigments (Reynolds, 1965). Several methods were applied for the measurement of visual browning changes in food products (Hendel et al., 1950, 1955; Notter et al., 1958; Stephenson et al., 1958). These methods are based on three main steps: (1) extraction of soluble color materials in case of solid foods; (2) clarification of the resulting extract, or direct clarification in liquid foods; (3) colorimetric measurement of the clarified extract. The methods proposed in the literature differ in the extraction–clarification step, and in the wavelength used for the optical density measurement.

Widely used clarification methods in citrus juices and citrus products were based upon the dilution of single strength juice with an equal volume of acetone (Curl, 1949; Joslyn, 1957; Bakal and Mannheim, 1966) or alcohol

Department of Food Engineering and Biotechnology, Technion—Israel Institute of Technology, Haifa, Israel.

Table I. Effect of Clarification Method on Browning Index (Average ± Standard Deviation) at 420 nm of Fresh and Heat-Treated (100 °C, 2 h) Citrus Juices

No.	Clarification method	Orange juice		Grapefruit juice	
		Fresh	Heated	Fresh	Heated
1	Acetone	0.165 ± 0.003	0.311 ± 0.003	0.115 ± 0.004	0.274 ± 0.003
2	Alcohol	0.145 ± 0.001	0.289 ± 0.003	0.103 ± 0.001	0.237 ± 0.001
3	Lead acetate	0.140 ± 0.002	0.235 ± 0.003	0.111 ± 0.002	0.202 ± 0.003
4	Centrifugation + alcohol (proposed procedure)	0.079 ± 0.001	0.195 ± 0.001	0.055 ± 0.001	0.186 ± 0.001
5	Millipore	0.077 ± 0.001	0.185 ± 0.001	0.054 ± 0.001	0.180 ± 0.001

(Joslyn, 1957; Karel and Nickerson, 1964). Clarified extracts for color measurements were obtained by filtration of the juice treated with those organic solvents. Meydav (1975) applied saturated lead acetate solution (AOAC, 1970) for precipitation of pulp and cloud particles prior to colorimetric determination of hydroxymethylfurfural. Karel and Nickerson (1964) used a Millipore filter (1.1 μm) for clarification of citrus juice prior to browning measurement. This procedure removes all suspended cloud particles yielding a clear serum suitable for direct colorimetric measurement. However, compared to the other clarification procedures mentioned above, the Millipore method is time consuming and inconvenient for routine work.

In the course of kinetic studies of browning deterioration in citrus products, presently undertaken by the authors, it was observed that the present clarification procedures using organic solvents suffer from serious accuracy drawbacks, especially when measuring of small differences in browning was required. This investigation was undertaken with the aim of evaluating the presently used clarification procedures and to propose a modification suitable for routine work, whereby higher accuracy in browning colorimetric determination can be obtained.

EXPERIMENTAL SECTION

Browning determination methods were evaluated in Valencia orange and Seedless Marsh grapefruit juices: (a) freshly extracted; (b) severely browned (heating in boiling water bath for 2 h).

The three clarification procedures based on previous methods were as follows. (1) Citrus juice was diluted with an equal volume of acetone, centrifuged at 800g for 5 min in an MSE centrifuge, and filtered through filter paper (Whatman No. 42). During filtration the funnel was covered with a watch glass to prevent loss of solvent (only with acetone). Removal of pulp by centrifugation prior to the filtration step increased substantially the filtration rates. (2) Juice was diluted with an equal volume of 95% ethyl alcohol, centrifugated at 8000g for 20 min, and filtered as outlined in step 1. (3) Four milliliters of $Pb(OAc)_2$ saturated solution was added to 25 ml of juice and the volume was made up to 50 ml with distilled water. Further clarification was carried out as above.

In addition to these three methods a modified clarification procedure described below was carried out. (4) Single strength citrus juice was centrifuged at 800g (2000 rpm) for 20 min to remove pulp and coarse cloud particles. The supernatant was diluted 1:1 with 95% alcohol and filtered through Whatman No. 42 filter paper, to obtain a fully clarified extract.

As a reference the following method was used. (5) Juice was clarified by using a 0.45-μm Millipore filter (Karel and Nickerson, 1964).

Transmittance spectra (Beckman D.B.) and browning indexes (absorbance at 420 nm; Beckman D.U. with Gilford attachment for digital readout) of all clear extracts were compared with the Millipore filtered serum (method no. 5). Six replicates of each clarification procedure were used for absorbance measurements.

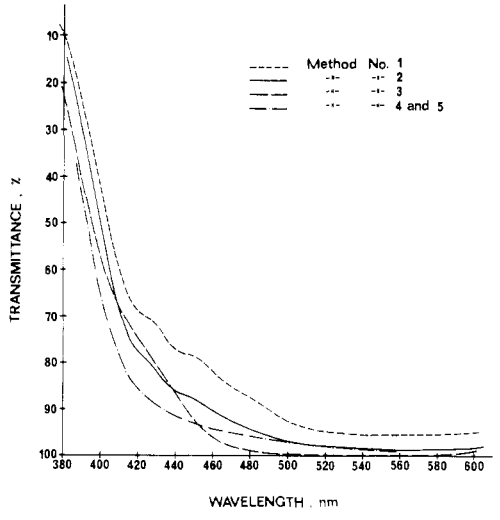

Figure 1. Effect of clarification procedure on transmittance spectra of fresh orange juice extracts.

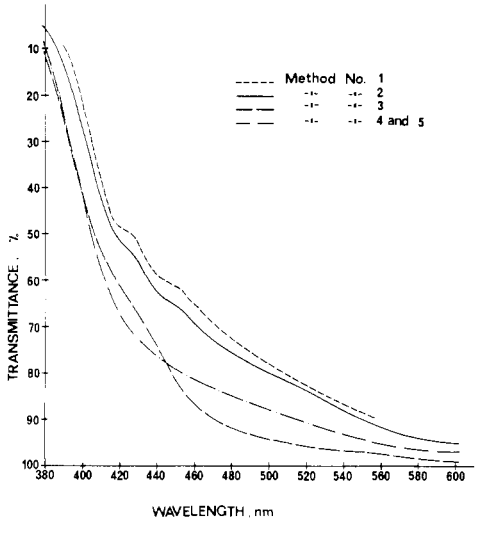

Figure 2. Effect of clarification procedure on transmittance spectra of heated orange juice extracts.

RESULTS AND DISCUSSION

Browning indexes of fresh and browned citrus juice extracts are summarized in Table I. Transmittance spectra of fresh and severely browned orange juice are given in Figures 1 and 2.

Compared with the Millipore filtered serum, all the transmittance spectra show that all clarification procedures, except that of step 4, resulted in alterations in light absorption characteristics. With acetone (procedure 1), the clarified orange juice spectra were shifted to much higher absorption values, and distinctive shoulders ap-

Table II. Effect of Clarifying Agents on Browning Index Alterations in Clear Orange Serum Obtained by the Millipore Method (Procedure 5)

No.	Clarification treatment	Fresh	Treated
1	Millipore	0.077	0.185
2	Millipore + acetone	0.066	0.170
3	Millipore + alcohol	0.077	0.185
4	Millipore + lead acetate	0.121	0.218

peared at about 425 and 450 nm (Figures 1 and 2). It may be deduced that these spectral differences are caused by some carotenoids extracted from juice pulp, as the spectral pattern resembles the characteristic transmittance of orange juice carotenoids (Petrus and Dougherty, 1973; Meydav, 1975). Slight spectral shifts were also found in acetone-clarified grapefruit juices, but none of the shoulders were observed. The less pronounced variations may be attributed to much lower concentration of carotenoids (Bauernfeind, 1958), and their absorption peaks (380, 400, and 425 nm; Sinclair, 1972). A similar, but less accentuated pattern, was observed with the alcohol-clarified juices (step no. 2).

Precipitation by lead acetate caused a severe deviation in transmittance spectra from the exponential curve typical of brown pigments (Meschter, 1954), thus excluding the possibility of its application for browning determinations.

Contrary to the deviations obtained by the commonly reported procedures (1, 2, and 3), a remarkable agreement was found, both in absorption indexes (Table I) and in spectral patterns (Figures 1 and 2) between the proposed method (step 4) and the Millipore filtered serum (step 5). Thus, it can be seen that removal of carotenoids from citrus juice (by pulp sedimentation) prior to final clarification is necessary. Moreover, it should be emphasized that heat treatment induced changes in carotenoid composition and content (Singleton et al., 1961; Meydav, 1975) may be attributed to browning changes while directly clarifying with organic solvents.

Browning indexes obtained in the Millipore filtered orange serum, subsequently treated by the clarifying agents (Table II), show that, even excluding the effect of carotenoids, only alcohol treatment resulted with no absorbance alterations. The decrease in browning indexes when acetone was used suggests that some precipitation of the browning substances may occur. Thus, acetone may affect the browning index determination in two (opposite in nature) ways: (a) the extraction of carotenoids; (b) slight precipitation of browning pigments.

In conclusion, it was found that browning determinations in citrus products by the clarification methods reported in the literature interfered with carotenoid extraction. A modified clarification procedure, which eliminates this interference, is proposed. Browning indexes obtained by this procedure were almost identical with results derived by the laborious and time-consuming Millipore filtering method. Furthermore, the procedure is suitable for routine laboratory analysis and its repeatability, as expressed by the standard deviation (Table I), is significantly better, in comparison to the widely applied acetone clarification procedure.

LITERATURE CITED

Association of Official Analytical Chemists, "Official Methods of Analysis", 10th ed, Washington, D.C., 1970, Method 31.021 d.

Bakal, A., Mannheim, H. C., *Isr. J. Technol.* **4**, 262–267 (1966).

Bauernfeind, J. C., Symposium on Carotenoids in Fruits Juices and Concentrates, Bristol, Juris-Verlag, Switzerland, 1958, p 265.

Curl, A. L., *Food Res.* **14**, 9–14 (1949).

Hendel, C. E., Baily, G. F., Taylor, D. H., *Food Technol.* **4**, 344–347 (1950).

Hendel, C. E., Silveira, V. G., Harrington, W. O., *Food Technol.* **9**, 433–438 (1955).

Hodge, J. E., *Symp. Foods: Chem. Physiol. Flavors, Proc.*, 465–491 (1967).

Joslyn, M. A., *Food Res.* **22**, 1–14 (1957).

Karel, M., Nickerson, J. T. R., *Food Technol.* **18**, 1214–1218 (1964).

Labuza, T. P., *Crit. Rev. Food Technol.* **3**, 217–240 (1972).

Meschter, E. E., in Symposium on Color in Foods, Farrel, K. T. et al., Ed., National Academy of Sciences–National Research Council, Washington, D.C., 1954.

Meydav, S., Ph.D. Thesis (in Hebrew), Technion, Israel Institute of Technology, 1975.

Notter, G. K., Taylor, D. H., Brekke, J. E., *Food Technol.* **12**, 363 (1958).

Petrus, D. R., Dougherty, M. H., *J. Food Sci.* **38**, 659–661 (1973).

Reynolds, T. M., *Adv. Food Res.* **14**, 167–263 (1965).

Sinclair, W. B., "The Grapefruit", University of California, Division of Agricultural Sciences, 1972.

Singleton, V. L., Gortner, W. A., Young, H. Y., *J. Food Sci.* **26**, 49–52 (1961).

Stephenson, R. M., Sano, T., Harris, P. R., *Food Technol.* **12**, 622 (1958).

Received for review August 17, 1976. Accepted November 29, 1976.