An Improved Method To Determine Nonenzymic Browning in Citrus Juices

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Two nonenzymic browning measurement methods were evaluated with canned and bottled orange and grapefruit juices, namely freeze-drying and solvent extracting. The freeze-dry method functioned effectively with nonpigmented grapefruit juice but was subject to considerable interference with pigmented juices. On the basis of several studies, a simple three-step solvent extraction procedure is proposed to evaluate the extent of nonenzymic browning. Variables found critical include solvent, clarification process, and time and temperature effects.

Color is an important quality factor in the marketing of citrus juices. However, detrimental changes in color, primarily caused by nonenzymic browning, reduce consumer acceptance for these products. Juice composition, packaging container, processing conditions, temperature, and storage time during warehousing and marketing affect the extent of nonenzymic browning (Marshall et al., 1986). Extensive research (Nomura, 1955; Joslyn, 1957; Clegg, 1964; Wolfrom et al., 1974; Varsel, 1980; Lee and Nagy, 1988) has been conducted on nonenzymic browning of citrus juices, but the mechanisms for brown pigment formation have not been completely elucidated.

An important part of browning research is to have a simple, reliable method that will quickly determine the amount of brown pigment formed under varying conditions. Methods utilized to quantify browning in citrus juice (Curl, 1949; Karel and Nickerson, 1964; Meydav et al., 1977; Kanner et al., 1982) were found by us to be either too time-consuming and/or subject to considerable variability. The objective of our research was to develop a method that objectively yields a "browning index" value representative of the pigments that contribute to the undesirable brown tint of citrus juices. To this end, our requirements for a satisfactory browning method were simplicity, good sensitivity, and reproducibility. Several different approaches were tried, and the one that best fit our requirements is presented.

EXPERIMENTAL SECTION

Samples. Commercially processed single-strength orange and grapefruit juices were purchased from a local processing company. The commercial samples were packed in tin-plated cans with enamel-coated lids (177 mL; 48 cans/case) and glass bottles (208 mL of grapefruit juice; 296 mL of orange juice; 24 bottles/case). Juice samples were taken directly from production lines and placed in a laboratory locker at 5 °C. Recently harvested oranges and grapefruit were also obtained from groves located at the Citrus Research and Education Center, Lake Alfred.

Methods and Instrumentation. *Freeze-Dry.* Orange and grapefruit juices were freeze-dried on a Thermovac Model FD-ULT-6 (Thermovac Industries Corp., Copiague, NY). Fifty milliliters of single-strength juice was frozen as a shell in a 500-mL round-bottom flask by rotation in a bath of liquid nitrogen and then dried for about 16 h under vacuum (20 Pa). The dried residue was extracted with 30 mL of methanol and filtered through Whatman No. 42 filter paper and the filtrate brought to 50-mL volume with methanol. The 50-mL sample was placed in a freezer overnight (−10 °C) and passed through a 0.45-μm filter (Magna nylon 66 membrane filter) the following day. The clarified, filtered sample was read at 420 nm with a Bausch and Lomb Spectronic 88 using 13 mm cuvettes (10-mm light path). The analog signal from the spectrometer was measured with a Fluke Model 75 four-digit multimeter for improved accuracy. Readings were recorded to three significant figures.

Alcohol Extraction. Alcohols tested were methanol, 95% ethanol, 1-propanol, and 2-propanol. Single-strength juices, subjected to varying alcohol and temperature treatments, were centrifuged for 15 min at 1000*g*, filtered through Whatman No. 42 filter paper and/or 0.45-μm filter, and read at 420 nm. The varying extraction and preparation procedures will be discussed more extensively in Results and Discussion.

RESULTS AND DISCUSSION

Freeze-Dry. The freeze-dry method was developed to isolate brown pigments prior to separation by HPLC (Rouseff et al., 1987a,b). Freeze-drying gently removes water from the sample and, thus, allows the dried residue containing the brown pigments to be selectively extracted with organic solvents. Brown pigments are poorly extracted from a freeze-dried sample with nonpolar to weakly polar solvents, e.g., petroleum ether, hexane, methylene chloride, and ethyl ether, but are effectively extracted with strong polar solvents, e.g., methanol (Fisher, 1987). Although laborious, the freeze-dry method demonstrated good sensitivity and reasonable reproducibility for nonpigmented grapefruit juice (Table I). The juice of white-fleshed grapefruit contains virtually no colored pigments (Gross, 1977), and therefore, color interference at 420 nm is insignificant. However, when we analyzed commercial orange juice samples, absorbance values were noticeably higher than grapefruit juice values (Table I). Absorption at 420 nm by orange juice samples stored at 0 °C was about 4-fold higher than grapefruit juice stored at this same nonbrowning temperature. The increased absorbance at 420 nm by orange juice is due to carotenoids (Petrus and Nagy, 1984). Alcoholic solutions of orange juice show major absorbances at 425, 443, and 465 nm (due mainly to the carotenoids present) and, therefore, would distort the value representing browning (absorbance at 420 nm).

Alcohol Extraction. The procedure of Meydav et al. (1977) to determine browning of citrus juices is widely used, but we found the procedure to be imprecise and subject to variation caused by the sample preparation, temperature, and filtration procedures. However, the simplicity of this procedure warranted further studies to improve sensitivity and reproducibility. To this end, we

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Table I. Browning Values of Canned and Glass-Packed Orange and Grapefruit Juices Stored for 15 Weeks at Varying Temperatures[a]

temp, °C	grapefruit juice		orange juice	
	canned	glass-packed	canned	glass-packed
0	0.119 ± 0.002	0.108 ± 0.002	0.453 ± 0.010	0.413 ± 0.003
20	0.116 ± 0.008	0.152 ± 0.004	0.464 ± 0.009	0.452 ± 0.001
30	0.120 ± 0.005	0.213 ± 0.003	0.460 ± 0.005	0.468 ± 0.001
40	0.320 ± 0.057	0.392 ± 0.023	0.630 ± 0.008	0.698 ± 0.023
50	0.501 ± 0.007	0.821 ± 0.005	1.021 ± 0.003	0.996 ± 0.004

[a] Samples prepared by freeze-dry method; values (mean ± SD) represent three replications; browning values determined by absorbance at 420 nm.

investigated the effects of solvent, temperature, time, and clarification procedures. Our method entailed the following:

Step 1. Commercially processed single-strength orange or grapefruit juice (10 mL) is centrifuged at 1000*g* for 15 min.

Step 2. Three milliliters of the supernatant fluid is removed and placed in a 10-mL centrifuge tube. Three milliliters of alcohol is added and the resultant solution mixed and placed in an ice bath for 15 min to accelerate flocculation of the finely suspended, colloidal particles.

Step 3. The sample is recentrifuged at 1000*g* for 15 min, the clear supernatant fluid removed with a Pasteur pipet and placed in a cuvet, and the absorbance read at 420 nm (browning index).

This three-step procedure, while appearing simple, required several studies to verify conclusions. The vast majority of methods that determine browning initially begin by diluting single-strength juice (either before or after centrifugation) with an equal volume of a polar, water-miscible solvent, e.g., acetone (Curl, 1949) or 95% ethanol (Joslyn, 1957; Karel and Nickerson, 1964; Meydav et al., 1977). The function of the solvent is 3-fold: First, it increases the solubility of the brown pigments. Second, it dehydrates the finely suspended colloidal materials of the cloud causing flocculation. Third, it should minimize extraction of the more lipid-soluble carotenoids.

Turbidity of citrus juice is attributed to "cloud" whose approximate composition is 25–28% lipids, 30–35% protein, 2–5% cellulose and hemicellulose, 4–6% pectin, and 25–30% hesperidin, nucleic acids, ash, and other juice cellular materials (Scott et al., 1965; Baker and Bruemmer, 1969; Klavons and Bennett, 1985, 1987). Because cloud (turbidity) contributes to density readings at 420 nm, effective removal is a prerequisite for an accurate browning determination.

A series of tests were performed with four different alcohols to evaluate brown pigment solubilization, cloud flocculation, and carotenoid interference (Table II). Fresh hand-squeezed white grapefruit juice (Marsh seedless) showed the lowest absorbance values because this method of juice expression is gentle and yields the smallest amount of cloud. No significant differences in absorbance means were observed with methanol, ethanol, and 2-propanol, but 1-propanol showed a significantly higher absorbance value (5% level). Freshly processed grapefruit juice (Marsh seedless white) showed higher absorbance values with all extracting alcohols. It is important to note that the processes for production of commercial juice yield a more turbid product than from hand-expressed juice. Highly efficient extraction machines, finishing equipment, concentration units, and pasteurizing treatments cause extensive maceration of tissue and, thus, produce multiphase systems composed of many components ranging in solubility from substances in true solution to those existing in suspension (Albrigo and Carter, 1977; Nordby and Nagy, 1980). The lowest mean absorbance value for freshly processed grapefruit juice was recorded for methanol. However, it was not significantly different from ethanol but was different from 1-propanol and 2-propanol. The marked differences in solvent effects were noted with glass-packed grapefruit juice stored at 40 °C for 15 weeks (column aged). Each alcohol was significantly different from the others; methanol yielded the lowest absorbance reading.

Table II. Alcohol Effects on Browning Absorbance Values[a]

solvent	grapefruit juice			orange juice		
	FS[b]	FP[c]	aged[d]	FS[b]	FP[c]	aged[d]
methanol	0.070[w]	0.134[w]	0.541[w]	0.098[w]	0.167[w]	0.627[w]
95% ethanol	0.072[w]	0.137[w,x]	0.565[x]	0.134[x]	0.182[x]	0.642[x]
2-propanol	0.070[w]	0.147[y]	0.598[y]	0.112[y]	0.255[y]	0.787[y]
1-propanol	0.086[x]	0.142[x,y]	0.582[z]	0.126[z]	0.337[z]	0.781[y]

[a] Samples prepared by alcohol extraction method; absorbance values at 420 nm represent mean of four replications; mean separation within columns (w, x, y, z) by Newman-Keuls range test, 5% level. [b] Fresh-squeezed juices of Marsh grapefruit and Valencia oranges. [c] Freshly processed, glass-packed juices; samples taken directly from processing line. [d] Glass-packed juice aged for 15 weeks at 40 °C.

Orange juice showed higher absorbance values than grapefruit juice because of the added contribution of carotenoids. Fresh-squeezed Valencia orange juice yielded significantly different absorbance values with the four alcohols (Table II). Also, freshly processed orange juice showed significantly different absorbance readings for the four alcohols. The mean methanol absorbance value was lowest, whereas the 1-propanol value was highest with the freshly processed juice. A significant difference between methanol and ethanol was noted for aged, bottled orange juice. Both methanol and ethanol values differed significantly from 2-propanol and 1-propanol. Table II indicates that methanol is the best alcohol for this extraction procedure because it yielded lower absorbance values and was less affected by the presence of carotenoids.

Passage of an alcohol-diluted juice through Whatman filter paper (No. 1 or 42) to obtain a clarified solution is commonly used in browning determination (Joslyn, 1957; Meydav et al., 1977). However, we have found that filtration adds to the variability of absorbance readings. Passage of a solution of fine flocculant material (cloud) through Whatman No. 42 filter paper will not cause retention of all finely dispersed colloidal materials. Filtration simply breaks up the flocculant material, and a false impression is obtained that the filter retained the flocculant and a clarified solution ensued. We have consistently noted that when flocculant samples were passed through Whatman No. 42 filter paper, readings were always higher and more variable than by simple centrifugation (Table III).

Examination of well-known browning methods as proposed by Curl (1949), Joslyn (1957), and Meydav et al. (1977) indicates no specific time for the alcohol–juice or acetone–juice mixture to flocculate before filtration and

Table III. Centrifugation versus Filtration on Browning Values[a]

	grapefruit juice				orange juice			
	filtered[b]		centrifuged		filtered		centrifuged	
treatment	mean	CV[c]	mean	CV	mean	CV	mean	CV
hand-squeezed	0.096	1.6	0.066	1.5	0.099	3.4	0.097	2.4
fresh-processed	0.164	5.9	0.141	1.5	0.226	4.3	0.167	2.0
aged	0.600	6.9	0.533	0.8	0.619	1.8	0.607	1.3

[a] Absorbance values at 420 nm. [b] Filtered through Whatman No. 42 filter paper at a vacuum of ca. 34 kPa. [c] Coefficient of variation in percent.

Table IV. Effects of Sample Preparation Time and Temperature on Browning Values of Bottled Grapefruit Juice

	control juice[a]		aged juice[b]	
time, min	ice bath (0 °C)	room temp (24 °C)	ice bath (0 °C)	room temp (24 °C)
5	0.230 ± 0.005[c]	0.480 ± 0.019	0.326 ± 0.005	0.339 ± 0.001
10	0.160 ± 0.003	0.454 ± 0.028	0.316 ± 0.005	0.334 ± 0.004
15	0.143 ± 0.002	0.330 ± 0.018	0.302 ± 0.002	0.333 ± 0.003
20	0.128 ± 0.003	0.305 ± 0.011	0.301 ± 0.001	0.330 ± 0.002
25	0.115 ± 0.003	0.284 ± 0.002	0.300 ± 0.002	0.322 ± 0.004
30	0.110 ± 0.001	0.184 ± 0.003	0.300 ± 0.003	0.319 ± 0.003

[a] Grapefruit juice obtained directly from processing line and immediately placed in a locker at 5 °C. [b] Grapefruit juice aged at 40 °C for 6 weeks. [c] Browning values reported as absorbance at 420 nm; values (mean ± SD) represent four replications.

density reading and no defined preparation temperature (we can only assume sample preparation was carried out at room temperature). However, we have found time and temperature of sample preparation to be very critical variables in browning evaluation. To systematically study these effects, experiments were conducted with control and aged orange and grapefruit juices packed in glass containers. All samples were initially subjected to the following approach: (1) Juice samples (10 mL) were centrifuged at 1000*g* for 15 min, and (2) 3 mL of the supernatant was removed, placed in a second centrifuge tube, and mixed thoroughly with 3 mL of methanol. Experimental variation occurred in the following steps: (3) The diluted samples were placed either in an ice bath (0 °C) or in a controlled room-temperature (24 °C) chamber, (4) samples were removed from the ice bath or room-temperature chamber at time periods of 5, 10, 15, 20, 25, and 30 min, and (5) all samples were centrifuged for 15 min at 1000*g* and the absorbance of the supernatant was read at 420 nm.

Experiments with grapefruit juice (Table IV) show that when control samples were placed in an ice bath, lower absorbance values were recorded when compared to room-temperature samples. Absorbance values also decreased with time. Aged grapefruit juice showed similar effects with temperature and time; however, absorbance values after about 15 min decreased slightly and appeared to plateau. A similar pattern emerged with bottled orange juice (Table V).

It is evident from Tables IV and V that temperature affects flocculation of finely suspended colloidal particles. Flocculation is more extensive when samples are placed in an ice bath and, thus, would tend to yield a more accurate assessment of the amount of brown pigments present. Samples placed at room temperature show higher absorbance values and tend to exaggerate the extent of browning in juices.

Although absorbance decreases with preparation time (or time interval for flocculation), 15 min was selected as a compromise based on time efficiency and the attainment of reasonably accurate browning values. Time and temperature are more critical in citrus juices that have not been subject to extensive heat treatments or long storage periods. The cloud is apparently more stable in freshly processed juice and, therefore, requires a longer period to flocculate. In aged juice, however, storage effects (temperature, storage time) have apparently exerted destabilizing conditions on the cloud with the result that shorter time periods are necessary for flocculation. Excellent discussions on juice cloud stability may be found in works by Baker and Bruemmer (1969), Krop and Pilnik (1974), and Baker (1980).

Table V. Effects of Sample Preparation Time and Temperature on Browning Values of Bottled Orange Juice

	control juice[a]		aged juice[b]	
time, min	ice bath (0 °C)	room temp (24 °C)	ice bath (0 °C)	room temp (24 °C)
5	0.177 ± 0.004[c]	0.241 ± 0.008	0.429 ± 0.004	0.447 ± 0.003
10	0.172 ± 0.001	0.219 ± 0.007	0.424 ± 0.004	0.427 ± 0.006
15	0.162 ± 0.001	0.218 ± 0.003	0.412 ± 0.005	0.422 ± 0.002
20	0.160 ± 0.002	0.191 ± 0.007	0.402 ± 0.003	0.417 ± 0.002
25	0.157 ± 0.003	0.185 ± 0.004	0.400 ± 0.001	0.416 ± 0.003
30	0.146 ± 0.001	0.183 ± 0.002	0.395 ± 0.005	0.400 ± 0.004

[a] Orange juice obtained directly from processing line and immediately placed in a locker at 5 °C. [b] Orange juice aged at 40 °C for 6 weeks. [c] Browning values reported as absorbance at 420 nm; values (mean ± SD) represent four replications.

CONCLUSION

The three-step, alcohol extraction procedure has been extensively researched. It entails centrifugation of the sample, addition of alcohol, chilling in an ice bath, recentrifugation, and reading the absorbance at 420 nm. It has been used to record nonenzymic browning reaction rates in both orange and grapefruit juices. This method has proven more effective than other proposed methods and should prove useful to quality-control laboratories because of its speed, simplicity, and reproducibility.

Registry No. Methanol, 67-56-1; ethanol, 64-17-5; propanol, 71-23-8; 2-propanol, 67-63-0.

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Effect of Processing on the Phytic Acid Content of Bengal Grams (*Cicer arietinum*) Products

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The effect of processing on the phytic acid content of brown and white varieties of Bengal grams (*Cicer arietinum*) has been studied. Soaking, boiling, roasting, and frying invariably result in the loss of phytic acid. Loss of phytic acid in presoaked grams increases with resting time. The loss during the soaking and resting period of presoaked grams may be attributed to enzymic activity. Immature brown grams contain much less phytic acid than dried mature brown grams, but the loss of phytic acid on boiling and roasting is greater in the former than in the latter. Soaking of white grams in sodium bicarbonate solution reduces the loss of phytic acid on soaking and heat treatments.

Legumes contain a significant amount of phytic acid (Makower, 1970; Thompson and Erdman, 1982; Chen and Pan, 1977; Chang and Schimmer, 1977). These are used as traditional supplements to staple cereals in Pakistan as a source of good-quality proteins. A wide variety of legumes known as pulses are grown in Pakistan. Bengal gram (*Cicer arietinum*) is the most commonly used legume. It is consumed as a curry or in snack foods.

Phytic acid has antinutritional properties because of its ability to chelate several metals and thereby reduce their bioavailability, resulting in mineral deficiencies and various diseases (Harrison and Mellanby, 1939; Reinhold et al., (1973). It is decreased significantly during the processing of cereals and legumes (Beal and Mehta, 1982; Harland and Harland, 1980).

Previously the effect of processing on the phytic acid content of wheat products has been reported (Khan et al., 1986). This paper deals with the effect of processing on the phytic acid content of Bengal grams (both brown and white varieties) for the preparation of various traditional food products.

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EXPERIMENTAL SECTION

Materials and Methods. *Description of Products. Brown Grams. Boiled Grams.* The grams were cleaned and soaked overnight in water. The water was drained off, and the grams were boiled in fresh water until they become soft enough to be eaten. These are consumed as such or mixed with boiled, peeled, and sliced potatoes after spicing with salt, chillies, lemon, etc. These are also used in the preparation of curry with or without meat and fried rice.

Roasted Grams. The grams were cleaned and roasted in a sand bath until the outer skin (hulls) split. These are consumed both with or without hulls as a snack food.

Presoaked and Roasted Grams. The grams were soaked in water overnight. The water was removed, and the grams were covered with moist muslin cloth. The grams were roasted in sand/salt bath after an interval of 2–6 h. These are consumed after the sand and salt particles are rubbed off.

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