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CONTROL OF ENZYMATIC BROWNING IN RAW FRUIT JUICE BY FILTRATION AND CENTRIFUGATION[1]

G. M. SAPERS[2]

Eastern Regional Research Center, ARS
U.S. Department of Agriculture
600 E. Mermaid Lane
Philadelphia, PA 19118

ABSTRACT

Filtration and centrifugation were investigated as means of preventing enzymatic browning in minimally processed fruit juices. The capacity of raw apple, grape and pear juices to undergo browning was associated with particulate fractions that could be removed by filtration with Bentonite and/or diatomaceous earth, microfiltration or ultrafiltration, depending on the commodity and cultivar. Centrifugation prevented browning in pear juice and in Granny Smith, Golden Delicious, and Red Delicious but not McIntosh apple juice, provided that foam was excluded and the relative centrifugal force was sufficient. A method for the preparation of nonbrowning, cloudy juices by recombination of juice supernatant or ultrafiltration permeate with particulates, heat-treated to inactivate polyphenol oxidase, was investigated.

INTRODUCTION

Conventionally, fruit and vegetable juices are preserved by pasteurization, which inactivates degradative enzymes and kills spoilage microorganisms but also may impart cooked flavors to heat-sensitive products. The food industry has given increasing attention in recent years to meeting consumer demands for "freshness" in processed foods by pursuing the development of minimally processed products (Albrecht 1986; Shewfelt 1987). Such characteristics might be achieved in raw fruit juices, cold-sterilized by membrane filtration, or preserved by refrigeration, if enzymatic processes associated with quality loss during storage such as enzymatic browning could be prevented (Borgstrom 1954; Moyer and Aitken 1980). Previously, we have investigated the use of various ascorbic acid (AA) derivatives, polyphenol oxidase (PPO) inhibitors, chelating agents, and cyclodextrins in delaying but not eliminating browning in juices during short-term storage (Sapers *et al.* 1989a,b,c).

An alternative approach to this problem is suggested by reports that, in many fruits, PPO activity is largely associated with particulate fractions (Mayer and Harel 1979; Vamos-Vigyazo 1981). The separation of such particulates from raw juices might be a sufficient step to eliminate or greatly reduce enzymatic browning during prolonged storage. Furthermore, with a molecular weight in excess of 30,000 daltons (although considerably smaller molecular masses have been reported in a few cases) (Vamos-Vigyazo 1981), even soluble forms of PPO might be separated from raw juices by ultrafiltration. Therefore, the objective of this study was to determine whether the capacity of raw fruit juices to undergo enzymatic browning during storage could be removed by centrifugation or filtration. Such treatments might form the basis of novel minimal processes for raw juices.

METHODS AND MATERIALS

Juice Preparation

To test the hypothesis that browning in raw juices could be prevented by removal of particulates, a model system was devised comprising raw juices, treated to remove particulate fractions and then stored under conditions favoring rapid oxidation. Juices were prepared from ripe apples, pears and grapes obtained at local food stores. Red and Golden Delicious apples were provided by ARS's Appalachian Fruit Research Station in Kearneysville, WV. The apples were cut into wedges with a kitchen appliance, dipped in 1% AA for ca. 30 s to prevent browning during juicing (omitted with Granny Smith apples because of their lesser tendency to brown), and drained. The wedges were juiced in an Acme Supreme Juicerator (Acme Juicer Mfg. Co., Lemoyne, PA), lined with a 6 × 56.5 cm strip of Whatman No. 1 filter paper. An additional 100 ppm AA was mixed into the freshly prepared juice to prevent browning during subsequent filtration and centrifugation steps (i.e., for several hours). Pear juice was prepared by the same procedure except that the fruit was sliced into cross cuts before dipping in 1% AA, draining and juicing. Grapes were cut in half before juicing with the Juicerator. Portions of these juices were treated by various filtration and centrifugation procedures; untreated portions containing AA, added during cutting and juicing, were used as controls.

Freshly prepared raw juices were analyzed for AA by the HPLC procedure of Liao and Seib (1990) to determine treatment residues. The juices were diluted

1:100 with an extraction solution containing 0.38% metaphosphoric acid and 70% mobile phase (with acetate omitted).

Filtration of Juices

In experiments to determine the effects of filtration on browning, the freshly prepared raw juices, with or without the addition of 0.1–2.0% Celite Analytical Filter Aid (CAFA; Manville Products Corp., Denver, CO), Bentonite (Sigma Chemical Co.), Celite 545 (Manville Products Corp.), talc (U.S.P., J. T. Baker Chemical Co., Phillipsburg, NJ), and silica gel (TLC, Mallinckrodt Chemical Works, St. Louis, MO), were filtered through Whatman No. 541 paper under suction. In separate experiments carried out with Granny Smith and Red Delicious juice, two other silica-derived adsorbents (BRITESORB® L900 and DP4250, The PQ Corp., Valley Forge, PA) were added at levels of 200–10,000 ppm. The suspensions were stirred for 10 min and then filtered through Whatman No. 541 paper under suction. In one trial, 0.4% Tween 80 (polyoxyethylene sorbitan monooleate, Sigma Chemical Co.) was dispersed in Golden Delicious juice prior to CAFA addition and filtration. A 5% slurry of Bentonite in water, dispersed with a Polytron homogenizer (Brinkmann Instruments Co., Inc., Westbury, NY), was added to some juice samples at levels corresponding to 0.1–0.5% Bentonite. The juice was then stirred for 5 min, mixed with 2% CAFA, and filtered, as described above. Juices that had been clarified by CAFA addition and filtration through Whatman No. 541 paper or by centrifugation (see below) were subjected to microfiltration through a Falcon 0.22 μm cellulose ester membrane or to ultrafiltration with a Minitan tangential flow system (Millipore Corp., Bedford, MA) through polysulfone membranes having molecular weight cut-off (MWCO) values of 30,000, 100,000 and 300,000 daltons.

Centrifugation

To determine the effects of centrifugation on the capacity of apple and pear juices to brown, 25 mL portions of raw juice were centrifuged in 30 mL polycarbonate Oak Ridge style centrifuge tubes at speeds of 1000–28,000 rpm (127–100,095 $\times$ g) for 10 min in a F-28/36 fixed-angle rotor of a Sorvall RC 28S Supraspeed Centrifuge (Du Pont Company, Wilmington, DE); all centrifugation was done at 4C. Since the presence of foam in freshly prepared juice was believed to interfere with centrifugation, some trials were carried out with juice added to centrifuge tubes by pipet rather than by graduated cylinder so that foam could be excluded.

Heat Inactivation of PPO in Juice Pellets

Juice particulates were isolated as pellets by centrifuging 25 mL portions of freshly prepared juice for 10 min at 28,000 rpm and decanting the juice supernatants. The pellets were drained, rinsed with water, suspended in 5 mL water added to the centrifuge tubes, and heated for 5 min in a boiling water bath, at which time the suspension temperature was 87C. The heated pellet suspension was cooled and centrifuged for 10 min at 28,000 rpm. The new pellets were drained and dispersed in juice supernatant or ultrafiltration permeate, obtained by passing the juice supernatant through a 30,000 dalton MWCO membrane, with a serrated Teflon pestle tissue grinder (Thomas Scientific, Swedesboro, NJ) and evaluated for browning, as described below.

Evaluation of Capacity to Brown

Portions of treated juices and untreated controls (25 mL in 50 mL beakers containing 25 mm magnetic stirrer bars, covered with Parafilm M to prevent evaporation), were stirred at 300 rpm for as long as 14 h at room temperature (ca. 20C) on a multipoint stirrer (Cole-Palmer Instrument Co., Chicago, IL) to accelerate enzymatic browning if it were to occur. Samples were observed at frequent intervals for the onset of browning. The presence or absence of browning could be determined unambiguously by visual observation. No attempt was made to compare treatments on the basis of the extent of browning or time of onset since these were subject to sample-to-sample variability. Rather, the success of a treatment in preventing browning was judged on a "go, no-go" basis by the absence of browning in treated samples after at least 6 h of storage at 20C with stirring, usually followed by one or more days at 4C without stirring, when controls turned brown within 1 or 2 h at 20C. In some experiments, samples were stored at 4C without stirring, and observations were made daily to determine the presence or absence of browning.

RESULTS AND DISCUSSION

Residual AA in Raw Juices

Raw apple (Granny Smith, Golden Delicious, Red Delicious) and pear (Anjou, Bartlett) juices, prepared from wedges or slices dipped in 1% AA, were found by HPLC analysis to contain about 500 ppm AA (range of 436–695 ppm), irrespective of commodity or cultivar. When 2% AA was used as the dip, the juices contained about 1100 ppm AA (range of 847–1521 ppm). These levels were sufficient to delay browning in the raw juices until after the completion of filtration and centrifugation treatments.

Effects of Filtration on Browning

Preliminary observations of raw Granny Smith and Golden Delicious apple juices, clarified by the addition of CAFA (a diatomaceous earth) and filtration, indicated that such juices did not undergo enzymatic browning during subsequent storage. To confirm and extend this observation, we investigated the ability of filtration with other filter aids and related products to eliminate the capacity of apple juice to brown (Table 1, Expt. A). CAFA proved to be the only material tested that appeared to prevent browning; i.e., juice filtered with CAFA did not brown after 3 h at 20C with stirring, followed by 36 h at 4C, when controls browned within 1 h at 20C. The BRITESORB® adsorbents, which were developed for the absorption of proteins from fruit juices, were ineffective in preventing browning in Granny Smith and Red Delicious juice.

Further experiments indicated that a level of CAFA addition of 2% was required for consistent prevention of browning (Table 1, Expt. B). Unfiltered juices

TABLE 1.
EFFECT OF FILTRATION ON ENZYMATIC BROWNING IN GOLDEN DELICIOUS APPLE JUICE[1]

Expt	Treatment[2]	Onset of browning (minutes at 20 C)
A	Unfiltered control	50
	Filtered	75
	Filtered with 1% CAFA	> 180[3]
	Filtered with 1% Celite 545	75
	Filtered with 1% talc	120
	Filtered with 1% silica gel (TLC)	120
B	Unfiltered control	10
	Filtered	40
	1% CAFA	50
	Filtered with 1% CAFA	120
	2% CAFA	40
	Filtered with 2% CAFA	> 180[3]
C	Filtered	5
	Filtered with 2% CAFA	> 180[3]
	Filtered with 0.4% Tween 80 + 2% CAFA	5

[1] Filtered through Whatman No. 541 paper with suction.

[2] CAFA = Celite Analytical Filter Aid.

[3] No browning after additional 36 h at 4 C.

containing CAFA browned readily, demonstrating that the absence of browning in juices filtered with CAFA resulted from the removal of PPO and/or PPO substrates and not from the inhibition of PPO by the filter aid. The addition of Tween 80, a detergent known to solubilize bound PPO (Takeo 1965), prior to CAFA addition and filtration yielded a filtrate that underwent rapid browning (Table 1, Expt. C). These results suggest that the effectiveness of the CAFA-filtration treatment in preventing browning depends on the separation of particulate-bound PPO from the juice. Apparently, the other silica products did not interact with particulates to which PPO is bound, facilitating their separation.

Studies with other juices demonstrated that the CAFA-filtration effect is not generally applicable but depends on the commodity, and even the cultivar (Table 2). The treatment was effective with Granny Smith (no browning after 22 h at 20C) and Red Delicious juice (no browning after 7½ h at 20C followed by 16 h at 4C) but not McIntosh juice. Browning in pear juice could be greatly delayed but not usually prevented by the CAFA-filtration treatment. Presumably, the size

TABLE 2.
VARIATION IN THE RESPONSE OF FRUIT JUICES TO FILTRATION[1]

		Onset of browning (minutes at 20 C)	
Fruit	Variety	Unfiltered control	Filtration with[2] 2% CAFA
Apple	Granny Smith	30	> 1320
	Red Delicious	1	>450[3]
	McIntosh	150	150
Pear	Anjou (sample 1)	30	180
	Anjou (sample 2)	90	>360[3]
	Bartlett	30	240
	Bosc	45	150
Grape	Thompson Seedless	5	> 180[3]

1 Filtered through Whatman No. 541 paper with suction.

2 CAFA = Celite Analytical Filter Aid.

3 No browning after additional 16 h at 4 C for juices of Red Delicious apple and Thompson seedless grape, and after 96 h at 4 C for Anjou pear juice (sample 2).

distribution or filtration characteristics of particulates to which PPO is bound will vary with commodity and cultivar.

Preliminary experiments indicated that browning in raw Golden Delicious apple juice could be prevented by addition of 0.5% Bentonite, followed by filtration through Whatman No. 541 paper; the speed of filtration could be improved by combining Bentonite with CAFA. Further studies demonstrated that McIntosh apple, Anjou pear and Bosc pear juices, which did not respond to filtration with CAFA alone, would not brown if pretreated with Bentonite before CAFA addition (Table 3). The amount of Bentonite required to prevent browning varied with each juice but never exceeded 0.5%. Under the conditions of this experiment, filtration was rapid,, and the filtered juices were only very slightly turbid. Bentonite is used as fining agent to remove haze-forming proteins from juices (Van Buren 1989). Our results indicate that Bentonite acts by coagulating particulates rather than by inactivating PPO.

Juices that did not respond to the CAFA-filtration treatment were subjected to microfiltration and ultrafiltration to determine the minimal filtration treatment required to prevent browning (Table 4). Filtration through a 0.22 μm membrane prevented browning in McIntosh apple juice but not in Bosc pear juice. Ultrafiltration through a 300,000 dalton MWCO membrane was effective with the latter juice. It is apparent that the particles removed by these treatments are many times larger than the molecular masses reported for PPO in apple and other fruits (Mayer

TABLE 3.
EFFECT OF BENTONITE ON BROWNING IN FILTERED APPLE AND PEAR JUICES

	Onset of browning (minutes at 20 C)					
	Juice					
	McIntosh apple		Anjou pear		Bosc pear	
Treatment[1]	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Unfiltered control	120	90	30	60	45	60
Filtered	120	90	150	90	45	120
Filtered, 0.1% Bentonite	180	120	540	>840	90	540
Filtered, 0.2% Bentonite	>840	120	>720	>840	240	>840
Filtered, 0.5% Bentonite	>840	>840	>720	>840	>780	>840

[1] 2% Celite Analytical Filter Aid added prior to filtration through Whatman No. 541 paper with suction.

TABLE 4.
EFFECTS OF MICROFILTRATION AND ULTRAFILTRATION ON BROWNING IN APPLE AND PEAR JUICE

Treatment	Onset of browning (minutes at 20 C) McIntosh apple	Bosc pear
Unfiltered control	150	45
Filtered with 2% CAFA	150	150
Filtered through 0.22 μm membrane[1]	>360	180
Filtered through 300,000 MWCO dalton membrane[1]	>360	>360[2]
Filtered through 30,000 MWCO dalton membrane[1]	>360	>360[2]

[1] Prefiltered through Whatman No. 541 paper with 2% CAFA before membrane filtration.

[2] No browning after additional 96 h at 4 C.

and Harel 1979; Vamos-Vigyazo 1981). Therefore, virtually all of the PPO in these juices must be bound to particulates.

While it has long been recognized that PPO in fruits is largely insoluble (Vamos-Vigyazo 1981) and that PPO is absent in clarified apple and pear juices (Biedermann 1956), there appears to have been no effort by juice processors to exploit these facts. Processes to obtain light-colored, cloudy apple juice by spraying the fruit with ascorbic acid solution prior to milling and then extracting, straining, deaerating, and pasteurizing the juice were developed in the 1940's (Pederson 1947; Atkinson and Strachan 1949). These processes depend on rapid handling of the crushed apples and juice to avoid product darkening. Various clarification and filtration or centrifugation procedures have been employed by processors to obtain clear juices (Smock and Neubert 1950; Moyer and Aitken 1980; Bump 1989; Kilara and Van Buren 1989). Although the ability of such procedures to remove preformed pigments resulting from enzymatic browning is recognized (Kilara and Van Buren 1989), their ability to prevent browning apparently has been overlooked.

The cold sterilization of fruit juices by filtration was first investigated and used in Europe more than 50 years ago (Charley 1939; Smock and Neubert 1950; Borgstrom 1954). Juices prepared by this process retain the characteristic flavor of the fresh fruit and are free of cooked taste but may be unstable due to the presence of active enzymes (Borgstrom 1954). More recently, ultrafiltration has been used to clarify fruit juices (Kirk *et al.* 1983; Swientek 1986; Thomas *et al.* 1986; Bump 1989). The potential use of such systems for cold sterilization has been recognized (Barefoot *et al.* 1989). The influence of ultrafiltration on juice quality has been investigated (Drake and Nelson 1987; Padilla and McLellan 1989); however, because these studies employed heat-pasteurized products, no conclusions could be drawn about the exclusion of PPO or other enzymes by the ultrafiltration system. Heatherbell *et al.* (1977) observed haze and sediment formation during storage of unpasteurized juices that had been filtered through a 50,000 dalton MWCO membrane. They did not establish whether small amounts of enzymes or enzyme degradation products passing through the membrane were responsible. Kim *et al.* (1989) reported high recovery of added pectinase in apple juice retentates under high-temperature, short-time ultrafiltration conditions. Köseoğlu *et al.* (1990, 1991) employed ultrafiltration through 50,000 and 100,000 dalton MWCO membranes to remove degradative enzymes from raw citrus and vegetable juices which were subsequently cold sterilized and combined with heat-pasteurized pulp. They demonstrated the absence of pectinesterase activity in ultrafiltration permeates. Since the occurrence of enzymatic browning in fruit juice ultrafiltration permeates would have been obvious in the many ultrafiltration studies reported in the literature, it is highly unlikely that these permeates contained active PPO. Our data suggest that filtration through such highly retentive membranes might not be required to remove PPO from raw juice.

Effects of Centrifugation on Browning

Preliminary studies suggested that the capacity of Granny Smith apple juice to undergo browning could be eliminated by centrifuging at speeds of 8000 rpm (8200 $\times$ g) or higher. Subsequently, we recognized the importance of excluding foam produced during juicing from the centrifuge tubes (Table 5). Apparently, foam disrupted by centrifugation generates a low density particulate fraction containing bound PPO that remains in the supernatant. We also observed that the capacity of McIntosh apple juice to brown was not eliminated by centrifugation. The success of microfiltration with this juice (Table 4) indicates that the failure of centrifugation to prevent browning is due to the presence of PPO bound to low density particulates rather than to soluble PPO. Since the same situation may occur with other apple cultivars or in other fruit juices not studied herein, any attempt to use centrifugation to control browning in juices should be evaluated for each of the individual cultivars that may be processed. The centrifugation speed required to sediment particulates to which PPO is bound also varied with cultivar (Table 6). When care was taken to exclude foam, browning in Red Delicious juice could be controlled by centrifuging at 4000 rpm (2043 $\times$ g) while Golden Delicious juice required 6000 rpm (4596 $\times$ g).

Centrifugation in an atmosphere of inert gas to prevent aeration has been used

TABLE 5.
EFFECTS OF FOAM ON CONTROL OF BROWNING IN APPLE AND PEAR JUICE BY CENTRIFUGATION

Juice	Treatment[1]	Onset of browning in supernatant (minutes at 20°C)
Bosc pear	Control	30
	Centrifuged (foam included)	150
	Centrifuged (foam excluded)	>420
Red Delicious apple	Control	90
	Centrifuged (foam included)	240
	Centrifuged (foam excluded)	>600
McIntosh apple	Control	90
	Centrifuged (foam included)	240
	Centrifuged (foam excluded)	240

[1] Centrifuged 10 min at 28,000 rpm at 4 C.

to partially clarify apple juice (Moyer and Aitken 1980). According to Smock and Neubert (1950), centrifugation at 9000 × g will produce a cloudy juice that will remain free from sediment during storage. They suggested that high speed centrifuges be used before filtration to reduce the load on filters. There is no indication in the literature that the effect of centrifugation on browning described herein was recognized previously, probably since centrifuged juices were subsequently pasteurized.

TABLE 6.
EFFECT OF CENTRIFUGATION SPEED ON BROWNING IN APPLE JUICE

		Onset of browning (minutes at 20 C)	
Speed (rpm)[1]	Max. RCF[2] (x g)	Red Delicious	Golden Delicious
None	0	45-60	60-90
2000	511	90	90
4000	2043	>360	150
6000	4596	>420[3]	>360[3]
28,000	100,095	>420[3]	>420[3]

[1] Juices centrifuged for 10 min at 4 C.

[2] Relative Centrifugal Force.

[3] No browning after additional 72-96 hrs at 4 C.

Preparation of Nonbrowning Cloudy Juices

Particulate fractions in some fruit juices may make important contributions to product appearance, body and flavor. Therefore, the removal of such fractions by filtration or centrifugation to preclude browning would not be feasible. In this study, we have attempted to prepare a cloudy, "cold-blanched" fruit juice product by centrifuging the raw juice, filtering the supernatant through a 30,000 dalton MWCO membrane, heating the pellet to inactivate particulate-bound PPO, and recombining the supernatant or permeate and heated particulates. A similar approach was employed by Köseoğlu *et al.* (1990, 1991) for citrus and vegetable juices. According to Walker (1964), apple PPO is completely destroyed at 80C. We found that heating McIntosh and Winesap juice pellets, suspended in a small volume of water, to 87C completely eliminated their capacity to cause browning in reconstituted juice (Table 7). Had the heated particulates been recombined with supernatant instead of permeate, the McIntosh product but not the Winesap would have browned during storage. Earlier experiments with Red and Golden Delicious juices indicated that cloudy products prepared with supernatants and heated particulates would not brown (data not shown). Heat treatment did not adversely affect the dispersibility of the pellets in juice ultrafiltrate. Heat treatments to inactivate PPO in isolated juice particulates might be compatible with the goal of freshness, provided that the natural turbidity, body and flavor of the juice are not compromised. A major advantage of such treatments would be the collateral destruction of microorganisms in the juice particulates. The reconstitution of juice from heat-treated particulates and cold-sterilized supernatant or permeate, under aseptic conditions, could yield a sterile, shelf-stable product with the quality attributes of a fresh juice.

TABLE 7.
BROWNING IN APPLE JUICE RECONSTITUTED FROM ULTRAFILTRATE AND HEATED PELLET

	Onset of browning (days at 4 C)	
Treatment	McIntosh	Winesap
Control	1	4
Supernatant[1]	2	>6
Permeate[2]	>7	>6
Permeate and heated pellet[3]	>7	>6

[1] Centrifuged 10 min at 28,000 rpm.

[2] Through 30,000 dalton MWCO membrane.

[3] Pellet heated 5 min in boiling water bath.

Research is continuing on the extension of these approaches to other commodities and to the control of other enzymatic reactions that limit the storage life of fresh juice products.

CONCLUSIONS

The capacity of apple and pear juices to undergo enzymatic browning resides in particulate fractions that can be separated from the juices by filtration. The response of the juice to specific filtration procedures depends on the commodity and cultivar; browning in all juices tested would be eliminated by ultrafiltration through a 300,000 dalton MWCO membrane.

The capacity of McIntosh apple juice to brown was not removed by centrifugation at 28,000 rpm (100,085 $\times$ g) for 10 min. Centrifugation at lower speeds was sufficient to prevent browning in other apple juices tested, provided that foam was excluded from the sample before centrifuging.

A nonbrowning, cloudy apple juice can be prepared by recombining heat-treated juice particulates with juice supernatant or ultrafiltration permeate.

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