

THE RETARDATION OF ENZYMATIC BROWNING IN AVOCADO PUREE AND GUACAMOLE

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ABSTRACT

Enzymatic browning in avocado puree and guacamole was evaluated by reflectance measurements for several varieties of avocado with varying amounts of chemical additives. Paste from Booth 8 had a greater tendency to brown than that from Lula. Browning at 75°F was effectively retarded without flavor change by 30 mg percent of sodium bisulfite or 200 mg percent ascorbic acid. Lemon juice or citric and hydrochloric acid adjustment of the puree from pH 6.6 to 5.1 accelerated browning of Booth 8. Combination of sodium bisulfite and ascorbic acid were particularly effective in preventing browning in all avocado preparations under all handling conditions - refrigerated, frozen and thawed, or freeze-dried and reconstituted.

INTRODUCTION

The avocado, although a food staple in the tropics and a popular salad fruit in the United States, is difficult to preserve fresh or process successfully. During freezing and freeze-drying investigations presently in progress at this laboratory, the extreme susceptibility of avocado flesh to enzymatic browning became an important operational problem. During the preparation of avocado puree and guacamole, the polyphenol substrates are brought into intimate contact with polyphenolase enzymes and oxygen, resulting in undesirable darkening of the avocado flesh within several hours.

Oxidative browning is traditionally retarded by the inclusion of sulfur dioxide-containing compounds and/or ascorbic acid. It was therefore deemed useful to study the effect of type and concentration of several browning retarding agents upon Florida avocados. As a result of this study, it is now possible to alleviate enzymatic browning in avocado flesh after pureeing or subsequent freezing, freeze-drying, storage, and reconstitution without adversely affecting the quality of the processed product.

LITERATURE REVIEW

Enzymatic browning as an important deteriorative mechanism in fruits and vegetables has been a topic of intensive investigation for decades. As a result, effective means for preventing browning exist and some understanding of the complex reactions involved has accumulated (2, 6).

The efficacy of sulfur dioxide in retarding browning is thought to be due to the formation

of addition products with the polyphenolase enzyme, thus interfering with the catalytic activity. Also, both sulfur dioxide and ascorbic acid function as reducing agents which reverse the oxidative activity of the enzyme (5). As with most enzyme systems, there are important modifying influences of pH, temperature, ionic species, etc. which are in need of clarification for each food system (1). Avocado polyphenolase has received a good share of attention in studies primarily concerned with isolation and mechanistic aspects but not with the browning phenomenon in the whole flesh (3). Utilization research with avocado products has explored various functional additives (7). The browning retardation effect of ascorbic acid has been noted and utilized in guacamole preparation (4), but not quantitized.

EXPERIMENTAL METHODS

Avocados obtained from Dade County groves and matured at 55°F were placed at 70°F to promote even ripening. Puree was prepared by slicing the washed fruit into quarters, removing the seed, skin, and any blemished portions. The flesh was rapidly passed through a stainless steel screw expeller fitted with a 0.18 inch screen. Guacamole was produced by adding 4g lemon juice, 8g comminuted onion, 1.5g salt, and 0.15g oregano per 100g puree.

The appropriate additives were added to 100g portions of the puree or guacamole with thorough mixing. The samples and controls were placed in 10 cm glass petri dishes, the surface smoothed with a spatula, and covered; care taken not to contact or disturb the smoothed surface.

The development of surface browning was followed in a 75°F room using a Photovolt reflector meter (Photovolt Corp., N. Y., N. Y.) with no filter, set at a reading of 100 with a light green color standard (Rd, 50.1; a, -16.3; b, 6.4) and reading 36 with an olive drab standard (Rd, 9.8; a, -0.5; b, 20.4, Hunter notations).

The sensing head was inverted over the center of the petri dish cover for reading. The times at which browning was just noticeable and at which darkening became unacceptable were noted. After 8 hours at 75°F the total change in reading was recorded, denoted as "A8," and used as a measure of the extent of browning.

To determine the effect of freezing and freeze-drying upon browning, samples were prepared in petri dishes as described, immediately placed in a -10°F freezer, and were either held frozen or were freeze-dried at a shelf temperature of 150°F and then stored dry at -10°F. For measurement, samples were either thawed by agitating the dish in 120°F water for about 20 minutes or reconstituted to original weight with distilled water at 75°F.

Table 1. Effect of Browning Retardants on Avocado Puree and Guacamole at 75°F

Treatment	Time in hours for:	
	Detectable Browning	Unacceptable Browning
<u>Booth 8 Puree</u>		
Control	3	4
NaHSO ₃ , 30 mg%	*	*
Citric acid 0.2%	2	3
Citric acid 0.4%	2	3
Lemon juice 2%	2	3
Lemon juice 4%	2	3
Ascorbic acid 100 mg%	5	6
<u>Lula Puree</u>		
Control	5	6
Control Refrigerated @ 45°F	30	54
Lemon juice 4%	6	7
Citric acid 0.2%	8	9
NaHSO ₃ , 30 mg%	*	*
<u>Booth 8 Guacamole</u>		
Control	2	3
NaHSO ₃ , 30 mg%	*	*
Frozen & Thawed	2	3
Frozen & Thawed + NaHSO ₃ , 30 mg%	*	*
Freeze-dried & Reconstituted	2	3
Freeze-dried + 30 mg% NaHSO ₃ + 100 mg% Ascorbic Acid	*	*

*No detectable browning after 24 hours.

Table 2. Reflectometer reading changes in avocado puree and guacamole with various additives during 8 hours at 75°F.

TREATMENT		8
	<u>Booth 8</u>	<u>pH</u>
Control		6.6
NaHSO ₃	10 mg%	"
	30 mg%	"
	50 mg%	"
	75 mg%	"
	100 mg%	"
Na ₂ S ₂ O ₅	10 mg%	"
	30 mg%	"
	50 mg%	"
	75 mg%	"
	100 mg%	"
K ₂ S ₂ O ₅	10 mg%	"
	30 mg%	"
	50 mg%	"
	75 mg%	"
	100 mg%	"
Ascorbic Acid	100 mg%	6.4
	200 mg%	6.2
	500 mg%	5.3
	750 mg%	4.9
	1000 mg%	4.6
Citric Acid	200 mg%	5.1
	400 mg%	4.9
Lemon Juice	1%	6.2
	2%	5.7
	4%	5.1
	7.5%	4.5
	10%	4.3
	<u>Lula</u>	
adjusted to pH 5.1 with:		5.1
lemon juice		"
citric acid		"
hydrochloric acid		"
Control		6.6

RESULTS AND DISCUSSION

It was evident quite early in the study that if an arbitrary decrease in photometer reading could not be used to define browning. Initial readings (at zero time) varied several units due to differences in geometry and the effect of additives, i.e. oregano, lemon juice. Varieties and batches differed in initial color (i.e. 67-76) and this difference was accentuated by the need to trim blemished portions from some fruit. Blemishes were close to the skin in the dark green outer flesh, resulting in greater retention of yellow

inner flesh after trimming.

Despite these variations, readings were consistent within experimental batches and agreed well with the occasional subjective ranking of degree of darkening performed by lab personnel. Over the surface of a single sample the reading did not vary. This was not the case when the puree was hand mashed, hence mechanical pureeing was employed. In general, readings above 65 indicated acceptable color retention, 65 to 63 detectable browning, and below 63 the darkening was deemed unacceptable. The period between detectable and unacceptable browning was 1 hour, although total browning time was dependent upon avocado variety, additives, and protective agents (Table 1). Refrigeration of unprotected puree at 45°F decreased the browning rate by a factor of 10.

Initial experiments were designed to contrast various sources and concentrations of sulfur dioxide, and concentrations of ascorbic acid and lemon juice. All sulfur dioxide-containing compounds above 50 mg% imparted a slight sulfite off-taste. Sodium bisulfite was the most effective compound at 30 mg% (Table 2). Thus, 30 mg% NaHSO₃ was chosen for further study. Ascorbic acid was effective at 200 mg% but not at 100 mg%. Lemon juice at 2 and 4% was of no value and actually speeded up browning during the first 4 hours (Table 1), although after 8 hours the control sample read as low (Table 2). Citric acid demonstrated the same effect. All experiments so far described were conducted with Booth 8 avocado. Later in the season, Booth 8 was replaced by Lula avocado. Lula browned much less rapidly. Also, samples acidified with lemon juice browned less rapidly than the control (Table 1). It is not too surprising that a different browning response was observed since in morphology and flavor these two varieties are quite different.

In view of the effectiveness of sodium bisulfite and ascorbic acid, these substances were used together in an attempt to demonstrate a synergistic or additive effect. In Booth 8 puree, the addition of 10 mg% NaHSO₃ plus 100 mg% ascorbic acid was more effective (Table 3) than either compound alone (Table 2). Bisulfite at 10 mg% partially decreased the browning observed and added lemon juice, and 30 mg% completely prevented it (Table 3). In view of the pH dependence of bisulfite (greater effectiveness at low pH) it is probable that the tendency to brown with added lemon juice was overcome by increased efficacy of bisulfite at the lower pH.

Table 3. Effect of combined additives on darkening of Booth 8 avocado puree reflectometer reading change in 8 hours at 75°F.

Additive + amount		Δ 8
<u>NaHSO₃ mg%</u>	<u>Ascorbic acid mg%</u>	
0 (control)	0	30*
10	0	23*
20	0	12*
30	0	8
10	100	8
10	200	7
30	100	4
30	200	1
20	50	9
20	100	8
30	50	6
	Lemon juice %	
10	2%	13*
10	4%	10*
30	2%	7
30	4%	2

* Denotes Unacceptable darkening.

Table 4. Darkening of avocado products as affected by processing and additives
reflectometer reading change in 8 hours at 75°F.

TREATMENT	Δ 8
<u>Booth 8</u>	
puree, fresh	30*
frozen & thawed	28*
freeze-dried & reconstituted (58) ¹	16*
guacamole, fresh	
frozen & thawed	19*
freeze-dried & reconstituted (59)	13*
guacamole + 30 mg% NaHSO ₃	
fresh	7
frozen & thawed	2
freeze-dried (60)	0
guacamole + 30 mg% NaHSO ₃ +100 mg% Ascorbic acid	
fresh	5
frozen & thawed	3
freeze-dried & reconstituted (59)	0
<u>Lula</u>	
puree control	12*
guacamole control	14*
guacamole + 20 mg% NaHSO ₃	5
guacamole + 200 mg% Ascorbic acid	4
guacamole + 20 mg% NaHSO ₃ + 200 mg% Ascorbic acid	3

(1) indicate initial reconstituted reading

* Denotes unacceptable darkening.

Experiments performed with guacamole exhibited the same trend. Bisulfite and ascorbic acid effectively retarded browning in either the fresh, frozen-thawed, or freeze-dried-reconstituted product (Table 4). Guacamole from Lula avocados browned less than the Booth 8 preparation (Table 4). In both cases, sodium bisulfite and ascorbic acid retardation behaved as in the puree experiments. One rather unexpected result was observed in the freeze-dried guacamole. The initial reading after reconstitution was much lower than in any other circumstance. The color was dark green in contrast to the light green of the initial or frozen thawed samples. Unprotected samples proceeded to brown as expected, whereas samples containing adequate bisulfite and ascorbate maintained the dark green color (Table 4). This darkening was not objectionable nor accompanied by off-flavor. A milder freeze-drying temperature (80°F) did not lighten the color. Further research relating to this phenomenon is presently in progress.

Incorporation of 30 mg% sodium bisulfite retarded enzymatic browning in avocado puree and guacamole indefinitely. In fact, bacterial decomposition was noted in bisulfite protected samples which still retained their normal color after 24 hours at 75°F. The inclusion of 100 to 200 mg% ascorbic acid was of marginal value, if bisulfite was also used.

As a significant aid to browning prevention in processed avocado products, it is recommended that sodium bisulfite and/or ascorbic acid be incorporated into the puree or guacamole prior to freezing or freeze-drying. In this manner, the processor and consumer will gain appreciable leeway in product handling.

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