Characteristics of Clouding Substances in Guava Puree

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This work investigates the composition and characteristics of cloud in guava puree prepared at various heating temperatures. The cloud content in guava puree was initially at 0.1% and increased greatly with increased heating temperature due to the polymerization of components (i.e., pectin and protein) in the puree. The cloud content in guava puree was significantly correlated with the change of its turbidity. Guava puree cloud was found to be composed of 43–45% protein, 25–26% carbohydrate, 5–8% pectin, 3–5% crude fiber, 6.5–9.3% ash, and 0.18% crude fat. The analyses of protein pattern and amino acid composition showed that the protein in cloud was of a low molecular weight, with a high isoelectric point and a compact structure; thus, it could combine with pectin and become suspended in the guava puree. The amount of divalent cation increased with increasing heating temperature, indicating that divalent cations could promote aggregation of components in puree. Phenolic compounds also contribute to the turbidity and sediment in puree.

Keywords: *Guava puree; cloud; protein; pectin; phenolics; turbidity*

INTRODUCTION

Guava fruit contains high-quantity pectin (ca. 705–804 mg/100 g) (Jagtiani et al., 1988). Guava pectin has a high methoxy index and is believed to impart a viscous property to the guava puree or juice (Ferro et al., 1969). The pectic substances have been shown to combine with protein or polyphenolic compounds to form a cloud suspension (Hoff et al., 1980). The high viscosity and cloudy appearance which are the special properties of guava puree can be changed during thermal processing and storage of guava puree, thus resulting in quality loss (Yen et al., 1992a). Yen et al. (1992b) also reported that the decrease in viscosity and turbidity of guava puree during storage is due to the de-esterification of pectin by pectin esterase.

In general, the substances causing the turbidity and sedimentation of juice include polysaccharides (pectin, cellulose, starch), protein, polyphenol (tannin), sugars, metal ions, etc. (Binnig, 1992). During the extraction of juice from fruit, pectin is released from the cell wall. Since the pH of juice is lower than the isoelectric point of protein, the positively charged protein is surrounded by the negatively charged pectin. Due to the interruption of the negative charge of the outer pectin molecule, the cloud suspended in the juice is more stable. The pectin contains neutral sugars, rhammnose, arabinose, glucose, and fructose in branch chains that can bind to the protein with a hydrogen bond and make the cloud more stable (Shomer, 1991). Polyphenols not only come from the fruit, but also are polymerized from small phenolic compounds or aldehydes in the puree by oxygen or by heating. The polyphenols can bind with protein, pectin, and starch to form the precipitate (Cheynier et al., 1988). The major function of metal ions is to form a stable complex with phenolic compounds and aldehydes (Cheynier et al., 1988).

Although the physical and chemical nature of cloud in citrus juice has been studied by Klavons et al. (1991, 1994), the basic information concerning the properties of the clouding substances in guava puree is not available. The objective of this work was to study the cloud content, composition, and characteristics of guava puree prepared at different heating temperatures.

MATERIALS AND METHODS

Preparation of Guava Puree. Fresh fully ripe guava (*Psidium guajava* L. cv. Chung shan) fruit was obtained from the Kaoshiung area of Taiwan. Guava puree was prepared according to the method described by Yen et al. (1992). The pH of the guava puree was pH 4.2 with 0.34% acidity. The guava puree was adjusted to pH 3.9 (acidity 0.58%) with citric acid as for the processing condition of commercial juice processing plants.

Both acidified and unacidified guava puree samples were pasteurized by heating at 60–62 °C for 30 s and 88–90 °C for 30 s in a plate heat exchanger, and then chilled with ice water to reduce the temperature of the heated juice to below 15 °C. The puree was packed in a 500 mL plastic bottle and stored at −40 °C until use. Before analysis, the sample was thawed at 15 °C for 2 h and then stored at 4 °C for analysis.

Preparation of Cloud. The cloud content of guava puree was analyzed by the method of Klavons et al. (1991). Pulp was removed from all samples by a low-speed centrifugation 9360*g* for 10 min. The pulpless puree (15 mL) was centrifuged at 27000*g* for 15 min, producing a supernatant with an optical density (OD) at 600 nm of less than 0.05. The supernatant was decanted, and the cloud pellet was redissolved in 10 mL of deionized water by vortexing. This process was repeated, and the total cloud weight of each sample was obtained by freeze-drying it to constant weight.

Chemical Determinations. The percentages of crude fat, crude fiber, and ash in guava puree cloud were determined according to AOAC (1984) methods 14.060, 14.064, and 14.063. The protein content of the guava puree cloud was determined according to the method of Bradford (1976) using bovine serum albumin as standard. The neutral sugars in the cloud were measured by a resorcinol/sulfuric acid micromethod (Monsigny et al., 1988). Total pectin in cloud was extracted according to

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Table 1. Effect of Processing Conditions on the Cloud and Its Component Content of Guava Puree[a]

components	unheated		60 °C, 30 s		88 °C, 30 s	
	N[b]	A	N	A	N	A
total cloud weight	110.38 ± 3.00^a	116.98 ± 3.02^a	118.15 ± 3.77^b	128.31 ± 3.85^a	138.89 ± 4.11^a	146.76 ± 6.76^a
cloud protein	48.08 ± 0.88^a	52.02 ± 1.72^a	52.77 ± 3.17^a	57.50 ± 2.50^a	61.24 ± 2.14^a	66.08 ± 2.88^a
cloud neutral sugar	28.61 ± 1.09^a	29.62 ± 1.78^a	29.88 ± 1.88^a	30.72 ± 0.82^a	35.42 ± 1.30^a	37.25 ± 1.15^a
cloud ash	7.15 ± 0.20^b	8.26 ± 0.20^a	8.74 ± 0.50^a	9.28 ± 0.33^a	10.94 ± 0.32^b	13.65 ± 0.55^a
cloud pectin	5.65 ± 0.07^b	6.86 ± 0.10^a	7.00 ± 0.32^a	7.92 ± 0.20^a	8.54 ± 0.43^b	10.44 ± 0.44^a
cloud tannin	6.46 ± 0.25^a	6.76 ± 0.35^a	7.68 ± 0.28^b	8.54 ± 0.38^a	9.95 ± 0.35^b	11.11 ± 0.41^a
cloud fiber	3.91 ± 0.10^a	4.34 ± 0.14^a	5.08 ± 0.18^b	5.77 ± 0.27^a	6.47 ± 0.50^a	7.12 ± 0.32^a
cloud fat	0.16 ± 0.01^b	0.19 ± 0.00^a	0.22 ± 0.03^a	0.24 ± 0.04^a	0.26 ± 0.02^a	0.29 ± 0.02^a

[a] Units: mg/100 g of guava puree. Values are expressed as mean ± standard deviation ($n = 4$). In each treatment, values in the same row with different superscripts are significantly different ($P < 0.05$). [b] N, unacidified; A, acidified.

the method of Ishii and Yokotsuka (1972); the pectin content of the extracts was analyzed by the *m*-hydroxydiphenyl method (Kintner and Van Buren, 1982). The tannin content in cloud was analyzed using the modified vanillin assay (Price et al., 1978), and the results were expressed as catechin equivalents.

Phenolic Composition of Cloud. Dried cloud (1 g) was extracted with acidic methanol (methanol:0.3% HCl ratio = 60:40, v/v) at room temperature for 1 h by shaking. After centrifugation at $2270g$ for 25 min, the supernatant upper layer was adjusted to 5 mL. The solution was filtered through a Millipore 0.45 μm filter and injected into the HPLC. The phenolic composition of the methanolic extract was determined by reverse-phase high-performance liquid chromatography (Hitachi), using an LiChrospher 100 RP-18 column (250×4 mm, 5 μm, E. Merck). Phenolic compounds were eluted in a gradient of 5% acetic acid (solvent A) and acetonitrile (solvent B) at a flow rate of 1 mL/min according to the following program: 0–20 min, 13% B; 20–60 min, linear gradient from 13 to 40% B; 60–70 min, linear gradient from 40 to 0% B. The chromatograph was monitored at 280 nm using a UV–vis detector (Hitachi, Model L-4200). Calculation of concentrations was based on the external standard method (Spanos et al., 1990).

Analysis of Amino Acid Composition. The cloud was first hydrolyzed with 6 N HCl in a vacuum at 110 °C for 24 h. The amino acids in the hydrolysates were determined by HPLC and precolumn derivatization with *o*-phthaldialdehyde (OPA). A Hitachi L-6200 liquid chromatogram equipped with a Lichrospher 100 RP-18 column (125×4 mm, 5 μm, E. Merck) and a fluorescence detector (Hitachi F1300, excitation at 340 nm, emission at 450 nm) was used. The mobile phase used for the separation of OPA-amino acids consisted of two eluents: 50 nM sodium acetate, pH 5.7, containing 0.5% tetrahydrofuran (solvent A); and methanol (solvent B). The gradient profiles were as follows: 0–5 min, 17% B; 5–8 min, 17–24% B; 8–14 min, 24% B; 14–23 min, 24–50% B; 23–28 min, 50% B; 28–34 min, 50–67% B; 34–34.5 min, 67% B. A 10 μL portion of the derivative was injected into the HPLC at constant flow of 1.2 mL/min, and the column temperature was maintained at 37 °C.

Mineral Analysis. Cloud (0.1 g) was dissolved in 3 mL of digesting solution (HNO_3:HCl:H_2SO_4 ratio = 4:1:1, v/v/v) and heated to clear solution. The digested solution was adjusted to 50 mL with deionized water and then analyzed by inductive by coupled plasma emission spectroscopy (ICP, Applied Research Laboratories, France). The concentration of minerals was calculated from the standard curve.

Electrophoretic Analysis. The cloud was dissolved in 0.1 M Tris-HCl buffer (pH 6.8). The upper clear solution was added slowly with 75% saturated ammonium sulfate and allowed to stand for 30 min. The mixture was centrifuged at $10000g$ for 1 h, and then the precipitate was redissolved in 0.1 M Tris-HCl buffer. The solution was filtered through a Whatman no. 1 filter, and the filtrate was dialyzed at 4 °C overnight. After centrifugation, the supernatant was used for electrophoresis.

The 15% polyacrylamide gel slabs were prepared and run with SDS using the discontinuous buffer system of Laemmli (1970). Electrophoresis at 100 V was carried out for 1.5 h, after which time the gel was fixed and stained with a solution of Coomassie Brilliant Blue R-250 in methanol/water/acetic acid (5:5:1, v/v/v), followed by excess dye washing in water/methanol/acetic acid (7:2:1, v/v/v). The molecular weights of proteins in the cloud were determined by comparing the relative mobilities of protein bands with those of standard proteins.

Statistical Analysis. All experiments were run in duplicate, and analyses of all samples were run in duplicate and averaged. Statistical analyses were carried out using the SAS (SAS Institute Inc., 1985) software package. Analyses of variance were performed by ANOVA procedures. Significant differences between the means were determined by Duncan's multiple range tests.

RESULTS AND DISCUSSION

Composition of Cloud. The effect of processing conditions on the cloud composition of guava puree is shown in Table 1. The content of cloud increased with concomitantly higher temperatures. The cloud content of unheated and 60 and 88 °C heated guava puree was 110, 118, and 139 mg %, respectively. The increase of cloud content might be due to the coagulation of puree component due to the heat treatment, especially the encapsulation of pectin to protein (Shomer, 1988). The acidified guava puree also had higher cloud content. The cloud content of unheated and 60 °C heated guava puree with acidification was 117 and 128 mg %, respectively. The binding of pectin and protein increases under acidic conditions (Binning, 1992); thus, the acidifying treatment stabilized the cloud in guava puree. The cloud contains 43–45% protein and 5–8% pectin. The cloud also contains 25–26% sugars, which may be due to the fact that the branch chain of pectin can bind other neutral sugars, such rhammnose, fructose, and glucose (Van Buren, 1991). The other components in cloud are found to be 5–7% tannin, 3–5% cellulose, 6.5–9.3% ash, and 0.18% crude fat. All components in cloud increased proportionally with heating temperature, and the acidified puree had a higher content of cloud component. From these results it can be seen that the concentration of cloud increased with heating temperature, although a similar ratio between each component was maintained. This indicates that heat treatment increases the reaction between each component and causes the increase of turbidity in the puree.

Major Minerals in Cloud. As shown in Table 2, the major minerals in cloud are K, Ca, Mg, Na, and Zn. Potassium had the highest concentration while zinc had the lowest. Although cloud contained a high amount of potassium, it was not affected by the processing conditions. However, the divalent ions were clearly altered with heating temperature, and the concentration of divalent cations in cloud of heated puree was greater

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Table 2. Amount of Major Minerals in Cloud of Guava Puree[a]

minerals (ppm)	unheated		60 °C, 30 s		88 °C, 30 s	
	N[b]	A	N	A	N	A
potassium	10437 ± 263^a	10450 ± 225^a	10499 ± 201^a	10467 ± 453^a	10505 ± 295^a	10480 ± 250^a
calcium	1624 ± 53^a	1799 ± 94^a	1984 ± 70^a	2146 ± 60^a	2333 ± 70^a	2412 ± 50^a
magnesium	539 ± 16^a	564 ± 16^a	668 ± 20^a	690 ± 16^a	742 ± 12^a	771 ± 24^a
sodium	259 ± 8^a	266 ± 7^a	238 ± 8^a	240 ± 7^a	225 ± 15^a	231 ± 5^a
zinc	26 ± 0.5^b	31 ± 1^a	59 ± 1^b	64 ± 1^a	74 ± 2^b	88 ± 3^a

[a] Values are expressed as mean ± standard deviation ($n = 4$). In each treatment, values in the same row with different superscripts are significantly different ($P < 0.05$). [b] N, unacidified; A, acidified.
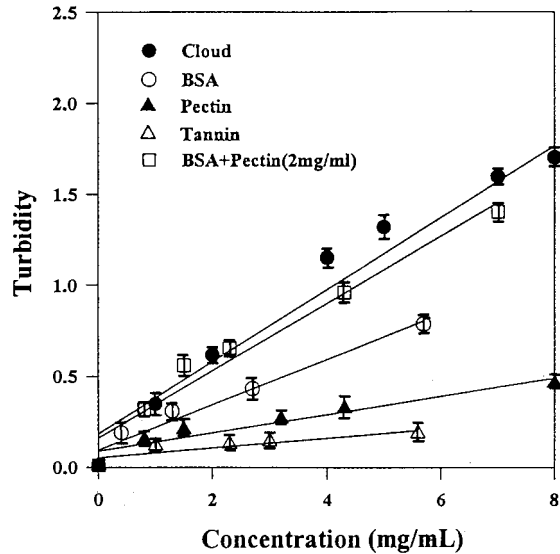
Figure 1. Turbidity of cloud from guava puree and its major components under different concentrations.

than that in unheated puree. There is no significant difference ($P > 0.05$) in mineral content between acidified and unacidified puree, except zinc. The heat treatment increased the binding between divalent cations and low methoxy pectin and polyphenols in the soluble portion of puree. This resulted in an increase of intramolecular coagulation in the soluble portion and an increase in the amount of cloud (Heatherbell, 1984). Therefore, divalent cations may play an important role in the coagulation of the cloud nucleus.

Effect of Cloud and Its Components on Turbidity. Figure 1 shows the changes in turbidity of cloud and its components under different concentrations. There is a good correlation ($r^2 = 0.9710$) between the turbidity and the concentration of cloud. Among the components in cloud, protein caused the higher turbidity, followed by pectin and then tannin. The combination of protein and pectin showed a synergistic effect on the formation of turbidity, and the turbidity of the combination is similar to the turbidity of cloud. Therefore, protein is the major contributor to the turbidity of puree, and tannin is insignificant to the formation of turbidity.

Amino Acid Composition of Cloud and Soluble Fraction of Puree. As can be seen in Table 3, the major amino acids formed in protein of cloud protein are aspartic acid and/or asparagine (Asx), glutamic acid and/or glutamine (Glx), and glycine. The total amino acid content in the cloud of acidified puree was greater than that in unacidified puree. Acidification can increase the content of polar amino acids, such as Asx, Glx, and serine, but not of the nonpolar amino acids. The total amino acid concentration in a soluble fraction of unacidified and acidified puree was 31.51 and 25.61

Table 3. Amino Acid Composition in Cloud and Soluble Fraction of Unheated Guava Puree[a]

amino acid	cloud (g/100 g)		soluble fraction (mg/100 mL)	
	N[b]	A	N	A
Asx[c]	10.11	10.60	11.05	10.73
Glx[c]	3.66	4.07	6.87	5.22
Ser	1.98	2.42	0.69	0.69
His	0.71	0.75	1.85	1.37
Gly	6.26	6.86	0.44	0.41
Thr	0.32	0.37	0.55	0.48
Arg	1.11	1.31	0.42	0.33
Ala	2.80	2.79	4.12	3.32
Tyr	0.57	0.60	4.99	2.52
Met	0.33	0.43	0.18	0.15
Val	0.20	0.39	0.11	0.08
Phe	0.44	0.47	0.08	0.07
Ile	0.25	0.28	0.07	0.06
Leu	1.22	1.53	0.03	0.10
Lys	2.66	2.93	0.06	0.08
total	32.62	35.85	31.51	25.61

[a] The data are mean values of duplicates. [b] N, unacidified; A, acidified. [c] Asx, aspartic acid or asparagine; Glx, glutamic acid or glutamine.

Table 4. Effect of Processing Conditions on Amino Acid Composition in Soluble Fraction of Guava Puree[a]

amino acid (mg/100 mL)	unheated		60 °C, 30 s		88 °C, 30 s	
	N[b]	A	N	A	N	A
Asx[c]	22.10	21.46	26.86	22.52	26.10	26.22
Glx[c]	13.73	10.53	17.93	13.12	11.28	10.35
Ser	1.78	1.38	3.20	2.32	4.99	5.46
His	3.69	2.74	4.54	3.61	12.01	12.74
Gly	0.88	0.82	0.78	0.87	0.91	0.89
Thr	1.09	0.96	1.17	0.99	2.31	2.29
Arg	0.83	0.67	0.98	1.04	1.45	1.01
Ala	8.24	6.64	10.59	7.95	18.28	20.16
Tyr	5.98	5.04	6.42	5.15	6.55	6.32
Met	0.37	0.29	0.45	0.38	0.45	0.59
Val	0.21	0.17	0.26	0.22	0.51	0.55
Phe	0.15	0.13	0.16	0.14	0.71	0.85
Ile	0.14	0.11	0.18	0.14	0.43	0.63
Leu	0.05	0.20	0.26	0.21	0.32	0.22
Lys	0.11	0.15	0.33	0.21	0.21	0.29
total	59.35	51.28	74.11	58.86	86.51	88.58

[a] The data are mean values of duplicates. [b] N, unacidified; A, acidified. [c] Asx, aspartic acid or asparagine; Glx, glutamic acid or glutamine.

mg/mL, respectively. Thus, acidification increased the total amino acid in cloud that could be transferred from the soluble portion of puree. From this result, it can be seen that acidification both enhances the coagulation and increases compactness of molecular structure. The increase of polar amino acids in cloud will help the binding of protein and sugars, which also could result in an increase of cloud content (Waters and Wallace, 1993). The results also indicate that the cloud contained higher amounts of glycine, leucine, and lysine than found in the soluble fraction of puree. Since glycine can increase the helical configuration of protein, it can form

Table 5. Phenolic Composition and Quantity of Guava Puree and Its Cloud[a]

	puree (mg/L)		cloud (mg/100 g)	
compounds	unheated	heated[b]	unheated	heated
gallic acid	26.55 ± 0.55^b	42.16 ± 0.72^a	10.01 ± 0.42^b	11.13 ± 0.58^a
catechin	20.28 ± 0.50^b	26.63 ± 0.44^a	72.07 ± 1.51^a	72.88 ± 1.57^a
chlorogenic acid	8.08 ± 0.06^a	6.85 ± 0.13^b	46.51 ± 1.64^a	21.25 ± 0.79^b
caffeic acid	2.28 ± 0.10^a	2.07 ± 0.06^b	4.92 ± 0.20^b	5.75 ± 0.10^a
epicatechin	3.60 ± 0.05^a	Tr[c]	42.81 ± 0.60^a	39.51 ± 0.45^b
coumaric acid	1.95 ± 0.05^b	2.46 ± 0.09^a	6.63 ± 0.29^b	9.15 ± 0.37^a
rutin	120.43 ± 6.81^a	62.49 ± 2.09^b	39.95 ± 1.27^a	18.75 ± 0.36^b
cinnamic acid	2.00 ± 0.01^a	0.72 ± 0.01^b	7.50 ± 0.35^a	2.73 ± 0.13^b

[a] Values are expressed as mean $\pm$ standard deviation ($n = 4$). For puree and cloud, values in the same row with different superscripts are significantly different ($P < 0.05$). [b] Heated at 88 °C for 30 s. [c] Tr, trace.
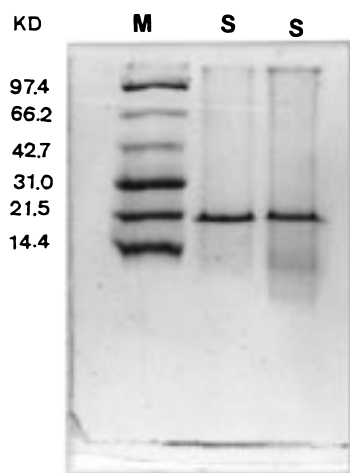

Figure 2. Distribution of protein molecular weight in the cloud of guava puree.

a more compact global protein structure. A protein with high amounts of alkali amino acids will raise its isoelectric point. Thus, in an acidic condition, the protein is more positively charged (pH < pI). This will enhance the binding of protein and sugars and result in a stable cloud state (Ishii et al., 1979). Therefore, the great differences of protein in cloud and in the soluble fraction of puree are due to the molecular structure and isoelectric point of the proteins.

Heat treatment not only caused the increase of soluble protein in the soluble fraction and the protein in puree cloud, but also caused the increase of total amino acids in these two samples (Table 4). However, the amino acid content was not influenced by acidification, especially with the increase of heating temperature.

Molecular Weight of Protein in Cloud. The molecular weight of protein in cloud distributed at 21 kDa (Figure 2), which shows it to be a low molecular weight protein. Since the major proteins in cloud have both a low molecular weight and a high isoelectric point, it can be encapsulated by pectin to form stable complexes (Heatherbell, 1984).

Phenolic Compounds in Guava Puree and Its Cloud. The content of tannin in cloud increased with the increase of heating temperature (Table 1), and Cheynier et al. (1988) indicated that tannin can be polymerized from phenolic compounds. Therefore, the analysis of phenolic compounds in puree and cloud will be helpful in revealing the relationship between phenolic compounds and tannin by heat treatment. From the HPLC profile (data not shown), guava puree and its cloud contained large amounts of rutin and catechin.

The rutin content in heated puree is significantly ($P < 0.05$) lower than that in unheated puree (Table 5),

which might be due to degradation of the glycoside bond by heat treatment (Naim et al., 1988). Furthermore, the degraded phenolic compounds polymerized to tannin. However, the content of gallic acid and coumaric acid in heated guava puree was greater ($P < 0.05$) than that of unheated puree. The increase of these phenolic compounds might be caused by their precursors after heat treatment (Fiddler et al., 1967; Risch et al., 1987). Chlorogenic acid, epicatechin, and cinnamic acid are easily degraded by heat treatment (Chen, 1993); thus, their concentration was lower after heat treatment. The cloud contained high amounts of catechin, chlorogenic acid, epicatechin, and rutin, at 31.3, 20.2, 18.6, and 17.1%, respectively, in unheated puree. The cholorgenic acid and rutin in cloud reduced about 50% after heat treatment. This clearly explained that the increase of tannin in cloud after heat treatment is mainly due to the degradation and coagulation of these two components.

CONCLUSIONS

Based on the results of this study, protein was shown to be the major component of clouding substances in guava puree. It may also be a controlling factor in the degree of cloudiness of guava puree. Acidification and heat treatment of guava puree increase the content of cloud. This may also be due to the increased binding ability of molecules in puree subjected to these two treatments. Divalent cations in cloud of guava puree increased with heat treatment, which could enhance the aggregation of components in the puree. The cloud contained greater amounts of glycine and alkali amino acids than formed in the soluble fraction of puree. This indicates that the protein structure in cloud was more compact and with higher isoelectric point. The cloud was surrounded by pectin molecules, forming a large net structure. However, both cloud and supernatant in puree contained high amounts of polar amino acids, which can be suspended in puree. Heat treatment reduced the content of phenolic compounds in both puree and cloud, but the tannin content in cloud increased due to the polymerization of polyphenol by heat treatment. Thus, the turbidity and sedimentation of puree might also be due to the existence of phenolic compounds.

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