

# Studies on Extraction and Storage of guava pulp

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**Abstract:** The investigation was conducted on studies processing of red fleshed guava (*Psidium guajava* L.). To study the effect of processing on antioxidant composition of guava pulp at ambient and cold storage (7°C). Processing and extraction of guava pulp by HTST heating at 80°C for 20 min and LTLT heating at 48°C for 1 hr heat processing treatment. The heat treatments enhanced the total phenolic content, flavonoid as well as total anthocyanin content. The effect of storage period on antioxidant composition of guava pulp reported result associated with anthocyanin content data revealed that there is decreasing trend was recorded in anthocyanin content during storage of guava pulp. HTST heating at 80°C for 20 min had longer storage life of guava pulp than LTLT heating at 48°C for 1 hr. The total phenolic content was also found to decrease from initial

135.76 mg GAE/g to 126.48 mg GAE/g after 60<sup>th</sup> days of storage of guava pulp at ambient temperature, while after 150<sup>th</sup> days of storage of guava pulp in cold storage decline in total phenolic content was observed from initial 135.76 mg GAE/g to 124.36 mg GAE/g. Also, the total flavonoid content was also found to decrease from initial 23.90 mg CE/g to 10.26 mg CE/g after 60<sup>th</sup> days of storage of guava pulp at ambient temperature, while after 150<sup>th</sup> days of storage of guava pulp in cold storage decline in total flavonoid content was also observed from initial 23.90 mg CE/g to 16.32 mg CE/g.

Decreasing trends in contents of antioxidant activity was recorded during storage of pulp at ambient temperature, while antioxidants activity of guava pulp was recorded during cold storage 7<sup>o</sup>

C. Extraction of red fleshed guava pulp by HTST heating accelerate retention of antioxidant activities during storage.

**Keywords:** Guva, pulp, storage

## INTRODUCTION

Guava (*Psidium guajava* L.) is widely cultivated in tropical and sub-tropical countries and finds increasing popularity all over the world. It is one of the most common fruits in India, gains importance next to mango, banana and citrus and is popularly known as “apple of tropics” (Gull *et al.*, 2012). It has a good source of vitamins C and pectin besides as fair amount of vitamin A. It is also a rich source of minerals like iron, calcium, and phosphorus and has many vitamins like ascorbic acid, pantothenic acid, vitamin A, carotenoids such as B-carotene and lycopene, and niacin. Single common guava fruit contains

about four times the amount of vitamin C as an orange (Misra and Seshadri, 1968). The fruit are very nutritious and rich in carotenoids, phenols, dietary fibers (Viraj and Pillai, 2012) and are known for high antioxidant activity (Porat *et al.*, 2009), The high level of antioxidants pigments like carotenoids and polyphenols present in guava increases its dietary value (Chen and Yen, 2006). White and pink flesh guavas fruits had high hydrophilic antioxidant activity and compounds for phenolic and vitamin C pointed that regular consumption of guava might be beneficial to health (Thaipong *et al.*, 2006), help to reduce

the incidence of degenerative diseases such as arthritis, arteriosclerosis, cancer, heart disease, and brain dysfunction. The fresh fruit is preferred for consumption but seasonal availability limits consumption of fruit throughout the year.

With the changing consumer attitudes, demands and emergence of new market products, it has become imperative for products to develop products, which have nutritional as well as health benefits. The fresh fruit has limited shelf therefore it is necessary to utilize the fruit for making different products to increase its availability over an extended period and to stabilize the price during the glut season. Guava can be consumed fresh or can be processed into juice, nectar, pulp, jam, jelly, slices in syrup, fruit bar or dehydrated products, as well as being used as an additive to other fruit juices or pulps (Leite *et al.*, 2006). These products have good potential for internal as well as external trade. The utilization of guava for preparation of beverages and intermediates moisture products has not been explored much. Guava pulp can be used as base for the preparation of these products.

## MATERIALS AND METHODS

The present study was carried out in the Department of Horticulture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani during year 2012-2013 and 2013-2014. Washed guava fruits were cut into thick slices with a stainless steel knife. Slices were divided in two lots. The slices were then subjected to two heat treatments to soften fruit slices as below.

1. Low temperature long time (LTLT) - 48°C for 1 h
2. High temperature short time (HTST) - 80°C for 20 min.

Each of this softened fruit slices were passed through fruit pulper to extract pulp. The pulp was collected in a stainless steel vessel and pulp yield noted. The resultant pulp was heated for 90°C for 1 min. and packed in clean and sterilized bottles, upturned and sealed. The pulp was analyzed for antioxidant (total phenols, total flavonoid, anthocyanin and antioxidant activity) content.

## Extraction of red fleshed guava pulp

The best treatment from the above experiment with highest yield and antioxidant activity was then selected for the Extraction of pulp. Washed, sliced guava subjected to HTST (80°C for 20 min.) was used for pulp extraction study. The heated soften slices was passed through pulper. The pulp yield was determined by weighing after extraction of pulp. The extracted pulp was heat processed at 90°C for 1 min. and packed in clean sterilized pouches, upturned and sealed. The pulp was then stored at ambient temperature and cold storage (7°C) for storage study and analyzed antioxidant (anthocyanin, total phenolic, total flavonoid) contents and total antioxidant activity

## Total anthocyanin

The total monomeric anthocyanin content was determined on a UV-visible spectrophotometer by the pH differential method (Wrolstad, 2004) using talo buffer, pH 1.0 (0.025 m) and sodium acetate buffer pH 4.5 (0.4 m). Samples were diluted in pH 1 and pH 4.5 buffers and absorbance measurements were made at 420 and 700 nm using 1 cm path length cuvettes. The pigment content was calculated and expressed as cyaniding 3-glucoside (cyd 3-glu) per kg FW, using an extinction coefficient (e) of 26.900 L / cm/mol and molecular weight of 449.2 g/mol.

Absorbance = (A 420 - A 700 nm) pH 1.0 - (A 420 - A700 nm) pH 4.5  
 Monomeric anthocyanin (mg/L) = A X MW X DF X 1000/Wt. of sample

The final concentration and anthocyanins was calculated based on total volume of extract and weight of sample.

## Total phenolic

Total polyphenolic content in guava samples was estimated spectrophotometrically folin ciocealtau reagent (FCR) as described by Singleton *et al.* (1999) using gallic acid as standard to the 100 ul of the suitably diluted sample extract, 2.9ml of deionized water and 0.5ml folin ciocealtau reagent and 2.0ml of 20 % Na<sub>2</sub>CO<sub>3</sub> solution was added. The mixture was allowed to stand for 90 min and absorption was measured at 760nm against water as blank. The amount of total, phenolic was expressed gallic acid. Two parallel determination of each

sample were performed and average values were calculated.

### Total flavanoids

Total flavanoids were measured by a colorimetric assay developed by Zhishen *et al.* (1999). One ml of sample extract was added to a 10 ml volumetric flask containing 4ml distilled water. Then 0.3 ml 5% NaNO<sub>2</sub> was added to this mixture and allowed to stand for 5 min at room temperature. Thereafter 0.3 ml of 10% A.CU 6H<sub>2</sub>O was added and the mixture was allowed to stand for 6min at room temperature. Two ml of, N NaOH was added and solution was diluted to desired volume (10ml) with distilled water. The absorbance of the solution versus blank at 510nm was measured immediately. The results were expressed as catechin equivalent (CE, using a standard curve (absorbance versus concentration, prepared from authentic catechin.

### Antioxidant activity assays

#### *Ferric reducing antioxidant power (FRAP)*

FRAP assay developed initially to measure ferric reducing ability of blood plasma (Benzie and Strain, 1996) has now been widely employed in a variety of plant and food samples. The FRAP assay also takes advantages of the electron transfer reaction, wherein a ferric salt, Fe (TPTz) 2 III, is used as an oxidant under acidic conditions, pH 3.6 FRAP assay was performed according to the procedure described by Benzie and Strain (1996) with some modifications FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions is known concentration. Working reagent was prepared freshly each day by mixing 300 mm acetate buffer pH 3.6, 10mm TPTz in 40 mm HCL and 20mm FeCl<sub>3</sub> in ratio 10:1:1 (v:v:v). Briefly 3ml of FRAP reagent was mixed with 100 sample reagent mixing at a wavelength of 593nm.

#### *Cupric reducing antioxidant capacity (CUPRAC)*

CUPRAC stands for cupric reducing antioxidant capacity. This method recently developed by Apak *et al.* (2004) measures the copper (II) or cupric ion reducing ability of poly phenols. This

is a simple and widely applicable antioxidant capacity index for dietary polyphenols, vitamin C and E. It makes use of the copper (II) neocuproine (CUUI)-NC) reagent as the chromogenic oxidizing agent.

The method comprises mixing of the antioxidant solution with a copper (II) chloride solution, a neocuproine alcoholic solution and an ammonium aquarist buffer at pH 7 and subsequent measurement of developed absorbance at 450nm after 30min. Normal sample measurement is as follows.

To a test tube were added 1 ml each of copper (II) chloride solution (10<sup>-2</sup>m) neocuproine solution (NC) of 7.5x10<sup>3</sup>m and ammonium acetate (NH<sub>4</sub> Ac) buffer (pH 7) solution. Antioxidant sample (or standard) solution (x ml) and H<sub>2</sub>O (1.1-x ml) were added to the initial mixture so as to make the final volume 4.1 ml. The tubes were stoppered and after one hour, the absorbance at 450 nm was recorded against a reagent blank. The standard calibration curve of each antioxidant compound was constructed in this manner as absorbance versus concentration. The molar absorptivity of the CUPRAC method for each antioxidant was found from the slope of the calibration line concerned and the antioxidant activity was expressed as umol Trolox/g.

### Statistical analysis

The statistical analysis was carried out as per methods suggested by Panse and Sukhatme (1967) for completely randomized design (CRD).

## RESULT AND DISCUSSION

### Effect of processing on antioxidant composition of guava pulp

The physicochemical composition of red fleshed guava pulp extracted by hot break method and cold break method are presented in Table 1. In the preliminary experiment we investigated two heat treatments (HTST and LTLT) for their effect on extraction of antioxidants in increasing the recovery of antioxidants (phenolics, flavonoid and anthocyanins). Extraction temperature plays a critical role in preserving pulp quality (Hayati R, 1987). Significant difference was observed between the two heat treatments with

regard to their physico chemical and antioxidant composition (total phenolic, flavonoid and anthocyanin content). High anthocyanin content 1014.70 µl/L was found in HTST treated guava slices, while yield of 1006.28 µl/L was observed in LTLT treated guava slices. The phenolic content was also found high in HTST treated guava slices 138.18 mg GAE/g in comparison to 135.76 mg GAE/g in LTLT treated guava slices. The high value of flavonoid content (25.42 mg CE/g) was found in HTST treated guava slices in comparison to 23.90 mg CE/g in LTLT treated guava slices.

**Table 1: Effect of processing on physico-chemical and antioxidant activities of red fleshed guava pulp**

Attributes	LTLT	HTST
TSS (%)	14.3	13.7
pH	5.07	5.12
Titrateable acidity (%)	0.49	0.48
Ascorbic acid (mg/100g)	136.42	139.46
Anthocyanin (µl/L)	1006.58	1014.70
Phenol (mg GAE/g)	135.76	138.18
Flavonoid (mg CE/g)	23.90	25.42

Overall processing of guava by HTST heat processing treatment significantly improved the antioxidant composition of the guava pulp. The heat treatments enhanced the total phenolic content, flavonoid as well as total anthocyanin content. The attempt was done with the extraction of red fleshed guava pulp. After storage study the red fleshed guava used for the extraction of pulp and storage study at ambient and cold storage (7°C), the guava pulp extracted by two methods LTLT (48°C for 1 h) and HTST (80°C for 20 min.). Enzyme deactivation systems, known as hot break units raise the processing 85°C and over so as to deactivate the enzyme as quickly as possible and therefore, preserve the product's naturally.

### Effect of storage period on antioxidant composition of guava pulp

The effect of storage period on antioxidant (anthocyanin, total phenol and flavonoid) composition of red fleshed guava pulp at ambient temperature and low temperature 7°C are presented in Table 1. The total anthocyanin content in guava pulp was 1006.58 µl/L and there

was a substantial decrease in the anthocyanin content with advancement of storage period. After 60<sup>th</sup> days of storage at ambient temperature anthocyanin content was 956.34 µl/L, whereas after 150<sup>th</sup> days of storage of guava pulp in cold storage at 7°C, anthocyanin content was found to decrease from initial 1006.58 µl/L to 984.54 µl/L. The result associated with anthocyanin content data revealed that there is decreasing trend was recorded in anthocyanin content during storage of guava pulp. HTST heating at 80°C for 20 min had longer storage life of guava pulp than LTLT heating at 48°C for 1 hr. Findings also reported by (Kalt *et al.*, 2000) extraction of fruit at 60°C resulted in higher recovery of anthocyanin and antioxidant property compared to 25°C room temperature. (Chaovalikit and Wrolstad 2004) reported more than 75 per cent of anthocyanin was destroyed during storage. Period of storage is more specific for colour alteration and anthocyanin degradation (Marti *et al.*, 2001), degradation followed first order kinetic during storage (Brenes *et al.*, 2005), increase in per cent polymeric colour followed by near zero order kinetic (Wicklund *et al.*, 2005), anthocyanin degradation influenced during processing and storage temperature. Where in the degradation of individual anthocyanin significantly decreased during storage dependent on storage temperature accelerates the anthocyanin degradation and increase in pH accelerated the destruction in anthocyanin.

The total phenolic content was also found to decrease from initial 135.76 mg GAE/g to 126.48 mg GAE/g after 60<sup>th</sup> days of storage of guava pulp at ambient temperature, while after 150<sup>th</sup> days of storage of guava pulp in cold storage decline in total phenolic content was observed from initial 135.76 mg GAE/g to 124.36 mg GAE/g.

The total flavonoid content was also found to decrease from initial 23.90 mg CE/g to 10.26 mg CE/g after 60<sup>th</sup> days of storage of guava pulp at ambient temperature, while after 150<sup>th</sup> days of storage of guava pulp in cold storage decline in total flavonoid content was also observed from initial 23.90 mg CE/g to 16.32 mg CE/g. Results revealed that decreasing phenolic and flavonoid compounds during storage are in agreement

**Table 2: Effect of processing and days of storage on antioxidant composition of guava pulp at ambient temperature and cold storage**

Storage (days)	Ambient temperature			Cold storage (7°C)		
	Anthocyanin	Total phenol	Total flavonoid	Anthocyanin	Total phenol	Total flavonoid
0	1006.58	135.76	23.90	1006.58	135.76	23.90
30	984.21	131.61	20.38	1003.60	134.50	22.26
60	956.34	126.48	10.26	1001.39	131.08	21.14
90	-	-	-	996.28	129.65	19.46
120	-	-	-	990.62	127.42	17.21
150	-	-	-	984.54	124.36	16.32

with (Patthamakanokporn *et al.*, 2008) and (Lim *et al.*, 2008) increase in unripe samples but decrease in ripe samples (Viraj and Pillai, 2012) ranged the total phenol and total flavonoid content. (Zahidah *et al.*, 2013) also reported the antioxidant and antimicrobial activities of leaves and seeds of pink guava and (Fidrianny *et al.*, 2012) on red guava leaves to isolate antioxidant substances.

**Table 3: Effect of processing and days of storage on antioxidant activity of guava pulp at ambient temperature and cold storage**

Storage (days)	Ambient temperature		Cold storage (7°C)	
	Antioxidant activity		Antioxidant activity	
	FRAP	CUPRAC	FRAP	CUPRAC
0	11.12	5.27	11.12	5.27
30	9.30	3.76	10.03	3.75
60	7.12	2.82	9.22	3.46
90	-	-	8.76	3.11
120	-	-	8.09	2.86
150	-	-	7.24	2.66

The effect of storage period on antioxidant activity by FRAP and CUPRAC assay of guava pulp are presented in Table 18. Total AOX is a unique parameter that quantifies the ability of a complex biological sample to scavenge free radicals. High 11.2  $\mu\text{mol Trolox/ml}$  total AOX of guava pulp by FRAP assay was noticed at ambient temperature. With the advancement of storage period after 60<sup>th</sup> days of storage decreased values of total AOX was found from initial 11.2 Trolox  $\mu\text{mol /ml}$  to 7.12 Trolox  $\mu\text{mol /ml}$ , whereas after 150<sup>th</sup> days of storage of guava pulp in cold storage the value was decreased from initial 11.2  $\mu\text{mol Trolox/ml}$  to 8.09 Trolox  $\mu\text{mol /ml}$ .

High 5.27  $\mu\text{mol Trolox/ml}$  total AOX of guava pulp by CUPRAC assay was noticed at ambient temperature. With the advancement of storage period after 60<sup>th</sup> days of storage decreased values of total AOX was found from initial 5.27 Trolox  $\mu\text{mol /ml}$  to 2.82 Trolox  $\mu\text{mol /ml}$ , whereas after 150<sup>th</sup> days of storage of guava pulp in cold storage the value was decreased from initial 5.27  $\mu\text{mol Trolox/ml}$  to 2.66 Trolox  $\mu\text{mol /ml}$ . Antioxidant activities of red fleshed guava pulp decreasing trend was observed during storage are in agreement with (Patthamakanokporn *et al.*, 2008) decreased trend observed during storage and (Lim *et al.*, 2006). (Gil *et al.*, 2000), (Perez Viantle *et al.*, 2004) losses in antioxidant activity were lower than 20 per cent and did not exceed 30 per cent loss through the storage period of for any mixture. (Abdullah *et al.*, 2012) and (Zahidah *et al.*, 2013), measure the activity of hydrophilic antioxidant, antioxidant activity of pink pulp clone (Thaipong *et al.*, 2006) and pink-fleshed guava with ethanol and methanol extraction (Khalid *et al.*, 2011).

## CONCLUSION

Decreasing trends in contents of antioxidant activity was recorded during storage of pulp at ambient temperature, while antioxidants activity of guava pulp was recorded during cold storage 7°C.

Extraction of red fleshed guava pulp by HTST heating accelerate retention of antioxidant activities during storage.

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