Annals of Plant and Soil Research 24(4):529-535 (2022)
https://doi.org/10.47815/apsr.2022.10204

Effect of pasteurization methods on physicochemical constituents and optimization of blends for anthocyanin rich guava nectar

AJIT KUMAR SINGH*, V.R. SAGAR AND RAJU KUMAR

Division of Food Science and Postharvest Technology, ICAR-Indian Agricultural Research Institute
New Delhi-110012

Received: May, 2022; Revised accepted, July, 2022

ABSTRACT

An investigation carried out to standardize the pasteurization method for the extraction of maximum pulp/juice from guava and grapes and a process for development of anthocyanin rich guava nectar. The pulp of guava variety Allahabad Safeda and juice of coloured grape variety, Pusa Navrang was extracted and used in different proportions for optimization of blends of extracted pulp/ juice from both the fruits for preparation of anthocyanin rich guava nectar without deterioration of guava flavor. Among the different pasteurization methods in respect of irradiation, microwave with 600 W microwave power level, followed by pasteurization at 70^{0}C as thermal treatment, found to be the best methods for maximum recovery of the extracted pulp/ juice and nutritional quality over other methods. After pasteurization, the recovery of pulp 62.3%, TSS 14.42^{o}B and ascorbic acid 193.0 mg/100ml was recorded in guava and whereas the juice recovery 68.7 %, TSS (20.53^{o}B), ascorbic acid (6.76 mg/100ml) and anthocyanin content (38.38 mg/100ml) was recorded in coloured grapes, when the fruits were treated with 600 W microwave power level. However, in case of thermal pasteurization at 70^{o}C the recovery of pulp 55.8%, TSS 12.4^{o}B and ascorbic acid 149.33 mg/100ml was recorded in guava and juice recovery 63.0%, TSS (16.47^{O}B), ascorbic acid (6.26mg/100ml) and anthocyanin content (33.48 mg/100ml) was recorded in coloured grapes. With regards to optimization of different blend proportions, for development of anthocyanin guava nectar, a blend of 50:50 of guava pulp and coloured grapes juice was found to be best proportion in preserving the colour, flavour, taste and overall quality of developed anthocyanin guava nectar with having guava dominant flavour.

Key words: Pasteurization, anthocyanin-rich guava nectar, total antioxidant activities, colour grape

INTRODUCTION

Among the tropical fruits, guava (*Psidium guajavas*) and grape (*Vitis venefera*) have a good production potential and are suitable for the production of a variety of value-added products with good taste and flavour. Guava contains very high amount of vitamin C, which helps in improving immunity and protects us from common infections and pathogens. The high content of vitamin C in guava makes it a powerhouse in combating free radicals and oxidation that are key enemies to many degenerative diseases. Ripe guava fruits are highly perishable when stored at room temperature, but it can be processed into various commercial products, including pulp, paste, canned slices in syrup and juice. Among these products, guava juice has become economically important in the market because of natural rich in nutrients and used as an alternative to other beverages such as soft drinks, tea and coffee (Akesowan and Choonhahirun 2013). Coloured grapes have excellent pigment colour due to the presence of anthocyanins, phenols and betalains in their pulp. Both anthocyanins and phenols are the promising antioxidants. Which have excellent therapeutic properties and can prevent or reduce the incidence of a wide range of diseases or disorders associated with free-radicals or radical oxygen species (ROS) like Parkinson's disease, hypertensive cerebra-vascular injury, cataract genesis, ocular hemorrhage, atherosclerosis, various types of cancers, gastro-intestinal disorders hypoxia, disorders related to alcoholism and ultimately ageing (Rasines-Perea and Teissedre 2017). Fruit beverages are increasingly gaining popularity throughout the world due to nutritive and therapeutic value over synthetic beverages, as synthetic drinks contain only water (about 88%), carbohydrates (about 12%) and provide about 48 Kcal (Sethi and Sethi, 2006). Thus, fruit-based beverages are far more superior than many synthetic drinks. If synthetic drinks are replaced by fruit beverages, it would be a boon

*Corresponding Author email.- 718713ajit@gmail.com, Division of Sample Surveys, ICAR-IARI, New Delhi-110012

to the consumers as well as to the fruit growers. Moreover, fruit pulps and drinks have great export potential in the fruit processing industry because fresh fruits have limited shelf-life due to the perishable nature of the fruits, they require immediate processing to avoid post-harvest losses and in addition to that fruits colour pigment and flavors constituents which are bound mainly to pulp particles can only be obtained by pressing. Therefore, the main objective of this work was to standardize the thermal process and utilize the fruits of guava and grape rich in vitamin C and colour pigments for making anthocyanin rich guava nectar to increase their availability over an extended period and stabilize the prices during the glut season.

MATERIALS AND METHODS

The fully mature fruits of guava cv. Allahabad Safeda and coloured grape (PusaNavrang) were obtained from the Division of Fruits and Horticultural Technology, IARI, New Delhi's Main Fruit Plantation. The guava and colour grapes were washed with tap water to remove adhering dirt and dust before drying under a fan. The guava fruits were manually sliced with a stainless steel knife, and the colour grapes (whole) were grated with an apple grater. Irradiation (0.5, 1.0, and 1.5 kGy), microwave (300, 600, and 900 W for 1min., 30 &10 sec.) and thermal (70, 80, 90°C based on POD inactivation) treatments were applied to grated guava and grape, as well as a control treatment for both fruits.

The guava pulp/juice was then extracted using the fruit pulper. The grape was extracted (500-4000 IbIn2) using a Johnston Automation fruit pulper (Manufacture: Bajaj Process Pack Ltd. India). The extracted pulp/juice was sieved through a 30 mesh sieve to remove any seed and skin to obtain the fine pulp/juice. The grape was extracted (500-4000 IbIn2) by using fruit pulper Johnston Automation (Manufacture: Bajaj Process Pack Ltd.d India). To obtain the fine pulp/juice, the extracted pulp/juice was sieved through a 30 mesh sieve to remove any seed and skin. The juice/pulp obtained from both fruits was weighed in order to determine the percentage juice/pulp.as follows:

$$\text{Recovery of Juice/Pulp(\%)} = \frac{\text{Weight of juice/pulp} \times 100}{\text{Total fruit weight}}$$

The extracted guava pulp and grape juice were blended in various proportions (60:40, 50:50, 40:60), along with 100 % guava and grape as control samples. All of the blends, as well as the control (100 % guava and grape), were used to make anthocyanin-rich guava nectar by following the standard nectar recipe (20 % pulp, 0.3 % acidity, and 15o Brix) and heating to 85°C for one minute to find the best proportion of both blending materials without sacrificing guava flavor or other nutritional parameters. A digital handheld refractometer (0-50°B, Atago Japan) was used to determine the total soluble solids (TSS) of the sample. Titratable acidity was determined by titrating a known aliquot of the sample against a standard sodium hydroxide solution while using phenolphthalein as an indicator. Ranganna (2000) have been defines formalized Ascorbic acid was measured as mg 100-1 ml by titrating the samples against 2,6-dichloropheno-lindophenol dye Ranganna (2000) To determine reducing and total sugars, the Shaffer-Somogyi micro-method was used (Ranganna,2000). The total monomeric anthocyanin content was determined using the pH differential method (Wrolstad *et al.*, 2006). The Folin-Ciocalteu method was used to determine total phenols (AOAC, 2016). The total antioxidant power was calculated using the FRAP method (Bezie *et al.*, 2018). Amerine *et al.,* 2020) described the Hedonic procedure for sensory evaluation of powder. The experiment was repeated twice, with three replications for each treatment. SAS was used to perform statistical analysis of variance on anthocyanin-rich guava nectar.

RESULTS AND DISCUSSION

Effect of pasteurization methods on recovery of juice/pulp

The recovery of guava pulp increased with increase in irradiation dose and it was maximum in the grated fruits treated with dose of 1.0 kGy (Table-1). While it was maximum (62.02%) in microwave power level of 900 W and 57.81% at a thermal temperature of 80°C, respectively. In case of grape, the juice recovery was maximum (62.7%) when the fruits were treated with 1.0 KGy irradiation, and it was 68.7% at microwave power level of 600 W and 64.9%at a thermal temperature of 90^{0}C, respectively. Amongst different methods tried,

Table 1: Effect of pasteurization methods on physico-chemical constituents of guava pulp and grape juice

Pasteurization method	Dose	Fruits and Parameters									
		Guava					Grape				
		Juice Recovery (%)	TSS (°B)	Titratable acidity (%)	Ascorbic acid (mg/100g)	Juice Recovery (%)	Pomace (%)	TSS (°B)	Titratable acidity (%)	Ascorbic acid (mg/100g)	Anthocynin (mg/100g)
Irradiation	0	54.02±1.48^{a}	11.83±00^{a}	0.32±00^{a}	291.00±0.0^{a}	56.7±0.0^{b}	43.3±0.0^{b}	19.20±0^{b}	0.35±0.0 ^{a}	8.0±0^{b}	40.12±0^{a}
	0.5	55.00±00^{a}	12.14±0.5^{a}	0.32±0.01^{a}	283.00±2.00^{b}	61.5±0.77^{a}	37.8±1.40^{c}	19.47±0.54)^{b}	0.36±0.01^{a}	7.00±0.00^{ab}	35.57±0.25^{b}
	1.0	56.77±1.00^{b}	12.17±0.9^{a}	0.33±0.00^{a}	252.67±1.52^{c}	62.7±1.03^{a}	35.4±0.94^{a}	20.±0.03^{a}	0.37±0.01^{b}	6.5±0.50^{a}	32.16±0.35^{c}
	1.5	56.30±1.50^{b}	12.51±0.33^{a}	0.34±0.02^{a}	231.33±2.08^{d}	62.4±0.63^{a}	36.1±0^{ac}	20.15±0.26^{a}	0.37±0.00^{b}	(6.0±0.00) ^{a}	30.40±0.40^{d}
LSD at 5%		2.20	0.95	0.02	3.07	1.4	1.8	0.57	0.0172	1.41	0.55
Microwave	0	55.00±00^{ac}	11.83±00 c	0.32±00^{a}	291.00±00^{a}	56.67±0.0)^{c}	43.33±0.0^{a}	18.20±0^{b}	0.35±0^{a}	8.0±0.0^{b}	40.12±0^{a}
	300	57.71±1.07 ^{a}	13.05±0.5 a	0.33±0.01^{a}	213.33±2.08^{b}	65.48±0.93^{b}	32.71±0.82^{b}	19.77±0.32 ^{b}	0.37±0.01^{ab}	7.13±0.15^{c}	40.50±0.0^{a}
	600	62.37±0.65^{b}	14.47±0.3^{ab}	0.33±0.01^{a}	193.50±1.80^{c}	68.79±1.05^{a}	30.01±0.84^{c}	20.53±0.45 ^{a}	0.37±0.01 ^{b}	6.76±0.05^{ac}	38.38±0.0^{b}
	900	61.02±2.97ab	13.64±0.72^{a}	0.34±0.02^{a}	183.77±2.28^{d}	67.78±1.12^{a}	30.30±0.72^{c}	20.88±0.68^{a}	0.37±0.00^{a}	6.44±0.38^{a}	37.48±0.40^{c}
LSD at %		3.04	0.89	0.02	3.36	1.69	1.29	0.8274	0.018	0.39	0.60
Thermal	0	55.00±0.0 ^{a}	11.83±00^{a}	0.32±00 a	291.00±00) ^{a}	56.67±0.0^{c}	43.33±0.0^{b}	16.20±0^{a}	0.35±0^{b}	8.0±0.0^{a}	40.12±0.0^{a}
	70	55.85±1.54 ^{ab}	12.40±0.5^{ab}	0.35±0.01^{ab}	149.33±1.10^{b}	63.05±0.65^{b}	35.50±1.02^{c}	16.47±0.08^{c}	0.37±0.01^{a}	6.26±0.25 ^{b}	33.48±0.40^{b}
	80	57.81±1.55^{b}	12.23±0.6^{ab}	0.35±0.02^{ab}	139.03±1.62^{c}	64.36±1.00^{a}	35.39±0.93^{a}	16.57±0.08^{c}	0.38±0.01^{c}	5.50±0.20^{c}	31.40±0.40^{c}
	90	56.89±1.88^{ab}	12.89±0.1^{b}	0.35±0.01^{b}	131.00±1.96^{d}	64.99±0.42^{a}	36.12±0.92^{a}	16.74±0.04^{b}	0.38±0.01^{b}	4.50 ±0.20^{d}	29.50±0.40^{d}
LSD at 5%		2.72	0.77	0.03	2.62	1.19	1.84	0.11	0.019	0.36	0.65

microwave recovery of juice was significantly higher than control and other methods. This might be due to enhanced degradation of bound cell wall of the fruits. Similar findings have been reported earlier in orange fruits treated with different irradiated doses (Betancourt *et al.*, 2009).

Effect of pasteurization on TSS, Acidity, total Anthocyanin, phenol content, ascorbic Acid, reducing and total reducing sugar and Sensory Evaluation

TSS increased with increasing concentrations of irradiation doses, microwave, and thermal pasteurisation compared to control in both the fruit grated material and the fruit juice. The increase in TSS could be attributed to water evaporation, which caused juice concentration and may have contributed to the increase in TSS. There was no statistically significant difference in total soluble solids between the thermal pasteurization treatments. The acidity of the pulp increased as the doses of irradiation, microwave, and thermal pasteurization were increased. The increased acidity in the treated material had a significant effect on the guava and grape control samples. (Kathiravan *et al.* (2014) reported similar findings in thermal pasteurization while preparing ready-to-drink beetroot juice. Pasteurization methods had a significant impact on the ascorbic acid content of guava and grape pulps. The ascorbic acid loss increased with increasing pasteurization doses in all three treatments (irradiation, microwave, and thermal). The decrease in ascorbic acid could be attributed to its heat-labile nature in the fruits at various pasteurization temperatures. Because of the degradation of some anthocyanin quality due to heat exposure of heat-sensitive material, the anthocyanin content of grape juice decreased slightly as pasteurization doses increased. There was, however, no statistically significant difference in total monomeric anthocyanin content between the temperature treatments. Furthermore, in the irradiation, microwave, and thermal treatments, the quantity of pomace content decreased with increasing pasteurization, and it was highest in control samples, followed by samples treated at 70°C. (Fig.1)

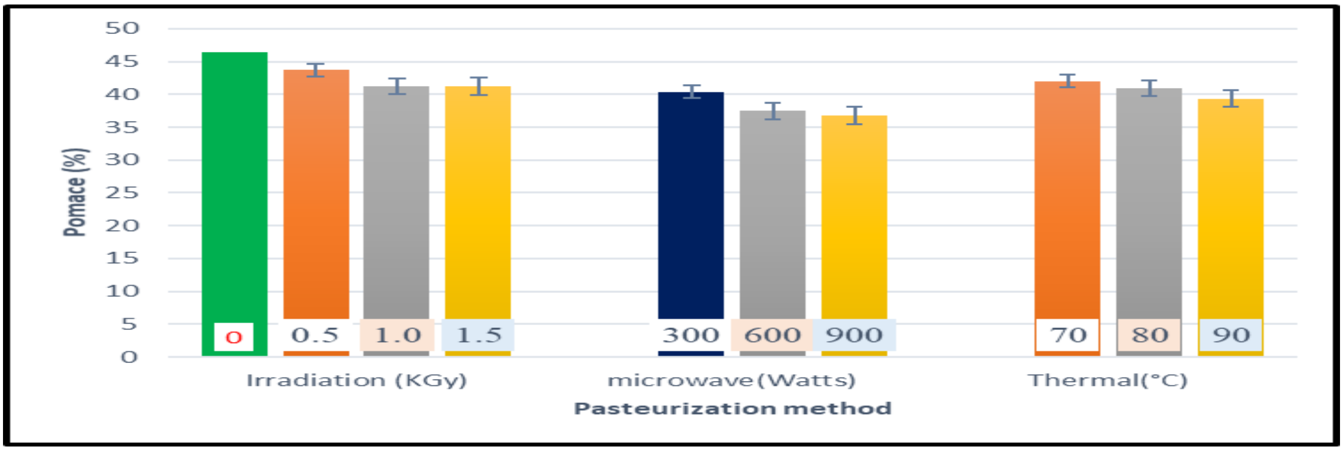

Fig 1: Effect of pasteurization method on pomace content during extraction of guava and grape juice

Grape juice reached 12.15° Brix in a 50:50 proportion and then declined (Table 2). The same pattern was observed when guava juice was added to grape juice (100%). This could be due to the higher TSS of grape to guava pulp balancing the sweetness. Shrestha *et al.*, 2020) observed similar results in pomegranate juice after heat treatment of apple pulp (Haya *et al.*, 2019). The addition of guava proportion to grape and grape to guava proportion resulted in an increase in acidity and a decrease in ascorbic acid. This may be due to higher content of ascorbic acid in guava to lower content of ascorbic acid to grape. Jan and Masih (2012 have been observed a similar increase in acidity in pineapple juice blended with carrot and orange juice. However, rising titratable acidity could be attributed to the production of acidic components as a result of sugar breakdown during the fermentation process (Umelo *et al.*, 2014). In terms of ascorbic acid, the loss of ascorbic acid may also be due to high-temperature heat processing of juice blends (Bhattacherjee *et al.* 2011).

Table 2: Effect of different proportion of guava and grape pulp/juice on nutritional quality of anthocyan in rich guava nectar

Blend	TSS (%)	TA (%)	Ascorbic acid (mg/100g)	Total monomeric anthocyanins (mg/100ml)	Total phenols (mg/100ml)	Reducing sugar (%)	Total sugars (%)
Guava- 100	9.50	0.32	255.0	0.0	23.80	2.90	4.60
GCG -90	9.60	0.33	226.0	1.0	23.86	3.05	5.05
GCG -80	9.70	0.33	215.0	2.5	24.10	3.70	5.47
GCG -70	9.80	0.45	195.0	8.6	24.85	3.90	6.20
GCG -60	10.10	0.50	160.0	18.20	25.10	4.10	6.60
GCG -50	10.15	0.55	155.0	25.25	25.90	5.60	7.05
CG -100	18.00	0.35	8.00	40.02	26.15	6.35	7.30
CGG -90	17.60	0.34	12.91	38.15	25.35	5.85	7.18
CGG -80	17.55	0.33	22.52	35.27	24.85	5.20	7.0
CGG -70	17.30	0.31	25.63	33.15	23.20	5.0	6.5
CGG -60	16.85	0.30	33.45	32.25	22.50	4.85	5.70
CGG -50	16.80	0.30	40.65	29.65	22.10	4.60	5.0
CD at 5%	0.33	0.01	4.00	0.62	0.23	0.02	0.05

G= guava colored grape, GCG=guava: coloured grape

Total anthocyanins, reducing sugars, and total sugars were found to increase as the proportion of guava to grape increased up to 50:50. This could be due to the nutrients' decreasing and increasing proportions to each other up to a certain point. In terms of overall organoleptic evaluation, GCG 60:40 and G:CG 50:50 had the highest overall acceptability score of 8.4, which was on par with pure guava (G-100), which had a score of 8.5 (Table 3). In terms of colour and flavour, GCG 60:40 received the highest scores of 7.72 and 8.04, respectively, putting it on par with CGC coloured grape 60:40. The G-100 beverage received the highest test score (7.83), followed by the GCG 60 blends (8.0). Due to different blending ratios, the sensory score varied significantly. However, a 50:50 guava:grape ratio was discovered to be the best for having the highest sensory score in terms of colour, flavour, taste, and overall acceptability, as similar findings revealed (Vaghashiya *et al.*,2015). The sensory colour, taste, aroma, and overall acceptability score of blended guava nectar prepared using various proportions of guava and aloevera juice/ pulp maximum score (9) in the blend using T4 (70 ml guava and 30 ml aloevera), which is statistically followed by T5 (60 ml guava and 40 ml aloevera) score (8) and T6 (50 ml guava and 50 ml (8). In blended nectar prepared with T9, a minimum score of (5) occurs (20 ml of guava and 80 ml of aloevera.

Table 3: Effect of different proportion of guava and grape pulp/juice on sensory evaluation of anthocyanin-rich guava nectar

Blend	Colour	Flavour	Taste	Overall
Guava-100	7.5	7.5	7.6	7.53
GCG-90	7.5	7.5	8.10	7.7
GCG-80	8.4	8.2	8.20	8.23
GCG-70	7.80	7.9	8.20	7.91
GCG-60	8.5	8.20	8.30	8.31
GCG-50	8.50	8.40	8.52	8.48
CG-100	7.0	7.6	7.6	7.40
CGG-90	7.5	7.8	7.6	7.60
CGG-80	8.00	8.2	8.10	8.10
CGG-70	8.3	8.4	8.20	8.3
CGG-60	8.4	8.4	8.5	8.43
CGG-50	8.5	8.60	8.5	8.43
CD at 5%	0.26	0.24	0.20	0.30

Microbial load of blended guava and grape nectar (50:50) during storage

The growth of acid-tolerant bacteria (ATB), yeast, and mould (Y & M) that appear to be thermally sensitive was the primary cause of blended nectar spoilage. The initial mean populations of ATB and Yand M in blended nectar were 5.690.09, 5.540.01, 5.680.03, and 5.530.01 cfu/ml, respectively. The total coliforms test resulted in a negative result. Thermal and microwave pasteurisation were proven to be effective in increasing the shelf life of nectar by inhibiting the growth of bacteria, yeast, and mould. The blended nectar remained free microorganism for the entire four-month period of storage. Similar to the studies of Naresh *et al.* (2015) in mango juice ionizing radiation, the initial microorganism load was drastically decreased by gamma irradiation at 0.5kGy, but the population subsequently increased. Among these, microwave treatment was found to be the most effective at inactivating pathogens of microbial cells interrupted by electromagnetic fields conjugating with critical molecules such as protein or DNA.

Microwave pasteurisation was found to be a comparatively better method of pasteurisation for guava and coloured grapes than thermal and irradiation pasteurisation for retaining the nutritional, functional, and sensory qualities of anthocyanin-rich guava nectar. In terms of microbial quality, both the thermal and microwave pasteurised blends were safe under refrigerated (4°C) conditions for up to four months. In the chemical composition of fruit juice/pulp, there was a wide variation in juice/pulp recovery (53.54-62.12 percent), pomace content (37.88-46.46 percent), ascorbic acid (8.0 mg/100 gm grape), (291 mg/100 gm), and anthocyanin from 0- 40.20 mg/100 ml. GCG-(Guava 50: Coloured Grape 50 percent) was the best of twelve different combinations of nectar prepared from different blends, with an overall sensory score of 8.6, followed by followed by GCG (60:40) at 8.0, respectively. The 50:50 guava/colored grape blend ratio was found to be the most acceptable for the preparation of anthocyanin-rich guava nectar with maximum retention of anthocyanin, guava flavour, and other nutrient.

REFERENCES

Akesowan A. and Choonhahirun, A. (2013). Effect of enzyme treatment on guava juice production using response surface methodology. *Journal of Animal and Plant Sciences* **23:** 114-20.

Amerine, Maynard Andrew, and Vernon L. Singleton. (2020.) "Wine." *Wine*. University of California Press,

AOAC. *Official Methods of Analysis* (16th Edn.) (2016.) Association of Official Analytical Chemists Washington, DC, USA, pp. 768-800.

Benzie, Iris FF, and Malegaddi Devaki: (2018) The ferric reducing/antioxidant power (FRAP) assay for non-enzymatic antioxidant capacity. Concepts, procedures, limitations and applications." Measurement of antioxidant activity and capacity: recent trends and applications Wiley, New York **11**: 77-106.

Betancurt, P. Ares, M.I., Montalban, A., Arcia, P., Borthagaray, M.D. and Curutchet, A. (2009). Effect of ´ irradiation as quarantine treatment on citrus fruit quality*. Canada Indian And Northern Affairs Canada,* Rio de Janeiro, Brazil.

Bhattacherjee, A.K., Tandon, D.K., Dikshit, A. and Kumar, S.(2011).Effect of pasteurization temperature on quality of aonla juice during storage. *Journal of Food Science and Technology* **48**: 269-73.

Jan, A. and Masih, D. (2012). Development and quality evaluation of pineapple juice blend with carrot and orange juice. *International journal of scientific research.***2**: 1-8.

Kathiravan, T., Nadanasabapathi, S. and Kumar, R .(2014). Standardization of process condition in batch thermal pasteurization and its effect on antioxidant, pigment and microbial inactivation of Ready to Drink (RTD) beetroot (*Beta vulgaris* L.) juice. *Int. Food Res.* **21**: 1305-12.

Shrestha, L., Kulig, B., Moscetti, R., Massantini, R., Pawelzik, E., Hensel, O., and Sturm, B. (2020) Optimisation of physical and chemical treatments to control browning development and enzymatic activity on fresh-cut apple slices. *Foods*, ***9***(1) :76.

Hayat, A., Devi, M. P., and Chakrabarty, S. (2019). Quality of lemon nectar blended with ginger a study*. International Journal Conservation Science* **7** (5),3008-3015.

Ranganna, S. (2000) *Handbook of Aanalysis and Quality Control for Fruit and Vegetable Products*, Tata McGraw-Hill Pub. Co., New Delhi, pp. 124-25.

Rasines-Perea, Z. and Teissedre, P. L: (2017) Grape polyphenols effects in human cardiovascular diseases and diabetes. *Molecules.***22**: 68-76

Rivas, A., Rodrigo, D., Martinez, A., Barbosa-Cánovas, G. V. and Rodrigo, M. (2006). Effect of PEF and heat pasteurization on the physical–chemical characteristics of blended orange and carrot juice. *LWT*-Food Science and Technology, **39**(10), 1163-1170.

Sethi, V. and Sethi, S: (2006) *Processing of Fruits and Vegetables for Value Addition*. Indus Publishing.

Umelo, P.S., Bergamaschi, K.B., Tiveron, A.P., Massarioli, A.P. and Oldoni, T. L.C., Zanus, M.C., Pereira, G.E. and Alencar, S.M.D. (2014) Phenolic composition and antioxidant activity of agroindustrial residues. *Ciencia Rural* **41**: 1088-93.

Vaghashiya J. M (2015). Study on preparation of health drink by blending Aloe vera, bitter gourd, aonla and guava. M.Sc. Thesis, Department of Postharvest Technology, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, pp 84.

Wrolstad, R.E., Durst, R.W., Giusti, M.M. and Rodriguez-Saona, L.E. (2006) Analysis of anthocyanin in nutraceuticals. In: *Quality Management of Nutraceuticals*. (Chp. 4 pp. 42-62), ACS Symposium Series No. 803, Washington DC, American Chemical Society.