DEVELOPMENT OF AN ANTIOXIDANT RICH BEVERAGE USING SOURSOP FRUIT AND GINGER EXTRACT

Gammana Widana Sashika **Madurangi,** Katugampalage Don Prasanna Priyantha **Gunathilake***
Department of Food Science & Technology, Faculty of Livestock, Fisheries & Nutrition
Wayamba University of Sri Lanka, Makandura, Gonawila, Sri Lanka
*E-mail: kdppgunathilake@yahoo.com

Abstract
There has been a growing demand for antioxidant rich food, as a solution for many oxidative stress related diseases. Various research findings have suggested that soursop and ginger are rich in antioxidants.
The present study mainly focused the formulation and storage study of beverage from soursop fruit by blending with ginger extract. Five levels of ginger extracts (1%, 3%, 5%, 7%, and 10%) were tested for the formulation by 25-semi trained-panellists using 9-point-hedonic-scale. Physico-chemical, proximate composition and antioxidant propertiesof soursop pulp, ginger extract and final formulation were determined.Changes in phenolic content, physicochemical and microbiological quality and antioxidant activity of the beverage were evaluated weekly foreight weeks storage at room temperature.
Results showed that formulation with 10% ginger extract was the most favourable in terms of sensory properties and high in antioxidant activity. The DPPH radical scavenging activity in the beverage were 70.06% ± 2.53, while soursop pulp, and ginger extract have 75.72%±1.67, 70.47% ± 1.61 values respectively. Total phenolic content of soursop,ginger and beverage were recorded as 149.25±5.02 mg-GAE/g, 156.38±8.57 mg-GAE/g, and 168.33±2.66 mg-GAE/g respectively. Titratable acidity, pH, and TSS of the final beverage were 0.61g CAE /100ml, 3.7, and 11.3°. Total plate counts and yeast and mould counts were zero during storage. Therefore, the beverage was microbiologically safe for consumption within 8 weeks. During storage, pH and TSS of the beverage were reduced whereas acidity increased.
This study concludes that 10% ginger extract can be used to formulate an acceptable, antioxidant rich soursop-ginger blended beverage.

Keywords: Antioxidant; beverage; DPPH; Ginger; Soursop; Extract

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1. INTRODUCTION

Soursopis a fruit with an acidic taste, closely related to custard apple. It is a small evergreen tree, member of the custard apple family; Annonaceae.*Annonamuricata* or soursop which originated from tropicalMexico, Central America, Caribbean, South America and sub-Saharan African countries now have spread and is grown in many countries, including some areas in South-East Asia such as Indonesia, Malaysia, Philippines, and Vietnam (Morton, 1987).However,soursop(*A. muricataL.*), cherimoya (*A.cherimola Mill*), custard apple (*A. reticulata L.*), and sweetsop (*A. squamosa L.*) species of Annona are abundant in home gardens in all agro-ecological regions of Sri Lanka (Heenkenda et al., 2009).Soursop is aless studied fruit tree species, which is mostly confined to home gardens. Soursop fruit can be consumed as fresh fruit or as juiceand the other plant parts are also a source of medicinal and other industrial products such as beverages, wine, jellies, jam and fruit-butter preserves and puree (Pinto et.al., 2005). Therefore, there is a potential for this fruit in the food processing industry.In the recent past, the soursop fruit was claimed in the global media as a cancer fighting fruit as it was found to be 10,000 times more effective than chemotherapy. Soursop consists of annonaceousacetogenins, which inhibits the development of damaged cells just before they could become cancerous (Mishra et al., 2013).Besides being a cancer remedy, soursop is a broad range antimicrobial agent for both bacterial and fungal infections, effective against internal parasites and worms, lowers high blood pressure and is used for depression, stress and nervous disorders(Buddhadasa et al., 2015).

Ginger (*ZingiberOfficinale*) has been traditionally used from ancient time for varied human ailments in different parts of the world, to aid digestion and treat stomach upset, diarrhea, and nausea (Gunathilake & Rupasinghe, 2015) . Moreover, ginger is largely used for functional beverages because some pungent constituents and other zingiberaceous plants have potent antioxidant (Gunathilake et al.,2013)and anti-cancer activities (Kundu et al., 2009), hypolipidaemic and hypocholesterolamic properties (Gunathilake et al., 2013).This bioactive properties of ginger are attributed to the presence of certain pungent constituents such as [6]-gingerol and [6]-shogaols (Gunathilake & Rupasinghe, 2013).Epidemiological studies as well as *in vitro*and *in vivo*studies strongly suggest that foods containing phytochemicals with antioxidation potential have strong protective effects against major disease risks including cancer and cardiovascular diseases (Pandey and Rizvi, 2009).Therefore, soursop ginger blended beverage would be a remedy for people who suffering from age related diseases, since it offers, enhanced health benefits with good sensory quality (Sirtori, et al., 2009). Blending different fruit juices with bioactives of ginger could enhance the physiological effects. The profile of phenolic phytochemicals determines the functionality of the whole food through additive or synergistic interaction of phenolic phytochemicals (Liu, 2003). Based on the above, this present study aims to prepare an acceptable quality RTS beverage using soursop fruit pulp with acceptable amountof ginger extract for the commercial preparation ofsoursop ginger blended RTS beverage with higher antioxidant properties.

2. MATERIALS AND METHODS

2.1Soursop Juice preparation

This research was carried out in the laboratory of Department of Food Science andTechnology of Wayamba University of Sri Lanka.Freshly rippedsoursop fruits were collected from Pannala area of Sri Lanka. Fruits were washed thoroughly in running water and were weighed, cut into halves, peeled, deseededand the pulp was stored in -18°C for further use.

2.2Ginger Extract preparation

Gingerextract was prepared using hot water extraction method described by Gunathilake & Rupasinghe (2014).

2.3 Preparation of blended soursop,ginger ready to serve beverage.

Frozen soursop pulp was thawed and water was added in 1:2 ratios. Soursop pulp water mixture was blended and filtered using muslin clothes. Sodium meta-bisulphite (50 ppm) was added as a chemical preservative and sugar was added to reach the TSS of 10°. Citric acid was added to get the pH below 4.0. Ginger extract was added to the formulations at levels of 1%, 3%, 5%, 7% and 10% and the product was heated to 100°C 2min. Then heatedfruit juice mixture was hot filled in to sterilized glass bottles and was sealed. Then, sealed bottles were sterilized stored at 28°C.

2.4 Sensory Evaluation

A sensory analysis was conducted to find out whether the addition of different levels of ginger extract have any effect for the consumer preference for the soursop, ginger blended RTS beverage and to determine the most preferred ginger level for the recipe. Panelists were instructed to rank the samples rendering to 9 point hedonic scale and their preference for the given attribute according to the Meilgaard et al., (1991).

2.5 Physico-Chemical Analysis

Samples were analyzed in triplicate for pH using a standardized pH meter (Model AA cells, pH meter, Japan) and total soluble solids by using a hand held refractometer (Model ATAGO, N-50B brix 0-50%, Japan). The total acidity was measured according to the method described in AOAC (1999).

2.6 Proximate Analysis

Proximate analysis was carried out for soursop fruit pulp and for the final beverage formulation.Protein, fat, ash, fiber and moisture contents were determined based on methods described in AOAC (1999). Carbohydrate content of fruit and beverage was determined by difference.

2.7Determination of Total phenolic content (TPC)

The TPC of each extract was determined using the method described by Singleton (1999) with small modifications described by Gunathilake & Rupasinghe (2014). In brief, 1.5 mL of Folin-Ciocalteu's reagent (10 times diluted) was added to test tubes containing 0.3 mL of standards or sample extract, and the contents were mixed thoroughly by vortexing. The reaction was neutralized by adding 1.2 mL of sodium carbonate (7.5 % W/V) to each tube and vortexed well. Tubes were allowed to stand at room temperature in the dark for 35 min. The absorbance was measured at 765 nm by spectrophotometer (model UV-2602, Labomed, inc, USA), using aqueous 80% (v/v) methanol as blank. The content of total phenolics in each extract was determined using a standard curve prepared for gallic acid and expressed as milligrames of gallic acid equivalents (GAE) per gram of fresh fruit.

2.8Determination of Antioxidant activity (DPPH radical scavenging assay)

The DPPH radical-scavenging assay was used todetermine the antioxidant activity of extractsaccording to a previously established procedure(Hatano et al, 1988) with slightmodifications.Extract solution (0.1 mL) in 95% methanol at variousconcentrations (10, 50, 100 ppm) was mixed with 3.0mL of methanolic solution of DPPH (0.004% (w/v).The reaction mixture was incubated for 30 min in thedarkness at room temperature. The absorbance of theresulting solution was measured at 517 nm with spectrophotometer (model UV-2602, Labomed, inc, USA).Methanol instead of sample solutionwas used as a control.

2.9 Determination of Microbial quality

Total Plate Count and Yeast and Mold Count were determined according to the method described byAOAC(1995).

2.10 Shelf life Determination

Within the 8weeks period of storage, pH value, Acidity, brix, total phenolic content ,total antioxidant activity were measured in weekly. Microbiological analysis were done in two weeks intervals.

2.11 Statistical analysis

The experimental design for the beverage formulation was a completely randomized design with three replicates for the responsevariables of the fruit juice blends and beverage. For the sensory data, the statistical analysis was performed byFriedman test with confidence interval of 95% usingMINITAB 15 software. For the analysis of variance (ANOVA), the general linear model (GLM) procedure of SAS 9.2.

3. RESULTS AND DISCUSSIONS

3.1Physico-chemical properties

Physico-chemical properties of the soursop pulp,ginger, final beveragehave shown inTable 3.1.

Table 3.1: Physico- chemical properties of soursoppulp,ginger, final beverage

	Soursop pulp	Ginger Extract	Final beverage
pH	4.36±0.73	5.23±0.84	3.70±0.015
TitrableAcidity	0.85±0.0.18 g/100ml(Citric acid equivalents)	0.08±0.0057 g/100ml(acetic acid equivalents)	0.60±0.007 g/100ml(citric acid equivalents)
TSS	15.7 ±1.66 °	0.3±1	11.3 ±0.57 °
		-	-

Table 3.2: Antioxidant properties of soursoppulp, ginger, final beverage

	Soursop pulp	Ginger	Final beverage
Total Phenolic content (mg GAE/1g)	149.25±5.020	156.38±8.570	168.33±2.665
DPPH method %	75.72±1.67	70.47 ± 1.61	70.06 ± 2.53

It can be illustrated that pH, acidity and TSS values of the final beverage formulation has reached standards given by the Sri Lanka Standard Institute (SLSI).According toSLSI standards, pH value should be lower than 4 in RTS drink which is suitable to overcome microbial growth. Normally, Brix value of RTS is maintained 10 to 15. Average Brix value of raw soursop pulp was 15.7° Brix value can be reduced by adding water to reach the required limit in RTS preparation.The recommended TSS for commercial RTS production is 15°Brix (SLS 729:1985). Therefore TSS of RTS beverage formulations was adjusted at the time of preparation. So the final beverage could reach 11.3° brix value. Titrable acidity of final RTS0.60±0.007 g/100ml (citric acid equivalents) which is similar to the commercialrecommendation of acidity for RTSpreparationis vital to preserve natural flavor of the fruit as well as to inhibit microbial growth.This can be attributed partly to the contribution of the inherent acid naturally present in the soursop pulp. The inherent acidity of soursop pulp is mainly attributed to citric acid.

3.2Antioxidant properties

According to the results, total phenolic content of the final beverage has obtained a higher value (168.33mg GAE/1g) than soursop pulp (149.25mg GAE/1g).It might be due to synergistic activity of incorporated gingerextract (156.38mg GAE/1g)and soursop pulp.Antioxidant activity referred as DPPH radical scavenging activity has slightly reduced in the beverage when compared to the soursop pulp. It may be due to the processing losses of antioxidant compounds.As shown by the Figure 3.1 reveals that DPPH radical scavenging activity has increased with the level of ginger extract incorporated to T1-T6 beverage formulations. Moreover, the highest radical scavenging activity has obtained by the T6 formulation(10% ginger added beverage), which is around 39% increment when compared to the control.It has revealed that the antioxidant compounds inherent to ginger could enhance the total antioxidant activity of the final beverage formulation.

Table 3.2: Antioxidant properties of soursoppulp, ginger, final beverage

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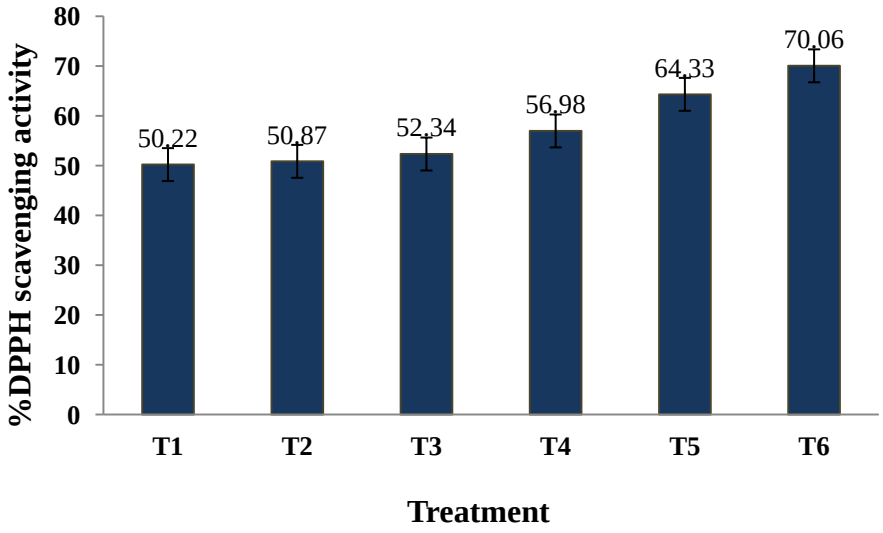

Figure 3.1:% DPPH scavenging activity of T1 – T6 formulations

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3.3 Proximate analysis

According to the above results, moisture of final beverage has increased while other components such as ash, fat, protein, fiber and carbohydrate have reduced when compared to soursop pulp. The fat content of final RTS is significantlylower than soursop pulp.It would be an additional benefit of the beverage.

3.4 Sensory Evaluation

Median scores of the sensory data is shown in the Figure 3.2.According to the results, there is no significant difference in median scores of treatments, for color and appearance attributes. But for the attributes of flavor, sourness and overall acceptability there were significant difference ($p<0.05$) among the treatments. Treatment T6 that is 10% ginger extract incorporated formula has received the highest cores for flavor, sourness and overall acceptability. Therefore, T6identified as the best sample for most of the attributes and hence was selected for the final beverage formulation.

Table 3.3: proximate composition of soursop fruit pulp and final beverage

	Soursop pulp (%)	Final beverage (%)
Moisture	82.96±0.56	87.56±0.60
Ash	0.58±0.01	0.52±0.0.01
Fat	0.38±0.01	0.05±0.0.005
Protein	0.69±0.01	0.64±0.0.005
Fiber	0.95±0.005	0.90±0.01
Carbohydrate	14.43±0.44	10.5 ±0.1

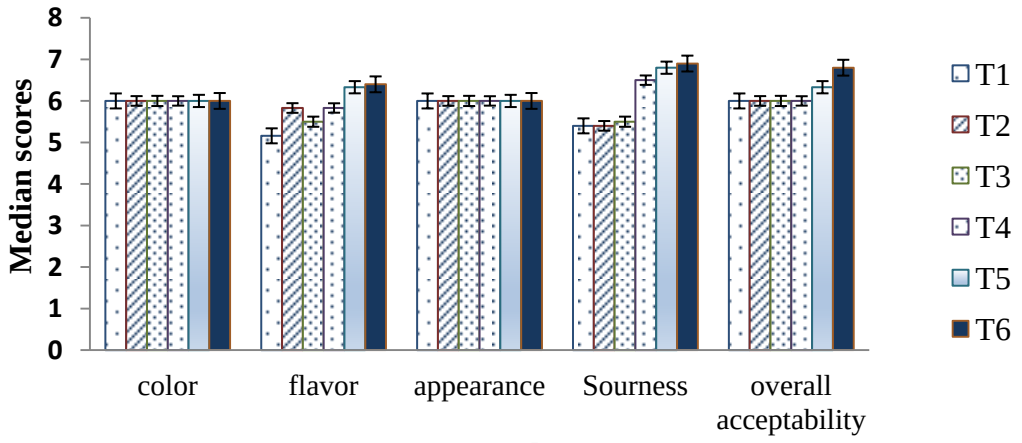

Figure 3.2:Median scores for the sensory attributes of T1 – T6 formulations

Table 3.4: Probability values and rank sums obtained by T1 – T6 formula for sensory attributes.

		Rank sums					
Attributes	p value	T1	T2	T3	T4	T5	T6
Color	0.153	84.5	83.0	70.5	88.0	92.5	106.5
Flavor	0.022*	63.5	86.5	79.0	90.0	102.5	103.5
Appearance	0.023*	64.5	86.5	93.5	98.0	103.5	102.0
Sourness	0.003*	80.5	83.0	90.5	93.0	99.5	103.5
Overall acceptability	0.012*	75.5	68.5	79.5	89.0	102.5	106.5

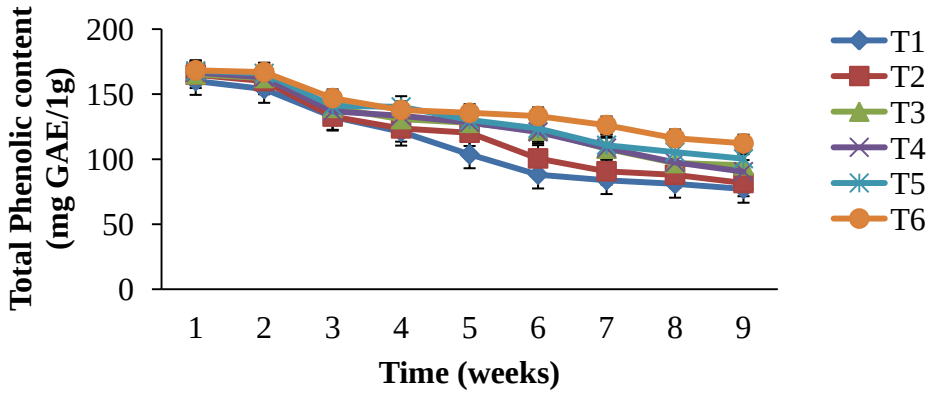

Figure 3.3: Changes in Total phenolic content of T1-T6 formulations during storage.

When considering probability values obtained by the T1-T6 formulations, there can be seen a significant difference among treatments for the attributes offlavour, appearance, sourness and overallacceptability.T6 formulation has obtained highest rank sums for colour, flavour, sourness and overall acceptability.

3.5 Microbial analysis

Throughout the storage study total plate count and yeast and mold count were remained <10 cfudue to weakening of bacterial growth by lower pH due to pH of the beverage andthermal death during processing. Carter et al,(2004) have reported that many products that could safely be maintained sterile by a pasteurization process alone could be particularly preserved by the addition of sodium metabisulphite. The sulphites inhibit yeasts, moulds and bacteria (Doughari and Elmahmood, 2007). Therefore all beverage formulations (T1-T6) are safe for consumption within 8 weeks period in ambient storage conditions.

3.6 Shelf life study

Titratable Acidity and pH of T1-T6 formulations were within the level of 0.6-0.8 acidity g/100 mL (citric acid equivalent) and 3.3-3.7 respectively during thestorage time. TSS content of beverage samples found to be non-significant among treatments throughout the study and was within the range of 11.3°-10.8°. Figure 3.3 has shown the Changes in Total phenolic content during storage.Total phenolic content significantly ($p<0.05$) differed between the treatments and reduced the storage (Figure 3.3). The highest mean value of 168.33 mg GAE/1g was obtained in the RTS beverage with 10% of ginger extract. The total phenolic content of all treatments have reduced gradually with the time may be due to autooxidation during ambient storage conditions.

4. CONCLUSION

This study concludes that 10% ginger extract can be used to formulate an antioxidant rich soursop ginger blended beverage.All tested parameters for the selected RTS product was in accordance with the commercial recommendation for the RTS beverages by the Sri Lanka Standard Institute. RTS could be stored at ambient temperature for a minimum period of 8 weeks.

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