



DEVELOPMENT AND NUTRITIONAL ANALYSIS OF SELECTED RTS BEVERAGE DEVELOPED FROM FERMENTED CARROT AND SOUR ORANGE BLEND DURING STORAGE

Afreen .S.M.M.S¹

Department of Agricultural Chemistry,
Eastern University,
Sri Lanka.

Premakumar.K²

Department of Agricultural Chemistry,
Eastern University,
Sri Lanka.

Himaya .S.M.M.S³

Department of Agricultural Chemistry,
Eastern University,
Sri Lanka.

ABSTRACT

Nowadays, the Ready-To-Serve (RTS) beverages are becoming very popular and are in good demand in urban areas. Preparation of RTS with vegetables and fruit juices combination is yet to become popular. Therefore, a study was designed to develop a RTS beverage by blending juices of fermented Carrot with Sour- Orange juices with sugar, citric acid and distilled water, considering the recommendations of Sri Lanka standards for RTS beverages. Carrot was fermented by using culture. Yoghurt preserved the Carrot as it has Lactic acid bacteria. Four formulations of the blends of Fermented Carrot juice with Sour-Orange juices at the ratios of (100:0, 60:40, 50:50 and 40:60) were prepared. Preparation of RTS using fermentation is a method of Preservation without the addition of chemical preservatives. Best combination was selected based on the sensory evaluation and stored at refrigerated Temperature (5°C). The selected combination was evaluated for its chemical qualities, organoleptic characteristics and microbial safety. The results of the physico- chemical properties showed that ascorbic acid (3.5mg/100ml), pH (3.23), TSS (14.5°Brix) and total sugar (13.5%) were decreased and titrable acidity (0.48%) was increased in the samples during storage. The blend had good sensory attributes without any significant lose in the quality attributes and the findings of microbial studies showed no total plate counts in the formulated beverages at the end of 10 weeks storage at 5°C refrigerated condition. Therefore, it is safe for consumption up to 70 days of storage.

KEY WORDS: Ready-to-serve beverages, fermentation, sensory evaluation, Chemical quality.

INTRODUCTION

This research work was designed to reduce seasonal wastage of the fruit and Vegetables thereby increasing their shelf life through the process of development of RTS beverages. Fruit and Vegetable sector has much potential to contribute to increase the level of national income, export revenue, generate new employment opportunities, increase farm income and enhance the nutrition and health of the people. Beverages are considered to be an excellent medium for the supplementation of nutraceutical components for enrichment. Beverages from fruits and vegetables are important in human diet (Kahn, 1998). Presently ready-to-serve beverages have been increasingly gaining popularity among masses. Fermentation is one of the oldest forms of food preservation technologies in the world. Natural fermentation occurs when microorganisms are allowed to react with susceptible organic substrates. Fermented foods play an important role in providing food security, enhancing livelihoods and improving the nutrition and social well-being of millions of people around the world. Blending of yoghurt with carrot juice would produce a nutritionally rich food (Ikken et al., 1998 and Raum, 2003). Carrot (*Daucus carota*) is highly nutritional, and an important source of β -carotene besides its appreciable amount of vitamins and minerals often used for juice production (Demir et al., 2004). Sour-Oranges (*Citrus aurantium*) are excellent sources of free citric acid, natural sugar, calcium and phosphorus. Juice blending is one of the best methods to improve the nutritional quality of the juice. Blending could lead to the production of delightful and delicious beverages with improved organoleptic quality and high nutritive value. Therefore, the study was conducted to find out the most suitable combination of RTS beverages with fermented Carrot juices and Sour-orange juices and to assess the physico-chemical, sensory properties of RTS beverage during storage.

MATERIALS AND METHODS

Formulation of RTS Beverage

Carrots were washed and cleaned and immersed in warm sterilized water for 15 minutes to kill the soil bacteria if any. They were mashed after peeling the skin to speed up the fermentation. Then one teaspoon yoghurt was applied to the mashed carrots. The fermentation was carried out in aerobic condition by covering the vessel using muslin cloth and kept at room temperature for 24-48 hours. The fermented juice was filtered through muslin cloth. Fresh and matured sour oranges were selected, washed, peeled off, cut into two halves, juice was extracted manually and extracted juice was filtered through muslin cloth to get clear juice. According to the recommendations of Sri Lanka standards, required amount of sugar and citric acid were dissolved in water to prepare sugar syrup up to boiling stage, cooled to TSS of 14° - 20°Brix and

mixed with fermented carrot and Sour-Orange juices at correct quantity to get the RTS beverage. Then homogenized, heated at 85°C for 20 minutes for pasteurization and cooled for 10 minutes. 0.07g Sodium metabisulphite (SMS) was added, filled in pre-sterilized bottles and sealed. The bottles were sterilized in hot water bath at 80°C for 30 minutes, cooled. The best combination of RTS was selected on the sensory evaluation and the selected RTS was stored at refrigerator at 5°C for 10 weeks.

The RTS beverages combinations were

T1 - 100:0 fermented carrot juice and sour orange juice (control)

T2 - 60:40 fermented carrot juice and sour orange juice

T3 - 50:50 fermented carrot juice and sour orange juice

T4 - 40:60 fermented carrot juice and sour orange juice

Sensory analysis

In sensory evaluation, the samples were subjected to seven-point hedonic scale test where score 1 is for “dislike very much” and 7 for “like very much” and the acceptability of samples was judged by 30 semi trained panelists to determine sensory preference. The sensory characteristics such as colour, taste, aroma, consistency and overall acceptability attributes of the RTS beverages were judged by the panelists.

Chemical Analysis

Titration acidity, ascorbic acid, pH and total sugar were determined for the consumer accepted RTS beverage of fermented Carrot juice with Sour-orange juice using AOAC methods for 10 weeks at 2 weeks interval. The TSS of each sample was analyzed using hand held refractometer (Model ATAGO-S-28E) and the values were expressed as °Brix. The acidity in each sample was determined according to standard procedure given in AOAC (2006). The pH of each sample was recorded with the help of digital pH meter (Model HANNA HI 98130). The ascorbic acid content was estimated by using detective dye, DCPIP (2, 6-dichlorophenol indophenol) according to the standard method of AOAC (2006).

Microbial Analysis

Microbial Analysis was carried out by total plate count method using nutrient agar. Each RTS beverage formulations were used to prepare a series of dilutions (10° to 10-3) and microbial counts were taken in three replicates.

Statistical Analysis

Results of the sensory evaluation and chemical analysis were analyzed statistically by ANOVA using SAS statistical analysis package and mean separation for chemical test was done by Duncan's Multiple range Test (DMRT). Standard errors were calculated using MINITAB 14 statistical package. Data of sensory evaluation were analyzed using the Tukey's test.

RESULTS AND DISCUSSION
Sensory qualities of Freshly Made RTS Beverages

The statistical data regarding sensory evaluation of freshly made RTS beverage from Fermented Carrot juice blend Sour Orange juice formulations are given in the Table1. It showed that there were significant differences among treatments.

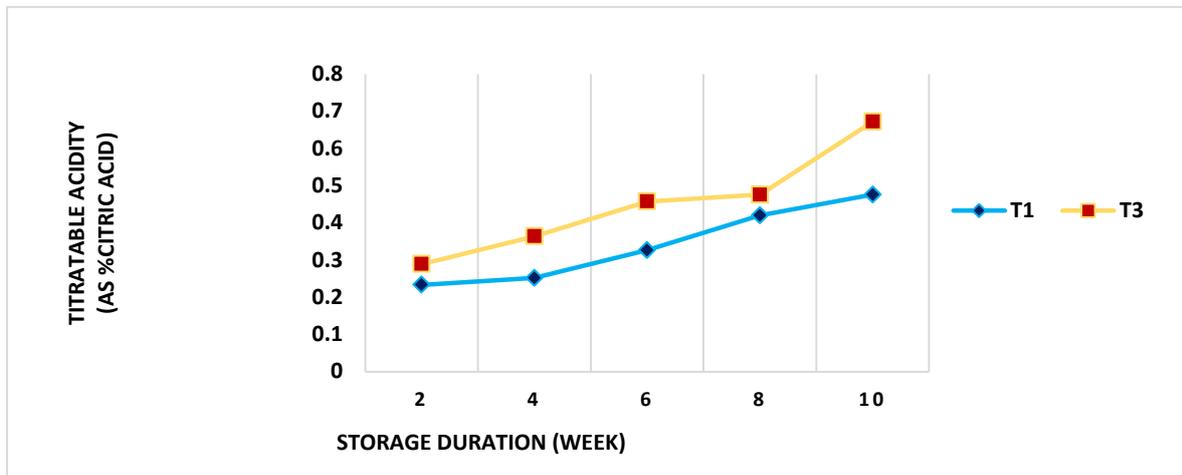


Table 1. Organoleptic Scores of fresh RTS beverage formulations

Beverage Formulations (Treatments)	Quality attributes				
	Color	Taste	Aroma	Consistency	Overall acceptability
Fermented Carrot juice blend Sour Orange juice RTS beverage					
T ₁	6.40±0.123 ^a	3.26±0.135 ^d	2.96±0.148 ^d	6.53±0.092 ^d	5.76±0.133 ^b
T ₂	5.83±0.136 ^b	5.60±0.149 ^b	4.46±0.124 ^c	5.73±0.126 ^c	4.10±0.182 ^c
T ₃	4.60±0.189 ^c	6.60±0.091 ^a	5.76±0.114 ^b	4.60±0.103 ^b	6.50±0.115 ^d
T ₄	3.63±0.176 ^d	4.36±0.169 ^c	6.53±0.092 ^a	2.96±0.140 ^a	3.20±0.139 ^a

Values are means of 30 replicates ± standard error
 Values with different letters are significantly different at p<0.05

Sensory parameters were measured using seven point hedonic scales

The results of organoleptic evaluation of the formulated RTS beverages showed that the combinations of Fermented carrot juice blend Sour-Orange juice (50:50) were found to be highly acceptable when compared with the other formulations in terms of sensory attributes like taste, and overall acceptability. The blends T3 along with their control were used for storage study.

Chemical Qualities of Selected RTS Beverages during Storage
Titrable Acidity

According to the figure1 gradual increase in Titrable acidity of the Fermented Carrot juice with Sour Orange juice blend RTS beverage was observed during storage which reaches the maximum value of 0.68%. In the same way Safdar et al. (1999) observed gradual increase in acidity during storage of tomato concentrate at three different temperatures.

The rise in acidity may be explained by the fact that the concentration of weakly ionized acid and their salts increased during storage. Similarly, Majumdar et al. (2010) reported that acidity increased from 0.25-0.36g/100ml during storage of cucumber-basil juice.

Figure.1. Changes in Titrable acidity of RTS beverage blend of Fermented Carrot juice with Sour- Orange juice during storage

Total sugar

Results pertaining to the total sugar of fermented Carrot juice with Sour Orange juice blend of RTS beverages during storage are shown in Figure 2. According to DMRT, total sugar decreased significantly (p<0.05) throughout the storage period. Fennema (1996) discussed that high polymerization of sugars at high temperature is the reason for the decrease in Total Sugar. Majumdar et al. (2010) also reported decreasing trend in non-reducing sugars from 8.27-7.59 percent in bottle gourd-basil juice during storage of six months.

Figure.2. Changes in Total Sugar of RTS beverage blend of Fermented Carrot juice with Sour- Orange juice during storage

Total sugar in all treatments had the decreasing trend with the storage period because hydrolysis of polysaccharides and oxidation of sugars. Nilugin and Mahendran, (2010) were also found that the significant reduction in total sugar throughout the storage period at ambient temperature in the RTS beverages.

Ascorbic acid

The ascorbic acid content of the RTS beverages showed a decrease in figure 3 with the advancement of storage period of 2nd, 4th, 6th and 10th weeks. This may probably due to the fact that ascorbic acid being sensitive to oxygen, light and heat might be easily oxidized in presence of oxygen by both enzymatic and non-enzymatic catalyst. These findings are in conformity with the studies of Jain et al, (2005) in Indian gooseberry juice blends, and Mapson, (1970). Barwal et al., (2005) reported that the ascorbic acid is decreased by pasteurization. Jawaheer et al (2003) also reported that another principal cause of ascorbic acid decrease might be

residual oxygen present in the head space of the container (assuming glass ware was impervious to oxygen).

Figure.3. Changes in Ascorbic acid of RTS beverage blend of Fermented Carrot juice with Sour- Orange juice during storage

TSS

The changes in TSS of fermented Carrot Juice and Sour Orange Juice Blend RTS beverages during storage was shown in Table 2. The decrease in TSS may be due to comparatively high pH which was conducive for the growth of microorganism and consumption of sugar by microorganisms to carry out fermentation. Similarly, Sahu et al.(2005) reported that decrease in TSS of whey based mango-herbal beverage during the storage of two months whereas Dhaliwal and Hira (2001) observed no significant changes in TSS of four different combinations of carrot juice with two levels each of beet root (5 and 10%) and black carrot (10 and 20%). The recommended TSS for commercial RTS production is 15°Brix (SLS 729:1985).

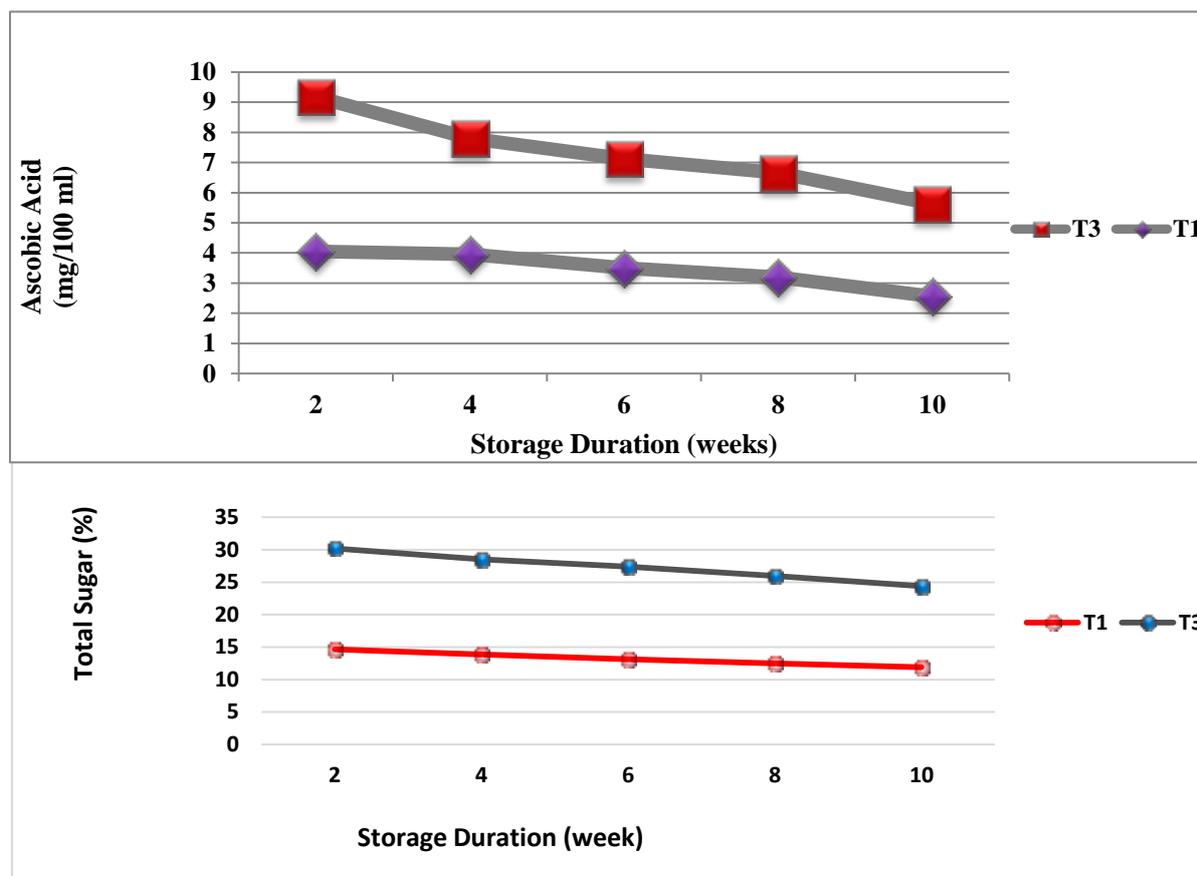


Table 2. Changes in Total Soluble Solids of RTS Beverage

Storage Duration (Weeks)	Blend of Fermented Carrot juice with Sour Orange juice RTS formulation	
	T1	T3
2	15.00 ± 0.577 ^a	16.00 ± 0.000 ^a
4	14.80 ± 0.115 ^b	15.80 ± 0.115 ^a
6	14.66 ± 0.067 ^b	15.50 ± 0.057 ^a
8	14.40 ± 0.115 ^b	15.00 ± 0.115 ^a
10	14.00 ± 0.000 ^a	14.50 ± 0.289 ^a

Values are means of 3 replicates ± standard error
The mean values with the same letters are not significantly different from each other at 5% level based on DMRT

pH

Statistical data pertinent to the pH of RTS beverage formulations during storage is indicated in the Table 3. There was a negligible change in pH of Fermented Carrot juice blend Sour Orange juice

RTS formulation with the increase of storage period. A similar study that described the pH of thermally processed Valencia and Navel orange juice found no significant modifications during storage at 4 and 10 0C (Bull et al., 2004). Rivas et al. (2006) reported no pH variations, in thermally treated juice (blended orange and carrot juice) during refrigerated storage at 2 and 12 0C.

Table.3. Changes in pH of RTS beverage during storage

Storage Duration (Weeks)	Blend of Fermented Carrot juice with Sour Orange juice RTS formulation	
	T1	T3
2	3.33 ± 0.033 ^a	3.25 ± 0.000 ^a
4	3.30 ± 0.006 ^a	3.24 ± 0.003 ^b
6	3.20 ± 0.057 ^a	3.24 ± 0.000 ^a
8	3.20 ± 0.000 ^b	3.23 ± 0.003 ^a
10	3.20 ± 0.000 ^b	3.23 ± 0.000 ^a

Values are means of 3 replicates ± standard error
The mean values with the same letters are not significantly different from each other at 5% level based on DMRT

Sensory Evaluation of RTS Beverages during Storage

The changes in sensory attributes are shown in Table 4. The significant changes were noticed among the control and treatment in both formulations. The results indicated that during storage there was a slight decrease in colour of RTS formulations. The change in colour parameter may be due to maillard reactions between sugars and amino acids (Gonzalez and Leeson, 2000). The blends of Fermented carrot juice with Sour-Orange juice made with the ratio of 50:50 were rated as higher than the Control (100:0) for taste. The results showed a decrease in taste which may be due to the degradation of ascorbic acid and furfural production. Baljeet et al. (2013) reported that mean value of 7.5

for taste in the freshly prepared whey based pine apple and bottle gourd mixed herbal beverage decreased to 4.9 after 20 days of storage.

RTS with 50:50 fermented carrot juice and sour orange juice (T3) got higher mean value of 5.5±0.16a while RTS with 100:0 fermented carrot juice and sour orange juice got the mean value of 5.1±0.14a for overall acceptability. There was significant reduction in overall acceptability of all RTS beverage formulations at 10 weeks of storage. Din et al (2011) also reported a decrease in overall acceptability of beverages prepared from different ratios of bitter gourd during storage. According to the results of sensory evaluation 50:50 fermented carrot juice and sour orange juice (T3) had good sensory qualities after 10 weeks storage.

Table 4. Organoleptic Scores of RTS beverage formulations at 10 weeks.

Treatment	Color	Taste	Aroma	Consistency	Overall Acceptability
T1	5.3±0.18 ^a	2.5±0.16 ^b	2.1±0.18 ^b	5.4±0.12 ^a	5.1±0.14 ^a
T3	3.2±0.17 ^b	5.6±0.14 ^a	5.2±0.16 ^a	3.1±0.18 ^b	5.5±0.16 ^a

The values are means of 30 replicates ± standard error

The means with the same letters are not significantly different from each other at 5% level based on Tukey's Test

Microbial analysis of RTS beverage during storage

It is clear that there were no microbial growth in the RTS beverages. The raise in microbial load on 70th days of storage was negligible and safe for consumption, and also echoed by Nagpal and Rajyalakshmi (2009), in their study on RTS beverages from bael and citrus fruit blends. The acid environment was maintained as a result of fermentation and it has prevented microbial growth in Fermented Carrot. But at 70th days of storage very mild growth of microorganism (Bacteria) was observed in control (T1) due to their higher pH and no microbial growth was observed in T3. As the pH increased throughout the storage period considerable number of CFU could be observed after 2 months of storage. This result is supported by Barwal et al. (2005a) on processing and development of ready to serve drink from bitter gourd fruit.

Since in the present study all these safety precautions were taken care of, there were no microbial growth in the RTS beverages and those products were safe for consumption up to 10 weeks of storage in terms of microbial quality.

CONCLUSION

The RTS beverages prepared by using Sour-Orange juice with Fermented carrot juices are nutritious and also safe for consumption since there is no addition of artificial colour, flavour, and artificial preservative. Consumers expect the food products to be healthy, tasty and functional. It showed that the blending of vegetable and fruits juice was highly acceptable and the quality was also found to be good.

On the basis of the results of this study it was concluded that formulation of mixed (blend) juice from Sour-orange and fermented carrot is possible to satisfy consumer taste and preferences. It was accomplished that the blend of 50 % fermented carrot juice with 50 % Sour-orange juice (T3) were most effective blended RTS Beverages with minimum change in TSS, pH, Titrable Acidity, Total Sugar and vitamin C. Sensory scores were also higher than other formulation (control) and RTS beverage of 50 % fermented carrot juice with 50 % Sour- orange juice (T3) received highest score for overall acceptability. Therefore, RTS beverage with 50:50 fermented carrot juice and

sour orange juice (T3) can be stored at refrigerator for 10 weeks without any significant loss in the quality characteristics.

REFERENCES

- Baljeet, S. Y., Ritika, B.Y. and Sarita, R. (2013). Studies on development and storage of whey-based pineapple (*Ananas comosus*) and bottle gourd (*Lagenaria siceraria*) mixed herbal beverage. *International Food Research Journal*, 20(2): 607-612.
- Barwal, V.S., Singh, T.K. and Alkesh.(2005a). Studies on processing and development of ready to serve drink from bitter gourd fruit. *Journal of Food Science and Technology*, 42(3): 217-220.
- Bull, M.K., Zerdin, K., Howe, E., Goicoechea, D., Paramanandhan, P., Stockman, R., Sellahewa, J., Szabo, E.A., Johnson, R.L. and Stewart, C.M. (2004). The effect of high pressure processing on the microbial, physical and chemical properties of Valencia and Navel orange juice. *Innovative Food Science and Emerging Technologies* 5: 135-149.
- Demir, N., J. Acar and K.S. Baheci (2004). The use of commercial pectinase in fruit juice industry. Part 3: Immobilized pectinase for mash treatment, 47: *Journal of Food Engineering* 275-280.
- Dhaliwal, M. and K. C. Hira, (2001) "Effect of storage in physico-chemical and nutritional characteristics of carrot, beetroot and black carrot juice," *Journal of Food Science and Technology*, 38(3): 343-347.
- Din A, Bukhari S. A. H., Salam A., and Ishfaq. B. (2011). Development of functional and dietetic beverage from bitter gourd. *International Journal of Food Safety*. 13: 355-360.
- Fennema, O. R. (1996). *Food chemistry*, (3rd Ed). CRC Press, United States of America: 157-412.
- Ikken, Y., I. Cambero. M. Marin., A. Martner., I. Hars. And P. Morales. (1998). Ant mutagenic effect of fruit and vegetable aqueous extracts against N-nitrosamine evaluated by the Ames test. *Journal of Agricultural and Food Chemistry*, 46:5194-5200.
- Jain, S. K. and Khurdiya, D.S (2005). Vitamin C enrichment of fruit juice based ready-to-serve beverages through blending of Indian gooseberry (*Emblica officinalis* Gaertn) juice. *Plant Foods for Human Nutrition*, 59(2), 63-66.
- JAWAHEER B, GOBURDHUN D & RUGGOO A. Effect of processing and storage of guava into jam and juice on the ascorbic acid content. *Plant Food for Human Nutrition*. 2003; 58: 1-12.
- Khan, A and R, Anderson. (2003). Insulin potentiating factor (IPF) present in foods, spices, and

- natural products. *Pakistan Journal of Nutrition*. 2(4):254-257.
12. Majumdar, T.K., C.R. Vasudish, K.S. Premavalli and A.S. Bawa. (2008). Studies on processing and storage stability of ash gourd-mint leave juice. *Journal of Food Processing and Preservation*, 34:549-556.
 13. Majumdar, T.K., D.D. Wadikar and A.S. Bawa. (2010). Development, stability and sensory acceptability of cucumber-basil juice blend. *African Journal of Food, Agriculture, Nutrition and Development* .10:4093-4104.
 14. Mapson, L.W (1970). Vitamins in fruits, In: Hulme, A.C (Edn.) *The Biochemistry of Fruits and their Products* Academic Press, London, 1: 369-384.
 15. Nagpal, S. Rajyalakshmi, P. (2009). Quality and Storage of RTS Beverage from Bael and Citrus Fruit Blends. *Beverage and Food World*, April: 24-26.
 16. NILUGIN SE & MAHENDRAN T. Preparation of Ready-To-Serve (RTS) beverage from Palmyrah fruit pulp. *Journal of Agricultural Science, Eastern University, Sri Lanka*. 2010; 5: 80 – 88.
 17. Raum, R. (2003) *Microbiological quality of health foods and organic foods*. *Netherlands Milk and Dairy Journal*, 14:130–134.
 18. Rivas, A., Rodrigo, D., Martinez, A., Barbosa-Canovas, G.V. and Rodrigo, M. (2006). Effect of PEF and heat pasteurization on the physical-chemical characteristics of blended orange and carrot juice. *Food Science and Technology* 39: 1163-1170.
 19. Safdar, N., Asrar, M., Abdullah, N., Ziaur, R. and Shah, W. (1999). Studies on the effect of storage condition on ascorbic acid acidity and pH of tomato concentrate. *Pakistan Journal of Food Sciences*
 20. Sahu, C., S. Patel, and P.L. Chowdhry. (2005) "Technology for manufacture of whey based mango herbal beverage," *International Journal of Food Science and Technology*. 42(5), 421-424.