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Quality evaluation of artificially sweetened millet flour incorporated pomegranate RTS beverage

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Abstract

The study aimed to develop a pomegranate beverage by substituting sucrose with an artificial sweetener sucralose and incorporating germinated flour from foxtail and little millet to enhance nutritional quality. The developed beverage was analyzed for physicochemical, bioactive, and sensory parameters. The result showed that substituting 100 % sucrose with sucralose had less total soluble solid content. Total and reducing sugar content was significantly high in control samples compared to sucralose-added treatments. However, total polyphenols and antioxidant activity were 20 and 18.50 % less in sucralose treatments, respectively, compared to the control. Incorporating germinated millet flour did not significantly increase the bioactive compounds of the pomegranate beverage. Further, substituting sucrose with sucralose was not accepted organoleptically, however, there was no change in the taste due to the incorporation of germinated millet flours. Hence, the substitution of sucrose with sucralose depends on the fruit type in the preparation of RTS (Ready-to-serve) beverage and the incorporation of germinated millet flours depends on the concentration to enhance the bioactive compounds of the beverage.

Key words: Pomegranate RTS beverage, sucralose, bioactive compounds, germinated millet flour

Introduction

Fruits are known for their ability to reduce the risk of the development of various diseases due to the bioactive compounds present in them. Bioactive compounds such as phenolic acids, flavonoids, oligosaccharides, dietary fiber, vitamins and minerals are present in high amount in fruits. Pomegranate (*Punica granatum* Linn.) is grown widely in tropical and subtropical regions in India. It is also suitable to grow under arid and semi-arid climatic conditions (Kulkarni *et al.,* 2005). About 3.18 MMT of pomegranate was produced in India during 2019-20 (static.pib.gov.in). Pomegranate is known for its health benefits as it is a rich source of polyphenols having very high antioxidant activity. A fresh pomegranate juice showed a total polyphenols content of 583.72 mg Gallic Acid Equivalent/100mL with antioxidant activity of 0.39 mM Ascorbic Acid Equivalent/mL (Fawole and Opera, 2013). Therefore, consuming its juice provides enough antioxidants required for a healthy lifestyle. At the same time, the high intake of cane sugar/sucrose is also rising among the population, which is again a concern due its detrimental effect on human health. There is a positive relationship between obesity and sucrose consumption. High sucrose intake is one factor responsible for obesity (Gulati and Mishra, 2014). Therefore, reducing sucrose intake is essential to reduce the calorie intake without compromising the intake of fruit beverages.

On the other hand, consumers always look for new flavours or ingredients in fruit beverages. It should be nutritious as well as acceptable organoleptically. So, the development of any fruit juice with new ingredients without compromising taste or nutrition is a challenge. In addition, millets are known for their health benefits for ages. In India, sorghum, pearl millet, finger millet, foxtail millet, kodo millet, little millet, proso millet and barnyard millet are grown and consumed in different regions. However, in the past 3-4 decades the consumption of millets has declined to <15% compared to the consumption before 40 years, which may be due to shift in the dietary habits or the availability of many processed foods (Kane-Potaka *et al.,* 2021). Therefore, Indian government has emphasized increased cultivation of millets as well as consumption owing to its nutritional quality and high water use efficiency. The year 2023 is declared as an International Year of Millets by Food and Agriculture Organization. Hence, an attempt was made to develop a pomegranate RTS beverage by incorporating millet flour and by replacing the sucrose with artificial sweetener sucralose. The developed beverage was evaluated for physicochemical parameters and nutritional quality during storage. The samples were analyzed for physicochemical, nutritional and sensory parameters.

Materials and methods

Whole foxtail and little millet were procured from local market. Refined sugar was procured from the supermarket. Sucralose from ProFoods^{TM} was procured online through Amazon India. Pomegranate fruits were procured from the local market. The chemicals were procured from Himedia Laboratories Pvt. Ltd (Bengaluru, India), Sisco Research Laboratories Pvt. Ltd (Mumbai, India) and Sigma Aldrich-Merck (St. Louis, Missouri, USA).

Preparation of beverage: First, the millets were subjected to germination by soaking the seeds in water overnight. The soaked seeds were spread in a thin layer on a wet blotting paper on a perforated stainless-steel tray covered by another similar tray. Subsequently, trays were kept at a temperature of 28±1 °C and 90±1 % relative humidity for 48 h for germination. Germinated millets were dried in hot air oven at 50±1 °C for 4-5 h and ground into fine flour using a blender (Usha Int Ltd, New Delhi, India). Pomegranate fruits free from infestation were selected. The arils were separated from the fruit and juice was extracted by subjecting to grinding using a laboratory scale mixer grinder (Amar Deep, 1.5 H.P, 2800 rpm, 230v). The juice was filtered using cheesecloth to remove seeds. The juice concentration, sucralose and millet flour concentration was standardized based on the sensory score obtained in the preliminary evaluation. Juice percentage was standardized as 30 %.

The concentration of millet flour and sucralose was standardized for 1 % and 17 ppm, respectively. The selected treatments, as well as the control, were prepared according to the procedure shown in Fig. 1. To avoid oxidation of bioactive compounds during storage, ascorbic acid was added at a concentration of 0.05% as an antioxidant, and sodium benzoate preservative was added at a concentration of 120 ppm as per the Food Safety and Standard Authority of India (FSSAI) safety limit for fruit beverages. The prepared beverages were stored under cool and dry conditions. Analysis was carried out for a period of 90 days at an interval of 15 days.

Physicochemical Analysis: Total Soluble Solids (TSS) of RTS was recorded by using digital refractometer (ATAGO Pocket refractometer PAL-3, Japan). The acidity of fruit pulp and beverage was estimated as per the method described by Ranganna

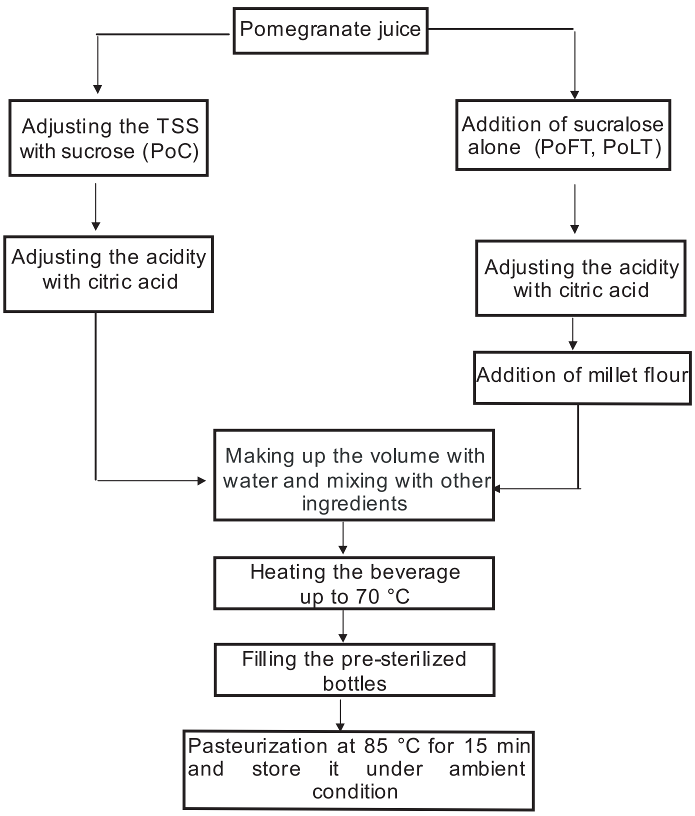

Fig 1. Flowchart for the preparation of pomegranate RTS beverage with the incorporation of millet flours. PoC= pomegranate juice + sucrose; PoFT= pomegranate juice + germinated foxtail millet flour + sucralose; PoLT= pomegranate juice + germinated little millet flour + sucralose.

(1986). Total and reducing sugars in RTS beverages was analyzed as per the method described by Lane and Eynon (1923).

Ascorbic acid: Ascorbic acid was estimated by visual titrimetric method using 2,6- dichlorophenol indophenol dye as described by Ranganna (1986).

Total polyphenols: Total polyphenols content in the beverage was determined as per the method described by Panwar *et al.* (2016). Briefly, 2 g of sample was extracted with 80 % methanol in distilled water (DW). Folin-Ciocalteu reagent (0.2 mL) was added to 0.5 mL of the extract taken in a test tube to which immediately 3.3 mL of DW and 1 mL of saturated sodium carbonate were added before storing the sample for 30 min under dark. The absorbance of the sample was read at 700 nm (T70 Dual Beam UV-VIS Spectrometer, PG Instruments Ltd, UK) and calculated using a standard calibration curve (R^2-0.999) made using gallic acid (0–0.1 mg/mL). The results were expressed as milligram gallic acid equivalent (GAE) per 100g.

Free radical scavenging activity by DPPH method: Free radical scavenging activity was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as per the method described by Kang and Saltveit (2002). One millilitre of methanol (80 %) extracted sample was taken in a test tube. Acetate buffer (10 mM) followed by 0.2 mM DPPH solution were added in the test tube. Absorbance of the solution was read at 517 nm (T70 Dual Beam UV-VIS Spectrometer, PG Instruments Ltd, UK) after 30 min of incubation under dark. The results were expressed as milligram ascorbic acid equivalent antioxidant capacity per gram (AEAC).

Reducing power assay by FRAP method: The reducing power assay was assessed as per the method described by Benzie and Strain (1996). Briefly, 2 g of the sample was extracted with 80% methanol. To 0.4 mL of aliquot, 3.6 mL of FRAP reagent was added and the absorbance was read at 593 nm after incubation at room temperature for 30 min. The results were expressed as milligram ascorbic acid equivalent antioxidant capacity (AEAC) per gram.

Total anthocyanins: Total anthocyanins content in pomegranate beverage was estimated as per the method illustrated by Ranganna (1986). About 0.5 g of juice sample was taken in a centrifuge tube, to which about 8 mL of extraction mixture containing 95 % ethanol and 1.5 N HCl (85:15 v/v) was added. The sample was covered and kept overnight at refrigerated condition. Then, the extract was filtered using filter paper; the filtrate was made up to volume with extraction mixture and read at 535 nm.

Sensory evaluation: Sensory evaluation was performed by research scholars of the laboratory. The samples were given with the codes and were evaluated by the participants after 2-3 h of having breakfast. Participants were asked to assess the beverages based on colour, flavour, mouthfeel, taste and overall acceptability using a 9-point hedonic scale consisting of dislike extremely = 1, dislike very much = 2, dislike moderately= 3, dislike slightly = 4, neither like nor dislike = 5, like slightly = 6, like moderate = 7, like very much = 8and like extremely = 9 (Larmond, 1977).

Statistical analysis: All the analysis was conducted in triplicates. Statistical analysis was carried out by the one-way analysis of variance (ANOVA) and Fisher's LSD values were calculated at P 0.05. Statistical analysis was performed using SPSS IBM 22.0 statistical software.

Results and discussion

Effect of millet flour and sucralose incorporation on physicochemical parameters in pomegranate RTS beverage: The TSS of any fruit beverage is an important quality parameter. Even though maintaining a stable TSS throughout the storage period is difficult but with minimal changes will result in an organoleptically well-accepted product. The effect of the addition of sucralose and millet flour on TSS and titratable acidity are presented in Figs. 2 and 3. The TSS in control sample (PoC) was significantly high due to the obvious reason that sucrose was used for adjusting the TSS of the beverage whereas the replacement of sucrose with artificial sweetener sucralose had very less TSS of 6.4 and 6.2 °Brix in PoFT and PoLT, respectively. However, towards the end of storage period, TSS reduced to 14.7, 4.8 and 4.4 °Brix in PoC, PoFT and PoLT, respectively. Decrease in TSS content during storage may be attributed to hydrolytic changes in carbohydrates in the beverage (Kishore *et al.,* 2011). When sucrose was replaced with artificial sweeteners (aspartame and acesulfame potassium) in cornelian cherry juice, a reduction from 15-25 to 10 °Brix was observed (Moldovan and David, 2020). Acidity contributes significantly for the sensory acceptance of any fruit beverage. Initially, the titratable acidity was found to be 0.34 % in PoFT and 0.32 % in PoC and PoLT treatments as the acidity was adjusted with citric acid during preparation. Towards the end of storage period, a stable acidity was observed in all the treatments with a slight increase in PoC (0.38 %). Alighourchi and Barzegar (2009) made a similar observation in a reconstituted pomegranate juice. Titratable acidity increased to 0.29 from 0.28 % in squeezed pomegranate juice stored for 72 h (Alkuraieef and Al-Juhani, 2022).

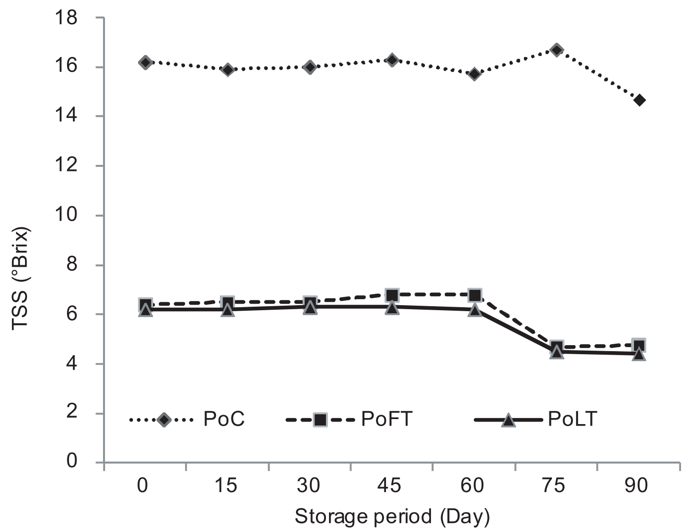

Fig 2: Changes in TSS of PoC, PoFT and PoLT treatments of pomegranate RTS beverage during storage. Values are expressed as mean and error bars represent standard deviation (n=3)

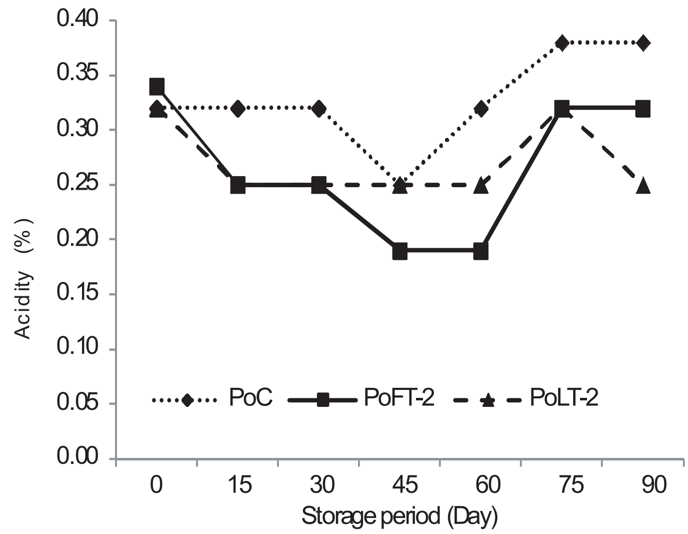

Fig 3: Changes in acidity of PoC, PoFT and PoLT treatments of pomegranate RTS beverage during storage. Values are expressed as mean

Changes in total sugars, reducing and non-reducing sugars during the storage period are presented in Table 1. Significantly high total and reducing sugar content was observed in control samples. It is almost the double the values compared to PoFT and PoLT initially. High total and reducing sugar content in PoC is due to addition of only sugar whereas sucralose alone was used in PoFT and PoLT. Increased total sugar content was observed in all the treatments after 15 days of storage, which almost remained the same up to 60 days. Eventually, a decreasing trend was observed up to 90 days of storage period. However, at the end of the storage period, increased total sugars and reduced sugar content were observed in all the treatments and decreased non-reducing sugars. Hydrolysis of polysaccharides such as starch, cellulose, pectin, etc. and their conversion into monosachharides might have contributed to the increased sugar content in the beverage.

Effect of millet flour and sucralose incorporation on bioactive compounds in pomegranate RTS beverage: Pomegranate fruit contains a very good amount of bioactive compounds such as phenols, flavonoids, anthocyanins, ascorbic acid and other compounds in its juice, peel and kernel (Montefusco *et al.*, 2021). Further, pomegranate is known to have high antioxidant activity, which benefits human health. The bioactive compounds of different treatments during storage are presented in Table 2 and 3. In pomegranate RTS beverage, initial ascorbic acid content was found to be 68.00, 64.00 and 56.00 mg/100g in PoC, PoFT and PoLT, respectively. Towards the end of storage period, highest reduction of about 65.30 % was observed in PoC whereas in PoFT and PoLT, 52.50 and 47.14 % reductions were observed, respectively. Montefusco *et al.* (2021) found ascorbic acid content of 42-95 mg/100 g of pomegranate fruit juice obtained from four different cultivars. The reduction of ascorbic acid content in pomegranate beverages may be attributed to its oxidation process during storage.

Anthocyanins are water-soluble pigments that come under flavonoids. Pomegranate fruits are rich source of anthocyanins. PoC showed highest anthocyanin content throughout the storage period compared to PoFT and PoLT treatments in this experiment. At the end of the storage period, lowest retention of anthocyanins was found in PoLT (66.57 %), whereas in PoC and PoFT high retention of 82.81 and 84.63 %, respectively, was observed. Montefusco *et al.* (2021) found anthocyanin content ranged from 21 to 61 mg/100g of 100 % fresh pomegranate juice among four different cultivars. About 138 mg/100g of anthocyanin was found after 100 days of fruit set in the pomegranate variety Ganesh by Kulkarni *et al.* (2005). Total anthocyanin content in reconstituted

Table 1. Total, reducing and non-reducing sugar content in PoC, PoFT and PoLT treatments of pomegranate RTS beverage during storage

Storage period (Day)	Total sugars (%)			Reducing sugars (%)			Non-reducing sugars (%)		
	PoC	PoFT	PoLT	PoC	PoFT	PoLT	PoC	PoFT	PoLT
0	8.50a±0.07	5.13b±0.03	4.26c±0.02	7.63a±0.14	2.59b±1.92	2.56b±0.02	0.86±0.11	2.54±1.89	1.69±0.03
15	14.36a±0.0	6.50b±0.01	5.53c±0.01	13.34a±0.36	3.78c±0.03	4.30b±0.05	1.01b±0.36	2.72a±0.04	1.23b±0.04
30	14.60a±0.0	6.63b±0.02	5.61c±0.03	13.40a±0.24	3.80c±0.02	4.31b±0.03	1.20b±0.24	2.82a±0.03	1.30b±0.05
45	15.85a±0.25	5.05c±0.02	5.28b±0.0	16.47a±0.18	4.95b±0.02	5.22b±0.02	0.43a±0.10	0.09b±0.03	0.05b±0.02
60	15.09a±0.08	6.25b±0.01	6.07c±0.05	14.15a±0.14	6.01b±0.02	5.97b±0.05	0.93a±0.19	0.23b±0.01	0.10b±0.05
75	13.96a±0.07	5.92b±0.01	5.76c±0.01	13.41a±0.24	5.77b±0.06	5.65b±0.02	0.55a±0.18	0.15b±0.05	0.12b±0.03
90	14.15a±0.07	5.97b±0.02	5.82c±0.03	13.55a±0.0	5.87b±0.0	5.73c±0.02	0.61a±0.07	0.10b±0.02	0.08b±0.02

Values are mean ± standard deviation (n=3). Values with different superscript letters in a row for different parameters differ significantly.

Table 2. Ascorbic acid and anthocyanin content in PoC, PoFT and PoLT treatments of pomegranate RTS beverage during storage

Storage period (Day)	Ascorbic acid (mg/100g)			Anthocyanins (mg/100g)		
	PoC	PoFT	PoLT	PoC	PoFT	PoLT
0	68.00a±3.46	64.00a±3.46	56.00b±3.46	90.90a±0.15	80.13b±0.26	76.85c±0.15
15	60.00a±0.0	62.00a±3.46	54.00b±0.0	90.50a±0.11	77.77b±0.20	71.94c±0.11
30	54.00b±0.0	60.00a±0.0	50.00c±3.46	81.73a±0.15	74.17b±0.11	69.98c±0.11
45	50.00b±3.46	58.00a±3.46	48.00b±0.0	81.11a±0.17	73.84b±0.20	69.49c±0.11
60	44.00b±3.46	50.00a±3.46	42.00b±0.0	80.55a±0.06	73.75b±0.10	69.03c±0.10
75	36.00b±0.0	42.00a±0.0	40.00a±3.46	80.39a±0.11	72.60b±0.06	67.98c±0.15
90	23.60b±0.0	30.40a±0.0	29.60a±0.0	75.28a±0.23	67.82b±0.11	51.16c±0.10

Values are mean ± standard deviation (n=3). Values with different superscript letters in a row for different parameters differ significantly.

Table 3. Total polyphenols, antioxidant and free radical scavenging activity in PoC, PoFT and PoLT treatments of pomegranate RTS beverage during storage

Storage period (Day)	Total polyphenols (mg GAE/100g)			DPPH (mg AEAC/g)			FRAP (mg AEAC/g)		
	PoC	PoFT	PoLT	PoC	PoFT	PoLT	PoC	PoFT	PoLT
0	251.89a±0.57	206.39b±1.51	198.48c±2.28	1.05a±0.01	1.09a±0.02	0.94b±0.02	1.26b±0.0	1.31a±0.0	1.31a±0.0
15	246.28a±1.71	202.43b±1.14	196.17c±2.49	1.01a±0.01	1.03a±0.01	0.90b±0.02	1.26b±0.0	1.30a±0.0	1.30a±0.0
30	241.67a±0.57	200.79b±1.98	196.83c±1.71	0.99b±0.0	1.04a±0.0	0.94c±0.01	1.25b±0.0	1.30a±0.0	1.29a±0.0
45	239.69a±0.57	199.47b±0.57	195.84c±0.0	0.97b±0.01	1.04a±0.01	0.92c±0.01	1.25c±0.0	1.29a±0.0	1.29b±0.0
60	235.73a±0.57	198.15b±0.57	192.54c±1.14	0.94b±0.01	0.96a±0.01	0.89c±0.01	1.24b±0.0	1.28a±0.0	1.22c±0.0
75	220.90a±1.14	195.84b±0.0	186.28c±0.57	0.90ab±0.01	0.99a±0.09	0.80b±0.01	1.22b±0.0	1.27a±0.0	1.21c±0.0
90	213.97a±0.57	189.91b±0.0	176.06c±0.0	0.85b±0.01	0.91a±0.01	0.77c±0.01	1.20b±0.0	1.22a±0.0	1.19c±0.0

pomegranate juice showed a significant reduction of about 91 % at the end of 210 days of storage from an initial concentration of 190.90 mg/L. Anthocyanin degradation is attributed to many factors, such as temperature, oxygen, enzymes, pH etc. (Alighourchi and Barzegar, 2009). Therefore, a slight decrease in anthocyanin pigment in the beverages may be attributed to oxidation and also due to condensation of pigments with ascorbic acid. Choi *et al.* (2002) observed similar degradation of monomeric anthocyanin pigments in blood orange juice.

Total polyphenol content was found to be significantly high in PoC (251.89 mg GAE/100g) compared to PoFT (206.39 mg GAE/100g) and PoLT-2 (198.48 mg GAE/100g). At the end of the storage period, 92 % of total polyphenol content was retained in PoFT, whereas PoLT and PoC showed retention of 88.70 and 84.94 %. The results are in accordance with Rashid *et al.* (2021) where high retention of phenols was observed in sucralose-added phalsa diet squash compared to control samples. The reduction of phenols in all the samples may be attributed to the oxidation of phenols throughout storage.

The antioxidant activity of fruits and vegetables is mainly due to the radical scavenging activity of phenolic compounds. In this study, the antioxidant activity was significantly high in PoC (1.05 mg AEAC/g) and PoFT (1.09 mg AEAC/g). However, at the end of the storage period, appreciable amount of 81, 83.5 and 82 % retention of antioxidant activity was observed in PoC, PoFT and PoLT, respectively. In case of radical scavenging activity, significantly high activity was resulted in PoFT (1.31 mg AEAC/g) and PoLT (1.31 mg AEAC/g) which, upon increasing the storage period, >90% of radical scavenging activity was

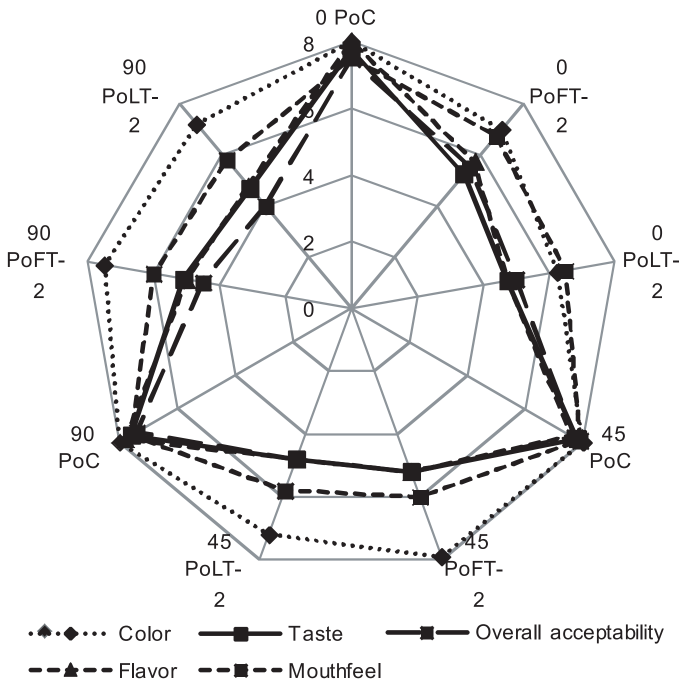

Fig 4. Mean score of germinated foxtail (PoFT) and little millet (PoLT) flour incorporated pomegranate RTS beverage for sensory attributes along with control (PoC) during storage (9 point hedonic scale)

retained in all the treatments. As mentioned above, pomegranate fruit juice is a rich antioxidant source. Therefore, even after 90 days of storage high amount of antioxidant activity was observed.

Evaluation of sensory parameters in pomegranate RTS beverage: Sensory evaluation of pomegranate RTS beverage evaluated at 0, 45 and 90 days interval period is presented in Fig. 4. One of the strategies to reduce the calorie intake without compromising the intake of fruit beverage is development of low-calorie fruit beverages with acceptable sensory parameters. In the pomegranate RTS beverage, sugar was replaced by an artificial sweetener, sucralose. During initial storage period, PoC scored high for all the sensory parameters compared to PoFT and PoLT treatments. Colour was not affected by the addition of sweetener; instead, adding millet flour slightly changed the colour. However, the taste was affected by the replacement of sugar with sucralose. But, since the addition of sucralose was less, it might have resulted in a reduced score for taste. At the end of the storage period, PoFT and PoLT scored 4.5 and 4.0, respectively for overall acceptability.

Evaluation of orange/pomegranate juice containing different sweeteners (no added sugar, sugar, sucralose, stevia and monk fruit extract) by 196 consumers resulted that consumer discriminated the sensory perception for the beverages with sugar substitutes especially stevia which indicated that consumers' perception of health consciousness is related to the sensory parameter of beverage also (Reis *et al.*, 2017). Sensory evaluation of passion fruit juice samples prepared with sucrose, sucralose, aspartame, stevia, cyclamate/saccharin and neotame showed that aspartame and sucralose can become a good substitute for sucrose at a concentration of 0.054 and 0.015 g/100 mL of juice (Rocha and Bolini, 2015). In the current study, addition of sucralose at a concentration of 17 ppm might not have contributed required sweetness by the panelists as they had not developed a taste for it.

The demand for bioactive compound-rich beverages has become high due to increased awareness about health benefit of intake of bioactive compounds. Pomegranate fruits are a rich source of polyphenols and antioxidants. Similarly, developing new flavoured beverages is also a need of the hour as consumers demand. The results of the study showed that germinated foxtail and little millet flour should be incorporated at higher concentrations into pomegranate RTS beverages to improve the bioactive compounds. Although millet flour did not alter the taste of the beverage, the replacement of sucrose with sucralose was not accepted by the panelists. However, the addition of sucralose can reduce the sucrose content in the beverage significantly, which is beneficial from the health point of view. Hence, combination of different alternate sweeteners and replacement of sucrose at lower levels may be tried to obtain a highly acceptable pomegranate RTS beverage.

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