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Original Research Paper

Optimizing the juice formulation for a pomegranate based functional beverage using D-optimal mixture design of response surface methodology

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ABSTRACT

Mixed beverages are prepared by the addition of two or more juices having a pleasant taste and dominant flavor. The optimization of level of pomegranate juice with lime and ginger juice for development of functional beverages was performed using D-optimal mixture design of response surface methodology. The levels of three independent factors were considered as pomegranate juice (70-85%), lime juice (10-20%) and ginger juice (5-10%). The different responses were considered for experiment as physicochemical (color value, total anthocyanin, total phenolics, antioxidant activity, ascorbic acid) and sensory (hedonic score and overall acceptability) properties. The optimization was applied to the selected range of the variables and responses. The selected optimized solution with maximum desirability (0.977) for independent factors were prepared and further evaluated for validating predicted values. Based on the validation experiments, functional beverage containing 85% pomegranate juice, 10% lime juice and 05% ginger juice was found acceptable which provides total phenolics (236.2 mg/L GAE), anthocyanin (4.61 mg/100 mL AAE), ascorbic acid (1.80 mg/100 g); and having antioxidant activity (7.54 mg/100 mL) on wet basis. When the developed RTS compared nutritionally and organoleptically with RTS drink from pomegranate juice (control) showed 40% increase in antioxidant activity, 20% increase in ascorbic acid, 5% decrease in calorific value and higher hedonic score as well as score for overall acceptability. Furthermore, this blended RTS drink could be safe to consume or stored for the period of 90 days at low temperature (5°C).

Keywords: Beverage, mixture design, numerical optimization, pomegranate, sensory analysis

INTRODUCTION

Mixed or blended beverages are the combination of two or more fruit juices resulted to improved aroma, taste, nutritional value and importantly dominant flavor. The most significant affecting factor for mixed or blended beverages is sugar to acid ratio which improves the consumer acceptability of the beverages. The mixing of fruit juices to beverages not only improves the nutrition but also the sensorial characteristics (Bhalerao et al., 2020).

Pomegranate (*Punica granatum*) is store house of different biological active components which provide health benefiting properties so, popularly known as super fruit. The demand for pomegranate fruit and its processed products is increasing now a days because of high phenolic components, anthocyanin content and antioxidant activity (Dadashi et al., 2013). Lime (*Citrus aurantifolia*) is an acidic fruit rich in Vitamin C and also phytochemical substances such as flavonoids, carotenoids, phenolic acid, essential oil etc. Ginger (*Zingiber officinale*) is a popular aromatic perennial plant and known for its medicinal properties like anti-inflammatory, reduction in cholesterol level, blood thinning properties which helps in management of heart diseases etc. The unique taste and aromatic flavor of lime and ginger juice could enhance the sensorial acceptance of fruit beverages (Hariharan & Mahendran, 2016).

The evaluation of sensorial characteristics while developing functional beverages for countering the effect of individual juice in blend is very important which will also give an idea about the overall acceptability and likening of the products (Shrivastava & Chakraborty, 2018). The ratings of the sensory attributes on the predefined scale get the overall impression of the products (Kilcast, 2010). Response surface methodology is one of the statistical techniques which is effectively use for optimizing complex process and successfully applied to determine the optimum formulation. It helps to reduce number of

experiments and gives effective and selective experimental design.

Therefore, the present investigation aimed to develop functional beverage using pomegranate, lime and ginger juice and optimize the level of these juices in beverage using D-optimal mixture design of RSM in order to improve its sensory as well as nutritional properties.

MATERIALS AND METHODS

Preparation of fruit juices

Fully mature pomegranate fruit of cv. Bhagawa, harvested after 165 days of maturity was procured form the experimental field of ICAR-National Research Centre on Pomegranate, Solapur, India. The pomegranate juice having TSS of 14.93 °brix, titrable acidity 0.35%, total phenolics 1732.2 mg/L GAE, antioxidant capacity 38.33 mg/100 mL AAE, ascorbic acid 16.25 mg/100 g, anthocyanin 22.87 mg/100 mL, reducing sugar 14.02%, total sugar 15.25% and colour value (L*: 18.65; a*: 41.95; b*: 24.54) was prepared using manual juice extractor (LavoHome), filtered and stored at refrigerated temperature (Catania et al., 2020). However, lime and ginger were purchased from the local market of Solapur.

Lime and ginger juice was prepared as per the method explained by Hariharan & Mahendran (2016). The prepared lime juice having TSS of 7.5 °brix, titrable acidity 1.29%, total phenolics 615.53 mg/L GAE, antioxidant capacity 48.33 mg/100 mL AAE, ascorbic acid 56.66 mg/100 g, anthocyanin content 2.55 mg/100 mL, reducing sugar 11.03%, total sugar 13.74% and colour value (L*: 64.91; a*: -5.27; b*: 24.52).

Whereas, ginger juice was prepared from ginger rhizomes were peeled and thoroughly washed in clean water. The filtered ginger juice having TSS of 6.7 °brix, acidity 0.74%, total phenolics 708.86 mg/L GAE, antioxidant capacity 55.0 mg/100 mL AAE, ascorbic acid 20.83 mg/100 g, anthocyanin 2.88 mg/100 mL, reducing sugar 11.02%, total sugar 13.06% and colour value (L*: 52.22; a*: 4.67; b*: 17.17). The functional beverage was prepared by blending individual juice at desired proportion.

Formulation of functional beverage and experimental design

The optimization of level of pomegranate, lime and ginger juice for the preparation of functional beverage was done using D-optimal mixture design of response surface methodology (Meilgaard, 2007). Three independent variables, namely pomegranate (A), lime (B) and ginger (C) juice were considered for design of the experiment. The volume fraction for these independent variables used in the present study was decided based on the preliminary trials as pomegranate juice 70-85 % (A); lime juice 10-20 % (B); and ginger juice 5-10 % (C). The sum of the fractions (A, B and C) in each mixture was 100. The responses where physicochemical properties include colour, bioactive components, antioxidant activity and sensory characteristics.

Physicochemical characteristics

Analysis for colour: Colour (L*, *a** and *b** values) of functional beverage samples were determined by using colour difference meter (Lab Scan XE, Hunter Color Lab) (Hunter & Harold, 1987).

Analysis for bioactive components: The total phenolics of samples were determined by Folin-Ciocalteu procedure given by Singleton & Rossi (1965). The total anthocyanin contents were estimated by pH differential method using two buffer systems such as potassium chloride buffer (pH 1.0, 0.025 M) and sodium acetate buffer (pH 4.5, 0.4 M) (Steed & Truong, 2008). The FRAP (ferric reducing antioxidant power) assay was estimated according to Benzie & Strain (1996). The ascorbic acid estimation was determined using the titrimetric method with 2, 6- dichlorophenolindophenol reagent as per the Association of Official Analytical Chemists (2010) with a slight modification.

Sensory evaluation

Rating of sensory characteristics by a semi-trained panel: The sensory properties of pomegranate based functional beverage were conducted by a semi-trained panel as per the procedure described by Chakraborty et al. (2015). The panelists were requested to score the each formulation according to 5-point hedonic scale for the attributes such as appearance, aroma, taste, after-taste, mouthfeel and overall acceptability.

Scale for sensory (S) : 1-Poor; 2-Fair; 3-Good; 4-Very good; 5-Excellent

Consumer rating of sensory attributes: The sensory attributes of functional beverage were rated according to 9-point hedonic scale (1 = dislike extremely,

5 = neither like nor dislike, and 9 = like extremely) (Amerine et al., 2013) by panel members.

Storage study

The optimized sample of pomegranate based functional beverage was studied for the change in physico-chemical and sensory properties for the period of 90 days.

Principal component analysis

The data obtained for physico-chemical analysis and sensory properties of pomegranate juice based functional beverage was employed for principal component analysis (PCA). The multivarient data analysis was performed using Past 4.03 software.

Statistical analysis

The experiment design for optimization by D-optimal mixture design of RSM was performed using the Design Expert 11 statistical package (Stat-Easy Co., Minneapolis, MN, USA). One-way ANOVA and significance tests were performed in SPSS software (IBM®, SPSS® Statistics 20, USA).

RESULTS AND DISCUSSION

The experimental design with range of independent variables and values for different responses is given in Table 1. The regression analysis was applied to fit the response surface model for each characteristic and calculated including all the linear such as pomegranate juice (A), lime juice (B) and ginger juice (C) and their interactions. The mean value and fitted model for the individual responses is given in Table 2. The regression coefficient for each physicochemical and sensory property is shown in Table 3. The insignificant terms ($p>0.05$) were all removed to obtain fitted response surface model. The degree of fitness is measured by R^2. The model is said to be fitted best when the R^2 value reaches to 1.

RSM analysis of physicochemical properties of functional beverage

Total phenolics: The mean value and degree of fitness value for total phenolics was 220.62±0.92 mg/L GAE and 0.9981 (Table 2). The model F-value of 351.23 implies that, cubic model is significant. The coefficient of the first order equation showed that a significant effect of level of independent variables as well as their interactions on the total phenolics of the functional beverage. The pomegranate juice element had the greatest influence on the total phenolics when compared to the level of addition of lime and ginger juice. From regression analysis, the level of pomegranate juice along with lime and ginger individually showed positive significant effect on the total phenolics of the beverage samples (Table 3). The increase in the level of pomegranate juice followed by ginger and lime juice resulted to increase in the total phenolics of the functional beverage.

The total phenolics in pomegranate juice (1732 mg/L GAE) was found higher than lime (615.53 mg/L GAE) and ginger juice (708.86 mg/ L GAE). Pomegranate juice is rich in total phenolics as compared to orange juice is also reported by Singh & Bunkar (2015). The blending of pomegranate with lime juice resulted to increase in total phenolics (González-Molina et al., 2008).

Anthocyanin: The mean value for anthocyanin of beverage sample was 3.95±0.19 mg/100 mL AAE. The F-value of 29.02 showed the fitted quadratic model is significant and having degree of fitness (R^2) as 0.9355 (Table 2). From the equation it was confirmed that as the level of pomegranate juice increased for the preparation of functional beverage, there was also increase in the anthocyanin of the beverage. This is due to presence of anthocyanin at greater extent in pomegranate juice when compared to the lime and ginger juice. The significant effect of independent variables on the anthocyanin more dominantly as compared to the their interactions (Table 3).

Anthocyanin is a component responsible for the deep intensive red colour of the pomegranate juice and it's obvious that the red colour of functional beverage was due to the addition of pomegranate juice instead of lime and ginger juice. The presence of anthocyanin in pomegranate juice was studied by Gaikwad et al. (2018). Moreover, the mixed fruit beverage rich in anthocyanin content from pomegranate was developed by Bhalerao et al. (2020).

Antioxidant activity: The mean value for antioxidant activity of pomegranate juice based functional beverage is 6.95± 0.07 mg/100 mL. The F-value of the 135.72 indicates the quadratic model is significant. The presence of pomegranate, lime and ginger juice in the beverage is positively correlated with the antioxidant activity of the beverage. The R^2 value of 0.9855 showed the degree of fitness which is close to

Table 1 : Colour value, hedonic score, overall acceptability and physicochemical characteristics of the different formulations of functional beverages obtained from D-optimal mixture design

Sample No.	Independent variables			Physicochemical characteristics							Sensory characteristics	
	A: Pomegranate (mL)	B: Lime (mL)	C: Ginger (mL)	Total phenolics (mg/L GAE)	Anthocyanin (mg/100 mL AAE)	Antioxidant activity (mg/100 mL)	Ascorbic acid (mg/100 mL)	L*	a*	b*	Hedonic score	OAA
S1	85	10	5	235.62±0.6	4.51±1.2	7.5±0.7	1.77±0.3	53.43±0.1	21.04±0.2	19.21±0.2	8.5±1.3	4.81±0.2
S2	77.47	15.45	7.07	225.51±0.6	4.29±1.2	7.16±0.7	1.75±0.5	54.02±0.2	18.12±0.3	15.07±0.3	8±1.3	4.29±0.2
S3	70	20	10	192.2±0.3	2.87±1.5	6.08±0.9	1.85±0.5	56.91±0.1	17.78±0.3	13.96±0.4	6.5±1.2	2.51±0.5
S4	78.04	11.95	10	226.74±0.3	4.37±1.7	7.2±0.8	1.76±0.4	51.52±0.3	27.08±0.2	14.00±0.2	7±1.2	2.80±0.3
S5	72.56	19.67	7.76	205.53±0.2	2.96±0.9	6.49±1.2	2.08±0.3	46.77±0.1	14.88±0.1	16.24±0.3	7±1.1	2.73±0.3
S6	73.09	16.90	10	211.65±0.5	3.47±1.3	6.50±1.5	1.78±0.3	56.41±0.1	18.11±0.2	14.03±0.2	7.5±1.1	3.82±0.2
S7	85	10	5	236.41±0.5	4.6±0.6	7.65±0.9	1.62±0.2	54.06±0.1	20.45±0.3	19±0.1	8.5±1.2	4.67±0.2
S8	77.22	17.77	5	223.82±0.7	4.35±0.8	7.06±1.3	1.76±0.5	52.32±0.1	20.65±0.2	16.98±0.1	7.5±1.2	3.79±0.1
S9	81.95	10	8.04	231.84±0.5	4.46±0.9	7.38±1.0	1.66±0.2	55.41±0.1	20.96±0.1	15.32±0.3	8.5±1.3	4.53±0.2
S10	75	20	5	218.7±0.9	3.95±1.2	6.75±0.8	2.13±0.5	58.78±0.2	13.56±0.2	16.2±0.4	7±1.3	2.86±0.5
S11	81.95	10	8.04	231.84±0.6	3.88±1.1	7.35±1.2	1.66±0.3	54.46±0.2	21.08±0.2	13.68±0.1	8±1.2	4.31±0.3
S12	75	20	5	219.87±0.6	3.98±1.0	6.73±1.1	2.12±0.2	58.51±0.3	13.21±0.3	16.2±0.4	7±1.2	2.75±0.3
S13	75.56	14.43	10	220.76±0.3	3.91±0.8	6.82±1.1	1.62±0.1	54.41±0.1	21.28±0.3	14.29±0.2	7.5±1.1	3.75±0.2
S14	70	20	10	194.85±0.2	2.76±0.8	6.08±1.3	2±0.1	56.90±0.1	16.39±0.2	13.52±0.2	7±1.1	2.79±0.5
S15	81.79	13.01	5.19	229.7±0.5	4.5±0.9	7.31±1.3	1.62±0.2	51.52±0.2	24.48±0.1	19.03±0.1	8.5±1.3	4.60±0.4
S16	77.47	15.45	7.07	224.83±0.4	4.3±1.2	7.09±1.9	1.8±0.3	52.68±0.3	17.14±0.1	19.48±0.3	8±1.3	4.38±0.4

OAA: overall acceptability

Table 2 : Analysis of the fitted model equations for the physicochemical and sensory characteristics of functional beverage

	Responses	Model	Mean ±SD	R^2	F- value	p-value	Polynomial equation
Physicochemical characteristics Sensory characteristics	Total phenolics (mg/L GAE)	Cubic	220.62±0.92	0.9981	351.23	<0.0001	+235.98A+187.86B+1932.85C+48.31AB -3078.36AC-3092.38BC+3064.53ABC-63.27AB(A-B)+1555AC(A-C)+1500.38BC (B-C)
	Anthocyanin (mg/100 mL AAE)	Quadratic	3.95±0.19	0.9355	29.02	<0.0001	+4.53A+2.35B+8.25C+3.87AB- 6.66AC – 7.12BC
	Antioxidant activity (mg/100 mL)	Quadratic	6.95± 0.07	0.9855	135.72	<0.0001	+7.54A+5.96B+5.54C+1.21AB+1.68AC+1.17BC
	Ascorbic acid (mg/100 mL)	Special Cubic	1.82±0.07	0.9019	13.80	0.0004	+1.70A+3.18B+1.42C-2.56AB+0.3067AC-3.01BC+5.78ABC
	L*	Cubic	54.26±0.51	0.9884	57.03	<0.0001	+53.75A+81.11B+2070.47C-62.80AB-3716.76AC 3562.14BC+3661.51ABC-8.21AB(A-B)+2009.30AC(A-C)+1407.64BC(B-C)
	a*	Cubic	19.14±0.54	0.9916	78.41	<0.0001	+20.77A-11.74B-387.99C+67.67AB+858.80AC+770BC 1126.12ABC+10.33AB(A-B)-579.54AC(A-C)-227.30BC(B-C)
	b*	Linear	16.02±1.38	0.800	11.81	0.00012	+18.35A+16.92B+6.84C
	Hedonic Score	Quadratic	7.63±0.25	0.9049	19.03	<0.0001	+8.58A+5.02B-2.89C+3.24AB+11.33AC+19.67BC
	OAA	Cubic	3.71±0.11	0.9920	83.0	<0.0001	+4.74A-0.9336B-230.68C+7.91AB+396.38AC+438.66BC-383.41ABC-1.21AB(A-B)-173.38AC(A-C)-233.77BC(B-C)

A: pomegranate juice; B: lime juice; C: ginger juice; OAA: overall acceptability

Table 3 : The regression coefficient for dependent variables of functional beverage with different levels of pomegranate, lime and ginger juice

Regression coefficients	A-pomegranate juice	B-lime juice	C-ginger juice	AB	AC	BC	ABC	AB(A-B)	AC(A-C)	BC(B-C)
Total phenolics (mg/L GAE)	235.976*	187.857*	1932.85*	48.3096	-3078.36	-3092.38	3064.53*	-63.2693	1555	1500.38
Anthocyanin (mg/100 mL AAE)	4.52899*	2.35454*	8.25115*	3.8665*	-6.65896	-7.11652				
Antioxidant activity (mg/100 mL)	7.54096*	5.96176*	5.54002*	1.20524*	1.67953	1.16605				
Ascorbic acid (mg/100 mL)	1.69985*	3.17823*	1.41639*	-2.56076*	0.306667	-3.01071	5.77653			
L*	53.7513*	81.1135*	2070.47*	-62.7981*	-3716.76*	-3562.14*	3661.51*	-8.21365	2009.3*	1407.64*
a*	20.7665*	-11.7379*	-387.991*	67.672**	858.799	769.999	-1126.12	10.3266	-579.539	-227.3
b*	18.3514*	16.9214*	6.83585*							
Hedonic score	8.57841*	5.02487*	-2.89236*	3.24398*	11.3298	19.6682*				
OAA	4.73904*	-0.93358*	-230.675*	7.91355	396.384	438.657*	-383.408	-1.21461	-173.377	-233.774*

* Significant at $p < 0.05$; OAA: overall acceptability

the unity and it is desirable for fitting of the model. The quadratic equation obtained after response represented the significant effect of all three independent variables including their interactions (Table 2). The presence of phenolic compounds, anthocyanin in pomegranate juice, ascorbic acid in lime juice and phenolics in ginger juice resulted to the antioxidant activity of the functional beverage. The regression coefficient analysis (Table 3) emphasized the pomegranate juice is the element influenced significantly on the antioxidant activity followed by the lime and ginger juice.

The addition of pomegranate juice in increasing amount into functional beverage confirmed the increase in antioxidant activity of the functional beverage. The significant higher level of total antioxidant activity (8.81 mg AEAC/100 mL) was recorded in RTS from pomegranate juice when compared to grape juice RTS (4.54 mg AEAC/100 mL) by Amin et al. (2018). Similarly, increase in antioxidant activity when increasing level of pomegranate juice in beverage blended with other fruits juice was investigated (Bhalerao et al., 2020; Singh & Bunkar, 2015).

Ascorbic acid: The R^2 and mean value of ascorbic acid content is 0.9019 and 1.82±0.07 mg/100 mL, respectively. The F-value of 13.80 showed the model fitted is significant. The increase in the level of lime juice caused increase in the ascorbic acid content of the beverage samples. The regression coefficient showed that, the positive effect of the independent variable whereas, the interaction of pomegranate-lime juice and lime-ginger juice showed negative effect (Table 3).

The ascorbic acid content is known to be higher in lime juice as compared to the pomegranate and ginger juice. Therefore, the lime juice is the element which significantly contributing for the ascorbic acid content of the functional beverage. A similar investigation reported the higher content of Vitamin C of the blended juice with the increase in percent of lemon and amla juice in the beverage contains pomegranate juice (Bhalerao et al., 2020; González-Molina et al., 2009; Singh & Bunkar, 2015).

Colour value: The mean values for 'L*' (54.26±0.51), 'a*' (19.14±0.54) and 'b*' (16.02±1.38). The 'L*' value representing the lightness of the beverage was more dominantly influenced by the lime juice, whereas

the redness of beverage was measured in terms of 'a*' value and significantly affected by the level of pomegranate juice in the beverage. The predictive model fitted for the 'L*' and 'a*' value are cubic model and linear model for the 'b*' value (Table 2). The F-value of 57.03, 78.41 and 11.81 for 'L*', 'a*' and 'b*' showed the predicted models are significant. The increase in the level of lime juice leads to increase in 'L*' value (lightness) of the beverage; and increase in pomegranate juice level caused increase in 'a*' value (redness) whereas, the 'b*' value (yellowness) of beverage increased with increasing level of ginger juice (Table 2).

The level of independent factor and their interaction was significantly affected the 'L*' and 'a*' value of the beverage (Table 3). The red colour of the mixed fruit beverage was reflected by the higher 'a*' value and it was due to the addition of pomegranate juice was reported by Bhalerao et al. (2020).

RSM analysis of sensory characteristics of the functional beverage

The sensory acceptability of the beverage by the consumer (untrained members) was evaluated using 9-point hedonic scale and it was dominated by the taste and appearance of the beverage. The range for hedonic score was 6.5 to 8.5. The beverage containing higher per cent of pomegranate juice was preferred more because of the sweet taste and intense red colour of pomegranate juice. The blending of pomegranate juice with lime and ginger juice helped to overcome the problem of bitter-sour taste of lime juice and pungent taste of ginger juice and improved the overall liking of the beverage. Based on the 9-point hedonic scale, S1, S7 and S9 received highest hedonic score (Table 1).

The overall acceptability (OAA) of beverage was determined by semi-trained panelist using 5-point scale, quality rating test. The range of OAA for beverage was 2.51 to 4.81. The samples preferred by panelist was S1 prepared with higher percent of pomegranate juice (85%) because of sweet taste and attractive colour. But, the contradictory trend was observed with the beverage sample made with higher level of lime and ginger juice. The higher acidity of lime juice contributed to the sour-bitter taste of the beverage. Moreover, the pungent taste of ginger juice resulted to the string spicy flavor of the beverage (Table 1). Increased proportion of lime juice (>10%) and ginger juice (>5%) in S3, S4, S5, S10, S12 and S14 resulted to reduced score of OAA (>3) by the panelists.

The predictive quadratic and cubic models were fitted with F-value of 19.03 and 83.0 for hedonic score and OAA of beverage respectively (Table 2). The R^2 value of 0.9049 and 0.9920 showed that the predicted models were significant. The polynomial equation for both hedonic score and OAA confirmed the effect of different levels of independent variables and their interactions had significant effect on the sensory properties of the beverage. The regression coefficients (Table 3) defined the increasing level of ginger and lime juice in beverage preparation had negative impact on hedonic score and OAA of beverage, respectively.

The increasing amount of pomegranate juice in a beverage had a significant effect on the higher score for overall acceptability of blended juice was recorded and pointed that the beverage from blended juices were more preferred over the beverage from individual one (Singh & Bunkar, 2015). The incorporation of ginger juice at higher level in the RTS beverage leads to pungency smell and reduced score for the flavor (Balachandran et al., 2006). Similarly, a report mentioned about the use of pomegranate juice helped to reduce the astringency effect of amla in mixed fruit beverage (Balachandran et al., 2006). The taste of beverage from amla with grape was also enhanced by incorporation of pomegranate juice (Jain & Khurdiya, 2004).

The overall liking and acceptability of sensory properties of beverage could be improved significantly and many authors stressed on the use of mixture design tool for obtaining best optimization of different combinations of juices blends such as pomegranate, amla and muskmelon (Benzie & Strain, 1996), black cherry, concord grapes and pomegranate (Lawless et al., 2013), mango, orange and lemon (Kumar et al., 2010).

Principal component analysis (PCA)

The variability within each physicochemical property has been explained by two principal components (PC1 & PC2). The PCA for physicochemical properties showed the first principal component had the highest eigenvalue of 5.699 and accounted for 63.328 % of the variability in the data set (Table 4).

Table 4 : Eigen value, % variance and loadings feature for the PC1 and PC2 for physicochemical and sensory properties of functional beverage

Characteristics		PC1	PC2
	Eigen value	5.699	1.285
	% variance	63.328	14.285
Physicochemical	Total phenolics (mg/L GAE)	0.38798	0.13455
	Anthocyanin (mg/100 mL AAE)	0.37445	0.16603
	Antioxidant activity (mg/100 mL)	0.40024	0.077828
	Ascorbic acid (mg/100 mL)	-0.32074	0.46491
	L*	-0.13958	0.24946
	a*	0.27004	-0.6074
	b*	0.24846	0.53233
Sensory	Hedonic score	0.38483	0.11151
	OAA	0.37943	0.07185

PC: principal component; OAA: overall acceptability

Whereas, the eigenvalue and % variance for second principal component was recorded as 1.285 and 14.285%, respectively. The colour value in terms of 'L*', 'a*' and 'b*' for different formulations were lies in the different quadrant of the principal component (Fig. 1). From the PCA biplot, the 'b*' value is in the same quadrant for sample S1, S7 and S16. More the amount of pomegranate juice resulted to intense reddish colour of the beverage which indicates the presence of anthocyanin content. Moreover, the higher the level of lime juice in beverage leads to increase in lightness value and ascorbic acid content of the beverage (S10 and S12). The 'a*' value lies in the opposite quadrant of 'L*' value due to the higher amount of ginger juice for S4, S13, S15 etc. The chemical properties such as total phenolics, antioxidant activity and anthocyanin for beverage S1, S7 and S16 lies in the same quadrant of PC1. It is mainly due to the higher per cent of pomegranate juice in the formulation which is responsible for higher nutritional value for sample S1, S7 and S16 (Fig. 1).

The sensory characteristics such as overall acceptability (OAA) and hedonic score for beverage S1, S7 and S16 were lies in the same quadrant of PC1. Regarding loadings, the PC1 showed positive loading values for all the responses except L* value and ascorbic acid content of beverages whereas, PC2 showed a negative loading value for the 'a*' calour value of the beverage (Table 4).

The PCA for sensorial attributes showed the first PC 1 had the highest eigenvalue of 0.775 and

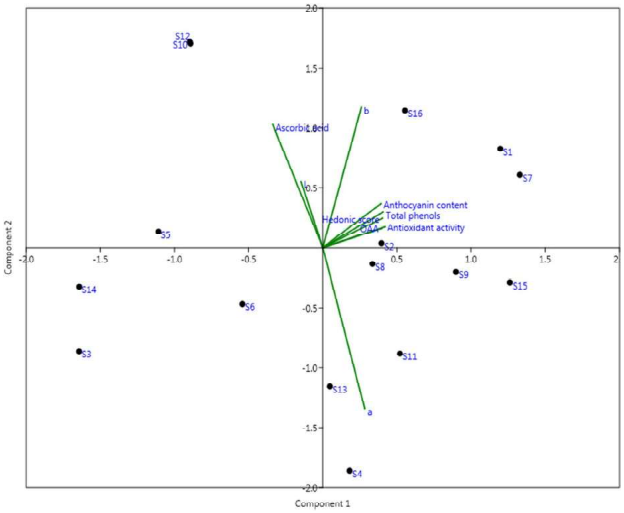

Fig. 1: Biplot between principal components (PC1 and PC2) in a rotated space generated from PCA of physicochemical and sensory characteristics of different formulations

accounted for 96.49% of the variability in the data set (Table 5). The eigen value and % variability for PC 2 was 0.0142 and 1.774%, respectively. The sensory attributes for beverage were accounted with respect to appearance, aroma, taste, after-taste and mouth-feel. The variability within each sensorial attributes has been explained by two principal components (PC1 & PC2). The beverage S1, S7 and S15 containing pomegranate juice in higher amount as compared to other formulations lies in the positive quadrant of PC1 which shown the higher sensory score for the aroma, appearance and taste (Fig. 2). The beverage formulation with lime and ginger juice higher than

10 and 5 per cent, respectively scored higher for the mouth-feel and aftertaste sensory attributes by the panelist and these lies in the negative quadrant of PC2 (Fig. 2). The data for loading feature represented the positive value for all the sensory attributes by PC1 and negative loading value by PC2 for mouth-feel and after taste of beverage samples (Table 5).

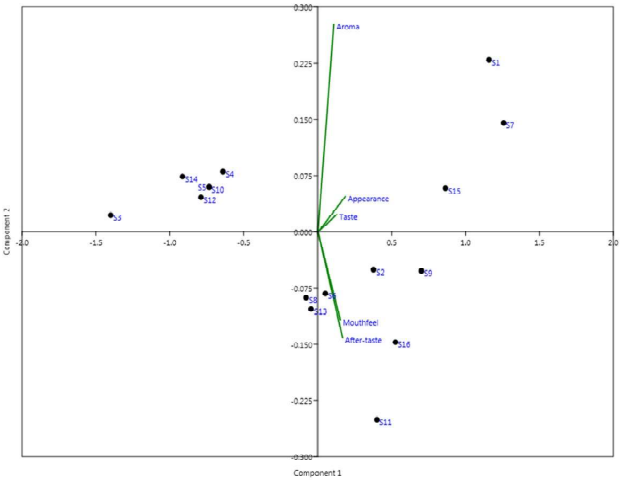

Fig. 2 : Biplot between principal components (PC1 and PC2) in a rotated space generated from PCA of sensorial attributes of different formulations

Table 5 : Eigen value, % variance and loadings feature for the PC1 and PC2 for sensorial attributes of functional beverage

Parameter	PC1	PC2
Eigen value	0.775503	0.0142612
% variance	96.496	1.7745
Appearance	0.50285	0.12022
Aroma	0.29162	0.82233
Taste	0.3429	0.041148
After-taste	0.45293	-0.42666
Mouth-feel	0.41059	-0.35013
OAA	0.41328	0.054757

PC: principal component; OAA: overall acceptability

Optimization of the beverage

The selected optimized solution with maximum desirability (0.977) for independent factors were prepared and further evaluated for validating predicted values. The predicted and experimental value for the optimized solution for the preparation of pomegranate based functional beverage is given in Table 6. Based on the validation experiments, functional beverage containing 85% pomegranate juice, 10 % lime juice and 05 % ginger juice was found overall acceptable which provides total phenolics (236.2 mg/L GAE), anthocyanin (4.61 mg/100 mL AAE), antioxidant activity (7.54 mg/100 mL), ascorbic acid (1.80 mg/100 g), L* (53.7), a* (21.1), b* (19.2), hedonic score (8.61) and overall acceptability (4.83) (Table 6). It confirms the maximum desirability for preparation of functional beverage from 85% pomegranate, 10% lime and 5% ginger juice (Fig. 3).

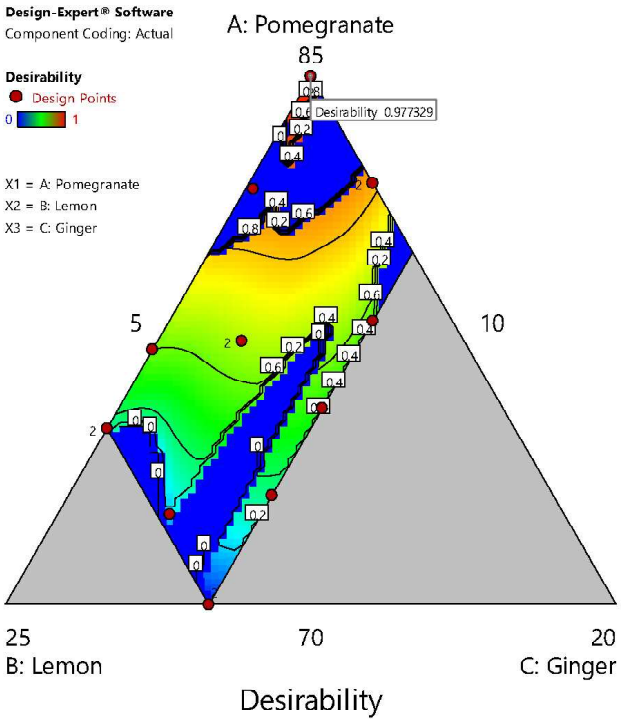

Fig. 3 : The counter plot for the optimized solution for pomegranate based functional beverage

The optimized sample of pomegranate based blended RTS drink when compared nutritionally and organoleptically with RTS drink from pomegranate juice (control) showed 40% increase in antioxidant activity, 20% increase in ascorbic acid, 5% decrease in calorific value and higher hedonic score as well as score for overall acceptability. The increase in antioxidant activity and ascorbic acid content resulted to fulfill the requirement of Vitamin C content and reduction in calorie value without affecting the sensorial properties is the desirable characteristics of RTS drink for health-conscious consumers (Table 7).

Table 6 : Optimized solutions with predicted and actual experimental values for functional beverage

Desirability: 0.977 Pomegranate (85%): Lime (10%): Ginger (05%)		
	Predicted [a]	**Experimental [a]**
Total phenolics (mg/L GAE)	235.976	236.2
Anthocyanin (mg/100 mL AAE)	4.52899	4.61
Antioxidant activity (mg/100 mL)	7.54096	7.54
Ascorbic acid (mg/100 mL)	1.69985	1.80
L*	53.7513	53.7
a*	20.7665	21.1
b*	18.3514	19.2
Hedonic Score	8.57841	8.61
OAA	4.73904	4.83

[a]No significant ($p<0.05$) difference between predicted and experimental values; OAA: overall acceptability

Table 7 : Effect of blending of lime and ginger juice with pomegranate on chemical constituents of functional beverage

Sample	Antioxidant activity (mg/100 mL)	% increase	Ascorbic acid (mg/100 mL)	% increase	Anthocyanin (mg/100 mL)	% decrease	Energy value	% decrease
Control	5.38	—	1.52	—	5.59	—	56	—
Optimized functional beverage	7.54	40.14	1.82	19.73	4.61	17.53	53.08	5.21

Table 8 : Changes in physicochemical and sensory properties of optimized functional beverage during storage periods

	0 day	30 day	60 day	90 day
Total phenolics (mg/L GAE)	236.2±0.3^{a}	218.86±0.3^{b}	187.2±0.2^{c}	157.1±0.5^{d}
Anthocyanin (mg/100 mL AAE)	4.61±0.1^{a}	4.22±0.4^{ab}	3.89±0.1^{b}	3.43±0.6^{c}
Antioxidant activity (mg/100 mL)	7.54±0.2^{a}	5.33±0.2^{b}	5±0.3^{b}	4.01±0.5^{c}
Ascorbic acid (mg/100 mL)	1.80±0.2^{a}	1.62±0.1^{ab}	1.33±0.2^{b}	1.07±0.7^{c}
L*	53.7±0.1^{a}	51.52±0.4^{b}	50.46±0.2^{c}	50.29±0.5^{c}
a*	21.1±0.5^{a}	21.62±0.5^{a}	21.88±0.4^{a}	21.42±0.6^{a}
b*	19.2±0.3^{a}	13.55±0.3^{b}	12.56±0.3^{c}	12.19±0.5^{c}
Hedonic Score	8.61±0.1^{a}	7.5±0.1^{b}	6.5±0.1^{c}	6.5±0.1^{c}
OAA	4.83±0.3^{a}	4.28±0.3^{b}	3.75±0.3^{c}	3.05±0.5^{c}

OAA: overall acceptability; Values are expressed as mean±SD of three independent determinations. Values in rows followed by the same letter are not significantly different at $p \leq 0.05$ as measured by Duncan's test

Storage study of the optimized functional beverage

The optimized sample of functional beverage with 85% pomegranate, 10% lime and 5% ginger juice was stored at refrigerated temperature for the period of 90 days and evaluated for its physicochemical and sensory properties at the interval of 30 days. It was observed that, over the period of 90 days, there was a slight degradation of the chemical constituents of the

beverage even though it seems a significant difference (Table 8).

Total phenolics, anthocyanin and ascorbic acid were reduced from 236.2 to 157.1 mg/L GAE, 4.61 to 3.43 mg/100 mL AAE and 1.80 to 1.07 mg/100 mL, respectively. The colour value with respect to 'a*' value remained unchanged whereas 'L*' and 'b*' value of beverage sample reduced unnoticeably. The hedonic score and overall acceptability were also found decreased due to change in sensorial attributes such as taste and colour. These findings are in consonance with the previous study reported about the degradation of nutritional composition and sensory score of guava-lime-ginger RTS stored for 180 days (Selvi et al., 2013) and ginger-lime RTS for 90 days (Deen, 2022).

CONCLUSION

The optimization resulted to the best combination of 85% pomegranate juice, 10% lime juice and 05% ginger juice for the preparation of nutritious, overall acceptable functional beverage. Furthermore, this functional beverage could be safe to consume or stored for the period of 90 days at refrigerated conditions. It was concluded that, a functional beverage could be prepared from pomegranate juice along with lime and ginger juice which provides total phenolics (236.2 mg/L GAE), anthocyanin (4.61 mg/100 mL AAE), antioxidant activity (7.54 mg/100 mL) and ascorbic acid (1.80 mg/100 g). The optimized sample of pomegranate based blended RTS drink showed 40% increase in antioxidant activity, 20% increase in ascorbic acid, 5% decrease in calorific value and higher hedonic score as well as score for overall acceptability as compared to RTS from pomegranate only. The blending of the pomegranate, lime and ginger juice could be a best method for the development of functional beverage with improved nutritional value and sensory properties.

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