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Optimization of Nutritional Drink of Pomegranate, Orange and Ginger Juices using Response Surface Methodology

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Abstract

The main aim of this work is to enhance the nutritional and functional properties of blended juice using response surface methodology (RSM). In this study the levels of juice (50-75 ml pomegranate juice, 25-50 ml orange juice and 3-5 ml ginger juice) were optimized using RSM. The product was optimized on the basis of physico-chemical, textural and sensory attributes. Based on RSM experiments, it could be concluded that a formulation having 75 ml pomegranate, 50 ml orange and 3 ml ginger juice with viscosity index 4.60 g.sec, consistency of 7.36 g.sec, cohesiveness of 487.45 g and overall acceptability 7.29 out of 9.00 was the best among all combinations. The antioxidant activity, total phenol, titratable acidity, pH, total soluble solids, vitamin-C, total sugar, potassium, calcium and magnesium content in the optimised product were 41.23% DPPH inhibition, 195 mg/100 ml TAE, 0.59%, 3.4, 14.38%, 42.23 mg/100 ml and 8.34%, 2380 mg/L, 65 mg/L, 126 mg /L, respectively.

Keywords: Antioxidant activity; Vitamin-C; Response surface methodolgy; Overall acceptability

Introduction

Fruits and vegetables are necessary for good health and for all age categories as these are important portion of a healthy diet. The importance and nutritional quality of fruits are due to its colour, unique taste, smell, enriched minerals, vitamins and other beneficial components [1]. Fruits like apple, pomegranate, grapes, orange, blueberries, cranberries, gooseberries, strawberries and carrots are the major sources of bioactive compounds and antioxidant also. Studies showed that the pomegranate juice contains higher amount of antioxidant levels than the other fruits [2,3]. Pomegranate (*Punica granatum L.*) contends many phenol compounds including flavanoids (anthocyanins, catechins and other complex flavanoids) and hydrolyzable tannins (punicalin, pedunculagin, punicalagin, gallagic and ellagic acid esters of glucose). The 92% of its antioxidant activity is based on these phenol compounds present in the pomegranate fruits [4]. While the orange (*Citrus cinensis*) is a widely consumed fruit, mainly used for its fresh flavour and it is a very good source of natural antioxidant and possess health benefits due to the presence of antioxidants like vitamin-C [5,6]. The many varieties of citrus are consumed as fresh fruit and some citrus is used for preparation of citrus products, like fresh juice, concentrated juice, tea and vinegar [7]. Hesperidin present in citrus fruits has a hypotensive effect [8], whereas narirutin exhibits the same antioxidant activity as butylated hydroxyanisole [9]. Ginger (*Zingiber officinale*) is a rhizomatous plant widely cultivated all over the India, Bangladesh, Jamaica, Taiwan and Nigeria. It is used as carminative, pungent and stimulant spice under the conditions of indigestion, stomach-ache, malaria, fever, etc. Ginger forms an important part of many pharmacopoeial ayurvedic (Indian system of medicine) formulations. The blending of the juice is the best methods to enhance the nutritional quality and the vitamin and mineral content of the juice depend on the types of fruits and vegetables used [10]. Thus blended drinks are a good method to the development of new products which gives new taste and improvement in the quality of nutrition. This improvement can be attributed to the mix of two or more kinds of fruit juices, to enhance the vitamins, mineral and the nutritional value of the product [11]. In previous study Deka and Sethi [12] reported that two or more fruits juice may be blended in various proportions for the preparation of nectar, ready-to-serve (RTS) beverages etc. Kim et al. [13] used the modified distance design to optimize and developed a functional drink utilizing citrus peel extract. Karangwa et al. [14] used RSM to optimize the processing parameters for clarified blended carrot-orange juices and improved its carotene content. The sensory and nutritional quality of bottlegourd, lemon and basil leaves juice is optimize by using RSM [15]. Siti Mazlina et al. [16] also used the RSM to optimize the quality of star fruit juice from different varieties of star fruit. RSM reduce the number of trials needed to evaluate multiple parameters and their interactions, thus, it takes less time compared to other methods. Therefore RSM, Central Composite Rotatable Design (CCRD) was used as a tool to design an optimized formulation for enhancing the nutritional and functional properties of blended juice [17]. CCRD drastically reduces the number of trials for more than two independent variables. In order to utilize the nutritional, functional and medicinal properties of fruits and vegetables, the juices of pomegranate, orange and ginger were blended in different proportions with a view to enhancing the bioactive constituents.

Materials and Methods

Fruits

The fully matured ripened, freshly harvested pomegranate (*Punica granatum* L.), orange (*Citrus cinensis*) and well developed ginger rhizomes (*Zingiber officinale*) were procured from the local market of Varanasi, India.

Juice preparation

Fruits pomegranate (2 kg), orange (2 kg) and ginger (0.5 kg) were

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washed with clean running water to remove dust particles and to reduce the microbial load on the surface of the fruits and ginger rhizomes. Peeled orange fruits were crushed in screw type juice extractor machine (Bajaj Process Pack Maschinen Private Ltd., Noida, India) for extraction of juice. Pomegranate fruits were cut into pieces and arils were separated. These arils were passed through the juicer (Model: HL1631/J, Made: Philips, Himachal Pradesh, India) for extraction of juice. Ginger were sliced with the help of stainless steel knives and crushed with mixer cum juicer (Model: HL1659, Made: Philips, Himachal Pradesh, India) for the extraction of juice. The juices were kept for 30 min in incubator (Model: LTI-700, Made: Eyela, Japan) at 4 ± 2°C for sedimentation. Then the clear juice was siphoned off. The juice was filtered through muslin cloth, after that clear juices were blended in the ratio of 75 ml (58.59%) pomegranate, 50 ml (39.06%) orange and 3 ml (2.35%) ginger juice.

Physico-chemical analysis

The determination of antioxidant activity of blended juice was done by 2, 2-diphenyl-2-picryl hydrazine (DPPH) inhibition method [18] and the total phenol contents of the blended juice were determined by modified Folin-Ciocalteau method [19]. The vitamin-C content of the juice was estimated by visual titration method with 2, 6-dichlorophenol-indophenols dye solution [20] and the total sugar was determined according to Lane and Eynon method [21]. The moisture content was determined by oven-dry method [22]. Ash content of the blended juice was determined by the standard procedure as given in AOAC [23] and crude protein was determined by Kjeldhal method [24] while crude fat was estimated using the automatic SOCS PLUS (SCS 4, Pelican Equipments, Chennai, India) by employing the standard method prescribed by AOAC [25]. The analysis of mineral contents was done with atomic absorption spectrophotometer (AAS) model UNICAM-969, UK.

Textural analysis

The textural properties of blended juice were analyzed using back extrusion rig probe. Textural properties were measured by a Texture Analyzer (TA-XT *Plus*, Stable Micro Systems, Surrey, UK) using the following parameters: pre-test speed=1 mm/s, test speed=1 mm/s, post speed=5.0 mm/s, distance=80 mm and trigger force of 5 g. Mean value was used to obtain a force-time curve by means of the textural parameters at 27°C. The textural properties of blended juice were analyzed in terms of consistency, cohesiveness and viscosity index.

Sensory evaluation

A sensory score card suggested by Amerine et al. [26] with little modification was adapted to analyze the sensory characteristics of the developed blended juice. Sensory were evaluated by a panel of 7 semi-trained members from Centre of Food Science and Technology, Banaras Hindu University, Varanasi for colour, flavour and overall acceptability of the blended juice. The tests were performed using 9-point hedonic scale, where 9 were 'like extremely' and 1 was 'dislike extremely'. Sensory evaluation was done at 27°C.

Experimental plan

Numerical optimization technique of the Design–Expert software (8.0.3) was used for simultaneous optimization of the multiple responses. The design of experiments is mathematical and statistical techniques for designing experiments and evaluating the effects of factors. The experiments were performed and responses were fitted in the design. After each individual experiment, responses were analyzed to assess the effect of independent variables on them. The first order or second order polynomial equation (Eq.1) examines the statistical significance of the model and the following form was fitted to the responses:

$$Y = \beta_o + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_i X_i^2 + \sum_{i=1}^{3} \sum_{j=i}^{4} \beta_j X_i X_j \qquad (1)$$

Where,

Y	=	response variable
$\beta o, \beta_i, \beta_i \ \& \beta_j$	=	regression coefficient
X_i, X_j& X_{ij}	=	coded independent variables

Response Surface Methodology (RSM) which involves design of experiments, selection of levels of variables in experimental runs, use of fitting mathematical models and finally selection of variables levels by optimizing the response [27] was employed in the study. A CCRD was used to design the experiments comprising of three independent processing parameters (Table 1). Twenty sets of experiments were performed taking into account 3 factors viz., levels of pomegranate, orange and ginger juice. The variables taken for present research work were concentration of pomegranate, orange and ginger juices, in the range of 50-75 ml, 25-50 ml and 3-5 ml, respectively. There were 6 experiments at centre point to calculate the repeatability of the method [28]. Responses obtained after each trials were analyzed to visualize the interactive effect of various parameters on physico-chemical, sensory attributes and textural properties of blended juice. It studies the interaction between all factors and quickly arrives at the optimum conditions of factors for desirable responses.

Results and Discussion

The present study was carried out for the optimization and the effect of these juices on nutritional and functional properties of blended

Factors					Responses						
A	B	C	VC	DPPH	TPC	VI	CH	CO	CL	FL	OAA
62.5	37.5	4	39.42	30.50	180	4.93	16.47	459.34	6.5	7.2	6.70
75	25	3	32.1	43.50	201	3.56	17.83	464.56	7.4	7.8	7.36
50	25	3	35.23	31.10	160	2.15	12.57	430.34	6.6	7.7	7.00
62.5	37.5	2.3	39.33	32.50	179	4.12	16.25	460.54	6.8	8.5	6.90
75	25	5	32.76	42.60	205	3.87	18.78	469.43	7.8	6.6	7.12
62.5	37.5	4	36.23	29.50	175	4.04	15.68	462.67	7.4	7.3	6.50
62.5	37.5	4	39.78	30.20	165	4.99	15.62	466.34	6.8	7.3	6.60
62.5	58.52	4	40.23	26.00	169	5.76	16.26	479.12	6.0	7.2	6.65
83.5	37.5	4	38.32	47.00	230	3.79	16.81	506.25	8.5	7.1	7.90
50	25	5	35.34	32.00	159	2.54	12.88	443.36	6.2	6.5	6.60
50	50	5	36.34	30.00	175	5.43	13.23	468.23	6.0	6.6	6.52
75	50	5	40.13	38.50	192	4.89	17.46	486.56	7.2	6.6	7.40
62.5	16.47	4	29.12	35.00	182	3.58	16.27	447.13	6.8	7.2	7.12
62.5	37.5	4	39.78	31.00	179	4.43	15.67	449.34	6.2	7.1	6.30
75	50	3	42.23	41.23	195	4.75	17.83	482.32	7.5	7.8	7.29
50	50	3	39.87	25.00	172	5.11	13.46	471.76	6.0	7.9	6.56
62.5	37.5	5.6	35.43	32.60	174	4.67	16.67	448.45	6.3	5.9	6.60
41.47	37.5	4	41.45	26.00	135	3.25	12.67	466.64	6.3	7.2	6.40
62.5	37.5	4	38.29	36.31	181	4.17	16.53	436.78	6.4	7.2	6.50
62.5	37.5	4	39.12	32.80	178	4.56	16.89	445.34	6.89	7.3	6.70

Where; A = Pomegranate juice, B = Orange juice, C = Ginger juice, VC = Vitamin-C, DPPH= % Antioxidant activity inhibition, TPC = Total phenol content, VI = Viscosity index, CH = Cohesiveness, CO = Consistency, CL = Colour, FL = Flavour, OAA = Overall acceptability.

Table 1: Experimental runs and actual values of factors used in central composite rotatable design.

juice. This was based on the physico-chemical (antioxidant activity, total phenol and vitamin-C), textural (viscosity index, consistency and cohesiveness) and sensory (colour, flavour and overall acceptability) characteristics of the blended juice.

Proximate nutritional composition of optimized blended juice

The nutritional quality of juices were improved with the blending of pomegranate, orange and ginger juice. The average contents of antioxidant activity, total phenol, vitamin-C, total sugar, fat, protein, moisture, ash, potassium, calcium and magnesium contents of the blended juice were 41.23%, 195 mg/100 ml TAE, 39.42 mg/100 ml, 8.3%, 0.8%, 1.5%, 85.05%, 0.58%, 2380 mg/L, 65 mg/L, 126 mg/L, respectively.

Effect of process variables on physico-chemical properties of blended juice

Antioxidant activity: The quadratic equation obtained by the response surface analysis (RSA) of the data showing the effect of pomegranate juice (A), orange juice (B) and ginger juice (C) is as follows:

$$\text{Antioxidant activity}=31.65+6.08 * A-2.17* B+0.18 * C+0.22 *A * B-1.19* A * C+0.28* B * C+2.16 * A^2+0.038 * B^2+0.76 * C^2 \quad (2)$$

where,

A=pomegranate juice ; B= orange juice ; C=ginger juice.

The coefficient of determination (R^2) was 0.92. The "Pred R-Squared" of 0.68 was in reasonable agreement with the "Adj R-Squared" of 0.85. "Adeq Precision" was 14.22 and greater than 4, which is the desirable value indicating an adequate signal. Antioxidant activity of the blended juice was found to be in the range of 25- 47% DPPH inhibition (Table 2). The quadratic model for antioxidant activity was found to be significant (p<0.05).

It could be seen from Figure 1a that antioxidant activity was mainly affected by the level of pomegranate juice and orange juice. The antioxidant activity of the blend juice increases with the increase in the level of pomegranate juice but as the level of orange juice increases, the antioxidant activity decreases. Ginger juice also enhances the antioxidant properties of mixed juice. Gonzalez-Molina et al. [29] reported the pomegranate juices possess 3-fold higher antioxidant activity than other fruit juices. Thus the blending of pomegranate juice with orange juice was found to have a synergistic effect in antioxidant capacity.

Constraints	Goal	Lower limit	Upper limit
Pomegranate juice (ml)	maximize	50	75
Orange juice (ml)	is in range	25	50
Ginger juice (ml)	is in range	3	5
Vitamin -C (mg/100ml)	maximize	29.12	42.23
% DPPH inhibition	maximize	25	47
Total phenol (mg/100TAE)	maximize	135	230
Viscosity index (g.sec)	is in range	2.15	5.76
Consistency (g.)	is in range	430.34	506.25
Cohesiveness (g.)	is in range	12.567	18.778
Colour	maximize	6	8.5
Flavour	maximize	5.9	8.5
Overall acceptability	maximize	6.3	7.9

Lower weight = 1, upper weight = 1 and importance= 3

Table 2: Levels of responses fixed for optimization of blend juices.

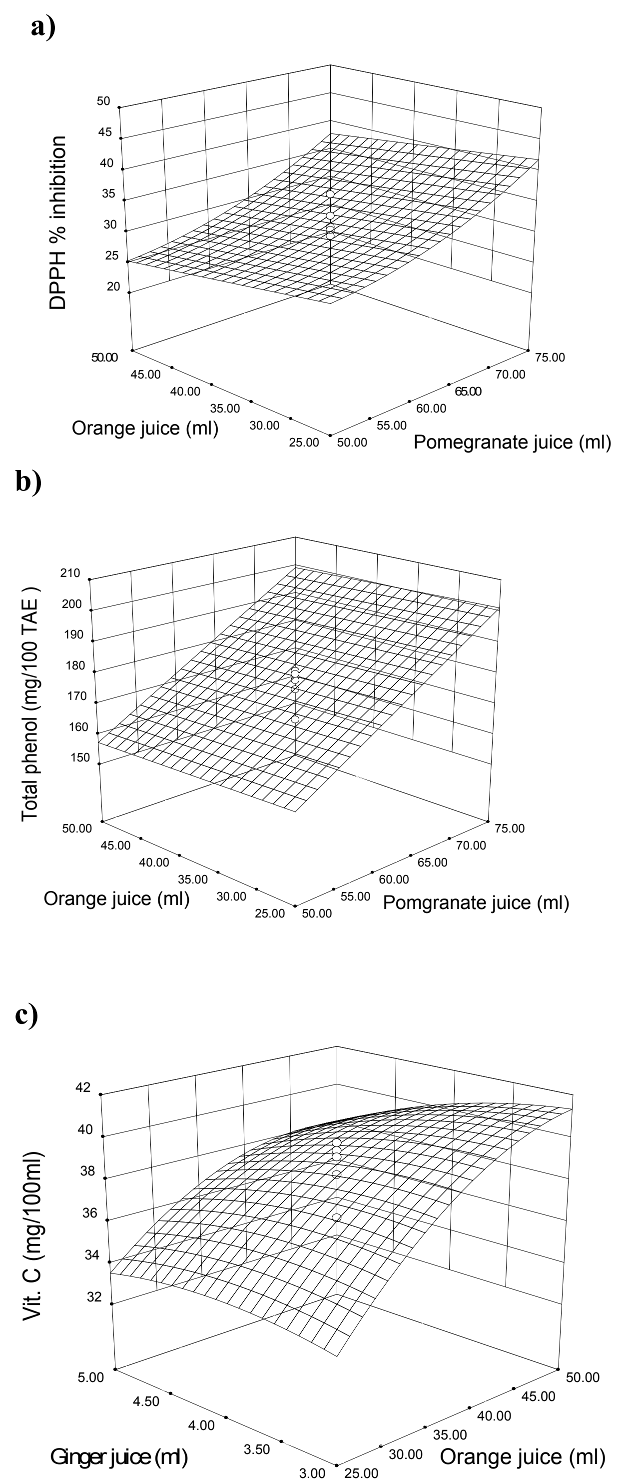

Figure 1: Effect of process variables on physico-chemical properties of blended fruit juice; **a)** Pomegranate and orange juice Vs DPPH % inhibition, **b)** Pomegranate and orange juice Vs total phenol content, **c)** Orange and ginger juice Vs vitamin-C.

Total phenol: The linear equation obtained by the RSA of the data

Factor	Responses								
	DPPH	TPC	V C	CO	VI	CH	CL	FL	OAA
Intercept	31.65	179.30	38.79	453.44	4.52	15.79	6.70	7.20	6.55
A	6.08	21.00	-0.35	11.41	0.20	1.96	0.64	-4.99	0.37
B	-2.17	-0.94	3.06	11.35	0.86	-6091	-0.19	0.022	-0.081
C	0.18	-0.40	-0.84	-0.13	0.15	0.10	-0.081	-0.68	-0.079
AB	0.22	-	1.48	-3.92	-0.45	-	0.034	-	0.091
AC	-1.19	-	0.25	-0.047	-0.032	-	0.059	-	0.039
BC	0.28	-	-0.80	-2.15	-0.030	-	-0.034	-	0.089
A^2	2.16	-	0.29	10.80	-0.38	-	0.26	-	0.22
B^2	0.038	-	-1.55	2.56	0.024	-	-0.096	-	0.12
C^2	0.76	-	-0.59	-0.49	-0.073	-	-0.043	-	0.077

Table 3: Coefficient of estimates of coded factors for different levels of juice level in blend juices.

showing the effect of A, B and C could be presented as follows:

$$\text{Total phenol}=179.30+21.00 * A-0.94* B -0.40 *C \quad (3)$$

The coefficient of determination (R^2) was 0.82. The "Pred R-Squared" of 0.70 was in reasonable agreement with the"AdjR-Squared"of 0.79. "Adeq Precision" was 17.78 which was greater than 4, indicating an adequate signal. Hence, the model could be used to navigate the design space. The coefficient of estimation is presented in Table 3. The total phenol content is expressed as tannic acid equivalent (TAE) in milligrams per gram of sample and it ranged from 135-230 (mg/100 ml TAE) for the 20 trials conducted as shown in Table 1.

The response surface plot as shown in Figure 1b, shows that with increasing level of pomegranate juice, a significant increase in the total phenol content of product was found. The total phenol content in pomegranate and orange juice is 243.89 mg gallic acid /100 ml and 55.4 to 28 mg/100 ml TAE respectively. Gonzalez-Molina et al. [29] reported that blending of pomegranate and lemon juice was done to gain increased total phenol content.

Vitamin C: The quadratic equation obtained by the RSA of the data showing the effect of A, B and C is as follows:

$$\text{Vitamin-C}=38.79 -0.35* A+3.06 * B-0.84 * C+1.48 *A * B+0.25 * A * C-0.80 * B * C$$

$$+0.29 * A^2 -1.55 * B^2-0.59 *C^2 \quad (4)$$

The coefficient of determination (R^2) was 0.93. The "Pred R-Squared" of 0.74 was in reasonable agreement with the "Adj R-Squared" of 0.87. "Adeq Precision" was 15.38 and greater than 4, which is the desirable value indicating an adequate signal. The vitamin-C of the blended juice was found to be in the range of 29.12 - 42.23 mg/100 ml (Table 2).

Figure 1c shows that vitamin-C increases as the level of orange juice increases in mixed juice while ginger juice also had slightly possitive effect on vitamin-C content of the blended juice. The orange juice have high amount of vitamin-C content 30 to 60 mg/100 ml while pomegranate juice contain 9 to 15 mg/100 ml. A similar study reported that the vitamin-C content in the mixture increased with the percentage of the lemon juice added to pomegranate juice [30].

Effect of process variables on textural properties of blended juice

Viscosity index: The quadratic equation obtained by the RSA of the data showing the effect of A, B and C is as follows:

$$\text{Viscosity index}= 4.52+0.20* A+0.86 * B+0.15 * C-0.45* A* B -0.032 *A *C -0.030 *B* C-0.38 *A^2+0.024 * B^2-0.073 * C^2 \quad (5)$$

The coefficient of determination (R^2) was 0.92. The "Pred R-Squared" of 0.69 was in reasonable agreement with the "Adj R-Squared" of 0.85. "Adeq Precision" was 14.64 which is higher than 4, indicating an adequate signal. Hence, this model could be used to navigate the design space. The coefficient of estimation is presented in Table 3. The viscosity index ranges from 2.15 to 5.76 g. sec (Table 2).

The response surface plot (Figure 2a) shows that with the increase in the level of pomegranate juice, the viscosity index initially increases. However, the viscosity index tends to increase with increasing levels of orange juice in the formulation. Higher levels of pomegranate juice

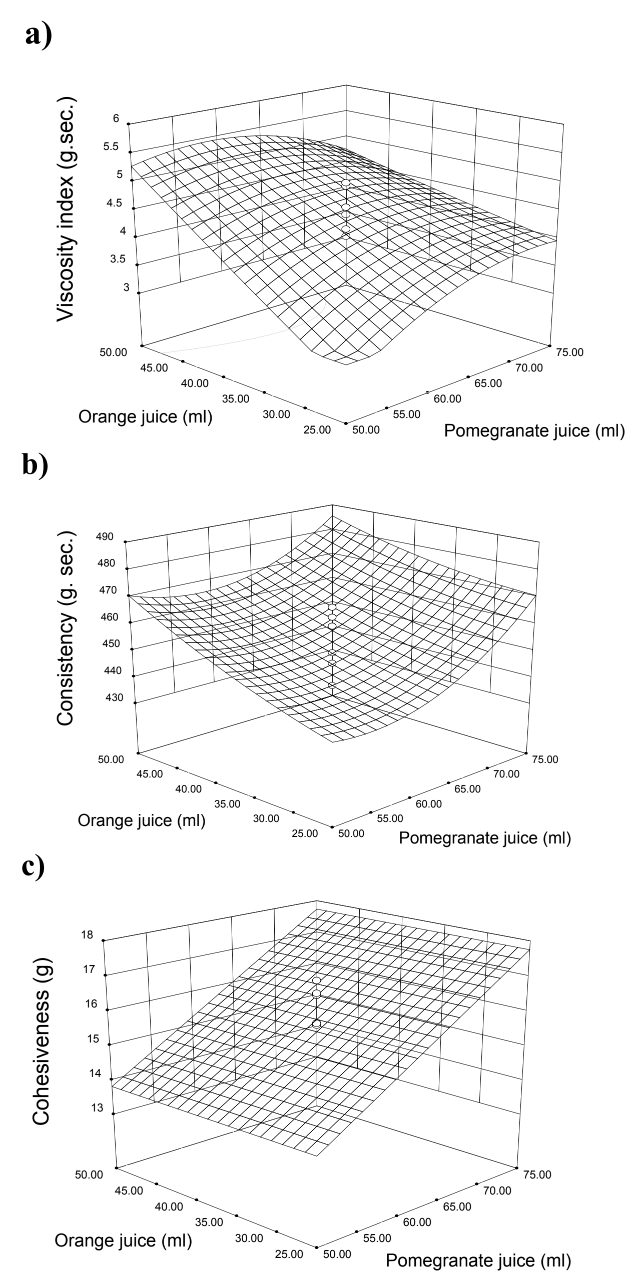

Figure 2: Effect of process variables on textural properties of blended fruit juice; a) Pomegranate and orange juice Vs viscosity index, b) Pomegranate and orange juice Vs consistency, c) Pomegranate and orange juice Vs cohesiveness.

and orange juice exerted more positive effect on the viscosity index than the levels of ginger juice, a significant increase in viscosity index was observed with an increase in the levels of orange juice in the formulation. Pomegranate juice contains 17 brix of total soluble solids while orange juice boasts of 12.02 brix. Thus, the blending of these fruits juices enhance the soluble solids that cause increases in viscosity resulting from molecular movements and interfacial film formation [31].

Consistency: The quadratic equation obtained by the RSA of the data showing the effect of A, B and C is as follows:

$$\text{Consistency} = 453.44+11.41*A+11.35*B-0.13*C-3.92*A*B-0.047*A*C-2.15*B*C+10.80*A^2+2.56*B^2-0.49*C^2 \quad (6)$$

The coefficient of determination (R^2) was 0.86. The "Pred R-Squared" of 0.56 was in reasonable agreement with the "Adj R-Squared" of 0.74. "Adeq Precision" was 9.85 and greater than 4, which is the desirable value indicating an adequate signal. The consistency of the blended juice was found to be in the range of 430.34-506.25 g.sec (Table 2). The linear model for consistency was found to be significant ($p<0.05$).

It could be seen from Figure 2b that the consistency of the product increased with the increase in the level of pomegranate juice as well as orange juice. Results were on expected lives as increase in solids level would normally lead to increase in consistency of blended juice. The pomegranate and orange juice contain total soluble solids 17 to 12 brix respectively thus the blending of these juices increase the consistency. Hobani [32] reported as the concentration of pomegranate juices increases, the consistency of mixed juices also increases significantly.

Cohesiveness: The linear equation obtained by the RSA of the data showing the effect of A, B and C could be presented as follows:

$$\text{Cohesiveness}=15.79+1.96*A-6.916E-003*B+0.10*C \quad (7)$$

The coefficient of determination (R^2) was 0.86. The "Pred R-Squared" of 0.56 was in reasonable agreement with the "Adj R-Squared" of 0.75 "Adeq Precision" was 9.85 which was greater than 4, indicating an adequate signal. Hence, the model could be used to navigate the design space. The cohesiveness of blended juice ranged from 12.56 and 18.77g for the 20 trials conducted as shown in Table 1, The coefficient of estimation is presented in Table 3.

The response surface plot as shown in Figure 2c, shows that with increasing level of pomegranate juice a significant increase in the cohesiveness of product was found. Teng et al. [33] found that the increases in starch concentration and temperature the cohesiveness also increases.

Effect of process variables on sensory properties of blended juice

Colour: The quadratic equation obtained by the RSA of the data showing the effect of A, B and C is as follows:

$$\text{Colour} = 6.70+0.64*A-0.19*B-0.081*C+0.034*A*B+0.059*A*C-0.034*B*C+0.26*A^2-0.096*B^2-0.043*C^2 \quad (8)$$

The coefficient of determination (R^2) was 0.87. The "Pred R-Squared" of 0.62 is in reasonable agreement with the "Adj R-Squared" of 0.75. "Adeq Precision" was 14.64 which is higher than 4, indicating an adequate signal. Hence, the model could be used to navigate the design space. The minimum and maximum colour score varied from 6.0 to 8.5 on a 9-point hedonic scale (Table 1). The quadratic model for colour was found to be significant ($p<0.05$). The coefficient of estimation is presented in Table 3.

Figure 3a shows the effect of the levels of pomegranate juice on the colour score of blended juice. It was analysed that with the increased levels of pomegranate juice, the appearance of colour increased, whereas the increased concentration of ginger juice showed no effect on the colour of the blended juice.

Pomegranate acceptability by consumers and processors depends

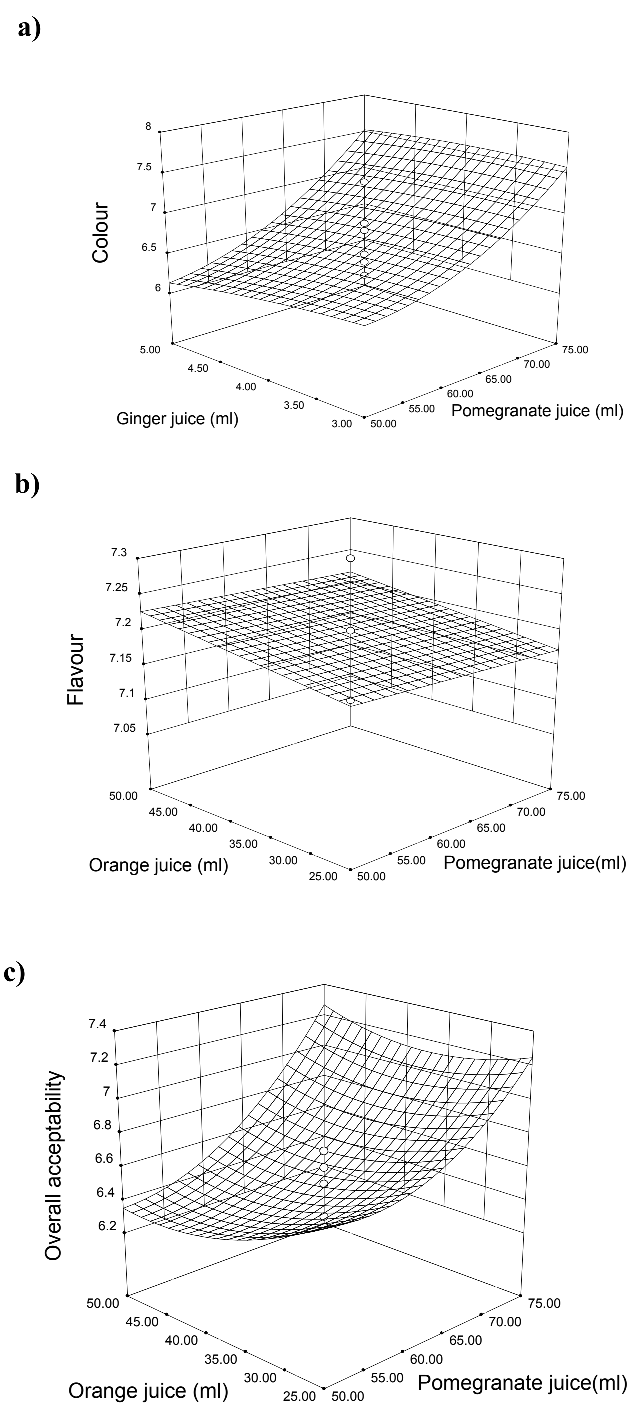

Figure 3: Effect of process variables on sensory properties of blended fruit juice; **a)** Pomegranate and ginger juice Vs colour, **b)** Pomegranate and orange juice Vs flavour, **c)** Pomegranate and orange juice Vs overall acceptability

Factors						Responses						Desirability
A	B	C	VC	DPPH	TPC	VI	CH	CO	CL	FL	OAA	
75.00	50.00	3.00	42.5201	39.4638	199.752	4.60721	17.6414	487.457	7.36081	7.89595	7.29819	0.772899
75.00	50.00	3.02	42.4682	39.5128	199.774	4.613	17.6422	487.424	7.36151	7.8792	7.29563	0.768966
74.86	50.00	3.00	42.3752	39.5973	199.811	4.61787	17.6415	487.143	7.3486	7.89587	7.28891	0.768515
75.00	50.00	3.04	42.5102	39.3693	199.736	4.61644	17.6414	487.404	7.36192	7.86913	7.29414	0.767199
75.00	48.71	3.00	42.5062	39.3362	199.73	4.55783	17.6418	485.971	7.39237	7.89368	7.28184	0.76593
74.67	50.00	3.00	42.4011	39.4678	199.78	4.63335	17.6464	486.7	7.33148	7.89429	7.27567	0.763487
75.00	50.00	3.07	42.5008	39.2974	199.722	4.62416	17.6483	487.357	7.36276	7.84619	7.29088	0.763157
74.56	50.00	3.00	42.1648	39.775	199.888	4.64159	17.6425	486.441	7.32112	7.89613	7.26818	0.76095
75.00	47.33	3.00	42.4983	39.2892	199.441	4.50558	17.6501	484.441	7.42386	7.89126	7.26727	0.759403
75.00	50.00	3.11	42.4917	39.2276	199.647	4.63139	17.6529	487.311	7.36347	7.82424	7.28793	0.759271

Table 4: Optimized solutions of juice blending with desirability by Design Expert 8.0.3.

basically on a combination of several quality attributes as rind colour, sugar content, acidity, and flavour. The blending of kinnow juice (87%), pomegranate juice (10%) and ginger juice (3%) recorded higher score for colour 7.27 [34].

Flavour: The linear equation obtained by the RSA of the data showing the effect of A, B and C is as follows:

Flavour = 7.20-4.992E-003*A+0.022* B-0.68 * C (9)

The coefficient of determination (R^2) was 0.97. The "Pred R-Squared" of 0.96 is in reasonable agreement with the "Adj R-Squared" of 0.97. "Adeq Precision" was 51.53 which is greater than 4, indicating an adequate signal. "Adeq Precision" measures the signal to noise ratio. A ratio of 7.94 indicates an adequate signal and therefore this model could be used to navigate the design space. Hedonic flavour score ranged from 5.9 to 8.5 (Table 1). The coefficient of estimate presented in Table 3 showed that the quadratic model was significant (P<0.05).

The response surface plot presented as Figure 3b shows the effect of levels of pomegranate juice and orange juice. The level of ginger juice increases the flavour score decrease significantly. Balachandran et al. [35] reported the characteristic flavour of ginger, oleoresin, present in the ginger responsible for the pungent flavour. Thus the increase the concentration of the ginger juices directly affects the flavour of the mixed juices.

Overall acceptability: The quadratic equation obtained by the RSA of the data showing the effect of A, B and C on overall acceptability (OA) score is shown below:

OA = 6.55 + 0.37 *-0.081 * B- 0.079 * + 0.091 * A * B +0.039* A*C+0.089 *B * C+ 0.22 *A^2 + 0.12 * B^2 + 0.077 * C^2 (10)

The coefficient of determination (R^2) was 0.93. The "Pred R-Squared" of 0.72 is in reasonable agreement with the "Adj R-Squared" of 0.87. "Adeq Precision" was 13.40 which is higher than 4, indicating an adequate signal. Hence, the model can be used to navigate the design space. The overall acceptability score ranged from 6.3 to 7.9 (Table 1). The coefficient of estimation is presented in Table 3. The coefficient estimates of overall acceptability score shown in Table 3 showed that quadratic model terms (A, A^2, and B^2) were significant (p<0.05).

The response surface plot presented as Figure 3c shows the effect of pomegranate and orange juice on OA score of blended juice. Increasing the levels of pomegranate juice produces a significant effect on OA score but higher the amounts of orange juice had the negative effect on OA score. The overall acceptability of the blended pomegranate, orange and ginger juices were found to be superior as compared to juices prepared from individual fruit.

Conclusion

The optimized level of pomegranate, orange and ginger juice for the manufacture of the nutritional and functional is predicted based on score of antioxidant activity, total phenol content, vitamin-C, viscosity index, consistency, cohesiveness, colour, flavour and overall acceptability score using RSM. Out of 10 suggested formulations, the formulation no. 1 had better overall rating of 7.29 than all other formulations and the desirability was 0.77, the highest amongst all other formulations (Table 4). Hence, the formulation with 75 ml pomegranate juice, 50 ml orange juice and 3 ml ginger juice was considered to be the most appropriate for manufacturing nutritional and functional drinks with the predicted scores of antioxidant activity 39.46%, total phenol 199.75 mg/100 ml TAE, vitamin-C 42.52 mg/100 ml, potassium 2380 mg/L, calcium, 65 mg/L, magnesium 126 mg /L, viscosity index 4.60 g.sec, consistency 487.45 g, cohesiveness 17.64 g, colour 7.3 and flavour 7.8.

References

1. Cassano A, Drioli E, Galaverna G, Marchelli R, Di-Silvestra G, et al. (2003) Clarification and concentration of citrus and carrot juices by integrated membrane processes. J Food Eng 57: 153-163.
2. Gil MI, Tomas-Barberan FA, Hess-Pierce B, Holcroft DM (2000) Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J Agric Food Chem 48: 4581- 4589.
3. Hong MY, Seeram NP, Heber H (2008) Pomegranate polyphenols down-regulate expression of androgen-synthesizing genes in human prostate cancer cells overexpressing the androgen receptor. J NutrBiochem 19: 848-855.
4. Afaq F, Saleem M, Krueger CG, Reed JD, Mukhtar H (2005) Anthocyanin and pathways hydrolyzable tannin-rich pomegranate fruit extract modulates MAPK and NF-kappa B and inhibits skin tumorigenesis in CD-1 mice. Int J Cancer 113: 423-433.
5. Gardner PT, White TAC, McPhail DB, Duthie GG (2000) The relative juices contributions of vitamin-C, carotenoids and phenolics to the antioxidant potential of fruit. Food Chem 68: 471- 474.
6. Campos FM, Chaves JBP, Azeredo RMCD, Mata GMSC, Pinheiro-Santana HM (2010) Adequate handling conditions to preserve vitamin-C and carotenoids in tomatoes. J Food Qual 33: 230-245.
7. Chung SK, Kim SH, Choi YH, Song EY (2000) Status of citrus fruit production and view of utilization in Cheju. Food Ind Nutr 5: 42-45.
8. Son HS, Kim HS, Kwon TB, Ju JS (1992) Isolation, purification and hypotensive effects of bioflavonoids in *Citrus sinensis*. J Korean Soc Food Nutr 21: 136-142.
9. Kim MR, Kim WC, Lee DY, Kim CW (2007) Recovery of narirutin by adsorption on a non-ionic polar resin from a water-extract of *Citrus unshiu*peels. J Food Eng 78: 27-32.

10. De Carvalho JM, Maia GA, Figueiredo RWD, Brito ESD, Rodrigues S (2007) Development of a blended non alcoholic beverage composed of coconut water and cashew apple juice containing caffeine. J Food Qual 30: 664- 681.

11. Jain SK, Khurdiya DS (2004) Vitamin-C enrichment of fruit juice based ready-to-serve beverages through blending of Indian gooseberry Juice. Plant Food Hum Nutr 59: 63-66.

12. Deka BC, Sethi V (2001) Preparation of mixed fruit juice spiced RTS beverages. Ind Food Pack 42: 58-61.

13. Kim JK, Baik MY, Hahm YT, Kim BT (2010) Development and optimization of a drink utilizing citrus (*Citrus unshiu*) peel extract. J Food Process Eng 35: 557-571.

14. Karangwa E, Khizar H, Rao L, Nshimiyimana DS, Foh MBK, et al. (2010) Optimization of processing parameters for clarification of blended carrot- orange juice and improvement of its carotene content. Advance J Food SciTechnol 2: 268- 278.

15. Majumdar TK, Wadikar DD, Vasudish CR, Premavalli KS, Bawa AS (2011) Effect of storage on physico- chemical microbiological and sensory quality of bottlegourd-basil leaves juice. American J Food Technol 6: 226- 234.

16. SitiMazlina MK, Abdul Ghani LA, NurAliaa AR, SitiAslina H, Rozita O (2008) Comparison on optimization of star fruit juice using response surface methodology between two Malaysian star fruit varieties. Pertanika J SciTechnol 16: 1-13.

17. Seung SK, Chang KH, Joung KH, Kim AJ (2008) Optimization of preparation conditions of Houttuyniacordatabeverage containing *Saururuschinensis*and *Polygonatumodoratum*by sensory evaluation and response surface methodology. J East Asian Soc Diet Life 18: 1072-1080.

18. Kim DO, Lee KW, Lee HJ, Lee CY (2002) Vitamin C equivalent antioxidant capacity of phenolic phytochemicals. J Agric Food Chem50: 3713-3717.

19. Arnous A, Makris DP, Kefalas P (2001) Effect of principal polyphenol components in relation to antioxidant characteristics of aged red wines. J Agric Food Chem 49: 5736-5742.

20. AOAC (1984) Official Methods of Analysis of the Association of Official Analytical Chemists, Washington, DC.

21. Lane JH, Eynon L (1923) Determination of reducing sugar by Fehling's solutions with methylene blue as indicator. J Soc Chem Ind 42: 32-36.

22. AOAC (1990) Official methods of analysis (15thedn) Arlington, VA: AOAC International.

23. AOAC (1995) Official methods of analysis (16thedn) Arlington, VA: AOAC International.

24. AOAC (2000) Official methods of Analysis of the Association of Official Agricultural Chemists, Association of Analytical Chemists, Washington, DC.

25. IDF (2001) Determination of total nitrogen content- Part 1 Kjeldahlmethod.IDF standard 201, Brussels, Belgium.

26. Amerine MA, Pongborn RH, Roescler EB (1965) Principles of Sensory Evaluation of Food. Academic Press, New York, USA.

27. Derringer G, Suich R (1980) Simultaneous optimization of several response variables. J QualTechnol12: 214-219.

28. Montgomery DC (2001) Design and analysis of experiments. (5thedn) Wiley, New York, USA.

29. Gonzalez-Molina E, Moreno DA, García-Viguera C (2008) Genotype and harvest time influence the phytochemical quality of fino lemon juice (Citrus limon (L) Burm F) for industrial use. J Agric Food Chem 56: 1669-1675.

30. Gonzalez-Molina E, Moreno DA, García-Viguera C (2009) A new drink rich in healthy bioactive combining lemon and pomegranate juices. Food Chem 115: 1364-1372.

31. Akbulut M, Coklar H, Ozen G (2008) Rheological characteristics of juniperrusdrupecea fruit juice (pekmez) concentrated by boiling. Food SciTechnolInt 14: 321-328.

32. Hobani AI (1994) Rheological properties of pomegranate juices. J King Saud Univ 6: 203-218.

33. Teng LY, Chin NL, Yusof YA (2011) Rheological and textural studies of fresh and freeze thawed native sago starch-sugar gels. J Food Hydrocolloids 31: 156-165.

34. Bhardwaj RL, Mukherjee S (2011) Effect of fruit juice blending ratios on kinnow juice preservation at ambient storage condition. African J Food Sci 5: 281-286.

35. Balachandran S, Kentish SE, Mawson R (2006) The effect of both preparation method and season on the supercritical extraction of ginger. Sep PurifTechnol 48: 94-105.