



Optimization of Ingredients using Response Surface Methodology (RSM) for Development of Instant Mango Smoothie Mix

SAHANA HEVLIN S., LAHARI S., JANIFER RAJ X.* , DAYASHANKAR GUPTA AND CHAUHAN O.P.

Defence Food Research Laboratory, DRDO, Mysore - 570011, India

*Corresponding author E-mail: janiferxavier@gmail.com

Abstract: Optimization of ingredients for the development of instant mango smoothie mix based on spray dried powders as major ingredients was carried out. Effect of the ingredients on pH, total soluble solids (TSS) and overall acceptability (OAA) was investigated based on Response Surface Methodology (RSM). The optimized formulations contain (g/100g) spray dried mango powder (40 g), spray dried milk powder (20 g), strawberry powder (15 g), chia seeds (15 g) and sugar (10 g) with overall acceptability (OAA) of 8.6 in 9 point Hedonic scale while the pH and TSS was found to be 4.9 and 28.7, respectively. Experimental design used for optimization was central composite design (CCD) and those obtained were analyzed using Analysis of variance (ANOVA) and a desirability profile for ingredients was derived. Main effects of spray dried mango powder and milk powder were found to influence response studies such as pH, TSS and OAA. A significant ($p < 0.05$) main effect of spray dried mango and milk powder. Developed product was analyzed for physico-chemical parameters such as proximate composition, color values, total phenolic content (mg GAE/g), total flavonoids content (mg CE/g), antioxidant activity (DPPH) and reducing sugar content. Microbiological load based on total plate count (CFU/g) was found desirable up to 48h at 37° for developed products which were stored in four layered packaging material. Secondary data collection from 100 respondents using an online questionnaire indicated the growing interest of consumers for instant smoothie mix.

Keywords: Instant smoothie mix, Response surface methodology, Physico-chemical parameters, Antioxidant activity, Secondary data collection.

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1. Introduction

The demand in the modern food industry is towards longer shelf-life and product variations leading to quality innovation in food engineering. The increasing world population and prioritization of food safety has led researches on an alternative approach in food preservation. A conventional method, spray drying, has gained back recognition in fulfilling those demands and emphasized on low operational expenditures in comparison to other heating methods, specifically eight times more economical than freeze drying and four times more economic than vacuum drying (Rodriguez-Hernandez *et al.*, 2005). Spray drying is a drying method that produces droplets of liquid feed into powdered products. The conversion involves atomization of liquid feed, undergoing heat treatment to reduce its moisture content to the desired level (Shabde *et al.*, 2010). The capability of spray drying enveloped in its high nutrient and flavor retention with rapid moisture evaporation during the conversion of liquid feed material into dried powdered form, leads to high powder stability and resistance to microbiological and oxidative degradation (Tan *et al.*, 2011). Commercialization value of the spray drying process depends on process yield, end product characteristics and production cost (Shishir *et al.*, 2017).

Spray-drying is a unit operation by which a liquid product is atomized in a hot gas current to instantaneously obtain a powder. The spray-drying process has been developed in connection with the manufacture of dried milk. The initial liquid feeding the spray-dryer can be a solution, an emulsion or a suspension (Gharsallaoui *et al.*, 2007). The resulting dried product conforms to powders, granules or agglomerates, the form of which depends upon the physical and chemical properties of the feed and the dryer design and operation (Filkova *et al.*, 2007). The characteristics of spray-dried fruit juice and pulp powders depend on spray-drying conditions including concentration of drying aid used, inlet air temperature, feed flow rate, feed characteristics etc. (Fazaeli *et al.*, 2012). Spray-dryers come in different patterns including co-current, counter current and mixed flow. Concurrent spray-dryers (where the feed droplets travel in the same direction as that of the drying gas flow) are most common and widely used dryers when compared to other systems (Zbicinski *et al.*, 2002).

Beverages from fruits and vegetables are important in the human diet, as they are pleasant and satisfying, due to their aesthetic and refreshing qualities. However, most of the carbonated beverages are thirst quenchers and will

not provide any nutrients. Thus there is plenty of scope for the development of ready to reconstitutable beverages of high nutritional value using locally available spray dried fruit powders. Even though many processed products are available, dehydrated products always have an upper hand in the consumer preferences. Among all dehydrated products, instant mixes have gained popularity in recent years, by way of providing convenience, reduced drudgery, extensible shelf life and easy to carry so that it reduces the transportation cost (Premavalli *et al.*, 2005).

The main aim of the present study was to develop a ready - to - reconstitute smoothie mix using spray dried powders such as milk, mango and strawberry. Product optimization was carried out using Response surface methodology to optimize the ingredients namely milk powder and spray dried mango powder. The best combination among them was decided based on the overall acceptability score (OAA) based on sensory analysis using the 9-point Hedonic scale. Further physico - chemical, proximate, nutritive, bio - chemical and microbiological studies were performed.

2. Materials and Methods

2.1. Raw Materials

All raw materials and ingredients required for the development of the product were purchased from M/S Mevive International Food Ingredients Company, Coimbatore, Tamil Nadu, India and the milk powder was bought from local markets of Mysore, Karnataka.

2.1.1. Reagents and Chemicals

All the chemicals used in this study were purchased from M/s. HiMedia Laboratories Pvt. Ltd (Mumbai, India), and SRL chemicals (Chennai, India).

2.2. Methods

2.2.1. Preliminary Trials

On an experimental basis, several smoothie variants were made using different spray dried fruit powders. Among those, four different fruit powders were chosen based on the sensory scores in the order mango > banana > strawberry. Mango and strawberry have been designated as the final spray dried fruit powders to be used for statistical optimization.

2.2.2. Optimization of Instant Mango smoothie using Response surface Methodology (RSM)

The RSM (Response Surface Methodology) is a group of mathematical and statistical methods that can be applied to develop, enhance, and optimize processes and is helpful in design, development, and formulation of new products. Quality and process improvement have grown in importance which relies heavily on statistical methodologies such as statistical process control (SPC) and experiment design. It is most effective when quality improvement occurs early in the product and process development cycle. RSM is frequently used directly to achieve quality improvement goals such as decreased variability and improved product and process performance.

2.2.3. Experimental design

The central composite design (CCD) of response surface methodology (RSM) was employed in this study to optimize the two most essential operating variables: Milk powder (% w/w) and mango powder (% w/w). Therefore, various ranges of variables were tested as the preliminary analysis, and the increments were continued until appreciable changes in the process responses (pH, OAA, TSS, and color) were noticed. The optimized mango and milk powder was eventually obtained by analyzing three dependent parameters as responses: pH, OAA, and TSS. Analysis of variance (ANOVA) was used to examine the interactions between the process factors and responses. The quality of the fit of the polynomial model was represented by the coefficient of determination R^2 , and the Fisher's F-test was utilized in the same system to determine its statistical significance. Model terms were assessed using the P-value (probability) with a 95 percent confidence level.

2.2.4. Fitting the model

In developing the regression equation, the test factors were coded according to the following equation:

$$x = \frac{X_i - X_0}{\Delta X_i} \quad (1)$$

where x is the dimensionless coded value, X_i is the actual value of variables, X_0 is the actual value of variables at the center point, and ΔX is the step change value. The experimental results were fitted with a second-order polynomial function:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2 \quad (2)$$

where, Y is the predicted response, b_0 the model constant, b_1 , b_2 the linear coefficient, b_{11} , b_{22} the squared coefficient and b_{12} the interaction coefficient.

2.3. Physico-chemical Analysis of the Developed Instant Mango Smoothie

The optimized formulation of instant mango smoothie was selected and subjected to different physico-chemical, nutritive value and microbiological safety analysis.

2.3.1. Water Activity

Measurement of water activity was carried out using a water activity meter (Aqualab series 3 manual version 5). Water activity was measured in duplicates for 24 h.

2.3.2. Color Measurements

Color parameters (L^* : brightness, a^* : red - green, and b^* : yellow - blue) were measured for prepared Mango smoothie powdered mix and reconstituted mango smoothie mix by using (Color meter, Hunter lab, Color management company, Virginia, United States).

2.3.3. pH

The pH of a food product determines the time and temperature at which it must be submitted to effectively eliminate microbiological and enzymatic activity, ensuring the final food product stability. The darker the color, the higher the pH. For pH, two readings were taken and measured using a handheld pH meter at room temperature (23-25°C).

2.3.4. Viscosity

It is defined as the internal friction of liquid or ability to resist flow. Internal friction in a fluid is demonstrated experimentally by looking at a liquid that has been ferociously agitated to form a vortex. In a large beaker, 400ml of sample is taken and the probe is adjusted by immersing it in the sample, adjusting the rpm, and running it. The probe rotates in accordance with the rpm and displays the results on the screen. The given value is recorded as Centipoise.

2.3.5. Total soluble solids (TSS)

Total soluble solids are solids that dissolve in a liquid. Sugar is a common total soluble solid. The index of refraction determines the total soluble solids content

of a solution. This is measured with a Hand refractometer and is known as the degrees Brix (ATAGO PAL-BX/RI).

2.3.6. Titratable Acidity

A solution of 0.1 N NaOH was prepared and used as a burette solution. 5g of sample was weighed, crushed with distilled water, and the contents were filtered using normal filter paper. The filtrate was collected and distilled with 100 ml of distilled water. In a conical flask, place 10ml of the aliquot and phenolphthalein indicator was added; titrate was added against NaOH until the pale pink color was obtained. Take note of the titer value that was obtained.

$$\text{Acidity(\%)} = \frac{\text{Titer Value} \times \text{Normality of NaOH} \times \text{Volume mada up} \times 100}{\text{Volume of aliquot} \times \text{weight of sample} \times 1000}$$

2.3.7. Bulk and Tapped Density

Approximately 2 g of powder was transferred to the 10 mL graduated cylinder. The bulk density was calculated by dividing the mass of the powder by the volume occupied in the cylinder.

For the Tapped density (qt), the cylinder was tapped steadily and continuously on the surface by hand until there was no further change in volume (Jinapong *et al.*, 2008).

2.3.8. Reconstitution Ratio (Rehydration)

The process of rehydrating dried foods by immersing them in water or another liquid to restore them to their original size, shape and texture. It is a measure of water absorption by the dehydrated product, calculated using the following equation as given by Basantpure *et al* (2003).

$$\text{Rehydration ratio} = X/Y$$

Where, X = Final weight after reconstitution

Y = Initial weight before reconstitution

2.4. Proximate Composition

Moisture, protein, fat, ash, crude fiber and carbohydrate content of Instant Mango smoothie were determined by following standard methodology (AOAC 2019).

2.5. Antioxidant activity

2.5.1. Determination of antioxidant activity by DPPH method

Antioxidant capacity of the developed product was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and ferric reducing antioxidant property (FRAP) following the method of Brand-Williams *et al.* (1995) and Benzie and Strain (1999), respectively.

2.5.2. Determination of Total phenolic content (TPC)

Total phenolic contents were determined based on Folin–Ciocalteu colorimetric method as described by Jithendran *et al.* (2021). They were expressed as gallic acid equivalents (mg GAE mg/100 g). Total phenolic content was calculated as the sum of extractable and hydrolysable fractions.

2.5.3. Estimation of Total flavonoid content (TFC)

The total flavonoid content of the extracts was determined using the procedure described by Sagolsem *et al.* (2021).

2.6. Determination of Reducing and Total sugars

The reducing and total sugars in the extracts were determined using the 3, 5-Dinitrosalicylic acid (DNS) method presented by Saquib *et al.* (2011).

2.7. Determination of Ascorbic acid (Vitamin-C) content

Ascorbic acid content in the extracts were determined using 2, 6 dichlorophenol-indophenol dye, where ascorbic acid reduces the indicator dye to a colorless solution. At the endpoint of titrating an ascorbic acid-containing sample with dye, excess unreduced dye is a rose-pink color in the acid solution. The titer of the dye can be determined using a standard ascorbic acid solution (Dilrukshi and Senarath, 2021).

3. Sensory Analysis

Sensory analysis is a scientific field that studies experimental design and statistical analysis principles to the use of human senses in the evaluation of consumer products. The discipline necessitates panels of human faculty members who test the products and record their responses. Hedonic rating refers to pleasant or unpleasant perceptions. It can be used to assess product acceptability. The samples were displayed to the panel, and a 9 rating scale

ranging from “like extremely” to “dislike extremely” was assigned. The finished product was evaluated by a trained panel of ten people. The sensory evaluation parameters were chosen based on the nature and composition of the sample kept for evaluation. The panel was given an appropriate scale of varying levels of acceptability perception ranging from 9 points for ‘extremely excellent’ to 1 point for ‘dislike extremely’.

The sensory properties thus evaluated by the panelists were Mean = X/N , where X is the total score sum and N is the total number of attributes.

4. Statistical Analysis

All the analytical assays were conducted in triplicates. Mean and standard deviation (SD) were calculated using Microsoft Excel 2010, from the replicated data for all the parameters. Data obtained after conducting design expert experiments in 16 experiments, data was assessed statistically for central composite design (CCD). All the results were statistically processed using Statistica Version 7.0 (Statsoft, USA).

5. Results and Discussions

5.1. Preliminary Trials

Variants of smoothie mix were prepared using spray dried powders of banana, strawberry, mango and dehydrated butterfly pea powder in combination with spray dried yogurt and milk powder. Among the smoothie variants, spray dried mango based smoothie was finalized based on the highest overall acceptability (OAA) score (8.6) carried out by 15 semi - trained panelist members.

5.1.1. Optimization of ingredients using response surface methodology (RSM)

Table 1: Experimental design matrix in terms of actual, coded factors, and the observed values for the response OAA, pH and TSS.

Independent Variables	Units	Symbol	Coded Levels				
			$-\alpha(axial)$	-1	0	+1	$+\alpha(axial)$
Mango powder	%	X_1	30.17	40.0	60.0	80.0	83.0
Milk Powder	%	X_2	13.8	20.0	35.0	50.0	56.2

Runs	Mango powder	Milk Powder	OAA Observed	Predicted	Residuals	pH Observed	Predicted	Residuals	TSS Observed	Predicted	Residuals
1	40.00000	20.00000	8.6	8.352086	0.247914	4.9	5.025627	-0.125627	28.7	28.07643	0.62357
2	40.00000	50.00000	8.2	8.072879	0.127121	5.5	5.540319	-0.040319	34.4	35.05273	-0.65273
3	80.00000	20.00000	7.7	7.947026	-0.247026	3.4	3.623412	-0.223412	40.3	39.42288	0.87712
4	80.00000	50.00000	7.8	7.939385	-0.139385	4.2	4.292972	-0.092972	41.7	42.21620	-0.51620
5	31.71573	30.50000	8.3	8.566368	-0.266368	5.4	5.332605	0.067395	28.7	28.64503	0.05497
6	88.28427	30.50000	8.4	8.127944	0.272056	3.6	3.426230	0.173770	42.4	42.62086	-0.22086
7	60.00000	9.28680	8.2	8.200784	-0.000784	4.2	4.075807	0.124193	32.4	32.99863	-0.59863
8	60.00000	51.71320	7.6	7.589071	0.010929	5.3	5.226047	0.073953	39.2	37.98130	1.21870
9 (C)	60.00000	30.50000	7.5	7.413057	0.086943	5.2	5.019623	0.180377	30.6	33.22074	-2.64074
10(C)	60.00000	30.50000	7.6	7.413057	0.186943	5	5.019623	-0.019623	32.9	33.22074	-0.32074
11(C)	60.00000	30.50000	7.5	7.413057	0.086943	4.9	5.019623	-0.119623	33.8	33.22074	0.57926
12(C)	60.00000	30.50000	7.2	7.413057	-0.213057	4.8	5.019623	-0.219623	31.2	33.22074	-2.02074
13(C)	60.00000	30.50000	7.4	7.413057	-0.013057	5.1	5.019623	0.080377	35.8	33.22074	2.57926
14(C)	60.00000	30.50000	7.5	7.413057	0.086943	4.9	5.019623	-0.119623	34	33.22074	0.77926
15(C)	60.00000	30.50000	7.4	7.413057	-0.013057	5.2	5.019623	0.180377	32.2	33.22074	-1.02074
16(C)	60.00000	30.50000	7.2	7.413057	-0.213057	5.1	5.019623	0.080377	34.5	33.22074	1.27926

Ingredients for product formulation were optimized based on the experimental layout of RSM with 2 independent factors and 3 dependent responses. One replication with no blocks was designed resulting in 16 combinations. After product formulation, responses including pH, TSS, OAA were studied. In the present study, the relationship between response functions such as OAA, pH & TSS. Process variables (ingredients) were finalized from previous OAA sensory scores of variants prepared using different spray dried powders. The predicted and actual response values from 16 runs based on the experimental design are shown (Table 1). OAA ranged from 7.2 to 8.6 in a 9 point Hedonic scale while pH and TSS ranged from 3.4 to 5.5 and 30.6 to 42.4, respectively. The highest OAA was observed at runs number one with mango at 40 % and milk powder at 20 % along with other ingredients as shown (Table 2). pH was found to be 4.9 while TSS was 28.7 for the optimized composition based on RSM.

Table 2: Composition of Smoothie Mix

Ingredients	Quantity (g/100 g)
Spray dried mango powder	40.0
Spray dried milk powder	20.0
Strawberry powder	15.0
Chia seeds	15.0
Sugar	10.0
TOTAL	100

5.1.2. Fitting the model for OAA

The second order polynomial model equation was obtained by fitting the results obtained as shown in Eq.3.

$$\text{OAA (Y)} = 13.931 - 0.155x_1 - 0.0933x_2 - 0.00117x_1^2 + 0.00107x_2^2 + 0.00023x_1x_2 \quad (3)$$

$$\text{pH (Y)} = 2.808 + 0.0008x_1 + 0.0693x_2 - 0.0008x_1^2 - 0.00081x_2^2 + 0.00013x_1x_2 \quad (4)$$

$$\text{TSS (Y)} = 23.982 - 0.00845x_1 + 0.01899x_2 + 0.00302x_1^2 + 0.0050x_2^2 + 0.00349x_1x_2 \quad (5)$$

ANOVA results indicated that the model is significant (Table 3). The p value of F test ($p < 0.05$) determined the significance of each regression coefficient. In the present study, a close relationship was observed between the observed experimental values and predicted values which indicated the satisfactory nature of the model. R^2 values (coefficient of regression) which determines the fit of the model was 0.85 for OAA, 0.95 for pH and 0.90 for TSS values of the developed products. The model was confirmed significant at the level of 0.05 % probability level with R^2 and adjusted R^2 values $> 80\%$ indicating the aptness of the model.

Table 3: Regression analysis (ANOVA) for OAA, pH and TSS of instant mango smoothie

Regression	SS	DF	MS	F-value	p-value (Prob>F)
OAA R²: 0.84626; Adj: 0.76938					
Mango powder(L)	0.139033	1	0.139033	3.07657	0.109964
Mango powder(Q)	1.735538	1	1.735538	38.40455	0.000102
Milk powder(L)	0.040327	1	0.040327	0.89237	0.367099
Milk powder(Q)	0.503653	1	0.503653	11.14499	0.007511
1L by 2L	0.019267	1	0.019267	0.42634	0.528515
Error	0.451910	10	0.045191		
Total SS	2.939375	15			
pH R²: 0.95046; Adj: 0.92568					
Mango powder(L)	3.365177	1	3.365177	117.3243	0.000001
Mango powder(Q)	0.815242	1	0.815242	28.4228	0.000332
Milk powder(L)	0.687355	1	0.687355	23.9641	0.000627
Milk powder(Q)	0.294853	1	0.294853	10.2798	0.009392
1L by 2L	0.006266	1	0.006266	0.2185	0.650238
Error	0.286827	10	0.028683		
Total SS	5.789375	15			
TSS R²: 0.90948; Adj: 0.86423					
Mango powder(L)	164.2361	1	164.2361	65.22572	0.000077
Mango powder(Q)	11.5737	1	11.5737	4.59647	0.057651
Milk powder(L)	46.7788	1	46.7788	18.57802	0.001537
Milk powder(Q)	11.1692	1	11.1692	4.43582	0.061440
1L by 2L	4.5712	1	4.5712	1.81542	0.207594
Error	25.1797	10	2.5180		
Total SS	278.1784	15			

5.1.3. Effect of ingredients on OAA, pH and TSS of the Instant Mango smoothie

Main effects of Spray Dried Mango powder ($p=0.000$) and Milk powder ($p=0.001$) in a quadratic form was found to have a significant effect on OAA while main effects of Spray Dried Mango powder ($p<0.05$) and milk powder ($p<0.05$) was found to be significant in linear fashion for TSS. Furthermore, the effects of tested ingredients affect pH in linear and quadratic fashion as indicated by the Pareto chart (Figure 1) and p values of ANOVA analysis (Table 3). Two way interactive effects of the ingredients tested were not found to be significant ($p>0.05$) on the responses OAA, pH and TSS (Figure 1) (Table3).

The Pareto chart of standardized effects displays the absolute values from largest to smallest. On the pareto chart, bars that cross the reference line are statistically significant while the reference line is indicated in red color. Hence, bars that represented factors that spray dried mango and milk powder crossed

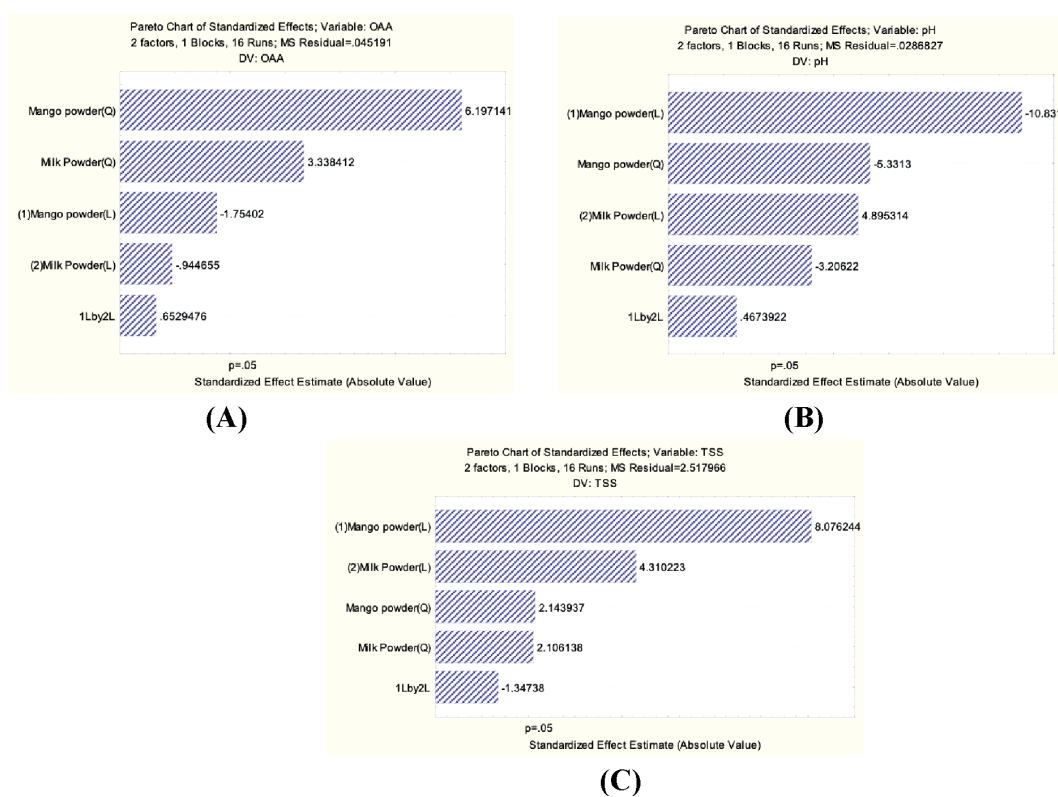


Figure 1: Pareto chart of standardized effects to analyze central composite design of (A) OAA, (B) pH and (C) Total soluble solids (TSS)

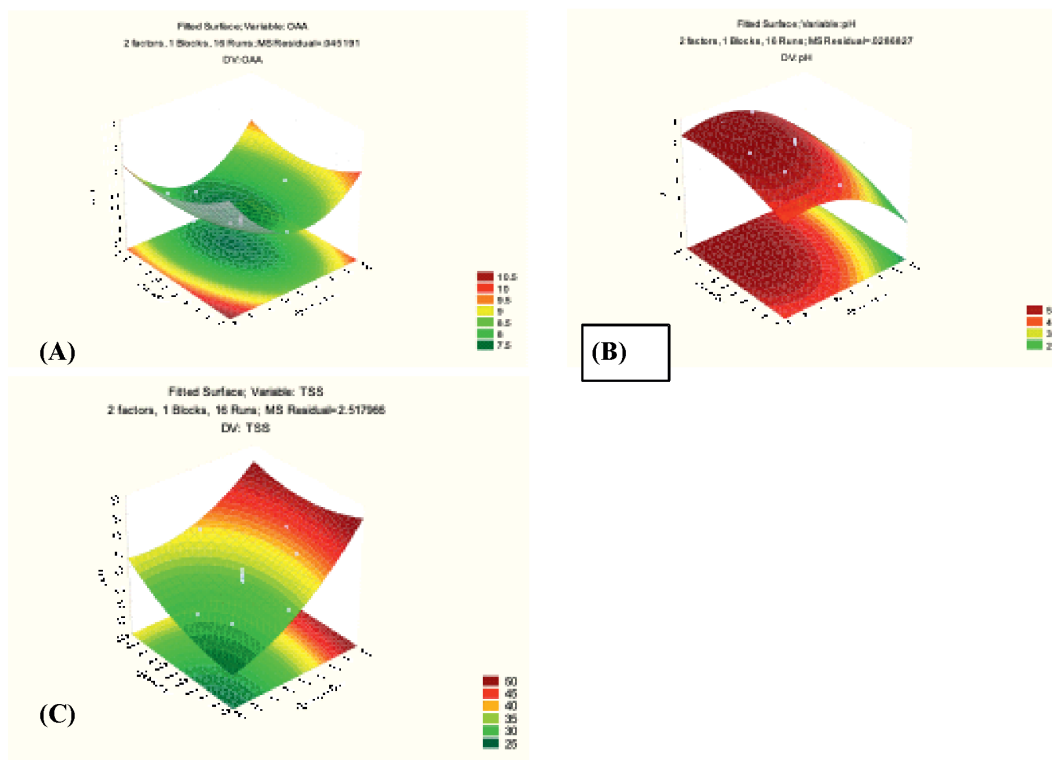


Figure 2: 3D Response surface graphs of (A) OAA, (B) pH and (C) Total soluble solids (TSS)

the reference line were found to be statistically significant at $p < 0.05$ level at the current model terms (Figure 1). 3D response surface graphs are the graphical representation of the regression equation and shape of the surface plots indicating the linear and quadratic effect of the tested independent variables on the dependent responses (OAA, pH and TSS) (Figure 2). Ready-to-reconstitute mango smoothie was reconstituted into smoothie in 5 minutes. Reconstitution in the ratios of 1:1 and 1:3 resulted in unacceptable consistency. The product with a reconstitution ratio of 1:2 was middling and viscous.

Effect of factors on OAA, pH and TSS and desirability function with changes in ingredients such as mango and milk powder is shown (Figure 3). The horizontal dotted line on the response graph shows the highest OAA achieved by this model (8.6). The horizontal line on the desirability graph indicates the maximum achieved desirability (0.78680). The vertical lines correspond to the approximate optimal value of the factors i.e the parameters at which the maximum OAA and desirability is achieved: 51.71 (% w/w) mango powder

and 31.72 (% w/w) milk powder; at this specified levels OAA was 8.6. The desirability function between 0 and 1 indicates the degree of performance for the predicted response with the given factors corresponding value and the mean value of the remaining factors. Contour plot for desirability based on quadratic fit for OAA, pH and TSS is shown (Figure 3).

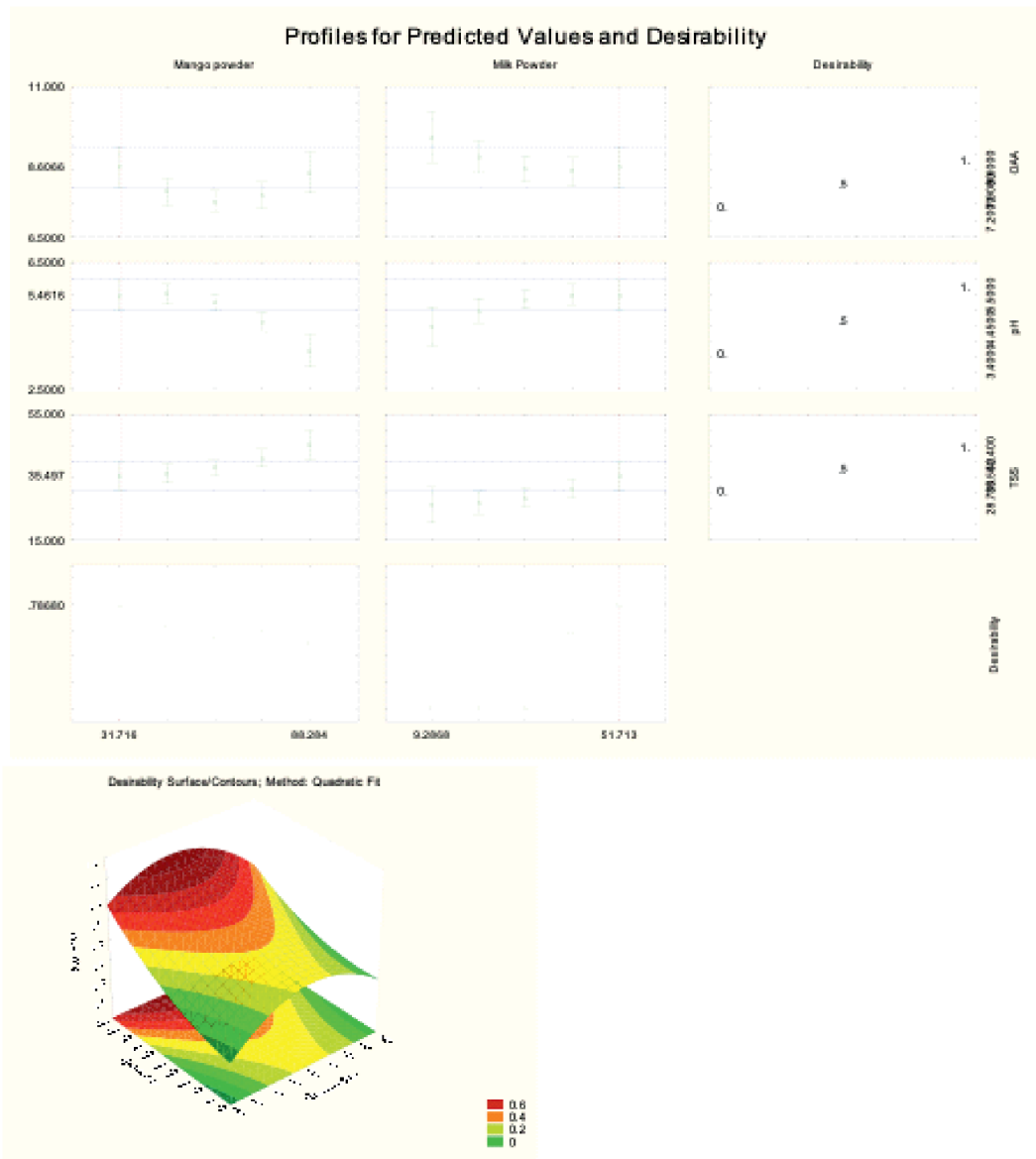


Figure 3: Effect of factors on OAA, pH and TSS and desirability function with changes in ingredients such as mango and milk powder

5.2. Proximate composition of the Instant mango smoothie mix

Proximate analysis refers to a method for determining the levels of macronutrients in food samples. Instant Mango Smoothie mix was evaluated for moisture, fat, protein, ash, fiber and carbohydrates (Table 4). Bunkar *et al.* (2014) optimized the process for manufacturing of an instant *kheer* mix based on pearl millet. The 3-factor Central Composite Rotatable Design optimized the ingredients such as dairy whitener, pearl millet, and powdered sugar. Pearl millet dry *kheer* mix got reconstituted into *kheer* in approximately 5 minutes, compared to 40–45 minutes for the traditional rice-based *kheer*. The pearl millet *kheer* mix was composed of moisture, fat, protein, carbohydrate, and ash at 2.80, 4.38, 5.84, 5.88, and 1.10 percent, respectively.

Table 4: Proximate composition and physico-chemical and of Instant mango smoothie

Parameter		Instant mango milk smoothie mix
Moisture (%)		2.51±0.288
Fat (%)		1.84±0.30
Protein (%)		2.25±0.25
Fibre (%)		34.15±2.11
Ash (%)		2.68±0.30
Carbohydrates (%)		90.63±0.80
pH		4.073±0.01
TSS		32.3±0.1
Acidity		1.3333±0.57
Total flavonoid content (mg CE/g)		2.45 ± 0.40
Total phenolic content (mg GAE/g)		19.67 ± 0.58
DPPH (% inhibition)		67.18 ± 8.98
Reducing sugars (%)		20.747 ± 0.39
Total sugars (%)		14.052 ± 0.69
Ascorbic acid (mg/100g)		73.33 ± 11.54
Water activity	Before reconstitution	0.468±0.01
	After reconstitution	0.972 ±0.004
Color	L*	43.08 ± 0.91
	a*	3.14 ± 0.19
	b*	29.16 ± 0.98
Values are mean ± standard deviation (S.D) of 3 replicates of mango milk smoothie		

The moisture level of food products is a crucial determinant of their quality and long-term usage since it encourages the growth of microorganisms, which degrades food quality by causing early damage. Kokani *et al.* (2019) reported that instant brown rice kheer mix had moisture content of 3.6 % . The moisture content of instant mango smoothie powder was 2.51 ± 0.288 percent. Moisture content can affect physical attributes of powder products, including hardness and coagulation. Body stores fat as a concentrated energy source, which is then used when the supply of carbohydrates is exhausted. As a cushion and protector of internal organs, such as the heart, kidneys, lungs, and intestine, fat also plays a crucial role (Mohammed *et al.*, 2017). Instant mango smoothie developed in the present study had a fat content of 1.84 ± 0.30 percent. According to Dilrukshi *et al.* (2021), fat content of instant green smoothie powder was 1.96 % which was similar to our results. Proteins are nitrogen-containing substances that are formed by amino acids. They serve as the major structural component of muscle and other tissues in the body. In addition, they are used to produce hormones, enzymes and hemoglobin. Proteins can also be used as energy; however, they are not the primary choice as an energy source. For proteins to be used by the body they need to be metabolized into their simplest form, amino acids. Protein content of developed instant mango smoothie was 2.25 ± 0.252 percent which was found to be similar to the protein content of instant mango drink powder (Akther *et al.* , 2020). Total ash content is the reflection of the mineral composition of food samples . Ash content of instant mango smoothie was found to be 2.68 ± 0.30 % . Panda *et al.* (2023) reported that 10 % horse gram extract incorporated smoothie had 0.81 ± 0.05 . Ash content of developed instant mango smoothie powder concentration suggests that it is a superior source of minerals. The fiber content of instant smoothie was 34.15 ± 2.11 . Fiber is the portion of plant-based food and is crucial in maintaining a healthy weight, preventing hypertension, balancing blood cholesterol levels, and controlling blood sugar. Carbohydrate content of the optimized instant Mango smoothie mix was 90.63 ± 0.80 . The result revealed that the instant smoothie mix is rich in carbohydrate content and capable of providing energy (388.08 kcal/100 g)

5.3. Physico-chemical characterisation of the developed product

5.3.1. CIE Color values

Color plays an essential role in the appearance of food materials. It acts as an indicator of quality, which influences the surface, taste perception, and

product appearance. The value of the parameter L^* in the analyzed instant mango smoothie mix was 43.08 ± 0.91 . The parameter redness was indicated as a^* (3.14 ± 0.19) whereas yellowness was represented by b^* (29.16 ± 0.98) (Table 4).

5.3.2. pH and Acidity

In food analysis, titratable acidity and pH are two related concepts that deal with acidity. Acid nature does not make it more susceptible to spoilage and pathogenic organisms which can be encountered by acidification. Panda *et al* (2023) reported that 10 percent Horsegram incorporated smoothie possessed 5.97 pH. With reference to Dilrukshi and Senarath (2021) initial pH of the fresh and instant green smoothies were 4.22 ± 0.020 and 4.44 ± 0.0385 respectively. pH of instant mango smoothie mix was found out to be 4.073 ± 0.01 (Table 4). Since it was below pH 4.6 pathogens such as *Clostridium botulinum* neither produce botulinum toxin nor germinate and grow (Dilrukshi and Senarath, 2021). Total titratable acidity of instant mango smoothie was 1.33 ± 0.57 . Titratable acidity of the roselle powder and pineapple flavored roselle powders ranged between 3.19 and 4.23 % malic acid (Gbadegesin *et al.*, 2017).

5.3.3. Total Soluble Solids

Brix measures the sugar content of foods and total soluble solids was found to be 3.23 ± 0.1 . Our findings are quite similar to the unflavored roselle sample reported by Gbadegesin *et al.*, (2017). Brix value of instant mango smoothie supplemented with *Moringa olifera* ranged 15– 18° B (Aderinole, 2018).

5.3.4. Water activity

Water activity is an important quality parameter for a dehydrated product to determine the shelf stability. Water activity of the instant mango smoothie powder was indicated as 0.468 ± 0.01 before reconstitution and 0.972 ± 0.004 after reconstitution. The instant green smoothie powder water activity was found to be 0.197 (Dilrukshi and Senarath, 2021).

5.3.5. Bulk and Tapped density

Quality parameters of the instant powder such as bulk and tapped density were assessed to determine the powder properties. The measured powder properties indicated that it had 13.5 ± 0.35 and 10.5 ± 0.5 bulked and tapped density, respectively (Table 4). Caliskan and Dirim (2012) reported that freeze dried pumpkin powder had a bulk density of 0.113 ± 0.006 g/ml. The higher bulk

density is important for storage and packaging of the powder and products with higher bulk density are easy to store in small containers when compared to low bulk density products.

5.3.6. Viscosity

Viscosity increases with increasing particle volume and small particle size (Kristensen *et al.*, 1997). Viscosity of the instant mango smoothie was found to be 107.2 ± 0.91 Cp. The viscosity of cream soup with less tempeh powder was 174.4 ± 4.08 Cp (Lo *et al.*, 2022).

5.4. Phenols, flavonoids and antioxidant activity

Bioactive components of foods are phenols and flavonoids compound with health promoting properties (Xavier *et al.*, 2024). Total phenol content observed in instant mango smoothie was 19.6 ± 0.40 GAE mg/100 g and total flavonoid content was 2.45 ± 0.40 mg QE/100 g. Antioxidant activity of instant mango smoothie was measured by *in vitro* assay (DPPH radical scavenging activity). It was found to be $(67.18 \pm 8.98$ mg TE/100 g) (Table 4). Panda *et al.* (2023) reported that horse gram added smoothie had a DPPH value ranging from 28.18 ± 2.86 to 38.20 ± 5.04 based on percent of horse gram content. Obilana *et al.* (2018) produced quick beverage powder by extruding malted pearl millet (Babla) and found that it has an antioxidant capacity of 1.78 μ mole TE/g. Kumar *et al.* (2021) studied the antioxidant nature of wheat grass-based optimized instant soup mix compared to commercial soup mix. Polyphenols, total flavonoids, and DPPH antioxidant activities for optimized wheat grass-based instant soup mix were 5.43 mg/g, 1.13 mg/g, and an IC_{50} activity of 12.06 mg/g for commercial soup mix.

5.5. Reducing sugar and total sugar content

Reducing sugars and total sugars of the instant mango smoothie were 14.052 ± 0.69 and 20.747 ± 0.39 g/100 g, respectively (Table 4). Gbadegesin (2017) reported that unflavored roselle powder had 2.26 percent reducing sugar and 25 percent pineapple incorporated sample had 7.90 percent.

5.6. Vitamin C content

Vitamin C is a crucial determinant of the dehydrated products because it is susceptible to oxygen, light and heat (Santos and Silva, 2008). Instant mango smoothie contained vitamin C content of 73.33 ± 11.54 mg/g (Table 4). According to Gbadegesin (2017), as the ratio of pineapple powder to roselle

powder increases, the value dropped dramatically. One possible explanation for this could be the vitamin C content that was lost during the oven drying of pineapple. Vitamin C concentration was found to be 148 ppm in the fresh green smoothie sample and 129.5 ppm in the instant green smoothie sample (Dilrukshi and Senarath, 2021).

5.7. Microbiological quality parameters

For microbiological analysis, total plate count is widely used as an indicator of quality of foods. When compared between yogurt and milk powder, the best results were shown by milk powder with nil bacterial colonies. The microbiological parameters of the instant mango smoothie on storage at 37°C is shown (Table 5). A similar study was carried by Goyal *et al* (2021) on optimization of instant foxtail millet based khichdi by using response surface methodology and evaluation of its shelf stability. The product was microbiologically tested at regular intervals and found to be stable throughout the storage period. Another study by Jain *et al* (2016) on process optimization for formulation of *Trigonella foenum graecum* and *Gymnema sylvestre* added vegetable cereal mix using response surface methodology used pour plate method to determine the total microbial load of the optimized vegetable cereal mix. The coliform count and yeast and mold counts were determined using MacConkey agar and Sabouraud dextrose agar, respectively.

Table 5: Microbiological parameter of the functional Instant Mango milk smoothie

	Total Plate Count (CFU/g)	
	24 ± 3 h 37° C	48 ± 3 h 37° C
10 ⁻¹	Nil	Nil
10 ⁻²	Nil	Nil
10 ⁻³	Nil	Nil
10 ⁻⁴	Nil	Nil
10 ⁻⁵	Nil	Nil
10 ⁻⁶	Nil	Nil

6. Secondary data collection using online questionnaire

Monteiro *et al.* (2013) analyzed preferences of consumer attitude towards purchasing intent for ready to drink orange juice and nectar in Brazil. The results showed that the price, brand, and product information had a strong positive influence on purchase of ready-to-drink orange juice and nectar. The ideal label, according to the majority of participants, should have a low

price, a market leading brand, and the information such as “no preservatives/natural.” These findings could aid in the strategic planning of consumer education. In the present study through an online questionnaire we analyzed the preferences of consumers towards different varieties of smoothies. Garrett ranking technique and percentage method was used to analyze the data of sample size 100. Results showed that easily available and healthy in nature are the most influencing factors that impacted consumer consumption and their preference decision.

Out of 100 respondents, ninety-seven (97 percent) knew about smoothies. A total of 100 (55 % male and 45 % female) responses were recorded in the online questionnaire survey. Among the 100 respondents, 76 percent were between the age group of 18-24, 10 percent were between the age group of 26-35, 7 percent were between the age group of 36-45, 2 percent were 45 years of age and above and the rest 5 percent were 18 years of age or less. High number of respondents (68 %) had completed their bachelor degree. The questionnaire was analyzed using three criteria viz. familiarity and knowledge about Instant smoothie, opinion on variety of varieties of smoothies and future preference of consumers on their consumption. Familiarity and knowledge level indicated that 22 percent consumers opted for instant smoothie while the rest 78 percent preferred fresh smoothie. Highest preference was recorded for mango smoothie (59 %), followed by strawberry smoothie (56 %) and banana smoothie (37 %).

7. Conclusions

It is possible to draw conclusion from the current research that the developed instant mango smoothie mix can be considered as a convenience food. In the present fast-paced world, where time and healthcare are the most valuable assets, customers are forced to make quick eating decisions. However, the customer frequently fails to make the best meal choices and ends up eating unhealthy food that is readily available. To address this issue, healthy smoothies with the goodness of milk and fruits are the best replacement for junk food. Quick reconstituting products without the cooking step may be helpful in saving time required for preparation of fresh smoothies. Furthermore, consumers would find the product developed in the present study as light weight, easy to handle, highly convenient to prepare, and nutritious.

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