Optimization of seabuckthorn fruit yogurt formulation using response surface methodology

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Abstract The response surface methodology was used to optimize the formulation of seabuckthorn fruit yogurt. The independent variables were proportions of seabuckthorn fruit syrup and skimmed milk powder. The responses were counts of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, taste and viscosity of the product. Statistical analysis revealed that fruit syrup and skimmed milk powder significantly affected all the responses. Contour plots for each response were used to generate an optimum area by superimposition. Optimum formulation conditions of fruit syrup (15 %) and skimmed milk powder (12.5 %) are recommended for the blend formulation yielding an acceptable and good quality seabuckthorn fruit yogurt. Model validation was conducted using separate experiments at optimum conditions. The experimental values were found to be in close agreement to the predicted values and were within the acceptable limits indicating the suitability of the model in predicting quality attributes of seabuckthorn fruit yogurt. The resultant product also exhibited more amounts of fat, protein, carbohydrate and antioxidants viz., vitamin C, E, carotenoids, phenols and anthocyanins when compared to a commercial one.

Keywords Yogurt • Seabuckthorn • Response surface methodology • *Streptococcus thermophilus* • *Lactobacillus bulgaricus* • Viscosity

Introduction

Yogurt is a fermented milk product obtained through lactic fermentation of milk with *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*. Yogurt is now widely consumed mainly due to its availability in variety of flavors. The fruit enriched yogurt adds variety to the consumer to select. Seabuckthorn (*Hippophae rhamnoides*), is a deciduous shrub with yellow or orange fruits (Li and Schroeder 1996). It is a unique plant currently being domesticated in several countries like China, Russia, Germany, Finland, Romania, France, Nepal, Pakistan and India. The plant is reported to have considerable medicinal value (Li and Wang 1998), being useful for the treatment of skin disorders resulting from bed confinement, stomach and duodenal ulcers, cardiovascular diseases and perhaps growth of some tumors. Seabuckthorn berries are among the most nutritious and vitamin-rich fruit found in the plant kingdom because of presence of several natural antioxidants viz., vitamin C, E, carotenoids, anthocyanins and phenols (Chauhan et al. 2001). The supplementation of yogurt with the fruit will enhance its nutritional quality and also yogurt with the therapeutic value.

Addition of seabuckthorn fruit syrup and skimmed milk powder (SMP) in milk influences the sensory, physical and functional characteristics of yogurt. Several variations in sensory, physical and functional properties of yogurt can be achieved depending on modification and combination of the different variables mentioned above. There is, however, not much reports in the literature to explain how seabuckthorn fruit syrup and skimmed milk powder addition affect the sensory, physical and functional quality of yogurt. The aim of this study was to find out optimum proportions of seabuckthorn fruit syrup and skimmed milk powder for

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Table 1 Real and coded values for the independent variables proportions of seabuckthorn fruit syrup (X_1) and skimmed milk powder (X_2) for preparation of seabuckthorn fruit yogurt

Experiment no.	Proportions of seabuckthorn fruit syrup in blend		Proportions of skimmed milk powder in blend	
	Real value (g) ^a	Coded value (X_1)	Real value (g) ^a	Coded value (X_2)
1	12.0	−1	7.5	−1
2	18.0	+1	7.5	−1
3	12.0	−1	12.5	+1
4	18.0	+1	12.5	+1
5	10.7	−1.414	10.0	0
6	19.2	+1.414	10.0	0
7	15.0	0	6.4	−1.414
8	15.0	0	13.5	+1.414
9	15.0	0	10.0	0
10	15.0	0	10.0	0
11	15.0	0	10.0	0
12	15.0	0	10.0	0
13	15.0	0	10.0	0
14	15.0	0	10.0	0

^a For 100 g of fruit yogurt by keeping whole milk (100 mL), gelatin (0.5 %) and yogurt cultures (2 %) as constant variables for all experimental runs

making seabuckthorn fruit yogurt (SFY) that should have desirable sensory properties i.e. taste, physical properties i.e.

viscosity and functional properties i.e. probiotic bacterial counts.

Materials and methods

Raw materials

Pure freeze dried culture of *S. thermophilus* (NCDC 075) and *L. bulgaricus* (NCDC 008) were procured from National Dairy Research Institute, Karnal, India. These cultures were maintained in 10 % skimmed milk powder (SMP), which was autoclaved at 115° C for 15 min. Standardized cow milk and skimmed milk powder of Nandini brand was procured locally from Mysore Cooperative Dairy, Karnataka, India. Gelatin (Food grade) was purchased from local market. Seabuckthorn berries (*Hippophae rhamnoides* L.var. *rhamnoides*) were brought from Field Research Laboratory, Leh, India.

Optimization of fruit yogurt formulation

Response surface methodology (RSM) is a commonly employed tool in analyzing experimental data resulting in the optimization of processes or products (Madamba 1997). The RSM was used to derive the optimum formulation conditions using a **two** parameter five level Central Composite Rotatable Design (CCRD) which dictated 14 experimental runs. Table 1 shows the real and coded values of the independent variables used to determine the optimum formulations of SFY. The independent variables affecting the

Table 2 The experimental data (Mean ± standard deviation of triplicate analyses) for response surface analysis of the effect of ingredients on the quality of seabuckthorn fruit yogurt

Experiment no.	Proportions of seabuckthorn fruit syrup in blend (g)	Proportions of skimmed milk powder in blend (g)	<i>S. thermophilus</i> count cfu/mL (Y_1) ^a	<i>L. bulgaricus</i> count cfu/mL (Y_2) ^a	Taste (Y_3) ^b	Viscosity (CP) (Y_4)
1	12.0	7.5	3.0±0.15	2.0±0.36	6.8±0.39	1320.5±1.67
2	18.0	7.5	6.1±0.26	5.0±0.45	7.6±0.29	4825.6±2.01
3	12.0	12.5	4.0±0.20	3.0±0.57	6.6±0.40	5410.8±1.94
4	18.0	12.5	6.5±0.27	5.2±0.76	7.4±0.17	11600.3±2.49
5	10.7	10.0	2.8±0.31	1.7±0.20	6.4±0.22	2925.8±2.32
6	19.2	10.0	7.0±0.40	5.8±0.25	8.0±0.20	11120.9±1.89
7	15.0	6.4	4.8±0.56	3.9±0.31	7.2±0.19	1100.2±2.50
8	15.0	13.5	5.8±0.45	4.9±0.25	7.0±0.26	12125.5 ±3.09
9	15.0	10.0	5.7±0.21	4.5±0.28	8.6±0.26	3710.7±3.15
10	15.0	10.0	5.5±0.28	4.6±0.34	8.5±0.22	3800.8±2.96
11	15.0	10.0	5.6±0.15	4.6±0.34	8.6±0.31	3915.5±2.49
12	15.0	10.0	5.4±0.32	4.5±0.28	8.5±0.23	3800.8±2.96
13	15.0	10.0	5.6±0.15	4.7±0.20	8.6±0.31	3800.8±2.96
14	15.0	10.0	5.5±0.28	4.6±0.34	8.5±0.33	3915.5±2.49

^a In 10⁸

^b 9 like extremely, 1 dislike extremely

Fig. 1 Process flow chart for preparation of seabuckthorn fruit yogurt

quality of the end product were the proportions of seabuckthorn fruit syrup (X_1) and skimmed milk powder (X_2). A total of 14 runs were conducted. Proportions of seabuckthorn fruit syrup varied from 10.7 g to 19.2 g and skimmed milk powder varied from 6.4 g to 13.5 g for 100 g of fruit yogurt. Factors such as counts of *S.thermophilus* and *L.bulgaricus* (cfu/mL), taste and viscosity (CP) were used as quality attributes of SFY. The responses were represented as Y_1 (*S.thermophilus* count), Y_2 (*L.bulgaricus* count), Y_3

(Taste) and Y_4 (Viscosity). It was assumed that Y_K (where $K=1, 2, 3, 4$) is a function of the two independent variables as shown by Eq. (1).

$$Y_K = f(X_1, X_2) \quad (1)$$

The exact mathematical representation of the function (f) is either unknown or extremely complex. However, a second

Table 3 Analysis of variance (ANOVA) data for the responses in the optimization of seabuckthorn fruit yogurt blend formulation

Source of variation	df	Sum of squares			
		<i>S.thermophilus</i>	<i>L.bulgaricus</i>	Taste	Viscosity
Linear	6	4.12	6.99	21.61	8.52
Two factor	5	3.94	6.62	21.61	7.98
Quadratic	3	0.31 ^a	0.63 ^a	0.15 ^a	1.60 ^a
Cubic	1	0.26	0.48	0.036	6.35
Pure error	33	2.85	4.23	2.32	8.19
Correlation coefficients (R)		0.9468	0.9165	0.9162	0.9538

^a Significant at 95 %

Table 4 Regression coefficients of the second order polynomial representing the relationship of the response and independent variables in the optimization of seabuckthorn fruit yogurt blend formulation

Coefficients	<i>S.thermophilus</i> (Y ₁)	<i>L.bulgaricus</i> (Y ₂)	Taste (Y ₃)	Viscosity (Y ₄)
β_0	5.59 ^a	4.64 ^a	8.59 ^a	3823.33 ^a
β_1	1.44 ^a	1.36 ^a	0.47 ^a	2660.56 ^a
β_2	0.35 ^a	0.33 ^a	−0.09	3307.09 ^a
$\beta_{1,1}$	−0.37 ^a	−0.50 ^a	−0.70 ^a	1342.40 ^a
$\beta_{2,2}$	−0.20 ^a	−0.18 ^a	−0.75 ^a	1137.40 ^a
$\beta_{1,2}$	−0.13	−0.18	−0.00	671.25 ^a

^a Significant at 95 % level

order polynomial of the form of Eq. (2) was assumed to relate the response i.e.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad (2)$$

Where, β_0 , β_1 , β_2 , β_{11} , β_{22} and β_{12} are regression coefficients and X_1 and X_2 are the coded levels of independent variables. Based on RSM, this equation was used to evaluate the linear, quadratic and interactive effects of independent variables on the chosen response. The responses for each run (Table 2) were subjected to multiple regression analysis.

Development of seabuckthorn fruit yogurt

Seabuckthorn fruit yogurt was prepared using the method suggested by Sukumar-de (1960). Process flowchart for the preparation of SFY is presented in Fig. 1. The cow milk was blended with SMP and gelatin (0.5 %), pasteurized at 85 °C for 30 min and cooled to 37 °C. The fruit syrup was prepared from seabuckthorn berries by crushing them in blender for pulp extraction, straining, adjusting the pulp pH to 5.4 (with food grade Sodium bicarbonate), blending this pulp (50 g) with required quantity of sugar syrup (prepared by dissolving 50 g sugar and 12.5 mL water for 100 g fruit syrup and finally boiling the contents to 70° brix) and it was then added to milk and mixed thoroughly with high speed stirring. The blends were inoculated with 2 % (v/v, *S.thermophilus* and *L.bulgaricus* in 1:1 ratio) of 24 h old culture and incubated at 37±2 °C in the same cup (100 mL) until pH 4.4 was obtained.

Chemical analysis

The pH scan 2 (Elico Instruments Private Limited, India) was used to measure the final pH of samples. The chemical analysis of SFY like total solids, fat and acidity (ISI 1960), protein (Sathe 1999), ash (AOAC 1984) and carbohydrate (calculated based on difference) were determined. Vitamin C

was determined by using 2,6-dichlorophenol-indophenol titration method (Ranganna 1986), vitamin E was estimated by using ferric chloride reagent, spectrophotometrically at 536 nm (UV-Visible Spectrophotometer 1601, Shimadzu, Columbia, MD, USA) (Desai 1984), total carotenoids (Arya et al. 1979), anthocyanins (Clydesdale and Francis 1976) and phenols (Singleton and Rossi 1965) were determined spectrophotometrically at 450, 535 and 765 nm's, respectively.

Enumeration of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*

IDF (1988) methods were used for enumeration of *S.thermophilus* and *L.bulgaricus*. The *S. thermophilus* agar (M 948) and *L.bulgaricus* agar (M 927) procured from Himedia Laboratories Limited, Mumbai were used. The samples were diluted in mixture of tryptone (0.05 %) and peptone (0.05 %) water. The agar plates of *S.thermophilus* were incubated aerobically at 37 °C for 48 h, while plates of *L.bulgaricus* were incubated at 37 °C for 72 h under micro anaerobic conditions. After incubation, typical colonies of *S.thermophilus* and *L.bulgaricus* were counted. Plates with 30–300 colonies were only used in counting and the counts were averaged for three replicates and expressed in cfu/mL.

Taste

Seabuckthorn fruit yogurt samples were prepared in 100 mL cup using the procedure described in Fig. 1 and stored overnight at 2–5 °C before subjecting them to sensory evaluation. The samples were served at 10 °C. A panel of 15 judges was formed on the basis of their knowledge of the product and their willingness to participate on a regular basis after obtaining agreement

Fig. 2 Contour plots for (a) *S. thermophilus* counts, (b) *L. bulgaricus* counts, (c) Sensory taste score and (d) Viscosity as function of proportion of seabuckthorn fruit syrup (g) and skimmed milk powder (g) and (e) Superimposed contour plot for significant responses for these parameters as affected by proportions of seabuckthorn fruit syrup (g) and skimmed milk powder (g)

(A)

(C)

(B)

(D)

(E)

among the judges on the characteristics of good quality yogurt and what they meant by the terminology for sensory evaluation. The panelists included faculty and research scholars of the Biochemistry and Nutrition Division, Defence Food Research Laboratory, Mysore. The panelists rated the SFY samples by assigning numerical value for quality attribute i.e. taste on 9-point scale (Like extremely 9, Dislike extremely 1).

Viscosity

Viscosity (the condition or property of being viscous) is most commonly evaluated parameter while determining yogurt consistency/thickness. It was measured using a RV Brook Field Viscometer (Stoughton, USA) in 100 mL fruit yogurt samples at room temperature (Gassem and Frank 1991). Samples were stirred for 40 s before measurement. Readings were converted into centipoise units. All viscosity values were measured at 10 rpm with spindle No.5.

Statistical analysis

Data and graphical analysis and response optimization were carried out using Statistical Software i.e. Design Expert (version 6.0.10, Stat-Ease Inc., Minneapolis, MN, USA). The paired comparisons of means were performed using Duncan's test (Steel and Torrie 1980). All analyses were carried out in triplicate.

Results and discussion

The experimental data (Table 2) for the responses were fitted into Eq. (2) to estimate the function, f , in Eq. (1). Four response surface models were obtained for the counts of *S.thermophilus* and *L.bulgaricus*, taste and viscosity. Table 3 shows the ANOVA data for response variables and their significance at 95 % confidence level along with correlation coefficients. A high correlation coefficient ($R>0.9162$) explained the goodness of fit of the experimental data in the response surface models of counts of *S.thermophilus* and *L.bulgaricus*, taste and viscosity. This function showed that over 91.62 % of the total variation

was accounted for or all the four response surface models fitted the data well and were adequately explained.

The regression coefficients obtained for the second order polynomials (SOP) of the four responses are shown in Table 4. These coefficients are necessary in developing the multiple regression equations for the responses to coded levels of the variables. The contour plots of four responses were developed as a function of the two independent variables (proportions of seabuckthorn fruit syrup and skimmed milk powder) according to their significance to the response. The contour plot for counts of *S.thermophilus* and *L.bulgaricus* as a function of proportion of seabuckthorn fruit syrup and skimmed milk powder is shown in Fig. 2a and b. Figure 2c and d show the contour plots of taste and viscosity as a function of proportions of seabuckthorn fruit syrup and skimmed milk powder. The optimum area (Fig. 2e) was obtained by superimposing (graphical method) the contour plots from which the optimum formulation was obtained (Madamba 1997).

Streptococcus thermophilus and *Lactobacillus bulgaricus* counts

The coefficients of the first order terms for the equation Y_1 and Y_2 (Table 4) with coded variables indicated that *S.thermophilus* and *L.bulgaricus* counts of the product increased with seabuckthorn fruit syrup and skimmed milk powder incorporation in the blend. Seabuckthorn fruit syrup was found to have maximum influence on bacterial counts followed by skimmed milk powder. This may be because of presence of prebiotic promoter of dietary fibre in seabuckthorn products that might have exerted prebiotic activity by selectively stimulating the growth and activity of lactic acid bacteria in the fruit yogurt during fermentation (Gibson and Roberfroid 1995; Van-loo et al. 1999). The *S.thermophilus* and *L.bulgaricus* counts of yogurt were reported to increase with increase in proportion of mango pulp (Kumar and Mishra 2003).

$$Y_1 = 5.59 + 1.44 X_1 + 0.35 X_2 - 0.37 X_1^2 - 0.20 X_2^2$$

$$Y_2 = 4.64 + 1.36 X_1 + 0.33 X_2 - 0.50 X_1^2 - 0.18 X_2^2$$

Table 5 Criteria and outputs of the numerical optimization of the responses for seabuckthorn fruit yogurt

Criteria	Goal	Limit Optimized levels	
X_1 : Seabuckthorn fruit syrup (g)	Is equal to =15	12–18	15
X_2 : Skimmed milk powder (g)	Is to maximize	7.5–12.5	12.5
Y_1 : <i>S.thermophilus</i> cfu/mL	Is to maximize	2.50–7.46	5.74
Y_2 : <i>L.bulgaricus</i> cfu/mL	Is to maximize	1.6–6.03	4.80
Y_3 : Taste	Is to maximize	6.25–8.88	8.09
Y_4 : Viscosity (CP)	Is to maximize	1097.63–12128.0	7194.0

Table 6 Predicted and actual values of the responses at optimum formulation conditions of seabuckthorn fruit yogurtCounts in cfu/mL in 10⁸^b Mean ± standard deviation of triplicate analysis

S.no	Response variables	Predicted values	Experimental values ± SD ^b
1.	<i>S.thermophilus</i> ^a	5.74	5.72±0.33
2.	<i>L. bulgaricus</i> ^a	4.80	4.80±0.60
3.	Taste	8.09	8.12±0.62
4.	Viscosity (CP)	7194.0	7199.0±3.14

Taste

The coefficients of term of the SOP equation (Y_3) (Table 4) showed that the taste of SFY increased with an increase in proportion of seabuckthorn fruit syrup due to incorporation of this fruit syrup at optimum proportions, which might had brought desired sweetening effect to the product and it also further helps in increasing the taste score to a maximum extent. It was reported that the increase in proportion of mango pulp in the blend during its development enhanced the overall acceptability score of the final product (Kumar and Mishra 2003).

$$Y_3 = 8.59 + 0.47 X_1 - 0.70X_1^2 - 0.75X_2^2$$

Viscosity

From the regression coefficients of Y_4 (Table 4), it is clear that an increase in the skimmed milk powder proportion in the blend increased the viscosity of the fruit yogurt. Increase in viscosity was reported as a result of incorporation of significant amount of skimmed milk powder to the blend (Krasaekoopt et al. 2004). Addition of skimmed milk powder to the blend

lead the final product in producing the thick viscous yogurt as a result of formation of more protein network in the gels (Tamime and Deeth 1980).

$$Y_4 = 3823.33 + 2660.56 X_1 + 3307.09 X_2 + 1342.40 X_1^2 + 1137.40 X_2^2 + 671.25 X_1X_2$$

Optimization of fruit yogurt

Numeric and graphic optimizations were carried out for the process parameters of the SFY. The desired goals for each variable and response were chosen as summarized in Table 5. The limit for each variable was narrowed down to obtain an optimal region. Each goal was chosen to be is: equal to and also to maximize based on the lactic acid bacterial counts, taste score and viscosity of the developed product because at this level the highest beneficial bacterial counts viz., *S.thermophilus* & *L.bulgaricus* of the product can be achieved; tasty, delicious and thick consistency product can be obtained. As a whole the sensory, physical and functional quality characteristic of the product could be improved to a greater extent. The optimized levels from Table 5, which shows that the software generated one optimum conditions

Table 7 Nutritional properties of seabuckthorn fruit yogurt in comparison with other commercial fruit yogurts

Parameters	Seabuckthorn fruit yogurt	Pineapple fruit yogurt	Grape fruit yogurt
Total solids (%)	28.9±0.22 ^a	26.7±0.34 ^b	27.5±0.21 ^{ab}
Acidity (%)	0.86±0.02 ^a	0.88±0.05 ^a	0.87±0.06 ^a
Fat (%)	5.4±0.82 ^a	5.1±0.74 ^a	5.2±0.69 ^a
Protein (%)	7.9±0.40 ^a	7.1±0.53 ^a	7.2±0.66 ^a
Carbohydrates (%)	14.5±0.51 ^a	13.3±0.49 ^a	14.1±0.59 ^a
Ash (%)	0.86±0.03 ^a	0.80±0.06 ^a	0.82±0.04 ^a
Vitamin C (mg/100 g)	20.8±0.62 ^a	0.70±0.21 ^b	0.61±0.32 ^b
Vitamin E (mg/100 g)	12.0±1.84 ^a	ND	3.7±0.96 ^b
Total carotenoids (mg/100 g)	1.4±0.62 ^a	0.06±0.02 ^b	0.04±0.01 ^b
Total anthocyanins (mg/100 g)	0.05±0.01 ^a	0.00±0.00 ^b	0.00±0.00 ^b
Total phenols (mg/100 g)	24.4±1.87 ^a	2.2±0.90 ^b	2.6±0.71 ^b

Mean values in the same row bearing a common superscript do not differ significantly ($p>0.05$)

ND not detectable

of independent variables with the predicted values of responses, in the seabuckthorn fruit syrup=15 g per 100 g and skimmed milk powder is at maximum level=12.5 g per 100 g for achieving the maximum *S.thermophilus* and *L.bulgaricus* counts=5.74 and 4.8 cfu/mL; maximum taste score=8.09 and maximum viscosity=7194.0 CP.

From the set of constraints and outputs given in Table 5, contour plots of relevant and statistically significant responses were generated, and the overlaying of those plots is displayed in Fig. 2e. The shaded area in Fig. 2e represents the X_1 - X_2 domain satisfying the imposed criteria. The optimum processing conditions could be drawn from this shaded area and specific goals were achieved. For example, shaded area in Fig. 2e determines the following criteria and goals: seabuckthorn fruit syrup=15 g per 100 g, skimmed milk powder =12.5 g per 100 g, *S.thermophilus* and *L.bulgaricus* counts=5.74 and 4.8 cfu/mL, taste score=8.09 and Viscosity=7194.0 CP.

Verification of second order polynomial model

The SFY was prepared using the derived optimum formulation conditions to check the validity of SOP model. The experimental values for counts of *S.thermophilus* and *L. bulgaricus*, taste score and viscosity were determined and compared with the predicted values of the SOP (Table 6). The experimental values (mean of three trials) were found to be in close agreement to the predicted values and were within the acceptable limits indicating the suitability of the model in predicting quality attributes of SFY.

Nutritional properties of SFY

The nutritional properties of SFY samples in comparison with other commercial fruit yogurt samples are shown in Table 7. The result shows that the seabuckthorn fruit yogurt exhibited more amounts of antioxidants viz., vitamin C, E, carotenoids, phenols and anthocyanins when compared to commercial fruit yogurt samples. This may be due to presence of comparatively more amounts of antioxidative nutrients in seabuckthorn fruit, which might have increased the concentration of these constituents in the final product. The vitamin E content was found to be non detectable in commercial pineapple fruit yogurt samples, this may be because of absence of vitamin E in the pineapple pulp from which the fruit yogurt had been prepared. Therefore this type of fruit yogurt can be recommended as a nutritive antioxidant rich probiotic food for the consumers because of presence of antioxidants and beneficial lactic acid bacteria in the final product. Similar results of antioxidants rich nutritive fruit yogurt was reported after incorporating juices of *Euterpe oleracea* (Coisson et al. 2005).

Conclusion

Fourteen different runs according to the CCRD were used to study the quality parameters of SFY at different proportions of seabuckthorn fruit syrup and skimmed milk powder. The response surface methodology was used to optimize the formulation conditions using counts of *S.thermophilus* and *L.bulgaricus*, taste and viscosity as responses. The SOP models for counts of *S.thermophilus* and *L.bulgaricus*, taste and viscosity were statistically significant. By superimposing the contour plots, an optimum formulation i.e. proportion of seabuckthorn fruit syrup 15 g and skimmed milk powder 12 g for 100 g of SFY was recommended with predicted responses close to experimental values. The SFY sample prepared using the optimized blend formulation compared well with the commercial fruit yogurt sample in nutritional properties.

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