The Bioscan 11(3): 1805-1811, 2016 (Supplement on Agronomy)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

OPTIMIZATION OF INGREDIENTS AND INCUBATION TEMPERATURE FOR THE PREPARATION OF GUAVA SEED POWDER FORTIFIED YOGHURT USING RESPONSE SURFACE METHODOLOGY

AJAY KUMAR MAURYA[1]*, RAJENDRA KUMAR PANDEY[2], DIPTI RAI[1] AND MANJU TIWARI[1]
[1]Centre of Food Science and Technology
[2]Department of Animal Husbandry and Dairying, Banaras Hindu University, Varanasi, 221 005, U.P., INDIA
e-mail: ajay.cft@gmail.com

KEYWORDS
Dietary fibre
Guava seed powder
Yoghurt
Syneresis

Received on :
09.06.2016

Accepted on :
29.08.2016

***Corresponding author**

ABSTRACT

The response surface methodology (RSM) was used to optimize the formulation of guava seed powder fortified yoghurt (GSPFY). The independent variables were proportions of guava seed powder (0.8 - 2 g/100 g), yoghurt culture (2 – 4 %), and incubation temperature (37- 42ºC). The analyses show that the GSPFY has firmness 172.89 g, fermentation time 5.10 hr, whey separation was 3.17 % during the optimization process. The optimized yoghurt was also high in dietary fibre (0.82 %), antioxidant activity (54.58 %) and total phenolic content (25.03 %). From the analysis of variance, the R^2 of all response variables is more than 0.77 that indicates that a high proportion of variability was explained by the model.

INTRODUCTION

Functional foods are developed specifically to promote health or reduce the risk of diseases. Examples of functional foods include foods that contain specific minerals, vitamins, fatty acids or dietary fibres. Prebiotics are non-digestible substances, such as fructooligosaccharide (FOS) and inulin, which provide beneficial physiological effect on the host by selectively stimulating the favourable growth or activity of a limited number of indigenous bacteria (FAO/WHO, 2001).

Yoghurt is one of the dairy products, which should continue to increase in sales due to diversification in the range of yoghurt-like products, including reduced fat content yogurts, probiotic yoghurts, yoghurt shakes, drinkable yoghurts, yoghurt mousse, yoghurt ice-cream, *etc*. (Fiszman and Salvador, 1999). The key to market growth is a continuous evaluation and modification of the product to match consumer expectations. For a long time, yogurt by itself has been recognized as a healthy food, due to the beneficial action of its viable bacteria that compete with pathogenic bacteria for nutrients and space (Tamine and Robinson, 1985).

Guava seed meal is a very promising source of bioactive compounds. It is a very potential source of antioxidant, antimicrobial and anticarcinogenic compounds, minerals and functional properties. Fontanari *et al*. (2008) obtained the value of 67 g/100g for total dietary fibre for guava seed powder and also suggested that new products based on fibres obtained from residues of this fruit can be formulated to prevent diseases, especially those related to the gastrointestinal tract and the cardiovascular system. According to Gamal *et al*. (2011) guava seeds waste not yet used for any beneficial purpose, while the proximate composition of defatted guava seeds meal contain 11.52 % protein, 0.54 % oil and 79.62 % crude fibre.

Since consumer concerns are related to both nutritional and sensory aspects, several authors studied texture characteristics of yoghurts due to the addition apple and wheat fibre (Staffolo *et al*., 2004), Orange fibre (Gracia Perez *et al*., 2005), Coconut cake (Ndife *et al*., 2014).

The aim of this study was to add value to a waste product (guava seeds) and find out the optimum proportions of guava seed powder, yoghurt culture, and incubation temperature for making guava seed powder fortified yoghurt (GSPFY) that has desirable sensory, textural profile and other nutritional properties similar to that of the control yoghurt.

MATERIALS AND METHODS

Guava seed powder (GSP)

Guava (*Psidium guijava L.*) overripe fruits were purchased from the orchard of Banaras Hindu University, Varanasi, UP, India. Fruits were crushed in fruit mill (C120, Bajaj process pack maschinen Pvt. Ltd. Ghaziabad, India) and then pulp was extracted from helicoidal juice extractor (C117, Bajaj process pack maschinen Pvt Ltd) to obtain guava pomace.

The seeds were cleaned, washed in running water and were dried in vaccum (VORP5030, Sonar, Delhi, India) at 60 ºC until constant weight. The dried seeds were reduced to ûne powder in electric grinder.

Starter culture

The mixed culture (NCDC 145) containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* obtained from the National Collection of Dairy Culture, NDRI, Karnal was used as starter culture. The starter culture was maintained in autoclaved reconstituted skimmed milk (12 g/100 ml) by sub-culturing once in a fortnight for attaining high activity. The activated cultures were used after three successive transfers for the preparation of stock cultures for GSPFY making (Raju and Pal, 2014).

Preparation of guava seed powder fortified yoghurt (GSPFY)

Fresh, Amul milk containing 6 % fat and 9 % SNF was procured from the market of Varanasi, India. Different level of GSP (g/100g), yoghurt culture (% v/v) and fermentation temperature (ºC), were the variables tested for the preparation of GSPFY, according to the experimental design outlined in Table 1. Twenty batches containing 3 sets of yoghurt each was prepared in 100 ml plastic cups. Sugar (5 g/100g) was also added in the milk. The preparation was mixed thoroughly and heated at 90 ºC for 10 min followed by cooling and kept for incubation at the experimental temperature (37-42 ºC). After incubation, yoghurt samples were stored at 4ºC for further study (Tamime and Robinson, 1985).

Experimental design and statistical analysis

Response surface methodology which involves design of experiments, selection of levels of variables in experimental runs, fitting mathematical models and finally selecting variable levels by optimizing the response (Khuri and Cornell, 1987) was employed in the study. The statistical analysis for guava seed powder fortified yoghurt production was performed by using Design Expert (2015, V 9.0.2; Stat- Ease Inc., Minneapolis, MN, USA) software. A central composite design (CCD) was used to design the experiments comprising of three independent variables (Table 1). Twenty experiments were performed taking into account three factors, *viz*., guava seed powder, yoghurt culture and temperature. There were six experiments at centre point to calculate the repeatability of the method (Montgomery, 2001). Texture, overall acceptability, firmness, fermentation time and whey separation of the yoghurt were taken as the responses of the design experiment .The full quadratic equation of the response variables for yoghurt was derived by using RSM as Eq. 1.

$$Y = \hat{a}_0 + \hat{a}_1A_1 + \hat{a}_2B_2 + \hat{a}_3C_3 + \hat{a}11A_1^2 + \hat{a}22B_2^2 + \hat{a}33C_3^2 + \hat{a}12A_1B_2 + \hat{a}13A_1C_3 + \hat{a}23B_2C_3 \;..... \quad (1)$$

Where, Y = responses; $\hat{a}_0$ = constant; $\hat{a}_1$, $\hat{a}_2$, $\hat{a}_3$ = linear regression;

$\hat{a}_{11}$, $\hat{a}_{22}$, $\hat{a}_{33}$ = interaction regression; A_1, B_2, C_3 = variables.

Sensory evaluation

Sensory evaluation of the produced yoghurt was conducted among 20-semi trained panellists in CFST, BHU, Varanasi, UP, India. Samples of 5 ml were put and served in a plastic container and coded alphabetically. The semi trained panellists evaluated all samples on 9 point hedonic scale (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much and 9 = like extremely) for texture and overall acceptability. Panellists were also served with a glass of water to neutralize the taste before analyzing the next sample (Yang and Li, 2010).

Texture profile analysis (TPA)

Textural attribute such as firmness was determined by back extrusion method using a texture analyzer, (TA-XT plus, M/s Stable Micro Systems ltd, Godalming, United Kingdom) fitted with a 25 kg load cell and was calibrated with 5 kg standard dead weight prior to use. For determining the firmness, the pasteurized and cooled yoghurt mix was filled up to 5 cm in a pre-sterilized glass beaker and incubation was carried out. The beakers were tempered at 25 ºC for 2 h prior to analysis. The probe (A/BE 35) was penetrated up to 10 mm (20 % compression) into the set GSPFY at a cross head speed of 1.0 mm/s. The probe displaced the material by compression followed by back-extrusion, so that the fluid flowed upwards through the concentric annular space. From the resulting force-time curves, firmness *i.e*., the force for compression (g) was calculated using the (Exponent Lite XT PLUS, Ver. 4.0.13.0 lite). All measurements were done in triplicate per each sample (Raju and Pal, 2014).

Whey separation

Yoghurt (100 g) was taken on filter paper and kept over a glass beaker in refrigerator for 12 h to separate the watery fluid from the yoghurt (Yadav *et al*., 2010). The watery fluid collected in glass beaker was measured using the measuring cylinder, and the percent syneresis was calculated on weight basis as follows:

$$\text{Syneresis}(\%) = \frac{\text{Volume of whey collected after drainage}}{\text{Volume of curd sample before drainage}} \times 100$$

Antioxidant activity

The DPPH radical scavenging activity of GSPFY was determined according to the methods of Brand-William *et al*. (1995) with some modifications. The antioxidant activity of optimized GSPFY sample was defined as the ability of the extract to scavenge 1, 1-diphenyl-2- picrylhydrazyl (DPPH) radicals. A 0.1 mM DPPH radical solution in 95 % ethanol was prepared. 1 ml of ethanolic DPPH solution (8 mg/ml) was mixed with 0.2 g of yoghurt sample, vortexed well and incubated for a night at (30 ± 20 ºC). In blank 0.2 ml distilled water was used instead of sample. The samples were centrifuged (3-30k, Sigma laborzentrifugen, Osterode, Germany) for 10 min at 4000 rpm, and the absorbance of samples was measured at 517 nm using UV-visible spectrophotometer (UV-1800 240V, Shimadzu, Japan). The antioxidant activity was expressed as:

Percentage (%) DPPH scavenging = [(absorbance of blank-absorbance of sample)/ (absorbance of blank) × 100]

Total phenolic content (TPC)

The total phenolic content of the optimized GSPFY was determined by the Folin-Ciocalteau method of Cliffe *et al*. (1994) with some modification. 5 grams per 50 ml of sample was filtered with what's man no.1 paper. 0.5 ml of the sample was added to 2.5 ml of 0.2 N up to 25 ml using distilled water. The above solution was then kept for incubation at room temperature for 2 hours at room temperature (30± 20 ºC). Absorbance was measured at 760 nm using 1 cm cuvette UV-1800 spectrophotometer. Gallic acid was used to prepare standard curve. The total phenolic content was expressed in mg of Gallic acid equivalent (GAE)/g of extract.

RESULTS AND DISCUSSION

Effect on the sensory evaluation

The effect of guava seed powder, yoghurt culture and incubation temperature on the sensorial quality of the GSPFY is represented by the quadratic model and is also aided by the equation 2 and 3. Sensory analysis helps deûning the product characteristics which are important with respect to customer acceptance of the product (Yaakob *et al.*, 2012).

Texture

The quadratic equation obtained by the response surface analysis of the data showing the effect GSP (A), yoghurt culture (B) and incubation temperature (C) on the texture and overall acceptability resulted in the following equation respectively in terms of coded factors:

$$\text{Texture} = +7.82 + 0.45A + 0.41B + 0.096C + 0.025AB + 2.244E\text{-}015AC - 0.050BC - 0.10A^2 - 0.086B^2 + 0.020C^2 \quad (2)$$

The response surface plot for texture is presented in fig. 1. With the interaction effect of guava seed powder (Fig. 1), the texture score of yoghurt increased significantly ($p<0.0001$) as the level of yoghurt culture increases from 2 to 4 % in the samples. The texture score was the highest (8.6) at 2 g, 4 % and 42 ºC and lowest (6.7) at 0.8 g, 2 % and 37 ºC of guava seed powder, yoghurt culture and temperature, respectively. The hedonic score for texture varied from 6.7 to 8.6 (Table 1).

The coeficient estimate for the variables has been presented in Table 3. All the variables *i.e.* guava seed powder, yoghurt culture and temperature had significant ($p<0.0001$) positive effect on texture of the yoghurt sample at linear level and guava seed powder and yoghurt culture had significant ($p<0.01$) negative effect at quadratic level. Interactive effect of guava seed powder with yoghurt culture was positive.

Fadela *et al.* (2009), who reported that the *Lactobacillus bulgaricus* not only acts as lactic acid producing agent but also known as texturizing agent even with a small amount. Therefore, the increase in texture may be due to the increasing level of yoghurt culture as well as guava seed powder which binds the water content.

The ANOVA data showed that the model is significant and lack of fit is not significant (Table 2). The high values of coefficient of determination for texture ($R^2 = 0.99$) indicates that 99 % of the variability in the response could be explained by the model and adjusted R^2 of 0.98 also showed that the model is highly significant. A lack of fit value of 0.60 is found to be not significant relative to pure error.

Overall acceptability

$$\text{Overall acceptability} = +7.66 + 0.28A + 0.25B - 0.025C + 0.013AB - 0.11AC - 0.012BC - 0.25A^2 - 0.20B^2 - 0.11C^2 \quad (3)$$

The coefficient of determination (R^2) was 0.78 indicating that 78 % of the variability in the response could be explained by the model. The "Pred. R-Squared" of 0.45 was in reasonable agreement with the "Adj R-Squared" of 0.59. A lack of fit value of 0.14 is found to be not significant. Hence, this model could be used to navigate the design space. The average overall acceptability score of guava seed powder fortified yoghurt varied from 6.2 to 7.92 (Table 1).

The response surface plot for overall acceptability is presented in fig. 2. As the level of guava seed powder increased (0.8 to 2 g), the overall acceptability score increased ($p<0.05$; Fig. 2). This may be due to interaction effect with yoghurt culture.

Table 1: Experimental results (central composite design) for the optimization of guava seed powder fortified yoghurt (GSPFY)

Run	Factors A:Guava seed powder (g/100g)	B:Yoghurt culture(%)	C:Temperature (ºC)	Responses Texture	Overall acceptability	Firmness (g)	Time (hr)	Whey separation (%)
1	2	2	37	7.5	6.9	166.46	5.3	2.8
2	2	4	37	8.5	7.9	164.12	5.25	3.2
3	2.4	3	39.5	8.3	7.4	179.45	5	2.7
4	1.4	3	35.29	7.7	7.7	156.78	5.58	3.15
5	0.8	2	42	7	6.4	156.12	5.31	4.1
6	1.4	3	39.5	7.9	7.82	173	5.05	3.25
7	2	2	42	7.8	7	177.78	5.2	2.75
8	1.4	3	39.5	7.8	7.31	174.3	5	3.45
9	1.4	3	39.5	7.9	7.45	173.1	5.1	3.25
10	1.4	3	39.5	7.76	7.92	173.67	5.1	3.31
11	2	4	42	8.6	7.6	178.25	5.1	3.8
12	1.4	4.68	39.5	8.2	7.3	169.71	5.18	3.1
13	0.8	2	37	6.7	6.2	148.67	5.59	4.2
14	1.4	3	39.5	7.87	7.68	173.98	5.1	3.2
15	1.4	3	43.7	8	7.2	175.56	5	3.35
16	1.4	3	39.5	7.72	7.73	176.78	5.1	3.2
17	0.8	4	42	7.7	7.3	164.32	5.23	3.5
18	1.4	1.31	39.5	6.9	7.1	165.83	5.3	3.25
19	0.39	3	39.5	6.7	6.7	140.21	5.55	3.9
20	0.8	4	37	7.6	6.8	150.56	5.45	2.5

Table 2 : Analysis of variance (ANOVA) of the response variables

Responses	Source	DF[1]	Mean square	F value	P value[2]	R^2 (%)	R^2 (%) adj
Texture	Model	9	0.6	115.51	< 0.0001	0.99	0.98
	Lack of fit	5	4.58E-03	0.76	0.6		
	Pure error	5	5.83E-03				
	Total	19					
Overall acceptability	Model	9	0.39	3.89	0.02	0.78	0.59
	Lack of fit	5	0.15	2.8	0.14		
	Pure error	5	0.053				
	Total	19					
Firmness (g)	Model	9	256.49	52.18	< 0.0001	0.98	0.96
	Lack of fit	5	7.91	4.1	0.07		
	Pure error	5	1.92				
	Total	19					
Fermentation Time (hr)	Model	9	0.072	14.32	< 0.0001	0.92	0.86
	Lack of fit	5	8.30E-03	4.74	0.06		
	Pure error	5	1.75E-03				
	Total	19					
Whey separation (%)	Model	9	0.39	16.52	< 0.0001	0.94	0.88
	Lack of fit	5	0.039	4.37	0.07		
	Pure error	5	8.87E-03				
	Total	19					

[1]DF = degree of freedom, [2]Significant at $p < 0.05$

Table 3 : Coefficient of estimates for different factors of GSPFY optimization

Factor	Coefficient of estimates				
	Texture	Overall acceptability	Firmness (g)	Time (hr)	Whey separation(%)
Intercept	7.82	7.66	174.16	5.07	3.27
A - GSP	0.45*	0.28*	9.73*	-0.12*	-0.28*
B - Yoghurt culture	0.41*	0.25*	1.08*	-0.042*	-0.081
C - Temperature	0.096*	-0.025	5.73*	-0.13*	0.13*
AB	0.025	0.013	-1.49	0.008	0.47*
AC	2.26E-15	-0.11	0.53	0.031	-0.044
BC	-0.05	-0.012	1.14	0.001	0.22*
A^2	-0.10*	-0.25*	-5.21*	0.076*	0.033
B^2	-0.086*	-0.20*	-2.40*	0.063*	-0.011
C^2	0.02	-0.11	-2.97*	0.081*	0.015

Significant at $p < 0.05$

Table 4 : Optimized solutions with predicted responses for GSPFY using design expert software 9.0.2

Number	Guava seed powder(g)	Yoghurt culture(%)	Temperature °C	Texture	Overall Acceptability	Firmness (g)	Time (hr)	Whey separation(%)	Desirability
1	1.4	3.86	39.45	8.12	7.73	172.89	5.09	3.17	0.833
2	1.4	3.86	39.34	8.11	7.72	172.88	5.09	3.17	0.832
3	1.4	3.85	39.37	8.1	7.72	172.97	5.09	3.18	0.831
4	1.4	3.88	39.4	8.11	7.72	172.97	5.09	3.18	0.832
5	1.4	3.9	39.3	8.12	7.72	172.61	5.09	3.16	0.832

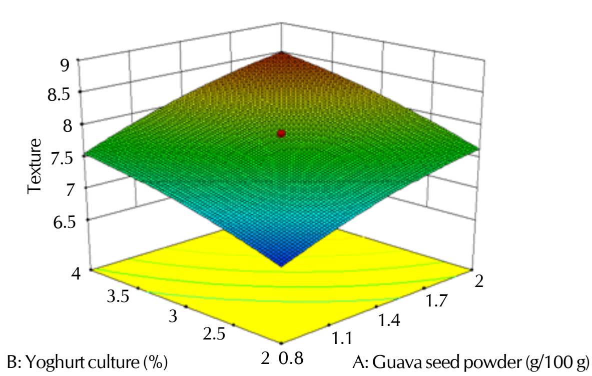

Figure 1: Response surface plots showing interaction effect of variables on texture of GSPFY

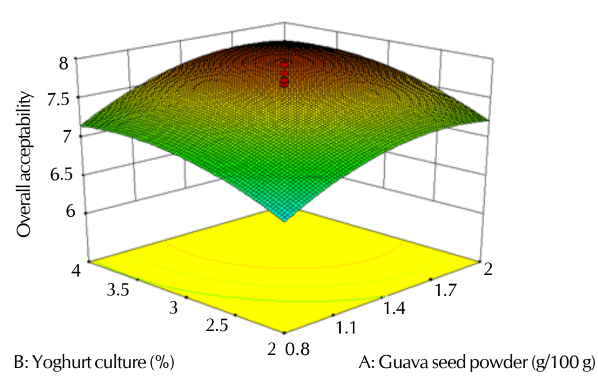

Figure 2: Response surface plots showing interaction effect of variables on overall acceptability of GSPFY

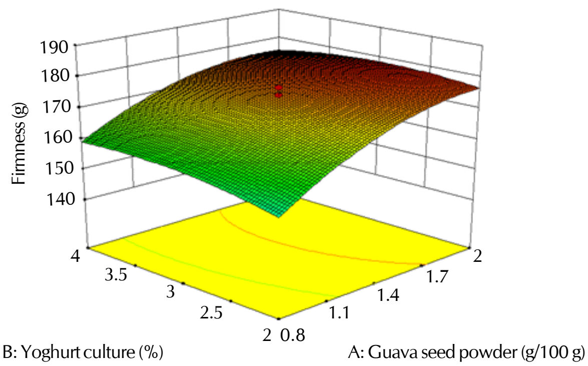

Figure 3: Response surface plots showing interaction effect of variables on firmness (g) of GSPFY

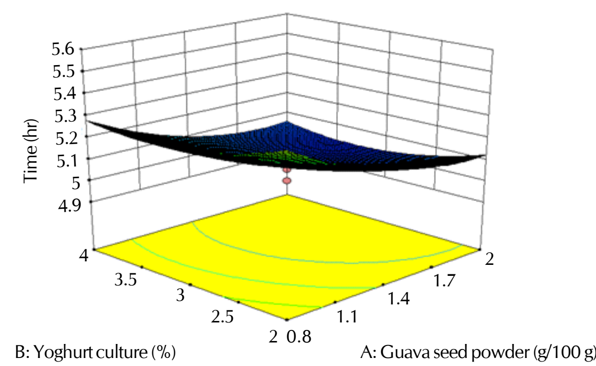

Figure4 : Response surface plots showing interaction effect of variables on fermentation time (hr) of GSPFY

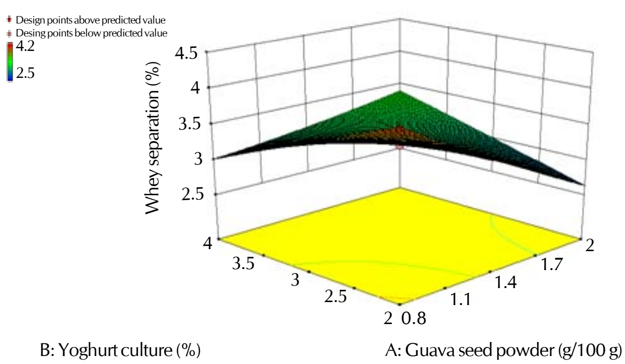

Figure 5: Response surface plots showing interaction effect of variables on whey separation (%) of GSPFY

From table 3 it is clear that there is a positive effect of guava seed powder and yoghurt culture on overall acceptability, however incubation temperature has a negative effect. Yaakob *et al.* (2012) studied the ingredient and processing levels for the production of coconut yoghurt using response surface methodology and reported similar findings. Kumar *et al.*, 2013 also studied effect of Tulsi extract on ice cream and found the same results.

Effect on firmness

The quadratic equation obtained by the response surface analysis of the data showing the effect GSP (A), yoghurt culture (B) and incubation temperature (C) on the firmness resulted in the following equation in terms of coded factors:

$$\text{Firmness} = +174.16 + 9.73A + 1.08B + 5.73C - 1.49AB + 0.53AC + 1.14BC - 5.21A^2 - 2.40B^2 - 2.97C^2 \quad \ldots\ldots\ldots\ldots\ldots (4)$$

The response surface plot for firmness is presented in fig. 3. The firmness increased significantly ($p<0.0001$) with increasing level of guava seed powder (0.8 to 2.4 g) and yoghurt culture (2 to 4 %), irrespective of the temperature level from 37 to 42 ºC. The firmness of the samples ranged from 140.21 g to 179.45 g (Table 1) depending upon the level of independent factor used for the formulation of GSPFY.

The ANOVA data showed that the model is significant and lack of fit is non significant (Table 2). The high values of coefficient of determination ($R^2 = 0.98$) and adjusted R^2 of 0.96 also showed that the model is highly significant. A lack of fit value of 0.074 is found to be non significant relative to pure error.

The coeûcient estimate for the variables has been presented in table 3. All the variables *i.e.* guava seed powder, yoghurt and temperature had significant ($p<0.01$) positive effect on firmness of the yoghurt sample at linear level and all the variables had significant ($p<0.01$) negative effect at quadratic level. Interactive effect of guava seed powder with temperature and yoghurt culture with temperature was positive, while guava seed powder with yoghurt culture was observed negative.

The increased firmness is related to an improvement of the texture and makes the yoghurt less susceptible to rearrangements within its network, shrinkage and whey expulsion (Brennan and Tudorica, 2008). The firmness of yoghurt is directly dependent on its total solids and specifically protein content and the type of proteins. Higher protein content would cause a higher degree of cross linkage of the gel network, resulting in a much denser and more rigid gel structure (Tamime, 2006). Foda *et al.* (2007) had also reported that addition of turmeric powder increased yoghurt firmness as it was also rich in dietary fibre.

The highest firmness was recorded 179.45 g at 2.4 g, 3 and 39.5 ºC of guava seed powder, yoghurt culture and temperature respectively (Table1).

Effect on fermentation time

The regression equation obtained in terms of coded factors for the effect of variables Guava seed powders (A), yoghurt culture (B) and incubation temperature (C) on the fermentation time resulted in the following equation (5):

$$\text{Fermentation time} = +5.07 - 0.12A - 0.042B - 0.13C + 8.750E\text{-}003AB + 0.031AC + 1.250E\text{-}003BC + 0.076A^2 + 0.063B^2 + 0.081C^2 \quad \ldots\ldots (5)$$

The fermentation time of the samples ranged from 5 to 5.59 hr (Table 1). Response surface plot showed that shorter fermentation time was obtained at higher incubation temperature. The response surface plot for fermentation time is presented in fig. 4. Figure 4 shows interaction effect of yoghurt culture with guava seed powder, as the level of yoghurt culture increased (2 to 4 %), the fermentation time decreased significantly.

It can be explained by the fact that higher fermentation temperature can promote the metabolic activity of starter bacteria (Wu *et al.*, 2009). Dietary fibre present in guava seed powder act as a prebiotic and promotes the growth of yoghurt culture and thus reduce the fermentation time. The fermentation time score was the lowest (5 hr) at 1.4, 3 % and 43.70 ºC of guava seed powder, yoghurt culture and temperature level, respectively (Table 1).

The effect of yoghurt culture was also negative, as indicated by the negative coefficient of estimate (Table 3). Table 3 depicts that effect of GSP was negative for fermentation time. Mishra and Mishra, (2014) also reported that higher fructooligosaccharide (FOS) concentration resulted in a shorter fermentation process. Moreover, FOS is considered as a prebiotic and can stimulate the growth and activity of probiotic bacteria (Oliveira *et al.*, 2011).

The ANOVA data showed that the model is significant and lack of fit is not significant (Table 2). The high values of coefûcient of determination (R^2 = 0.92) indicates that 92 % of the variability in the response could be explained by the model and adjusted R^2 of 0.86 also showed that the model is highly signiûcant.

Effect on whey separation

Whey separation (wheying-off) is defined as the expulsion of whey from the network which then becomes visible as surface. Wheying-off negatively affects consumer perception of yoghurt (Lucey *et al.*, 1998).

The quadratic equation obtained by the response surface analysis of the data showing the effect GSP (A), yoghurt culture (B) and incubation temperature (C) on the whey separation resulted in the following equation (6) in terms of coded factors:

$$\text{Whey separation} = + 3.27 - 0.28A - 0.08B + 0.13C + 0.47AB - 0.044AC + 0.22BC + 0.033A^2 - 0.011B^2 + 0.015C^2 .... (6)$$

The high values of coefficient of determination (R^2 = 0.94) indicates that 94% of the variability in the response could be explained by the model and the adjusted R^2 of 0.88 showed that the model is highly significant. A lack of fit value of 0.07 is found to be non significant relative to pure error (Table 2).

The response surface plots for whey separation score presented in fig. 5. Figure 5 shows interaction effect of guava seed powder with yoghurt culture, as the level of guava seed powder increased (0.8 to 2 g) the whey separation score decreased. It may be due to the water holding capacity of dietary fibre present in guava seed powder, which absorb the moisture and decrease whey separation.The whey separation was lowest (2.7 %) at 2.4 g, 3 % and 39.5 ºC of guava seed powder, yoghurt culture and temperature level, respectively (Table 1).

Table 3 depicts that GSP and yoghurt culture has negative coefficient of estimate on whey separation. There was a slight increase in whey separation as the temperature was increased. Garcia-Perez *et al.* (2005) also reported that syneresis decreased due to increased water holding of fibre that absorbed the whey released by the gel structure. Foda *et al.* (2007) had also reported that the rate of syneresis gradually decreased with increasing turmeric powder concentrations from 0.1 to 1.0 %. Guven *et al.* (2005) investigated that the whey separation of yoghurt decreased with increase in inulin concentration

Optimization

The numerical optimization technique of the Design-Expert software (9.0.2) was used for simultaneous optimization of the multiple responses. The constraints have been listed in Table 1. Responses obtained after each trial were analyzed to visualize the interactive effect of various parameters on sensory attributes and firmness, fermentation time and whey separation of yoghurt. Optimized solutions obtained from the Design Expert software for the yoghurt is presented in Table 4. Out of 6 suggested formulations, the formulation No. 1 had better overall acceptability score of 7.73 than all other formulations and also the desirability was 0.833 which was the highest amongst all other formulations (Table 4).

ACKNOWLEDGMENT

Financial support for this research was provided by the Department of Biotechnology, New Delhi, India.

REFERENCES

Brand-Williams, M. E., Cuvelier, C. and Berset. 1995. Use of free radical method to evaluate antioxidant activity. Food Sci. and Techno. **28:**25-30.

Brennan, C. S., and Tudorica, M. 2008. Carbohydrate-based fat replacers in the modification of the rheological, textural and sensory quality of yoghurt: comparative study of the utilisation of barley beta-glucan, guar gum and inulin. *Int. J. Food Sci. and Techno*. **43:** 824-833.

Cliffe, S., Fawer, M. S., Maier, G., Takata, K. and Ritter, G. 1994. Enzyme assays for the phenolic content of natural juices. *J. Agri. and Food Chem*. **42:** 1824-1828.

Fadela, C., Abderrahim, C. and Ahmed, B. 2009. Sensorial and physico-chemical characteristics of yoghurt manufactured with ewe's and skim milk. *World J. Dairy Food. Sci*. **4:** 136-140.

FAO/WHO. 2001. Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria. Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report.

Fiszman, S. M. and Salvador, A. 1999. Effect of gelatine on the texture of yogurt and of acid-heat-induced mil gels. Euro. *Food Res. and Techno.* **208:** 100-105.

Foda Mervat, I., Abd El- Aziz, M., Awad, A. A. 2007. Chemical, Rheological and Sensory evaluation of yoghurt supplemented with turmeric, *Int. J. Dairy Sci.* **2(3):** 252-259

Fontanari, G. G., Jaco, M. C., Souza, G. R., Batistuti, J. P., Neves, V. A., Pastre, I. A. and Fertonanim, F. L. 2008. DSC studies on protein isolates of guava seeds Psidium guajava. *J. Therm. Anal. and Calori.* **93(2):** 397-402.

Gamal, F. M., Samira, S. M. and Fakhriya, S. T. 2011. Antioxidant, Antimicrobial, and Anticarcinogenic Properties of Egyptian Guava Seed Extracts, *Nat. and Sci*. **9(11):**

Garcia-Perez, F. J., Lario, Y., Fernandez-Lopez, J., Sayas, E., Perez-Alvarez, J. A. and Sendra, E. 2005. Effect of orange fiber addition on yoghurt color during fermentation and cold storage. *Col. Res. Appl.* **30(6):**457-463.

Guven, M., Yasar, K., Karaca, O. B. and Hayaloglu, A. A. 2005. The effect of inulin as a fat replacer on the quality of set-type low-fat yogurt manufacture. *Int. J. Dairy Techno.* **58:** 180-184.

Khuri, A. I. and Cornell, J. A. 1987. Response surfaces, design and analysis. Marcel Dekker Inc, New York.

Kumar, S., Rai, D. C. and Singh, D. 2013. The Functional, Rheological and Sensory Attributes of Tulsi (Holy Basil,*Ocimum Sanctum*) Extract Based Herbal Ice Cream. *The Bioscan*. **8(1):** 77-80.

Lucey, J. A. and Singh, H. 1998. Formation and physical properties of acid milk gels: a review. *Food Res. Int.* **30(7):** 529-542.

Mishra, S. and Mishra, H. N. 2014. Optimization of the prebiotic & probiotic concentration and incubation temperature for the preparation of synbiotic soy yoghurt using response surface methodology. Food Sci. and Techno. xxx 1-10.

Montgomery, D. C. 2001. Design and analysis of experiments, 5th edn. Wiley, New York.

Ndife, J., Felicia, I. and Rabiu, G. 2014. Production and quality assessment of functional yoghurt enriched with coconut. I*nt. J. Nut. and Food Sci*. **3(6):** 545-550.

Oliveira, S. P. R., Florence, R. C. A., Perego, P. Oliveira, N. M. and Converti, A. 2011. Use of lactulose as prebiotic and its influence on the growth, acidification profile and viable counts of different probiotics in fermented skim milk. *Int. J. Food Micro.* **145(1):** 22-27.

Raju, P. N. and Pal, D. 2014. Effect of dietary fibers on physico-chemical, sensory and textural properties of Misti Dahi. *J Food Sci Techno.* **51(11):** 3124-3133.

Staffolo, M. D., Bertola, N., Martino, M. and Bevilacqua, Y. A. 2004. Influence of dietary fibre addition on sensory and rheological properties of yoghurt. *Int. Dairy J.* **14(3):** 263-268.

Tamime, A. Y. 2006. Fermented milks. Oxford: Blackwell.

Tamine, A. Y. and Robinson, R. K. 1985. Yogurt science and technology. Oxford: Pergamon Press. pp. 365-373.

Yaakob, H., Rashidah, A. N., Daud, K. S., Malek, A. R. and Rahman, A. R. 2012. Optimization of ingredient and processing levels for the production of coconut yoghurt using response surface methodology. *Food Sci. and Biotech*. **21(4)**: 933-940.

Yadav, D. N., Rajan, A., Sharma, G. K. and Bawa, A. S. 2010. Effect of fiber incorporation on rheological and chapatti making quality of wheat flour. *J. Food Science and Technology*. **47(2):**166-173.

Yang, M. and Li, L. 2010. Physicochemical, textural and sensory characteristics of probiotic soy yogurt prepared from germinated soybean characteristics of probiotic soy yogurt. *Food Techno. and Biotech*. **48(4):** 490-496.