Original Research Article

OPTIMIZED CONDITIONSFOR THE PRODUCTION OF YOGURT FROM SOYA BEAN (*Glycine max*) AND CINNAMON (*Cinnamomum verum*)

ABSTRACT

This study aimed at producing functional soy bean yoghurt by optimizing the production conditions using response surface methodology. The quantity of ferment (LYOFAST Y439A) (50–100 g) and quantity of cinnamon (10-40 g) were optimized using central composite design. Responses (global acceptability and scavenging activity) obtained from experimental runs were fitted into second order polynomial regression model. Also, the multiple optimization technic was used to obtain the compromised optimum condition. The optimized yoghurt was evaluated for its pH and proximate composition using standard methods. The optimum conditions for the production of this yoghurt was as thus: 69.64 g for quantity of ferment and 10 g for quantity of cinnamon, 100 g soya beans, 100 g of sugar and 1 L of water soybeans. Soy bean yoghurt made from optimized conditions had a global acceptability of 6.8, scavenging activity of 34 %. Also, protein, fat, carbohydrate, calcium, sodium magnesium and energy value was respectively 17.1%, 2.91%, 17.93%, 144 mg/100 g, 159.91 mg/100g, 159.91 mg/100g, 63.18 mg/100g and 166.31 kcal/100g. Thus, acceptable yoghurt with functional properties can be obtained from soya beans and cinnamon.

Keywords: *Cinnamon; soy beans; sensory evaluation; optimization; yoghurt*

1. INTRODUCTION

The risingcompetitions in the food market impulse to provide nutritious food with interestingtherapeutic properties and flavor. Yogurt is a conventional food known for its nutritional,therapeutic and sensory properties and isthe utmostpreferred and popular vehicle for probiotic microorganisms through the intestinal tract[1]. It is briefly defined as a clot formed by fermentation and precipitation of milk proteins [2]. In recent years, there has been a significant increase in the popularity of yogurt as a functional food [1]. Functional food can be defined as dietary items that, besides providing nutrients and energy, beneficially modulate one or more targeted functions in the body, by enhancing a physiological response and/or by reducing the risk of diseases [3]. The recognized functional food ingredients include protein, fiber, vitamins, minerals and antioxidants, which can be found in grains, legumes and cereals, Vegetables and fruits [4].

Among these foods, soybeans (*Glycine max* L) are broadly cultivated and considered as high-quality food because they contain high amounts of protein, fat, essential amino acids and phytochemicals, including tocopherols, isoflavones, anthocyanins and saponins [5]. When ingest, it bringsa number of health benefits, such as anti-inflammatory, anti-mutagenicity,DNA damage reduction, and inhibition of low-density lipoprotein oxidation[6]. These effects are also establish in soy bean yoghurt (soymilk), soy sauce, soy sprouts, and tofu. Soymilk is the most current healthy food drink and is a good source of bioactive compoundsand high-quality proteins. In developing countries, it is a low-cost source of good quality protein and energy [7]. It is obtained by soaking and grinding whole soybean. This is later followed by the fermentation process by *Lactobacillus*, *Streptococcus* and *Leuconostoc*[8]. Nonetheless, some LAB has been reported to grow slowly or poorly in soymilk and produce low levels of organic acids. Therefore, to improve the healthy property and growth of probiotic bacteria, fermented foods generally needs to be supplemented with various prebiotics such as raffinose or inulin or a combination of glucose and raffinose. This approach leads to new products with better properties and different components.

Besides, cinnamon (*Cinnamomum verum*) is derived from a tree belonging to the family Lauraceae. Cinnamon is high in polyphenols, proanthocyanidins, antioxidant activity, and is a great source of iron, manganese, calcium, and fiber. Many studieshad reported the antioxidant, antidiabetic, anti-inflammatory, anti-thrombosis and analgesic effects of cinnamon [9]. Moreover, it has as main component cinnamaldehyde, which has antibacterial properties [2]. Akarca et al.[10] showed that cinnamon powder at 0.3%, 1% and 2.5% to pasteurized milk had less amount of microbes during the storage period. Also, cinnamon has distinct aroma, spicy, flavor, and sweet properties, which has favor it usage in food beverage, confectionery and sweet industries. It is used as flavoring agent in soft drinks. Research work done by Khadka[1], showed that yoghurt could be formulated from cow milk and *Cinnamon oleoresin*. However, to the best of our knowledge there is little or no reports on the determination of optimum condition for the formulation of yoghurt from soybeans and cinnamon. Accordingly, can the determination of optimum condition required for the production of yoghurt from soybean and cinnamon provide an acceptableproduct with functional properties? This study aimed to produce a fermented soybean yogurt with improved functional properties.

2. MATERIAL AND METHODS

2.1 Materials

Soya bean grains (*Glycine max*), sugar, and cinnamon(*Cinnamomum verum*)were obtained from the local market of Dschang (West region of Cameroon). Lactic acid starterbacteria LYOFAST Y439A (produced by LaboratoiresHumeau, France, www.humeau.com) was purchased and used as the ferment.LYOFAST Y439A is a culture of EPS-producing *Streptococcus thermophilus* and *Lactobacillus delbrueckiissp bulgaricus*. The starter was reconstituted using skim milk.

2.2 Methods

2.2.1 Optimization of production condition of yogurt from soya bean and cinnamon

The central composite design with two blocs was used to design the experiments due to it specificity (allowing a more precise estimate of the output of the process) [11]. The objective of optimization was to maximize the global acceptability and scavenging activity. Based on the literature review and preliminary test, the experimental domains (Table1)waslatter used to obtain a set of 14 combinations of experiments made up of four factorial points, six central points and four axial points (Table 2). The number of experiments (N) was calculated using the following formula [12].

$N=2k +2k +n_o$

Where: k is the number of variables;n_o is the number of trials in the center.

Table 1. Levels of different process variables used in the optimization process

		Level				
Factors	**Symbol**	-α (1.41)	-1	0	+1	+ α (1.41)
Quantity of ferment	X_1	39.65	50.00	75.00	100.00	110.35
Quantity of cinnamon	X_2	15.00	10.0	27.50	40.00	45.18

Table2. Experimental and experimentation matrix for optimization process

Trial number	Ferment (X_1)	Cinnamon (X_2)
1	75.00 (0.00)	27.50 (0.00)
2	75.00 (0.00)	45.18 (+1.41)
3	39.65 (-1.41)	27.50 (0.00)
4	75.00 (0.00)	27.50 (0.00)
5	75.00 (0.00)	27.50 (0.00)
6	75.00 (0.00)	10.0 (-1.00)
7	110.35 (+1.41)	27.50 (0.00)
8	75.00 (0.00)	27.50 (0.00)
9	50.00 (-1.00)	15.00 (-1.00)
10	100.00 (+1.00)	15.00 (-1.00)
11	50.00 (-1.00)	40.00 (+1.00)
12	75.00 (0.00)	27.50 (0.00)
13	75.00 (0.00)	27.500 (0.00)

14	100.00 (+1.00)	40.00 (+1.00)

2.2.2 Production of milk and yogurt from soybean

It has to be noted that the different ingredient ratios (Table 2) was used for the production thus at the end of this step we has 14 different sets of yoghurt. The production of soymilk and yoghurt was done using the modified method of Denkovaand Murgov[13]**.** Precisely, after weighing 100g of grain using a ST-400 type balance, they were washed to remove impurities such as dust and surface bacteria. Then, soaked in one liter of water for 8-10h, to soften the seed membrane, facilitate absorption and thus release certain anti-nutrients such as lectins. It was then followed by de-hulling, grinding to obtained colloidal solution of soybeans. The latter was sieved and filtrated; the sieving was done using a sieve whose pore diameter varied around 0.5mm in order to eliminate the chalky taste and insoluble fibers. Then it was filtered using a cotton cloth with a diameter of approximately 0.01mm in order to reduce all traces of solid particles. The soy milk was pasteurized using an electric heater and a clean pot at a temperature of 90-110°C for 1h with the primary aimed of destroying all microorganisms, contaminants and anti-nutrients naturally present in soy, and thus makes soy milk digestible. The pasteurized milk was cooled down to 42± 1°C and the ferment was latter added and the air tight sterilized bottles and incubate at of 42°Cfor 6-7h. Ferment cultures were grown up for 18 h at 37°C previously inoculation with sterilized skim milk media (10% w/v skim milk in distilled water, sterilized at 110°C / 10 min). This procedure permitted a reliable concentration of ferment cultures of about 10^8cfu/ml for respectively starter culture, verified by plate counting.

2.2.3 Evaluation of responses for the optimization process

2.2.3.1 Sensory evaluation analysis

The panel constituted 60 untrained individual of both sex of age varying between 20≤X≥50. The panelists were informed on the conditions to be respected during the session (silence) and how to evaluate the product based on the different forms of presentation of the samples.
The tasters were asked to evaluate the acceptability of the yogurt samples that werecoded using a three letter. Just before each test session, panelists were given orientation about the procedure of sensory evaluation. The health status of the panelist was also considered during panelist selection (not suffering from colds and allergies that affect their sensitivity for the product). Panelists were asked to rinse their mouth with tap water that was provided to them, before the next serving. The panelist were asked to rank the products on the basis of visual color, texture/consistence, aroma and global acceptability (overall acceptability) using a nine point hedonic scale rated from 1(dislike extremely) to 9 (like extremely). All sensory evaluation was performed at ambient room temperature with equalized light intensity levels, free from disturbing noises and with a continually circulating air. It should be noted that, the sensory parameter that was consider the response was the overall acceptability.

2.2.3.2 Evaluation of scavenging activity

The free radical scavenging activity of milk samples was measured using DPPH (1,1 diphenyl 2, picryl hydrazyl) assay [14].Yogurtsample (100 µL) was mixed with 900 µL of DPPH solution (0.2 mM) prepared in methanol. The mixture was allowed to incubate at room temperature for 30 min. After incubation period, 1 mL of chloroform was added and centrifuged at 3000 x g for 5 min. The absorbance of clear solution was measured at 517 nm. A 100 mM of DPPH prepared in methanol was used as a control. The percentage inhibition of DPPH free radical (scavenged %) was calculated based on reading of control solution by employing the following equation:

Scavenging activity (%) = [(absorbance of the control – absorbance of the sample)/absorbance of the control] x 100.

2.2.4 Modeling, validation of model and optimum conditions

The behavior of this system was explained by the following second-degree polynomial equation:
$Y = I + ax_1 + bx_2 + cx_3 + dx_1^2 + ex_1x_2 + fx_1x_3 + gx_2^2 + hx_2x_3 + ix_3^2 + \varepsilon$
Where Y is the measured response
I is a constant,

a, b and c linear coefficients,
d, g and i are square coefficient ,
e, f and h are interaction coefficient ,
x_1, x_2, x_3, x_1^2, x_1x_2, x_1x_3, x_2^2, x_2x_3, x_3^2 are levels of independent variables
ε is the error.

The validation of the model was done using R-square (R^2), absolute mean deviation analysis (AMDA)and bias factor (B_f). Concerning the validation of the optimum conditions, the level of significance of the experimental and predicted result of responses were compared and also the desirability.

2.2.5 Evaluation of pH and proximate composition of the optimized yoghurt

It has to be noted that, at the end of the optimization produce, the product obtained using the compromised condition was term optimized yoghurt.

2.2.3.1 Determination of pH

The pH was carried out using seven excellence pH meter (Mettler Toledo) with model 4.0.1 serial No B719086801.The pH meter was calibrated with reference standards buffers of pH 4.01, 7.00 and 10.01. The pH meter was powered and the electrode dipped into the solution of the standards followed by sample reading at ambient temperature and the values obtained were recorded for all the samples.

2.2.3.2Determination proximate composition

The moisture, ash, protein, fatand mineral (Ca, Mg and Na)content of the sample was determined using the method as outlined in AOAC[15]. The carbohydrate content was determined by method of difference as described by Ihokoronye and Ngoddy[16]. This method was used for all the different samples. The values were calculated using equation below.

Carbohydrate (%)= 100 - % (ash + protein + fat + moisture)

Evaluation of the energy value was determined by indirect calculation method proposed by **James** [17]**.** The three groups of nutrients, which provide the body with energy, are carbohydrates, fats and proteins. One gram of carbohydrate (C) was assumed to give 4 Kcal energy, one gram of fat (F) 9 Kcal energy and one gram of protein (P) 4 Kcal.
Energy value (kcal/100g) = (% protein x 4) + (% crude fat x 9) + (% total carbohydrate x 4). Where: % P = Protein content (%), % F = Fat content (%), %C = total carbohydrate (%).

2.2.4 Statistical Analyses

Using experimental design tools, the analysis of variance (ANOVA) was used to determine the influence of each factor as well as the degree of significance of each of its effects. It examines the statistical significance of each factor on the global acceptability and scavenging activity. The regression equations were subjected to the Fisher test to determine the coefficient of determination R^2. The calculations were performed with Minitab version 18. The accepted confidence level was $p < 0.05$. Graphical representations of the response surfaces and contour plots were made using Sigma Plot version 11.0 (c) systat software.

3. RESULTS

3.1Optimization of the production conditions of yoghurt from soybean and cinnamon

The results of the effect of the quantity of ferment and cinnamon on the global acceptability and scavenging activity of the product are shown in Table 3.The global acceptability varies from 5.63 to 7.01 and the scavenging activity from 25.68% to 35.01%.

Table 3. Experimentation matrix and experimental values for the global acceptability and scavenging activity of the yoghurt

Trial Number	Ferment (X_1)	Cinnamon (X_2)	Global acceptability		Scavenging activity (%)	
			experimental values	Predicted values	experimental values	Predicted values
1	75.00 (0.00)	27.50 (0.00)	6.30	6.27	34.43	34.90
2	75.00 (0.00)	45.18 (+1.41)	5.70	5.77	31.96	31.59
3	39.65 (-1.41)	27.50 (0.00)	6.50	6.38	33.07	32.72
4	75.00 (0.00)	27.50 (0.00)	6.10	6.27	34.43	34.90
5	75.00 (0.00)	27.50 (0.00	6.10	6.27	34.43	34.90
6	75.00 (0.00)	9.8 (-1.00)	7.01	6.78	35.01	34.65
7	110.35 (+1.41)	27.50 (0.00)	6.13	6.09	28.45	28.09
8	75.00 (0.00)	27.50 (0.00)	6.20	5.96	34.69	32.88
9	50.00 (-1.00)	15.00 (-1.00)	6.28	6.49	31.10	31.46
10	100.00 (+1.00)	15.00 (-1.00)	5.96	6.12	29.81	30.17
11	50.00 (-1.00)	40.00 (+1.00)	5.60	5.60	30.92	31.28
12	75.00 (0.00)	27.50 (0.00)	5.91	5.96	33.69	32.88
13	75.00 (0.00)	27.500 (0.00)	6.10	5.96	31.69	32.88
14	100.00 (+1.00)	40.00 (+1.00)	5.63	5.57	25.68	26.04

3.2Pareto chart of the effects of factors on the global acceptability and scavenging activity of the soybean yoghurt

Figure 1shows the Pareto plots for the responses. It can be observed that the factors that significantly ($p< 0.005$) the global acceptability was the quantity of cinnamon while for the scavenging activity was the quadratic effect of the quantity of ferment and cinnamon. The individual effect of the quantity of ferment and cinnamon.

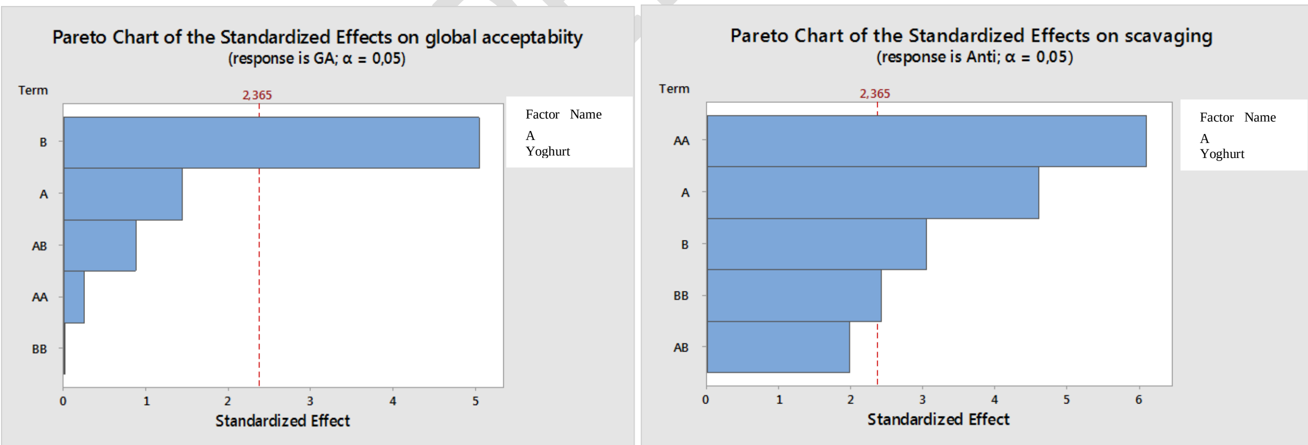

Figure 1. Pareto chart of the effects of quantity of ferment and cinnamon on the global acceptability and scavenging activity

3.3Proposed model for the global acceptability and scavenging activity

The equations below are the proposed model for the various responses. It can be highlighted that, the global acceptability was negatively influence by the individual effect of the quantity of ferment, cinnamon, quadratic effect of the quantity of cinnamon and positively influence by the quadratic effect of the quantity of cinnamon and interaction effect of the quantity of cinnamon and ferment. The scavenging effect on the other hand was positively influence by the individual effect of the quantity of ferment and cinnamon and negatively influence by quadratic effect of the quantity of cinnamon, quantity of ferment and the interaction effect of the he quantity of cinnamon and ferment.

$$\text{Global acceptability} = 7.63 - 0.0073X_1 - 0.0501X_2 - 0.000030\,X_1^2 + 0.000009X_2^2 + 0.000280\,X_1 * X_2$$

$$\text{Scavenging activity} = 10.09 + 0.561\,X_1 + 0.464\,X_2 - 0.003598\,X_1^2 - 0.00569\,X_2^2 - 0.00317\,X_1X_2$$

3.4Contour plot showing the optimum global acceptability and scavenging activity of the yoghurt

Figure 2 shows the contour plots for the yoghurt's global acceptability and scavenging activity. It reveals that the shaded zone of 6.4 to 7 (Figure 2) was the maximizing optimum global acceptability. Also, from the contour plot below (Figure 2), it can be observe that the maximizing scavenging activity is 34 %.

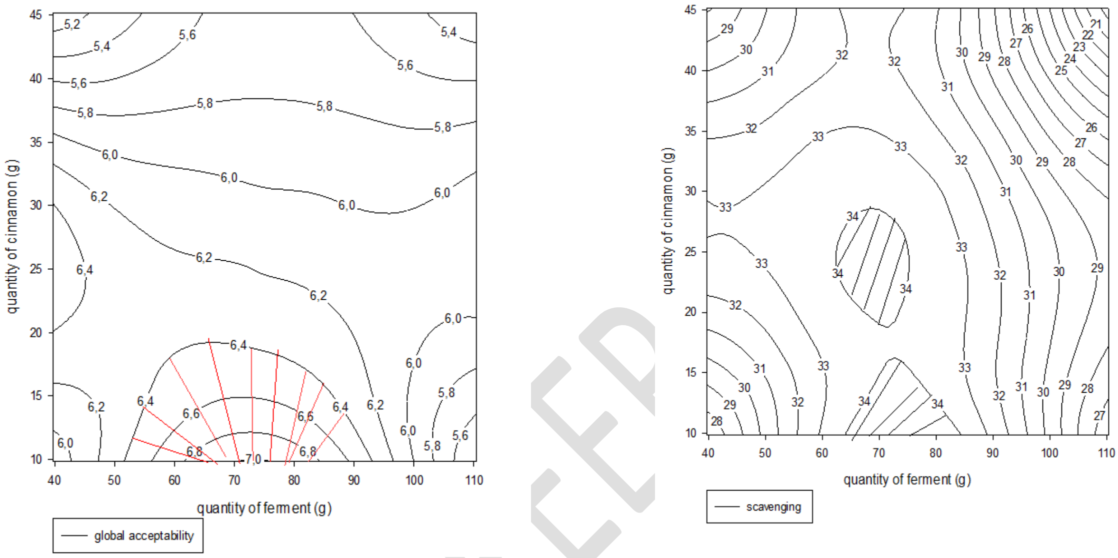

Figure 2. Contour plot showing the optimum zone for yoghurt (a) global acceptability (b) and scavenging activity

3.5Verification of the individual optimum conditions of the responses and validation of the model

It should be noted that, the global acceptability has as optimum conditions 39.64g (quantity of ferment), 9.8 g (quantity of cinnamon). The scavenging activity on the other hand has as optimum condition 69.64g and 9.8 g respectively for quantity ferment and cinnamon. Pasteurization time 150 mins and fermentation temperature 42°C. Verifying these conditions (Table4), led to no significant differences between the experimental values of the responses and the predicted optimum values provided by the model. Also, all the responses had good validation parameters' values for the model as it is in the accepted range of one, zero, > 75% and between 0.75 and 1.25 respectively for desirability, AMDA values, coefficient of determination (R^2) and Biaisfactor.

Table 4. Verification of the optimum conditions for the global acceptability and scavenging activity

	Individual responses	
	Global acceptability	**Scavenging activity**
Model predicted value	6.70 ± 0.00^a	34.35 ± 0.00^a
Experimental values	6.8 ± 0.09^a	34.00 ± 0.17^a
Desirability	0.99	0.93

AMDA	0.00	0.02
Bias factor	0.85	0.77
Coefficient of determination (R^2)	83.86%	92.75%

Values are mean ± standard error of double determinations. Different superscript within the same column differ significantly ($p < 0.05$) using Duncan multiple range test.

3.6Multiple optimizations

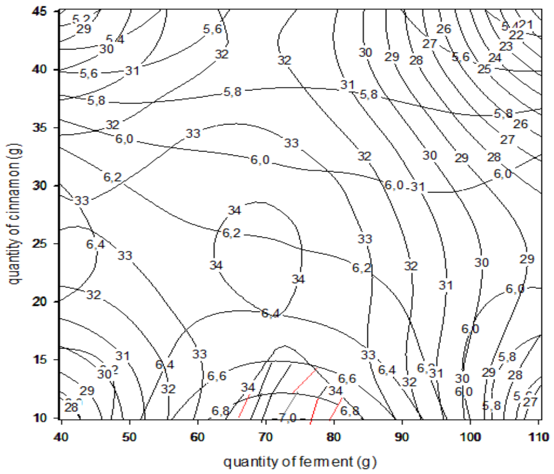

Figure 3. Overlaid contour plots of global acceptability and scavenging activity of the yoghurt

Multiple responses enable building of an appropriate response surface model that group all the responses and then trying to find a set of operating conditions for all responses by keeping them in desired ranges. Here (Figure3), the shaded regions is the compromised zone for all the responses. From this zone, the optimum condition was as thus: 69.64 g for quantity of ferment and 10 g for quantity of cinnamon. Manipulating under this condition gave global acceptability and scavenging activity that were not significantly different from the model predicted value with a good desirability (Table 5).

Table5. Verification of compromised optimum condition

	Global acceptability	Scavenging activity
Model predicted value	6.60 ± 0.00[a]	33.58 ± 0.00[a]
Experimental values	6.8 ±0.02[a]	34.00 ±0.29[a]
Desirability	0.85	

Values are mean ± standard error of double determinations. Different superscript within the same column differ significantly ($p < 0.05$) using Duncan multiple range test.

3.7Response surface analysis for global acceptability and scavenging activity of the yoghurt

The response surface plot analysis showing the effect of the individual factors on global acceptability and scavenging activity of yoghurt is shown in Figure 4. It can be inferred that, both the global acceptability and scavenging activity increases as the quantity of ferment and cinnamon increases up to a maximum where it drops with increasing quantity of this factors.

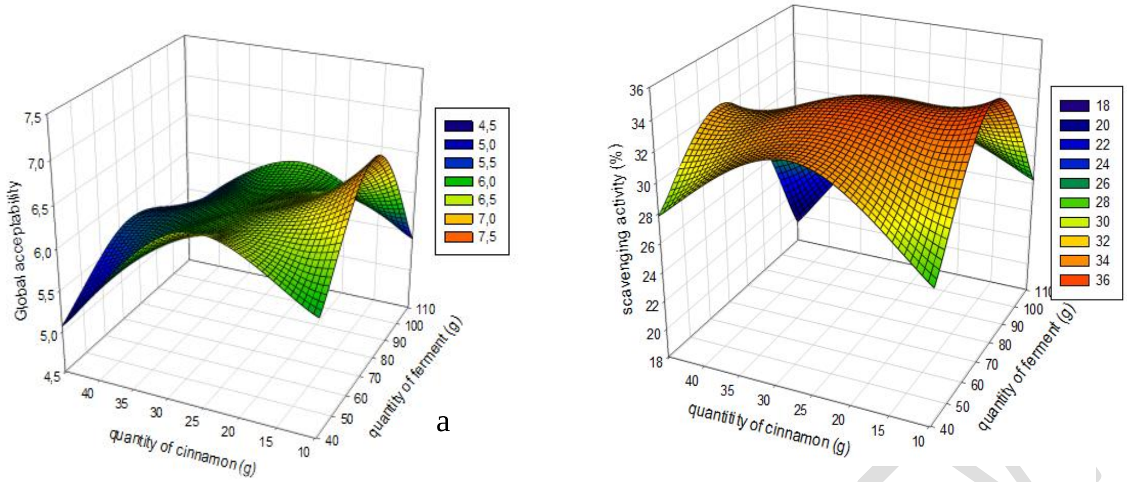

Figure4. Response surface plot showing the effect of quantity of ferment and cinnamon on the global acceptability (a) and the scavengingactivity(b)

3.8pH and proximate composition of the optimized yoghurt

Table 6 shows the proximate composition of the optimized yogurt sample.

Table 6. Proximate composition and pH of the optimized yogurt sample

Parameters	Amount
Proteins (% in dry sample)	17.10 ± 0.01
Fat (% in dry sample)	2.91 ± 0.02
carbohydrate(% in dry sample)	17.93 ± 0.01
Calcium(mg/100g in dry sample)	144.00 ± 0.01
Sodium (mg/100g in dry sample)	159.91 ± 0.01
Magnesium (mg/100g in dry sample))	63.18 ± 0.02
Ash content (% in dry sample)	1.00 ±0.02
Moisture content (% in freshsample)	61.07 ± 0.83
Energy value (kcal/100g)	166.31 ± 0.02
pH	4.30 ± 0.00

4. DISCUSSION

The overall acceptability and scavenging activity of the yoghurt increases as the quantity of cinnamon and ferment increase, this is because cinnamon has antioxidant effects, colour and flavouring properties [18]. Similar observations were made by Güneş and Bilgi[2] on the effect of cinnamon on microbiological, chemical and sensory analyses of probiotic yogurt. The ferment on the other hand contents *Lactobacillus*, *Streptococcus* and *Leuconostoc*which help in the fermentation process [8]. This approach leads to new products with better properties and different components, such as bioactive peptides. Another outcome of fermentation is the reduction of unwanted constituents present in raw materials, such as allergens or phytic acid [19]. Fermented foods are considered for their capacity, not only to prevent their alterations and to extend shelf life, but especially for their potential benefits on human health. The advantage of this process is to improve the quality of the original product, such as flavours, aroma and appearance. Fermentation is also used to facilitate the hydrolysis of oligosaccharides and proteins present in plants that are not digested by the human gut [20]. Moreover, the action of fermented bacteria on soy proteins can lead to release of antioxidant bioactive peptides, bearing beneficial properties both for the maintenance of food quality and for human health [21]. This observation is in the same line with that of Tonolo et al.[21] on antioxidant properties of fermented Soy during shelf life.

Multiple optimizationsare useful approach to optimization of multiple responses as it uses the simultaneous optimization technique popularized by Derringer and Suich[22]. Their procedure makes use of desirability functions. Here, the desirability was closer to 1 thus the condition is satisfied [23]. Also, the fact that there was no significant difference between the experimental and the predict results showed that the model is good [24].

The results for the validation of the model shows that there is no significant difference between the experimental and the predict results of these responses thus the model is good [24]. This is further justified by their desirability values which is a function which helps to find a combination of the experimental factors that provides a good result for multiple response variables. When this function is zero (0), it means the condition is not satisfied and when equal to 1 it means full satisfaction. Thus full satisfaction is obtained as these values were closer to 1. The percentage absolute mean deviation analysis (AMDA) obtained was almost zero which is the accepted value for this parameter and the Bias factors (Bf) onthe other hand was between 0.75 and 1.25 [25]. Also, the high coefficient of determination shows that the experimental values are equivalent to the predicted theoretical model values since the R^2 statistic is higher than 75% [26].

The fat, ash, carbohydrate, lipid, moisture, calcium, sodium and magnesium content obtain in the optimized yogurt is in the range of Madu et al. [27] on physical and proximate compositions of selected milk products. The presence of minerals such as calcium, magnesium, and sodium in the product indicates that this it can be a source of these minerals. Minerals in the formulated diet can maintain health, cell growth and proliferation, metabolism, development and immunological functions. The determination of dairy product's pH gives an indication of the hygiene and determines sample acidity and alkalinity. The acidic pH value of the sample can be attributed to the action of lactic acid bacteria (bacteria growth) which causes acidity increase [27]. Similar pH value was obtained by Khadka[1] on preparation and shelf life study of cinnamon oleoresin incorporated yoghurt.

CONCLUSION

From this investigation, the optimum condition required for the production of yoghurt from soya bean and cinnamon is 69.64 g for quantity of ferment and 10 g for quantity of cinnamon for a 100g soya beans, 100g of sugar and 1L of water. Globally, this study shows that acceptable yoghurt with functional properties can be obtained from soya beans and cinnamon. Thus the consumption of this product is highly encourage to the population. We recommend the integration of the optimized yoghurt into the dietary habits of the populations due to itsgood nutritional and therapeutic potentials.

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