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Ingredient Optimization of Functional Low-Fat Spread Using Response Surface Methodology

Suman Kharb[1,2], Dilip Kumar Thompkinson[2] and Harshita Kumari[1]*

[1]James L. Winkle College of Pharmacy, University of Cincinnati, Cincinnati, OH, USA

[2]Dairy Technology Division, National Dairy Research Institute, Karnal, Haryana, India

***Corresponding Author:** Harshita Kumari, James L. Winkle College of Pharmacy, University of Cincinnati, Cincinnati, OH, USA.

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Abstract

There is increasing consumer demand for fortified low-fat spreads with favorable sensorial and rheological characteristics. Fortification is achieved by the addition of ingredients that can modify functional properties of formulation. In this paper, we have created a new low-fat fortified formulation comprising of a lipid phase which contained milk fat, soy oil, and mustard oil. The resultant formulation was further optimized to provide 11.73% omega-3 fatty acids which could assist in reducing cholesterol levels in consumers. To enhance the sensory and rheological attributes, response surface methodology (RSM) was used to optimize the contents of ingredients: skim milk powder (SMP) (15 - 25%), xanthan gum (XG) (0.2 - 0.6%), carboxymethylcellulose (CMC) (0.2 - 0.6%), and inulin (4 - 8%), and processing temperature (70 - 80°C). The individual and interactive effects of all variables on the sensory and rheological attributes were investigated (50 trials) and an optimum formulation was determined to contain 15.02% SMP, 0.20% XG, 0.6% CMC, and 4.25% inulin with a processing temperature of 71.13°C.

Keywords: *Response Surface Methodology Omega-3; Inulin; Skim Milk Powder; Whey Protein Concentrate*

Abbreviations

RSM: Response Surface Methodology; (RSM), SMP: Skim Milk Powder; XG: Xanthan Gum; CMC: Carboxymethylcellulose (CMC)

Introduction

The ease of accessibility to information through online methods has led to increased dietary awareness among consumers. More so, there is utmost interest in low-fat food materials which can have added functional attributes [1-3]. Dairy is one of the most important as well as most used ingredient in a variety of food products due to its nutritional value; however, several studies have been published wherein low-fat diet is recommended to control health conditions, such as, diabetes [4], polycystic ovarian syndrome/PCOS [5], cardiac [6] etc. Butter and spreads are vital food products in most countries; hence, low fat versions of these food products with functional ingredients are in much need.

In spreads, milk, vegetable oils, or a blend of both can be used as a fat source. The popularity of butter, a milk-fat-based spread, has decreased owing to its high cost, low spreadability, and high saturated fat and cholesterol contents. In response to rising consumer demand for spreads that are healthy and palatable with good textural properties, recent research efforts have focused on the production of modified butter and fat spreads of various types. To enhance the nutritional value and spreadability of butter, milk fat has been blended with vegetable oils, and a variety of high-fat, low-fat, and very-low-fat spreads have been reported. Low-fat dairy spreads, which fall into the category of fat spreads, are also known as yellow-fat spreads [7]. Spreads with at least 15% fat are w/o emulsions, whereas low-fat spreads with decreased fat concentrations are typically o/w emulsions [8].

Changes in the eating patterns of health-conscious consumers has resulted in a demand for foods with health-beneficial functional attributes. Although there are many definitions, functional foods can be categorized as containing functional ingredients to promote health and prevent diseases caused by nutritional deficiency. Research attention has been focused on the disease prevention or treatment abilities of various functional ingredients in food product formulations, including dietary fiber, omega-3 and omega-6 fatty acids, antioxidants, vitamins, and minerals. It has been found that replacing saturated fatty acids with omega-3 fatty acids can lower plasma cholesterol levels and consequently reduce coronary heart disease mortality. Omega-3 fatty acids are typically found in fish oil or vegetable oils such as canola, soy, or flaxseed oil. As efficient dietary fibers with prebiotic properties, primarily related to the specific enhancement of bifidobacteria in the stomach [9], both inulin and oligofructose have been incorporated into new food products. Notably, inulin also has gel-forming capabilities, which can be exploited to mimic the texture and macroscopic properties of fat. As the replacement of fat by inulin does not alter mouth feel and structure, inulin has potential as a fat substitute in reduced-fat or low-fat spreads [10]. Nevertheless, food manufacturers have typically focused on the nutritional or technological properties of inulin [11]. Antioxidants, which can prevent or delay cell damage by free radicals, can be obtained from natural or artificial sources. Antioxidants are abundant in fruits and vegetables, and specific antioxidants, particularly vitamin E, are also found in vegetable oils. Fat spreads are an excellent medium for fat-soluble antioxidants, and synergistic effects can be achieved. In developed countries, food is commonly fortified with essential functional ingredients. However, in developing Asian and African countries, there remains a need for highly fortified foods to address issues such as extreme malnutrition. For example, sufficient levels of calcium as a functional ingredient may decrease the risk of osteoporosis in the elderly [12], as supplementation with calcium (in conjunction with vitamin D) can decrease bone loss and fracture risk [13]. Moreover, increased calcium intake can decrease blood pressure in both elderly and hypertensive subjects.

Based on health requirements, attention has mainly been focused on formulating fat spreads with better characteristics than butter, less fat than margarine, and high contents of polyunsaturated fatty acids. As a result, spreads with varying quantities of fat, moisture, protein, and other additives have been developed. Furthermore, the production of low-fat spreads using vegetable oils, milk fat, milk and vegetable proteins, stabilizers, and preservatives has been investigated. In 2019, Espert and co-workers [14] made reduced fat spread based on anhydrous milk fat and cellulose ethers. Soon after, in 2000, Reddy and co-workers [15] have reported a low-fat dairy spread using chakka and butter. Chakka is obtained from drainage of whey from curd and is used in preparation of low-fat dairy product. Notably, current consumer demands necessitate the development of low-fat spreads containing protein and enriched with dietary fiber, minerals and omega-3 fatty acids. Herein, we report optimization of a new low-fat spread which consists of all three: protein, dietary fibers and omega-3 fatty acids.

Materials and Methods

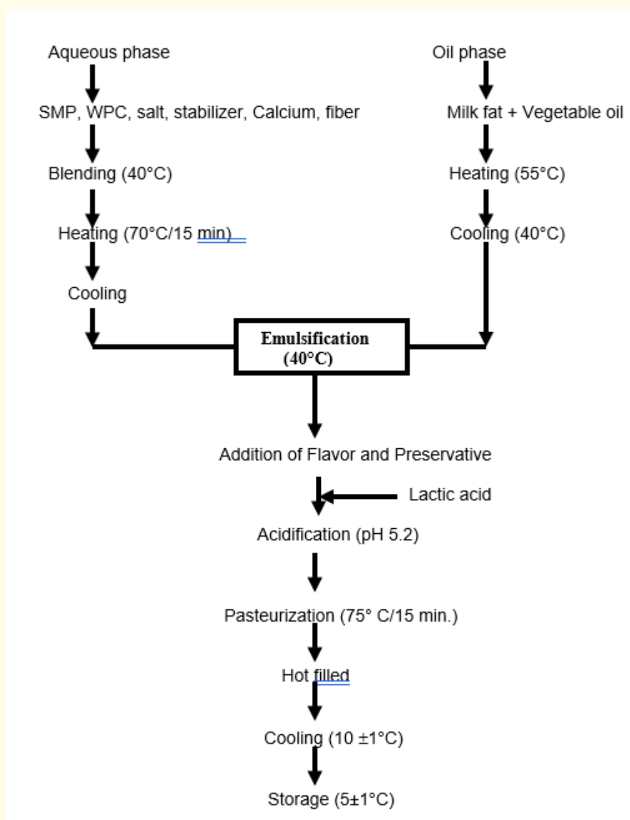
Materials

Skim milk powder (SMP) and WPC were used as protein sources in the low-fat table spreads. SMP was obtained from Modern Dairies (India), and WPC was obtained from Mahaan Protein, Ltd. (India). Milk fat and vegetable oils were used as fat sources. Milk fat (clarified ghee) was produced at the National Dairy Research Institute (NDRI, Karnal, India). Refined and deodorized soybean oil (Gemini Brand, India) and refined mustard oil (Dhara Barad, India) were used as vegetable oils for preparing suitable fat blends. Inulin (Raftiline ST, Orafti

Active Food Ingredients, Belgium) was used as a dietary fiber source. XG; Lucid Colloids, India) and CMC; Central Drug House Pvt. Ltd., India) were used as hydrocolloids. Iodized salt (Tata Chemical, India) was purchased from a local market in India. Lactic acid (AR, 88.0%, S.D. Fine Chem. Ltd., India) was used as an acidulant.

Methodology

Scheme 1 shows an overview of the procedure used to prepare w/o-type emulsions as low-fat table spreads. In the schematic, we show the oil and aqueous phases were prepared with varying proportions of ingredients that were optimized through RSM method. The aqueous phase comprised of dry ingredients which were blended in water before emulsification, whereas the oil phase was in liquid state. A suitable low-fat oil phase comprising an appropriate level of omega-3 fatty acids (11.73%) with an omega-6/omega-3 ratio of 1.67%, was determined by varying proportions of milk fat and vegetable oils (soybean and mustard oil) and tested via GLC (gas-liquid chromatography). The suitable aqueous phase, on the other hand, was determined by testing varying percentages of ingredients. Specifically, following range of ingredients and temperature were tested through RSM method with a central composite rotatable design (CCRD): milk protein: 15% - 25% SMP, whey protein: 2% (fixed), stabilizer/hydrocolloids: 0.2% - 0.6% XG and CMC, and dietary fiber: 4% - 8% inulin, processing temperature (70 - 80°C). The CCRD design (A statistical design. Central composite rotatable design is most commonly used factorial used in RSM) had 50 experiments that were carried out in randomized order and included 32 factorials, 10 axial and 8 replicates; see SI, table S1).



Scheme 1: *Schematic diagram of the manufacturing process of the low fat spread.*

Analytical methods

Physicochemical attributes

The gravimetric method outlined in AOAC was used to determine the moisture and ash content of the optimized spread [16]. The Mojonnier method was used to determine the fat content of the optimized spread [17]. The lactose and protein contents of the optimized spread were determined using the Lane-Eynon method [18,19]. The colour of final formulation low fat spread sample was measured by taking multiple readings and then calculating the average value, using a Colorflex Model (Hunter Lab, USA) fitted with dual beam xenon flash lamp equipped with the Universal Software (version 4.10). The instrument was first calibrated using standard black glass and white tile as specified by the manufacturer. The Data was presented in terms of lightness (L*), ranging from zero (black) to 100 (white), redness (a*), ranging from +60 (red) to -60 (green) and yellowness (b*), ranging from +60 (yellow) to -60 (blue) in values of the international colour system, when presenting the samples to the instrument in a standard, repeatable manner.

Sensory attributes

Sensory evaluation is a useful tool for determining the acceptability of food. Expert panelist were trained beforehand. A panel of ten trained judges recruited from the faculty of the Dairy Technology Division at NDRI (Karnal, India) assessed the sensory characteristics of the prepared low-fat spreads using a nine-point hedonic scale, as defined by Shone., *et al*. [20] which ranged from 1 (dislike extremely) to 9 (like extremely). Before evaluation, experts were trained for 30 minutes on how to perform the test. Assessments of color, flavor, body and texture, spreadability and overall acceptability were carried out at weekly intervals by the panelists. The panelists tested color through visibility, flavor through taste, body and texture through physical state (liquid/semi-solid) and the spreadability by uniformly spreading the product over a slice of bread at 5 ± 1°C.

Rheological attributes

The rheological characteristics of the prepared spreads were evaluated using a TAXT2i texture analyzer (Stable Micro Systems, UK) equipped with a 25 kg load cell (maximum). The spreadability and hardness were tested at 5°C using a P 25 cylindrical aluminum probe which is attached to the texture analyzer. The analyzer was calibrated with a 5 kg load cell and a force was applied to the product to a depth of 25.0 mm.

Consumer acceptability

In order to study the consumer's acceptance of the spread, it was served to large numbers of consumers representing both sexes and all age groups and their comments were recorded in the consumer appraisal card.

Results and Discussion

The effects of selected ingredients (SMP, XG, CMC, and inulin) and temperature on the sensory and rheological properties of functional low-fat spreads were investigated using RSM. In the CCRD, 50 trials including 32 factorial points, 10 axial points, and 8 repeats were conducted in a randomized order (SI, table S1).

Effect of ingredients on sensory attributes of functional low-fat spreads

Color

Color was evaluated by visible judging. The color of final formulation based on the sensory score in the end was evaluated by instrument (Colorflex Model (Hunter Lab, USA).

The functional low-fat spreads had color and appearance scores in the range of 5.0 - 7.6. The formulation with 31.89% SMP, 6% inulin, 0.4% XG, and 0.4% CMC processed at 75°C received the lowest score, whereas that with 15% SMP, 0.2% XG, 0.6% CMC, and 4% inulin pro-

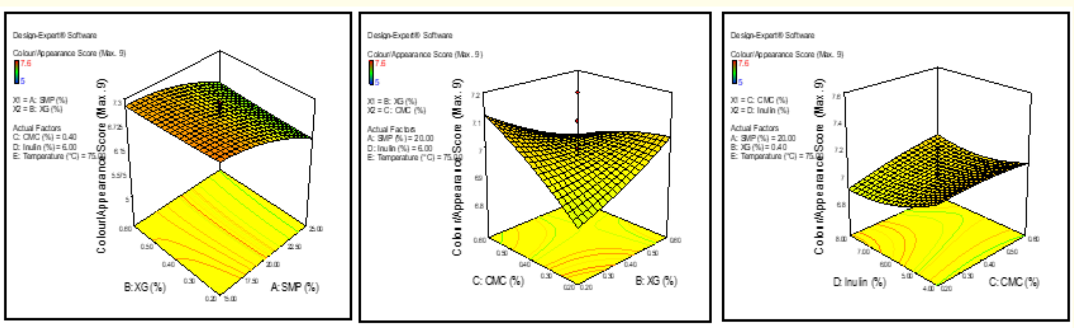
Run order	A SMP	B XG	C CMC	D Inulin	E Temp.
46	15	0.2	0.2	4	70
32	25	0.2	0.2	4	70
30	15	0.6	0.2	4	70
15	25	0.6	0.2	4	70
10	15	0.2	0.6	4	70
11	25	0.2	0.6	4	70
39	15	0.6	0.6	4	70
13	25	0.6	0.6	4	70
18	15	0.2	0.2	8	70
29	25	0.2	0.2	8	70
19	15	0.6	0.2	8	70
48	25	0.6	0.2	8	70
03	15	0.2	0.6	8	70
16	25	0.2	0.6	8	70
21	15	0.6	0.6	8	70
09	25	0.6	0.6	8	70
41	15	0.2	0.2	4	80
20	25	0.2	0.2	4	80
36	15	0.6	0.2	4	80
34	25	0.6	0.2	4	80
17	15	0.2	0.6	4	80
37	25	0.2	0.6	4	80
02	15	0.6	0.6	4	80
43	25	0.6	0.6	4	80
22	15	0.2	0.2	8	80
12	25	0.2	0.2	8	80
24	15	0.6	0.2	8	80
26	25	0.6	0.2	8	80
38	15	0.2	0.6	8	80
44	25	0.2	0.6	8	80
27	15	0.6	0.6	8	80
23	25	0.6	0.6	8	80
49	8.1	0.4	0.4	6	75
04	31.8	0.4	0.4	6	75
31	20	-0.07	0.4	6	75
35	20	0.87	0.4	6	75
50	20	0.4	-0.075	6	75
06	20	0.4	0.87	6	75

05	20	0.4	0.4	1.24	75
42	20	0.4	0.4	0.75	75
01	20	0.4	0.4	6	63.10
47	20	0.4	0.4	6	86.89
28	20	0.4	0.4	6	75
14	20	0.4	0.4	6	75
45	20	0.4	0.4	6	75
33	20	0.4	0.4	6	75
08	20	0.4	0.4	6	75
25	20	0.4	0.4	6	75
07	20	0.4	0.4	6	75
40	20	0.4	0.4	6	75

Table S1: *Experimental design matrix (CCRD) for ingredients.*

cessed at 70°C received the highest score. As shown in SI, table S2, regression analysis of the data gave a coefficient of determination (R^2) of 0.92, which is higher than the recommended minimum value of 0.85 [21] for the sensory qualities of food products, and the adequate precision (23.71) was significantly higher than the table value of 4.00. In addition, an increase in the SMP level resulted in a highly significant ($p < 0.01$) decrease in the color score (SI, table S2). Similar trends were observed for inulin and the processing temperature, whereas the effects of XG and CMC were not significant. Furthermore, at high levels of inulin, the spread became grainy in appearance. Similarly, Guven., *et al*. [22] found that increasing the inulin content had a negative effect on the color/appearance score of ice cream. In addition, Rønn., *et al*. [23] reported that a temperature sweep that is higher than the mean imparted low-fat spreads with graininess.

Furthermore, SMP with XG was found to significantly affect the color and appearance (Figure 1a). When the amount of XG was increased, the score gradually increased, whereas this trend was reversed when the amount of SMP was increased, and the interactive effect was significant ($p < 0.05$). Goel., *et al*. [24] reported that increasing solid, not fat (SNF) levels in low-fat spreads containing more than 8% SNF derived from nonfat dry milk had adverse effects on appearance. The addition of both CMC and XG tended to increase the appearance score. However, the color and appearance were negatively affected by the interaction between these two variables (Figure 1b). The interactive effect of XG and temperature was also negative, although not significant. Figure 1c reveals that as the levels of CMC and inulin were increased, the color/appearance score also increased, and the interactive effect of these variables was positive but not significant.



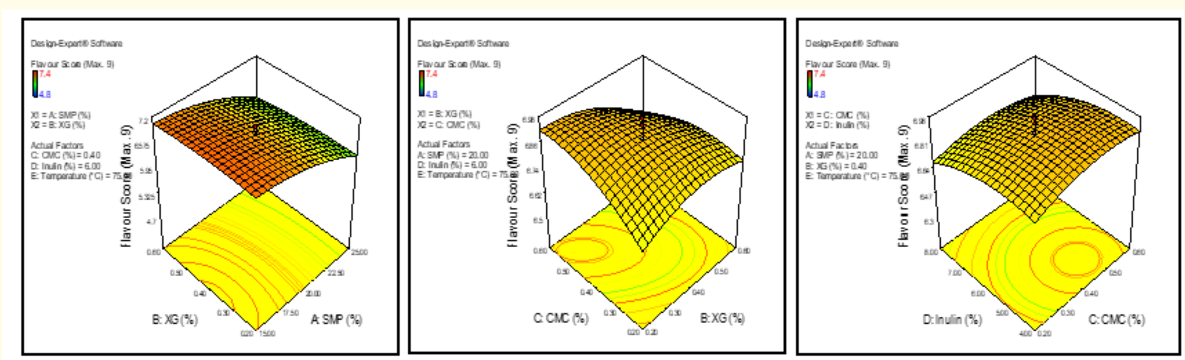
1(a) effect of SMP and XG 1(b) effect of XG and CMC 1(c) effect of CMC and inulin

Figure 1: *Response surface plot for color and appearance as influenced by varying ingredient levels.*

Flavor

Flavor is a key criterion for determining product quality and thus acceptability. The flavor score of the formulated table spreads ranged from 4.8 to 7.4. The formulation with 31.89% SMP, 0.4% XG, 0.4% CMC, and 6% inulin processed at 75°C had the lowest score, whereas that with 15% SMP, 0.2% XG, 0.6% CMC, and 4% inulin processed at 70°C received the highest score, indicating that the flavor score is adversely affected by higher SMP levels. The regression analysis gave an F value that was higher than the tabulated value and an R^2 value of 0.9811 (SI 2). Furthermore, the adequate precision value of 44.13 was higher than the table value of 4.00. Based on the coefficient values for the linear terms, SMP had the most significant negative effect on flavor, followed by inulin, processing temperature, and XG. According to Patel and Gupta [25], adding 5%-10% SMP to a spread improves the flavor and texture characteristics, whereas adding greater amounts SMP has a negative impact on the mouthfeel. The linear term of CMC had a significant ($p < 0.05$) positive effect on the flavor score. According to Deshpande and Thompkinson [26], spreads containing gelatin and/or CMC exhibit a pleasant taste, good consistency, and spreadability over a wide temperature range.

Figure 2a reveals that increasing the level of SMP from 15% to 20% resulted in a slight increase in the flavor score. However, this effect was attenuated by the negative coefficient ($p < 0.01$) of the quadratic term. Adding CMC and XG individually improved the flavor score, but the flavor score decreased when these ingredients were combined (Figure 2b). This reduction in flavor score upon the addition of combined stabilizers (XG and CMC) is consistent with previous findings. According to Malkki., *et al*. [27] the addition of a mixed stabilizer reduces the perceptible intensities of volatile and nonvolatile components. Furthermore, this decrease may be affected by the type of stabilizer used [28,29]. A significant negative interaction between CMC and inulin was observed (Figure 2c). Guven., *et al*. [22] reported that increasing the inulin content negatively affects the taste and flavor score of yogurt. Similarly, Vashishitha [30] found that a dietary formulation with a higher level of inulin received a lower sensory score.



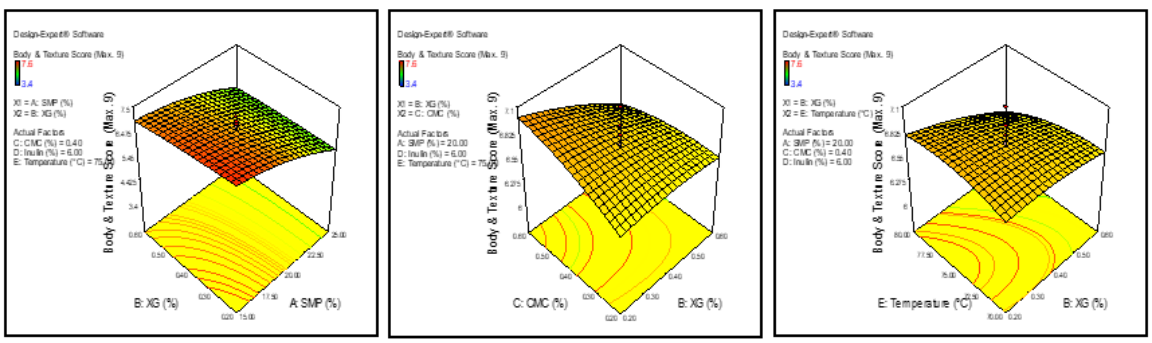
2(a) effect of SMP and XG 2(b) effect of XG and CMC 2(c) effect of CMC and inulin

Figure 2: *Response surface plot for flavor as influenced by varying ingredient levels.*

Body and texture

The consistency of a product is determined by its body and texture. The score for this attribute varied from 3.4 to 7.6. The lowest score was given to the formulation with 31.89% SMP, 0.4% XG, 0.4% CMC, and 6% inulin processed at 75°C, whereas the highest score was obtained by the formulation with 15% SMP, 0.2% XG, 0.6% CMC, and 4% inulin processed at 70°C. The R^2 value for the body and texture parameter was 0.95, which was much higher than the minimum desired value of 0.85, indicating that the model was satisfactory. The adequate precision value of 25.307 was higher than the minimum value of 4.00 required to achieve a satisfactory model (SI 2). For the linear terms, the regression coefficients revealed negative effects at higher levels of SMP, XG, and inulin, whereas a positive effect was observed for CMC (SI 2). The quadratic term for the processing temperature had a significant negative effect on the body and texture score. As the processing temperature increased, the amount of liquid fat also increased, which affected the product viscosity. Floury., *et al*. [31] reported that the oil and aqueous phases both show temperature-dependent viscosities, which decrease at higher temperatures, thus affecting the body and texture of the final product.

The combined interaction of SMP and XG had a significant positive effect on the body and texture characteristics (Figure 3a), which could be attributable to the enhanced water-holding capacity of milk protein. Spreads with MSNF contents of 10% - 12% have previously been found to have better body and texture [25,32]. As shown in figure 3b, increasing the level of XG decreased the body and texture score, whereas increasing the level of CMC resulted in a higher score, although the interactive effect was negative. The processing temperature was observed to have a negative interactive effect with XG (Figure 3c), which may be due to temperature-dependent changes in the conformation of XG [33].



3(a) effect of SMP and XG 3 (b) effect of XG and CMC 3(c) effect of XG and temperature

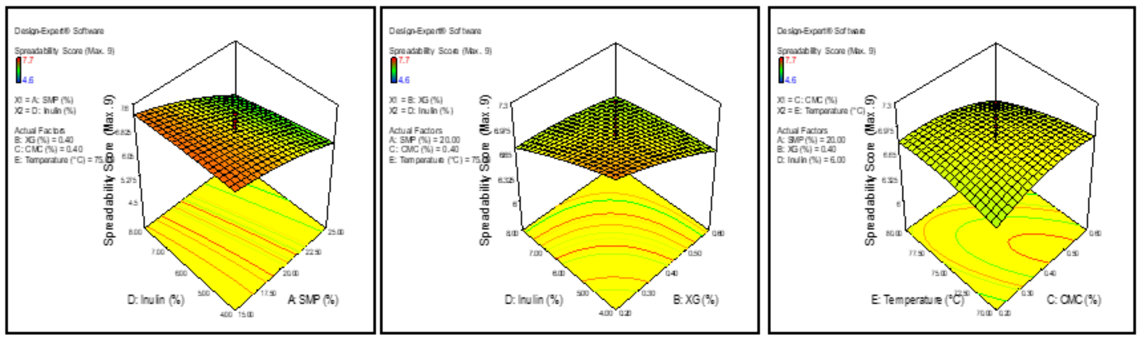
Figure 3: *Response surface plot for body and texture as influenced by varying ingredient levels.*

Spreadability

Advantageously, fat spreads can remain spreadable at low temperatures, and achieving spreadability is essential for low-fat table spreads. The spreadability score of the low-fat spreads varied from 4.6 to 7.7. The lowest score was obtained for the formulation with 31.89% SMP, 0.4% XG, 0.4% CMC, and 6% inulin processed at 75°C. The highest score was given to the formulation with 15% SMP, 0.2% XG, 0.6% CMC, and 4% inulin processed at 70°C. As shown in SI table S2, an R^2 value of 0.8942 was achieved, which is higher than the minimum desired value of 0.85, thus indicating that the model was highly significant ($p < 0.01$). The adequate precision value of 15.206 was higher than 4; hence, the model can be used to navigate the design space. Based on the regression coefficients for the linear and quadratic terms, increasing the levels of all the ingredients had a negative effect on the spreadability score.

In the case of SMP and inulin, a negative interactive effect was observed, as shown in figure 4a. Similarly, Suman and Thompkinson [34] found that increasing the levels of milk protein and dietary fiber in low-fat spreads resulted in decreased spreadability. However, the interactive effect of inulin and XG was found to be positive (Figure 4b). The interactive effect of temperature and CMC was also significant

($p < 0.05$), with an increase in the CMC level at higher processing temperatures decreasing the spreadability score, as shown in figure 4c. This negative effect could be due to a decrease in the solution viscosity at higher temperatures [35].



4(a) effect of SMP and inulin 4(b) effect of XG and inulin 4(c) effect of CMC and temperature

Figure 4: *Response surface plot for spreadability as influenced by varying ingredient levels.*

Overall acceptability

The overall acceptability score of the formulated table spreads varied from 4.0 to 7.6. The highest and lowest scores were given to the formulations a (25% SMP, 0.6% Xantum gum, 0.2% CMC, 4% inulin, 80°C processing temperature) and b (20% SMP, 0.4% Xantum gum, 0.4% CMC, 1.2% inulin, 75°C processing temperature), respectively. The R^2 value for the regression model was 0.97 and the F value was 47.08, which confirmed the very high statistical significance ($p < 0.01$) of the model. **Based on t**he linear terms, all the compositional variables, except the processing temperature, had significant ($p < 0.01$) effects on the overall acceptability score (SI, table S2). However, in terms of the quadratic terms, only CMC exhibited a positive effect, which may be due to its effect on color and appearance, flavor and body, and texture (SI, table S2). In contrast, the effect of the processing temperature was negative ($p < 0.05$). Notably, the effect of SMP was six times greater than that of inulin.

The response surface plot (Figure 5a) revealed that the overall acceptability score of the product decreased as the SMP level increased. In contrast, increasing the XG level above the center point increased the score. However, SMP and XG showed a significant positive interactive effect on the overall acceptability score. The opposite trend was observed for SMP with inulin (Figure 5b). In addition, the interaction of XG and inulin increased the overall acceptability score when SMP, CMC, or temperature was taken as the center point (Figure 5c).

Rheological attributes

Hardness/Firmness

Hardness, which is an indicator of product firmness and the ease of spreading, was evaluated using the peak force (N) measured during sample deformation. The hardness of the formulated table spreads varied from 138.45 to 3147.49g at 5°C. The formulation with 31.89% SMP, 0.4% XG, 0.4% CMC, and 6% inulin processed at 75°C had the highest hardness value. The lowest hardness value was observed for the formulation with 15% SMP, 0.2% XG, 0.6% CMC, and 4% inulin processed at 70°C. Based on the F value (10.98), the regression coefficients were highly significant ($p < 0.01$) (SI, table S2). Furthermore, the high adequate precision value (15.57) indicated a satisfactory quadratic model fit, which was verified by the R^2 value of 0.88. The regression coefficients revealed that all the ingredients had significant

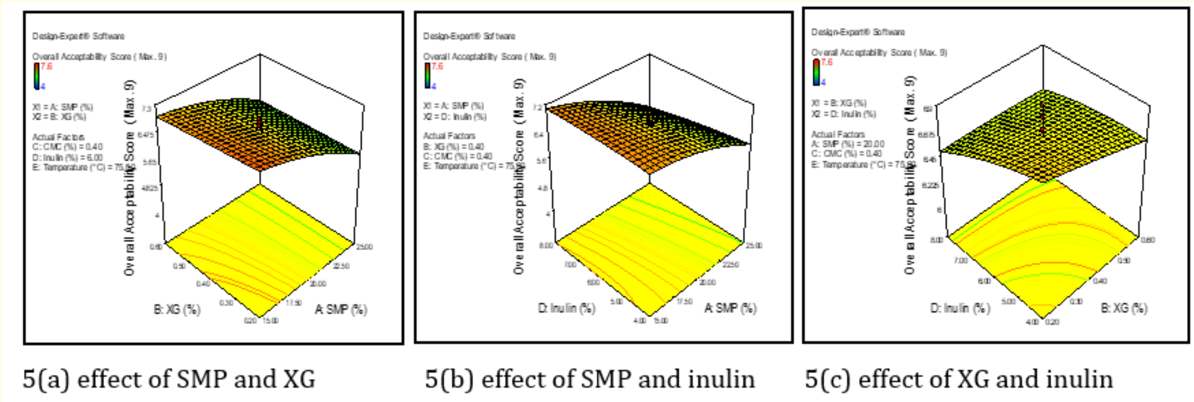


Figure 5: *Response surface plot for overall acceptability as influenced by varying ingredient levels.*

effects on the spread hardness at 5°C, with highly significant positive effects observed for the linear terms of SMP, XG, temperature, and inulin.

As depicted in figure 6a, the interaction between SMP and XG was significant ($p < 0.01$), with the firmness increasing at higher levels of SMP (~25%) and XG (~0.6%). Similarly, Prajapati [36] found that increasing the MSNF content of spreads caused a significant increase in hardness ($p < 0.01$), likely because the water-binding ability was enhanced. At higher concentrations, the combination of CMC and XG also increased the firmness of the spread, as shown in figure 6b, which could be attributed to an increase in the binding capacity of these hydrocolloids in the environment within the spread. The results were consistent with the previous findings of Patel and Gupta [25]. The combination of CMC with inulin or temperature decreased the firmness (Figure 6c), although these effects were not significant.

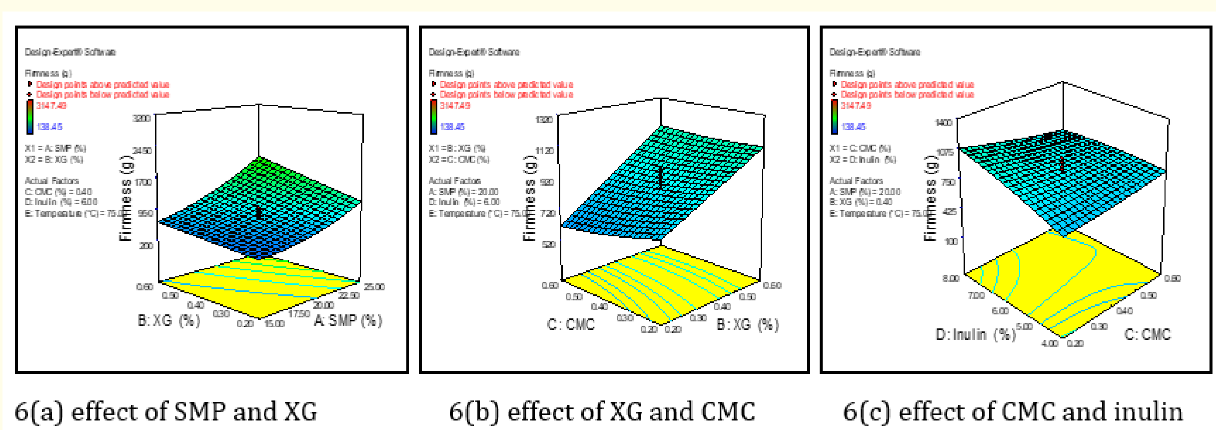
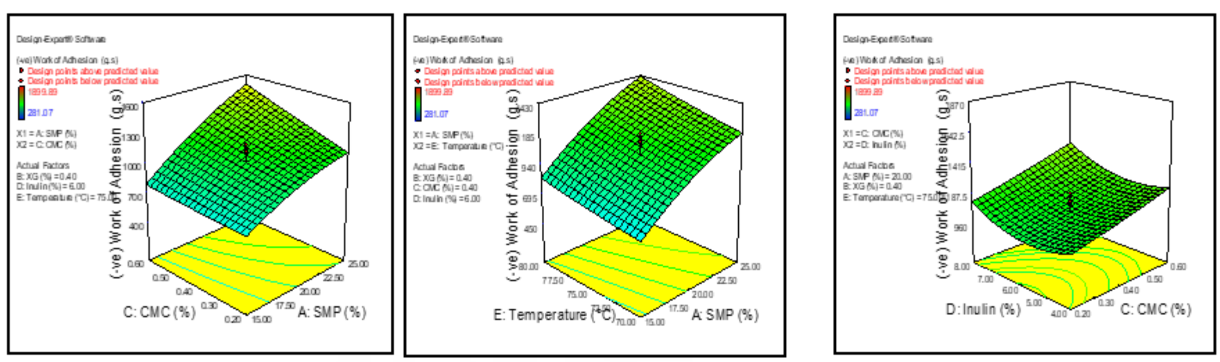


Figure 6: *Response surface plot for hardness/firmness as influenced by varying ingredient levels.*

Work of adhesion

The work of adhesion, which ranged from -281.07 to -1899.89 g.s., is related to stickiness and provides a measure of textural quality. The formulation with 25% SMP, 0.6% XG, 0.6% CMC, and 4% inulin processed at 80°C was the most adhesive, whereas that with 15% SMP, 0.2% XG, 0.2% CMC, and 4% inulin processed at 70°C was the least adhesive. The adequacy of the quadratic model was indicated by relatively high R^2 (0.85) and adequate precision (13.9) values (Table 1). The regression coefficients were positively correlated with the linear terms for SMP, XG, CMC, and temperature (SI, Table S2). Based on the linear terms, SMP and XG had significant ($p < 0.01$) effects on the adhesion. Furthermore, the interaction between XG and SMP had a positive effect ($p < 0.05$) at levels of 0.4% and 20%, respectively. In addition, the work of adhesion increased at higher levels of CMC, indicating a positive nonsignificant quadratic effect.

A positive interactive effect ($p < 0.05$) was observed for SMP and CMC (Figure 7a). After an initial decrease, the work of adhesion was increased by the combined effect of CMC and SMP at higher levels. Similar trends were obtained for CMC and SMP with temperature (Figure 7b), with increased adhesion observed as the levels of CMC, SMP, and the processing temperature increased. In contrast, higher levels of XG and inulin decreased the work of adhesion. Based on the linear terms, CMC and inulin had nonsignificant positive effects on the work of adhesion (Figure 7c). The work of adhesion decreased up to an inulin content of 6% but increased when the inulin content was increased further.



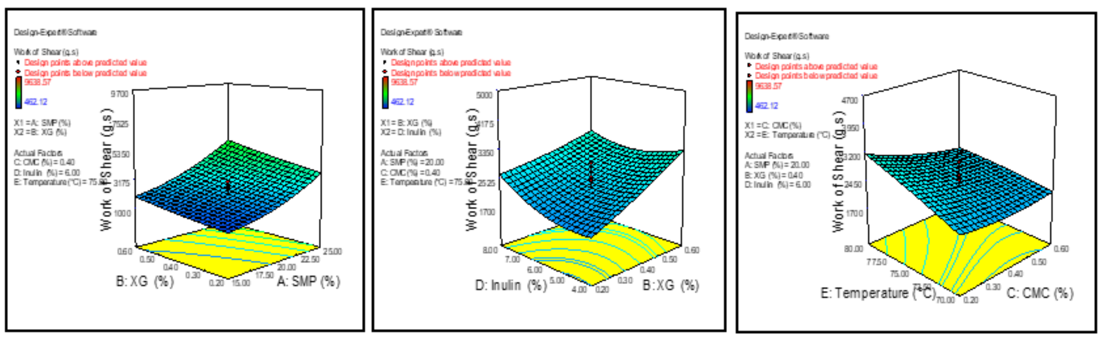
7(a) effect of SMP and CMC 7(b) effect of SMP and temperature 7(c) effect of CMC and inulin

Figure 7: *Response surface plot for work of adhesion as influenced by varying ingredient levels.*

Work of shear

The work of shear, which is the amount of force required for shearing, was evaluated as the resistance offered by the sample during probe penetration. The work of shear values ranged from 462.12 to 9638.57 g.s. The formulation with 15% SMP, 0.2% XG, 0.2% CMC, and 4% inulin processed at 70°C yielded the lowest work of shear value, whereas that with 31.89% SMP, 0.4% XG, 0.4% CMC, and 6% inulin processed at 75°C had the highest work of shear value. The regression analysis gave an F value of 9.60 (SI table S2), indicating that the model was highly significant ($p < 0.01$). The R^2 value was 0.86 and the adequate precision value was 14.168, confirming that the model could be used to navigate within the design space.

The regression coefficients for the linear terms showed that SMP and XG positively affected ($p < 0.01$) the work of shear (SI table S2). In addition, the quadratic term for SMP was also significant ($p < 0.01$). The interaction between SMP with XG had a positive nonsignificant effect (Figure 8a). The interaction of XG with inulin increased the shear (Figure 8b), whereas CMC and temperature had a nonsignificant negative interactive effect (Figure 8c). Thus, although the response observed for the work of shear was similar to that observed for hardness, the relationship with the level of each ingredient was essentially linear, with the greatest effect observed for SMP followed by inulin.



8(a) effect of SMP and XG 8(b) effect of XG and inulin 8(c) effect of CMC and temperature

Figure 8: *Response surface plot for work of shear as influenced by varying ingredient levels.*

Stickiness

As a sensory characteristic of semisolid foods, stickiness describes the sensation felt by the tongue and mouth [37,38], the intensity of which is related the tendency of the product to adhere to various areas in the mouth. The stickiness of the formulated table spreads ranged from -55 to -632.9g. The lowest value was obtained for a formulation with lower levels of ingredients (15% SMP, 0.2% XG, 0.2% CMC, and 4% inulin processed at 70°C), whereas the highest value was obtained for a formulation with higher levels of ingredients (25% SMP, 0.2% XG, 0.2% CMC, and 8% inulin processed at 80°C). The R^2 value was 0.859, the adequate precision value was 13.90, and based on the F value, the model was adequate for predicting the response (SI table S2). The regression coefficients for the linear terms indicated that all the ingredients except CMC had a significant ($p < 0.01$) positive effect on the stickiness. In contrast, the stickiness increased as the CMC concentration increased, which could be due to an increase in the water-binding ability of the product. These findings are consistent with the work of Patange [39], who reported that the use of higher quantities of CMC as a stabilizer instead of carrageen or pectin increased the stickiness of ghee-based low-fat spreads. Furthermore, increasing the level of inulin was observed to result in a stickier product. Similarly, Gel-Nagar., *et al*. [40] found that inulin-enriched yog-ice-cream samples exhibited greater stickiness, which was attributed to the formation of a viscous gel matrix.

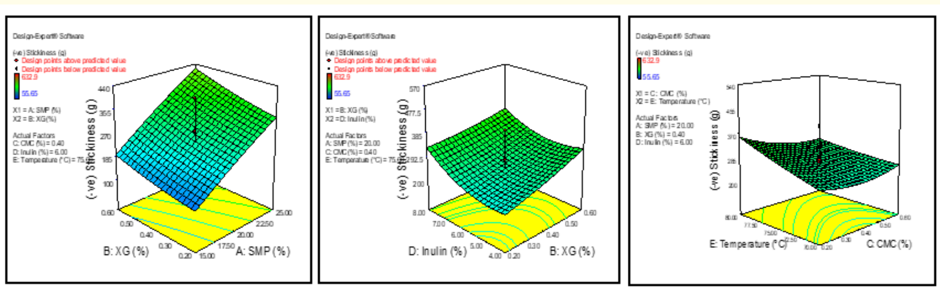
Based on the quadratic terms, the other ingredients had positive effects on the stickiness, although the effect of SMP was not significant, likely because the binding of fat via protein-lipid interactions increases stickiness. In previous studies, the stickiness of refrigerated spreads was found to decrease considerably with increasing MSNF levels [41,42]. The interactive effect of SMP and XG increased the stickiness of the spread (Figure 9a). A similar trend was observed for XG with inulin (Figure 9b), whereas CMC with inulin or temperature exhibited the opposite trend (Figure 9c). However, none of the interactive effects were statistically significant.

Optimal formulation

Based on the above results, data analysis was performed in the Design Expert 7.0.1 package to determine the optimal ingredient levels for producing a highly acceptable functional low-fat spread. The limits of all the constraints and the criteria used to determine the best

Experiment No.	Color/ appearance	Flavor	Body/texture	Spreadability	Overall acceptability	Hardness/ firmness	Work of adhesion	Work of shear	Stickiness
Intercept	6.993**	6.904**	6.738**	6.897**	6.677**	887.58	2439.42	2439.42	269.03
A-SMP	-0.370**	-0.417**	-0.728**	-0.606**	-0.638**	464.96**	1421.02**	1421.02**	107.55**
B-XG	0.0007	-0.042**	-0.142**	-0.0184**	-0.073**	220.67**	540.49**	540.49**	39.91**
C-CMC	0.040	0.027*	0.077*	-0.024	0.086**	-33.51	-206.37	-206.37	-15.21
D-INULIN	-0.058*	-0.073**	-0.181**	-0.165**	-0.123**	88.41*	192.37	192.37	41.23**
E-TEMP.	-0.084**	-0.056**	-0.047	-0.061	-0.045	114.94**	301.95*	301.95*	33.16**
A2	-0.168**	-0.183**	-0.239**	-0.130**	-0.199**	144.11**	445.39**	445.39**	-7.19
B2	0.016	-0.063**	-0.044	-0.041	-0.009	1.84	65.08	65.08	7.42
C2	-0.018	-0.061**	-0.062	-0.112*	0.013	15.91	107.69	107.69	21.60*
D2	0.060**	-0.068**	-0.036	-0.033	-0.039	-28.35	294.59*	294.59*	38.70**
E2	0.043	-0.094**	-0.084*	-0.590	-0.048*	-52.33	-39.51	-39.51	4.18
AB	0.059*	-0.017	0.102*	0.027	0.069*	121.04*	153.82	153.82	7.41
AC	0.058	-0.026	0.051	-0.109	0.040	36.24	30.85	30.85	11.84
AD	0.01	0.050**	-0.138**	-0.128*	-0.132**	-37.92	-55.59	-55.59	23.02
AE	-.016	-0.039*	-0.039	-0.113*	-0.083**	45.97	85.39	85.39	5.81
BC	-0.090**	-0.070**	-0.141**	-0.084	--0.217**	35.61	64.02	64.02	13.33
BD	0. 078*	0.019	0.079	0.109	0.069*	-75.78	-218.81	-218.81	14.55
BE	-0.009	0.029	-0.094*	-0.099	-0.110**	54.19	58.13	58.13	2.24
CD	0.041	-0.015	-0.056	0.009	0.003	-93.47	-252.12	-252.12	-20.62
CE	-0.035	-0.110**	-0.133**	-0.119*	-0.187**	-49.45	-177.28	-177.28	-17.80
DE	0.028	0.009	-0.047	-0.074	0.040	8.31	172.14	172.14	-1.75
R^2	0.925	0.981	0.950	0.894	0.970	0.88	0.86	0.86	0.85
F-value	17.95	62.50	27.700	12.25	47.08	10.98	9.60	9.60	8.83
Adequate precision	23.719	44.13	25.30	15.20	33.160	15.57	14.16	14.16	13.90
Press	2.580	0.730	5.520	8.39	2.570	7.83	7.13	7.13	5.23

Table S2: *Regression coefficient estimates based on scores for various sensory attributes.*
***Significant at p < 0.01, *Significant at p < 0.05.*



9(a) effect of SMP and XG 9(b) effect of XG and inulin 9(c) effect of CMC and temperature

Figure 9: *Response surface plot for stickiness as influenced by varying ingredient levels.*

formulation are presented in table 1. The goal was to maximize the sensory response while keeping the variables within their experimental ranges. Using this approach, the optimal formulation was found to contain 15% SMP, 0.20% XG, 0.60% CMC, and 4.25% inulin with a processing temperature of 71.13°C. For this optimal formulation, the predicated sensory ratings for color and appearance, flavor, body and texture, spreadability, and overall acceptability were 7.59, 7.3, 7.62, 7.58, and 7.57, respectively.

Constraint	Goal	Lower limit	Upper limit	Importance
SMP	Is in range	15.00	25.00	3
XG	Is in range	0.20	0.60	3
CMC	Is in range	0.20	0.60	3
Inulin	Is in range	4.00	8.00	3
Temperature	Is in range	70.00	80.00	3
Color/appearance	Maximum	5.00	7.60	3
Flavor	Maximum	5.00	7.40	3
Body/texture	Maximum	3.40	7.60	3
Spreadability	Maximum	4.60	7.70	3
Overall acceptability	Maximum	4.00	7.60	3

Table 1: *Criteria for formulation optimization.*

To test the validity of the optimized formulation, the corresponding sample was prepared in triplicate and the sensory characteristics were evaluated. A comparison of the predicted responses with the actual responses revealed a good correlation (SI table S3). When the t-test for two samples was performed assuming equal variance, the t value for each parameter was smaller than the table value, indicating that the observed values were similar to the predicted values. Thus, the combination of 15% SMP, 0.20% XG, 0.60% CMC, and 4.25% inulin with a processing temperature of 71.13°C was confirmed to produce the optimal sensory attributes for formulating an acceptable low-fat spread. The characteristics of the low-fat spread made with the optimized solution were further evaluated using physicochemical and microbiological tests, as summarized in table 2.

Comparison with market sample

The formulated table spread with functional attributes was compared with a popular brand of table spread available in the local market. Both the samples were subjected to sensory, rheological and physical evaluation for the purpose of comparative assessment. The

Attributes	Predicted	Actual	t value
Color/appearance	7.59	7.62	0.32
Flavor	7.30	7.36	0.69
Body/texture	7.62	7.65	0.19
Spreadability	7.58	7.61	0.44
Overall acceptability	7.57	7.58	0.70

Table S3: *Predicted and actual response values for the optimal spread formulation.*
*#Values predicted using design expert version 7.0.1, @ Actual values (average for the optimized product), *The t values were nonsignificant at $p < 0.05$, Table value: $t = 4.303$.*

Constituents	Formulated spread (g/100g)
Moisture (g)	38.00
Fat (g) ω3 (g) ω6:ω3 ratio	35.00 4.10 1.67
Protein (g)	7.50
Lactose (g)	11.50
Ash (g)	3.20
Dietary Fiber (g)	3.90
Stabilizer (g)	0.80
Calcium (mg)	222.20
Energy (kcal/100 g)	406.60
Water activity	0.958
Microbiological analysis	
Total viable count (log/ml)	2.00
Yeast & mold count (cfu/ml)	Nil
Coliform count (cfu/ml)	Nil
Rheological analysis at 5°C	
Hardness (g)	537.23 ± 4.42
Work of shear (g.s.)	1817.11 ± 285.14
Work of adhesion (g.s.)	-405.51 ± 19.74
Stickiness (g)	-159.56 ± 10.73

Table 2: *Proximate composition of the formulated table spread.*

results presented in SI, table S4 indicate that the formulated spread had high flavor score (7.76) as compared to market sample (5.36). The market spread was criticized for bland flavor as compared to the experimental spread, which had strong spicy flavor resulting in higher flavor score. Notably, the developed/optimized spread had remarkably improved spreadability over the market sample (score, 7.80 and 5.43). The overall acceptability scores of the experimental spread and market sample were 8.13 and 6.40 respectively. The developed spread was found to be significantly superior in all the sensory attributes as compared to market sample.

The differences in spreadability and body and texture scores of formulated and market sample observed are also supported by instrumentally measured texture parameters at 5°C. The hardness of the formulated spread was appreciably lower (537.23g) than that of market sample (1237g). Similar trend was observed for the work of shear and work of adhesion parameters. These results are indicative of firmer body and better spreadability of the product developed during this investigation as compared to the market sample. However, the stickiness value of the formulated product was lower (-159.56g) than that of the market sample (-346.14g). The lower stickiness value are indicative of the presence of higher PUFA rich vegetable oil in the formulated table spread resulting in greater lubricating effect during the test. This makes the product less sticky and therefore easily spreadable even at refrigerated temperature. Our findings are in corroboration with the findings of Deshpande [42]. He observed that the extruder friction value indicative of stickiness was lower in spread containing vegetable oil. The enhanced spreadability of the developed spread may be due to air incorporation during blending and emulsification might have played some role in imparting and improving spreadability to the product as observed by Kulkarni and Rama Murthy [43] and Patange [39].

The assessment of water activity attributes (SI table S4) reveals that the table spread developed during this investigation had lower water activity (0.958) than market sample (0.967). The higher water activity of commercial spread suggests a higher chance of spoilage of spread and less stability. The increase in water activity, increase the chance of growth of pathogen microorganism like *Clostridium botulinum* type E* and *Yersinia enterocolitica* [44]. Color value assessment (in terms of L*, a* and b*) of formulated table spread has higher L* and b* parameters, whereas a lower a* parameter (which indicate redness of the spread) than the market sample (SI table S4).

Properties		**Formulated spread**	**Market spread**	t_{cal}
Sensory	Colour/appearance	8.10	6.26	4.76**
	Flavour	7.76	5.36	7.85**
	Body & texture	7.80	5.43	7.20**
	Spreadability	8.30	6.6	10.57*
	Overall acceptability	8.13	6.4	6.65**
Rheological	Firmness (g)	537.23	1237	5.81**
	Work of adhesion (g.s)	-405.51	-758.33	7.59**
	Work of shear (g.s)	1817.11	3725.66	5.50**
	Stickiness (g)	-159.56	-346.14	6.21**
Colour (CIELAB) value	L*	83.76	81.92	12.40*
	a*	5.34	5.64	6.08**
	B*	29.91	17.62	88.69*
Water activity (25°C)	a_w	0.958	0.967	0.68^{ns}

Table S4: *Comparison between developed spread and market spread.*
***Significant at 1% level, * Significant at 5% level; 't'- table value 4.30.*

Conclusion

Based on the findings of this study, the following conclusions can be drawn. The selected fat blend was a good source of omega-3 fatty acids and maintained a beneficial omega-6/omega-3 ratio. XG and CMC worked well in combination to produce a spread with good body and texture, spreadability, and overall acceptability. Inulin was found to be a suitable source of dietary fiber for incorporation into table spreads in terms of functionality and texture. The optimal combination of ingredients for the formulation was 15% SMP, 0.20% XG, 0.6% CMC, and 4.25% inulin with a processing temperature of 71.13°C. The developed table spread, which had good physicochemical, sensory, and rheological characteristics, was superior to commercial fat spreads available in the Indian market. The formulated table spread may have good market potential with respect to both sensory attributes and cost in addition to offering functional qualities that can provide beneficial health effects.

Interpretive Summary

Fortification refers to adding ingredients that provide additional nutritional benefit due to ingredients' properties. Current study pertains to the formulation of a functional low-fat spreads wherein a lipid phase was prepared using a fat blend containing milk fat, soy oil, and mustard oil and was optimized to provide 11.73% omega-3 fatty acids. A response surface methodology/RSM was used to optimize the contents of ingredients and to enhance sensory and rheological attributes. The individual and interactive effects of all variables on the sensory and rheological attributes were investigated (50 trials) and an optimum formulation was determined.

Conflict of Interest

There is no conflict of interest.

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