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Formulation and Optimization of New Spreads Based on Olive Oil and Honey: A Response Surface Methodology Box-Benken Design

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Abstract

Bread spread is one of the fundamental foods in human diets. Generally, cheese spread, butter, chocolate spread, and margarine are the most consumed. In the last decade, a new concept alimentary has been integrated, it was low fat spread or functional spread. This work is an attempt to formulate and optimize new low-fat spreads based on olive oil and honey using a response surface methodology box-benken design. To optimize its stability and its textural properties under the effects of three factors, beeswax content, stirring time, and stirring speed. Results revealed that the best mixture was the formulation that contained 1% beeswax, 79% honey, and 20% olive oil, formulated under 6.39 min of time stirring at 15,428 rpm speed. The beeswax was the major factor showing the highest effect on all the properties of spreads.

Keywords

Beeswax, Olive Oil, Honey, Spread, Emulsion Stability, Textural Properties

1. Introduction

The purpose of many food processing operations is to create microstructures that give the product the desired properties. The artificial structured foods use texturing processes such as, for example, emulsification processes, foaming, spread kneading, etc. The final product has a complex microstructure held together by binding forces between the various phases. In addition to important properties such as stability and flavour, the control of the microstructure of artificial structured foods is often the quality-determining factor. The market need for zero or low-fat food products has become increasingly important and so new ingredients and knowledge are needed in order to manipulate these types of products, such as margarine, spreads, salad dressings. Spreads are semi-solid foods rich in fat which should flow easily when deformed. They are classified as water/oil (W/O) emulsions with different formulations consisting of fat, water, emulsifiers, stabilizers, salt, antioxidants, and other ingredients [1].

On the other hand, Bread is an important part of the diet in many parts of the world [2]. It is seldom eaten in a completely pure form, but usually in combination with other foodstuffs. Spreads are mainly applied to perform these two functions: adhesion of the "top layer" to the bread and lubrication. Moreover, some spreads are designed to serve specific nutritional purposes and to contribute to a healthy diet. The growth of the "functional foods" categories in the market stimulated and pushed spread manufacturers for development. As a result, some functional spreads were introduced. Like low fat spreads, cholesterol-lowering spreads, reduced sugar spreads, and enriched antioxidants spread. Cholesterol-lowering spreads are reduced in satured fat content, have been created by using some functional vegetable oil (use olive oil) instead of palm oil or butter.

In the development of a new functional spread, the knowledge of the structural behaviour of the oleogel in the formulation is fundamental to obtaining a product with adequate textural and physical-chemical properties.

Based on these considerations, the aim of this present work was to investigate the application of olive oil and honey to obtain functional spread with reduced satured fat content and enriched in antioxidants using beeswax as an emulsifier. The formulation was based on the use of reponse surface methodology RSM.

The choice of these two food matrices is based to their high nutritional values and their benefits on human health [3] [4]. Virgin olive oil is well known for its high content of phenolic substances, amount of oleic acid, and tocopherols that are thought to have health-promoting properties [5].

As for honey which is produced by bees from the nectar of flowers and honeydews, is a natural complex food with high nutritional value [6] [7] [8]. The major sugars in honey are monosaccharides, namely glucose, and fructose, it also contains maltose, sucrose, and other sugars in small quantities [9] [10]. It has been shown that fructose is absorbed more slowly from the gastrointestinal tract than glucose. For that reason, fructose is an insulin-independent monosaccharide found in honey and it is rapidly taken up by the liver when honey is consumed. Also, honey could be utilized as an appropriate sweetener alternative for the type II diabetic diet [4] [11].

2. Materials and Methods

2.1. Samples

The present study was carried out on monovarietal virgin olive oil from the Tunisian cultivars, namely Chetoui, planted in the north of Tunisia. The honey samples were derived from eucalyptus which came from the north of Tunisia.

Natural beeswax was provided by the Central Laboratory for Analysis and Testing.

2.2. Preparation of Spreads

As described in our previous research, we tried to formulate stable olive oil and honey-based spreads using beeswax as an emulsifier based on the stability of the product and the results of certain rheological parameters. Then, a sensory analysis was performed. Results showed that samples which have high honey content are preferable for consumers; those which are rich in oil hide an acidic and bitter taste. Hence olive oil (organic phase) was fixed at a concentration of 20% [12]. However, the continuous phase is a mixture of honey and beeswax at different concentrations. Three spread formulations have been prepared separately which differ only in the nature of the honey: derived from eucalyptus, thyme, and polyfloral origins.

2.3. Optimization of the Spreads Preparation by Response Surface Methodology (RSM)

The experimental design method as a subset of scientific investigation is a popular and widely used research approach. It seeks to determine and establish existing links between two sets of variables which are the factors and the responses, in order to optimize the organization of experimental trials to obtain the maximum information from a minimal number of experiments with the best possible precision. This objective can be achieved by establishing a causal relationship between independent variables.

For the present study, the Box-Behnken design method was used and which required three levels (−1, 0, +1) to fit the second-order model. Results analysis was performed by Design-Expert software (Version 11, Stat-Ease, Inc., Minneapolis, USA). The response surface methodology (RSM) was applied to determine optimum conditions for the new formulation spread based on olive oil and honey to optimize its stability and its rheological properties under the effects of t factors namely, Wax content, stirring time, and stirring speed.

Parameters such as Wax content (%), Stirring time (min), and Stirring speed (rpm) were taken as variables (Table 1) whereas Stability, Peroxide value, hardness, adhesion, and cohesion as responses.

Table 1. Natural and coded values of the independent variables used in the three levels RSM design implemented to optimize Spread formulations.

Coded values	Natural values		
	Wax Content X_1 (%)	Stirring time X_2 (min)	Stirring speed X_3 (rpm)
−1	1	3	15,000
0	3	6	16,500
+1	5	9	18,000

Based on the number of operating variables investigated in this study, the experimental results obtained will be fitted to the second-order polynomial regression model expressed as follows:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum_i \sum_j \beta_{ij} \cdot X_i \cdot X_j + \varepsilon$$

where Y represents the response: β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients; ε is the residual value between calculated and experimental data; X_i and X_j define the independent variables and n is the factors number. "i" (value is equal to −1, 0 or +1).

The use of the matrix thus formed has led to the performance of 16 separate tests where the experimental conditions vary in a programmed manner. The repetition of the central point (experiment 8, 9, 12, 16) was carried out in order to determine the experimental error (analysis of variance), an error which is incorporated in the calculation of the estimate of the effects of the factors (Table 2).

Fitting procedures, coefficient estimates, and statistical analysis were performed using Design-Expert software. The analyses of variance (ANOVA) were used to assess the significance of the model generated and of all the terms that

Table 2. Experimental values of different responses measured for spread new formulation based on Eucalyptus honey.

N°	Coded variables			Natural variables		
	Wax Content (%)	Stirring time (min)	Stirring speed (rpm)	Wax Content (%)	Stirring time (min)	Stirring speed (rpm)
1	1	0	1	5	6	18,000
2	0	1	−1	3	9	15,000
3	1	1	0	5	9	16,500
4	−1	0	1	1	6	18,000
5	0	−1	−1	3	3	15,000
6	−1	0	−1	1	6	15,000
7	1	0	−1	5	6	15,000
8	0	0	0	3	6	16,500
9	0	0	0	3	6	16,500
10	−1	−1	0	1	3	16,500
11	0	1	1	3	9	18,000
12	0	0	0	3	6	16,500
13	0	−1	1	3	3	18,000
14	1	−1	0	5	3	16,500
15	−1	1	0	1	6	16,500
16	0	0	0	3	6	16,500

make up the model, as well as the lack of fit. The method for testing statistical significance was performed by calculating the p-value from the F-value, considering the existence of significance for $P < 0.05$).

Only the statistically significant terms were used in the construction of the theoretical model. Coefficient of determination (R^2), adjusted coefficient of determination (R_{adj}^2), and adequate precision were used to estimate the adequacy of the polynomial equation to the response (Albuquerque, Pinela, Barros, Oliveira, & Ferreira, 2020a).

2.4. Physicochemical Analysis

2.4.1. Stability Test

15 g (F_0) of each sample was transferred to test tubes (internal diameter 30 mm, height 500 mm) which were tightly sealed with plastic caps and then centrifuged for 30 min at 5000 rpm (Hettich, Roto silent/K, Germany). The weight of the precipitated fraction (F_1) was measured, and the emulsion stability was characterized as follows:

$$\text{Stability}(\%) = (F_1/F_0) \times 100$$

Centrifugation was repeated three times to separate the oily fraction. Thus, the oily phase and the precipitate were recovered for the rest of the analyses 12.

2.4.2. Peroxide Value

The peroxide value, expressed in meq O_2/Kg body fat, was determined for the oily phase of the spread as described by ISO 3960: 2001.

2.4.3. Determination of Textural Properties

The texture is the set of rheological and structural properties perceptible by mechanical, tactile, visual, and auditory receptors. It represents a property of the food perceived on the sensory level and which evolves from the bite until the formation of the food bolus suitable for swallowing [13].

The texture is determined using a Texturometer, which allows compression tests to be carried out using a flat-ended probe (diameter of 30 mm). Texture analysis is performed using an "LLOYD X instrument TA plus" texturometer. One carries out a test of two successive compressions with: Sample height (H) = 35 mm, Speed (V) = 2 mm/s and Compression ratio (Ψ) = 50%.

The samples are kept at a temperature of 20˚C before the test. The parameters given by the texturometer are analyzed by expert design software. The criteria used are as follows: Hardness (N), Adhesion (N mm), and Cohesion.

3. Results and Discussion

3.1. Model Adequacy

Box-Behnken design was used to evaluate the influence of three factors Wax content (%), Stirring time (min), and Stirring speed (rpm). whereas Stability, Peroxide value, hardness, adhesion, and cohesion as responses are shown in **Table 3**. The regression coefficients determined by ANOVA for each model and

Table 3. Model statistical parameters and coefficients.

Coefficient	Stability (%)	Peroxide value (meq O_2 KG)	Firmness (N)	Cohesiveness	Adhesiveness (Nmm)
P value	0.0004	0.0061	<0.0001	<0.0001	<0.0001
X_1	−5.84	0.1087	40.98	9.71	17.66
X_2	0.3496	0.2557	−2.08	0.0511	−0.8959
X_3	−1.88	0.1425	−4.93	1.27	−2.12
X_{12}	−4.58	0.0012	0.6327	0.0209	0.2726
X_{13}	−1.58	0.1375	0.925	1.01	0.3986
X_{23}	−4.28	0.0375	1.23	0.0184	0.5279
X_1^2	−3.83	0.0576	21.29	4.8	9.17
X_2^2	−2.26	0.0162	−1.95	0.009	−0.8403
X_3^2	−1.96	−0.0101	1.31	0.0089	0.5635
F value	25.43	9.65	979.73	572.58	979.73
R^2	0.9745	0.93354	0.9993	0.9988	0.9993
Adj R^2	0.9361	0.8334	0.9983	0.9971	0.9983
Lack of fit	0.0694	0.5715	0.108	0.0781	0.108

the statistical parameters F-values, coefficient of determination (R^2), adjusted R^2, and lack of fit values are summarized in Table 3. The high F value for all responses revealed that the model obtained was statistically significant. The R-square of all responses was found between 0.933 and 0.993, which revealed a high accuracy of the models. Also, the Adjusted R-square was found to be acceptable for all responses, indicating a high degree of correlation between the experimental and predicted values. Besides, the p-value of lack of fit was insignificant for all responses (P > 0.05), indicating that the model fits the experimental data accurately. Statistical parameters show that the relationship between independent variables and different responses is adequately represented by the model.

3.2. Emulsion Stability

In order to successfully make a spread without the oily phase topped in the form of droplets, the key step was to introduce an ingredient that proves its role as much as an emulsifier with an adequate concentration and to play on certain parameters (a know the time and speed of agitation) when preparing this formulation. The main goal to achieve is to produce a spread with maximum stability.

In this present study, beeswax was chosen as an emulsifier for our spread since this property is currently well approved by the literature [14] for beeswax. Beeswax was approved as an excellent oleogelator [15]. The effects of these different independent variables (% beeswax, stirring speed, and stirring time) on the stability of our spread are displayed in **Table 3**. The Model F-value of 25.43 implies the model is significant. There is only a 0.04% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case, X_1, X_3, X_1X_2, X_2X_3, X_1^2, X_2^2, C^2 are significant model terms. The stability of the different formulations varies from 79.7% up to 98.55%.

The stability of the different formulations varies from 79.7% up to 98.55% shown in **Figure 1**.

We note that trials with maximum beeswax concentration exhibited the greatest instability (trial 1 and trial 3, 79.9% and 79.7% respectively). However, the tests with the minimum beeswax concentration are the most stable samples. Samples 10 and 15 differ only in the stirring time which is minimum for test 10 (3 min) and maximum for test 15 (9 min). We then conclude that the stirring time is a determining variable in the stability of the spread. Likewise, samples 4 and 6 differ only in the stirring speed which is minimum for test 6 and maximum for test 4. Then we conclude that the stirring speed is a determining variable of the stability of the spread. The stability results of these 4 samples are significantly different. Test 15 with maximum stirring time and the medium stirring speed was shown to be the most stable.

Beeswax with the minimum concentration set for (1%) was sufficient to bind the liquid phase to the oily phase. It only takes 9 minutes and a stirring speed of

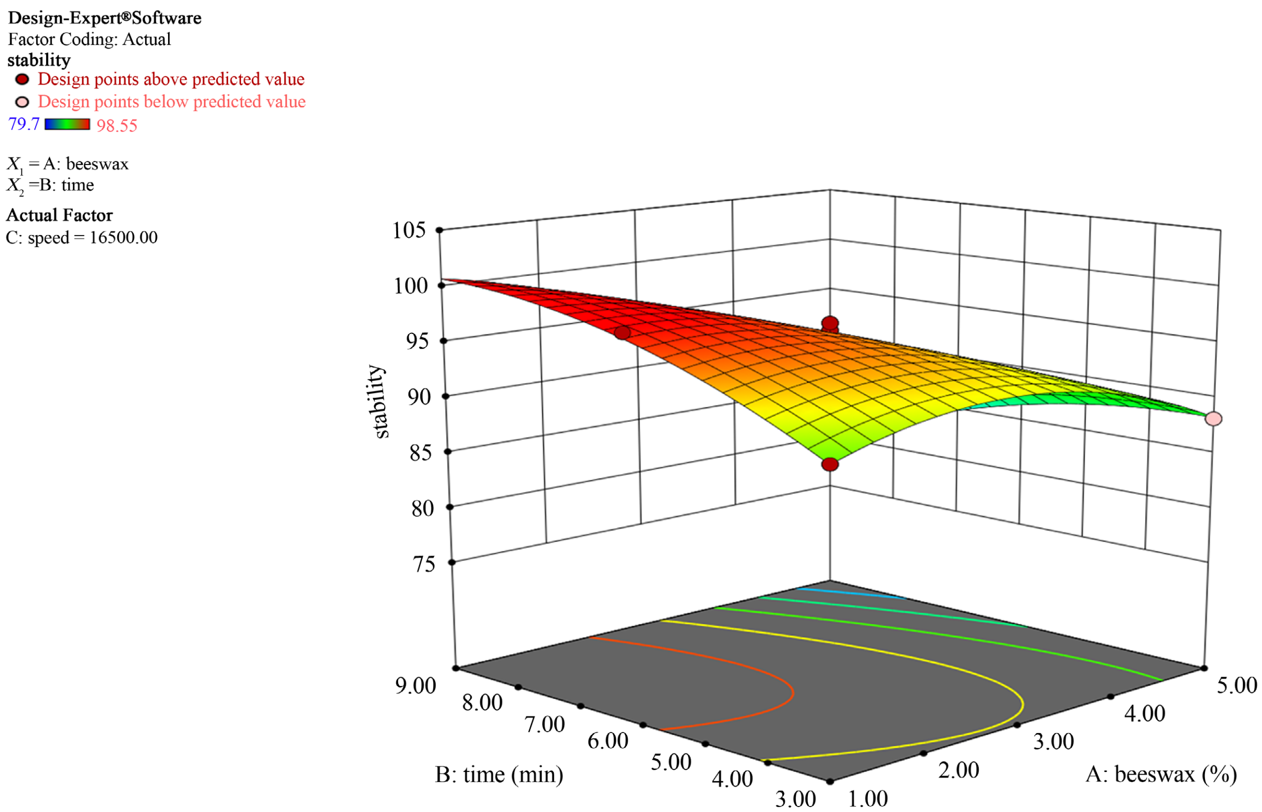


Figure 1. Change in stability depending on the processing factors.

16,500 rpm to strengthen this bond and avoid the overcoming of the oily phase. This clearly indicates that the stirring time and the stirring speed are two variables that have a synergistic effect on the stability of the spread.

3.3. Peroxide Value

Oxidation of lipids is the major problem in the fat food industry; it affects thenutrinnionnal quality and mechanical properties of the product [16]. Peroxide value is a measure of the oxidative rancidity of oil and is expressed as milliequivalent of peroxide oxygen combined with one kilogram of oil (meq/kg oil). All the prepared spreads were found to be highly oxidative stable with the highest PV of 1.37 meq/kg oil.

The effect of processing conditions on the peroxide value is shown in **Table 3**. The Model F-value of 9.65 implies the model is significant. There is only a 0.61% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case, X_1, X_2, X_3, X_1X_3 are significant model terms. It's shown from analysis of variance (ANOVA) that time stirring (X_2) is the major parameter affecting the peroxide value of mayonnaise followed by speed stirring (X_3), beeswax concentration (X_1), and finally the interaction between beeswax concentration and speed stirring (X_1X_3).

The stirring time promotes the oxidation of lipids. This is due to contact with oxygen during stirring and the increase in the temperature of the mixture under the mechanical effect of stirring, so the longer the stirring time, the more oxygen incorporation and the greater the increase of temperature [17].

This low peroxide value, low than 3 meq/kg oil as clear in **Figure 2**, indicates that the emulsion produced is not rancid [18]. Some research proved that using natural wax such as beeswax and carnauba wax to make oleogels protect them from oxidation. The circulation of oils is reduced by gelling oils. The slower circulation of oil in oleogel led to slower oxidation and to a lower peroxide value [19].

3.4. Textural Measurements

3.4.1. Firmness

As described above, spread formulations prepared to contain 20% olive oil, and the rest, the continuous phase, is formed by eucalyptus honey and beeswax.

Honey and olive oil are known as viscous products [20] [21] while beeswax (the % in different formulations varied from 1 to 5 maximum) is solid [22]. The firmness values of spreads varied between 95.5 and 184.9 N. Beeswax percentage (X_1), stirring time (X_2), and stirring speed (X_3) are significant terms in the model equation (**Table 3**). Variation in firmness spreads as a function of X_1, X_2 and X_3 are shown in **Figures 3(a)-(c)**. The quadratic terms of beeswax percentage (X_1) had a positive effect on firmness (**Figure 3(a)**). This indicates that as the beeswax percentage was increased, firmness increased; the minimum firmness values were attributed to the spread formulations with 1% beeswax and the maximum firmness values were obtained with 5% beeswax. Therefore, these results indicated

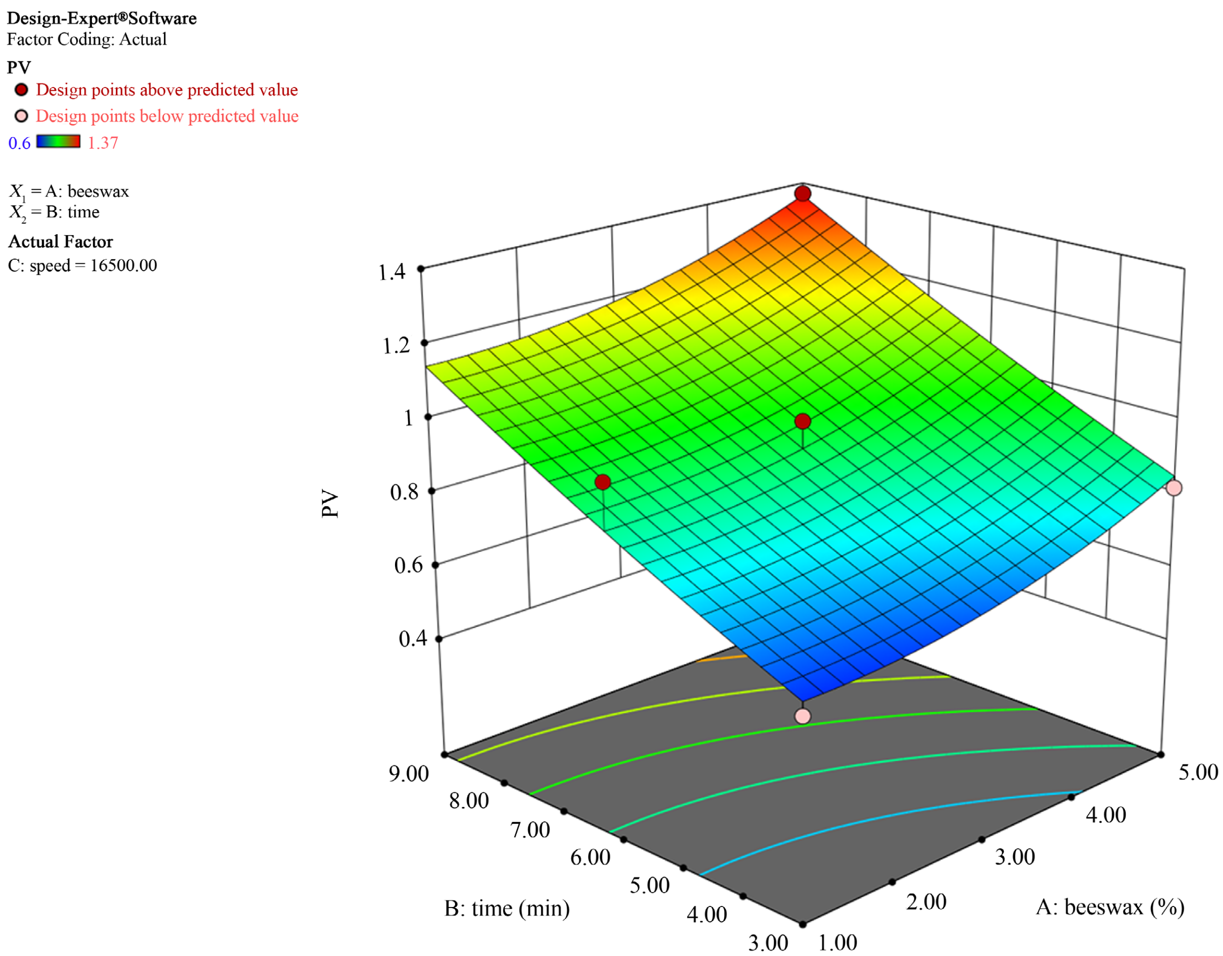
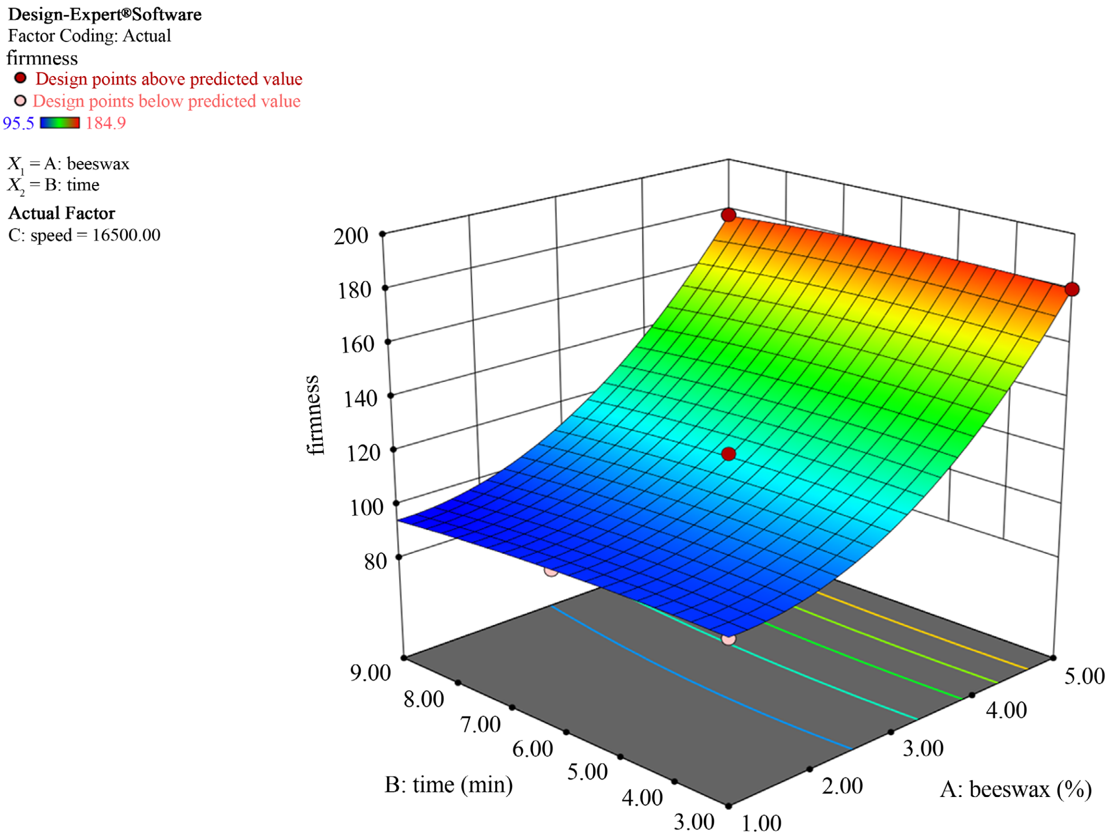


Figure 2. Change in peroxide value depending on the processing factors.

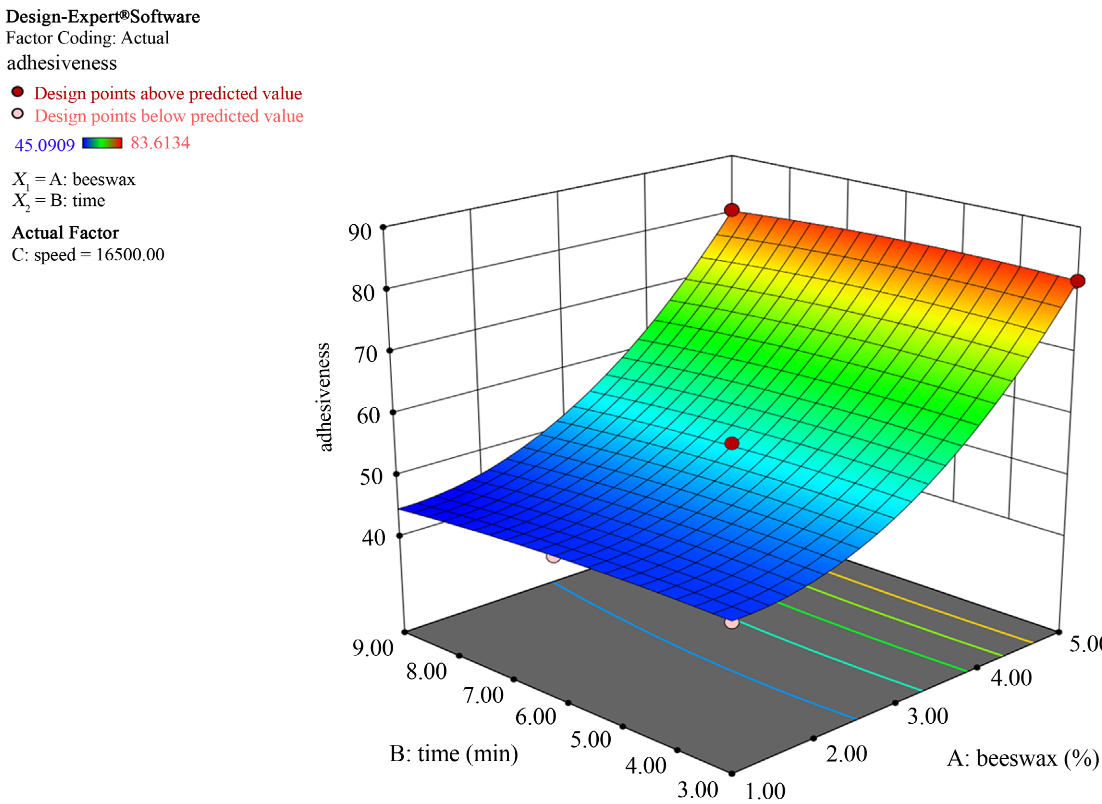
that beeswax can be used as an effective emulsifying agent to make olive oil and honey form a stable three-dimensional network structure, thereby forming an emulsion state.

Earlier research studies on spread formulations have mentioned the importance of adding emulsifiers with optimal concentrations for good spreadability. In their study, [23], used xanthan gum and distilled monoglycerides in order to improve the textural properties of pistachio oil spreads. Xanthan Gump was also used in the low-fat spread formulation prepared by [24]. Actually, beeswax is used as a potential emulsifying agent for the construction of food W/O emulsion. It is used mainly in order to produce oleogels [14] [15] for applications in beef heart patties to replace animal fat or to make edible oil-loaded beeswax microparticles as ingredients for functional food products [25]. It was demonstrated a strong correlation between the hardness of products and the content of beeswax introduced. Thanks to their solid state, beeswax was also used in the manufacture of food packaging [26] [27].

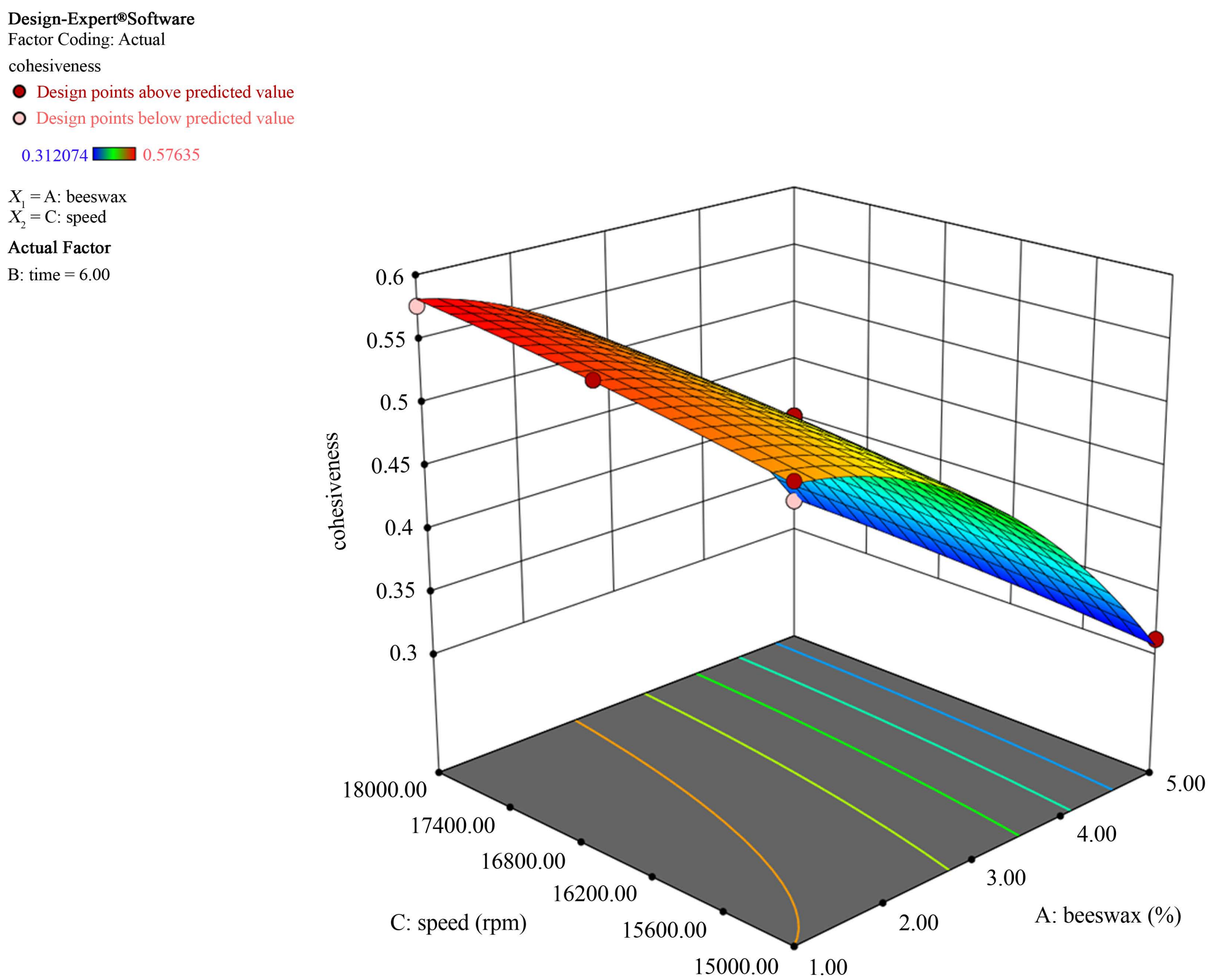
The quadratic terms of Stirring time (X_2) and stirring speed (X_3) had a little negative effect on firmness (**Figure 3(b)** and **Figure 3(c)**). This indicates that as the stirring time or/and the stirring speed was increased, firmness decreased.


Design-Expert®Software
Factor Coding: Actual
firmness
Design points above predicted value
Design points below predicted value
95.5
184.9
X1 = A: beeswax
X2 = B: time
Actual Factor
C: speed = 16500.00
firmness
200
180
160
140
120
100
80
9.00
8.00
7.00
6.00
5.00
4.00
3.00
B: time (min)
1.00
2.00
3.00
4.00
5.00
A: beeswax (%)

(a)


Design-Expert®Software
Factor Coding: Actual
adhesiveness
Design points above predicted value
Design points below predicted value
45.0909
83.6134
X1 = A: beeswax
X2 = B: time
Actual Factor
C: speed = 16500.00
adhesiveness
90
80
70
60
50
40
9.00
8.00
7.00
6.00
5.00
4.00
3.00
B: time (min)
1.00
2.00
3.00
4.00
5.00
A: beeswax (%)

(b)



(c)

Figure 3. Change in textural properties (a)-(c) depending on the processing factors.

The beeswax percentage greatly affects the spread firmness; the effect of stirring time and stirring speed is relatively less important. Whereas interactions between variables X_1/X_1, X_1/X_3, X_2/X_3 were found insignificant. The ANOVA results revealed that beeswax percentage/stirring time ratio (P = 0.5049), beeswax percentage/stirring speed ratio (P = 0.2086) and stirring time/stirring speed ratio (P = 0.1114) were insignificant model terms of firmness of spreads (Table 3).

3.4.2. Cohesiveness

Cohesiveness is the extent to which a material can be deformed before it ruptures.

The Model F-value of 572.58 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case, X_1, X_2, X_3, X_1 X_3, x_1^2 are significant model terms. Indeed, beeswax concentration is the major parameter affecting the cohesiveness of spreads.

Glibowski *et al.* found that the fat partial replacement with oil content caused a significant decrease in the spread cohesiveness. Cohesiveness is a determination of intermolecular strength. Thus, it seems that the strong interactions among solid particles such as sugar and beeswax as matrix-forming agents in the formulation of these spreads during stirring were formed [1]. It is shown in Figure 3(b) that the maximum cohesiveness is obtained for a minimum concentration of beeswax which is similar to a maximum concentration of honey.

3.4.3. Adhesiveness

Adhesiveness is a work necessary to overcome the attractive forces between the surface of the food and the surface of the other materials with which the food comes in contact [27]. These values of adhesiveness ranged between 45.09 - 83.61 Nmm. The Model F-value of 979.73 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case X_1, X_2, X_3, X_1^2, X_2^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. It is shown in Figure 3(c) that the beeswax concentration dominates the variation of the adhesiveness parameter.

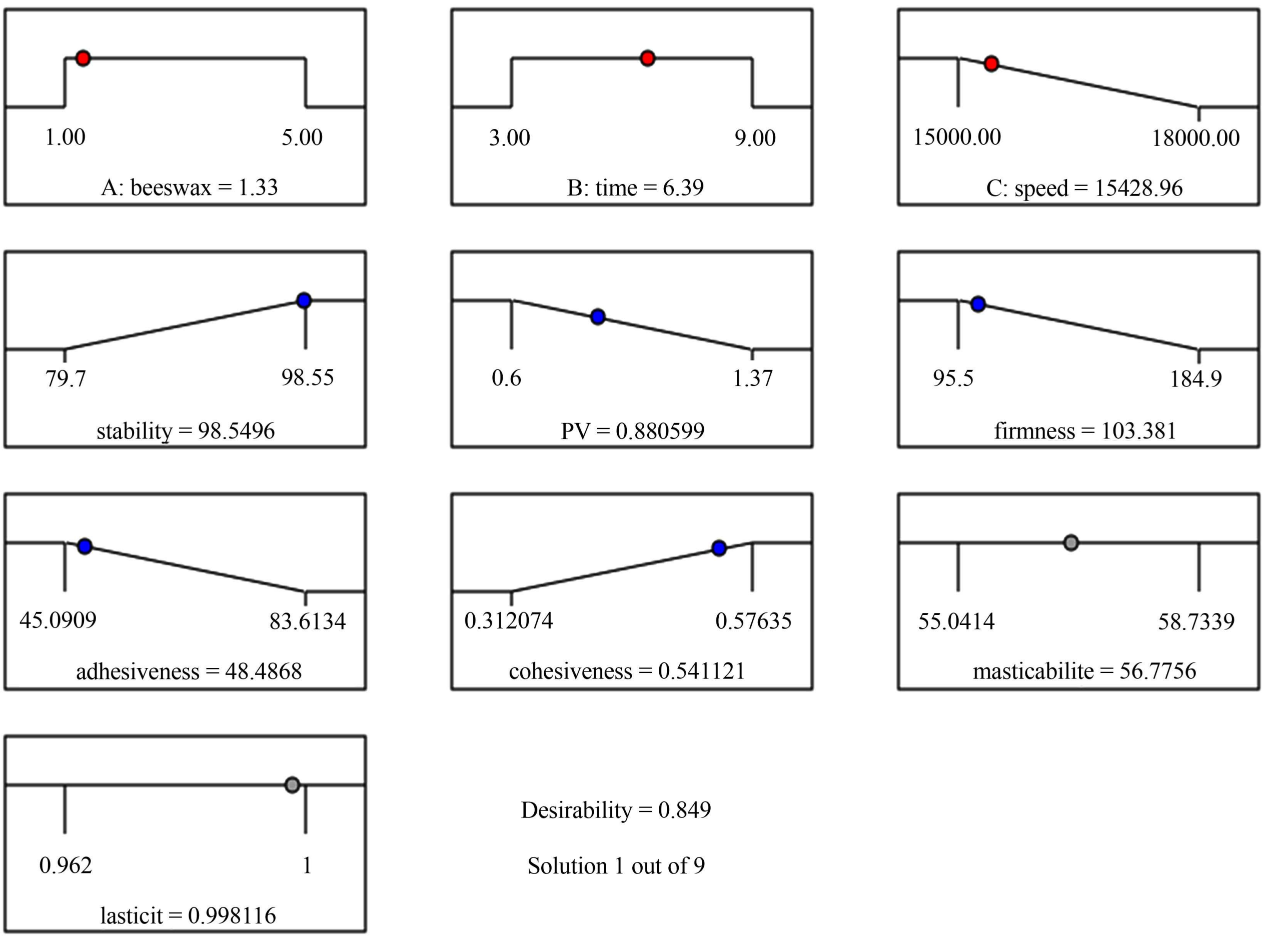


Figure 4. Desirability function approach.

The adhesiveness increased as the content of the emulsifier was increased. An increase of this parameter by increasing emulsifier content might be attributed to the high viscosity of the aqueous phase and low particle size of spreads. A decrease in particle size by increasing the concentration of XG stabilizer has been previously reported by the literature [26] [27] showed that the additional emulsifier was able to coat the surface area and resulted in the formation of a greater number of smaller particles.

3.5. Fitting the Best Formulation

This study attempted to lift the technological barrier hindering the development of spreads containing vegetable oils with no solid fat using different levels of beeswax. Box-Benken design was used to optimum the spread formulation. All formulations presented significant differences in terms of stability, peroxide value, and textural profile attributes. The optimum spread formulation was chosen with optimum textural properties, maximum stability, and lowest PV. As shown in **Figure 4**. The optimum had 1.33 beeswax content and 6.39 min of time stirring at 15,428 rpm speed. This optimum was found with 0.84 desirabilities.

4. Conclusions

From the results of the present work, it can be concluded that the tentative formulation and optimization of low-fat spread based on olive oil and honey was successful. Box-Benken design was used to optimum the spread formulation. All formulations presented significant differences in terms of textural analysis, stability, and PV. The optimum formulation was 1% beeswax, 79% honey and 20% olive oil.

By summarizing the results of our previous work and the results of this work, we confirm the formulation of functional spread with high emulsion stability and good textural properties.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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