



## Use of sunflower protein in snack bars

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The production of new healthy low-carbohydrate, high-protein plant-based snacks is one of the food industry's major trends. Alternative plant protein source, such as sunflower protein isolate, appears to be a promising ingredient for protein bars. Sunflower protein isolate is a secondary product with a high added value obtained from sunflower meal after the extraction of sunflower oil from seeds. One of the advantages of the sunflower protein compared to other plant proteins (such as soy, pea, and wheat) is the high content of helianthinin, which makes it highly digestible (up to 95%) and low allergenic, thus, helping avoid bloating, heartburn and upset stomach. It also has a high content of chlorogenic acid, making it an excellent antioxidant. This work aimed to determine the effect on nutritional, physicochemical, and organoleptic properties of snack bars if enriched with sunflower protein powder. Crude protein, fat, fiber, and moisture were assessed. Snack bars containing sunflower protein had low-moisture, were low in lipids, free of antinutrients, and were a good source of proteins. The results demonstrated that using isolate and concentrate, obtained from sunflower meal, in snack bars is a practical alternative to other plant proteins, which are often allergens. Not only does it help maintain the physicochemical characteristics of the product, but it helps improve the nutritional value as well.

### 1. Introduction

The global protein bar market size was \$4.66 billion in 2019 and is expected to reach \$7.03 billion by 2027. Driven by a rising number of fitness and health-conscious people North America dominates the market with key players Mondelez International Inc., Glanbia Plc., General Mills Inc., and The Kellogg's Company (Fortune Business Insights, 2020). Weight management, healthy eating, improving muscle mass are the main responses consumers choose snacks based on natural ingredients with no added sugar, artificial sweeteners, dyes, or preservatives (Mordor Intelligence, 2020). There is also a trend for developing deserts for vegans (Cimini A., Moresi M., 2019). Snack bars should contain 8 to 10 g of protein (up to 50% of the total energy of the bar), be rich in essential vitamins and minerals (Commission Directive 96/8/EC, 1996). Replacement of conventional sugary snacks with high-protein alternatives may be an effective strategy for the prevention of diabetes and effective weight loss (Gulati S. et al., 2017). The organic products market has grown rapidly over the past years driven by ethics, environmental concerns, and weight management (Vigar V. et al., 2020). The main challenge for the industry is to bring biologically active substances in organic food products in an acceptable for consumers form as well as to meet the rigorous standards for certified organic production (Popa M. E. et al., 2019).

One of the most popular oilseed crops is the sunflower, and the Russian Federation was the second producer having 11 million tons of sunflower in 2019 (Nuseed, 2019). Sunflower seed is widely used for oil production, however wide industrial application of oil extraction secondary products in animal fodder is limited because of the high fiber content. Sunflower meal is a secondary product of oil production obtained in approximately 300 thousand tons per year, is a promising source for protein extraction (Makarova A. et al., 2017). Sunflower protein compared to other plant proteins (such as soy, pea, and wheat) has a high content of helianthinin, which makes it highly digestible (up to 95%) and low allergenic, thus, helping avoid bloating, heartburn, and upset stomach. It also has a high content of chlorogenic acid, making it an excellent antioxidant (Salgado P. R., et al. 2011).

Several reports are showing the possibility to use secondary products of sunflower oil extraction for enriching muffins (Grasso S., Liu S., Methven L, 2020), obtaining gluten-free bread (Zorzi C. Z. et al., 2020), developing

cookies (Liang S. & Were L.M., 2018), however, no data were published regarding the application of sunflower protein isolate and concentrate in high protein energy bars formation.

Dates are rich in carbohydrates (74%) and low in lipids (0.2%) as well as in protein (2.3%), thus the addition of plant protein would be beneficial for obtaining a balanced snack in terms of nutritional value (Al-Shahib W., Marshall R. J., 2003). Comparing to other natural sweet confections, dates in a blend with nuts can be a good source of dietary fibers (Idowu A. T. et al., 2020)

The novelty of the present project consists of combining the functionality of the product intended for physically active people (high protein content) with its high nutritional value, safety, and acceptable, high sensory quality. Although numerous formulations have been made until now, for some of the bars on the market acceptable taste is not yet achieved. In processing new products, it is crucial to optimize both the sensory and functional properties of the product for proper acceptability and exceptional quality. The assumption was to get bars also with high nutritional value. Therefore, the study aimed to develop high-protein bars using organic ingredients such as sunflower protein concentrate and isolate, prebiotic-inulin, and pro-health additives (dried fruits and nuts).

The objective of this study was to characterize the influence of sunflower protein isolate and concentrate on chemical composition, flavor, the texture of low-carbohydrate high-protein bars by using response surface methodology (RSM), which is widely used for optimizing not only process parameters but also independent variables (Tur A. V. et al, 2017). The results of this research will be beneficial for meeting not only the consumer's growing demands but also the food industry representatives.

## 2. Materials and Methods

### 2.1 Ingredients and chemicals

Commercially available date paste, roasted cashew nuts, hazelnut, coconut flour, inulin powder, dry coconut milk powder, freeze-dried cherries were purchased from a local market. Unpurified sunflower protein concentrate was obtained from LLC "Green Proteins" (Moscow, Russian Federation) with the following characteristics (%): crude protein 59.0; crude fat 8.6; carbohydrates 18.0. Unpurified sunflower protein isolate was obtained from LLC "Sunprotein" (Altai Region, Russian Federation) with the following characteristics (%): crude protein 82.0; carbohydrates 5.5; moisture 5.5. Chemicals (analytical grade) were purchased from Sigma Aldrich.

### 2.2 Development of snack protein bars

Nuts were chopped to 0.5 cm size and mixed with other ingredients to form a blend according to Table 1. After hand mixing, compressed sheeting was done, then the sheet was frozen for 2 hours at -18°C. Then the sheet was cut into bars of 3 cm width, 1 cm height, and 5 cm in length. Each bar of approximately 75 g was packed individually in film. The amount of date paste, roasted cashew, and hazelnut, freeze-dried cherries, dry coconut milk powder, inulin powder, cinnamon, salt remained constant. Sunflower protein concentrate and isolate were varied according to the model created by response surface methodology (Table 2 and 3). Control bars were prepared without sunflower protein.

Table 1: Sunflower protein bar formulation

Ingredients	Quantity, g
Date paste	1000
Roasted cashew nuts	170
Roasted hazelnut	150
Freeze-dried cherries	50
Inulin powder	50
Dry coconut milk powder	35
Dry coconut flour	25
Cinamon	10
Salt	5
Sunflower protein concentrate	as per table 3
Sunflower protein isolate	as per table 3

## 2.1 Experimental design

Response surface methodology (RSM) was applied for the experiment design and statistical analysis. Central composite rotatable design was used to optimize the levels of sunflower protein concentrate ( $X_1$ ) and isolate ( $X_2$ ), and their effect on dependent variables: crude protein ( $Y_1$ ), crude fat ( $Y_2$ ), crude fiber ( $Y_3$ ), moisture ( $Y_4$ ). Maximum and minimum levels were searched out by conducting early trials. 13 date protein bars were made using an experimental design with 2 variables having 3 levels. In total 8 formulations related levels of variations and 5 correspond to center point replicates.

Table 2: Central composite rotatable design experimental design and levels of independent variables

Levels of independent variables used	Sunflower protein concentrate, (%)	Sunflower protein isolate, (%)
-1	3	2
0	6	4
+1	9	6

Table 3: Experimental design for sunflower protein bars formulation

	Sunflower protein concentrate, (%)	Sunflower protein isolate, (%)
•		
1	9	6
2	3	6
3	9	2
4	3	2
5	10.24	4
6	1.76	4
7	6	6.83
8	6	1.17
9	6	4
10	6	4
11	6	4
12	6	4
13	6	4

## 2.2 Chemical analysis

Developed date protein bars were stored at 25 °C. Crude protein, crude fat, crude fiber, and moisture content of developed bars were determined on a dry matter basis (George W., Latimer, J. R., 2019).

## 2.3 Sensory analysis

Separate sensory descriptors were defined for each tested bar. Based on the quality characteristics, the sensory panel indicated an overall sensory quality (low–high) for each sample. The task of the assessors was to determine the intensity of each of quality features and to put their assessment on an unstructured, 10 cm graphic scale. Bars were coded, portioned into equal weight (approximately 2 g) and placed in plastic boxes covered with lids, and kept at room temperature for 30 min before analysis. The assessment was attended by people who agreed to participate in the study and declared that they are consumers of high-protein bars. The study was conducted with a convenience sample of 20 people (10 women and 10 men) aged between 19 and 40 years inhabitants of Moscow, Russian Federation. The consumers received a sample of the product along with the questionnaire. Their task was to assign the appropriate degree of desirability using the hedonic 9-point scale (Peryam D. R., Pilgrim F. J., 1957).

## 2.4 Statistical analysis

The “Matlab” (The MathWorks, Inc., USA) was considered for analysis of experimental results. Central composite rotatable design was chosen to estimate the calculated responses. In order to fit the model to the experimental data multiple regression analysis was used and represented as a second-degree polynomial equation (1):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2, \quad (1)$$

where  $Y$  – measured parameter,  $\beta_0$  - regression coefficient,  $\beta_1$  - regression coefficient for  $X_1$ ,  $\beta_2$  - regression coefficient for  $X_2$ ,  $X_1$  - coded level of sunflower protein concentrate,  $X_2$  - coded level of sunflower protein isolate. The equation represents the relationship between the predicted response and independent variables. The adequacy of the developed models was evaluated using F-ratio and lack of fit test.

### 3. Results and discussion

#### 3.1 Proximate composition of protein bars

The proximate analysis includes the determination of crude protein, crude fat, crude fiber, the moisture of protein bar samples. Table 4 shows mean values for determined crude protein, crude fat, crude fiber, moisture. The crude protein ranges from  $8.9 \pm 0.5$  to  $15.9 \pm 0.2\%$ , crude fat from  $12.8 \pm 0.3$  to  $13.6 \pm 0.3\%$ , crude fiber from  $10.5 \pm 0.2$  to  $12.1 \pm 0.2\%$ , moisture content of protein bar sample ranges from 17.9 to  $18.5 \pm 0.2\%$ . The addition of sunflower protein isolate and concentrate led to a significant increment in crude protein content. Control bars with no added sunflower protein represented the lowest content of crude protein, crude fat, and fiber. The highest content of crude protein was in bar • 1. Bar • 10 had the highest crude fat and moisture content. Bar • 12 had the highest crude fiber content.

Table 4: Crude protein ( $Y_1$ ), crude fat ( $Y_2$ ), crude fiber ( $Y_3$ ), moisture ( $Y_4$ ) in protein bars (%)

	Crude protein (%)	Crude fat, (%)	Crude fiber, (%)	Moisture, (%)
1	$15.9 \pm 0.5$	$13.47 \pm 0.3$	$11.95 \pm 0.2$	$18.52 \pm 0.2$
2	$12.31 \pm 0.5$	$12.96 \pm 0.3$	$10.87 \pm 0.2$	$18.22 \pm 0.2$
3	$12.49 \pm 0.5$	$13.41 \pm 0.3$	$11.69 \pm 0.2$	$18.29 \pm 0.2$
4	$8.91 \pm 0.5$	$12.89 \pm 0.3$	$10.61 \pm 0.2$	$17.95 \pm 0.2$
5	$14.95 \pm 0.5$	$13.57 \pm 0.3$	$12.06 \pm 0.2$	$18.47 \pm 0.2$
6	$9.87 \pm 0.5$	$12.85 \pm 0.3$	$10.54 \pm 0.2$	$18.05 \pm 0.2$
7	$14.81 \pm 0.5$	$13.22 \pm 0.3$	$11.46 \pm 0.2$	$18.41 \pm 0.2$
8	$10.02 \pm 0.5$	$13.19 \pm 0.3$	$11.15 \pm 0.2$	$18.10 \pm 0.2$
9	$12.41 \pm 0.5$	$13.21 \pm 0.3$	$11.30 \pm 0.2$	$18.26 \pm 0.2$
10	$13.00 \pm 0.5$	$14.00 \pm 0.3$	$12.00 \pm 0.2$	$19.10 \pm 0.2$
11	$12.51 \pm 0.5$	$13.50 \pm 0.3$	$10.70 \pm 0.2$	$17.80 \pm 0.2$
12	$11.92 \pm 0.5$	$12.80 \pm 0.3$	$12.10 \pm 0.2$	$18.50 \pm 0.2$
13	$13.11 \pm 0.5$	$13.90 \pm 0.3$	$11.50 \pm 0.2$	$18.10 \pm 0.2$

#### 3.2 Optimisation of protein level using response surface methodology

Dates are rich in carbohydrates and fiber, being a potential energy source for athletes (Ayad A. A. et al., 2020), however their protein-deficient leads to further ingredient optimization for developing a balanced in terms of nutritional value energy bar. Functional ingredients, such as freeze-dried cherries, dried coconut milk, coconut flour, cashew, and hazelnut as well as probiotic inulin have been selected for designing an excellent bar profile. Sunflower concentrate and isolate have been selected for protein levels variation in terms of economic aspects, as well as due to growing demand for organic vegan energy bars.

There are several methods for response optimisation by means of finding the best levels of input variables. One of them is drawing surface plots of the fitted models (Box G. E., Draper N. R., 1987). In food research studies the RSM methodology allows to optimize the ingredients level (Aguilera Y. et al, 2019), product improvers (Collar C. et al, 2007), and process conditions for product development (Sparkman K. t al, 2019) by checking the factors impact, as well as complex interaction, on the model through multiple regression analysis.

In this research three coded levels of sunflower protein concentrate ( $X_1$ ) and isolate ( $X_2$ ) at different rates were used to draw surface plots in Matlab software based on RSM responses estimation of independent variables and fitted second-order polynomial model. Maximum crude protein content as well as crude fat, fiber and moisture were targeted for the optimization process.

Table 5: Regression coefficients for crude protein, crude fat, crude fiber and moisture of date bars enriched with sunflower protein isolate and concentrate

Terms of model equations	Crude protein ( $Y_1$ )	Crude fat, ( $Y_2$ )	Crude fiber, ( $Y_3$ )	Moisture, ( $Y_4$ )
Constant	12.585	13.485	11.523	18.356
$X_1$	1.794	0.256	0.538	0.149
$X_2$	1.698	0.021	0.119	0.112

$X_1X_2$	0.002	-0.002	-	-
$X_1^2$	-0.091	-0.144	-0.117	-0.051
$X_2^2$	-0.088	-0.146	-0.117	-0.052

The surface plots (Figure 1) depicted that the maximum crude protein (Figure 1a), fat (Figure 1b), fiber (Figure 1c) and moisture (Figure 1d) content were achieved by adding 9% of sunflower protein concentrate and 6% sunflower protein isolate. The effect of linear terms of  $X_1$  and  $X_2$  of date bars is significant for all  $Y_1$ - $Y_4$  parameters (Table 5).

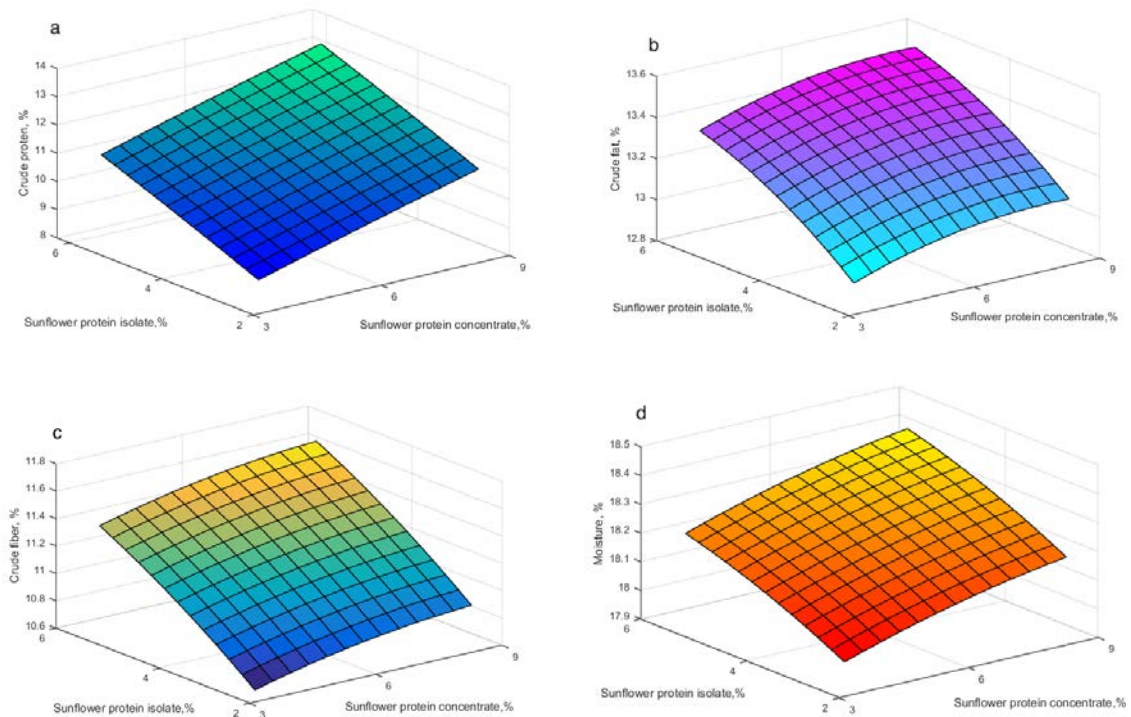


Figure 1: Effect of sunflower protein concentrate and isolate on crude protein (a), crude fat (b), crude fiber (c) and moisture (d) content

The  $X_1^2$  and  $X_2^2$  quadratic terms were also found significant for crude fat and fiber content. An insignificant effect of two variables interaction ( $X_1X_2$ ) was shown on crude protein and crude fat content. Crude protein of date bars was expectedly affected by independent variables  $X_1$  and  $X_2$ .

The optimized variable levels for the target values of physicochemical parameters were as follows: sunflower protein concentrate ( $X_1$ ) = 9%, sunflower protein isolate ( $X_2$ ) = 6%.

### 3.3 Sensory quality and consumer acceptance

In order to determine the dependence of added sunflower protein content on sensory quality of bars, formulations 1, 3, 4, 5, 8, 13 as well as control bar with no added sunflower protein were selected for consumers test.

The obtained bars were varied in terms of the intensity of the taste sensation (Figure 2). The highest intensity of these sensory quality parameters was recorded in the case of the control bar. All of the bars got high marks of the overall quality indicator (above 6 c.u.). The highest marks of the overall quality among all evaluated products were obtained by the control bar without sunflower protein (9 c.u.). The greatest positive impact on the overall sensory quality was caused by coconut, cherry, sweet and smoothness (bar • 5). Protein enrichment of bars may cause changes in the sensory evaluation of these products. It was expected that the addition of sunflower protein powder might negatively influence the overall quality of bars, however the addition of sunflower was sensible only in bar • 4 (4 c.u.), which didn't influence the overall quality of the product (8 c.u.) and the texture of bars were characterized as smooth.

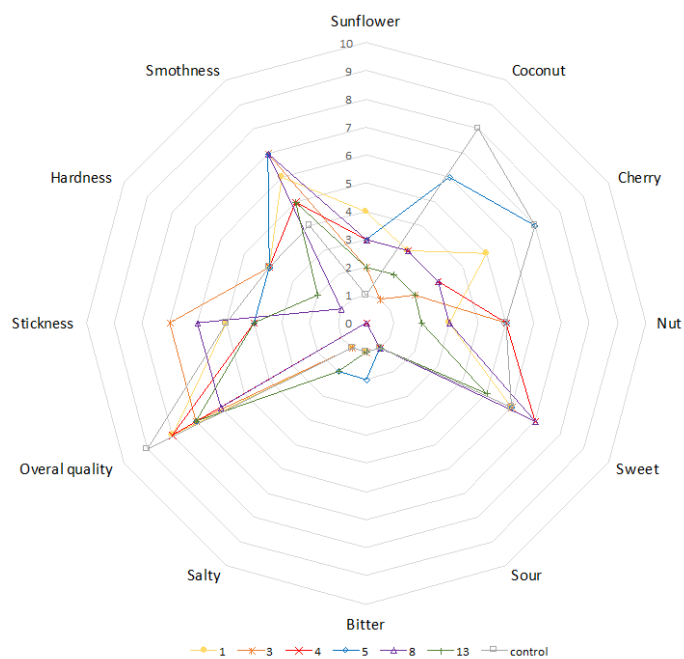


Figure 2: Results of sensory evaluation of sunflower protein bars, (Quantitative descriptive profile method, n=10). Explanatory: 1 – 9% concentrate, 6% isolate; 3 – 9% concentrate, 2% isolate; 4 – 3% concentrate, 2% isolate; 5 – 11% concentrate, 4% isolate; 8 – 6% concentrate, 1.2% isolate; 13 – 6% concentrate, 4% isolate; control – no added sunflower protein

There was no significant differentiation between the 6 variants of date bars with added sunflower protein in the sensation of individual sensory quality parameters. The bars were characterized by high intensity of taste and coconut flavor. The bars were smooth, sticky, and soft consistency, with a low intensity of bitter or sour taste. These date bars can be commercialized. The cost of production of these bars is given in Table 6.

Table 6: Cost analysis of sunflower protein bars

Ingredients	Price (\$ per kg)	Quantity (g/100g bar)	Quantity (g/75g bar)	Ingredient cost (\$ per 75g bar)
Date paste	1.84	66.7	50.0	0.0919
Roasted cashew nuts	7.35	11.3	8.5	0.0625
Roasted hazelnut	7.88	10.0	7.5	0.0591
Freeze-dried cherries	4.60	3.3	2.5	0.0115
Inulin powder	2.63	3.3	2.5	0.0066
Dry coconut milk powder	5.12	2.3	1.8	0.0090
Coconut flour	2.63	1.7	1.3	0.0033
Cinamon	0.66	0.7	0.5	0.0003
Salt	0.39	0.3	0.3	0.0001
Sunflower protein concentrate	3.94	6.7	5.0	0.0197
Sunflower protein isolate	10.50	4.0	3.0	0.0315
<b>Total cost</b>	<b>47.50</b>			<b>0.3</b>

#### 4. Conclusions

In this research, the development of energy bars for athletes, based on date paste, enriched with functional ingredients such as freeze-dried cherries, dried coconut milk, coconut flour, cashew and, hazelnut as well as probiotic inulin was studied. In order to find an optimal combination of sunflower protein concentrate and isolate for a high-protein energy bar, response surface methodology was applied. It has been found that the protein level of date bars could considerably be improved by incorporating 9% sunflower protein concentrate and 6% isolate without affecting any sensory characteristics. Cost analysis showed the potential for high-protein energy

bars enriched with low allergic sunflower protein production. The results of this research will be beneficial for meeting not only the consumer's growing demands but also for the food industry representatives.

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