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Optimal Ultrasound-Assisted Process Extraction, Characterization, and Functional Product Development from Flaxseed Meal Derived Polysaccharide Gum

Muhammad Nadeem Akhtar [1], Zarina Mushtaq [2], Nazir Ahmad [2], Muhammad Kamran Khan [2], Muhammad Haseeb Ahmad [2], Abdullah Ijaz Hussain [3] and Muhammad Imran [2,*]

1 Department of Food Science, Nutrition and Home Economics, Government College University, Faisalabad 38000, Pakistan; nadeemgcuflc@gmail.com
2 Institute of Home and Food Sciences, Faculty of Life Sciences, Government College University, Faisalabad 38000, Pakistan; zee_ft@hotmail.com (Z.M.); nazirahmad83@gmail.com (N.A.); mk.khan@gcuf.edu.pk (M.K.K.); haseeb1828@gmail.com (M.H.A.)
3 Department of Chemistry, Faculty of Physical Sciences, Government College University, Faisalabad 38000, Pakistan; abdullahijaz@gcuf.edu.pk
* Correspondence: imran@gcuf.edu.pk; Tel.: +92-335-2020050

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Abstract: Flaxseed (*Linum usitatissimum* L.) has several health-promoting applications as dietary food ingredient supplementation, owing to presence of high quality of oil, polyunsaturated fatty acids, high dietary fiber and protein contents. The presence of different anti-nutritional components, for example cyanogens (HCN) and tannins in meal, limits its application for food purposes. The study was conducted to observe the effect of ultrasound-assisted extraction on polysaccharide gums (PSG) yield using response surface methodology. The selected variables were sonication temperature (°C), water to meal ratio, sonication amplitude level (%), sonication pH, and sonication time (min). Ultrasound-assisted extraction significantly reduced the anti-nutritional components like HCN and tannins. The extracted PSG yield from partially defatted flaxseed meal (PDFM) samples varied to a minimum of 7.24% to a maximum of 11.04% when extraction temperature (°C) and amplitude level (%) varied from −1 to +1 and keeping all other variables constant at mean value. Physiochemical and functional properties of extracted PSG were studied. Yoghurt with different treatment combinations were prepared by supplementing flaxseed-derived PSG as stabilizer ranging from 0.25% to 1.5%, keeping baseline samples without PSG as control. Functional properties of PSG-supplemented yoghurt such as pH, syneresis, and viscosity were determined to assess the influence of PSG supplementation on yoghurt quality. In the organoleptic behavior of PSG-supplemented yoghurt, no adverse effect on the flavor have been observed, but the textural properties vary significantly among different treatments. Overall, the acceptability of 1% PSG-supplemented yoghurt was significantly higher than other treatments.

Keywords: flaxseed; response surface methodology; sonication; PSG; functional food; characterization

1. Introduction

Flaxseed (*Linum usitatissimum* L.) belongs to genus *Linum* and the family Linaceae, and has diversification in physical appearance as smoothed and oval, 4–6 cm long, pale yellow to darker shading, and glossy appearance [1]. It is mainly cultivated in temperate to sub-tropical regions of the world. Flaxseeds have several health-promoting applications as dietary food ingredient supplementation, owing to presence of high quality of oil, polyunsaturated fatty acids, high dietary fiber and protein contents [2]. Flaxseed oil is the main product utilized in the food industry. The main by-product of the flaxseed oil industry is flaxseed meal. The presence of different anti-nutritional components, for example cyanogens (HCN) and tannins in meal, limits its application for food purposes [3]. Nevertheless, the partially defatted flaxseed meal has been identified as one of the major sources of the plant derived gums that may be utilized as thickeners, emulsifiers, and stabilizers in frozen desserts, yoghurt, cream cheese, ice cream, and other processed foods [4]. Numerous methods are being utilized for extraction of gums from partially defatted meal that involve solvent and process variations. Polysaccharide gums present in meal are normally extracted by utilizing hot water as solvent [5]. The gum yield and physicochemical characteristics are mostly affected by different extraction parameters including extraction pH, extraction temperature, extraction time, solvent/meal ratio, and type and quality of raw material [6].

Among the different innovative technologies being utilized for extraction of phytochemicals, ultrasound is used to limit the handling time, improve quality, and confirm the safety aspects of extracted material. The use of sonication in the extraction of phytochemicals improves their functional and quality characteristics and imparts a positive effect on the food products [7]. Sonication process is utilized for the extraction of a variety of phytochemicals such as gums, herbal oil, proteins, etc. Similarly, it is also used for the extraction of different bioactive materials such as antioxidants, phytochemicals, etc., from different sources [8]. Sonication is a fast and economical extraction technique for improved extraction of phytochemicals. In the literature, it has been found that the sonication process is 1.5 times more efficient than conventional extraction processes [9,10]. Sonication works on the principle of cavitation, which enhances the mass transfer and as a result yields superior quality of material in less time. The sonication process has a number of advantages over conventional extraction processes, such as extraction being achieved in less time, less effect on the extraction product, evasion of the organic solvents (most do not have Generally Recognized As Safe (GRAS) status), and extraction at minimum temperature, making it more suitable for utilization on an industrial scale [11].

The supplementation of plant-based polysaccharide gums (PSG) in the human diet have significant health-promoting effects. The possible health-promoting effects of plant-based PSG such as anti-diabetic, laxative, anti-hypercholesterolemic, hypoglycemic, hypolipidemic, and atherogenic can be achieved only by utilizing them with certain levels in food products [12]. Such types of nutritional and health-promoting benefits can be achieved when these bioactive components are supplemented in different food products such as dairy products. Stabilizers and gums extracted from plant materials are frequently utilized in dairy products to improve functional characteristics such as texture, mouth feel, viscosity, and storage stability [13]. However, these components might not be used beyond a certain limit as increasing their concentration beyond a certain level in food products results in adverse quality changes in supplemented products [14].

The eminent properties of flaxseed-derived PSG render it most suitable to be supplemented in food products [12]. Thus, there is a constant need to search for new and innovative methods that yield higher quality of gums with desirable characteristics at an economical cost. The main objectives for current research were the optimization of sonication extraction and characterization of PSG extracted from partially defatted flaxseed meal (PDFM), and development of functional and nutrient-rich dairy yoghurt using extracted flaxseed-derived PSG.

2. Materials and Methods

2.1. Partially Defatted Flaxseed Meal (PDFM) and Characterization

For current study, the flaxseed (*Linum usitatissimum* L.) cv. Chandni were chosen as major raw material. Mini Oil Presser model 6YL-550 (Henan Best Grain and Oil Machinery Engineering Co., Ltd., Zhengzhou, China) was used to obtain PDFM. Moisture content of PDFM was determined by Method No. 44-15A as described in American Association of Cereal Chemists (AACC) [15]. Ash content was determined by Method No. 08-01 of AACC from PDFM sample using a muffle furnace made by PCSIR (Islamabad, Pakistan; model MF-1/02) at 550 °C [15]. Kjeldahl's method was utilized for determination of crude protein as mentioned in Association of Official Analytical Chemists (AOAC) manual in its Method No. 990.03 [16]. Soxhlet's apparatus model H-2 1045 (Perstorp Analytical, Tecator AB, Hoganas, Sweden) was utilized for estimation of crude fat as mentioned in AACC manual, Method No. 30-10 [15]. Estimation of crude fiber was made according to the method as described in manual of AOAC Method No. 978.10 [16]. Method of differentiation was used for determination of nitrogen free extract (NFE) as described below.

$$\text{NFE (\%)} = 100 - (\text{MC\%} + \text{Ash\%} + \text{Crude fat\%} + \text{Crude protein\%} + \text{Crude fiber\%}).$$

Cyanogens present in the form of HCN in PDFM samples were determined by alkaline titration method in accordance with the procedure outlined in AOAC [16]. Tannins in PDFM was determined by the method as adopted by Schanderi [17].

2.2. Ultrasound-Assisted Extraction of PSG from PDFM

Ultrasound- (model VCX 750, Sonics & Materials, Inc., Newtown, CT, USA) technology-assisted process was used for the extraction of PSG from PDFM using distilled water as the solvent (Figure 1). PDFM samples were weighed (100 ± 0.1 g) by digital weight machine model Kern 440_35N (KERN & Sohn GmbH, Balingen-Frommern, Germany) for each run. The influence of sonication temperature (°C), water to meal ratio, sonication amplitude level (%), sonication pH, and sonication time (min) were studied by applying the Box–Behnken design for optimal extraction of PSG from PDFM samples. Table 1 represents the coded values of experiment. The pH of extraction was continuously monitored by using a pH meter and was adjusted accordingly by 0.2 molar solution of NaOH and HCl, respectively. Similarly, a digital thermometer was used to control the temperature of media within ±1.5 °C. A 40-mesh size screen was used for filtration of extracted solution after completion of extraction and gum was separated by using two volumes of 95% ethanol. Centrifugation was used to separate the PSG and then PSG were precipitated and dried in a benchtop laboratory freeze dryer. The extracted gum yield was calculated as the percentage of PDFM.

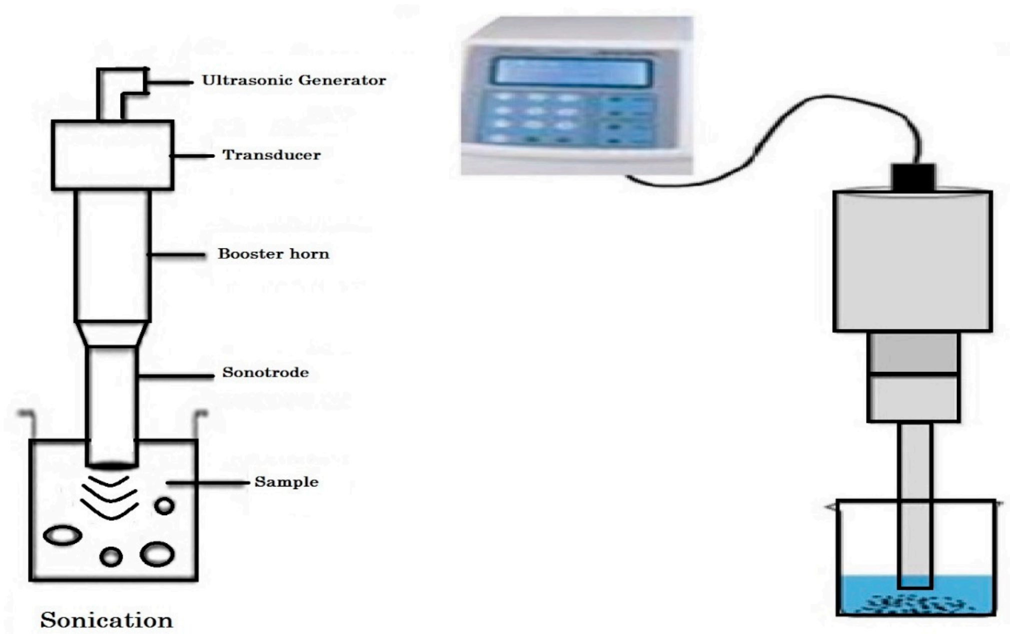

Figure 1. Schematic diagram of Sonicator.

Table 1. Coded and actual levels of independent variables for optimization of flaxseed-derived polysaccharide gums (PSG) yield as determined by the Box–Behnken design.

Sonication Variables	Coded Levels		
	−1	0	1
Extraction temperature (°C)	50	70	90
Water to meal ratio	10	15	20
Amplitude level (%)	30	50	70
Sonication pH	5	7	9
Sonication extraction time (min)	10	20	30

2.3. Physico-Chemical Characterization of PSG

The extracted PSG was characterized with respect to its proximate and anti-nutritional composition by adopting the methods as elaborated in Section 2.1. A Hunter Laboratory Tristimulus colorimeter was used for determination of color of the freeze-dried PSG samples [18]. A rheometer with controlled shear rate was used for evaluation of shear stress at 25 °C [19].

2.4. Functional Characterization of PSG Samples

Aviscometer was used for determination of viscosity of 1% (*w*/*v*) PSG solution. The method in [20] was utilized for determination of foaming properties such as foam stability (%) and foaming capacity. Similarly, emulsion properties such as emulsion stability index (ESI) and emulsifying activity index (EAI) of PSG were estimated according to the procedure in [21] with a little variation.

2.5. Development and Characterization of PSG Supplemented Functional Yoghurt

Yoghurt samples by varying the concentrations of PSG were prepared as described by [22]. Extracted flaxseed-derived PSG as stabilizer was supplemented by varying concentrations. Different treatment combinations were prepared by supplementing flaxseed-derived PSG as stabilizer ranging from 0.25% to 1.5%, keeping baseline samples without PSG as control. Yoghurt samples were filled in polypropylene cups and were stored at 4 °C for further study. The methods in [23] were used for determination of pH and syneresis of yoghurt. A viscometer was used for determination of viscosity of different treatments at 4 °C.

2.6. Sensory Evaluation of PSG Supplemented Functional Yoghurt Samples

Organoleptic behavior of PSG-supplemented yoghurt samples was carried out by experienced and untrained assessors as instructed by [24]. A 9-point hedonic scale was used for sensory evaluation of prepared treatments being 1 = extremely dislike to 9 = extremely like. Optimization of PSG level was made on the basis of organoleptic properties of the supplemented yoghurt.

2.7. Statistical Analysis

The quadratic equation was used to describe the behavior of the Box–Behnken design. MATLAB software package (MATLAB 7, The MathWorks, Natick, MA, USA, 2004) was used to analyze the PSG yield for determination of level of significance [25]. Three sonication runs were carried out for each treatment and the average was used as yield with standard deviation. Level of significance of average values of product data analysis was determined by the Duncan's multiple range (DMR). Organoleptic behavior and storage stability of product were accomplished in triplicate and 5% level of significance was used to determine to significant differences among treatments.

3. Results and Discussion

3.1. Partially Defatted Flaxseed Meal (PDFM) Characterization

It was necessary to characterize PDFM to explore its potential for food product development. The mean compositional values of PDFM are represented in Table 2 Some of the anti-nutritional compounds present in the raw PDFM samples such as cyanide (HCN) and tannin are also presented in Table 2. Both compounds are considered anti-nutritional as HCN is considered to be a pyridoxine inhibitor while tannin is a protein inhibitor. Comparatively higher amounts of HCN (242.25 ± 0.76 mg/kg) and tannin (176.24 ± 0.46 mg/100 g) were present in the PDFM samples. It was obvious from Table 2 that PDFM samples were rich in different nutrients such as protein (33.19 ± 0.68%), crude oil (8.14 ± 0.38%), ash content (6.08 ± 0.23%), and carbohydrates (35.93 ± 0.66%). It is considered to be a rich source of some of the vital nutrients such as fiber, PUFA, protein, and minerals [2].

Table 2. Comparison of chemical characterization of Flaxseed meal and PSG.

Analyzed Parameter	Flaxseed Meal	PSG
Moisture (%)	5.12 ± 0.27	5.89 ± 0.37
Crude protein (%)	33.19 ± 0.68	12.94 ± 0..24
Crude oil (%)	8.14 ± 0.38	0.78 ± 0.13
Ash (%)	6.73 ± 0.23	12.06 ± 0.42
NFE (%)	35.93 ± 0.66	68.33 ± 0.76
HCN (mg/kg)	242.25 ± 0.76	24.21 ± 0.64
Tannin (mg/100 g)	176.24 ± 0.46	42.81 ± 0.15

Values represent the mean ± standard deviation; n = 3.

3.2. Ultrasound-Assisted Optimal Extraction of PSG

The effect of different factors such as sonication extraction temperature (°C), water to meal ratio, amplitude level (%), sonication extraction pH and, sonication extraction time (min) for optimal PSG extraction was studied by using a three-level–five-factors Box–Behnken design. Total 46 sonication extraction runs were carried out as determined by the Box–Behnken design. The PSG yield obtained by different sonication extraction runs are illustrated in Table 3. It is clear from the table that the PSG yield varied from 7.24 ± 0.21% to 11.04 ± 0.28%. The variation in percent PSG yield showed that there was a significant effect of sonication extraction conditions on PSG yield.

Table 3. Percentage of flaxseed PSG yield as carried out by the Box–Behnken design.

Sonication Run	Sonication Variables					PSG Yield (%)
	Sonication Temperature (°C)	Water/Meal Ratio	Amplitude Level (%)	Sonication pH	Sonication Time (Minutes)	
1	70 (0)	15 (0)	50 (0)	7 (0)	20 (0)	8.93 ± 0.21
2	50 (−1)	15 (0)	50 (0)	7 (0)	10 (−1)	7.78 ± 0.18
3	90 (1)	15 (0)	50 (0)	9 (1)	20 (0)	10.85 ± 0.21
4	70 (0)	20 (1)	70 (1)	7 (0)	20 (0)	10.37 ± 0.17
5	70 (0)	15 (0)	50 (0)	5 (−1)	10 (−1)	8.41 ± 0.31
6	90 (1)	15 (0)	50 (0)	7 (0)	10 (−1)	9.87 ± 0.22
7	70 (0)	10 (−1)	30 (−1)	7 (0)	20 (0)	8.01 ± 0.23
8	50 (−1)	15 (0)	50 (0)	9 (1)	20 (0)	9.04 ± 0.17
9	70 (0)	20 (1)	50 (0)	7 (0)	10 (−1)	8.98 ± 0.21

Table 3. *Cont.*

Sonication Run	Sonication Variables					PSG Yield (%)
	Sonication Temperature (°C)	Water/Meal Ratio	Amplitude Level (%)	Sonication pH	Sonication Time (Minutes)	
10	70 (0)	15 (0)	50 (0)	7 (0)	20 (0)	8.94 ± 0.24
11	70 (0)	15 (0)	70 (1)	7 (0)	10 (−1)	9.91 ± 0.08
12	70 (0)	20 (1)	50 (0)	9 (1)	20 (0)	10.31 ± 0.27
13	70 (0)	15 (0)	70 (1)	7 (0)	30 (1)	10.39 ± 0.09
14	70 (0)	10 (−1)	50 (0)	9 (1)	20 (0)	9.91 ± 0.23
15	70 (0)	20 (1)	50 (0)	7 (0)	30 (1)	9.72 ± 0.17
16	70 (0)	15 (0)	30 (−1)	7 (0)	30 (1)	8.41 ± 0.14
17	90 (1)	20 (1)	50 (0)	7 (0)	20 (0)	10.34 ± 0.07
18	90 (1)	15 (0)	30 (−1)	7 (0)	20 (0)	8.97 ± 0.09
19	70 (0)	10 (−1)	50 (0)	7 (0)	30 (1)	9.21 ± 0.27
20	50 (−1)	15 (0)	70 (1)	7 (0)	20 (0)	8.98 ± 0.22
21	70 (0)	10 (−1)	70 (1)	7 (0)	20 (0)	9.94 ± 0.18
22	70 (0)	20 (1)	30 (−1)	7 (0)	20 (0)	8.47 ± 0.25
23	70 (0)	15 (0)	50 (0)	7 (0)	20 (0)	8.97 ± 0.24
24	70 (0)	20 (1)	50 (0)	5 (−1)	20 (0)	8.95 ± 0.19
25	70 (0)	15 (0)	50 (0)	7 (0)	20 (0)	8.96 ± 0.17
26	50 (−1)	15 (0)	30 (−1)	7 (0)	20 (0)	7.24 ± 0.21
27	90 (1)	10 (−1)	50 (0)	7 (0)	20 (0)	9.84 ± 0.09
28	90 (1)	15 (0)	70 (1)	7 (0)	20 (0)	11.04 ± 0.28
29	50 (−1)	10 (−1)	50 (0)	7 (0)	20 (0)	7.91 ± 0.14
30	50 (−1)	15 (0)	50 (0)	5 (−1)	20 (0)	7.85 ± 0.18
31	50 (−1)	15 (0)	50 (0)	7 (0)	30 (1)	8.47 ± 0.19
32	50 (−1)	20 (1)	50 (0)	7 (0)	20 (0)	8.38 ± 0.12
33	70 (0)	15 (0)	30 (−1)	5 (−1)	20 (0)	7.93 ± 0.17
34	70 (0)	15 (0)	30 (−1)	7 (0)	10 (−1)	7.87 ± 0.23
35	70 (0)	15 (0)	50 (0)	7 (0)	20 (0)	8.95 ± 0.21
36	70 (0)	15 (0)	70 (1)	9 (1)	20 (0)	10.98 ± 0.27
37	90 (1)	15 (0)	50 (0)	5 (−1)	20 (0)	9.76 ± 0.19
38	70 (0)	15 (0)	30 (−1)	9 (1)	20 (0)	8.98 ± 0.08
39	70 (0)	10 (−1)	50 (0)	5 (−1)	20 (0)	8.64 ± 0.17
40	70 (0)	15 (0)	70 (1)	5 (−1)	20 (0)	9.87 ± 0.24
41	70 (0)	15 (0)	50 (0)	7 (0)	20 (0)	8.97 ± 0.15
42	70 (0)	15 (0)	50 (0)	9 (1)	10 (−1)	9.81 ± 0.19
43	70 (0)	10 (−1)	50 (0)	7 (0)	10 (−1)	8.71 ± 0.23
44	70 (0)	15 (0)	50 (0)	9 (1)	30 (1)	10.37 ± 0.28
45	70 (0)	15 (0)	50 (0)	5 (−1)	30 (1)	9.14 ± 0.18
46	90 (1)	15 (0)	50 (0)	7 (0)	30 (1)	10.36 ± 0.21

3.3. Fitting the Experimental Model

A number of indicators were employed to examine the authenticity of second-order polynomial response model, including coefficient of determination (R^2), coefficient of the variation (CV), and model significance (F-value). A regression model was employed to calculate the predicted values of PSG yield and comparison was made with actual values of PSG yield. The relationship between the described variations and the total variations is called coefficient of determination (R^2) as described in [26].

The calculated values of coefficient of determination (R^2) and adjusted coefficients of determination (Adjusted R^2) were found to be 0.9976 and 0.9957, respectively, while authenticity and suitability of the applied second order polynomial model is provided by adequate precision 93.3695 of the response model. The calculated value of R^2 for PSG yields close to 1 was the indication that the response model described 99.76% of the variation, as seen in the current experimentation. If the CV value was lower than 5%, the model would be consistent as indicated by [27], which appeared to be correct in the current research, as the CV for PSG yield was 0.66%. The significance of the model can also be evaluated by the adequate precision that might be explained by a deviation from the response. A deviation ratio higher than 4 was required. The current experimental ratio of 93.370 indicated satisfactory results. This model finds its application to be utilized for current experimentation.

Analysis of variance (ANOVA) was used to evaluate the effects of independent variables as linear, interaction, quadratic, and residual coefficients. It was clearly understood from the results that there was a significant effect of model effects to PSG yield (Table 4). The linear and quadratic effects showed highly significant behavior than that of interaction effects ($p \leq 0.01$). The behavior of different model effects noticed for extraction of PSG yield from PDFM was linear > quadratic > interaction. It was also very much clear from Table 4 that linear coefficients and quadratic coefficient were mostly significant.

Table 4. Analysis of Variance (ANOVA) for PSG yield.

	SOV	DF	*F*-Value	*p*-Value	Remarks
Linear	Model	20	522.38	<0.0001	Significant
	A-Temperature	1	3923.57	<0.0001	Significant
	B-Water/meal ratio	1	186.15	<0.0001	Significant
	C-Amplitude level	1	4036.62	<0.0001	Significant
	D-pH	1	1560.68	<0.0001	Significant
	E-Time	1	371.10	<0.0001	Significant
Interaction	AB	1	0.0597	0.8089	Non-Significant
	AC	1	7.23	0.0126	Significant
	AD	1	0.6635	0.4230	Non-Significant
	AE	1	2.65	0.1158	Non-Significant
	BC	1	0.0597	0.8089	Non-Significant
	BD	1	0.5374	0.4703	Non-Significant
	BE	1	3.82	0.0619	Significant
	CD	1	0.2389	0.6293	Non-Significant
	CE	1	0.2389	0.6293	Non-Significant
	DE	1	1.92	0.1784	Non-Significant
Quadratic	A^2	1	3.52	0.0725	Significant
	B^2	1	35.11	<0.0001	Significant
	C^2	1	21.18	0.0001	Significant
	D^2	1	338.50	<0.0001	Significant
	E^2	1	23.07^B	<0.0001	Significant
Residual		25	-	-	
Lack of Fit		20	17.41	0.0025	
Pure Error		5	-	-	
Cor Total		45	-	-	

The second-order polynomial model equations obtained for PSG yield in terms of coded variables are as follows:

$$\begin{aligned} Y = {} & 8.95 + 0.9613A + 0.2094B + 0.975C + 0.6062D + 0.2956E + 0.0075AB + 0.0825AC \\ & -0.025AD - 0.05AE - 0.0075BC + 0.0225BD + 0.06BE - 0.015CE \\ & -0.0425DE + 0.039A^2 + 0.1231B^2 + 0.0956C^2 + 0.3823D^2 + 0.0998E^2 \end{aligned}$$

where,

A = Extraction Temperature (°C)
B = Water to Meal Ratio
C = Amplitude Level (%)
D = pH
E = Time

Similarly, the second-order polynomial model equations obtained for PSG yield in terms of actual variables are as follows:

$$\begin{aligned} Y = {} & 7.51859 + 0.032365\ Temp - 0.147125\ WMR + 0.010406\ Amp - 1.00115\ pH \\ & +0.007771\ Time + 0.000075\ Temp \times WMR + 0.000206\ Temp \times Amp \\ & -0.000625\ Temp \times pH - 0.00025\ Temp \times Temp - 0.000075\ Amp \times Time \\ & -0.002125\ pH \times Time + 0.000097\ Temp^2 + 0.004925\ WMR^2 \\ & +0.000239\ Amp^2 + 0.095573\ pH^2 \ \ + 0.000998\ Time^2 \end{aligned}$$

where,

Temp = Extraction Temperature (°C)
WMR = Water to Meal Ratio
Amp = Amplitude Level (%)

3.4. Single Factor Analysis

The effect of sonication extraction factors such as sonication temperature (°C), water to meal ratio, sonication amplitude level (%), sonication pH, and sonication time (min) on PSG yield was evaluated by regulating the levels from −1 to +1 for every extraction variable. The sonication temperature (°C) showed a positive effect on extraction of PSG. Increasing trend in PSG yield was observed by increase in sonication extraction temperature (°C) when all other extraction factors were set at mean values. It is clearly understood from the results that the sonication extraction temperature (°C) significantly affects the process variable. Similar results were observed in the case of amplitude level (%) when all other factors are kept at mean values. However, water to meal ratio showed a different trend on PSG yield. Up to the mean value of water to meal ratio, a very slow increase in PSG yield were observed, but a slightly higher gum yield was observed when the water to meal ratio was increased from 0 to +1. Sonication extraction time (min) also showed a positive influence when all other factors were kept in mean range. A different trend was observed when studying the effect of sonication pH on the PSG yield. Slow increase in PSG yield was noticed at initial level, but when pH was further increased from mean value there was an abrupt increase in flax gum yield.

3.5. Analysis of Mutual Interaction Effect

Response surface methodology (RSM) was utilized for standardization of the PSG extraction conditions, i.e., sonication extraction temperature (°C), water to meal ratio, amplitude level (%), sonication extraction pH, and sonication extraction time (min). It was very difficult to estimate PSG yield by varying mutual responses by response model. Maximum PSG yield was obtained by varying the responses of two extraction variables and keeping the other three variables at mean coded values.

Upon studying the mutual responses of sonication extraction temperature (°C) and amplitude level (%), keeping all other variables at mean level, a decreasing trend was observed in PSG yield. These results showed significant behavior toward PSG yield. The PSG yield 7.24% to 11.04% by varying sonication extraction temperature (°C) and amplitude level (%) from −1 to +1. The minimum and maximum PSG yield were observed by varying these two responses and keeping the other three responses at mean value. The results have been graphically represented in Figure 2. When mutual effects of water to meal ratio and sonication extraction time (min) were studied, keeping the other three variables at mean level, marginally significant behavior in PSG yield was observed. Meanwhile all other mutual interactions showed a non-significant behavior towards extraction yield of PSG. Very limited published data have been available in support of PSG yield through response surface methodology that supports our results.

Previously, the whole seeds were utilized for aqueous extraction of PSG or mucilage. The results of the current study were in line with earlier research carried out under specific extraction conditions that effect the PSG yield from defatted meal. The study conducted by [28] optimized the conditions for aqueous extraction of seed gums as extraction temperature of 85–90 °C, water to seed ratio 13, and extraction pH 6.5–7.0. Similar research carried out by [29] assessed the effects of independent factors, for example extraction temperature (45–100 °C), water to seed ratio (4–24), and extraction pH (3–7), on the dependent variables such as gum yield, protein content, and apparent viscosity by using response surface methodology. The study concluded that there were two major factors that significantly affect the gum yield, those being extraction temperature and extraction pH, while the other independent variable, i.e., water to seed ratio, had minor influence on gum yield. According to [29], maximum gum yield was obtained at an extraction temperature range of 85 to 90 °C, extraction pH 6.5 to 7.0, and by keeping water to seed ratio 14.

It was indicated from previous studies and current experimentation that at elevated extraction temperatures (°C), gum viscosity associated with the seeds continued to decrease and make seeds less sticky. All this facilitates the extraction and increases the yield [30]. Studies have also shown that the yield of PSG was strongly influenced by the effect of the time–temperature relationship [31]. On the other hand, by keeping temperature (°C) and time (min) constant, the gradual increase in the PSG yield was due to water to meal ratio. This might be due to the fact that the extra fluid triggers the gums out of the seeds [32].

Studies have shown that it is not feasible to use higher temperature because beyond a certain temperature limit, as PSG yield may adversely be affected [28]. Similarly, there was also a limit of water to meal ratio at 25, as beyond this there was not much increase in PSG yield and functional properties of gums were adversely affected, limiting its applications in food industry.

According to current experimentations and previous research, the optimal parameters for PSG yield were found as extraction temperature 80 °C and water to meal ratio up to 20. These parameters resulted in equilibrium yield of about 10% of the dry weight of meal [33]. Similar effects of extraction pH were also observed by [28,30,31] in different seed gums. On the other hand, the results of current experimentations were in contradiction to the research of [34–36], who observed maximum seed gum yield in basic environment. Keeping in view the economic and commercial aspects, there is much potential in oilseed meal for commercial gum extraction and use as a new source of food additive as hydrocolloids.

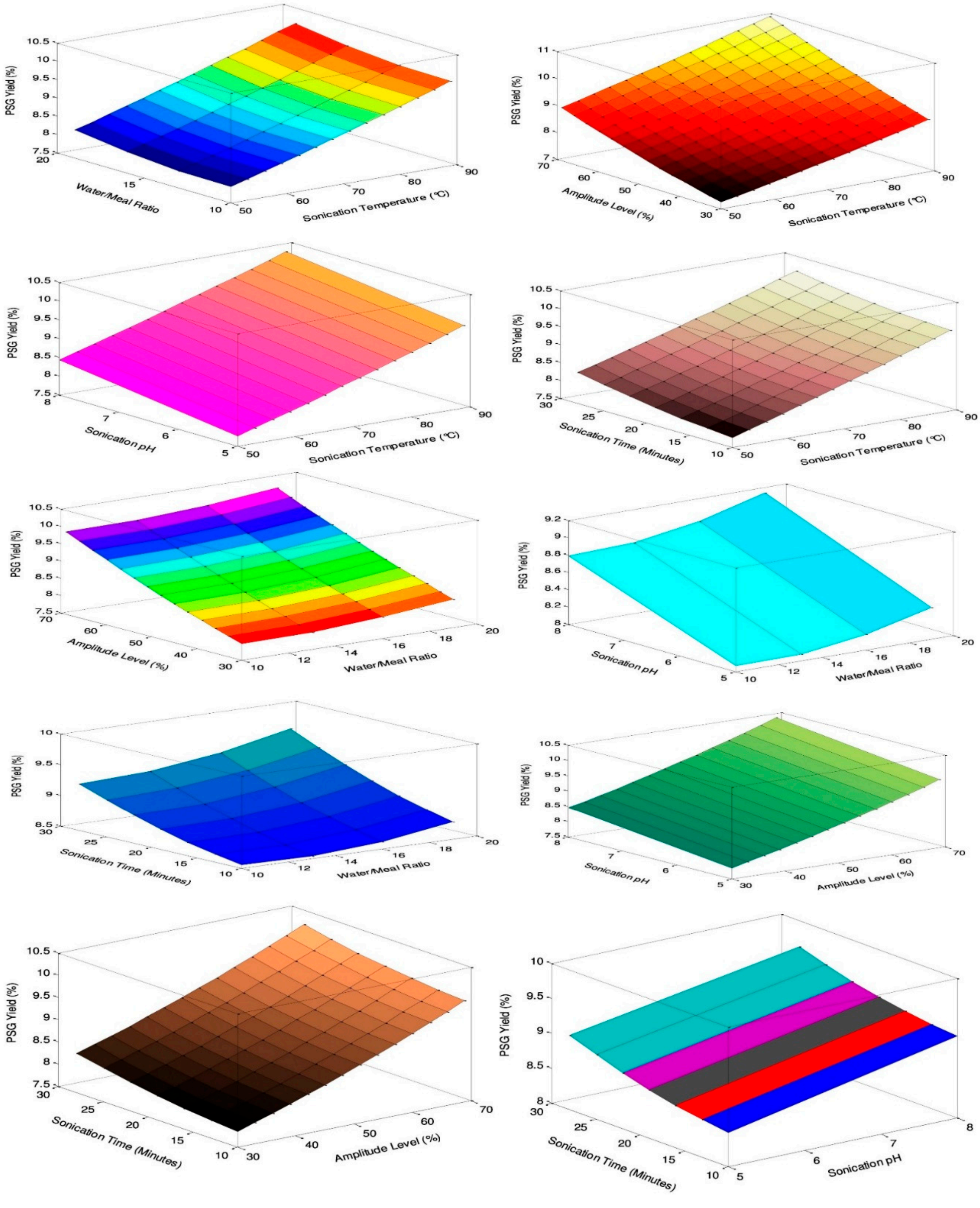

Figure 2. Effects of mutual interactions on PSG yield.

3.6. Physico-Chemical Characterization of PSG

The chemical composition of flaxseed-derived PSG has been shown in Table 2. Compared to the corresponding meal sample, it is clear that gums have higher quantities of proteins, carbohydrate, and ash. The authors of [37] also found similar results while studying the effect of temperature on gum extraction from flaxseed. Similarly, the study carried out by [38] regarding the influence of temperature on gum yield observed that the gum extracted at higher temperature contained higher level of fat, protein, and ash contents than gum extracted at ordinary temperature. Higher extraction temperature increased the yield of gum, eased in alcoholic separation, and improved functional properties of extracted gums. The presence of toxic compounds in oilseed meals limits its commercial applications for food purposes. These toxic and anti-nutritional compounds, mostly polyphenolic compounds, are mainly responsible for binding of the digestive enzymes, vitamins, and dietary proteins. Extraction variables, mainly extraction temperature, seem to link in the reduction of cyanogenic compounds [39]. Similarly, tannin contents were also reduced during extraction of PSG due to high extraction temperature.

The color of extracted PSG powder and PSG solution was measured by a Hunter Laboratory Tristimulus colorimeter. The result showed that PSG powder showed a higher L value (L = 5.66) than the PSG solution (L = 4.74). A higher value of redness in PSG powder (a = 1.13) compared with PSG solution (a = 0.48) and a little bit difference in yellow color of PSG powder (b = 3.71) to PSG solution (b = 3.11) were observed. Higher in lightness and low in redness and yellowness make it suitable for utilizing in different food products [4]. This might be because of the fact that during freeze-drying, there was no involvement of heat, and color changes were much less compared with that for drying methods. The similarity in the color content of PSG powder and PSG solution samples indicate that PSG powders retain similar and widespread use as that of PSG solutions.

3.7. Functional Properties PSG

3.7.1. Solubility and Swelling Power

Solubility and swelling power are a solid illustration of absorption of water by gums. The solubility of PSG varied from 20 to 50 g water/100 g PSG when evaluated at ordinary temperature. These findings are similar with that of earlier studies. Similar results were also observed by [40], that the water absorption capacity of PSG varied from 15–35 g water/g PSG, which was in line with earlier studies on guar gum (22 g water/g PSG) [38]. Similarly, solubility and swelling power were interlinked with each other as an increase in solubility also resulted in an increase in swelling power.

3.7.2. Viscosity

A decreasing trend was observed in the viscosity of 1% PSG solution with high shear rate (Figure 3A). The viscosity of the PSG solution depends upon different factors, including composition and chemical content of PSG. Composition of PSG varies due to cultivar and seasonal variations [4]. In the current study, the viscosity gradually decreased with increase in shear rate, which might be due to the fact that all kinds of polysaccharides show shear thinning properties. Almost similar findings were observed by [4] using 1% *w/w* PSG solution, and by [41] while studying the viscosity of mustard mucilage solution. Weak gelling properties and high viscosity is mainly contributed by neutral fractions of flax gums [40]. The authors of [37] compared the viscosity of different gum solutions and found that at 0.3% (*w/v*) flaxseed gum solution showed almost half the viscosity compared with other gums like locust bean gum and guar gum solution, although that could be altered by changing the temperature, solution concentration, and pH. Keeping in view the current study and previous research, it can be concluded that the PSG could be utilized as a stabilizing agent in products with high viscosity, mainly in food, pharmaceutical, and cosmetic products.

3.7.3. Foaming Properties

Flaxseed-derived PSG have shown positive influences on the foaming properties in food systems due to high viscosities of PSG [42]. Therefore, due to their excellent foaming properties and moderately high viscosity, flaxseed-derived PSG have a great potential to be used in food systems [4]. The foaming properties of flaxseed-derived PSG have been presented in Figure 3B. The foaming stability (%) of current study was reasonably higher than observed by [4] in flaxseed-derived PSG. These foaming properties of PSG also play a role in the strength of protein networks [43]. This might be due to the fact that PSG molecules are likely to remain to the surface and thus lower the interfacial tension, which is involved in stabilizing the foams [44]. Conversely, the foam volume decreased with an increase in holding time. There was significant reduction in the foam stability with 60 min holding time. Therefore, due to its higher foam stability, PSG is effective in improving the textural properties of food.

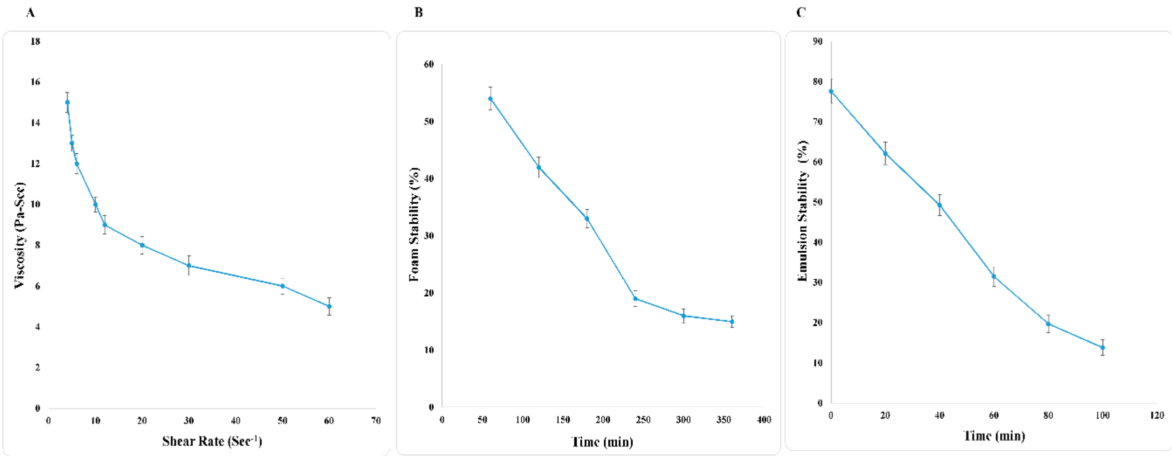

Figure 3. Functional properties of PSG (**A**) Effect of shear rate on viscosity; (**B**) Effect of time on foam stability and (**C**) Effect of time on emulsion stability.

3.7.4. Emulsion Properties

The emulsion properties, such as emulsion stability index (ESI) and emulsion activity index (EAI), of the 1% PSG solutions have been shown in Figure 3C. The EAI of flaxseed-derived PSG have been shown in the range of 50 to 80 m^2/g. The present study was in close relationship of the study of [4] who evaluated the responses of different drying techniques on properties of PSG. Similar results were also obtained by [45] when comparing the functional properties of different types of polysaccharides. A high emulsion stability index (ESI) was observed in the sample even after 60 min of whipping, but a gradual decreasing trend was observed in the ESI during 100 min of holding time. Another study [46] elaborated that the emulsion stability properties of PSG were contributed to by high viscosity and intrinsic proteineous materials. The interaction between proteineous matter and ionic hydrocolloids at appropriate pH would be responsible for increasing in emulsification properties and corresponding emulsion stability.

3.8. Characterization of PSG-Supplemented Yoghurts

The values of different physicochemical analysis of flaxseed-derived-PSG-supplemented yoghurt are shown in Table 5. The analysis results have shown that viscosity, syneresis, and pH were significantly affected by different levels of flaxseed PSG (Table 5).

Table 5. Functional attributes of flaxseed-derived-PSG-supplemented yoghurt.

Treatment	Viscosity (cp)	Synersis (%)	pH
T_0	502 ± 3	13.58 ± 1.07	3.97 ± 0.68
T_1	548 ± 2	12.02 ± 1.24	4.16 ± 0.54
T_2	593 ± 4	11.30 ± 1.12	4.30 ± 0.59
T_3	641 ± 2	9.95 ± 0.94	4.42 ± 0.61
T_4	688 ± 2	8.63 ± 0.87	4.65 ± 0.66
T_5	735 ± 3	7.04 ± 1.01	4.78 ± 0.52
T_6	776 ± 5	5.87 ± 0.95	4.91 ± 0.63

T_0 = Control yoghurt; T_1 = Yoghurt with 0.25% PSG; T_2 = Yoghurt with 0.5% PSG; T_3 = Yoghurt with 0.75% PSG; T_4 = Yoghurt with 1.0% PSG; T_5 = Yoghurt with 1.25% PSG; T_6 = Yoghurt with 1.5% PSG.

3.8.1. Viscosity

The result of the viscosity of yoghurt supplemented with different levels of flaxseed-derived PSG and control have been shown in Table 5. The observation indicated that all yoghurt treatments show non-Newtonian and shear thinning (pseudoplastic) behaviors. In a study conducted by [47] using xanthan gum with locust bean gum in 50:50 ratio, the preparation of stirred-type yoghurt showed increasing behavior in the consistency co-efficient and a decreasing in the liquid separation. The increase in viscosity was mainly contributed to by the synergistic effects of PSG and milk protein [20]. Different hydrocolloids have been used to control the texture and stabilize in different dairy products [48]. Hydrocolloids normally function at very low concentration and play a role in viscosity enhancement and hinder the sedimentation of dispersed particles. It was generally concluded that proteins and hydrocolloids (mainly polysaccharides) were the main contributing factors to the microstructural properties of foods [49].

3.8.2. Syneresis

Syneresis is one of the main quality determination factors in yoghurt, which is perceived as accumulation of liquid/whey on the surface of yoghurt. It is mainly because of the contraction of the 3D arrangement of protein (casein) network that resulted in separation of whey proteins from yoghurt. PSG in yoghurt are involved in the formulation of thicker gel network compared to the control, so syneresis decreased with an increase in level of supplementation because of the capability of PSG to absorb water. Supplementation of PSG in yoghurt had a significant effect on syneresis (Table 5). It was clear from the results that the lowest percentage of syneresis was observed in the case of yoghurt prepared from the 1.5% gum concentration. It was also observed from our data that with an increase in the supplementation level of PSG in yoghurt, there was a significant reduction in syneresis (%). The decrease in syneresis (%) was due to the water absorbing capacity of hydrocolloid used. The results are in agreement with [50].

3.8.3. pH

The mean values of the pH of yoghurt supplemented with different levels of flaxseed-derived PSG have been depicted in Table 5. A minor increase in the values of pH were observed of supplemented yoghurt with PSG concentration. These observed values of pH were within the normal range and were very much in agreement with the study carried out by [51,52], which concluded that PSG supplemented levels and types have no effect on the pH and acidity of dairy products.

3.9. Sensory Evaluation of PSG-Supplemented Yoghurt

The organoleptic properties of flaxseed-derived-PSG-supplemented yoghurt are presented in the Figure 4. No adverse effect on the flavor of PSG-supplemented yoghurt has been observed. No objectionable flavor was observed in any treatment, but the textural properties vary significantly among different treatments. Flaxseed-derived PSG possessed most of the physiochemical and functional properties sought after by the food industry. Flaxseed-derived PSG have good thickening, gel forming, foam enhancing, emulsifying, suspension forming, clarifying, and rehydrating properties. The current research was very much in agreement with the study of [22,53].

From these observations, it can be concluded that the use of flaxseed-derived PSG as stabilizer at a concentration of 1% in supplemented yoghurt enhanced their rheological and sensory properties. The above studied results are in favor of utilization of flaxseed-derived PSG in the production of different food products, especially in fermented dairy and dairy-like products.

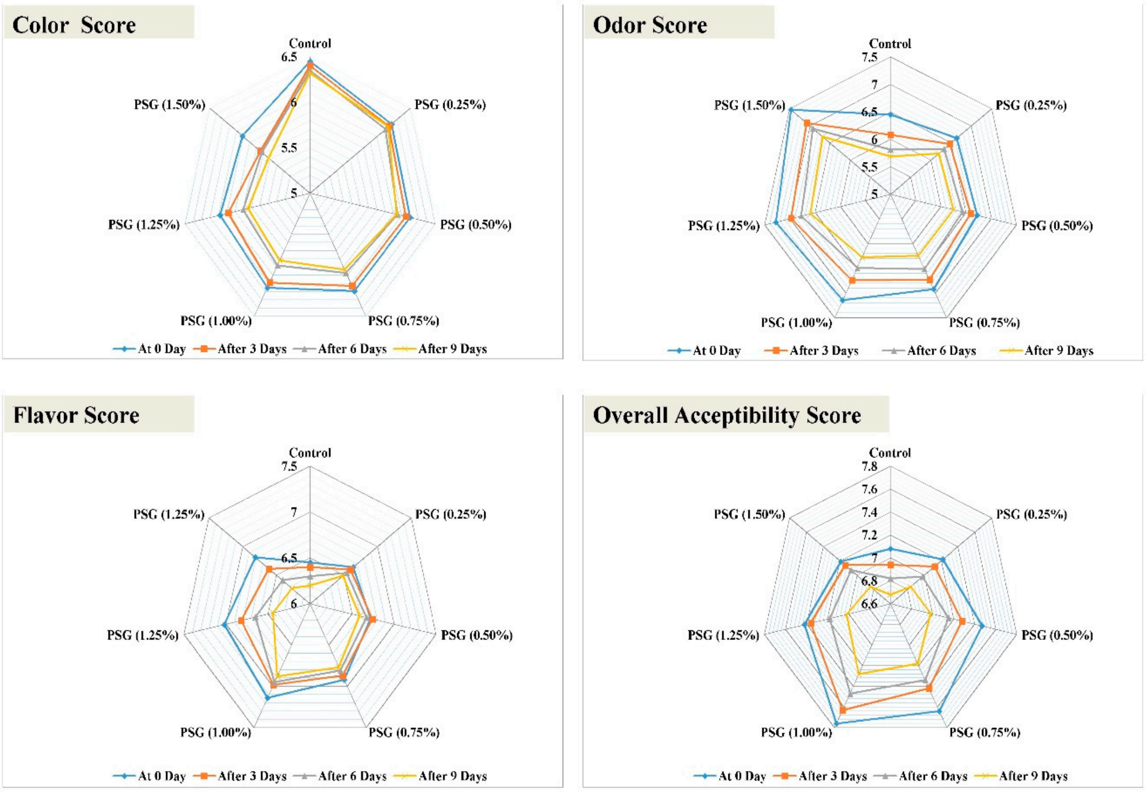

Figure 4. Sensory properties of flaxseed-derived-PSG-supplemented yoghurt at different storage interval.

4. Conclusions

It was concluded from the present study that different sonication operating factors were statistically significant for the extraction of PSG. PSG extraction yield was estimated by using second-order polynomial models. The functional properties of PSG in a solution depend upon the polysaccharide and other types of materials present in solution. Along with the chemical characterization, the solution stability, viscosity, emulsifying and foaming properties studied explore its potential as a new food additive. Supplementation of flaxseed-derived PSG gums in dairy products such as yoghurt helped to improve physiochemical, functional, textural, and organoleptic properties. Further exploration of extraction methods and nutritional and therapeutic potential of flaxseed gums should be conducted.

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