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Physical and sensory properties of peanut sauce prepared through fermentation process

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Abstract
Defatted peanut cake is the valuable by product obtained after extraction of oil and it is rich in protein. Peanut sauce was prepared using defatted peanut cake and wheat as a basic raw materials which are soaked and roasted, respectively prior to the fermentation process. The experiment was conducted as per the Response Surface Methodology (RSM). The Central Composite Rotatable Design (CCRD) with quadratic model was used in designing the experiment and the effects of defatted peanut cake percentage and brine fermentation time on the important physical (viscosity and specific gravity) and sensory properties (colour, taste, flavour and overall acceptability) of peanut sauce were studied. The results showed that the viscosity ranged from 1.82 cP to 2.94 cP and specific gravity ranged from 1.1048 to 1.1447. Overall acceptability of peanut sauce was found 6.08 to 6.85 out of 9 point Hedonic Scale. Low p-values associated with F-values for the viscosity, specific gravity and overall acceptability (p<0.001) indicated that the models were significant except colour, taste and flavor. Therefore, the models for viscosity, specific gravity and overall acceptability were found to be both well-fitted and reliable. However, it should be noted that the models for color, taste and flavor may not have demonstrated statistical significance.

Keywords: Defatted peanut, peanut sauce, fermentation, Koji fermentation, Moromi fermentation

1. Introduction
Groundnut (*Arachis hypogea* L.), that is considered to be the 'King of Oilseeds', is an annual plant herb (legume) comes from the pea family of Fabaceae. It is also known as peanut, earthnut, monkey-nut and goobers in U.S. and British terms (Biswas and Bhattacharjee, 2019). Peanuts are majorly used for the oil production. It is estimated that around 65% of groundnut produced in the world is crushed to extract oil and the remaining largely goes to make other edible products. However, as per estimates, in India, around 82% of peanut produced is used for edible oil production, 12% as seed and 6% as feed (Mehrotra, 2011) [9].

Following an extraction of oil, the residual cake is also processed mostly for animal feed, but is also used for human consumption. Excluding oil, the by-products of peanut also contains several other functional compounds like proteins, fibers, polyphenols, antioxidants, vitamins and minerals which may be additional as a useful ingredient into many several processed foods (Arya *et al*., 2015) [3]. In general, the resultant cake contains about 43 to 65% protein and 6 to 20% fat and different group of vitamin B depending upon the method of extraction (Desai *et al*., 1999) [7].

The utilization of defatted peanut meal in food products offers a promising approach to increase groundnut protein consumption. It can be incorporated as a low-fat groundnut concentrate, composite flour, or functional ingredient in various food items such as bakery products, breakfast cereal flakes, snack foods, multipurpose supplements, infant and weaning foods and extruded meals (Purohit and Rajyalakshmi, 2011). Furthermore, defatted peanut flour is high protein content and nutritional value make it suitable for use in the production of extruded snack products (Davara *et al.*, 2022) [6].

Fermented foods and beverages contribute significantly to the diets of many people throughout the world. Oilseed and cereal cake fermentation products are especially popular in Southeast Asia and parts of Africa (Beuchat *et al*., 1978) [4]. With globalization, the sauce has now reached the international realm where the condiment can be found in commercial and home kitchens in both the East and the West (Lian *et al*., 2019) [8].

Fermentation is a biochemical reaction that extracts energy from carbohydrates without using oxygen. Under anaerobic conditions, bacteria and/or yeast break down sugar into alcohol, acid and/or gas to produce ethanol, carbon dioxide and lactic acid, which increase product shelf life and impart desirable sensory properties. The desirable flavour of fermented foods is predominantly due to the acid, sugar and volatile flavour compounds (Schilling and Campbell, 2021) [13]. Fermentation also adds value to food. The fermented foods are highly priced in the global market due to their unique flavours and textures. It increases nutrient bioavailability and digestibility of foods like legumes (Anonymous, 2023) [1].

So far, to the best of our knowledge, there is a lack of information for physical and sensory properties of the peanut sauce. In view of this, the research work was done to generate the information about the interaction between process parameters on physical as well as sensory property of peanut sauce prepared through fermentation technology.

2. Materials and Methods

2.1 Raw materials and microbial cultures

Defatted peanut cake and wheat are the basic raw materials required in the preparation of peanut sauce which were procured from the local suppliers. The *Aspergillus oryzae* and *Saccharomyces rouxii* were purchased from MTCC, Chandigarh, Panjab (India) and *Pediococcus halophilus* was purchased from ATCC, USA. All the chemical used in the analysis were of analytic grade.

2.2 Roasting of wheat and Soaking of defatted peanut cake flour

The cleaned wheat grains were roasted at 180 ℃ for few minutes (5-10 min) till all grains were roasted appropriately, it was then cooled to room temperature and crushed slightly to obtain approximately 4 to 5 pieces per kernel. Defatted peanut cake was converted to coarse powder. Then it was soaked at room temperature for 12 h to increase its moisture content up to 60% (wb). Soaked samples were then autoclaved at 121 °C for 30 min and cooled before it is used for fermentation process.

2.3 *Koji* fermentation

For koji production, cooked defatted peanut cake flour and pieces of roasted wheat were mixed thoroughly in the plastic tray sterilized with alcohol as per the proportion fixed for the different treatments. The mixture was then inoculated with 0.1% (w/w) of *Aspergillus oryzae*. Each tray was loaded with the mixture to a 3-5 cm thickness and covered with muslin cloth and then incubation at 45±3 ℃ for 6 days. The preparation of koji was completed in 6 days when the culture began turning greenish yellow in color.

2.4 Brine fermentation

For brine fermentation (*moromi* fermentation), The *koji* mass as obtained after *koji* fermentation was transferred to glass jar equipped with hand operated stirring facility and 20% NaCl (Brine) solution was added in the mass. The ratio of *koji* mass to brine solution was kept as 1:2. The desired concentration of *Pediococcus halophilus* was added in the *koji* mass filled in the glass jar and then covered with muslin cloth and lid is closed and allowed to undergo fermentation at 37±3 ℃ for incubation. After one week of incubation, the desired concentration of *Saccharomyces rouxii* was added in the mixture and kept for the period as decided for the different treatments. Mixture was stirred one to two times in a day.

2.5 Refining process

The thick slurry called as *moromi mass* obtained after brine fermentation was centrifuged at 5800-6000 rpm for 15 min to separate peanut sauce from the slurry. The fermented peanut sauce as obtained after centrifugation was filtered using muslin cloth and pasteurized at 85 ℃ for 15 min to stop the microbial growth. The pasteurized peanut sauce was stored in the sterilized glass bottles and kept at 4 ℃ for further analysis.

2.6 Determination of viscosity

Viscosity of peanut sauce was determined by glass Oswald viscometer at room temperature (Ranganna, 2000) [12]. Viscometer was placed vertically on the stand to measure the viscosity and 20 ml of sauce was piped in to lower bulb. Sauce was pulled in on the other bulb to a point above the mark of bulb level then released and when it crossed the mark stop watch was started. When the sauce hit a mark below the bulb, the watch was paused. The sauce was passed through the viscometer's capillary section and the time taken to move it through the viscometer's capillary section using the stop watch was noted. Distilled water was used as a reference.

$$\text{viscosity (cP)} = \frac{\text{Dj}}{\text{Dw}} X \frac{\text{Tj}}{\text{Tw}} \quad \ldots (1)$$

Where,

Dj = Density of sauce Tj = Time taken by sauce to flow

Dw = Density of water Tw = Time taken by water to flow

2.7 Determination of specific gravity

Specific gravity of peanut sauce was estimated according to method suggested by Ranganna (2000) [12]. In this the sauce was placed in to the measuring cylinder up to the 50 ml mark and measure the weight of the sauce. Specific gravity of peanut sauce was calculated by equation given below.

$$\text{Specific gravity} = \frac{\text{Density of sauce}}{\text{Density of water}} \quad \ldots (2)$$

Density of sauce was calculate by equation given below.

$$\text{Density of sauce} \left(\frac{\text{g}}{cm^3}\right) = \frac{\text{Weight of peanut sauce (g)}}{\text{volume of measuring cylinder(ml)}} \quad \ldots (3)$$

2.8 Determination of sensory properties

All the samples were judged for its sensory parameters like colour, taste, flavor and overall acceptability on the given score sheet based on the degree of preference by 9-point Hedonic Scale method given by Ranganna (2000) [12]. Commercial available soy sauce was used as a control sample. The sensory analysis was conducted by twelve trained and semi-trained panelists comprising of staff and graduate students of the Department of Processing and Food Engineering, College of Agricultural Engineering and Technology, Junagadh Agricultural University, Junagadh. All the samples were given to panelists randomly after coding. Potable water was provided to the panelists for palates cleansing between sample testing.

2.9 Experimental design and statistical analysis

The experiment was designed as per the Central Composite Rotatable Design (CCRD) of Response Surface Methodology (RSM) (Anderson and Whitcomb, 2005) [2]. A two-factor five-level Central Composite Rotatable Design (CCRD) with quadratic model was employed with two independent variables, *viz*., defatted peanut cake (10, 21.72, 50, 78.28 and 90%) and brine fermentation time (30, 52, 105, 158 and 180 days). Table 1 shows treatment details with coded and uncoded variables.

Response factors were the main physical and sensory indices, namely viscosity (cP), specific gravity, colour, taste, flavor and overall acceptability. The following second order quadratic equation was used to express the responses as a function of the independent variables (using coded levels). Quality response functions:

$$Y=b_0+b_1X_1+b_2X_2+b_{11}X_1^2+b_{22}X_2^2+b_{12}X_1 \ldots (4)$$

Where Y is the response calculated by the model; X_1 and X_2 are the code of independent variables, *i.e.* defatted peanut cake and brine fermentation time, respectively; b_0 is constant term, b_1 and b_2 are linear, b_{11} and b_{22} are quadratic and b_{12} is interaction coefficients, respectively.

The second order polynomial coefficients were calculated by using the software package Design Expert version 11 to estimate the responses of the dependent variable.

Table 1: Treatment details

Treatment No.	Coded variables		Uuencoded variables	
	X_1	X_2	Defatted peanut cake (%)	Brine fermentation time (days)
1	-1	-1	21.72	52
2	1	-1	78.28	52
3	-1	1	21.72	158
4	1	1	78.28	158
5	-1.41	0	10.00	105
6	1.41	0	90.00	105
7	0	-1.41	50.00	30
8	0	1.41	50.00	180
9	0	0	50.00	105
10	0	0	50.00	105
11	0	0	50.00	105
12	0	0	50.00	105
13	0	0	50.00	105
14	0	0	50.00	105

3. Results and discussion

Table 2 shows experimental data of physical properties (viscosity and specific gravity) and sensory properties (colour, taste, flavor and overall acceptability) observed for different treatments. The complete data were fitted to quadratic models as the quality response functions described in Eq. (3.4). The resulting model parameters are presented in Table 3. The models for viscosity, specific gravity and overall acceptability demonstrated low p-values associated with F-values (p<0.001), indicating their statistical significance. However, it is important to note that the models for color, taste and flavor did not show the same level of significance. Therefore, the models for viscosity, specific gravity and overall acceptability were considered fitted and reliable, while the models for color, taste and flavor may not have been statistically significant. The adequacy of fit of the models were confirmed (except colour, taste and flavor) by the high R^2 for all responses ($R^2 \geq 0.90$). Moreover, the relatively low CV (<10%) suggested sufficient repeatability of experimental results.

3.1 Physical properties of peanut sauce

3.1.1 Viscosity

The observations of viscosity of peanut sauce samples as prepared through fermentation process is presented in Table 2. From the table, it can be observed that the viscosity of peanut sauce ranged from 1.82 cP to 2.94 cP. The maximum viscosity (2.94 cP) was observed for the treatment No. 6 while the minimum viscosity (1.82 cP) was observed for the treatment No. 5. Fig. 1(a) shows the interactive effect of defatted peanut cake and brine fermentation time on viscosity of peanut sauce. Which indicated the increase in viscosity as the percentage of defatted peanut cake was increased. The viscosity was expected to be decreased up to 82 days of brine fermentation time. With further increase in brine fermentation time, it was observed to be increased. The increase in the viscosity was also reported by Park *et al.*, (2019) [10] during fermentation of milk. It is also indicated that the viscosity of peanut sauce increased as brine fermentation time was increased up to 180 days.

Table 2: Physical and sensory properties of peanut sauce

Tr. No.	Physical properties		Sensory properties			
	Viscosity (cP)	Specific gravity	Colour	Taste	Flavour	Overall acceptability
1	2.04	1.1202	6.52	7.13	6.52	6.42
2	2.73	1.1389	6.68	6.68	6.74	6.54
3	2.25	1.1400	6.82	7.48	7.20	6.85
4	2.58	1.1200	6.94	7.35	6.19	6.81
5	1.82	1.1084	7.02	6.81	6.81	6.82
6	2.94	1.1232	6.76	6.63	6.52	6.79
7	2.29	1.1447	5.82	6.13	5.88	6.08
8	2.52	1.1400	6.88	6.39	7.06	6.66
9	2.13	1.1341	7.06	6.28	7.19	6.82
10	2.22	1.1352	7.18	6.30	6.38	6.75
11	2.14	1.1320	6.71	6.29	6.13	6.69
12	1.95	1.1321	6.12	6.25	6.63	6.80
13	2.08	1.1301	6.00	6.27	5.94	6.67
14	1.99	1.1373	6.47	6.33	6.25	6.71

Table 3: Analysis of variance (ANOVA) and regression coefficients for response surface quadratic model of physical and sensory properties of peanut sauce

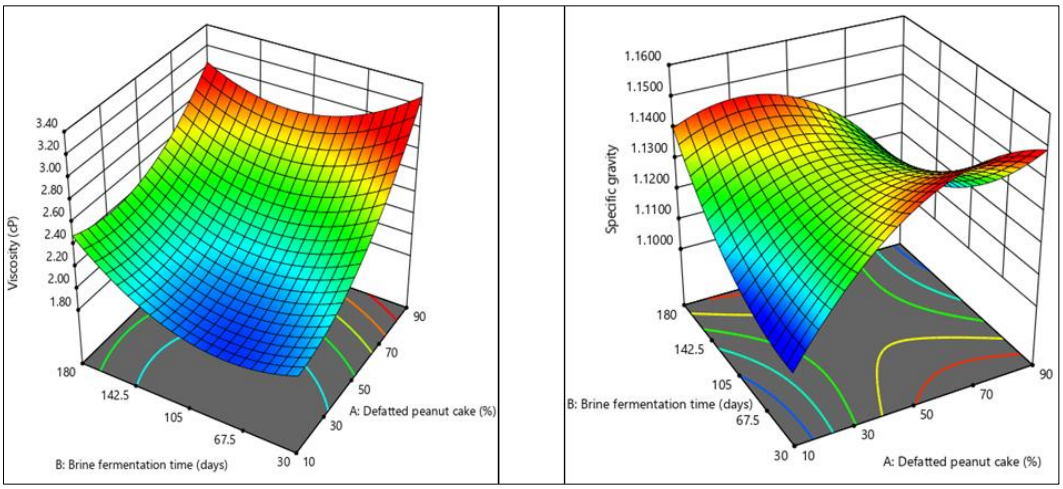
Source	Viscosity (cP)	Specific gravity	Colour	Taste	Flavour	Overall acceptability
Intercept	2.09	1.13	6.59	6.29	6.42	6.74
Linear terms						
X_1	+0.3267***	+0.0025*	-0.0104	-0.1050	-0.1508	0.0047
X_2	+0.0471	-0.0007*	0.2578	0.1750	0.2254	0.1900***
Interaction terms						
X_1X_2	-0.0913	-0.0097	-0.0106	0.0788	-0.3081	-0.0400
Quadratic terms						

X_1^2	+0.1481**	-0.0087**	0.1817	0.3837*	0.1485	0.0494
X_2^2	+0.1622**	+0.0046**	-0.0880	0.1531	0.0492	-0.1681***
Indicators for model fitting						
R^2	0.9249	0.9197	0.3874	0.6169	0.4643	0.9447
Adj-R^2	0.8779	0.8696	0.0045	0.3774	0.1294	0.9102
Pred-R^2	0.6853	0.5777	-0.3654	-1.7162	-0.5456	0.7918
Adeq Precision	12.6004	13.1773	3.4155	4.0220	4.1499	17.4070
F-value	19.70	18.33	1.01	2.58	1.39	27.34
Lack of fit	NS	NS	NS	S	NS	NS
C.V. %	4.95	0.3183	6.19	5.28	6.21	0.94

X_1= defatted peanut cake, X_2= brine fermentation time, ***Significant at p<0.001, **Significant at p<0.01, *Significant at p<0.05, S = Significant, NS = Non-significant

3.1.2 Specific gravity

The data of specific gravity of peanut sauce is presented in Table 4.1. It is ranged from 1.1084 to 1.1447. Maximum specific gravity (1.1447) was found for the treatment No. 7 and the minimum specific gravity (1.1084) treatment No. 5. Fig. 1(b) shows the interactive effect of defatted peanut cake and brine fermentation time on specific gravity of peanut sauce, which indicated the increase in specific gravity as the percentage of defatted peanut cake was increased up to 76% and decreasing trend was observed with further increase in defatted peanut cake percentage. It is also indicated that the specific gravity increased as brine fermentation time was increased up to 180 days.



(a) (b)

Fig 1: Response surface curves for the physical properties of peanut sauce as a function of defatted peanut cake percentage and brine fermentation time. (a) Viscosity; (b) specific gravity

3.2 Sensory properties of peanut sauce

3.2.1 Colour

Colour of peanut sauce ranged from 5.82 to 7.18 out of 9 point Hedonic Scale. The maximum score for colour of peanut sauce (7.18) was observed for the treatment No. 10 and minimum sensory score for colour (5.82) of peanut sauce was found for the treatment No. 7. The response surface curve of variation in the colour of peanut sauce as a function of percentage of defatted peanut cake and brine fermentation time is shown in Fig. 2(a). Which indicated the decrease in sensory score of colour as the percentage of defatted peanut cake was increased up to its maximum level, i.e. 90%. The sensory score of colour of peanut sauce increased with increase in brine fermentation time up to 139 days and further decreased with increase in brine fermentation time up to its maximum level, i.e. 180 days.

3.2.2 Taste

The data of sensory score of taste of peanut sauce ranged from 6.13 to 7.48 out of 9 point Hedonic Scale. The maximum sensory score of taste (7.48) was observed for the treatment No. 3 and the minimum sensory score of taste of peanut sauce (6.13) was observed for the treatment No. 7. Fig. 2(b) shows the interactive effect of defatted peanut cake and brine fermentation time on taste of peanut sauce. This graph indicated the decrease in taste as the percentage of defatted peanut cake and brine fermentation time were increased up to 56% and 70%, respectively with further increase in percentage of defatted peanut cake and brine fermentation time up to its maximum level, the taste was observed to be increased.

3.2.3 Flavour

The experimental data of the sensory score of flavour of peanut sauce ranged from 5.88 to 7.20 out of 9 point Hedonic Scale. The maximum (7.20) and minimum (5.88) sensory score of flavour of peanut sauce was observed for the treatment No. 3 and treatment No. 7, respectively. Fig. 2(c) describes the interaction effect of defatted peanut cake and brine fermentation time on flavour of peanut sauce. Which shows the sensory score of flavour decreased with an increase in defatted peanut cake up to its maximum level (90%). Sensory score of flavour of peanut sauce decreased with increase in brine fermentation time up to 118 days and increased with further increase in brine fermentation time up to its maximum level (180 days).

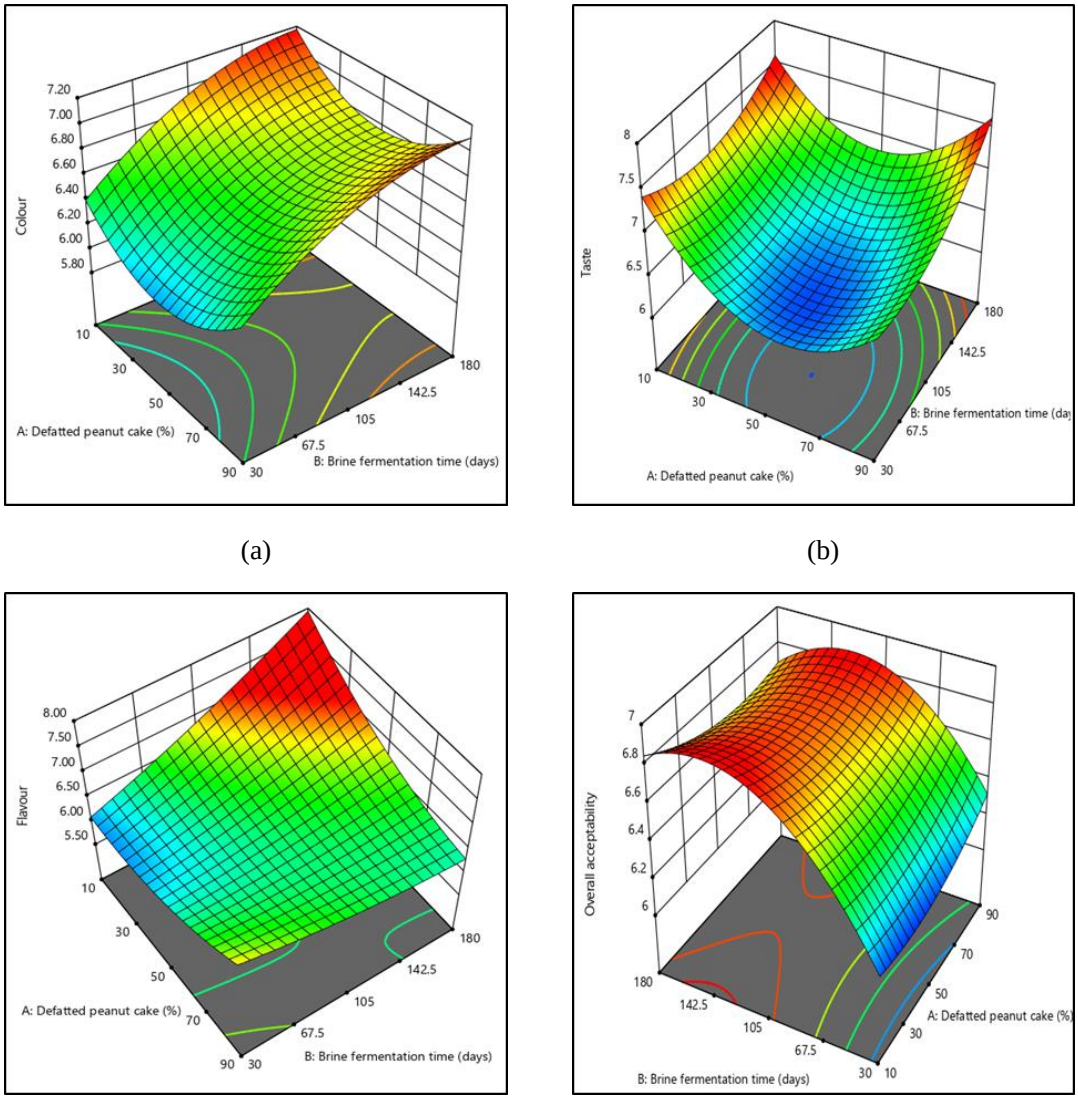


Fig 2: Response surface curves for sensory properties of peanut sauce as a function of defatted peanut cake percentage and brine fermentation time. (a) Colour, (b) Taste, (c) Flavour and (d) Overall acceptability.

3.2.4 Overall acceptability of peanut sauce

Overall acceptability of peanut sauce was ranged from 6.08 to 6.85 out of 9 point Hedonic Scale. The maximum Overall acceptability (6.85) was observed for the treatment No. 3. The minimum overall acceptability (6.08) was found for the treatment No. 7. Fig. 4.2(d), indicated the decrease in overall acceptability as the percentage of defatted peanut cake was increased up to 30% and with further increase in percentage of defatted peanut cake up to its maximum level, i.e. 90%, the overall acceptability was observed to be increased. Overall acceptability was found to be increased with an increase in brine fermentation time up to 143 days and thereafter, it was decreased with further increase in brine fermentation time up to its maximum level, i.e. 180 days.

4. Conclusion

The research findings revealed notable trends in the viscosity and specific gravity of peanut sauce in relation to the amount of defatted peanut cake used and the duration of brine fermentation. The viscosity and specific gravity of the sauce were observed to increase with an increase in the proportion of defatted peanut cake. However, as the brine fermentation time increased (up to 82 days for viscosity and up to a maximum of 180 days for specific gravity), a decrease in viscosity and specific gravity was observed. The treatment combination of 10% defatted peanut cake and 105 days of brine fermentation time resulted in the minimum viscosity of 1.82 cP and minimum specific gravity of 1.1084. On the other hand, the treatment combination of 21.72% defatted peanut cake and 158 days of brine fermentation time achieved the maximum scores for taste (7.46), flavour (7.20), and overall acceptability (6.85). These findings suggest that specific combinations of defatted peanut cake and brine fermentation time can significantly impact the sensory attributes and physical properties of the peanut sauce.

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