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OPTIMIZATION OF PLANT SEED FORTIFIED YOGURT

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ABSTRACT: *Sunflower (Helianthus annuus) and sesame seeds (Sesamum indicum) represent high natural sources of protein, fatty acids, vitamin B and bio active components, which causing nutritional enrichment. Comparing with control yogurt, enriched yogurt has high protein, has desirable pH value. The sample was incorporated with 1%,1.5%,2% sunflower seed and 1%,2%,3% sesame seeds. Milk fat varies with0.5%,3%,4.5%.Lactobacillus bulgarigus and Streptococcus thermophiles were used for the fermentation. Protein content, fat and pH were measured. Fat content of the final product varies with different fat content milk. Control sample was prepared along with these. There is a increase in fat and protein content compared to the control sample.*

Key words: Sunflower seed, Sesame seeds, analysis.

I. INTRODUCTION

Yogurt is one of the most popular fermented dairy products in the world, made by adding live bacteria to milk. It has been eaten for thousands of years and is frequently used as part of a meal or snacks; in addition, yogurt contains beneficial bacteria and may function as a probiotic, providing a variety of health benefits above and beyond plain milk. Most yogurt is white and thick, but many commercial brands are artificially coloured. The primary sensory attributes of yogurt include texture, colour and flavour. Yogurt is typically characterised as a smooth, viscous gel with a characteristic taste. Its popularity is not only due to various health claims and therapeutic effects but also for its sensory properties.

Consumption of yogurt helps improve diet quality because it is an excellent source of protein, vitamin B2 and B12, folate, niacin, calcium, magnesium, phosphorus and zinc. Yogurt is also a rich source of bioactive peptides released during lactic fermentation by lactic acid bacteria Probiotics exert several health benefits, including improving lactose digestion, immune and gastro-intestinal, the viable count of each probiotic bacteria is very important to provide health benefits to the host.

1.1 SUNFLOWER SEED

Sunflower is the one of the three most cultivated oil crops in the world. It contains around 50-54 % of fat and 20-23% of protein. In this around 30 to 40% are rich in linoleic acid and 20% are poor in oleic acid. Sunflower seeds are mainly used for their oil, but as with other oil seeds, the meal left behind after oil extraction is a valuable product because of its high protein content. Sunflower seeds are attractive due to their high protein content and extensive availability. Compared to other vegetable protein sources, sunflower seeds contain low or no anti-nutritional factors and, except for lysine present at low concentration, their amino acid composition conforms to the food and agriculture organisation outline of human requirements. Sunflower protein consists largely of albumins and globulins and, consequently, has a high intrinsic solubility. Nowadays, the main market outlet for sunflower protein relies on animal feed. This chapter discusses sunflower proteins, principally seed proteins, including composition, structure, stability, and biochemical and molecular characterization.

1.2 SESAME SEEDS

Sesame (*Sesamum indicum* L.) is one of the world's most important crops. It has been considered as a healthy food providing good source of energy. Sesame seeds contains 35-57% oil, 20-25% protein, 20-25% carbohydrate and 5-6% ash. Seeds are rich source of beneficial bioactive compounds and endogenous antioxidants mainly phenolic lignans, tocopherols and phytosterols. Regular consumption of sesame seeds helps to low and blood pressure and protects the liver from oxidative damage due to the presence of sesamin and sesamolin. Actually. The major uses of sesame in human food are: oil production, snack manufacture and garnish of bakery products. Raw sesame seeds increased titratable acidity,

protein and fat content, amino acids especially some essential amino acids and omega fatty acids. Bacterial counts of sesame enriched yogurt increased and the best sensory scores was obtained with 2% sesame supplementation. Due to the huge popularity of yogurt among consumers, producers and manufacturers continue to develop new yogurt assortments with value-added ingredients. Nutraceutical functions and positive effects of sesame seeds were previously reported. It is important to combine the nutritional value of sesame and the health benefits of probiotic culture in yogurt. Our study evaluated the effects of ground sesame and on

stirred yogurt quality parameters (pH, titratable acidity and syneresis) and, the acceptability of sesame yogurt by consumers.

II. MATERIALS AND METHODS

This chapter deals with the materials and methods used for preparation of sunflower and sesame seed powder and various process involved in manufacturing of the yogurt.

2.1 MATERIALS

Sunflower seeds, white sesame seeds, were purchased from local market of erode. *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* culture in form of freeze dried was obtained from Milky mist dairy industry and UHT milk of (0.5, 3, and 4.5) % fat was purchased locally from local sources.

2.2 PREPARATION OF YOGURT

Stirred yogurt manufacturing process was applied for the production of yogurt. UHT milk of (0.5,3,4.5) % fat was taken as per the trials, since usage of UHT milk there is no process of pasteurization, milk is heated to 45ºC using induction heating for 1 minute, and yogurt culture was added in ratio of 0.1g for 100ml of milk, then sample is incubated at 45ºC for a period of 2 hrs. (Approx. the pH reaches above 4), sample is cooled at 4ºC for 2-3 hrs. The ground sunflower seeds and sesame seeds were added in different concentrations of 1%,1.5%,2% and 1%,2%,3% and in different combinations were added and stirred gently

2.3 SUNFLOWER SEED POWDER PREPARATION

Hulled sunflower seeds were selected, it is then washed 2 to 3 times to remove dust and other foreign particles, then dried using for about for 4 hours at 60ºC.

Hulled sunflower seeds

↓

Washing the seeds using clean water for 2 – 3 times

↓

Spread in a tray

↓

Drying using tray drier at 60º C for 4 hrs

↓

Sunflower seeds

↓

Ground the seeds using mixer

↓

Sieved using sieve shaker

↓

Sunflower seed powder (355 microns)

Fig 2.1 sunflower seed preparation

2.4 SESAME SEED POWDER PREPARATION

Sesame seeds were washed using clean water for 2-3 times to remove dust and other foreign particles and then spread in a tray so that there will be even drying, for about 4 hours at 60 ◦ Celsius in tray dryer.

White sesame seeds

↓

Washing the seeds using clean water for 2 – 3 times

↓

Spread in a tray

↓

Drying using tray drier at 60 ◦ Celsius for 4 hrs

↓

Sesame seeds

↓

Ground the seeds using mixer

↓

Sieved using sieve shaker

↓

Sesame seed powder (850 microns)

Fig 2.2 sesame seed powder preparation

2.5 ANALYSIS

2.5.1 pH

The pH was measured using a digital pH meter. The instrument was calibrated using standard calibration solutions of pH 7.

2.5.2 Titratable Acidity (As Lactic Acid)

The acidity was determined by titrating 5 ml squash sample and 5 ml of water, against 0.1 N sodium hydroxide using phenolphthalein as an indicator. The pink colour was marked the end point of the titration.

The percentage titratable Acidity was calculated using the formula.

$$\text{Titratable acidity} = \frac{V*N*0.09}{\text{Weight of sample}} *100$$

Where: V= volume of sodium hydroxide used to titrate (ml)

N= normality of the sodium hydroxide solution (N)

W= weight of sample (g)

2.5.3 Moisture content

Moisture of the sample was determined by using hot air oven. Empty Petri plates were weighed. About 5 g of the sample was transferred to the Petri plates. The Petri plates were placed in the oven at 100˚C and weight was taken until concordant value was obtained.

2.5.4 Total solid content

Total solid was determined using a directly forced air oven drying method (Younus et al., 2002). The value was obtained using the formula:

% Total solids = 100 – moisture (%)

2.5.5 Total Ash Content

The ash content was determined by using a muffle furnace. The ashing porcelain dishes were placed into a muffle furnace for 30 min at 550˚C. The dishes were removed and cooled in a desiccator for about 30 minutes to room temperature each dish was weighed to the nearest g. About 2.5 g of oat flour sample was added into each dish. The dishes were placed on a hot plate and the temperature was slowly increased until smoking ceases and the samples become thoroughly charred. The dishes were placed inside the muffle furnace at 550˚C for 6 hr. and removed from the muffle furnace and then placed in desiccators for 1 hr. to cool

(Obi et al., 2010). Finally, the weight of total ash was calculated by difference and expressed as a percentage using the formula

$$\text{Total ash}(\%) = \frac{W2-W}{W1-W} *100$$

Where: W=weight of grams of an empty dish, g

W1= weight in grams of the dish plus the dried test material, g

W2= weight in grams of the dish plus ash, g

2.5.6 Protein

Biuret method was used to determine the protein content of squash samples. Standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard, '0'serves as blank and 0.2 ml of sample was taken in test tubes. Volume of all test tube was made up to 1ml using water. 4ml of biuret reagent was added and mixed thoroughly. The test tube was kept at room temperature for 30 minutes. Absorbance value of the solution was read at 550nm against the blank in spectrophotometer. Standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis was drawn. From the graph the amount of protein present in the sample (%) was calculated.

2.5.7 Syneresis

To measure the syneresis of yogurt sample, A25-g aliquot of each sample was placed on a funnel that was covered with Whatman filter paper no.1 and filtered during storage at 4 ◦ C. The syneresis was expressed as the percent weight of the whey. Separated from the initial sample and calculated using the following formula:

$$\text{Syneresis (\%)} = \frac{\text{separated whey(g)}}{\text{Initial sample weight}} *100$$

2.5.8 Fat

A 5 g sample of yoghurt was weighed with 0.01 g accuracy into a Cowbell Milky butyrometer (O - 6%). 16 ml of sulphuric acid (H_2S0_4) and 1 ml of isoamyl alcohol were added. The butyrometer was capped tightly and turned up and down repeatedly, to have a homogeneous mixture and to have the proteins dissolved entirely. The butyrometer then was immersed in a 65 ± 2^0 water bath for 5min. Following heating, the butyrometer was centrifuged for 5 min at1000 rpm. After removing from the centrifuge, the lower level of the fat column in the butyrometer was set at the start point of the scale by means of the cap. The reading was taken by the lower meniscus of the scale.

2.5.9 Carbohydrates

Weigh 100mg of the sample into a boiling tube. Hydrolyse by keeping it in boiling water bath for 3 hours with 5mL of 2.5 N-HCl and cool to room temperature. Neutralise it with solid sodium carbonate until the effervescence ceases. Make up the volume to 100mL and centrifuge. Collect the supernatant and take 0.5 and 1mL aliquots for analysis. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1mL of the working

standard. '0' serves as blank. Make up the volume to 1mL in all the tubes including the sample tubes by adding distilled water. Then add 4mL of anthrone reagent. Heat for eight minutes in a boiling water bath. Cool rapidly and read the green to dark green colour at 630nm.Draw a standard graph by plotting concentration of the standard on the *X*-axis versus absorbance on the *Y*-axis. From the graph calculate the amount of carbohydrate present in the sample tube.

2.5.10 Sensory evaluation

Sensory attributes such as colour, odour, taste, texture, and overall acceptability of the product as fruit bar were evaluated as recommended by Ranganna [9] by Hedonic rating test. A panel consisting of 14 members was selected to evaluate the sample through properly planned experiments. The panellists were selected from the students of Department of Food Technology, Kongu engineering college, perundurai. The requirement for panel membership are (i) good health, (ii) average sensitivity, (iii) high degree of personnel integrity, (iv) intellectual curiosity and interest in sensory evaluation, (v) ability to concentrate and learn, and (vi) availability and willingness to spend time in evaluation and submission to periodic test for acuity and consistency. These tests aim at finding differences in specific quality of characteristics between different stimuli and also direction and/or intensity of the differences. Periodically the panel is given refresher training. Color attribute was judged by visual observation. It also includes size, shape, uniformity, maturity, and absence of defects. Texture is the property of food, which is associated with the sense of feel or touch experienced by finger or the mouth. Texture attribute is best indicated by sensation caused by contact with hard and soft parts of the mouth. Samples were served to the panellists and they were asked to rate the acceptability of the product through sensory methods. Different attributes viz., colour, odour, taste, texture, and overall acceptability were rated on the basis of the 9 points of the hedonic scale ranging from 1 (extremely dislike/ most undesirable) to 9 (extremely like/most desirable). A test proforma was also supplied to the panellists' at the time of evaluation. It is given here, 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely.

III. RESULTS AND DISCUSSION

3.1 EXPERIMENTAL DESIGN

Experimental methods are widely used in research as well as in industrial settings. However, sometimes for very different purpose the primary goal in scientific research is usually, to show the statistical significance of an effect that a particular factor exerts on the dependent variable of interest. Design of experiments techniques provides an efficient means to optimize the process. The mixture simplex lattice design was used to find optimum combination of constituents.

Table 1: Response surface methodology

Run	Sunflower seed powder(g)	Sesame seed powder(g)	Milk fat (%)	pH	Protein (%)	Titratable Acidity (%)	Syneresis (%)	Fat (%)
1	1	2	4.5	4.5	11.37	0.896	35	5.6
2	1.5	3	4.5	4.62	13.2	1.16	37	6
3	1.5	2	3	4.5	12.3	0.95	33	4.4
4	1.5	2	3	4.555	12	0.998	34	4.6
5	2	1	3	4.2	11.94	0.646	33	4.3
6	1.5	2	3	4.555	12.01	1.19	34	4.5
7	1	2	0.5	4.5	11.9	0.858	24	1.7
8	1.5	2	3	4.6	12.3	0.932	33	4.4
9	1.5	1	4.5	4.3	11.61	0.653	36	5.5
10	1	1	3	4.2	10.62	0.608	30	3.6
11	1.5	3	0.5	4.665	13.26	1.253	25	2.7
12	1.5	2	3	4.5	12	0.876	33	4.4
13	2	2	4.5	4.554	12.57	0.865	36	6.1
14	2	2	0.5	4.552	12.51	0.889	35	5.6
15	1.5	1	0.5	4.3	11.43	0.601	37	6
16	2	3	3	4.6	13.2	1.253	33	4.4
17	1	3	3	4.662	12.66	1.195	34	4.6

3.2 ANALYSIS OF PROCESSING CONDITIONS

3.2.1 EFFECT OF SUNFLOWER AND SESAME SEED ON FAT CONTENT

Table 2: ANOVA for Quadratic model: FAT

Source	Sum of squares	df	Mean square	f-value	p-value	
Model	30.56	9	3.40	224.11	< 0.0001	significant
A-sunflower seed	0.0054	1	0.0054	0.3568	0.5691	
B-sesame seed	0.3520	1	0.3520	23.23	0.0019	
C-milk fat	9.53	1	9.53	629.26	< 0.0001	
AB	0.0025	1	0.0025	0.1650	0.6967	
AC	0.0003	1	0.0003	0.0225	0.8850	
BC	0.0606	1	0.0606	4.00	0.0856	
A^2	0.0464	1	0.0464	3.06	0.1235	
B^2	0.0038	1	0.0038	0.2501	0.6323	
C^2	0.0005	1	0.0005	0.0304	0.8666	
Residual	0.1061	7	0.0152			
Lack of fit	0.0741	3	0.0247	3.09	0.1524	not significant
Pure error	0.0320	4	0.0080			
Cor total	30.66	16	3.40			

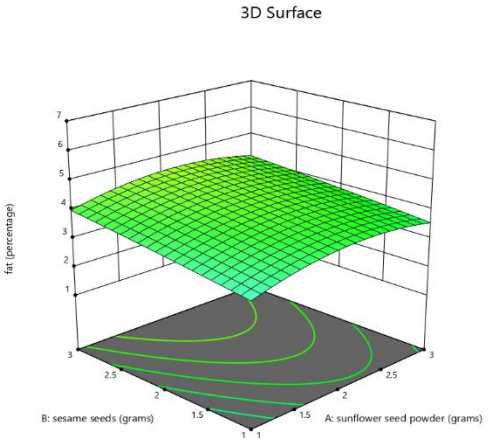

Fig. 3.1: Effect of sunflower and sesame seed on fat content

From the graph we inferred that linear increase in fat content of the yogurt for 1 to 2 grams of sunflower seed of 4 to 4.5 % with respect to milk fat and decreases after 2.25 to 3 grams of sunflower seed from 4.5 to 4 % fat. In case of sesame seed powder sample shows linear increase from 3to 4% of 1 to 3grams of sesame seed powder. Hence both sunflower seed powder and sesame seed powder positively affect the fat content of the final sample. But in the sunflower seed powder fat content starts to decrease when sunflower seed exceeds 2.25 grams.

3.2.2 EFFECT OF SUNFLOWER AND SESAME SEED ON PROTEIN CONTENT

Table 3: ANOVA for Quadratic model: Protein

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	7.36	3	2.45	51.68	< 0.0001	significant
A-sunflower seed powder	1.68	1	1.68	35.49	< 0.0001	
B-sesame seeds	5.64	1	5.64	118.99	< 0.0001	
C-milk fat	0.0271	1	0.0271	0.5722	0.4629	
Residual	0.6167	13	0.0474			
Lack of Fit	0.5111	9	0.0568	2.15	0.2398	Not significant
Pure Error	0.1057	4	0.0264	**F-value**	**p-value**	
Cor Total	7.97	16		51.68	< 0.0001	

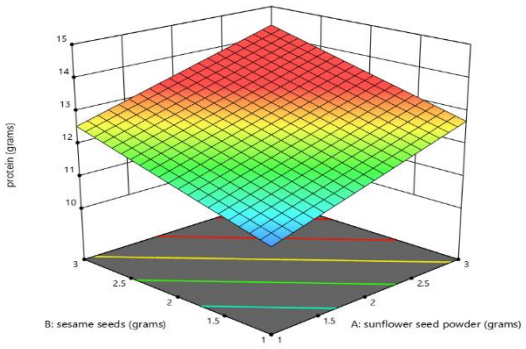

Fig. 3.2: Effect of sunflower and sesame seed on protein content

In case of protein there is a gradual increase from 10.9 to 12 grams of protein at 1 to 2 grams of sunflower seed powder respectively. Similarly in case of sesame seed powder protein content found to be gradually increases from 10.6 to 13.26 grams at 1 to 3 grams of sesame seed powder. Hence the addition 2 grams of sunflower seed powder and 2 grams of sesame seed powder give better results for the protein content.

3.2.3 EFFECT OF SUNFLOWER AND SESAME SEED ON TITRATABLE ACIDITY

Table 4: ANOVA for Quadratic model: Titratable Acidity

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	0.7284	9	0.0809	9.36	0.0038	significant
A-sunflower seed	0.0075	1	0.0075	0.8672	0.3827	
B-sesame seeds	0.2430	1	0.2430	28.11	0.0011	
C-milk fat	0.0007	1	0.0007	0.0756	0.7913	
AB	0.0001	1	0.0001	0.0116	0.9174	
AC	0.0006	1	0.0006	0.0701	0.7988	
BC	0.0048	1	0.0048	0.5545	0.4807	
A^2	0.0113	1	0.0113	1.30	0.2912	
B^2	0.0006	1	0.0006	0.0698	0.7992	
C^2	0.0153	1	0.0153	1.77	0.2248	
Residual	0.0605	7	0.0086			
Lack of Fit	0.0025	3	0.0008	0.0575	0.9795	not significant
Pure Error	0.0580	4	0.0145			
Cor Total	0.7889	16				

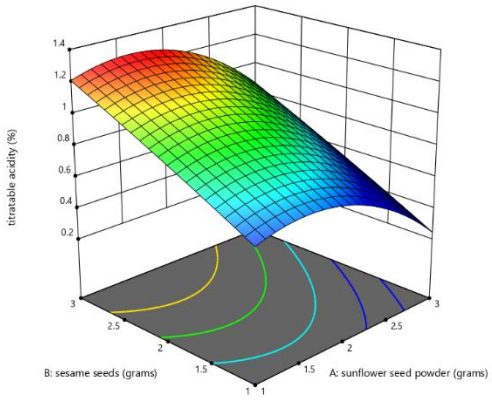

Fig. 3.3: Effect of sunflower and sesame seed on Titratable acidity

It is found that gradual increase in TA from 0.6 to 0.7 % at 1 to 1.5 grams of sunflower seed powder. Then it starts to decrease from 0.7 to 0.6% at 1.5 to 2 grams of sunflower seed powder. While coming to the sesame seed powder there is a gradual increase in TA from 0.6 to 1.253% at 1 to 3 grams of sesame seed powder. Addition of sesame seed powder positively affects the titratable acidity of the sample.

3.2.4 EFFECT OF SUNFLOWER AND SESAME SEED ON SYNERESIS

Table 5: ANOVA for Quadratic model: Syneresis

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	280.21	3	93.40	94.47	< 0.0001	significant
Asunflower seed	15.13	1	15.13	15.30	0.0018	
B-sesame seeds	3.13	1	3.13	3.16	0.0988	
C-milk fat	261.96	1	261.96	264.95	< 0.0001	
Residual	12.85	13	0.9887			
Lack of Fit	11.65	9	1.29	4.32	0.0863	not significant
Pure Error	1.20	4	0.3000			
Cor Total	293.06	16				

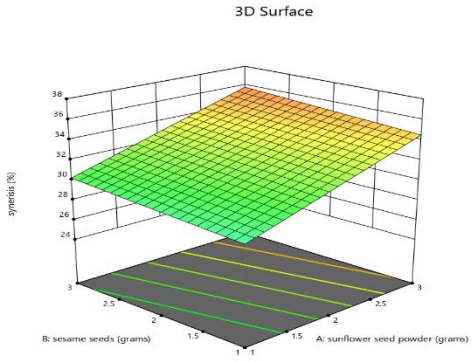

Fig. 3.4: Effect of sunflower and sesame seed on Syneresis

While coming to the syneresis there is a gradual increase from 28 to 37% at 1 to 2 grams of sunflower seed powder. In case of sesame seed powder there is a increase in syneresis value from 28 to 30 %. Both sunflower seed powder and sesame seed powder did not positivley affect the syneresis value**.**

3.2.5 EFFECT OF SUNFLOWER AND SESAME SEED ON pH

Table 6: ANOVA for Quadratic model: pH

Source	**Sum of Squares**	**Sum of Squares**	**Mean Square**	**F-value**	**p-value**	
Model	0.3234	0.3234	0.0359	22.47	0.0002	Significant
A sunflower seed	0.0064	0.0064	0.0064	3.97	0.0864	
B-sesame seeds	0.0871	0.0871	0.0871	54.43	0.0002	
C-milk fat	3.753E-06	3.753E-06	3.753E-06	0.0023	0.9627	
AB	0.0001	0.0001	0.0001	0.0625	0.8097	
AC	0.0000	0.0000	0.0000	0.0152	0.9055	
BC	0.0000	0.0000	0.0000	0.0192	0.8937	
A^2	0.0081	0.0081	0.0081	5.04	0.0597	
B^2	0.0351	0.0351	0.0351	21.92	0.0023	
C^2	0.0014	0.0014	0.0014	0.8665	0.3829	
Residual	0.0112	0.0112	0.0016			
Lack of Fit	0.0042	0.0042	0.0014	0.7991	0.5555	not significant
Pure Error	0.0070	0.0070	0.0017			
Cor Total	0.3346	0.3346				

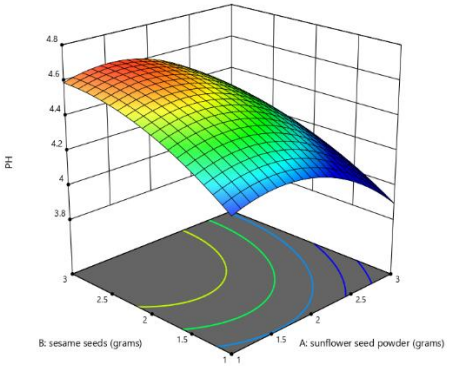

Fig. 3.5: Effect of sunflower and sesame seed on pH

Yogurt pH was significantly affected by sesame supplementation. Yogurt bacteria metabolize carbohydrates for growth and energy producing various organic acids. pH ranges from 4.65 to 4.5 then it is decreased until 4.2. There is no significant difference between the control yogurts.

3.3 SENSORY ANALYSIS

Sensory analysis was conducted using 9-point hedonic scale and the average values are as follows

Table 7: Sensory table

Attributes	Optimized sample	Control sample
Taste	8	7
Texture	7	8
Color	7	7
Flavor	8	6
Overall acceptability	8	7

3.4 ANALYSIS FOR FINAL PRODUCT

Table 8: Final product analysis

Analysis	**Results**
pH	4.55
Fat	4%
Protein	11.4%

Carbohydrates	4.87%
Titratable acidity	0.93%
Total ash	0.8%
Moisture content	74%
Total solids	26%
Syneresis	30%

IV. CONCLUSION

Sunflower seeds and sesame seeds are oil seeds which have been used for oil production over a long period of time. These seeds are good source of protein, amino acids, polyphenols and flavonoids. Thus the yogurt conditions were optimized using response surface methodology to assess the different combination of seed powder, milk fat, titratable acidity, syneresis index and protein. Determination of optimal conditions and predicted values was based on the desirability 0.810. Optimized sample was found to be 1g sunflower seed powder, 2g sesame seed powder, 3%milk fat.

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