

**EVALUATION OF PHYTOSTEROL IN SESAME SEED OIL AND STUDY ITS EFFECTS ON FERMENTED DAIRY PRODUCT****Abilasha R<sup>1</sup>, Geetha P<sup>2\*</sup> and Arivazhagan R<sup>2</sup>**<sup>1</sup>Post Graduate, Department of Food Process Engineering, <sup>3</sup>School of Management, SRM University, Kattankulathur-603203<sup>2</sup>Assistant Professor, Department of Food Process Engineering, <sup>3</sup>School of Management, SRM University, Kattankulathur-603203

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**ABSTRACT:** *Phytosterols are group of steroid alcohols and esters which possess the health benefits to lower total and LDL blood cholesterol by preventing cholesterol absorption from the intestine. Many food companies in developed countries are incorporating phytosterols in wide variety of dairy products. Dairy products being widely consumed in India offer a great scope of utilizing the cholesterol lowering benefits of phytosterols by its enrichment in most commonly consumed dairy products. In general, Sesame (*Sesamum indicum*) is one of the rich sources of phytosterols. The objective of the study was to determine the level of phytosterols present in sesame and to use it as functional food ingredient to reduce LDL cholesterol through fermented dairy product such as shrikhand. This method comprised of extraction, alkaline saponification, and prior to HPLC analysis. The phytosterol were observed at 205nm. Different levels such as 4%, 8%, 12% and 16% of sesame seed oil were incorporated into shrikhand and various physico-chemical analyses such as carbohydrate, fat, protein, ash, acidity and pH. The colour values  $L^*$ ,  $a^*$ ,  $b^*$ , phytochemical analysis and antioxidant activity were studied. Statistical analysis also revealed that the increase of sesame seed oil in shrikhand differ significantly ( $p < 0.05$ ) in all the physico-chemical parameters. Based on the results, it was concluded that 8% of sesame seed oil incorporated shrikhand sample shown high nutritional value, antioxidant property and presence of phytosterol compared with control sample. Hence it is suitable for human consumption and serves as food supplements for hypocholesterolemic effects.*

**KEYWORDS:** Fermented Dairy Product; Low Cholesterol; Phytosterol; Sesame Seed Oil

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**INTRODUCTION**

According to World Health report 2002, cardiovascular diseases (CVDs) will be the largest cause of death by 2020 in India. A high blood cholesterol level is one of the major factors contributing to the increased CVDs in people. Increase in coronary heart diseases throughout India, phytosterols as functional food ingredient will be a new approach to reduce LDL cholesterol through dairy products. Sesame seed (*Sesamum indicum*) is one of the most abundant sources of the phytosterols present in the extraction of lipid fraction. Phytosterols make up the largest proportion of unsaponifiable matter fractions of lipids. Plant fats and oil contain phytosterols as naturally occurring bio active compounds. The most important natural sources of plant sterol in human diets are oils, although they were also found in a range of seeds, legumes vegetables [4]. In plants, more than 200 different type of phytosterol have been reported. The most abundant being  $\beta$ -sistosterol, Campesterol and stigmasterol were found in corn, cotton seed, peanut, sesame seed and linseed oils [8]. Current analytical methods for phytosterol determination were generally time consuming as they include lipid extraction,

saponification, extraction of unsaponifiable matter and derivatization of sterols [12]. It is also one of the phytochemical compounds which also have the activity of the antioxidant and anticancer activity [10]. There are two studies showing that 1–2 g/day of sterols or stanols in yoghurts are effective at lowering LDL cholesterol in patients with moderate primary hypercholesterolemia [12]. Earlier, Researchers have attempted to improve the sensory and nutritive characteristics of shrikhand by adding fruit pulp is the new approach and to study the effect on the quality characteristics of shrikhand [11]. In the past few years the beneficial health effect attributes to phytosterol have prompted interest in quantifying these compounds in different foods. Based on the above information, the present study has been attempted to determine presence of phytosterol in the sesame seed and also to study the effect of sesame seed oil in shrikhand by various analysis such as proximate, microbial and antioxidant activity.

## **MATERIAL AND METHODS**

### **Sample Collection**

The sesame seed sample was purchased from the guduvanchery super market, Chennai, Tamilnadu and 6 ketocholestanol (Internal standard) was collected from sigma-aldrich, India.

### **Sample preparation**

Clean whole sesame seed of 50 g weighed and grounded into fine powder.

### **Lipid Extraction**

The lipid extraction was done by procedure given by El.bagtegi et.al (2007). Briefly the extraction of the lipid was done using soxhlet apparatus. 5g sesame seed was extracted in soxhlet using hexane solvent for 6 h. The solvent in the sample removed using rotary evaporator, then the sample were transferred to test tube then it was dried under reduced pressure 400mmHg using vaccum oven. The sample was stored in refrigerator until saponified.

### **Saponification for phytosterols**

Saponification was done by procedure given by [17]. Briefly the extract was taken and added with 500µl of 50% KOH(w/v) and 4 ml of 1% ethanolic pyrogallol (w/v) in the test tube and the sample was sprinkled with 2.5ml of internal standard (50µg 6-Ketocholestanol dissolved with 2.5 ml of ethanol). The tubes were kept for 30 min at 70°C in a water bath. The tubes were cooled on ice and 2ml water along with 5 ml hexane was added. The tube was shaken vigorously and then tubes were kept for centrifuge for 10 min at 2000 rpm. The hexane layer was removed and it contains the unsaponifiable matter, the layer was dried under reduced pressure 400mmHg in vaccum oven. The extract sample was stored at 0°C for further analysis.

### **Analysis of phytosterol using HPLC**

The sample of 0.1mg was dissolved with 1.5ml of hexane for the HPLC analysis. The sample was filtered before injecting to the HPLC. For phytosterol analysis, the 5.00µl of the sample was injected to the Luma column C-18 with mobile phase acetonitrile 80% and methanol 20% at a flow rate of 0.5ml/min where the column was maintained at 30°C. The chromatogram were detected using photodiode detector at 205nm.

### **Preparation of shrikhand**

The preparation of the shrikhand was done by using the standard method [5]. The standardized milk with 4.5% fat and 8.5% Solid nonfat was purchased from the super market in Chennai. The milk was heated at 90°C and then cooled to 30 °C then 0.5% of previous day dahi culture was added and incubates for 10-12 h followed by draining the whey by transferring the sample in to muslin cloth and hanged for 8h. The semisolid mass left after drainage called chakka, base for the preparation of shrikhand. The 40% of sugar was added to the chakka then the mixture was stirred properly to get smooth paste consistency. Shrikhand was prepared by incorporation of different level of sesame seed oil such as 4, 8, 12, and 16 % and various analyses were carried out for identity suitable consumption and presence of phytosterol. The products were aerobically packed in the polystyrene cups and stored in the refrigerated condition for further studies.

### **Sensory analysis**

The sensory analysis of the developed product was done by using 9 point hedonic scale method [17]. On the basis of sensory parameters such as color and appearance, flavor, body and texture, overall acceptability the level of 8% shrikhand was selected as the optimum level.

### **Proximate composition and color analysis**

The moisture, protein, fat, acidity and ash content were determined by standardized procedure [2]. The pH was determined by using digital pH meter. The energy was determined by calculation method [9]. The color analysis of the sample was determined by hunterlab colorflex calorimeter.

### **Microbial analysis**

The microbial analysis such as total plate, yeast and mold count was performed by the IS 5402: 2002 and IS 5403: 1999 [12] respectively.

### **Antioxidant activity**

The antioxidant activity of the product was analyzed by DPPH assay method [10].

### **Statistical Analysis**

The statistical significance of data was analysed by one-way and two-way ANOVA without replication by SPSS software Windows version 20.0. Randomized block design as used as experimental design in this study. Critical difference required for determination for statistical significance between treatments was determined as suggested by [17]. Results were presented in average of five samples with standard error (SE) and statistical significance was set at  $p < 0.05$ .

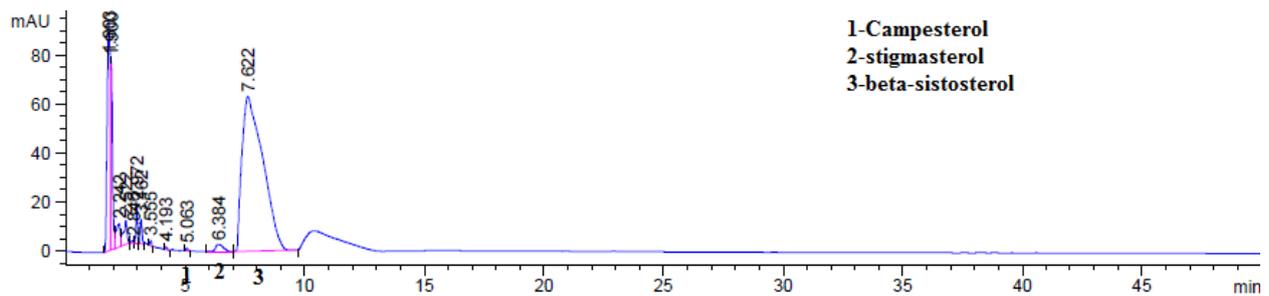
## **RESULT**

### **Analysis for phytosterol**

In decade, few functional food ingredients had been created more interest in the phytosterol, Whereas most clinical studies have involved relatively high doses of phytosterols (2–7 g/day)

using enriched foods.[1 &13] has suggested that much lower levels of phytosterols, such as those that occur naturally in diets rich in plant foods, maybe effective in reducing cholesterol absorption.

In the present study, the phytosterol in the sesame seed was analysed using HPLC. The phytosterols were measured via alkaline hydrolysis. The phytosterol were observed at 205nm. The HPLC result of the phytosterol analysis was shown in fig 1. From the graph, it has been clearly revealed that a large peak was established at the retention time of 7.62 min and it was sitosterol. The two small peaks were established at the retention of 5.063 and 6.382 min was determined as campesterol and stigmasterol.



**Fig 1: HPLC analysis of phytosterol at 205nm.**

### Physico - chemical analysis

The sesame seed oil plays a dominant role in the nutrition profile of the shrikhand. The mean value of the proximate parameters of shrikhand containing different level of sesame seed oil was shown in the table 1. Compare to control sample, the incorporated shrikhand shows a higher fat level ranges from 5.23 – 9 g/100g that attributes due to the sesame seed oil contains fat and the presence of phytosterol was in the form of lipid in sesame seed oil. Effect of fat concentration on shrikhand differ significantly ( $p < 0.05$ ), thus it can be recommended as a good source for fat. Thus increase the smoothness of the shrikhand. The mean value of moisture content ranges from 49.4-54% (d.b). The higher in moisture content in the shrikhand may attribute due to less removal of whey while preparation of chakka and also the effect of concentration of sesame seed oil on shrikhand differ significantly ( $p < 0.05$ ) on the level of moisture content. The pH of the shrikhand sample was ranges from 5-6. From the table, it shows that it is low acid food. The pH and acidity plays an important role in the growth of microorganism and shelf life of the product. The ash content of the shrikhand ranges from 0.68-0.8% due to the incorporation of sesame seed oil. The higher ash content may be attributed due to inorganic matter present in the sesame seed oil. The mean values of total sugars was ranges from 20-25 thus have significant effect ( $p < 0.05$ ) has the level of incorporation increases. The values obtained were compared with the PFA standard value given for shrikhand. . The mean values for the color analyses of sesame seed oil shrikhand were shown in the table 3. Sesame seed oil incorporated shrikhand showed a higher  $a^*$  and decline in  $L^*$ ,  $b^*$  values. From the values given in table the shrikhand shows a creamish yellow color.

**Table 1: proximate composition for shrikhand containing different level of sesame seed oil (Mean±SE)\***

Parameters	Level of sesame seed oil incorporated (%)					
	Control	4	8	12	16	PFA Standard
Moisture (%)	49.4±0.25 <sup>a</sup>	52.6±0.45 <sup>b</sup>	53.8±0.2 <sup>c</sup>	55.9±0.89 <sup>d</sup>	56.2±0.01 <sup>d</sup>	42
Ash (%)	0.68±0.56 <sup>a</sup>	0.72±0.10 <sup>b</sup>	0.78±0.6 <sup>c</sup>	0.8±0.56 <sup>d</sup>	0.8±0.10 <sup>d</sup>	0.9
Protein ((g/100g)	5.78±0.14 <sup>a</sup>	5.80±0.34 <sup>a</sup>	5.93±0.7 <sup>b</sup>	6.0±0.21 <sup>b</sup>	6.12±0.39 <sup>c</sup>	10.5
Acidity	0.9±0.14 <sup>a</sup>	0.9±0.12 <sup>a</sup>	0.9±0.24 <sup>b</sup>	0.9±0.32 <sup>c</sup>	0.9±0.44 <sup>d</sup>	1.4
pH	5.2±0.03 <sup>a</sup>	5.2±0.23 <sup>a</sup>	5.2±0.33 <sup>a</sup>	5.2±0.67 <sup>b</sup>	5.2±0.04 <sup>b</sup>	6
Fat (g/100g)	5.23±0.90 <sup>a</sup>	6.89±0.04 <sup>a</sup>	7.92±0.58 <sup>a</sup>	8.54±0.64 <sup>b</sup>	9.67±0.33 <sup>c</sup>	5
Total Sugars	19.24±0.1 <sup>a</sup>	20.39±0.4 <sup>a</sup>	22.26±0.09 <sup>b</sup>	23.0±0.90 <sup>b</sup>	25.90±0.89 <sup>c</sup>	58
TS (%)	48.34±0.02 <sup>a</sup>	50.12±0.89 <sup>a</sup>	51.23±0.42 <sup>a</sup>	52.3±0.08 <sup>a</sup>	53.45±0.04 <sup>b</sup>	

\*(Mean±SE) with different superscripts differs significantly ( $p < 0.05$ ),  $n = 5$  for each treatment

**Table 2: color analysis for shrikhand containing different level of sesame seed oil (Mean±SE)\***

Color Parameters	Level of sesame seed oil incorporated (%)					
	Control	4	8	12	16	
L*	83.04±0.03 <sup>c</sup>	82.30±0.02 <sup>b</sup>	81.75±0.06 <sup>b</sup>	81.31±0.20 <sup>b</sup>	80.89±0.12 <sup>a</sup>	
a*	-0.64±0.89 <sup>a</sup>	-0.71±0.23 <sup>a</sup>	-1.09±0.43 <sup>b</sup>	-1.16±0.34 <sup>c</sup>	-1.34±0.03 <sup>c</sup>	
b*	13.55±0.54 <sup>c</sup>	13.43±0.44 <sup>b</sup>	13.36±0.34 <sup>b</sup>	13.20±0.22 <sup>a</sup>	13.10±0.09 <sup>a</sup>	

\*(Mean±SE) with different superscripts differs significantly ( $p < 0.05$ ).  $n = 5$  for each treatment

### Sensory Analysis

The mean value for the sensory values was shown in table 2. The color and appearance scores showed a decreasing trend with increase in sesame seed oil level of incorporated up to 16% where the shrikhand with sesame seed shows a creamish yellow color, soft and smooth in appearance. The effect of color and appearance differ significantly ( $p < 0.05$ ) as the level of sesame seed oil increases. Flavor and sweetness of Shrikhand showed a similar pattern and it shown that the shrikhand incorporated shows a bitter flavor and low sweet taste. The body and texture scores was also shows a significantly ( $p < 0.05$ ) decreased trend as the level increases. The incorporated shrikhand shows a grainy as well as pasty texture than the control shrikhand. The overall acceptability was significantly higher ( $p < 0.05$ ) in shrikhand incorporated with 8% sesame seed oil compare to other sample. From the result, it was concluded that 8% sesame seed oil incorporated sample shows high acceptance score compared to all other incorporated samples.

**Table 3: Sensory attributes for shrikhand containing different level of sesame seed oil**

Sensory attributes	Level of sesame seed oil incorporated (%)				
	Control	4	8	12	16
Appearance	8.0±0.15 <sup>c</sup>	8.2±0.1 <sup>c</sup>	8.2±0.5 <sup>b</sup>	7.5±0.67 <sup>b</sup>	7.4±0.45 <sup>a</sup>
Flavor	8.3±0.49 <sup>d</sup>	8.3±0.5 <sup>d</sup>	8.2±0.2 <sup>c</sup>	7.1±0.07 <sup>b</sup>	6.5±0.05 <sup>a</sup>
Taste	8.6±0.21 <sup>c</sup>	8.4±0.03 <sup>c</sup>	8.5±0.1 <sup>c</sup>	7.6±0.04 <sup>b</sup>	6.9±0.78 <sup>a</sup>
Body and Texture	8.5±0.49 <sup>c</sup>	8.1±0.19 <sup>b</sup>	8.0±0.4 <sup>b</sup>	6.5±0.4 <sup>a</sup>	6.5±0.09 <sup>a</sup>
Overall acceptability	8.6±0.41 <sup>c</sup>	8.4±0.3 <sup>c</sup>	8.6±0.6 <sup>c</sup>	7.6±0.67 <sup>b</sup>	6.9±0.60 <sup>a</sup>

\*(Mean±SE) with different superscripts differs significantly ( $p<0.05$ ).  $n=5$  for each treatment

### Microbial Analysis

The safe use of the shrikhand can be recommended on the basis of their microorganism content. TPC, YMC was selected as a representative and samples were tested for their presence. The growth of the microorganism in the sample was tabulated in the table 4. The colonies formed was ranges between 1.7-1.14 log cfu/g. A significantly ( $p<0.05$ ) decrease trend was observed in the determination of total plate count as the level of sesame seed oil increases. The yeast and mold count was not observed.

**Table 4: Microbial analyses for shrikhand containing different level of sesame seed oil (Mean±SE)\***

Microbial analysis	Level of sesame seed oil incorporated (%)				
	Control	4	8	12	16
Total plate count(log cfu/g)	1.70±0.08 <sup>c</sup>	1.56±0.02 <sup>c</sup>	1.34±0.01 <sup>b</sup>	1.23±0.02 <sup>a</sup>	1.14±0.07 <sup>a</sup>
Yeast and mold	Nil	Nil	Nil	Nil	Nil

\*(Mean±SE) with different superscripts differs significantly ( $p<0.05$ ).  $n=5$  for each treatment

### Antioxidant Assay

The mean value for the antioxidant activity was shown in the table 5. The percentage of inhibition increase as the level of incorporation of sesame seed oil increases thus show that the Shrikhand contains antioxidant activity. The  $Ic_{50}$  value of the sesame seed incorporated sample was in the range of 0.614 mg/ml where the  $Ic_{50}$  of the control sample was 3.703 mg/ml. The differ in the  $Ic_{50}$  value shows that the sesame seed oil contain antioxidant property that attributes due to the presence of the phytosterol in the sesame seed oil. The effect of antioxidant activity on all the shrikhand differ significantly ( $p<0.05$ ).

**Table 5: Effect of sesame seed on antioxidant activity of shrikhand (Mean±SE)\***

	Level of sesame seed oil incorporated (%)				
	Control	4	8	12	16
$Ic_{50}$ (mg/ml)	3.703±0.07 <sup>d</sup>	2.377±0.02 <sup>c</sup>	0.641±0.23 <sup>c</sup>	0.641±0.12 <sup>b</sup>	0.623±0.11 <sup>a</sup>

\*(Mean±SE) with different superscripts differs significantly ( $p<0.05$ ).  $n=5$  for each treatment

## DISCUSSION

Phytosterols are group of steroid alcohols and esters which possess the health benefits to lower total and LDL blood cholesterol by preventing cholesterol absorption from the intestine. Many food companies in developed countries are incorporating phytosterols in wide variety of dairy products. India is yet to develop phytosterol enriched dairy products. Dairy products being widely consumed in India offer a great scope of utilizing the cholesterol lowering benefits of phytosterols by its enrichment in most commonly consumed dairy products. In this study the sesame oil incorporated shrikhand which was enriched with phytosterol was prepared.

The mean values of physico-chemical parameters such as fat, Carbohydrate, Ash and Moisture shows a significantly increase ( $p < 0.05$ ) trend with level of sesame seed oil increases. The probable reason may be due to the higher protein, Fat, Carbohydrate, Ash and total sugars of the sesame seed oil than the chakka. Increase in fat content increase the smoothness of the shrikhand. It was found that increase in fat improves the smoothness and palatability of the shrikhand [6]. Comparability the same result was also found when the shrikhand was prepared with papaya pulp [11]. On the other hand the acidity and pH was remain same thus doesn't show many difference compare to the control.

A significantly ( $p < 0.05$ ) decrease trend was observed in the determination of total plate count as the level of sesame seed oil increases. The Antioxidant activity of the developed product increase compare to the control sample thus because of the phytosterol present in the sesame seed oil. The same has been observed the phytosterol and sesamol in the sesame seed as the antioxidant activity [10]. Thus shows that the product contains health benefit.

Furthermore, the significantly decreasing trend in the sensory parameters has been observed increase in the of sesame seed oil level. The same result was observed in development of shrikhand with chiku pulp and orange pulp combination by [15]. The same result was observed in developing the shrikhand with apple pulp [16].

## CONCLUSION

The present study confirmed that sesame seed oil can be used as the food ingredient without adversely affecting the quality of the product. The product prepared with 8% of the sesame seed oil was selected as optimum on the basis of sensory parameters. The developed product contains high antioxidant property and it also contain phytosterol which as the hypocholestrimic activity than the control. The nutritional profile of the product has increased and is also poses the health beneficial effect. The product can be stored for three weeks in refrigerated temperature ( $4 \pm 1^\circ\text{C}$ ).

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