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Preparation of functional Shrikhand with pomegranate fruit peel extracts

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Abstract

A study was undertaken to develop functional shrikhand incorporating aqueous and ethanol extracts of dried and fresh pomegranate fruit peels at various levels. An optimum level of 20 per cent aqueous and 15 per cent ethanol extracts were finalized in comparison with control based on the sensory evaluation. Physico-chemical characteristics were highly significant ($P \leq 0.01$) between control and treatments. Calorific value of functional shrikhand was almost similar to the control. In comparison, dried peel extracts had higher TPC and antioxidant activity than the fresh peel extract. The ethanol extracts of pomegranate peel incorporated functional shrikhand had higher antioxidant activity than the aqueous extracts. The antimicrobial activity of the added fruit peel extracts reduced the total bacterial and yeast and mould counts than that of control. Based on shelf-life studies, cost of production and added health benefits, it was concluded that, 20 per cent aqueous extracts of pomegranate fruit peel incorporated shrikhand can be introduced as a functional dairy product.

Keywords: Functional shrikhand, pomegranate fruit peel extracts, analysis

Introduction

Functional foods are becoming an important trend in food industry as they are known to impart positive effects on human health. Of that, dairy sector is of principal importance to Indian economy, as dairy products are likely to remain important dietary components because of their nutritional value. About 50% of the total milk produced in India is converted into a variety of traditional dairy products such as ghee, makkhan (33%) dahi/yoghurt/shrikhand (7%), khoa (7%) and chhana/paneer (3%) using processes such as coagulation, desiccation and fermentation (Aneja *et al.*, 2002, Bhardwaj, 2013 and Vijay Ranjan *et al.*, 2018) [2, 5, 39].

The use of fresh and sweet buffalo milk is recommended for the preparation of shrikhand (De, 1980) due to higher yield with enriched minerals compared to cow milk shrikhand (Chopade *et al.*, 2011). The term shrikhand derived from the Sanskrit word "Shikharini" meaning 'Nature' (Swapna and Chavannavar, 2013) is a semi-soft, sweetish sour, whole milk product prepared from lactic acid fermented curd. The curd is partially strained to remove the whey, which produces a solid mass called chakka, the basic ingredient for shrikhand.

Fruits and vegetable processing industries in India generate substantial quantity of wastes which are becoming a significant nutritional, economical, and environmental problem. On the other hand, these fruit peels and pomace are the cheapest raw materials and are a rich source of minerals, polyphenols, antioxidants and dietary fibres. Pomegranate (*Punica granatum L.*) is a tropical fruit that is loaded with essential nutrients and bioactive compounds that is being used in herbal medicine for years. This fruit peel extracts possess noteworthy antioxidant compounds such as tannins and flavonoids, which are responsible for exceptional wound healing, antibacterial and antiviral qualities (Sorrenti *et al.*, 2019).

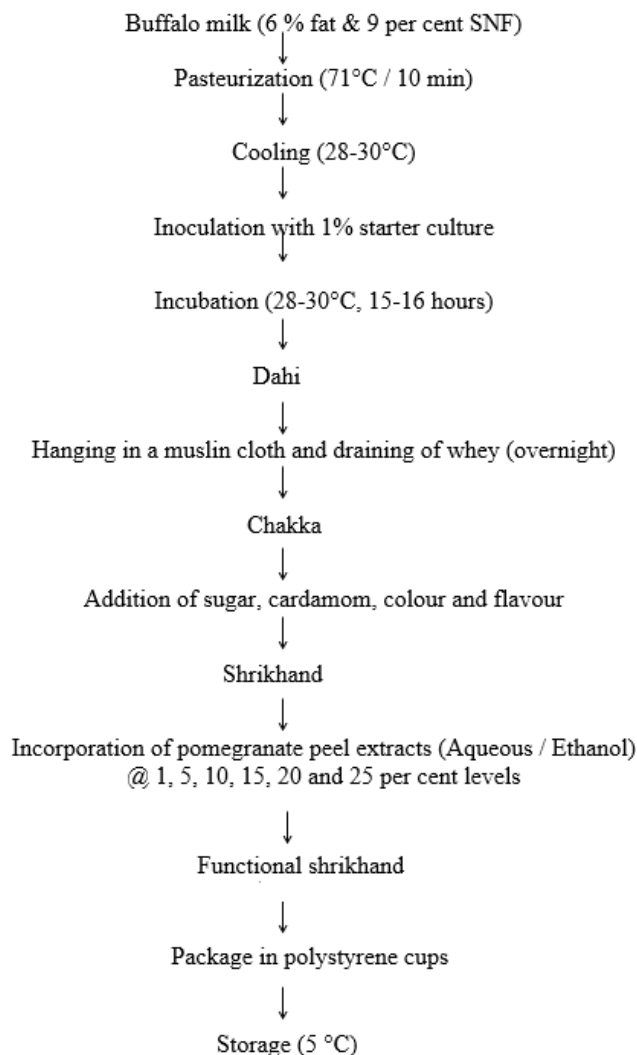
Since, fruit peel extract incorporated dairy product like shrikhand is a new effort and there are very minimal studies, this study was proposed to develop a functional shrikhand incorporating pomegranate peel extract.

Materials and Methods

Fresh buffalo milk (6% fat and 9% SNF) procured from the dairy farm maintained at Community Cattle Care Centre at College of Food and Dairy Technology, TANUVAS, Koduveli, Chennai were used for this study. Pomegranate fruit peel extract was prepared as per the procedure outlined by Singh and Immanuel, 2014 [33] and stored at 4 °C.

Preparation of functional shrikhand

Shrikhand was prepared as per the protocol outlined by De, 1980 [13]. The standard freeze dried dahi cultures (NCDC 159 and NCDC 160) obtained from the National Collection of Dairy



Sensorial analysis

Sensory evaluation of control and functional shrikhand incorporated with pomegranate peel extracts was carried out using 9- point hedonic scale (Amerine *et al.*, 1965) [1]. All the samples were appropriately coded before subjecting for sensory evaluation. Based on the sensory evaluation, optimum levels of the aqueous and ethanol extracts of the pomegranate fruit peels to be incorporated in functional shrikhand were identified. The sensory analysis, physicochemical properties, chemical composition and shelf-life studies were carried out for the functional shrikhand consisting of optimum levels of fruit peel extracts (20% aqueous or 15% ethanol) during the different storage periods *viz.* 0, 5, 10, 15 and 20 days at 5 °C.

Physicochemical properties

The functional shrikhand were examined for physicochemical parameters *viz.* pH, titratable acidity and chemical composition as per the standard procedures mentioned in IS: 95321-1980 [20], and the AOAC, 18th Edition, 2006. The calorie value of the developed product also calculated.

Determination of phenolic content and antioxidant activity

The total phenolic content of functional shrikhand was determined according to Follin - Ciocalteu method adopted by Singleton and Rossi, 1965 [34]. The Gallic acid standards that have been prepared were measured by using spectrophotometer at 765 nm wavelength. Total phenolic content was expressed as gallic acid equivalent (GAE).

The total antioxidant activity of functional shrikhand was evaluated as per the DPPH method adopted by Brand-Williams *et al.* (1995) [9]. The reduction was determined by the decrease in absorbance at 517 nm. The scavenging activity was calculated as % scavenging effect using the following equation:

$$\text{Scavenging Effect (\%)} = \frac{A_0 - (A_1 \text{ sample})}{A_0 \text{ control}} \times 100$$

Where, A_0 is the absorbance of the control at 30minute

A_1 is the absorbance of the sample at 30minute

Microbial analysis

Microbiological analysis *viz.* standard plate count, coliform count and yeast and mould counts were also carried out for the functional shrikhand incorporated with pomegranate peel extracts as per the standard protocol described in BIS: 1981, SP: 18 (Part XI). The statistical analysis was carried out using the Software of Statistical Package for Social Sciences (SPSS 16.0) as per the standard procedure adopted by Snedecor and Cochran (1994) [34].

Results and Discussion

In this present investigation, efforts were made to amalgamate pomegranate fruit peel extracts (both aqueous and ethanol extracts) in shrikhand and analyze their effects on sensorial, physicochemical, microbiological, antioxidant properties and properties, phenolic content of the functional shrikhand.

Sensory analysis of functional shrikhand incorporated with aqueous and ethanol extracts of pomegranate fruit peels

The average sensory scores of the functional shrikhand incorporated with aqueous and ethanol extracts of fresh and dried pomegranate peels at different levels (1, 5, 10, 15, 20 and 25 per cent) were presented in Tables 1 and 2.

From the statistical analysis, it has been concluded that aqueous extracts of fresh and dried pomegranate peels could be incorporated up to 20 per cent level and ethanol extracts of the same could be incorporated up to 15 per cent level in the shrikhand. The results were in close resemblance with the finding of Nigam *et al.* (2009) [26], Narayanan and Lingam (2013) [25] and Bhoyar *et al.* (2014) [6] who also revealed that up to 20 per cent incorporation was acceptable by sensory analysis. Further, El-Said *et al.*, 2014 [14] revealed that increasing the percentage of the added pomegranate peel extracts beyond 20% resulted in decrease in the viscosity of the stirred yoghurt, there by affected the body and texture.

Table 1: Sensory evaluation of functional Shrikhand incorporated with aqueous extracts of pomegranate fruit peels at different levels (Mean \pm SE)

Sensory Attributes	Inclusion level (%)	Types of functional Shrikhand	
		FPAE	DPAE
Colour and Appearance	Control	8.80 \pm 0.00 ^c	8.82 \pm 0.02 ^c
	1	8.69 \pm 0.22 ^c	8.63 \pm 0.11 ^c
	5	8.58 \pm 0.21 ^c	8.55 \pm 0.10 ^c
	10	8.33 \pm 0.14 ^b	8.40 \pm 0.10 ^b
	15	8.23 \pm 0.13 ^b	8.23 \pm 0.11 ^b

	20	8.13±0.13 ^b	8.15±0.11 ^b
	25	6.07±0.07 ^a	6.23±0.08 ^a
F value		66.34 ^{**}	139.82 ^{**}
Flavour	Control	8.80±0.00 ^c	8.60±0.00 ^c
	1	8.52±0.06 ^d	8.36±0.09 ^c
	5	8.40±0.06 ^c	8.24±0.09 ^b
	10	8.28±0.07 ^c	8.11±0.13 ^b
	15	8.15±0.08 ^b	8.05±0.14 ^b
	20	8.05±0.08 ^b	8.03±0.13 ^b
	25	6.17±0.11 ^a	6.28±0.12 ^a
F value		240.90 ^{**}	81.29 ^{**}
Sweetness	Control	8.80±0.00 ^d	8.82±0.02 ^d
	1	8.51±0.09 ^c	8.40±0.10 ^c
	5	8.39±0.09 ^c	8.29±0.10 ^b
	10	8.24±0.08 ^c	8.18±0.10 ^b
	15	8.08±0.06 ^b	8.08±0.10 ^b
	20	7.98±0.07 ^b	8.02±0.16 ^b
	25	6.26±0.17 ^a	6.15±0.10 ^a
F value		125.77 ^{**}	107.57 ^{**}
Body and Texture	Control	8.82±0.02 ^d	8.77±0.02 ^d
	1	8.55±0.06 ^d	8.57±0.09 ^c
	5	8.45±0.06 ^c	8.45±0.08 ^b
	10	8.32±0.06 ^c	8.32±0.08 ^b
	15	8.22±0.07 ^b	8.22±0.06 ^b
	20	8.08±0.08 ^b	8.08±0.07 ^b
	25	6.40±0.14 ^a	6.20±0.13 ^a
F value		164.38 ^{**}	179.95 ^{**}
Overall Acceptability	Control	8.78±0.02 ^c	8.75±0.02 ^c
	1	8.47±0.09 ^c	8.58±0.09 ^c
	5	8.36±0.09 ^b	8.47±0.10 ^c
	10	8.22±0.07 ^b	8.37±0.10 ^b
	15	8.13±0.07 ^b	8.22±0.12 ^b
	20	8.05±0.08 ^b	8.14±0.12 ^b
	25	6.28±0.28 ^a	6.13±0.13 ^a
F value		65.10 ^{**}	115.89 ^{**}

Mean of six observations; ** Highly Significant ($P \leq 0.01$)

Means bearing different superscripts in a same column differ significantly ($p < 0.01$) for each sensory attributes

FPAE and DPAE - Aqueous extracts of fresh and dried pomegranate peels

FPEE and DPEE - Ethanol extracts of fresh and dried pomegranate peels

Table 2: Sensory evaluation of functional shrikhand incorporated with ethanol extracts of pomegranate fruit peels at different levels (Mean# ± SE)

Sensory Attributes	Inclusion level (%)	Types of functional Shrikhand	
		FPEE	DPEE
Colour and Appearance	Control	8.75±0.02 ^c	8.80±0.03 ^c
	1	8.50±0.13 ^b	8.67±0.12 ^b
	5	8.30±0.11 ^b	8.57±0.11 ^b
	10	8.20±0.11 ^b	8.42±0.10 ^b
	15	8.00±0.23 ^b	8.32±0.10 ^b
	20	6.05±0.25 ^a	6.22±0.14 ^a
F value		46.73 ^{**}	105.76 ^{**}
Flavour	Control	8.78±0.02 ^c	8.83±0.03 ^c
	1	8.32±0.08 ^b	8.30±0.06 ^b
	5	8.22±0.08 ^b	8.19±0.06 ^b
	10	8.10±0.08 ^b	8.07±0.06 ^b
	15	7.95±0.07 ^b	7.95±0.06 ^b
	20	6.17±0.17 ^a	6.43±0.25 ^a
F value		122.68 ^{**}	62.76 ^{**}
Sweetness	Control	8.75±0.05 ^c	8.82±0.02 ^c
	1	8.28±0.06 ^b	8.32±0.14 ^b
	5	8.17±0.06 ^b	8.22±0.14 ^b
	10	8.05±0.06 ^b	8.09±0.11 ^b
	15	7.93±0.06 ^b	8.00±0.12 ^b
	20	6.30±0.26 ^a	6.15±0.15 ^a
F value		64.87 ^{**}	75.33 ^{**}
Body and Texture	Control	8.78±0.02 ^c	8.80±0.03 ^c
	1	8.35±0.09 ^b	8.33±0.13 ^b
	5	8.24±0.09 ^b	8.27±0.14 ^b
	10	8.12±0.09 ^b	8.15±0.14 ^b
	15	8.00±0.10 ^b	7.98±0.17 ^b

	20	6.27±0.27 ^a	6.48±0.36 ^a
F value		55.27 ^{**}	23.00 ^{**}
Overall Acceptability	Control	8.82±0.02 ^c	8.82±0.03 ^c
	1	8.34±0.14 ^b	8.28±0.06 ^b
	5	8.24±0.14 ^b	8.17±0.06 ^b
	10	8.10±0.11 ^b	8.05±0.06 ^b
	15	8.02±0.12 ^b	7.93±0.06 ^b
	20	6.22±0.14 ^a	6.35±0.26 ^a
F value		72.78 ^{**}	64.85 ^{**}

Mean of six observations

Means bearing different superscripts in a same column differ significantly ($p < 0.01$) for each sensory attributes^{**} Highly Significant ($P \leq 0.01$)

Sensory analysis of functional shrikhand incorporated with optimum levels of pomegranate fruit peel extracts during storage period

From the Tables 3 and 4, it was statistically revealed that there was a highly significant difference ($P \leq 0.01$) between the different storage periods at 5°C for the control as well as functional shrikhand incorporated with aqueous and ethanol extracts of pomegranate fruit peels at optimum level (20% and 15% respectively). Further, there was a drastic reduction in sensorial score on 20th day and was not accepted by the

sensory panel. Hence, this clearly indicated that the control and functional shrikhand could be stored at 5 °C up to 15 days only.

These results were supported by Nigam *et al.* (2009) [26], Kumar *et al.* (2011) [23], Narayanan and Lingam (2013) [25] and Singh and Immanuel (2014) [33] in respect to sensory scores during storage. Singh and Immanuel (2014) [33] reported that the fruit peels of pomegranate and orange might prove to be a better substitute in place of synthetic antioxidants in extending the shelf life of food products.

Table 3: Sensory evaluation of functional shrikhand with optimum level (20 per cent) of aqueous extracts of pomegranate fruit peels during storage at 5 °C (Mean# ± SE)

Sensory Attributes	Storage period (Days)	Control	Types of functional shrikhand	
			FPAE _{20%}	DPAE _{20%}
Colour and Appearance	0	8.73±0.08 ^b	8.31±0.08 ^b	8.44±0.11 ^b
	5	8.63±0.08 ^b	8.16±0.08 ^b	8.31±0.07 ^b
	10	8.50±0.07 ^b	7.84±0.20 ^b	8.14±0.09 ^b
	15	8.27±0.10 ^b	7.78±0.26 ^b	7.98±0.08 ^b
	20	6.92±0.27 ^a	6.58±0.12 ^a	6.83±0.31 ^a
F value		27.96 ^{**}	20.54 ^{**}	16.39 ^{**}
Flavour	0	8.65±0.04 ^b	8.46±0.10 ^c	8.43±0.10 ^b
	5	8.53±0.05 ^b	8.34±0.10 ^c	8.31±0.10 ^b
	10	8.43±0.05 ^b	8.18±0.08 ^b	8.15±0.08 ^b
	15	8.30±0.07 ^b	7.83±0.17 ^b	7.83±0.17 ^b
	20	6.25±0.31 ^a	6.75±0.17 ^a	6.67±0.42 ^a
F value		46.83 ^{**}	28.75 ^{**}	10.92 ^{**}
Sweetness	0	8.55±0.24 ^b	8.52±0.09 ^c	8.49±0.04 ^c
	5	8.45±0.24 ^b	8.41±0.09 ^b	8.31±0.05 ^b
	10	8.32±0.23 ^b	8.23±0.07 ^b	8.18±0.04 ^b
	15	8.08±0.22 ^b	8.03±0.04 ^b	8.03±0.03 ^b
	20	6.75±0.17 ^a	6.72±0.16 ^a	6.58±0.20 ^a
F value		11.18 ^{**}	54.31 ^{**}	62.94 ^{**}
Body and Texture	0	8.79±0.09 ^c	8.65±0.08 ^b	8.61±0.07 ^b
	5	8.59±0.08 ^c	8.55±0.08 ^b	8.46±0.06 ^b
	10	8.38±0.06 ^b	8.40±0.08 ^b	8.33±0.06 ^b
	15	8.12±0.04 ^b	8.22±0.10 ^b	8.18±0.08 ^b
	20	6.75±0.17 ^a	6.93±0.27 ^a	6.75±0.17 ^a
F value		65.75 ^{**}	24.78 ^{**}	59.14 ^{**}
Overall Acceptability	0	8.67±0.05 ^b	8.64±0.16 ^c	8.54±0.14 ^c
	5	8.57±0.05 ^b	8.46±0.12 ^b	8.39±0.11 ^b
	10	8.42±0.06 ^b	8.24±0.12 ^b	8.19±0.11 ^b
	15	8.23±0.08 ^b	8.03±0.10 ^b	8.00±0.09 ^b
	20	6.73±0.25 ^a	6.65±0.17 ^a	6.75±0.17 ^a
F value		41.63 ^{**}	34.48 ^{**}	31.66 ^{**}

Mean of six observations

Means bearing different superscripts in a same column differ significantly ($p < 0.01$) for each sensory Attributes^{**} Highly Significant ($P \leq 0.01$)

Table 4: Sensory evaluation of functional shrikhand with optimum level (15 per cent) of ethanol extracts of pomegranate fruit peels during storage at 5°C (Mean# ± SE)

Sensory Attributes	Storage period (Days)	Control	Types of functional shrikhand	
			FPPE _{15%}	DPEE _{15%}
Colour and Appearance	0	8.73±0.08 ^b	8.65±0.04 ^b	8.67±0.05 ^b

	5	8.63±0.08 ^b	8.53±0.05 ^b	8.57±0.05 ^b
	10	8.50±0.07 ^b	8.43±0.05 ^b	8.42±0.06 ^b
	15	8.27±0.10 ^b	8.30±0.07 ^b	8.23±0.08 ^b
	20	6.92±0.27 ^a	6.25±0.31 ^a	6.73±0.25 ^a
F value		27.96 ^{**}	46.83 ^{**}	41.63 ^{**}
Flavour	0	8.65±0.04 ^b	8.32±0.07 ^b	8.27±0.06 ^b
	5	8.53±0.05 ^b	8.22±0.07 ^b	8.14±0.07 ^b
	10	8.43±0.05 ^b	8.12±0.07 ^b	8.02±0.07 ^b
	15	8.30±0.07 ^b	7.98±0.06 ^b	7.75±0.16 ^b
	20	6.25±0.31 ^a	6.72±0.24 ^a	6.81±0.25 ^a
F value		46.83 ^{**}	27.60 ^{**}	16.50 ^{**}
Sweetness	0	8.55±0.24 ^b	8.28±0.06 ^b	8.30±0.05 ^b
	5	8.45±0.24 ^b	8.18±0.06 ^b	8.19±0.05 ^b
	10	8.32±0.23 ^b	8.08±0.06 ^b	8.07±0.05 ^b
	15	8.08±0.22 ^b	7.95±0.04 ^b	7.80±0.16 ^b
	20	6.75±0.17 ^a	6.69±0.24 ^a	6.89±0.28 ^a
F value		11.18 ^{**}	29.70 ^{**}	14.54 ^{**}
Body and Texture	0	8.79±0.09 ^c	8.60±0.06 ^b	8.52±0.05 ^b
	5	8.59±0.08 ^c	8.50±0.06 ^b	8.42±0.05 ^b
	10	8.38±0.06 ^b	8.35±0.05 ^b	8.29±0.05 ^b
	15	8.12±0.04 ^b	8.17±0.07 ^b	8.13±0.03 ^b
	20	6.75±0.17 ^a	6.73±0.08 ^a	6.93±0.32 ^a
F value		65.75 ^{**}	39.28 ^{**}	18.62 ^{**}
Overall Acceptability	0	8.67±0.05 ^b	8.59±0.11 ^b	8.28±0.06 ^b
	5	8.57±0.05 ^b	8.40±0.11 ^b	8.24±0.06 ^b
	10	8.42±0.06 ^b	8.28±0.09 ^b	8.12±0.06 ^b
	15	8.23±0.08 ^b	7.88±0.18 ^b	7.83±0.18 ^b
	20	6.73±0.25 ^a	6.78±0.25 ^a	6.69±0.24 ^a
F value		41.63 ^{**}	20.18 ^{**}	22.65 ^{**}

Mean of six observations

Means bearing different superscripts in a same column differ significantly ($p < 0.01$) for each sensory attributes** Highly Significant ($P \leq 0.01$)

Physico-chemical properties and chemical composition of functional shrikhand with optimum levels of pomegranate fruit peel extracts during storage at 5 °C

Table 5 and 6 show the mean \pm SE values of physico-chemical properties and chemical composition of functional Shrikhand with pomegranate fruit peel extracts during storage at 5 °C. Significant ($p < 0.05$) to highly significant ($p < 0.01$) differences were observed in control as well as treatments between storage periods.

As storage periods increased, pH of the products was reduced, whereas, the titratable acidity was increased, but both were in acceptable range up to 15 days of storage at 5°C. The reason for low pH value of functional shrikhand than that of control might possibly be due to the addition of fruit peel extracts in shrikhand. The results were in accordance with the findings of Patel and Chakraborty (1985) [27] and David (2015) [11] who obtained pH values of 4.1 and 4.36 respectively in shrikhand. The studies of Boghra and Mathur (2000) [8], Kulkarni *et al.* (2006) [22] and Prajapati *et al.* (1993) [28] also supported the results and they reported the same range of pH (4.2 to 4.4 and 4.3 to 4.35 respectively) in shrikhand during their analysis. There was a negative correlation between pH and titratable

acidity in shrikhand. The results were in accordance with the findings of Prajapati *et al.* (1993) [28] who reported the same range of titratable acidity (1.24 to 1.26% LA) in shrikhand. The results were also in accordance with the legal standards of IS: 95321-1980 [20].

Chemical composition

Based on the statistical analysis, there is a high significant ($P \leq 0.01$) difference between control and treatments with regard to the moisture. The moisture content of control shrikhand (50.23%) in this study was in accordance with the finding of Mehta Meena (2013) [24], who observed 45 to 52% of moisture in shrikhand collected from Mumbai city. Similarly, the observations in treatments were in close resemblance with the finding of Sonawane *et al.* (2007) [36] who worked on strawberry pulp incorporated shrikhand. There was no significant difference in the crude protein content between control and functional shrikhand, which was in close resemblance with the findings of Sharma and Zariwala (1978) [31] and Boghra and Mathur (2000) [8]. There was no fibre content in control as well as fruit peel extracts added functional shrikhand.

Table 5: Physicochemical properties of functional Shrikhand incorporated with optimum levels of pomegranate peel extracts during storage at 5 °C (Mean \pm SE)

Types of Shrikhand	pH					F value
	Storage periods (Days)					
	0	5	10	15	20	
Control	4.40±0.31 ^b	4.35±0.16 ^b	4.30±0.11 ^b	4.24±0.31 ^b	3.40±0.15 ^a	3.53 [*]
FPAE _{20%}	4.36±0.14 ^b	4.33±0.33 ^b	4.16±0.17 ^b	4.00±0.31 ^b	3.25±0.02 ^a	4.09 [*]
DPAE _{20%}	4.33±0.33 ^b	4.13±0.18 ^b	4.05±0.19 ^b	3.93±0.32 ^b	3.18±0.07 ^a	3.34 [*]
FPEE _{15%}	4.25±0.26 ^b	4.22±0.19 ^b	4.11±0.24 ^b	4.05±0.17 ^b	3.33±0.17 ^a	3.26 [*]
DPEE _{15%}	4.27±0.26 ^b	4.22±0.19 ^b	4.13±0.24 ^b	3.98±0.23 ^b	3.17±0.11 ^a	4.45 ^{**}
Titratable acidity (% LA)						

Types of shrikhand	0	5	10	15	20	F value
Control	1.16±0.17 ^a	1.20±0.14 ^a	1.36±0.17 ^a	1.50±0.08 ^a	1.83±0.02 ^b	4.21 ^{**}
FPAE _{20%}	1.28±0.09 ^a	1.38±0.07 ^a	1.57±0.12 ^a	1.67±0.08 ^b	1.79±0.06 ^c	4.71 ^{**}
DPAE _{20%}	1.31±0.07 ^a	1.33±0.08 ^a	1.52±0.13 ^a	1.64±0.09 ^b	1.69±0.08 ^c	2.94 [*]
FPEE _{15%}	1.28±0.08 ^a	1.29±0.06 ^a	1.43±0.11 ^a	1.46±0.09 ^a	1.76±0.06 ^b	5.60 ^{**}
DPEE _{15%}	1.27±0.08 ^a	1.30±0.05 ^a	1.44±0.11 ^a	1.46±0.09 ^a	1.73±0.07 ^b	4.83 ^{**}

#Mean of six observations

Means bearing different superscripts in a same row differ significantly

** Highly Significant ($P \leq 0.01$)*Significant ($P \leq 0.05$)**Table 6:** Chemical composition of functional shrikhand incorporated with optimum levels of pomegranate fruit peel extracts (Mean# ± SE)

Types of Shrikhand	Chemical composition (%)					
	Moisture	Crude Protein	Crude fibre	Ether extract (fat)	Total ash	Nitrogen free extract
Control	50.23±0.09 ^a	10.97±0.12	Nil	13.02 ±0.01	0.98±0.00	75.03±0.25
FPAE _{20%}	59.64±0.59 ^c	10.95±0.93	Nil	12.98±0.42	0.96±0.19	75.11±0.21
DPAE _{20%}	58.37±0.33 ^c	10.96±0.24	Nil	12.99±0.12	0.97±0.14	75.08±0.07
FPEE _{15%}	52.37±0.62 ^b	10.95±0.17	Nil	12.94±0.31	0.97±0.12	75.14 ±0.17
DPEE _{15%}	52.38±0.74 ^b	10.96±0.23	Nil	12.99±0.09	0.98±0.19	75.07 ±0.25
F value	93.45 ^{**}	0.00 ^{NS}	Nil	0.03 ^{NS}	0.01 ^{NS}	0.05 ^{NS}

#Mean of six observations

Means bearing different superscripts in a same column differ significantly

**Highly Significant ($P \leq 0.01$)

NS – Non significant

The fat content was found to be decreased in functional shrikhand compared to that of control, which might be due to the incorporation of fruit peel extracts in functional shrikhand. With regard to total ash content, there was no significant difference between control and treatments. This result was in close resemblance with the legal standard specified (0.9%) for shrikhand (IS: 95321-1980 and FSSR, 2011) [20]. In case of Nitrogen free extract (NFE), there was no significant difference between the control and functional shrikhand. The NFE content of functional shrikhand increased at minor level on addition of fruit peel extracts.

significant difference ($P > 0.05$) between control and functional shrikhand as the energy value was reduced in fruit peel extracts incorporated functional shrikhand. Among the treatments, 20 per cent DPAE incorporated functional shrikhand had the highest calorific value (461.07±4.15 kcal/100g), whereas, the calorie value was lowest (460.82±3.92 kcal/100g) in 15 per cent FPEE incorporated functional shrikhand. The calorie value of the control shrikhand was higher than the functional shrikhand. The reason might be the difference in the moisture content and other chemical composition of the products prepared.

Calorific value

Considering the calorific value (Table 7), there was no

Table 7: Calorific value of functional Shrikhand incorporated with optimum levels of pomegranate fruit peel extracts (Mean# ± SE)

Types of Shrikhand	Calorific value (kcal / 100 g)
Control	461.18±4.25
FPAE _{20%}	461.06±4.14
DPAE _{20%}	461.07±4.15
FPEE _{15%}	460.82±3.92
DPEE _{15%}	461.03±4.11
F value	0.00 ^{NS}

#Mean of six observations

Means bearing different superscripts in a same column differ significantly

NS – Non significant

Total phenolic content and antioxidant activity

The mean ± SE values of total phenolic content of functional shrikhand were ranged from 7.78±0.36 to 19.74±0.34 mg/g. These results were almost similar to the findings of Ashoush and Gadallah (2011) [4]. The observations were in accordance with the finding of El-Said *et al.*, 2014 [14] who revealed that the TPC of aqueous and methanolic extract of PPE for stirred yoghurt were in the range of 13.98 to 14.81 mg and 14.83 to 15.80 mg Gallic acid/g respectively.

Regarding the antioxidant activity, the ethanol extracts of pomegranate peel incorporated functional shrikhand had higher activity than that of aqueous extracts. The results were almost similar to the findings of Singh *et al.* (2002) and Hegazy and Ibrahim (2012) [19] who reported that the solvent

played a vital role in the extraction of the plant constituents and also reported about the use of pomegranate peel extracts as dietary supplements as they had high potential antioxidant activity.

With respect to the storage study, both the phenolic content and antioxidant activity in functional shrikhand decreased day by day which might be due to loss of these activities on advancement of storage period. These results were almost similar to the findings of Hala *et al.* (2010) [18] who reported that the decrease of total phenolic content and % of RSA might be due to highly unstable compounds that undergo numerous enzymatic and chemical reactions during food storage.

Table 9: Total phenolic content and antioxidant activity of functional Shrikhand with optimum levels of pomegranate fruit peel extracts during storage at 5 °C (Mean# ± SE)

Types of Shrikhand	Total phenolic content (mg/g)					F value
	Storage period in days					
	0	5	10	15	20	
Control	0	0	0	0	0	-
FPAE _{20%}	16.02±0.65 ^b	15.30±1.53 ^b	15.17±0.48 ^b	14.83±0.27 ^b	10.85±0.71 ^a	4.20**
DPAE _{20%}	17.95±1.01 ^b	16.75±1.77 ^b	16.66±1.36 ^b	15.48±0.78 ^b	11.06±0.28 ^a	4.32**
FPEE _{15%}	17.45±0.13 ^c	15.60±0.39 ^b	14.07±0.14 ^b	13.28±0.32 ^b	10.08±0.36 ^a	75.77**
DPEE _{15%}	19.74±0.34 ^c	15.90±0.63 ^b	13.56±0.43 ^b	12.98±0.33 ^b	10.15±0.25 ^a	73.51**
Antioxidant activity (DPPH)%						
Types of Shrikhand	0	5	10	15	20	F value
Control	0	0	0	0	0	-
FPAE _{20%}	52.43±0.05 ^b	51.26±0.08 ^b	49.33±0.37 ^b	48.79±1.38 ^b	33.72±2.72 ^a	6.03**
DPAE _{20%}	58.50±0.76 ^b	56.50±0.81 ^b	53.33±1.87 ^b	51.50±1.95 ^b	36.83±2.20 ^a	7.71**
FPEE _{15%}	54.20±0.35 ^d	52.89±0.05 ^c	52.25±0.11 ^b	51.87±0.18 ^b	40.70±0.32 ^a	30.59**
DPEE _{15%}	64.30±0.19 ^c	63.98±1.92 ^c	63.27±0.70 ^c	62.75±0.69 ^b	48.89±0.31 ^a	4.98**

#Mean of six observations

Means bearing different superscripts in a row differ significantly

**Highly Significant ($P \leq 0.01$)*Significant ($P \leq 0.05$)

While comparing the fresh and dried pomegranate fruit peel extracts, dried peel extracts had higher TPC and antioxidant activity than fresh peel extracts. The results were almost similar to the findings of Samsukhidir (2012) [30] who reported that DPPH method of dried pomegranate peel had higher scavenging effect (70.28%) compared to fresh pomegranate peel (55.77%).

Microbial analysis of functional Shrikhand during storage at 5 °C

From the Table 8, it was known that the total plate count and yeast and mould count were increased both in the control and functional shrikhand, as the storage days increased, but were within the normal range up to 15th day of storage. The antimicrobial activity of the added fruit peel extracts also reduced the total bacterial and yeast and mould counts than

the control. These results were almost similar to the findings of Salunke *et al.* (2005) [29], Khan and Haneef (2011) [21] and Gullon *et al.* (2016) [17], who reported that pomegranate peel extracts (aqueous, methanolic and ethanolic) had strong bactericidal action against *Salmonella sp.*, *E. coli*, *L. monocytogenes*, *S. aureus*, coliform and yeast and mould count. There were no coliforms in control and functional shrikhand.

In addition, Dahham *et al.* (2010) [12] and Fawole *et al.* (2012) [15] reported that methanolic peel extracts showed strong broad-spectrum activity against Gram +ve and Gram-ve bacteria and fungi than the aqueous extracts. This peel extract had shown highest antimicrobial activity compared to other extracts. These were correlated well with the findings of this study.

Table 8: Microbial analysis of functional shrikhand incorporated with optimum level of aqueous and ethanol extracts of fruit peels during storage at 5 °C (Mean# ± SE)

Types of shrikhand	Storage period (Days)	Microbial counts (log ₁₀ cfu/g)		
		Total plate count	Coliforms	Yeast and mould
Control	0	6.26±0.12 ^a	Nil	Nil
	5	6.52±0.19 ^a		2.12±0.08 ^a
	10	6.73±0.13 ^b		2.25±0.08 ^a
	15	6.89±0.21 ^b		2.41±0.03 ^a
	20	7.19±0.13 ^c		2.95±0.05 ^b
F value		3.20*	Nil	8.79**
FPAE _{20%}	0	6.23±0.09 ^a	Nil	Nil
	5	6.48±0.18 ^a		2.14±0.03 ^a
	10	6.66±0.12 ^b		2.28±0.06 ^a
	15	6.81±0.21 ^b		2.38±0.04 ^b
	20	7.09±0.12 ^c		2.91±0.04 ^c
F value		3.58*	Nil	13.06**
DPAE _{20%}	0	6.10±0.16 ^a	Nil	Nil
	5	6.24±0.23 ^a		2.11±0.01 ^a
	10	6.52±0.14 ^a		2.28±0.06 ^a
	15	6.67±0.12 ^a		2.43±0.09 ^b
	20	6.98±0.13 ^b		2.81±0.04 ^c
F value		4.73**	Nil	8.51**
FPEE _{15%}	0	6.10±0.14 ^a	Nil	Nil
	5	6.15±0.19 ^a		2.10±0.04 ^a
	10	6.48±0.11 ^a		2.24±0.08 ^a
	15	6.53±0.14 ^a		2.32±0.08 ^a
	20	7.00±0.10 ^b		2.68±0.04 ^b
F value		4.29**	Nil	5.13**

DPEE _{15%}	0	5.95±0.20 ^a	Nil	Nil
	5	6.15±0.23 ^a		2.07±0.03 ^a
	10	6.35±0.21 ^a		2.13±0.06 ^a
	15	6.48±0.16 ^b		2.32±0.14 ^a
	20	6.79±0.08 ^c		2.75±0.11 ^b
F value		3.37 [*]	Nil	5.95 ^{**}

#Mean of six observations

Means bearing different superscripts in a row differ significantly

**Highly Significant ($P \leq 0.01$)*Significant ($P \leq 0.05$)

Cost of production for functional Shrikhand

Table 9 shows the cost of production per kg of control and aqueous / ethanol extracts of pomegranate fruit peel incorporated functional Shrikhand at optimum level were Rs.181, Rs.185 and Rs.365 respectively.

The cost of production of control shrikhand was lower (Rs.181/kg) when compared to the functional shrikhand. Among the functional shrikhand, 20 per cent aqueous extracts of fruit peels incorporated functional shrikhand had lower cost

of production (Rs.185/kg) than 15 per cent ethanol extracts of fruit peels incorporated functional shrikhand (Rs.365/kg), which was due to higher cost solvent used in the later in this study.

Because of the higher antioxidant activity, total phenolic content, lower cost of production and added functional attributes, aqueous extracts of fruit peels (20 per cent) incorporated functional shrikhand could be introduced as value added functional dairy product in human diet.

Table 9: Cost of production per kg of functional Shrikhand incorporated with optimum levels of pomegranate fruit peel extracts

Quantity of Ingredients	Cost of Ingredients (Rs.)	Types of Shrikhand		
		Control (Rs.)	Aqueous extract incorporated functional Shrikhand (Rs.)	Ethanol extract incorporated functional Shrikhand (Rs.)
Buffalo milk (3.2 lit/kg)	45 / lit.	144	144	144
Starter culture	3.30 / lit.	10	10	10
Sugar (400g/kg)	45 / kg	18	18	18
Cardamom	10g	3	3	3
Fruit peel	0	0	0	0
Solvent	180/120ml (Ethanol)	0	0	180
Processing cost	-	5	9	9
Polystyrene cups	1 / cup	1	1	1
Cost per kg of different types of shrikhand (Rs.)		181	185	365

Conclusion

Shrikhand is an indigenous fermented milk product bearing much benefits over fluid milk such as digestibility and palatability, distinct taste, richness, delicacy, diversity and fairly longer shelf-life. Due to the presence of phytochemicals in pomegranate fruit peel, incorporation of those extracts to food make it functional and beneficial to health by acting as an antioxidant, anti-cancer and anti-inflammatory properties. It is concluded that, the functional shrikhand incorporated with 20 per cent aqueous (FPAE, DPAE) and 15 per cent ethanol (FPEE, DPEE) extracts of fresh and dried pomegranate peels were found to be the best when compared to the control, based on the sensory evaluation. While comparing the fresh and dried fruit peel extracts, dried peel extracts had higher TPC and antioxidant activity. Owing to the higher antioxidant activity, total phenolic content, lower cost of production and added functional attributes, 20 per cent aqueous extracts of fruit peels incorporated shrikhand can be introduced as value added functional dairy product in human diet.

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