

ANALYSIS OF FUNCTIONAL SHRIKHAND INCORPORATED WITH ORANGE FRUIT PEEL EXTRACTS

A. Agalya¹, T.R. Pugazhenth², Rita Narayanan³,
A. Bharathidhasan⁴ and V. Sowmya¹

College of Food and Dairy Technology
Tamil Nadu Veterinary and Animal Sciences University
Koduveli, Chennai, Tamil Nadu, India

ABSTRACT

Shrikhand is a traditional fermented dairy product with added nutritional profile. The present study encompasses on analyzing the functional properties viz. phenolic content, antioxidant activity, microbial quality and calorific value of functional shrikhand incorporated with orange fruit peel extract. The statistical analysis inferred that extracts of orange contained phenolic content in the range of 7.78±0.36 to 16.25±0.94 mg/g during storage. The ethanol extracts incorporated functional shrikhand presented higher antioxidant activity (17.74 to 34.25%) than that of aqueous extracts (14.35 to 33.64%) of orange peel. On further comparison, the dried fruit peel extracts offered more TPC and antioxidant activity (16.25±0.94 to 10.85±0.21 and 34.25±0.05 to 22.80±0.31%) than fresh fruit peel extracts (15.85±0.39 to 7.78±0.36 and 28.58±0.15 to 14.35±1.09) respectively. The orange fruit peel incorporated functional shrikhand can be stored up to 15 days at 5°C without affecting the overall sensory acceptability with enhanced antioxidant activity and total phenolic content and it could be used as dairy supplement in human diet.

Keywords: Functional Shrikhand, fruit peel extracts, total phenolic content, anti-oxidant activity

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*Corresponding author; email: drtrptry@gmail.com

¹ Research Scholar

² Professor, Department of Livestock Products Technology (Dairy Science), Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, India

³ Professor and Head, Department of Food Processing Technology, College of Food and Dairy Technology, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Koduveli, Chennai, India

⁴ Associate Professor, Post Graduate Research Institute in Animal Sciences, TANUVAS, Kattupakkam, Chennai, India

INTRODUCTION

Shrikhand is one of the most popular dahi based indigenous Indian desserts prepared by fermenting milk to develop curd, draining of watery portion to obtain a solid mass *chakka* and addition of sweetener and flavors (De, 1980). Owing to diverse antioxidant molecules like milk caseins and whey proteins (Suetsuna *et al.*, 2000; Pihlanto, 2006) and presence of low molecular thiols in dairy based foods (Niero *et al.*, 2015),

dairy sector has began introducing medicinal plants and extracts to further improve the technological and therapeutic properties of milk products. Considering these, dairy industry has made a strategy in incorporating food-processing byproducts such as fruits and vegetable wastes as an economical and sustainable source of raw material in milk products (Iriundo-DeHond *et al.*, 2018).

Antioxidants that are induced in foods might be from natural or synthetic source. While later exhibiting toxic effects when consumed, frequent intake of natural antioxidants could lower the prevalence of cancer, hypertension, diabetes, and cardiovascular diseases (Marles and Farnsworth, 1994). There were several studies that reported natural antioxidants and phenolics in fruits and vegetables. Of those, a study made by Alenisan *et al.* (2017) concluded that plant-based antioxidants retarded the oxidation process and prolonged the shelf life of dairy products. Therefore, the present study utilized orange fruit peel to develop functional shrikhand.

Orange (*Citrus sinensis*) contains high natural antioxidants with chief flavonoids such as limonene, hesperidin, narirutin, naringin and eriocitrin (Schieber *et al.*, 2001)

Orange, though being 60% of the total citrus world production, the peels which represent between 50 to 65% of total weight of the fruit was not processed further usefully. Additionally, it had been found that orange peel was rich in secondary metabolites and phytochemicals that could be profusely used as drugs or as food supplements (Bruneton, 1999; Amabeoku, 2009; Kumar *et al.*, 2011).

Since, fruit peel extract incorporated dairy product like shrikhand is a new effort and there are very minimal studies, by considering the above, a novel attempt was made to incorporate the aqueous and ethanolic extracts of fresh and dried orange peels as economical raw material in the preparation of functional shrikhand.

MATERIALS AND METHODS

Farm-fresh buffalo milk (6 % fat and 9 % SNF) was obtained from the dairy farm maintained at Community Cattle Care Centre at College of Food and Dairy Technology, TANUVAS, Koduvelli, Chennai. The fresh and dried orange fruit peel extracts were prepared according to the standard formulation (Singh and Immanuel, 2014) and stored at 4° C.

Preparation of functional shrikhand

Shrikhand was prepared as per the procedure delineated by De (1980). The high quality cane sugar and cardamom were cleaned and ground to fine flour consistency just before use in value added shrikhand preparation. The standard freeze dried dahi cultures (NCDC 159 and NCDC 160) were procured from National Collection of Dairy Cultures (NCDC), National Dairy Research Institute (NDRI), Karnal.

Buffalo milk (6 % fat & 9 per cent SNF)

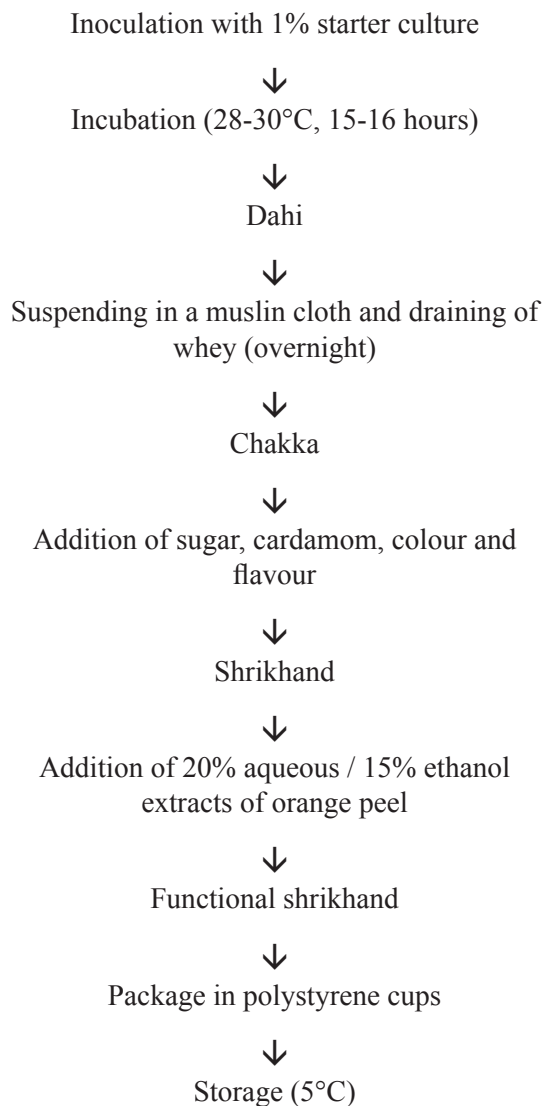


Pasteurization (71°C / 10 min)



Cooling (28-30°C)





Determination of total phenolic content

The total phenolic content of functional shrikhand was analysed in the control as well as orange peel extracts incorporated functional shrikhand till 20 days of storage period at 5 days of interval according to Follin-Ciocalteu method adopted by Singleton and Rossi

(1965). One hundred microliters of sample, dissolved in 70% ethanol or water, were mixed with 2 ml of 2% sodium carbonate solution containing 100µl Folin-Ciocalteu reagent (Folin-Ciocalteu: Methanol, 1:1, v/v) and incubated for 30 min. The optical absorbance (OD) was measured at 760 nm. 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).

Determination of antioxidant activity

The total antioxidant activity of functional shrikhand was evaluated as per the DPPH procedure adopted by Brand-Williams *et al.* (1995). DPPH radical scavenging activity assay assessed the ability of the extract to donate hydrogen or to scavenge free radicals. DPPH radical is a stable free radical and when it reacts with an antioxidant compound which can donate hydrogen, it will be reduced to diphenyl picryl hydrazine (DPPH).

An aliquot of 100 µl of the sample solution was mixed with 2.9 ml of 60 µM DPPH in methanol solution and vortexed. The reaction mixture was left in the dark for 30 min, after which the absorbance was measured at 517 nm. Methanol was used as blank. Initially, the solution was deep violet in colour, which was changed to light yellow. The change in colour was due to the reduction of DPPH with the antioxidant compounds present in the peels of orange. 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 - Absorbance of the control at 30 minute

A_1 - Absorbance of the sample at 30 minute

Microbial analysis

Microbiological analysis viz. standard plate count, coliform count and yeast and mould counts for control and selected optimum levels of orange peel extracts (20 percent aqueous or 15 percent ethanol) added functional shrikhand were carried out as per the standard technique described in BIS: 1981, SP: 18 (Part XI).

Calorific value

Calorific value of functional shrikhand incorporated with optimum levels of orange peel extracts was calculated.

Statistical analysis

The data obtained were evaluated statistically using the Software of Statistical Package for Social Sciences (SPSS 16.0) as per the standard procedure adopted by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Both the control and functional shrikhand incorporated with fresh and dried orange peel extracts at optimum levels viz. 20% (for aqueous) and 15% (for ethanolic) extracts (FOAE, DOAE, FOEE and DOEE) were analyzed for the total phenolic content and antioxidant activity during different

storage periods viz. 0, 5, 10, 15 and 20 days at 5°C.

Total phenolic content of functional shrikhand

Total phenolic content (TPC) was not present in control shrikhand. The mean \pm SE values of TPC for the functional shrikhand ranged from 7.78 \pm 0.36 to 16.25 \pm 0.94 mg/g (Table 1), which was similar to the findings of El-Said *et al.* (2014), who discovered that the TPC of aqueous extract of fruit peel extract for stirred yoghurt was from 13.98 to 14.81 mg Gallic acid/g and for methanolic extract, it was ranged from 14.83 to 15.80 mg Gallic acid/g. In some cases, change in trend (aqueous extract had higher TPC than ethanol extracts) was also noticed, which might be due to the difference in variety of the fruits used in this study. Kennas and Amellal-Chiban (2019) also reported that the solvent influences the extraction of phenolics from fruit peels.

Antioxidant activity of functional shrikhand

As illustrated in Table 2, the mean values of antioxidant activity of functional shrikhand ranged from 14.35 to 33.64 (aqueous extracts) and 17.74 to 34.25% (ethanol extracts) respectively. In average, the ethanol extracts incorporated functional shrikhand had higher antioxidant activity compared to aqueous extracts, which was correlated to the findings of Kennas and Amellal-Chiban (2019) who showed that the highest DPPH scavenging activity was recorded for the ethanolic extract (76.75 \pm 2.59 μ g/mL) of fruit peel compared to methanolic extracts.

Hegazy and Ibrahim (2012) showed that the solvent played a fundamental role in

Table 1. Total phenolic content of functional shrikhand incorporated with optimum levels of different types of fruit peel extracts during storage at 5°C (Mean ± SE) (n=6)

Types of Shrikhand	Storage period in days					F value
	0	5	10	15	20	
Control	0	0	0	0	0	-
FOAE _{20%}	13.26±0.45 ^b	12.38±1.65 ^b	11.89±0.71 ^b	11.57±0.40 ^b	7.78±0.36 ^a	4.36**
DOAE _{20%}	14.05±0.07 ^c	13.08±0.15 ^b	12.74±0.22 ^b	12.61±0.15 ^b	10.85±0.21 ^a	20.48**
FOEE _{15%}	15.85±0.39 ^d	13.25±0.14 ^c	12.36±0.08 ^b	12.25±0.31 ^b	10.05±0.24 ^a	33.90**
DOEE _{15%}	16.25±0.94 ^b	14.98±0.24 ^b	13.74±0.27 ^a	13.65±0.26 ^b	12.22±0.18 ^a	7.03**

FOAE_{20%} & DOAE_{20%} -Aqueous extracts of fresh & dried orange peels @ 20% respectively

FOEE_{15%} & DOEE_{15%} -Ethanol extracts of fresh & dried orange peels @ 20% respectively

Means bearing different superscripts in a row differ significantly (P<0.05)

** Significant (P≤0.01)

Table 2. Antioxidant activity (DPPH %) of functional shrikhand incorporated with optimum levels of different types of fruit peel extracts during storage at 5°C (Mean# ± SE) (n=6)

Types of Shrikhand	Storage period in days					F value
	0	5	10	15	20	
Control	0	0	0	0	0	-
FOAE _{20%}	21.08±0.26 ^b	20.63±0.10 ^b	20.23±0.18 ^b	19.93±0.39 ^b	14.35±1.09 ^a	3.73*
DOAE _{20%}	33.64±0.51 ^b	32.65±0.80 ^b	31.89±0.10 ^b	30.85±0.33 ^b	22.80±0.31 ^a	10.19**
FOEE _{15%}	28.58±0.15 ^c	23.58±0.34 ^b	22.89±0.05 ^b	22.52±0.49 ^b	17.74±0.10 ^a	8.60**
DOEE _{15%}	34.25±0.05 ^b	33.99±0.04 ^b	33.85±0.23 ^b	33.25±0.07 ^b	22.85±0.16 ^a	18.08**

Means bearing different superscripts in a row differ significantly (P<0.05)

**Highly Significant (P ≤ 0.01)

the extraction of the plant constituents. Beyond that, a study by Jayaprakasha *et al.* (2003) supported that the supplementation of natural antioxidant agents in shrikhand improved the storage life of the products.

With regard to the storage period, both antioxidant and phenolic contents in functional shrikhand showed gradual decrease as advancement of the storage days, which might be due to loss of these activities during storage period. These results were in accordance with the studies of Hala *et al.* (2010) and El-Batawy *et al.* (2014) who reported that the total phenolic count and % of RSA gradually decreased in all the treated samples during cold storage. Hala *et al.* (2010) concluded that this might be attributed to the transformation of phenolic content which are highly unstable compounds and undergo numerous enzymatic and chemical reactions during food storage.

Moreover, while comparing the fresh and dried fruit peel extracts, dried peel extracts had higher TPC and antioxidant activity in both fruits. These results were almost similar to the findings of Samsukhidir (2012) 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

Table 3 and 4 depicts the mean \pm SE values of microbial counts for the control and the functional shrikhand incorporated with 20% aqueous and 15% ethanolic extracts of orange peel. The study revealed that as the storage days increased, the standard plate counts and yeast and mould counts were proliferated in control as well as functional shrikhand, but

were within the normal range up to 15th day of storage. It was further discovered that the antimicrobial activity of the added fruit peel extracts reduced the total bacterial count. Additionally, control and functional shrikhand reported nil coliform counts.

These findings were in accordance with the studies of Dahham *et al.* (2010), Fawole *et al.* (2012), Gullon *et al.* (2016), Yadav and Rai (2018) and Kumar *et al.* (2019). Dahham *et al.* (2010) and Fawole *et al.* (2012) testified that methanolic peel extracts showed strong broad-spectrum activity against Gram +ve and Gram-ve bacteria and fungi than the aqueous extracts. Gullon *et al.* (2016) testified that fruit peel flour had strong bactericidal action against *Salmonella sp.*, *E. coli*, *L. monocytogenes* and *S. aureus*. Additionally, Khan and Hanee (2011) supported the present study that the antimicrobial activity of the added fruit peel extracts reduced the coliforms count. The observations were also in accordance with the finding of Kumar *et al.*, 2011 who reported that the citrus peel extract could be considered to be as equally potent as the antibiotics, such as metacillin and penicillin, which helped in controlling the microbial growth.

Calorific value of functional shrikhand

From the Table 5, it was found that among the treatments, aqueous and ethanolic extracts of fresh orange peel had high energy value (461.02 to 461.05) than dried orange peel extracts (460.86 to 460.97). And, the calorie value of control shrikhand was higher (461.18) than the functional shrikhand. The reason might be the difference in the moisture content and other chemical composition of the products prepared.

Table 3. Microbial analysis of functional shrikhand incorporated with optimum level (20 %) of aqueous extracts of orange peels during storage at 5°C (Mean# ± SE) (n=6)

Types of shrikhand	Storage period (Days)	Microbial counts (log ₁₀ cfu/g)		
		Total plate count	Coliforms	Yeast and mould count
Control	0	6.26±0.12 ^a	Nil	Nil
	5	6.52±0.19 ^a	Nil	2.12±0.08 ^a
	10	6.73±0.13 ^b	Nil	2.25±0.08 ^a
	15	6.89±0.21 ^b	Nil	2.41±0.03 ^a
	20	7.19±0.13 ^c	Nil	2.95±0.05 ^b
	F value	3.20*	Nil	8.79**
FOAE₂₀ %	0	6.15±0.16 ^a	Nil	Nil
	5	6.17±0.20 ^a	Nil	2.22±0.02 ^a
	10	6.45±0.10 ^a	Nil	2.41±0.01 ^a
	15	6.48±0.12 ^a	Nil	2.51±0.07 ^b
	20	6.89±0.10 ^b	Nil	2.88±0.06 ^c
	F value	3.52*	Nil	5.58**
DOAE₂₀ %	0	6.08±0.14 ^a	Nil	Nil
	5	6.20±0.21 ^a	Nil	2.20±0.02 ^a
	10	6.39±0.10 ^a	Nil	2.40±0.12 ^a
	15	6.45±0.12 ^a	Nil	2.49±0.08 ^b
	20	6.82±0.09 ^b	Nil	2.83±0.06 ^c
	F value	3.09*	Nil	5.18**

Means bearing different superscripts in a column differ significantly (P<0.05)

** Significant (P≤0.01)

* Significant (P≤0.05)

Table 4. Microbial analysis of functional shrikhand incorporated with optimum level (15 %) of ethanol extracts of fruit peels during storage at 5°C (Mean# ± SE) (n=6)

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	Nil	2.12±0.08 ^a
	10	6.73±0.13 ^b	Nil	2.25±0.08 ^a
	15	6.89±0.21 ^b	Nil	2.41±0.03 ^a
	20	7.19±0.13 ^c	Nil	2.95±0.05 ^b
	F value		3.20*	Nil
FOEE_{15%}	0	6.06±0.14 ^a	Nil	Nil
	5	6.19±0.21 ^a	Nil	2.14±0.05 ^a
	10	6.36±0.20 ^a	Nil	2.24±0.08 ^a
	15	6.35±0.18 ^a	Nil	2.32±0.08 ^a
	20	6.79±0.09 ^b	Nil	2.69±0.04 ^b
	F value		2.78*	Nil
DOEE_{15%}	0	6.03±0.17 ^a	Nil	Nil
	5	6.17±0.23 ^a	Nil	2.17±0.06 ^a
	10	6.35±0.21 ^a	Nil	2.22±0.08 ^a
	15	6.40±0.14 ^a	Nil	2.30±0.08 ^a
	20	6.79±0.09 ^b	Nil	2.60±0.05 ^b
	F value		2.83*	Nil

Means bearing different superscripts in a column differ significantly (P<0.05)

** Significant (P≤0.01) *Significant (P≤0.05)

Table 5. Calorific value of functional shrikhand incorporated with optimum levels of orange peel extracts (Mean# ± SE) (n=6)

Types of shrikhand	Calorific value (kcal / 100 g)
Control	461.18±4.25
FOAE _{20%}	461.05±4.13
DOAE _{20%}	460.86±3.95
FOEE _{15%}	461.02±4.10
DOEE _{15%}	460.97±4.05
F value	0.00^{NS}

Means bearing different superscripts in a same column differ significantly (P<0.05)

NS - Not significant

CONCLUSION

From the study conducted, it could be inferred that dried fruit peel extracts expressed more TPC (16.25±0.94 to 10.85±0.21 mg/g) and antioxidant activity (34.25±0.05 to 22.80±0.31%) than fresh fruit peel extracts (15.85±0.39 to 7.78±0.36 mg/g and 28.58±0.15 to 14.35±1.09% respectively). The storage studies endorsed that the functional shrikhand can be stored up to 15 days at temperature of 5°C without affecting their overall sensory acceptability due to presence anti-microbial property of the fruit peels. Owing to the higher antioxidant activity, total phenolic content and added functional attributes, 20 per cent aqueous extracts of fruit peels incorporated shrikhand can be introduced as a robust dairy supplement in human diet.

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