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Prolonging shelf life of enriched shrikhand under map: effect on chemical and microbiological qualities

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Abstract

The investigation was aimed at developing enriched shrikhand by blending WPC at 3,4,5 and 6 per cent and showed no significant difference between the colour and appearance scores at all the levels of WPC incorporation compared to control and as well as between the treatments. Maximum overall acceptability score of 8.20 was awarded to the sample prepared by using WPC at 5 per cent level was found to be significantly higher when compared to control and higher levels WPC in the treated sample that led to higher per cent of total solids in the finished product and the sample was subjected to Modified Atmospheric Packaging (MAP). It does not lead to any significant changes in the moisture and fat. The lower FFA and acidity in samples could be due to the antimicrobial properties of CO₂ gas.

Keywords: shrikhand, WPC and modified atmospheric packaging

1. Introduction

Fermented milks are known for their excellent nutritive and therapeutic properties, particularly when selected cultures of lactic acid bacteria are used as starters for fermenting milk products. Over the years they have become an indispensable supplement to the staple food consumed every day. Fermented milks like dahi has been very popular in India. It has been estimated the nearly 8 per cent of the total milk produced in India is utilized for the preparation of fermented milk products (Aneja, *et.al.*, 1997).

Shrikhand has originated in western India. It is obtained from dahi by partial drainage of whey, to which sugar, fruits and nuts are added. It derives its name from Sanskrit word "Shikharini". The production of shrikhand is so far limited to sweetmeat halwais, individual houses and small dairies. The traditional method involves the preparation of curd or dahi, preparation of chakka by draining off whey from the curd by suspending for about 12-18 hours in muslin cloth bags and blending additive like sugar, flavor and color.

In traditional method of preparation of shrikhand, most of the whey proteins are lost during whey drainage in chakka making stage. Whey contains about 20 per cent of milk proteins of high biological value. There is a need for popularizing the production and consumption of this highly nutritious byproduct, introducing improved methods of manufacture, storage and marketing of the product so as to meet the required standards of quality, nutritive value and organoleptic preferences of the people in different regions of the country.

The total world production of whey is approximately 85 million tonnes in which India's contribute approximately 8 million tonnes of the total global production (Alak and Karunakar, (2012) ^[1]). On average, the manufacturers of one tonne of cheese results in the production of 8 tonnes of Whey, In India, it is estimated that about 1000 million kgs of Whey is annually derived as a byproduct.

Presence of several nutritionally important constituents having excellent functional characteristics enhances opportunities for wide range of application of whey and whey constituents in the food industry. Presently, whey protein concentrates constitutes a small proportion (10 per cent) of protein utilization in food industry. More product formulation work is needed to move WPC into the general market place.

Whey protein concentrate is defined by US Food and Drug Administration (FDA) as "substance obtained by the removal of sufficient non-protein constituents from whey so that the finished dry product contains not less than 25 per cent proteins". WPC represents a protein

source of excellent nutritional and functional importance. WPC and isolates are Generally Recognized As Safe (GRAS) and therefore can be used as ingredients by standards of identity.

Modified Atmosphere Packaging may be defined as the enclosure of food products in gas barrier packaging materials, in which gaseous environment has been changed. This relies on mixtures of the atmosphere gases O₂, CO₂ and N₂ in different concentrations than those in air to retard deterioration processes in foods. The technologies thus, rely on gases that are safe, common, cheap, and readily available and usually not considered chemical additives. The proper use of food, gas mixture and package materials and types has been the subject of most of MAP foods.

There is only scanty information available literature on the modified atmosphere packaging to enrich and enhance the shelf life of shrikhand and similar products. Hence, investigation has to be taken to increase the shelf life of shrikhand by employing novel processing techniques like modified atmosphere packaging without affecting its physicochemical, microbiological, textural and organoleptic properties.

2. Materials and methods

Fresh Cow milk was procured from KVAFSU Dairy Farm, Hebbal; Bangalore was used for shrikhand preparation. Skim milk- Fresh cow milk preheated to 35 °C was subjected to centrifugal separation using Alfa Laval Cream Separator (AE 108) at Students Experimental Dairy Plant (SEDP) of the Dairy Science College, KVAFSU, Hebbal, Bangalore. Skim milk thus obtained was used for the preparation of shrikhand. Cream - Fresh cow milk preheated to 35 °C was subjected to centrifugal separation using Alfa Laval Cream Separator (AE 108) at Students Experimental Dairy Plant (SEDP) of the Dairy Science College, KVAFSU, Hebbal, and Bangalore. Cream obtained with 65 per cent fat was used for standardization of raw milk to desired fat level in milk for the preparation of shrikhand. Skim milk powder (SMP) "Sagar" brand spray dried SMP procured from the local market was used to make up the Solids- Not-Fat (SNF) content of milk for the preparation of shrikhand. Sugar was procured from local market.

Starter culture: Mixed strains of *Lactococcus lactis* and *Lactococcus lactis var diacetylactis* (1:1) procured from the Dairy Microbiology Department of the Dairy Science College, KVAFSU, Hebbal, Bangalore was used for culturing milk.

Chemicals

Analytical grade chemicals (Merk) procured from local market, Bangalore were used in the present study.

Preparation of control and enriched shrikhand

The control and enriched shrikhand were prepared as per procedure followed by Bogra and Mathur (2000) with slight modification. Shrikhand was prepared from fresh cow milk, which was standardized using cream, and SMP to 4.50 per cent fat and 8.50 per cent SNF. The standardized milk was heated to 95 °C for 5 minutes, cooled to 30°C and inoculated with *Lactococcus lactis* and *Lactococcus lactis var diacetylactis* (1:1) at the rate of 2.00 per cent of the volume of milk. It was then incubated for 16 hours at 30°C. The curd thus obtained was hanged in a muslin cloth for 12 hours for the removal of whey. The resultant semisolid mass called

chakka was then added with good quality ground sugar at the rate of 40.00 per cent of the weight of chakka and kneaded to get a smooth consistency. For the manufacture of enriched shrikhand, WPC was added to chakka at the rate of 3.00, 4.00 5.00 or 6.00 per cent.

Process optimization to enhance the shelf-life of shrikhand by Modified Atmosphere Packaging

For packaging shrikhand under MAP, Carbon-dioxide (CO₂) and Nitrogen (N₂) were used individually and in combinations.

For the present study, Carbon-dioxide (CO₂) and Nitrogen (N₂) were used individually and in combination as mentioned below:

Sl.no.	Code	Gas combination	Percentage
1	G1	CO ₂	100
2	G2	N ₂ +CO ₂	50:50
3	G3	N ₂	100

The gas cylinders were connected to the MAP gas mixer and the supply of gas to the mixer is set at 8 bar pressure.

Packaging material

The packaging material used for packaging shrikhand samples was procured from Shakthi Packaging, Bangalore. The packaging material had two layers of polyethylene terephthalate/polyethylene (PET/PE, 12/54µm). This packaging material was specifically chosen for its low microwave absorption properties. The thickness of the material was 66 µm. the size of the packaging material was 20X14 cm (LXB).

Analytical Equipments

Laboratory equipments

Analytical equipments such as Hot air oven (Scientific Industries, Bangalore), Autoclave (York Scientific Industries, Delhi), Digital pH meter (Elico, Model-LI 127, Bangalore), Bacteriological incubator (Serwell Industries Inc., Bangalore), Water bath, (Kemi, Model KWB 180, Mumbai), Electronic balance (Mettler-Toledo India Pvt. Ltd., Mumbai), etc. were used for chemical and microbiological analysis.

Thermocouple

Multi-channel digital thermocouple (M/S Electronic India Pvt. Ltd., Model 441E, Bangalore) was used to measure the temperature at 4 to 5 locations of microwave exposed shrikhand samples. Temperature measurements of the samples were made outside the tunnel.

Laminar airflow chamber

Laminar airflow chamber (Labline Instruments, Bangalore) was used for microbial analysis of shrikhand samples. The chamber-working surface was cleaned with cotton swab dipped in 95 per cent ethyl alcohol and was sterilized by UV light for 20 minute prior to microbial analysis.

Modified Atmospheric Packaging (MAP) Machine

Shrikhand samples were subjected to MAP using MAP machine. The machine had the following two units: (figure 2 and diagram 2 and 3).

The Modified Atmospheric Packaging Machine consists of following important components. The schematic diagram of the system is given in figure 1 and diagram 1 and 2.



Fig 1: Vacuum/gas filling and packaging machine

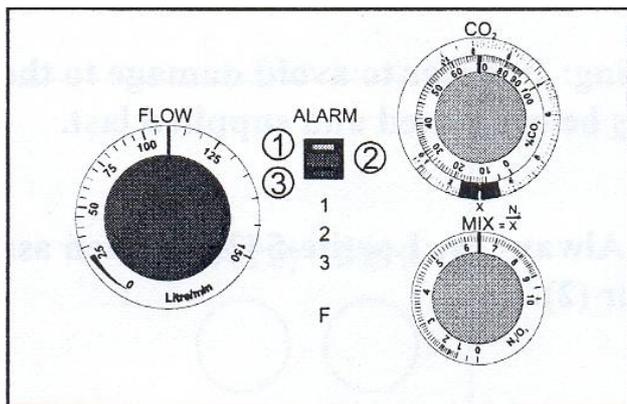


Diagram 1: MAP Gas Mixer

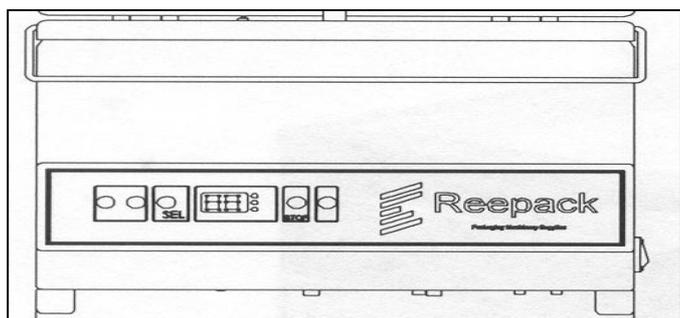


Diagram 2: Vacuum/Gas filling and packaging machine

1. Gas mixer - Manufactured by PBI/Dansensor A/S, Denmark, Model: Map Mix90003/150V/F
2. Vacuum/gas filling and packaging machine – Manufactured by Reepack packaging machinery supplies, Denmark, Model: RV 50

Procedure for mixing of gases and packaging

The entire setup consisted of the following units:

1. Gas cylinder
2. MAP gas mixer (with controls)
3. Vacuum/gas filling and packaging machine (with controls)

1. Gas cylinder: The gas cylinder of N₂ and CO₂ were connected to the MAP gas mixer for modified atmospheric packaging purpose.

2. MAP gas mixer: The MAP gas mixer was used to mix N₂ and CO₂ gases in appropriate proportion for modified atmospheric packaging of shrikhand as mentioned below:

1. Turn the “Flow” knob for setting of gas flow in liters/minutes
2. For filling 100 per cent N₂, set the CO₂ knob to “zero” and then set the “Mix” knob to 10 = 100 per cent N₂
3. For filling 100 per cent CO₂, set the N₂ knob to “Zero” and then set the CO₂ knob to 100 per cent
4. For mixing 50 per cent N₂+50 per cent CO₂, first set the CO₂ knob to 50 per cent, read the value of “X” that shows 5. Now calculate MIX, i.e. N₂/X. The required percentage of N₂ is 50, therefore 50/5 = 10. Now set the “Mix” knob to 10.

3. Vacuum/gas filling and packaging machine: The machine is programmed using the control panel in the front as mentioned below:

- A. First select “Vacuum” and set it to 100 per cent by using increase or decrease button on the control panel
- B. Select “Gas” option, then set it to 100 per cent for filling the gas from the MAP mix machine
- C. Finally select “Seal” option and set it to 4.5 seconds and
- D. Ensure the MAP mix machine is connected to this vacuum/gas filling and packaging machine.

Now, keep the package containing shrikhand and insert the mouth of the package in to the “nozzle” provided inside the chamber of vacuum/gas packaging machine. Close the top cover. The machine starts and the following operations are carried out automatically:

1. Aspiration: The pump creates vacuum as per set value by removing air from chamber and package containing shrikhand.
2. Gas injection: Injection of gas (as per setting) through the nozzle in to the pouch takes place immediately after completing of vacuum creation cycle.
3. Sealing: Immediately after the gas injection cycle, the pouch is hermetically sealed by the sealing bars.
4. Air re-entry: The cover reopen automatically allow the air to the re-enter in to the chamber.

Statistical Analysis

The results are the average of three replications was statistically analyzed by using ANOVA technique (Zar, 2003) for one way analysis with independent samples.

4. Result and Discussion

4.1 Effect of different levels of WPC on the quality of shrikhand

4.1.1 Effect of WPC on chemical composition of enriched shrikhand

The effect of various levels of WPC on chemical composition of shrikhand is depicted in Table 1.

There was a significant decrease in the moisture and fat per cent of treated sample that ranged from 41.25 to 40.34 and 6.55 to 6.46 respectively, as the level of incorporation of WPC increased from 3 to 6 per cent. The decrease in the moisture and fat per cent of treated samples was mainly due to the significant increase in per cent total solids from 58.07 to 59.66. Whereas, a significant increase in the protein per cent of treated shrikhand from 8.39 to 9.64 was also observed. The incorporation of WPC was mainly responsible for this increase in protein per cent of treated shrikhand and there was a non-significant level increase in the per cent acidity of shrikhand samples as the level of incorporation of WPC increased from 3.00 to 6.00 per cent level.

Table 1: Effect of various levels of WPC on chemical composition of enriched shrikhand

Per cent WPC	Moisture	Fat	Protein	Total Solids	Acidity
	Per cent				(% LA)
Control	41.93 ^a	6.62 ^a	7.08 ^a	58.07 ^a	1.170
3	41.25 ^b	6.55 ^b	8.39 ^b	58.75 ^b	1.172
4	40.86 ^c	6.52 ^b	8.51 ^c	59.14 ^c	1.175
5	40.60 ^d	6.50 ^b	8.56 ^d	59.40 ^d	1.103
6	40.34 ^e	6.46 ^b	8.64 ^e	59.66 ^e	1.184
C D	0.110	0.02	0.021	0.23	NS

Values are average of three trials

NS=Non-significant

The milk solids were effectively replaced with WPC in milk bread and its physicochemical and sensory attributes were found to be enhanced (Jayaprakasha, 2001) [8]. Puranik and Gupta (1997) [10] have reported successful utilization of 7.00 per cent WPC-60 or 10.00 per cent WPC-50 in the formulation of cake batter. Busra *et al.*, (2014) [4], revealed that, WPC and buttermilk powder addition improved dough properties in terms of dough stability. Protein content of the flat breads increased up to 14.60 per cent with WPC usage.

4.1.2 Effect of various levels of WPC on the sensory characteristics of enriched shrikhand

The enriched shrikhand was prepared by blending WPC at 3.00, 4.00, 5.00 and 6.00 per cent levels and showed no significant difference between the colour and appearance scores at all the levels of WPC incorporation compared to control and as well as between the treatments. There was a significant increase in the body and texture score (8.31) of enriched shrikhand prepared by blending WPC at 5.00 per cent level compared to control (7.65). Hence, incorporation of WPC at 5.00 per cent level was found to be optimum. At higher levels of WPC incorporation product becomes viscous and thick in its consistency. Hence this could be the reason for awarding significantly lower score for the shrikhand sample prepared by using WPC at 6.00 per cent level.

The maximum flavour score was awarded to control (8.0) and minimum (6.91) for the sample with 6 per cent WPC. But, samples prepared by using WPC upto 5.00 per cent level were showed the no significant effect on the flavour scores. The lower flavour score of the samples may be attributed to the lower per cent of fat (Table-2) in the samples at higher levels of WPC incorporation.

Table 2: Effect of various levels of WPC on the sensory characteristics of enriched shrikhand

Per cent of WPC	Colour and Appearance	Body and Texture	Flavour	Overall Acceptability
Control (0%)	7.80	7.65 ^a	8.00 ^a	8.00 ^a
3	7.83	7.70 ^a	7.92 ^a	7.81 ^a
4	7.86	7.80 ^a	7.90 ^a	7.83 ^a
5	7.88	8.31 ^b	7.85 ^a	8.20 ^b
6	7.73	7.00 ^c	6.91 ^b	6.50 ^c
CD	NS	0.23	0.18	0.28

Values are average of three trials

NS=Non significant

Maximum overall acceptability score of 8.20 was awarded to the sample prepared by using WPC at 5.00 per cent level was found to be significantly higher when compared to control. This could be due to the fact that better functional properties

of WPC particularly in improvement of body and texture and better consistency. Thapa and Gupta (1992) [15] reported that the processed cheese prepared with added WPC at 20.00 per cent level showed decline in appearance score ($p < 0.05$) in addition to browning of the product. The addition of WPC increases the level of proteins in the product, enhances its hydrophilic properties and improves its viscosity (Tamine and Robinson, 2007) [14].

There was significant improvement in the body and texture scores of shrikhand prepared by incorporating WPC at 3.00, 4.00 and 5.00 per cent level than that of control. At 5.00 per cent level of incorporation, a significant increase in the body and texture scores was observed due to its good mouth feel, smooth and firm consistency, soft body and textural characteristics without any free moisture. But at higher levels of WPC (6.00 per cent), judges felt that shrikhand was highly viscous and drier and hence awarded lower body and texture scores for the shrikhand containing 6.00 per cent WPC. The decline in the body and texture scores may be ascribed to incorporation of higher levels WPC in the in treated sample (Table-1) that led to higher per cent of total solids in the finished product. These results are in confirmation with the findings of Sharma *et al.*, (1998), who reported that a dried whey product used at lower levels (2.00 to 5.00 per cent) in dairy products contributed to the desirable mouth feel attributes. Addition of whey concentrate to more than 5.00 per cent level brought about deterioration in the quality of shrikhand in terms of flavour, consistency, body and taste (Giram *et al.*, 2001) [7].

Processed cheese spread with good melt ability, desired characteristics with improved spread ability can be prepared by using dried WPC at levels up to 4.50 per cent of cheese solids (Suneeta *et al.*, 2007) [13].

4.2 Modified Atmosphere Packaging

4.2.1 Storage studies on chemical composition of shrikhand

4.2.1.1 Effect of modified atmosphere packaging on the chemical composition of enriched shrikhand stored at ambient temperature ($30 \pm 1^\circ\text{C}$)

Modified atmospheric packaging technique was used to pack the enriched shrikhand by using 100% CO₂ (G₁), 50% N₂ and 50% CO₂ (G₂) and 100%N₂ (G₃).

There was no significant difference in the moisture, fat and protein per cent of control and treated samples up 3rd days of storage at $30 \pm 1^\circ\text{C}$ (Table 3). This implies MAP does not lead to any significant changes in the moisture, fat and protein contents of the enriched shrikhand. But, there was a significantly higher acidity values were recorded for control from day 1 to 3 days of storage when compared to all the MAP samples. The control sample recorded maximum acidity of 1.293 and 1.390 per cent lactic acidity on 1st and 2nd day of storage at $30 \pm 1^\circ\text{C}$, afterwards product was rejected but G₁, G₂ and G₃ recorded 1.260, 1.330 and 1.302 per cent lactic acidity of acceptable range on 3rd day of storage. The lower acidity in the MAP samples could be due to the lower microbial counts. Similarly, FFA content of control was recorded significantly higher than the MAP samples stored at 1st and 2nd day. Control sample recorded 0.321 and 0.440 $\mu\text{.eql/g}$ on 1st and 2nd day respectively, whereas, G₁, G₂ and G₃ recorded 0.312, 0.381 and 0.351 $\mu\text{.eql/g}$ on 3rd day of storage. The lower FFA could be due to the lower enzymatic activities in MAP samples than the control. Similar results were also reported by Arunkumar, (2006) [3] and Karthikeyan, (2005) [9].

Table 3: Effect of modified atmosphere packaging on the chemical composition stored enriched Shrikhand at 30±1°C

1 st day					
Treatment	Moisture	Fat	Protein	Acidity	FFA
	-----Per cent-----			(%LA)	($\mu\text{equl/g}$)
Control	39.60	6.75	8.75	1.293 ^a	0.335 ^a
G ₁	39.53	6.71	8.72	1.211 ^b	0.235 ^b
G ₂	39.41	6.77	8.76	1.230 ^b	0.281 ^b
G ₃	39.48	6.78	8.73	1.242 ^b	0.285 ^b
CD	NS	NS	NS	0.021	0.052
2 nd day					
Control	39.19	6.81	8.81	1.390 ^a	0.440 ^a
G ₁	39.13	6.75	8.75	1.218 ^b	0.221 ^b
G ₂	39.08	6.80	8.79	1.261 ^b	0.311 ^b
G ₃	39.11	6.79	8.74	1.321 ^b	0.320 ^b
CD	NS	NS	NS	0.08	0.020
3 rd day					
Control	Spoiled				
G ₁	39.01	6.81	8.78	1.260	0.312
G ₂	38.92	6.84	8.83	1.330	0.381
G ₃	38.97	6.82	8.76	1.342	0.392

G₁, G₂ and G₃ shrikhand were spoiled on 4th day

Values are average of three trials

NS = Non-significant

G₁ = Enriched shrikhand packed under 100% CO₂

G₂ = Control shrikhand packed under 50% N₂ + 50% CO₂

G₃ = Enriched shrikhand packed under 100% N₂

4.2.1.2 Effect of modified atmosphere packaging on the chemical composition of enriched shrikhand stored at refrigeration temperature (7 ± 1° C)

Modern packaging technique (MAP) are used to pack the enriched shrikhand by using 100% CO₂ (G₁), 50% N₂ and 50% CO₂ (G₂) and 100%N₂ (G₃).

The moisture per cent of shrikhand samples were varied from 39.46 to 39.58 on 1st day of storage, on 2nd day 39.36 to 39.43 and on 3rd day 39.23 to 39.29. There was no significant difference in the moisture per cent of control and treated samples up 35 days of storage at 7±1°C (Table 4 and 5). Similarly, There was no significant difference in the fat and protein per cent of control and treated samples up 35 days of storage at 7 ± 1° C. This implies MAP does not lead to any significant changes in the moisture, fat and protein contents of the enriched shrikhand. But, there was a significantly higher acidity values are recorded for control from day 7th to 35th day of storage when compared to all the MAP samples. The control sample recorded significantly maximum acidity of 1.231 and 1.251, 1.265, 1.277 and 1.283 per lactic acidity on 7th, 14th, 21st, 28th and 35th day of storage respectively. The MAP samples G₁, G₂ and G₃ recorded significantly lower acidity values could be due to the lower microbial counts (Table-7). The control sample was rejected on 35th day of storage, but G₁, G₂ and G₃ was recorded satisfactory acidity values up to 56th day of storage (Table-6).

Table 6: Effect of modified atmosphere packaging on the chemical composition stored enriched shrikhand at 7±1 °C

49 th day					
Treatment	Moisture	Fat	Protein	Acidity	FFA
	-----Per cent-----			(%LA)	($\mu\text{equl/g}$)
G ₁	38.89	6.99	9.01	1.274	0.258
G ₂	38.84	7.11	9.13	1.287	0.334
G ₃	38.91	7.09	9.09	1.293	0.340
CD	NS	NS	NS	NS	NS
56 th day					
G ₁	38.84	7.05	9.07	1.280	0.267
G ₂	38.79	7.17	9.20	1.293	0.346

Table 4: Effect of modified atmosphere packaging on the chemical composition stored enriched shrikhand at 7±1 °C

7 th day					
Treatment	Moisture	Fat	Protein	Acidity	FFA
	-----Per cent-----			(%LA)	($\mu\text{equl/g}$)
Control	39.58	6.74	8.74	1.231 ^a	0.320 ^a
G ₁	39.51	6.70	8.71	1.210 ^b	0.200 ^b
G ₂	39.38	6.78	8.76	1.228 ^b	0.279 ^b
G ₃	39.46	6.77	8.74	1.242 ^b	0.289 ^b
CD	NS	NS	NS	0.019	0.092
14 th day					
Control	39.43	6.79	8.80	1.251 ^a	0.335 ^a
G ₁	39.38	6.73	8.76	1.230 ^b	0.219 ^b
G ₂	39.30	6.86	8.89	1.247 ^b	0.288 ^b
G ₃	39.36	6.84	8.83	1.249 ^b	0.294 ^b
CD	NS	NS	NS	0.021	0.270
21 st day					
Control	39.29	6.85	8.86	1.265 ^a	0.349 ^a
G ₁	39.20	6.78	8.83	1.244 ^b	0.228 ^b
G ₂	39.17	6.93	8.95	1.256 ^b	0.297 ^b
G ₃	39.23	6.89	8.88	1.258 ^b	0.301 ^b
CD	NS	NS	NS	0.019	0.032

Values are average of three trials

NS = Non-significant

G₁ = Enriched shrikhand packed under 100% CO₂

G₂ = Control shrikhand packed under 50% N₂ + 50% CO₂

G₃ = Enriched shrikhand packed under 100% N₂

Table 5: Effect of modified atmosphere packaging on the chemical composition stored enriched shrikhand at 7±1 °C

28 th day					
Treatment	Moisture	Fat	Protein	Acidity	FFA
	-----Per cent-----			(%LA)	($\mu\text{equl/g}$)
Control	39.17	6.90	8.91	1.277 ^a	0.353 ^a
G ₁	39.08	6.84	8.89	1.255 ^b	0.235 ^b
G ₂	39.01	6.98	8.99	1.262 ^b	0.300 ^b
G ₃	39.15	6.94	8.93	1.269 ^b	0.307 ^b
CD	NS	NS	NS	0.006	0.037
35 th day					
Control	39.06	6.96	8.94	1.283 ^a	0.368 ^a
G ₁	39.00	6.90	8.92	1.261 ^b	0.244 ^b
G ₂	38.97	7.01	9.03	1.275 ^b	0.315 ^b
G ₃	39.01	7.00	8.98	1.278 ^b	0.321 ^b
CD	NS	NS	NS	0.019	0.078
42 nd day					
Control	Spoiled on 36 th day				
G ₁	38.95	6.94	8.96	1.267	0.249
G ₂	38.90	7.06	9.08	1.281	0.321
G ₃	38.97	7.04	9.03	1.289	0.331
CD	NS	NS	NS	NS	NS

Values are average of three trials

NS = Non-significant

G₁ = Enriched shrikhand packed under 100% CO₂

G₂ = Control shrikhand packed under 50% N₂ + 50% CO₂

G₃ = Enriched shrikhand packed under 100% N₂

G ₃	38.86	7.14	9.15	1.299	0.351
CD	NS	NS	NS	NS	NS
G₁, G₂ and G₃ sample were spoiled on 57th day					

Values are average of three trials

NS = Non-significant

G₁ = Enriched shrikhand packed under 100% CO₂

G₂ = Control shrikhand packed under 50% N₂ + 50% CO₂

G₃ = Enriched shrikhand packed under 100% N₂

Fandos *et al.*, (2000) [6] evaluated the shelf life quality of cameros cheese packed in different gas environments viz., 20 per cent N₂/80 per cent CO₂, 40 per cent N₂/60 per cent CO₂, 50 per cent N₂/50 per cent CO₂, 100 per cent CO₂ and control. They reported that increase in FFA, soluble nitrogen and decrease in pH in control as compared to experimental samples during storage at 34°C. Among experimental sample, the 100 per cent CO₂ showed very less changes in the above constituents as compared to rest of the samples. Same results were also reported by Arunkumar, (2006) [3] and Karthikeyan, (2005) [9].

4.2.2 Microbial quality of modified atmosphere packaging

4.2.2.1 Effect of modified atmosphere packaging on the microbial quality of enriched shrikhand stored at 30 ± 1°C

The untreated shrikhand sample (control) had significantly high total counts of log₁₀ 3.64 cfu/g compared to treated G₁ (log₁₀ 2.40 cfu/g), G₂ (2.70 cfu/g) and G₃ (2.80 cfu/g) on 1st of storage. Similarly, control had significantly higher yeasts and mould counts of 1.84 cfu/g compared to G₁ (1.20 cfu/g), G₂ (21.38 cfu/g) and G₃ (1.60 cfu/g). This could be due to the antimicrobial properties of gases (CO₂ and N₂).

Table 7: Effect of modified atmospheric packaging on the microbiological quality of shrikhand stored at 30±1°C

Treatments	Counts expressed in log ₁₀ cfu/g							
	1 Day		2 Day		3 day		4 day	
	TC	YM	TC	YM	TC	YM	TC	YM
Control	3.63	1.84	4.28	1.82	Spoiled			
G ₁	2.40	1.20	2.60	1.40	3.10	1.80	Spoiled	
G ₂	3.70	1.38	4.00	1.60	4.26	1.75	Spoiled	
G ₃	3.80	1.60	4.20	1.72	4.50	1.90	Spoiled	
CD	1.23	0.64	1.68	0.42	1.60	0.16		

Values are average of three trials

Table 8: Effect of modified atmospheric packaging on the microbiological quality of shrikhand stored at 7±1°C

Treatments	Counts expressed in log ₁₀ cfu/g																	
	7 Day		14 Day		21 Day		28 Day		35 Day		42 Day		49 Day		56 Day		63 Day	
	TC	YM	TC	YM	TC	YM	TC	YM	TC	YM	TC	YM	TC	YM	TC	YM	TC	YM
Control	2.80 ^a	1.28 ^a	2.82 ^a	1.35 ^a	2.96 ^a	1.42 ^a	3.00 ^a	1.52 ^a	3.50 ^a	1.92 ^a	Spoiled on 36th day							
G ₁	1.36 ^b	1.01 ^b	1.41 ^b	1.09 ^b	1.63 ^b	1.20 ^b	1.80 ^b	1.30 ^b	1.97 ^b	1.36 ^b	2.44	1.38	3.00	1.40	4.03	1.68	Spoiled	
G ₂	1.82 ^b	1.06 ^b	1.90 ^b	1.10 ^b	1.94 ^b	1.18 ^b	2.02 ^b	1.36 ^b	2.24 ^b	1.47 ^b	2.55	1.50	3.26	1.63	4.48	1.72	Spoiled	
G ₃	1.90 ^b	1.08 ^b	1.96 ^b	1.33 ^b	2.10 ^b	1.66 ^b	2.40 ^b	1.77 ^b	2.68 ^b	1.83 ^b	2.72	1.90	3.48	1.92	4.63	1.98	Spoiled	
CD	1.44	0.28	1.41	0.33	1.33	0.22	1.20	0.22	1.75	0.56								

Values are average of three trials

G₁ = Enriched shrikhand with modified atmospheric package (100% CO₂)

G₂ = Enriched shrikhand with modified atmospheric package (50 N₂+ 50% CO₂)

G₃ = Enriched shrikhand with modified atmospheric package (100% N₂)

4.2.3 Sensory characteristics of modified atmosphere packaging

4.2.3.1 Effect of modified atmosphere packaging on the sensory characteristics of enriched shrikhand stored at 30±1 °C

There was no significant change in the colour and appearance, body and texture and packaging scores of control and all other

G₁ = Enriched shrikhand with modified atmospheric package (100% CO₂)

G₂ = Enriched shrikhand with modified atmospheric package (50 N₂+ 50% CO₂)

G₃ = Enriched shrikhand with modified atmospheric package (100% N₂)

Control sample recorded significantly higher total counts up to 2nd days of storage than those of treated spreads.(Table7) and afterwards control was rejected on 3rd day due to spoilage. Whereas, treated samples G₁, G₂ and G₃ was found acceptable counts up to 3 days of storage. Reported results revealed the potentiality of MAP in increasing cheese sensorial and microbial shelf life by carefully designing for an individual cheese (Sadegh *et al.*, 2012) [11].

4.2.2.2 Effect of modified atmosphere packaging on the microbial quality of fresh shrikhand stored at refrigeration temperature 7±1°C

The control had significantly high total counts of 2.80 cfu/g compared to treated G₁ (1.36 cfu/g), G₂ (1.82 cfu/g) and G₃ (1.90 cfu/g) on 7th day of storage (Table 8). Similarly, control had significantly higher yeasts and mould counts of 1.28 cfu/g compared to G₁ (1.01 cfu/g), G₂ (1.06 cfu/g) and G₃ (1.08 cfu/g). This was mainly because of the antimicrobial properties of the MAP samples. This trend was continued upto 35th day of storage of control sample and as well as for the all the MAP samples, whereas, the total and yeast and mould counts of the treated samples G₁, G₂ and G₃ was found acceptable up to 56th day of storage at refrigeration temperature. Gonzalez *et al.*, (2000) found no significant differences among the combinations of CO₂/N₂ (20% CO₂ / 80% N₂, 40% CO₂/60% N₂, 50% CO₂/50% N₂, and 100% CO₂) stored at 3-4°C studied in retarding the lipolysis of Cameros cheese.

treated samples stored at 30± 1 °C on 1st day of storage (Table 9). But, there was significant improvement in the flavour scores of all MAP samples compared to control. Within the treated samples G₃ awarded slightly higher flavour score compared to G₂ and G₃. Maximum overall acceptability score was awarded to sample G₁ (88.01) and it was significantly higher than the control (82.00). On 2nd day, also same trend

was continued, that there was no significant change in the colour and appearance, body and texture and packaging scores of control and all other treated samples stored at $30\pm 1^\circ\text{C}$ on 2nd of storage (Table 10). But, there was significant improvement in the flavour scores of all MAP

samples compared to control. Whereas control was awarded significantly lower scores for all the sensory attributes except packaging on 3rd day. The judges rejected control sample on 3rd of storage at $30\pm 1^\circ\text{C}$.

Table 9: Effect of modified atmosphere packaging on the sensory characteristics stored enriched shrikhand at $30\pm 1^\circ\text{C}$

1 st Day					
Treatment	Colour and appearance (Max. 15)	Body and texture (Max. 30)	Flavour (Max. 50)	Package (Max. 5)	Overall Acceptability (Max. 100)
Control	13.00	25.90	40.37 ^a	4.50	82.00 ^a
G ₁	13.70	26.80	42.98 ^b	4.50	88.01 ^b
G ₂	13.68	26.46	42.77 ^b	4.50	87.41 ^b
G ₃	13.50	26.17	41.20 ^b	4.50	85.37 ^b
CD	NS	NS	1.83	NS	3.17

Values are average of three trials

NS = Non-significant

G₁ = Enriched shrikhand packed under 100% CO₂

G₂ = Control shrikhand packed under 50% N₂ + 50% CO₂

G₃ = Enriched shrikhand packed under 100% N₂

Table 10: Effect of modified atmosphere packaging on the sensory characteristics stored enriched shrikhand at $30\pm 1^\circ\text{C}$

2 nd Day					
Treatment	Colour and appearance (Max. 15)	Body and texture (Max. 30)	Flavour (Max. 50)	Package (Max. 5)	Overall Acceptability (Max. 100)
Control	11.87	21.50	36.45 ^a	4.50	74.32 ^a
G ₁	12.51	24.95	41.43 ^b	4.50 ^b	83.03 ^b
G ₂	12.32	24.92	41.25 ^b	4.50	82.99 ^b
G ₃	12.10	24.87	39.90 ^b	4.50	81.37 ^b
CD	NS	NS	1.61	NS	2.91

Values are average of three trials

NS = Non-significant

G₁ = Enriched shrikhand packed under 100% CO₂

G₂ = Control shrikhand packed under 50% N₂ + 50% CO₂

G₃ = Enriched shrikhand packed under 100% N₂

Table 11: Effect of modified atmosphere packaging on the sensory characteristics stored enriched shrikhand at $30\pm 1^\circ\text{C}$

3 rd Day					
Treatment	Colour and appearance (Max. 15)	Body and texture (Max. 30)	Flavour (Max. 50)	Package (Max. 5)	Overall Acceptability (Max. 100)
Control	Spoiled				
G ₁	12.39	23.99	40.80	4.50	81.68
G ₂	12.13	23.95	40.52	4.50	81.10
G ₃	12.00	23.87	39.00	4.50	79.37
CD	NS	NS	NS	NS	NS

G₁, G₂, G₃ samples were Spoiled on 4th Day

Values are average of three trials

NS = Non-significant

G₁ = Enriched shrikhand packed under 100% CO₂

G₂ = Control shrikhand packed under 50% N₂ + 50% CO₂

G₃ = Enriched shrikhand packed under 100% N₂

But, within the treated samples G₃ awarded slightly higher flavour score compared to G₂ and G₃ upto 3rd day of storage (Table 11). Maximum overall acceptability score was awarded to sample G₁ (81.68) and it was significantly higher than the G₂ (81.10) and G₃ (79.37). On 4th day, all the samples were awarded poor sensory scores for all the sensory attributes due to poor colour appearance, dry body and coarse texture and presence of off-flavour in the products. Again this could be due to the growth of microbial counts (Table 7). Reported results revealed the potentiality of MAP in increasing cheese sensorial and microbial shelf life by carefully designing for an individual cheese (Sadegh *et al.*, 2012) [11].

4.2.3.2 Effect of modified atmosphere packaging on the sensory characteristics of enriched shrikhand stored at $7\pm 1^\circ\text{C}$

There was no significant change in the colour and appearance, body and texture and packaging scores of control and all other treated samples stored at $7\pm 1^\circ\text{C}$ on 7th day of storage (Table 12). But, there was significant improvement in the flavour scores of all MAP samples was observed compared to control. Within the treated samples G₃ awarded slightly higher flavour score compared to G₂ and G₃. Maximum overall acceptability score was awarded to sample G₁ (91.25) and it was significantly higher than the control (86.14). On 14th day also same trend was continued, that there was no significant change in the colour and appearance, body and texture and packaging scores of control and all other treated samples

stored at $7\pm 1^\circ\text{C}$. But, there was significant improvement in the flavour scores of all MAP samples was observed compared to control. Even though control was awarded lower sensory scores on 35th day it was accepted due to the better sensory scores (Table 14). But, on 42nd day control was

awarded significantly lower scores for all the sensory attributes due to inferior sensory quality and hence, on the same day control sample was rejected. But, within the treated samples G₃ awarded slightly higher flavour score compared to G₂ and G₃ up to 56th day storage at refrigeration temperature.

Table 12: Effect of modified atmosphere packaging on the sensory characteristics stored enriched shrikhand at $7\pm 1^\circ\text{C}$

7 th Day					
Treatment	Colour and appearance (Max. 15)	Body and texture (Max. 30)	Flavour (Max. 50)	Package (Max. 5)	Overall Acceptability (Max. 100)
Control	12.50	26.04	43.10 ^a	4.50	86.14 ^a
G ₁	13.11	26.97	46.67 ^b	4.50	91.25 ^b
G ₂	13.10	26.78	46.48 ^b	4.50	90.86
G ₃	12.89	26.13	46.19 ^b	4.50	89.71 ^b
CD	NS	NS	3.09	NS	3.57
14 th Day					
Control	12.10	25.88	42.15 ^a	4.50	84.63 ^a
G ₁	13.10	26.71	46.19 ^b	4.50	90.50 ^b
G ₂	12.90	26.45	46.10 ^b	4.50	89.95 ^b
G ₃	12.55	26.00	45.87 ^b	4.50	88.92 ^b
CD	NS	NS	7.72	NS	4.29

Values are average of three trials

NS = Non-significant

G₁ = Enriched shrikhand packed under 100% CO₂

G₂ = Control shrikhand packed under 50% N₂ + 50% CO₂

G₃ = Enriched shrikhand packed under 100% N₂

Table 13: Effect of modified atmosphere packaging on the sensory characteristics stored enriched shrikhand at $7\pm 1^\circ\text{C}$

21 st Day					
Treatment	Colour and appearance (Max. 15)	Body and texture (Max. 30)	Flavour (Max. 50)	Package (Max. 5)	Overall Acceptability (Max. 100)
Control	11.95	25.69	41.98 ^a	4.50	84.12 ^a
G ₁	12.86	26.33	45.91 ^b	4.50	89.60 ^b
G ₂	12.57	26.10	45.87 ^b	4.50	89.04 ^b
G ₃	13.32	25.80	45.59 ^b	4.50	88.21 ^b
CD	NS	NS	3.61	NS	4.09
28 th Day					
Control	11.27	25.34	40.80 ^a	4.50	82.39 ^a
G ₁	12.50	26.01	45.70 ^b	4.50	88.71 ^b
G ₂	12.32	25.91	45.66 ^b	4.50	88.39 ^b
G ₃	12.09	25.56	45.38 ^b	4.50	87.53 ^b
CD	NS	NS	4.58	NS	5.14

Values are average of three trials

NS = Non-significant

G₁ = Enriched shrikhand packed under 100% CO₂

G₂ = Control shrikhand packed under 50% N₂ + 50% CO₂

G₃ = Enriched shrikhand packed under 100% N₂

Table 14: Effect of modified atmosphere packaging on the sensory characteristics stored enriched shrikhand at $7\pm 1^\circ\text{C}$

35 th Day					
Treatment	Colour and appearance (Max. 15)	Body and texture (Max. 30)	Flavour (Max. 50)	Package (Max. 5)	Overall Acceptability (Max. 100)
Control	11.50	24.90	40.10 ^a	4.50	81.10 ^a
G ₁	12.32	25.89	45.60 ^b	4.50	88.31 ^b
G ₂	12.11	25.67	45.46 ^b	4.50	87.74 ^b
G ₃	11.93	25.30	45.00 ^b	4.50	86.73 ^b
CD	NS	NS	4.90	NS	5.63
42 nd Day					
Control	Spoiled on 36 th day				
G ₁	12.12	25.61	45.41	4.50	87.64
G ₂	11.95	25.37	45.20	4.50	87.02
G ₃	11.83	25.03	44.97	4.50	86.33
CD	NS	NS	NS	NS	NS

Values are average of three trials

NS = Non-significant

G₁ = Enriched shrikhand packed under 100% CO₂

G₂ = Control shrikhand packed under 50% N₂ + 50% CO₂

G₃ = Enriched shrikhand packed under 100% N₂

Table 15: Effect of modified atmosphere packaging on the sensory characteristics stored enriched shrikhand at 7±1 °C

49 th Day					
Treatment	Colour and appearance (Max. 15)	Body and texture (Max. 30)	Flavour (Max. 50)	Package (Max. 5)	Overall Acceptability (Max. 100)
G ₁	11.92	25.40	45.20	4.50	87.02
G ₂	11.75	25.18	45.00	4.50	86.43
G ₃	11.63	24.92	44.76	4.50	85.81
CD	NS	NS	NS	NS	NS
56 th Day					
G ₁	11.61	25.19	44.89	4.50	86.19
G ₂	11.44	24.87	44.69	4.50	85.50
G ₃	11.32	24.61	44.45	4.50	84.88
CD	NS	NS	NS	NS	NS
G₁, G₂, G₃ samples spoiled on 57th day					

Values are average of three trials

NS = Non-significant

G₁ = Enriched shrikhand packed under 100% CO₂

G₂ = Control shrikhand packed under 50% N₂ + 50% CO₂

G₃ = Enriched shrikhand packed under 100% N₂

On 56th day, maximum overall acceptability score was awarded to sample G₁ (86.19) and it was significantly higher than the G₂ (85.5) and G₃ (84.88). But On 57th day all the samples were discarded due to spoilage (Table 15). Because, all MAP samples were awarded poor sensory scores for all the sensory attributes due to poor colour appearance, dry body and coarse texture and presence of off-flavour in the products. This could be due to the higher microbial counts (Table 8). Fandos *et al.*, (2000) [6] evaluated the shelf life quality of cameros cheese packed in different gas environments viz., 20 per cent N₂/80 per cent CO₂, 40 per cent N₂/60 per cent CO₂, 50 per cent N₂/50 per cent CO₂, 100 per cent CO₂ and control. They concluded that 40 per cent N₂/60 per cent CO₂ and 50 per cent N₂/50 per cent CO₂ gas environment are the most effective for extending the shelf life of Cameros cheese and retaining good quality sensory characteristics.

4.3 Cost of production of Effect of modified atmosphere packaging enriched shrikhand

The total production cost of control was Rs.5349.16 and for G₁, G₂ and G₃ shrikhand (including loss during storage) were

Rs.5401.93, Rs.5399.48 and Rs.5397.52. The cost of shrikhand per kg was estimated to be Rs.137.16 for control and Rs. 138.51, Rs. 138.45 and Rs.138.39 for G₁ (CO₂), G₂ (50% N₂+50% CO₂) and G₃ (100% N₂), respectively. The difference in the cost was accounted primarily by the fixed cost as capital investments on MAP (CO₂, and N₂).

Karthikeyan., (2005) [9], studied the total production cost was Rs. 88.74, Rs. 88.87 and Rs. 88.99 per kg of paneer for packed under 100% N₂, 50% N₂+50% CO₂ and 100% CO₂ respectively and was slightly higher than control paneer (Rs.85.99). The fixed cost as capital investments on MAP equipments and difference in the operating cost (power and MAP gases) used for MAP was accounted for the higher rate of paneer. Though the capital investment was higher, the corresponding benefit was very substantial in terms of increased shelf-life. In addition, the substitution of brine with coating could allow us to gain a double advantage: both preserving the product quality and reducing the cost of its distribution, due to the lower weight of the package (Conteet *et al.*, 2009) [5].

Table 16: Cost of manufacturing modified atmosphere packaged shrikhand

Heads	Milk-100 kg (Fat-4.50%, SNF-8.50%), yield-39 kg shrikhand					
	Rs/Kg	Qty used	Control(Rs)	G ₁ (Rs)	G ₂ (Rs)	G ₃ (Rs)
Expenditure						
a) Raw material cost						
Cow milk	30.00	100.00	3000.00	3000.00	3000.00	3000.00
Sugar	36.00	11.00	396.00	396.00	396.00	396.00
Sub-total			3396.00	3396.00	3396.00	3396.00
b) Operating cost						
I- Service cost						
Power(@Rs. 6.0/kWh)	6.00	0.75/1.5	4.50	9.00	9.00	9.00
Rate of gas/filling(including loss) N ₂ @ Rs. 0.163, N ₂ +CO ₂ @ Rs. 0.175 CO ₂ @ Rs 0.187/pouch		180.00		33.66	31.50	29.34
Steam (@ Rs. 2/kg steam)	2.00	12.00	24.00	24.00	24.00	24.00
Water (@ Rs. 0.10/lit)	0.10	400.00	40.00	40.00	40.00	40.00
Wash Chemicals (@ Rs.0.25/kg)	0.25	18.00	4.50	4.50	4.50	4.50
Maintenance (@ Rs 0.75)	0.75	18.00	13.50	13.50	13.50	13.50
Sub-total			86.50	124.66	122.50	120.34
II-Packaging cost	200.0	3.90	780.00	780.00	780.00	780.00
III-Marketing cost						
Storage and distribution	2.00	18.00	36.00	36.00	36.00	36.00
Sales promotion	0.75	18.00	13.50	13.50	13.50	13.50
Sub-total			49.50	49.50	49.50	49.50
IV-Salary/Wages (Rs. 100/day)			225.00	225.00	225.00	225.00

VI- Interest on capital(14% of Rs. 4176)			584.64	584.64	584.64	584.64
Fixed cost						
VII-Equipment Depreciation (10000+8000+35000+326010+10000)			2.63	16.21	16.21	16.21
VIII-Rent of building			120.00	120.00	120.00	120.00
Fixed cost			122.63	136.21	136.21	136.21
c) Production Cost (a+b)			5244.27	5296.01	5293.85	5291.69
d) Loss during storage (2% for control and Gas shrikhand)			104.89	105.92	105.88	105.83
e) Total Production Cost (c+d)			5349.16	5401.93	5399.48	5397.52
3. Production cost of shrikhand/kg			137.16	138.51	138.45	138.39

- G₁ = Enriched shrikhand packed under 100% CO₂
 G₂ = Control shrikhand packed under 50% N₂ + 50% CO₂
 G₃ = Enriched shrikhand packed under 100% N₂

Conclusion

In present investigation shrikhand was enriched with different levels of WPC at 3.00, 4.00, 5.00 and 6.00 per cent levels and showed no significant difference between the colour and appearance scores at all the levels of WPC incorporation compared to control and as well as between the treatments.

Maximum overall acceptability score of 8.20 was awarded to the sample prepared by using WPC at 5.00 per cent level was found to be significantly higher when compared to control and higher levels WPC in the treated sample that led to higher per cent of total solids in the finished product and which enhances functional and nutritional properties of shrikhand. Shrikhand was subjected to MAP to increase the shelf life of enriched shrikhand. The untreated shrikhand sample (control) had significantly high microbial total counts and also had significantly higher yeasts and mould counts. But, treated samples recorded significantly lower counts which could be due to the antimicrobial properties of CO₂ and N₂ gases. The control had significantly high total counts and as well as yeast and mould counts compared to treated samples stored under refrigeration temperature. Again this could be the antimicrobial properties of the MAP. Whereas, the total and yeast and mould counts of the treated samples G₁, G₂ and G₃ was found acceptable up to 56th day of storage at refrigeration temperature.

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