Intl. J. Ferment. Food. 9(1): 01-12, June 2020

DOI: 10.30954/2321-712X.01.2020.1

Spice Oil as Flavour Adjunct in *Bhapa dahi* (Steamed *Dahi*) and its Impact on Shelf-life Extension of Product

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Received: 16-01-2020 **Revised:** 17-04-2020 **Accepted:** 19-05-2020

ABSTRACT

'*Bhapa dahi*' meaning steamed *dahi*/yogurt, is a popular Bengali fermented dairy based delicacy popular in West Bengal. Spices and their extract have been used as food additives to improve the flavor, aroma and even extend the shelf-life of food. Besides these, the spice extract might confer antimicrobial properties and exert therapeutic virtues too. Three types of spice essential oils viz. cinnamon, clove and cumin were tried out as adjunct flavour in mango flavoured *Bhapa dahi* and its influence on the microbial quality with emphasis on Lactic Acid Bacteria (LAB) count and shelf-life of product was studied. It is recommended to utilize clove oil at level of 0.04 % by weight of the base mix (viz., *chakka* and sweetened condensed milk) to prepare mango flavoured *Bhapa dahi*. Clove oil was superior over cinnamon (@ 0.02 % by weight) and cumin oil (@ 0.02 % by weight) as flavour adjunct in mango flavoured *Bhapa dahi*. Use of such additive not only enhanced the sensory score of the fermented product, but also enhanced its shelf-life by about 14 days (vs. control product) when packaged in polypropylene pack and stored under refrigerated condition; the growth of LAB was somewhat restricted in the fermented product during refrigerated storage.

Keywords: *Bhapa dahi*, Lactic acid bacteria, packaging, composition, sensory quality, shelf-life

'*Bhapa Doi*' is a popular Bengali fermented dairy based delicacy prepared at household level in West Bengal. *Bhapa* means steamed and *doi* means *dahi*/yogurt. It is basically steamed sweet *dahi*. So far, the organized dairies have not taken up its commercial production due to lack of published scientific information. Patel *et al.* (2016) had standardized the process of producing mango flavoured *Bhapa dahi* from a base mix comprising of a blend of partly skimmed sweetened condensed milk (SCM) and *chakka*; the recommended proportion of these two ingredients being 1:1; mango pulp was used at level of 18.0 % by weight of base mix. Today Lactic Acid Bacteria (LAB) is a focus of intensive international research for its pivotal role in invariably all fermented dairy foods, possibly exerting therapeutic effects in human subjects.

Spices and herbs have been used as food additives, since ages, to improve the flavor, aroma and even extend the shelf-life of food. Besides these, they may possess medicinal value and might exert

How to cite this article: Chaudhary, A., Jana, A. and Balakrishnan, S. (2020). Spice Oil as Flavour Adjunct in *Bhapa dahi* (Steamed *Dahi*) and its Impact on Shelf-life Extension of Product. *Intl. J. Ferment. Food*, **9**(1): 01-12.

Source of Support: None; **Conflict of Interest:** None

antimicrobial properties (Shelef, 1983; Zaika, 1988). The antibacterial activity of spices differ depending on the type and form (fresh, dried, extracts) of spice used in the food formulation (Nanasombat and Lohasupthawee, 2005). Sesquiterpenes in clove is a potent anti-carcinogenic agent; the antimicrobial component is eugenol (Bullarman *et al.* 1977; Zheng *et al.* 1992). Cinnamicaldehyde is the major antimicrobial compound in cinnamon; it also inhibits mold growth and restricts mycotoxin production (Bullarman *et al.* 1977; Deans and Ritchie, 1987). Cumin contains fatty oil (i.e. petroselic acid) which exerts antimicrobial effect. Cuminaldehyde, carvone, limonene and linalool and even limnonene, eugenol, pinene contributes to the antimicrobial activity of cumin (Derakhshan *et al.* 2008).

Polystyrene and polypropylene materials are commercially used for packaging of *dahi, shrikhand, Misti dahi,* etc. (Pal, 2003). A good oxygen barrier will help to protect the product from oxidation, while a good light barrier will help to avoid light-induced oxidation. The present investigation utilized three types of spice essential oils as a flavour adjunct in the manufacture of mango flavoured *Bhapa dahi,* with possible shelf-life extension. Two types of packaging materials were studied to evaluate the shelf-life of *Bhapa dahi.*

MATERIALS AND METHODS

Ingredients

The dairy ingredients used in the manufacture of *Bhapa dahi* were milk and sweetened condensed milk (SCM). Tea special milk (Amul brand – homogenized milk having 4.6 % fat, 8.5 % SNF) was procured from Amul Shoppe, Anand to prepare *chakka* (i.e. partly drained *dahi*). Partly skimmed SCM (Nestle Co., brand Milkmaid) was procured from Granary Superstore, Anand.

The non-dairy ingredients used were starter culture, mango pulp and spice oil. Lyofast Y170 E Sacco yoghurt culture (freeze dried lactic cultures from M/s. Cadorago Co., Italy) was used. One UC corresponded to use of culture @ 0.015 % by weight of milk; 1 UC could ferment 100 L milk.

Canned Alphonso mango pulp (M/s. Madhu Co., Ahmedabad) was obtained from Anand local market. Clove essential oil (EO) and cinnamon EO were obtained from M/s. Ayruveda Essentials, Indore, Madhya Pradesh, whereas cumin EO was obtained from M/s. Landmark Enterprise, Unjha, Gujarat.

Equipment/Appliances

Hobart mixer (M/s. Hobart, Corporation, Ontario, Canada, Model No. N 50) was used to blend the base mix. The base mix (blend of *chakka*, sweetened condensed milk and mango pulp) was subjected to steaming in a pressure cooker (Make – Marlex, 10 L capacity) to obtain gelled set curd - *Bhapa dahi*.

Manufacture of *Bhapa dahi*

Bhapa dahi was prepared as per the process standardized by Patel *et al.* (2016) with slight modification. The modification entailed varying the steaming period and incorporation of EO of clove, cinnamon and cumin at requisite levels. Use of 7.0 L of 'tea-special milk' yielded on an average 2.12 kg of *chakka* having 26.13 % TS and 5.0 kg of *Bhapa dahi* (45.0 % TS). The quantity of base mix plus flavouring taken for steaming in one lot was 750.0 g.

Incorporation of essential oil of spices in *Bhapa dahi*

Three types of EO of spices (clove @ 0.04, 0.06, 0.08 %; for cinnamon and cumin @ 0.02, 0.04, 0.06 % by weight of base mix) were studied. The experiment was replicated five times. At a time, 5.0 kg of *Bhapa dahi* was prepared.

Packaging materials and protocol of packing

Rigid re-closable transparent polyvinyl chloride (PVC) trays (200 μm thick) and polypropylene (PP) cups (1150 μm thick) with reclosable lids were used for packaging of *Bhapa dahi*. The PVC trays were procured from M/s. Industrial Plastic Forming Co., Mumbai, while PP cups were procured from M/s. Welcome Plastics Co., Anand, Gujarat. Both the packages were subjected to sterilization in the UV chamber (M/s. Philips, UV light - 80W) at a distance of 2.0 cm from the UV light source for 2 h.

Storage of *Bhapa dahi*

The *Bhapa dahi* samples packaged in PVC trays and PP cups were stored in a refrigerator maintained at 7±2°C. The study was conducted up to 35 days, sensory evaluation and chemical analysis was carried out at 7 days interval; some *Bhapa dahi* samples got spoilt earlier than this period.

Analyses of product

The *Bhapa dahi* was analyzed for fat (modified Gerber fat test, Puntambekar, 1968), protein (Jayaraman, 1981), TS (Mojonnier method, Milk Industry Foundation, 1959), ash (BIS, 1989) and acidity (BIS, 1973). The total carbohydrate content of the product was obtained by difference.

Sensory evaluation of product: The 100.0 point score card for *dahi* suggested by Ranganadham and Gupta (1987) was used for scoring *Bhapa dahi.* The product was taken out from refrigerator, tempered at 23±1ºC for 35 min. and then served to ten judges comprising of staff members of Dairy Technology department. All the samples were coded.

MICROBIAL ANALYSIS

The microbial analysis of fresh (0 day) as well as stored (7±2ºC, up to 35 days) *Bhapa dahi* was carried out. The Standard plate count (SPC) was performed utilizing SPC Agar (Wehr and Frank, 2004). The LAB count of the product was performed utilizing Lactic Count Agar (Downes and Ito, 2001). The Yeast and Mold Count (YMC) of product was performed utilizing Potato Dextrose Agar, adjusted to 3.5 pH using 10.0 % sterile tartaric acid solution (APHA, 1992). The coliform count was performed using Violet Red Bile Agar (FSSA, 2012). All the agar used were of Hi Media, Mumbai.

STATISTICAL ANALYSIS

The mean value of each attribute under study obtained from duplicate samples were subjected to statistical analysis using 'Completely Randomized Design' (CRD) with equal number of observations (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Preparation of mango and spice flavoured *Bhapa dahi*

All the ingredients [i.e. *chakka,* partly skimmed sweetened condensed milk (4.20 % fat, 70.55 %TS) and flavouring] were weighed accurately as per the desired formulation. Mango pulp (26.15 %TS, 0.65 % citric acid) was incorporated in the base mix (*chakka* and SCM; 1:1 w/w) at the rate of 18.0 % and essential oil (EO) of spice was added, as per the formulation. Such mixture was blended in a Hobart mixer for 15-20 min. The blended mixture was then filled in stainless steel pail and subjected to steaming in a pressure cooker (without applying weight on cooker lid) for 10.0 min. leading to a set, firm gel (*Bhapa dahi*). The product was cut into pieces and transferred to sterilized package, allowed to cool to room temperature and subsequently shifted to a refrigerator (7±1ºC) till its usage.

Sensory quality of *Bhapa dahi* as influenced by essential oil of spice

The preference for purchase of any food, including fermented food, is based on its sensory acceptability. Such sensory perception stems from the mixed sensation of flavour from compounds produced by fermentation of milk as well as compatible flavourings used, if any. It was the aim of the investigation to find out which out of the three EOs would render the *Bhapa dahi* containing mango pulp, the most acceptable one.

Each EO would impart a flavour, characteristic of the pertinent spice in question. Additionally, the level of EO to be used in product as flavour adjunct would depend on the strength of the flavouring compound present in a specific spice. Hence, there was a need to ascertain the optimal usage level of the specific EO in question.

(I) Sensory scores of Mango flavoured *Bhapa dahi* as influenced by cinnamon oil

Based on the preliminary trials, it was decided to use cinnamon oil as adjunct flavouring at levels of 0.02, 0.04 and 0.06 % by weight of the base mix plus mango pulp.

Flavour: There was a linear decrease in the flavour score of *Bhapa dahi* as the level of cinnamon EO increased from 0.02 to 0.06 %. The flavour score of *Bhapa dahi* made using 0.02 % EO (i.e. 42.36 out of 45.0) was significantly ($P < 0.05$) greater than the scores associated with the remaining three *Bhapa dahi* samples, inclusive of control. The latter three products had flavour scores that was at par with each other (Table 1).

For any spice extract to be used as a food additive, its optimal usage level is necessary; excess quantity would render the product having harsh flavour, while lower level would impart mild flavour. Thabet *et al.* (2014) recommended use of 0.3 % of cinnamon oil as flavouring in *Labneh* concentrated yogurt as compared to use of higher level (i.e. 0.5, 0.8 %).

Body and texture, Color and appearance, Acidity scores: The body and texture score, color and appearance as well as acidity scores of *Bhapa dahi* samples made using three levels of cinnamon EO were at par with each other (Table 1). None of these sensory attributes were affected by the presence of cinnamon oil.

Total sensory score: The total sensory score of *Bhapa dahi* made using cinnamon oil at 0.02 % level was maximum. This showed that presence of such level of cinnamon oil enhanced the sensory perception of the resultant mango flavoured product. Such score was significantly ($P < 0.05$) higher than the scores associated with other three samples, including control. The total sensory score of control and *Bhapa dahi* containing 0.04 % EO was at par with each other (Table 1).

Thabet *et al.* (2014) reported a linear decrease in the total sensory score of *Labneh* with increasing level of essential oil added in product making.

(II) Sensory scores of Mango flavoured *Bhapa dahi* as influenced by clove oil

Use of different essential oil of spice as adjunct flavour in mango flavoured *Bhapa dahi* would have varied impact on the sensory quality of resultant product. Since each spice oil has its characteristic unique flavour, which might or might not be compatible with mango flavour in fermented product.

Table 1: Sensory scores of *Bhapa dahi* as influenced by the presence of cinnamon oil

Sensory attributes	*Bhapa dahi* containing cinnamon oil at levels (%)				C.D. (0.05)
	0	0.02	0.04	0.06	
Flavour (45)	37.78±0.73^{b}	42.36±0.48^{a}	37.55±0.93^{b}	37.50±0.95^{b}	1.07
Body & texture (30)	26.80±0.28	26.80±0.13	26.75±0.11	26.50±0.75	NS
Colour & appearance (10)	8.65±0.16	8.75±0.34	8.72±0.23	8.64±0.33	NS
Acidity (10)	8.17±0.30	8.15±0.21	8.09±0.26	7.99±0.32	NS
Total score$^{\#}$ (100)	86.40±0.85^{b}	91.06±0.75^{a}	86.11±1.05^{bc}	85.63±1.89^{c}	1.64

 # Total score includes score of 15.0 for bacterial quality.

Table 2: Sensory scores of mango flavoured *Bhapa dahi* as influenced by the presence of clove oil

Sensory attributes	*Bhapa dahi* containing clove oil at levels (%)				C.D.(0.05)
	0	0.04	0.06	0.08	
Flavour (45)	37.12±0.82^{c}	38.82±0.74^{b}	43.22±0.17^{a}	35.89±0.23^{d}	0.77
Body & texture (30)	25.58±0.40	25.68±0.72	25.82±0.21	25.42±0.44	NS
Colour & appearance (10)	8.42±0.11	8.63±0.17	8.82±0.28	8.60±0.21	NS
Acidity (10)	8.03±0.33	8.05±0.25	8.26±0.08	7.80±0.19	NS
Total score$^{\#}$ (100)	84.15±0.81^{c}	86.18±1.34^{b}	91.12±0.38^{a}	82.71±0.40^{d}	1.11

 # Total score includes score of 15.0 for bacterial quality.

Flavour: The flavour score of *Bhapa dahi* containing clove EO at level of 0.06 % was highest (43.22 out of 45.00); such score was significantly (P<0.05) superior over the pertinent scores of rest of the samples, including control (Table 2). The flavour score of *Bhapa dahi* containing EO at levels of 0.04 and 0.06 % was significantly (P<0.05) greater than the score associated with control product. *Bhapa dahi* made using maximum level of EO (0.08 %), however, had the least flavour score. Such score was significantly (P<0.05) lower than the flavour score associated with rest of the product samples (Table 2). *Bhapa dahi* containing 0.08 % EO was perceived to be too harsh in flavour, with unpleasant aftertaste and hence had the least flavour score.

The flavour score of *Labneh* containing EO (sage, thyme, marjoram) decreased with an increase in the concentration (i.e. 0.2, 0.5 and 1.0 ppm) of EO; best flavour score was associated with product made using 0.2 ppm (Otaibi and Demerdash, 2008).

Body and texture, Color and appearance and Acidity scores: Since the level of clove oil used was miniscule (i.e. 0.04 to 0.08 %), it failed to exert any marked influence on the colour and appearance, body and texture and even acidity scores of the resultant *Bhapa dahi* samples. The control as well as experimental *Bhapa dahi* samples had identical color and appearance, body and texture as well as acidity scores (Table 2).

Total sensory score: Owing to the superiority in the flavour score of *Bhapa dahi* made using clove EO at 0.06 % level over rest of the samples (Table 2), the total sensory score also exhibited similar trend. The maximum total sensory score (i.e. 91.12 out of 100.0) of such *Bhapa dahi* (clove EO @ 0.06 %) was significantly (P<0.05) higher than the scores associated with the remaining *Bhapa dahi* samples. The total sensory score of *Bhapa dahi* samples, based on the level of EO in product, was in decreasing order as follows: 0.06 % > 0.04 % > control > 0.08 % (Table 2). The difference in the total sensory score between any two *Bhapa dahi* samples was found to be statistically significant (P<0.05).

Based on the above findings, it is recommended to use clove EO at the rate of 0.06 % by weight of base mix plus mango pulp, in the preparation of *Bhapa dahi.* Comparing the sensory score for flavour of *Bhapa dahi* collated in Table 1 and 2, it is clearly evident that presence of clove oil as adjunct flavour (i.e. score of 43.22) was more desirable than use of cinnamon oil (score of 42.36 out of 45.0) at their optimal usage level.

(III) Sensory scores of Mango flavoured *Bhapa dahi* as influenced by cumin oil

As was noted earlier for cinnamon and clove oils, incorporation of cumin oil at three levels (viz., 0.02, 0.04 and 0.06 %) led to marked changes in the sensory scores for flavour as well as total sensory score of the resultant *Bhapa dahi* samples (Table 3).

Flavour: The flavour score (i.e. 42.36 out of 45.0) of *Bhapa dahi* containing 0.02 % cumin EO was highest which differed significantly (P<0.05) from the scores of remaining *Bhapa dahi* samples, including control. However, the flavour score of control and product containing 0.04 % EO was at par with each other; such values were significantly (P<0.05) superior than the value associated with *Bhapa dahi* made using 0.06 % EO (Table 3).

Table 3: Sensory scores of mango flavoured *Bhapa dahi* as influenced by the presence of cumin oil

Sensory attributes	*Bhapa dahi* containing cumin oil at levels (%) of				C.D. (0.05)
	0	0.02	0.04	0.06	
Flavour (45)	38.07+0.73^{b}	40.53+0.53^{a}	38.15+0.12^{b}	37.28+0.21^{c}	0.60
Body & texture (30)	26.60+0.39	25.90+0.59	26.57+0.37	26.45+0.14	NS
Colour & appearance (10)	8.60+0.27	8.64+0.18	8.70+0.32	8.55+0.21	NS
Acidity (10)	8.21+0.49	8.13+0.16	8.10+0.17	7.99±0.16	NS
Total score$^{\#}$ (100)	86.48+1.45^{b}	88.21+0.33^{a}	86.52+0.53^{b}	85.27+0.44^{c}	1.10

 # - Total score includes score of 15.0 for bacterial quality.

There was a linear decrease in the flavour score of *Bhapa dahi* when cumin EO was used at incremental higher levels (≥ 0.04 %). Harsh flavour of cumin oil, including slight unpleasant aftertaste was evident when using higher levels. Such undesirable flavour perception led to decreased flavour score of such pertinent products.

Thabet *et al.* (2014) also reported decrease in the total sensory score of *Labneh* when using cumin oil at incremental higher levels (i.e. 0.3 to 0.8 % by weight). The presence of dill EO and caraway EO, each used at level of 2.0 µL/100 mL, enhanced the taste and odor of salt-free *labneh*; the product had more pronounced flavour and was more refreshing as compared to control product (Zaky *et al.* 2013).

Body and texture, Color and appearance and Acidity scores: Use of three levels of cumin EO in *Bhapa dahi* did not have any marked influence on the color and appearance, body and texture as well as acidity scores of the resultant products (Table 3). Such non-significant effect on the pertinent attributes was anticipated since the level of EO used was quite low.

Total sensory score: The total sensory score (i.e. 91.06 out of 100.0) of *Bhapa dahi* using cumin EO at the least level (i.e. 0.02 %) was maximum. Such score was significantly ($P < 0.05$) higher than the scores associated with rest of the *Bhapa dahi* samples, including control. *Bhapa dahi* prepared using 0.04 % cumin oil had total sensory score that was similar to the score of control product (Table 3).

Looking closely, it is evident that the total sensory score of *Bhapa dahi* followed exactly the same trend as shown by the flavour score. This is obvious since flavour score (maximum score of 45.0) had the highest contribution to the total sensory score of the fermented product (100.0).

Based on the above investigation it is recommended to utilize EO of cinnamon, clove and cumin at levels of 0.02, 0.06 and 0.02 % respectively in the preparation of mango flavoured *Bhapa dahi* with improvement in their sensory acceptability. Since *Bhapa dahi* containing clove EO (@ 0.06 %) had the highest flavour as well as total sensory score from amongst the three EOs used, the *Bhapa dahi* prepared using such EO, packaged in two type of packages, was studied for the changes during refrigerated storage and feasible shelf-life extension.

Composition of *Bhapa dahi*

So far there are no standards laid down by FSSA for *Bhapa dahi*. However, the composition of *Bhapa dahi* has been compared with the standards laid down for *shrikhand* as per FSSA; both the products are prepared utilizing *chakka* as the base material. All the *Bhapa dahi* samples complied with FSSA (2011) requirements for fat-on-dry matter (FDM), protein-on-dry matter (PDM), sugar (sucrose, dry basis) and acidity. However, the *Bhapa dahi* samples did not conform to the requirements laid down for total solids (TS, minimum 58.0 %) and total ash (maximum 0.9 % on dry basis) prescribed for *shrikhand*. However, FSSA (2011) specifies maximum of 3.5 % ash content for *chakka*; *Bhapa dahi* had ash content well below such value. The moisture content of control and experimental *Bhapa dahi* was in the vicinity of 56.00 % (Table 4). The FDM content of all the products was greater than 16.50 % (FDM of minimum 10.0 % is specified for *shrikhand*). Since the level of incorporation of spice EO was very low, such addition did not have any marked influence on any of the constituents of *Bhapa dahi,* except for protein content. The protein content of *Bhapa dahi* containing cumin oil (@ 0.02 %) was higher than the values associated with rest of the product samples owing to the least moisture content associated with such product (Table 4).

Changes in the quality characteristics of *Bhapa dahi* during refrigerated storage

The data presented in Table 5 depicts the changes that occurred in the moisture content of experimental (containing clove oil) and control (without clove oil) *Bhapa dahi* during storage, packaged in two different packaging materials (i.e. PP, PVC). Control *Bhapa dahi* could be stored for only up to 28 days, while product containing clove oil remained acceptable till 35th day of storage.

Table 4: Proximate composition of control and experimental *Bhapa dahi* containing spice oil at their optimum level

Constituents	*Bhapa dahi* prepared using spice oil			
	Control (without spice oil)	Cinnamon @ 0.02%	Clove @ 0.06 %	Cumin @ 0.02%
Moisture, %	56.06	56.15	56.06	55.01
Fat, %	7.34	7.35	7.38	7.45
FDM, %	16.70	16.76	16.79	16.55
Protein, %	8.28	8.29	8.31	9.14
Carbohydrate,%	27.29	27.19	27.23	27.38
Ash, %	1.03	1.02	1.03	1.02
Acidity, % LA	0.91	0.92	0.91	0.91

The values are average of 3 replications.

Table 5: Changes in the moisture content of stored *Bhapa dahi* as influenced by the presence of spice oil and the type of packaging material

Storage period (P, days)	Moisture content (%) of experimental *Bhapa dahi*		Av. for storage period (P)	Moisture content (%) of control *Bhapa dahi*		Av. for storage period (P)
	PP	PVC		PP	PVC	
0	56.11	56.11	56.11^{a}	56.72	56.72	56.72^{a}
7	55.13	53.79	54.46^{b}	54.70	54.52	54.60^{b}
14	53.67	52.92	53.29^{bc}	53.85	52.79	53.31^{cd}
21	52.96	52.22	52.58^{c}	53.20	52.69	52.94^{de}
28	52.39	51.63	52.01^{cd}	52.63	51.57	52.09^{e}
35	52.01	51.23	51.61^{d}	—	—	—
Av. for product (T)	53.71	52.98		54.21	53.65	
Factors	**SEm**	**CD (0.05)**	**CV%**	**SEm**	**CD (0.05)**	**CV%**
P	0.47	1.37		0.360	1.06	
T	0.27	NS	2.16	0.227	NS	1.63
P × T	0.66	NS		0.509	NS	

PP – Polypropylene, PVC – Poly vinyl chloride.

The period of storage (P) had a significant (P<0.05) influence on the moisture content of two types of *Bhapa dahi.* The moisture content of control *Bhapa dahi* showed significant (P<0.05) reduction during first week (0 and 7th day) as well as during second week (7th and 14th day) of storage. In case of experimental *Bhapa dahi,* the decline in the moisture content of product was significant (P<0.05) only during the first week of storage. The change in the moisture content during the entire period of storage was 7.88 and 9.36 % for experimental *Bhapa dahi* stored in PP and PVC respectively; such changes in the values for control *Bhapa dahi* were 7.77 and 9.98 % respectively. The treatment (T, type of packaging) and the interaction P × T failed to exert any marked influence on the moisture content of *Bhapa dahi* (Table 5).

According to Patel *et al.* (2016), the moisture content of stored *Bhapa dahi* tended to decrease with progress of storage; the initial and final moisture content at 24th day of storage was 52.44 and 49.89 % respectively. Kamruzzaman *et al.* (2002) reported that *dahi* samples packaged in plastic cups lost their weight due to surface evaporation of water during its refrigerated storage.

Changes in the titratable acidity of product

The values depicted in Table 6 indicates that there was

Table 6: Changes in the titratable acidity of stored *Bhapa dahi* as affected by the presence of spice oil and the type of packaging material

Storage period (P, days)	Acidity (% LA) of experimental *Bhapa dahi*		Av. for storage period (P)	Acidity (% LA) of control *Bhapa dahi*		Av. for storage period (P)
	PP	PVC		PP	PVC	
0	0.924	0.924	0.924^{f}	0.924^{e}	0.924^{e}	0.924^{e}
7	0.939	0.948	0.943^{e}	0.939^{d}	0.939^{d}	0.939^{d}
14	0.969	0.975	0.972^{d}	0.966^{c}	0.969^{c}	0.967^{c}
21	0.987	0.999	0.993^{c}	0.984^{b}	0.999^{b}	0.987^{b}
28	1.035	1.041	1.038^{b}	1.029^{a}	1.038^{a}	1.033^{a}
35	1.059	1.065	1.062^{a}	—	—	—
Av. for product (T)	0.985	0.992		0.968	0.972	
Factors	**SEm**	**CD (0.05)**	**CV%**	**SEm**	**CD (0.05)**	**CV%**
P	0.004	0.01		0.002	0.006	
T	0.002	NS	1.21	0.001	0.004	0.56
P × T	0.006	NS		0.003	NS	

PP – Polypropylene, PVC – Poly vinyl chloride.

a gradual rise in the titratable acidity of *Bhapa dahi* samples (control as well as experimental) throughout the refrigerated storage period.

The storage period (P) had a significant (P<0.05) influence on the acidity of control as well as experimental *Bhapa dahi*. The increase in the acidity of the two type of *Bhapa dahi* samples was found to be significant (P<0.05) at 7 days interval of storage, till the end of storage. Neither the type of package (T) nor the interaction P × T had any marked effect on the acidity of experimental *Bhapa dahi*. However, the type of packaging material (T) had a significant (P<0.05) influence on the acidity of control *Bhapa dahi*; the product packaged in PP maintained its acidity better than did PVC. Such an increase in the acidity of *Bhapa dahi* (control as well as experimental) at incremental storage period (P) (7 days interval) was found to be significant (P<0.05) at each interval of storage (Table 6).

Unlike the present finding for control *Bhapa dahi*, Arding (2015) did not notice any marked difference in the acidity of strawberry yoghurt packaged in Low Density Poly Ethylene (LDPE) and multi-layered Ethylene Vinyl Alcohol (EVOH) during their refrigerated storage.

The increase in acidity of fermented milk products, even during refrigerated storage is a usual phenomenon, probably due to continued lactic fermentation by the starter and non-starter flora present in the product. Patel *et al.* (2016) also noted an increase in the acidity of *Bhapa dahi* stored for up to 24 days kept in stainless steel container. The acidity value noted for *Bhapa dahi* when fresh and stored (refrigeration) for 24 days was 1.05 and 1.15 % lactic acid respectively. Yeganehzad *et al.* (2007) and Dimitrova and Kartalska (2015) reported a marked increase in the acidity of yoghurt during refrigerated storage of up to 3 weeks.

Changes in the total sensory score of *Bhapa dahi*

As was evident for most of the individual sensory scores (i.e. flavour, body and texture - not shown here), the total sensory score also showed a linear decrease throughout the refrigerated storage in case of control and experimental *Bhapa dahi* samples. The period of storage (P) had a significant (P<0.05) influence on the total sensory score of two types of *Bhapa dahi*. The type of packaging material (T) as well as interaction P × T did not show any marked influence on the total sensory score of the two *Bhapa dahi* samples (Table 7). Based on sensory acceptability, the experimental

Table 7: Changes in the total sensory score (out of 100.0) of stored *Bhapa dahi* as affected by the presence of spice oil and the type of packaging material

Storage period (P, days)	Total score (out of 100) of experimental product		Av. for storage period (P)	Total score (out of 100) of control product		Av. for storage period (P)
	PP	PVC		PP	PVC	
0	90.01	90.19	90.10[a]	87.01	87.20	87.10[a]
7	87.38	86.72	87.05[bc]	86.15	86.10	86.12[a]
14	85.96	85.41	85.68[c]	85.89	85.06	85.47[a]
21	81.99	82.70	82.34[d]	77.73	76.65	77.18[b]
28	78.94	78.18	78.56[e]	75.43	73.40	74.41[b]
35	76.28	75.32	75.79[f]	—	—	—
Av. for product(T)	83.42	83.08		82.44	81.68	
Factors	**SEm**	**CD (0.05)**	**CV%**	**SEm**	**CD (0.05)**	**CV%**
P	0.543	1.58		1.615	4.76	
T	0.314	NS	1.60	1.021	NS	4.82
P × T	0.769	NS		2.284	NS	

PP – Polypropylene, PVC – Poly vinyl chloride.

Bhapa dahi (containing clove oil) and control *Bhapa dahi* had shelf-life of 35 and 28 days respectively.

In case of experimental *Bhapa dahi*, except for similar total sensory score observed on 7th and 14th day of storage, the decline in scores at other successive intervals was found to be statistically significant (P<0.05). However, in case of control *Bhapa dahi*, the decline in total sensory score was non-significant up to 14th day of storage. Subsequently, the decline in the score was found to be significant (P<0.05) up to 21st day, after which the score remained fairly constant (i.e. till 28th day). The decrease in the total sensory score of control and experimental *Bhapa dahi* during the entire span of study was to the tune of 17.05 and 18.88 % respectively (Table 7).

A decline in the sensory scores of mango flavoured *Bhapa dahi* has also been reported by Patel *et al.* (2016). The total sensory score (out of 100.0) of *Bhapa dahi* when fresh and on 24th day of refrigerated storage was 94.17 and 69.00 respectively. Such decline in the total sensory score was due to decrease in the flavour score; such pertinent scores (out of 45.0) were 43.29 when fresh and 30.07 at the end of storage period.

The total sensory score of control as well as experimental *Labneh* (23.4 % TS) containing 0.3 % of cinnamon oil decreased gradually up to 24th day of refrigerated (6^{o}C) storage (Thabet *et al.* 2014). The salt-free *Labneh* containing added dill/caraway EO (2.0 µL/100 mL) could be satisfactorily stored at 7^{o}C for up to 28 days (Zaky *et al.*, 2013).

Changes in the microbial count of stored *Bhapa dahi*

In general, excessive Standard Plate Count (SPC) is derogatory to the microbial quality of dairy foods (e.g. milk and milk products). However, in case of fermented dairy foods like *dahi*/yoghurt, *Labneh*, *Misti dahi*, *Shrikhand*, *Bhapa dahi*, etc. survival of LAB as well as probiotic bacteria in high numbers (> 10^6/g of product) is highly desirable (Yadav *et al.* 2007). Hence, under such circumstances, the SPC of fermented dairy foods would exceed the LAB count of such food. Presence of high count of SPC in *Bhapa dahi* would be indicative of higher LAB count (if strict hygiene has been exercised), which is a desirable feature for this product in terms of therapeutic virtues including gut health (Soomro *et al.* 2002).

Lactic Acid Bacteria: The period of refrigerated storage had a significant (P<0.05) influence on the LAB count of control as well as experimental *Bhapa dahi* samples. In case of experimental *Bhapa dahi*, initially there was a marginal increase in the LAB count up to 14th day; thereafter there was a significant (P<0.05) decline in such count till 21st day, followed by

Table 8: Lactic acid bacteria count of stored *Bhapa dahi* as influenced by presence of clove oil and type of package

Storage period (P, days)	LAB count (log_{10}cfu/g) of experimental product		Av. for storage period (P)	LAB count (log_{10}cfu/g) of control product		Av. for storage period (P)
	PP	PVC		PP	PVC	
0	9.73	9.75	9.74^{bc}	9.86	9.89	9.87^{b}
7	9.80	9.82	9.81^{ab}	10.02	10.01	10.01^{a}
14	9.88	9.92	9.90^{a}	10.16	10.13	10.14^{a}
21	9.65	9.52	9.58^{cd}	9.82	9.75	9.78^{b}
28	9.51	9.35	9.43^{de}	9.59	9.61	9.60^{c}
35	9.35	9.25	9.30^{e}	—	—	—
Av. for product(T)	9.65	9.60		9.89	9.88	
Factors	**SEm**	**CD (0.05)**	**CV%**	**SEm**	**CD (0.05)**	**CV%**
P	0.070	0.20		0.047	0.13	
T	0.040	NS	1.79	0.029	NS	1.17
P × T	0.099	NS		0.067	NS	

PP – Polypropylene, PVC – Poly vinyl chloride.

a gradual decrease in count up to 28th day. However, towards the end of storage period (i.e. between 28th to 35th day) there was a marked (P<0.05) decline in the LAB count; the count was 9.30 log_{10}cfu/g at 35th day of storage as compared to log count of 9.74 per g in the fresh product (Table 8). In case of control *Bhapa dahi* also, initial storage period registered a significant (P<0.05) increase in LAB count till 7th day; such high count continued to remain so till 14th day (Table 8). Further storage resulted in significant (P<0.05) decrease in LAB count of product till the end of storage (i.e. 28th day). This implied that the initial increase (14 days period) in LAB count (as log_{10}cfu/g) of control and experimental *Bhapa dahi* was 2.74 and 1.64 % respectively; similarly the decline in the LAB count (log_{10} cfu/g) from 14th day onwards was 5.62 and 6.45% respectively for control and experimental *Bhapa dahi.*

Standard Plate Count: The period of refrigerated storage had a significant (P<0.05) influence on the SPC of control as well as experimental *Bhapa dahi*. In case of experimental *Bhapa dahi*, initially there was a marginal increase in the SPC up to 14th day; thereafter there was a significant (P<0.05) decrease in SPC count till 21st day. With further storage, there was a gradual decrease in the SPC up to 28th day. However, towards the end of storage period (i.e. between 28th to 35th day) there was a significant (P<0.05) decline in the SPC; the count was 9.80 log_{10}cfu/g at this period compared to initial count of 10.17 log_{10}cfu/g (Table 9). In case of control *Bhapa dahi* also, initial storage period registered a significant (P<0.05) increase in SPC till 7th day; such high count remained stable till 14th day (Table 9). Further storage resulted in significant (P<0.05) decrease in SPC of product till the end of storage (i.e. 28th day). This implied that the initial increase in SPC (log cfu/g) of control and experimental *Bhapa dahi* was 2.41 and 2.26 % respectively; similarly the decline in the SPC (log cfu/g) from 14th day onwards was 7.72 and 6.12 % respectively (Table 9).

The type of packaging materials (T) did not have any marked influence on the SPC of two types of *Bhapa dahi.* However, the interaction P × T had a significant (P<0.05) influence only in case of experimental *Bhapa dahi*. At 21st day of storage for experimental *Bhapa dahi*, PP was found to be bacteriologically superior over PVC as packaging material (log count of SPC was greater for PP; higher LAB count led to higher SPC). In case of control *Bhapa dahi*, the interaction P × T did not have any marked influence on the SPC of product (Table 9).

Table 9: Standard Plate Count of stored *Bhapa dahi* as influenced by the presence of clove oil and the type of package

Storage period (P, days)	SPC (log_{10}cfu/g) of experimental *Bhapa dahi*		Av. for storage period (P)	SPC (log_{10}cfu/g) of control *Bhapa dahi*		Av. for storage period (P)
	PP	PVC		PP	PVC	
0	10.10	10.24	10.17^{bc}	10.35	10.36	10.35^{b}
7	10.23	10.34	10.28^{ab}	10.47	10.49	10.48^{a}
14	10.35	10.45	10.40^{a}	10.58	10.63	10.60^{a}
21	10.15	9.95	10.05^{c}	10.06	10.01	10.03^{c}
28	10.04	9.88	9.96^{c}	9.88	9.82	9.84^{d}
35	9.89	9.72	9.80^{d}	—	—	—
Av. for product(T)	10.12	10.09		10.27	10.26	
Factors	**SEm**	**CD (0.05)**	**CV%**	**SEm**	**CD (0.05)**	**CV%**
P	0.044	0.13		0.043	0.12	
T	0.025	NS	1.08	0.027	NS	1.04
P × T	0.063	0.18		0.061	NS	

PP – Polypropylene, PVC – Poly vinyl chloride.

The SPC of control as well as cinnamon oil (0.3 %) containing *Labneh* (both having 23.4 per cent TS) increased up to 8th day of refrigerated (6°C) storage and thereafter it showed a decline in the count up to 24th day. The initial increase in the SPC (expressed as log cfu/g) till 8th day of storage was to the tune of 0.89 and 1.00 % respectively in case of control and spice containing *Labneh* (Thabet *et al.* 2014). Likewise, Otaibi and Demerdash (2008) noted an initial increase in SPC of control *Labneh* as well as *Labneh* containing thyme oil (0.2 ppm) (23.3 % TS) up to 7th day of refrigerated (5°C) storage; extended storage period up to 21st day led to a decline in the SPC in both the product. The decline in the SPC, counted from 7th to 21st day of storage, was 13.42 and 14.38 % respectively for control and experimental *Labneh*.

Fig. 1: Photograph of packaged mango flavoured *Bhapa dahi* incorporating spice oil

CONCLUSION

Based on the result of present investigation, it is recommended to utilize essential oil of clove at level of 0.06 % by weight of the base mix plus mango flavouring for producing mango flavoured *Bhapa dahi*. The presence of clove oil served as a flavour enhancer for mango flavoured product and aided in shelf-life extension. The LAB count of *Bhapa dahi* containing clove oil was somewhat lower than that of control product, when fresh and during refrigerated storage. The experimental *Bhapa dahi* containing clove oil and control product remained sensorily acceptable up to 35 and 28 days respectively at refrigerated (6°C) storage condition. The essential oil of clove was found to be superior over use of cumin or cinnamon essential oils in producing *Bhapa dahi*.

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