

Original Research Article

<https://doi.org/10.20546/ijcmas.2020.905.137>

Study of Formulation, Sensory Evaluation and Microbiological Study of Camel and Buffalo Milk based Khoa Burfi Blended with Watermelon Seeds

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ABSTRACT

Keywords

Khoa, Burfi,
Sensory Properties,
Standard Plate
Count, Yeast and
Mould Count,
Coliform Count

Article Info

Accepted:

10 April 2020

Available Online:

10 May 2020

The present study was aimed to estimate the sensory properties and microbiological study of camel and buffalo milk based khoa burfi. Four treatment samples were developed by using different combination with watermelon seeds in selected camel and buffalo milk khoa viz. Control (without watermelon seeds incorporation) and treatments T1, T2 and T3 with 10%, 20% and 30% watermelon seeds incorporation respectively. On the basis of sensory scores, the khoa burfi prepared by incorporation of 10% watermelon seeds level scored maximum for all the sensory attributes such as appearance, color, flavor, taste, overall acceptability and was selected for pursuing the storage studies. The selected camel and buffalo milk khoa burfi were subjected to refrigerated ($4 \pm 1^\circ\text{C}$) storage temperature and quality characteristics were evaluated at every 3 days interval upto a period of 15 days. The microbial load of standard plate count, increased significantly high ($P < 0.01$) in the control and all treatment khoa burfi whereas the initial yeast and mould count was nil up to 6th day of storage than increased significantly ($P < 0.05$). No coliform counts were observed.

Introduction

In the western world, camel milk is experiencing a novel awareness in these days and even the FAO has stepped in promoting

camel milk (Ramet, 2001). Camel milk is considered to have anti-cancer (Magjeed, 2005), hypo-allergic (Shabo *et al.*, 2005) and anti-diabetic properties (Agrawal *et al.*, 2003). High content of unsaturated fatty acids

contributes to its overall dietary quality (Karray *et al.*, 2005; Konuspayeva *et al.*, 2008). Camel milk is rich in chloride. Chlorides contents ranged between 0.20 and 0.28 g per 100 g, respectively and the mean value (g per 100 g) was 0.26 ± 0.01 for chlorides (Khaskheli *et al.*, 2005).

Buffalo milk has a high fat content and can be preserved naturally for longer periods due to high peroxidase activity. Buffalo milk contains more calcium, better calcium: phosphorous ratio and less sodium and potassium compared to cow milk, making it a better nutritional supplement for infants. Buffalo milk is preferred for the preparation of western and traditional (indigenous) milk and dairy products and is superior in nutritional terms.

In India 46 per cent of total milk production consumed as liquid milk and 54 per cent is converted into milk products (www.nddb.org/statistics/milkproduction).

Amongst the traditional milk products, khoa is an important indigenous heat coagulated, partially dehydrated milk product, popular in large section of population throughout the country. The chemical composition of khoa include 20-25% humidity, 25-37% fat, 17-20% protein, 22-25% lactose, 3.6-3.8% ash and 100-103 ppm iron depending on whether it is made from cow, buffalo or mixed milk (Moulick and Ghatak, 1997). It contains relatively large amounts of building proteins, bone forming minerals and energy giving fat and lactose. Most fat-soluble vitamins A, D, E and K are also expected to be retained. Above all, milk conversion to Khoa is the best milk preservation method for a relatively longer period of time without the use of any natural or chemical preservatives.

In India, burfi is most popular khoa based milk sweet, white to light cream in colour with firm body and smooth to granular

texture. Burfi was prepared by many research workers using various fruits like ber (Kathalkar, 1995), papaya and sapota (Khedkar *et al.*, 2007), mango (Kadam *et al.*, 2009), orange (Thaware *et al.*, 2009), fig (Matkar & Deshmukh, 2007) etc. These fruits enhance the acceptability of burfi to the masses as well as choosy classes. Other ingredients are also incorporated in different proportions to meet the special needs of flavor, body and texture.

Watermelon (*Citrullus lanatus*) being a very famous fruit in Rajasthan, refreshing and diuretic properties of its red flesh present inside, together with its pleasant taste, make it a popular choice for producing juices and salads or for vegetable and raita making. The watermelon contains important carotenoids such as β -carotene, carotene and Lycopene which are important in neutralizing free radicals in the body (Oseni & Okoye, 2013), high in proteins and fats and can find applications as a protein source in various food formulations and preparation (El-Adway & Taha, 2001). This fruit is a rich natural source of lycopene (Perkins-Veazie *et al.*, 2001). Intake of lycopene containing-products has been associated with a reduced incidence of coronary heart disease and some types of cancer (Giovannucci, 2002). Watermelon seeds are a good source of low-molecular-weight polypeptides i.e. globulin, glutenin and albumin. Seeds are also rich in aspartic acid, glutamic acid and serine (Tabiri *et al.*, 2016).

Nowadays, incorporation of fruit seeds in Khoa Burfi is gaining popularity amongst consumers due to typical, highly liked flavour and nutritional value. A new range of product in dairy industry, value addition as a supplement of different nutrients and high impact of growth and immune protective on the health of consumer are the advantages of developing this type of product.

Therefore, an attempt was made to manufacture Khoa Burfi from Camel and Buffalo milk. However, a very little work has been reported about use of Watermelon Seed in value addition of Khoa Burfi. Considering these above facts in view the present research work was planned with the specific objectives to value addition of Khoa Burfi using Watermelon Seeds as growth and immune protective additive.

Materials and Methods

Material collection and sample preparation

Fresh camel milk was collected from camel dairy maintained at ICAR-NRC on Camel, Bikaner and fresh buffalo milk was collected from buffaloes maintained under the 'Buffalo Unit' of Dept. of LPT, CVAS, RAJUVAS, Bikaner. All samples were collected manually in sterile bottles and were kept under chilled condition to perform the different experiments.

Formation and accessibility of camel and buffalo milk based khoa burfi blended with or without watermelon seeds

Formation of khoa was done by using different ratio of camel and buffalo milk. Best result was obtained on the basis of high yield, consistency of khoa and low cost of production by combination of 50% camel milk and 50 % buffalo milk. On the basis of evaluation for quality parameters like yield, consistency of khoa, cost of production, sensory evaluation and physico-chemical characteristics, optimum ratio of admixture of camel and buffalo milk was determined.

Burfi was prepared as per the method described by Reddy (1985). Received milk was preheated at 35-40°C before filtration. Then milk was filtered in order to remove the visible dust and dirt particle. The process

involved standardization of camel and buffalo mixed milk to 6 per cent fat and 9 per cent SNF, taken in an iron karahi and heated on gentle fire. At the time of boiling, milk was stirred with the help of a khunti in a circular manner. The stirring-cum-scrapping process was continued till a pasty consistency was reached. Then temperature was lowered upto 77-79°C. At this stage, watermelon seeds as per treatment and sugar @ 30 per cent of khoa were added. Finally this mixture was heated on a low fire with stirring till the desired texture was obtained. It was then spread in a tray and allowed to cool. After setting, camel and buffalo milk khoa based watermelon seeds burfi was cut into rectangular blocks and stored at refrigeration ($4 \pm 1^\circ\text{C}$) followed by packaging.

Product development

Various levels of watermelon seeds powder incorporated camel and buffalo milk based khoa burfi by inclusion of 10% watermelon seeds powder, 20% watermelon seeds powder and 30% watermelon seeds powder were used for preparation of treatment burfi under investigation.

T₀ – 100 parts of buffalo and camel milk khoa by weight + 0 Parts of

Watermelon seeds powder,

T₁ – 90 parts of buffalo and camel milk khoa by weight + 10 Parts of

Watermelon seeds powder,

T₂ – 80 parts of buffalo and camel milk khoa by weight + 20 Parts of

Watermelon seeds powder,

T₃ – 70 parts of buffalo and camel milk khoa by weight + 30 Parts of

Watermelon seeds powder.

Sensory evaluation

The samples of khoa burfi were subjected to sensory evaluation on 8 point hedonic scale

by a panel of eight semi-trained members from academic staff and students of the department for various sensory attributes viz., appearance & colour, flavour, body & texture and overall acceptability using 8 point descriptive scale where '8' denotes 'Excellent' and '1' denotes 'extremely poor'. Khoa burfi samples were presented in plastic plates. All samples were marked with digital code, and the order of presentation of samples was randomized for each panelist.

Microbial tests

All samples were assessed for microbial status, i.e. standard plate counts, coliform count and yeast and mould count as per standard procedures. The drawing of the representative sample of the khoa burfi and its preparation for the microbiological examination was carried out under the standard procedure.

Standard plate count

The standard plate counts of khoa burfi samples was evaluated by using method described in IS: 5402 (1969). The 11 g of khoa burfi sample aseptically weighed and transferred into a sterile 99 ml dilution blank and mixed well. The samples were properly diluted by serial dilution by using 9 ml phosphate buffer. Then from 2nd, 3rd and 4th dilution of khoa burfi samples were used for plating.

One ml diluent from each sample was taken in duplicate into the sterile petriplates with the help of sterile pipettes. Then the standard plate count agar media was added to these Petriplates and properly rotated so as to mix the content well. The plates were allowed to solidify. All the solidified plates were incubated at 37°C for 48 hrs in an incubator in an inverted position and the number of colonies developed was recorded as cfu/g.

Coliform counts

The serial dilutions prepared for standard plate count were used for coliform count. The diluents from 1st and 2nd dilutions of khoa burfi samples were used for plating. The 1 ml diluents from each was taken in duplicate in petriplates and then 10-15 ml violet red bile agar media was added and mixed well. The plates were allowed to solidify. The plates were again overlaid with the same violet red bile agar media and allowed to solidify. Then the plates were incubated at 37°C for 24 hrs in an incubator. The number of coliform colonies was recorded as cfu/g. The coliform colonies were with dark red centered and pinkish periphery.

Yeast and mould count

The yeast and mould count of khoa burfi samples were determined by using method described in IS: 5403 (1969). The serial dilutions prepared for standard plate count were used for enumeration of yeast and mould count. The diluents from 1st and 2nd dilutions of khoa burfi samples were used for plating. One ml each was taken in duplicate in petriplates and the Potato Dextrose Agar (PDA) was used by adjusting pH 3.5 by using 10% sterilized tartaric acid solution. After solidification, the agar plates were incubated at 25°C for 5 days. At the end of incubation period count of the colonies of yeast and mould were recorded as cfu/g.

Statistical analysis

All the experiments of study were repeated three times and samples were drawn in duplicate. Data collected during the present investigation were subjected to statistical analysis by adopting appropriate methods of analysis of variance as described by Snedecor and Cochran (1994).

Wherever, the variance ratio were found significant at 5 per cent and highly significant at 1 per cent levels of probability, the significance of mean differences were tested by Duncan's New Multiple Range Test (Duncan's Range Test) as modified by Kramer (1957).

Results and Discussion

Sensory evaluation

The result of sensory evaluation of camel and buffalo milk based khoa burfi blended with watermelon seeds have been presented in Table 1 and figure 2. The result of sensory evaluation for different treatments in Table 1 indicate that the panelists, on average, prefer the treatment T₁ (10% watermelon seed powder incorporated camel and buffalo milk khoa burfi) for appearance/colour, flavor, body/texture and overall acceptability.

A highly significant difference ($p < 0.01$) was observed between samples for flavor and overall acceptability whereas a significant difference ($p < 0.05$) was observed for appearance and colour except body and texture which was found to be non-significant as shown in Table 2. Thus it may be concluded that the different levels of watermelon seeds significantly affect the all sensory quality of camel and buffalo milk based khoa burfi except body and texture.

The results of sensory evaluation in present study are in accordance with the results reported for sweet orange burfi (Wadewale, 2010), date burfi (Pawar, 2011), ash gourd burfi (Nikam, 2012), pineapple burfi (Bankar *et al.*, 2013), figure millet burfi (Kapare, 2017) and green peas burfi (Lahankar, 2017) in which 10% incorporation of respective ingredient was selected as best with respect to other treatments.

Microbial analysis

Most of the physico-chemical changes like acidity development, change in pH etc., are affected by the presence and growth of various microorganisms. Therefore the stored samples of khoa burfi were subjected to microbiological analysis for standard plate count (SPC), yeast and mould count (YMC) and coliform count. The changes observed in microbial quality of the khoa burfi, prepared with or without incorporation of watermelon seeds powder during assessment at a regular interval of 3 days under refrigerated temp ($4 \pm 10C$) during storage study have been presented for standard plate counts and yeast and mould counts under refrigerated storage condition for 0, 3, 6, 9,12 and 15 days. No coliform counts were observed during this storage duration.

Standard plate count

The major spoilage of khoa burfi is due to the growth of microbes. Hence, the SPC of khoa burfi samples were studied. The data related to standard plate count (SPC) of samples have been shown in Table 3 and depicted in figure 3.

During storage of khoa burfi at refrigerated temperature, a highly significant ($P \leq 0.01$) increase in SPC up to 15th day was observed and there after the product was found unacceptable due to visible mould growth.

The recorded results are in concord with the findings of Palit and Pal (2005) for burfi, Prabha (2006) for dietetic burfi and Londhe (2006) for peda. Increase in SPC of burfi samples during storage had been also reported by several other researchers viz. Sachdeva and Rajorhia (1982), Bhatele (1983), Reddy (1985), Mandokhot and Garg (1985), Mishra and Kuila (1988) in various other products as well.

However, Kumar *et al.*, (1997) did not observe any microbial growth in peda packaged under modified atmosphere packaging (MAP).

The data related analysis of variance for standard plate count of camel and buffalo milk khoa burfi revealed a highly significant difference ($P < 0.01$) for between treatment and between period whereas non-significant difference for interaction between treatment and period was observed as shown table 4.

Yeast and mould count

For most of the intermediate moisture Indian dairy foods such as Peda, Burfi, Kalakand, etc. mould growth tends to be a major problem and often most important single factor limiting their shelf life. Hence, yeast and mould counts were studied. The mean \pm SE values of yeast and mould counts of samples and storage periods has been presented in the table 5 and figure 4 whereas analysis of variance in table 6.

The numbers of the fungal colonies obtained during present investigation are similar to various workers who had analyzed the milk products like Peda, Burfi and Kalakand (Biradar *et al.*, 1985), Dwarkanath and

Srikanta (1977), Sachdeva and Rajorhia (1982) who reported increase in yeast and mould count during storage of burfi at $30 \pm 2^\circ\text{C}$ and $7 \pm 2^\circ\text{C}$.

The data related to analysis of variance for yeast and mould count of camel and buffalo milk khoa burfi, presented in table 6 revealed a highly significant difference ($P < 0.01$) that was observed between period and between treatment but the interaction between treatment and period was non-significant for yeast and mould count.

Coliform count

The growth of coliform count shows unhygienic production of the khoa burfi. Hence, the coliform counts of khoa burfi samples were studied. The product was found to be free from coliforms and during storage period 0th, 3rd, 6th, 9th, 12th and 15th day of refrigerated storage there was no coliform count observed. Similar studies were conducted to evaluate the coliform count and no coliform was reported by other workers (Gupta *et al.*, (2010), Venkata *et al.*, (2017) and Vasava *et al.*, (2018)). The coliform count of burfi was 1.61×10^4 cfu/gm observed (Dwarkanath and Srikanta, 1977).

Table.1 Effect of various levels of watermelon seed on sensory quality of camel and buffalo milk burfi (mean \pm SE)

Type of khoa	Flavour	Body and texture	Appearance and colour	Overall acceptability
T ₀	7.0 ^b \pm 0.408	6.5 \pm 0.289	7.0 ^{ab} \pm 0.408	6.84 ^{bc} \pm 0.207
T ₁	7.5 ^b \pm 0.289	7.0 \pm 0.408	7.5 ^b \pm 0.289	7.34 ^c \pm 0.188
T ₂	6.5 ^b \pm 0.289	6.0 \pm 0.408	6.5 ^{ab} \pm 0.289	6.34 ^b \pm 0.188
T ₃	5.5 ^a \pm 0.289	5.8 \pm 0.479	6.0 ^a \pm 0.408	5.75 ^a \pm 0.218

Note– Means bearing different superscript in a column (small letter) differ significantly.

Table.2 Analysis of variance of sensory quality (between treatments) for camel and buffalo milk based khoa burfi blended with watermelon seeds

Parameter	D.F.	Mean square	Level of sig.
Flavour	3	2.9166	S**
Body and texture	3	1.2291	NS
Appearance and colour	3	1.6666	S*
Overall acceptability	3	5.520833	S**

** = Highly Significant (P<0.01), * = Significant (P<0.05) and NS = Non-significant

Table.3 Standard plate count log (cfu/g) (mean ± SE) of camel and buffalo milk khoa burfi

Day	T ₀	T ₁	T ₂	T ₃	Overall
Day 0	3.72 ± 0.041	3.76 ± 0.028	3.82 ± 0.101	3.97 ± 0.08	3.81^a ± 0.042
Day 3	3.79 ± 0.089	3.86 ± 0.025	3.91 ± 0.063	4.06 ± 0.117	3.91^{ab} ± 0.046
Day 6	3.91 ± 0.087	3.95 ± 0.013	4.01 ± 0.122	4.14 ± 0.083	4.00^{bc} ± 0.047
Day 9	4.01 ± 0.108	4.06 ± 0.068	4.12 ± 0.126	4.22 ± 0.122	4.10^{cd} ± 0.054
Day 12	4.12 ± 0.103	4.15 ± 0.062	4.21 ± 0.071	4.30 ± 0.109	4.20^{de} ± 0.046
Day 15	4.17 ± 0.052	4.22 ± 0.109	4.25 ± 0.120	4.34 ± 0.084	4.25^e ± 0.045
Overall	3.95^A ± 0.052	4.00^{AB} ± 0.044	4.05^B ± 0.052	4.17^C ± 0.047	

Note:- A- Means bearing different superscript in a column (small letter) and in a row (capital letter) differ significantly. T₀– camel and buffalo milk khoa without any seed powder incorporation, T₁ – camel and buffalo milk khoa with watermelon seed powder (10%), T₂ – camel and buffalo milk khoa with watermelon seed powder (20%), T₃ – camel and buffalo milk khoa with watermelon seed powder (30%)

Table.4 Analysis of variance for SPC (Standard plate count)

Source	D.F.	Mean square	Level of sig.
Between period	5	0.335313	S**
Between treatment	3	0.172596	S**
Interaction between treatment and period	15	0.000915	NS
Error	48	0.025509	

** = Highly Significant (P<0.01), * = Significant (P<0.05) and NS = Non-significant

Table.5 Yeast and mould count log (cfu/g) (mean ± SE) of camel and buffalo milk based khoa burfi blended with watermelon seeds

Day	T ₀	T ₁	T ₂	T ₃	Overall
Day 0 to 6	NIL	NIL	NIL	NIL	0^a
Day 9	0.73 ± 0.039	0.75 ± 0.026	0.82 ± 0.057	0.91 ± 0.056	0.80^b ± 0.029
Day 12	0.86 ± 0.028	0.88 ± 0.042	0.93 ± 0.047	0.99 ± 0.054	0.92^c ± 0.024
Day 15	0.92 ± 0.017	0.98 ± 0.015	1.03 ± 0.026	1.08 ± 0.107	1.00^d ± 0.03
Overall	0.42^A ± 0.103	0.44^{AB} ± 0.107	0.46^{BC} ± 0.114	0.50^C ± 0.123	

Note:- A- Means bearing different superscript in a column (small letter) and in a row (capital letter) differ significantly.

Table.6 Analysis of variance for Yeast and mould

Source	D.F.	Mean square	Level of sig.
Between period	5	3.018224	S**
Between treatment	3	0.02156	S**
Interaction between treatment and period	15	0.00464	NS
Error	48	0.003591	

** = Highly Significant (P<0.01), * = Significant (P<0.05) and NS = Non-significant

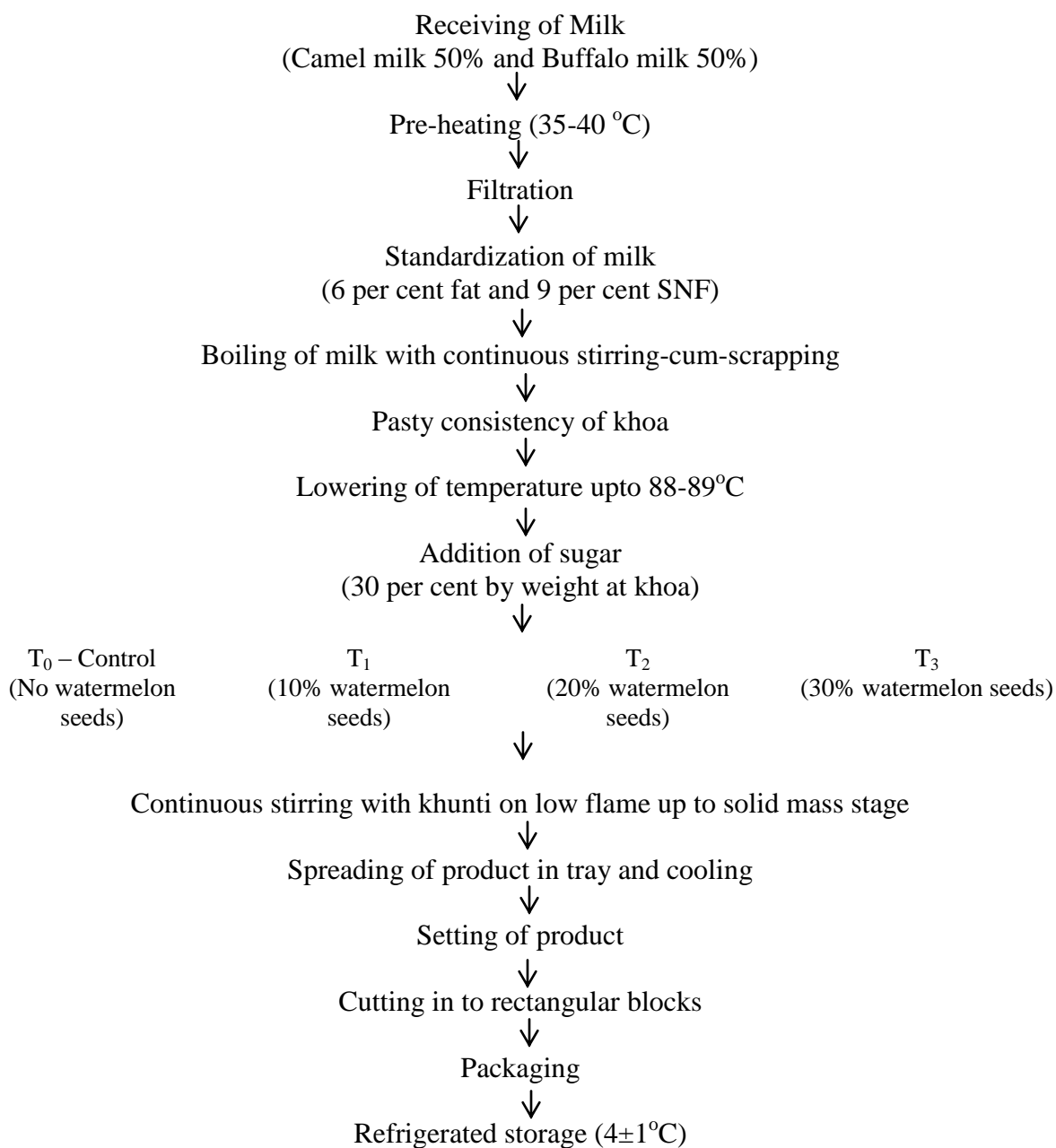


Figure.1 Flow diagram for preparation of camel and buffalo milk based khoa burfi blended with or without watermelon seeds

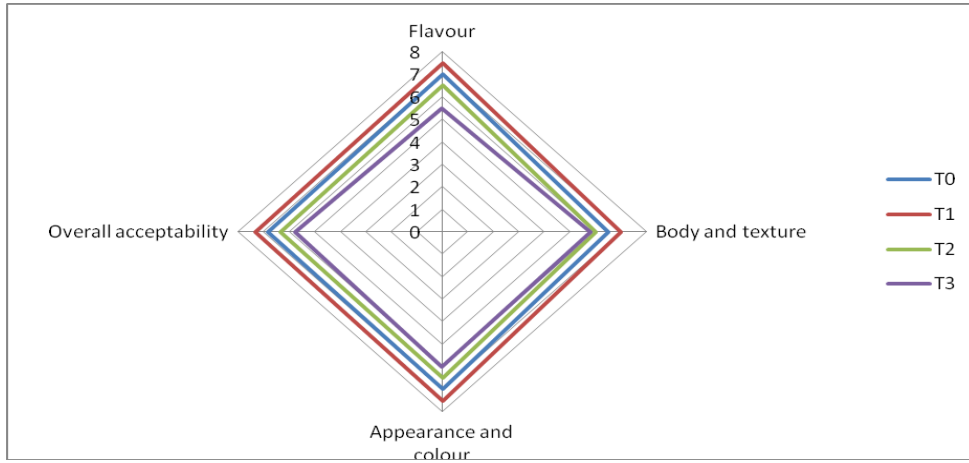


Figure.2 Effect of various levels of watermelon seed on sensory quality of camel and buffalo milk burfi

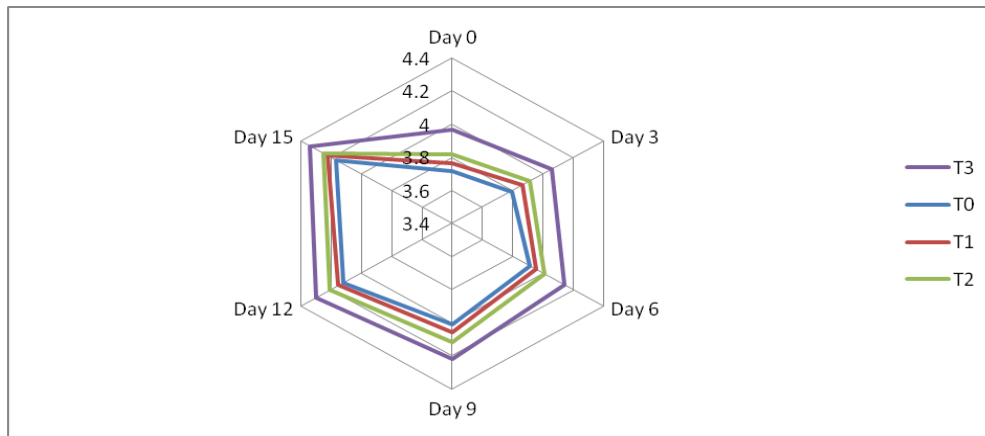


Figure.3 Standard plate count of camel and buffalo milk based khoa burfi blended with watermelon seeds

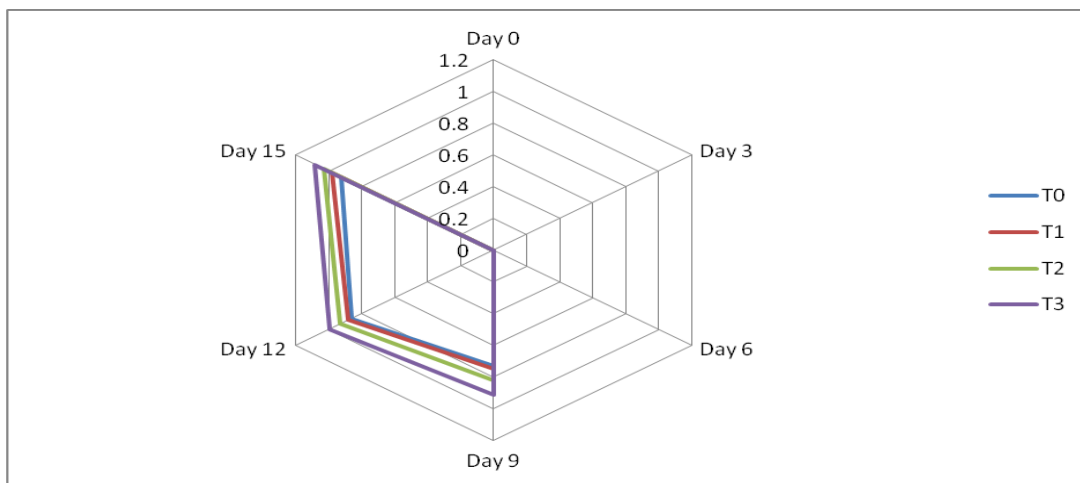


Figure.4 Yeast and mould count of camel and buffalo milk based khoa burfi blended with watermelon seeds

Thus from the present study it may be concluded that the inclusion of watermelon seeds enhanced the sensory quality (flavour, color/appearance and overall acceptability) and overall acceptability of camel and buffalo milk khoa burfi.

It also concluded that watermelon seeds incorporated khoa burfi significant increase in nutritional properties and consumption of watermelon seeds as an adjunct in khoa based products will positively benefit the consumers.

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How to cite this article:

Jorawar Singh, Basant Bais, Rakesh Ranjan, Rajani Joshi, Diwakar, Parma Ram and Ajay Sharma. 2020. Study of Formulation, Sensory Evaluation and Microbiological Study of Camel and Buffalo Milk based Khoa Burfi Blended with Watermelon Seeds. *Int.J.Curr.Microbiol.App.Sci.* 9(05): 1231-1242. doi: <https://doi.org/10.20546/ijcmas.2020.905.137>