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#### Kakan AV

Department of Dairy  
 Technology, College of Dairy  
 Technology, Warud Pusad,  
 Maharashtra Animal & Fishery  
 Sciences University, Nagpur,  
 Maharashtra, India

#### Changade SP

Department of Dairy  
 Technology, College of Dairy  
 Technology, Warud Pusad,  
 Maharashtra Animal & Fishery  
 Sciences University, Nagpur,  
 Maharashtra, India

#### Shaikh Adil

Department of Dairy  
 Technology, College of Dairy  
 Technology, Warud Pusad,  
 Maharashtra Animal & Fishery  
 Sciences University, Nagpur,  
 Maharashtra, India

#### Suvaratan Ranvir

Dairy Chemistry Division,  
 National Dairy Research  
 Institute, Karnal, Haryana,  
 India

#### Correspondence

##### Shaikh Adil

Department of Dairy  
 Technology, College of Dairy  
 Technology, Warud Pusad,  
 Maharashtra Animal & Fishery  
 Sciences University, Nagpur,  
 Maharashtra, India

## Effect of different levels of paneer whey on physico-chemical and microbial characteristics of milk bread

Kakan AV, Changade SP, Shaikh Adil and Suvaratan Ranvir

#### Abstract

Present study was conducted to evaluate the effect of addition of five different percentages (control 0%, 25%, 50%, 75% and 100%) of *paneer* whey on physico-chemical and microbial characteristics of milk bread. *Paneer* whey contains about 6.36% total solids, was utilized for preparation of milk bread samples by the straight dough method. The total solids content and pH of milk bread increased while moisture content and alcoholic acidity was decreased significantly ( $p < 0.05$ ) with the increase in level of *paneer* whey. The ash content of milk bread was increased non-significantly. The increase in total protein content was significant with highest value (12.53%) noted for 100% pw milk bread. The treatments control (T1), T2 and T3 were at par with each other for alcoholic acidity (%  $H_2SO_4$ ). The microbiological analysis of milk breads on the day of production for SPC showed a non-significant statistical difference. The yeast and mold count (CFU/gm) for treatment T5 (100pw:0w) was found statistically significant and higher over all other treatments. The results show scope for addition of *paneer* whey for enhancing nutritive value of bread and also in mitigating the problems of whey disposal.

**Keywords:** Physico-chemical, microbial, milk bread, *Paneer* Whey

#### Introduction

The consistent growth in the annual production of cheese and coagulated milk products represents the generation of extremely large quantities of whey.

Global production of liquid whey from cheese and casein amounted to 186 million MT in 2008 with an annual average growth of approximately 2% between 2002 and 2008 (USDEC, 2003) [34]. Raju *et al.* (2005) [33] reported that about 4.84 million tonnes / annum of whey are generated in India, out of which 80% is obtained from *channa* or *paneer*.

Today, the annual global increase of whey production is equivalent to 2%, which is parallel to annual increase in milk production. As a consequence, many researchers have been studying alternative possibilities to utilize whey more economically, especially in the production of valuable raw material rather than the manufacture of whey powder (Bozanic *et al.* 2014) [5].

The world production of whey is higher than 160 million tons per year with 1-2% annual growth rate. Out of the total production of whey, some 70% is used in the production of various products, while 30% is used either as pig feed, fertilizers or is disposed of in rivers and seas. In spite of all the above mentioned, whey is considered one of the biggest pollutants in the food processing industry (Blazic *et al.* 2017) [4].

Million tonnes of whey is disposed into rivers, lakes, other water bodies, wastewater treatment plants or into land represents a significant loss of resources. It causes serious pollution problems (Canli, 2005) [8]. Jarita and Kulkarni (2009) [22] recorded that the disposal of each liter of whey costs about 35 paise. Khamrui and Rajorhia (1998) [24] indicated that treating 5 lakh liters of whey in the sewage would cost \$ 10,000 per day for primary treatment.

In India, the production of whey is scattered, unorganized and the production levels vary from small (20-50 liter / day) to large scale (50,000 –1, 00,000 liter/ day); hence may not be processed at one location. Nevertheless, India cannot allow the whey solids to go waste though it is unaffordable with available technologies, which are beyond reach of the medium and small-scale dairy enterprises. It is essential to fill up the gap between its production and its disposal.

Whey may be defined broadly as the serum or watery part of milk remaining after separation of the curd, which results from the coagulation of milk proteins by acid or proteolytic enzymes (Panesar *et al.*, 2007) [30]. Butylina (2007) [7] suggested that sour and sweet whey are the two types of whey. The type and composition of whey mainly depends upon the processing techniques used for casein removal from liquid milk. Whey contains approximately 6% total solids of which 70% is lactose and 11% is whey protein (Zall, 1984) [35]. The pH of sweet and sour whey ranges from 5-7 and 4-5 respectively (Zall, 1992) [36].

Bread has always been one of the most popular and appealing food products due to its superior nutritional, sensorial and textural characteristics, ready to eat convenience as well as cost competitiveness (Giannou and Tzia, 2007) [13]. The use of different additives has become a common practice in the business of baking. The proteins from dairy sources, like whey proteins, are safe and natural food additives that exhibit thickening functions similar to hydrocolloids, starches, and other thickeners in food systems (Hudson *et al.*, 2000) [17]. Incorporation of dairy ingredients into the dough improves the baking quality and can be more beneficial than chemical additives because of its high nutritional value and natural origin.

The use of whey solids in the appropriate manner is likely to provide low cost solids to the bakers besides resulting in newer/high nutritional value products. Therefore, the present project was designed to utilize *paneer* whey from the dairy sector in the production of milk bread and to study its effect on physico-chemical and microbial characteristics of milk bread samples.

### Materials and methods

A composite sample of fresh *paneer* whey was obtained from the Department of Dairy Technology and used during the research work. The whey was prepared using citric acid of commercial grade @ one percent as a coagulant. The whey collected was clarified and separated twice at  $40 \pm 5^\circ\text{C}$  using power operated centrifugal separator and filtered through double layered muslin cloth. The *paneer* whey obtained was used in place of water for dough making at four different levels viz., 25, 50, 75 and 100 percent. Refined wheat flour, sugar, hydrogenated fat, salt, baker's yeast, vanilla essence were procured from local market of Pusad, MS, India.

### Evaluation of Physico-Chemical Characteristics of Raw Materials

The quality assessment of the main ingredients i.e. refined wheat flour and *paneer* whey was performed (Table 1). Sieved refined wheat flour was analyzed for moisture, total ash, acid insoluble ash, gluten contents and alcoholic acidity as per methods described by BIS (I.S. 1155-1968, Part IV). The total protein content was estimated by AOAC method 920.87. Pretreated *paneer* whey was analyzed for total solids, pH, titrable acidity and protein content. The total soluble solid was estimated using hand refractometer as described in SP: 18 Part XI (1981) [19]. Titrable acidity, pH and protein were analyzed as described in SP: 18 Part XI (1981) [19].

### Method for Milk Bread Making

The milk bread was prepared by following "Straight dough process", AACC 2000 [1]. During each replication, 1500 g refined wheat flour was taken and divided into five lots of 300 g each to apply five different treatments.

Control (T1) = 100 percent water

T2 = 75 percent water + 25 percent *paneer* whey

T3 = 50 percent water + 50 percent *paneer* whey

T4 = 25 percent water + 75 percent *paneer* whey

T5 = 100 percent *paneer* whey

Each treatment was replicated five times.

Various steps followed during the preparation of milk bread are as follows (Fig. 1) and material balance explained in Table 2:

For making the dough, 1500 g cleaned refined wheat flour was taken in the trough along with all the dry ingredients and mixed uniformly followed by addition of molten fat. The mixture was then divided into five lots for five different treatments. The dried mix was thoroughly blended together with the various proportions of whey and water as shown in the Table 2 using food processor for 10 minutes for formation of dough. The proofing of dough obtained from each treatment was done in two steps. The baking of the proofed dough in G.I moulds was done at  $230 \pm 5^\circ\text{C}$  for  $30 \pm 2$  min to achieve light brown color of the crust. After baking the milk bread samples were taken out from baking oven and allowed to cool at ambient temperature for  $30 \pm 2$  minutes in the mould itself. The cooled milk bread samples were taken out in the stainless steel tray for further studies.

### Physico-Chemical Characteristics

A sample of 1cm x 1cm x 1cm was drawn from five different locations (top, bottom, middle, head end, tail end) in the milk bread loaf obtained through all treatments. This sample was transferred to sterilized, air tight glass container and stored at  $25 \pm 5^\circ\text{C}$  for further analysis and tested for Moisture and Total Solids(%), Ash(%), Acid Insoluble Ash (%), Total Protein (%), Alcoholic Acidity (%) and pH of aqueous extract as per methods given in SP: 18 Part V, 1982 [20].

For microbial studies, milk bread samples were aseptically ground using mortar and pestle. Every time 11 g sample was weighed and transferred to 99 ml sterile phosphate buffer aseptically. Further dilution to  $10^3$  levels was carried out by serially transferring 1 ml of diluted sample to 9 ml sterile phosphate buffer blanks.

### Standard Plate Count

Standard Plate Count of each sample was determined by plating  $3^{\text{rd}}$  dilution of sample suspension in duplicate on Plate Count Agar and incubated at  $37 \pm 1^\circ\text{C}$  for 48 h on lines of Asghar (2009) [3].

### Yeast and Mold Count

The yeast and mould count was estimated by plating third dilution of sample suspension on Potato Dextrose Agar. The pH of the medium was adjusted to 3.5 using 10% tartaric acid for each 100 ml media and added to plate before pouring sample. The plates were incubated at  $30 \pm 1^\circ\text{C}$  for 120 h and elaborate count was recorded as prescribed by Asghar (2009) [3].

### Results and Discussion

#### Effect of different levels of *paneer* whey on physico-chemical properties of milk bread

The milk bread was obtained by replacing water with different proportions of *paneer* whey by following "Straight Dough Process" (AACC, 2000) [1]. The mean values of five replications for various physico-chemical parameters are presented in Table 3 and graphical illustration is given in figure 2. The results recorded are discussed below;

### Moisture

It is obvious from the statistical results that moisture content of milk breads obtained from different treatments was significantly affected with increasing level of *paneer* whey. The control samples showed maximum moisture content ( $36.83 \pm 0.03\%$ ) while the minimum moisture content ( $34.72 \pm 0.02\%$ ) was recorded in milk bread sample testing 100 percent *paneer* whey (T5). There was a gradual decrease in the moisture content of milk bread with an increase in level of *paneer* whey. Decrease in the moisture content of various treatments may be due to the increase in total solids content because of addition of *paneer* whey. It might also be attributed to gradual decrease in water absorption due to the addition of whey as stated by Kaur *et al.* (2002)<sup>[23]</sup> and Constandache (2005)<sup>[9]</sup>. Results recorded are in harmony with Gawali (1999)<sup>[12]</sup>, who observed that due to addition of whey the water absorption was reduced from 60.3 to 55.8 percent. Similarly in the present study to moisture content of milk bread was reduced with increase in level of *paneer* whey and observations are supported by Brar *et al.* (2002)<sup>[6]</sup> and Rao *et al.* (1974)<sup>[21]</sup>. Results recorded are in harmony with Wasnik (2016) for *Paneer* whey based jelly confection, as author reported that decrease in the moisture content from treatment T1 to T5 may be due to the increase in total solids content because of addition of *paneer* whey and total solids present in it.

### Total Solids

It is observed from Table 3 that there are statistically significant differences for all the treatments in respect to total solids content of milk breads. The total solids content of milk bread samples received from different treatments was ranging from  $63.16 \pm 0.03$  percent to  $65.27 \pm 0.02$  percent. The highest TS level of 65.27 percent was observed in milk bread prepared by using 100 percent *paneer* whey (T5) and lowest 63.16 percent was noted in control (T1). This variation may be due to addition of *paneer* whey in different proportions. In the present study *paneer* whey used was testing  $6.36 \pm 0.01$  percent total solids. Further this may be attributed with low water absorption as reported by Gawali (1999)<sup>[12]</sup>. Based on these results it was inferred that the moisture content varied with the variation in the total solids content. The results observed are in line with Jain *et al.* (1974)<sup>[21]</sup> and Hamzah and Wong (2011)<sup>[16]</sup>.

### Ash

It is observed from the Table 3 that ash content increases with increase in level of *paneer* whey. Statistically all the treatments have shown non-significant differences. The ash content recorded for various treatments was  $1.01 \pm 0.03\%$  (control);  $1.04 \pm 0.03\%$  (T2);  $1.09 \pm 0.04\%$  (T3);  $1.16 \pm 0.06\%$  (T4) and  $1.24 \pm 0.04\%$  (T5) respectively. The increase in ash content may be due to high mineral content of *paneer* whey. In the present study, refined wheat flour and *paneer* whey used were testing 0.94 percent and 0.61 percent ash respectively. Similar results were observed by Oloyae *et al.* (2006)<sup>[29]</sup> and Maloma *et al.* (2011)<sup>[26]</sup>. Similar values were observed by Wasnik (2016) for *Paneer* whey based jelly confection.

### Acid insoluble ash

A significant difference for the acid insoluble ash was noticed between Control (T1) and T2, T3, T4 and T5. However the treatments T2 (25pw:0w), T3 (50pw:50w) and T4 (75pw:25w) are statistically at par and have shown equal

effect but these treatments are significantly varied from T5 (100pw:0w). The highest acid insoluble ash (0.16%) was observed for treatment T5 and the lowest (0.09%) was recorded in control (T1). The values recorded are similar to the values observed by Ndife *et al.* (2011)<sup>[28]</sup> and Pastuszka *et al.* (2012)<sup>[32]</sup>.

### Total protein

The average values for total protein content of milk breads obtained from control and various proportions of *paneer* whey are depicted in Table 3. It is revealed from results that there is a significant difference between the treatments and the highest total protein content was found in T5 (12.53%) treatment and the lowest total protein content was found in control T1 (11.53%) treatment. It can be observed from the Table that the values for total protein content increased slightly with the increase in proportions of *paneer* whey in different treatments. In the present study *paneer* whey testing 0.35 percent protein was used thus the increase in total protein content could be attributed to the protein content of whey. The increase in total protein content due to addition of whey was supported by Wasnik (2016), Kaur *et al.* (2002)<sup>[23]</sup> and Constandache (2005)<sup>[9]</sup>.

### Alcoholic acidity

The average values of five replications recorded for alcoholic acidity (%  $\text{H}_2\text{SO}_4$ ) for different treatments of milk bread are given in Table 3. Statistical analysis shows that difference in control T1, T2 and T3 treatments is non-significant and statistically at par with each other. However these treatments differ significantly from T4 and T5 treatments. The difference between values of T4 and T5 is non-significant. The highest alcoholic acidity ( $0.90 \pm 0.08\%$   $\text{H}_2\text{SO}_4$ ) was recorded for 100 percent *paneer* whey (T5) treatment while the lowest alcoholic acidity ( $0.61 \pm 0.03\%$   $\text{H}_2\text{SO}_4$ ) was noted for control (T1). The results obtained indicate that as the level of *paneer* whey incorporation in milk bread increased, the alcoholic acidity of milk breads also increased gradually. Similar results were observed by Dewitt (1998)<sup>[10]</sup> and Drobot *et al.* (2007)<sup>[11]</sup>.

### PH

The mean pH values for milk breads obtained from different treatments are given in Table 4. The values recorded were statistically analyzed and the differences among the values of treatments were highly significant. The highest pH value (5.87) was recorded for control (T1) while lowest value (5.12) was of 100 percent *paneer* whey treatment (T5). The results recorded indicate that there was decrease in pH with an increase in proportion of *paneer* whey. This may be due to increase in level of *paneer* whey which increases the acidity of the milk breads. In the present investigation *paneer* whey used had 0.38 percent acidity which resulted in increase of acidity and decrease in pH values of milk breads. The similar pH values for bakery products were reported by Jarita and Kulkarni (2007)<sup>[24]</sup>.

### Effect of different levels of *paneer* whey on microbiological quality of milk bread

The effect of adding *paneer* whey level at different levels in milk bread on standard plate count and yeast and mold count was studied and the results are presented in Table 4 and illustrated in fig. 3.

### Standard plate count

The SPC (CFU/gm) was recorded on the day of production using Plate Count Agar for second dilution. The average values of five replications for different treatments for Standard Plate Count are presented in Table 4. It is seen from the Table that the statistical difference between treatments Control (T1), T2, T3 and T4 is non-significant. Treatment T5 (100pw:0w) has recorded significantly higher counts over all other treatments. The Standard Plate Count was found to be the lowest ( $1.54 \times 10^3$  CFU/gm) for control (T1) and highest ( $2.38 \times 10^3$  CFU/gm) for T5 (100pw:0w). There was an increase in Standard Plate Count of treatments with the increase in level of *paneer* whey. The lactose content in *paneer* whey might have favored the growth of the microorganisms in the milk bread samples containing *paneer* whey. Similar results were observed by Papinwar (2010) [31] and Latif *et al.* (2005) [25].

### Yeast and mold count

The Yeast and Mold count (CFU/gm) for second dilution of various treatments of milk bread was recorded on the day of production using Potato Dextrose Agar. The average values of Yeast and Mold count for different treatments after five replications are presented in Table 4. It is observed from the Table 4 that counts for treatment T5 (100pw:0w) are statistically significant and higher over all other treatments. The statistical difference between treatments Control (T1), T2, T3 and T4 is non-significant. The Yeast and Mold count was found to be the lowest ( $9.03 \times 10^3$  CFU/gm) for control (T1) and highest ( $12.5 \times 10^3$  CFU/gm) for 100 percent *paneer* whey treatment (T5). The milk breads were prepared using 20 percent sugar in the dough formula, which might have favored mold growth. Similar results were observed by Latif *et al.* (2005) [25], Oloyae *et al.* (2006) [29] and Maloma *et al.* (2011) [26].

**Table 1:** Composition of Raw Materials

Parameters	Refined wheat flour	Paneer whey
Moisture (%)	12.09 ± 0.08	93.64 ± 0.01
Total solids (%)	87.90 ± 0.08	6.36 ± 0.01
Protein (%)	10.06 ± 0.01	0.35 ± 0.02
Total ash (%)	0.94 ± 0.02	0.61 ± 0.11
Acid insoluble ash (%)	0.11 ± 0.01	--
Wet gluten (%)	29.28 ± 0.64	--
Dry gluten (%)	9.72 ± 0.17	--
Alcoholic acidity(%H <sub>2</sub> SO <sub>4</sub> )	0.10 ± 0.22	--
Acidity (% L.A)	--	0.38 ± 0.01
pH	--	5.61 ± 0.25

**Table 2:** Material balance used for milk bread making

Raw Materials	Control (T1)	T2	T3	T4	T5
Refined wheat flour(g)	300	300	300	300	300
Fat (g)	9.0	9.0	9.0	9.0	9.0
Sugar (g)	60	60	60	60	60
Salt (g)	3.0	3.0	3.0	3.0	3.0
Yeast (g)	9.0	9.0	9.0	9.0	9.0
SMP (g)	2.0	2.0	2.0	2.0	2.0
Calcium propionate (g)	1.5	1.5	1.5	1.5	1.5
Improvers (g)	0.650	0.65000	0.650	0.650	0.650
<i>Paneer</i> Whey (ml)	-	45	90	135	180
Water (ml)	180	135	90	45	-

**Table 3:** Effect of Different Levels of *Paneer* Whey on Physico-Chemical Properties of Milk Bread

Treatments	Moisture (%)	Total solids (%)	Ash (%)	Acid insoluble ash (%)	Total protein (%)	Alcoholic acidity (%H <sub>2</sub> SO <sub>4</sub> )	pH
Control (T1)	36.83±0.03 <sup>a</sup>	63.16±0.03 <sup>a</sup>	1.01±0.03 <sup>a</sup>	0.09±0.01 <sup>a</sup>	11.53±0.02 <sup>a</sup>	0.61±0.03 <sup>a</sup>	5.87±0.04 <sup>a</sup>
T2	36.30±0.04 <sup>b</sup>	63.69±0.04 <sup>b</sup>	1.04±0.03 <sup>a</sup>	0.12±0.02 <sup>b</sup>	11.74±0.02 <sup>b</sup>	0.67±0.05 <sup>a</sup>	5.66±0.03 <sup>b</sup>
T3	35.72±0.01 <sup>c</sup>	64.27±0.01 <sup>c</sup>	1.09±0.04 <sup>a</sup>	0.13±0.01 <sup>b</sup>	12.02±0.02 <sup>c</sup>	0.80±0.07 <sup>a</sup>	5.51±0.10 <sup>c</sup>
T4	35.24±0.02 <sup>d</sup>	64.75±0.02 <sup>d</sup>	1.16±0.06 <sup>a</sup>	0.13±0.01 <sup>b</sup>	12.26±0.03 <sup>d</sup>	0.85±0.09 <sup>b</sup>	5.36±0.07 <sup>d</sup>
T5	34.72±0.02 <sup>e</sup>	65.27±0.02 <sup>e</sup>	1.24±0.04 <sup>a</sup>	0.16±0.01 <sup>c</sup>	12.53±0.01 <sup>e</sup>	0.90±0.08 <sup>b</sup>	5.12±0.02 <sup>e</sup>

\*Mean of 5 replications.

**Table 4:** Effect of Different Levels of *Paneer* Whey on Microbial Quality of Milk Bread

Sr. No.	Treatments	Standard plate count (CFU/gm)	Yeast and mold count (CFU/gm)
1	Control (T1)	$1.54 \times 10^3 \pm 0.10^b$	$9.03 \times 10^3 \pm 0.10^a$
2	T2	$1.68 \times 10^3 \pm 0.13^b$	$9.51 \times 10^3 \pm 0.10^a$
3	T3	$1.63 \times 10^3 \pm 0.17^b$	$9.58 \times 10^3 \pm 0.10^a$
4	T4	$1.60 \times 10^3 \pm 0.12^b$	$10.2 \times 10^3 \pm 0.10^a$
5	T5	$2.38 \times 10^3 \pm 0.07^a$	$12.5 \times 10^3 \pm 0.10^b$

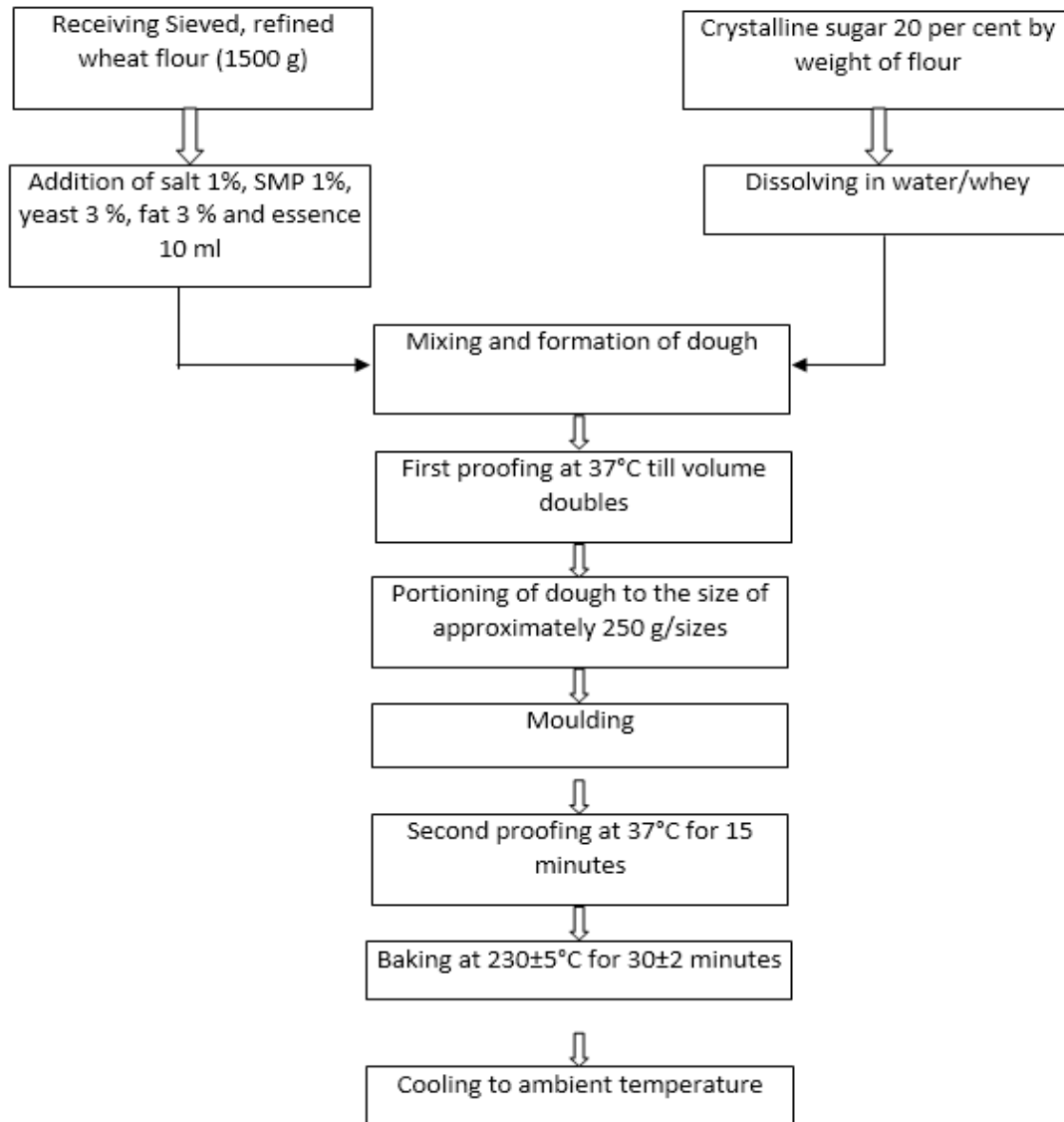


Fig 1: Flowchart of method for milk bread making

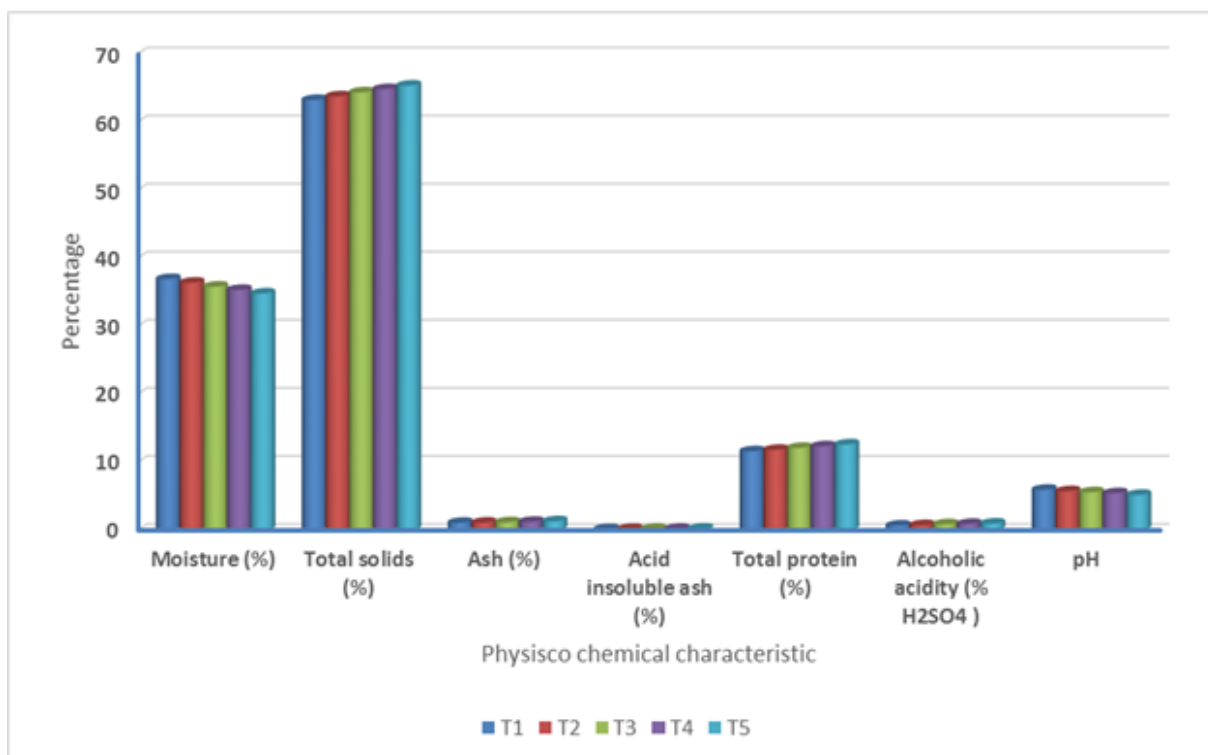
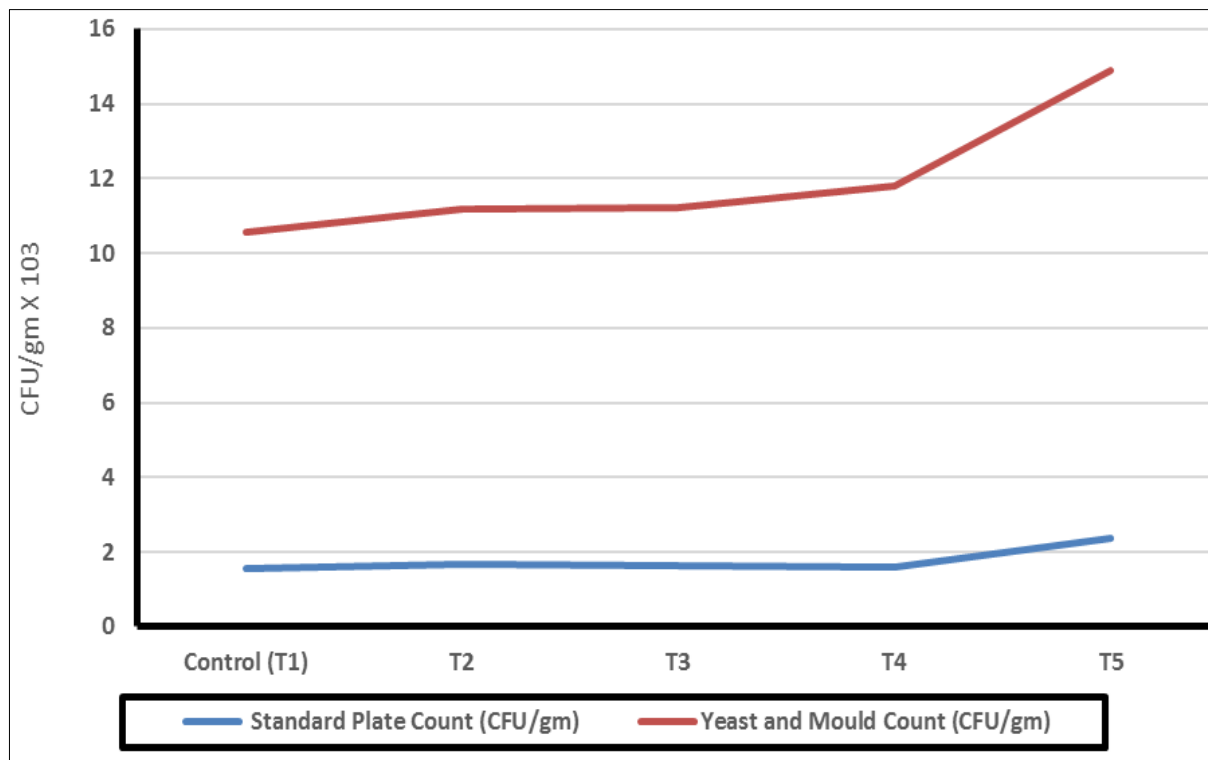


Fig 2: Effect of different levels of *paneer* whey on physico-chemical properties of milk bread



**Fig 3:** Effect of different levels of paneer whey on microbiological quality of milk bread

### Conclusion

A decrease in the values for moisture and pH were recorded with the increase in level of *paneer* whey. The use of *paneer* whey resulted in marginal increase in the total solid, ash and total protein content of milk breads. In microbial analysis as the level of *paneer* whey increased the count (cfu/gm) was found to increase. Replacement of water with *paneer* whey not only improves the nutritional attributes of milk breads but will also contribute to the economy of operation of dairy plants by reducing the cost of effluent treatment and helps to overcome the pollution problems caused by whey drainage.

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