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Studies on Physico-chemical and Textural Analysis of Paneer using Lactobionic Acid as an Acidulant

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ABSTRACT: In recent years, the functionalization and marketing of bioactive milk compounds evolved as an important remunerative sector in the dairy industry. Lactose is among the most vital components of bovine milk and it also has various functional derivatives such as lactitol, lactulose, siallylactose and lactobionic acid. Lactobionic acid (LBA) was first found in Caucasian sea yoghurt and is composed of polyhydroxy gluconic acid which is bond to galactose sugar. In recent times, LBA is widely recognized in the food sector for its acidulant, antioxidant and prebiotic properties. This research work was carried out to develop the paneer with the addition of different levels of LBA. The acidulant property of LBA was optimized in paneer preparation by combination with the citric acid *viz.*, 0.5%, 1% and 1.5% LBA. The addition of different levels of LBA had a positive effect on the physico-chemical, yield and textural parameters of paneer. In the present study, it was observed that there was a significant change was observed in yield, total solids recovery percentage and also textural parameters. LBA added paneer increases the yield percentage from 15.71 to 18.57% with a total solids recovery percentage from 56.55 to 65.36%. According to the textural analysis, LBA significantly influenced the hardness of paneer. Therefore, the results of this study indicated that paneer prepared with the addition of lactobionic acid yielded maximum total solids recovery percentage and best textural attributes.

Keywords: Lactobionic Acid, Paneer, Yield, Total solids recovery, Acidulant.

INTRODUCTION

Milk is a good source of calcium, protein, lactose and minerals thus it is a nutrient-rich complete food. However, the traditional and contemporary view of the role of milk has been remarkably expanded beyond the horizon of the nutritional subsistence of humans. Aside from the nutritional values of milk, biologically active compounds from milk such as lactose, casein and whey proteins are increasingly important for physiological and biochemical functions that have crucial impacts on human metabolism and health. Lactose is a major carbohydrate source present in milk and it's derivatives are widely used in food and pharmaceutical industries for instance, lactitol, lactulose and lactobionic acid (Fox, 2011). Currently, there is an increasing concern on the inclusion of the functional compound LBA in the development of dairy products for its multi-functional properties.

Lactobionic acid (LBA) is a sugar acid consisting of gluconic acid and galactose sugar by partial oxidation of lactose. Lactobionic acid (LBA) is an aldobionic acid, which consists of gluconic acid bonded to galactose sugar with nine free hydroxyl groups through an ether linkage. The nomenclature of LBA is 4-O-β -galactopyranosyl-D-gluconic acid (Gutierrez *et al.*, 2012). The molecular weight of LBA is 358.3 g/mol and the pKa is 3.6. pKa is defined as an acid dissociation constant, higher pKa value means weak acid while a lower pKa value means the acid is strong. Therefore, LBA is weak acid when compared to lactic acid and citric acid. It has a higher water solubility property with a moisture content of 4-5%. The calcium lactobionate salt exhibits 40,000 times higher water solubility than calcium carbonate and 10 times higher than calcium lactate (Alonso *et al*., 2013). The Food and Drug Administration (FDA, 2018) recommends LBA salt to be safe for human consumption when used in sufficient quantities to achieve the desired result on food. LBA salt is suitable for use in food, particularly as a firming agent in foods such as puddings. LBA acts as an antioxidant, acidulant, stabilizer, gelling and prebiotic substance (Cardoso *et al*., 2019). Ribeiro *et al.* (2016) developed dairy gel by using LBA as an acidulant. The findings revealed that it is important that the addition of twice the concentration of LBA is required to achieve the same pH using Glucono-δ-lactone (GDL) (0.25 % GDL = 0.5 % LBA) and LBA produces firm gels and has the potential to decrease souring time and retain aroma.

Paneer is a traditional heat acid-coagulated dairy product. Paneer preparation accounts for approximately 7% of the total milk production in India. Due to lifestyle habits, paneer is a major protein source for the vegetarian population and is utilized by a vast number of people. The shelf life of paneer is about a day at room temperature and for about 7 days under refrigeration conditions (Chaudhary *et al*., 2019). There are numerous techniques employed to develop paneer that are enriched with functional characteristics. Apart from citric acid, other acidulants are employed to improve the total solids recovery, yield percentage, shelf life and acceptable level of paneer.

To understand the acidulant property of LBA in a better way, this present investigation was carried out to study the optimal inclusion level of LBA as an acidulant in paneer preparation.

MATERIALS AND METHODS

Sources of raw materials. Milk was procured from the Dairy plant of the College of Food and Dairy Technology. Citric acid and LBA of HiMedia Lab. Private Ltd, Mumbai, India, was used as an acidulant in paneer preparation.

Methods

1. Preparation of paneer: The paneer was prepared as the method suggested by Kumar *et al*. (2019) with minor modifications based on the needs of the research study.

Flow chart for preparation of paneer using different levels of citric acid and LBA as an acidulant (Kumar *et al*., 2019).

Milk (3.5 % Fat, 8.5 % SNF)
↓
Heated to 90°C without holding
↓
Cool down to 85°C by keeping in cold water bath
↓
Addition of acidulants (citric acid and LBA) at 85°C
↓
Coagulum was allowed to settle for 5 minutes
↓
Whey was drained through a muslin cloth
↓
Coagulum was pressed in hoops by applying pressure of 0.08 kg/cm^2 for 15 minutes
↓
Paneer was immersed in chilled water (4°C)
↓
Packed in LDPE pouch and stored at 4°C

Preliminary trials were conducted to optimize the level of LBA addition in paneer preparation. Based on the trials, 2% LBA does not initiate the coagulation process in milk while coagulation of milk occurs at 5% LBA concentration.

Table 1: Preliminary trials for inclusion of LBA in paneer preparation.

Types of Paneer Sample	Citric acid (%)	LBA (%)	Physical character of paneer
C	2	-	Hard
T_1	1.5	0.5	Hard
T_2	1	1	Soft
T_3	0.5	1.5	Too Soft
T_4	-	2	No coagulation occurred
T_5	-	3	No coagulation occurred
T_6	-	4	Sticky & breakage of coagulum
T_7	-	5	Soft coagulum

C-Control, T-Trial

To minimize the inclusion level of LBA, it was combined with citric acid to prepare paneer. The inclusion level of LBA and citric acid listed in Table 2 and Fig. 1 illustrates the developed paneer samples.

Table 2: Optimized proportion of LBA and citric acid.

Types of Paneer sample	Citric acid (%)	LBA (%)
Control	2	-
P_1	1.5	0.5
P_2	1	1
P_3	0.5	1.5

P-Paneer

Control (2% citric acid added paneer)

P_1 (1.5% citric acid + 0.5% LBA added paneer)

P_2 (1% citric acid + 1% LBA added paneer)

P_3 (0.5% citric acid + 1.5% LBA added paneer)

Fig. 1. Paneer prepared with different levels of LBA.

2. Physico-Chemical analysis of paneer: The prepared paneer samples were analyzed for determination of moisture, fat, protein, ash, titratable acidity and pH. Moisture content, fat, protein, ash, acidity and pH values were estimated according to AOAC (2016).

3. Total solids recovery percentage of developed paneer: The percent recovery of milk solids in paneer was determined based on a percentage of yield by using the following equation

$$\text{Total solids recovery (\%)} = \frac{\text{Yield (\%)} \times \text{Total solids in paneer}}{\text{Total solids in milk}}$$

4. Textural analysis of developed paneer: The developed paneer samples were analyzed for textural properties *viz.*, hardness, adhesiveness, springiness, cohesiveness, gumminess and chewiness using Stable Micro System Texturometer model TA-HD plus texture analyzer. The paneer sample was subjected to compression testing with a contact force of 10 g which moved at a return speed of 5 mm/sec and a return distance of 10 mm. All textural attributes were measured at room temperature. Six trials were done for each paneer sample and the mean average value of the readings was calculated.

5. Statistical analysis: All parameters were conducted in six trials and an analysis of variance was performed using IBM SPSS® for windows® software as per the standard procedure given by Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

1. Physico-chemical analysis of paneer prepared with different levels of LBA: From Table 3, the statistical results indicated that a highly significant ($P \leq 0.01$) difference was observed among the different paneer samples in moisture and protein content. It was observed that the moisture and protein content of paneer increased with an increase in the concentration of LBA.

Moisture and protein content gradually increased with increasing concentrations of LBA. This could be due to a higher hygroscopic property of LBA than citric acid. The protein content of control and LBA-added paneer was also found to increase. The increase in protein might be due to the solid retention property of LBA through hydrogen bonds with water molecules as suggested by Cardoso *et al.*, (2019).

The total ash and fat content of paneer did not significantly differ with the different levels of LBA addition.

Table 3: Physico-chemical analysis of paneer prepared with different levels of LBA.

Types of paneer sample	Moisture (%)	Fat (%)	Protein (%)	Total Ash (%)
Control	$56.80^{a} \pm 0.046$	20.95±0.10	$17.07^{a} \pm 0.08$	1.31 ± 0.085
P_1	$56.98^{b} \pm 0.003$	20.95±0.09	$17.08^{a} \pm 0.08$	1.27 ± 0.007
P_2	$57.28^{c} \pm 0.016$	20.95±0.21	$17.11^{b} \pm 0.10$	1.36 ± 0.006
P_3	$57.32^{c} \pm 0.009$	20.95±0.11	$17.11^{b} \pm 0.12$	1.42 ± 0.032
F value	**96.774****	**0.639^{NS}**	**12.764****	**1.948^{NS}**

@Average of six trials

**- Statistically highly significant ($P \leq 0.01$), NS- Non significant ($P > 0.05$)

2. Yield and Total solids recovery percentage of paneer prepared with different levels of LBA: In general, the average yield of paneer from one liter of milk is about 16-17%. The effect of acidulants in paneer plays a vital role to minimize solids losses in whey and recover maximum total solids and achieve a desirable yield value. From Table 4 and Fig. 2, the yield of paneer using citric acid was 15.71%, which increased to a maximum of 18.57% in the paneer using different levels of LBA. The average yield in all these paneer samples was highly significant from one another. The maximum yield percentage was observed in paneer samples P_2 and P_3 than in the control sample. The total solid content in paneer was increased progressively directly proportional to the yield value. The percentage of solids recovery in the control paneer was 55.88% while 56.98%, 65.73% and 66.80% in P_1, P_2 and P_3 samples.

The yield of paneer depends on the TS content of milk, type of acidulants and amount of moisture retained in paneer. The results of the table are comparable with the report of Deshmukh *et al*. (2009) who also concluded that moisture and protein content had a direct effect on the yield percentage of paneer. This was also in agreement with Merrill and Singh (2011) who reported that cheese yield was increased by 1.5 to 2 times than control due to the addition of LBA.

Table 4: Yield and Total solids recovery of paneer prepared with different levels of LBA.

Types of paneer sample	Yield (%)	Total solids recovery (%)
Control	15.71[a] ± 0.010	55.88[a] ± 0.020
P_1	16.01[b] ± 0.003	56.98[b] ± 0.003
P_2	18.35[c] ± 0.160	65.73[c] ± 0.010
P_3	18.57[c] ± 0.023	66.80[d] ± 0.010
F value	**343.322****	**8607.949****

@Average of six trials
**- Statistically highly significant (P≤0.01), NS- Non significant (P>0.05)

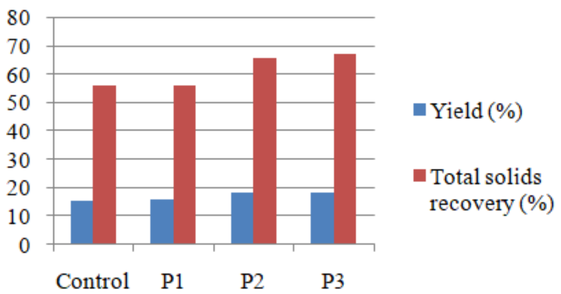

Fig. 2. Yield and Total solids recovery percentage of paneer.

3. Textural analysis of paneer prepared with different levels of LBA: Textural properties of paneer had a strong influence on acceptance. Paneer has a smooth and spongy body with a close texture, whereas buffalo milk paneer has a firm and spongy body and a close texture. The texture profile attributes differ significantly with the level of LBA.

Table 5 revealed that there was a highly significant difference in textural parameters of LBA-added paneer when compared with the control. The textural parameters *viz*. hardness, cohesiveness, gumminess, chewiness and resilience of different levels of LBA-added paneer was decreased but springiness and adhesiveness was increased when compared to the control sample. It was related to hardness and cohesiveness and was mutually exclusive with chewiness. Hence, inclusion of LBA reduced the hardness of the product thereby resulting in reduced gumminess compared to control. Chewiness is a measure of both gumminess and springiness and any reduction in gumminess of the product affects the chewiness.

Khan and Pal (2011) reported that the strength of the coagulant has a direct effect on the body and texture of paneer. In this present study, the moisture content of the treated paneer formed a softer coagulum which had higher moisture retention that has a direct effect on the hardness of paneer. The findings of the current study can be correlated with Chaudhary *et al*. (2019) who treated paneer with GDL and observed similar behavior as LBA- treated paneer samples. Due to the same pKa values of GDL and LBA.

Fig. 3. Textural analysis of paneer.

Table 5: Textural analysis of paneer prepared with different levels of LBA.

Types of paneer sample	Textural parameters						
	Hardness (g)	**Adhesiveness (g sec)**	**Springiness**	**Cohesiveness**	**Gumminess (N)**	**Chewiness (N)**	**Resilience**
Control	3574.95^{d}±0.09	-7.36^{d}±0.08	0.40^{a}±0.06	0.69^{b}±0.08	28.36^{d}±0.07	6.94^{c}±0.12	0.31^{c}±0.05
P_1	2630.86^{c}±0.09	-7.67^{c}±0.06	0.42^{b}±0.05	0.68^{b}±0.08	20.29^{c}±0.08	6.51^{b}±0.08	0.30^{bc}±0.04
P_2	2445.27^{b}±0.08	-8.22^{b}±0.08	0.43^{b}±0.08	0.66^{a}±0.08	11.58^{b}±0.09	6.51^{b}±0.08	0.29^{ab}±0.10
P_3	2139.36^{a}±0.09	-8.32^{a}±0.07	0.44^{b}±0.06	0.65^{a}±0.06	11.11^{a}±0.08	6.12^{a}±0.07	0.27^{a}±0.10
F value	**4908898601****	**4032.094****	**6.611****	**8.879****	**108620.10****	**1393.80****	**7.292****

@Average of six trials
**- Statistically highly significant (P≤0.01), NS- Non significant (P>0.05)

CONCLUSIONS

In conclusion, the yield and total solids recovery percentage of paneer were enhanced by the inclusion of different levels of LBA. There was a highly significant (P<0.05) increase in moisture, protein, yield and total solids recovery percentage and also changes in textural parameters of LBA added paneer samples compared to control. However, the paneer prepared in this present study with 1% LBA and 1% citric acid had a maximum yield, total solids recovery percentage, and textural properties than the control sample. From the result of the present study, it may be concluded that the inclusion of LBA for the preparation of paneer could be successfully utilized in the future to improve the yield and total solids recovery percentage.

FUTURE SCOPE

This research work hasput a light to utilize LBA as an acidulant in paneer production. Thus presents a promising intervention in improving the yield of paneer, total solids recovery and textural attributes.

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Conflict of interest. None.

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