

Standardization and optimization of process parameters for manufacturing of yak milk paneer.

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ABSTRACT

In the current study, the physicochemical and sensory quality of yak milk paneer (cottage cheese) were studied and also aimed to determine the optimum processing condition by using the response surface methodology technique. The nineteen experiments were generated in design expert software, the calcium chloride (0-0.20%), Citric acid (0.5-2.5%) and coagulation temperature (72-90°C) were taken as independent variables while the moisture, yield, texture, sensory score, titratable acidity taken as a responses for the optimization process. The coefficient of determination, R² values for moisture, yield, texture, sensory score, titratable acidity was found to be greater than 0.80. Optimization by the application of the desirability function method resulted in a quantity of calcium chloride, citric acid and coagulation as 0.13%, 0.50% and 78.42°C, respectively. The yak milk and milk products are a sustainable income source of high-altitude region peoples so the optimized process conditions of yak milk paneer may help to small scale processors to improve the quality and yield of products.

Keywords: Yak milk, Paneer, coagulation, standardization

RESUMEN

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INTRODUCTION

Milk and milk products are making a major contribution in the alimental culture of the Himalayan people (Rai et al. 2016). At the high elevation (>2,100 m) of mountain regions of the Darjeeling hills of West Bengal, Jammu and Kashmir, Himachal Pradesh, Sikkim and Arunachal Pradesh in North east part of India, the mountains of neighboring countries i.e Nepal, Bhutan and Tibet in China, only milk products of yaks are a major source of nutritional sustainability. In the social, economic, and nutritional well-being of the Himalayan region people, Indigenous Yak milk products have significantly contributed to sustaining live. The globally population of domestic Yaks are nearly 14.2 million, of which 71 thousand Yak animals are available in India (Kandeepan and Sangma, 2011). Yak milk and its process products are largely intake in these difficult mountain areas (Rasul et al. 2019). By the people of the Sikkim, the different varieties of indigenou fermented milk products are manufacturing and locally it is called Dahi, Mohi, Gheu, Maar, Chhurpi, Chhu, Somar, Philu and Shyow (Rai et al. 2016). Yak milk churpi is heat coagulated and fermented products, which coagulated with old curd after boiling.

A similar product such as milk paneer is a heat and acid coagulated product and has good quality and quantity of protein source and easily are available at a lower cost. Milk paneer protein is having a higher biological value of 80% to 86 % and plays a significant role in the human digestion system (Kumar et al. 2014). Other than protein, it contains fat and fat-soluble vitamins as well as few minerals, especially calcium and phosphorus. Milk paneer contains approximately moisture, fat, protein, carbohydrates, and minerals of 53–55%, 23–26%, 17–18 %, 2–2.5 %, 1.5–2.0 % respectively (Singh et al. 2013). The product can be stored for one day at ambient temperature and can extend the shelf life up to one week if stored in a refrigeration condition.

Generally, Milk is heated at 85-90 °C and cool down to 72-75 °C and coagulated by using the food-grade Citric, tartaric, lactic acid and sour whey. Whey is drained after coagulation and pressed to coagulum and cut into sliced and further dipped into chilled water for improving the body and texture of paneer (Goyal and Goyal, 2016). Further, the quality and yield of paneer were influenced by the total solids, heat treatment given to milk, quantity and type of coagulant, temperature and pH of coagulation etc. Ghosh et al. (2019) reported that temperature of milk is the main requirement of paneer because it affects the sensory and microbiological quality of paneer. Ahuja et al. (2012) reported that the pH and concentration of coagulation solution also affect iso-electric pH of milk and resulted to influence the yield and losses of solids into the whey. Similarly, the initial total solids and fat contents have a

significant role in the texture formation of paneer. So, each parameter has a significant role in the quantity and quality of paneer. However, there are no recent studies on the physicochemical properties and optimization of yak milk paneer as function of temperature and acid coagulants. Therefore, in this study, the physicochemical and sensory quality of yak milk paneer were studied at various levels of temperature, concentration of coagulants and also aimed to determine the optimum processing condition by using the response surface methodology technique.

MATERIALS AND METHODS

The fresh raw yak milk was collected from the farmers of East Sikkim district of Sikkim, India and stored at a chilled condition for further experiments and testing. The food grade acidulant citric acid, calcium chlorides were purchased from High Tech Pvt. Ltd., Siliguri, West Bengal, India.

Experimental plan: Several preliminary experiments were carried out for determination of minimum and maximum range of independent variables for experiment plan. In this design, the calcium chloride (CaCl_2), citric acid and coagulation temperature were considered as an independent parameters and responses are paneer yield, moisture content, texture, titratable acidity, and sensory score. The nineteen experiments (generated by using the Central Composite Rotatable Design (CCRD) three level three factor) in design expert software (Statease Inc., Minneapolis, MN 55103). A total number of 19 experiments were carried out in which five were repeated at a central point.

Yak milk paneer preparation: The fresh one kg of raw yak milk was filtrated through a twofold muslin cloth for eliminate of the dirt, dust particles and other foreign impurities such as hairs. The quantity of CaCl_2 was added in yak milk as per the experimental design (Table 1) and heated to 95°C for five minutes and then cool down as per the experiment plan (Table 1). The yak milk was stirred slowly for mixing and to protect from sticking to the bottom of the container during the heating. The milk was coagulated by using the citric acid solution according to the experiment plan. The coagulated mass was filtered, drain the whey and the coagulated mass was encased in a cotton cloth and then pressed in manual paneer pressing machine for 20 minutes. Further, the wrapped pressurized paneer was dipped into chilled water at 4°C for two hours for improvement of texture and body (Figure 1). The product was packaged in flexible plastic film (LDPE) bags and stored at deep freezer for further experiments. A total 19 experiments were performed for optimization of yak milk paneer and analyzed the responses.

Physical and chemical analysis: For various physico-chemical parameters of the Yak milk and the 19 Yak milk paneer samples were analyzed; moisture and total solids by gravimetric method, fat by Gerber method, pH by digital pH meter, and titratable acidity by the standard procedure described in Shanaziya et al. 2018.

Paneer yield: The yield of the yak milk paneer was noted as the total quantity of yak milk taken upon the weight of the paneer obtained after each experiment (Eq 1).

$$Yyd (\%) = \frac{W_p}{W_{ym}} \times 100 \quad (1)$$

Where Yyd is Yield of the paneer (%), the weight of yak milk paneer is W_p and the weight of yak milk is W_{ym} .

Table 1. Experimental design with actual and coded values of independent variables.

| Exp No | Independent variables | | |
|--------|--|----------------------------------|---|
| | X ₁ : CaCl ₂ (%) | X ₂ : Citric acid (%) | X ₃ : Coagulation temperature (°C) |
| 1 | 0.10 (0) | 1.50 (0) | 80(0) |
| 2 | 0.10 (0) | 1.50(0) | 80(0) |
| 3 | 0.00 (-1) | 2.50(+1) | 90 (+1) |
| 4 | 0.20 (+1) | 0.50(-1) | 70 (-1) |
| 5 | 0.10(0) | 1.50(0) | 70(-1) |
| 6 | 0.10(0) | 1.50(0) | 80(0) |
| 7 | 0.00 (-1) | 1.50(0) | 80(0) |
| 8 | 0.20(+1) | 2.50(+1) | 90 (+1) |
| 9 | 0.20(+1) | 2.50(+1) | 70(-1) |
| 10 | 0.10(0) | 1.50(0) | 80(0) |
| 11 | 0.00 (-1) | 2.50(+1) | 70(-1) |
| 12 | 0.20(+1) | 0.50(-1) | 90(+1) |
| 13 | 0.10(0) | 1.50(0) | 80(0) |
| 14 | 0.10(0) | 1.50(0) | 90(+1) |
| 15 | 0.00(-1) | 0.50(-1) | 70(-1) |
| 16 | 0.00(-1) | 0.50(-1) | 90(+1) |
| 17 | 0.10(0) | 0.50(-1) | 80.00(0) |
| 18 | 0.10(0) | 2.50(+1) | 80(0) |
| 19 | 0.20(+1) | 1.50(0) | 80(0) |

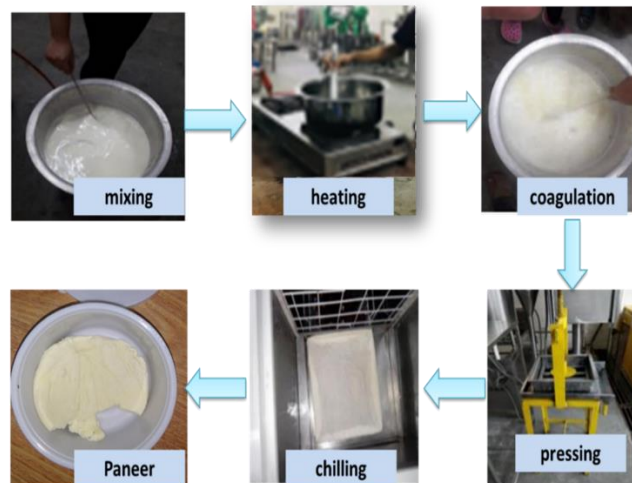


Fig.1. Preparation of yak milk paneer at Milk Pilot plant.

Texture analysis: Textural properties of each yak milk paneer samples were determined in TA-HD Plus Texture Analyzer (Model TAXT2i, Exponent Stable Micro Systems, United Kingdom) and obtained textural responses viz, hardness, cutting and puncture force. The pre and post speed were 1 mm/s during the analysis, respectively (Mishra et al. 2016).

Sensory score: The fresh produced yak milk paneer (19 different paneer) samples were assessed organoleptically by a panel of 30 semi-trained judges from the Department of Processing and Food Engineering, College of Agricultural Engineering and Post harvest Technology, Central Agricultural University Imphal, Ranipool, Gangtok, Sikkim for appearance, flavor, body and texture and overall acceptability. The judges were also asked to note their observations on nine-point hedonic scale score cards. The test samples of paneer stored at $8\pm 1^{\circ}\text{C}$ were tempered at 15.5°C for 1 h before presenting to the judges under code numbers (Singh et al. 2013).

Analysis and Optimization: The response surface methodology techniques were used for the analysis of responses variables. In this design, experiments were randomized in order to minimize the effects of unexplained variability in the observed responses due to extraneous factors by using the design expert software. The function was assumed to be approximated by a second-degree polynomial equation (2):

$$Y_k = b_{k0} + \sum_{i=1}^n b_{ki}X_i + \sum_{i=1}^n b_{kii}X_i^2 + \sum_{i \neq j=1}^n b_{kij}X_iX_j \quad (2)$$

Where, b_{k0} indicate the value of fitted response at the center point of the design, i.e. point (0,0,0), and b_{ki} , b_{kii} , and b_{kij} were the linear, quadratic and cross-product regression terms, respectively. The processing conditions were optimized by using the optimization tool of design expert software. The target of individual independent variables and responses was fixed in the form of weightage (maximum and minimum) and the target values were decided based on the physicochemical and sensory characteristics of paneer sample while the overall acceptability of product was set maximize from the consumer point of view.

Statistical analysis: Experimental data are reported as means of experiment results. The significant test ($p < 0.05$) was conducted for each response by analysis of variance (ANOVA) using Design Exert software (Trial version 8.0.7.1, Stat-Ease Inc., Minneapolis, MN, USA). The linear, quadratic and interaction terms were analyzed and reported in the study.

RESULTS AND DISCUSSION

The proximate composition of Yak milk (Fat: 4.1g and solids not fat: 7.6g in per 100 mL respectively) was obtained. The milk has a slightly higher value of the fat and poor value of the Solid Not Fat content than cow milk.

Effect of process variable on responses: A summary of the interaction, quadratic and linear terms for the yak milk paneer is shown in Table 2. The linear term of X_1 variable is highly significant ($p < 0.5$) for all responses (moisture content, titratable acidity, paneer yield, sensory score and texture attributes) while the quadratic term of X_1 is significant for moisture content, titratable acidity and sensory score at 1% and 5 % level of significance. The titratable acidity, paneer yield and moisture content and sensory score were significantly influenced by linear and

quadratic term of X_2 variables, respectively. The interaction term of calcium chloride (X_1) and citric acid (X_2) was important for titratable acidity and paneer yield, while the other linear and quadratic term were found most significant for the yield of yak milk paneer. The coefficient of determination (R^2) of the developed regression equations for all the dependent variables of the Yak milk paneer experiments was obtained above 0.80, with the lack of fit being non-significant ($p > 0.05$). This results indicated that regression equation was adequate, possessed no significant lack of fit, and showed high values for the R^2 for all responses. The concentration level of calcium chloride was significantly found on responses viz. moisture content, paneer yield, titratable acidity, sensory score and textural properties. While the paneer yield and titratable acidity were significantly influenced at an increased level of citric acid solution (Table 2). From Table 2, it was observed that the yield of paneer has highest at level of CaCl_2 , Citric acid and coagulation temperatures of 0.20%, 0.50% and 70°C respectively. The overall effect of the processing conditions on the responses of Yak milk paneer are shown in Table 3.

Effect of process parameter on 3D response graph: The response surface graphs for responses variables (moisture content, paneer yield, textural properties, sensory scores and texture attributes of the Yak milk paneer) are shown in Figure 2 and Figure 3. Figure 2 showed that moisture content of Yak milk paneer was varied from 55.34 to 73.49% for entire experiments of increasing the concentration level of calcium chloride (0 to 0.20%) and citric acid (0.50 to 2.50 %). The addition of citric acid and CaCl_2 positively changed the moisture content of the yak milk paneer. The higher moisture content (73.49%) of paneer was noticed at 2.50 % and 0% level of citric acid and calcium chloride, in the experiments, respectively. The moisture content of yak milk paneer has significantly decreased at increasing a level of calcium chloride (Figure 2). This might be due to the fact that more interaction between the protein and calcium and resulted in higher water leached from paneer and became hard texture. In case of the yield of paneer, the effect of the independent parameters was created a significant changes in the responses. The coagulants and their concentrations had a significant effect on the yield of paneer. The Yak milk paneer yield is varied with the acidification of milk. It is mainly depends on the initial quality of milk i.e. titratable acidity, the combination of Fat and SNF, amount of load and duration of pressing, strength and temperature of acid coagulants and residence time of coagulum (Khan and Pal, 2011; Arvind *et al.*, 2019).

The effect of interaction between CaCl_2 and citric acid on the yield of paneer is graphically represented in Figure 2. From Figure 2, observed that the addition of citric acid and CaCl_2 significantly affect the yield of the paneer. Similarly, reduced in citric acid level less than 1 % and resulted to decline the paneer yield and noticed a higher yield at higher concentration of coagulant. This might be due to the denaturation of protein particles during the heating and coagulation process (Khan and Pal, 2011). The solids of the paneer were decreased with increasing in the strength of coagulants agent and observed at higher strength of citric acid. The results agreed with previously reported finding by Khan *et al.*, (2014).

The textural attributes viz. cutting force and puncture force obtained in the experiment and ranged from 0.01 to 1.78 N and 0.01 to 0.05 N, respectively. The surface plot of cutting and punctured force as a function of citric acid and calcium chloride is presented in Figure 2. The Figure 2 demonstrated that cutting and puncture decreased

and progressively increased with increasing level of calcium chloride and citric acid while the temperatures, changes in milk not produce any significant changes in the paneer. The higher forces were obtained at a maximum level of citric acid due to protein denaturation and become hard (Singh *et al.* 2015).

The titratable acidity significantly varied from 0.02 to 0.07 % lactic acid at different concentration of coagulants citric acid (0.50 – 2.5 %) and calcium chloride (0-0.20%). Figure 3 shows the graphical representation of the effect of the interaction term of CaCl₂ and citric acid on the titratable acidity. The interaction effect of citric acid and calcium chloride was significantly affecting the titratable acidity and observed to increase with increasing levels. The maximum titratable acidity value was noticed at higher concentration of coagulant acid. This might be due to the change in the pH of the milk after addition of coagulants (Kumar *et al.* 2014).

The textural properties of yak milk paneer sample are highly important for sensory acceptability of the product. The overall acceptability scores for yak milk paneer samples were judged and given score from 2 to 8.1. Sensory scores of the Yak milk paneer significantly ($p < 0.05$) changed by concentration of citric acid and calcium chloride solution. The sensory score was maximum for paneer made with citric acid and calcium chloride level of 0.50 % solution and 0.20%, respectively. The higher amount of citric acid and a lower concentration of CaCl₂ was given a lower score and paneer is a poor texture (Khan *et al.*, 2014). The sensory score was increased with increasing level of calcium chloride concentration at a minimum level of citric acid (0.50%) (Figure 3). The similar findings were reported by Shanaziya *et al.* (2018) and Kumar *et al.* (2008) in the paneer samples.

Optimization and validation: After the completion of the analysis of data, optimization of the experimental data done by setting the value of the response variable to a certain goal of maximum, in range or minimum value. The optimum process condition was obtained on the basis of following criteria; maximum yield, texture and sensory score, minimum acidity value and in range of moisture content. The level of independent parameters of yak milk paneer was optimized by using design expert software based on its desirability and obtained three optimized combinations (Table 4).

From Table 4, we can see that the desirability value of response number 1, 2 and 3 were highest 0.66. Although, all three experiments conditions were similar, the optimized conditions acquired for the given criteria were 0.13 %, 0.50%, and 78.42°C of CaCl₂, Citric acid and coagulation temperature, respectively on the basis of maximum desirability 0.66. These optimum conditions was used for preparation of yak paneer samples and analyzed the response variables. These experimental values compared with software predicted values. There is no significant difference was noticed between experiment and predicted conditions of process.

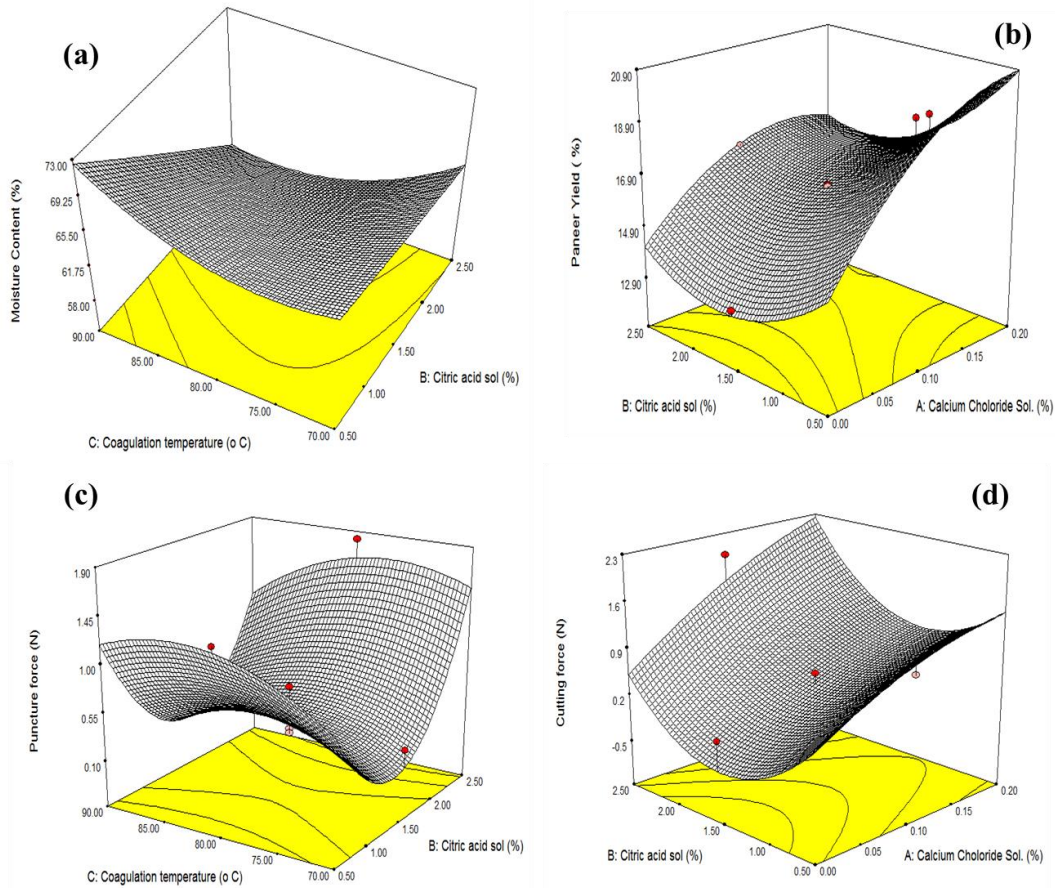


Fig. 2. Effect of independent variables on the response of (a) moisture content, (b) paneer yield, (c) puncture force and (d) cutting force.

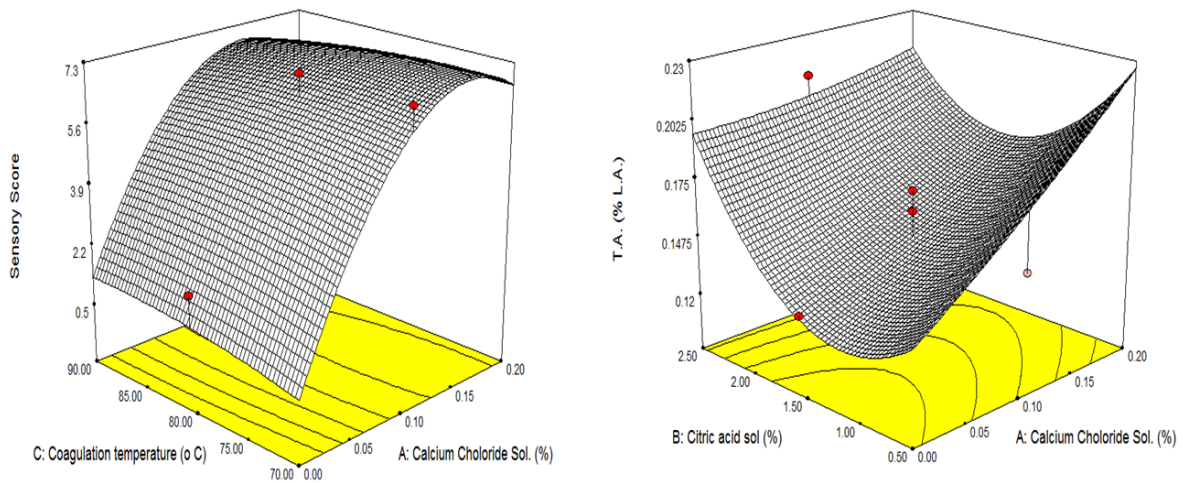


Fig. 3. Effect of independent variables on the response of (a) sensory score (b) titrable acidity

Table 2. ANNOVA analysis of the responses of yak milk paneer process

| Responses | M _c | TA | Yd | S.S | T.S. | |
|--------------------|----------------|-------------|------------|-------------|----------------|---------------|
| | | | | | Puncture Force | Cutting force |
| Model | -9.36 | +0.34 | -44.143 | -8.59240 | -21.28247 | -8.41372 |
| x_1 | -137.61 | +0.18 | +61.52 | +109.248 | +9.815 | +12.107 |
| x_2 | +10.63 | -0.014 | -9.93 | -5.506 | -1.57836 | -2.978 |
| x_3 | +2.15 | -7.78E-003 | +1.367 | +0.311 | +0.548 | +0.255 |
| x_1x_2 | +14.10 | +0.100 | -7.062 | +1.2500 | +1.250 | +1.60 |
| x_1x_3 | -1.920 | +0.000 | -0.021 | -0.375 | +7.50E-003 | -0.0900 |
| x_2x_3 | -0.013 | +0.000 | +0.060 | +0.0375 | -0.0182 | +0.0140 |
| x_1^2 | +840.96 | -1.025 | -136.40 | -270.618 | -22.154 | -1.685 |
| x_2^2 | -3.085 | +4.74E-003 | +1.566 | +0.79381 | +1.01845 | +0.61314 |
| x_3^2 | -0.0132 | +4.742E-005 | -9.08E-003 | -2.061E-003 | -3.26E-003 | -1.716E-003 |
| <i>p</i> model | <0.0001 | 0.0018 | <0.0001 | <0.0001 | 0.0013 | 0.0003 |
| <i>F</i> value | 25.88 | 8.68 | 22.44 | 20.38 | 9.37 | 13.51 |
| R ² | 0.96 | 0.89 | 0.95 | 0.95 | 0.90 | 0.93 |
| Adj R ² | 0.92 | 0.79 | 0.91 | 0.90 | 0.80 | 0.86 |
| LOF | ns | Ns | ns | ns | Ns | Ns |

*Significant at 5 % level; **significant at 1 % level; ns: Non-significant; LOF: lack of fit; Adj: adjusted.

Mc: moisture content, T.A.: titratable acidity, Yd: Yield of paneer, SS: Sensory score and TS: textural studies

Table 3. Analysis of variance for the overall effect of the processing conditions on the preparation of Yak milk paneer.

| Independent parameters | Responses | | | | | |
|------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| | M _c | TA | Yield | Sensory | TS | |
| | | | | | Puncture | Cutting force |
| x_1 | <0.0001*** | 0.0002*** | <0.0001*** | <0.0001*** | <0.0001*** | <0.0001*** |
| x_2 | 0.0537* | 0.0015*** | 0.0003*** | 1.0000 ^{ns} | 0.2195 ^{ns} | 0.0927 ^{ns} |
| x_3 | 0.0522 ^{ns} | 0.3901 ^{ns} | 0.8986 ^{ns} | 1.0000 ^{ns} | 0.9784 ^{ns} | 0.3749 ^{ns} |

*, **, *** Significant at $p < 0.10$, $p < 0.05$, and $p < 0.01$, respectively.

Mc: moisture content, T.A.: titratable acidity, Yd: Yield of paneer, SS: Sensory score and TS: textural studies

Table 4. Optimization values of responses of yak milk paneer

| Exp. No. | X ₁ | X ₂ | X ₃ | Y ₁ | Y ₂ | Y ₃ | Y ₄ | Y ₅ | Y ₆ | Y ₇ |
|----------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 1 | 0.13 | .50 | 78.42 | 60.14 | 10.03 | 1.47 | 1.03 | 0.0308 | 7.92 | 0.660 |
| 2 | 0.13 | .50 | 78.30 | 60.24 | 10.01 | 1.46 | 1.02 | 0.0309 | 7.91 | 0.660 |
| 3 | 0.13 | .50 | 79.45 | 59.78 | 10.05 | 1.51 | 1.026 | 0.0304 | 7.90 | 0.659 |

Where, X₁- CaCl₂ (%), X₂- Citric acid (%), X₃- Coagulation temperature (°C), Y₁- Moisture content (%), Y₂- Yield (%), Y₃- Puncture force (N), Y₄- Cutting force (N), Y₅- Titratable acidity (%), Y₆- Sensory score, Y₇- Desirability

CONCLUSIONS

The Yak milk and product are important for the sustainable livelihood of high altitude peoples. In the social, economic, and nutritional well-being of the Himalayan region people, Indigenous Yak milk products have significantly contributed to sustaining life. The present investigation is directed to improve the physical and chemical quality, yield, sensory and textural properties of Yak milk paneer through the optimization process with using response surface methodology. The independent parameters viz. citric acid, calcium chloride, and temperature were significantly ($p < 0.05$) affect the responses of yak milk paneer. No significant effect of heating temperature on hardness was observed during the process. The best optimized conditions obtained for the given criteria were 0.13 % (CaCl₂), 0.50% (Citric acid), and 78.42°C (Coagulation temperature) based on maximum desirability 0.66. The best way is the Yak milk paneer for industrial and small-scale productions.

Abbreviations

- RSM: Response surface methodology
- CCRD: Central Composite Rotatable Design
- w/v: weight by volume
- LDPE: Low density polyethylene
- CaCl₂ : calcium chloride
- ANOVA: Analysis of variance
- R²: Coefficient of determination
- SNF: Solid not fat
- N:Newton

Conflict of Interests: The authors report no conflict of interests.

Author's contribution: All authors contributed to the study conception and design. RKR contributed to technical and conceptual content and technical writing of the paper. Material preparation, data collection and analysis were performed by IB and SW. All authors read and approved the final manuscript.

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Compliance with Ethical Standards: This article does not contain any studies with human participants or animals performed by any of the authors.

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