Pak. j. sci. ind. res. Ser. B: biol. sci. 2025 **68B**(2) 184-196

Development and Quality Evaluation of Cheese Spread Enriched with Branched chain Amino Acids

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(received September 13, 2022; revised February 4, 2024; accepted February 12, 2024)

Abstract. Cheese spread is a soft, spreadable, unripened, diacetyl flavoured and creamy white with various ingredients such as fruits, vegetables, meats, spices, etc. Branched chain amino acids (BCAA) - leucine, isoleucine and valine are essential amino acids that are metabolized directly in muscles and offer energy fuel to their performance. A contemporary study is designed to develop and evaluate the physico-chemical and sensory attributes of cheese spread supplemented with branched chain amino acids. Cheese spread was made and branched chain amino acids (BCAA) were added with the percentage of 1%, 2%, 3% and 4% in treatments T_1, T_2, T_3 and T_4 respectively. The product was then analyzed for physico-chemical analysis, energy value calculation, colour determination, texture and sensory analysis on the 1st, 15th and 30th day of study, data obtained was subjected to statistical analysis to see the outcome; multiple mean comparison and response surface methodology was used. Results showed that a reduction in pH, acidity, protein, fat and moisture content was observed after the BCAA addition and storage study. L and a value increased but the b value decreased after BCAAs concentration gradually increased the lowest values were observed in the control group. For sensory evaluation, a score of fatty after taste and bitterness taste increased after adding BCAA value for colour decreased from T_0 to T_4 and each passing day. An increase in scores of co-hesiveness and adhesiveness was also observed. Overall, the maximum score was shown by T2 on day 1 and other days compared to all treatments.

Keywords: cheese spread, nutritional deficiencies, cheese curd, proximate, sensory analysis, BCAA

Introduction

Cheese is a dairy product produced in various textures, forms and flavours by coagulation of milk protein casein (Gulzar *et al*., 2015). Cream cheese spread is a soft, spreadable cheese that is structurally diversified from other types of cheese. It is a fresh, soft, un-ripened, diacetyl flavour with a smooth consistency and a creamy white colour. It is highly nutritious, rich in milk proteins (whey and casein) and fat. It can have additional ingredients, such as fruits, vegetables, meats and spices (Thage *et al*., 2005).

The double burden of malnutrition greatly affects the health infrastructure in both developed and developing nations through various forms, *i.e.*, stunting, wasting and micro-nutrient deficiencies, especially among children and lactating women. There is a need to address these deficiencies with value-added, supplemented and fortified products by considering consumer psychology and economics. Value-added nutrient-rich milk based on cheese spread and specialized cheese is needed to deal with the wastage of milk in our country and the widespread nutrition-related ailments (Brestenský *et al*., 2018).

During the manufacturing of cheese, whey is drained to give desirable consistency to cheese after curd formation. However, unfortunately during cheese making, essential (lysine, isoleucine, valine, leucine, tryptophan, etc.) and non-essential amino acids (alanine, aspartic acid, glutamic acid, serine, etc.) are lost in whey (Banks, 2004). These amino acids that are lost during processing can be added to cheese spread during manufacturing to regain the product's amino acid profile. These amino acids have an essential role in energy metabolism and other physiological events. Essential amino acids, such as valine, leucine and isoleucine, also called branched chain amino acids (BCAA), which are

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directly metabolized in muscles. They ensure the energy needed for performing work (Brighenti *et al*., 2008).

Branched chain amino acids (BCAAs) promote the synthesis of muscle protein and promote catabolism of protein; therefore, enrichment of BCAA is suggested as a potential therapy for patients with critical ailments. BCAAs also have positive effects in controlling redox state as BCAA-enriched diets increase mice's longevity by induction of mitochondrial biogenesis and reduction of oxidative stress with signaling mechanisms (Mattick *et al*., 2013). Furthermore, BCAA supplementation reduces oxidative stress, improves glucose metabolism and protein synthesis in liver cirrhosis patients. Supplementation of BCAAs can affect protein synthesis rate, redox state and intracellular signaling networks; all of these can have far-reaching ameliorative benefits (Brestenský *et al*., 2015). BCAA supplementation reduces the risk of hepatocellular carcinoma, improves muscle mass, nutritional status, complication free survival and quality of life. It can down regulate protein metabolism leading to improved nitrogen balance and clinical outcomes (Dam *et al*., 2018; Les *et al*., 2011).

The maximum and minimum dosage of BCAA is not established so far; however, the daily recommended amount of leucine and isoleucines the valine is in a ratio of 40:20:20 mg/Kg body weight supported by research evidence. Consumption of BCAA is recommended in combination rather than leucine alone because of the depletion of other BCAA in the body (Brestenský *et al*., 2015). No toxicity of BCAA is reported yet, even at high doses (Thage *et al*., 2005). Cheese spread is novel because of its unique functional attributes and currently no addition of BCAA is done in cheese. By keeping in view the above mentioned scenario the present study was designed to develop and enrich cheese spread with BCAA. The evaluation of the physico-chemical and sensory characteristics of cheese spread was also carried out.

Materials and Methods

This study was conducted in the Dairy Science and Technology Laboratory, National Institute of Food Science and Technology at the University of Agriculture, Faisalabad, Pakistan. It presents a comprehensive exploration of standard procedures adopted for the formation and analysis of cheese spread enriched with branched chain amino acids. Milk for cheese spread was obtained from Animal Husbandry Farm in the University of Agriculture, Faisalabad, Pakistan, while required chemicals and reagents were acquired from Sigma Aldrich.

Cheese preparation. Five batches of cheese spread were made (1 batch for each treatment). 2 Kg of milk was utilised for each batch. Milk was standardized to 12% protein and 5% protein for the preparation of cheese spread. Then it was homogenized (12-14 MPa at 50-55 °C), pasteurized (72-75 °C for 30-90 s) and cooled down to the desired setting temperature (30 °C). At this temperature, the starter culture (mesophilic culture) was added to the milk at the rate of 3.6 g/100 L (w/v). The milk with starter culture was incubated for half an hour at 30 °C in the incubator. After 30 min rennet (CHY-MAX® Extra, Chr. Hansen's high quality 100% chymosin-based rennet) was added to milk containing starter culture at the rate of 10 ppm and left undisturbed till the formation of cheese curd in incubator. The curd was taken out from the incubator for approximately four and a half hour and cutting was done using a cutter. Then the curd was heated at 40-42 °C for 10-15 min to separate the whey (Ong *et al*., 2020; Lucey, 2003).

Enrichment of branched chain amino acids in cheese spread. The mixture of BCAA was prepared by a combination of three amino acids leucine, valine and isoleucine in different ratios (Table 1). After removing the whey, the curd was blended by adding BCAA mixture in different proportions as listed in Table 2. BCAA mixture was added by following standard procedure and the cheese spread samples were stored at 4 °C prior to analysis.

Physicochemical analysis. ***Acidity and pH.*** Cheese spread was assessed for avidity and pH according to the methods of AOAC (Latimer, 2019). The pH meter probe was dipped into a slurry and a stable reading for each sample was noted in triplicate. The acidity of cream cheese spread was determined by the standard acid-base titration method.

Proximate analysis. The proximate composition of the cheese spreads, including moisture, protein, fat and carbohydrates which was determined using established methods (AOAC official methods of analysis). Moisture content was determined by drying samples at 105 °C until a constant weight was achieved. Protein content was estimated using the Kjeldahl method, while fat content was determined using the Soxhlet extraction method. Carbohydrate content was calculated by difference (Latimer, 2019).

Table 1. Preparation of amino acids mixture

Amino acid	Abbreviation used	Ratio in mixture
L-leucine	Leu	2
L-valine	Val	1
L-isoleucine	Lle	1

Table 2. Proportions of BCAA mixture to be used in cheese spread

Treatment	BCAA (%)
T_1	0
T_2	1
T_3	2
T_4	3
T_5	4

Energy value calculation. The energy value of the cheese spreads was determined using a bomb calorimeter (Model: CalorMaster Pro, AnalyticaTech Inc., USA) following the principles of calorimetry. This instrument measures the heat generated when a sample is completely burned in a controlled environment. The heat released during combustion is directly proportional to the energy content of the sample (Cohen *et al*., 2017).

Calculation. Energy value (Kcal/g) = (Heat released by sample)/(Mass of sample)

Colour analysis. Mini-scan portable colourimeter (Hunter Associates Laboratory Inc., Reston, USA) used for measured colour. Colour measurements were made using the Commission International de l'Éclairage (Wadhwani and McMahon, 2012). L*, a* and b* values using illuminant D65. The L* value is an indicator of luminosity (the degree of lightness from black to white). The a* value is an indicator of green (-) and red (+), whereas b* is an indicator of blue (-) and yellow (+). Because combining a* and b* gives a better indication of colour than their individual values, we calculated hue angle as the inverse tangent of the ratio b*/a*. The petri dish was placed directly on the colourimeter sensor. The colour intensity (C), the hue angle (hab) and total color difference (ΔE) in comparison to an untreated control were calculated, where hab=0° for red hue and hab°=90° for a yellow hue and the results were expressed as: C=(a*2+b*2) 0.5; hab=arctan (b*/a*); ΔE=[(L-L0)2+(a-a0)2+(b-b0)2]0.5. The colour was expressed as a whiteness index (WI) based on the formula [19]: WI=100-[(100-L*)2+a*2+b*2]0.5 where L0, a0 and b0 were the L, a and b values of the reference sample which is here the control samples.

In the colour analysis method using the Mini-scan portable colourimeter (Hunter Associates Laboratory Inc., Reston, VA), the colour measurements were conducted according to the guidelines set by the Commission Internationale de l'Éclairage (CIE) using illuminant D65. The colourimeter measures the colour attributes of the samples, including L*, a* and b* values, which provide information about luminosity, red-green colour component and blue-yellow color component, respectively.

Procedure. *Sample preparation.* Before conducting colour measurements, the cheese spread samples were appropriately prepared and conditioned. It is essential to ensure that the samples are uniformly presented to the colourimeter to obtain accurate and representative color readings.

Colourimeter setup. The mini-scan portable colourimeter was equipped with the necessary settings, including the illuminant D65, to ensure consistent lighting conditions during colour measurement.

Measurement. A petri dish containing the cheese spread sample was placed directly on the colorimeter sensor. The colorimeter scans the sample and records the L*, a* and b* values.

Hue angle calculation. The hue angle (hab) was calculated using the inverse tangent of the ratio b*/a*, providing information about the dominant hue of the colour.

Colour intensity calculation. The colour intensity (C) was calculated using the formula C=(a*^2+b*^2)^0.5, representing the colour saturation.

Total colour difference calculation. The total colour difference (ΔE) in comparison to an untreated control sample was calculated using the formula ΔE=[(L-L0)^2 +(a-a0)^2+(b-b0)^2]^0.5, indicating how much the color of the sample deviates from the reference.

Whiteness index calculation. The colour was also expressed as a whiteness index (WI) using the formula WI=100-[(100-L*)^2+a*^2+b*^2]^0.5. This index provides information about the perceived whiteness of the sample.

Sample preparation/conditioning. The specific details regarding the sample preparation and conditioning are not explicitly mentioned in the provided information, it's common practice to ensure that the samples are properly presented and conditioned to avoid any external factors that could influence colour measurements. This may include ensuring uniform spreading of the cheese spread in the petri dish and allowing any temperature related effects to stabilize before measurement.

The quality and accuracy of color measurements are influenced by proper sample handling, lighting conditions and instrument calibration. Therefore, careful attention to these factors is crucial to obtaining reliable and meaningful colour data.

Texture analysis. Texture analysis was done using a texture analyzer as described by (Mohamed and Shalaby, 2016). The texture profile tests of the cream cheese spreads were performed the day after production (1 day), in triplicate, in a Texture Analyzer CT3 (Brookfield Engineering Labs, Middleboro, USA), equipped with a cylindrical acrylic geometry (TA11/100) with 25.4 mm in diameter. The spreads were pre-homogenized in the original packaging (20×52 mm) at 10 °C and analyzed inside the container itself. Hardness, adhesiveness, gumminess and stringiness (length and work) parameters were obtained directly from the equipment program (Texture Expert for Windows, version 1.19). The samples were compressed in two cycles with 20% deformation, with a rest of 5 s between cycles and speed of 1 mm/s, while the cohesiveness of cheese spread was calculated by the area under the second compression cycle divided by the first. It describes how well a food retains its form between the 1st and 2nd chew.

Sensory evaluation. Sensory evaluation of cheese spread was done with 9 points hedonic scale according to the method prescribed by (Mohamed and Shalaby, 2016). For sensory evaluation of cheese spread a panel of judges consisting of well-trained professionals consisting of ten persons was selected. Freshly prepared cheese spread samples were placed at room temperature in airtight jars for sensory evaluation. Mineral water was placed near the samples to keep the taste of mouth neutral. Performa was developed by considering the hedonic scale having 9 categories from extremely dislike (1 point) to extremely like (9 points). The acceptance test was conceded for consistency parameters (1=less smooth; 9=homogenous), flavour (1=undetectable; 9=highly acceptable), appearance of cheese spread (1=very rough; 9=very smooth), colour (1=colourless and gray; 9=bright) fatty after taste (1=negligible; 9=very fatty) and bitterness (1=no; 9=bitter), texture and spreadability (1=not spreadable; 9=very good). The highest score indicated the highest degree of preference for a parameter among treatments.

Storage study. Cheese spread was stored at 4 °C for 30 days to assess the effect of storage on physico-chemical and sensory parameters of cheese spread enriched with BCAAs. These parameters were evaluated on the 1st, 15th and 30th day of storage.

Storage conditions. *Temperature*. The cheese spread samples were stored at a temperature of 25 °C±2 °C. This temperature range was chosen to reflect typical storage conditions in both domestic and commercial settings.

Humidity. The storage environment maintained a relative humidity of 50%±5%. Stable humidity conditions are essential to prevent moisture-related changes that could affect the sensory and physical attributes of the cheese spread.

Light exposure. To assess the potential impact of light exposure on the cheese spread samples, they were stored in opaque containers that minimized exposure to external light sources. This was done to prevent color changes and the degradation of sensitive compounds due to light-induced reactions.

Duration. The cheese spread samples were stored for a duration of 30 days. Multiple time points were selected for analysis to monitor changes in various parameters over the storage period.

Sampling protocol. At specific intervals during the storage period (weekly), representative samples were withdrawn from each storage condition for analysis. Samples were handled carefully to avoid any disturbances that could affect their quality and attributes.

Statistical analysis. Each experiment was repeated at least 3 times in order to ensure the accurarcy of the results of all analysis. The obtained data were subjected to one-way analysis of variance (ANOVA) with a Tukey Post Hoc Test was performed to investigate significant differences among the five cream cheeses by following the methods of Montgomery (2017). Response surface methodology aimed to evaluate the effect of supplementing branched chain amino acids (BCAA) and storage days interval on cheese's physico-chemical characteristics. Therefore, they were selected for further optimization. The assessment criteria were based on the maximum desirability in quality parameters of cheese spread. pH, acidity, L-value, a-value and b-value were selected and identified on the responses of cheese spread based on the previous findings. The methodology was designed with two levels under 2k tests (k: number of factors) in triplicate for each parameter. In the experimental field, two values (lowest and highest) were kept constant. The predictive model was run in a central composite design to select the most significant RSM factors (response surface model). All the calculations are based on this general equation:

$$Y=\beta_o+\beta_1X_1+\beta_2X_2+\beta_{12}X_1X_2+\beta_1^2X_1^2+\beta_2^2X_2^2$$

where:
Y=dependent variable; β=intercept; X_1=BCAA conc. (Independent variable); X_2=Days (independent variable); and β_1, β_2 and β_{12} are regression coefficients.

Tests are undertaken in central composite design. Optimization of two factors (BCAA concentration and storage days) was carried out by using Central Composite Design (CCD) (Lujan-Moreno *et al*., 2018). The variations in responses were not seen linear and CCD permits the curvature of responses. However, a quadratic model was proposed in developing the RSM. Hence in CCD, only two major factors (BCAA concentration and storage days) were proposed while ignoring the cheese spread temperature. While keeping the number of factors as low as possible, the quadratic model was recommended. Hence only two factors were used. This proposed CCD observed center point with factorial analysis. Each factor was set to equidistance from the center to a factorial point, which is ±1. The distance of the center point is ±α from the star point value with α>1. This obtained experimental design consists of ten test values. The highest value in BCAA concentration and days interval is denoted as +1, which is 4% and 30 days, respectively. Whereas the lowest value in BCAA concentration and days interval is denoted as -1, which is 0% and 0 days, respectively Table 3.

Responses of factorial experimental design with two levels for cheese spread. The first experimental analysis was carried out for each parameter and the best-suited factors were selected, which gave the most effective responses. The results for each response were given in Table 4. The concentration of amino acids and the storage time duration equally affected the cheese spread significantly. The BCAA and storage time has a significant negative effect on pH, whereas a significant positive impact on acidity and total solids. The color values varied significantly. The lightness of cheese spread had a significant negative effect. Whereas the color a and b values had a significant impact on cheese spread positively.

Results and Discussion

The main goal of the research was to examine the impact of BCAA on the consistency of the spread of cheese. Five cheese spread treatments with varying concentrations of BCAA were prepared. Different analyzes such as physico-chemical, sensory and texture analysis confirm the consistency of cheese spread.

Proximate analysis. The excellent spreading properties of cheese spread could be attributed to either the product's fat or moisture content or to a mix of these. Fig. 1 presents the moisture content of cheese spread, which revealed that the addition of branched chain amino acids has a significant effect on moisture content. On day 0, T_0 showed the highest moisture content (59.16%), whereas T_4 showed the lowest moisture content (58.04). The decreased moisture content was observed among all the treatments as the storage time increased. T_2 revealed the highest decrease in moisture content during the storage interval as compared to other treatments. Similar findings were also seen in a study conducted by Gulzar *et al.* (2015) who studied the nutritional and functional properties of fruit based cheese spread. The improper packing of cheese spread attributed to decreased moisture values as in this scenario; it starts losing moisture during storage after rind formation. Suleiman *et al.* (2011) also reported the decreased moisture content in processed cheese of three months old and it was ranged between 49.33-54.17%.

Table 3. Tests undertaken in the two-level full factorial design

Test number	X_1=Branched chain amino acid concentration	X_2=Days interval
1	+1	0
2	0	0
3	+1	-1
4	-1	-1
5	0	-1
6	-1	+1
7	+1	+1
8	0	0
9	-1	0
10	0	+1
-1	0	+1
0% amino acid	2% amino acid	4% amino acid
0 day	15 day	30 day

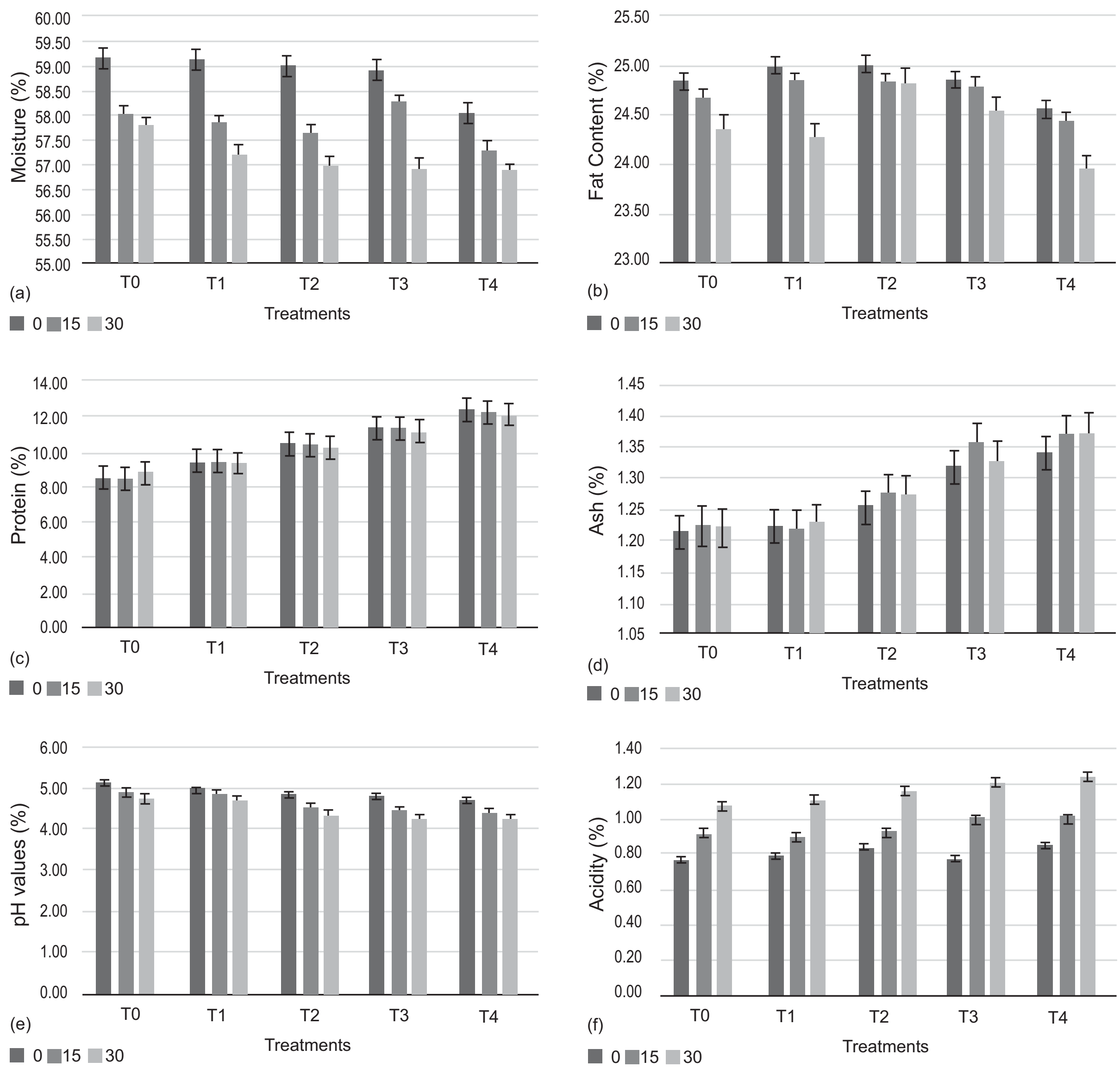

Fig. 1. Mean comparison for Physico-chemical analysis of cheese spread.

The results of fat content analysis of cheese spread have been presented in Fig. 1. Fat content in all cheese spread samples ranges from 23.94% in T_4 at day 30 to 25.01% in T_2 at day 0. The value of fat content has decreased as the storage days increases. The lipid oxidation during storage might be responsible for the decrease in fat content. Fats are broken down into smaller components, like free fatty acids, as a result of this process. These fatty acids have the ability to sublime, which causes the cheese to gradually lose some of its fat content. The highest decrease (0.78%) was observed in T_1 from 24.99% to 24.26%. The reduction in fat content is compatible with findings from previous findings of (Licitra *et al*., 2000).

Protein is an essential parameter that directly influences the texture and flavour of cheese spread. The influence of storage time and different concentrations of branch chain amino acids in cheese spread was analyzed. Fig. 1 depicts the significant difference in crude protein content. Maximum (12.42%) and minimum (8.51%) values of protein content were witnessed in the T_4 at 0 days and T_0 after 15 days, respectively. The increase in values of protein content might be due to the loss of moisture during storage. Due to branch chain amino acids in cheese spread, the increased nitrogen percent was observed among the treatments expressed as percent crude protein. These findings are well-matched by the results described by Brestensky *et al.* (2018).

Table 4. Effect of each factor on cheese spread responses

Responses	BCAA concentration (X_1)	Days interval (X_2)	BCAA: days interval (X_1X_2)
pH	-*	-	-
Acidity	+*	+*	+
Moisture	-	+*	-
Total solids	+*	-	+
L-value	-	-*	-
a-value	+	+	+
b-value	+	+	-

*= significant

Ash in all dairy items is accepted as a reason for different nutrients and minerals and little in caloric density. The highest (1.38%) and lowest (1.21%) values of ash content were seen in the T_4 after 30 days and T_0 at 0 days, respectively. The increase in ash content could be credited to the increasing salt concentration with the tenderizing of cheese spread. Moreover, gradual rise in ash content over time may be due to moisture loss, proteolysis and microbiological activity, which concentrate minerals and nonvolatile chemicals. The results of the present study are in accordance with Kaminarides *et al.* (2019). Moreover, Macdougall *et al.* (2019) observed significant changes in textural, rheological and microstructure properties in cream cheese spread with the help of confocal laser scanning microscopy.

Total solids. The total solids of food material are inorganic deposits staying after the natural portion has been consumed after burning. The minimum value of total solids (33.89) is obtained in T_0 after 0 days and the maximum total solid (38.60) is found in T_4 after 30 days. The increase in total solids might be due to decreased moisture content and an increase in the fat and protein ratio of cheese.

pH and acidity. The BCAA concentration has a stabilizing effect for maximizing the strength of cheese spread at low pH. Fig. 1 demonstrates the mean values of pH with the interaction of storage days and treatments. The highest pH value of 5.11 was observed at 0 days in treatment T_0 and the lowest value of 4.23 was deemed to occur after 30 days in treatment T_3. The decrease in pH after 30 days was observed as 0.8, 1, 1.2, 1.25 and 0.9 in T_0, T_1, T_2, T_3 and T_4 respectively. A clear decrease in pH values was seen as the storage interval increased. T_3 showed a 0.56 drop in pH which is the highest decrease as compared to others. Acidity also affects the pH values of cheese spread as high acidity pH values are low and reported by (Feeney *et al*., 2002).

The acidity of cheese spread was measured at the storage time of 0, 15 and 30 days. The impact on the acidity of all treatments, storage days and interaction of storage days and treatments were studied. Fig. 1 demonstrates the significant effects of treatments and storage on cheese spread acidity. The impacts of treatment and storage time on contact are also highly important. After storage intervals, a gradual decline in acidity values was observed. Among the treatments, a decrease in acidity was also observed. Mean acidity values show that maximum acidity (1.24) is found in the T_4 after 30 days and a minimum acidity value (0.77) after 0 days is reported in T_0. These findings showed that acidity is rising directly with increasing BCAA levels. These values are in line with what is defined by Banks (2004).

Colour analysis. The L-value, a-value and b-value of cheese spread were studied at 0, 15 and 30 storage days. The mean value of L-value, a-value and b-value is shown in Fig. 2. The maximum (94.13) and minimum (90.42) L-value of cheese spread were found in T_2 after 30 days and in T_2 after 15 days. Maximum a-value (0.54) and minimum a-value (0.11) were found in T_4 after 30 days and T_0 after 0 days. The maximum (4.93) and minimum (2.27) b-value of cheese spread were found in T_2 after 15 days and in T_4 after 30 days. Treatments do not significantly affect the L-value. T_0 seems to be a little darker, which might be due to the presence of more soluble BCAA components in T_2 and T_3 as compared to T_0. The higher value of soluble BCAA makes the cheese less dark and shinier.

Sensory evaluation. Creamy colour cheese gives a characteristic good appearance to the product. Fig. 3 presents the effect of storage and BCAA addition of sensory properties of cheese spread. It shows that the score for colour reduced after increasing the concentration of BCAA and it also reduced on the 15th and 30th day. The minimum value at day 0 was obtained by T_4 (2.67) and the maximum value was 7 on the same day. Similarly, the maximum score on day 15th and 30th for T_0 and minimum for T_4. The colour of the control cheese spread (T_0) was a little yellowish and the addition of BCAA resulted in the whitish color of the cheese spread.

It was observed that maximum (9) and minimum (7) fatty after taste values are found in the T_1 on day 0.

Considering the addition of BCAA, the emulsifying property of BCAA plays a significant role in increasing values of fatty after taste. It gives a better texture and flavour in the mouth, while with storage days, a slight reduction in fatty after taste was observed after each passing day (Muir *et al*., 1997). Fig. 3 represents the effect of the addition of BCAA and storage study on acidity. Study respondents reported a slight increase in the score of acidic flavours when the percentage of BCAA increased gradually. However, this effect was non-significant concerning days and treatments (Khiabanian *et al*., 2020). Bitternes is considered mouthfeel and detected by receptors present on the tongue. Bitterness score was recorded minimum at day 0 in T_1 and it increased with the addition of BCAA and T_4 treatment scored highest (6.67). An increasing trend of bitterness was also observed after each passing day. Respondents found T_2 satisfactory (6 scores) and 2% concertation of BCAA acceptable. These findings are similar to the results of García Gómez *et al.* (2021).

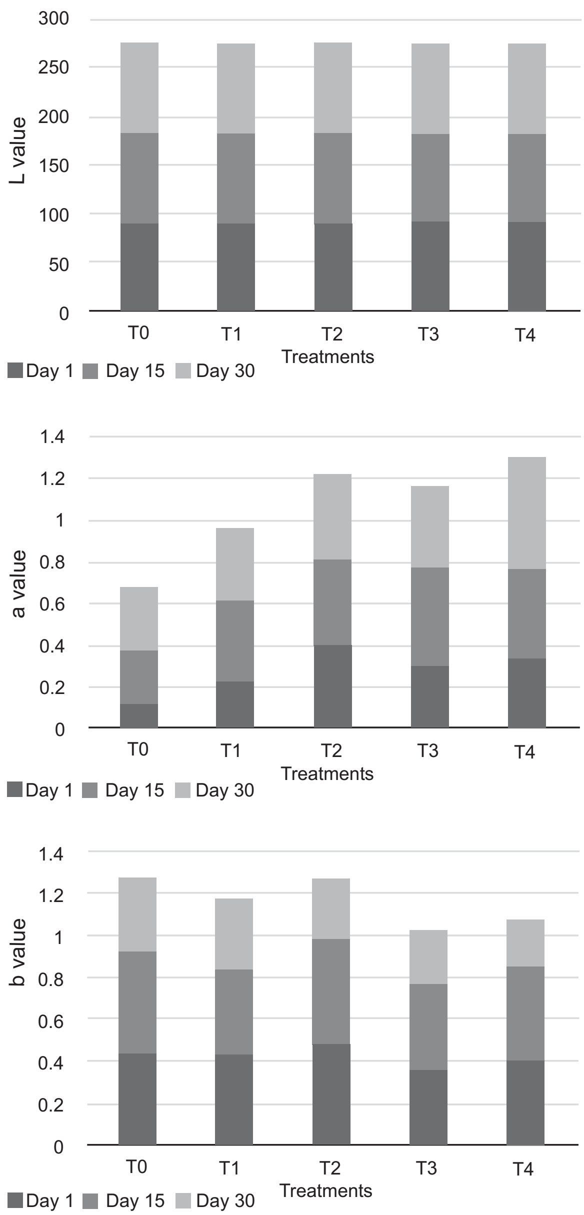

Fig. 2. Mean comparison for color (L, a, b value).

Spread ability is one of the main properties of cheese spread that distinguishes it from non-spreadable cheese varieties. The score demonstrated an inverse relationship between spread ability and BCAA concentration. The maximum spread ability score was obtained by the control sample on all days. It increased in T_0, T_1 and T_2 with storage and reduced in T_3 and T_4. The score for cohesiveness increased with each passing day and BCAA concentration increased. Products with high cohesiveness are considered as of poor quality. T_2 scored a minimum score for cohesiveness at the start of the study even better than control.

The stickiness of food is related to adhesiveness. It is evident from Fig. 3 that adhesiveness increased as well when the percentage of BCAA was more. Control samples performed better and scored less concerning this parameter. Among treatments with added BCAA T_1 performed best. Overall acceptability of prepared products serves as a quality indicator. With time, the overall acceptability score decreased, however, this difference was not statistically important. It shows that cheese spread remains acceptable without added stabilizers and preservatives for up to one month. Future explorations need to focus on the effect of BCAA enriched cheese spread organoleptic and textural properties with more storage days.

Response surface methodology. ***Predictive modeling in pH of cheese spread.*** Table 5 represents the predictive and adjusted R^2 values with precision accuracy. It is evident from the predictive modeling that pH had a significant effect on both factors. This table shows the relation of each element with its responses, significant/non-significant model developed. The predicted R-squared value of 0.8841 is in reasonable agreement with the adjusted R-squared of 0.9307. Whereas adequate precision measures the signal to noise ratio that describes a ratio greater than 4 is desirable. The ratio of 20.746 indicates a sufficient signal. This predictive model for pH is used to navigate the design space.

Table 5. R-square values for cheese spread responses.

Parameter	R^2	Adjusted R^2	Predicted R^2	Adequate precision (desired <4)	St. Dev	Mean
pH	0.9555	0.9307	0.8841	20.746	0.071	4.64
Acidity	0.9812	0.9707	0.9531	27.214	0.027	0.97
Moisture	0.9060	0.8538	0.6685	12.431	0.31	57.94
Total solids	0.9851	0.9768	0.9657	37.118	0.20	36.13
Lightness (L)	0.3952	0.0593	-0.4922	3.886	1.16	92.04
A	0.8460	0.7604	0.4461	9.886	0.053	0.35
B	0.8733	0.8029	0.7319	10.900	0.37	3.86

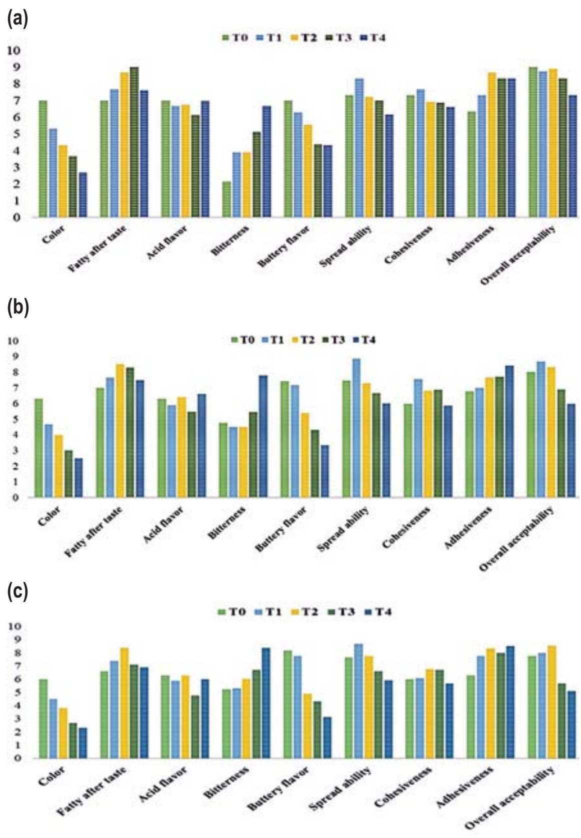

Fig. 3. Sensory properties of cheese spread enriched with BCAA at 0 days **(a)**, at 15th day **(b)** and 30th day **(c)**.

According to response surface plotting, 10 models were obtained in process optimization. Among all the predicted models, the most suitable model with the highest desirability was chosen. The desirability represents the maximum relation between the response (pH) of cheese spread and factors (BCAA and days). As shown in Fig. 3. the desirability increases as the concentration of BCAA increases to some extent beyond 3%. The desirability decreases as pH declines and causes the soft texture of cheese spread the current finds are in accordance with the findings of Huppertz *et al.* (2005).

Table 11 shows the range of cheese spread pH change during storage and concerning BCAA concentrations with the interaction of storage days and treatments. The highest pH value of 5.11 was observed at 0 days in treatment T_0 and lowest value of 4.23 seemed to occur after 30 days in treatment T_3. Table 6 elaborated the analysis of variance for response surface model analysis of the pH of cheese spread. Table 11 shows that a rise in BCAA concentration is desirable for maintaining the pH.

Predictive modeling for the acidity of cheese spread. The predicted R-squared value of 0.9812 is in agreement with the value of adjusted R_2 (0.9707). This shows pH is significantly affected by independent variables; branched-chain amino acids concentration and storage days. Adequate precision greater than 4 is desirable and adequate precision for acidity is 27.214 falls in this criteria and is a sufficient signal (Table 5).

The desirability represents the maximum relation between the response (acidity) of cheese spread and factors (BCAA and days). As shown in Table 11 the

Table 6. Analysis of variance for response surface model analysis of the pH of cheese spread

Source	Sum of squares	df	Mean square	F-value
Model	0.97	5	0.19	38.62*
A-Conc.	0.47	1	0.47	93.54
B-Days	0.46	1	0.46	91.75
AB	0.013	1	0.013	2.58
A^2	0.018	1	0.018	3.66
B^2	8.003E-003	1	8.003E-003	1.59
Residual	0.045	9	5.038E-003	
Total	1.02	14		

desirability increases as the concentration of BCAA increases the desirability decreases as acidity rises and causes the soft texture of cheese spread. Table 7 illustrates the analysis of variance for response surface model analysis of acidity of cheese spread. The current study strongly agreed with Akarca *et al.* (2023), they studied the mozzarella cheese made from buffalo milk for its functional and textural properties. Table 11 shows the range of cheese spread acidity change during storage and concerning BCAA concentrations. The calculated model for acidity for both factors in single and in their combined effect explained 75-98% of the observed values. Acidity and pH are maintained if the concentration of BCAA is increased. The findings of the present research are supported by several studies on cheese textural and functional properties with a special focus on pH and acidity (Alhebshi *et al*., 2022; Nazir *et al*., 2022; Khiabanian *et al*., 2020).

Predictive modeling in L-value of cheese spread. Table 5 demonstrates values of R^2, Adjusted R^2, Predicted R^2 and Adequate precision are 0.3952, 0.0593, -0.4922, and 3.886 respectively. Analysis of variance for response surface model analysis of lightness of cheese spread shows an significant impact of L-value on cheese spread (Table 8). Table 11 demonstrates that the L value was lowest at day 0 and 0% concentration of BCAA. However, it increased with passing days and contraction of BCAA.

Predictive modeling in a-value of cheese spread. Table 9 shows the values of R^2, Adjusted R^2, Predicted R^2 and adequate precision are 0.8460, 0.7604, 0.4461 and 9.886 respectively. Table 11 demonstrates the range of a-value during storage and BCAA concentrations. The actual value for a-value is close to the predicted value, showing that our model is a good fit. Table 11 showed that the a-value was minimum at day 0 and it increased with days and treatments (from T_1 to T_4).

Predictive modeling in b-value of cheese spread. According to the analysis of variance for response surface model analysis of color b-value of cheese spread it is evident that it significantly effects the color characteristics of cheese spread (Table 10). Table 5 depicts that the R-squared value of 0.8733 is with the adjusted R-squared of 0.8029. The value for adequate

Table 7. Analysis of variance for response surface model analysis of acidity of cheese spread

Source	df	Sum of squares	Mean square	F-value
Model	5	0.35	0.070	93.93*
A-Conc.	1	0.024	0.024	32.39
B-Days	1	0.32	0.32	426.11
AB	1	4.805E-003	4.805E-003	6.46
A^2	1	5.952E-005	5.952E-005	0.080
B^2	1	3.413E-003	3.413E-003	4.59
Residual	9	6.692E-003	7.436E-004	
Total	14	0.36		

*=Significant

Table 8. Analysis of variance for response surface model analysis of lightness of cheese spread

Source	Sum of squares	df	Mean square	F-value
Model	7.96	5	1.59	1.18*
A-Conc.	0.66	1	0.66	0.49
B-Days	3.83	1	3.83	2.83
AB	3.29	1	3.29	2.43
A^2	0.098	1	0.098	0.072
B^2	0.086	1	0.086	0.064
Residual	12.19	9	1.35	
Total	20.15	14		

*=Significant

Table 9. Analysis of variance for response surface model analysis of color a-value of cheese spread

Source	Sum of squares	df	Mean square	F-value
Model	0.14	5	0.028	9.89*
A-Conc.	0.069	1	0.069	24.82
B-Days	0.048	1	0.048	17.09
AB	0.000	1	0.000	0.000
A^2	8.571E-003	1	8.571E-003	3.08
B^2	0.012	1	0.012	4.45
Residual	0.025	9	2.785E-003	
Total	0.16	14		

*=Significant

Table 10. Analysis of variance for response surface model analysis of color b-value of cheese spread

Source	Sum of squares	df	Mean square	F-value
Model	8.30	5	1.66	12.40*
A-Conc.	1.06	1	1.06	7.95
B-Days	4.07	1	4.07	30.40
AB	0.17	1	0.17	1.25
A^2	6.193E-003	1	6.193E-003	0.046
B^2	3.00	1	3.00	22.38
Residual	1.20	9	0.13	
Total	9.51	14		

*=Significant

precision is 10.900, that described a ratio greater than 4 is desirable. Table 11 shows the b-value of cheese spread, depicting its value every day during storage rather than selected days (0, 15th and 30th). It presents the comparison of the actual trend of a value of BCAA than the predicted one. Both trends are close to each other, demonstrating that the model is a good fit. Table 11 shows that the b-value decreased with the addition of BCAA and storage study. Maximum value b-value was obtained at day 0.

Table 11. Mean values of pH, Acidity, Lightness, a value and b value with storage days

Treatment	Days			SEM
	0	15	30	
		pH		
T_0	5.11±0.05	4.87±0.06	4.74±0.05	0.0564
T_1	4.92±0.04	4.84±0.06	4.67±0.06	0.0403
T_2	4.83±0.04	4.53±0.02	4.33±0.06	0.0729
T_3	4.79±0.02	4.41±0.02	4.23±0.02	0.0824
T_4	4.71±0.04	4.39±0.04	4.24±0.04	0.0810
SEM	0.0359	0.0567	0.0588	
		Acidity		
T_0	0.77±0.02	0.91±0.03	1.07±0.05	0.0454
T_1	0.79±0.02	0.90±0.03	1.11±0.04	0.0476
T_2	0.84±0.02	0.93±0.06	1.16±0.04	0.0491
T_3	0.76±0.04	1.00±0.06	1.21±0.05	0.0662
T_4	0.85±0.04	1.00±0.05	1.24±0.04	0.0580
SEM	0.0114	0.0151	0.0191	
		Lightness		
T_0	90.47±0.34	92.46±0.35	93.77±0.28	0.4892
T_1	91.23±0.65	93.25±0.39	91.98±0.54	0.3332
T_2	92.59±0.44	90.42±0.95	94.13±0.57	0.5744
T_3	91.04±0.82	91.01±0.44	93.00±0.41	0.3705
T_4	92.02±0.43	92.50±0.56	90.66±0.57	0.3146
SEM	0.2352	0.3072	0.3541	
		a value		
T_0	0.11±0.03	0.25±0.03	0.31±0.02	0.0294
T_1	0.21±0.02	0.39±0.03	0.35±0.01	0.0288
T_2	0.38±0.04	0.42±0.02	0.41±0.03	0.0100
T_3	0.29±0.02	0.47±0.02	0.39±0.03	0.0258
T_4	0.32±0.02	0.43±0.02	0.54±0.03	0.0314
SEM	0.0255	0.0206	0.0213	
		b value		
T_0	4.37±0.06	4.80±0.10	3.50±0.26	0.1970
T_1	4.23±0.15	4.23±0.06	3.33±0.15	0.1546
T_2	4.77±0.06	4.93±0.15	2.93±0.15	0.3225
T_3	3.57±0.15	4.07±0.25	2.50±0.20	0.2385
T_4	3.97±0.15	4.43±0.21	2.27±0.15	0.3330
SEM	0.1105	0.0954	0.1325	

T_0=Control; T_1=1% branched chain amino acid added; T_2=2% branched chain amino acid added; T_3=3% branched chain amino acid added; T_4=4% branched chain amino acid added.

Conclusion

Cheese spread is a valuable product from an industrial and nutritional point of view. It can help in the reduction of malnutrition and other ailments. Moreover, economic benefits for industry, as well as the country, can be obtained as well. Branched-chain amino acids can be used as a source to increase the nutritional value and health benefits of cheese spread. In the present study treatment, T2 performed better in sensory evaluation and was liked more by panelists. There is a need to explore further the effect of BCAA on cheese quality concerning other parameters.

Acknowledgement

The present study was performed with the support of the National Institute of Food Science and Technology, University of Agriculture Faisalabad. This project was funded by Technology, Development Fund, Higher Education Commission, Pakistan.

Conflict of Interest. The authors declare that they have no conflict of interest.

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