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Original Article

Papaya (*Carica papaya*) latex- an alternative to rennet for cottage cheese preparation

Md. Shohel Rana[1], Md. Raihanul Hoque[1], Md. Obaidur Rahman[1], Raihan Habib[1] and Mohammad Shohel Rana Siddiki[1,#]

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AFFILIATIONS

[1]*Department of Dairy Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.*

CORRESPONDENCE:

[#] Mohammad Shohel Rana Siddiki, *Department of Dairy Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.*
E-mail: msrsiddiki@bau.edu.bd

ABSTRACT

Objective: The study was conducted to standardize the desire level of papaya latex on quality of cheese prepared from cow milk.
Materials and methods: Cheese sample was prepared using whole milk collected from Dairy Farm, Bangladesh Agricultural University. This experiment was conducted on five treatments from cow milk named as sample CC_1: cow control; A_1: 2 drops (0.06 gm); B_1: 3 drops (0.10 gm); C_1: 4 drops (0.14 gm); D_1: 5 drops (0.16 gm) of papaya latex. All experimental cheeses were judged by a panel of judges for organoleptic evaluation using a score card. The total solids and ash content of the different types of cheese were determined by oven drying method according to AOAC. Fat per cent, protein and acidity were determined by Babcock method, procedure and titrating with N/10 sodium hydroxide solution, respectively described by Aggarwala and Sharma.
Results: There was significant difference within the overall physical score of different samples except color score. Overall score of sample B_1 was highest (93.67±1.53) and score of sample D_1 was lowest (72.33±12.01). In case of chemical analysis, the highest protein value was 17.14±0.34% found in B_1 and lowest value was 14.94±0.16% found in D_1. The highest fat value of sample B_1 was 23.00±1.00% and lowest value was 16.00±1.00% found in C_1. Highest carbohydrate was found in A_1 (9.44±2.25) and lowest in D_1 (5.02±0.04). Total solids and moisture content of cheese differed significantly ($P<0.01$) among the sample. On the other hand, non-significant difference was found in acidity.
Conclusion: Cheese from cow milk, time on curd coagulation have significant difference ($P<0.01$) but non-significant difference was found in yield. Highest yield was found in sample CC_1 (200.00±5.00 gm/kg) and lowest yield was found in B_1 (193.33±2.89 gm/kg).

KEYWORDS

Cheese; Coagulation; Cow; Latex; Papaya; Rennet

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INTRODUCTION

Cheese is one of the most common and widely consumed fermented and important dairy products due to its' excellent dietary source of high-quality protein, vitamins and minerals such as absorbable dietary calcium (Walther et al., 2008). Cheese is broadly used from the ancient periods with hundred of verities.

Milk and protein coagulant are the essential ingredients in cheese making. Coagulation of milk is the primary step for the development of cheese flavor and texture (Ozcan and Kurdal, 2012). It relies on specific enzymatic proteolytic degradation of milk components especially protein to improve textural properties and the nutritional value of cheeses. In most cheese production uses rennet as the coagulant (Guinee and Wilkinson, 1992). Other coagulating agents as for example plant extracts that have not only the capacity to coagulate milk and but also proteolytic activity which can be used as a substitute of rennet to make cheese (Roseiro et al., 2003). Preparation of cheese form papain has been practiced in Indonesia since the Dutch colonial era (Rahman, 2014). Normally, the latex of papaya (*Carica papaya L.*) raw fruits are the source of papain which has strong milk clotting activity and also particularly proteolytic. It requires a free sulfhydryl group for its catalytic activity. The papain, a photolytic enzyme isolated from papaya latex is capable to break down polypeptides which are organic molecule consist of amino acids (Amri and Mamboya, 2012).

The cheese which is manufactured by using papain enzyme as coagulant is Dangke, it originates from Enrekang, a regency of The South Sulawesi province of Indonesia. Rahman (2014) mentioned that, traditionally the steps of Dangke production are fresh cow milk is heated until it reaches 70ºC and added with papaya sap which contains papain enzyme. After the curds are coagulated, it was sundered and molded into cheese forms. This crude enzyme from plant latex are suitable for preparing softer cheeses as compared with rennet and is less expensive than other coagulants. In addition, the use of animal rennet may be confined for religious reasons (e.g., Judaism and Islam) (Roseiro et al., 2003). The major purpose of the present study was to utilize the plant resources in Bangladesh, notably papaya tree for its enzyme, known as papain, which is found in the latex of the un ripened yet matured fruit for use as alternative milk coagulant rather than employing the conventional rennet for the production of fresh un-ripen cheese upon optimizing the process parameters of cheese production.

MATERIALS AND METHODS

Duration and Site of the experiment: During the period April to September, 2016 the milk samples were collected from the Dairy Farm, Bangladesh Agricultural University (BAU) and the experiment was conveyed at the Dairy Chemistry, Microbiology and Technology Laboratory of the Department Dairy Science, BAU, Mymensingh

Preparation of crude enzyme: Fresh papaya was collected from the healthy plant tree early in the morning and washed with alcohol. Papaya punched with syringe, and allowing the milky latex to drain and latex collected drop by drop. The papaya latex served to coagulate the milk protein and formed curds.

Cheese preparation: For each trial, 2.5 liters of milk was used. Milk was boiled at 81˚C. After cooling it to 40˚C, calcium chloride ($CaCl_2$) was added at 0.03%, and was allowed to stand for 10 min. Then bacterial culture was added to the boiled milk. Then starter culture (composed of *S. thermophilus, L. bulgaricus* and *S.lactis)* was added to the boiled milk. Generally 1% starter culture (sour *dahi*) was used. The milk with starter culture was mixed thoroughly and ripened for 1 h at 40˚C. Lactic Acid Bacteria (LAB) were responsible for converting lactose into lactic acid, thereby reducing the pH of the solution. At that time, the milk was allowed to ripen for 1 h up reaches appropriate pH level. After addition of starter culture, papaya latex (papain) at A_1: 2 drops (0.06 gm); B_1: 3 drops (0.10 gm); C_1: 4 drops (0.14 gm); D_1: 5 drops (0.16 gm) and CC_1: cow control 0.3 gm/kg of cheese milk (Habib et al., 2012) rennet was added for coagulation of milk. Required quantity of papaya latex (papain) was added by thoroughly mixing.

After addition of papaya latex the milk was allowed to stand for 3-4 h to complete coagulation. After coagulation the curd was cut into cubes with the size of 1/4 to 5/8 inch using stainless steel wire knives. After cutting of coagulated curd it was allowed to continuous stirring and scalding for removing whey water from the curd and gaining appropriate pH (4.6). The curd was cooked at 46˚C. The curd was stirred constantly at this step to avoid uneven cooking or overcooking, and the cooking only took 20 min. After stirring and scalding, the whey was allowed to drain out from the coagulated curd and the curd was collected when most of the whey had gone. When all of the curd was milled, salt was added at about 1% by weight. Then the curd was hooped and pressed into a

stainless steel dices to form cheese block by forcing pressure. After collection of cheese it was kept in refrigerator for further use.

Analysis of the cheese samples: By using a score curd for organoleptic evaluation of all experimental cheese samples were judged by a panel of judges.. The main object of judging was to evaluate whether there prevailed any significant differences in cheeses of different groups in terms of color, finish, body and texture and flavor. For the different type of cheese samples, oven drying method (AOAC, 2000) was used to determine the total solids and ash; and they were expressed as % by weight. Babcock method, described by Aggarwala and Sharma (1961) was used to determine fat percent and protein was determined by Kjeldahal procedure. Acidity was measured by titrating with N/10 NaOH (sodium hydroxide) solution using the procedure of Aggarwala and Sharma (1961).

Statistical analysis: The statistical model was Completely Randomized Design (CRD) and statistical analysis was done using Statistical Package software (SPSS). The differences among means of sample were compared with the help of a Least Significant Difference test. Any difference with a significant level below 0.05 has been highlighted with an asterisk.

RESULTS AND DISCUSSION

Physical parameter

Flavor: The statistical analysis among cheese samples (**Table 1**) showed significant difference ($P<0.01$). Highest flavor score was recorded in case of sample B_1 (3 drops papaya latex). On the other hand, lowest score was seen in case of sample D_1 (5 drops papaya latex). This result indicates that judges appreciated the 3 drop in cheese. The flavor of cheese is the result of the interaction of enzymes from the milk; starter bacteria; ripening from the rennet and accompanying lipases, and secondary flora. Result of this experiment also agrees with the work of Mijan et al. (2010) who reported that the flavor of cheese manufactured from cow milk was 40.1.

Body and texture: The statistical analysis showed a significant difference within body and texture score of different samples (**Table 1**) ($P<0.01$). Highest body and texture score was recorded in case of sample CC_1 (0.28 gm rennet). On the other hand, lowest score was observed in case of sample D_1 (5 drops papaya latex). Body scores are accompanied by the conversion of part of the protein to water soluble compounds mainly proteases and peptones. The result is in similar with the findings of Mijan et al. (2010) and also similar with the result of Rasheed et al. (2016), where body and texture score of cheese was 25-27.

Finish: Within finish score of different samples was differed significantly ($P<0.05$) (**Table 1**). Highest finish score was recorded in case of sample B_1 (3 drops papaya latex). On the other hand, lowest score was seen in case of sample D_1 (5 drops papaya latex). This result indicates that judges appreciated the 3 drops in cheese due to better appearance, smooth surface, free from cracks and practically free from molds. The result is in agreement with the findings of Mijan et al. (2010).

Color: The cheese color was yellowish-white manufactured from cow milk. Non-significant difference showed within color score of different samples (**Table 1**) .Highest color score was recorded in case of sample B_1(3 drops papaya latex). On the other hand, lowest score was recorded in case of sample A_1 (2 drops papaya latex). This result indicates that judges appreciated the 3 drops in cheese. The result is also consistent with Mijan et al. (2010) who found color score of cheese to be 8.2.

Overall score: Significant difference was found within overall score of different samples (**Table 1**). Highest score was recorded in case of sample B_1 (3 drops papaya latex). On the other hand, lowest score was recorded in case of sample D_1 (5 drops papaya juice). The overall score of sample B (3 drops papaya latex) was 93.67 ± 1.53. This result indicates that judges appreciated the 3 drops in cheese manufacturing.

Chemical parameters

Fat content: The amount of fat content of cheese samples are demonstrated in **Table 2**. Statistically, differences were highly significant among those mean values ($P<0.01$). It was found that sample B_1 (23%) contains highest score on the other hands sample C_1 contains lowest score. Previous studies have mentioned that sodium chloride may inhibit lipolysis in cheese (Wolf et al., 1983). Though, within the range 0.5% - 3.0% (w/w) of NaCl had no influence of NaCl on lipolysis (Rulikowska et al., 2013). The results for cow milk cheese coincide with Mijan et al. (2010) and Rasheed et al. (2016) who found 23.5%.

Protein content: Differences of protein contents of cheese samples (**Table 2**) were significant ($P<0.05$) among those mean values. It was found that sample CC_1 contains highest score than the others. Proteolytic enzymes such as papain are responsible for the formation of intermediate size nitrogenous products, such as

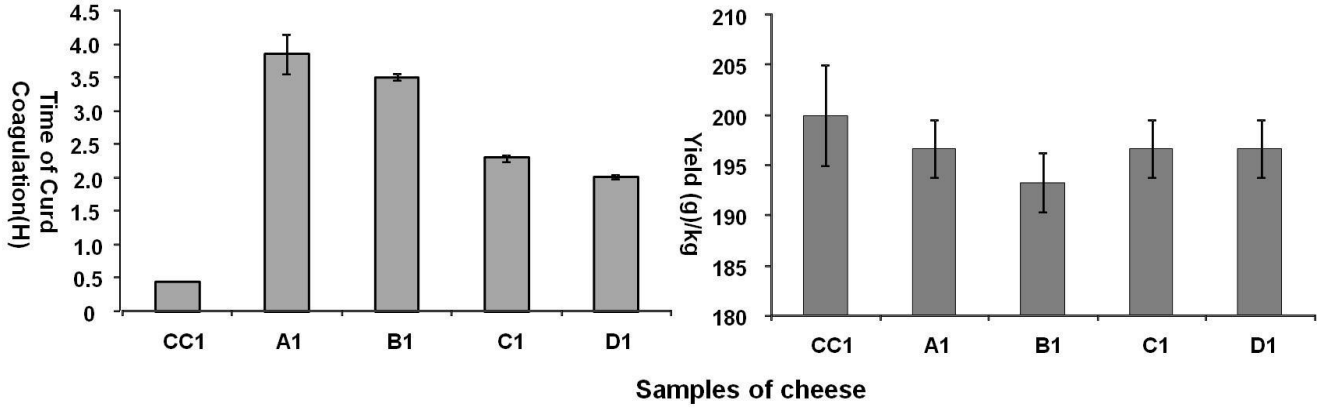

Figure 1: Effect of rennet (control group) and papaya latex on curd coagulation of different type of cheese prepared from cow milk. CC_1= 0.28 g rennet, A_1=2 drops papaya latex,B_1=3 drops papaya latex,C_1=4 drops papaya latex, D_1= 5 drops papaya latex.

Table 1: Overall score obtained by different types of cheese Samples from cow milk

Sample No.	Flavor (45) Mean±SD	Body and Texture (30) Mean±SD	Finish (15) Mean±SD	Color (10) Mean±SD	Total (100) Mean±SD
CC_1	43.33^{a}±0.58	28.00^{a}±0.00	12.11^{a}±0.58	9.00±0.00	92.60^{a}±0.58
A_1	43.00^{a}±1.73	27.33^{a}±0.58	10.67^{c}±0.58	8.33±0.58	89.33^{b}±1.53
B_1	44.33^{a}±0.58	27.67^{a}±0.58	12.33^{ab}±0.58	9.33±0.58	93.67^{a}±1.53
C_1	41.67^{b}±1.53	27.67^{a}±0.58	11.33^{b}±0.58	9.00±0.00	89.67^{b}±2.52
D_1	30.33^{c}±6.81	23.00^{b}±5.20	10.00^{d}±0.00	9.00±1.00	72.33^{c}±12.01
LSD	3.398	2.486	0.543	0.606	5.861
L S	**	**	*	NS	**

***=Significant at 1% level,*=Significant at 5% level, LS=Level of Significance,LSD = Least Significant Difference, NS=Non significant.^{abcd}=means with the different superscripts differed significantly within the same column. Where,CC_1=0.28 g rennet, A_1=2 drops papaya latex, B_1=3 drops papaya latex, C_1=4drops papaya latex, D_1= 5 drops papaya latex*

Table 2: Chemical composition of different types of cheese samples

Sample No.	Fat(%) Mean±SD	Protein(%) Mean±SD	CHO(%) Mean±SD	TS (%) Mean±SD	Ash(%) Mean±SD	Acidity(%) Mean±SD	Moisture(%) Mean±SD
CC_1	19.50^{c}±0.50	19.33^{a}±2.09	9.35^{a}±1.38	51.47^{a}±3.51	2.19^{b}±0.22	0.50±0.07	48.09^{b}±4.16
A_1	21.00^{b}±1.00	16.77^{c}±2.03	9.44^{a}±2.25	52.24^{a}±1.90	2.46^{b}±0.33	0.56±0.08	48.20^{b}±1.32
B_1	23.00^{a}±1.00	17.14^{b}±0.34	8.80^{b}±0.52	52.48^{a}±1.07	2.70^{a}±0.10	0.61±0.01	47.57^{b}±1.07
C_1	16.00^{d}±1.00	17.03^{b}±0.85	7.23^{c}±0.13	42.45^{b}±1.03	2.70^{a}±0.10	0.61±0.01	57.55^{a}±1.03
D_1	19.00^{c}±1.00	14.94^{d}±0.16	5.02^{d}±0.04	42.50^{b}±0.84	2.50^{ab}±0.10	0.61±0.02	57.83^{a}±0.36
LSD	0.968	1.437	1.267	2.038	0.205	0.047	2.173
LS	**	*	**	**	*	NS	**

***=Significant at 1% level, *=Significant at 5% level, LSD = Least Significant Difference, NS=Non significant, LS = Level of Significance,^{abcd}=means with the different superscripts differed significantly within the same column. Where,CC_1= 0.28 g rennet, A_1=2 drops papaya latex,B_1=3 drops papaya latex,C_1=4 drops papaya latex, D_1= 5 drops papaya latex.*

proteoses, peptones, polypeptides, peptides and free amino acids. Enzymes of micro-organisms work on these and other substances to produce amino acids, amines, fatty acids, esters, aldehydes, alcohols and ketones (Fox and McSweeney, 1996). Cheese serves as a store house of essential amino acids, having similar proportion of that is present in milk. Mijan et al. (2010) who reported that the cheese protein content prepared from cow milk was 20.5%. Protein content in cheese may be varied, rely on protein content in initial milk, processing or effect of papain.

Carbohydrate (CHO) content: Significant difference (P<0.01) was found within the carbohydrate content of different samples (**Table 2**). The higher carbohydrate content was in sample B_1 (9.44%) than the others. Lactose is the main carbohydrate of milk. In addition to lactose, milk contains little amounts of glucose, gelatos, and other saccharides. When milk is coagulated, most percentage of the lactose is drained out in the whey and less percentage remaining in the curd. The result of this study is almost similar to the results of Mijan et al. (2010)

and Islam (2006) who found 5.45% and 5.5% carbohydrate, respectively.

Total solids (TS) content: Differences were highly significant ($P<0.01$) among the mean values of total solids content of cheese (**Table 2**). It was found that sample B_1 contains highest 52.48 % TS than the others. The weight loss in cheese during ripening has been imposed mainly to the loss of moisture (Buffa et al., 2003). The uptake of salt also affects the loss of moisture (Melilli et al., 2006). The result is in agreement with Mijan et al. (2010) and Yalman et al. (2017) found 52.9% and 50.6% respectively.

Ash content: Statistically, there was significant difference ($P<0.05$) within the ash content of different cheese samples (**Table 2**). It was found that sample B_1 and C_1 contains highest 2.70% ash than the others. Ash is always higher than the milk from it made due to salt added in cheese, which increase the amount of ash percentage in cheese. The result is similar to the result of Mijan et al. (2010) found 2.7% ash in the cheese prepared from cow milk.

Acidity content: The acidity percentage of cheese samples are demonstrated in **Table 2**. Statistically, there was no significant difference within the acidity content of different types of cheese samples. Acidity of different cheese sample CC_1, A_1, B_1, C_1 and D_1 were 0.50±0.07, 0.56±0.08, 0.61±0.01, 0.61±0.01, and 0.61±0.02. The result is slightly lower than that of Mijan et al. (2010) who reported 0.7% acidity in cheese prepared from cow milk.

Moisture content: There was significant difference ($P<0.01$) within the moisture content of different cheese samples (**Table 2**). The highest moisture was observed in sample D_1 and the lowest in C_1. Moisture content of different cheese sample CC_1, A_1, B_1, C_1 and D_1 were 48.09±4.16, 48.20±1.32, 47.57±1.07, 57.55±1.03 and 57.83±0.36.The result is in similar trend with Islam (2006) who found 46.8% moisture content of cows' milk cheese whereas Mijan et al. (2010) found 47.4% .

Effect of rennet (control group) and papaya latex on curd coagulation for cheese prepared from cow milk

Time of curd coagulation (Hour): A significant difference ($P<0.01$) was found within the different cheese samples (**Figure 1**). It was found that sample CC_1 took lowest time in curd coagulation than the others. The samples were allowed for 30 min with undisturbed condition. Otherwise, the curds will be broken into pieces too small to filter efficiently with cheese cloth. Experimental samples took too much time on curd coagulation.

Yield (g)/kg: No significant difference recorded within the yield of different cheese samples (**Figure 1**). It was found that sample CC_2 contains highest yield than the others. Cheese yield is a very important parameter because the higher the recovered percentage of solids results in the greater amount of cheese obtained and therefore profitable in economic terms (El-Gawad and Ahmed, 2011). The reduction in yield is imposed to the highly proteolytic nature of plant based milk coagulants. Mahajan and Chaudhari (2014) who found 234.9 gm/kg for cheese made from milk coagulating action of papain enzyme in slightly similar with the result of this study.

CONCLUSION

Milk coagulation is the major step in the development of cheese texture and flavor which rely on specific enzymatic proteolytic degradation of milk components specially proteins. Papain could be used in the dairy industry as a alternative of rennet. This research aim to conduct to evaluate the quality of cheese prepared from cow milk by using different concentration of papaya latex. Time of curd coagulation and yield observed, physical and chemical analysis was performed to evaluate the effect of different level of papaya latex on quality of cheese. Analysis from the obtained results in this research work of all parameters, it was fund that the sample "B" was superior than other samples regarding to the physical tests such as flavor, color finish, body and texture score and chemical qualities on the basis of total solid, protein and fat content.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHORS' CONTRIBUTION

MS Rana and MSR Siddiki conceived the study and study design. R Habib and MR Hoque analyzed data. MS Rana and MSR Siddiki wrote and edited the manuscript. All authors were involved in revising the manuscript and approved the final manuscript.

REFERENCES

1. Aggarwala AC, Sharma. RM, Laboratory Manual of Milk Inspection, 4th edition. Asia Publishing House, New Delhi, India. 1961; p. 14.
2. Amri E, Mamboya F. Papain, a plant enzyme of biological importance: A Review. American Journal of Biochemistry and Biotechnology. 2012; 8(2):99-104. https://doi.org/10.3844/ajbbsp.2012.99.104
3. AOAC. Association of official analytical chemists. official methods of analysis, 16th Edn., Washington, DC, USA. 2000; p. 20–49.
4. Buffa M, Guamis B, Saldo J, Trujillo AJ. Changes in water binding during ripening of buffalo milk. Indian Journal of Dairy Science. 2003; 49(5):350-352.
5. El-Gawad MAMA, Ahmed NS. Cheese yield as affected by some parameters review. Acta Scientiarum Polonorum Technologia Alimentaria. 2011; 10(2):131-153
6. Fox PF, McSweeney PLH. Proteolysis in cheese during ripening. For Reviews International. 1996; 12:457-509.
7. Guinee TP, Wilkinson MG. Rennet coagulation and coagulants in cheese manufacture. International Journal of Dairy Technology, 1992; 45(4):94–104. https://doi.org/10.1111/j.1471-0307.1992.tb01791.x
8. Habib R, Wilbey RA, Grandison AS. Pressing technique and its effect on the quality of Dhaka cheese. International Journal of dairy Technology. 2012; 65(3):379–386. https://doi.org/10.1111/j.1471-0307.2012.00827.x
9. Mahajan RT, Chaudhari GM. Plant latex as vegetable source for milk clotting enzymes and their use in cheese preparation. International Journal of Advanced Research. 2014; 2(5):1173-1181.
10. Melilli C, Barbano DM, Caccamo M, Tuminello L, Carpino S and Licitra G. Interaction of brine concentration, brine temperature and presaltingon salt penetration in Ragusano cheese. Journal of Dairy Science, 2006; 89(5):1420-1438. https://doi.org/10.3168/jds.S0022-0302(06)72211-1
11. Mijan MA, Haque MA, Habib MA, Wadud MA. Evaluation of quality of mozzarella cheese. The Bangladesh Veterinarian. 2010; 27(1):36–42.
12. Ozcan T, Kurdal E. The effects of using a starter culture, lipase, and protease enzymes on ripening of mehalic cheese. International Journal of Dairy Technology. 2012; 65(4):585–593. https://doi.org/10.1111/j.1471-0307.2012.00868.x
13. Rahman S. Studi pengembangan dangke sebagai pangan lokal unggulan dari susu di kabupaten enrekang. Jurnal Aplikasi Teknologi Pangan. 2014; 3(1):20–25.
14. Rasheed S, Qazi IM, Ahmed I, Durrani Y, Azmat Z. Comparative Study of Cottage Cheese Prepared from Various Sources of Milk. Proceedings of the Pakistan Academy of Sciences: Pakistan Academy of Sciences B. Life and Environmental Sciences. 2016; 53(4):269–282.
15. Roseiro LB, Barbosa M, Ames JM, Wilbey RA. Cheese making with vegetable coagulants use of Cynara L. for the production of ovine milk cheeses. International Journal of Dairy Technology. 2003; 56(2):76-85. https://doi.org/10.1046/j.1471-0307.2003.00080.x
16. Rulikowska A, KilcawleyK N, Doolan IA, Alonso-Gomez M, Nongonierma AB, Hannon JA, Wilkinson MG. The impact of reduced sodium chloride content on Cheddar cheese quality. International Dairy Journal, 2013; 28(2):45-55. https://doi.org/10.1016/j.idairyj.2012.08.007
17. Walther B, Schmid A, Sieber R, Wehrmüller K Cheese in nutrition and health. Dairy Science Technology. 2008; 88:389-405. https://doi.org/10.1051/dst:2008012
18. Wolf ID, Raper NR, Rosenthal JC. USDA activities in relation to the sodium issue: 1981-83. Food Technology. 1983; 37(9):59-63.
19. Yalman M, Güneşer O, Yüceer YK. Evaluation of some physical, chemical and sensory properties of kasar cheese and its processed and analogue types. Journal of Agricultural Sciences. 2017; 23:63-75.

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