International Journal of Food and Fermentation Technology
Citation: *Int. J. Food Ferment. Technol.*, **13**(01): 47-56, June 2023
DOI: 10.30954/2277-9396.01.2023.5

ASSOCIATION FOR AGRICULTURE ENVIRONMENT AND BIOTECHNOLOGY

RESEARCH PAPER

Shelf-life Evaluation of Quarg- type Cheese Incorporated with Encapsulated *Terminalia arjuna*

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Paper No.: 276 **Received:** 23-02-2023 **Revised:** 25-05-2023 **Accepted:** 03-02-2023

ABSTRACT

Quarg-type cheese incorporated with encapsulated *Terminalia arjuna* was stored at 5 ± 1 °C to analyse the storage stability of the product. During the period of storage, the samples were analysed for its physico - chemical properties like moisture, pH, instrumental colour characteristics. As the storage period proceeded an increase in the acidity and reduction in moisture was observed. During the storage period colour L* values decreased and both a* and b* values increased. The antioxidant activity was estimated by % DPPH inhibition activity and during storage period the activity gradually decreased from 95.49 ± 3.46% to 86.13 ± 1.49%. The total phenolic content also decreased as the days of storage increased. The proteolysis analysed by tyrosine value indicated that in optimized sample the proteolysis was rapidly occurring as compared to control sample. At the end of storage period control sample had tyrosine value of 27.40 ± 0.14 whereas optimized sample had a value of 40.30 ± 0.09. An increase in the total viable count and yeast and mold count was observed in the microbial analysis conducted for both control and optimized samples during the storage period. The textural properties were analysed during the storage period which indicated a reduction in hardness in optimized samples. The adhesiveness increased in the optimized samples when compared to control samples. All the analysis carried out indicated positive and negative results in terms of storage stability.

Keywords: *Terminalia arjuna,* pH, moisture, tyrosine, storage, Quarg-type, cheese

Terminalia arjuna (commonly known as *Arjuna*) is a majestic tree that has a significant part in the Indian ayurvedic medical system (Soni & Singh, 2019). Decoctions made from Arjuna bark were suggested by Chakradatta, the eminent ancient physician who recommended its uses with milk (as *kshirpaka*) or with ghee (*ghrita*) (Chopra *et al.* 1958). Due to the presence of a significant number of active phytoconstituents, Arjuna has traditionally been widely employed in medicinal formulations for a variety of ailments. The bark is used as an astringent, cooling aphrodisiac, cardiotonic, and tonic in the Indian traditional system of medicine for fractures, ulcers, spermatorrhoea, leucorrhoea, diabetes, cough, tumor, asthma, inflammation, and skin disorders. Primarily, administration of drugs manufactured with Arjuna bark supports normal cardiac function, supplies energy to the heart muscles, enhances platelet function, and aids in maintaining a stable blood pressure level (Dwivedi *et al.* 2007; Paarakh, 2010).

Quarg (Quark) is a soft, spreadable type of fresh cheese. It is popular throughout central Europe (e.g., Germany, Poland, and Austria). Kvarg, Tvarog, Tworog, Twarog, Sauermilchquark, and Speisequark

How to cite this article: Aparnna, V.P., Singh, S. and Chauhan, A.K. (2023). Shelf-life Evaluation of Quarg- type Cheese Incorporated with Encapsulated *Terminalia arjuna*. *Int. J. Food Ferment. Technol.*, **13**(01): 47-56.

Source of Support: IoE Scheme; **Conflict of Interest:** None

are other regional names for this product. Quarg is milky white to slightly yellowish in colour; smooth, uniformly soft, mildly acidic and elastic in texture with clean flavor. Due to the high moisture content (~82%, w/w), the shelf-life is limited to 2-4 weeks at <8°C. Fresh acid- or acid/rennet-curd cheeses are commonly made by adding starter culture and a trace quantity of rennet to skim milk. Under these circumstances, the milk slowly undergoes quiescent acidification, resulting in the production of a gel with a pH close to 4.6- 4.8. After stirring, the gel is concentrated using one of many processes, such as centrifugation or ultrafiltration (UF), which entail the removal of whey or permeate. The finished product can be immediately chilled and packed. Quark cheese is well-suited for processing into fresh cheese recipes or a variety of cuisines (e.g., cheesecakes, sauces, desserts).

Multiple studies have been carried out to improve the functional properties of quarg cheese. The researches carried out to improve the properties of quarg cheese includes, Incorporation of vegetable oil to substitute the milk fat content (Shekhar 2014), addition of encapsulated probiotic bacteria to improve the flavor of cheese (Kadiya *et al.* 2014) and studies on addition of dietary fibre like inulin and oat fibre in quarg cheese (Gahane, 2008). There have also been numerous studies carried out to evaluate the storage stability of functional quarg-type cheese. They were mostly conducted to analyse the effect of processing conditions on the physico-chemical properties of cheese during the storage period (Sachdeva *et al.* 1993). Addition of MicroGARD® 100* and nisin to quarg cheese was also carried out in order to extend the shelf-life of the product (Kadiya, 2009; Shashikant and Kanawjia, 2013). Spice addition to extent the shelf-life was also done by Belewu *et al.* (2005) and Patange *et al.* (2018). The studies related to herb incorporation and its effects in fresh cheese varieties like quarg has not been carried out yet.

The present study was carried out to evaluate the storage stability of functional quarg-type cheese incorporated with *Terminalia arjuna* in encapsulated form. To our current knowledge there is no literature available on the same.

MATERIALS AND METHODS

Production of encapsulated Arjuna bark powder

Encapsulation of Arjuna herb extract

Encapsulation of Arjuna was done according to the method carried out by Sawale *et al.* (2017a) with slight modifications. The wall materials used were Maltodextrin and Sodium Alginate.

Preparation of emulsion

Maltodextrin (MD) (8.0g/100ml) and Sodiumalginate (SA) (1.5%) was dissolved in distilled water separately. To completely dissolve the wall materials, they were stirred over boiling water bath and were cooled. The solutions were kept overnight at 37 °C. Aqueous solution was made by mixing 100 ml of (MD) with (SA). Arjuna extract (2g/100ml) was added to the wall material solution and mixed using magnetic stirrer for an hour. This solution further mixed using emulsifier (T25 IKA, Ultra- Turrax®, Germany) at 4000 rpm for 5 mints. The emulsion was tray dried at 50°C and powdered to obtain encapsulated powder. The obtained powder was stored at 5°C until further use.

Production of Quarg Cheese incorporated with encapsulated Arjuna bark powder

Quarg cheese was made by reconstituting skim milk powder in pasteurized water and heating it to 32- 35 °C. The skim milk was added with NCDC culture of mesophilic bacterial strains namely *Lactococcus lactis* sub sp. *lactis* and *Leuconostoc citrovorum* at the rate of 2% and kept in incubator at 37°C for 2 hours. When the pH had reached 6.3, microbial rennet was added in a small amount and cheese milk was incubated for 14 – 16 hours at 32-35°C. After incubation period pH decreased to 4.3- 4.4. The cheese curd was cut using knife and after a 10-minute hold, the curd is heated to 60°C for 10 min. After cooking the curd is cooled down and de-wheying was carried out by

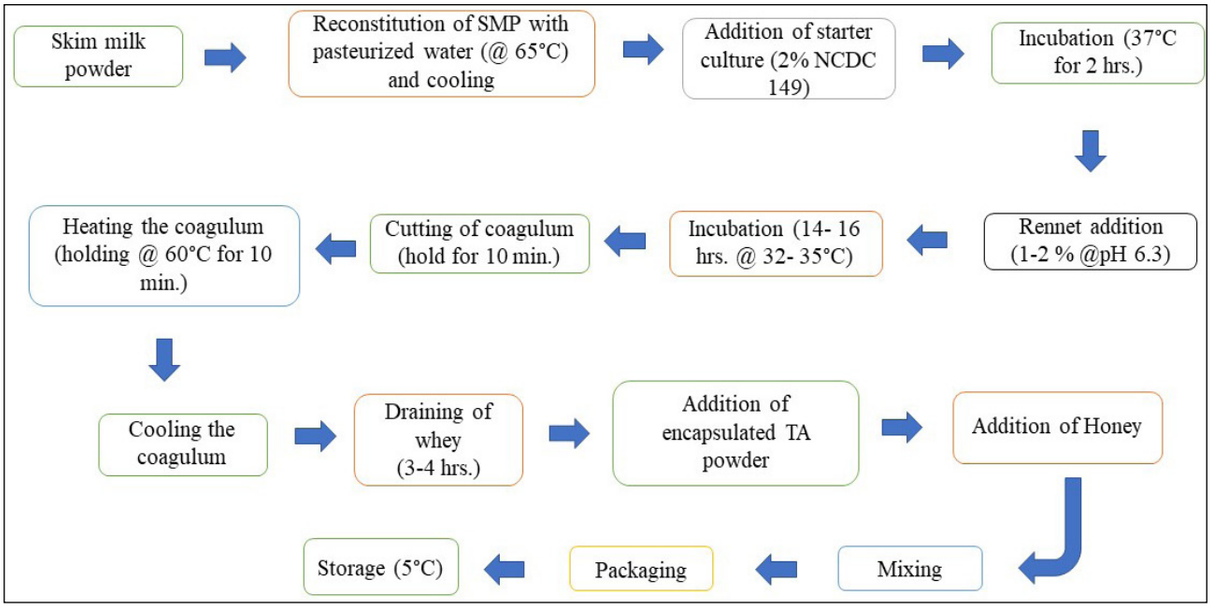

Fig. 1: Process diagram for encapsulated Arjuna incorporated Quarg-type cheese

transferring the cheese curd to muslin clothes. The cheese curd was hung for approximately 3- 4 hours until the required moisture is retained in the cheese. The cheese was mixed with optimized rates of Arjuna and honey and mixed together. The quarg cheese produced was stored in polyethylene cups at 4°C.

Textural analysis

Textural parameters of product like hardness and firmness were analysed using Texture Analyzer (TA. XT plus texture profile analyser, Stable Micro Systems, UK) fitted with a 25 Kg load cell. The product was subjected to application of force to a depth of 10 mm by a P 25 Cylindrical Aluminium probe attached to the texture analyser fitted with a 5 kg load cell. All the tests were carries out at room temperature (37±1ºC). Texture parameters like hardness, adhesiveness, cohesiveness, springiness and gumminess were measured using the instrument. Triplicate measurements were made for each sample. Parameters measured consisted of firmness, work of adhesion, work of shear and stickiness by using the Texture Expert for Windows software version 1.20 (Stable Micro Systems).

Table 1: Test conditions for Texture Analysis

Mode	Measure force in compression
Option	Return to start
Pre-test speed	2.0 mm/s
Test speed	2.0 mm/s
Post-test speed	2.0 mm/s
Trigger Force Auto	5g
Data acquisition rate	200.00 PPS

Instrumental Colour characteristics

The colour of samples was measured with a Hunter colour lab equipped with a measuring head (diameter 127 mm). Colour was measured using the CIE L*a*b* scale and illuminant. Numerical values of a* and b* were converted into hue angle (h_) and Chroma (C*) that represents the hue and the saturation index. Results were expressed as L* (luminosity), hue angle (h_) and Chroma (C*). The hunter L, a, b colour scale is more visually uniform than the xyz colour scale. The three terms L, a, b indicates as follows: L scale: Light vs. dark where a low number (0-50) indicates dark and a high number (51-100) indicates light. a scale: Red vs. green where a positive number indicates

red and a negative number indicates green. b scale: Yellow vs. blue where a positive number indicates yellow and a negative number indicates blue. The L value for each scale therefore indicates the level of light or dark, the a* value redness or greenness, and the b value yellowness or blueness. All three values are required to completely describe an object's colour.

Microbial Analysis

The product sample was examined for the total plate count; yeast and mold count, coliform count during storage period of 15 days. The microbial analysis was carried out according to method suggested by Gahane, (2008).

To Prepare standard dilution, 1ml of sample was taken and transferred to test tube with 9ml of normal saline solution (0.9% NaCl). The samples were serially diluted up to 10-9 dilution. The test tube containing samples were properly mixed.

Standard plate count

Plate Count Agar (pH 7.0±0.1) as nutrient medium was used to estimate the total number of viable bacteria in the sample. The prepared plates were incubated at 37°C for 48 h.

Yeast and mold count

The yeast and mold counts were estimated using Potato Dextrose Agar (pH 3.5±0.1). The microbial plates were incubated at 30°C for 3 days and counts were expressed as log cfu/ml of sample.

Coliform count

Pour plate method employing Violet Red Bile Agar (pH 7.4±0.1) was used to enumerate coliform count in the product. The prepared plates were incubated at 37°C for 48 h. Colonies with dark red colouration were counted and they were expressed as coliform per g of sample.

Sensory Evaluation

Sensory attributes of the product samples were analysed by semi-trained sensory panel consisting of 10 judges from Department of Dairy science and Food Technology, BHU, Varanasi. The panellists evaluated the sensory attributes like colour & appearance, body and texture, aroma, flavor and overall acceptance. The score was given according to the 9-point hedoic scale

Tyrosine value

The extent of proteolysis in the storage samples was determined by estimating the tyrosine value according to the method suggested by Chatterjee *et al.* (2016). 1. Trichloroacetic acid (TCA): 0.72 N solution was prepared by dissolving 117.64 g of the reagent in 1 L of distilled water. 2. Sodium carbonate solution: 75 g of anhydrous sodium carbonate was dissolved in distilled water and diluted to 500 mL. 3. Folin's phenol reagent: Folin's phenol reagent was diluted 1:2 with distilled water prior to use. Five grams of sample was added with 10 mL of distilled water followed by 10 mL of 0.72 N TCA continued with vigorous shaking. The samples were incubated at 37 ºC for 10 min and precipitated proteins were filtered through Whatman No. 42 filter paper. To 5 mL of the TCA soluble filtrate, 3 mL of distilled water, 10 mL of the sodium bicarbonate reagent and 3 mL of Folin's phenol reagent was added. The sample is again incubated at 37 ºC for 10 min for colour development. A spectrophotometer is used at 650 nm to measure the intensity of colour developed. A stock solution was prepared using L-tyrosine and the standard curve with regression equation was obtained using the absorbances against their tyrosine concentration. The equation obtained was $y = 0.0152x + 0.0215$, $R^2 = 0.99854$

DPPH radical scavenging activity

The DPPH radical scavenging assay was estimated according to Dudonne *et al.* (2009) with some modifications to estimate the free radical scavenging activity. Methanolic extract of sample was prepared by centrifuging 1 gram of sample with 10 ml of methanol at 6000 rpm for 10 minutes and the supernatant was collected. 1 ml of the standard DPPH solution prepared (4mg/10ml methanol) was added

to 5 ml of sample (supernatant) and was dispersed thoroughly using vortex. The sample was dark incubated for 30 mints. The absorbance was taken in UV-VIS spectrophotometer at 517 nm against blank solution. % DPPH inhibition = Absorbance of blank− Absorbance of sample Absorbance of sample × 100.

Total Phenolic Content

The total phenolic content of the extracts was determined by the FolinCiocalteau method with some modifications. 0.5 ml of sample (methanolic extract as mentioned in DPPH) was added to 0.5 ml of Folin-Ciocalteau reagent (FCR) (1 ml FCR in 9ml distilled water), followed by 1 ml of 7.5% of Sodium Carbonate solution. The above solution was then kept in dark incubation for one hour. Absorbance was measured at 760 nm using UV-1800 spectrophotometer. The standard calibration curve of Gallic acid (0 - 800 mg/L) was produced and phenolic content was expressed in mg of Gallic acid equivalents (GAE)/ g of extract.

RESULTS AND DISCUSSION

Effect of storage on sensory attributes of quarg-type cheese

During the storage period of 15 days, it was observed that all the sensory parameters score for both Quarg cheese incorporated with Arjuna bark powder (QA) and Control quarg cheese samples (QC) gradually decreased. The storage caused softening of both the cheese samples which can be due to absorption of moisture in the duration of storage. Addition of Arjuna and honey gave better sensory scores to QA samples throughout the storage period as compared to QC samples. The sensory score of QA and QC for parameters namely colour and appearance, body and texture, flavor, aroma and overall acceptability during the storage period is shown in Table 2.

Effect of storage on colour values

The changes in moisture and colour parameters during storage period is shown in Table 3. It was observed that there was a gradual decrease in moisture and pH during the storage period. The reduction in moisture can be due to migration of moisture, release of free whey outside the cheese matrix (Gahane, 2008). The microbial action on cheese steadily increases, leading to decreased pH during the storage period. Colour L* values reduced for QA samples during the storage period from 69.31 ± 0.33 to 66.53 ± 0.28. Colour a* values increased from 10.05 ± 0.45 to 10.42 ± 0.88 in QA and from -1.35 ± 0.06 to -1.78 ± 0.44 in QC (Table 3). Sawale *et al.* (2017b) also observed an increase in a* values during the storage period of Arjuna incorporated flavoured milk.

Table 2: Sensory attributes of Arjuna herb incorporated Quarg cheese during storage

Sensory attribute	Days	QA	QC
Colour and appearance	0	7.85 ± 1.4	6.97 ± 3.2
	5	7.5 ± 1.08	7.25 ± 0.71
	10	7.5 ± 0.37	7.1 ± 0.71
	15	6.98 ± 1.6	6.5 ± 0.85
Aroma	0	7.72 ± 0.78	7.15 ± 0.5
	5	7.67 ± 0.46	7.07 ± 0.25
	10	7.55 ± 0.89	6.98 ± 0.05
	15	7.53 ± 0.13	6.14±0.19
Flavor	0	7.54 ± 0.25	7.02 ± 1.9
	5	7.37 ± 0.05	6.78 ± 0.97
	10	7.30 ± 0.36	6.35 ± 0.08
	15	7.04 ± 0.65	6.27 ± 0.45
Body & Texture	0	7.81 ± 0.19	7.77 ±0.98
	5	7.69 ± 0.36	7. 56 ± 0.31
	10	7.38 0.12	7.43 ± 0.59
	15	6.98 ± 0.39	7. 38 ± 0.37
Overall acceptability	0	8.12 0.37	8.02 ± 0.45
	5	8.04 ± 1.02	7. 93 ± 0.89
	10	7.96 ± 0.03	7.45 ± 0.39
	15	7.87 0.36	7.05 0.28

Data presented as mean ± SD (n= 3).

Colour b* values in QA and QC increased during the storage period. In QA it was 16.55 ± 0.95 on first day and 16.68 ± 0.14 by 15th day whereas in QC b* values were observed as 9.86 ± 0.11 on first day and 10.22 ± 0.10 on the final day of analysis. This indicates increase in yellowness with increase in storage period. Shekhar, (2014) stated that the increase in yellowness

of quarg during storage was related to the increasing yeast and mold count, titratable acidity and physico-chemical changes.

Table 3: Moisture content and colour characteristics of Arjuna herb incorporated Quarg cheese during storage

Parameter	Day	Sample	
		QA	QC
Moisture	0	60.82 ± 0.45	59.98 ± 1.20
	5	59.2 ± 0.14	57.34 ± 0.23
	10	56.7 ± 0.12	54.05 ± 0.98
	15	55.3 ± 0.37	53.15 ± 0.12
L*	0	69.31 ± 0.33	89.84 ± 0.95
	5	68.4 ± 0.24	92.74 ± 1.45
	10	68.1 ± 0.45	91.84 ± 0.13
	15	66.53 ± 0.28	91.93 ± 0.06
a*	0	10.05 ± 0.45	-1.35 ± 0.06
	5	10.01 ± 0.98	-1.32 ± 0.25
	10	10.13 ± 0.23	-1.33 ± 0.12
	15	10.42 ± 0.88	-1.78 ± 0.44
b*	0	16.55 ± 0.95	9.86 ± 0.11
	5	16.49 ± 0.37	11.23 ± 0.03
	10	16.53 ± 0.34	10.71 ± 0.17
	15	16.68 ± 0.14	10.22 ± 0.10
pH	0	4.72 ± 0.28	4.56 ± 1.74
	5	4.69 ± 0.43	4.53 ± 0.97
	10	4.63 ± 0.07	4.52 ± 0.21
	15	4.46 ± 0.16	4.43±0.39

Data presented as mean ± SD (n= 3).

Effect of storage on antioxidant activity

Arjuna contains high antioxidant activity and it is generally observed that as storage prolongs the activity decreases. The changes in antioxidant activity during the storage period is shown in Fig. 2. There was a decrease in the antioxidant activity of the samples as the storage period increased. The percent DPPH inhibition activity of QA decreased from 95.49 ± 3.46 % to 86.13 ± 1.49 %. Pankaj *et al.* (2013) also observed reduction in antioxidant activity of ghee incorporated with Arjuna ethanolic extract during a storage period of 10 days.

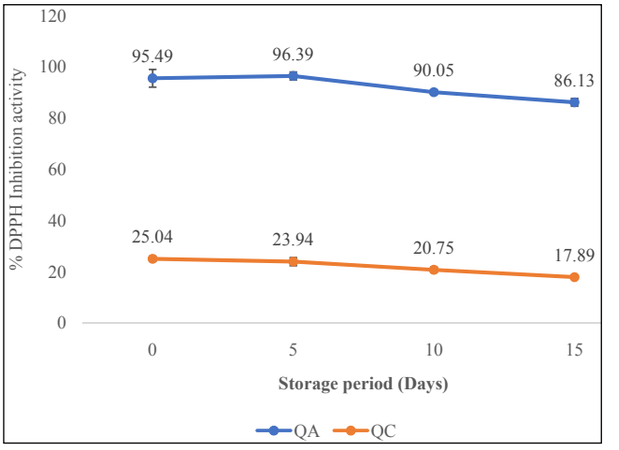

Fig. 2: Antioxidant activity of encapsulated Arjuna incorporated Quarg-type cheese during storage

Effect of storage on polyphenol content

The total phenolic content was in QA and QC samples were estimated and expressed as mg/g gallic acid equivalent as shown in Fig. 3.

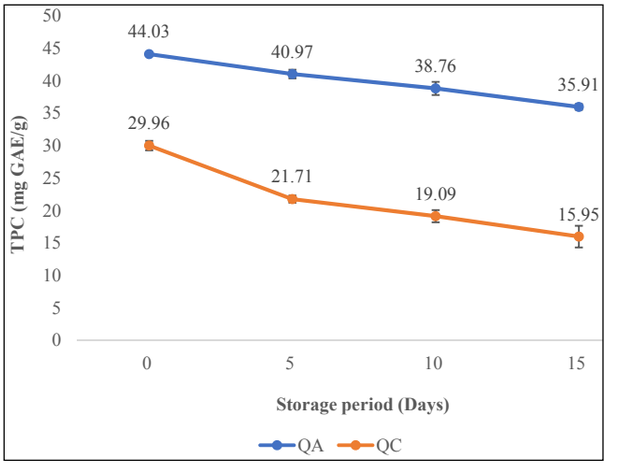

Fig. 3: Total phenolic content of encapsulated Arjuna incorporated Quarg-type cheese during storage

There was a steady decrease in the phenolic content as the storage period increased. The phenolic content in samples incorporated with Arjuna reduced from 44.03 ± 0.09 to 35.91 ± 0.45 (Table 4). The QA samples phenolic content reduction was in relation to observations made by Sawale *et al.* (2017b) and Mahmood *et al.* (2020), that Arjuna incorporated

products had reduction in their total phenolic content during the storage period. Sawale *et al.* (2017b) and Luca *et al.* (2014) mentioned that the slower rate of polyphenol content reduction during the storage period is possible because encapsulation reduces the rate of loss of phenolic compounds.

Table 4: Effect of storage on microbiological parameters

Microbiological parameter	Storage days	Sensory score	
		QA	QC
Yeast & Mold Count (cfu /g)	0	1×10^9	Nil
	5	3×10^8	1×10^7
	10	4×10^6	3×10^9
	15	6×10^6	5×10^6
Coliform count (cfu/g)	0	Nil	Nil
	5	Nil	Nil
	10	Nil	Nil
	15	1×10^6	Nil
Standard plate count (cfu /g)	0	3×10^8	3×10^7
	5	6×10^6	7×10^7
	10	7×10^5	8×10^7
	15	6×10^7	7.7×10^7

Data pesented as mean ± SD (n= 3).

Effect of storage on tyrosine value

Tyrosine value is considered a measure of degree of proteolysis which greatly affect the acceptability of the product. The analysis was carried out and results were plotted against standard tyrosine solution (y = 0.0152x + 0.0215, R^2 = 0.99854). The results obtained was expressed as µg/ml of filtered extract. Mulvihill and McCarthy, (1994) observed that the reasons which significantly influenced the proteolytic activity were denatured proteases (which can rearrange resulting in proteolytic activity) and high plasmin content. The samples QA and QC showed a significant increase in the tyrosine value as the storage days increased. It can be seen that QA samples had greater rates of proteolysis as compared to QC samples. The QA sample had high tyrosine value at the end of storage life (40.30 ± 0.09) as compared to QC samples (27.40 ± 0.14). This concludes that Arjuna and honey addition highly influenced the rate of proteolysis in the quarg cheese. Production of proteolytic enzymes by yeast and molds can be regarded as a reason for increase in tyrosine value (Kharb, 2007). Shekhar, (2014) also observed a significant increase in tyrosine value of both control samples of quarg cheese and samples added with oils. The comparative data for tyrosine value of QA and QC is shown in Fig. 4.

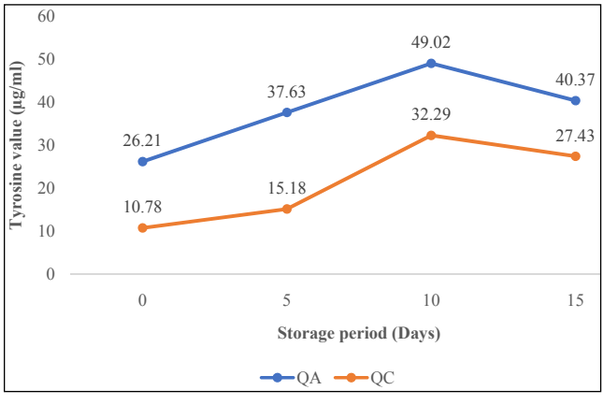

Fig. 4: Tyrosine value of encapsulated Arjuna incorporated Quarg-type cheese during storage

Effect of Storage on textural properties

The storage period had a significant effect on the textural properties of QA and QC. The hardness values decreased from 52.01 ± 1.09 to 50.07 ± 0.09 in samples incorporated with Arjuna and honey. The adhesiveness was found to increase with increasing days of storage in QA. This may be due to the addition of honey. On the other hand, QC samples had a decrease in the adhesivess values from 32. 03 ± 0.35 to 26.97± 0.17. QC samples had increased cohesiveness values as compared to QA throughout storage period. The cohesiveness observed at day 15 for QA was 0.46 ± 0.06 as compared to 0.63 ± 0.09 in QC. Gumminess values were seen to decrease along with increasing storage days.

Effect of storage on microbial quality of cheese

Quarg cheese has a high moisture content and an acidic character, microbes may thrive in it because these conditions are ideal for their multiplication. Both QA and QC samples were analysed throughout the storage period for standard plate count, coliform

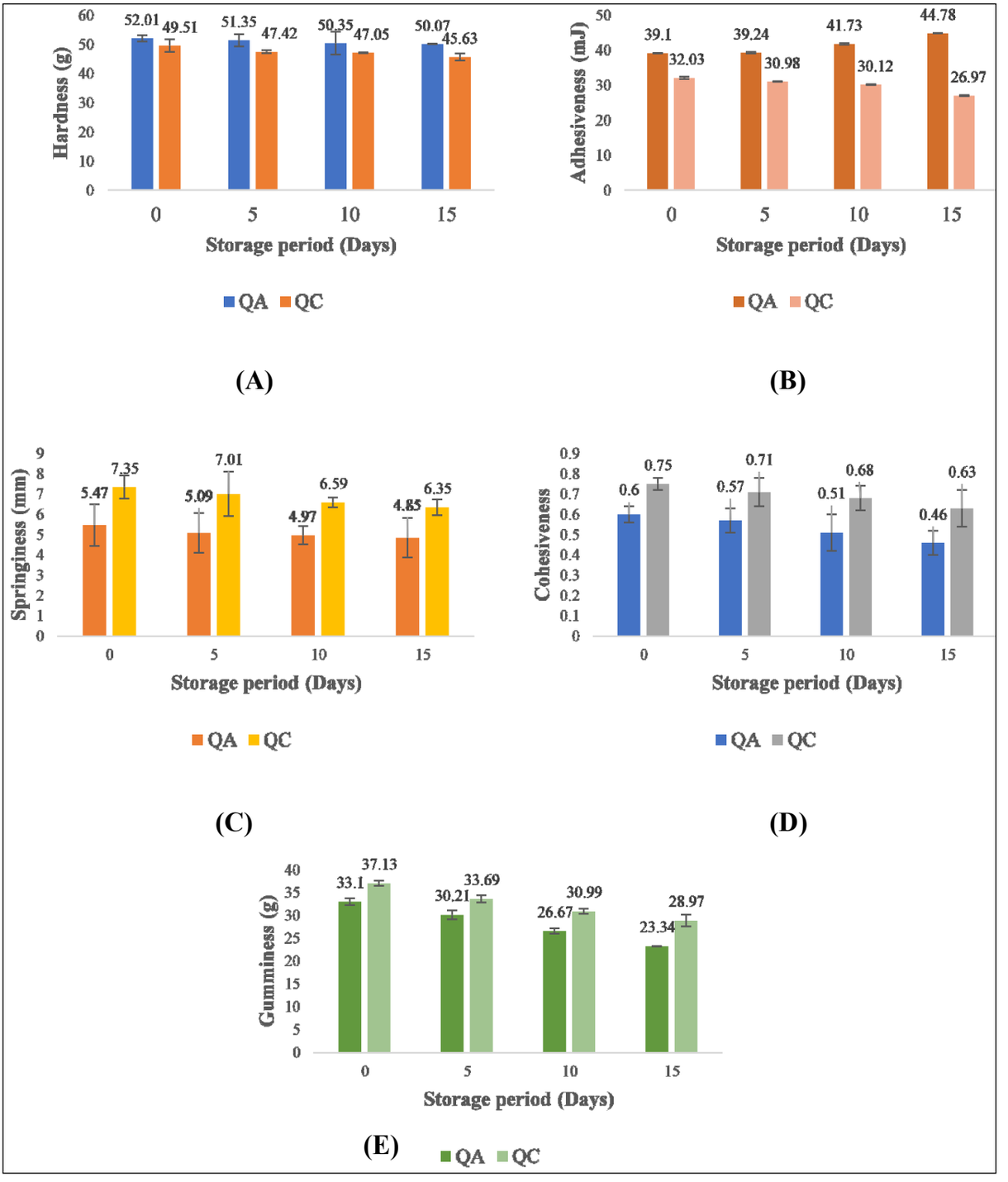

Fig. 5: Changes in hardness, adhesiveness, springiness, cohesiveness, and gumminess during storage period is shown in **(A)**, **(B)**, **(C)**, **(D)**, (E)respectively

count and yeast and mold count. The samples had an increase in the microbial load as the days of storage increased (Table 4). The increase in standard plate count was from 1 × 109 to 6 × 106 in QA samples during the 15 days storage period. Patange *et al.* (2018) also noticed a significant rise in the standard plate count with storage of Quarg cheese. It was observed that addition of honey had increased the yeast and mold count as compared to control samples without added honey.

Significant increase in yeast and mold count during storage of Quarg cheese was also reported by Shekhar, (2014) and Patange *et al.* (2018). Engel *et al.* (1980) revealed that yeasts play a significant role as spoilage microorganisms in Quarg; yeasty flavor formed when yeast cell counts reached 105 cells/g. He also investigated the development of yeasts in Quarg stored at 6°C and discovered that yeast spoiling of Quarg might occur during storage at 6°C within the specified shelf-life (18 d). Shashikant and Kanawajia, (2013) employed microGRAND®-100* as a bio preservative in Quarg cheese, and it was discovered that it was highly effective in extending the shelf-life of Quarg cheese from 21 days to 42 days, a 100 percent increase compared to the control. Quarg cheese has a high moisture content and an acidic character, yeast and mold may thrive on its surface because these conditions are ideal. Quarg cheese's shelf-life is severely limited by this aspect.

CONCLUSION

Quarg- type cheeses due to their high moisture content generally have low shelf-life (around two weeks). The current study examined the product storage stability of encapsulated Arjuna herb incorporated quarg cheese using physico-chemical and microbial analysis. The results showed that addition of arjuna herb to the product was not helpful in terms of extending the shelf-life of the product even though the sensorial attributes did not fall drastically throughout the storage period. The chemical analysis like DPPH and phenolic content analysis showed a reduction in the activity of functional compounds along with tyrosine values showing a higher breakdown in the cheese incorporated with *arjuna* herb. The microbial analysis did not show any growth of coliform bacteria but had a significant increase in the total plate count. The textural attributes also showed that as the storage period prolonged the product quality decreased. There is great potential to improve the shelf-life of quarg cheese by addition of nisin and other compounds that can decrease the growth of unwanted microflora. Along with this improvement in the packaging material used for the product could greatly help extent the shelf-life.

Acknowledgements

The authors gratefully acknowledge to Institution of Eminence (IoE) scheme, Banaras Hindu University, Varanasi (U.P.) India, for financial support under the Incentive to Seed Grant under IoE Scheme (Dev. Scheme No 6031 & PFMS Scheme No 3254).

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