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Physicochemical, microbiological, and sensory characterization of Halloumi cheese fortified with garlic (*Allium sativum)* and pepper (*Piper nigrum*) †

Aravindi Nipunika Gamage, Rajivini Jeyasiri, Dinelka Dananji Hettiarachchi, Sachini Sandaranga Munasinghe and Nadeesha Dilrukshi*

Department of Livestock and Avian Sciences, Faculty of Livestock, Fisheries and Nutrition, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP) 60170, Sri Lanka

* Correspondence: nadeeshadilrukshi@wyb.ac.lk

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Abstract: This study aimed to develop a Halloumi cheese using cow's milk and fortify it with garlic and pepper to evaluate its physicochemical, microbiological, and sensory properties. The developed Halloumi cheese showed a significant increase ($P<0.05$) in its total solids, protein, fat, and ash content and a significant decrease ($P<0.05$) in moisture and pH during a 35-day storage period. The textural properties of the cheese were significantly affected ($P<0.05$) by the storage period. The microbiological shelf life of the Halloumi cheese was 21 days in refrigerated conditions. In sensory evaluation, the cheese fortified with the spice powder mixture received higher consumer acceptance than nonfortified Halloumi cheese.

Keywords: Cheese; Halloumi; microbiology; physicochemical; sensory

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1. Introduction

Halloumi is the traditional cheese of Cyprus. It is widely popular in Cyprus and other countries of the Eastern Mediterranean. Recently it has gained international acceptance and recognition [1]. Halloumi cheese is a semi-hard to hard unripen cheese, with a unique sensory and texture profile obtained by cooking (90 – 95℃) the pressed curd in the whey for more than 30 minutes. The color varies from white (if a mixture of ovine and caprine milk is used) to yellowish (if bovine milk is used) [2]. This fresh Halloumi cheese has a characteristic aroma, its texture is elastic and compact with no holes and it is easy to cut. Halloumi has a greater melting point than usual cheese; therefore, it is one of the few cheeses that can be grilled or fried [3]. Traditional Halloumi cheese has been produced from raw ovine or caprine milk. However, the rising demand has allowed readily available bovine milk to prepare Halloumi. The compositional changes due to use of bovine milk inevitably have made some modifications to the traditional Halloumi cheese-making procedure [1]. The objective of this study was to produce Halloumi cheese from cow's milk with added value, fortified with garlic and pepper, and assess its sensory, microbiological, and physicochemical characteristics.

2. Methods

2.1. Materials

The whole cow milk was purchased from a local milk collecting centre (Nestle, Sanda-lankawa, Makandura, Sri Lanka). Salt, pepper, and garlic were purchased from the local supermarket (Pannala, Sri Lanka). $CaCl_2$ (DCI Calcium Chloride, 32 to 33% wt/vol) and rennet (chymosin ≥1800 μ/g) were obtained from J.K.Tradelink (Pvt) Ltd, in Sri Jayawar-denepura Kotte, Sri Lanka.

2.2. Manufacture of Halloumi cheese fortified with garlic and pepper

The Halloumi cheese fortified with garlic and pepper was manufactured according to the procedure described by Mehyar et al. [4]

2.3. Physicochemical Analysis

The Halloumi cheese samples were prepared according to the method described by Milci et al. [2]. The total solid and moisture content of the Halloumi cheeses was determined by the Oven drying method (AOAC, 2000; method 925.23 and method 948.12 respectively). The fat content was determined by the Soxhlet method (AOAC, 2000; method 963.15). The ash content was determined by ashing the sample to constant weight at 550^0C for 2 hours (AOAC, 2000; method 942.05). The Kjeldahl method was used to determine the protein content (AOAC, 2000; method 978.02). The pH of the cheese samples was measured using a digital pH meter (ST 3000- Ohaus Corporation –USA).

Texture profile analysis of fortified Halloumi cheese samples (hardness, gumminess, cohesiveness, and chewiness) was evaluated using a TX 700 texture analyzer (Shimadzu, Kyoto, Japan) according to the methods of Zheng et al. [5].

Hunter Lab color meter (KONICA MINOLTA INC. Osaka, Japan) was used to determine L* (lightness), a* (green-red value), and b* (blue-yellow value). The relevant values were auto-generated by a software [6]. The differences between the samples were evaluated using individual parameters (L* a* b*), and the total color difference (ΔE *) according to the method described by Nedomová et al. [6].

2.4. Microbiological analysis

10g of cheese was blended with 90 mL of sterile peptone water in a stomacher to obtain a homogeneous mixture. Serial dilutions down to 10^{-6} were made employing 1 mL transfers into 9 mL of sterile peptone water [7]. Counts of total bacteria, *Escherichia coli*, yeasts and molds, *Staphylococcus aureus* and lactic acid bacteria were enumerated by the spread plate technique using plate count agar, Mac-Conkey agar, potato dextrose agar, mannitol agar and de Man, Rogosa and Sharpe (MRS) agar respectively. The total bacteria were determined at 35 ± 1°C using an incubation period of 48 ± 2 h. *Escherichia coli* was determined at 35 ± 1 °C for 24 ± 2 h. Yeasts and molds was incubated at 35°C ± 1°C for 48 ± 2 h. Lactic acid bacteria was incubated at 35 ± 1°C for 72 ±2 h and *Staphylococcus aureus* was determined at 35 ± 1 °C for 24 ± 2 h. All microbiological analyses were performed in 40 days period with 10 days of intervals [4].

2.5. Sensory evaluation

A sensory assessment was conducted on two types of cheese samples: Nonfortified Halloumi cheese (control sample) and Halloumi cheese fortified with garlic and pepper (fortified sample). Based on a 9-point hedonic scale, the evaluation was performed by 30 untrained panelists who rated the samples' appearance, aroma, color, before taste, after taste, texture, and overall acceptability.

2.6. Statistical analysis

In this experiment, triplicate samples were taken for all the analyses. The Significance was evaluated using Analysis of variance (ANOVA) in the physicochemical and microbiological properties of the Halloumi cheese samples. All statistical calculations were performed using SPSS software version 16.0. A level of confidence of 95% ($P<0.05$) was used. The results from the sensory panel were analyzed using XLSTAT software.

3. Results and Discussion

3.1 Physicochemical characteristics

Chemical composition is one of the most significant factors influencing cheese production [8]. According to Table 1 the moisture content and pH values of fortified Halloumi cheese significantly decreased (P<0.05) during the storage period. The decrease in the moisture content of produced cheeses during the storage period might be due to loss of moisture and a decrease in the pH content might be due to the increase in lactic acid formation with storage time. The pH fluctuations in the current study seem to be consistent with Milci et al. [2]. The total solid, protein, fat and ash content of the cheese samples were found to be significantly increased (P< 0.05) during the storage period.

Table 1. Chemical composition of Halloumi cheese fortified with garlic & pepper powder during storage at 10 ± 1 °C for 35 days.

Component	Storage Period				
	7th Day	**14th Day**	**21st Day**	**28th Day**	**35th Day**
Moisture (%)	$63.86^{a} \pm 0.78$	$63.22^{ab} \pm 0.12$	$62.88^{ab} \pm 0.22$	$62.17^{bc} \pm 0.40$	$61.49^{c} \pm 0.62$
Total Solid (%)	$45.79^{c} \pm 0.77$	$46.52^{c} \pm 0.12$	$47.87^{b} \pm 0.67$	$48.91^{ab} \pm 0.24$	$49.62^{a} \pm 0.22$
Fat (%)	$13.64^{c} \pm 0.27$	$14.01^{c} \pm 0.20$	$14.74^{b} \pm 0.19$	$15.23^{ab} \pm 0.35$	$15.85^{a} \pm 0.10$
Protein (%)	$17.97^{d} \pm 0.22$	$18.42^{cd} \pm 0.22$	$18.89^{bc} \pm 0.19$	$19.26^{ab} \pm 0.33$	$19.84^{a} \pm 0.14$
Ash (%)	$2.14^{cd} \pm 0.31$	$2.28^{cd} \pm 0.07$	$2.78^{abc} \pm 0.58$	$3.22^{ab} \pm 0.17$	$3.53^{a} \pm 0.11$
pH	$6.09^{a} \pm 0.06$	$5.87^{ab} \pm 0.05$	$5.85^{abc} \pm 0.23$	$5.54^{bcd} \pm 0.11$	$5.22^{d} \pm 0.10$

Values are mean ± SD (n = 3). Means with different superscript lowercase letters in the same row are significantly different (p<0.05).

The cheese's texture is assumed to be one of the significant determinants of the consumer acceptability of cheese [5]. It was observed that fortified Halloumi cheese hardness (from 4.20N to 6.11N), gumminess (from 1.99N to 3.57N), and chewiness (from 2.31N to 3.34N) significantly increased (P<0.05) and cohesiveness (from 0.85 to 0.74) significantly decreased (P<0.05) during the storage period (Table S1). These findings contradict Ayyash et al. [9], who reported no significant differences (P > 0.05) in hardness, cohesiveness, and gumminess among the experimental cheeses during the entire 56-day storage period. The increase in hardness and chewiness values of developed Halloumi cheese in the current study may be due to a decrease in cheese moisture content. The gumminess in Halloumi cheese was related to moisture content and hardness. Also, water, protein, and fat are the main factors affecting cheese hardness [6]. Cheese cohesiveness usually decreases as cheese moisture content decreases [10].

Table 2. Color Properties of Halloumi cheese fortified with garlic & pepper powder during storage at 10 ± 1 °C for 35 days.

Color Property	Storage Period				
	7th Day	**14th Day**	**21st Day**	**28th Day**	**35th Day**
L*	$72.16^{a} \pm 0.40$	$68.03^{b} \pm 1.89$	$63.60^{c} \pm 1.60$	$61.62^{cd} \pm 0.45$	$60.21^{d} \pm 0.10$
a*	$3.26^{bcd} \pm 0.11$	$3.36^{bcd} \pm 0.25$	$3.93^{abc} \pm 0.35$	$4.23^{ab} \pm 0.40$	$4.83^{a} \pm 0.55$
b*	$26.90^{a} \pm 0.40$	$28.30^{b} \pm 0.45$	$29.31^{c} \pm 0.95$	$33.30^{c} \pm 1.32$	$35.72^{d} \pm 0.43$
ΔE*	2.07	2.24	5.99	4.78	2.11

Values are mean ± SD (n = 3). Means with different superscript lowercase letters in the same row are significantly different (p<0.05).

The results in Table 2 indicate L* values of fortified Halloumi cheese decreased significantly (P<0.05) during storage. In The fortified Halloumi cheese, due to the moisture loss during storage, the bright white color intensity reduced and the color became dull yellow, which can be attributed to the decrease in L* values [11]. During the storage period, the fortified Halloumi sample's a* and b* mean values significantly increased (p<0.05). The results were in agreement with Nedomová et al. [6]. An increase in b* values could be explained by a loss of moisture during storage [10]. Over the 35-day storage period, the fortified Halloumi cheese's total color difference (ΔE*) increased from 2.07 to 2.11. Notably, a significant color difference (5.99) was observed on the 21st day of storage.

3.1. Microbiological analysis

3.2.1. Growth pattern of microorganisms

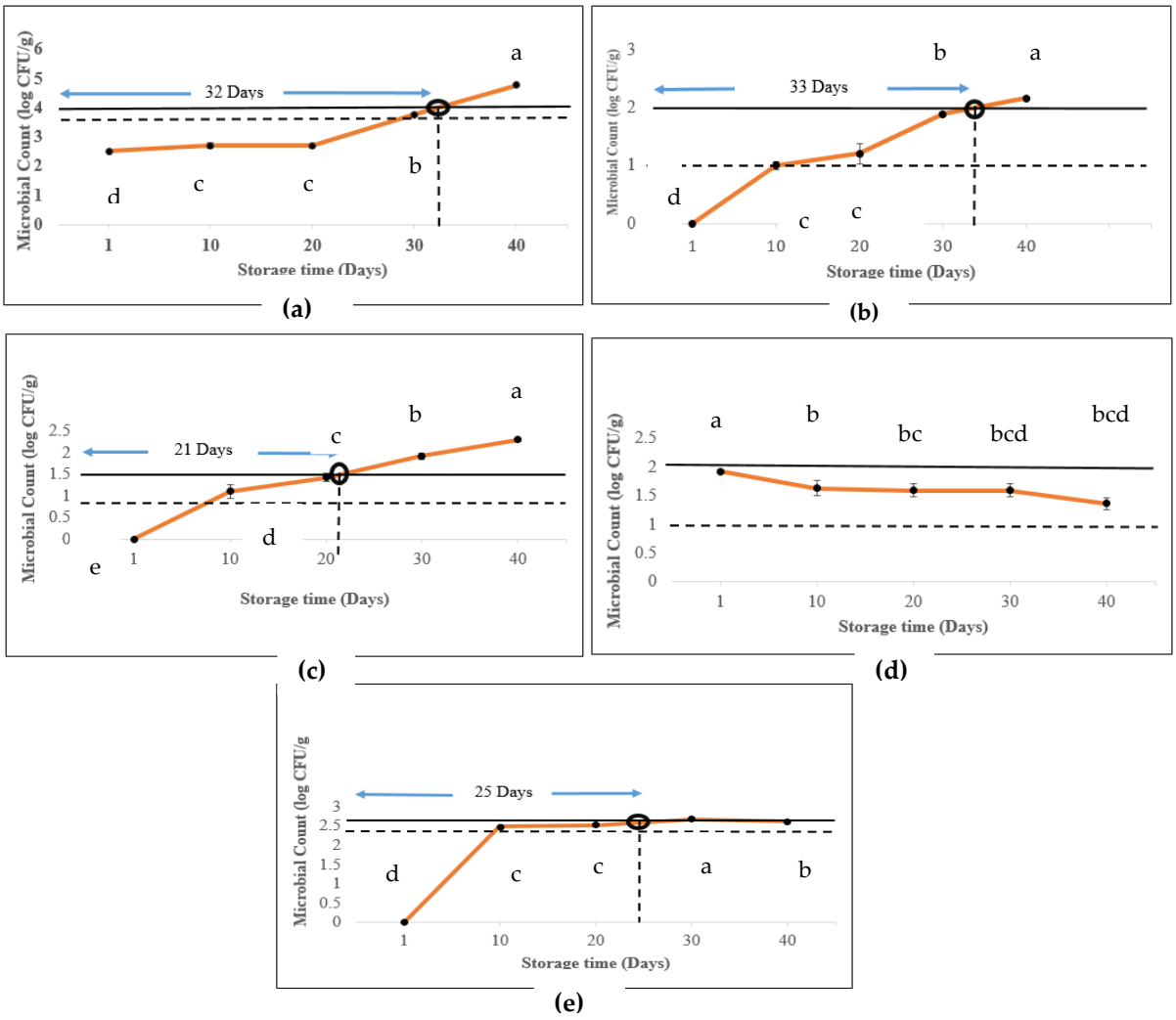

Figure 1. Growth curves of total bacteria (a), *Escherichia coli* (b), yeasts and molds (c), *Staphylococcus aureus* (d), and Lactic acid bacteria during storage time.(— Maximum permissible limit,- -Minimum permissible limit, — Count of microorganism). Values are mean ± SD (n=3). Means with different superscript lowercase letters in the same raw are significantly differenet ($p<0.05$).

According to the Figure 1 and Table S2, the total bacteria count increased slowly in the early stages of storage and exhibited a sharp increase after that reaching 4.78 ± 0.02 log CFU/g at the end of 40 days of storage period. These results agree with the findings of Kamleh *et al.* [7]. *Escherichia coli* count in fortified Halloumi cheese samples increased significantly ($p<0.05$) as the storage progressed. However, the total coliforms count in the Halloumi cheese sample was lower than those mentioned by Kamleh *et al.* [7]. Processing environment, biofilm development in the post-heating equipment or sources and heat treatment failure could be the reasons for this significant growth of *E. coli*. The growth of yeasts and molds was significantly different ($p<0.05$) during the 40 days of storage time. The results differ from that reported by Papademas and Robinson, [1] in fresh Halloumi cheese made from bovine milk. The reason for this significant growth of yeasts and molds may be the increased acidity throughout the storage time of Halloumi cheese. *Staphylococcus aureus* count decreased significantly ($p<0.05$) during the 40 days of storage time. Similarly, reported a significant decrease ($p<0.05$) in *Staphylococcus aureus* count during the storage period of 28 days [12]. The count of lactic acid bacteria was not significantly different ($p>0.05$) during the storage.

3.2.2. Microbiological shelf life

In the current study, total bacteria demanded 32 days to reach its 4.0 log CFU/g maximum permissible limit [13] (Figure 1a), *Escherichia coli* demanded 33 days to reach its 2.0 log CFU/g maximum permissible limit [13] (Figure 1b), yeasts and molds exceeded its 1.5 log CFU/g maximum permissible limit [13] at 21 days of storage (Figure 1c), *Staphylococcus aureus* demanded more than 40 days to reach its 2.0 log CFU/g maximum permissible limit [13] (Figure 1d) and lactic acid bacteria exceeded its 2.6 log CFU/g maximum permissible limit [13] at 25 days of storage (Figure 1e).

3.3. Sensory characteristics

Figure 2 illustrates that the fortified Halloumi cheese sample scored the highest mean scores for overall flavor (53.4%), before (53.8%) and after (54.2%) taste, texture (52.9%) and aroma (53.0%). In comparison, the participants preferred the control sample over the fortified sample for its color (49.1%) and appearance (52.3%).

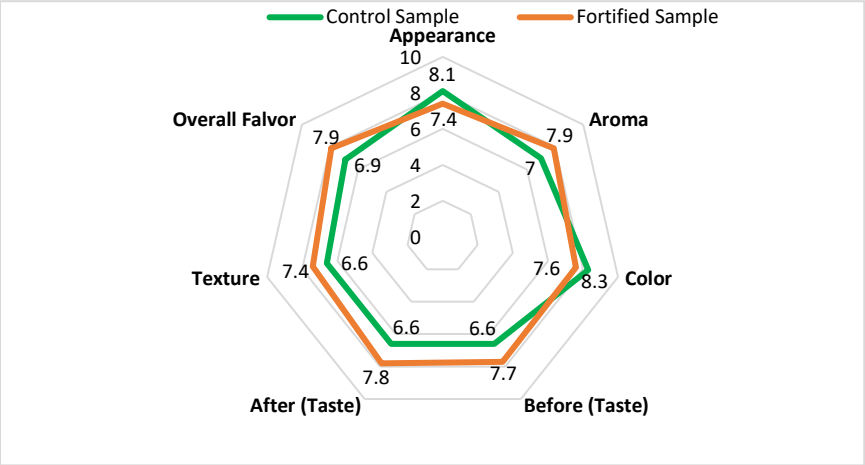

Figure 2. Mean sensory evaluation scores for fortified and nonfortified (control) Halloumi cheese sample

4. Conclusion

This study indicated that the fortified Halloumi cheese sample's pH value and moisture content significantly declined and total solid, fat, protein and ash contents were significantly increased. The microbiological shelf life of functional Halloumi cheese fortified with garlic and pepper was detected as 21 days at 10 ± 1°C of storage, which was demanded by yeasts and molds as the shortest time to exceed its maximum permissible limit in the Halloumi cheese. In sensory evaluation, the spice powder mixture fortified Halloumi cheese had higher consumer acceptance than the control unfortified Halloumi cheese.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Textural Properties of fortified Halloumi cheese during storage; Table S2: Microbial count of total bacteria, *E. coli*, yeasts & molds, *S. aureus* and LAB during storage time

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Papademas, P. and Robinson, R.K., 2000. A comparison of the chemical, microbiological and sensory characteristics of bovine and ovine Halloumi cheese. *International Dairy Journal*, *10*(11), pp.761-768.
2. Milci, S., Goncu, A., Alpkent, Z.A.F.E.R. and Yaygin, H., 2005. Chemical, microbiological and sensory characterization of Halloumi cheese produced from ovine, caprine and bovine milk. *International Dairy Journal*, *15*(6-9), pp.625-630.
3. Papademas, P. and Robinson, R.K., 1998. Halloumi cheese: the product and its characteristics. *International Journal of Dairy Technology*, *51*(3), pp.98-103.
4. Mehyar, G.F., Al Nabulsi, A.A., Saleh, M., Olaimat, A.N. and Holley, R.A., 2018. Effects of chitosan coating containing lysozyme or natamycin on shelf-life, microbial quality, and sensory properties of Halloumi cheese brined in normal and reduced salt solutions. *Journal of Food Processing and Preservation*, *42*(1), p.e13324.
5. Zheng, Y., Liu, Z. and Mo, B., 2016. Texture profile analysis of sliced cheese in relation to chemical composition and storage temperature. *Journal of Chemistry*, *2016*.
6. Nedomová, Š., Kilián, L., Pytel, R., & Kumbár, V. (2017). Effect of ripening time on colour and texture properties in cheese. *Potravinarstvo Slovak Journal of Food Sciences*, *11*(1),pp. 296–301.
7. Kamleh, R., Olabi, A., Toufeili, I., Najm, N.E.O., Younis, T. and Ajib, R., 2012. The effect of substitution of sodium chloride with potassium chloride on the physicochemical, microbiological, and sensory properties of Halloumi cheese. *Journal of Dairy Science*, *95*(3), pp.1140-1151.
8. Kye, N.Y.F.A.R. (2004) 'Cheese technology', 57(2), pp. 91–98.
9. Ayyash, M.M., Sherkat, F., Francis, P., Williams, R.P.W. and Shah, N.P., 2011. The effect of sodium chloride substitution with potassium chloride on texture profile and microstructure of Halloumi cheese. *Journal of Dairy Science*, *94*(1), pp.37-42.
10. Diezhandino, I., Fernández, D., Sacristán, N., Combarros-Fuertes, P., Prieto, B. and Fresno, J.M., 2016. Rheological, textural, colour and sensory characteristics of a Spanish blue cheese (Valdeón cheese). *LWT-Food Science and Technology*, *65*, pp.1118-1125.
11. Wrolstad, R.E. and Smith, D.E., 2017. Color analysis. *Food analysis*, pp.545-555.
12. Al-Nabulsi, A.A., Osaili, T.M., AbuNaser, R.A., Olaimat, A.N., Ayyash, M., Al-Holy, M.A., Kadora, K.M. and Holley, R.A., 2020. Factors affecting the viability of Staphylococcus aureus and production of enterotoxin during processing and storage of white-brined cheese. *Journal of dairy science*, *103*(8), pp.6869-6881.
13. Kamleh, R., Toufeili, I., Ajib, R., Kanso, B. and Haddad, J., 2012. Estimation of the shelf-life of Halloumi cheese using survival analysis. Czech Journal of Food Sciences, 30(6), pp.512-519.

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