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Effect of Natural Whey Culture on the Quality Characteristics of Edam Cheese made from Pasteurized Milk

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ABSTRACT

The goal of study was to evaluate the influence of different levels of Natural Whey Culture (NWC; 2, 3 and 4%) on the physicochemical, free amino acids, organic acids, textural, microbiological and sensorial characteristics of Edam cheese produced from pasteurized milk in various stages of ripening (1, 15, 30 and 45 days). The results showed that, titratable acidity, moisture, ash, WSN/TN and NPN/TN% were higher ($P \leq 0.05$), whereas protein, and fat contents were lower in Edam cheeses with NWC than in control Edam cheese. All individual amino acid levels were greater in Edam cheeses with 3 or 4% NWC than in control or Edam cheese samples with a starter culture. The concentrations of acetic, propionic, lactic and butyric acids were much higher levels in Edam cheeses with NWC than in other cheese treatments. Edam cheese with NWC was markedly higher values of textural evaluation than the values for the other cheese samples. Edam cheese with NWC was the highest LAB counts during ripening, followed by Edam cheese with starter culture and control Edam cheese was the lowest counts. Edam cheese with NWC received much higher sensory scores than the control cheese. Overall, Edam cheese with 4% NWC was higher in quality characteristics than cheese with a starter culture and was comparable to Edam cheese produced from raw milk.

***Keywords*:** Edam cheese, Natural whey culture, traditional cheeses, ripening

INTRODUCTION

In cheese manufacturing, the milk was acidifed by bacteria naturally occurring in raw milk, or selected (or defined) starter cultures, or natural whey cultures (NWCs) regularly reproduced in the factory that determines the cheese texture and flavor (Murtaza *et al.*, 2013). Bacterial cultures are an essential requirement in the manufacture of pasteurized cheeses and their function is to stimulate acid production during milk coagulation and curd development in the early stages of cheesemaking and produce flavour compounds during ripening (Cuffia *et al.*, 2019). Natural lactic bacteria in milk have better activity than starter added because they are better adapted to the conditions, and this makes the difference between the cheeses produced from raw and pasteurized milk (Moser *et al.*, 2018).

Natural whey cultures are undefined cheese starter cultures used in small cheese-making plants for the manufacture of most traditional cheeses worldwide. The whey resulting from the previous raw cheese making process is incubated at a defined temperature (35-52°C)/ 1-2 days for use in tomorrow's cheese mking to initiate milk acidification (Fox *et al.*, 2017). Their composition is extremely complex, and variable that can include mixtures of both mesophilic and thermophilic cultures and enzymes which play a significant in milk acidification and curd development (Bertani *et al.*, 2020). NWCs are commonly used for making pasta-filata cheese in Italy (De Candia *et al.*, 2007), hard cheeses in Argentina (Moser *et al.*, 2018) and Comté cheese in France (Turgay *et al.*, 2011).

In the production of semi-hard Edam cheese, unpasteurized cow's milk without starter cultures has traditionally been used, but it is currently produced from pasteurized milk with a mesophilic starter culture to standardize cheese quality. Edam cheese made with raw milk without lactic starter culture shows specific sensory characteristics distinguishable from the cheese made from pasteurized milk with a starter culture (Amiri *et al.*, 2021).

From a literature survey, there are no investigations on the use of NWC in Edam cheese production. Therefore, the objective of this research was to investigate the effects of different levels of NWC (2, 3, and 4%) on the physicochemical, free amino acids, organic acids, textural, microbiological and sensorial characteristics of Edam cheese produced from pasteurized milk over 45 days-ripening. The results were compared to traditional Edam cheese (unpasteurized milk, no starter culture) and Edam cheese produced from pasteurized with a mesophilic starter culture.

MATERIALS AND METHODS

Materials

Fresh cow's milk (10.60% total solids, 3.00% fat, 3.14% protein, 3.67 % lactose, 0.78% ash, and pH 6.6) was supplied by the dairy industry unit, Fac. of Agric., Fayoum Univ., Egypt. The starter cultures (FD-DVS CHN-11, mesophilic aromatic culture) and rennet powder were supplied by MIFAD Company for food additives, Cairo, Egypt. NaCl and $CaCl_2$were obtained from Emisal Company, Fayoum, Egypt, EL-Nasr Company, Cairo, Egypt, respectively.

Nature whey culture preparation

According to Moser *et al.*, (2018), whey (5.18 % total solids, 0.19 % fat, 3.67 % lactose, 0.71 % protein and pH 6.2) was taken from traditional Edam cheese-making after the scalding step, and immediately kept at 35ºC for 24 h for getting natural whey culture.

Manufacture of Edam cheese

Edam cheese was manufactured according to Hoffmann *et al.*, (2020) with some modification. Fresh cow's milk was

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pasteurized (72ºC/15sec), cooled to 32ºC, and 0.02% $CaCl_2$ was added. Milk was divided into four portions; the first one was inoculated with 1% starter culture, and the remaining portions were inoculated with 2.0, 3.0 and 4.0% NWC, respectively. Traditional Edam cheese was made from raw milk without starter culture as a control cheese. All treatments were left for 30 min to increase acidity by 0.01 %, and rennet (3g/100 kg milk) was added. After 30 min, cutting the curd was done. After 10 min, agitation started, and continued for 30 minutes reaching 37ºC. Draining off the whey, and washing the curd with warm water. The curd was pressed, and brine salted (16% brine solution) for 48 h at 12ºC then dried, coated, and ripened for 45 days.

Cheese samples were taken for all treatments at 1, 15, 30, 45days for physiochemical, microbiological, and sensorial analysis, and at 45days for free amino acids, organic acid contents, and textural evaluation. On sampling, the rind was removed from each cheese and about 100 g of a representative sample was grated to obtain a homogenous sample for further analysis.

Physiochemical analysis

Cheese samples were analyzed at 1, 15, 30, and 45 days of ripening for pH, titratable, protein, ash and fat. In addition, water soluble nitrogen and non-protein nitrogen were determined using the Kjeldahl method as described by AOAC (2005).

Free amino acids and Organic acids analysis

The individual free amino acids of cheese samples ripened for 45 days were measured as described by Peace and Gilani (2005). While, an analysis of organic acids was carried out according to Mato *et al.*, (2005).

Textural evaluation

The texture profile analysis of Edam cheese ripened for 45 days was determined by Texture Analyzer (Model CT310K Texture Analyzer, USA) providing parameters; hardness, cohesiveness, gumminess, chewiness and springiness.

Microbiological analysis

Microbiological analysis of Edam cheese samples was performed according to APHA (2015). Briefly, 10 gram of sample was aseptically homogenized in 90 ml of sterile peptone water (10 g/L casein peptone, 5 g/L NaCl and 20 g/L trisodium citrate dehydrate with pH 7.0) at 40ºC in a sterile plastic bag for 2 min. The decimal dilutions of the suspensions were carried out for microbiological analysis in 9 ml sterile peptone water. The dilutions were plated onto MRS agar (anaerobic incubation) for lactic acid bacteria, onto M17 agar for lactococci count, Nutrient agar for the total viable count, Potato Dextrose Agar (PDA) for yeasts, and molds, and MacConkey Agar for the enumeration of *Escherichia coli*. Nutrient agar and MRS agar plates were incubated at 37ºC for 48 h, M17 agar plates at 30ºC for 48 h, PDA agar plates at 25ºC for 72 h, and MacConkey Agar plates at 37ºC for 24-48 h. The results were reported as a log cfu/g.

Sensory evaluation

The sensory characteristics of Edam cheese samples at 1, 15, 30, and 45 days were assessed by twenty panelists according to Hamdy et al., (2021). The panelists were asked to evaluate Edam cheese samples using a 9-hedonic scale test for a taste evaluation.

Statistical analysis

The data presented as the mean ± standard deviation of each treatment and analyzed by one way ANOVA using SPSS Statistics software (SPSS Inc., Chicago, USA) and comparing means at $P \leq 0.05$ by LSD test.

RESULTS AND DISCUSSION

Physicochemical characteristics

Table (1) shows the main physicochemical characteristics of traditional and experimental Edam cheese samples during the ripening period. Significantly different values ($P \leq 0.05$) were found in physicochemical parameters in control Edam cheese compared to the other treatments. The results showed that, the highest amount of titratable acidity was found in Edam cheese manufactured from raw milk and that manufactured from pasteurized milk acidified with 4% NWC, which may be due to the increases in microbial activity. There was no statistically significant difference ($P \geq 0.05$) in titratable acidity of Edam cheese made with NWC and control sample. The increase in titratable acidity and reduction in pH of cheese samples during ripening is correlated with each other. Cheeses with NWC exhibited the highest values ($P \leq 0.05$) of moisture content than the other ones, on the other hand, protein, fat, and ash contents were significantly lower than Edam cheese produced from pasteurized with a starter culture. The higher moisture content in Edam cheese with added NWC could be explained by the presence of whey proteins in cheese whey holding water into the cheese matrix as reported by Sabikhi et al., (2014). Besides, pasteurization affected the retention of water in the cheese matrix. The high protein content in Edam cheese samples with NWC in comparison to Edam cheese produced from pasteurized milk could be explained by the proteolysis of whey protein caused by lactobacilli (Aljewicz *et al.,* 2014). Ash contents were not significant among different Edam cheese treatments. These results were in line with Gatti et al., (2014).

Evaluation of proteolysis

The average WSN/TN% and NPN/TN% in traditional and experimental Edam cheeses during the ripening are shown in Table (1). No signficant difference was found in WSN/TN or NPN/TN% of the experimental, and control Edam cheeses. Proteolysis intensity (WSN/TN and NPN/TN%) in the cheese samples showed that its amount increased during the ripening period in all cheeses, and the highest amount was observed in the Edam cheeses with 4% NWC on day 45, probably due to the higher activity of enzymes and/or bacteria (Azarnia *et al.,* 2010). Proteolysis in terms of WSNTN% proceeded similarly in the Edam cheeses acidified with NWC with regard to that observed in control cheeses. These results were in consist with Cuffia *et al.*, (2019). The high contents of WSN/TN and NPN/TN% were correlated to the growth of LAB and NSLAB cultures and their enzymes (Azarnia *et al.,* 2010). In addition, the higher WSN/TN fraction of Edam cheese was related to the action of residual coagulant, proteinases from milk and somatic cells, plasmin activity and microbial protease. Moreover, the higher moisture and acidity activate the action of chymosin on αs_1-casein and result in increasing WSN/TN occurring at the end of the ripening period (Hinz *et al.,* 2012), due to the bacterial proteinases of microflora (Aljewicz *et al.,* 2014).

Free amino acids

Table (2) shows the individual free amino acids contents of control and experimental Edam cheeses ripened for 45 days. Cheese samples contained 16 individual free amino acids for all the treatments studied. Tyr, Pro, Glu, Lys, Leu, and Phe were the main free amino acids in all treatments, representing around 65% of the total free amino acids content. Levels of all individual amino acids were higher in Edam cheeses with 3 or 4% NWC than in control and Edam cheese made with pasteurized milk.

This could be attributed to the higher proteolytic rate in Edam cheese with NWC due to the presence of mixed microflora and their enzymes (proteases, proteinases and peptidases) leading to the formation of large and small peptides and subsequent accumulation of free amino acids in cheese during ripening (Pachlová *et al.,* 2018). The total free amino acid content of Edam cheeses with 3 or 4% NWC was higher than that of the control sample. This could probably be attributed to a higher bacterial peptiolytic activity in this cheese. It is possible that the NWC contributes to the high proteolytic activity observed in cheeses. These findings were in consist with Garbowska *et al.,* (2020) and Sallami *et al.,* (2004).

Organic acids in cheese samples

Table (3) shows the main organic acids of traditional and experimental Edam cheeses ripened for 45 days. Acetic, propionic, lactic and butyric acids levels were found the major organic acids in all trials of Edam cheese samples ripened for 45 days. Generally, acetic acid levels were found the highest concentration in all cheese samples followed by propionic and lactic acids, while butyric acid was found the lowest concentrations in all Edam cheese samples. During ripening, non-starter lactic acid bacteria (NSLAB) might enhance the oxidation of lactic acid into acetic acid and carbon dioxide which contributes to the flavor of Edam cheese, hence the concentrations of acetic acid were considerably higher than other acids (Düsterhöft *et al.,* 2017).

The four organic acids levels in the experimental Edam cheese samples with NWC were higher than traditional Edam cheese or pasteurized Edam cheese with a starter culture, which might be due to the valuable content of the mixed microflora and their proteolytic and lipolytic activities as compared to that exist in raw milk or in the starter culture. Moreover, NWC produces additional total free amino acids by increased proteolysis that serves as precursors for the production of organic acid at much higher concentrations (Bertuzzi *et al.,* 2018).

Textural evaluation

Textural results of traditional and experimental Edam cheeses ripened for 45 days were shown in Table (4). The hardness values were lower in all the experimental cheeses than in the control cheese. Traditional Edam cheese was the hardest cheese among all experimental Edam cheese, while Edam cheese with starter culture had the lowest hardness value. Edam cheese with a higher level of NWC had lower values of hardness when compared to other Edam cheese with NWC. Usually high acidity, high protein content, and dry matter make cheese texture harder, and more resistant to deformation (see Table 1).

Control Edam cheese showed the lowest cohesiveness, whereas Edam cheese with NWC or starter culture showed the highest cohesiveness. The highest value of gumminess was found for Edam cheese with 4% NWC, the value was 18.04 N and the lowest value was found for Edam cheese with a starter culture, and the value was 9.06N. The cheese made with NWC has high values of chewiness as the texture is soft but the cheese made with starter culture has low chewiness values. The highest value of chewiness was found for 4% NWC, and the value was 15.99N.mm, the lowest value was found for the Edam cheese made with starter culture, and the value was 9.93 N.mm. This is expected as gumminess, and chewiness are secondary parameters derived from hardness, cohesiveness, and springiness. The highest value of springiness was found for Edam cheese with 3 or 4% NWC, and the value was 0.89 mm, and least value was found for control Edam cheese, the value was 0.80 mm. These findings were comparable to those obtained by Awad (2006) and Květoslava *et al., (*2021).

Microbiological analysis

Microbiological results of traditional and experimental Edam cheeses during the ripening are shown in Table (5). TVC in traditional Edam cheese was significantly higher ($P \leq 0.05$) in comparison with the corresponding counts in Edam cheeses with NWC or with starter culture on all 4 ripening days tested. LAB population was slightly higher ($P \leq 0.05$) in Edam cheese with NWC than in traditional Edam cheese, and declined thereafter in all treatments. During ripening, the highest LAB counts occurred in the Edam cheese with NWC, followed by Edam cheese with starter culture, and the lowest counts were determined in the control Edam cheese. *lactococci* counts in Edam cheese with starter culture were significantly higher ($P \leq 0.05$) than in raw milk cheese and cheese with NWC ($P \leq 0.05$), indicating that heat treatment inactivates microorganisms which provides a better medium for starter and adjunct culture growth.

Molds and yeasts were detected at low levels only in unpasteurized milk cheese samples at 15 days, and detected at 30 days in all cheese samples, and significantly increased at the end of ripening in pasteurized milk cheese samples. Pasteurization of milk reduce considerably the moulds counts in pasteurized milk cheeses, but during ripening, it increased and reached equal value with raw milk cheese. Coliform bacteria didn't observe in all Edam treatments as ripening progressed.

Sensory evaluation

Sensory evaluation of Edam cheeses during the ripening period of 45 days is presented in Table (6). No significant differences were found ($P \geq 0.05$) in appearance among Edam cheese samples. The taste, smell and texture scores of Edam cheese with NWC were significantly higher ($P \leq 0.05$) than that of control and Edam cheese with starter culture after 30 days of the ripening, which could be related to the rate of proteolysis and the formation of free amino acids. At the end of ripening period, Edam cheese with NWC was characterized by higher overall acceptability than traditional and Edam cheeses with a starter culture. The rate of proteolysis may be enhanced by the rich source of microflora in NWC affecting the microbiota of the Edam cheese matrix and their enzymes which in turn enhance the sensory properties of Edam cheese samples.

CONCLUSION

Results shows NWC was an effect on the physicochemical and sensory characteristics of Edam cheese produced from pasteurized milk. The acidification of milk with NWC resulted in cheeses with higher titratable acidity, moisture, ash contents, and thus, the lower amount of the other parameters. Lactic acid bacteria, and lactococci populations were higher in cheeses with NWC than those of the control cheese, indicating a higher rate of proteolysis, amino acids, and organic acids in cheese produced with NWC. Cheese acidifid with 4%NWC had better sensory attributes than control cheese. Finally, Edam cheese produce with pasteurized milk can be acidifid with 4% NWC which was found best in all aspects and had similar characteristics to those of control Edam cheese.

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تأثير بادئ الشر الطبيعي علي خصائص الجبن الايداد المصنع من لبن مبستر

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تهدف الدراسة إلى تقييم تأثير المستويات المختلفة من بادئ الشرش الطبيعي (2، 3 ، 4٪) على الخصائص الفيزوكيميائية، الأحماض الأمينية الحرة، الأحماض العضوية، القوام، الميكروبيولوجية، الحسية للجبن الايدام المصنع من اللبن المبستر أثناء مراحل التسوية (0 ، 1 ، 15 ، 30 ، 45 يوم). وأظهرت النتائج أن الحموضة والرطوبة ودلائل التحلل البروتيني كانت أعلى معنويا، بينما محتوى البروتين والدهون كان أقل معنويا في الجبن الايدام الناتجة من لبن مبستر مع بادئ الشرش الطبيعي مقارنة بالجبن الايدام الناتجة من لبن خام. وكانت مستويات جميع الأحماض الأمينية أعلى في الجبن إيدام المصنع ببادئ الشرش الطبيعي بنسبة 4٪ مقارنة بعينات جبن إيدام ناتجة من لبن خام او لبن مبستر مع البادئ التجاري. وكانت تركيزات الاحماض العضوية أعلى بكثير في جبن إيدام مع بادئ الشرش الطبيعي مقارنة بمعاملات الجبن الأخرى. وكانت جبن إيدام مع بادئ الشرش الطبيعي أعلى بشكل ملحوظ في تقييم القوام من قيم عينات الجبن الأخرى. ومن الناحية الحسية كانت الجبن المصنعة باستخدام بادئ الشرش الطبيعي أعلى بكثير من جبن الكنترول. وبشكل عام فالجبن الإيدام الناتجة من لبن مبستر مع إضافة 4٪ بادئ الشرش الطبيعي كانت أعلى في خصائص الجودة من الجبن المصنعه من لبن مبستر مع إضافة البادئ التجاري وكانت مشابهه للجبن المصنعه من اللبن الخام.