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CHEMICAL CHARACTERISTIES OF ITALIAN RICOTTA CHEESE AS INFLUENCED BY THE PROTEOLYSIS DURING RIPENING.

Mohamed,S. A. ;S.M. Hasan and S. T. Abusalloum

Food Science and Technology Department, Faculty of Agriculture, Omar Almukhtar University, Elbeida, Libya

ABSTRACT

The objective of the present work is to study the role of proteolysis being occurred in the Italian Ricotta Forte cheese throughout 20 months of ripening. Samples were taken for the examination after 1(day),and the after 1,2,4,6.12 and 19 months. Samples were analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), which showed that the level of bovine serum albumin decreased towards the end of the ripening. However,α-lactalbumin and β-lactoglobulin did not degrade rapidly during the ripening. Meanwhile,the levels of pH 4.6-soluble N (SN) as a % of total N (TN) and total free amino acids (FAA) increased towards the end of the ripening. This study improves our understanding about the compositional and proteolytic parameters of Ricotta Forte cheese.

INTRODUCTION

There is an increasing interest in traditional dairy products manufactured on a small scale, due to the difficulties in mimicking them on an industrial scale, and an increasing interest in artisanal foods (Baruzzi *et al*., 2000). Ricotta cheese is a heat/acid precipitated cheese that can be made from whey or a mixture of whey and whole or skim milk. Several cheeses are manufactured throughout the world using a combination of acid and heat for coagulation, including some forms of Queso Blanco (Central and South America), Paneer (India) and Ricotta (Italy). In different cheeses, proteolysis plays an important role in determining typical sensory characteristics and represents a significant indicator of quality (Gaiaschi *et al*., 2001). In soft rennet-coagulated cheeses, the main proteolytic agent is the residual couagulant, because the high moisture content and the absence of cooking, or low cooking temperatures, enhance its retention in the curd, and its activity on proteins (Noomen, 1978).

Ricotta is an unripened soft cheese that originated in Italy,but which is now popular in Greece and in Latin American countries such as Argentina. It remains, particularly, popular in southern Italy, where it is produced in many forms from various milks and milk fractions (Hough *et al*., 1999). Traditionally, Ricotta is manufactured from the whey of Mozzarella cheese; however, other types of milk fractions have been used including whey from other cheese varieties, whole milk, partially skimmed milk or a combination of these ingredients (Calabro, 1980).The objective of this research project was to study the proteolysis in Ricotta Forte cheese during ripening.

MATERIALS AND METHODS

Cheese samples

Samples of Ricotta Forte were obtained from an Italian factory. Cheese was made in two trials, and the ripening process was completed throughout 19 months. The samples (100 g) of Ricotta Forte cheese were taken at 1 day and then after 1, 2, 4, 6, 12 and 19 months of ripening , and were stored at –20 ºC.

Compositional analysis

Cheese samples were analysed for moisture according to (IDF, 1982), total protein (IDF, 1964), fat contents were determined according to Gerber method (Bradley *et al*., 1992) and salt (Fox, 1963). Each analysis was performed in triplicate. The pH of a 1:1 slurry of cheese in distilled water was determined using a pH-meter(corning pH/ion analyzer.corning, NYI after calibration with standard buffers pH 4.0 and 7.0).

Assessment of proteolysis

Proteolysis was assessed in cheese samples by measuring pH 4.6-soluble N (i.e., the proportion of total N, as measured by Kjehdahl, soluble in water at pH 4.6) using the method described by Fenelon *et al*. (2000), and expressing the results as a percentage

of total (pH 4.6-SN/TN). The pH 4.6 -soluble fractions of the cheese samples were prepared by a slight modification of the procedure of Kuchroo and Fox (1982), as described by Sousa and McSweeney (2001). Sodium dodecyl sulphate (SDS)-PAGE was used for the separation of polypeptides insoluble at pH 4.6 from Ricotta Forte cheeses according to the method described by Laemmli (1970), as modified by Singh and Creamer (1991). Levels of total free amino acids in the pH 4.6-soluble fractions of cheese were determined by the trinitrobenzenesulphonic acid (TNBS). Assay of Polychroniadou (1988) and individual free amino acids were determined in Ricotta Forte cheese after 1, 6 and 19 months of ripening using an amino acid analyzer as described by Fenelon *et al*. (2000).

Statistical analysis

One-way analysis of variance (ANOVA) of data for the chemical analysis (i.e. cheese composition, pH value, level of WSN as % TN and concentration of FAA) was conducted using SPSS Version 11.0 for Window XP (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Chemical Composition and pH-values of Ricotta Forte cheeses:

The moisture, NaCl, fat and protein contents (± standard deviations) and pH values of Ricotta Forte cheeses from trials 1 and 2 during ripening are presented in Table 1. Chemical compositions at day 1 were in the typical range of Ricotta cheese ready to consume, and were similar to the chemical composition of Ricotta cheese manufactured from whole milk, which contains approximately 72% moisture, 13% fat and 11% protein (Farkye, 2004). Variations in moisture, salt, fat and protein concentrations in the cheese

throughout ripening were observed. Moisture content decreased from 71.4 to 57.6 % and from 72 to 56.9 % in trials 1 and 2, respectively, throughout the period extending from the beginning and the end of ripening. These decrease was probably due to evaporation (since there was no control of relative humidity) during ripening, and resulted in higher levels of protein, fat and salt in both trials later in ripening. pH values of Ricotta Forte cheeses of trials 1 and 2 throughout ripening are shown in Table (1). The pH was about 3.41 and 3.49 at day 1, and increased during ripening, reaching 3.87 and 3.96 at the end of ripening in trials 1 and 2, respectively. These increases in pH during ripening were in agreement with the results of Baruzzi *et al*. (2000), who reported that the pH of Ricotta Forte increased from 3.9 to 4.5 after 2 months of ripening.

Table 1.Chemical composition (%) and pH values of Ricotta Forte cheese during ripening.

Results presented as mean ± standard deviation of triplicate analyses.

Assessment of proteolysis

Levels of pH 4.6-soluble nitrogen (SN) as a percentage of total nitrogen (TN) in Ricotta Forte cheeses of trials 1 and 2 during ripening are shown in Table(2). The levels of pH 4.6-SN/TN in trials 1 and 2 increased throughout ripening from 6.9 at day 1 to 13 % and 14.1%, respectively, at the end of ripening (19 months). Levels of pH 4.6-SN/TN were lower at the end of ripening in both trials, compared to most other varieties, which could be due to the fact that whey proteins are resistant to hydrolysis by chymosin and plasmin. Candioti *et al.* (2002) observed that whey proteins are strongly resistant to commercial rennet containing porcine pepsin, bovine pepsin and chymosin.

Table 2.Levels of pH 4.6-soluble nitrogen as a % of total N (4.6 SN/TN) during ripening of Ricotta Forte cheese.

Results presented as mean ± standard deviation of triplicate analyses.

SDS-PAGE electrophoretograms of the pH 4.6-insoluble fractions of Ricotta Forte cheese during ripening are shown in Figure(1). Assessment of primary proteolysis by SDS-PAGE of Ricotta Forte cheese throughout ripening showed some differences between the cheese samples. The intensity of the bovine serum albumin band (zone A, 66 kDa), the band at ~ 57 kDa and the band at ~ 84 kDa decreased in intensity during ripening, and these proteins were present at very low concentrations, or were absent towards the end of ripening. On the other hand, the major band on the SDS-PAGE electrophoretograms (zone B, ~ 17 kDa perhaps corresponding to the caseins) decreased slightly during ripening. β-Lactoglobulin and α-lactalbumin did not rapidly degrade during the ripening (zones C and D).

Figure1.Sodium dodecyl sulphate polyacrylamide gel electrophoretograms of pH 4.6-insoluble extracts of Ricotta Forte cheese during ripening.

Levels of free amino acids in Ricotta Forte cheese during ripening in trials 1 and 2 are given in Table(3). The concentrations of total FAA increased towards the end of ripening from 5.9 and 5.4 after 1 day of ripening to 20.6 and 19.4 mg Leu g^{-1} after 19 months of ripening in trials 1 and 2 respectively. This was not expected since Ricotta Forte cheese has high levels of whey proteins, which are resistant to hydrolysis by chymosin and plasmin and it could be due to the high moisture content and the long ripening period of Ricotta Forte cheese.

Table 3. Levels of total free amino acids during ripening of Ricotta Forte cheese.

Results presented as mean ± standard deviation of triplicate analyses.

Profiles of individual amino acids from Ricotta Forte after 1 day, 6 months and 19 months of ripening are shown in Figure 2. Concentrations of all free amino acids showed a clear trend, increase with the ripening time, as was expected, since, during proteolysis, free amino acids released by proteolytic agents, namely microbial peptidases. Amino acids, released from peptides by the action of peptidases, subsequently act as precursors for catabolic reactions, which produce many important volatile flavour compounds (see McSweeney and Sousa, 2000; Yvon and Rijnen, 2001). The concentrations of alanine, glutamic acid, leucine and lysine were higher than those of the other amino acids in Ricotta Forte cheese after 19 months of ripening, while tyrosine, aspartic acid, methionine and glycine were present at lower concentrations than the other amino acids at this ripening time. The same trend was observed by other authors for different varieties; McBrearty *et al*. (2001) reported that the concentration of leucine and glutamic acid was higher than other amino acids in Cheddar cheese after 6 months of ripening; Antonsson *et al*. (2003) also found that Herrgard cheese (semi-hard Swedish cheese) had large amounts of leucine, phenylalanine and glutamine after 6 months of ripening. Although the concentrations of lysine and histidine in Ricotta Forte cheese increased ~10 fold after 19 months of ripening, they were still not high compared to levels of glutamic acid, alanine and leucine which increased 5, 7 and 9 fold, respectively, while the concentrations of most of other amino acids increased 3-5 fold during ripening.

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تاثيرالتحلل البروتيني للجبن الايطالى ريكوتا Ricott Forti خلال التسويه

صلاح الناجي محمد, صلاح محمد حسن وسليمان طاهر بوسلوم
قسم علوم وتقنية ألأغذية – كلية الزراعة – جامعة عمر المختار- البيضاء- ليبيا

يهدف هذا البحث الي دراسة تحلل بروتين جبن الريكوتا اثناء الانضاج . جبن الريكوتا من ألأجبان التي تعتمد على حدوث التجبن في وجود الحرارة والحامض وتصنع من الشرش وعادة لاتجرى لها عملية انضاج (تسوية). عينة الجبن المستخدمة في هذا البحث صنعت في ايطاليا وأجريت لها عملية انضاج (تسوية) لمدة 20 شهر وتم أخذ العينات للتحليل على فترات متفاوتة بعد يوم , أسبوع ,1 ,2 ,4 ,6, 12 , 19 شهر من الانضاج .

أوضحت النتائج أن التركيب الكيماوي للريكوتا بعد التصنيع مباشرة (يوم) مطابقا لمواصفات جبن الريكوتا المصنعة في ايطاليا . اجراء عملية الفصل بالهجرة الكهربية أظهر نقص في مستويات سيرم الالبيومين في نهاية عملية الانضاج بينما لم يحدث تغير أثناء الانضاج لكلا من الفا لاكتوالبيومين وبيتا لاكتوجلوبيولين .

من ناحية أخرى لوحظت زيادة في النيتروجين الذائب وألأحماض ألأمينية الحرة الكلية أثناء ألأنضاج.

قام بتحكيم البحث

أ.د / طة عبد الحليم نصيب — كلية الزراعة – جامعة المنصورة
أ.د / الطاهرة محمد احمد عمار — كلية الزراعه – جامعة المنصورة