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EFFECT OF RIPENING TEMPERATURE ON PROTEOLYSIS AND ORGANOLEPTIC PROPERTIES OF EDAM-TYPE CHEESE

Marian Kujawski, Grażyna Cichosz, Ewa Podhajna, Barbara Sańko
Chair for Food Safety Development, University of Warmia and Mazury in Olsztyn, Poland

ABSTRACT

The effect of ripening temperature on changes in pH, dynamics of proteolysis and organoleptic properties of cheese was studied. The Dutch-type cheese ‘Hetman’ packed in thermoshrinkable foil and produced in a dairy plant at Kolno was used in the studies. The degradation of paracasein and changes in organoleptic properties were examined during six weeks of cheese ripening at 8, 12 and 15°C.

The pH value was found to increase with ripening temperature, proving intensification of enzymatic changes. An elevated temperature of ripening intensified proteolysis, enabling the production of cheese with required organoleptic properties in a shorter period of time. The optimal ripening temperature was found to be 12°C, whereas the temperature of 15°C caused the formation of amino acid degradation products that negatively influenced the organoleptic properties of “Hetman” cheese.

Key words: organoleptic properties, ripening time, proteolysis, ripening cheeses.

INTRODUCTION

The ripening cheeses do not have typical organoleptic properties immediately after hooping and salting. These are developed only during the cheese ripening. One of the most important biochemical processes determining the taste and texture of a cheese is proteolysis, which include microbiological, enzymatic and physico-chemical processes [5]

The proteolytic system of lactic acid bacteria consists of two functional enzyme classes: (1) proteinases that hydrolyse native and denatured proteins and (2) peptidases that catalyse the hydrolysis of peptides formed in the result of proteinase actions [18].

The action of enzymes of lactic acid bacteria is mainly based on the breakdown of oligopeptides and peptides to di- and tripeptides and amino acids, which can pass through the cytoplasmatic membrane into the cell. Their further breakdown upon the action of highly specific enzymes, *ie* aminopeptidases and dipeptidases enables the growth of bacterial cells [3]. Following the death and autolysis of the cells, intracellular enzymes are released what intensifies peptolysis, particularly of peptides formed from α_s-fraction. Upon the action of peptidases, low molecular weight peptides and amino acids are released which are important for the organoleptic properties of ripening cheeses [8].

The time of cheese ripening is quite different for different cheeses, being dependent on the acidity and water content determining the enzyme activities. The process of ripening of the most of hard cheeses is a long lasting process, thus expensive, since the financial expenses for the raw material purchase, processing, and treatments during ripening are refunded after a relatively long time. This fact is the reason of the interest in the methods for acceleration of cheese ripening, yet maintaining comparable organoleptic and physico-chemical properties of the final product.

Different methods are known for intensification of proteolysis during cheese ripening. The use of proteolytic enzymes or the mixture of proteases and lipases is an efficient method, but too expensive because enzymes are mostly lost in whey, thus limiting its utilisation. In addition, the enzymatic preparations, including encapsulated ones, destabilize the balance of flavour compounds because of different substrate specificity [4]. The addition of free amino acids or growth stimulants intensifies the multiplication and biochemical activity of the starter culture, but often encounters a risk for overacidification of cheese curd [6]. On the other hand, the use of heat-shocked cells besides traditional starter (or bacterial cell concentrate) protects against overacidification but requires high financial expenses [16]. The cheapest and simplest method for intensification of proteolysis is undoubtedly the use of higher temperatures for cheese ripening. Taking the above into consideration, the studies were carried out on the influence of different ripening temperatures on the extent of physico-chemical changes and flavour characteristics of Edam-type cheese.

OBJECTIVE

The objective of the present work was to evaluate: (1) the changes in the acidity of cheeses vacuum-packed in thermoshrinkable foil, (2) the influence of ripening temperature on the extent and intensity of proteolysis in 'Hetman' cheese, and (3) the organoleptic properties of cheese, depending on ripening temperature.

MATERIALS AND METHODS

Material. Rennet ripening cheese 'Hetman' was used for the studies. The cheese was produced from milk after thermization, pasteurization and bactofugation in a dairy plant "Kurpianka" at Kolno, using a continuous technological line. Saltpetre, coagulating enzyme and starter culture Flora Danica were added during the cheese production.

Packing. The cheeses were packed in thermoshrinkable foil type BK 1L for packing fresh gassing cheeses. After sampling, each block of cheese for ripening at required temperature was foil-repacked in vacuum conditions. After marking, the cheeses ripened at 8, 12 and 15°C. The studies were carried out in triplicate.

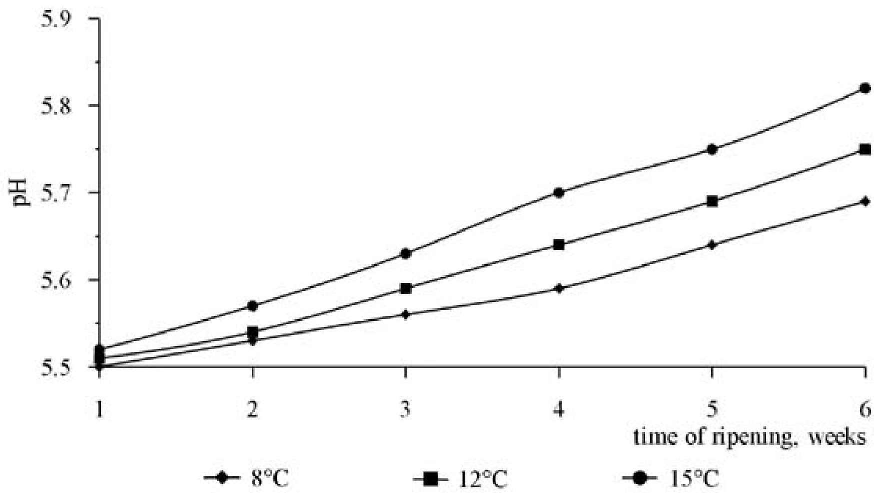
Analytical methods. The following analyses were carried out during six weeks of cheese ripening at 8, 12 and 15°C: (a) pH value [according to PN-73/A-86232], (b) organoleptic properties [according to PN-68/A-86230; NZ/CZML/A-106], (c) the degree of paracasein degradation by measuring the changes in the content of individual nitrogen forms: total nitrogen, soluble nitrogen using the Sode-Mogensten method, and amino acid nitrogen determined by the Sirks' method (the results were expressed in percentages of the total nitrogen) [9], and (d) free amino groups using the method with TNBS [15].

RESULTS AND DISCUSSION

In this work, the influence of different temperatures on the rate, extent and intensity of proteolysis was determined. The content of soluble nitrogen is a measure of the extent of ripening that is mostly dependent on the action of chymosin, whereas the content of amino acid nitrogen is a measure of the ripening intensity that is determined by microorganisms and enzymes they synthesise.

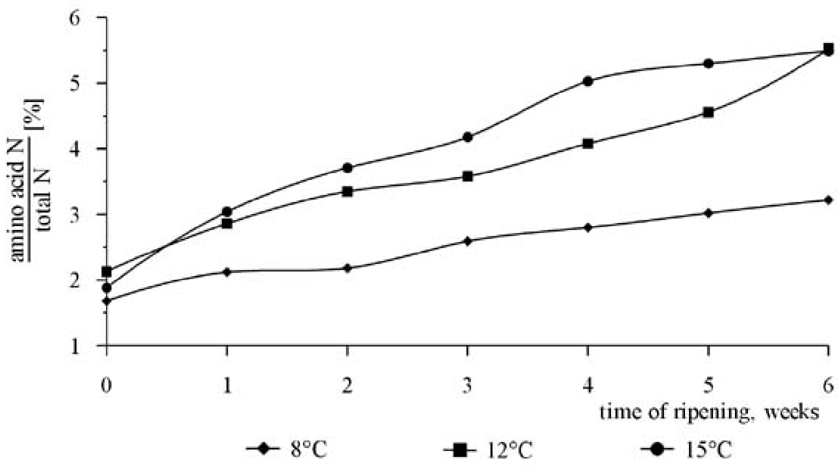
The changes in pH value prove that the enzymatic degradation occurs during ripening (Fig. 1). A clear relationship was observed between the ripening temperature and the acidity of cheese. Despite similar acidity of cheeses after salting (the pH values were 5.50, 5.51 and 5.52 for respectively 8, 12 and 15°C), the hydrogen ion concentration was the lower, the higher was the temperature during cheese ripening. After six weeks of ripening the respective pH values were 5.69, 5.75 and 5.82 (Fig. 1). This proves that the enzymatic processes were intensified at higher temperatures.

Figure 1. Changes in "Hetman" cheese acidity (pH) depending on temperature



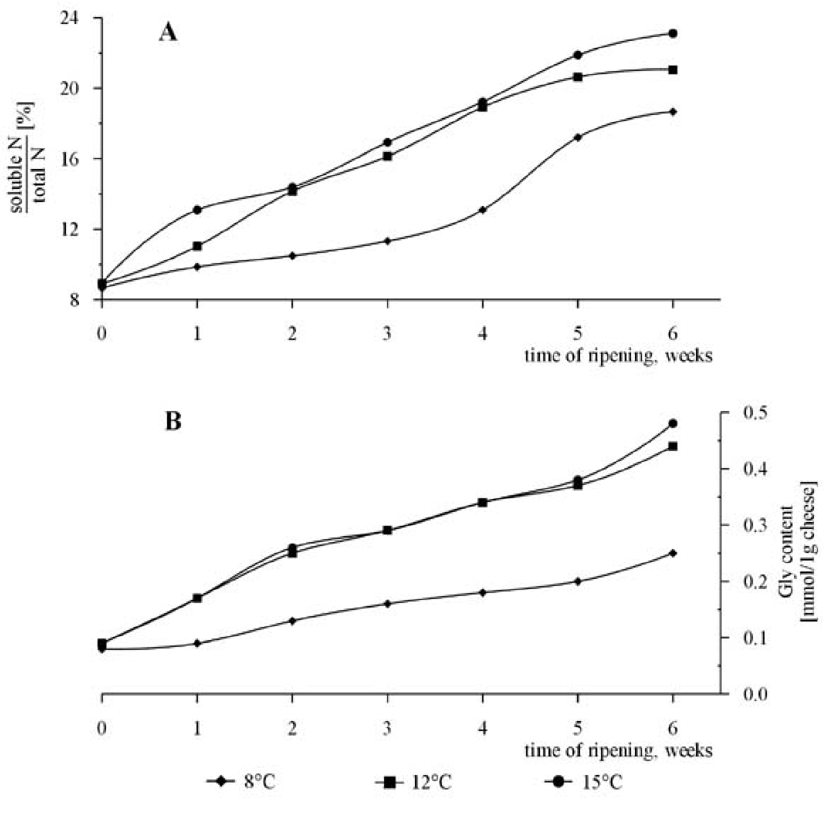
Considerable differences were observed in the dynamics of proteolytic changes during cheese ripening at 8, 12 and 15°C. It was found that the amount of amino acids was the greater the higher was the ripening temperature. The greatest differences were observed between the contents of amino acid nitrogen after six weeks of ripening at 8 and 15°C. The average content of these compounds was by 41.3% higher for cheeses ripening at 15°C than those ripening at 8°C, being respectively, 5.49 and 3.22% of the total nitrogen (Fig. 2).

Figure 2. Intensity of "Hetman" cheese ripening [amino acid nitrogen/total nitrogen] depending on temperature



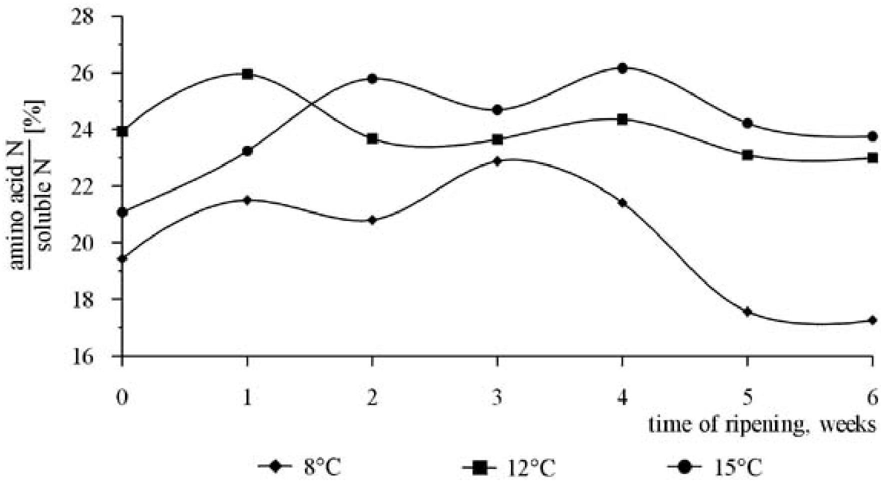
Similar relationships were observed for the increase in soluble nitrogen compounds (Fig. 3A). The percentage content of these compounds (expressed in per cent of total nitrogen N_t) increased from 8.92% after salting to 23.11% after 6-week ripening for cheeses ripening at 15°C, whereas it increased from 8.90% after salting to 21.05% after 6-week ripening for cheeses ripening at 12°C. The cheeses ripening at 8°C showed slower rate of increase in low molecular weight compounds and their maximum content after six weeks of ripening was 18.66% N_t.

Figure 3. Extent of "Hetman" cheese ripening depending on temperature.
A – Kjeldahl's method [soluble nitrogen/total nitrogen]
B – method with TNBS [free amino groups]



A comparable percentage of amino acid nitrogen was found for the fraction of soluble nitrogen in cheeses after the first week of ripening at all examined temperatures (Figure 4). At 15°C, the rate these compounds were increasing was quicker and maintained on a similar level until the fourth week of ripening, decreasing during the last two weeks of ripening, most probably, because of degradation of amino acids through decarboxylation, deamination and desulfuration [1,10,14]. Beginning with the fourth week of ripening at 8°C, the percentage of amino acid nitrogen in the soluble nitrogen fraction also decreased. The results obtained for chemical analysis of paracasein degradation during cheese ripening are in agreement with the literature data [11,19]. The fraction of soluble nitrogen compounds, that includes free amino acids, peptides of the molecular weight below and above 1.4 kDa, is about 25% of the total nitrogen in ripened cheese [GOSHI et al., 1986; VISSER et al., 1983]. This value can be referred to cheeses ripening at 12 and 15°C. The content of soluble nitrogen was only 18.66% of the total nitrogen for other cheeses (8°C/6 weeks).

Figure 4. Dynamics of "Hetman" cheese ripening [amino acid nitrogen/soluble nitrogen] depending on temperature



The content of soluble nitrogen is on principle assumed as the indicator of cheese ripeness. Polychroniadou [15] and Kuchroo et al. [13] proved that the content of soluble nitrogen in ripening cheeses correlates with the amount of free amino groups determined with TNBS method ($r = 0.857$ at $\alpha = 0.01$). We obtained similar value for the correlation coefficient ($r = 0.915$, $\alpha = 0.01$) in this work. During ripening, it was observed a continuous increase in the content of free amino groups (expressed in millimoles of glycine, mmol Gly/g cheese), being a measure of the ripening intensity. In the method used, the reaction of free amino groups with TNBS is exploited, allowing monitoring cheese proteolysis (Fig. 3B).

The cheeses ripening at 8°C were characterised by a slow rate of degradation changes, reaching the level of amino groups greater by 277.8% (from 0.09 mmol Gly/g to 0.25 mmol Gly/g cheese) than the initial value only in week six of ripening. Particularly rapid degradation of protein was observed for cheeses ripening at 12 and 15°C, for which the increase in amino groups by about 280% compared to the initial value was noted as soon as in week three of ripening. The course of proteolysis for these both groups of cheeses was comparable. After six weeks of ripening at 12°C, the free amino groups increased to the value of 0.44 (by about 490% compared to the initial value of 0.09 mmol Gly/g cheese) and to 0.48 (by about 530%) after six weeks of ripening at 15°C.

The surface growth of undesired microflora was not observed on evaluating organoleptically the cheeses ripening at 8, 12 and 15°C. The plastic foil used for packing protected well against harmful microorganisms and strict compliance with hygiene eliminated recontamination during sampling (Tables 1,2 and 3).

Table 1. Organoleptic evaluation of Dutch-type cheese ("Hetman", dairy plant at Kolno) during ripening at 8°C

Time of ripening	Consistency	Eye formation	Colour	Taste and smell
Week 1	Hard, elastic	Eyeless	Not much intensive, uniform in the whole mass	Not enough salty, slightly acid
Week 2	Hard, elastic	Eyeless	Natural, uniform in the whole mass	Slightly acid and salty, mild
Week 3	Hard but elastic, slightly crumbly	Eyeless	Natural, uniform in the whole mass	Mild, slightly sweet, delicate
Week 4	Flexible, elastic, proper	Single irregular eyes	Natural uniform in the whole mass	Perceptible cheese flavour, slightly salty, mild
Week 5	Flexible, elastic, proper	Single irregular eyes	Natural, uniform in the whole mass	Cream-like, distinct features of Dutch-type cheese flavour
Week 6	Flexible, compact	Single irregular eyes	Natural uniform in the whole mass	Cheesy, clean, typical Dutch-type cheese flavour
During six-week ripening of the cheese it was not found neither the changes on the cheese surface (smooth, soft, outer layer not damaged) nor the growth of undesired microflora and fungi				

Table 2. Organoleptic evaluation of Dutch-type cheese ("Hetman", dairy plant at Kolno) during ripening at 12°C

Time of ripening	Consistency	Eye formation	Colour	Taste and smell
Week 1	Hard, elastic	Eyeless	Not much intensive, uniform in the whole mass	Not enough salty, slightly acid, weakly sensed
Week 2	Hard, elastic	Eyeless	Natural, uniform in the whole mass	Delicate, not enough salty, cheese flavour perceptible
Week 3	Elastic, slightly crumbly	Single irregular eyes	Natural, uniform in the whole mass	Mild, slightly sweet, delicate
Week 4	Flexible, elastic, proper	Regular eyes with fissures	Natural, uniform in the whole mass	Cheesy, clean, typical for Dutch-type cheese
Week 5	Flexible, elastic, proper	Regular eyes with fissures	Natural, uniform in the whole mass	Typical for ripen cheese, slightly piquant
Week 6	Flexible, soft	Regular eyes typical for the cheese studied	Natural, uniform in the whole mass	Cheesy, clean, intensive, slightly piquant
During six-week ripening of the cheese it was not found neither the changes in the cheese surface (smooth, soft, outer layer undamaged) nor the growth of undesired microflora and fungi				

Table 3. Organoleptic evaluation of Dutch-type cheese ("Hetman", dairy plant at Kolno) during ripening at 15°C

Time of ripening	Consistency	Eye formation	Colour	Taste and smell
Week 1	Hard, elastic	Eyeless	Not much intensive, uniform in the whole mass	Not enough salty, slightly acid, plain
Week 2	Hard, elastic	Eyeless	Natural, uniform in the whole mass	Mild, not enough salty, cheese flavour perceptible
Week 3	Loose, flexible	Single irregular eyes	Natural, uniform in the whole mass	Cheesy, clean, Dutch-cheese typical
Week 4	Flexible, elastic, proper	Single regular eyes with fissures	Natural, uniform in the whole mass	Cheesy, clean, slightly sweet
Week 5	Flexible, soft	Regular eyes typical for the cheese type	Natural, uniform in the whole mass	Intensive, slightly piquant, ripen cheese flavour
Week 6	Flexible but splitting, slightly sticky	Regular eyes typical for the cheese type	Natural, uniform in the whole mass	Very intensive, bitterness perceptible, piquant
During six-week ripening of the cheese it was not found neither the changes in the cheese surface (smooth, soft, outer layer undamaged) nor the growth of undesired microflora and fungi				

In the initial stage of ripening all cheeses were characterised by slightly acid taste and crumbly consistency. The cheeses ripening at 15°C had proper elastic structure with small number of regular eyes already in week three of ripening. Properly soft and elastic consistency and small irregular eyes in cheeses ripening at 8 and 12°C were found only in week four of ripening. Typical taste of Dutch cheese (mild, slightly sweet) was observed in week three of ripening at 15°C (at the content of amino acid nitrogen of 4.18% of the total nitrogen). Relatively high water content of Dutch cheeses and elevated temperature of ripening intensify the activity of proteolytic enzymes, causing the formation of substances responsible for organoleptic properties. The cheeses ripening at 12°C had the proper taste and smell in week four of ripening, when the content of amino acid nitrogen was 4.08% of the total nitrogen, and the cheeses ripening at 8°C had the proper flavour only in week five of ripening (amino acid nitrogen was 3.02% of the total nitrogen).

The organoleptic studies carried out after six weeks of ripening allowed to state that good flavour characteristics and proper consistency, structure and eye formation were characteristic of cheeses ripening at 8 and 12°C. On the other hand, the cheeses ripening at 15°C were found to have unfavourable changes in taste, smell and consistency.

Higher activity of bacterial enzymes in the second stage of the ripening process was connected with the presence of higher amounts of available peptides which were formed in the result of the rennet action. Low temperature of ripening inhibits the activity of bacterial enzymes, what the results obtained in the present work prove (Fig. 3B and 4). Irrespective of the increases in the content of amino acid nitrogen, a part of free amino acids could be degraded by bacterial enzymes to aliphatic alcohols, aldehydes and ketones and to volatile sulfur compounds [10]. Because of the presence of phosphopyridoxal in cheeses, free amino acids might be decarboxylated with the formation of amino compounds [12]. The above-mentioned compounds, extremely important in the pool of flavour substances, are formed upon so-called secondary microflora (*Lactobacillus, Propionibacterium, Enterococcus)* and technologically harmful microflora (*Clostridia, E. coli, Micrococcus*) present in a cheese.

The organoleptic evaluation proved the relationship between flavour of Dutch-type cheeses and free amino acid content. Lower temperatures of ripening (8 and 12°C) ensure proper activities of coagulating enzyme, proteinases and bacterial peptidases what guarantees that typical organoleptic properties of cheese are obtained. On the other hand, higher temperatures of ripening intensify the enzymatic processes, but make possible the growth of harmful microflora responsible for free amino acid degradation, what negatively determines the flavour of a cheese.

Concluding, it should be stated that the use of higher ripening temperatures intensifies both the chymosin activity and the activity of proteinases and bacterial peptidases. However, because of simultaneous activation of non-starter microflora, mostly heat-resistant, this method for cheese ripening acceleration is quite risky and possible for use exclusively in the production of cheeses from milk of the highest microbiological quality.

CONCLUSIONS

On a basis of the studies performed and the results obtained we can draw the following conclusions:

1. The increase in pH is the greater the higher is temperature of ripening what proves intensification of the enzymatic changes in cheese.
2. An elevated temperature of ripening intensifies proteolysis and peptidolysis of cheese.
3. The taste and smell of Edam-type cheeses are dependent on the changes in the content of free amino acids, and the products of degradation of low molecular weight nitrogen compounds negatively modify the organoleptic properties of "Hetman' cheese ripening at 15°C.
4. An elevated temperature is an efficient but, at the same time, risky method for shortening the ripening time of cheese demanding milk of the highest quality.
5. The method with TNBS for determining the content of free amino groups may be used instead of labour- and time consuming analysis of the content of soluble nitrogen compounds determined by the Kjeldahl's method.

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Marian Kujawski, Grażyna Cichosz, Ewa Podhajna, Barbara Sańko
Chair for Food Safety Development
University of Warmia and Mazury in Olsztyn
Heweliusza 1, 10-724 Olsztyn
phl/fax: (+48 89) 523 34 43
e-mail: kujawski@uwm.edu.pl

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