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THE EFFECT OF FREEZING AND PRESSURE OF 50 MPA AND 100 MPA ON THE PROTEOLYTIC ACTIVITY OF ENZYMES IN EDAM CHEESE

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K e y w o r d s: cheese, frozen, high pressure, ripening, proteolytic activity.

A b s t r a c t

This study examined the effects of pressure treatment at 50 MPa / 0.5 h and 100 MPa / 0.5 h, at 18 ± 2°C, on the proteolytic activity of edam cheese subject to freezing after week 1, 4, 6 and 8 of ripening. The aim of the study was to test the utility of high pressures in cheese ripening under model conditions.

Sensory and chemical analysis was performed on Edam cheeses after varied periods of ripening. The chemical analysis involved determination of active acidity of cheese, water and salt content and the content of different fractions of nitrogen compounds. Subsequently, the cheeses were frozen in a freezer (freezing rate 1 cm h^{-1}) or in an alcohol bath (freezing rate 0.1 cm h^{-1}), in order to speed up the lysis of starter cells. Extracts of frozen cheeses were subjected to high pressure treatment. Proteolytic activity of enzymes was determined by the Westhoff method in frozen, frozen and pressurised and control cheese samples after 1 and 2 weeks of incubation at 30°C.

The results of the sensory and chemical analysis displayed the normal course of the ripening process. The proteolytic activity of enzymes increased as the cheese ripened. No significant difference was found between the proteolytic activity in extracts from control cheese and in those from frozen and pressurised cheese. Freezing the cheese after 8 weeks of ripening lowered the activity of proteolytic enzymes. The proteolytic activity of enzymes in extracts from frozen cheeses after 8 weeks of ripening, pressurised at 50 MPa / 0.5 h was higher than in those subjected to the pressure of 100 MPa / 0.5 h. Pressure treatment of extracts from frozen cheeses at 50 MPa or 100 MPa did not result in expected acceleration of proteolysis.

WPŁYW MROŻENIA ORAZ CIŚNIENIA 50 MPA I 100 MPA NA AKTYWNOŚĆ PROTEOLITYCZNĄ ENZYMÓW W SERZE EDAMSKIM

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S ł o w a k l u c z o w e: ser, mrożenie, wysokie ciśnienie, aktywność proteolityczna.

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A b s t r a k t

Badano wpływ ciśnienia 50 MPa / 0,5 h i 100 MPa / 0,5 h, w temperaturze 18 ± 2°C, na aktywność proteolityczną enzymów sera edamskiego, poddanego mrożeniu po 1, 4, 6 i 8 tygodniach dojrzewania. Celem było sprawdzenie, w warunkach modelowych, możliwości zastosowania wysokich ciśnień do przyspieszenia procesu dojrzewania serów.

Sery edamskie o zróżnicowanym stopniu dojrzałości poddawano ocenie sensorycznej i analizie chemicznej. Analiza chemiczna obejmowała oznaczenie kwasowości czynnej sera, zawartości wody, soli i poszczególnych frakcji związków azotowych. Następnie sery mrożono w zamrażalniku lodówki (prędkość zamrażania 1cm h^{-1}) lub w łaźni alkoholowej (prędkość zamrażania 0,1 cm h^{-1}), w celu przyspieszenia lizy komórek starterowych. Ekstrakty z serów mrożonych poddawano działaniu wysokiego ciśnienia. W ekstraktach z serów mrożonych, mrożonych i poddanych ciśnieniu oraz kontrolnych (niemrożonych) oznaczano aktywność proteolityczną enzymów met. Westhoffa po 1 i 2 tygodniach inkubacji w temp. 30°C.

Wyniki oceny sensorycznej i analizy chemicznej serów wykazały prawidłowy przebieg procesu dojrzewania. Aktywność enzymów proteolitycznych wzrastała w miarę dojrzewania sera. Nie stwierdzono statystycznie istotnych różnic między aktywnością proteolityczną w ekstraktach z sera kontrolnego i ekstraktach z serów mrożonych poddanych wysokiemu ciśnieniu. Mrożenie sera po 8 tygodniach dojrzewania spowodowało obniżenie aktywności enzymów proteolitycznych. Aktywność proteolityczna enzymów w ekstraktach z serów mrożonych po 8 tygodniach dojrzewania, poddanych ciśnieniu 50 MPa / 0,5 h była wyższa niż poddanych ciśnieniu 100 MPa / 0,5 h. Poddanie ekstraktów z mrożonych serów ciśnieniu 50 MPa lub 100 MPa nie spowodowało oczekiwanego przyspieszenia procesu proteolizy.

Introduction

High-pressure technology is a non-thermal method of food preserving and processing, consisting in applying, at room temperature, pressure of 100–1 000 MPa. This method has aroused much interest, since the application of high pressure makes it possible to adjust the speed of enzymatic reactions in food and to eliminate undesirable microorganisms. The application of high pressure also facilitates the creation of new products with much more favourable organoleptic characteristics and nutritive value. High pressure technology has already been applied in the production of jams and fruit jellies, juices, desserts, yogurts and sauces. High pressure is also used for fish preparations, seafood, as well as meat products (JANKOWSKA 2001).

Abundant research has been conducted on the possibility of applying high pressure technology in cheese making (CAPELLAS et al., 2001, KOŁAKOWSKI et al. 1998, MESSENS et al. 1999, 2000, 2001, SALDO et al. 2002, TRUJILLO et al. 2002). YOKOYAMA et al. (1993) found that exposure of cheddar cheese to pressure of 50 MPa at 25°C reduced its ripening time from 6 months to 3 days. In addition, MESSENS et al. (2000, 2001) treating smear-ripened cheese and paillardin cheese at pressure of 50 MPa / 8 h, found a more intense course of the proteolytic process in comparison with traditionally ripening cheese, particularly in the external part of cheese. On the other hand, they did not find

any significant changes in the course of the ripening process in gouda cheese subjected to pressure of 50 MPa / 3 days, in comparison to the control cheese (MESSENS et al. 1999). These results are consistent with the results of research conducted by KOŁAKOWSKI et al. (1998), who applied pressure in the range of 50–500 MPa / 4 h. Similarly, SHEEHAN et al. (2005) did not prove any influence of pressure treatment at 400 MPa / 5 min at 21°C on the course of proteolysis and rheological properties of mozzarella cheese. According to O'REILLY et al. (2002, 2003), the course of the ripening process in cheese subjected to pressure depends on the type of cheese, value and duration of the pressure applied, as well as the temperature. On the other hand, JUAN et al. (2008), while examining sheep's milk cheese pressure-treated at 300 MPa / 10 min, observed that the course of proteolysis depended on the day of cheese ripening process on which high pressure was applied. The application of high pressure at earlier stages of ripening resulted in higher changes in the course of the cheese ripening process.

In view of the possibility of using high pressure technology to accelerate the process of cheese ripening, the aim of the research undertaken was to determine the effect of freezing and subsequent pressurizing at 50 MPa and 100 MPa on changes in the activity of proteolytic enzymes of edam cheese.

Materials and Methods

Edam cheese, of varied ripening time – 1, 4, 6, 8 weeks – was divided into cuboid pieces with dimensions of 5 cm x 10 cm x 12 cm, packaged into freezing bags and frozen at -35°C by two methods. Slow freezing was applied for 24 hours in a freezer (freezing rate 1 cm h^{-1}) and fast freezing in an alcohol bath – for 2.5 h (freezing rate 0.1 cm h^{-1}). Cheese samples were subsequently defrosted at 18 ± 2°C. Water extracts obtained from cheese were pressurized at 50 MPa / 0.5 h or 100 MPa / 0.5 h using high-pressure apparatus produced by Unipress Equipment.

Cheese was subjected to chemical analysis and sensory evaluation. Chemical analysis included determination of water content, active acidity of cheese, salt content, total content of nitrogen compounds (KREŁOWSKA-KUŁAS 1993), content of nitrogen compounds soluble in water extract of cheese, nitrogen compounds soluble at 4.6 pH (SODE-MOGENSEN 1948), amino acid nitrogen compounds (STADHOUDERS 1960) and non-protein nitrogen compounds (SCHOBER et al. 1961).

A sensory evaluation of the cheeses was carried out by the scoring method by a team of 6 people trained in making sensory evaluations of edam cheese by a competent person (internal training). The following attributes were taken

into account: taste, flavour, texture, colour and eye formation. A 5-point scale was used.

Proteolytic activity of enzymes (2% TCA and 12% TCA) was determined after 1 and 2 weeks of incubation at 30°C in aqueous extracts from frozen cheese, in pressurised extracts from frozen cheese and in control extracts (from non-frozen cheese), according to a modified method of WESTHOFF et al. (1993). An activity unit (a.u.) was assumed to be the amount of the enzyme which under established conditions of reaction resulted in the growth in absorbance by 0.01.

In order to determine the effect of ripening time and the type of extract on proteolytic activity of enzymes, the results were analysed statistically with the use of two-factorial analysis of variance without repetitions, at the level of significance of $p = 0.05$. Moreover, the results were analysed statistically after 8 weeks of ripening with the use of single-factorial analysis of variance at the level of significance of $p = 0.05$. The mean values and standard deviations were also calculated. All the analyses were made in 3 repetitions, except for determinations of nitrogen compounds, which were made in 2 repetitions. The statistical analysis was performed with the use of Microsoft Excel 2003 for Windows 2003.

Results and Discussion

On the basis of the chemical analysis performed, it was found that water content in cheese amounted to about 41%, which was consistent with the standard. The acidity of cheese after week 1 of ripening was 5.18 pH; it decreased in the course of the ripening process to reach the value of 5.42 pH after 8 weeks (Table 1).

Table 1

Chemical analysis of cheese

Time of ripening cheese (weeks)	Moisture (%)	Acidity (pH)	NaCl (%)	Total nitrogen compounds (%)
1	41.38 ± 0.12	5.18 ± 0.01	1.21 ± 0.02	4.64 ± 0.03
4	40.94 ± 0.40	5.30 ± 0.01	1.18 ± 0.01	4.75 ± 0.04
6	41.02 ± 0.31	5.35 ± 0.01	1.14 ± 0.01	4.54 ± 0.04
8	41.48 ± 0.18	5.42 ± 0.01	1.16 ± 0.01	4.48 ± 0.06

A low content of NaCl in cheese, amounting to about 1.2 %, was very favourable for health reasons, since a high consumption of NaCl is one of most frequently listed factors for hypertension.

Total nitrogen content in cheese was at the level of about 4.6% (Table 1). During the ripening process, a growth of the nitrogen compound forms was observed, namely of nitrogen compounds soluble in water extract of cheese, nitrogen compounds soluble at 4.6 pH, amino acid nitrogen compounds and non-protein nitrogen compounds, which demonstrated the proper course of the cheese proteolysis process (Table 2).

Table 2

Analysis of nitrogen compounds in cheese

Time of ripening cheese (weeks)	Non-protein nitrogen compounds (% $N_{og.}$)	Nitrogen compounds soluble at 4,6 pH (% $N_{og.}$)	Amino acid nitrogen compounds (% $N_{og.}$)	Nitrogen compounds in cheese extract (% $N_{og.}$)
1	4.09 ± 0.11	6.47 ± 0.14	2.95 ± 0.42	8.19 ± 0.58
4	5.02 ± 0.58	10.32 ± 1.46	4.31 ± 0.41	16.42 ± 0.65
6	8.21 ± 0.49	13.22 ± 0.28	7.05 ± 0.57	24.23 ± 1.09
8	11.97 ± 0.35	14.51 ± 0.72	11.16 ± 0.72	34.42 ± 0.38

The sensory evaluation of cheese indicated that it was characterized by a typical, clear taste and smell, elastic consistency and proper holes (Table 3).

Table 3

Sensory evaluation of cheeses

Quality factors	Severity coefficient (a)	Time of ripening cheese (weeks)					
		4		6		8	
		mean evaluation score (b)	product ($a \cdot b$)	mean evaluation score (b)	product ($a \cdot b$)	mean evaluation score (b)	product ($a \cdot b$)
Flavour	0.25	3.67 ± 0.52	0.92	4.33 ± 0.52	1.08	4.67 ± 0.52	1.17
Taste	0.30	3.83 ± 0.75	1.15	4.33 ± 0.52	1.30	4.83 ± 0.41	1.45
Texture	0.15	3.33 ± 0.52	0.50	4.17 ± 0.75	0.63	4.50 ± 0.53	0.68
Colour	0.10	4.17 ± 0.75	0.42	4.67 ± 0.52	0.47	4.67 ± 0.52	0.47
Eye formation	0.20	3.33 ± 0.81	0.67	4.67 ± 0.52	0.93	4.67 ± 0.52	0.93
Sum total	1.00	–	3.66	–	4.14	–	4.70

The statistical analysis with two-factorial variance without repetitions, at the significance level of $p = 0.05$, revealed a significant effect of cheese ripening time on the proteolytic activity of enzymes in all the extracts under examination, except for the cheese frozen in an alcohol bath, in which no statistically significant differences were found between the proteolytic activity of enzymes after 6 and 8 weeks of ripening (Table. 4–11).

Table 4
Proteolytic activity of enzymes in extract from the cheese frozen in a freezer (2%TCA). Time of incubation – 1 week

Extract type	Time of ripening cheese (weeks)			
	1	4	6	8
	proteolytic activity (a.u./g cheese)			
k	$19.58^{ac} \pm 0.86$	$28.85^{df} \pm 0.23$	$41.56^{gi} \pm 0.32$	$42.80^{jl} \pm 0.46$
m	$20.61^{ab} \pm 0.26$	$32.84^{de} \pm 0.28$	$39.89^{gh} \pm 0.22$	$37.62^{ik} \pm 0.62$
m + 50 MPa	$23.02^{e} \pm 0.60$	$33.66^{f} \pm 0.21$	$39.95^{i} \pm 0.62$	$38.06^{l} \pm 0.41$
m + 100 MPa	$22.86^{ac} \pm 0.31$	$31.58^{df} \pm 0.29$	$46.45^{gi} \pm 0.35$	$36.93^{jl} \pm 0.41$

k – control extract from unfrozen and unpressurised cheese
m – extracts from frozen cheeses
m + 50 MPa – extracts from frozen cheeses, pressurised at 50 MPa
m + 100 MPa – extracts from frozen cheeses, pressurised at 100 MPa
The same letters denote absence of statistical differences at $p = 0.05$

Table 5
Proteolytic activity of enzymes in extract from the cheese frozen in a freezer (2%TCA). Time of incubation – 2 weeks

Extract type	Time of ripening cheese (weeks)			
	1	4	6	8
	proteolytic activity (a.u./g cheese)			
k	$25.62^{a} \pm 0.38$	$42.98^{b} \pm 0.52$	$78.04^{c} \pm 0.52$	$86.54^{d} \pm 0.12$
m	$26.61^{a} \pm 0.12$	$47.17^{b} \pm 0.51$	$73.15^{c} \pm 0.37$	$72.88^{d} \pm 1.02$
m + 50 MPa	$26.03^{a} \pm 0.44$	$46.57^{b} \pm 0.30$	$69.00^{c} \pm 0.68$	$78.73^{d} \pm 0.17$
m + 100 MPa	$25.98^{a} \pm 0.44$	$47.99^{b} \pm 0.20$	$72.25^{c} \pm 0.28$	$74.92^{d} \pm 0.10$

Explanations as in Table 4
The same letters denote absence of statistical differences at $p = 0.05$

Table 6
Proteolytic activity of enzymes in extract from the cheese frozen in an alcohol bath (2% TCA). Time of incubation – 1 week

Extract type	Time of ripening cheese (weeks)			
	1	4	6	8
	proteolytic activity (a.u./g cheese)			
k	$19.58^{ad} \pm 0.86$	$28.85^{eh} \pm 0.23$	$41.56^{il} \pm 0.32$	$42.80^{lo} \pm 0.46$
m	$19.57^{ac} \pm 0.71$	$33.10^{eg} \pm 0.65$	$45.11^{ik} \pm 0.66$	$39.06^{lm} \pm 0.02$
m + 50 MPa	$20.85^{bc} \pm 0.24$	$31.82^{gf} \pm 0.11$	$46.65^{jk} \pm 0.77$	$44.68^{mo} \pm 0.30$
m + 100 MPa	$19.71^{bd} \pm 0.30$	$33.48^{fh} \pm 0.45$	$43.31^{jk} \pm 0.44$	$40.25^{mo} \pm 0.71$

Explanations as in Table 4
The same letters denote absence of statistical differences at $p = 0.05$

Table 7
Proteolytic activity of enzymes in extract from the cheese frozen in an alcohol bath (2% TCA). Time of incubation – 2 weeks

Extract type	Time of ripening cheese (weeks)			
	1	4	6	8
	proteolytic activity (a.u./g cheese)			
k	$25.62^{ab} \pm 0.38$	$42.98^{cd} \pm 0.52$	$78.04^{ef} \pm 0.52$	$86.54^{gh} \pm 0.12$
m	$25.25^{ab} \pm 0.05$	$43.08^{cd} \pm 0.83$	$74.39^{ef} \pm 0.29$	$79.50^{gh} \pm 0.49$
m + 50 MPa	$29.22^{a} \pm 0.43$	$50.94^{c} \pm 0.38$	$73.23^{e} \pm 0.69$	$81.54^{g} \pm 0.04$
m + 100 MPa	$29.05^{b} \pm 0.39$	$43.45^{d} \pm 0.28$	$73.26^{f} \pm 0.05$	$72.76^{h} \pm 0.63$

Explanations as in Table 4
The same letters denote absence of statistical differences at $p = 0.05$

Table 8
Proteolytic activity of enzymes in extract from the cheese frozen in a freezer (12%TCA). Time of incubation – 1 week

Extract type	Time of ripening cheese (weeks)			
	1	4	6	8
	proteolytic activity (a.u./g cheese)			
k	$21.70^{ab} \pm 0.28$	$26.98^{cd} \pm 0.42$	$38.51^{ef} \pm 0.49$	$42.50^{gh} \pm 0.46$
m	$23.81^{ab} \pm 0.26$	$29.22^{cd} \pm 0.06$	$37.09^{ef} \pm 0.49$	$32.35^{gi} \pm 0.15$
m + 50 MPa	$22.37^{a} \pm 0.42$	$29.79^{c} \pm 0.65$	$41.37^{e} \pm 0.35$	$38.15^{g} \pm 0.07$
m + 100 MPa	$22.04^{b} \pm 0.17$	$27.38^{d} \pm 0.35$	$38.27^{f} \pm 0.14$	$36.81^{h} \pm 0.37$

Explanations as in Table 4
The same letters denote absence of statistical differences at $p = 0.05$

Table 9
Proteolytic activity of enzymes in extract from the cheese frozen in a freezer (12%TCA). Time of incubation – 2 weeks

Extract type	Time of ripening cheese (weeks)			
	1	4	6	8
	proteolytic activity (a.u./g cheese)			
k	$24.96^{a} \pm 0.83$	$36.81^{b} \pm 0.29$	$66.27^{c} \pm 0.76$	$80.99^{d} \pm 0.45$
m	$26.79^{a} \pm 0.46$	$34.73^{b} \pm 0.57$	$69.67^{c} \pm 0.15$	$70.38^{d} \pm 0.30$
m + 50 MPa	$29.68^{a} \pm 1.12$	$40.62^{b} \pm 1.22$	$65.48^{c} \pm 0.24$	$73.28^{d} \pm 0.30$
m + 100 MPa	$29.97^{a} \pm 0.38$	$37.03^{b} \pm 0.44$	$68.28^{c} \pm 0.84$	$70.39^{d} \pm 0.57$

Explanations as in Table 4
The same letters denote absence of statistical differences at $p = 0.05$

Table 10
Proteolytic activity of enzymes in extract from the cheese frozen in an alcohol bath (12% TCA). Time of incubation – 1 week

Extract type	Time of ripening cheese (weeks)			
	1	4	6	8
	proteolytic activity (a.u./g cheese)			
k	$21.70^{ab} \pm 0.28$	$26.98^{de} \pm 0.42$	$38.51^{gh} \pm 0.49$	$42.50^{gh} \pm 0.46$
m	$21.60^{ac} \pm 0.26$	$31.10^{df} \pm 0.64$	$39.22^{gi} \pm 0.22$	$38.23^{gi} \pm 0.09$
m + 50 MPa	$20.08^{ab} \pm 0.51$	$34.37^{de} \pm 0.43$	$44.23^{gh} \pm 0.47$	$38.58^{gh} \pm 0.29$
m + 100 MPa	$19.76^{b} \pm 0.51$	$27.81^{e} \pm 0.56$	$37.86^{h} \pm 0.12$	$32.99^{h} \pm 0.62$

Explanations as in Table 4
The same letters denote absence of statistical differences at $p = 0.05$

Table 11
Proteolytic activity of enzymes in extract from the cheese frozen in an alcohol bath (12% TCA). Time of incubation – 2 weeks

Extract type	Time of ripening cheese (weeks)			
	1	4	6	8
	proteolytic activity (a.u./g cheese)			
k	$24.96^{a} \pm 0.83$	$36.81^{b} \pm 0.29$	$66.27^{c} \pm 0.76$	$80.99^{c} \pm 0.45$
m	$22.61^{a} \pm 0.46$	$37.91^{b} \pm 0.27$	$73.38^{c} \pm 0.60$	$73.20^{c} \pm 1.06$
m ± 50 MPa	$23.13^{a} \pm 0.43$	$37.39^{b} \pm 0.68$	$72.14^{c} \pm 0.73$	$72.27^{c} \pm 0.39$
m ± 100 MPa	$23.03^{a} \pm 0.63$	$31.43^{b} \pm 0.80$	$69.69^{c} \pm 1.07$	$71.50^{c} \pm 0.63$

Explanations as in Table 4
The same letters denote absence of statistical differences at $p = 0.05$

An analysis of nitrogen compounds soluble in 2% TCA, and in 12% TCA revealed that the proteolytic activity of enzymes in aqueous cheese extracts increased during the 6-week period of cheese ripening (Table 4–11).

It was also observed that in cheese ripening for 6 and 8 weeks, the increase of proteolytic activity of enzymes determined after 2 weeks of incubation was more rapid.

For example, in week 2 of incubation, the proteolytic activity of enzymes in the extract from control cheese after 4 weeks of ripening increased by 14.13 a.u./g of cheese, after 6 week of ripening – by 36.48 a.u./g of cheese, and after 8 weeks of ripening – by 43.74 a.u./g of cheese (Table 4, 5).

In the cheese ripening process, due to the lack of an easily available source of sugar-derived carbon, bacteria of cheese starters are subjected to autolysis and release intracellular peptidases. The presence of aminopeptidases, as well as di-, tri- and sometimes carbopeptidases were established in cells of *Lactoccoccus* strains (CICHOSZ 2004). The aim of freezing cheese in the experiment

was to facilitate lysis of cheese starter bacteria, which resulted in higher susceptibility of released enzymes to high pressure.

However, the two-factorial analysis of variance without repetitions at the significance level of $p = 0.05$ did not reveal any significant differences between the proteolytic activity of enzymes in extracts from the control cheese and extracts from pressurised frozen cheeses (Table 4–11). A statistically significant difference was only found between the proteolytic activity in the extract from control cheese and the extract from frozen cheese pressurised at 50 MPa, after 1 week of incubation (Table 6).

Moreover, in order to examine the effect of the type of extract on the proteolytic activity of enzymes after 8 weeks of cheese ripening, a single-factorial analysis of variance was carried out. It revealed statistically significant differences at the level of $p = 0.05$ between the proteolytic activity of enzymes in frozen cheese extracts after 8 weeks of ripening, pressurised at 50 MPa, and the proteolytic activity of enzymes in extracts from the same cheese pressurised at 100 MPa. The proteolytic activity was higher in extracts pressurised at 50 MPa than in those pressurised at 100 MPa. The tendency was observed in the cheeses frozen in both an alcohol bath and a freezer (Table 4–11). No statistically significant difference at the significance level of $p = 0.05$ was found between the proteolytic activity of enzymes determined after 2 weeks of incubation, in extracts from cheeses frozen in an alcohol bath, pressurised at 50 MPa and the proteolytic activity of enzymes pressurised at 100 MPa (Table 11). These observations are consistent with the research conducted by KOŁAKOWSKI et al. (1998), who found a higher proteolytic activity of enzymes in camembert cheese subjected to a pressure of 50 MPa in comparison with cheese pressurized at 100 MPa. Additionally, O'RELLY et al. (2002, 2003) established that a more intensive course of the proteolysis process in cheddar cheese subjected to the pressure of 50 MPa / 72 h / 25°C could result from improved proteolytic activity of enzymes caused by high pressure.

Statistically significant differences at the significance level of $p = 0.05$ were also found between the proteolytic activity of enzymes in extract from 8-week control cheese and the proteolytic activity of enzymes in extracts from frozen cheeses after 8 weeks of ripening and subsequently pressurised (Table 4–11).

Nevertheless, the activity of proteolytic enzymes in extracts from frozen cheeses subjected afterwards to high pressure was lower than the activity of enzymes in the extract from the control cheese, which suggests an unfavourable effect of low temperature on the activity of enzymes in cheese after 8 weeks of ripening. The freezing applied in the study probably "damaged" the proteolytic enzymes. An analysis of compounds soluble in 2% TCA and 12% TCA, after 8 weeks of cheese ripening also showed significant differences at the level of $p = 0.05$ between the proteolytic activity of enzymes in extracts from control cheeses and the enzyme activity in extracts from frozen cheeses, with the

proteolytic enzyme activity in frozen cheese extract depending on the cheese freezing method applied. Smaller changes in the enzyme activity were caused by rapid freezing. The proteolytic activity of enzymes in cheeses subjected to slow freezing in a freezer, determined after 1 week of incubation, decreased by 12.1%, and that determined after 2 weeks of incubation – by 15.78%, whereas the proteolytic activity of enzymes in cheeses subjected to fast freezing in an alcohol bath decreased by 8.74% and 8.13%, respectively (Table 4–7).

Summing up, exposing cheese to freezing in order to induce lysis of starter culture cells and afterwards applying a high pressure of 50 MPa / 30 min and 100 MPa / 30 min did not result in the expected growth of activity of proteolytic enzymes.

Numerous studies indicate that high pressure technology provides great prospects for improving cheese production. However, the development of optimal parameters requires further research.

Conclusions

1. An increase in the activity of proteolytic enzymes was noted during the cheese ripening process.

2. Pressure treatment of extracts from frozen cheese at 50 MPa / 0.5 h and 100 MPa / 0.5 h had no significant effect on the growth activity of proteolytic enzymes.

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