METHOD FOR CHARACTERIZING THE EXTENT OF CHEESE RIPENING

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ÖSSZEFOGLALÓ

Az érzékszervi minősítés mellett kipróbáltuk a sajtérés nyomonkövetésére a vízoldható frakciók méretkizárásos kromatográfiáját (HPSEC) Pannónia és Trappista mintáknál. A λ = 214 nm-en és λ = 230 nm-en mért kromatogramok alkalmasak a Trappista és Pannónia sajtok érésének nyomonkövetésére. A módszer segítséget nyújthat elsősorban hosszabb érlelésű sajtoknál az érési körülmények optimalizálásában, új technológiák kidolgozásánál valamint a termékminősítésben.

1. INTRODUCTION AND OBJECT

During cheese ripening primary and secondary changes can be distinguished. They together result in accumulation of lactic, fatty and free amino acids. Secondary changes are catalysed specifically by enzymes of micro-organism origin, that result in the formation of end products typical of each particular cheese variety. These chemical and concomitant physical changes are related mainly to the progressive hydrolysis of protein, to peptides and gradual accumulation of amino acids.
The "primary proteolysis" effect a change in fraction of casein and the "secondary" one can be typified by products which are present in the water-soluble fraction.
For the investigations samples of Pannonia and Trappist cheese of different age (ripe, green and overripe) were applied.
The suitability of size-exclusion chromatography (HPSEC) was investigated for characterising the degree of cheese ripening.

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2. MATERIALS

Cheese samples:	*Pannonia*		*Trappista*
Origin (Milk factory):	*Zalaegerszeg*		*Szekszárd*
Ripe samples	*10 pieces*		*10 pieces*
Samples after various ripening periods (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks)	*10 pieces*		*10 pieces*
Condition of ripening	*Main r.: 22-24 °C, 5 weeks, 65% r.v.p.*	*Post r.: 10 °C, 5 weeks, 75% r.v.p.*	*16-18 °C, 10 weeks, 70% r.v.p.*

The cheese samples for HPLC stored -20 °C temperature before preparation of water-soluble fraction.

Methods of Investigations

1. Size-Exclusion HPLC Separation

** Preparation of water-soluble fraction of cheese (Kaminogawa et.al 1986)*

Grating (cheese grater)

⇓

Dispersing in 0.05M sodium citrate buffer (pH 4.0) to give a ratio of 1:2 (cheese/buffer, w/v)

⇓

After mixing (Moulinex mixer, 5 min.), warming to 40°C and holding at this temperature for 1 hr

⇓

Removing the fat and unextractable protein (centrifugation, 0°C, 4000 rpm, 30 min)

⇓

Supernatant pH adjusted (pH=4.6)

⇓

Removing insoluble protein (centrifugation, 4°C, 7000 rpm, 30 min)

⇓

Supernatant freeze-drying

✱ *Separation of water-soluble fraction of cheese*

Size-exclusion chromatography (HPSEC) was performed with a BECKMAN SPHEROGEL TSK-2000 SW column (10 μm, 7,5 mm x 30 cm) and a HEWLETT PACKARD 1090 system according to the method of Vijayalaksmi et al 1986.

Parameters of separation:

• *flow rate*	*1.0 ml/min*
• *eluent:*	*0.05M phosphate buffer pH 5.0*
	+35% methanol
	+ 0,1% trifluoracetic acid
• *oven temperature*	*25 ℃*
• *max. pressure*	*40 - 60 bars*
• *injection volume*	*25 μl*
• *detection (DAD, λ)*	*214, 230, 254, 280 (nm)*

Molecular Weight Markers: M.W. Range 2512-16949 (Pharmacia) [Stock sol. 1 mg/ml]
Stock sol. from freeze - dried samples 20 mg/ml.

2. Organoleptic tests
Hungarian standards (MSZ 12280-87, MSZ 12277-87).

Methods of evaluation

1. Quantitative analysis of HPLC

The peak areas of the chromatograms were determined. Data of the ripe samples were analysed by one-way analysis of variance. For a chosen peak-with well-known molecular weight-the correlation of the area and time of ripening was analysed by regression analysis. The ripening time was estimated from the peak areas by stepwise variable selection.

2. Organoleptic tests

The organoleptic tests were evaluated by means of a special software.

3. RESULTS AND DISCUSSION

The ripped samples of both cheeses were acceptable according to the organoleptic tests. The total scores of the organoleptic test changed positively correlated with time of ripening.

I. Size-Exclusion HPLC Separation

The chromatographic profile of the water-soluble peptide fractions is characteristic at each wavelength (Figs 1.,2.,3.).
It is very difficult to identify the peptide fragments on the chromatogram at λ=280 nm, because of the number of peaks, and their variability.
Chromatograms at λ=230 nm and λ=214 nm are simpler because some peaks are merged.
We used the peak purity program of the HPLC system to show the merged peaks.

From the ratios of peak areas we may conclude to the amino acid compound of fragments, because the aromatic compounds have a maximum absorption at λ=280 nm and the peptide bonds at λ=214 nm.

Comparing the spectra of ripe Trappist samples we reached the following conclusions:

- *the first three peptide fractions (13300-4200 D) have the same ratio of aromatic and non-aromatic amino acids*
- *the 2600-1600 D fractions are merged at λ=230 nm and their aromatic compounds are smaller than those at the first three peaks.*
- *in the smallest fraction (1100 D) the amount of the aromatic compounds is the highest.*

We searched those peptide fractions, the amount of which were positively correlated with the time of ripening.

All characteristic peaks of chromatograms - at λ=214 nm and λ=230 nm – of Trappist cheese were significantly correlated with ripening time.

As for the Pannonia cheese, only two smaller peptide fragments are correlated with ripening time (Table 1.).

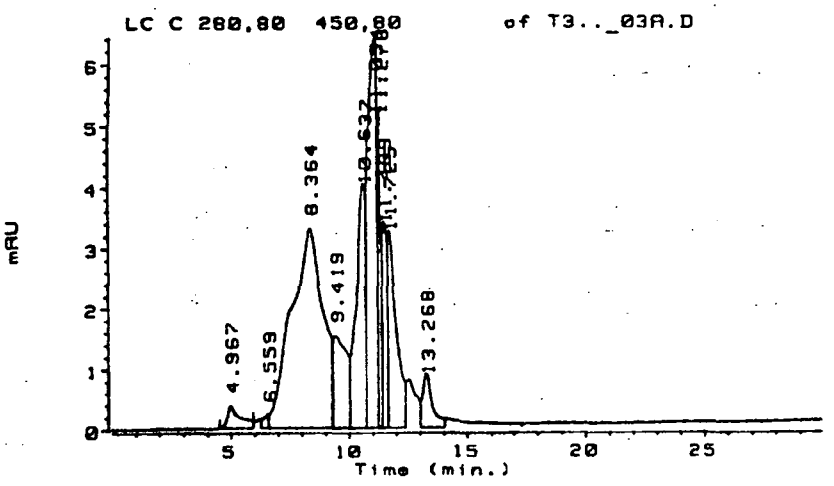

Fig. 1. Ripe Trappist sample (280 nm)

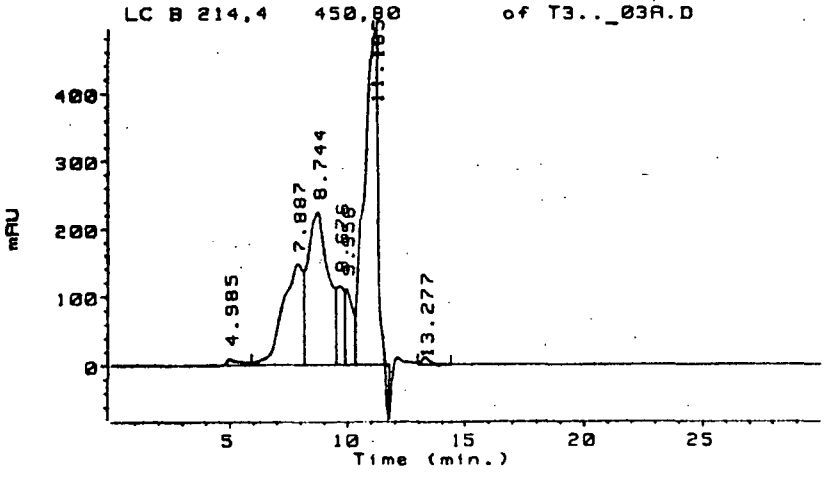

Fig. 2. Ripe Trappist sample (214 nm)

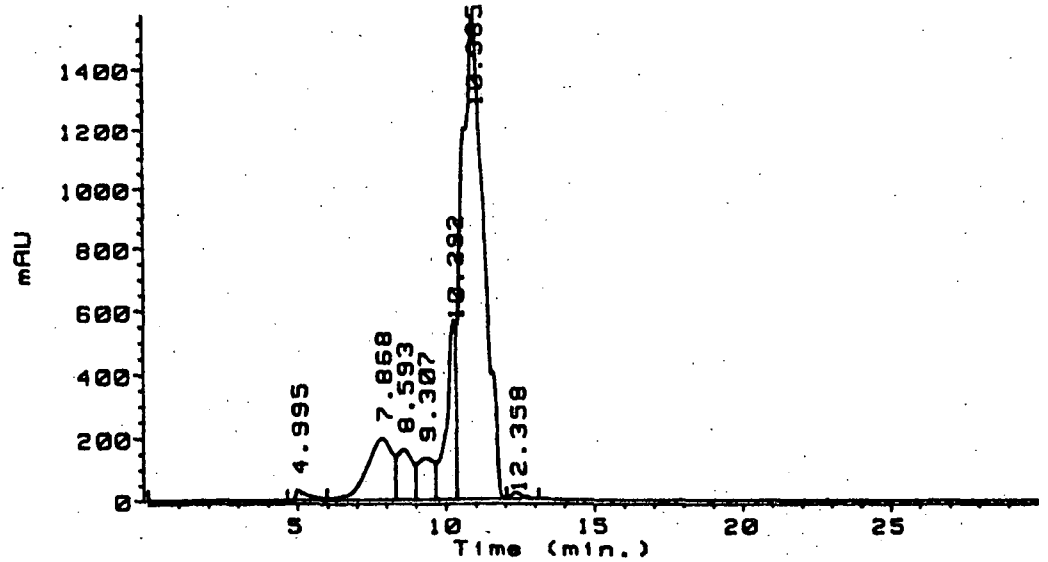

Fig. 3. Ripe Pannonia sample (214 nm)

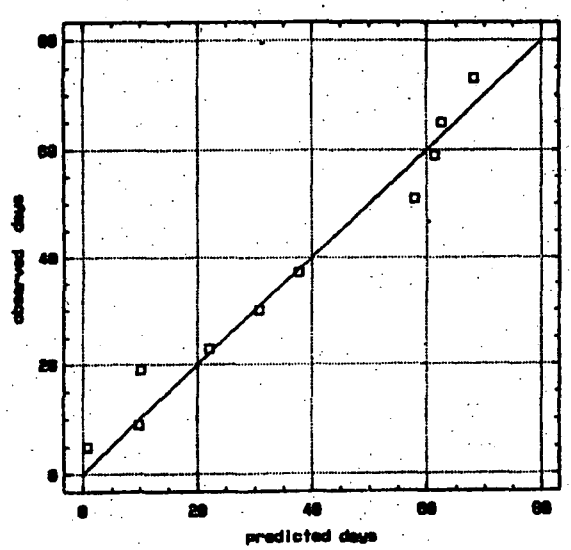

Fig. 4. Predicted and ovserved value of ripening days at Pannonia cheese

Table 1: Result of regression analysis

	Trappist			
M.W. (D)	*Y=aX + b* *Y= peak area* *X= ripening time*		*Standard Deviation*	*Correl. Coeff.*
	a	*b*		*(r)*
13300	*68.178*	*1397*	*1128.01*	*0.8213*
8400	*71.078*	*2753*	*441.884*	*0.9675*
4200	*41.816*	*336*	*253.494*	*0.9691*
2000	*32.81*	*8776*	*336.86*	*0.9183*
	Pannonia			
2000	*9921*	*730136*	*170732*	*0.8273*
1300	*-2057*	*1104*	*7760.31*	*0.9636*

For the regression analysis we used ten data.

We tried to calculate the ripening time from the peak areas by stepwise selection. The predicted and observed values with 95% intervals for Pannonia samples can seen be in Fig. 4.

The chromatograms at λ=230 nm and at λ=214 nm are suitable for the measurement of the ripeness of Trappist and Pannonia cheeses.
The Trappist is a semi-hard cheese, so the extent of ripening might be evaluated by measuring any characteristic peaks of the water-soluble fraction.
The Pannonia is a hard cheese, so the deepness of the ripening might be evaluated by measuring the peak of water-soluble fraction of M.W. 2000D

REFERENCES

Vijayalaksmi, M. A., Lemieux, L. and Amiot ,J.: (1986): J. Liq. Chromatogr. 9, 3559.

Kaminogawa, S., Yan, T.R., Azuma, N. and Yamauchi, K.(1986):: J. Food Science 51, 1253.