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REGULAR ARTICLE

DETERMINATION OF PROTEINS IN YOGHURT

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ABSTRACT

The aim of this work was to introduce method for detection of protein content in yoghurts. The analysis was focused on determination of major milk proteins which include caseins and lower amounts of whey proteins, in particular α-lactalbumin a β-lactoglobulin. 10 samples from the market were analyzed by means of reversed phase high-performance liquid chromatography. Liquid chromatograph Alliance 2695 with PDA detector 2996 was used. The separation was performed on C18 column X Bridge TM, 150 x 3.0 mm, 3.5 μm. Mobile phase contained water, acetonitrile and trifluoroacetic acid. Average value of determined proteins were: α_S-CN 47.1 ± 2.6 %, β-CN 44.0 ± 3.8 %, κ-CN 9.0 ± 5.2 %, 96.7 ± 8.4 % LA and LG 14.7 ± 15.3%.

Keywords: RP-HPLC, protein, casein, α-lactalbumin, β-lactoglobulin

INTRODUCTION

Determination of individual caseins and whey proteins in dairy products has been a topical analytical issue for several recent years, since it provides important information which indicate the composition of milk and dairy products. The information acquired can then serve

for assessing dairy products adulteration and thus for customer protection **(Veloso *et al.*, 2002; Rodríguez *et al.*, 2010)**.

Yoghurt is made from both cow's milk and from milk goat's and sheep's. Yoghurts made from sheep's milk products are considered very good and popular for its unique organoleptic qualities (pleasant smell, taste and creamy texture). Sheep's milk is still considerably more expensive than cow's milk, leading some dairy farmers to the confusion of these two types of milk in order to increase their income. In order to ensure the authenticity of yoghurt used chemical, immunological and physico-chemical analytical methods. There are several publications on the detection of sheep's, goat's and cow's milk yoghurt, but still within the EU there are no regulations for the reference method for detection and evaluation of adulteration yoghurt **(Kaminarides and Koukiassa, 2002)**.

The aim of this work was to introduce method for detection of caseins and whey proteins content in yoghurts by means of the RP-HPLC method (reversed phase high-performance liquid chromatography).

MATERIAL AND METHODS

Samples

10 samples from the market were analyzed by the RP-HPLC method. The samples were cooled to 4-6 °C up to the analysis. Parallel analysis of the samples was performed.

Sample No. 1: Cream-line plain yoghurt, dairy works Kunín
Sample No. 2: Activia plain yoghurt
Sample No. 3: Zott plain yoghurt
Sample No. 4: Plain set yoghurt with probiotic culture BiFi, Hollandia
Sample No. 5: South-Bohemian plain yoghurt, Agro-la
Sample No. 6: Greek yoghurt
Sample No. 7: Plain yoghurt Klasik
Sample No. 8: Plain yoghurt Bio
Sample No. 9: Plain yoghurt Bifido
Sample No. 10: Plain yoghurt Light

Chemicals

Acetonitrile and dichloromethane (Merck, Germany), trifluoroacetic acid, 2-mercaptoethanol and standards α_s-casein, β-casein, κ-casein, α-lactalbumin and β-lactoglobulin (Sigma Aldrich, Germany), acetic acid, hydrochloric acid (Penta, Czech Republic), Tris (hydroxymethyl)-aminomethan (Bio-Rad Laboratories, Richmond, CA).

Sample preparation

The 5 g of yoghurt was weighed down, put into a tube and skimmed (4000 rot/10 min). The skimmed whey was separated from casein and frozen to -18 °C. Caseins were rinsed by an aqueous solution of dichloromethane (1:1) and lyophilised **(López–Fandiño *et al.*, 1993)** using a lyophiliser of ALPHA 1–4 LSC (Christ, Germany).
Before the very determination, lyophilised casein was diluted in a solution of Tris-HCl (pH 6.8) and 2-mercaptoethanol. Next, the casein and whey protein samples were filtered through a nylon membrane filter (0.22 μm) into vials. Finally yoghurt pH was measured.

Conditions of HPLC determination

For α–lactalbumin and β–lactoglobulin determination the following appliances were utilized: a liquid chromatograph of Alliance 2695 with PDA 2996 detector (Waters, USA) and X Bridge TM C18, 150 x 3.0 mm, 3.5 μm column (Waters, Ireland). Column temperature for casein detection was 45 °C and for whey proteins 40 °C. Mobile phase A included water/acetonitrile/trifluoroacetic acid (TFA) in ratio of 95/5/0.1 (v/v/v) and mobile phase B included water/acetonitrile/TFA in ratio of 5/95/0.1 (v/v/v). Gradient elution and mobile phase flow rate of 0.4 $ml.min^{-1}$ were applied. The detection was performed at 205 nm. Casein analysis lasted for 30 minutes and whey protein analysis lasted for 35 minutes. Injection volume of caseins was 5 μl and of whey proteins was 10 μl.

Evaluation

Collection and evaluation of data in relation to RP-HPLC were performed in the Empower2 software (Waters, USA). Basic statistical characteristics (mean, standard

deviation, relative standard deviation, maximum value, minimum value) were computed using Microsoft Excel.

Validation and optimization of RP-HPLC for whey proteins

Optimization of HPLC analysis was performed using standard solutions of α–lactalbumin and β–lactoglobulin. Calibration curve for the α-lactalbumin was designed over a concentration range of 0.404–1.571 $mg.ml^{-1}$ ($y = 0.5835x - 0.215$; $R^2 = 0.989$). Calibration curve for the β–lactoglobulin was designed over a concentration range of 0.406–1.133 $mg.ml^{-1}$ ($y = 0.3635x + 0.076$; $R^2 = 0.9752$). The method sensitivity was detected using calibration slope.

The repeatability of the procedure was determined from the results of multiple measurements per sample (n = 7) and was determined as RSD 2.53% for α-lactalbumin and RSD 2.40 % for β-lactoglobulin. The repeatability of retention times was determined from the results of multiple measurements per sample (n = 12) and was determined as RSD 1.02 % for α-lactalbumin and RSD 0.33% for β-lactoglobulin. The limit of detection (LOD) was determined as 3 S/N (signal/noise ratio) 0.0045 $mg.ml^{-1}$ for α-lactalbumin and β-lactoglobulin. The limit of quantification (determined as 10 S/N) was 0.015 $mg.ml^{-1}$ for α-lactalbumin and β-lactoglobulin. Evaluation was performed using external standard and quantification was performed using timed groups.

Validation and optimization of RP-HPLC for caseins

Optimization of HPLC analysis was performed using standard solutions of α_{S1}–casein, β–casein and κ–casein. Individual peaks were together into the group and processing as one peak. In the case of κ-casein, peaks were summarized in the time range 10.00 – 12.50 min, for α_{S1}-casein 13.20 – 14.40 min and for β-casein 14.10 – 16.00 min. The repeatability of the procedure was determined from the results of multiple measurements per sample (n = 6) and was determined as RSD 4.6 % for α_{S1}-casein, RSD 6.7 % for β-casein and RSD 0.7 % for κ-casein. The limit of detection (LOD) was determined as 3 S/N (signal/noise ratio) 0.0045 $mg.ml^{-1}$ and the limit of quantification (determined as 10 S/N) was 0.015 $mg.ml^{-1}$ for α_{S1}-casein, β-casein and κ-casein. Evaluation was performed using external standard and quantification was performed using timed groups.

Chromatograms of the analyses are shown in Fig. 1–4.

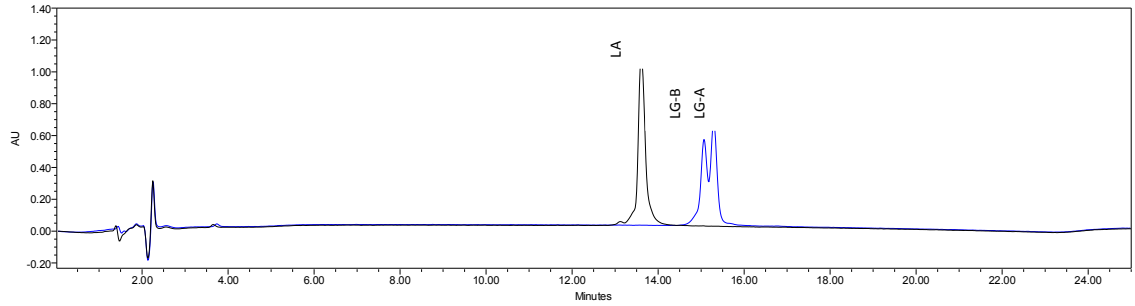

Figure 1 Chromatogram of α-lactalbumin and β-lactoglobulin standards

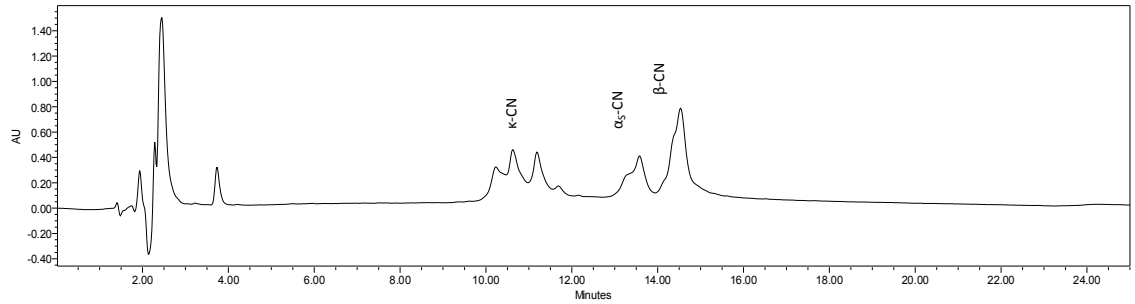

Figure 2 Chromatogram of caseins standards

RESULTS AND DISCUSSION

In Table 1 there is percentage representation of α_S-casein (α_S-CN), β-casein (β-CN), κ-casein (κ-CN), α-lactalbumin (LA), and β-lactoglobulin (LG). α_S-CN, β-CN, and κ-CN represent 100 % as well as the sum of LA a LG.

Table 1 Concentration of caseins, α-lactalbumin and β-lactoglobulin in yoghurts in %

Sample	1	2	3	4	5	6	7	8	9	10
α_S-CN	52.0	48.7	48.0	46.4	45.7	43.1	45.7	49.0	44.3	47.5
β-CN	48.0	38.8	52.0	42.4	42.5	43.2	45.6	42.2	41.0	44.3
κ-CN	*	12.5	*	11.2	11.8	13.7	8.7	8.8	14.7	8.2
α-LA	100	100	100	100	74.5	*	100	100	100	96.2
β-LG	*	*	*	*	25.5	*	*	*	*	3.9

*LOD < 0.45 %

Table 2 Basic statistics characteristic

%	average	min	max	SD
α_S-CN	47.1	43.1	52.0	2.6
β-CN	44.0	38.8	52.0	3.8
κ-CN	9.0	0	14.7	5.2
α-LA	96.7	74.5	100.0	8.4
β-LG	14.7	0	25.5	15.3

From the table it is clear that the casein content in individual samples did not differ. The whey protein was most clearly represented by α-lactalbumin, unlike β-lactoglobulin, which was in most cases below the detection limit. It is possible because β-lactoglobulin is thermolabile.

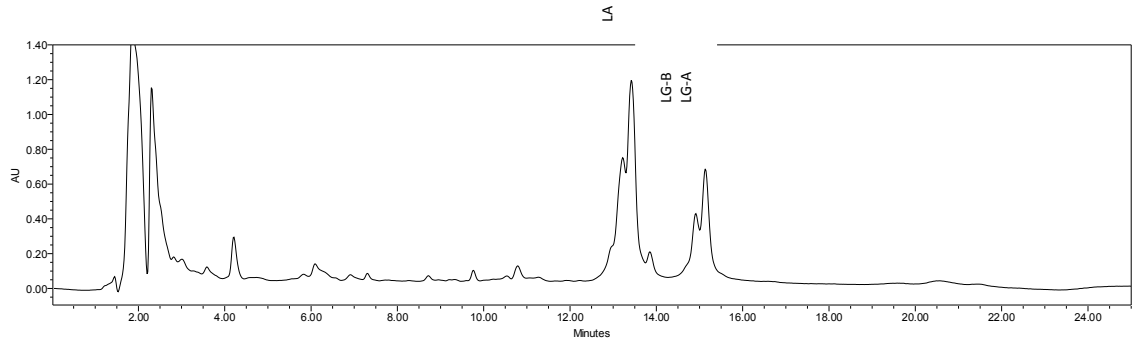

Figure 3 Chromatogram of α-lactalbumin and β-lactoglobulin samples

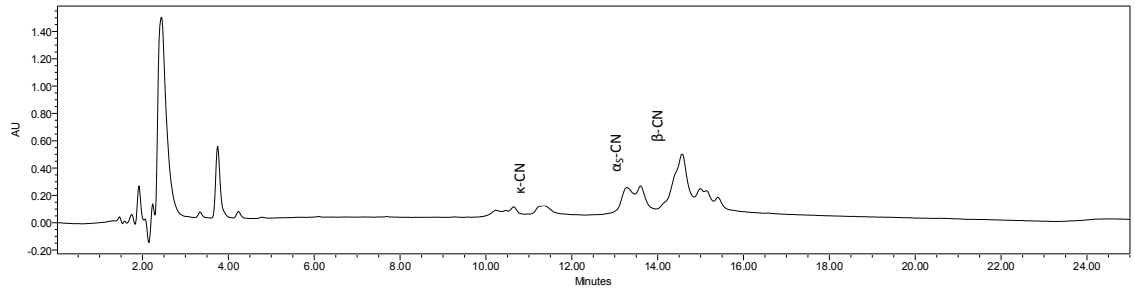

Figure 4 Chromatogram of caseins samples

In the chromatogram of whey proteins are identified and individual fractions LA and LG. **Czerwenka *et al.* (2010)** in his work deals with the identification of various fractions of whey proteins. On the basis of his work can be reliably identified β-lactoglobulin B and β-lactoglobulin A, which are clearly separated.

CONCLUSION

Whey proteins and caseins contents were determined in 10 yogurt samples using RP-HPLC. Average values of caseins were: α_S-CN 47.1 ± 2.6 %, β-CN 44.0 ± 3.8 % and the κ-CN 9.0 ± 5.2 %. Average values of LA were about 96.7 ± 8.4 % and LG were 14.7 ± 15.3 %. This method is suitable for the determination of caseins, α-lactalbumin and β-lactoglobulin in the yogurt.

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