

ORIGINAL RESEARCH PAPER

INFLUENCE OF CITRUS AND CELERY PECTINS ON PHYSICOCHEMICAL AND SENSORY CHARACTERISTICS OF FERMENTED DAIRY PRODUCTS

Mihaela Ivanova^{1*}, Nadezhda Petkova², Mina Todorova², Valentina Dobрева³, Radka Vlaseva¹, Panteley Denev², Dimitar Hadjikinov³, Valentin Bouvard⁴

¹University of Food Technologies, Technological Faculty, Department of Technology of Milk and Dairy Products, 26 Maritsa Blvd., Plovdiv, Bulgaria

²University of Food Technologies, Technological Faculty, Department of Organic Chemistry and Inorganic Chemistry, 26 Maritsa Blvd., Plovdiv, Bulgaria

³University of Food Technologies, Technological Faculty, Department of Environmental Engineering, 26 Maritsa Blvd., Plovdiv, Bulgaria

⁴IUT Lyon1, Département Génie Biologique, site de Bourg-en-Bresse, France

*Corresponding author: mihaela_18bg@abv.bg

Received: April, 27, 2020

Accepted: November, 20, 2020

Abstract: The aim of the current study was to improve the nutritional value, rheological quality and sensory characteristics of yoghurt prepared with high-esterified citrus and celery pectins. Comparative studies of physicochemical characteristics of yoghurt with incorporated pectin isolated from celery (*Apium graveolens* var. *rapaceum* D. C.) tubers and commercially available citrus pectin were conducted. The celery pectin extracted by ammonium oxalate was characterized as highly methoxylated (HM) with a degree of esterification (DE) 75 % and anhydrouronic acid content (AUAC) 66 %, respectively. The rate of lactic acid formation, rheological, and sensory characteristics of the resulting yoghurts were also studied. It was found, that the addition of high-esterified celery pectin in the milk in an amount of 0.3 % decreased syneresis of the acid coagulum and led to a slight increase in viscosity. The addition of pectins in the concentration from 0.1 to 0.3 % caused alterations in sensory perception, which was positively evaluated by the panellists. However, the highest scores from sensory evaluation of resulting yoghurts demonstrated these prepared with 0.2 % celery pectin. This study suggested that the highly esterified celery pectin presents an alternative source of soluble dietary fibre for the enrichment of yoghurt and the production of a functional product with potential health effects.

Keywords: FTIR spectra, high methylated celery pectin, high methylated citrus pectin, sensory evaluation, viscosity, yoghurt

INTRODUCTION

Bulgarian yoghurt is a traditional dairy product with proven health characteristics. The valuable nutritional and dietary qualities of Bulgarian yoghurt are mainly due to the *Lactobacillus bulgaricus* and have been confirmed by numerous scientific studies by Bulgarian and foreign scientists [1 – 3].

In recent years, there is a trend of increasing the biological value of lactic acid products through the incorporation of soluble dietary fibers [4 – 6]. Polysaccharides have been added in dairy products to improve their texture, rheology, physicochemical properties, and sensory attributes for meeting the consumer demands. Anionic polysaccharides such as pectin, stabilize the dairy matrix mainly based on the adsorption of polysaccharides on the surface of positively charged proteins below the isoelectric pH of proteins [7].

The pectin chains are composed of two main regions: homogalacturonan (HG) and rhamnogalacturonan (RG). Homogalacturonan consists of (1→4)-linked α -D-GalA residues, esterified with methanol at C-6 to a different extent, which results in low methoxylated (< 50 %) and high methoxylated (> 50 %) pectins. Some hydroxyl groups are partly acetylated at O-2 and/or O-3 positions. Their specific functional properties are mainly due to the degree of methoxylation and acetylation. Rhamnogalacturonan type I constituted of repeating units of alternating α -(1→2)-linked rhamnose and α -(1→4)-linked GalA residues. Some of α -L-rhamnose (Rha) residues are branched with side chains attached to O-4 and rarely at O-3 positions, such as β -D-galactans, (1→5)- α -L-arabinans and arabinogalactans [8 – 10]. The main raw materials for the production of pectin are citrus peels and apple pomace which are by-products from juice production. The content of pectin in citrus peel and apple pomace contains about 25 % and 12 % pectin, respectively [10].

However, pectins from alternative sources as a vegetable (celery, leek, tomatoes, and potatoes) also deserve attention due to its improved functional properties [11 – 14]. Celery tubers are rich, but underestimated source of pectin [15 – 17]. In our previous studies, pectin isolated from celery tubers by ultrasound-assisted extraction demonstrated promising emulsifying properties [15]. Celery pectin is characterized by a low degree of acetylation (4 %) and anhydrouronic acid content (AUAC) that can reach 75 % [18]. Moreover, low methoxylated and amidated celery pectin were successfully incorporated in the fermented dairy products [19, 20]. The addition of pectins in the manufacture of fermented dairy products is based on their ability to interact with the casein micelles in milk in the presence of calcium ions, forming stable gel structures in the pH range of 2.5 to 4.5 [21]. HM pectin (DE=61 %), were adsorbed at the protein surface at low pH (< 5.0) and low concentration leads to bridging [7].

The syneresis is an undesirable process that occurs during the storage of yoghurt. A practical method for increasing the viscosity and decreasing the syneresis is the use of hydrocolloids to yoghurt [7]. The highly esterified pectin incorporation results in the interaction with the casein of milk and the formation of a thicker coagulum. Pectins are usually added to protect protein in thermally treated fermented milk, as usual, its concentration is about 0.3 - 0.5 % [22]. To the best of our knowledge, any studies about the incorporation of high esterified pectin from celery tubers in yoghurt were not available. Therefore, we hypothesized that celery pectin may improve the quality of fermented products as well as citrus pectin. Moreover, the incorporation of pectins will

improve the nutrition and sensory properties of these products with the effect of dietary fibers.

Therefore, the aim of this study was to improve the nutritional value by the addition of soluble dietary fiber as high-esterified citrus and celery pectins and to investigate the influence of these pectins on physicochemical and sensory characteristics of the prepared yoghurt.

MATERIALS AND METHODS

Materials

Raw cow milk characterized by sensory, physicochemical and microbiological parameters, according to the requirements of European Union Regulation № 853/2004 was used in the current study. The fat content was standardized to 3.6 ± 0.1 %. Starter culture for yoghurt containing the specific strains (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) was provided by Lactina Ltd., Bulgaria. HM citrus pectin (Classic CM 203) with DE > 68 % was kindly provided by Herbstreith & Fox KG, Germany. For extraction of celery pectin, celery tubers (*Apium graveolens* var. *rapaceum* D.C.) with an origin the Netherlands were purchased from the local fruit market in Plovdiv, Bulgaria. All other reagents were analytical grade and used without further purification.

Methods

Pectin extraction from celery tubers

The fresh celery tubers were washed with tap water, cleaned and peeled. They were coarsely chopped and dried at 40 °C in a ventilated oven. Then, the plant material was finely ground in a laboratory homogenizer and passed through a 1 mm sieve. Before extracting the sample insoluble solids (AIS) were obtained from dry ground celery tubers. For this purpose, the celery tubers were washed with 70 % ethanol acidified with 2 % hydrochloric acid. Then AIS was extracted with two liters 2 % (w/v) aqueous ammonium oxalate at 85 °C for 45 min with continuous stirring on a magnetic stirrer and then filtered. The extraction process was performed in duplicate. The extract was filtered, cooled down and it was precipitated with two volumes of the acidified ethanol (0.05 % HCl). The coagulated polysaccharide was separated by filtration through nylon cloth, washed once again with 70 % ethanol to a neutral pH, and finally with 96 % ethanol, dried in an oven, at 50 °C, to a constant weight and finely ground. The yield of pectin was expressed on dry weight basis of AIS.

Pectin characterization

Moisture and ash content of isolated celery pectin and commercial citrus pectin were determined according to AOAC (2007) method [23]. The degree of esterification and anhydrouronic acid content were titrimetrically determined according to Food Chemical Codex [24]. The degree of acetylation was determined by the hydroxamic acid reaction, with β -D-glucose pentaacetate as a standard [12].

FTIR spectroscopy of pectin samples

FTIR spectra of citrus and celery pectin were recorded on a spectrometer Nicolet FTIR Avatar (Termo Science, USA) in KBr pellets in the range 4000 - 400 cm^{-1} at resolution 4 cm^{-1} and 132 scans were performed. The absorption was reported in wavenumbers (cm^{-1}). Spekwin32 software was used to display the spectra.

Yoghurt preparation and fermentation

A classical technology for the production of set type yoghurt in laboratory conditions was applied. As above-mentioned, milk samples were standardized at 3.6 % fat content. Different pectin (citrus or celery) in a concentration of 0.1; 0.2 and 0.3 % was incorporated in the milk together with 6 % sucrose before pasteurization, according to previous study where it was found that higher pectin quantities were associated with early syneresis in yoghurts with set type coagulum because of pectin's natural acidity [25]. Pectin-enriched samples were heated up to 80 °C, treated by a laboratory high-shearing device Polytron®PT45-80, (Kinematika, Switzerland with technical characteristics 220 V; 50 Hz; 1600 W) at speed 10,000 rpm. The samples were heated to 92 ± 2 °C and held at this temperature for 20 min. After cooling at 42 ± 2 °C, the samples were inoculated with yoghurt starter culture in the amount of 2 %. The samples were stirred and put into the plastic packagings with caps and then incubated at 42 ± 2 °C. The fermentation process was performed. After coagulation, the samples were cooled down to 4 ± 2 °C and stored at this temperature. A control sample was performed in the same way but without pectin addition.

pH and Titrable acidity

The changes in the amount of lactic acid and its biochemical transformation to lactic acid during the fermentation process were monitored by active acidity (pH) and titratable acidity, expressed as % lactic acid. Active acidity (pH) was potentiometrically measured using pH-meter - 7110 WTW (Germany). Titratable acidity (TA) was measured according to the Bulgarian National Standard (BNS) 1111-80 [26]. During fermentation, the pH and TA values were measured every 30 min.

Syneresis test

The structural characteristics of the coagulum after fermentation were determined by the amount of the separated serum, which characterizes the stabilizing capacity of the added pectins. Ten grams of yoghurt samples were centrifuged immediately after coagulation using centrifuge MLWT23 (Poland) at 3000 rpm for 20 min and then syneresis was calculated and expressed in %. 100 % stability of set type coagulum was taken when no serum was released. Any deviation from this percentage means greater coagulum instability and higher probability of serum release during storage of the product.

Viscosity

The viscosity of yoghurt was determined by rheoviscometer, at a temperature of 25 °C by Rotational viscometer – „Rheotest“ 2, Germany.

Sensory analysis

The sensory analysis test was performed according to the requirements of the BNS 12:2010 [27]. The sensory quality of experimental yoghurt was evaluated by a jury of trained panelists using a 10 point scale.

Statistical analysis

Statistical analysis was performed using Microsoft Excel 2010 (ANOVA). Multiple comparisons were made by LSD method. The results were presented as mean value \pm SD (n=3).

RESULTS AND DISCUSSION**Characteristics of celery and citrus pectins**

Pectin was successfully extracted from AIS of celery tubers using ammonium oxalate conventional extraction. The isolated celery pectin, as well as commercial citrus pectin, was characterized as high methoxylated ones with DE above 70% and the results were presented in Table 1.

Table 1. Characteristics of pectins from different plant sources

Sample	Characteristics of pectins					
	Yield [%]	Moisture [%]	Ash [%]	DE [%]	DA [%]	AUAC [%]
Celery pectin	29 \pm 1 ^a	5.5 \pm 0.2 ^a	3.2 \pm 0.2 ^a	75.8 \pm 0.2 ^a	1.8 \pm 0.1 ^a	65.9 \pm 3.0 ^a
Citrus pectin Classic CM 203	-*	5.2 \pm 0.1 ^a	2.0 \pm 0.2 ^b	73.2 \pm 0.2 ^b	1.5 \pm 0.1 ^b	78.0 \pm 1.2 ^b

a,b - Means with different letters within a row are significantly different ($p < 0.05$), DE – the degree of esterification, DA – the degree of acetylation, AUAC – anhydrouronic acid content, * - pectin is a commercial product and this information is not available from the producer

The isolated polysaccharide has similar characteristics to the citrus pectin. The close degree of esterification and AUAC suggested similar functional properties. The moisture content did not exceed 6 %, which is comparable to the reported values for pectin from other sources [28]. According to [28, 29] pectin powder should have lower moisture content for safe storage and prevent deterioration of pectin quality due to the production of pectinase as a result of microorganisms growth. Ash content was higher in celery pectin (3.2 %) in a comparison with commercial citrus pectin (2 %). Low ash content is recommendable for good gel formation of pectin, as its values should be below 10 % [28, 31]. In our case, isolated celery pectin demonstrated lower ash content together with citrus commercial pectin; therefore both pectins demonstrated perfect quality for food purposes. Moisture and ash content of isolated celery pectin was comparable to the reported values for ammonium oxalate-extracted pectin from roselle calyx [30] and acid extracted pectin from citrange (*Citrus sinensis* \times *Poncirus trifoliata*) albedos [31]. The celery pectin yields were 29 % dry weight that coincided with our previous studies [11, 16] it was comparable to pectin from orange peel (*Citrus sinensis*) [5]. AUAC was higher in citrus pectin. Celery pectin extracted by a conventional

method characterized by lower AUAC in comparison with celery pectin obtained after ultrasonic extraction 69 % [15]. The important characteristics of commercial pectin used as food additives are 65 % galacturonic acid content, which may be present as a free acid, methyl ester, in amidated pectin or acid amide form [32]. The degree of acetylation (1.8 %) of celery pectin is similar to citrus pectin (1.5 %). This was in accordance with acetyl content reported by [33]. The celery pectin isolated by conventional ammonium oxalate characterized by a high degree of esterification and lower AUAC values in comparison with our previous reports for ultrasound-assisted extraction [15]. Probably the decrease in DE is the cavitation process that can lead to depolymerization of the pectin chain and deesterification of the ester carboxylate group. The values of AUAC in citrus pectin were higher than these in celery pectin. However, both of the pectins were characterized with values above 65 % galacturonic content that is the essential characteristic for pectins to be used as food additives, according to FAO. Values of AUAC were comparable to the galacturonic acid content of pectin from apple (73.5 %) and citrus (72.1 %), grapefruit (74.86 %), navel orange peel (~70 %), fresh orange peel (66.4 - 70.1 %), jackfruit pectin (69.47 - 75.34 %) [28].

FTIR spectra

The FT-IR spectra of celery and citrus pectins were shown in Figure 1.

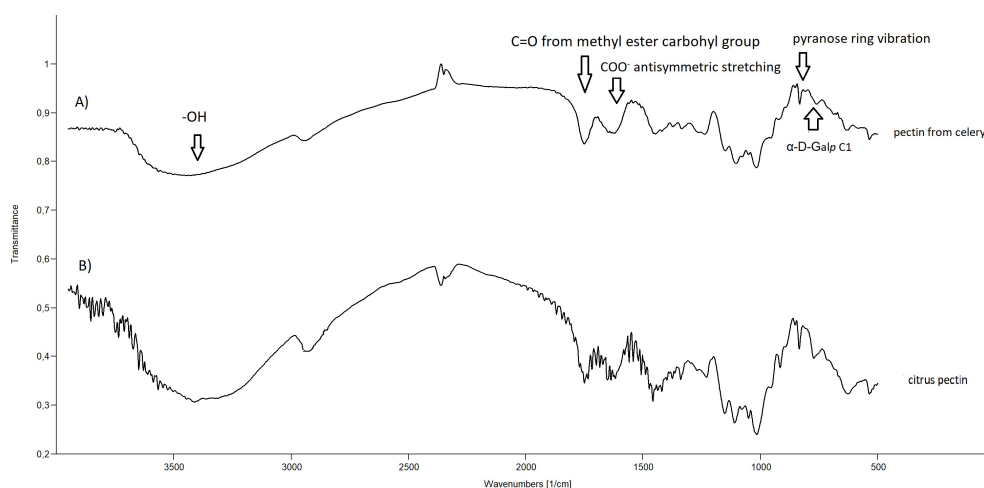


Figure 1. FTIR spectrum of pectins, where A) pectin from celery, B) pectin from citrus (commercial)

The typical bands for pectic polysaccharides were found in both spectra. The broad bands at 3430 cm^{-1} were assigned to the (O–H) stretching vibrations of hydrogen-bonded and free hydroxyl groups in the pectin molecule. The band at 2937 cm^{-1} was typical for (C–H) stretching of CH_2 groups. The bands at 1746 cm^{-1} were typical for C=O stretching vibration of methyl esterified carboxyl groups and the band at 1630 cm^{-1} corresponded to the absorption of the carboxylate anions (Figure 1). The presence of acetyl residue was observed at 1372 cm^{-1} due to the bending of C–H from CH_3 and a C–O stretching vibrations at 1232 cm^{-1} that correspond to the acetyl ($-\text{COCH}_3$) groups. The band at 1015 cm^{-1} associated with $\nu\text{C-O}$, $\nu\text{C-C}$ (C2-C3, C2-O2, C1-O1 pectin) stretching from the pyranose ring. The band at 920 cm^{-1} was assigned to $\rho(\text{CH})$ methyl

group in complex ester. Additionally, in the fingerprint region, C-O bending was observed at 950 cm^{-1} and two typical for α -D-galactopyranosyl residues in conformation C1 bands were observed at 888 cm^{-1} and 760 cm^{-1} . The detailed assignment of FTIR spectra confirmed the presence of typical functional groups in the pectin chain. Most of the bands in FTIR spectra coincided with some previous reports for celery, fumaria, and lemon pectins [12, 15, 31, 34].

Yoghurt characterization

The dynamics of lactic acid formation during the coagulation for milk with the addition of high-esterified pectin isolated from celery and commercial citrus pectin in quantity $0.1 - 0.3\%$ at temperature $43 \pm 2\text{ }^{\circ}\text{C}$ were presented in Table 2. From the obtained results (Table 2), it is clear that the initial pH and the titratable acidity are significantly related to the increase in the amount of pectin ($p < 0.05$). A similar trend in pH decrease was observed in lactic acid fermented yoghurt enriched with 0.1 to 1 % pectin-rich orange fiber [5, 6], as well as fruit containing yoghurt [3].

Table 2. Dynamics of acid formation, depending on the time of coagulation of milk with the addition of high-esterified pectin

	Control sample		Commercial pectin						Pectin from celery					
Pectin [%]	0		0.1		0.2		0.3		0.1		0.2		0.3	
Time [min]	pH	TA [$^{\circ}\text{T}$]	pH	TA [$^{\circ}\text{T}$]	pH	TA [$^{\circ}\text{T}$]	pH	TA [$^{\circ}\text{T}$]	pH	TA [$^{\circ}\text{T}$]	pH	TA [$^{\circ}\text{T}$]	pH	TA [$^{\circ}\text{T}$]
0	$6.55 \pm 0.10^{\text{aA}}$	$22 \pm 0.5^{\text{aA}}$	$6.40 \pm 0.04^{\text{aB}}$	$22 \pm 0.5^{\text{aA}}$	$6.36 \pm 0.05^{\text{aB}}$	$24 \pm 0.5^{\text{aB}}$	$6.34 \pm 0.09^{\text{aB}}$	$25 \pm 0.5^{\text{aB}}$	$6.39 \pm 0.07^{\text{aA}}$	$23 \pm 0.5^{\text{aA}}$	$6.34 \pm 0.07^{\text{aB}}$	$24 \pm 0.5^{\text{aB}}$	$6.25 \pm 0.07^{\text{aC}}$	$28 \pm 0.5^{\text{aC}}$
30	$6.30 \pm 0.05^{\text{bA}}$	$23 \pm 0.5^{\text{aA}}$	$6.42 \pm 0.03^{\text{bB}}$	$23.5 \pm 0.5^{\text{bA}}$	$6.35 \pm 0.04^{\text{bA}}$	$25.5 \pm 0.5^{\text{bB}}$	$6.30 \pm 0.04^{\text{bA}}$	$26 \pm 0.5^{\text{bB}}$	$6.38 \pm 0.05^{\text{bA}}$	$24 \pm 0.5^{\text{aA}}$	$6.33 \pm 0.04^{\text{bA}}$	$25 \pm 0.5^{\text{bB}}$	$6.23 \pm 0.05^{\text{bC}}$	$29 \pm 0.5^{\text{aC}}$
60	$6.26 \pm 0.04^{\text{cA}}$	$27 \pm 0.5^{\text{bA}}$	$6.24 \pm 0.07^{\text{cA}}$	$36 \pm 0.5^{\text{cB}}$	$6.10 \pm 0.08^{\text{cB}}$	$31.5 \pm 0.5^{\text{cC}}$	$6.06 \pm 0.07^{\text{cB}}$	$30 \pm 0.5^{\text{bC}}$	$6.12 \pm 0.06^{\text{cB}}$	$28 \pm 0.5^{\text{bA}}$	$6.07 \pm 0.09^{\text{cB}}$	$30 \pm 0.5^{\text{bC}}$	$6.00 \pm 0.04^{\text{cB}}$	$33 \pm 0.5^{\text{bD}}$
90	$5.95 \pm 0.06^{\text{dA}}$	$35.5 \pm 0.5^{\text{cA}}$	$6.05 \pm 0.08^{\text{dA}}$	$33.5 \pm 0.5^{\text{dB}}$	$6.04 \pm 0.04^{\text{dA}}$	$33 \pm 0.5^{\text{dB}}$	$5.88 \pm 0.05^{\text{dA}}$	$44 \pm 0.5^{\text{cC}}$	$6.04 \pm 0.07^{\text{dA}}$	$32 \pm 0.5^{\text{cB}}$	$6.04 \pm 0.08^{\text{dA}}$	$33 \pm 0.5^{\text{cB}}$	$5.98 \pm 0.07^{\text{dA}}$	$39 \pm 0.5^{\text{cD}}$
120	$5.62 \pm 0.10^{\text{eA}}$	$42 \pm 0.5^{\text{dA}}$	$5.73 \pm 0.07^{\text{eA}}$	$45 \pm 0.5^{\text{eB}}$	$5.76 \pm 0.06^{\text{eA}}$	$41.5 \pm 0.5^{\text{eA}}$	$5.75 \pm 0.08^{\text{eA}}$	$52 \pm 0.5^{\text{dC}}$	$5.78 \pm 0.09^{\text{eA}}$	$40 \pm 0.5^{\text{dA}}$	$5.74 \pm 0.07^{\text{eA}}$	$40 \pm 0.5^{\text{dA}}$	$5.88 \pm 0.08^{\text{eB}}$	$44 \pm 0.5^{\text{dB}}$
150	$5.46 \pm 0.09^{\text{fA}}$	$49.5 \pm 0.5^{\text{eA}}$	$5.60 \pm 0.04^{\text{fB}}$	$52.5 \pm 0.5^{\text{fB}}$	$5.30 \pm 0.07^{\text{fA}}$	$58 \pm 0.5^{\text{fC}}$	$5.33 \pm 0.07^{\text{fA}}$	$58 \pm 0.5^{\text{eC}}$	$5.47 \pm 0.10^{\text{fA}}$	$51 \pm 0.5^{\text{eA}}$	$5.35 \pm 0.07^{\text{fA}}$	$54 \pm 0.5^{\text{eB}}$	$5.76 \pm 0.07^{\text{fB}}$	$52 \pm 0.5^{\text{eB}}$
180	$5.12 \pm 0.04^{\text{gA}}$	$62 \pm 0.5^{\text{fA}}$	$5.23 \pm 0.04^{\text{gB}}$	$65 \pm 0.5^{\text{gB}}$	$5.00 \pm 0.08^{\text{gA}}$	$68 \pm 0.5^{\text{gC}}$	$4.95 \pm 0.04^{\text{gC}}$	$66.5 \pm 0.5^{\text{fD}}$	$5.07 \pm 0.07^{\text{gA}}$	$63 \pm 0.5^{\text{fA}}$	$5.20 \pm 0.08^{\text{gB}}$	$60 \pm 0.5^{\text{fE}}$	$5.22 \pm 0.08^{\text{gA}}$	$60 \pm 0.5^{\text{fE}}$
210	$5.01 \pm 0.07^{\text{hA}}$	$69 \pm 0.5^{\text{gA}}$	$4.83 \pm 0.08^{\text{hB}}$	$72.5 \pm 0.5^{\text{hB}}$	$4.72 \pm 0.08^{\text{hB}}$	$70.5 \pm 0.5^{\text{hA}}$	$4.78 \pm 0.08^{\text{hB}}$	$71.5 \pm 0.5^{\text{gA}}$	$4.88 \pm 0.08^{\text{hB}}$	$72 \pm 0.5^{\text{gB}}$	$4.98 \pm 0.07^{\text{hA}}$	$68 \pm 0.5^{\text{gA}}$	$4.80 \pm 0.04^{\text{hB}}$	$76 \pm 0.5^{\text{gC}}$

a-h - Means with different letters within a column are significantly different ($p < 0.05$)

A-D - Means with different letters within a row are significantly different ($p < 0.05$)

Lactic strains can ferment lactose into lactic acid, with an increase of acidity and a decrease in pH of yoghurt [5]. This tendency persisted until 90 min when the active lactic acid process reduced these differences and only significant differences in pH

value and titratable acidity were recorded in the samples with 0.3 % pectin added. The end of the fermentation process occurs at 210 min when all the samples have similar parameters except the fermented milk with the addition of 0.3 % pectin. It has significantly higher titratable acidity values ($p < 0.05$) due to the buffer capacity - pH values are similar but the titratable acidity is significantly higher ($p < 0.05$). Similar to the observation of [7] in our case the same tendency was observed: the acidity was incremented with the increase in the concentration of pectin.

Syneresis

The influence of the amount of high-esterified pectin on syneresis of lactic coagulum was examined. The amount of the separated serum was determined by a centrifugal test (Table 3).

Table 3. *Quantity of serum from the coagulum*

Characteristics	Control sample	Commercial citrus pectin			Pectin from celery		
Pectin concentration [%]	0	0.1	0.2	0.3	0.1	0.2	0.3
Quantity of serum [%]	25 ± 1^a	15 ± 1^b	6 ± 1^c	5 ± 1^c	13 ± 1^b	6.5 ± 0.5^c	4.5 ± 1^c

a,b,c - Means with different letters within a row are significantly different ($p < 0.05$)

The control sample demonstrated the highest values of separated serum (25 %). The results from the centrifugal test showed that the amount of the separated serum was decreased by increasing the amount of high-esterified pectin. Citrus and celery pectin in concentration 0.2 % significantly reduced the syneresis process. However, celery pectin decreased twice times the quality of separated serum even in the concentration of 0.1 %. The trend in these results was explained by [35], by the ability of the high-esterified pectin to be absorbed irreversibly and unabsorbed pectin incorporates into a friable network structure composed of casein micelles, covered with pectin. The adsorption of pectin on casein micelles strongly depends on pH and starts at pH~5.0.

These data give us grounds to recommend the use of highly-esterified pectin to enrich milk products with soluble dietary fibre and reduce the amount of separated serum for the studied interval of values.

Viscosity

An important structural-mechanical property of fermented products is the parameter dynamic viscosity. The viscosity (η) of fermented dairy products prepared with the amount and type of used pectin was determined in the range of variation of speed gradient (D) from 0.17 to 72.9 s⁻¹. Viscosity (η , Pa.s) of the analyzed samples, depending on the velocity gradient (or shear rate - D, s⁻¹) is presented (Figure 2).

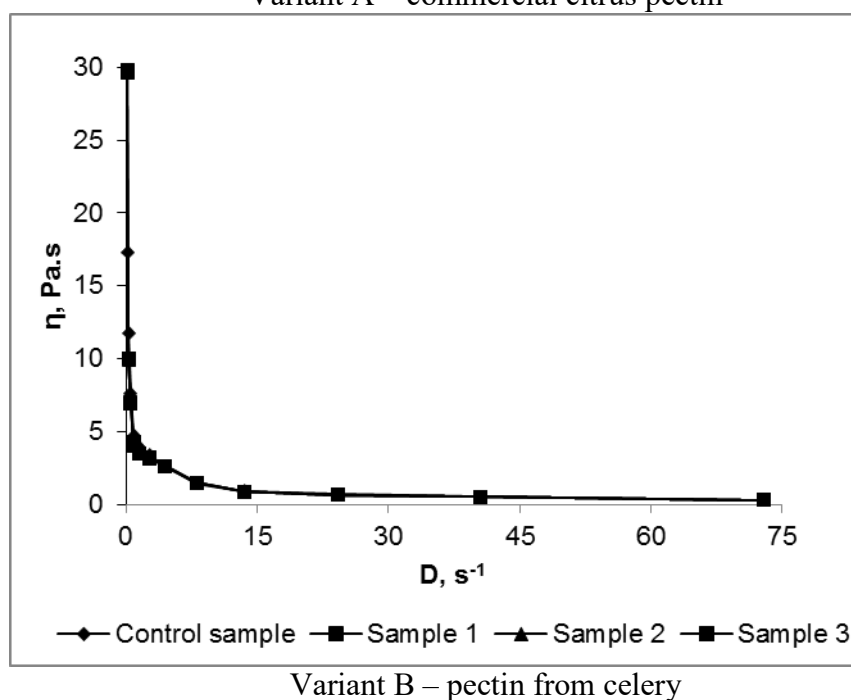
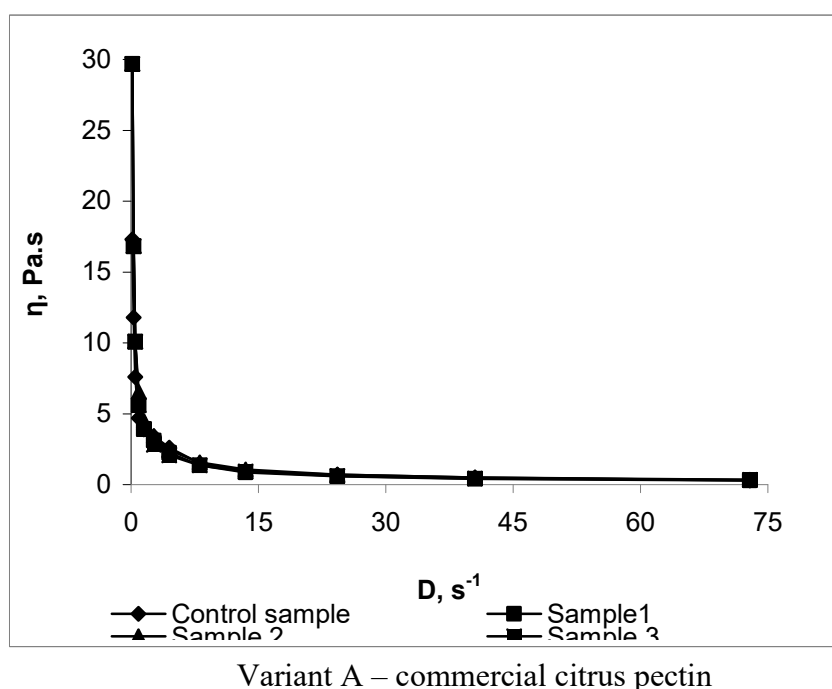


Figure 2. Dynamic viscosity on fermented dairy products with the addition of high-esterified pectin in the concentration 0.1 % (sample 1), 0.2 % (sample 2) and 0.3 % (sample 3) compared to the control sample

The viscosities of all samples decreased with increasing values of the velocity gradient (D, s^{-1}) at all concentrations of pectin. The results of the tested samples with celery pectin and citrus pectin showed that the values of the dynamic viscosity of the fermented milk products obtained with the addition of high-esterified pectin were similar to those of the control sample. A similar tendency was observed in the case with the incorporation of low esterified amidated pectin in fermented lactic acid products

[20]. The viscosity decreased to the highest extent after shear rate 10 s^{-1} . Our results were comparable to the reported viscosity studies of yoghurt showed a viscosity below $0.3 \text{ Pa}\cdot\text{s}$ were observed on the 0 days of storage [36]. It also reported that viscosity of yoghurt, produced at fermentation temperature 41°C , with starter culture dose 0.3% and fat content 3.50% after 1-day storage reached $\eta = 0.311 \text{ Pa}\cdot\text{s}$ [2]. However, in our study higher viscosity values in the low shear rate range from 0.17 to 10 s^{-1} were found only in samples with the highest concentration (0.3%) of high-esterified celery pectin – sample 3 (Figure 2 B). The viscosity of samples with citrus pectin and celery pectin at the beginning of measurements was between 28 - $25 \text{ Pa}\cdot\text{s}$ which was comparable to the viscosity of yoghurt with 0.3% orange pectin [5].

Sensory analysis

The sensory characteristics of yoghurt were significantly improved with the increase of pectin incorporation rate. The results of the sensory analysis were shown (Figure 3).

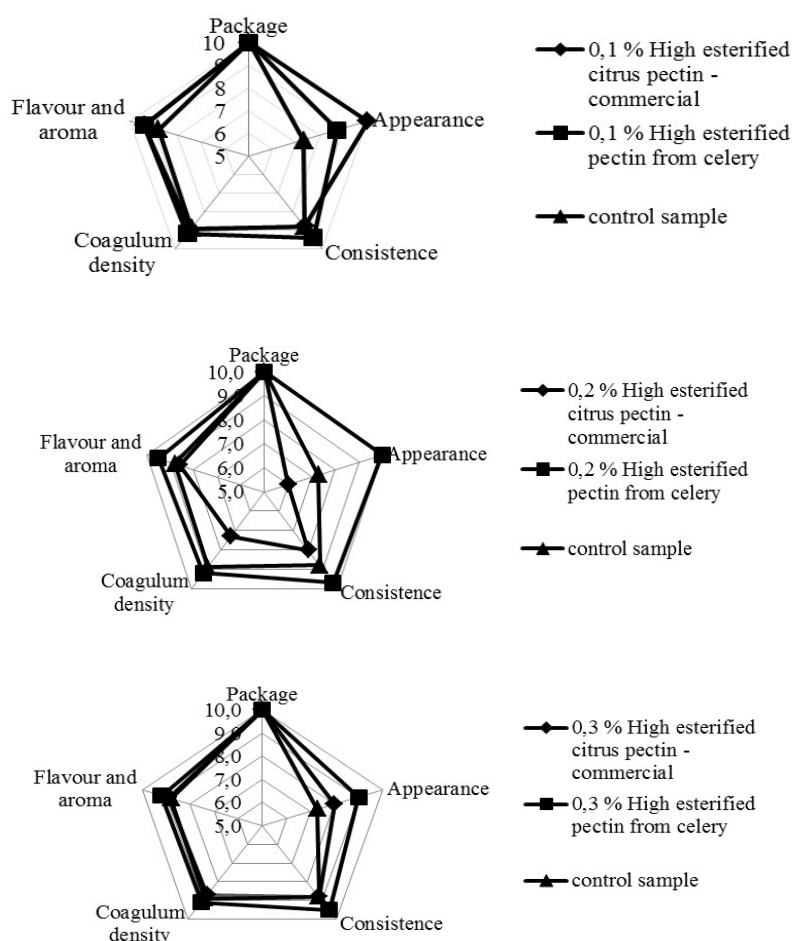


Figure 3. Sensory characteristics of lactic acid products with the addition of high-esterified commercial (citrus) and celery pectin

The improvement of the taste of yoghurt was proportional to the increase of pectin concentration. The addition of pectins in the concentration from 0.1 to 0.3% caused alterations in sensory perception in comparison with the control sample. Yoghurts

prepared with celery pectin demonstrated higher scores than other samples with citrus pectin. The highest scores from sensory evaluation of resulting yoghurts demonstrated these prepared with 0.2 % celery pectin. The results obtained showed that the increase of the concentration of the pectin incorporation in milk leads to a higher assessment of the panelists for the appearance and texture of the final product. A decrease in flavor was not reported, indicating that the high-esterified celery pectin can be used successfully for the reduction of syneresis in yoghurt.

CONCLUSIONS

To the best of our knowledge, this is the first detailed report for the incorporation of celery pectin in fermented lactic acid products and their comparison with products prepared with commercial citrus pectin. As a result of the addition of pectins yoghurt enriched with dietary fiber (pectin) was produced. It is found that yoghurt with the incorporation of highly esterified celery pectin in concentration 0.3 % was characterized with better sensory characteristics, shorter acidification time, and the lowest syneresis in a comparison with the control sample ($p < 0.05$). The comparative spectral, chemical, physicochemical, and sensory characteristics showed no significant differences in yoghurt obtained with celery pectin and citrus pectin ($p > 0.05$). Considering this, the isolated celery pectin can be successfully applied for the development of functional food formulations based on milk, with potential health benefits and improved rheological and sensory characteristics.

ACKNOWLEDGEMENTS

This research was financially supported by The Scientific Investigation Section Project 3/13-H, fund “Science”, University of Food Technologies, Plovdiv, Bulgaria. The authors also express their gratitude to Lactina Ltd. Bulgaria and Herbstreith & Fox KG, Germany for supplementation with lactic acid bacteria and pectin used in this study.

LIST OF NOTATIONS AND SYMBOLS

AIS – Alcohol Insoluble Solids
AOAC - Association of Official Agricultural Chemists
AUAC - Anhydrouronic Acid Content
BNS – Bulgarian National Standard
DAc – Degree of Acetylation
DE – Degree of Esterification
FAO - Food and Agriculture Organization
FTIR - Fourier Transform Infrared Spectroscopy
HM – High Methoxilated
LSD – Lowest Standard Deviation
TA – Titratable Acidity

REFERENCES

1. Vlaseva, R.: *Functional dairy products*, Academic Publishing of University of Food Technologies, Plovdiv, **2014**, 89-97;
2. Dabija, A., Oroian, M., Codină, G., Rusu, L.: Assessment the influence of the main technological factors on yogurt quality, *Scientific Study & Research Chemistry & Chemical Engineering, Biotechnology, Food Industry*, **2020**, **21** (1), 83-94;
3. Taneva, I., Zlatev, Z.: Total phenolic content and antioxidant activity of yoghurt with goji berries (*Lycium barbarum*), *Scientific Study & Research Chemistry & Chemical Engineering, Biotechnology, Food Industry*, **2020**, **21** (1), 125-131;
4. Vlaseva, R., Denev, P., Ivanova, M.: Application of dietary fibers in dairy products, *Food Science, Engineering and Technology*, **2011**, **1**, 79-82;
5. Arioui, F., Ait Saada, D., Cheriguene, A.: Physicochemical and sensory quality of yogurt incorporated with pectin from peel of *Citrus sinensis*, *Food Science & Nutrition*, **2016**, **5** (2), 358-364;
6. Kieserling, K., Vu, T.M., Drusch, S., Schalow, S.: Impact of pectin-rich orange fibre on gel characteristics and sensory properties in lactic acid fermented yoghurt, *Food Hydrocolloids*, **2019**, **94**, 152-163;
7. Yousefi, M., Jafari, S.: Recent advances in application of different hydrocolloids in dairy products to improve their techno-functional properties, *Trends in Food Science & Technology*, **2019**, **88**, 468-483;
8. Voragen, A., Coene, G., Verhoef, R., Schols, H.: Pectin a versatile polysaccharide present in plant cell walls, *Journal of Structural Chemistry*, **2009**, **20**, 263-275;
9. Renard, C., Crépeau, M., Thibault, J.: Structure of the repeating units in the rhamnogalacturonic backbone of apple, beet and citrus pectins, *Carbohydrate Research*, **1995**, **275**, 155-165;
10. Georgiev, Y., Ognyanov, M., Yanakieva, I., Kussovski, V., Kratchanova, M.: Isolation, characterization and modification of citrus pectins, *Journal of BioScience & Biotechnology*, **2012**, **1** (3), 223-233;
11. Petrova, I., Petkova, N., Kyobashieva, K., Denev, P., Simitchiev, A., Todorova, M., Dencheva, N.: Isolation of Pectic Polysaccharides from Celery (*Apium graveolens* var. *rapaceum* D. C.) and Their Application in Food Emulsions, *Turkish Journal of Agricultural and Natural Sciences*, **2014**, **2**, 1818-1824;
12. Ognyanov, M., Remoroza, C., Schols, H., Georgiev, Y., Petkova, N., Krystijan, M.: Structural, rheological and functional properties of galactose-rich pectic polysaccharide fraction from leek, *Carbohydrate Polymers*, **2020**, **229**, 1-48;
13. Yang, T., Ma M.: Extraction, structure, and emulsifying properties of pectin from potato pulp, *Food Chemistry*, **2018**, **244**, 197-205;
14. Szymanska-Chargot, M., Zdunek, A.: Use of FT-IR spectra and PCA to the bulk characterization of cell wall residues of fruits and vegetables along a fraction process, *Food Biophysics*, **2013**, **8** (1), 29-42;
15. Petrova, I., Petkova, N., Ognyanov, M., Simitchiev, A., Todorova, M., Denev, P.: Food emulsions with amidated pectin from celery (*Apium graveolens* var. *rapaceum* D.C.) tubers. *Agricultural Science and Technology*, **2017**, **2** (3), 246-250;
16. Murdzheva, D., Petkova, N., Vasileva, I., Todorova, M., Ognyanov, M., Ivanov I., Denev, P.: Accelerated modification of lowmethoxylated celery pectin, 12th International Conference on Polysaccharides-Glycoscience, October 19-21, Prague, Check Republic, **2016**, 213-217;
17. Siddiqui, I.: Studies on vegetables. Investigation of water, oxalate, and sodium hydroxide soluble celery (*Apium graveolens*) polysaccharides: pectic polysaccharides, *Journal of Agricultural and Food Chemistry*, **1990**, **38**, 70-74;
18. Hansen, K., Thuesen, A., Söderberg, J.: Enzyme Assay for Identification of Pectin and Pectin Derivatives, Based on Recombinant PectateLyase, *Journal of AOAC*, **2001**, **84** (6), 1851-1854;
19. Vlaseva, R., Ivanova, M., Bouvard, V., Hadjikinova, M., Hadjikinov, D., Dobрева, V., Petkova, N., Denev, P., Balabanova, T.: Comparative characteristics of the technological possibilities of low-esterified pectin from bulgarian celery and commercial product, Proceedings of the XIII National Conference "Natural Science" 2015, 25-27 September, Varna, **2016**, 39-43;
20. Vlaseva, R., Ivanova, M., Hadjikinova, M., Hadjikinov, D., Dobрева, V., Petkova, N., Denev, P., Dimitrov, D.: Comparative characterization of possibilities of using low-esterified and amidated

- pectin in fermented dairy products, *Journal of Microbiology, Biotechnology and Food Sciences*, **2016**, 6 (1), 649-651;
21. Marozienne, C., Kruif, G.: Interaction of pectin and casein micelles, *Food Hydrocolloids*, **2000**, 14 (4), 391-394;
 22. http://www.herbstreithfox.de/fileadmin/tmpl/pdf/broschueren/The_Specialists_for_Pectin_09.pdf
 23. AOAC: *International, Official methods of analysis*, 18th edition, Gaithersburg, Maryland, US: AOAC International **2007**;
 24. *** Food Chemical Codex: 5th edition, National academic press, Washington, D.C., N.Y. Press, USA, **2004**, 322-323;
 25. Vlaseva, R., Edgarian, M., D. Keskinova: Study of the technological compatibility of milk and pectin in the production of lactic acid products, *Scientific Works of University of Food Technologies*, **2003**, 50 (2), 221-225;
 26. Bulgarian National Standard BNS 1111-80: Milk and dairy products. Determination of titratable acidity, **1980**;
 27. Bulgarian National Standard BNS 12:2010. Bulgarian yoghurt, **2010**;
 28. Koh, P., Leong, C., Noranizan, M.: A Microwave-assisted extraction of pectin from jackfruit rinds using different power levels, *International Food Research Journal*, **2014**, 21 (5), 2091-2097;
 29. Salam, M., Jahan, N., Islam, M., Hoque, M.: Extraction of Pectin from lemon peel: Technology development, *Journal of Chemical Engineering IEB*, **2012**, 27 (2), 25-30;
 30. Nazaruddin, R., Noor Baiti, A., Foo, S., Tan, Y., Ayob, M.: Comparative chemical characteristics of hydrochloric acid- and ammonium oxalate-extracted pectin from roselle (*Hibiscus sabdariffa* L.) calyces, *International Food Research Journal*, **2013**, 20 (1), 281-284;
 31. Zouambia, Y., Ettoumi, K., Krea, Y., Moulai-Mostefa, N.: A new approach for pectin extraction: Electromagnetic induction heating, *Arabian Journal of Chemistry*, **2017**, 10 (4), 480-487;
 32. May, C.: Pectins in: *Handbook of Hydrocolloids*, (Editors: Phillips G.O., Williams P.A.), CRC, NewYork, **2000**, 169-188;
 33. Leroux, J., Langendorff, V., Schick, G., Vaishnav, V., Mazoyera, J.: Emulsion stabilizing properties of pectin, *Food Hydrocolloids*, **2003**, 17, 455-462;
 34. Dimopoulou, M., Alba, K., Campbell, G., Kontogiorgos, V.: Pectin recovery and characterization from lemon juice waste streams, *Journal of the Science of Food and Agriculture*, **2019**, 99 (14), 6191-6198;
 35. Tuinier, R., Rolin, C., Kruif, C., Electrosorption of Pectin onto Casein Micelles, *Biomacromolecules*, **2002**, 3, 632-638;
 36. Zamberlin, Š., Dubravka S., Mamula, P., Havranek, J., Pecina, M., Pogačić, T.: Viscosity of stirred yoghurt during storage, *Mljekarstvo*, **2007**, 57 (3), 209-218.