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Isolation of Pectic Polysaccharides from Celery (*Apium graveolens* var. *rapaceum* D. C.) and Their Application in Food Emulsions

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Abstract

Celery (*Apium graveolens* var. *rapaceum* D. C.) is rich source of biologically active substances, widely used worldwide in human nutrition. In the current research, pectic polysaccharide has been isolated from celery tubers by ultrasound-assisted extraction with an aqueous ammonium oxalate. The obtained pectin has been characterized as highly methoxylated (HM) with degree of esterification (DE) 75 % and anhydrouronic acid content (AUAC) 57 %, respectively. Furthermore, the rheological properties of this pectic polysaccharide have been investigated. The effect of pectin concentration – 0.4; 0.6; 0.8 and 1 % and oil phase 30, 40 and 50 % on dispersibility and stability of the resulting emulsions have been evaluated. It has been found that isolated pectic polysaccharide from celery tubers influence significantly on the rheological behavior of the emulsion and they characterized as non-Newtonians fluids. Because of the impressive emulsion stabilization properties of celery pectin as dietary fiber, we recommend its application for preparation of food emulsion with improved nutritional value and health benefits.

Keywords: Pectic polysaccharides, celery tubers, ultrasonic extraction, emulsions, rheology

Introduction

Celery (*Apium graveolens*) is a plant that belongs to *Apiacea* family. Three varieties of celery are cultivated worldwide: var. *rapaceum* (Mill.), var. *dulce* (Mill.) and var. *secalinum* (Alef.). The first mention variety is the most distributed and cultivated. It forms tubers with white color inside (Hermann, 1996). This vegetable is wildly used in human nutrition because of its culinary importance and rich nutrients content. Celery tuber contains 11.4 % dry matter, 1.55 % proteins, 0.33 % lipids, 2.25 % total carbohydrates, dietary fibers 4.23 % (Souci et al., 2000) - (lignin, cellulose, pectin (Siddiqui, 1990)), 0.94% minerals (mainly K, Mg, Fe), vitamins C, K, B1, B2 and PP (Aleksic, 1984; Kulier 1996).

As vegetable celery, finds enormous application in food industry, for culinary purposes and for healthy nutrition due to its chemical composition mentioned above (Marković et al., 2009).

The celery tubers are also source of pectin (Siddiqui, 1990; Hansen et al., 2001). Pectic substances are widely used in food industry as food additives (E440) with gelling and stabilizing properties in jams, jellies, marmalades, milks and confectionery products (Sakai et al., 1993). They are also applied as thickener and emulsifier (Rolin and Vries, 1990; Kochekov, 1992).

Pectin molecules are composed of several structural regions. The main simply arranged fragment is called homogalacturonan and it consists of linear chains of a(1→4)-D-galacturonic acid residues, that can be partly methyl-esterified at C-6 to different degrees (Rolin and De Vries, 1990): low methoxylated (< 50%) and high methoxylated (> 50 %). Some hydroxyl groups are partly acetylated at O-2 and/or O-3 (Schols & Voragen, 2003). The second fragment is rhamnogalacturonan I, constituted of linear repeating structure [→2)-α-L-Rhap-(1→4)-α-D-GalpA-(1→] (Renard et al., 1995; Voragen et al., 1995) (Figure 1). This structural fragment contains branched chains that consist of neutral sugar units connected to O-4 of L-Rha residues (Zykwinska et al., 2007)

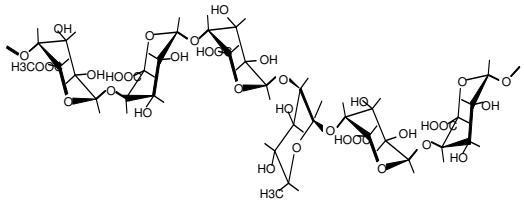

Figure 1. Fragment from the pectin backbone chain

Celery pectic polysaccharides are characterized with low degree of acetylation around 4 % in comparison of beet and sunflower pectin and anhydrouronic acid content (AUC) 75 % (Hansen et al., 2001).

Our earlier investigations showed that main pectic polysaccharide fraction obtained from celery tubers belongs to the pectic substances with average degree of esterification (DE) and good gelling strength (Denev et al., 2004). Pectins are applied in emulsion preparation used as stabilizer. The strong relationship was found between rheological properties (emulsion stability) and degree of esterification of the pectin (Popova et al., 1993; Einhorn-Stoll et al., 1996)

The emulsifying capacity of citrus and beet pectin were well known. Pectic substances isolated from these plants possess the ability to reduce the interfacial tension between an oil phase and a water phase and can be efficient for the preparation of emulsions (Leroux et al., 2003). To the best of our knowledge, the pectins isolated from celery tubers were not completely investigated. In comparison of citrus and apple pectins it is not commercially available and its application in food emulsion is not completely studied.

The aim of the current research was (1) to isolate and characterize pectic polysaccharides from celery tubers and (2) to investigate their emulsion-stabilizing properties.

Materials and Methods

All reagents and chemicals were analytical grade.

Fresh celery tubers were commercially obtained from local market in Plovdiv. The plant material was washed, peeled, sliced, ground and dried at 40 °C. The dried residue was finely ground in laboratory homogenizer and preliminary washed with 70 % ethanol acidified with 2 % hydrochloric acid. Fifty grams the achohol insoluble part from celery tubers were extracted with 2 L d H_2O, in which was previously dissolved 20 g ammonium oxalate, at 85 ºC for 45 min with continuous stirring under constant ultrasonic irradiation with frequency 35 kHz and then filtered. The extraction process repeated with 1 L 1% ammonium oxalate. The crude filtrates were obtained. They were cooled to the room temperature and were precipitated with two volumes with acidic ethanol (0.05% HCl) and left for one hour. The coagulated celery pectic polysaccharide were separated by filtration, washed once with 70% ethanol to a neutral pH and finally with 96% ethanol. Pectins were dried at 40ºC in a laboratory dryer.

The AUA content and the degree of esterification (DE) of pectic polysaccharides obtained from celery tubers was determined by the titrimetric method, according to the Food Chemical Codex (2004), slightly modified for using Hinton's indicator.

Fourier transformation infrared analysis (FT-IR). The IR-FT spectra of isolated celery pectic polysaccharides was recorded in KBr pellets on a Nicolet FT-IR Avatar Nicolet (Termo Science, USA) spectrometer in the range 4,000–400 cm^{-1} at resolution 4 cm^{-1} and absorbtion was reported in wavenumbers (cm^{-1}).

Emulsification. Isolated celery pectin were dissolved in water with initial stirring at room temperature, followed by heating at 40 °C until the solution became transparent.

Commercially available sunflower oil was obtained from local market and incorporated into emulsion preparations without further purification.

Emulsification was performed on a laboratory homogenizer POLYTRON® Kinematica GmbH PT-G 45/80, (Switzerland) for 30 sec at 523 $rad.s^{-1}$ and temperature 20°C. The oily phase for oil-in-water (o/w) model emulsions was sunflower oil at volume fraction 30, 40 and 50% v/v.

Rheological Measurements. Viscosity and shear stress of model emulsions were measured at various velocity gradients via digital viscometer "Brookfield" RV-DV II+Pro". Adapter for small samples equipped with a camera, cylinder and a cylindrical spindle SC4-27, water jacket SC4-13R was used for the samples. Effective viscosity and shear stress were calculated by specialized software „Rheocalc" 32. Range of shear rate was determined by preliminary experiments. The measurement of viscosity was carried out at different values of shear rate. After completion of the experiments, "Rheocalc" 32 automatically draws a graphical relationship between shear stress and shear rate. The resultant relation is approximated, which is used to determine the rheological model of model emulsions. This model, which produces the highest correlation coefficient was selected, and the effective viscosity is calculated by the program according to the equation of the selected rheological model.

Results

Isolation and characterization of isolated pectin from celery tubers.

For the first time ultrasonic irradiation procedure were applied to isolate pectic polysaccharides from celery (*Apium graveolens* var. rapaceum D. C.) Scheme for pectin extraction was presented on Figure 2.

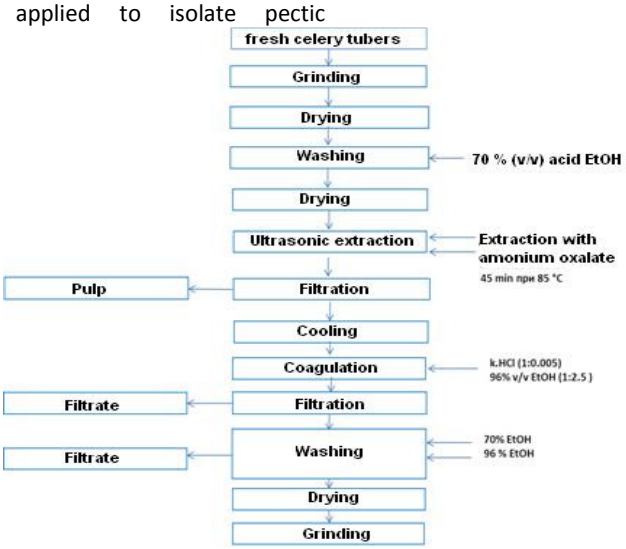

Figure 2. Ultrasound-assited extraction (UAE) process of pectic polysaccharides from celery tubers

Similar to Georgiev et al. 2012 to obtain a good starting material for further pectin extraction, the celery tubers were firstly washed with ethanol removing of alcohol-soluble low molecular weight substances and for inactivation of endogenous enzyme systems (Figure 2). The resulting pectin polysaccharide after extraction and clarifying procedure was dissolved in water and then precipitated again with two-volume 95 % ethanol. The pectins were white to slightly yellow color.

The characteristics of isolated pectic polysaccharides from celery tubers by ultrasound treatment were presented in Table 1.

Table 1. Characterization of pectic polysaccharides isolated from celery tubers by ultrasonic treatment

Substance	Yield, %	AUAC*, %	DE**, %
Pectic polysaccharides from tubers of celery	30,1	56,9	75,7

*anhydrouronic acid content (AUAC)
**degree of esterification

The isolated pectin was characterized with 30,1% yield. According to Panchev et al. (1994) and in our case the ultrasound treatment was found to intensify the extraction process of celery pectin. Near results for galacturonan yield from celery stalks were reported by Ovodova et al., 2009.

The DE of esterification was higher than the reported results from conventional extraction (Hansen et al., 2001) and microwave extraction (Denev et al., 2004) - (64 - 65 %). The celery pectic polysaccharide characterized with high anhydrouronic acid content - 56,9% (Table. 1).

The IR-FT sprectra of celery pectic polysaccharide contained band typical for pectin (Figure 3). A broad band at 3446 cm^{-1} was attributed to stretching of hydroxyl groups, an absorption at 1747 cm^{-1} was due to C=O stretching vibration of methyl-esterified carboxyl groups, the absorption at 1647 cm^{-1} was caused by C=O stretching vibrations of ionic carboxyl groups. The bands at 1149, 1103 and 1014 cm^{-1} indicated the pyranose structure of isolated substance. The observed in our spectra bands are closer to reported for apple pectin isolated by ultrasonic irradiation (Zhang et al., 2013).

Figure 3. IR-FT spectrum of isolated pectic polysaccharide under ultrasonic treatment

Emulsifying and Emulsion-Stabilizing Properties.

In the present study high methoxylated celery pectins were tested for their actions in model emulsions using a variation in the emulsion composition: concentration of pectin and oil). Structure and mechanical properties of the disperse system (oil and water phase and included in it hydrocolloids), significantly influenced the rheological behavior. The rheological measurements were used as an analytical tool to elucidate the structural organization and interaction of the constituents of the emulsion. The relationship between the viscosity and shear rate (D, s^{-1}) was used to assess the strength of the colloidal interactions between globules (Quemada and Berli, 2002).

Studies were conducted with model O / W emulsion obtained with different volume of oil phase (MF) - 30, 40 and 50% concentrations of pectic polysaccharides of celery (PPOC) 0.4; 0.6; 0.8 and 1% relative to the aqueous phase, without the addition of other stabilizing or emulsifying agent. The viscosity and shear stress of the model emulsions were measured at different values of shear rate. (D = 17 to 68, s-1). Basic structural and mechanical properties characterizing the rheological behavior and rheological type of M / V systems including pectic polysaccharides of celery were determined.

Fig.4. presented rheological curves of model emulsions with different concentrations of pectic polysaccharides of celery and oil phases, respectively, 30, 40 and 50% v/v. The rheological behavior of model systems with pectin celery characterized them as non-Newtonian fluids. In this non-Newtonian character is more pronounced when emulsions with a high ratio of oil phase and increases with increasing amount of pectic polysaccharides in them.

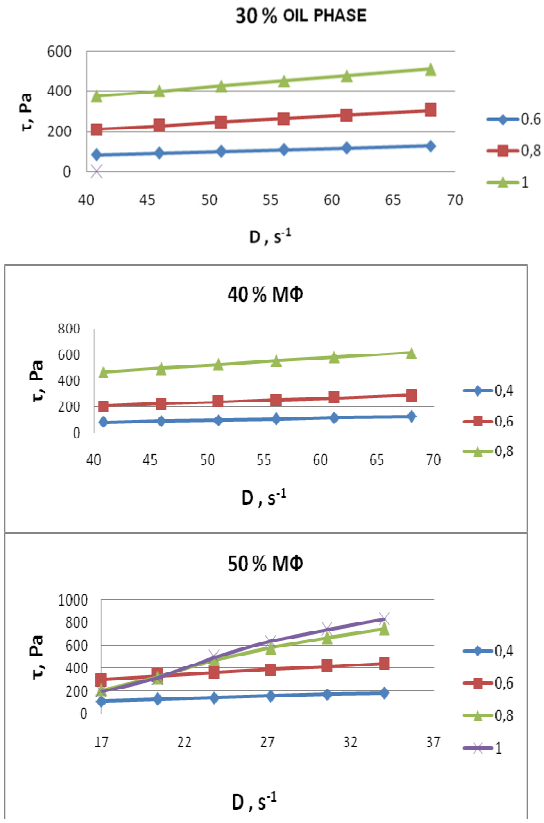

Figure 4. Rheograms of model emulsions at oil phase volume 30, 40 и 50% v/v

The results of the rheological tests showed that increasing the concentration of pectic polysaccharides of celery results in an improvement of the structure and rheological properties of the model systems. This is probably due to the properties and aggregation behavior of gelation. Pectic polysaccharides as hydrocolloid has surface active properties and structure the aqueous phase by reaction with water.

The volume of the oil phase also affected the rheological properties of model emulsion systems.

At low concentrations of pectic polysaccharides (0.4%), the impact was weak and slightly more strongly at higher concentrations (0.8% and 1%). Stable emulsion was not formed at the concentration of 0.4% pectic polysaccharides and 30% oil phase.

Experimental data revealed that to describe the rheological behavior of the emulsions with pectic polysaccharides of celery the Casson model (1) could be applied. The rheological characteristics of model emulsions were shown in Table 2.

$$\sqrt{\tau} = \sqrt{\tau_0} + \sqrt{\eta D} \qquad (1),$$

where:
τ – the shear stress, Pa;
τ_0 – the yield stress;
η – the plastic viscosity, Pa.s;
D – the shear rate, s^{-1}.

Table 2. Data on rheological characteristics of model emulsions

Model emulsions	$\eta_{пл}$, Pa.s	τ_0, Pa	R, %
30% oil phase			
volume v/v	132.0	0.37	99.9
- 0.6 % PPOC	238.9	2.25	99.9
- 0.8% PPOC	294.3	7.09	100.0
- 1.0% PPOC			
40% oil phase			
volume v/v	286.7	12.9	100.0
- 0.6% PPOC	303.0	11.1	100.0
- 0.8% PPOC	2082.0	4.16	97.8
- 1.0% PPOC			
50% oil phase			
volume v/v	217.0	2.5	99.8
- 0,6% PPOC	2019.0	8.78	97.9
- 0.8% PPOC	2762.0	6.23	97.6
- 1% PPOC			

The presence of yield stress determined the rheological type of model emulsions as plastic fluid. Viscosity (η, mPa.s) of the analyzed samples, depending on the velocity gradient (share rate) (Figure 5). The results presented in Figure 5 show that the viscosities of model emulsions in oil phase 40% decrease with increasing values of the velocity gradient (D, s-1) at all concentrations of pectin. In the emulsions at a concentration of 0.8% pectin viscosity decreased by 21% in 0.6% pectin, respectively, by 15%, and in 0.4% pectin by 10%.

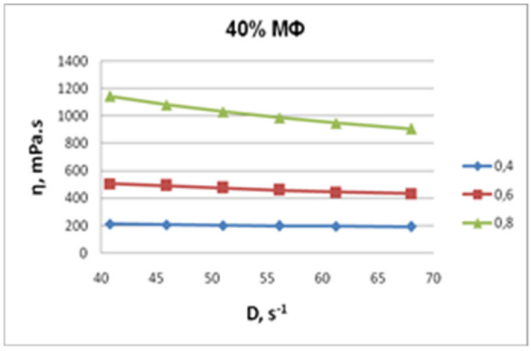

Figure 5. Dependence of the plastic viscosity on a share rate

Graphically presented dependence (Figure 6) reveals that the increase of the concentration of pectin in the aqueous phase of model emulsions at 0.6 to 0.8 leads to an increase of 232% of the values of the viscosity of model emulsions with 30% oil phase volume. For systems with an oil phase 40%, the increase is 207%.

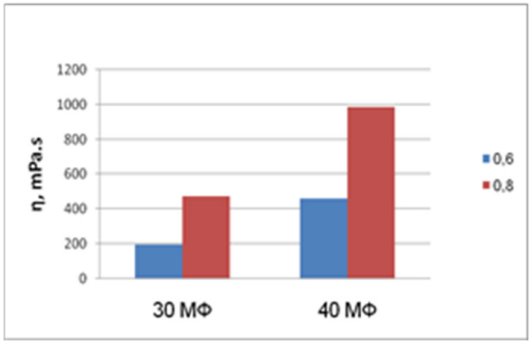

Figure 6. Dependence of the plastic viscosity on a share rate

Discussion

Pectin extraction using ultrasound is not commonly applied because of risk to break down the polymer (Panchev et al., 1988). But the method is classified as green and innovative and is preferred in pectin degradation and modification to obtained pectins with desirable rheological properties (Zhang et al., 2013).

The resulting celery pectic polysaccharides after the ultrasound-assited extraction characterized as highly methoxylated (HM) with DE 75 %. The results coinsided with reports of conventional extraction (Hansen et al., 2001). Therefore, the chosen extraction conditions under ultrasonic treatment minimized pectin degradation and increased the yield with 3% (our unpublished results). The DE of celery pectin did not degrease

after ultrasonic treatment. The results from IR-FT spectroscopy proved that the isolated substances is pectin, because of presence of typical bands around 1740, 1620 and 1240 cm^{-1} (Szymanska-Chargot, Zdunek, 2013). In our case the pyranose configuration also didn`t changed after ultrasound treatment. The same was observed for apple pectin obtained by ultrasound irradiation (Zhang et al., 2013).

The ionic interactions between homogalacturonan (HG) domains in pectic molecules were considered to explain many of its properties and biological functions (Ridley et al., 2001; Willats et al., 2001). Not only the degree of esterification, but also the distribution of methyl and acetyl groups onto HGs has a deep impact on those interactions (Ralet et al., 2008).

The research was carried to study rheological properties of emulsions prepared with highly methylated celery pectin isolated by ultrasound treatement. The concentration of pectic polysaccharide was varied in range 0,4; 0,6; 0,8 и 1% and the volume of oil phase– 30, 40 и 50%, respectively. The results showed that unstable emulsions were obtained with lower concentration of celery pectin 0,4% and 30% oil phase. Model emulsions demonstrated non-Newtonian behavior. This occurs when chains detangle the spherical coils and the droplets in the emulsions are deformed into ellipsoidal shapes, while aggregates are broken into their elements and start to form layers coincident with the plane of shear, therefore offering less resistance to flow (Brummer, 2006; Tadros, 2011).

The application of celery pectin in emulsions will improve not only the functional properties of the products, but also will enhance to the nutritional and healthy benefits for human. Especially, high-methylated pectins lower the cholesterol levels in blood, reduce the absorption of glucose in the serum of diabetics. Pectins and its structure fragments are part from group of dietary fibers, potential prebiotics, substances with anti-inflammatory, antitumor, and immunostimulating activity (Bhimanagoudaet al., 2006; Ovodova et al., 2009; Georgiev et al., 2012, Ognyanov et al., 2014).

Conclusion

To the best of our knowledge this is the first report for ultrasound-assited extraction of pectic polysaccharides from celery tubers. The resulting polysaccharide was characterized as high DE – 75.7 % and AUAC - 56,9 %. The structure was proven by IR-FT spectroscopy. The obtained highly methylated celery pectin were used for preparation of oil-in water model emulsions. It was established that it possessed emulsion-stabilizing properties due to the ability of pectic polysaccharides to structure water media. The rheological characteristics of the investigated emulsions depended on concentration of the used celery pectin, as well as on the volume of the dispersive phase. The celery pectin emulsions exhibited non-Newtonian behavior. The model that described the rheological behavior of the celery pectin emulsions was presented. The obtained results showed the potential application of celery pectin as emulsion stabilizer.

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