Article

Pectin from Fruit and Berry Juice Production By-Products, Determination of Physicochemical, Antioxidant and Rheological Properties

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Abstract: The by-products of the juice and cider industry in forms of pomace and peels are rich sources of pectin which further can be used in the food, cosmetic and non-food industries. The aim of the research was to use by-products from fruits and berries grown in Latvia: Rhubarb (*Rheum rhabarbarum*); Apples *(Malus domestica);* Plums *(Prunus domestica);* Red Currants *(Ribes rubrum);* Black Currants *(Ribes nigrum);* Gooseberries *(Ribes uva-crispa);* Sour Cherries *(Prunus cerasus);* Pumpkins *(Cuccurbita spp.)* for pectin extraction. Citric acid (0.1 N) was used for the hot water extraction. The yield of pectin (PY = 4.47 – 17.8 % DM) and physicochemical parameters of obtained pectin were determined: methoxy degree (ME = 4.27 – 8.13 %), esterification degree (DE = 45.16 % – 64.06 %), anhydrouronic acid content (AUA = 38.23.% - 84.74 %); moisture content (5.2 – 7.4 %), ash content (1.42 - 2.88 g 100 g^{-1}), total phenolic content (TPC = 2.076 – 4.668 GAE, mg L^{-1}) and antiradical scavening activity (DPPH Method (0.56 – 37.29 %)). Pectin analysis was performed by FT-IR and the rheological properties of pectin gels were determined.

Keywords: antiradical scavenging activity; esterification; gels; phenolics; viscosity; rheology

1. Introduction

Reducing food loss and waste in all fields of food production consumption has increased attention on the part of society. Food waste in primary food production is about 9.1 ± 1.5 MT, and 16.9±2.7 MT in processing, moreover, the fruit and berry juice industry is one of the largest agro-based industries. and, therefore, by-products occur – peel, pomace. The pomace contains up to 16 % of the mass of fruits being purposed for processing, moreover, it is still rich in biologically active substances such as fibres, vitamins, carotenes, organic acids, and macronutrients that play an important role in human health[1]–[4].

By-products from the juice and cider production industry are promising sources of pectin as new pectin resources are increasingly emerging, moreover, pectin from by-products can be further used in food as E440a (pectin) and E440b (amidated pectin), in cosmetics and non-food industries as a thickener, gelling ingredient, cation-binding agent; furthermore, it is a source of antioxidants, as polysaccharides from fruits and berries have a such chemical structure that provides anti-diabetic, immunomodulating, antitussive, astringent biological properties[5]–[9]. The consumption of pectin impacts blood cholesterol levels; removes toxins from the body and it regulates blood glucose levels [10][6], [11]–[13]. Pectin can form gel with simple gelling mechanism and that can be used for biomedical applications: drug delivery; tissue engineering, and wound dressing[14], [15].

Pectin is complex, an acid-rich polysaccharide from plant cell walls - multifunctional and versatile hydrocolloid [16]. Structure of pectin is formed of three main regions: homogalacturonan (HG), rhamnogalacturonan I (RG-I) and

 doi:10.20944/preprints202211.0499.v1

rhamnogalacturonan II (RG-II). HG region is composed of α- (1,4)-linked D-galacturonic acid (GalA) units that may be methyl-esterified at the C-6 carboxyl or acetylated at the O-2 and/or O-3 [5], [11], [17], [18]. Structure and properties are important for classification and functionality of pectin for food and cosmetics product design [19], [20].

The yield and composition of pectin depend on the plant source, harvest time, extraction technique, and conditions employed during pectin isolation and purification [21], [22]. Research of different extraction methods have been studied: conventional heating in acid, enzymatic extraction, microwave, ultrasound, combined methods, electromagnetic and autoclaving [19], [23]–[26]. Extraction of pectin with the conventional method is carried out with hot acidified water with inorganic (sulfuric, nitric, hydrochloric, etc.) or organic acids (tartaric, citric), (temperature above 60º C; pH range of 1.5 – 3; time for 0.5 – 6 h [23], [27]. Usage of these methods can rise the degradation of pectin and the method is considered time-consuming and can cause irreversible environmental pollution. To minimize the impact on the environment researchers found that pectin with higher molecular weight and viscosity can be obtained by using weak citric acid instead of mineral acid and the pectin extraction yields were higher than other methods [24], [28].

Commercially pectin is extracted from citric fruits, apples and sugar beets; though, several scientific reports have investigated pectin extraction from various plants: sunflower residues, lemon, passion fruit, pomegranate, melon peel, pistachio green hull, pomelo peel, cacao pod husks, pumpkin, black mulberry[6], [29]–[35].

Rhubarb (*Rheum rhabarbarum*) is early spring raw material in Latvia for juices and it has important economic value [36], [37]. For beverages juice of rhubarb is extracted with a cold press and, therefore, by-products as pomace are left, moreover, still rich in bioactive compounds such as: anthraquinones, stilbenes, flavonoids, tannins [36]. Plums (*Prunus domestica*) after apples, pears and peaches are the 3rd popular fruit grown in Europe and its production in Europe has reached 27700 tonnes and it is 25 % of whole world's plum production [38]. Chemical composition of plum fruits include: sugars, organic acids, tannins and dye extracts, pectin, vitamins and mineral salts [39]. Apples (*Malus domestica*) are widely grown in EU countries and used in food manufacturing for juice, cider, wine, distilled spirit and vinegar manufacturing. Apple market in 2021 has reached 7100 tonnes in Latvia [38]. The solid waste represents 20 to 35 % of the fresh weight of the apple fruit[29], [32], [40]. In pomace from juice industry there has been found pectic substances about 9.2 – 12.8 % [40]. The residue consists of peel, core, seed, calyx, stem and pulp. The most pectin substances come from the epi- mesocarp accounting up to 95.5 % of the solid waste[41], [42].

Therefore, the aim of the research was to minimize waste and biomass utilization in juice production industry by using different local fruit and berry by-products for pectin extraction with weak organic acid; and to determine yield, physical, chemical, antioxidant and rheological properties of extracted pectin.

2. Materials and Methods

By-products (BP) for the research were obtained from local juice and cider producers in from June to August, 2022. Cold-pressed juices were made by extractors that first crush and then press the fruit at a very low speed. Rhubarb (*Rheum rhabarbarum*), Apples (*Malus domestica*), Plums (*Prunus domestica*), Red Currants (*Ribes rubrum*), Black Currants (*Ribes nigrum*), Gooseberries (*Ribes uva-crispa*), Sour Cherries (*Prunus cerasus*) were used for extraction. Pumpkin (*Cucurbita pepo* L, Pink Banana Jumbo) BP – peel and pomace were obtained from Lat Eko Food Ltd., 2021. BP were stored at –20 ± 2ºC in plastic bags till extraction and experiments.

2.1. Sample Preparation and Extraction

BP were dissolved with 0.1M citric acid (1:2 (w/v); pH= 1 - 1.5), homogenized and conventional hot water extraction was done according to the Citric Acid Method (CA) with some modifications [43]. The resulting solutions, - prepared samples were heated at 90 °C for 60 min, cooled and centrifuged (*Sigma 4-16KS*) at 6000 rpm for 20 min. Collected

 doi:10.20944/preprints202211.0499.v1

supernatants were treated with absolute ethanol (1:2 (v/v)) at +4 ± 1°C for 14 – 16h. The pectin precipitates were collected by re-centrifugation, and then were washed twice with 80 % (v/v), and 90 % (v/v) ethanol, the obtained pectin was conventionally hot-air dried to constant moisture content ≤ 9%.

2.2. Pectin Yield, Ash Content and Moisture Content

The pectin yield was calculated (Formula 1).

PY (%) = Weight of dried pectin, (g)/ Weight of by-product, dry matter (DM), (g)*100 (1)

Ash content in extracted, dried pectin samples was determined according to AOAC 942.05 method [44]. Moisture content (%) of by-products and resulted pectin samples was determined according to Reference method ISO 712:2009(EN).

2.3. Equivalent Weight, Methoxyl Content, Degree of Esterification, Anhydrouronic Acid Content

Determination of pectin's equivalent weight (EW) was done and calculated (Formula 2) with method described by Virk and Sogi (2007) with some minor modifications [32]. Pectin (0.2 ± 0.02 g), ethanol (5 mL), sodium chloride (1.0 g), distilled water (100 mL), 5 - 6 drops of phenol red indicator were dissolved and titrated against standard 0.1 M NaOH until the colour of indicator changed (pH 7.5) to pink and persisted for at least 30 seconds.

$$EW = \frac{m * 100}{0.1 * V1}, \quad (2)$$

EW - equivalent weight, g mL^{-1}; m - weight of pectin sample, g; 0.1 - normality of alkali; V_1 - volume of alkali, mL.

Methoxyl content (ME) was determined by pectin saponification and titration of the liberated carboxyl groups. The neutralized solution obtained during the determination of equivalent weight was collected, and 25 ml of 0.25 N NaOH was added. The mixture was stirred thoroughly and kept at ambient temperature for 20 min. Then, 25 ml of 0.25 N HCl was added and titrated against 0.1 N NaOH to the end point. The calculation (Formula 3) of ME value:

$$ME, \% = \frac{3.1 * V_2 * N_A}{m}, \quad (3)$$

ME – methoxyl content, %; V_2 - volume of alkali, mL; N_A - normality of alkali; m - weight of pectin sample, g [34].

The degree of esterification (DE) was calculated (Formula 4):

$$DE, \% = 100 * \frac{V_2}{(V_1 + V_2)}, \quad (4)$$

Anhydrouronic acid content (AUA) was calculated from titration volumes from EW and ME content determination (Formula 5):

$$AUA\ (\%) = \frac{176 * N * V1 * 100}{m * 1000} + \frac{176 * N * V2 * 100}{m * 1000} \quad (5)$$

V_1 - volume of alkali after determination of pectin's EW, mL; V_2 - volume of alkali after determination of pectin's ME, mL; N – normality of alkali (0.1); m = weight of sample, g [34].

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2.4. Fourier Transform Infrared Spectroscopy (FT - IR) of Pectin Samples

The chemical structure of pectin extracted from BP was characterized by Fourier Transform Infrared Spectroscopy (FT-IR) with *Thermo Fischer*, Nicolet 6700, FT-IR Spectrometer for measuring all IR frequencies, Spectral Range (Standard) 7800 – 350 cm^{-1}, Wavenumber Precision 0.01 cm^{-1}.

2.5. Content of Total Phenolic Compounds, Antiradical Scavening Activity of Pectin

The content of total phenolic compounds (TPC) of the pectin extracts was determined according to the Folin-Ciocalteu method with some modifications [45]–[48].

20 ± 0.1 mg of pectin sample was extracted with acetone, ethanol and water solution (7:7:6) in volumetric 100 mL flask, in US bath for 10 min. 2.5 mL of Folin- Ciocalteu reagent (diluted 10 times with water) was added to 0.5 mL of extracted sample in test tubes and after 3 minutes 2.0 mL sodium carbonate (Na_2CO_3) solution (7.5 %) was added. The resulting solution was mixed and allowed to stand for 30 minutes at 20 ± 1º C in dark place. Absorption was read at 765 nm with JENWAY 630 Spectrophotometer. Gallic acid (0 – 100 mg L^{-1}) was used for calibration of a standard curve. The results were expressed as milligram Gallic acid equivalent per 100 g of dry weight (mg GAE 100 g^{-1} DW). Quantification was based on a standard curve (Formula 6).

$$y=0.0313\ x+0.055,\ R^2=0.999, \quad (6)$$

y - Total Content of Phenolics, TPC, mg GAE 100 g^{-1}, x - Absorption at λ=765 nm, R – coefficient of determination.

Antiradical scavenging activity of pectin samples was determined with DPPH method on the basis of scavenging activities of the stable 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical. The DPPH reagent (4 mg) was dissolved in MeOH (100 mL) for a solution concentration of 40 μL/mL. To determine the scavenging activity, 100 μL DPPH reagent was mixed with 100 μL of sample extract in a test tube and was incubated at room temperature for 30 min. After incubation, the absorbance was measured at λ=514 nm. The antiradical scavenging activity (A, %) of pectin material was expressed (Formula 7):

$$A = \frac{AbsC - AbsS}{AbsC} * 100, \% \quad (7)$$

A – Antiradical Scavening activity, AbsC – Absorbance of control sample, AbsS – Absorbance of pectin sample extract [39–41].

Folin - Ciocalteu's Phenol reagent, Sigma-Aldrich, Cat. No. F9252, Sodium carbonate, Merck, Cat No. 106392, Gallic acid monohydrate (3,4,5-Trihydroxybenzoic acid monohydrate), ACS reagent, ≥98.0% Sigma-Aldrich, CAS Number 5995-86-8 2,2-Diphenyl-1-picrylhydrazyl reagent - Sigma-Aldrich, CAS Number 1898-66-4 were used for TPC and Antiradical scavenging activity, DPPH method determination.

2.6. Rheology of Pectin Gel Samples

The ability of forming stable gel was measured for formulated solution of citric acid (7 ± 1 %), sucrose (29 ± 1 %), pectin (6 ± 1 %) and water (58 ± 1 %) for all samples [50]. Solution of pectin/ sucrose and citric acid were diluted in distilled water, mixed and heated at 95 ºC for 10 minutes; afterwards, cooled and stored at + 4 °C until rheological measurements.

Rheological properties of gels from pectin were determined with *Anton Paar Modular Compact Rheometer, Smart Pave 102*, Germany. G' (the storage modulus - the elastic component of the material) and G'' (the loss modulus), the viscosity η, loss factor, $\tan(\delta) = G''/G'$ were determined with shear rate from 0.1 to 100 s^{-1} at 25 °C[51], [52].

 doi:10.20944/preprints202211.0499.v1

Apple pectin (Merk, Sigma Aldrich, Germany) was used to compare physical, chemical and rheological properties.

All measurements were carried out for three independent samples (n=3) and the results were expressed as mean values ± standard deviation (SD). A mathematical analysis of the data has been performed using MS Excel Data Analysis, ANOVA, a Single-Factor, Correlation and Regression analysis were used. The protruding hypotheses have been tested with a p-value method and the factors have been evaluated as relevant if $p < \alpha = 0.05$. In the analysis of variance, the Tukey and Friedman test was used to justify the differences in the results between the studied samples.

3. Results

3.1. Extraction of Pectin and Pectin Yield

The yield of pectin (PY) from by-products (BP) after juice production is attached in Figure 1. PY was different and varied from by-product's source. The highest yield of pectin was obtained from apple BP (17.8 ± 2.6 %) and pumpkin BP (16.2 ± 1.8 %). PY from cherry BP 4.47 ± 1.12 %, red currants BP 6.53 ± 0.86 %, gooseberries BP 7.01 ± 0.65 %, black currants BP 11.9 ± 1.66 %, plums BP 9.64 ± 2.17 %, rhubarb BP 12.0 ± 2.4 %.

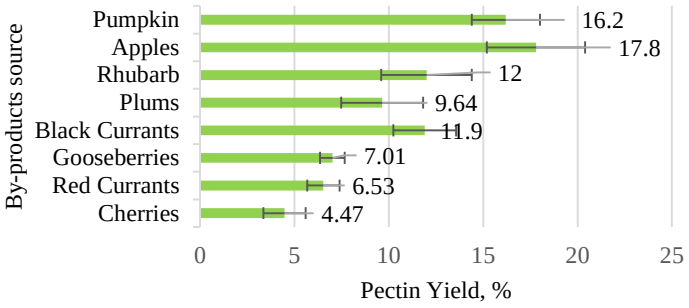

Figure 1. Pectin Yield from food production by-products extracted with hot water- citric acid

The data are presented as mean values (n=3)

Conventionally dried pectin from different BP (T = 40 ºC ±2 ºC) is attached in Figure 2.

Figure 2. Pectin obtained from juice extraction and primary food production by-products

 doi:10.20944/preprints202211.0499.v1

3.2. Ash and Moisture Content

The moisture content (Table 1) of extracted and conventionally dried pectin from by-products varied from 5.17 ± 0.12 % to 6.12 ± 0.16 %. Commercially produced pectin from apples has moisture content ≤10 %, therefore, it can be considered that the moisture content in extracted pectin doesn't enhance the growth of micro-organisms and production of pectinase enzymes and cannot further affect the pectin quality.

Ash content (Table 1) in extracted pectin samples ranged from 1.42 ± 0.06% to 3.22 ± 0.21 %. Pectin from apple Sigma-Aldrich CAS Nr. 9000-69-5 (residue ≤7 %). For good-quality gel formation from pectin, the maximum limit for ash content is 10 %. Therefore, the ash content in the research indicates the purity of pectin from by-products.

Table1. Properties of pectin obtained from juice extraction and primary food production by-products

Source	Moisture, %	Ash, g 100 g^{-1}
Cherries	6.0 ± 0.1	1.94 ± 0.05
Red Currants	6.4 ± 0.1	1.42 ± 0.06
Gooseberries	7.4 ± 0.2	2.24 ± 0.08
Black Currants	6.2 ± 0.1	2.68 ± 0.02
Plums	5.2 ± 0.1	2.11 ± 0.24
Rhubarb	7.2± 0.2	2.14 ± 0.08
Apples	6.5 ± 0.1	2.88 ± 0.14
Pumpkin	7.2 ± 0.1	2.65 ± 0.18

Mean ± SD; the data are presented as mean values (n=3)

3.3. Equivalent Weight, Methoxyl Content, Degree of Esterification, Anhydrouronic Acid Content

Equivalent weight (EW, g mol^{-1}), (Table 2) in extracted pectin samples ranged from 417.2 ± 134.0 g mol^{-1} in Rhubarb BPP to 1390 ± 469.0 g mol^{-1} in Cherry BPP, compared with commercial apple pectin from Sigma Aldrich 708.5 ± 24.5 g mol^{-1}. EW was significantly different between samples from different sources ($p>0.05$).

Degree of methyl esterification ((DE), Table 2) is the main information about pectin properties required for many applications [53], [54]. Many of the galacturonic acid residues have been esterified and, thereby, form methyl esters. Pectin with a degree of esterification DE > 50% is classified as high methoxyl (HM) pectin and low methoxyl (LM) pectins have a DE < 50%. The obtained pectin from BP is HM pectin as DE is >50 %, except pectin from plums BP (DE = 45.16 ± 3.74 %). LM pectin is gelled with calcium ions and independent on the presence of acid or high solids content[15], [19], [55].

Methoxyl (ME) content and anhydrouronic acids (AUA), mainly galacturonic acid (GalA) levels in pectin can affect the structure and texture of the pectin gel formed in pectin samples[56], [57]. ME in BPP (Table 2) ranged between 4.27 ± 1.04 % in BPP from cherries to 8.13 ± 0.45 % in BPP from black currants. AUA (Table 2) in pectin is recommended 65 % to be used as food additive or for pharmaceutical purpose as it is the indicator of the purity[12], [23]. This requirement limits the potential sources of food and pharmaceutical pectin. In samples from cherry BPP AUA was 38.23 %, for Sigma Aldrich Apple pectin and gooseberries BPP AUA also was < 65 %, 57.38 ± 1.47 and 52.70 ± 8.09, respectively. Pectin could also affect the viscosity of pectin solutions in further usage and pectin with lower DE could create a better condition for forming gels [14], [58].

Table 2. Properties of pectin obtained from juice extraction and primary food production by-products

Source of pectin	DE, %	EW, g mol^{-1}	ME, %	AUA, %

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Cherries	64.06 ± 3.83^{b}	1390.0 ± 69.0^{a}	4.27 ± 1.04^{a}	38.23 ± 11.05^{a}
Red currants	57.12 ± 4.07^{a}	716.9 ± 80.95^{b}	6.35 ± 1.84^{c}	64.11 ± 20.60^{b}
Gooseberries	57.14 ± 7.18^{a}	895.8 ± 316.8^{c}	5.53 ± 0.77^{b}	52.70 ± 8.09^{c}
Black currants	54.69 ±2.67^{a}	465.0 ± 72.04^{d}	8.13 ± 0.45^{e}	84.74 ± 8.48^{d}
Plums	45.16 ± 3.74^{c}	531.6 ± 95.8$^{e, d}$	5.15 ± 2.10^{b}	63.31 ± 9.49^{b}
Rhubarb	54.32 ± 0.44^{a}	417.2 ± 34.0^{d}	5.52 ± 2.85^{b}	78.63 ± 5.06^{d}
Apples	56.03 ± 3.03^{a}	581.0 ± 46.1^{e}	6.86 ± 0.77^{c}	69.36 ± 5.54^{b}
Pumpkin	51.11 ± 0.69$^{a, c}$	426.0 ± 8.7^{d}	7.61 ± 0.30$^{d, e}$	84.55 ± 2.35^{d}
Sigma Aldrich Pectin	56.69 ± 0.39^{a}	708.5 ± 24.5^{b}	5.73 ± 0.11$^{b, c}$	57.38 ± 1.47$^{c, b}$

Similar letters (a, b, c, d, e) indicate no significant difference among samples in column ($p>0.05$). Mean ± SD; the data are presented as mean values (n=3)

3.4. Fourier Transform Infrared Spectroscopy (FT - IR) of Pectin Samples

The chemical structure of pectin extracted from various sources of by-products was characterized by FT-IR and their spectra are presented in Figure 3. FT-IR spectra in the region between 800 and 1300 cm^{-1} are considered as the 'finger print' region for carbohydrates which allows an identification of major chemical groups specific for particular polysaccharides[34]. It can be observed that the samples extracted from by-products of berries, apples, pumpkin have the spectra in the region similar to those of apple pectin from Sigma Aldrich, therefore, the extracted polysaccharides obtained in this study were pectin. Bands in the region at 1760 – 1745 cm^{-1} are related to carbonyl groups, bands at 1640 – 162 cm^{-1} are due to carboxylate ion stretching, band between 3600 and 3000 cm^{-1} is attributed to the stretching of O-H groups of the galacturonic acid polymer[5], [24], [34].

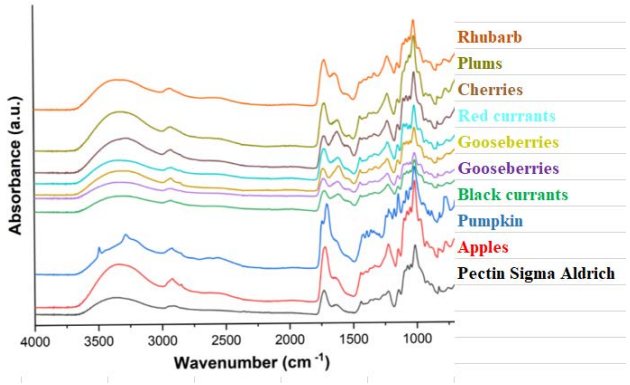

Figure 3. FTIR spectra of the pectin from by-products and control sample from Sigma Aldrich Pectin

3.3. Content of Total Phenolics, Antiradical Scavening Activity (DPPH Method)

Average content of total phenolic compounds (TPC) in pectin extracted from by-products is summarized in Table 4. The highest content of TPC was in pectin from black currants BPP (4.668 ±0.405 GAE, mg L^{-1}) and in pectin from plums BPP (3.102 ± 0.047 GAE, mg L^{-1}). Antiradical scavenging activity ranged from 37.29 ± 2.03 % in black currant BPP. Antiradical scavenging activity (DPPH method) was different in pectin samples, furthermore, it was higher in comparison with control sample – Sigma Aldrich Apple Pectin. There was found strong correlation between TPC content and antiradical activity (r = 0.88).

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Table 4 The Total Phenolic Content (TPC), Gallic acid equivalents (GAE, mg L^{-1}) of the pectin extracts and Antiradical scavenging activity (DPPH method).

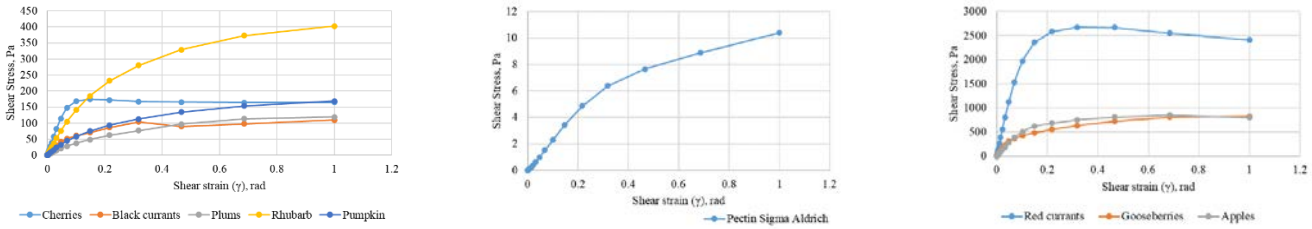
Source	TPC DW, GAE, mg L^{-1}	Antiradical scavenging activity, %
Sigma Aldrich Apple pectin	2.803± 0.002$^{b, c}$	0.56 ± 0.04^{e}
Apple BPP	2.398 ± 0.047^{b}	2.27 ± 0.82^{d}
Rhubarb BPP	2.173 ± 0.024^{b}	8.79 ± 0.44$^{c, b}$
Plums BPP	3.102 ± 0.047$^{c, b}$	11.86 ± 1.12^{b}
Red Currants BPP	2.516 ± 0.048^{b}	7.57 ± 0.88^{c}
Gooseberries BPP	2.657 ± 0.118^{b}	15.97 ± 1.23^{b}
Pumpkin BPP	2.076 ± 0.048^{b}	3.03 ± 1.01^{d}
Black Currants BPP	4.668 ± 0.405^{a}	37.29 ± 2.03^{a}

BPP – by-product pectin. Each pectin sample was prepared in three replications and subsequently analysed in triplicate. Similar letters (a, b, c, d, e) indicate no significant difference among samples in column ($p > 0.05$). Mean ± SD.

3.4. Rheology of Pectin Gel Samples

Pectin gels' gelling ability is important for textural regulation in foods with pectin [59]. The strength of the gel is affected by the pH of the product, the pectin's methoxyl content, and molecular weight [57]. Some studies reported that type of acids influenced the macromolecular and gelling properties of isolated pectin and citric acid was the least pectin degrading, depolymerizing and de-esterifying extracting agent. Low DE pectin could also affect the viscosity of pectin solutions in further usage and pectin with lower DE could create a better condition for forming gels. Higher viscosity of pectin gels may be as a result of smaller particles of solid fraction[51], [57], [60][30].

Shear stress (Figure 4) increased for all pectin gel samples with increasing the shear strain, γ (rad). The highest shear stress was observed for pectin gels from red currants BPP, gooseberries BPP and apples BPP. Shear stress was significantly different for all pectin gel samples in comparison with apple Sigma Aldrich Apple pectin.



(a) (b) (c)

Figure 4. Variation of Shear Stress (Pa) Profile with Shear Strain (γ) of pectin gels
a – Shear stress of gels from Cherries BPP; Black currant BPP; Plums BPP; Pumpkin BPP. b - Shear stress of gel from Sigma Aldrich Apple pectin. C - Shear stress of gels from Gooseberries BPP; Red currant BPP; Plums BPP; Apples BPP.

Complex viscosity (CV) (Figure 5) for BPP gels was different and according to results obtained they are classified in 4 groups. CV from pectin Sigma Aldrich was of the lowest viscosity in comparison to all pectin gel samples from other BPP. CV for all types of pectin gels was found to decrease with increasing the shear strain γ (Figure 5) and demonstrated better shear thinning flow property than that of Apple Pectin Sigma Aldrich gel; for Apple Pectin Sigma Aldrich gel it was observed that complex viscosity was the lowest (Figure 5c) while the highest complex viscosity was observed for samples of red currant BPP gel (Figure 5b).

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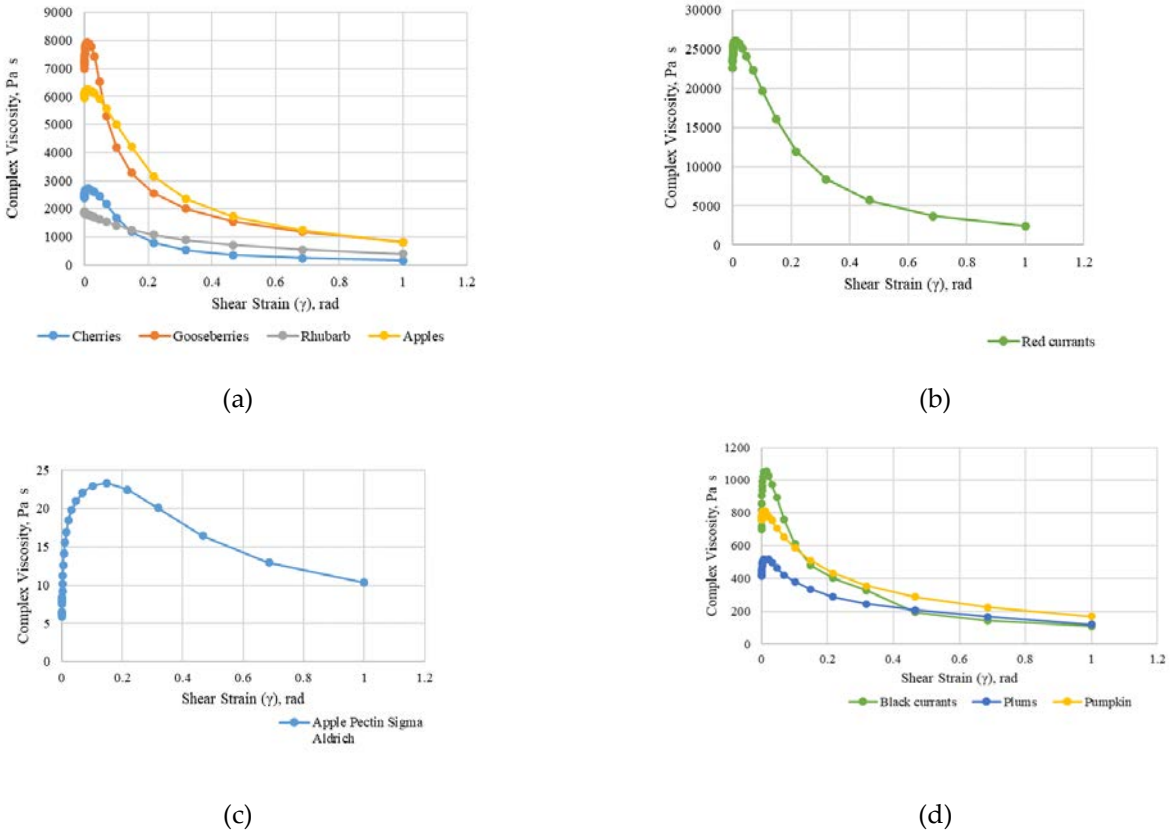

Figure 5. Complex Viscosity - Flow Behaviour of gel samples from by-product pectin
a - complex viscosity of gels from Cherry BPP, Gooseberry BPP, Rhubarb BPP, Apple BPP; b - complex viscosity of gels from pectin Red Currants BPP; c - complex viscosity of gels from Sigma Aldrich Apple pectin; d - complex viscosity of gels from pectin from Black Currants BPP, Plums BPP and Pumpkin BPP.

Storage modulus (G′, Pa) – the elasticity of pectin gels and loss modulus (G″, Pa) were determined, the results of G′, G″, and damping factor (tan δ, G″/G′) are presented in Figures 6, 7 and 8.

G′, elastisity of gels from BPP was higher (Figure 6) in comparison with Sigma Aldrich Apple pectin (Figure 6b). Storage Modulus or elasticity of gels from BPP remain more elastic, moreover, the gel from Red Currant BPP was of the highest elasticity (Figure 6a).

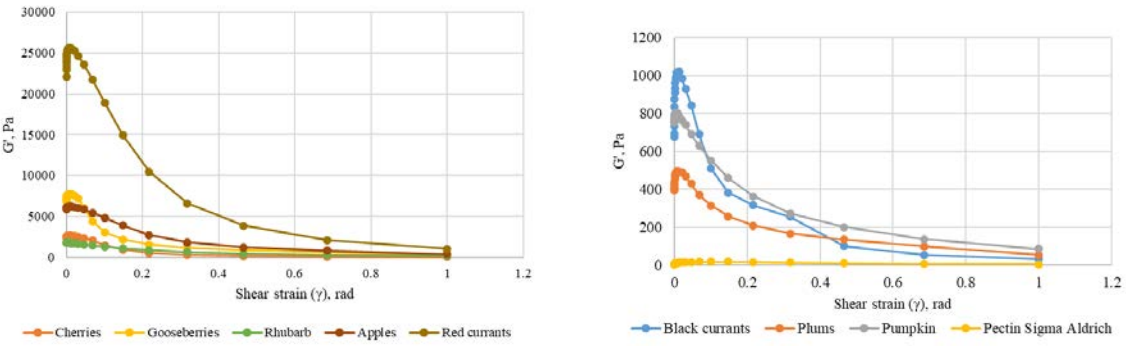

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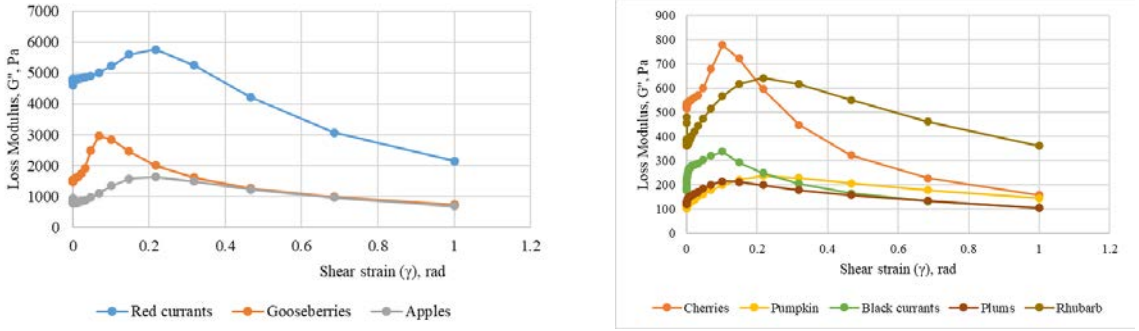
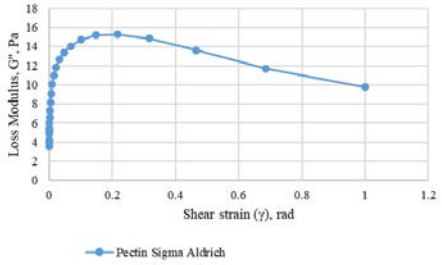
Figure 6. Storage modulus (G′, Pa) – the elasticity of pectin gels
a - Storage modulus of gels from Cherry BPP, Gooseberry BPP, Rhubarb BPP, Apple BPP; Red Currants BPP; b - Storage modulus of gels from Sigma Aldrich Apple pectin; Black Currants BPP, Plums BPP and Pumpkin BPP.

Viscous behaviour of pectin gel samples from BPP - loss modulus *G′′* (Figure 7) was different from samples with Sigma Aldrich Apple pectin; gel samples from Red Currant BPP was of the highest Los Modulus followed by Gooseberry BPP pectin gel and Apple BPP pectin gel. It was observed that *G′* > *G′′* in all pectin samples in the testing area, indicating that pectin gels from extractions remained in the gel network.



(a) (b)



c

Figure 7. Loss modulus – Viscous behaviour of gel samples from by-product pectin
a - Loss modulus of gels from, Gooseberies BPP, Apple BPP; Red Currants BPP; b - Storage modulus of gels from Rhubarb BPP, Cherries BPP; Black Currants BPP, Plums BPP and Pumpkin BPP; c - Loss modulus of gels from Sigma Aldrich Apple pectin.

The ratio G′/G″ determines the condition for weak and strong gels [51]. With the increase of angular frequency - shear strain, the damping factors – loss factors of all pectin gel samples increased (Figure 8), therefore, indicating that gels would change to liquid (damping factor >1) when higher angular frequency was applied. The G′/ G″ values of pectin solutions were almost similar to those of Sigma Aldrich Apple Pectin. A loss factor of 1 means the substance is at a transition between a liquid and a solid state, respectively, the gel point [51], [61].

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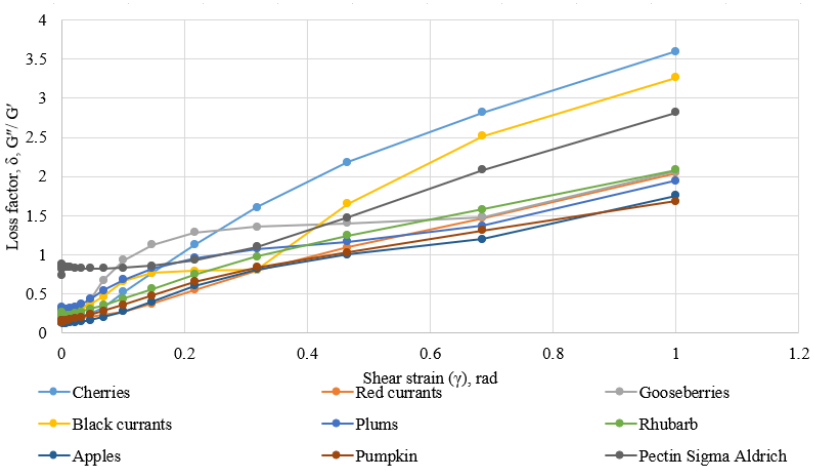

Figure 8. Loss factor of gel samples from by-product pectin

4. Discussion

Although more than 210 years have passed since the discovery of pectin, their chemical and structural properties are still under investigation due to the heterogeneity of this family of polymers. Many factors might influence the results of pectin yield in plant by-products: source of material, harvest time, pH of extracted solution, time and temperature set for the experiment. Apples and pumpkin by-products resulted in highest yields in pectin extraction.

The increased or decreased equivalent weight (EW), probably might depend from the number of free acids, the lower equivalent weight could be of partial degradation of pectin.

The methoxyl content (ME) – the number of moles of methyl alcohol in 100 mol galacturonic acids. in pectin has important role in determining the functional properties of pectin; the structure and texture of forming pectin gel, controlling the setting time[62].

Methods for determination the rheological properties are necessary for characterization of gels as they contain a certain fraction of particles, moreover, rheological behaviour of these suspensions is important for a wide range of industrial, natural, and biological products and processes[14].

Structure of gels depends on the interaction between pectin, sugar and acid. In our research all gel samples were prepared in equal concentrations of sucrose/ pectin/ citric acid. Commercially obtained pectin is in pure form without other natural substances, for example, pectic acids and sugars, and for better gelling it needs to add pure carbohydrates – sucrose [28]. Gelation ability of gels containing pectin depends on the concentration and molecular weight of pectin - a higher molecular weight promotes gelling [63], [64]. The fruit and berries BPP contain natural carbohydrates and there is no need to add them or add as much as it is added by using commercial pectin in powdered form [28], [65]. For pectin with high methoxy groups, solutions with high sugar concentration, such as sucrose or similar carbohydrates - dextrose, etc., are usually used in an appropriate amount, which act as a dehydrating agent for pectin molecules, thus allowing a closer connection between the polymer chains. Pectin gels from gooseberries BPP and Apple Pectin Sigma Aldrich formed stronger gel and the damping factor or loss modulus at the gel stage was higher for those samples.

It is observed that viscosity is dependent on polymer chain length and the degree of polymerization as well, diluting the polymer with too many additives may decrease viscosity and lead to a poor gelling ability [51]. Some researchers found that pectin with higher molecular weight and viscosity can be obtained by using citric acid as solvent instead of mineral acid. In our study results show that high complex viscosity can be obtained with weak organic acid [34], [66], [67].

The radical scavenging activity of pectin from different sources of by-products was assessed against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, based on electron transfer, involving the reduction of a coloured oxidant; all BPP samples were of high antiradical scavenging activity. Antioxidant activity is important for application of pectin for food products and biomedical products[68]. Depending on the number of aromatic rings, more than 8000 different phenolic compounds have been identified, moreover, phenolic compounds are reported to have strong antioxidant activity that can be associated to their ability to destroy oxygen derived free radicals, break radical chain reaction and/or chelate metals [69], [70].

5. Conclusions

By-products after juice production from fruits and berries are rich source for pectin extractions and its content can reach up to 17 %. Pectin is with high degree of esterification and low ash content. Rheological properties – texture and gel stability can be strongly linked to chemical composition - degree of esterification and methoxyl content. Pectin due to its high content of phenolics and antioxidant activity can be used in food industry and in cosmetics as gelling or binding agent.

Acknowledgment

This work has been supported by the European Regional Development Fund within the Activity 1.1.1.2 "Post-doctoral Research Aid" of the Specific Aid Objective 1.1.1 "To increase the research and innovative capacity of scientific institutions of Latvia and the ability to attract external financing, investing in human resources and infrastructure" of the Operational Programme "Growth and Employment" (No.1.1.1.2/VIAA/4/20/656).

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