INFLUENCE OF PROCESSING AND EXTRACTION TECHNOLOGIES ON PHYSICOCHEMICAL AND RHEOLOGICAL PROPERTIES OF PECTIN OBTAINED FROM PUMPKIN BY-PRODUCTS

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Abstract

Pumpkin (Cucurbita spp.) – a tropical fruit in the family Cucurbitaceae is widely grown worldwide – including Northern Europe region. During pumpkin processing by-products – pumpkin pulp and peel remain, still containing pectin which has a unique chemical structure and physical properties. Ultrasonic (US), microwave (MW), conventional hot water/ acid (HW) was used to determine the most efficient pectin extraction method. Pectin yield (PY) calculated on a dry matter (DM) basis reached 3.8% – 16.2 % in pumpkin by-product samples. Galacturonic acid (Gal A) content reached 75.34 g 100 g $^{-1}$, methoxy (ME) content - 9.55 %, equivalent weight was from 685 – 745, moisture content ≤10%, ash of extracted pectin ≤10 %. Glucose was the predominantly in pectin samples – in pectin from pumpkin peel - 31.65±9.11 g 100 g^{-1}. Fucose was found in small amounts in all pectin samples – 0.14 – 0.87 g 100 g $^{-1}$, Rhamnose 2.59 – 7.48 g 100 g $^{-1}$, Arabinose – 1.82 – 7.58 g 100 g $^{-1}$, Galactose, 2.42 – 17.11 g 100 g $^{-1}$, Xylose 0.23 - 2.23 g 100 g $^{-1}$, Mannose 0.24 – 1.38 g 100 g $^{-1}$, Glucuronic acid (GlcA) – 0.32 – 1.01 g 100 g $^{-1}$. The gelling properties and gel forming was different according to method of extraction and methoxy content in pectin samples - better gel forming was detected for pectin gels with pectin obtained from pumpkin pomace with US and MW. The results demonstrate that extraction without acid, assisted with microwaves and ultrasound can be effectively used as environmentally friendly methods for isolation of high-methoxylated pectin with high content of galacturonic acid (>50 %) from by-products after pumpkin processing. Pectin from pumpkin by-products further can be used as thickener, gelling agent, cation-binding agent for food and cosmetics industries.

Keywords: *esterification, galacturonic acid, gel, microwave, monosaccharides, texture, ultrasound*

1. INTRODUCTION

Reducing food loss and food waste has an increasing 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 (Stenmark *et al.*, 2016). During primary food production from vegetables by-products occur – peel, pomace, therefore about 18 – 21% of the fruit is discarded as peel or seeds still rich in biologically active substances such as fibres, vitamins, carotenoids, organic acids, micro and macronutrients that play an important role in human health and it has been found out that the concentration of bioactive compounds in by-products is higher than in the edible part of the fruit (Konrade *et al.*, 2016; Strati and Oreopoulou, 2014).

Pumpkin (*Cucurbita* spp.) by-products are promising sources of pectin, which can be further used in food as E440a (pectin) and E440b (amidated pectin), cosmetics and non-food industries as thickener and gelling agent, cation-binding agent, as well as sources of antioxidants as polysaccharides from pumpkins have such chemical structure that provides anti-diabetic, immunomodulating, antitussive, astringent biological properties (Bush, 2014; Simpson and Morris, 2014).

Pectin is complex, acid-rich polysaccharide from plant cell walls - multifunctional and versatile hydrocolloid (Bush, 2014; Bai *et al.*, 2020). Structurally, pectin is formed of three main regions: homogalacturonan (HG), rhamnogalacturonan I (RG-I) and 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 (Dranca, Vargas and Oroian, 2020). Structure and properties are important for functionality of pectin for food and cosmetics product design (Gawkowska, Cybulska and Zdunek, 2018).

The global pectin market was 887.67 million USD in 2020 and it is predicted to reach growth of 7.27 % to 2026 (Sedej *et al.*, 2011). Commercially pectin is extracted from citric fruits and apples, 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 (Gamonpilas *et al.*, 2021; Wandee *et al.*, 2021).

The yield and composition of pectin depend on the plant source, harvest time, extraction technique and conditions employed during pectin isolation and purification (Maran and Priya, 2015; Anh *et al.*, 2021). Research of different extraction methods and results of pectin yield have been studied: conventional heating in acid, enzymatic extraction, microwave, ultrasound, combined methods electromagnetic, autoclaving (Kratchanova, Pavlova and Panchev, 2004; George *et al.*, 2014; Sharma *et al.*, 2015). Extraction of pectin with 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) (Koubala *et al.*, 2008). Usage of these methods can rise the degradation of pectin and the method is considered as time consuming and can cause irreversible environmental pollution. To minimize the impact on the environment researchers found that pectin can be extracted with water from the middle lamella of the cell walls where it is located in plants (Taboada *et al.*, 2010). Pectin with higher molecular weight and viscosity can be obtained by using citric acid as solvent instead of mineral acid.

Therefore, the aim of research was to elaborate the most effective and environmentally friendly methods and materials for utilizing pumpkin by-products – peel and pomace obtained in food processing and to determine the yield of extracted pectin, physical, chemical and rheological properties – ability to form gels for further usage in food and cosmetics industries.

2. MATERIALS AND METHODS

Pumpkin (*Cucurbita pepo* L, Pink Banana Jumbo) by-products – peel (PP) and pomace (PO) were obtained from Lat Eko Food Ltd., 2021. By-products were washed, grinded with single speed food processor –*Robot-Coupe Blixer3*, France, dried with conventional air dryer *Essiccatori professionali B.Master, SR19390,* Italy, temperature +40 ºC, 36 – 48 h to moisture content 5 - 8 %. Drying, washing and grinding was done at the Institute of Horticulture, Latvia. After drying materials were grinded and sifted through 1.2 mm sieve, collected and kept in plastic boxes in dark place +20ºC ± 2 ºC till extraction and analyses.

2.1. Sample preparation and extractions, determination of pectin yield, PY

1. 20 g of dried pumpkin by-product sample was dissolved in a) distilled water (1:15, w/v), pH= 4 – 4.5 and b) with 0.1M citric acid (1:15, w/v), pH values pH=2.2 – 2.7.
2. Pumpkin by-products (fresh) were dissolved in a) distilled water (1:6 (w/v), pH=3 – 3.5 and b) with 0.1M citric acid (1:6 (w/ v), pH= 2 – 3.5.

Different extraction methods (MW – microwave, HW – hot water extraction; US – Ultrasound) were evaluated to obtain the highest yield of pectin from pumpkin by-products. All samples were prepared and analysed in triplicate (n=3).

Hot water extraction (HW) was done according to citric acid method (CA) with citric acid or without acid with some modifications (Sun, Chen and Zhu, 2020). The solutions of prepared samples were heated at 80 °C for 60 min, cooled and centrifuged (*Sigma 4-16KS,* Germany) at 6000 rpm for 15 min. Collected supernatants were treated with absolute ethanol at +4±1 °C for 14 – 16 h (1:2, v/ v). The pectin precipitates were collected by re-centrifugation, and then were washed twice with ethanol. The pectin was dried (DW≤9 %) at temperature from +35 ºC to +40 ºC.

Microwave (MW) heating extraction is an alternative extraction technique, where electromagnetic radiation is involved in the microwave frequency range in the sample to rotate and produce energy in solvent (Anh *et al.*, 2021). The samples were treated with microwave power 1000 W (MW1000) and 800W (MW800) for 10 min.

Ultrasound extraction (US) was performed in ultrasonic processor *UP200S Hielscher Ultrasound Technology*, Germany. The extraction was performed by continuous ultrasound waves at a frequency of 40 kHz temperature of US bath t=+80 ºC±2 ºC; time – 30 min (Sharma *et al.*, 2015; Zouambia *et al.*, 2017; Yang, Mu and Ma, 2018). After MW and US treatment samples were cooled to room temperature +22±2ºC and procedure was done as described for HW extraction.

The pectin yield was calculated (Formula 1):

PY (%) = Weight of dried pectin (g)/ Weight of pumpkin by-product (g) 100 (1)

2.2. Ash and moisture content

For further analyses extracted pectin samples were blended (from dried and fresh) according to method of extraction and source of samples (peel (PP) or pomace (PO)). Ash content in extracted pectin samples was determined according to AOAC 942.05 method (Thiex, Novotny and Crawford, 2012).

Moisture content (%) was determined according to Reference method LVS EN ISO 712:2010 and ISO 712:2009.

2.3. Equivalent weight (EW), methoxyl content (ME)

Determination of pectin's equivalent weight (EW) was done and calculated (Formula 2) with method described by Virk and Sogi (2007). Pectin (0.5 g), ethanol (5 mL), sodium chloride (1.0 g), distilled water (100 mL), and 3 – 4 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.

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

EW - equivalent weight, g mL^{-1};

m - weight of alkali, g;

0.1 - normality of alkali;

V - volume of alkali (mL) (Virk and Sogi, 2007).

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 30 min. Then, 25 ml of 0.25 N HCl was added and titrated against 0.1 N NaOH to the end point (Yusof *et al.*, 2020; Siddiqui and Chand, 2021). The calculation (Formula 3) of ME value was as following:

$$ME, \% = \frac{3.1 * V_1 * N_A}{m}, \quad (3)$$

ME – methoxyl content, %; V_1 - volume of alkali (mL), N_A - normality of alkali, m - weight of sample (g)

2.4. Degree of esterification, Galacturonic acid content and neutral sugar profile in pectin samples

The degree of esterification was determined using the titration method. 10 mL of extracted pectin sample solution (5 mg mL^{-1}) was mixed with phenolphthalein indicator (3–5 drops). The solution was titrated with NaOH (0.1 mol L^{-1}) until the colour of sample solution turned to pink and the volume of NaOH solution was recorded (V). Afterwards the mixture and 2 mL of NaOH solution (0.5 mol L^{-1}) were mixed uniformly and shaken vigorously for 15 min. Then the HCl solution (0.5 mol L^{-1}) was added when stirring until pink colour of indicator just disappeared. Then phenolphthalein indicator (3–5 drops) was added to the mixture and the solution was titrated with NaOH solution (0.1 mol L^{-1}) until the colour of sample solution turned to pink again. The dosage of NaOH solution was recorded (V_2) (George *et al.*, 2014; Xu *et al.*, 2015; Yu *et al.*, 2021).The calculation (Formula 4) of DE value was as following:

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

The galacturonic acid (GalA) content was determined 1) by colorimetric titration of carbazole sulfuric acid with some modifications (Rodsamran and Sothornvit, 2019).

1 mL of pectin solution was fully reacted with 5 mL concentrated sulfuric acid and hydrolysed 20 min at 75 ºC in a water bath. Afterwards, alcohol-based carbazole solution (200 µL, 0.15 %, w/v) was added to the cooled reaction mixture and it was left to colour in a dark environment for about 2 h. The absorbance was measured at 530 nm with *M501 Single beam Scanning UV/Visible Spectrophotometer (Camspec UV)*, United Kingdom. The Gal A content was calculated by a standard curve of D-(+)-Galacturonic acid (0 – 200 µg mL^{-1}), (Merk, Sigma Aldrich, Germany).

2) Neutral sugars and galacturonic acid of extracted pectin samples were analysed by acid hydrolysis followed by high-pH anion exchange chromatography with pulsed amperometry detection (HPAEC-PAD) and GC-MS analysis (triplicate runs) (Ruthes et al., 2017). Two-step sulfuric acid hydrolysis (72 % H_2SO_4, 3 h, followed by 1 M H_2SO_4, 100 °C, 3 h) was performed for the pectin samples. TFA hydrolysis (2 M TFA at 121 °C for 3 h) was performed on the extracts using 1.00±0.02 mg of dried pectin material. The hydrolysed monosaccharides were analysed by HPAEC-PAD on an ICS3000 system (Dionex, Sunnyvale, CA) using a Dionex CarboPac PA1 column at 30 °C at a flow rate of 1 mL min^{-1}. For the GC-MS analyses, the remaining hydrolysed samples were dried until complete acid removal, reduced with sodium borohydrate ($NaBH_4$) at 60 °C for 1 h and acetylated with pyridine and acetic anhydride (1:1 v/v; 100 µL) at 100 °C for 30 min.

2.5. Sensory evaluation and rheology of pectin gel samples

2.5.1. Pectin gel sample preparation

Structure of gels depends on the interaction between three components - pectin, sugar, and acid. 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 (Ptichkina, Markina and Rumyantseva, 2008, Danilova *et al.*, 1994). Solution of pectin/ sucrose and citric acid were diluted in distilled water and mixed and heated at 95 ºC for 20 - 30 minutes, cooled and stored at + 4 °C until sensory and rheological measurements.

2.5.2. Sensory evaluation of pectin gels

For testing gel samples were arranged in order to determine the degree of gel forming and strength described by Morten C. Meilgard (2016) with some modifications. Two experienced appraisers evaluated samples. Prepared samples were carried for degree of gel strength and was tested with Multi sample difference test- approach – evaluation by Analysis of variance (ANOVA). Scale used for evaluation: 1 to 9, where 1=very poor strength of gel, 5=weak strength of gel, 9=very strong gel (Meilgaard, 2016). Analyse results of evaluations were interpretated by significance effect of interactions between samples.

2.5.3. Rheology of pectin gels

Rheological properties of gels from different extracted 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. (Dabbaghi *et al.*, 2021; Zhang *et al.*, 2016).

Apple pectin (Merk, Sigma Aldrich, Germany) was used for comparison of physical, chemical, rheological and sensory 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, PY

Pectin (Figure 1) yield (PY) depending from extraction technique was determined. In our research we used citric acid or distilled water in extraction with hot water (HW). Samples of pumpkin by-products were extracted with assisted microwave energy 1000 W (MW1000) and 800 W (MW800) and ultrasound energy (US) without acid.

The yield of pectin (Figure 2) (PY) from fresh by-products varied from 3.6 % to 16.2 % and in dried by-products from 3.3 to 14.8 % depending on extraction type and by-product's source - the highest yield was in fresh pumpkin pomace (PO) in samples obtained with MW 1000 W – 16.2 ± 0.2 % and 11.2 ± 0.3 % with MW 800 W extraction. Extraction of dried PO resulted in PY with MW1000 extraction 14.8 to 16.2 % and with MW 800 from 10.5 to 11.2 % PY. US extraction of peel resulted PY from 8.1 to 8.28 %. Extraction with HW resulted in PY from pomace 3.4 to 7.9 % and from peel 3.3. to 6.4 % of pectin.

There were no significant differences in extracting pectin with hot water with or without acid ($p>0.05$).



Fig. 1. Pectin from pumpkin by-products before drying. **a** - pectin from pumpkin peel, **b** - pectin from pumpkin pomace

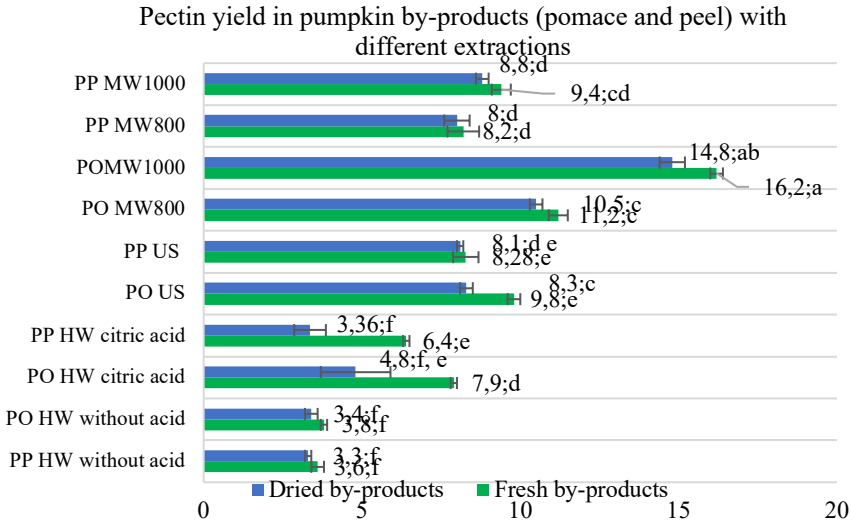


Fig. 2. Pectin yield (PY, %) from pumpkin by-products obtained using different extraction methods

Pumpkin pomace (PO); Pumpkin peel (PP); Hot water (HW); Ultrasound (US); Microwave (MW) assisted extractions; MW1000 - extraction power 1000 W, MW800 - extraction power 800 W; Similar letters (a, b, c, d, e, f) indicate no significant difference among samples ($p>0.05$). The data is presented as a mean (n=3).

3.2. Ash and moisture content

Moisture content of extracted and conventionally dried pectin from pumpkin by-products varied from 5.17 % to 8.64 %. Commercially produced pectin from apple 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 (Dhiman and Attri, 2017).

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, ash content in the research indicates the purity of pectin from pumpkin by-products.

3.3. Equivalent weight (EW), Methoxyl content (ME)

Equivalent weight (EW) (Table 1) in extracted pectin samples ranged from 760±54 to 968±77, compared with apple pectin (833 to 1666.30), lemon pomace pectin from 368 to 1632- it is lower (Azad *et al.*, 2014; Bush, 2014). EW was significantly different between samples with different extraction methods ($p>0.05$). Higher EW was with MW assisted extraction while lower EW with HW without acid.

Methoxyl content in pectin samples ranged between 7.55 % to 8.08 % in pectin from pumpkin peel and 8.05 % to 8.67 % in pectin from pumpkin pomace. Higher ME content in samples prepared using hot water/ acid (HW) extraction, while lower in samples obtained by US extraction.

Source	Extraction	EW	Moisture, %	Ash, g 100 g^{-1}	ME, %
Pumpkin peel	**US**	760±62^{a}	6.0±0.1	1.94±0.05	7.55±0.05
	MW1000	946±38^{c}	6.4±0.1	1.42±0.06	7.82±0.02
	MW800	820±57^{b}	7.4±0.2	2.24±0.08	7.84±0.05
	HW citric acid	944±84^{c}	6.2±0.1	2.68±0.02	8.02±0.02

	HW without acid	720±46[a]	5.2±0.1	2.11±0.24	8.08±0.05
Pumpkin pomace	**US**	792±56[b]	7.2±0.2	2.14±0.08	8.25±0.04
	MW1000	968±77[c]	6.5±0.1	2.88±0.14	8.67±0.03
	MW800	880±88[b, d]	7.2±0.1	2.65±0.18	8.05±0.02
	HW citric acid	956±68[c]	8.6±0.2	2.11±0.24	8.24±0.04
	HW without acid	760±54[a]	8.4±0.2	2.14±0.04	8.22±0.07

Table 1. Properties of pectin obtained from pumpkin by-products with different extractions

US- ultrasound, MW – microwave, HW – hot water. Similar letters (a, b, c, d) indicate no significant difference among samples in column ($p>0.05$). Mean ± SD; the data are presented as mean values (n=3)

3.4. Degree of esterification, Galacturonic acid content, neutral sugar profile in pectin samples

GalA content with UV/ Visible Spectrophotometer in pectin samples ranged from 74.2 g 100 g^{-1} to 80.4 g 100 g^{-1} in PO pectin and 67.3 g 100 g^{-1} to 81.1 g^{-1} in PP pectin (Figure 3). US and HW with acid extraction resulted in higher GalA content and GalA in PP pectin was of higher amount. Determination of GalA by acid hydrolysis (Figure 4) showed different results - different extractions showed the content of GalA from 43.84 g 100 g^{-1} to 75.34 g 100 g^{-1}.

Degree of esterification (DE) (Figure 3) varied from 56.8 % to 64.6 % in samples from PO and DE of pectin from PP was from 67.3 % to 70.0 % and it can be classified as of high degree of esterification. For commercial apple pectin DE is 50 – 75%. Pectin with HW without citric acid from pumpkin by-products showed lower DE, highest DE was obtained for pectin with US and MW800. The difference was likely to have occurred due to the different raw materials used – peel, pulp, extraction conditions and the microwave power, temperature during extractions and time of extraction. The esterification degree of pectin could also affect the viscosity of pectin solutions in further usage (Yoo *et al.*, 2012) and pectin with lower DE could create a better condition for forming gels.

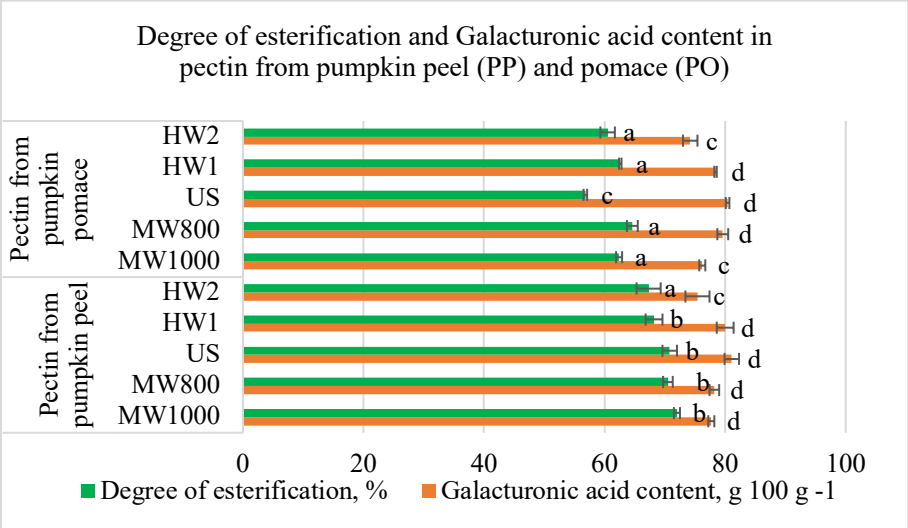


Fig. 3. Degree of esterification, % and galacturonic acid (GalA) content, % with UV/ Visible Spectrophotometer in pectin samples obtained from pumpkin by-products

HW1 – Hot water extraction with citric acid, HW2 – Hot water extraction without acid, MW800-microwave power 800W, MW1000 – microwave power 1000W, US – ultrasound assisted extraction,

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

Glucose is the main monosaccharide in pectin samples, in samples from PP its content is significantly higher than in PO ($p>0.05$) (Table 3). Arabinose was found from 1.82 ± 0.01 g 100 g^{-1} to 7.58 g 100 g^{-1}, rhamnose – 2.59 g 100 g^{-1} to 5.95 g 100 g^{-1}, fucose from 0.09 to 2.68 g 100 g^{-1}, galactose in pectin samples from 2.42 g 100 g^{-1} to 17.11 g 100 g^{-1}. The highest content of mannose was determined in pectin samples from pumpkin peel with US extraction -1.38 ± 0.02 g 100 g^{-1}. Xylose and GlcA was in very low concentrations (≤ 1.34 g 100 g^{-1}).

	Fuc, g 100 g^{-1}	Rha, g 100 g^{-1}	Ara, g 100 g^{-1}	Xyl, g 100 g^{-1}	Man, g 100 g^{-1}	GlcA, g 100 g^{-1}
PP US	0.26±0.02^{c}	3.53±0.82^{c}	5.17±0.12^{a}	0.84±0.02^{c}	1.38±0.02^{a}	0.85±0.02
PO US	0.23±0.04^{c}	5.17±0.19^{a}	2.51±0.44^{d}	2.23±0.01^{a}	0.78±0.01^{b}	1.01±0.05
PO MW800	0.18±0.01^{c,d}	5.06±1.12^{a}	2.53±0.09^{d}	0.45±0.02^{c}	0.41±0.12^{c}	0.76±0.12
PO MW1000	0.14±0.2^{d}	2.59±0.91^{d}	5.76±1.24^{a}	0.23±0.01^{c}	0.30±0.02^{c}	0.71±0.14
PP MW800	0.09±0.01^{e}	2.75±0.12^{d}	1.82±0.02^{e,d}	1.34±0.02^{b}	0.50±0.02^{c}	0.32±0.02
PP MW1000	0.22±0.02^{c}	4.86±1.24^{a}	5.50±1.09^{a}	0.56±0.04^{c}	1.13±0.02^{a}	0.97±0.05
PP HW2	2.68±0.17^{a}	4.47±0.96^{a}	3.52±0.09^{c,d}	0.38±0.02^{c}	0.52±0.01^{b}	0.34±0.01
PO HW 2	0.22±0.02^{c}	4.31±0.56^{a}	5.50±0.17^{a}	0.52±0.05^{c}	0.84±0.04^{b}	0.78±0.01
PO HW 1	0.87±0.02^{b}	7.48±1.44^{b}	7.58±0.10^{b}	0.70±0.02^{c}	0.44±0.03^{c}	0.71±0.08
PP HW 1	0.15±0.01^{d}	3.18±0.98^{d}	4.13±0.14^{c}	0.27±0.02^{d}	0.30±0.02^{c}	0.37±0.04
Apple pectin	0.60±0.12^{b}	5.95±1.24^{a}	4.27±1.02^{c}	1.31±0.05^{b}	0.24±0.01^{c}	0.66±0.02

Table 2. Neutral sugar profile of pumpkin pectin samples obtained with different extractions

*Apple pectin sample from Sigma Aldrich. Each pectin sample was prepared in duplicate and subsequently analysed in triplicate. Abbreviations: PP – pumpkin peel, PO - pumpkin pomace, US - ultrasound, MW - microwave, HW – hot water, HW1 – extraction without acid, HW2 – extraction with citric acid. Similar letters (a, b, c, d) indicate no significant difference among samples in column ($p > 0.05$). Mean ± SD; the data are presented as mean values (n=3)

GalA content (Table 3) in all pectin samples from pumpkin by-products with was >50 %, in comparison with apple pectin from Sigma Aldrich – 49.07±3.14. The data received by spectrophotometry resulted in higher Gal A content, though the content of Gal A is high in all pectin samples (>50 %).

	Glucose, g 100 g^{-1}	GalA, g 100 g^{-1}	Galactose, g 100 g^{-1}
PP US	20.86±2.14^{a}	63.84±1.14	13.27±2.12
PO US	19.65±1.10^{a}	56.89±12.14	11.53±3.14
PO MW800	6.92±0.14^{c}	75.34±14.04	8.35±2.11
PO MW1000	12.66±2.13^{d}	71.72±11.17	5.90±1.12
PP MW800	23.13±13.14^{a}	55.41±0.24	4.65±0.14
PP MW1000	12.40±5.14^{d}	61.93±12.13	12.44±3.33
PP HW2	17.93±2.12^{d}	62.68±10.18	7.48±0.62
PO HW2	15.60±3.11^{d}	63.38±6.04	8.84±2.67

PO HW1	21.20±12.14[a]	58.61±4.99	2.42±0.14
PP HW1	31.65±9.11[b]	55.18±2.18	4.77±0.94
Apple pectin*	34.62±4.14[b]	49.07±3.14	3.25±0.19

Table 3. Glucose, Galactose and Galacturonic acid (GalA) profile in pectin samples with different extractions

*Apple pectin sample from Sigma Aldrich. Each pectin sample was prepared in duplicate and subsequently analysed in triplicate. Abbreviations: PP – pumpkin peel, PO - pumpkin pomace, US - ultrasound, MW - microwave, HW – hot water, HW1 – extraction without acid, HW2 – extraction with citric acid, Similar letters (a, b, c, d) indicate no significant difference among samples in column ($p>0.05$). Mean ± SD; the data are presented as mean values (n=3)

3.5. Sensory evaluation and rheology of gel samples

Gel forming and stability was detected with sensory evaluation to determine components of texture-firmness and gel setting (Meilgaard, 2016). Degree of gel strength was tested with Multi sample difference test- approach – evaluation by analysis of variance (ANOVA). Scale used for evaluation: 1 to 9, where 1 = very poor strength of gel, 5- weak strength of gel, 9 =very strong gel (Meilgaard, 2016). Analyse results of evaluations were described by significance effect of interactions between samples.

Better gel forming was detected for pectin gels with pectin obtained from PO with US and MW1000 (score - 8.7 and 8.8 – ranged as "very strong" gel) samples (Figure 4). Gels with pectin from PP were ranked as gels of "weak" strength. There was a significant difference ($p>0.05$) between gel samples from different extracted pectin - the variances of the different gel samples were unequal ($F>F_{critical}$).

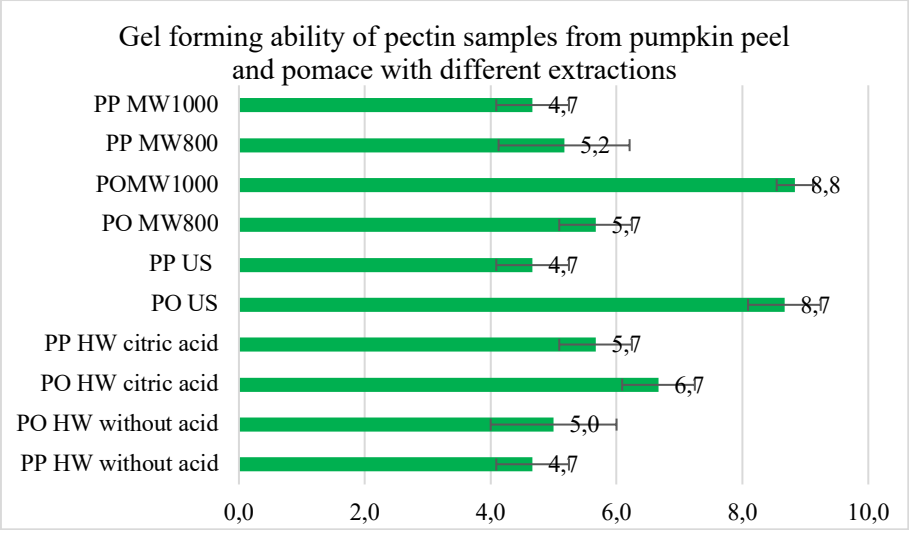


Fig. 4. Sensory evaluation of different extracted pectin gel samples from pumpkin peel and pomace

Each pectin sample was prepared in three replications and subsequently analysed in triplicate. Abbreviations: PP – pumpkin peel, PO - pumpkin pomace, US - ultrasound, MW - microwave, HW – hot water

Rheology for gel samples with pectin was used to characterize the mechanical and textural properties (Shear Stress, Complex Viscosity, Storage Modulus, Loss Modulus, Loss Factor) of extracted pectin samples with pectin concentration of 6 % (w/ v), at 25 °C by rheometer using an apple pectin as a control, and the results are illustrated in Figures 5 - 8.

Shear stress (Figure 5) increased for all pectin gel samples from pumpkin by-products, except for PP pectin with HW extraction without citric acid. The highest shear stress was observed for PO MW1000 – 116.53 Pa

The shear stress obtained and analysed for pectin gels is shown in Figure 5. The slope of the shear stress was observed to increase with increasing the shear strain, γ (rad)**.**

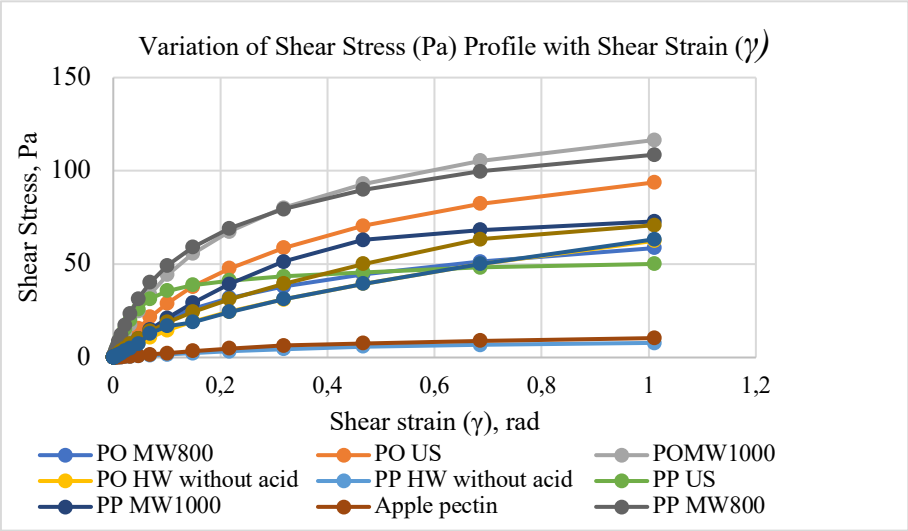


Fig. 5. The shear stress profiles of pectin gels

Complex viscosity (CV) (Figure 6) for apple pectin gel was of the lowest viscosity in comparison to all pectin gel samples from pumpkin by-products. The complex viscosity (CV) for all types of pumpkin pectin gels was found to decrease with increasing the shear strain γ (Figure 6) from 925 to 123.08 Pa demonstrating a better shear thinning flow property than that of apple pectin gel. For apple pectin gel it was observed that complex viscosity was the lowest from 6.48 Pa s to 23.33 Pa s while the highest complex viscosity was observed for samples PPMW800 and PP US at $\gamma = 0.0001$. As shear strain increased to 0.467 CV was significantly higher ($p > 0.05$) for all pumpkin pectin samples in comparison with apple pectin (CV = 6.42 Pa s), for PPMW800 CV = 192.67; PPUS = 97.941 Pa s. Samples PP MW800 and PP US were of the highest viscosity. A medium strong negative correlation between GalA content in pectin samples and complex viscosity was found, measured at 0.217 rad ($r = 0.51$, $p > 0.05$) and correlation was moderate between DE and CV of pectin gels ($r = 0.32$, $p > 0.05$).

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 7, 8 and 9.

G′ for all PP and PO pectin gel samples was higher (Figure 7) in comparison with apple pectin therefore storage modulus or elasticity of gels from pumpkin by-products remain more elastic.

Viscous behaviour of pectin gel samples from pumpkin by-products - loss modulus *G″* (Figure 8) was different from apple pectin gel samples, *G″* for US extracted pectin gel was of the highest indicator. It was observed that $G' > G''$ in all pectin samples in the testing area, indicating that pectin gels from PP and PO with HW, MW and US extractions remained in the gel network.

The ratio G'/G" determines the condition for weak and strong gels. With the increase of angular frequency - shear strain, the damping factors – loss factors of all pectin gel samples increased (Figure 9), 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 apple pectin. A loss factor of 1 means the substance is at a transition between a liquid and a solid state - the gel point (Alam *et al.*, 2020).

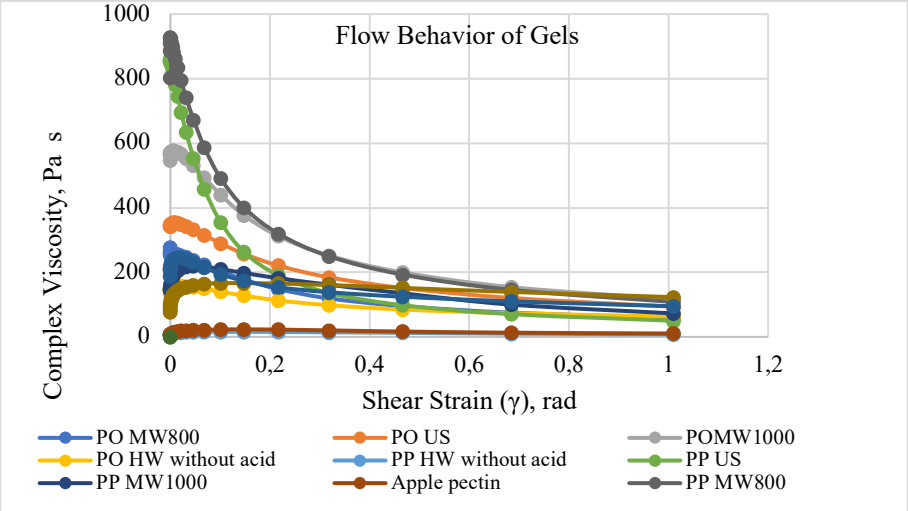


Fig. 6. Complex viscosity of pectin gel samples

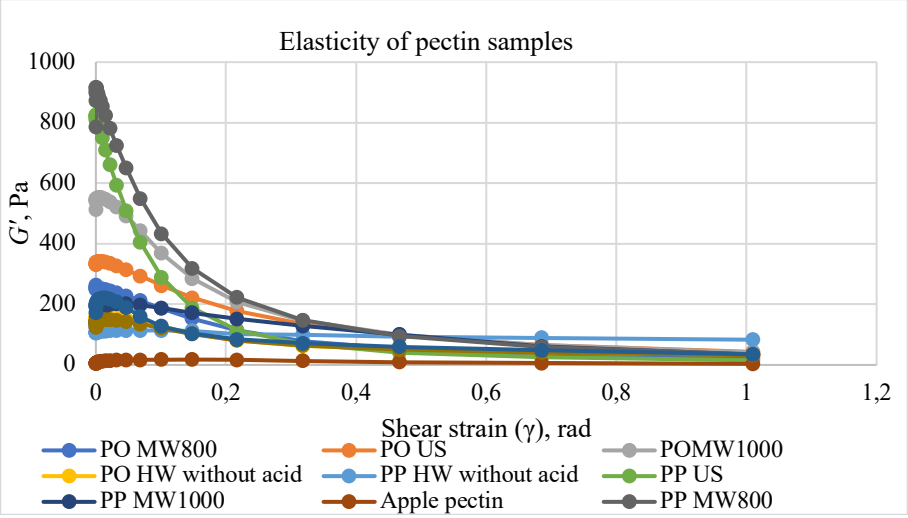


Fig. 7. Storage modulus – elasticity of pectin gel samples

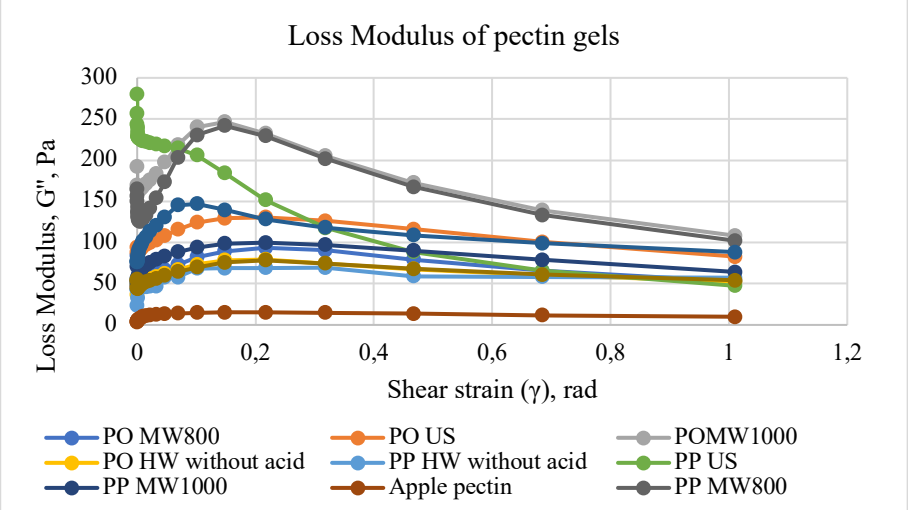


Fig. 8. Loss modulus – viscous behaviour of pectin gel samples

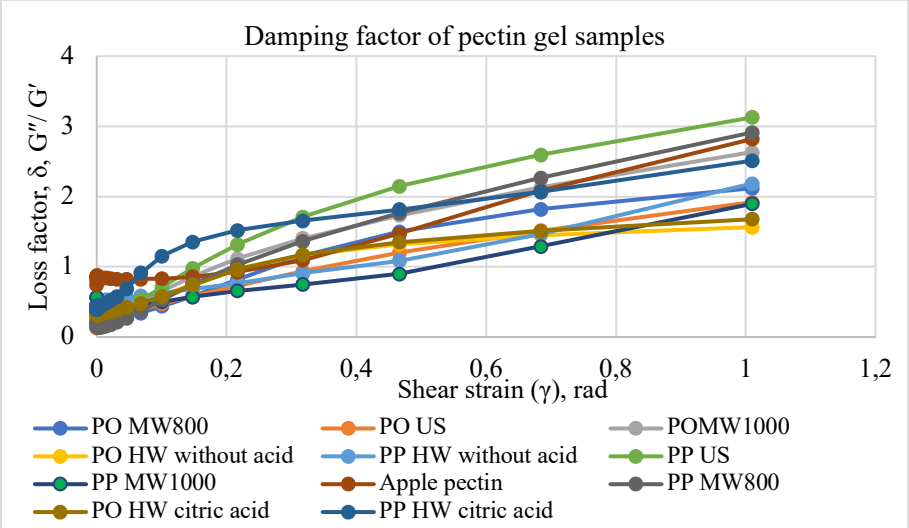


Fig. 9. Damping factor of pectin gel samples

4. DISCUSSION

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 (Perussello *et al.*, 2017). Our research showed higher increase in pectin yield with ultrasound and microwave assisted energy, therefore it is in agreement with previous researches of other authors that it might be due to the disruption of cell wall and release of cellular material at ultrasound and microwave power exposure (Kratchanova, Pavlova and Panchev, 2004). Sandarini (2020) found that the optimum extraction time leading to maximum pectin yield was 60 min, longer extraction time resulted to degradation of pectic substances (Sandarani, 2020). For our samples microwave treatment was 10 minutes. Maran and Priya (2015) studied pectin extraction from sisal waste with ultrasound extraction, results showed that the optimal conditions are: ultrasonic power of 61 W; temperature of 50 ºC; time of 26 min and solid/ liquid ratio of 1:28 g mL^{-1}, increase in the yield with the increase of ultrasonic power (up to 65 W) was observed and then decreased (Maran and Priya, 2015). The highest yield of pectin (PY=7.1 %) with MW from unutilised pumpkin after seed collection for oil extraction was observed at t=102.2 ºC, T=10 min (Ko, Aguedo and Hromádková, 2016). The increase in the pectin yield was a result of microwave heating, though the time and energy should be controlled to avoid the burning or overheating of the materials (Kratchanova, Pavlova and Panchev, 2004). Pectin's yield with MW from pumpkin cup and skin increased (to 24.21 % in pumpkin skin) by increasing the temperature to 90 ºC and by increasing the extraction time to 30 minutes, though the microwave energy was very low (20 W). Microwave heating over conventional systems has advantages: a rapid energy transfer, selective heating, faster throughputs, fast on and off switching, clean environment at the point of use and compact equipment (Ko, Aguedo and Hromádková, 2016). Kratchanova (2004) found that microwaves generate an internal heating of the sample that leads to a better material disruption and a higher diffusion of components out (Kratchanova, Pavlova and Panchev, 2004).

The increased or decreased equivalent weight (EW), probably might depend from the amount of free acids, the lower equivalent weight could be of partial degradation of pectin (Yang, Mu and Ma, 2018). Citric acid/ hot water extractions and microwaves resulted in higher EW while lower EW was with ultrasound assisted extractions.

The methoxyl content (ME) – the number of moles of methyl alcohol in 100 mol galacturonic acids. ME of pectin has important role in determining the functional properties,structure and texture of forming pectin gels, controlling the setting time (Bush, 2014; Siddiqui and Chand, 2021; Liu *et al.*, 2020). Composition of by-products and source – pomace and peel also showed different metoxyl content – for pomace ME was higher in comparison with peel.

Pectin is a complex polysaccharide that consists of multiple different monosaccharide residues mainly rich in galacturonic acid (GalA) and the amount of galacturonic acid residues in pectin is an important parameter in the quantitative and structural analysis (Bush, 2014). Galacturonic acid is an acidifying agent in foods (Takamine, Ogutu and Potato, 2007), therefore GalA content is important for further usage and purpose of pectin, commercial pectin from apples contains GalA ~ 50 %. Sedigheh (2021) found that the amount of galacturonic acid in pumpkin peel/ skin and cap 38.04 % and 46.6 % (Yazdanpanah, 2021). In our study GalA content was high in all pectin samples and there were not significant differences between pectin from peel and pomace ($p>0.05$).

Virk (2007) found that an increase in acid strength, decreased the galacturonic acid content (Virk and Sogi, 2007). 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 value of GalA means that the extracted pectin might have a high amount of protein, therefore can be predicted that pectin from pumpkin by-products has low content of protein (Azad *et al.*, 2014).

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. Initially, pectin is composed of at least 17 different monosaccharides, the largest of which is galacturonic acid (GalA), linear chains of it are the main feature of pectin - obstructed by neutral sugar units or short chains (Mohnen, 2008). The most common neutral sugars are galactose (Gal) and arabinose (Ara), which form complex chains with varying degrees of polymerisation (Chen, Fu, & Luo, 2016). Monosaccharides can be connected to each other using 20 different bonds. In GalA monomers, carboxyl groups or hydroxyl groups can be esterified with methyl and/ or O-acetyl group. O-acetyl-esterification occurs mainly at the O-3 position and occasionally at the O-2 position. The major constituent in pectin - homogalacturonan (HG) which is (1 4) linked, α-D-galacturonic acid and its methyl ester, it distinguishes pectin from all other polysaccharides. HG may contain independent α-D-xylose units (Yoo *et al.*, 2012).

Glucose and xylose are used to understand the content of cellulose, hemi-cellulose and starch: as cellulose and starch are linear chains of glucose, but xylose and glucose are the backbone of hemi-cellulose (Bai *et al.*, 2020). Pumpkin pectin obtained from peel showed higher content of glucose in comparison of that from pomace, therefore the attention should be paid for further experiments of pectin extraction as xylose content was higher in samples with ultrasound and microwave assistance (power 800 W).

Structure of gels depends on the interaction between three components - pectin, sugar, and acid. In our research all gel samples were prepared in equal concentrations of sucrose/ pectin/ citric acid. Gelation ability of gels containing pectin depends on the concentration and molecular weight of pectin - a higher molecular weight promotes gelling (Perera and Smith, 2013). 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 pumpkin pomace obtained with ultrasound extraction formed stronger gel and the damping factor or loss modulus at the gel stage was higher for samples with US extraction.

Dabbaghi (2021) 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 (Dabbaghi *et al.*, 2021). 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 without acid with microwaves and/ or ultrasound assisted energy.

5. CONCLUSIONS

Pumpkin (*Cucurbita pepo* L, Pink Banana Jumbo) by-products (peel and pomace) occurring after primary food production are a good source for pectin extractions as its content can reach up to 16 %, the extraction by microwave power > 800 W without acid resulted in high yields of pectin. Extracted pectin is with high degree of esterification and low ash content. Ultrasound and microwave assisted extractions could be used as an alternative method to extract pectin from pumpkin by-products with lower extraction temperatures, shorter extraction time, reduced energy and without application of acids for extraction. Rheological properties – texture and gel stability can be strongly linked to extraction method and therefore chemical composition - degree of esterification, GalA content, ME content. The reduce of waste products in primary food production from pumpkins can be solved by promoting value added product - pectin for further usage as gelling or binding agent in food industry or in cosmetics.

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