EXTRACTION OF PECTIN FROM APPLE POMACE

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Abstract. Pectins are biopolymers, which exist inside the cell walls. Apples are the richest source of pectin comparing to other fruits and vegetables. Pectin has many health benefits and might be used in regenerative medicine as drug delivery systems, as dietary fiber, as edible films, to neutralize cholesterol and to lose excess weight. Five cultivars grown in Latvia, Tukums Municipality, Pūre parish in the season of the year 2019: 'Zarja Alatau', 'Antonovka', 'Antejs', 'Sinap Orlovskij' and 'Alesja' were selected for extraction. The study was carried out with the aim to investigate the extraction possibilities of pectin from apple pomace using pH decreasing untraditional acidifying agent – lemon juice. Pectin from apple pomace was extracted using the hot acid extraction method. The content of pectin and sugars (glucose, fructose, sucrose, total sugars) was determined by the colorimetric method and HPLC Shimadzu Prominence LC-20 Refractive Index Detector (RID-10A). The highest level of pectin was determined in the cultivars 'Antejs' (4.9 g per 100 g), 'Sinap Orlovskij' (4.8 g per 100 g) and the lowest in 'Antonovka' (3.9 g per 100 g). The results demonstrated that the highest concentrations of total sugars of glucose, fructose and sucrose were in the cultivars 'Antejs' (Glucose 1.2, Fructose 6.6, Sucrose 4.1 = Total sugars 11.9 g per 100 g) and 'Alesja' (Glucose 1.2, Fructose 6.6, Sucrose 3.6 = Total sugars 11.4 g per 100 g), the lowest in 'Antonovka' (Glucose 0.9, Fructose 6.0, Sucrose 1.4 = Total sugars 8.3 g per 100 g). The best content of pectin and total sugars from all cultivars was detected in the apple cultivar 'Antejs'.

Keywords: apple pomace, pectin, sugars.

Introduction

Food and pharmaceutical industries make products for human life and health, but sometimes, the artificial agents – preservatives, flavours, colorants, sweeteners and binding agents, are very hard usable for internal organs, like the intestinal tract and liver, because there are possible toxic accumulations [1]. Therefore, one of the solutions is to use materials from natural sources, like heteropolysaccharides, such as pectins. Pectin is the binding material for the cell wall of plants present in the middle lamella and primarily made up of α-1.4-D-galacturonic acid units and is a complex substance of homogalacturonic acid, which contains the side chain of carbohydrates – arabinose, rhamnose, galactose, fucose, mannose and glucose [2-5]. The largest natural sources of pectin in nature are citrus peel 20-30 % and apple pomace (18-19 %), which are used for commercial extraction of pectin, but in nature exist other sources, such as gooseberry, strawberry pomace, sunflower heads, mango peel, banana peel, papaya and sugar beets [4; 5]. Pectin has been considered in the class of dietary fibers with a positive effect on digestive processes [6; 7]. It is used in food industries for confectionery products, jams, fruit conserves, jellies as a gelling agent, emulsifier, thickener, stabilizer [8; 9]. In pharmaceuticals, it reduces the glucose uptake [10; 11], cholesterol levels [12], it is used to prevent colon cancer [13] and has anti-inflammatory properties [14].

Worldwide 85 % of pectin is produced from citrus peels and 14 % from apple pomace [15], and in future its necessity in the global market will increase annually by 8.56 % until 2023. [16]. Extraction from plant cells is the most important process in pectin production. A water-insoluble protopectin form is found in the middle lamellae of plant cells, but water-soluble anionic polysaccharide is formed by fruit ripening or heating fruit substrate in an acidic environment [17; 18]. The regulation of pH is very important for pectin extraction. Most of the procedures aimed at pH decreasing have an adverse effect on human health, since they involve the use of strong acids, such as hydrochloric or nitric [19; 20]. Another method for reducing pH is to use not so dangerous substances as weak organic acids, like citric, tartaric, malic, acetic, lactic and phosphoric acids [21]. The best extraction parameters for powdered pectins from apple pomace are pH = 2.5, temperature = + 88 °C and time 120 min, if citric acid is used [21]. Most of commercial pectins are in powdered form, which means more time is needed for extraction and a mixture of 96 % ethanol is used. An alternative method could obtain pectin in soluble form, it reduces the cost of production, time and no need for purifying from 96 % ethanol. Before extraction of pectin pH is reduced to acidity level. pH can be reduced by adding powdered or liquid form of acidifying agents – organic or inorganic acids – to solution. The production of these acids in pure synthetic, natural form needs time and energy that cost extra money. Fruits or berries, which contain the same organic acids as citric, malic or tartaric acids, can be used as alternative acidifying agents. One of such fruits that can be used as a natural acidifying agent is lemon, which has a high content of citric acid. The aim of the present research was to investigate the extraction possibilities of pectin from apple pomace, using a pH decreasing untraditional acidifying agent – lemon juice.

Materials and methods

Apple origin and harvest

Five apple cultivars were harvested by hand in the early morning in Latvia, Tukums Municipality, Pūre parish in the season of the year 2019, and the stage of maturity was detected by Streif index [22] and Iodine Starch test [23] for 'Zarja Alatau' 0.10, 6.10, 'Antonovka' 0.12, 6.20, 'Antejs' 0.11, 6.00, 'Sinap Orlovskij' 0.15, 5.50 and 'Alesja' 0.16, 6.80. Apples were packed into polystyrene boxes and transported to the laboratory.

Preparation of the apples for extraction

Apples were prepared on the day of harvest and selected based on the absence of physiological and mechanical injuries and washed in tap water. The washed apples were cut into four pieces and the seeds within the core were removed. Sage BJE820 (Sage, UK) equipment was used for the separation of juice from the sample, and apple pomace was obtained.

Extraction method for pectin

Apple pomace was transferred into glass beaker and heated at temperature of + 88 ºC for 120 min in deionized water (Crystal E HPLC System, Adrona, Latvia) with the solid/liquid ratio 1:3 w/v. Heating was carried out using a digital hot plate with thermostat control (Biosan, MSH-300, Latvia). Lemon juice (obtained by Sage BJE820 equipment) was added to water to decrease pH to 2.5 and apple pomace was added to acidified water. After extraction, the solution of the apple pomace water mix was filtered through fivefold cheesecloth and allowed to cool down to + 21 ºC. The obtained filtrate was collected and then concentrated in a vacuum rotary evaporator Heidolph Laborota 4000 (Heidolph Instruments GmbH & CO. KG, Germany) at + 40 ºC to the end Brix % 9.0.

Determination of total pectin

Total pectin was determined according to the spectrophotometric method described by Mohamed [24]. 0.1g of samples were mixed with 300 mL of 0.05 M of the sodium salt of ethylene-diaminetetraacetic acid (EDTA-Na_2, Merck, Germany) and the pH was increased by adding 1M NaOH (Sigma-Aldrich, USA) to reach 11.5. After standing at the room temperature for 30 min, the pH was adjusted to 5.0 with 1M acetic acid (Sigma-Aldrich, USA). 0.1 g of enzyme – pectinase from *Aspergillus niger* (1U mg^{-1}, Sigma-Aldrich, Switzerland) was added to the acidifying solution and stirred for 1 hour. 1 hour later the solution was diluted to 500 mL with deionized water and filtered with Whatman No. 1. The first five filtrates were discarded. 2 mL of the filtrate were diluted to 50 mL with deionized water. 2 mL of the end solution were used for the colorimetric determination with JENWAY 6405 UV/VIS Spectrophotometer (Baroworld Scientifid Ltd., UK). The absorbance of total pectin was measured at the wavelength of 520 nm and the known amount of galacturonic acid (Sigma-Aldrich, Switzerland) ranged from 0.5-3.5 mg 2 mL^{-1}.

HPLC analysis of sugars

Glucose, fructose and sucrose were determined by a high-performance liquid chromatograph Shimadzu LC20 Prominence (Japan). The parameters of analysis of the chromatography system were set as follows: detector: refractive index RID-10A, column YMC NH_2, 4.6 mm x 250.0 mm, 5 µm (YMC CO., LTD, Japan); temperature 35 ºC; isocratic regime; mobile phase acetonitrile (HPLC grade, Sigma-Aldrich, USA) and deionized water (Crystal E HPLC System, Adrona, Latvia) (80:20); flow rate 1.0 mL min^{-1}, volume of injected sample 10 µL; total time of the analysis up to 15 min. Data for analysis was processed using Shimadzu LabSolution software (LCSolution version 1.21 SP1).

Determination of pH

The pH of water was measured directly using a potentiometric method with WTW inoLab pH 7110 (WTW GmbH, Germany).

Determination of moisture

Weight of 1g of apple pomace or extract in the moisture analyzer AND MX-50 (A&D Instruments Ltd., United Kingdom) was analysed 25 minutes at temperature 140 ºC.

Determination of total solids content (Brix %)

Total solids content was determined using the digital refractometer Krüss DR-201-95 (Krüss GmbH, Germany) and was expressed as Brix %.

Data processing/Statistical analysis

The data of the research were analysed by the statistical and mathematical methods (mean, standard deviation). The data were compared by the analysis of variance (ANOVA) and significance was defined at $P < 0.05$. For the data analysis the Microsoft Excel software of the version 2016 was used.

Results and discussion

For the extraction of pectin, with lemon juice as acidifying agent, no earlier reports exist, as well as using other fruits or berries. In nature lemons are fruits that have strong acidic properties and can be expected to decrease pH, if added to water [25; 26]. Acidity is formed in lemons due to the high content of citric acid 45.8-48.0 $g \cdot L^{-1}$ in lemon juice, comparatively, the grapefruit and orange juice contain less amount of citric acid, respectively 25.0 $g \cdot L^{-1}$ in grapefruit juice and 16.7-9.10 $g \cdot L^{-1}$ in orange juice [27].

Before adding the apple pomace, lemon juice was added to water to decrease pH to 2.5. Traditional standard time 120 min., pH 2.5, temperature + 88 ºC and 9.0 Brix % of total solids content were used to obtain pectin. The results after vacuum rotary evaporation showed that the highest level of pectin was determined in the cultivars 'Antejs' (4.9 g per 100 g), 'Sinap Orlovskij' (4.8 g per 100 g) and the lowest in 'Antonovka' (3.9 g per 100 g) (Fig.1).

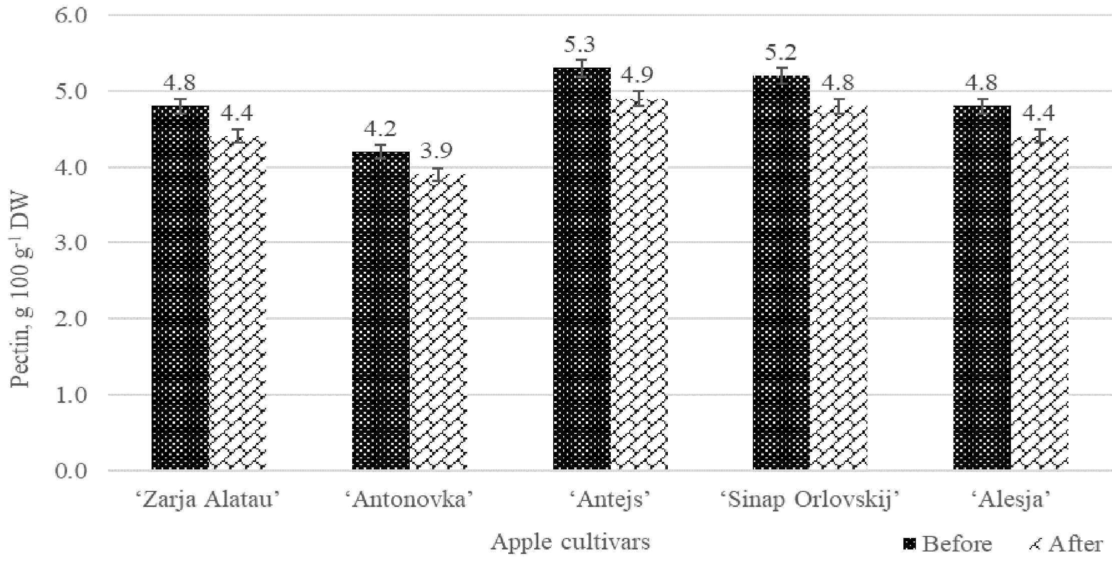

Fig. 1. **Content of pectin in apples and apple extracts**

The highest level of pectin before extraction was detected in 'Antejs' (5.3 g per 100 g), 'Sinap Orlovskij' (5.2 g per 100 g) and the lowest in 'Antonovka' (4.2 g per 100 g). Comparing the results before and after the extraction of pectin, it was detected that 92 % of substances were extracted into the apple extract. Respectively, the best apple cultivars with the highest pectin content were 'Antejs', where the content of pectin before extraction was 5.3 g per 100 g and 4.9 g per 100 g after extraction, and 'Sinap Orlovskij', where the content of pectin before extraction was 5.2 g per 100 g and 4.8 g per 100 g after extraction. The content of extracted pectin is not only dependent on the time, pH and temperature, but also on pectin in different apple varieties and by-products. *Bhushad et al.* reported and summarized other researchers' findings on the sources of the content of pectin, which differed in a wide range and varied from 3.50 to 14.32 % or 3.50 to 14.32 g per 100 g in apple pomace [28]. In the process of juice and cider production from apples, large amounts of residues – apple pomace (peel, calyx, stem, core and soft tissue) are formed [29]. Therefore, apple pomace is a good source not only for obtaining pectin, but also for other biological active substances. *Bhushan et al.* determined that apple pomace is rich in functional components – polyphenols, dietary fibers, vitamins, minerals and carbohydrates [30].

Traditionally, powdered pectin is in pure form without other natural substances, and for gelling it needs to add pure carbohydrates-sugar like sucrose. One of the advantages is that apple extract with pectin contains natural carbohydrates (Fig.2) and there is no need to add them or add as much as it is added by using powdered pectin.

The results demonstrated that the highest concentrations of total sugars of glucose, fructose and sucrose before extraction were detected in the cultivars 'Antejs' (Glucose 1.2, Fructose 6.6, Sucrose 4.1 = total sugars 11.9 g per 100 g) and 'Alesja' (Glucose 1.2, Fructose 6.6, Sucrose 3.6 = total sugars 11.4 g per 100 g), the lowest in 'Antonovka' (Glucose 0.9, Fructose 6.0, Sucrose 1.4 = total sugars 8.3 g 100 per g) (Fig.2).

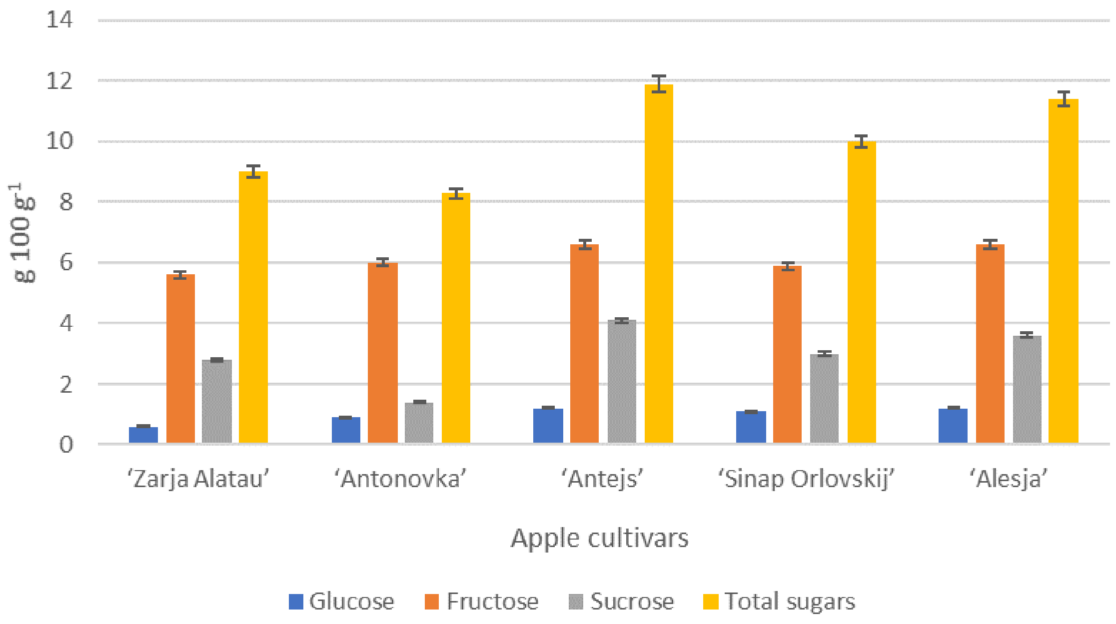

Fig. 2. **Content of glucose, fructose and sucrose in apples before extraction**

After extraction, 75 % of carbohydrates were dissolved from the apple pomace matrix in apple extract. The content of total sugars was detected and expressed as total solids content in Brix %, which means that in production of pectin from apple extract, the best apple cultivars 'Antejs' and 'Sinap Orlovskij' Brix is 9.0 %, by calculation of total sugars, it was detected that 8.9 Brix % of total solids were carbohydrates, respectively, the sum of glucose (1.0 g per 100 g), fructose (4.9 g per 100 g) and sucrose (3.0 g 100 per g). The rest of the total solids 0.1 Brix % were other biologic active water-soluble compounds, like polyphenols, pectin, dietary fibers, vitamins and minerals. From all examined cultivars the best content of pectin was detected in 'Antejs' and 'Sinap Orlovskij', which can be used to produce apple extract with pectin.

Conclusions

1. In the used non-tractional hot extraction process of pectin from apple pomace and lemon juice as acidifying agent it was found that it is possible to produce apple extract with pectin from different apple cultivars without strong inorganic acids or pure organic acids.
2. Apple cultivars 'Antejs' and 'Sinap Orlovskij' showed the highest content of pectin in apple extracts.

3. Regardless of the studied apple cultivars, using the hot extraction method with untraditional acidifying agent – lemon juice – showed that 92 % of pectin was extracted into the apple extract in the form of pectin.

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