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Pectin extraction from pomegranate peels with citric acid

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ABSTRACT

Pectins were extracted from pomegranate peels with citric acid, according to a central composite design with three variables: pH (2–4), temperature (70–90 °C), and extraction time (40–150 min). Fourier transform infrared (FTIR) spectroscopy was used to follow changes in material composition during the main steps of pectin extraction, and also to determine the degree of methyl esterification and galacturonic acid content of pectins produced under different conditions. Harsh conditions enhanced the extraction yield and the galacturonic acid contents, but decreased the degree of methoxylation. The optimum extraction conditions, defined as those predicted to result in a yield of galacturonic acid higher than 8 g/100 g while keeping a minimum degree of methoxylation of 54% were: 88 °C, 120 min, pH 2.5. Close agreement was found between experimental and predicted values at the extraction conditions defined as optimum.

1. Introduction

Pomegranate (*Punica granatum* L.) is one of the oldest known edible fruits, native to Persia, cultivated in ancient Egypt, Greece and Italy, then spread to several other regions [1]. The pomegranate arils are consumed as such or processed into juices and other products, whose consumption has been motivated by their alleged health benefits derived from their high antioxidant capacity [2–4]. The processing of pomegranate juice results in a pomace consisting of approximately 73 wt% peels [5]. Several studies have been focused on extraction of phenolic compounds from pomegranate peels and their active properties [6–8]. A recent study [9] was published on optimization of ultrasound assisted extraction of pectin from pomegranate peel, although the properties of pectin extracted from different extraction conditions have not been evaluated.

Pectin extraction is most commonly carried out using a dilute mineral acid, usually hydrochloric, sulfuric, or nitric acid, because of their lower price, and their ability to generate pectins enriched in homogalacturonic blocks as a result of significant hydrolysis of neutral sugar-containing rhamnogalacturonic regions at low pH and high temperature [10]. However, the main drawback of those mineral acids are their toxicity and the generation of environmentally unfriendly (corrosive) effluents, requiring special treatments to remove undesirable compounds from the pectin extracts so the final product can receive the GRAS (generally recognised as safe) status [11]. Pectin extraction yields from apple pomace, cocoa husks, and passion fruit peel with citric acid have been reported to be similar to those obtained with hydrochloric acid [12–14]. Moreover, organic acids have a lower hydrolyzing capacity than mineral acids (because of their lower dissociation constant), and are therefore expected to cause less de-polymerization of pectins [15].

Pectins can be characterised by different parameters, the most important being the degree of methoxylation or degree of methyl esterification (DM), which refers to the percentage of methoxylated C_6 atoms in the galacturonic acid backbone, and is closely related to the gelling properties [16]. DM may reach the equivalent of 14% methoxyl, which means degrees of esterification between 50 and 80%, for high methoxyl pectins (HMP), whilst pectins with methoxyl contents below 7%, corresponding to a DM below 50%, are regarded as low methoxyl pectins (LMP) [17]. The main difference between HMP and LMP refers to their gel formation mechanisms, since gel formation is the key to most pectin applications. Pectin gels are formed when the molecule chains are crosslinked, forming a three-dimensional network where water and co-solutes are retained [17]. In the case of HMP, crosslinks are formed by hydro-

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gen bonds and hydrophobic forces between methoxyl groups, as at high sugar contents and pH ≤3.5, whereas in LMP ionic crosslinks are formed between calcium ions and free carboxyl groups [18].

Another important parameter is the galacturonic acid content, which indicates the purity of the pectin extracted from pomegranate peels.

This study was carried out to evaluate the influence of pH, temperature, and time on pectin extraction from pomegranate peels with citric acid.

2. Materials and methods

2.1. Chemical composition of pomegranate peel powder and changes with extraction process

Pomegranate peels, provided by Bakkavor Foods Ltd. (London, UK), were oven-dried at 60 °C for 24 h, and milled to 0.5 mm in a Retsch Brinkmann ZM-1 centrifugal grinding mill (Retsch GmbH, Haan, Germany).

The ash, extractive, and Klason lignin contents of the pomegranate peel powder were determined according to methods TAPPI T413 OM-93 [19], TAPPI T204 cm-97 [20], and TAPPI T222 om-22 [21], respectively. Ashes were determined by heating the sample at a heating rate of 9.6 °C/min until 600 °C, keeping this temperature for 3 h, cooling it to 200 °C in no less than 1 h, and further cooling it in a desiccator. The extractives were determined by adding 150 mL of absolute ethanol to 4 g of sample, Soxhlet extraction (for no less than 24 refluxes in 4–5 h extraction), and drying at 105 °C for 1 h. Klason lignin was determined by adding 17 mL of 72% sulfuric acid solution to 1 g of dried, extractive-free and grounded sample, under stirring for 15 min. After 24 h (digestion), 306 mL of distilled water were added to dilute sulfuric acid to 4%, the mixture was transferred to volumetric flask connected to a condenser, heated to boiling for 4 h, cooled to room temperature, and vacuum filtrated. The precipitate was thoroughly washed in distilled water, and dried at 105 °C until constant weight. Holocellulose was determined on dried, extractive-free samples, according to Yokoyama, Kadla, and Chang [22], with minor modifications. 3 g of samples were added with 120 mL of deionized water. 2.5 g sodium chlorite (80% purity), and 1 mL glacial acetic acid were then added, and the dispersion was heated at 70 °C under stirring for 1 h; then, two subsequent additions of 2.5 g sodium chlorite and 1 mL acetic acid were made, keeping the dispersion at 70 °C under stirring, for 1 h (second addition) and 3 h (third addition). After cooling (in an ice bath for 30 min), the sample was vacuum filtered, the resulting precipitate (holocellulose) was thoroughly washed with deionized water, and dried in an oven at 105 °C. The dried holocellulose was then submitted to α-cellulose determination as described in TAPPI T203 cm-99 [23], with some modifications. 1 g of dried holocellulose was extracted with 15 mL of 17.5% NaOH for 2 min, grounded, added with 40 mL deionized water, and vacuum filtrated; the precipitate (α-cellulose) was thoroughly washed with deionized water, and dried in an oven at 105 °C. The hemicellulose (including pectin) contents were determined by difference between holocellulose and α-cellulose contents, according to Salim & Wahab [24]. All determinations were made in triplicate.

Fourier Transform Infrared (FTIR) spectra were collected from the pomegranate peel powder, the alcohol-insoluble residue (AIR), the extracted pectin, and commercial apple pectin (Sigma-Aldrich, 75% methyl esterification), in the frequency range of 4000–800 cm^{-1} (128 scans at 2 cm^{-1} resolution) on a Nicolet Magna-IR 860 FTIR spectrometer (Thermo Nicolet, Madison, WI, USA). Samples were placed on a GoldenGate diamond ATR accessory (Specac, Orpington, Kent). The empty crystal was used as reference. The changes in spectra were followed in an attempt to understand any changes which occurred during the pectin extraction process.

2.2. Preparation of the alcohol-insoluble residue (AIR)

The alcohol insoluble residue (AIR) was prepared according to Waldron and Selvendran [25], with some modifications. 100 g of milled peels were washed three times with ethanol to remove alcohol soluble components (firstly with 600 mL of boiling 70% v/v ethanol solution for 5 min, then with 600 mL of boiling absolute ethanol for 5 min, and the third time with 600 mL of absolute ethanol at room temperature for 5 min), then washed in 200 mL acetone. Between washings, the material was filtered through a

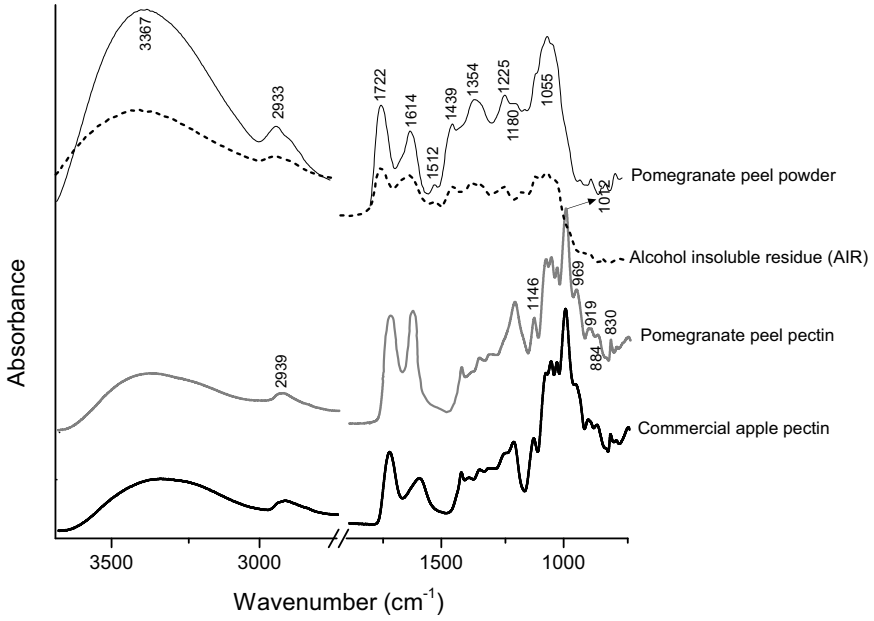

Fig 1. FTIR spectra of pomegranate peel powder, alcohol insoluble residue (AIR), pomegranate peel pectin (obtained at 88 °C, 120 min, pH 2.5), and commercial apple pectin (Sigma-Aldrich, 75% methyl esterification).