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Comparison of Structure and Emulsifying Activity of Pectin Extracted from Apple Pomace and Apricot Pulp

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Abstract: Pectins were obtained from apple pomace and apricot pulp by solubilization at (80-82°C) in acidified medium (HCl 0.5 N) at pH 1.5 for 60 min. Pectin content varies depending on the product and the solvent used for the precipitation. Pectins have high ash content (10.0-13.0%) and high galacturonic acid content (33.8-57%). Pectins precipitated with aluminum chloride were highly methylated (67.2% and 53.9%) for apricot and apple, respectively, while pectins precipitated with aluminum sulfate were poorly methylated (41.2% and 31.01%) for apple and apricot, respectively. Apricot pectins are rich in proteins (3.06 to 3.93%) and phenolic compounds (2.75 to 6.20 μg/mg) as compared to apple pectins. The studied pectins were depolymerized, with molecular weight ranged from 886.99 to 3388.65 g/mol. The emulsifying activity (EA) of the extracted pectins showed that they were endowed with a surfactant power represented by EA varying between 37.03 and 45.87. This activity is directly linked to pectin structure, particularly, the presence of proteins, phenolic compounds and low molecular weight. The stability of prepared emulsions is due to electrostatic repulsion of charged molecules (proteins and pectin).

Key words: Pectin · Apple · Apricot · Emulsifying activity · Emulsion stability

INTRODUCTION

Pectin is a food additive widely used in the food industry because its gelling, stabilizing, thickening and emulsifying properties [1-4]. Pectin is defined as a mixture of heteropolysaccharides. The polysaccharide structure is based on 1,4-linked α-D-galacturonic acid, interrupted by L-rhamnose residues with side-chains of neutral sugars, mainly L-rhamnose, L-arabinose and D-galactose [4]. Pectin is extracted from plant cell walls.

The main sources of pectin are co-products from the fruit juice and sugar industries, mainly citrus peels, apple pomace and sugar beet pulp. Hot acid extraction has the highest yield of pectin and is the most used process for industrial extraction of pectin [5]. Pectin structure and properties depend on its source and the extraction process [3]. Information on the extraction of pectin from apricot co-product is scarce.

The aim of this work was to study the yield and the structure of extracted pectins as a function of the product (apple and apricot) and the solvent of precipitation (aluminum salts: sulfate and chloride). To study the emulsion capacity as well as the relation between the structure and the emulsifying properties.

MATERIALS AND METHODS

Extraction of Pectins: Apple pomace and apricot pulp were obtained from Ngaous company (Ngaous, Algeria), specialized in the production of fruit juices and jams. Apple pomace and apricot pulp were dried in air-circulated oven at 70°C then packed in polyethylene bags for further usage. Extraction of pectins from the dried apple pomace and apricot pulp was carried out by hydrolysis in acid aqueous solution with HCl (0.5 N) adjusted to pH 1.5 at 80±1°C for 1 h. The solid liquid ratio was 1kg/50L [6]. After extraction, the pectin solution was filtered with a Whatman No. 4 filter paper. Concentrated pectin solution was precipitated with aluminum salts (sulfate and chloride). Pectin precipitate was then filtered with a Whatman No. 4 filter paper and dried in a vacuum dryer at 50°C and 40 cm Hg.

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Chemical Characteristics of Extracted Pectins

Ash Content: Ash content was determined according to AOAC [7] method 14.006.

Protein Content: Nitrogen content of pectins was determined by Kjeldahl method [7]. Protein content was calculated by multiplying the nitrogen content by a factor of 6.25.

Uronic Acid Content: The content of uronic acid calculated as a function of galacturonic acid was determined by titration and colorimetry using the metahydroxydiphenyl method (MHDP) [8].

Dosage of Methanol: Determination of methanol content was assessed following Klavons and Bennett method [9]. Methanol was measured after oxidation in aldehyde and reaction with 2-4 pentanebione (acetyl acetone). The reaction gives a yellow color, absorbed at 420 nm. The degree of esterification was estimated based on the formula used by Fertonani *et al.* [10].

Dosage of Phenolic Compounds: Polyphenols were estimated using Folin Ciocalteu method [11]. Oxidation of phenols changes the reagent into a mixture of blue oxide of tungsten and molybdenum. The color intensity is proportional to the oxidized phenolic compounds ratio. Absorbance was measured at 765 nm after 2 h of incubation. Polyphenols concentrations were deduced from calibration ranges based on gallic acid (0-200 μg/ml) and were expressed as microgram of equivalent gallic acid per milligram of extract (μgEGA/mg).

Determination of Molecular Weight and Intrinsic Viscosity: Determination of intrinsic viscosity was performed at 25°C. The studied pectin was solubilized in distilled water then filtered. The obtained filtrate was used to prepare solutions with concentrations varying between 0.003 and 0.048 g/ml. Intrinsic viscosity (ç) and molecular weight (M) were estimated using Mark Houwink-Sakurada equation [12]:

$$ç = K M^a$$

whereas k and a are constants.

Study of the Emulsifying Activity and Emulsion Stability of Extracted Pectin: Emulsifying activity and emulsion stability were assessed using Dalev and Simeonova [13] procedure. Pectins are able of emulsifying and stabilizing oil-water systems in presence of a bacteriocide (sodium azide 0.02%).

Determination of emulsifying activity is based on the ratio of the emulsified layer volume and the whole volume of the solution. In graduated tubes, emulsions were prepared by adding 3 ml of vegetable oil to 3 ml of pectin solution (0.5%, w/w), containing 0.02% sodium azide as a bacteriocide. The mixture was homogenized in a vortex mixer at maximum speed, for 3 min, at room temperature. Samples were then centrifuged at 527g, for 5 min, at 23°C. After centrifugation, the whole volume of the solution (W_V) and the emulsified layer volume were determined (ELV). Emulsifying activity (EA) was calculated as:

$$EA(\%) = \frac{ELV}{Wv} \times 100$$

Similar emulsion samples were prepared to study the emulsion stability (ES) after 1 and 30 days of storage at 4 and 23°C. Samples were centrifuged at 527g, for 5 min, at 4 and 23°C. The initial emulsified layer volumes (VEi) were measured. After each storage period. Samples were centrifuged and the remaining emulsified layer volumes were measured (VEr). Emulsion stability was calculated using the following relation [1]:

$$ES(\%) = \frac{VEr}{VEi} \times 100$$

All experiments were performed in triplicate.

RESULTS AND DISCUSSION

Extraction Yields: Figure 1 shows that apple pomace has the highest yield of pectin (27.50 et 20.06%), precipitated with aluminum chloride and sulfate, respectively. However, apricot pulp has lower yield of pectin (4.97 et 3.40%), precipitated with aluminum chloride and sulfate, respectively. Renard *et al.* [14] reported yields of the order of 15-20%. Massiot and Renard [15] found similar yields from apple pomace (15-27%). However, Marcon *et al.* [16] obtained a lower yield of pectin (7.2%) from apple pomace extracted at 75°C for 55 min, by solubilization with citric acid (5%) and precipitation with alcohol. The low pectin yield obtained from apricot pulp could be explained by the high enzymatic activity catalyzed by pectinolytic enzymes, mainly polygalacturonase (PG). peach and pear fruits contain similar amount of pectin as apricot and are rich in PG, whose activity increases during maturation, causing hydrolysis of pectin located in the middle lamella

Table 1: Composition of extracted pectins

Pectins types	P.P.C	P.P.S	P.A.C	P.A.S
Moisture content (%)	8.00±1.55	8.50±1.31	11.00±1.01	10.00±1.66
Ash content (%)	13.00±2.25	10.00±1.25	16.00±4.01	11.80±1.89
Protein content (%)	2.50±0.42	1.23±0.70	3.93±0.56	3.06±0.14
Polyphenols content	3.07±0.70	1.98±0.51	2.75±0.57	6.20±0.64
(µg/mg) Intrinsic viscosity	242.99±8.60	140.26±5.67	177.54±0.33	38.25±1.48
(ml/g) molecular weight (g/mol)	3388.65±21.7	1876.96±16.6	2723.39±31.1	886.99±12.3

P.A.C: pectin of apricot precipitated with aluminum chloride; P.A.S: pectin of apricot precipitated with aluminum sulfate; P.P.C: pectin of pomace apple precipitated with aluminum chloride; P.P.S: pectin of pomace apple precipitated with aluminum sulfate.

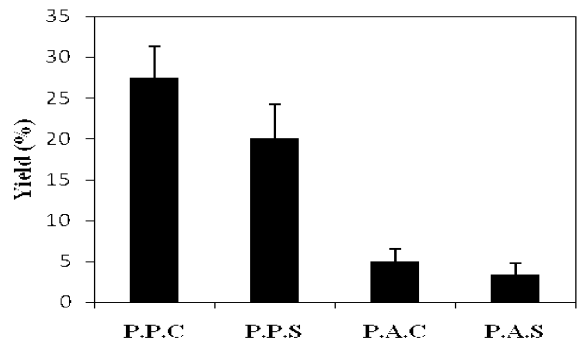

Fig. 1: Yield of extracted pectins
P.A.C: pectin of apricot precipitated with aluminum chloride; P.A.S: pectin of apricot precipitated with aluminum sulfate; P.P.C: pectin of pomace apple precipitated with aluminum chloride; P.P.S: pectin of pomace apple precipitated with aluminum sulfate.

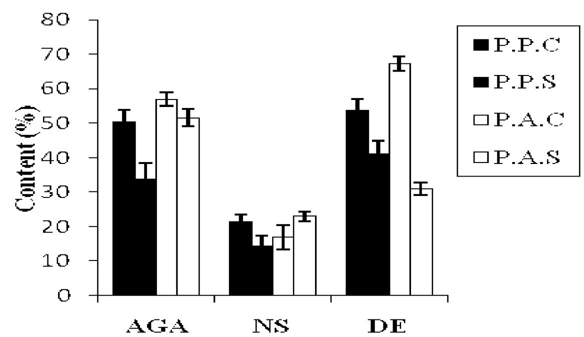

Fig. 2: Structural composition of extracted pectins
AGA: Anhydrous galacturonic acid; DE: Degree of esterification; NS: Neutral sugars; P.A.C: pectin of apricot precipitated with aluminum chloride; P.A.S: pectin of apricot precipitated with aluminum sulfate; P.P.C: pectin of pomace apple precipitated with aluminum chloride; P.P.S: pectin of pomace apple precipitated with aluminum sulfate.

and in the cell wall [17]. Grimplet [18] has also confirmed the presence of polygalacturonase enzymes in apricot, similar to enzymes found in peach. For apple, pectases activity, particularly PG are strongly inhibited due to its high flavonoids content (11.8%) [19]. Flavonoids high content of fruits such as apple and apricot plays an inhibitor role on pectin hydrolysis by decreasing depolymerisation during maturation and extraction [19].

Chemical Characteristics of Extracted Pectin: Protein content: Apple pectin presents low protein content (2.5 and 1.23%) precipitated with aluminum chloride and sulfate, respectively (Table 1). This is probably due to partial hydrolysis with HCl (pH 1.5) during solubilization. For apricot pulp, protein content was higher (3.93 and 3.06%) precipitated with aluminum chloride and sulfate, respectively. This could be due to the co-precipitation of proteins with pectins [20]. Massiot and Renard [15] obtained protein content varying between 2.1 and 7.5%. However Schieber *et al.* [19] found values of the order of 4% for pectins extracted from apple pomace. Protein content of 3.7% was extracted from beet pulp using similar experimental parameters (80-82°C, 60 min, pH 1.5 using HCl) [1].

Ash Content: Ash content of studied pectin varied between 10 and 16% (Table 1). Ash content increases with increasing the precipitation pH value and the concentration of aluminum sulfate. However, organic solvents such as alcohol lower ash content by increasing the lessivassion of the impurities during pectin precipitation.

Content of Galacturonic Acid and Neutral Sugars: The extracted pectin presented a high galacturonic acid content (AGA) (Fig. 2), slightly lower than standard values. This confirms the good choice of solubilization parameters, particularly pH 1.5. In fact, pH has an effect on AGA content. Lo Scalzo *et al.* [22] obtained high AGA content at pH 1.5. This suggests that pectin AGA content increases with pH decrease and it is related to enzymatic activity decrease, particularly polygalacturonase, where the optimal pH is 3.5 [17]. Many research papers obtained similar AGA values from apple pomace. Levigne *et al.* [5] obtained values varying between 29 and 52.8%. Schols *et al.* [23] reported values between 33.4 and 42.5%. However, Fertonani *et al.* [10] found much higher results varying between 53 and 75%. Studies outlined that polysaccharides such as pectins show qualitative and quantitative variations due to fruit variety, stage of maturity, geographical origin, storage conditions [21] and extraction parameters [12]. These parameters could activate or deactivate pectinases, such as polygalacturonase.

Results of neutral sugars (NS) were lower compared to AGA content (Fig. 2). This could be explained by polysaccharides changes during maturation and storage of fruits, as well as extraction parameters. Polysaccharides change during maturation, often implies pectin solubilization and loss in neutral sugars such as galactose and arabinose and a decrease of their molecular weight [24].

Degree of Esterification: Methanol content expressed as Degree of esterification (DE) is showed in Figure 2. The degree of esterification varies as a function of raw material and precipitation agent. Massiot and Renard [15]; Fertonani *et al.* [10] found similar methanol content (2.20 to 4.10%) for apple pectin precipitated using alcohol. In fact, apple pectin has a high degree of methylation during maturation [17]. Renard *et al.* [25] have obtained highly methylated pectin (60 to 84%) from apples. In general, co-products resulting from fruits processing contain pectinase, mainly pectin methyl esterase (PME) and polygalacturonase. These enzymes may activate during storage of raw material leading to pectin with low DE. On the other hand, deesterification could be due to chemical reasons and occurs at too low pHs and too high temperatures. The studied pectins were obtained by solubilization at 80-82°C and hydrolysis at pH 1.5. In addition, to blanching and drying of raw materials. Meanwhile, PME exists naturally in fruits and may affect the degradation of 5% of methoxyl groups of polygalacturonic chains esterified during maturation.

Study of the Emulsifying Activity and Emulsion Stability of Extracted Pectin

Emulsifying Activity: The emulsifying properties of extracted pectin from apple and apricot were studied as well as the possibility to maintain these emulsions stable during storage of 1 and 30 days at 4 and 23°C. Results of the emulsifying activity (EA) are shown in Figure 3. Emulsifying activity of all pectins varies between 37.04 and 45.80%. Results are similar to the literature [1, 3]. However, Huang *et al.* [26] found lower values (30.3%). Many factors may affect emulsifying activity of pectins, particularly the low molecular weight which reduces the superficial tension [1], extraction parameters such as temperature and time [1], proteins of pectin which are active at the interface oil-water [3, 27], the high DE, the presence of acetyl groups [28] and the presence of phenolic compounds having hydrophobic properties.

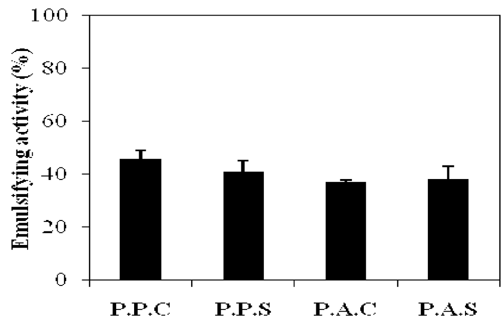

Fig. 3: Emulsifying activity of extracted pectins
P.A.C: pectin of apricot precipitated with aluminum chloride; P.A.S: pectin of apricot precipitated with aluminum sulfate; P.P.C: pectin of pomace apple precipitated with aluminum chloride; P.P.S: pectin of pomace apple precipitated with aluminum sulfate.

Emulsion Stability: The studied samples presented acceptable stability (Table 2). However, values were slightly irregular during storage mainly at temperature 4°C. Yapo *et al.* [1] found values of the same order of magnitude, but their pectin kept its stability during storage. Emulsion stability is due to the steric repulsion of aggregates by a hydrated layer [2]. Pectin stabilizes these emulsions at the interface by creating a layer around particles and allowing emulsion formation. The stabilization power of these emulsions is due to the link between charged molecules (pectins) and proteins [3] and to the low molecular weight of pectins (70 kg/mol) [4].

It was concluded that apple contains more pectin than apricot whatever the agent used for the precipitation. Aluminum salts produce pectins with a high ash content. These extracted pectins have a high AGA content. Precipitation using sulfate increases demethylation. Emulsifying activity of pectin extracted from depolymerized apple and apricot is directly linked to their structure.

Table 2: Emulsion stability of pectin solutions

Pectins types	P.P.C	P.P.S	P.A.C	P.A.S
Stability (1 day, 4°C)	88±11.3	91.2±5.4	92±2.8	80±0
Stability (30 days, 4°C)	85.2±10.9	78±2.8	82.1±2.9	80±0
Stability (1 day, 23°C)	69.7±8.9	81.5±2.1	85.1±1.3	76.5±4.9
Stability (30 days, 23°C)	69.7±8.9	78±2.8	85.2±1.1	80±0

P.A.C: pectin of apricot precipitated with aluminum chloride; P.A.S: pectin of apricot precipitated with aluminum sulfate; P.P.C: pectin of pomace apple precipitated with aluminum chloride; P.P.S: pectin of pomace apple precipitated with aluminum sulfate.

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