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Evaluation of the effect of age of cladode and solvent on yield of mucilage and pectin from cactus pads

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Abstract— Natural products and health foods have recently received a lot of attention both by health professionals and the common population for improving overall well-being. In this line, two distinctive water-soluble polysaccharide materials known as mucilage and pectin, that occur in Opuntia Ficus Indica (OFI) cladodes have been extracted. The mass yield of each polysaccharide was assessed according to the growth cladode and the solvent used for the precipitation, the efficiency of ethanol and acetone was compared for the precipitation of polysaccharides, the content of mucilage and pectin in the cladodes are not affected with the age of the cladode and shows a random distrubition. However, acetone showed a strong power for the precipitation of mucilage and pectins, the yields obtained for the mucilages are of the order of 0.32-0.6%, 0.19-0.37% and those obtained for pectins are of the order of 0.19-2.31% 0.07-1.479%, when using acetone and ethanol respectively. The great number of potentially active nutrients and their multifunctional properties make cactus cladodes perfect candidates for the production of health-promoting food and food supplements.

Index Terms— Sustainable plant ,Cactus, Cladode, opuntia Ficus Indica, mucilages, pectins, food supplements

1. Introduction

Sustainable plant and marine natural resources of biomass that exist in many regions of the world may be used as less expensive alternatives for producing added-value industrial polysaccharide gums. Among resources currently being sought for this purpose, indigenous plants from arid lands deserve special attention due to their agronomic advantages, such as the low input of water and energy needed for their commercial exploitation. Besides, desertification in arid regions has become a major concern worldwide, and encouragement of the sustainable use of the native flora of these regions may contribute to revert this problem. Along with its direct use as fruit or vegetable, cactus (Opuntia spp.) has been considered a potential source of an industrial hydrocolloid gum. Although the gum it is not yet commercially available, there is a genuine interest among companies to start producing it on a large scale.

Cactus (Opuntia ficus-indica) belongs to the family Cactaceae. Family Cactaceae is reported to contain about 130 genera and nearly 1500 species. Opuntias (Cactus) are now part of the natural landscape and the agricultural systems of many regions of the world. Some species are even naturalized weeds in countries such as South Africa and Australia where the environmental conditions are particularly favourable.

In many different countries the Opuntias and their products serve various purposes (as food, forage, energy, medicine, cosmetic, agronomic and others). It is indeed difficult to find more widespread and better exploited plant, particularly in the subsistence economy of arid and semi-arid zones, where farmers due to the lack of natural and productive resources, must look to those few species that can profitably survive and produce. Thus Opuntias have become an endless source of products and functions, initially as a wild plant and, later, as a crop for both a subsistence and a market-oriented agriculture [1]. Opuntias are particularly rich in fibrous compounds, which are mucilages and pectin [2-4].

The mucilage extracted from the cladodes of Opuntia sp. contains basically polygalacturonic acid (very similar to pectin structure), plus residues of some sugars, such as D-galactose, D-xylose, L-arabinose, L-rhamnose and D-galacturonic acid [5]. Some authors have suggested that Opuntia sp. mucilage has a functional component with industrial perspectives [6], and its extraction and characterization have also been reported [7]. Sepulveda et al. (2007) [7] among other authors, reported extraction methods for Opuntia mucilage. They used different methods that combine different pad/water ratios, extraction temperatures, and extraction times. For the precipitation of the mucilage, they assessed two types of alcohol and two water/alcohol ratios.

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They concluded that no differences were found in any of the measured variables among the different extraction or precipitation methods. Nopal mucilage is used in culinary applications, as an additive for food industry, for improving stability and compressibility ofbuilding materials, for cosmetic applications, for improving house paint as an adhesive for lime, as a flocculant agent for water purification, and otheruses [6] and in traditional treatments of diabetes, gastritis, hyperglycemia, etc [8].

Pectin is widely used in the food industry as a hydrocolloid gelling gum. Commercially, it is commonly derived from fruit waste mainly apple and citrus peel. Generically, the term ‘‘pectin’’ represents a family of structural polysaccharides which occur as constituents of the primary cell wall of plant cells and intercellular regions of higher plants where they function as hydrating agent and cementing material of the cellulosic network [9]. The main pectin component is a central, linear backbone chain composed of a-D-galacturonic acid units linked by (1→4) glycosidic bonds. This linear (or ‘smooth’) structure is interrupted by highly branched regions (‘hairy’ zones) [10-12]. Extracted pectin is widely used as functional food ingredient and it (or its EU code, E440) is listed among the ingredients of innumerable food products. Worldwide annual consumption is estimated at around 45 million kilograms, with a global market value of at least 400 million Euros [13]. The gelling properties of pectin, that are well known to home jam makers and industrial producers alike, were first described by Hennri Braconnot in 1825 [14].

Figure 1 is a schematic view of the molecular architecture of the cell wall. They are commonly produced during the initial stages of primary cell growth and make about one third of the cell-wall dry substances.

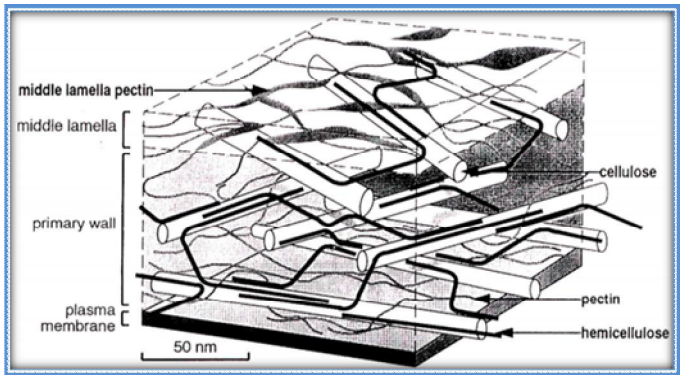

Fig. 1. *Simplified and Schematic Representation of the Architecture of the Cell Wall. Cellulose microfibrils appear crosslinked by hemicellulosic polymers. Pectins present in the middle lamella are Ca^{2+} crosslinked to each other (McCann and Roberts 1991)* [15].

The objective of this work was to evaluate the yields of mucilages and pectins of nopal OFI at different maturity stage and with two precipitation solvent in order to determine the optimum conditions of extraction of each polysaccharide.

2. Materials and Methods

A. Sample Preparation

OFI, a species of the cactus was collected from a farmhouse in the region of Berrechid Morocco. The cladodes were neatly washed with water in order to remove dirt from the materials used before mucilage and pectins extraction. Pads obtained are classified according to their weight in four stages (see table 1).

Table1. *cladodes ranked according to their weight*

Stage of growth	Weight (g)
1	<100
2	100-250
3	250-400
4	400<

The Opuntia ficus indica, were harvested during the summer of 2013 from July to August. Each sample was made up of 1 kg of nopal pads which were collected from several plants in the same sampling areas, but were at different maturity stages. The samples were transported to the laboratory and the pads were classified and separated, as has already been mentioned. All samples were analyzed in triplicate.

B. Mucilage and Pectins Extraction

The thorns of nopal pads were removed manually then washed with distilled water and disinfected using commercial 10% sodium hypochlorite solution in order to eliminate microorganisms. These samples were sectioned into small slices (1*1 cm) in order to facilitate the extraction of mucilage and pectins.

The fresh cactus pads were cut into small pieces (1*1 cm) with a kitchen knife. Cactus pieces were heated in water at 85°C for 20 min to inactivate enzymes and left to cool to ambient temperature; neutralized to pH 7.5 from initial pH 4.0 in order to induce de-esterification of methoxyl groups and filtered through a cloth filter to extract as much mucilage as possible[16]. Pectic polysaccharides were extracted from residue by water (2 × 2 hours at 85 °C) [17]. Water-soluble polysaccharides were extracted from cladodes by

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mixing the decided leaves with water (1:4 w/v). The water-soluble extract was concentrated on a rotary evaporator at 60°C and precipitated with 3 volumes of ethanol or acetone, The precipitates were recovred by centrifugation for 30 min at 5000g. The two series of bioflocculants were purified according to **Belbahloul et al. 2014 [18].**

Yield: It was determined on fresh weight and expressed as mucilage or pectins percentage (%).

3. Results and Discussion

Chemically, pectins are a family of complex heteropolysaccharides comprised by a diversity of carbohydrate residues. Like most other plant polysaccharides, pectins are polydisperse in composition and molecular size, that is, they are heterogeneous with respect to both chemical structure and molecular weight. Their composition varies with the source and conditions of extraction, location, and other environmental factors (**Chang et al.1994**) **[19]**. The main component common to most pectins is a backbone chain structure of α-(1→4)-linked D-galacturonic acid units interrupted by the insertion of (1→2)-linked L-rhamnopyranosyl residues in adjacent or alternate positions (**Aspinall 1980**) **[20]**. The yield of the extracted cactus pectin varied from 0.07 to 2.31% of the fresh weight of cladodes, depending on the extraction conditions used. The pectin yield that was produced under various extraction conditions are presented in **Figure.2**. The highest yield of pectin was 2.31%, which was obtained from stage 2 with acetone as coagulant. The precipitation with ethanol gave 1.48% of pectin as highest yield. There was no significant difference between the yields of pectins between growth stages of cladode.

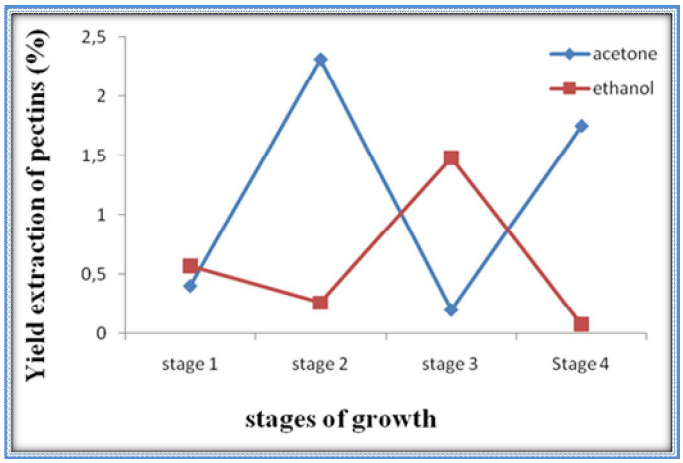

Fig. 2. *pectins yields variation according to the age of the cladode and solvent*

The occurrence of pectins in various Opuntia species from Mexico has been documented for almost three decades (**Villarreal et al. 1963**) **[21]**. **Table 2** shows data from such early studies in which the pectin contents found in eight species and varieties of Opuntia were compared. The yield of soluble pectin in these samples was within a wide range of 0.13% to 2.64% in wet basis.

Table 2: *Pectin yields in Some Opuntia Species and in Some Fruits*

Species	Total Pectin (%)		Soluble Pectin (%)	
	Wet Weight	Dry Weight	Wet Weight	Dry Weight
Opuntia ficus-indica var I[a]	1.91	13.84	1.418	10.28
O. ficus-indica var II[a]	1.10	8.39	0.478	3.65
O. spp. *(Blanca I)*[a]	0.95	7.6	0.482	4.02
O. spp *(Blanca II)*[a]	0.84	7.05	0.129	1.00
O. amylacea[a]	1.40	9.58	0.715	4.89
O. megacantha[a]	0.80	5.06	0.279	1.63
O. steptracantha[a]	0.97	6.59	0.365	2.21
O. robusta[a]	3.30	26.61	2.64	23.87
Apple pomace[b,c]	0.5-1.6	10-15	------	------
Citrus peel[c]	------	20-30	------	------
Sugar-beet pulp[b]	1.00	------	------	------

[a] Data from (Villarreal *et al.* 1963) [21]
[b] Data from (Renard and Thibault 1993)[11]
[c] Data from (Rolin 1993) [22]

Attention is drawn to the high pectin content found for O. robusta (known as "nopal camueso") in comparison with O. ficus indica and the other species. It is interesting to note that the pectin content in O. robusta is within the range reported for citrus peel (20% to 30% d.m.b.), an industrial source of pectin. At present, apple pomace and citrus peels are the main sources of commercially acceptable pectins. Other sources of pectins that have been considered are sugar beet and residues from the seed heads of sunflowers. From this comparison, it is clear that Opuntia spp. can be regarded as a promising commercial source of industrial pectins. Nevertheless, many other technical and economic considerations beside pectin content must be made carefully in order to appraise the viability of cactus pectin industrialization.

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Cactus mucilage may find applications in the food, cosmetics, pharmaceutical and other industries. It has indicated that cactus mucilage may work as a clarifying agent for drinking water in the same way that okra gum does. Also, the culinary properties of cactus mucilage as a fat replacer and a flavor binder have been emphasized. The extraction and purification with ethanol dissolves part of the chlorophyll present in the pads and produces a clear yellow mucilage powder, similar to other commercial gums used in the food industry. The mucilage yield obtained in each treatment was based on fresh weight as shown in **Figure 3**.

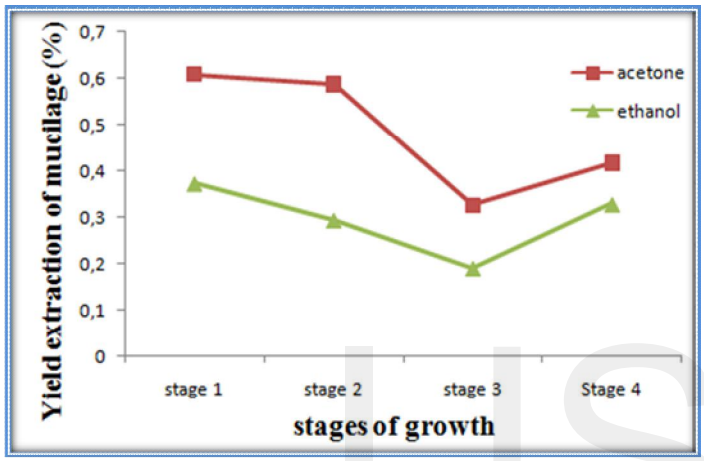

Fig. 3. *mucilage yields variation according to the age of the cladode and solvent*

The yield of recovered mucilage obtained in this study (ranged between 0.19% and 0.6% of f.w) was indeed lower than data on mucilage contents reported for O. ficus-indica of 1.5% (**Saenz et al. 2007) [7]** and higher than the 0.07% obtained by **Cardenas et al. (1997) [23].** Who pointed out that the content of mucilage in relation to the dry matter from the cladodes. However, the climatic conditions at the time of the pads collection (winter, cold and rain), may justify this high value, since the same authors indicate that such values could increase under favorable hydrated conditions, especially in the cells of the parenchyma where the water was stored. The effect of the pads/solvent relationship on the mucilage yield showed a big tendency to increase when the acetone was used for the precipitation. The best yields obtained were with cladodes stage 1 and 2. The highest value was reached in stage 1 of pads using acetone as coagulant (0.6%) while 0.37% by using ethanol. **Saag et al. (1975) [24],** working with other varieties of Opuntia reported, after a water extraction and precipitation with ethanol, yields of 0.53% (f.w.) for Opuntia monocantha and of 0.48% (f.w.) for Opuntia nopalea cochinillifera. The same authors pointed out that the yields depended on the climatic conditions and the crop age.

The result suggested that the change of precipitant solvent had highly significant effects on the yield of pectin, and the acetone had considerable effects on the yield of pectin when it was used to coagulate pectin from the pads. **Saenz et al. 2007 [7]** note that there are no significant differences in yield were observed between the alcohol type and the alcohol/water ratio used.

4. Conclusion

This research emphasized on pectin and mucilages extraction from cactus cladodes. The yield of pectins extracted in different stage did not present significant differences. Consequently, the use of acetone is recommended, since its commercial value is lower in comparison with ethanol. The best solvent of extraction of mucilage was using acetone as the precipitant solvent. This gave a yield of 0.6% higher than ethanol. The yield of mucilage and pectin is not influenced by the age of the pads. Pectin is widely used as a texturizer and stabilizer in a variety of foods and other industries. Despite its availability in a large number of plant species, commercial sources of pectin are very limited. Therefore, cactus appears to be a very promising new source of these biopolymers.

References

[1] Barbera, G., 1995.History, economic and agro-ecological importance. In : Agro-ecology, cultivation and uses of cactus pear. Eds. Barbera, G., Inglese, P. & Pimienta-Barrios, E. (eds.) FAO Plant production and protection paper 132:1-12.

[2] Burret F, Lebreton PH, Voirin B. 1982. Les aglycones flavonique de cactees: distribution, signification. J Nat Prod 45: 687–693.

[3] Salt Ta, Tocker JA, Adler JH. 1987. Dominance of Δ5-sterols in eight species of the Cactaceae. Phytochemistry 26: 731– 733.

[4] Lee JC, Kim HR, Jang YS. 2002. Antioxidant properties of an ethanol extract of the stem of Opuntia ficus indicavar. saboten. J Agric Food Chem50: 6490–6496.

[5] McGarvie D, Parolis H. 1979. The mucilage of Opuntia ficus-indica. Carbohyd. Res., 69(1): 171-179.

International Journal of Scientific & Engineering Research Volume 5, Issue 5, May-2014
ISSN 2229-5518

[6] Saenz C, Sepulveda E, Matsuhiro B. 2004. Opuntia spp. Mucilage´s: a functional component with industrial perspectives. J. Arid. Environ., 57(33): 275-290.

[7] Sepulveda E, Saenz C, Aliaga E, Aceituno C. 2007. Extraction and characterization of mucilage in Opuntia spp. J. Arid. Environ., 68(4): 534-545.

[8] Stintzing, F., Carie, R.. 2005. Cactus stems (Opuntia spp.): A review on their chemistry, technology and uses. Mol. Nutr. Food Res. 49,175 194.

[9] Lau, J. M., McNeil, M., Darvill, A. G., & Albersheim, P. 1985.Structure of backbone of rhamnogalacturonan. I. A pectic polysaccharide in the primary cell walls of plants. Carbohydrate Research, 137, 111–118.

[10] Oosterveld, A., Beldman, G., Searle-van Leeuwen, M. J. F., & Voragen, A. G. J. 2000.Effect of enzymatic deacetylation on gelation of sugar beet pectin in the presence of calcium. Carbohydrate Polymers,43, 249–256.

[11] Thibault, J. F., Renard, C. M. G. C., Axelos, M. A. V., Roger, P., & Crepeau, M. J. 1993.Studies of the length of homogalacturonic regions in pectins by acid-hydrolysis. Carbohydrate Research, 238, 271–286.

[12] Voragen, A. G. J., Pilnik, W., Thibault, J.-F., Axelos, M. A. V., & Renard, C. M. G. C. 1995.Food polysaccharides and their applications. In A. M. Stephen (Ed.), Food science and technology series(pp. 287–339). New York: Marcel Dekker.

[13] Savary, B. J., Hotchkiss, A. T., Fishman, M. L., Cameron, R. G., & Shatters,R.G. 2003. DevelopmentofaValenciaorangepectin methyl esterase for generating novel pectin products. In F. Voragen, H. Schols, & R. Visser (Eds.),Advances in pectin and pectinase research(pp. 345–361). The Netherlands: Kluwer Academic Publishers.

[14] Braconnot, H. 1825. Annales de chimie et de physique-Annals of Chemistry and Physics, 28(2), 173–178.

[15] McCann MC, Roberts K. 1991. Architecture of the primary cell wall. In: Lloyd CW, editor.The Cytoskeletal Basis of Plant Growth and Form. London: Academic Press. pp. 109–129.

[16] Adriana, C., Francisco, M.G., Marguerite, R. 2008. On the gelling behaviour of „nopal" (Opuntia ficus indica) low methoxyl pectin. Carbohydrate Polymers 73, 212–222.

[17] Yordan, G., Manol, O., Irina, Y., Veselin, K., Maria, K. 2012. Isolation, characterization and modification of citrus pectins. J. BioSci. Biotech. 1(3), 223-233.

[18] Belbahloul, M., Anouar., A, Zouhri, A. 2014. Low technology water treatment: Investigation of the performance of cactus extracts as a natural flocculant for flocculation of local clay suspensions. International Journal of Engineering Research & Technology (IJERT). Vol. 3 Issue 3, March.

[19] Chang, K.C., Dhurandhar, N., You, X. and Miyamoto, A. 1994. Cultivar/location and processing methods affect the quality of sunflower pectin. J. Food Sci.,59: 602.

[20] Aspinall, G.O. 1980. Chemistry of cell wall polysaccharides. In: The Biochemistry of Plants. Preiss, J. New York, Academic Press: 473.

[21] Villarreal F., Rojas P., Arellano, V. y Moreno J. 1963. Estudio químico sobre seis especies de nopales (Opuntia spp.). Ciencia mex. 22:59.

[22] Rolin, C. 1993. Pectin. In: Industrial Gums. Polysaccharides and Their Derivatives. Whistler, R.L. and BeMiller, J.N. San Diego, Academic Press Inc.: 258-293.

[23] Cardenas, A., Higuera-Ciapara, I., Goycoolea, F., 1997. Rheology and aggregation of cactus (Opuntia ficus indica) mucilage in solution. Journal of the Professional Association for Cactus Development 2, 152–157.

[24] Saag, L., Sanderson, G., Moyna, P., Ramos, G. 1975. Cactaceae Mucilage Composition. Journal of the Science of Food and Agriculture 26, 993–1000.