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Galacturonic acid in pectic substances of sunflower head residues: quantitative determination by HPLC

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Assuming the interest in low-methoxyl pectin from sunflower head residues lies mainly in the production of low-caloric foods a precise and fast method for the quantification of galacturonic acid would be helpful. An HPLC method involving enzymatic hydrolysis was compared with colorimetric and titrimetric procedures. The results showed that the quantitative data obtained by HPLC method (96·5%) were in good agreement with those from other methods (97·0 and 92·9%, respectively). Because the colorimetric analysis is expensive and highly time consuming and titrimetric procedure demands an accurate purification of the pectin, the HPLC method applied in this study proved to be useful for the galacturonic acid quantification.

INTRODUCTION

Pectic substances, commonly known as pectins, are basically complex mixtures of polysaccharides whose major component consists of α-D-galacturonic acid units linked by $\alpha(1 \rightarrow 4)$ glycosidic bonds. In this main chain β-L-rhamnose units are occasionally inserted through $(1 \rightarrow 4)$ and $(2 \rightarrow 1)$ glucosidic linkages and the carboxyl groups are partially esterified by methyl alcohol or neutralized with mono or divalent cations. Other neutral sugars such as arabinose, fucose, galactose, glucose, mannose and xylose may occur attached as side-chains.

Sunflower plant (*Helianthus annuus* L.), however, seems to be one of the few sources whose pectic substances show a much simpler structure consisting almost exclusively of galacturonic acid polymer (Zitko & Bishop, 1965; Miyamoto & Chang, 1992). Methoxyl content of sunflower heads pectin, mainly the oxalate-soluble fraction, is usually lower than other traditional sources of pectin. So, from the point of view of its utility, sunflower head residues remaining on soil after the seeds have been removed for the oil industry are a pectin source with an interesting potential food application. In fact, besides traditional gels (60% or more of sugar) other gellified products with a very low sugar content or even without it could be obtained with this pectin simply by incorporating small amounts of calcium. Having in mind the health guidelines for the reduction of sugar consumption in diets and the increasing demand for low-caloric foods, this naturally low-methoxyl pectin can be an important alternative to other conventional pectins (apple and citrus) obtained by de-esterification. Indeed, sunflower pectin is already attracting attention at the industrial production level.

Since Colin and Lemoine (1940) reported the presence of a special pectin in sunflower heads, several studies have been conducted in order to obtain a better understanding of its chemistry and physical/rheological properties (Lin *et al.*, 1975; Campbell *et al.*, 1978; Kim *et al.*, 1978; Sosulski *et al.*, 1978).

Following previous studies about physicochemical characterization of sunflower head pectin (Alarcão-Silva, 1990) and technological utilization in the manufacture of low-caloric gels (Alarcão-Silva *et al.*, 1992) a HPLC method has been studied to determine the galacturonic acid in the pectin hydrolysates. Despite some references to this analytical procedure applied to pectin in general (Voragen *et al.*, 1986; Schols *et al.*, 1989) as far as we know little work has been done concerning sunflower pectin. The results obtained by this method were compared with those from titrimetric and colorimetric methods.

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MATERIALS AND METHODS

Pectin was obtained from dried heads (without seeds) of sunflower (*Helianthus annus* L.) cultivar SC-010 harvested in Tapada da Ajuda, Lisboa (Portugal) in October 1992. The extraction of pectin was carried out according to the method of Lin *et al.* (1975) and slightly modified. In the present study only the oxalate-soluble pectin was considered since preliminary trials have shown that it was the most interesting for possible industrial use. This fraction was submitted to consecutive treatments of purification based essentially on National Researchu Council procedure as shown in Fig. 1.

All the chemicals were of reagent grade. D(+) galac-

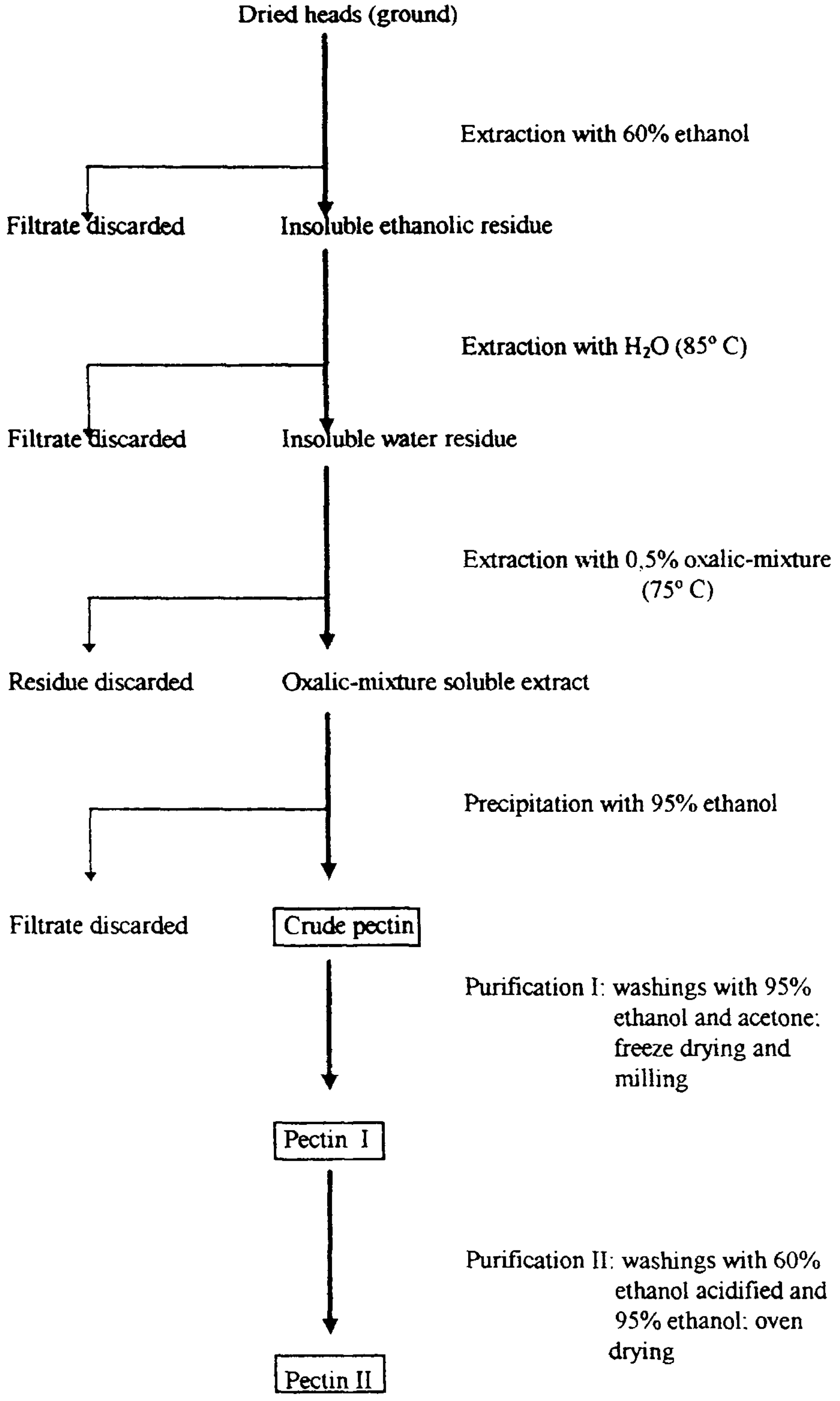

Fig. 1. Schema of sunflower pectin preparation (extraction and further treatments).

turonic acid standard was purchased from Sigma. The commercial enzymatic liquid preparations were Pectinex 3XL 30000 FDU 55°C/ml and Pectinex Ultra SP-L 26000 PG/ml (Novo- Nordisk Ferment, Ltd).

CHEMICAL ANALYSIS

Hydrolysis

Some experiments of acid hydrolysis were done using HCl and TFA at a concentration range from 0·2 to 2·0 mol dm^{-3} under various conditions of temperature (100–120°C) and time (1–3 h). Enzymatic hydrolysis was assayed during addition of 3 cm^3 of enzymatic solution to 250 mg of pectin dispersed in 5 cm^3 of acetate buffer pH 4·5 (0·1 mol dm^{-3}). The degradation was carried out for 48 h at 50°C. To avoid microbial growth, 0·5 cm^3 of toluene was added.

Liquid chromatography

A Waters HPLC system equipped with U6K injector, M6000 pump, R401 refractive index detector and M745 data module was used. The column was a μBondapak NH$_2$ 10 μm (300 × 3·9 mm) heated at 35°C. The mobile phase consisted of 0·015 mol dm^{-3} NaH$_2$PO$_4$ (pH 4·6) with a flow rate of 1·5 ml min^{-1}. Samples and standards were filtered through 0·45 μm HA Millipore membranes. Quantification was achieved by peak-height measurement and using the external standard method.

Other methods

Galacturonic acid content of sunflower pectin was also determined by titrimetric (National Research Council, 1972) and colorimetric *m*-hydroxydiphenyl methods as described by Di Giorgi *et al.* (1985). The contents of monosaccharides in the enzymatic preparations and in the pectin hydrolysates were analysed by the dinitrosalicylic acid (DNS) method (Marsden *et al.*, 1982) and the determination of total sugars in the non-hydrolysated pectin was carried out by the Munsen-Walker method (AOAC, 1984). Ash content was determined according to AOAC (1984).

RESULTS AND DISCUSSION

The HPLC technique used in the determination of acid sugars in complex mixtures of polysaccharides has not been successful due to the difficulty of releasing the units that constitute the polymer (Hicks *et al.*, 1985; Garleb *et al.*, 1991). Although several procedures have been reported (Albersheim *et al.*, 1967; Voragen *et al.*, 1982; Giangiacomo *et al.*, 1982; Forni *et al.*, 1985; Wei & Fang, 1990; Quemener & Thibault, 1990) the differences between the results had indicated that hydrolytic techniques should be significantly improved.

With the aim of obtaining the highest release of galacturonic acid from sunflower pectin, acid and enzymatic hydrolysis were carried out. The lower amounts of galacturonic acid (< 50%) as evaluated from acid hydrolysis showed that this procedure alone is not efficient for glycosidic linkage cleavage (Table 1). In contrast, both enzymatic preparations led to higher yields (no differences in data were found with the two enzymatic preparations) which indicated a complete depolymerization of polygalacturonic chain from pectin sunflower. These results revealed that enzymatic preparations containing pectolytic (pectin-transeliminase, polygalacturonase and pectinesterase) and a range of hemicellulolytic activities are especially appropriate for the efficient degradation of pectin. However, this procedure can only be applied on the quantification of uronic sugars; if all the glycidic fraction must be analyzed, a purification step of the enzymes should be carried out to eliminate contaminants, specially the low molecular weight carbohydrates present in solution.

On a dry weight basis quantitative data of the HPLC method were compared with the results from other methods commonly used for the galacturonic acid quantification (Table 2). Determinations were carried out in the two different stages of pectin purification. Both fractions of pectin are clean-up of contaminant carbohydrates (evaluated by the Munsen–Walker method) which suggests that for commercial use a high degree of purification might not be necessary. The main difference between the two fractions was in the ash content (4·6 and 1·2%, respectively for pectin I and II) and due to this fact the second step of purification must be carried out in the titrimetric method because the cations that are attached to galacturonic acid led to a lower quantification.

However, the colorimetric procedure allowed an accurate quantification of galacturonic acid in both pectins (I and II) but the analysis is slow and expensive. In the development of the HPLC method an amino-bonded phase column was chosen which could be used with polar samples due to its versatility. This column

Table 1. Galacturonic acid (%) released by acid hydrolysis of sunflower pectin I under various conditions

		Galacturonic acid (% of dry weight)			
		1 h (100°C)		3 h (120°C)	
		$\bar{x}$	s_x[a]	$\bar{x}$	s_x[a]
HCl	0·2 mol/dm^3	27·6	±1·85	50·25	±4·86
	2·0 mol/dm^3	35·81	±4·34	42·75	±3·50
TFA	0·2 mol/dm^3	27·27	±0·64	49·21	±4·20
	2·0 mol/dm^3	22·33	±0·58	41·05	±0·62

[a]Standard deviations ($n = 6$).

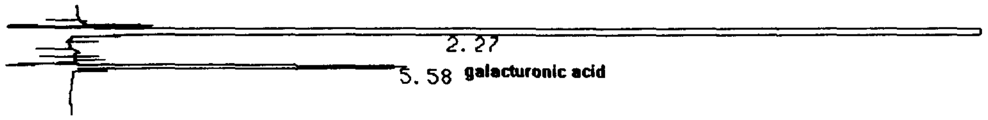

Fig. 2. Chromatogram of hydrolysed pectin. Column: μBondapak NH_2; flow-rate: 1·5 ml min^{-1}; mobile phase: 0·015 mol dm^{-3} NaH_2PO_4 (pH 4·6).

Table 2. Galacturonic acid contents of pectin isolated from sunflower head residues (% of dry weight)

Procedure	Pectin I		Pectin II	
	$\bar{x}$	s_x[a]	$\bar{x}$	s_x[a]
Titrimetric	57·1	±1·55	92·9	±2·2
Colorimetric	91·1	±2·95	97·0	±1·8
HPLC (enzymatic hydrolysates)[b]	89·0	±1·90	96·5	±3·4

[a] Standard deviations ($n = 6$).
[b] Enzymatic hydrolysis was carried out with Pectinex Ultra SPL.

can also be used as a weak anion exchanger with an acid mobile phase (acid causes the NH_2 groups to protonate, giving the surface a positive charge). For this reason, the mobile phase acetonitrile–0·015 mol dm^{-3} NaH_2PO_4 (70:30) (pH 7·0) had been assayed according to Wei & Fang (1990) in order to separate uronic sugars from neutral sugars but the enzymatic procedure (without previous purification of the enzymes) did not allow the quantification of neutral sugars because the enzymatic preparations available have a high level of carbohydrates. As the level of neutral sugar residues found in the pectin sunflower was very low (ca. 4% by DNS determination) only the galacturonic acid was quantified using the aqueous mobile phase (0·015 mol dm^{-3} NaH_2PO_4) which allows a baseline separation with improvement in peak tailing of this major compound as shown in Fig. 2.

CONCLUSION

Although the HPLC method used has allowed a precise quantification of galacturonic acid, further studies on HPLC must be developed in order to obtain a better knowledge of the pectin chemical composition from sunflower heads residues. The use of appropriate hydrolytic enzymes purified in order to eliminate interference would be essential either in this aspect or if we are to elucidate the molecular structure of polysaccharides.

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