Journal of Food Technology 1 (3): 102-105, 2003


# Solid Wastes of Tomato-Processing Industry (*Lycopersicon esculentum* "Hybrid Rome") as Renewable Sources of Polysaccharides

[1]Giuseppe Strazzullo, [1]Vincenzo Schiano Moriello, [1]Annarita Poli, [2]Barbara Immirzi, [3]Paola Amazio and [1]Barbara Nicolaus

[1]Istituto di Chimica Biomolecolare (ICB) CNR, via Campi Flegrei 34, 80078, Pozzuoli (Na), Italy [2]Istituto di Chimica e Tecnologia dei Polimeri (ICTP) CNR, via Campi Flegrei 34, 80078, Pozzuoli, Italy; [3]Istituto di Biochimica delle Proteine (IBP) CNR, via Pietro Castellino, Italy

**Abstract:** Polysaccharides from solid wastes of tomato-processing industry (*Lycopersicon esculentum* "Hybrid Rome") were extracted using a simple and rapid method, purified and chemically characterized. The yield of biopolymers represented 7,5 % of lyophilised biomass. Solid, clear, elastic and biodegradable films were obtained from this natural and renewable source of polysaccharides.

**Key words:** Environmental, polysaccharides, raw material, tomato-processing industry and biodegradable film

## Introduction

The food packaging industry is an important sector of the world economy. The main issue of the food industry is the management of waste matter and their possible recycling in order to obtain products with good added value (Rosales *et al.*, 2002). The food industry processes tons of tomatoes every year. The disposal of the refuse has a notable spin-off in terms of costs and pollution of the environment (Leoni, 1997).

Currently the drying of the discard constitutes the most common approach to produce fertilizers. With the new biotechnologies it is possible to manipulate these residue by converting them in a possible source of biotechnological products such as polysaccharides (Tong and Gross, 1990; MacDougall *et al.*, 1996; Gross, 1986, Maugeri *et al.*, 2002). These biopolymers are fundamental for their chemical-physical properties (emulsifying, viscoelasticity, polyelectrolyte, adherence, bio-compatible, stabilizer, etc.) and have the ability to interact with other polymers, such as proteins, lipids, as well as polysaccharides. (Steele *et al.*, 1997). The most important source of polysaccharides derives from vegetables and their cellular wall or reserve products and their content influences the rheological behaviour of fruit juices and vegetable purées (Siviero *et al.*, 1996).

All rheological properties can be influenced by the content of pectin and their degree of polymerization and esterification, cellulose, hemicellulose and other oligo and polysaccharides, which contribute and define the consistency and the viscosity of the material (Koch and Nevins, 1989; Porretta *et al.*, 1993).

We describe a rapid and simple method in order to recover high grade polysaccharides in excellent yield from solid wastes of tomato-processing industry (*Lycopersicon esculentum* variety "Hybrid Rome"). The polysaccharides obtained from this natural and renewable source are characterized and used to conceive and prepare useful biodegradable films.

## Materials and Methods

**Tomato wastes:** The food packaging industry " La Doria S.p.A Salerno Italy " kindly supplied the raw materials. On weight basis the raw materials, produced by mechanical tomato pressing for the production of pulp and puree tomatoes (*Lycopersicon esculentum* "Hybrid Rome"), represent about 2.2 % of the total processed tomato (Leoni, 1997). The wastes included peels, seeds, other solid parts, rotten and unripe tomatoes (5 kg approximately each supplier), were frozen with liquid nitrogen and then lyophilised under *vacuum*.

### Polysaccharide Extract

**We Performed two Protocols of Extractions:** Method A - the lyophilised biomass (20 g) was treated with 5 N KOH under stirring for 3 days and then centrifuged at 10.000 g for 40 min. After centrifugation the supernatant was precipitated with cold ethanol (v/v) at -20°C for a night. The pellet, collected after centrifugation, was dissolved in hot water, dialysed against tap water for 3 days and dried under *vacuum*. The polysaccharide obtained was named sample A.

Method B - following KOH treatment and after centrifugation the supernatant was partitioned with *n*-butanol (v/v), the extraction was repeated twice in order to obtain an uncoloured water phase. The pH was adjusted to pH 1 with 2 N HCl. The aqueous phase was further partitioned with *n*-butanol and then precipitated with cold ethanol (v/v) at -20°C for a night. The pellet, collected by centrifugation, was dissolved in hot water, dialysed against tap water for 3 days and dried under *vacuum*. The polysaccharide obtained, was named sample B. Samples A and B

were utilized for further analyses.

**Chemical-physical Properties:** Carbohydrate content was performed according to Dubois's method using glucose as a standard (Dubois *et al.*, 1956). Total protein content, nucleic acid and pyruvate were detected as described in Nicolaus *et al.*, 1999. Molecular weight of Sample A and B was estimated by gel filtration on a Sepharose CL-6B column (1x80 cm) using H2O/Pyridine/AcOH (500:5:2, by vol.) (Nicolaus *et al.*, 1999).
Hydrolysis of polysaccharides was performed with 2 M trifluoroacetic acid (TFA) at 120°C for 2 h. Monosaccharide composition was identified by TLC (thin layer chromatography) analysis and high pressure anion exchange-pulsed amperometric detector (HPAE-PAD). TLC was developed with the following solvent system: a) acetone/butanol/H2O (8:2:2, by vol.) for neutral sugars; b) ButOH/H2O/AcOH (3:1:1, by vol.) for acidic sugars. Sugars were visualized by spraying the plates with ?-naphthol. HPAE-PAD Dionex (USA) equipped with Carbopac PA1 column, was eluted isocratically with: a) 16 mM NaOH for neutral sugars, and b) 100 mM NaOH and 150 mM sodium acetate (NaAcOH) for acidic sugars (Maugeri *et al.*, 2002).
Optical rotation value was obtained on a Perkin-Elmer 243 B polarimeter at 25 °C in water.
1H-NMR spectra of Sample A (30 mg/ml D2O) were performed on a Bruker AMX-500 at 70 °C. Chemical shifts were reported in parts per million relative to sodium 2,2,3,3-*d*4-(trimethylsilyl) propanoate. (Pazur, 1994; Perlin and Casu, 1982).
Measurements of specific viscosity as a function of concentration of aqueous solutions of pectic polysaccharides were carried out using Cannon-Ubbelohde 75 suspended level viscometers at 25°C.
The gravimetric analysis was performed using Mettler Toledo Star System equipped with thermo analytical balance in a temperature range of 50° to 450°C with a temperature program of 10 min at 50°C following by 10°C/min.

**Biodegradable Film:** Biodegradable films were performed by solubilizing 50 mg of polysaccharide in 5 ml of water at room temperature. After dissolution, 5 mg of glycerol was added as plasticiser. The solution was poured onto Petri disk to allow evaporation of water. The biofilms were analysed with dynanometer test. The stress was applied by tensile dynanometer machine by Instron II USA.

## Results

The choice of methods for the purification of polysaccharides from tomato raw materials, obtained during the production of pulp and puree tomatoes (Leoni, 1997), has been made in order to remove cytoplasmic contaminants (proteins and low molecular weight species) as well as minimising physical and chemical modifications.
The extraction of polysaccharides is outlined in Fig. 1. From 20 g of lyophilised tomato raw material, 1.5 g of samples A (method A) were obtained, by means of method B the same amount of raw material provided 850 mg of samples B. Samples A and B were both partially characterized.
HPAE-PAD analysis performed on samples A and B showed the same neutral sugar composition: glucose (Glc): xylose (Xil): galactose (Gal): galactosamine (GalN): glucosamine (GlcN): fucose (Fuc) in a relative molar ratio of 1: 0.9: 0.5: 0.4: 0.2: *tr* (Fig. 2). The carbohydrate content for samples A was 100 % and 67 % for samples B. The uronic acid content was also different: 20 % in A and 10 % in B; galacturonic acid being major uronic acid detected in both samples. Moreover, samples A and B were protein free.
By size exclusion chromatography analysis a rather broad peak was obtained, indicating that the material was highly polydisperse. The chromatography elution profile on Sepharose CL-6B of polysaccharides indicated a molecular weight of 1 x106 Da; a calibration curve of standard dextrans as major peak for both samples was used. Sample A and B have [?]25D values of - 0,18 and - 0,15 respectively at concentration of 1 mg ml-1 in H2O (Table 1).
1H-NMR spectrum of sample A (Fig. 3) showed a complex profile. It exhibits five well resolved peaks in anomeric region (from ? 4.5 to ? 5.5; ? chemical shifts are expressed in ppm) at ? 4.94 (doublet, d); ? 5.06 (d); ? 5.09 (d); ? 5.26 (d); ? 5.27 (d) with the same value of constant coupling
J1-2 (3.8-4.0 Hz, due to a probably *gluco-galacto* configuration); three anomeric peaks at ? 5.07 (singlet, s); ? 5.18 (s); ? 5.30 (s), (J1-2 0.5-1 Hz) indicating the occurrence of a *manno* configuration; at upfield region a doublet at ? 1.20 indicative of presence of deoxy-sugars was observable. The eight anomeric signals indicated the presence of eight different monosaccharides, regarding type or glycosidic linkage position. The eight monosaccharides were labelled A to H with respect to increasing ? (Fig. 3). On the base of chemical shifts and coupling constant data A, D, F residues have probably a ? *manno* configuration; B, C, E, G, ? *gluco-galacto* configuration and H residue a ? *gluco-galacto* configuration.
The rheological properties were further characterized by studying the specific viscosity () of biopolymers A and B (Table 1). The measurements were performed on aqueous solutions of polysaccharides in the range 0,37 - 0,87 g/dl. The observed viscosity is related to the size and number of macromolecules in solution. As

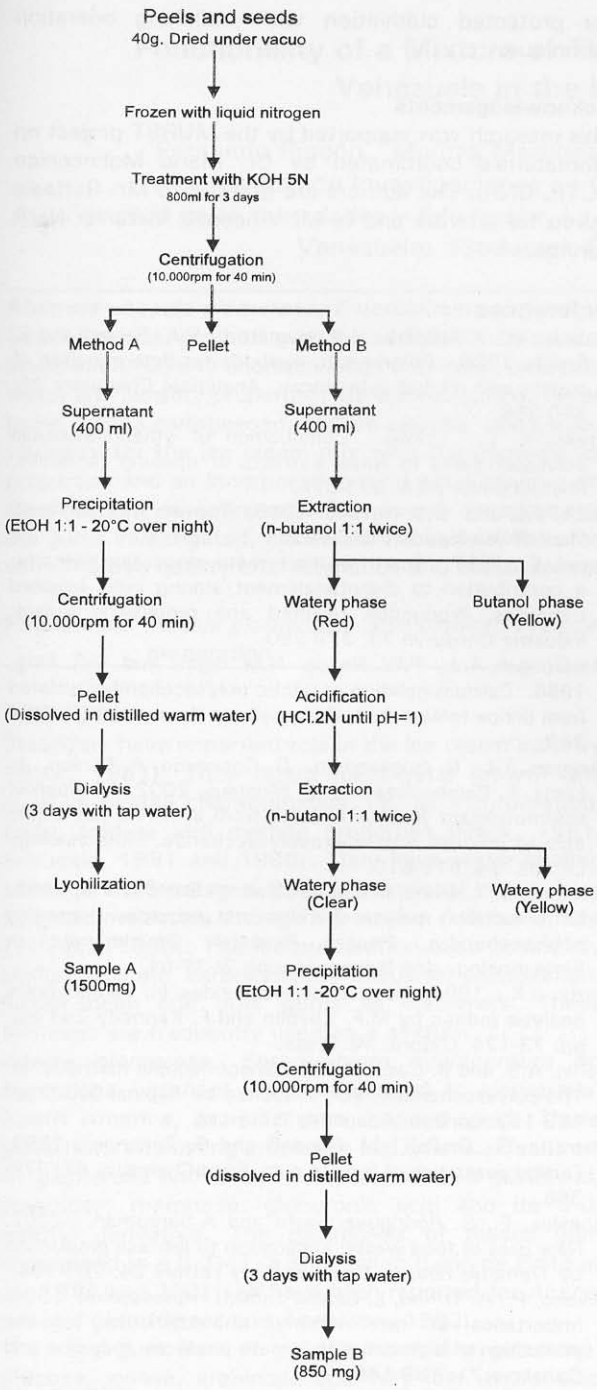


Fig. 1: Flow chart of polysaccharide extraction from tomato waste material.

concentration increases coils start to overlap and become entangled, with viscosity showing a more marked dependence on concentration (data not

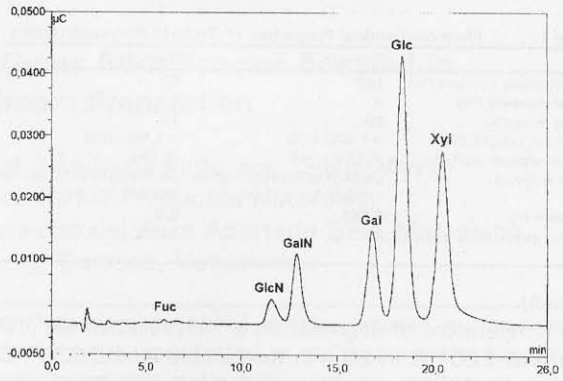


Fig. 2: The monosaccharide composition of Sample A using HPAE-PADchromatography analysis

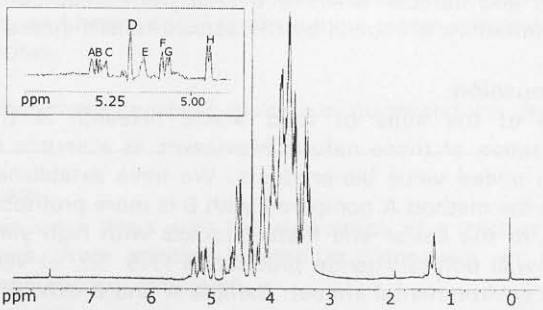


Fig. 3: $^{1}$H-NMR spectrum of Sample A, the anomeric region being expanded. The eight residues were labelled A to H following the increase in chemical shifts ($\delta$ in ppm)

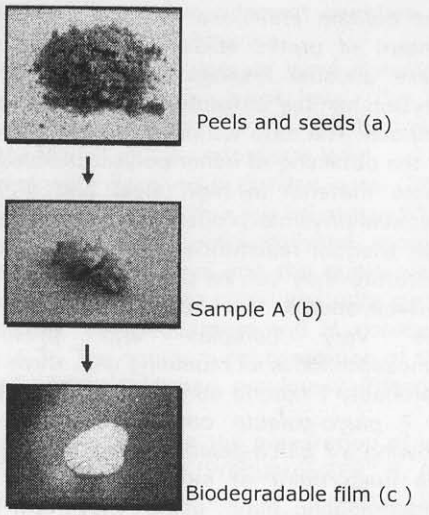


Fig. 4: (a) Tomato raw material from food packaging industry;
(b) Purified polysaccharides A;
(c) Biofilm obtained by using polysaccharide A and glycerol as plasticiser

Table 1: Physicochemical Properties of Tomato Polysaccharides

| | Sample A | Sample B |
|---|---|---|
| Carbohydrate content (%) | 100 | 67 |
| Protein content (%) | 0 | 0 |
| Uronic Acid (%) | 20 | 10 |
| Molecular weight (Da) | >1.000.000 | >1.000.000 |
| Optical rotation $(\alpha)^{D}_{25}$ | -0,189 | -0,154 |
| Sugar analysis | Glc/Xil/Gal/GalN/GlcN/Fuc 1/0,9/0.5/0,4/0,2/tr | Glc/Xil/Gal/GalN/GlcN/Fuc 1/0,9/0.5/0,4/0,2/tr |
| Viscosity (η) | 0,561 | 0,9 |
| Thermo gravimetric analysis (T°C) | 250°C | 230°C |

shown).

The temperature of degradation of polysaccharides (10 mg) was 250° C in 20 min for sample A and 230° C for sample B with a residue of about 5 mg (Table 1). Both polysaccharide A and B were used for the preparation of biodegradable films as described above. Films obtained were clear and elastic (Fig. 4); those with polysaccharide A compared with B were more solid and durable when recovered from small static deformations produced by the applied tensile stress.

## Discussion

One of the aims of food waste research is the utilization of these natural biomasses as a source of high added value bio-products. We have established that the method A compared with B is more profitable due to the easier and faster process with high yield cell-wall polysaccharide production (7.5 %), at very low environmental impact. Sample A and B exhibited the same sugar composition with a different carbohydrate content. The sugar analysis of polymers A and B revealed the presence of glucose and xylose as major components, and a low level of uronic acids, in contrast to that of the cell-wall pectic polysaccharides that contain arabinose in large amount and higher content of uronic acids (MacDougall *et al.*, 1996). These unusual findings are due to different minor polysaccharides extracted by the use of these new methods. The data we have presented are appropriate for the obtaining of minor polysaccharides from tomato waste material in high yield and with interesting chemical-physical properties such as high viscosity, high thermal resistance and high molecular weight. Therefore they can be used better than commercially available source. The structure of the polysaccharide A was very complex and presented eight monosaccharides as repeating unit, three of them with a probably ? *manno* configuration, four residues with an ? *gluco-galacto* configuration and one residue showing a ? *gluco-galacto* configuration.

The main point of interest was the formation of biodegradable films using these bio-polymers on addition of glycerol. The clear gel formed from polysaccharide A was more durable and elastic than that obtained with polysaccharide B. The films obtained can be used in different fields such as agriculture i.e. for protected cultivation with mulching operation technique.

## Acknowledgements

This research was supported by the MURST project on Biomaterials coordinated by Dr. Mario Malinconico (ICTP, CNR). The authors are grateful to Mr. Raffaele Turco for artwork and to Mr Vincenzo Mirra for NMR service.

## References

Dubois, M., K.A.Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1956. Colorimetric methods for determination of sugars and related substances, Analytical Chemistry 28: 350-356.

Gross, K. C., 1986. Composition of ethanol-insoluble polysaccharides in water extracts of ripening tomatoes. Phytochemistry 25: 373-376.

Koch, J.L. and D.J. Nevins, 1989. Tomato fruit cell wall. Plant Physiology 91: 816-822.

Leoni, C., 1997. "Scarti" in the tomato processing industry: a contribution to disentanglement among culls rejected tomatoes. Production rejected and processing waste. Industria Conserve 73: 278-290.

MacDougall, A.J., P.W. Needs, N.M. Rigby and S.G. Ring, 1996. Calcium gelation of pectic polysaccharides isolated from unripe tomato fruit. Carbohydrate Research 923: 235-249.

Maugeri, T.L., C. Gugliandolo, D. Cacciamo, A. Panico, L. Lama, A. Gambacorta and B. Nicolaus, 2002. A halophilic thermotolerant *Bacillus* isolated from a marine hot spring able to produce a new exopolysaccharide. Biotechnology Letters 24: 515-519.

Nicolaus, B., L. Lama, M.C. Manca and A.Gambacorta, 1999. Extremophiles: polysaccharides and enzymes degrading polysaccharides. Recent Research Development in Biotechnology and Bioengeenering 2: 37-64.

Pazur, J.K., 1994 . Neutral polysaccharides. In: Carbohydrate analysis (edited by M.F. Chaplin and F. Kennedy 2nd ed). pp: 73-124. Oxford: IRL Press.

Perlin, A.S. and B. Casu, 1982 . Spectroscopic methods. In: The polysaccharides, Vol. 1. (edited by Aspinal G.O.). pp: 133-193. London: Academic Press Ltd.

Porretta, S., G. Poli, M .Rondelli and G. Zanangeli, 1993. Quality evaluation of tomato pulp. Food Chemistry 47: 379-386.

Rosales, E., S. Rodriguez Couto and A.Sanroman, 2002. New uses of food waste: application to laccase production by *Trametes hirsuta*. Biotechnology Letters 24: 701-704.

Siviero, P., A. Trifino, L. Sandei and M. Franceschini, 1996. Importance of raw material characteristics for the production of high-viscosity tomato products. Industrie and Conserve 71: 139-146.

Steele, N.M., M.C. McCann and K. Roberts, 1997. Pectin modification in cell walls of ripening tomatoes occurs in distinct domains. Plant Physiology 114: 373-381.

Tong, C.B. and K.C. Gross, 1990. Stimulation of ethylene production by a cell wall component from mature green tomato fruit. Physiologia Plantarum 80: 500-506.