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Polysaccharides from Wastes of Vegetable Industrial Processing: New Opportunities for their Eco-Friendly Re-Use

Annarita Poli¹, Gianluca Anzelmo¹, Gabriella Fiorentino^{1,2},
Barbara Nicolaus¹, Giuseppina Tommonaro¹ and Paola Di Donato^{1,2}

¹C.N.R. National Research Council, Institute of Biomolecular Chemistry (ICB),

²University of Naples 'Parthenope',

Department of Environmental Sciences, Centro Direzionale,
Italy

1. Introduction

Polysaccharides are the most abundant natural biopolymers and represent by far the largest group of polymers produced in the world: indeed more than 150,000 M tons of polysaccharides per year are produced in comparison with about 140 M tons of synthetic polymers (Navard, 2005).

Polysaccharides are widely diffused in nature: they can be found in plants, animals and microorganisms, performing different fundamental biological functions. Depending on their functional role, they can be distinguished in: energy reserve substances (e.g. starch or inulin in plants, glycogen in animals), structural elements maintaining mechanical shape and rigidity of the living cells (cellulose, hemicellulose and pectin in plant cell wall, chitin in arthropod exoskeletons), and water-binding elements (agar, pectin and alginates in plants, mucopolysaccharides in animals).

About 99% of total natural polysaccharides are located in plants and vegetables that consequently represent a major renewable source of these biopolymers exploitable for different purposes. Indeed, polysaccharides are presently used in all sectors of human activities and in several application such as: food nutrients, food additives and feed production; material science concerning the formulation of polymeric materials for different biotechnological applications; health care for biocompatible materials, drug delivery or as source of biologically active molecules; sustainable energy production by means of biofuels generation (Persin et al., 2010).

The main and most abundant polysaccharides from plants and vegetables include starch, cellulose and hemicelluloses, inulin and pectins that, thanks to their useful physical-chemical properties such as emulsifying power, viscoelasticity, polyelectrolyte conformation, adherence, bio-compatibility, stabilizing power, *et cetera* are usually exploited in the above mentioned fields of application. In addition, several other polysaccharides with different structures and properties are continuously isolated and are under investigation in relation to their features and potential employment in several industrial sectors.

Plant polysaccharides are used in food production mainly as source of carbohydrates, monomer sugars, sweet syrups, sweeteners and dietary fibres. Starch from potato, corn and other starchy plants, is the main source of sweeteners such as maltose, glucose or fructose syrups in addition to other products including prebiotics, mannose, sorbitol and vitamin C, ethanol, *et cetera*. Inulin and related oligofructoses, from chicory and Jerusalem artichoke, are used as functional food ingredients and can contribute to reduce the risk of many diseases by stimulating the immune system, decreasing the levels of intestine pathogenic bacteria, and lowering the synthesis of triglycerides and fatty acids in the liver, *et cetera* (Kaur & Gupta, 2002). Pectin, from apple and citrus fruits, is widely used as functional food ingredient and it is listed among the ingredients of innumerable food products: its worldwide annual consumption is estimated to be around 45 million kilograms, with a global market value of to be least 400 million euros. Its industrial utilisation is based mainly on its gelling properties for the production of jams and jellies, fruit juice, confectionary products and bakery fillings; nevertheless, pectin is also used for the stabilisation of acidified milk drinks and yogurts (Willats et al., 2006). Non-starch polysaccharides isolated from fruits and vegetables (cellulose, hemicelluloses, pectins) are the main constituents of the so-called dietary fibres, which include a host of different polymers, highly variable in terms of molecular size, structure and monomeric composition. They are widely employed in the industrial food production also as additives, thickeners, emulsifiers, gelling and texturizing agents, fat and/or sugar replacers in low-calories food preparations, (Sanjay & Gross, 2001).

Natural polysaccharides from plants are non-toxic, biocompatible, biodegradable and water soluble. Such properties make them suitable for different pharmaceutical and biomedical uses. Indeed, several polysaccharides (starch, pectins, cellulose and others) find manifold medical applications as drug delivers, inert diluent for drugs, wound dressing, as constituents for scaffolds and implants in tissue engineering, *et cetera*. Medical uses of polysaccharides also rely on their direct beneficial effects on human health like for example blood cholesterol reduction displayed by pectins in a wide variety of subjects and experimental conditions (Sriamornsak, 2003). Dietary fibres positively affect health by decreasing intestinal transit time and increasing stools bulk, reducing blood total and/or LDL cholesterol levels, or reducing post-prandial blood glucose and/or insulin levels (Champ et al., 2003). In addition, it is noteworthy that plant polysaccharides can be considered also as an emerging class of bioactive molecules displaying anti-cancer, anti-oxidant, anti-bacterial, anti-virus and chemo-preventive effects. They could play important roles in several physiological and pathological conditions and in this context many researches have been carried out to assess their biological activity. Some interesting examples include polysaccharides extracted from mulberry leaves, showing anti-oxidant properties (tested as DPPH-scavenging activity) and good anti-bacterial effects against some common pathogens such as *B. subtilis*, *E. coli* and *S. aureus* (Wang & Jiang, 2010); high molecular weight polysaccharides from *Opuntia ficus-indica* accelerating re-epithelization in a model of dermal wound (Trombetta et al., 2006) or those from *Salvia chinensis* eliciting B lymphocytes *in vivo* (Liu et al., 2002) or even some pectic polysaccharides from ginseng that are able to rescue cell viability from rotavirus infection (Baek et al., 2010). Finally, polysaccharides extracted from plants such as *Aloe barbadensis* Miller, *Lentinus edodes*, *Ganoderma lucidum*, *Coriolus versicolor*, resulted to exert both anti-genotoxic and anti-tumor promoting activities *in vitro* models and thus might be considered as potential cancer chemo-preventive substances (Hyung et al., 1999).

Other potential medical applications of polysaccharides have been recently envisaged also in the fields of tissue engineering and regenerative medicine: thanks to their similarities with the extracellular matrix, to their chemical versatility as well as to their good biological performance (commonly they are used in nature as structural materials), these biopolymers (for instance starch, cellulose, arabinogalactan) are among the most studied and promising natural polymers that have been suggested for tissue engineering (Mano et al., 2007).

The well known self-assembly capabilities and stimuli responsiveness of polysaccharides make them suitable bio-materials for different applications in fields like biodegradable plastic production, nano-material science and the already discussed bio-compatible materials production for medical uses. Polysaccharides are used with or without chemical modifications that have been the object of several researches in order to obtain new biodegradable and sustainable materials (Gandini, 2008). Starch has been extensively exploited for the realisation of biodegradable plastic and resins that can be blown into film, injection moulded and thermoformed; it is also fermented to lactic acid producing polylactic acid polymers and co-polymers employed for biodegradable plastic production (Narayan, 1994); in addition, it is currently object of massive research efforts for the production of the so-called thermoplastic starch and the processing of foamed materials for loose-fill packaging (Gandini, 2008). Cellulose from wood pulp is mostly used for the production of paper and cardboard and the regeneration of fibres and films (for coatings, laminates, optical films), as well as for building materials, pharmaceuticals, foodstuffs, and cosmetics (Klemm et al., 2005); microfibrils are also employed in nano-material science for manufacturing nano-paper or as reinforcing elements in composite materials (Gandini, 2008); cellulose whiskers have been used as mechanical reinforcing agents for low-thickness polymer electrolytes for lithium battery applications, in the reference (Azizi Samir et al., 2005). Starch, cellulose and pectin have been together also the subject of intense research to assess their use as electroactive polymers. These latter are a new class of materials that can be potentially used for several applications as biosensors, environmentally sensitive membranes, artificial muscles, solar materials, *et cetera* (Finkenstadt, 2005).

The rising global energy requirements together with the depletion of fossil fuel reserves have highlighted the importance of developing technologies to exploit renewable energy sources and for clean carbon-neutral fuel productions, i.e. biofuels. Polysaccharides are renewable sources of monomer sugars to ferment for the production of bioethanol, one of the most used biofuel in the world. Starch from corn is nowadays one of the main sources of bioethanol, although also cellulosic biomasses are under investigation because they could allow ethanol production without displacing agricultural food crops like corn. Nevertheless, biological transformation of cellulose to ethanol is a technology still under investigation because of the harsh conditions required for the industrial process that make it still far from being efficient and costly effective (Chang, 2007).

2. Vegetable wastes: new polysaccharides' sustainable sources

Polysaccharides recovered from plants and vegetables represent a renewable source of food, chemicals, materials and energy. They are ubiquitous and constitute carbon-neutral feedstock for several production processes. For these reasons they may offer potential solutions to the actual need of a sustainable development thus fulfilling the increasing demand of energy or materials and at the same time facing the depletion of fossil reservoirs.

As mentioned above, different types of polysaccharides are currently extracted from a variety of vegetable sources in order to obtain biopolymers for multiple uses: all these processes have both economical and environmental costs, not only depending on the chemical and/or thermal treatments required for the extraction of the biopolymers, but also affected by the starting materials, mostly represented by feedstock (e.g. cereals or potatoes for starch; bran for cellulose; citrus fruits for pectins, *et cetera*).

Therefore, the search of alternative polysaccharide sources is crucial in order to reduce the constraints of food/non-food competition and to ensure the sustainable use of biomasses without negatively interfering with the food chain.

A good candidate to this purpose is represented by under-utilised biomasses such as residues of vegetable processing for food production. Indeed, transformation and packaging of fruits and vegetables by food industry generate every year a huge amount of wastes that represent a worldwide problem for both the environmental and the economical aspects. Processing of fruits and vegetables is wasteful due to materials discarded from wholesale markets and food waste stuff; in addition, by-products come from processing and packing procedures since only a fraction of the incoming biomass is effectively used for food production (Mahro & Timm, 2007). As an example, industrial processing of vegetables for juices production or canning generates huge amounts of wastes constituted by peels, seeds, pulps that account for 30-50 % of input materials; on the other hand selection for packing and preservation discards about 5-30 % of the original feedstock, mainly including fruits or vegetable that are unripe, damaged during transportation or that lack the required features for packing and selling. The management of such wastes represents a critical issue for food industries that process tons of feedstock every year. In fact, disposal of vegetable residues has a notable spin off both in terms of costs and environmental pollution, because of their high organic matter content. Usually, food industry by-products are disposed in landfills and only partially reused by composting or drying for animal feeding and land fertilizing. Nevertheless, modern eco-compatible technologies offer more efficient strategies to recycle these wastes in order to use them as a sustainable source for the extraction of value added-chemicals such as different kinds of polysaccharides useful for manifold applications. In addition, it is remarkable that many plant polysaccharides have not yet been discovered and therefore the further exploitation of alternative biomasses, like vegetable wastes, for the sustainable production of materials, drugs and energy can deeply be implemented in the next future.

2.1 Polysaccharides from vegetable wastes: exploitation of residues from industrial processing of fruits, legumes and cereals for food production

Several examples concerning the exploitation of vegetable wastes for the production of useful polysaccharides are available in literature: some of them are well established industrial processes, as in the case of pectin extraction, many others refer to new potentially valuable sources, largely unexplored that have stimulated research interest in relation to "green" extraction methods implementation, novel polysaccharide identification and investigation about their potential applications. In Table 1 selected examples of vegetable wastes rich in polysaccharides are reported, including some of the most diffused and abundant fruits, vegetable, legume and cereal crops processed by food industry. Data shown here refer in particular to isolation techniques used, extraction yields and polysaccharide chemical composition.

Waste source	Extraction technique	Total Yield (%)	Molecular Weight (KDa)	Monosaccharide Composition (% molar ratio or mg/g dry weight)	Reference
Apple pomace	EtOH (85%) at 70 °C; oxalic acid-ammonium oxalate (pH 4.6) at 85°C	7.7	233.4	GalA/Ara/ Gal / Glc/ Rha/Xyl (853.5/37.3/23.3/16.9/9.6/7.3) (mg/g dry weight)	Min et al., 2011
Apple pomace	Mixing in H ₂ O; homogenization; autoclave at 121 °C; β-glucanase (Viscozyme® L)	4.6	231.7	GalA/Ara/ Gal / Rha/Glc/ Xyl (693.2/78.04/26.2/ 15.47/14.8/13.2) (mg/g dry weight)	Min et al., 2011
Apple pomace	Phenol/acetic acid/H ₂ O; CDTA chlorbutol; 1M KOH; 4 M KOH	4.1	N.D.	Fuc/Ara/Xyl/Man/Gal/Glc/UronicA (5.6/1.2/25.3/6.9/12/46.2/2.8) (% molar ratio)	Watt et al., 1999
Orange peels	Microwave pre-treatment; drying at 60 °C; 0.5M HCl at 80 °C	18	64	GalA (665) (mg/g dry weight)	Kratchanova et al., 2004
Citrus peels	Enzyme extraction (protease, cellulase)	12.6	180	Rha/Fuc/ Ara/Xyl/Man/Gal/Glc/GalA (10/2/71/7/10/34/12/756) (mg/g dry weight)	Zykwinska et al. 2008
Citrus peels	Sequential Extraction: EtOH/H ₂ O	3.6 - 8.6	N.D.	Identified as pectin according to Yu & Love, (1996)	Wang et al., 2008
Bergamot peels (<i>Citrus bergamia</i> Risso)	Sequential Extraction: EtOH/H ₂ O (EtOH insoluble fraction)	45.2	N.D.	Glc/GalA/ Ara/Gal/Xyl/Man/Rha/Fuc (217.1/216.6/66.2/48.1/29.7/24.6/7.8/5.1) (mg/g dry weight)	Mandalari et al., 2006
Bergamot peels (<i>Citrus bergamia</i> Risso)	Sequential Extraction: EtOH/H ₂ O (EtOH soluble fraction)	29.8	N.D.	Glc/ Ara/Rha/Man/GalA/Gal/Xyl/Fuc (328.6/43.7/36.4/24.3/19.2/4.7/2.8/2.2) (mg/g dry weight)	Mandalari et al., 2006
Banana peels	Sequential Extraction: H ₂ O/ Chelating agent/ Acid solution	21.7	573-249	GalA/Rha/ Ara/Xyl/Man/Glc/Gal (69.1/ 0.5/1.7/ 0.5/ 1.2/ 4.3/ 1.0) (% molar ratio)	Happi Emaga et al., 2008
Plantain peels	Sequential Extraction: H ₂ O/ Chelating agent/ Acid solution	14.6	570-129	GalA/Rha/ Ara/Xyl/Man/Glc/Gal (48.3/0.5/9.0/6.5/2.7/6.6/4.0) (% molar ratio)	Happi Emaga et al., 2008
Peanut cakes	EtOH extraction	25.8	N.D.	Gal (main component)	Song et al., 2011
Agro-waste materials	Distilled H ₂ O, pH 11 under stirring	8.1 - 54.8	N.D	Cellulose, hemicelluloses, galactans, arabans, pentosans	Kuan & Liong, 2008
Soy hull	0.1 M HCl at 95°C; precipitation with 2-propanol, pH 3.5	28	N.D.	GalA (720) (mg/g dry weight)	Kalapathy & Proctor, 2001

Table 1. Exploitation of wastes from fruit and vegetable processing for polysaccharide productions. Abbreviations: Glc=glucose, Xyl=xylose, Gal=galactose, GalA=galacturonic acid, Fuc=fucose, Man=mannose, Ara=arabinose, Rha=ramnose, Uronic A= uronic acids.

Apple and citrus wastes are traditionally the main sources of commercial pectin (Thibault & Ralet, 2003). They are generated from processing industries for apple and citrus fruits after juice extraction that are among the foremost food industries, producing about $3\text{--}4.2 \times 10^6$ and 15.6×10^6 M tons per year of wastes, respectively (Min et al., 2011). Therefore, the exploration of proper disposal methods for apple pomace and citrus peels, the residue left after processing, has been the focus of several studies aimed at improving the extraction methods and to recover further biopolymers. Pectins are industrially obtained from apple pomace and citrus peels by means of acid extraction (i.e. oxalic, hydrochloric, nitric and sulphuric acid) at high temperatures (80–90°C). A new eco-friendly extraction technique was tested for apple pomace: based on combined physical and enzyme treatments (Min et al., 2011), it afforded amounts of pectin comparable to those obtained by means of classical chemical treatments (about 10–15% of dry matter waste). Besides pectin, other main components of apple pomace are the cell wall polysaccharides cellulose and xyloglucan. Recent investigations were focused on the pomace xyloglucan component that was isolated by means of alkaline extraction and was identified as a fucogalacto-xyloglucan. Such xyloglucan (after derivatisation procedures similar to cellulose conversion into methylcellulose, hydroxypropylcellulose, carboxymethylcellulose) might be useful as thickening agent, texture modifier or as a source of biologically active oligosaccharides (Watt et al., 1999).

Citrus fruits are particularly rich in pectin (ranging from 20 to 30% of dry matter waste) and the large quantities of citrus wastes generated by the fruit juice industry represent one of the most important raw material for the production of commercial pectin. Several varieties of citrus fruits (including lemon, lime, orange and grapefruit) have been investigated as polysaccharides sources and different extraction techniques have been tested: in Table 1 some examples found in literature regarding different fruit species and alternative isolation methods are reported. The yields and quality of the pectins extracted can be improved by means of pre-treatment with microwaves as shown by studies carried out on orange peels. Microwaves are able to destroy the plant tissue thus increasing the porosity and the water absorption capacity of the plant material that results in a considerable increase in the yield of extractable pectin and improvement of its parameters (e.g. degree of esterification, molecular mass and gel strength) (Kratchanova et al., 2004). Milder extraction methods have been tested on different wastes and have been applied also to citrus wastes: a remarkable example is represented by treatment with non-pectinolytic enzymes such as cellulases (which are able to degrade cellulose and the cellulose-like backbone of xyloglucan) as well as proteases (that are able to degrade proteins) acting synergistically to deconstruct the cellulose/xyloglucan and protein networks, thus facilitating pectins liberation from the cell walls. This method has been validated by extraction yields higher than those obtained by acid treatment, generally used for pectin extraction (Zykwinska et al., 2008). Sequential ethanol/water extraction has been used for different varieties of citrus cultivated in Taiwan and largely employed for juices production: about 33,000 hectares are dedicated to citrus cultivation in Taiwan and the production is about 534,000 tons/year. Peels are the primary by-product of citrus fruits processing for juice production and, if not properly treated, they become a possible source of environmental pollution. The total pectin (including alcohol and water soluble fractions) yields obtained with ethanol/water extraction were evaluated for eight varieties of Taiwan citrus species (i.e. *Citrus reticulata* Blanco, *C. tankan* Hayata, *C. reticulata* - *C. sinensis*, *C. grandis* Osbeck, *C. grandis* Osbeck CV, *C. microcarpa*, *C. sinensis* (L.), *C. limon*). As reported in Table 1, pectin yields from waste peels ranged from 3.6% (w/w) in the case of *Citrus tankan* Hayata to 8.64% (w/w) for *Citrus grandis* Osbeck (Wang et al., 2008). Another interesting example of citrus waste biomass

is represented by bergamot, *Citrus bergamia* Risso, residues: bergamot is used in Italy mostly for the extraction of its essential oil, obtained by wash-scraping the fruit. The annual Italian production of bergamot amounts to 25,000 tons and peel is an underutilized by-product of the essential oil and juice processing industry that likewise other citrus waste peels still contains exploitable biopolymers such as pectins. A sequential ethanol/water extraction has been used and it afforded 29.8% polysaccharide's yield in the 70% ethanol soluble fraction and a 45.2% yield in the alcohol insoluble fraction. Chemical analysis of the isolated fractions showed that both of them were mainly composed of pectic substances encasing the cellulose microfibrils (Mandalari et al., 2006).

Besides the above mentioned examples, other fruits and vegetable processing residues produced in huge amounts from important crops typical of different geographical areas, have been investigated for their polysaccharides content in order to determine their sustainability as profitable polysaccharides sources. In Table 1 some data are shown about banana and plantain processing wastes that were investigated for their content of neutral detergent fibre, acid detergent fibre, cellulose, hemicelluloses and pectin. These fruits are important food crops in tropical and subtropical regions and represent one of the main agricultural economic resources in African countries. The development of the processing industries based on these crops and the increasing production generated a significant amount of wastes that pose the problem of their disposal without causing environmental pollution. In fact banana and plantain wastes, mainly consisting of peels, represent the 40% of the total weight of fresh fruit. Different strategies have been proposed for the valorisation of these agro-wastes like the production of methane, alcohol, adsorbents for water purification, biomass, but the authors also point to their use as potential source of pectins and dietary fibres that represent about 50% (w/w) of dried banana and plantain peels. Such wastes, undergone to a sequential extraction treatment, showed to be an interesting polysaccharide source, with particular regard to pectin quality (Happi Emaga et al., 2008).

Residues remaining after processing of legumes and cereals could also be used for polysaccharides production, thanks to their fibre content. Peanut, for instance, is a major legume oilseed crop grown in many areas of the world, whose annual production totals approximately 29 million metric tons with China, India and the U.S. being the world's three largest producers (http://www.soyatech.com/peanut_facts.htm). The processing of peanut generates a residual cake that has been the focus of several researches aimed at the characterization of the functional ingredients and properties of these residues, with regard to their rheological properties, hypoglycemic capacity, water holding capacity, *et cetera*. Peanut cake is also particularly rich in fibres and therefore it was investigated by means of response surface methodology in order to assess the best polysaccharides extraction conditions. Crude polysaccharides extracted from defatted peanut cakes were also subjected to preliminary structural analyses and resulted to be mainly composed of α -galactose (Song et al., 2011), although further studies to assess their functional and biological properties are still required. Other potential sources of polysaccharides are okara (*Glycine max*), corn cob (*Zea mays sp.*), wheat straw (*Triticum sp.*) and rice husk (*Oryza sativa*), deriving from legumes and cereal processing: approximately 700,000 tons of okara are generated every year by the production of tofu in Japan, whereas 880 million tons of cereals are produced annually worldwide, of which 550 million tons are represented by wheat straw. Traditionally, these waste materials are used as bedding for animals and livestock feeding, burned in the fields, or added into soil as green fertilizer but in the cited study they were evaluated for their content of dietary fibres whose chemical, physicochemical and functional properties were studied for determining potential applications in foods. In particular the dietary fiber

fractions isolated from corn cob and okara showed the highest water- and oil-holding capacities, emulsifying activities and emulsion stabilities, thus suggesting possible applications as functional ingredients in foods (Kuan & Liong, 2008). Another legume processing waste that is generated in significant amounts is soy hull, a co-product of the soybean industry: it is available in large quantities and might be a good and inexpensive source of pectin (about 25%), recoverable by adapting the acid extraction method usually employed for industrial pectin production (see data in Table 1). By means of 0.1 M HCl treatment followed by alcohol precipitation, it is possible to recover from soy hull amounts of pectin comparable to those obtained from citrus fruits, usually employed as commercial feedstock for the production of this polysaccharide (Kalapathy & Proctor, 2001).

2.2 Polysaccharides from tomato, granadilla and lemon wastes: new opportunities from alkaline treatment of residues

In this context, the search of alternative biomasses to be used as renewable sources of polysaccharides stimulated the investigation of wastes from the industrial processing of fruits and vegetables. Special attention has been paid to residues of the industrial transformation of tomato and lemon, two examples of typical Italian crops destined to canning and liquors production, respectively, and granadilla, a fruit highly diffused in Southern America and used for fruit salads, beverages and the production of tropical fruit juices. Food production based on processing and preservation of fruits and vegetables, like tomato and lemon, is an important industrial sector for Italian economy, even though generating tons of wastes every year. Indeed, the total Italian tomato production totally amounts to nearly 9,000,000 tons/year (data from ISTAT, National Institute of Statistics, <http://www.istat.it>) mainly processed by food canning industry: about 1.8-2.3% (i.e. 162,000-207,000 tons) is constituted by peels and seeds that are discarded as wastes. The total amount of lemons harvested per year is about 570,000 tons (data from ISTAT), mainly used for the production of juices and liquors, another crucial sector of food industry in Italy, with particular regard to Southern Italy for “limoncello” liquor production. In contrast with other types of fruits, lemons have a small edible portion and consequently their processing produces larger amounts of waste material (lemon pomace) constituted by peels, pulps and seeds, thus representing a serious environmental issue. Notably, tomato and lemon wastes have been recently tested for their capability to promote and sustain microbial growth of biotechnologically useful microorganisms: by means of dialysis fermentation, using wastes as cheap growth media, it has been possible to produce either enzymes and biopolymers from extremophilic bacteria (Di Donato et al., 2011). Granadilla (*Passiflora ligularis*) fruits belong to the genus *Passiflora* (*Passifloraceae*) comprising about 500 species of herbaceous vines or trees largely distributed in the warm temperate and tropical regions of America and Africa, with a smaller number of species occurring in South-Eastern Asia, India, Malaysia, and Australia. Beyond their domestic use in fruit salads and in beverages, granadilla fruits can be processed industrially to produce tropical fruit juices: processing of fruits yields about 30% of juice, 52% of peels and 11% of seeds, whose disposal represents a considerable problem for the countries producing *Passiflora* juices.

All the selected wastes were treated by means of a newly developed extraction technique aiming at a more eco-friendly exploitation of residues (Strazzullo et al., 2003; Tommonaro et al., 2008). In recent years isolation of polysaccharides has been the focus of several research papers dealing with the implementation of technologies for their extraction from various natural sources (e.g. plants or fungi). Different approaches have been proposed including hot

water extraction, acid or alkaline extraction, ultrasound-assisted and microwave-assisted extraction. In general, hot-water extraction is the most widely used method for polysaccharide recovery, but it does not provide high yields, and requires long extraction times and high temperatures; using ultrasound or microwave assisted water extractions it is possible to accelerate the recovery process but without increasing the extraction yield, since a plenty of polysaccharides usually remain in the water extraction residues. A recent report by Huang et al. (2010) has been focused on the optimization of the alkaline extraction of polysaccharides from the fungus *Ganoderma lucidum* employing NaOH solutions at 60.1 °C: such conditions afforded an experimental yield value of 8.30%, close to the expected value of 8.21%, calculated by means of a response surface methodology design. Notably, comparable polysaccharides yields (7.5%) were previously obtained by means of KOH extraction at room temperature (Strazzullo et al., 2003; Tommonaro et al., 2008): this is a rapid method conceived to recover high grade polysaccharides in elevated yields with a lower environmental impact. The alkaline method has been implemented in a previous study dealing with the recovery of minor polysaccharides from tomato canning residues, and it has been successively applied also to granadilla processing wastes (Tommonaro et al., 2007). Below are reported data previously published about tomato and granadilla treatment by means of alkaline extraction that has enabled isolation of new polysaccharides possessing interesting biological activity and chemical-physical features useful for biotechnological applications (Tommonaro et al., 2008). Furthermore, results obtained applying the same method to lemon processing wastes are described in the following sections.

2.2.1 Extraction and chemical-physical analyses of polysaccharides from tomato, granadilla and lemon wastes

Tomato canning wastes (*Lycopersicon esculentum* variety “Hybrid Rome”) were kindly supplied by Fontanella Industry (Salerno, Italy). They mainly consisted of peels, seeds, rotten and unripe fruits. Fruits of granadilla (*Passiflora ligularis*) were grown in Perú in subtropical pedo-climatic conditions, at a temperature ranging from 15 to 20°C and an altitude of 1,800-2,200 m. The fruits were harvested at the end of April, corresponding to the peak of their ripening. Samples were brought to Italy and kept at -20°C before being analyzed. After peeling, pulp was removed, whereas peels were freeze-dried in order to prevent microbial alteration and used for the polysaccharides extraction procedures. Lemon wastes (*Citrus limon*), generated from industrial processing of lemons for juice extraction and liquor production, were kindly supplied by Solagri s.c. (Sant' Agnello di Sorrento, Naples, Italy). They are identified as “lemon pomace”, mainly constituted by albedo, exhausted peels, seeds and the remaining pulps after juice and essential oil recovery. Each waste sample was freeze-dried in order to prevent microbial alteration and the resulting solid dried material was finely ground to a homogeneous powder in a laboratory blender. Samples of the three selected wastes were treated by means of an eco-compatible method, implemented for the extraction of polysaccharides from industrial tomato wastes (Strazzullo et al., 2003; Tommonaro et al., 2008), subsequently applied to peels of granadilla fruits (Tommonaro et al., 2007) and more recently employed for lemon pomace. Briefly, wastes were treated with 5N KOH (1:20 g dry waste/mL KOH) for 72 hours, under stirring at room temperature. For tomato wastes, yields of polysaccharides extraction were determined at different KOH concentrations (0.5N, 1N, 2N and 5 N) and different extraction times (24, 48 and 72 hours), whilst for lemon pomace only 5N KOH was tested at different extraction times (24, 48 and 72 hours). After extraction, the suspensions were sieved and centrifuged at 10,000g for 40' in order to separate the solid

residues. The supernatants were then added with cold ethanol 96% (v/v) dropwise under stirring. The alcoholic solutions were stored at - 20°C overnight and then centrifuged at 10,000g for 1 h at 4 °C. The pellets, solved in hot water, cooled at room temperature and dialyzed for 3 days against tap water in Spectrapore dialysis tubes (10,000-14,000 MW cut-off), were finally frozen and lyophilized under vacuum. The extraction yields obtained by means of this procedure for the different wastes are reported in Table 2: the lowest value was registered in the case of granadilla peels (about 1%), followed by tomato wastes (7.5-10.0%) and the highest value (about 14%) was displayed by lemon pomace. The lyophilized samples thus obtained were utilized for chemical and physical analyses. First of all, aqueous solutions of extracted polysaccharides underwent colorimetric assays for the determination of carbohydrate content, according to the phenol/sulfuric acid method with glucose as standard (Dubois, 1956), total protein content, according to Bradford’s method (Bradford, 1976) and uronic acid content, according to Blumenkrantz and Asboe-Hansen’s method (Blumenkrantz & Asboe-Hansen, 1973). Nucleic acid content was also estimated spectrophotometrically by measuring the absorbance at 260 nm. All the extracted biopolymers were characterized by low protein and nucleic acid contents (< 5.0%) and high carbohydrate contents, corresponding to 100%, 75.5% and 65.5% in tomato, granadilla and lemon polysaccharides, respectively. The presence of uronic acids was notable in the lemon polysaccharide (27.5%), due to the high content of cell-wall pectins in lemon pomace matter (MacDougall et al., 1996), and lower amounts of uronic acids were detected in polysaccharides extracted from granadilla peels and tomato wastes (5.5% and 20%, respectively).

Parameters	<i>Lycopersicon esculentum</i> L. var. San Marzano (solid tomato wastes from processing industry) ^a	<i>Passiflora ligularis</i> called <i>granadilla</i> or passion fruits (peels) ^b	<i>Citrus limon</i> (lemon pomace from processing industry) ^c
Yield (%)	7.5-10.0	1.0	14.3
Molecular Weight (Da)	≥1,000,000	≥1,000,000	≈1,500,000
Carbohydrates (%)	100	75.5	65.5
Uronic Acids (%)	20	5.5	27.5
Proteins (%)	1.0	1.0	3.5
Nucleic acids (%)	1.0	<1.0	1.5
Monosaccharide ^e composition (% molar ratio)	Glc/Xyl/Gal/GalN/ GlcN/Fuc (1:0.9:0.5:0.4:0.2:tr)	Xyl/Glc/Gal/GalN/ uk/Fuc (1:0.5:0.2:0.06:0.05:tr)	Glc/GalA/Man/ Ara/Rha (1:0.7:0.6:0.5:tr)
TGA (°C)	250°C	280°C	295°C
[α] _D ²⁵ (1 mg/ml H ₂ O)	- 177.58	- 186.42	- 98.65 ^d

^a Data from Tommonaro et al. 2008; ^b Data from Tommonaro et al., 2007; ^c data from present study; ^d concentration 1.4 mg/ ml H₂O; ^eabbreviations for monosaccharide composition: Glc=glucose, Xyl=xylose, Gal=galactose, Fuc=fucose, GalA=galacturonic acid, Man=mannose, Ara=arabinose, GalN= galactosammine, GlcN= glucosamine, Rha=rhamnose, uk= unknown sugar, tr= trace.

Table 2. Physical-chemical characteristics of polysaccharides recovered from vegetable sources.

Isolated polysaccharides were then purified by means of gel filtration on a Sepharose DEAE CL-6B column, eluted with distilled H₂O (100 ml) and a linear gradient of NaCl (400 ml) from 0 to 1M at a flow rate of 0.3 ml/min. Fractions (5 ml) were collected, spotted on a pre-coated thin- layer chromatography (TLC) plate (silica gel F₂₅₄, 0.25 mm thick, Merk) and sprayed with α -naphthol to preliminarily identify carbohydrates-containing fractions. For each purification step, both protein and nucleic acid contents were verified, monitoring the absorbance at 280 nm and 260 nm, respectively. The fractions that resulted to contain higher amounts of carbohydrates, according to Dubois assays, were pooled and freeze-dried for further analyses. Polysaccharides from tomato wastes were purified as previously described, showing three main peaks: a first major peak with the highest carbohydrate content (90%) and a second peak (carbohydrate content corresponding to 50%) were eluted in water, while a third peak (carbohydrate content of 41%) was eluted in 0.2 M NaCl. Only the first pool underwent further analyses. Purification of raw polysaccharide from granadilla peels gave three sugar pooled fractions: the first pool, eluted in water, presented a high sugar content (75%), whereas the second pool, eluted in 0,1 M NaCl, and the third pool, eluted in 0,25 M NaCl, contained 21.5% and 6.5% of carbohydrates, respectively. Also in this case, only the first pool underwent further analyses. In the case of lemon pomace, two main peaks were collected: the first peak, with a carbohydrate content corresponding to 44%, was eluted in 0.55 M NaCl and the second peak, containing 10% of carbohydrates, was eluted in 0.7 M NaCl. Protein and nucleic acid contents were lower than 1% in both fractions, and only the first peak was further analysed.

Molecular weight of purified samples (at a concentration of 10 mg/ml in distilled H₂O) was estimated by gel filtration on Sepharose CL-6B column (1x80 cm), using H₂O as mobile phase at a flow rate of 0.3 ml/min. Fractions (3.5 ml) were collected and tested as described before. Molecular weight values were evaluated by interpolation of elution volumes of fractions with higher carbohydrate contents on calibration curves of standard dextrans (150,000, 670,000 and 1,800,000 Daltons, Fluka). In the case of polysaccharides extracted from tomato waste and granadilla peels, values greater than 1,000,000 Da were detected, whilst the lemon pomace polysaccharide resulted to have a molecular weight of approximately 1,500,000 Da.

In order to verify the monosaccharide composition, the purified polysaccharide fractions were hydrolysed with 2M trifluoroacetic acid (TFA) at 120°C for 2 hours. Sugar components were firstly detected by TLC, using a mix of acetone/butanol/H₂O (8:1:1, v/v/v) as mobile phase and standard monosaccharides for qualitative determination. Afterwards monosaccharide composition was defined by High Pressure Anion Exchange-Pulsed Amperometric Detector (HPAE-PAD, DIONEX) equipped with Carbopac PA1 column. Sugars were eluted isocratically with 16 mM NaOH and identified by comparison with reference standards. The results are reported in Table 2. In the case of tomato polysaccharide the following neutral sugar composition was found: glucose/ xylose/ galactose/ galactosamine/ glucosamine/ fucose in a relative molar ratio of 1:0.9:0.5:0.4:0.2:trace; in addition, the presence of galacturonic acid was also detected in the sample. The sugar components of granadilla peels polysaccharide were: xylose/ glucose/ galactose/ galactosamine/ unknown sugar/ fucose in the relative proportion of 1:0.5:0.2:0.06:0.05:trace, whilst in the case of lemon pomace polysaccharide, galactose/ galacturonic acid/ arabinose/ fructose/ rhamnose were identified in a relative molar ratio of 1:0.7: 0.6:0.5:trace. The determination of optical rotation values as well as thermogravimetric analyses (TGA) of polysaccharides were also performed, and the results are shown in Table 2. Optical rotation values were acquired with a Perkin-Elmer 243 B polarimeter at 25° C in water.

Thermogravimetical analyses were carried out using a Mettler TGA apparatus. Each sample (5 mg) was heated from 30 to 400°C at a heating rate of 20 °C/min under nitrogen. The degradation temperatures of tomato waste and granadilla peel polysaccharides were estimated to be approximately 250°C (Tommonaro et al., 2008) and 280°C (Tommonaro et al., 2007), respectively (Table 2).

The thermogravimetical analysis of lemon pomace polysaccharide showed a weight loss due to water presence, centered at 60-80°C (Fig. 1). From such temperatures up to 290°C, the biopolymer was very stable and started to decompose at 295°C.

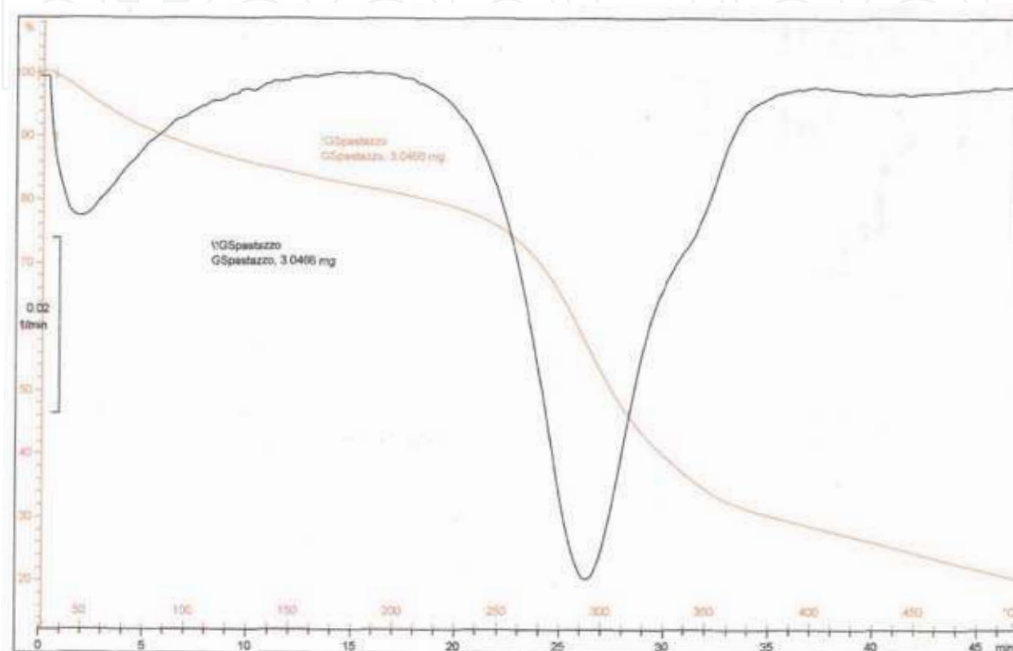


Fig. 1. Thermogravimetical analysis of lemon pomace polysaccharides.

Polysaccharide structures were further investigated by means of classical spectroscopic techniques such as NMR and IR spectroscopy. NMR spectra of purified samples (15 mg/ml D₂O) were performed on a Bruker AMX- 300 MHz (¹H-NMR) and 400 MHz (¹³C-NMR) at 70 °C. Samples were prepared according to Poli et al. (2009). Briefly, each sample was exchanged twice with D₂O with a lyophilization step in-between and finally dissolved in 500 µl of D₂O. Chemical shifts were reported in parts per million (ppm) with reference to sodium 2,2,3,3-d₄-(trimethylsilyl) propanoate and to CDCl₃ for ¹H and ¹³C-NMR spectra, respectively. The ¹H-NMR spectrum of the tomato waste polysaccharide showed a complex profile, with main signals at δ values as reported in Table 3. The anomeric region of the spectrum (from δ 4.5 to δ 5.5) exhibited eight peaks: five of them were well resolved doublets (d) with the same coupling constant value of J_{1-2} (3.8-4.0 Hz), probably due to a *gluco-galacto* sugar configuration, whereas the other three anomeric peaks, almost singlets (s) with a small J_{1-2} (0.5-1 Hz), indicated the occurrence of a *manno* configuration. The upfield region of the spectrum showed a doublet peak at δ 1.20 indicating the presence of deoxy-sugars in the polysaccharide. The eight anomeric signals suggested the incidence of eight different monosaccharides, with regard to the type or the glycosidic linkage position. The eight monosaccharides were labelled from A to H with respect to decreasing δ (Table 3). On the base of the chemical shifts and the coupling constant data, the residues A, D, F had probably an α -*manno* configuration, the residues B, C, E, G probably possessed an α -*gluco-galacto* configuration and the H residue had probably a β -*gluco-galacto* configuration.

Type ^b	δ ¹ H	Multiplicity	J ₁₋₂ ^c	Configuration
A	5.30	pseudo s	0.5-1 Hz	α-manno
B	5.27	d	3.8-4.0 Hz	α-gluco-galacto
C	5.26	d	3.8-4.0 Hz	α-gluco-galacto
D	5.18	pseudo s	0.5-1 Hz	α-manno
E	5.09	d	3.8-4.0 Hz	α-gluco-galacto
F	5.07	pseudo s	0.5-1 Hz	α-manno
G	5.06	d	3.8-4.0 Hz	α-gluco-galacto
H	4.94	d	3.8-4.0 Hz	β-gluco-galacto

Table 3. Chemical shifts and coupling constant of anomeric signals in ¹H-NMR spectrum of tomato polysaccharide*. ^aData from Strazzullo et al., 2003 and Tommonaro et al., 2008; ^blabels refer to signals with decreasing δ; ^c coupling constant; doublets (d); singlets (s).

The ¹H-NMR data relative to analyses of granadilla peel polysaccharide are reported in Table 4. The ¹H-NMR spectrum showed six well resolved peaks in the anomeric region: at δ 4.87 (pseudo singlet, J< 1.0 Hz); δ 4.94 (d, J=5.7 Hz); δ 5.08 (s); δ 5.27 (s); δ 5.47 (d, J=10.3 Hz); δ 5.60 (s) (Table 4). The signals in the upfield region of the spectrum indicated the presence of deoxy-sugars (δ 1.29) and acetamino sugars (δ 2.45-2.55). Remaining signals (δ ranging from 3.5 to 4.5) were due to ring protons confirming the occurrence of pyrosidic exose. The six anomeric signals, labelled in Table 4 from A to F, indicated the presence of six different monosaccharides, presented in the repeating unit. Chemical shifts and coupling constant values pointed out that the residues B, E and F probably had an α-gluco-galacto, a β-gluco-galacto and a β-manno configuration, respectively.

Type ^b	δ ¹ H	Multiplicity	J ₁₋₂ ^c	Configuration
A	5.60	s	-	-
B	5.47	d	10.3 Hz	α-gluco-galacto
C	5.27	s	-	-
D	5.08	s	-	-
E	4.94	d	5.7 Hz	β-gluco-galacto
F	4.87	pseudo s	< 1.0 Hz	β-manno

Table 4. Chemical shifts and coupling constant of anomeric signals in ¹H-NMR spectrum of granadilla polysaccharide*. ^aData from Tommonaro et al., 2007; ^blabels refer to signals with decreasing δ; ^c coupling constant; doublets (d); singlets (s).

The ¹H-NMR and the ¹³C-NMR analysis of raw lemon pomace polysaccharide resulted in a very complex signal pattern. The ¹H-NMR spectrum of lemon polysaccharide showed *inter alia* four signals in the anomeric region (Fig. 2) at δ 5.28 (pseudo singlet, J<1.0 Hz), δ 5.19 (d, J=8.7 Hz), δ 5.12 (pseudo singlet, J<1.0 Hz) and δ 4.67 (d, J=8.7 Hz). The signals in the upfield region of the spectrum indicated the presence of deoxy-sugars (δ 1.3). The four anomeric signals highlighted the incidence of four different monosaccharides, with regard to the type or the glycosidic linkage position. On the basis of the chemical shifts and coupling constant data three residues were supposed to have an α-configuration, whilst the fourth showed a β-configuration.

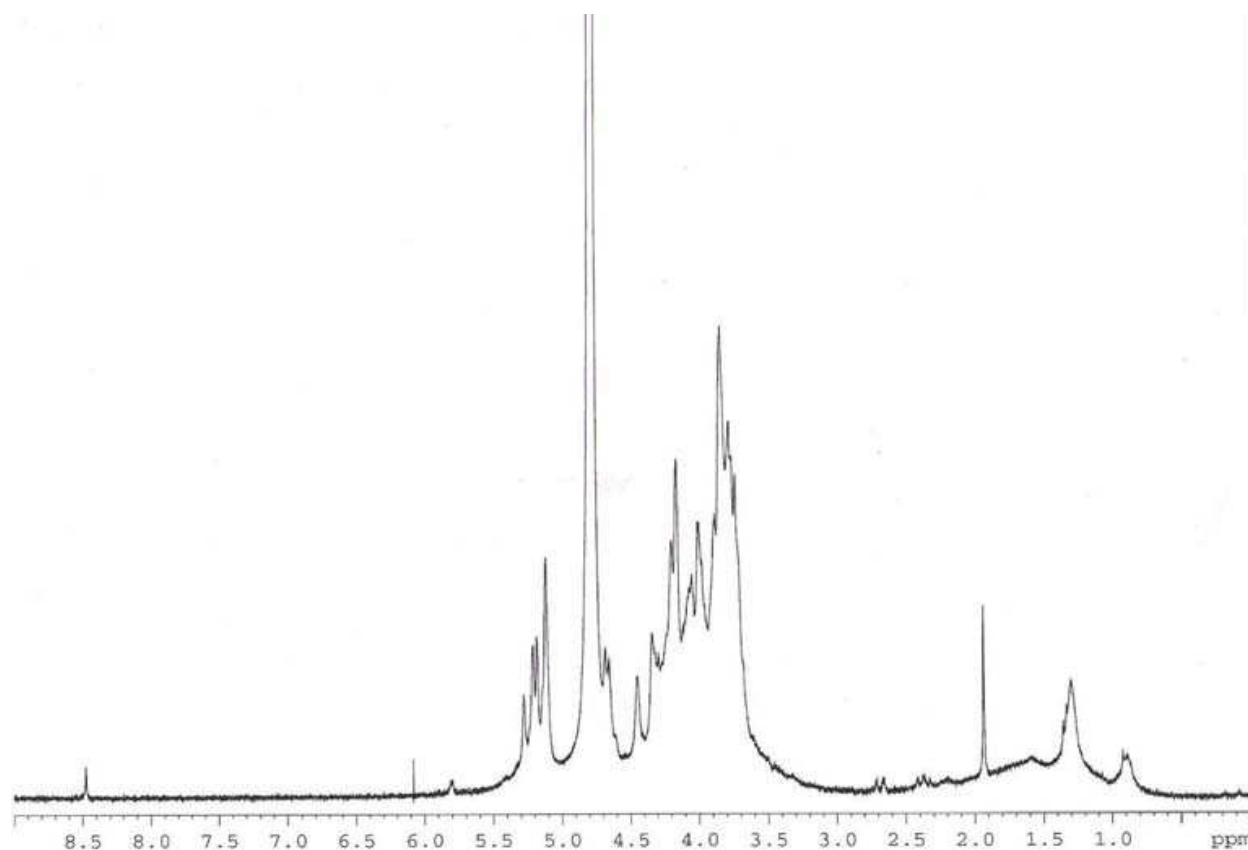


Fig. 2. ^1H NMR spectrum of the raw lemon pomace polysaccharide. Chemical shifts are reported in ppm relative to sodium 2,2,3,3- d_4 -(trimethylsilyl)propanoate.

The ^{13}C -NMR spectrum of raw lemon pomace polysaccharide was essentially characterised by four signals in the anomeric region at δ 105.55, 107.52, 107.99, 108.61, confirming the presence of four residues in the repeating unit (Fig. 3). A signal at δ 178.77 attributable to the carbonyl group (uronic acids) was also detected. Moreover, the presence of an arabino furanosidic residue was confirmed by the presence of signals belonging to ring carbons in the region at δ between 81 and 85 ppm.

More detailed information about the structure will be available when NMR spectroscopic studies will be completed.

Further insights in the structure of polysaccharides from tomato, granadilla and lemon wastes were obtained by means of infra red spectroscopy. Fourier Transform Infra Red spectra were recorded by a Perkin-Elmer Paragon 500 single-beam spectrophotometer. The powder sample was grounded with KBr, put under beam as diskette and the spectrum was collected after 16 scans under nitrogen.

In the infrared spectrum of tomato polysaccharide the bands relative to OH, CH and C=O stretching were distinguished at $3,400\text{ cm}^{-1}$, $2,929\text{ cm}^{-1}$ and $1,730\text{--}1,660\text{ cm}^{-1}$, respectively (Strazzullo et al., 2003). The IR spectrum ($400\text{--}4,000\text{ cm}^{-1}$) of granadilla peels polysaccharide was characterized by several bands typical of polysaccharide structure that can be attributed to functional groups present in the biopolymer (Tommonaro et al., 2007). The spectrum presented bands at $3,000\text{--}3,600\text{ cm}^{-1}$ corresponding to O-H stretching vibration and at $2,870\text{--}$

2,922 cm^{-1} corresponding to CH_2 asymmetric and symmetric stretching vibrations. The band at 1,635 cm^{-1} was attributed to a N-acetyl group of a sugar residue or to the stretching vibration of $\text{C}=\text{O}$. A broad absorption band attributable to $\text{S}=\text{O}$ was observed at 1,240 cm^{-1} . Bands at 1,159 cm^{-1} and 1,045 cm^{-1} were ascribed to the stretching vibrations of $\text{C}-\text{O}-\text{C}$ and $\text{O}-\text{H}$, respectively. Finally, the absorption occurring at about 900 cm^{-1} was evidence for the β -configuration of the glucan linkages of some residues. In the case of raw lemon pomace polysaccharide, the IR spectrum (shown in Fig. 4), although not well resolved, showed absorption bands at 3,397 cm^{-1} and at 2,925 cm^{-1} that could indicate the OH and CH stretching, respectively. The band at 1,636 cm^{-1} was attributed to the stretching vibration of $\text{C}=\text{O}$. In addition, signals at 1,452 cm^{-1} and at 1,084 cm^{-1} could correspondingly attributed to CH and OH deformation. The absorption occurred at about 855 cm^{-1} could denote the β -configuration presence in some sugar residues.

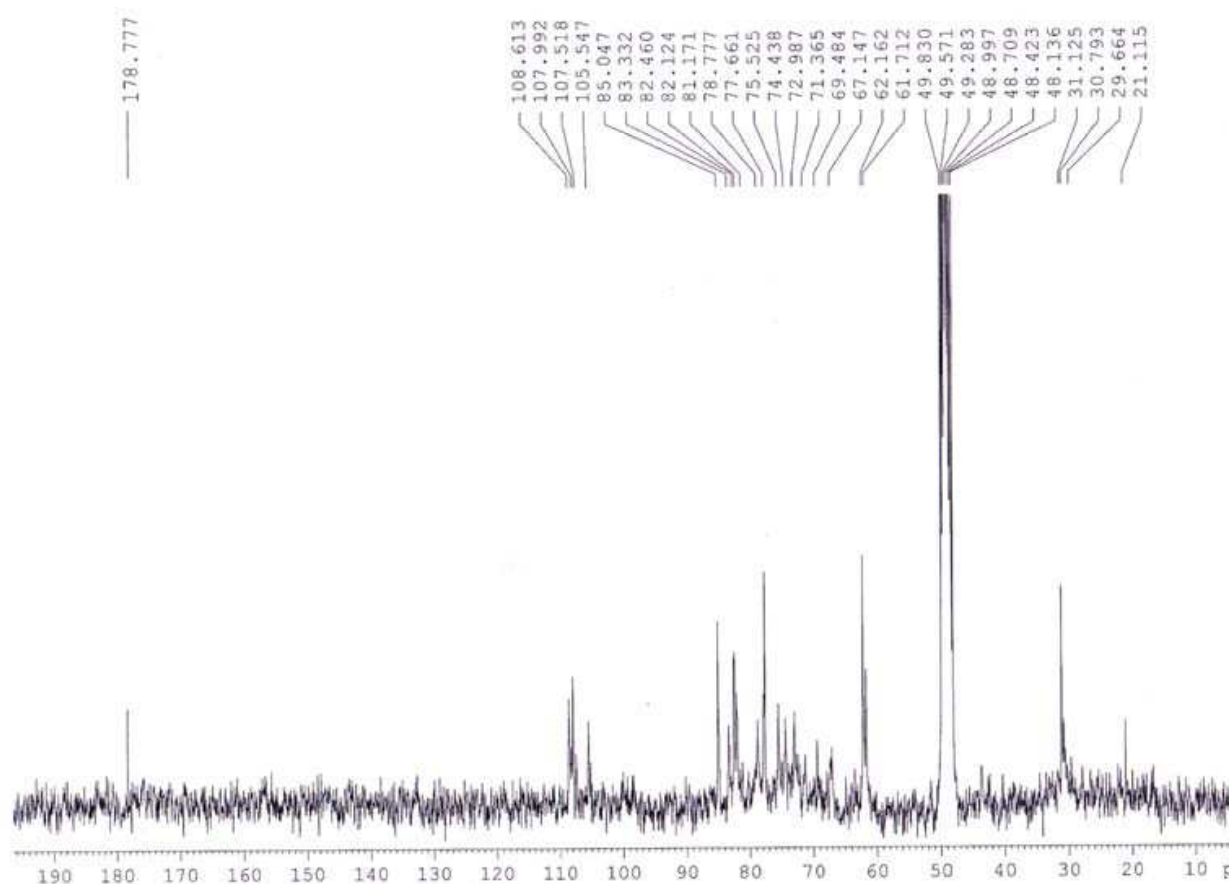


Fig. 3. ^{13}C -NMR spectrum of the raw lemon pomace polysaccharide. Chemical shifts are reported in ppm relative to CDCl_3 .

Specific viscosity (η) as function of concentration of polysaccharides aqueous solution was determined for all three waste s biopolymer by using Cannon-Ubbelohde 75 suspended level viscometers at 30°C. Rheological properties were also considered by studying the specific viscosity of each sample (at a concentration of 1%) with respect to the pH range obtained with different appropriate buffers at 30°C. In the case of the polysaccharide extracted from tomato wastes, the viscosity of aqueous solution reached the maximum value

of $\eta=1.7$ at a concentration of 4%, while the increase of pH did not affect the viscosity, reaching its highest value ($\eta=3.29$) at pH 3.0 with 1% polysaccharide solution in 50 mM citrate buffer. The measurement of viscosity (η) of granadilla polysaccharide was performed using different concentrations of aqueous solution of polysaccharide (0.5%; 1.0%; 2.0%; 2.5%; 3.0%, w/v). A linear correlation between concentration and viscosity of polysaccharide solution was observed, in particular the lowest viscosity (0.63 η) was registered at a concentration of 0.5% and the highest viscosity (1.4 η) at the maximum concentration tested (3.0%). For viscosity measurements at a given concentration of polysaccharide (1.0%) in a pH range (2.32-8.36), a linear increase of viscosity in relation to the increase of pH values was registered, with the highest viscosity (7.0 η) in citrate-phosphate buffer solution at pH 8.0. The measurement of viscosity (η) of lemon pomace polysaccharide was performed using 1.0% polysaccharide solution in a pH range (4.0-7.0) in 50 mM citrate-phosphate buffer. In this case the lowest value (0.58 η) was registered at pH 4.0, whereas the highest (0.77 η) at pH 7.0.

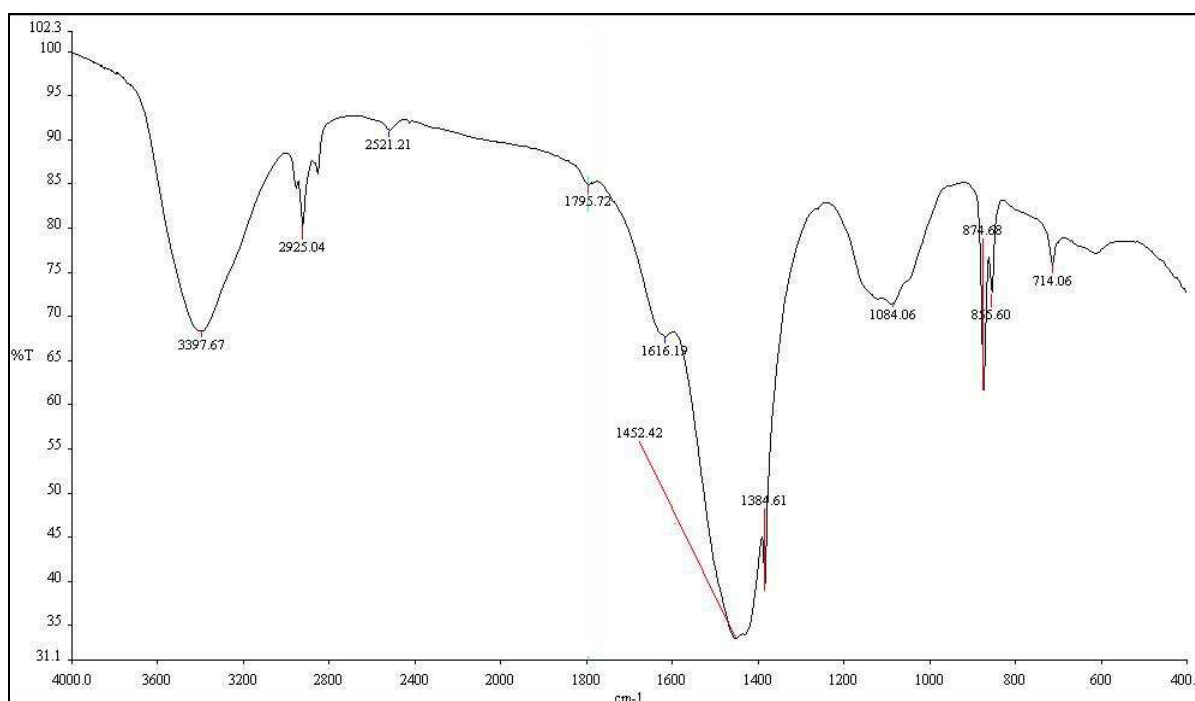


Fig. 4. IR spectrum of raw lemon pomace polysaccharide. Fourier Transform Infra Red spectrum was recorded by using a Perkin – Elmer Paragon 500 single-beam spectrophotometer.

2.2.2 Biological properties of polysaccharides from tomato, granadilla and lemon wastes

The anticytotoxic activity of polysaccharides as function of inhibition of Avarol (10 $\mu\text{g/ml}$) toxicity in brine shrimp (*Artemia salina*) bioassay was evaluated (Minale et al., 1974). Avarol (a natural toxic sesquiterpene hydroquinone isolated from *Dysidea avara* sponge) shows strong toxicity (LC_{50} 0.18 $\mu\text{g/ml}$) in brine shrimp bioassay, that gives results well correlated with cytotoxicity in the cancer cell lines such as KB, P388, L5178y and L1210 (De Rosa et al.,

1994). For this assay 10 ppm Avarol were used in order to obtain the death of all the brine shrimp larvae. Briefly, for each dose tested, surviving shrimps were counted after 24 h, and the data statistically analysed by the Finney program, which affords LD₅₀ values with 95% confidence intervals (Finney, 1971).

The biopolymer isolated from granadilla peels was found to be an anticytotoxic compound in this bioassay, increasing the value of Avarol LD₅₀ from 0.18 µg/ml up to 2.13 µg/ml when present at a concentration of 500 ppm (Table 5). Lemon pomace polysaccharide behaved in a similar way (Table 5), with a value of Avarol LD₅₀ of 2.32 µg/ml when present at a concentration of 500 ppm. Finally, the protective effect of the tomato waste polysaccharide against the toxic role of Avarol was more evident even at a lower concentration (5 ppm). Tomato polysaccharide in a concentration of 500 ppm caused an increase of Avarol LD₅₀ up to 11 µg/ml, thus resulting the most powerful anti-toxic agent (Table 5).

	500 ppm ^d	50 ppm ^d	20 ppm ^d	5 ppm ^d	LD ₅₀ ppm
Granadilla polysaccharide	28/30 ^e	26/30	n.d.	28/30	n.a.
Granadilla polysaccharide +Avarol 10 ppm	10/30	0/30	n.d.	0/30	2.13 ^f
Lemon pomace polysaccharide	29/30	30/30	30/30	n.d.	n.a.
Lemon pomace polysaccharide +Avarol 10 ppm	11/30	8/30	8/30	n.d.	2.32 ^f
Tomato waste polysaccharide	28/30	28/30	n.d.	26/30	n.a.
Tomato waste polysaccharide +Avarol 10 ppm	25/30	23/30	n.d.	11/30	11 ^f

Table 5. Inhibition of Avarol Toxic Activity in *Artemia salina* bioassay by granadilla peel^a, lemon pomace^b and tomato waste polysaccharides^c. ^aData from Tommonaro et al., 2007; ^bdata from present study; ^cdata from Tommonaro et al., 2008; ^dconcentration of polysaccharide in 1% DMSO; ^esurvivals/total larvae of *Artemia salina* after 24 h of incubation in artificial sea water; ^fvalues of 95% confidence intervals; n.d. not determinate; n.a. no activity.

Additional biological properties of tomato polysaccharide were studied by De Stefano et al. (2007) that reported the effects of this polymer on the J774 mouse monocyte/macrophage cell line. The authors pointed out that polysaccharide inhibited in a concentration-dependent manner the nitrite and ROS productions as well as the iNOS protein expression induced by LPS in stimulated macrophages. Incubation of cells with polysaccharide determined a significant decrease of nuclear factor-κB (NF-κB)/DNA binding activity which was correlated with a marked reduction of the iNOS mRNA levels. These results indicated that tomato polysaccharide inhibited NF-κB activation and iNOS gene expression by preventing the reactive specie productions, thus suggesting a role for this biopolymer in the control of oxidative stress and/or inflammation process.

2.2.3 Biotechnological aspects of tomato wastes polysaccharides

In order to verify the biodegradability of tomato waste polysaccharide, a growth test using a thermohalophilic bacterium *Thermus thermophilus* strain Samu-Sa1 isolated from

hot springs of Mount Grillo (Baia, Naples, Italy) was performed (Romano et al., 2004). Tomato polysaccharide was added as a sole carbon source (1g/L) in M162 medium (Degryse et al., 1978) modified with 2g/L NaCl at pH 7.2. For comparison, strain Samu-SA1 was grown on TH standard medium as described in Romano et al. (2004). The growth was monitored by measuring the absorbance at 540 nm and converted into cell dry weight by means of an appropriate calibration curve. The depletion of polysaccharide content was observed with Dubois method. After 30 h of batch incubation at 75°C (the optimal temperature of growth for strain Samu-Sa1), the tomato polysaccharide had been completely hydrolysed, as confirmed by the Dubois assay applied to the cell-free cultural broth. Moreover, the growth curves of strain Samu-Sa1 on polysaccharide medium were 2.6 fold higher than that obtained in TH medium (standard medium) with a yield of 1.2 g of dry cells/L.

Furthermore, a useful application of polysaccharides extracted from tomato processing industrial wastes and from granadilla peels is the development of biodegradable films by means of biopolymers and glycerol solutions (Strazzullo et al., 2003; Tommonaro et al., 2007). Applying the procedure previously described, solid, clear and elastic films were obtained from both polysaccharides, able to recover small deformations produced by tensile stress applied. These biofilms could have interesting biotechnological applications in different fields, such as agriculture, e.g. for protecting cultivations with mulching operation techniques. In the case of polysaccharide extracted from lemon pomace, no results were obtained using the same procedure for biofilm production and, therefore, further studies are needed.

3. Conclusion

The need for a more sustainable production of polysaccharides based on agricultural feedstock has stimulated the search for renewable sources of such biopolymers, that should not compete with the food chain.

Accordingly, waste biomasses produced by fruits and vegetable processing have shown to be promising raw materials either for polysaccharides industrial production and for the discovery of new ones. In fact, such waste biomasses are produced every year in huge amounts by food production based on transformation of fruits and vegetables: they comprise mainly peels, seeds and pulps in which many kinds of molecules and biopolymers are still retained after processing for juice extraction, canning, beverages production, *et cetera*.

Polysaccharides are the most abundant biopolymers that can be recovered from these food by-products and several examples are indeed available in literature concerning their exploitation for polysaccharide extractions. In this regard, the search for under-utilized waste biomass has been paralleled also by the implementation of processes and technologies that are safe for the environment and that allow a more complete exploitation of such wastes for a sustainable production of polysaccharides. In this section, besides remarkable examples present in literature, we also reported our results about the use of fruit and vegetable residues for the extraction of polysaccharides by means of a newly developed method based on alkaline treatment of waste biomasses. The investigated biomasses are representative of some significant food production based on fruits and vegetables cultivated in European and South American countries.

A new extraction methodology, based on alkali treatment of biomass at room temperature, has been implemented for tomato waste exploitation and has been successively employed for polysaccharides isolation from granadilla wastes and more recently also for lemon pomace and new unpublished data related to lemon pomace were reported here. By means of the alkali treatment new polysaccharides have been isolated from the selected waste vegetable biomass whose biochemical properties have been studied. Tomato waste polysaccharide, showing a structure similar to a xyloglucan biopolymer, was investigated for its biological activity and showed to be anticytotoxic in the Brine Shrimp bioassay; moreover it also exerted an interesting anti-oxidant activity, as confirmed by ROS inhibition assay, suggesting a role for this compound in the control of oxidative stress and/or inflammation. Biotechnological application for this biopolymer were suggested by its capability to form biofilms, that could be used in different fields such as agriculture, i.e. for protected cultivation with mulching operation technique. In addition, tomato waste polysaccharide biodegradability was assessed by using it as sole carbon source for microbial growth, thus highlighting also its employment for cheap biomass production. Granadilla and lemon polysaccharides, showing a xylan-like and a pectin-like structure, respectively, were also investigated for their rheological properties and for their biological activities, both confirming to be anticytotoxic compounds in the Brine Shrimp bioassay. Future perspectives of this research include further characterization of lemon polysaccharide properties, investigation of other vegetable biomass generated in significant amounts and useful for polysaccharide biopolymers production and finally improvement of extraction technologies. Incidentally, it can be underlined that the wastes investigated were used without any chemical or physical pre-treatment, therefore polysaccharide yields from vegetable wastes could be improved by employing physical (e.g. ultrasound or microwave treatment) or enzyme methods.

In conclusion, resources depletion and environmental concerns have triggered new regulations and growing awareness throughout the world, thus promoting the use of renewable resources to produce chemicals and energy. Accordingly, the production of useful biopolymers like polysaccharides starting from cheap and abundant renewable resources, such as residues of fruits and vegetables processed for food production, could contribute to the issue of waste management and to the implementation of the "biorefinery" strategy.

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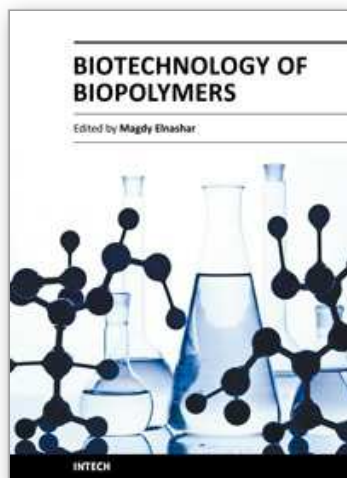
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The book "Biotechnology of Biopolymers" comprises 17 chapters covering occurrence, synthesis, isolation and production, properties and applications, biodegradation and modification, the relevant analysis methods to reveal the structures and properties of biopolymers and a special section on the theoretical, experimental and mathematical models of biopolymers. This book will hopefully be supportive to many scientists, physicians, pharmaceuticals, engineers and other experts in a wide variety of different disciplines, in academia and in industry. It may not only support research and development but may be also suitable for teaching. Publishing of this book was achieved by choosing authors of the individual chapters for their recognized expertise and for their excellent contributions to the various fields of research.

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Phone: +86-21-62489820
Fax: +86-21-62489821

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