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Re-use of Agro-industrial Waste: Recovery of Valuable Compounds by Eco-friendly Techniques

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Abstract: The global demographic expansion has determined a voracious demand for edible goods, thus also originating the primary issues about the disposal of waste and by-products with high environmental impact. Waste, by-products and effluents coming from industrial processing and agricultural procedures of vegetables and fruit can be defined as biomass, according to CE directive 2001/77. Those raw materials are currently used as compost, animal feed and biofuel production. In addition, these products can be used as starting substrates for the production of high value-added compounds according to the biorefinery concept. In this review, the biorefinery strategy was applied on waste coming from the industrial processing of lemon and tomato, two of the most abundant vegetables in Italy.

Keywords: *biomass; biorefinery, polysaccharides, tomato, lemon.*

1. Introduction

This review article highlights the biorefining approach to the reuse of vegetable biomasses that could provide valuable compounds, potentially useful for several industrial sectors. The main focus is on the agro-industrial waste, *i.e.*, residual biomass produced by food industries based on the processing of fruit and vegetables. Actually, these activities represent a pivotal industrial area for Italy and other European countries, but they produce high amount of waste that have to be managed as material with high environmental impact, thus requiring high economic cost for their disposal.

It is estimated that the food industry in Europe generates about 250 million tons/year of by-products, waste and effluents and the 6% of them is represented by fruits and vegetable [1]. Such waste derives from the selection of vegetables for the fresh consumption in wholesale markets and packaging industries of fresh vegetables for the supermarket selling. Waste and by-products include fruits that are damaged, unripe or lack the qualitative standards required for the market selling such as regularity in shape and color. Huge amounts of waste are also generated after the production of juices, canned goods, sauces, liquors, *etc*., as solid and liquid residues, that are usually disposed into landfills or are used as compost or animal feed.

All these wastes pose increasing disposal and potentially severe pollution problems because of their high organic matter content and, in addition, they represent a loss of valuable biomass and nutrients. However, within an approach aiming at the sustainability of all the supply chains related to the cultivation and processing of vegetables, it is

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essential to develop a strategy for waste minimization and value-adding to the agro-industrial waste and by-products, especially in relation to the large quantities of vegetables processed annually. This could be implemented by a new production strategy based on the exchanges of matter and energy between different production sectors, in which co-products of one industrial area become resources to other industrial processes. All these concepts are addressed in the broader model of biorefinery, *i.e.,* an industrial plant with zero emissions and sustainable transformation of biomass in a wide range of high added value products and energy [2].

Currently, different strategies of treatment are applied for the valorisation of the agro-industrial waste such as compost, (a product of humification of organic matter and it is considered a nutrient-rich, organic fertilizer and soil conditioner), animal feed (thanks to the high fibres content) and bioethanol production. Actually, vegetable waste, due to their high polysaccharides content, can be subjected to solid state fermentation for the production of ethanol that should be used as liquid fuel supplement and solvent in many industries. Other alternative strategies for the valorisation of agro-industry waste also include their exploitation as growth media for biotechnologically useful microorganisms and their related enzymes [3] or as source of biopolymers, *i.e.*, bioactive compounds, antioxidants and anti-inflammatory that should be used in several industrial area as food cosmetic and pharmaceutical industries [4]. Actually, agro-industrial residues represent a cheap chemical feedstock for extraction of useful products since they are rich in high value added compounds like lipids, fibres, natural pigments, carotenoids and antioxidant and bioactive molecules such as polyphenolic compounds.

According to these considerations, this review highlights the application of the biorefinery concept on waste obtained from the industrial processing of tomato and lemon, two of the most abundant vegetables present on Italian market.

Actually, the industrial transformation of tomatoes for the canning industry plays a central role for the Italian economy. The total tomato production amounts to nearly 9,000,000 tons/year and about 1.8-2.3% of them (*i.e.,* 162,000-207,000 tons) is discarded as waste constituted by unripe or damaged fruits, peels and seeds.

Citrus fruits processing is one of the foremost industrial activities in Italy, and they are estimated to produce about 3-4.2×106 tons per year of waste. Such residual biomass, that represent more than 60% in weight of the processed lemons, is constituted of peels and seeds, and by the so-called “pastazzo” *i.e.,* lemon pomace (the albedo part of the fruit) that is particularly rich in fibres.

Such wastes were used as sources of polysaccharides since such biomolecules find several application in different fields including as an example production of food nutrients, food additives and feed; formulation of polymeric materials for different biotechnological applications (*i.e.,* biocompatible materials), for drug delivery, as source of biologically active molecules; finally for sustainable energy production by means of biofuels generation.

2. Materials and Methods

Vegetable Waste: Tomato **(***Lycopersicon esculentum* var. ‘‘*Hybrid Rome*’’**)** by-products and waste (peels and seeds) derived from tomato canning process were kindly supplied by “Fontanella Industry” (Sa). Lemon (*Citrus limon*) by-products derived from processed lemons for juice and liquor production, constituted by peels, seeds and pomace, were supplied by “Solagri s.c.” (Sant’Agnello di Sorrento, Na). All waste were finely minced and lyophilized before extraction.

Extraction Procedure: 1 g of powdered material was suspended in 20 ml KOH 5N, and kept under stirring at room temperature for 72 hours; the resulting mixture was sieved and centrifuged at 10,000 rpm for 40 min. The solid part retained onto the sieve and the pellet collected after the centrifugation were dissolved in H_2O and neutralized with 1M HCl. The residue was filtered and successively lyophilized. The resulting powder was dissolved in H_2O and the polysaccharide was precipitated by adding cold ethanol (1/1, v/v) drop-wise under stirring. The alcoholic solution was kept at –20°C overnight and subsequently centrifuged at 10,000 rpm for 1 h at 4°C. The pellet was solved in hot water, cooled to room temperature and dialyzed (Visking Dialysis Membrane MWCO 12–14 kDa, GmbH, Germany) for 3 days against tap water and then freeze-dried. The lyophilized samples obtained were utilized for chemical and physical analyses as previously described [5].

3. Results and Discussion

The most widely used method for polysaccharide recovery is hot-water extraction, but besides being energy requiring (due to the high temperatures and long extraction times of the process) this method does not provide high extraction yields [5]. Notably, higher polysaccharides yields were previously obtained by means of a newly developed extraction technique [4] based on KOH extraction at room temperature. This method was considered more eco-friendly because of the lesser energy (since no heating step is needed) and solvent requirements; moreover as previously reported, it allowed recovery of minor polysaccharides that showed interesting biotechnological and biological features [5].

Table 1: Tomato Waste Polysaccharide Physicochemical Features [5].

Yield(%w/w dry waste)	7.5-10
Molecular Weight	1,000,000
Carbohydrates(% w/w)	~ 80
Uronic Acids (% w/w)	20
Proteins (% w/w)	<1.0
Nucleic Acids (% w/w)	<1.0
Monosaccharide Composition (% molar ratio)	Glc/Xyl/Gal/GalN/GlcN/Fuc (1:0.9:0.5:0.4:0.2:tr)
TGA	250°C
$[\alpha]^{25}$ (1 mg/ml) H_2O	-177.58
Viscosity	0.56

The structural features of the isolated polysaccharide were further investigated by means of spectroscopy analysis.
The ^{1}H-NMR analysis of the purified polysaccharide showed a complex profile with main signals at δ values as reported in Table 2 [5].

Table 2: Chemical Shifts and Coupling Constant of Anomeric Signals in ^{1}H-NMR Spectrum of Tomato Waste Polysaccharide[a] [5].

Type[b]	δ^1H	Multiplicity	$J_{1\text{-}2}$[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*

[a][9] ; [b]labels refer to signals with decreasing δ; [c] coupling constant; doublets (d); singlets (s).

The anomeric region of the spectrum (from δ 4.5 to δ 5.5) exhibited eight peaks suggesting the incidence of eight different monosaccharides: according to chemical shifts and the coupling constant data three have probably an α-*manno* configuration, four an α-*glucogalacto* configuration and the last probably a β-*gluco-galacto* configuration.

The polysaccharide showed a good viscosity, measured on aqueous solution, in agreement with its high molecular weight; moreover it also showed a good thermal since its temperature of degradation was around 250°C.

Moreover it was also used as building block for the production of bioplastic. This work was carried put in collaboration with Dr. Malinconico and Dr. Immirzi of the Institute of Polymer Chemistry and Technology of CNR (ICTP-CNR), that used it used to produce a biodegradable film [4] that could be potentially employed as mulching film in agriculture [6] as alternative to plastic materials, that are usually left on the field or burnt by farmers with negative consequences for the environment.

Finally, the polysaccharide was investigated for its biological properties focusing the attention on its anti-oxidant or anti-inflammatory action. Its ability to act as a free radicals scavenger was assessed by measuring its effect on the production of reactive oxygen species (ROS) and on iNOS protein expression stimulated by LPS in J774 mouse monocyte/macrophages. The isolated polysaccharide showed to be able to inhibit NF-kB activation of iNOS expression with consequent ROS decrease thus confirming a potential role for this compound in controlling oxidative stress and/or inflammation [7].

The lemon waste biomass was treated with KOH 2N for 72 h in order to extract the polysaccharide components as reported above. The fractions obtained were subjected to chemical and physical analyses and the results are reported in Table 2. The polysaccharide fraction showed a high molecular weight (about 1,500,000 Da) and a significant carbohydrates content, with nearly 30% uronic acids amount. The gross chemical composition of this polysaccharide, that showed a low content of both proteins and nucleic acids, was determined by acid hydrolysis of the polymer for the identification of main monomer sugars [5].

As reported in Table 3, galactose/ galacturonic acid/ arabinose/fructose/ rhamnose were the sugars identified in a relative molar ratio of 1:0.7:0.6:0.5:trace, therefore suggesting a galactomannan structure for this polysaccharide fraction.

Table 3: Lemon Waste Polysaccharide Physicochemical Features [5].

Yield (% w/w dry waste)	14.3
Molecular Weight	1,500,000
Carbohydrates (% w/w)	65.5
Uronic Acids (% w/w)	27.5
Proteins (% w/w)	3.5
Nucleic Acids (% w/w)	1.5
Monosaccharide Momposition (% molar ratio)	Gal/GalA/Man/Ara/Rha (1:0.7:0.6:0.5:tr)
TGA	295°C
$[\alpha]^{25}$ (1 mg/ml) H_2O	-98.65

The ^{1}H-NMR analysis of lemon pomace polysaccharide showed a very complex signal pattern with four signals in the anomeric region (Fig. 1) 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): according to the chemical shifts and coupling constant data, three residues were supposed to have an α-configuration, while the fourth showed a β-configuration.

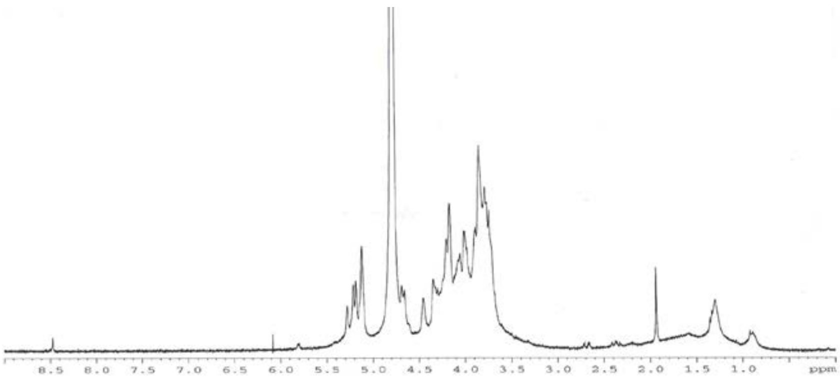

Figure 1: ^{1}H NMR Spectrum of the Raw Lemon Pomace Polysaccharide. Chemical Shifts are Reported in ppm Relative to Sodium 2,2,3,3-d_4-(trimethylsilyl)propanoate [5].

Also for this polysaccharide thermal stability was investigated, showing that also this biopolymer is significantly thermostable since it showed a with a degradation temperature of about 295°C.

Finally, the polysaccharide was investigated for its biological properties by means of the brine shrimp (*Artemia salina*) bioassay [8]. This test allowed to assess its anticytotoxic activity against Avarol, a natural toxic sesquiterpene hydroquinone isolated from *Dysidea avara* sponge that shows strong toxicity in brine shrimp bioassay. The obtained results are reported in Table 4.

When added alone, the lemon polysaccharide showed to be non-cytotoxic for the shrimp larvae till to a 500 ppm concentration (first row in Table 4); in the presence of 10 ppm Avarol, whose LD_{50} is 2.32 μg/ml, it was able to reduce larvae mortality up to about 30% when added at a concentration of 500 ppm. This results showed that lemon polysaccharide could act as a cytotoprotective agent.

Table 4: Inhibition of Avarol Toxic Activity in *Artemia salina* Bioassay [5]

	500 ppm[a]	**50 ppm**	**20 ppm**	**Avarol LD_{50} ppm**
Lemon waste polysaccharide	29/ 30[b]	30/30	30/30	*n.a.*
Lemon waste polysaccharide + Avarol 10 ppm	**11/30**	**8/30**	**8/30**	**2.32**[c]

[a] Concentration of polysaccharide; [b] survivals/total larvae of *Artemia salina;*[c] value of 95% confidence intervals; *n.a.* no activity

4. Conclusions

According to the new philosophies concerning sustainable industrial development generally referred to as 'biorefinery', vegetable biomass can be valorised by its conversion into the starting substrates for the production of energy and for the recovery of value added compounds.

The research activity reported herein dealt with the application of a new extraction methodology for the isolation of polysaccharides from two types of agro-industry waste that can be useful as food nutrients, food additives and in several applications, in particular for the formulation of polymeric materials. The recovered polysaccharides indeed possess interesting biological activity and promising biotechnological applications.

In particular, polysaccharide recovered from tomato waste exhibited interesting chemico-physical and rheological properties (such as a high molecular weight a high viscosity and thermostability) and promising biotechnological features since they could be considered as a building block for the formation of biofilms due to its ability to polymerize. Moreover, tomato waste polysaccharide showed to be able to act as potential anti-inflammatory agents being able to inhibit the production of ROS in tumor cell lines. With regard to lemon waste polysaccharide, it also showed a high molecular weight and an elevated thermostability. Also this biopolymers showed interesting biological properties as cytoprotective agent as confirmed by *Artemia salina* bioassay.

In conclusion, the exploitation of natural resources and the environmental pollution have produced an increase attention to the use of renewable resources to produce chemicals and energy. In this context, agro-industry waste biomass could be re-used as renewable source of useful bio-based chemicals, such as polysaccharides, thus contributing to provide new solutions to both waste management and development of biorefinery strategy.

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