FOOD INDUSTRY WASTE AS RESOURCE OF BIO-ACTIVE COMPOUNDS

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Abstract: Up-cycling of agro-alimentary industry waste is getting more attention from the scientists due to the fact that most of vegetable residues may be considered as a resource of high added value products, such as flavours, antioxidants, cosmetic excipients or active principles, drugs or drug adjuvants. This paper focuses on the opportunities opened by grapes (*Vitis vinifera*), one of the largest and most common fruit crop in Europe. Identification and/or isolation of bioactive natural products from this waste, as well as their chemical and/or enzymatic modification, are the main directions to obtain valuable natural compounds from up-cycled food industry waste.
Keywords: up-cycling, agro-alimentary waste, bio-active compounds, *Vitis vinifera*.

1. Introduction

Organic chemicals play an important role in our everyday lives. Since the middle of the 20th century, fossil oil and natural gas have been serving as the main raw material resources for chemicals production. Generally, almost all organic compounds can be derived from several basic building blocks, including syngas from methane, ethylene, propylene, butane, butylenes, butadiene and BTX (benzene, toluene, xylene). These building blocks are obtained from natural gas, petroleum and coal [1].

There is a growing interest in the replacement of fossil-based chemicals with biochemicals or "green" chemicals. Several factors, including awareness of finite petroleum resources, availability of renewable resources, environmental imperatives and recent advances in processing technologies, are driving chemical industry to shift the feedstocks from classic to renewable counterparts.

The advantages of using biomass include opportunities for less pollution, more biodegradable and sustainable products and, in some cases, lower costs. It has been found that many biomass derived chemicals have economical advantages, particularly for some functionalized chemicals. In addition, recent advances in processing technologies, especially in fermentation (such as: enzymatic engineering, metabolic engineering and genetic manipulation), may provide new opportunities for producing a wide variety of industrial products from renewable plant resources. A key to the chemical industry gradual shift toward the use of renewable resources is the implementation of the biorefinery concept. Similar to a petroleum refinery, a biorefinery integrates a variety of processing technologies to produce multiple bioproducts from various biomasses. Such an approach will help maximize the value of the biomass and minimize low or no value by-products [2].

The constant search for cheap and renewable bioresources led researchers to investigate the opportunities offered by the annual vegetable waste which accumulates worldwide: millions of metric tons of various pomace waste originate from viticulture, vegetal oil production, tomato processing, fruits processing, as well as from sugar or cassava production.

Up-cycling of agro-alimentary industry waste is getting more attention from the scientists due to the fact that most of vegetable residues may be considered as a resource of high added value products, such as flavours, antioxidants, cosmetic excipients or active principles, drugs or drug adjuvants.

Two different strategies are, basically, used to capitalize vegetable by-products: a) identification and isolation of bioactive natural products in vegetable waste as a possible resource of important compounds or enriched fractions and b) chemical and/or enzymatic modification of main compounds to obtain optimized analogues, food additives, drugs or cosmetics. Thus, this is an example of integrated approach between the query for waste up-cycling of agro-food industry and the search for bioactive products from renewable resources.

This paper focuses on the opportunities opened by grapes (*Vitis vinifera*), one of the largest and most common fruit crop in Europe, used mainly for wine production. After harvesting, destemming, crushing and pressing, mainly two different by-products accumulate: grape stems and grape pomace (skins, seeds and lees) in a global amount of aprox. 20% of the harvested grape.

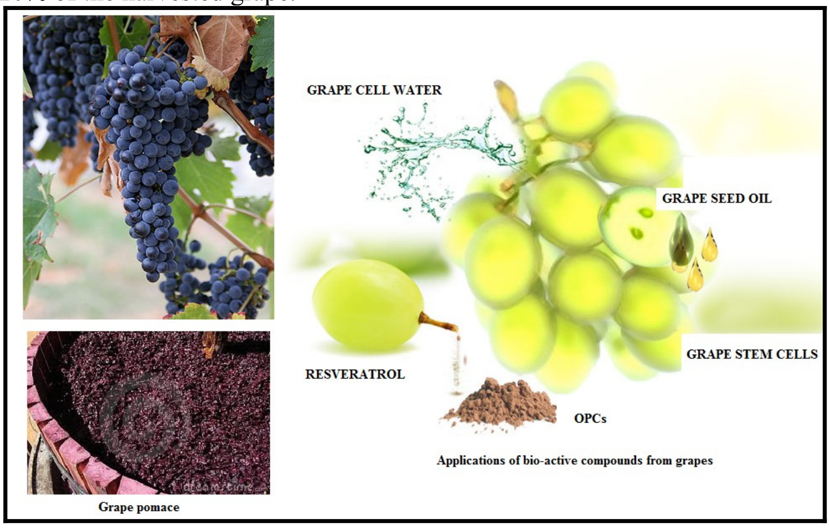

Fig. 1. Grapes, pomace and some applications of the bio-active compounds

Identification and isolation of bioactive natural products from this waste, as well as their chemical and/or enzymatic modification are the main directions to obtain valuable natural compounds from up-cycled food industry waste.

2. Bioactive natural compounds in grape by-products

2.1. Bioactive compounds from grape stems

As by-product from winemaking, grape pomace is frequently used for distillation and production of wine-derivatives alcoholic distillates, with an alcoholic concentration depending on the type of final product. In example, *tescovina* (Romania) has an alcohol content up to 45%, while italian *grappa* contains 30-37% alcohol. Unlike pomace, it seems there is no real use for grape stems other than animal food and composting.

Literature data [3] indicate that it is possible to isolate and identify antiproliferative compounds from an EtOAc (ethyl acetate) crude extract of grape stems, using chromatographic and spectroscopic analyses, including NMR spectra. Considering the amount in the mixture as criterion, the identified products were divided into two groups. Major compounds and their structures are listed in Fig. 2.

Oleanolic and betulinic acids are among them, as well as the stilbenoid *trans*-resveratrol and *trans*-ε-viniferin, a resveratrol dimer. A more polar antiproliferative constituent was also found, it was shown to be a mixture of sitosterol 6'-*O*-acylglucosides, as proved by GC-MS analysis of the methanolysis products. As a matter of fact, the glucose moiety was esterified by fatty acids (linoleic, linolenic, palmitic and stearic, in decreasing percentages). One of the main products was a mixture of inseparable analogues, namely 2,3-di-*O*-acyl-glicerol galactosides. Methanolysis followed by GC-MS analysis of the methylesters established the acyl residues. The optical rotation measurements on the galactoglycerol obtained from methanolysis allowed establishing the configuration of the stereogenic centre C-2 as *R* in this product and, consequently, as *S* in the natural substrate.

Further minor constituents with antiproliferative properties were isolated and identified from grape stems, their structures being shown in Fig. 3. They are daucosterol, gallic acid, catechin and gallocatechin.

All these compounds were found as pure constituents or inseparable mixtures. Submitted to specific tests [4, 5], they showed different growth inhibitory properties for tumoral cells. In example, one of the most effective compounds was betulinic acid, but *trans*-resveratrol showed interesting results also. Some other compounds showed weak antiproliferative activity, such as: ε-viniferin, gallic acid, oleanolic acid and gallocatechin.

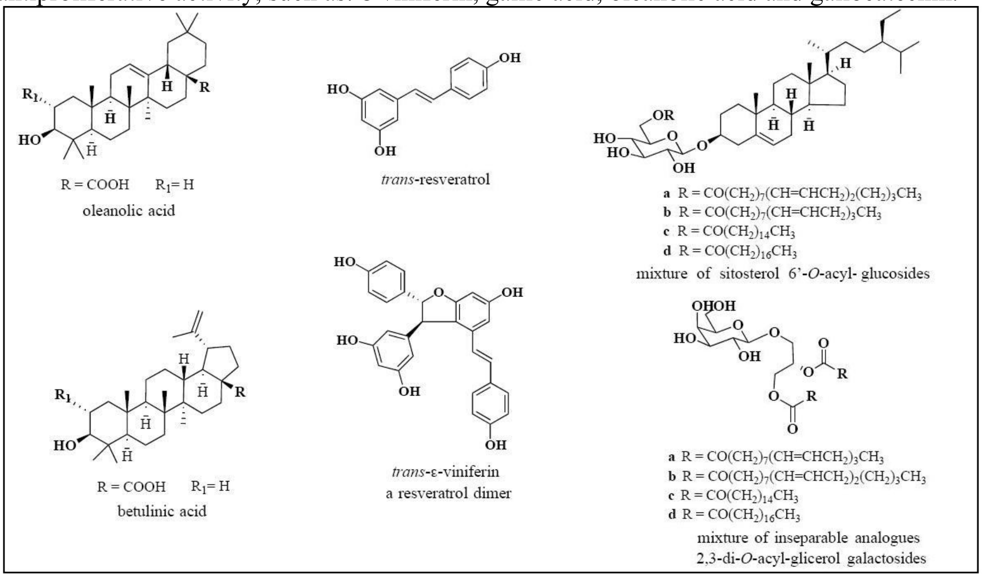

Fig. 2. Organic compounds with biologic activity obtained from grape stems

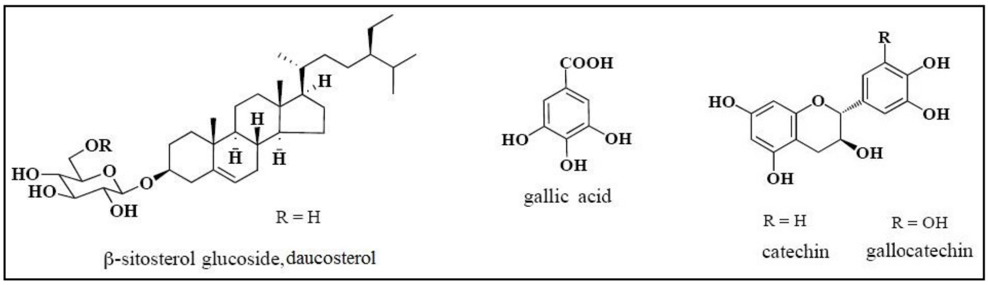

Fig. 3. Minor organic compounds with biologic activity obtained from grape stems

Almost all constituents isolated from grape stems are already known in literature for their benefic properties.

Resveratrol is also known as a grape phytoalexin and is considered one of the most important phenolic compounds in red wines [6], having the capability to prevent coronary syndrome, as well as tumors. It can be extracted from grape stems in an amount of 130 mg/kg dried stems (approx.).

Betulinic and oleanolic acids, as well as sitosterol 6'-*O*-acyl-glucosides and 2,3-di-*O*-acyl-glicerol galactosides have been proved to be effective antiproliferative principles towards tumors [7] or potent antitumor-promoting [8] and anti-inflammatory agents [9].

Gallic acid, catechin and gallocatechin are well-known as constituents of black or green tea and proved antioxidant [10] and anticarcinogenic properties [11].

2.2. Bioactive compounds from grape pomace

The aim of studying grape pomace was to isolate and identify compounds with antioxidant properties, known as "free-radical scavengers". These organic compounds have the ability to trap free radicals, action which would immediately inhibit the auto-oxidation cycle. Under oxygen deficient conditions, alkyl radical scavengers contribute to the stabilization of polymer chains, preventing their decay. Lactones and some phenols are known to be very effective as "free-radical scavengers" in low oxygen media.

A methanolic extract obtained from the destemmed grape pomace was analysed and main flavonols, flavonols glucosides, flavanols and their gallate esters, anthocyanins and low molecular weight pro-anthocyanins were identified [3]. Five pyrano-anthocyanins (less common compounds in pomace, normally found in aged wines) were also identified. These compounds and their structures are presented in Fig. 4.

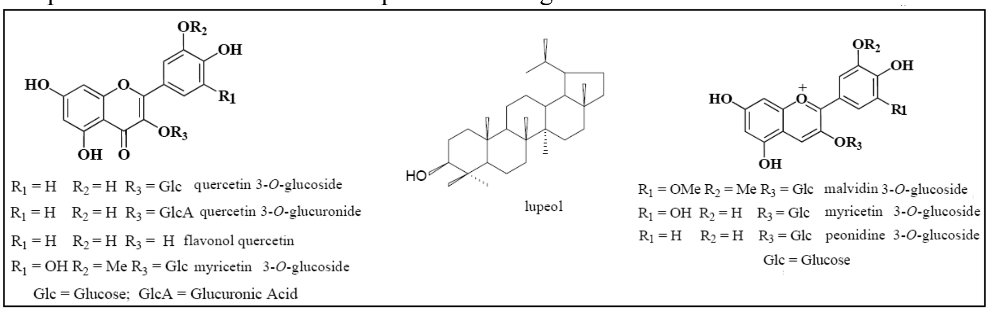

Fig. 4. Most important components with antioxidant properties from grape pomace

Quercetin 3-*O*-glucoside and quercetin 3-*O*-glucuronide resulted to be the major flavonol glycosides (in terms of amount in mixture), while malvidin 3-*O*-glucoside was found to be the main anthocyanin. By specific methods (flash-chromatography), members of the triterpenes family were identified, lupeol and oleanoic acid (Fig. 2), as well as the flavonol quercetin and daucosterol (Fig. 2).

Most of polyphenols identified in grape pomace are well known in the literature [12-14] due to their interesting biological properties, mainly for their antioxidant, free radical scavenging activity. It has been reported that the anthocyanin fraction from red wines was the most effective phenolic fraction in scavenging reactive oxygen species and in lipoprotein oxidation inhibition and platelet aggregation [15]. There are recent studies indicating lupeol as effective anti-inflammatory agent [16], apoptosis inducer [17] and inhibitor of tumoral cells proliferation [18].

The quantitative analysis of the main anthocyanins and flavonols shows that delphinidin 3-*O*-glucoside, myricetin 3-*O*-glucoside, quercetin 3-*O*-glucoside and quercetin, all bearing a catechol moiety, have the highest percentage in the corresponding mixture [3]. With respect to the crude alcoholic extract, these flavonoids are also consistently enriched, whereas a significant reduction in peonidine 3-*O*-glucoside and malvidin 3-*O*-glucoside, without *ortho*-dihydroxy moiety, is recorded.

2.3. Bioactive compounds from grape seeds

Oligomeric proanthocyanidins (OPCs) are naturally occurring substances present in a variety of food and vegetal sources, one of them being the red grape seeds. OPCs are unique members of the flavonols family (i. e., catechin, epicatechin and gallocatechin, Fig. 3) and have powerful antioxidant characteristics and excellent bioavailability. Clinical tests suggested that OPCs may be as much as fifty times more potent than vitamin C, in terms of antioxidative activity. In addition to this, they have the capability to strengthen and repair connective tissues, including at the cardiovascular system, to moderate allergic and inflammatory responses by reducing the histamine production and have antidepressant-like effect [19, 20].

OPCs are present in grape seeds in various amounts (in mg/100 g edible portion), as monomers (171), dimers (170) and trimers (33), depending on the type of grapes. Although they are not consumed as such, seeds are of great importance for wine producers (5 ounces of red wine contain 91 mg OPCs) [21, 22] and as bio-resource of flavonols for the pharmaceutical industry.

3. Study on the chemical and enzymatic modification of resveratrol

Most data on bioactive compounds isolated and identified in grape by-products concern *trans*-resveratrol. *Trans*-resveratrol (3,5,4'-trihydroxy-*trans*-stilbene, Fig. 2) is a stilbenoid found mainly in the skin of red grapes (resveratrol content in grapes skins and pomace was found to be stable, as shown by samples taken after fermentation and stored for a long period [23]) and in other fruits. Red wine contains very little of it, on the order of 0.1-14.3 mg/l [24]. Resveratrol has been also produced by chemical synthesis [25] and by biotechnological synthesis (metabolic engineered microorganisms) [26, 27].

Recent studies indicated resveratrol as an active principle in biochemical reactions. Thus, it was reported that it significantly extends the lifespan of some yeasts, worms and fruit fly [28, 29]. Many studies have been conducted to investigate its anti-tumoral activity in animals [24]. It was also shown that it can induce apoptosis in platelets and smooth muscles [30]. Resveratrol seems to act more effective on tumors it can contact directly, as esophageal tumors or melanoma [31]. Furthermore, it seems to have cardioprotective effects, moderate drinking of red wine being known to reduce the risk of heart disease (“the French paradox”). This effect is achieved by the following actions: inhibition of vascular cell adhesion molecule expression; inhibition of vascular smooth muscle cell proliferation; stimulation of endolethelial nitric oxide synthase (eNOS) activity; inhibition of platelet aggregation; inhibition of LDL peroxidation [32]. As for resveratrol antidiabetic activity, it was proved in human clinical trials [33] that it determines low blood sugar levels. Other benefic effects include neuroprotective, antiviral and anti-inflamatory activity. Still, resveratrol has been shown to be ineffective in inhibiting microbial and fungi proliferation [34].

Resveratrol, in common with other polyphenols, was found to be a strong topoisomerase inhibitor, sharing similarities to chemotherapeutic anticancer drugs, such as etoposide and doxorubicin [35]. Resveratrol has recently been evaluated as an antiproliferative agent towards prostate tumor cells [36, 37]. Compounds with antiproliferative properties against prostate carcinoma are intensively studied in order to obtain new anticancer drugs or adjuvants of currently used drugs. Various lipophilic analogues of resveratrol were obtained and some of them are presented in Fig. 5.

The most important analogue, 4’-*O*-acetylresveratrol (yield 40%), was synthesized through an enzymatic regioselective direct acylation in the presence of the enzyme *Candida antarctica* lipase (CAL) and vinyl acetate in *tert*-amylic alcohol [3].

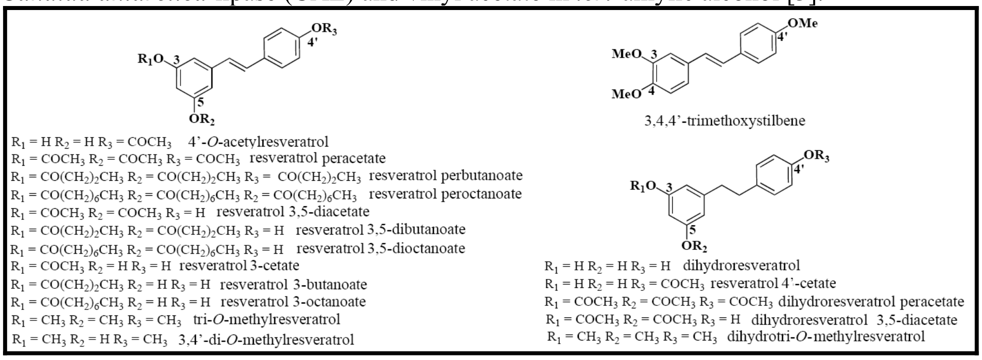

Fig. 5. Lipophilic analogues of resveratrol [3]

The observed regioselectivity of the enzyme towards position 4’ was used in order to obtain other resveratrol derivatives. One approach was based on a preliminary peracylation by conventional chemical reagents, such as anhydrides or acyl chlorides, followed by regioselective deprotection of the peracylated resveratrol. Resveratrol esters (peracetate, perbutanoate and peroctanoate) were subjected to lipase-catalysed alcoholysis, employing *n*-butanol as nucleophilic reagent. Butanolysis was more rapid, but less selective than direct acylation. In the presence of CAL, both 4’-deprotected and 5,4’-deprotected derivatives (and acylated butanol as by-product) were obtained.

The enzyme preferentially attacks functional groups bonded to C in position 4’ and this enabled synthesis of resveratrol 3,5-diacetate, 3,5-dibutanoate and 3,5-dioctanoate. Furthermore, these compounds may become a substrate for the CAL, thus affording the corresponding resveratrol 3-cetate, 3-butanoate and 3-octanoate.

Further chemical and enzymatic modifications on resveratrol aimed to use the central C=C double bond [38] and yielded in dihydroresveratrol, which was then directly acylated in the presence of CAL to afford resveratrol 4'- cetate. The dihydroresveratrol peracetate was submitted to the enzymatic butanolysis and afforded the derivative dihydroresveratrol 3,5-diacetate.

By simple chemical conversion, tri-*O*-methylresveratrol, 3,4'-di-*O*-methylresveratrol and dihydrotri-*O*methylresveratrol were obtained.

The influence of the methoxy groups position was evaluated through the synthesis of a resveratrol analogue, namely the 3,4,4'-trimethoxystilbene, having a modified substitution pattern of the stilbene skeleton. It was proven that the activity of the trimethoxystilbene analogue was comparable to that of resveratrol, thus suggesting that the substitution pattern is important for the biologic activity.

Other analogues showed potent inhibitory activity against porcine aortic endothelial cells [39], very important feature since the inhibition of angiogenesis in tumor cells is a new frontier in the fight against cancer [40]. Methylated analogues of transveratrol are also showing promising anti-cancer properties [41].

4. Conclusion

Taking into consideration data presented in this paper, it is obvious that food industry waste is a promising resource of bio-active compounds. Its availability and renewable character is giving research a new impulse to elaborate protocols for faster and/or easier isolation and identification of these valuable compounds, as well as for their chemo-enzymatic conversion. One key factor is the preservation, if not the enhancement, of the biologic activity of natural compounds throughout all processes during their functionalization. Using these bio-active compounds as flavours, antioxidants, cosmetic excipients or active principles, drugs or drug adjuvants is adding value and makes all efforts worthwhile. Despite these data, *Vitis vinifera* is not, regrettably, on the ***ePlantLIBRA*** (EC project number 245199) list of prioritised plants selected for the European Community's Seventh Framework Programme (FP7/2007-2013), although this database will provide a unique comprehensive information resource for expert users: scientists, epidemiologists, health professionals, health educators, food industry professionals, food regulatory authorities and policy makers.

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