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Protective effect of grape extract phospholipid vesicles against oxidative stress skin damages

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ABSTRACT

Grape extract rich in polyphenols (~129 ± 32 mg of gallic acid equivalents per g of dry extract) was obtained from the pomaces of Cannonau grapes by homogenization in an ethanol/water mixture. The efficacy of ultrasounds in speeding up the extraction kinetics of polyphenols was demonstrated. The extract was incorporated in liposomes and PEVs (penetration enhancer containing vesicles) with Labrasol® or Labrasol®/ethanol. All the vesicles were spherical and predominantly unilamellar: liposomes were large (~927 nm) and polydispersed (PI ~0.56), while PEVs were small (~140 nm) and fairly homogeneous (PI ~0.3). Moreover, PEVs were able to incorporate a high amount of the extract (~98% of the extract used for their preparation, 50 mg/ml). The formulations were highly cytocompatible and were able to promote the proliferation of keratinocytes and fibroblasts. In addition, thanks to their antioxidant activity, grape extract formulations provided a cytoprotective effect against oxidative stress damage.

Therefore, an efficient, environmentally-friendly extraction strategy is proposed to obtain an extract with high phenolic content from waste grape pomaces, which was incorporated in vesicular systems to maximize the antioxidant power in a cell model of oxidative stress.

1. Introduction

Epidemiological studies have suggested the beneficial effects of the daily consumption of vegetables and fruits on human health, reducing the occurrence of both minor and important chronic pathologies such as cardiovascular, neurodegenerative diseases and some types of cancer (Tedesco et al., 2015; Weisburger, 1998). This positive influence on human health underlines the importance of edible plants, fruits, and vegetables, thanks to their high content of fiber and active compounds. Among the latter, phenolics occur widely in about half of the plant species used as human food and especially in some fruits, such as grape, apple, blueberry and cranberry. Unfortunately, some of these valuable compounds remain in the fruit and vegetable by-products, such as peel, seeds, skin and stems. In recent years, a considerable scientific interest has been focused on the possible recovery of natural active compounds from several food waste, to be used for the production of pharmaceutical, nutraceutical and cosmetic products. Galanakis reported that the recovery process of high added-value components from food waste involves five sequential stages that, by different established and emerging technologies, lead to obtain real products and meet the goals of recapture (Galanakis, 2012). One interesting method to improve the extraction efficiency by using conventional "food friendly" solvents is the application of ultrasounds, which facilitate the tissue disintegration and the active molecule–solvent intimate contact (Galanakis and Schieber, 2014; Galanakis, 2013).

Grape is one of those fruits that contains high amounts of different kinds of beneficial molecules, such as anthocyanins, catechins, procyanidins, flavonol glycosides, phenols and stilbenes. As well recognized, grape is mainly used for winemaking, resulting in the accumulation of high quantities of pomaces (Marqués et al., 2013), mostly composed of waste seeds, skins and stems, which still contain these bioactive compounds (Portu et al., 2015) due to incomplete extraction (Drosou et al., 2015; Tournour et al., 2015).

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Among others, the grape pomaces may be considered one of the main, inexpensive sources to recover bioactive compounds.

The skin, as the external barrier of the human body, is continuously exposed to chemical, mechanical and physical environmental stress resulting in a high overproduction of free radicals and reactive oxygen species. Keratinocytes are the main actors controlling skin reactions against stress and inflammation by the release and up-regulation of pro-inflammatory activators, such as prostaglandin, cyclooxygenase, reactive oxygen species, nitric oxide and cytokines (Santoro and Gaudino, 2005). Moreover, they can modulate the leukocytes' function amplifying inflammatory reactions, which results in a final pathological condition. The accumulation of these reactive species may cause lipid peroxidation, modified gene expression and DNA damage, usually associated to the development of chronic inflammatory conditions and tumor initiation and promotion. Several studies demonstrated that an external intake of antioxidants may counteract the above mentioned cascade process, re-establishing the physiological conditions. Phenols are antioxidant, antiradical, antimicrobial, and anti-inflammatory compounds and they provide protection and prevention of chronic diseases and cancer. A suitable delivery system can improve the local bioavailability of these compounds, especially in stressed tissues. Indeed, pharmaceutical delivery systems allow active agents to overcome the stratum corneum barrier favoring their penetration and/or permeation in the skin. Liposomes are promising and effective drug delivery systems widely used in the cosmetic, food and pharmaceutical fields, thanks to their versatility and outstanding biocompatibility. Liposomes and modified-liposomes have been extensively studied as carriers for the topical application of drugs and natural active agents (Caddeo et al., 2013b; Manca et al., 2013).

This study is focused on the enhancement of the local bioavailability of grape pomace extract and its antioxidant activity for the protection of the skin from the harmful effects of oxidative stress. The extract obtained from the grape pomaces was evaluated for antioxidant activity and phenolic content, and incorporated in liposomes and modified vesicles, which were deeply characterized for physico-chemical properties. The ability of the extract-loaded vesicles to protect human keratinocytes and fibroblasts against hydrogen peroxide damage was also tested.

2. Material and methods

2.1. Materials

Lipoid S75 (S75), a mixture of soybean phospholipids (~70% phosphatidylcholine, 9% phosphatidylethanolamine and 3% lysophosphatidylcholine), triglycerides and fatty acids, was purchased from Lipoid (Ludwigshafen, Germany). Labrasol® (caprylcaproyl macrogol-8 glycerides, 30% of mono-, di- and triglycerides of C8 and fatty acids, 50% of mono and di-esters of PEG, 20% of free PEG 400; HLB 14; Lab) was obtained from Gattefossè (Saint Priest, France). Ethanol and all other products were of analytical grade and were purchased from Sigma–Aldrich (Milan, Italy).

2.2. Extract kinetic evaluation

Fresh pomaces from Cannonau red grapes were provided by Vini Gostolai S.r.l. (Oliena, Italy) in October 2014 and stored at −80 °C until use. Aliquots of 100 g of grape pomaces were dispersed in a mixture (900 ml) of ethanol/water (50:50 w/w) and homogenized. The extraction was performed using two different methods: in the first, the pomaces were left in the extraction mixture for 48 h under constant stirring, at room temperature (non-sonicated samples); in the second, during the extraction process, at scheduled times (1, 2, 4, 6, 8, 24 and 48 h), samples were sonicated for 1000 s (200 cycles, 5 on, 5 off, 15 microns of probe amplitude), with a high intensity ultrasonic disintegrator (Soniprep 150, MSE Crowley, London, UK). During the extraction procedure, 5 ml of each sonicated sample was withdrawn and centrifuged twice (20 min, 6000 rpm); the absorbance of the supernatant was read at 280 nm to measure the total phenolic index (TPI), while the antioxidant activity (AA%) was evaluated measuring the ability of the sample to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (see below). At the end of both extraction methods, the dispersions were centrifuged twice (20 min, 6000 rpm), the supernatants collected and evaporated under vacuum at 30 °C to remove ethanol and then lyophilized, producing a purple-brown powder.

2.3. Folin–Ciocalteu and DPPH assays

The total phenolic content of the grape pomace extract was measured according to the Folin–Ciocalteu colorimetric assay by using a UV spectrophotometer (Lambda 25, PerkinElmer, USA). 100 μl of the extract in ethanol (1 mg/ml), 100 μl of the Folin–Ciocalteu reagent and 800 μl of 20% (w/v) Na_2CO_3 aqueous solution were mixed and the absorbance was read at 765 nm after 30 min of incubation in the dark, at room temperature. The total phenolic content was calculated by means of a calibration curve built using gallic acid as a reference at different concentrations (0–0.125 mg/ml). Results, expressed as mg of gallic acid equivalents per g of dry extract, were the means of six determinations (Castangia et al., 2015a).

The AA% of the extract was assessed by measuring its ability to scavenge the DPPH radical. 25 μl of the extract in ethanol (1 mg/ml) were dissolved in 1975 μl of DPPH methanolic solution (40 μg/ml). The sample was incubated for 30 min at room temperature, in the dark. Then, the absorbance was measured at $\lambda = 517$ nm against blank. All the experiments were performed in triplicate. The AA% was calculated according to the following formula (Caddeo et al., 2013b; Manca et al., 2014a):

$$AA\% = \left[\frac{\left(ABS_{DPPH} - ABS_{sample}\right)}{ABS_{DPPH}}\right] \times 100.$$

2.4. Vesicle preparation

S75 (360 mg) and grape pomace extract (100 mg) were weighed in a glass vial and 2 ml of water (to prepare liposomes) or 2 ml of a mixture of Labrasol®/water (50:50 v/v) or Labrasol®/ethanol/water (45:5:50 v/v) (to obtain PEVs) was added. Samples were left overnight at room temperature to facilitate the swelling of the phospholipid, and then sonicated (15 + 15 cycles, 5 s on and 5 s off) with a Soniprep 150 ultrasonic disintegrator (MSE Crowley, London, UK) at 15 microns of amplitude.

Samples (1 ml) were purified from the non-incorporated extract by dializing (Spectra/Por® membranes, 3 nm pore size; Spectrum Laboratories Inc., Rancho Dominguez, USA) against water (2 l) at room temperature for 2 h (refreshing the water every 30 min). The AA% of the extract in vesicles was measured by the DPPH test, before and after the dialysis process. The entrapment efficiency (EE%) of the vesicles was expressed as the percentage of the AA% after dialysis versus that before dialysis.

2.5. Vesicle characterization

To observe the sample by transmission electron microscopy (TEM) a thin film was placed on a carbon grid, stained with phosphotungstic acid (1%) and examined with a JEM-1010 microscope