Agri-food industry by-products valorisation: a focus on pomegranate peel extracts used as a tannin-rich ingredient in different food areas, including winemaking

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Pomegranate peel, a by-product from juice production, is a rich source in bioactive compounds. Extraction will be performed with different green methodologies based on the type of application. Both the tannin and the pectic fraction of the extracts will be chemically characterized. Their addition in baked goods and their use as oenotannin in winemaking will be tested, to evaluate technological and antioxidant properties. All enriched/fortified products will be subjected to sensory analysis to determinate any variations after the addition of extracts.

**Valorizzazione di un co-prodotto dell’industria agro-alimentare: focus su estratti di buccia di melagrana utilizzati come ingredienti ricchi di tannini in differenti settori alimentari, inclusa la vinificazione**

Le bucce di melograno, co-prodotti derivanti dalla produzione di succo, sono una risorsa ricca di composti bioattivi. L’estrazione delle bucce avverrà con diversi metodi green a seconda del tipo di applicazione dell’estratto. Questi verranno caratterizzati rispetto alla frazione fenolica e polisaccaridica. Si testerà la loro aggiunta in prodotti da forno e come tannino enologico nel vino, per valutarne al meglio le proprietà tecnologiche e antiossidanti. Tutti i prodotti addizionati saranno soggetti a test sensoriali per valutare variazioni dopo l’aggiunta degli estratti.

**1. State-of-Art**

Pomegranate is receiving an increased attention because of its abundance in bioactive compounds with beneficial health properties. Since it was defined by the media as “superfruit”, its demand had a significant spike, followed by an increased demand of pomegranate-derived products such as juices, jams, flavored water and salad/dessert dressings (Kahramanoglu, 2019). Peel counts for about 50% of the fruit weight, and the production of this waste is estimated in ≈ 1.9 million metric tons in 2017. This waste needs to be address to other types of production because, if put in landfills, it represents a threat for the environment. It could be used as fertilizer, bio-adsorbent or animal feed as such, but since the peel is rich in valuable compounds (Valero-Mendoza *et al.*, 2023), studies on nutraceutical-technological uses are ongoing. Innovative extraction strategies are required, toward more sustainable procedures of by-product treatment. Hydrodynamic cavitation (HC) is an eco-friendly and cost-effective extractive technology, which allows good yields and easy scale-up of the process. It is based on the phenomenon of cavitation, which occurs when negative pressure is applied to a liquid, where small cavities (microbubbles) filled with gas are formed. When the pressure rises up again, bubbles collapse generating a local shock wave that disrupt the plant matrix in that area, causing extraction. This technique, not tested so far on pomegranate, is reported to well extract the pectin fraction from matrices (Presentato *et al.*, 2020). Also, low temperature, solid/liquid extraction will be performed, to exclusively extract the tannin fraction. This technique, based on the mass transfer process (diffusion) from a high concentration area to a low concentration area, allows a higher selectivity towards target compounds due to solvent selection and temperature control (Vorobiev and Lebovka, 2020). Maximization of phenols and absence of polysaccharides will be achieved with hydroalcoholic media and low temperatures, since polysaccharides are not soluble in these conditions.

**1.1 Pectin substances in pomegranate and possible applications**

Pomegranate peel could be considered a good source of polysaccharides, with crude fibers being accounting for about 21% of the peel and total carbohydrate the 86% (Al-Rawahi *et al.*, 2013). Polysaccharides of pomegranate are part of the dietary fibers and are mostly constituted by pectin material, but different extraction methods could bring to different yields and quality of the pectin. Key extraction parameters are extraction time, extraction temperature, DM/solvent ratio and pH. Health benefits of pomegranate pectin have been evaluated *in vitro* because soluble fibers are well-known to explicate a prebiotic effect; in fact, *B. breve* B632 and *L. plantarum* L12 strains grew well on pomegranate polysaccharides as a carbon source, in a comparable way to the strains fed on glucose (Khatib *et al.*, 2017). Moreover, pectin has important technological properties, such as thickening and gelling properties. To practically evaluate these aspects, the extract will be inserted in a bakery product, in substitution to the elements of the recipe that give structure to the final product, flour and sugar.

**1.2 Tannins in pomegranate and possible applications**

Pomegranate tannins are the most studied portion of the peel, responsible for antioxidants, antimicrobial and antiviral properties. Other health benefits are anti-inflammatory, anti-allergenic, anti-diabetic and anti-hyperlipidemic properties (Valero-Mendoza *et al.*, 2023). The most important class of tannins found are ellagitannins (hydrolysable), with some particular compounds unique of pomegranate, such as punicalagins and punicalins. Area of applications of pomegranate tannin-rich extract are numerous, from pharmaceutical and nutraceutical ingredients to food preservatives, food colorant, bio-stimulant for plant growth (Pathak, Mandavgane and Kulkarni, 2017).Innovative solutions of application are as additive in edible coatings (Salem *et al.*, 2022) and as oenotannin in wine-making (Canuti *et al.*, 2020). To further analyze the possible use of pomegranate tannin in enology, the characterized extract will be tested in different wine-making conditions.

**2. Objective**

Objective of this study is pomegranate peel valorization. The structure of the research and the different aims are:

1. **Application of green, innovative extraction methods**: hydrodynamic cavitation and low temperature alcoholic solid/liquid extraction. The derived extracts will be chemically characterized. Tannin fraction will be evaluated through HPLC-DAD-MS and pectin fraction through SEC, DLS, 1H-NMR analysis and HPAEC-PAD for sugar analysis.
2. **Application of the hydrodynamic cavitated extract in the bakery sector**: the use as thickening agent/sugar substitute in a vegan-gluten free product will be tested. Structural properties (weight, spread ratio, water activity, total humidity, color, and texture) will be also evaluated as well as sensory and antioxidant (DPPH and Folin-Ciocalteau assays) properties. Changes of these properties during time will be evaluated.
3. **Application of the alcoholic extract in the wine-making sector**: the use as oenotannin in red and white vinification will be tested. On wines will be conducted the following analysis at different times after bottling: color intensity, hue, monomeric anthocyanins content (HPLC-DAD), polymeric colored pigment (HPLC), total phenols (HPLC), anti-radical activity through DPPH assay, sensory evaluation.
4. **Data elaboration and PhD thesis writing**

Timing of he mentioned activities is proposed in table 1, with the Gantt diagram.

***Table 1***Gantt diagram for this PhD thesis project.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Activity Months | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** |
| A1) | ***Extraction and characterization*** |   |   |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 1) HC extract |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 2) Alcoholic extract |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
| A2) | ***Bakery application*** |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |   |  |   |   |   |  |  |  |  |
|  | 1) Analysis of bakery product  |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |  |  |  |   |   |  |  |  |  |
|  | 2) Sensory evaluation |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |   |  |   |   |   |  |  |  |  |
| A3) | ***Wine-making application*** |   |  |   |   |   |   |  |   |   |   |   |   |  |   |  |  |  |  |   |   |   |   |   |  |
|  | 1) Preparation of wines |   |  |   |   |   |  |  |   |   |   |   |  |  |   |  |  |  |  |   |   |   |   |  |  |
| 2) Analysis of wines  |   |  |   |  |  |   |  |   |   |  |  |   |  |   |  |  |  |  |   |   |  |  |   |  |
| ***3)*** Sensory evaluation |   |  |   |  |  |   |   |   |   |  |  |   |   |   |  |  |  |  |   |   |  |  |   |   |
| A5) | ***Thesis and Paper Preparation*** |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |  |   |   |   |   |   |   |   |

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