

Accelerated production of hesperidin-rich citrus pectin from waste citrus peel for prevention and therapy of COVID-19

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Abstract: Computational studies suggest that hesperidin, a flavonoid abundant in citrus peel, binding the three main cellular receptors of SARS-CoV-2 virus can act in the prophylaxis and treatment of COVID-19. Herein we urge the uptake of hydrodynamic cavitation industrial-scale reactors based on the low cost, reliable Venturi tube for the extraction of citrus pectin rich in hesperidin (and in other bioflavonoids including naringin) by very fast processing of waste orange peel or waste lemon peel in water only. A device able to process up to 500 kg of waste peels per session, similar to the one lately deployed in Italy for hydrodynamic cavitation-assisted brewing, is capable to provide 36,000 doses of 1000 mg hesperidin per day.

Keywords: Hesperidin; COVID-19; pectin; flavonoids; hydrodynamic cavitation; IntegroPectin

Recent computational studies suggest that hesperidin, a citrus flavonoid abundant in citrus peel, binding the three main receptors of SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2) virus is a promising drug to prevent or treat the related disease COVID-19 [1,2,3,4].

In a molecular docking study, scholars in Indonesia conclude that hesperidin has the highest affinity to bind the receptors (the lowest docking score for all three protein receptors) for three proteins involved in the cell infection from SARS Cov-2, the virus responsible of COVID-19, inhibiting the proteins responsible for viral infection and virus development [1].

In closer detail, hesperidin binds to three key protein targets: RBD-S, PD-ACE2, and SARS-CoV-2 protease, thereby preventing binding of the RBD (Receptor Binding Domain) of spike glycoprotein (RBD-S) to the ACE2 (Angiotensin Converting Enzyme-2) receptor at the protease domain (PD) (PD-ACE2) of the host cell, leading to the viral infection [1].

Remarkably, the same team reported that four other citrus flavonoids also abundant in orange and lemon peel, namely tangeretin, hesperetin (the aglycone of hesperidin), nobiletin and naringenin, show excellent affinity to the selected receptors, suggesting that all said citrus flavonoids may contribute to inhibit the viral infection and replication [1].

In another study, scholars in China reached similar conclusions [2]. In detail, the team analyzed all the proteins encoded by SARS-CoV-2 genes, compared them with other coronaviruses, such as SARS-CoV and MERS-CoV, and modeled the protein structures using said structures along with those of human relative proteins (human ACE2 and type-II transmembrane serine protease enzymes) as targets to screen three databases of approved drugs. These databases were the following: the database of traditional Chinese medicine and natural products (including reported common anti-viral components from traditional Chinese medicine), the database of commonly used anti-viral drugs (78 compounds), and the ZINC drug database of the Food and Drug Administration of the USA by virtual ligand screening method [2].

The method clearly showed that hesperidin targets the binding interface between Spike and ACE2 so that by superimposing the ACE2–RBD complex to the hesperidin–RBD complex, a distinct overlap of hesperidin with the interface of ACE2 was observed. This suggests that hesperidin may disrupt the interaction of ACE2 with RBD, leading the Chinese scholars to conclude that hesperidin, blocking the interface of ACE2 and Spike RBD binding, “could probably be used for treating SARS-CoV-2” [2].

In a further study, a molecular model was built of the 3-chymotrypsin-like protease (3CLpro) structure of the SARS-CoV-2, which is vital to virus replication and is considered as a promising drug target [3]. The study carried out virtual screening to identify readily usable therapeutics derived from the previous progress of specific inhibitors development for the SARS-CoV enzyme that can be conferred on its SARS-CoV-2 counterpart.

Results showed that the flavonoid glycosides diosmin (a pre-approved drug) and hesperidin (an approved drug) obtained from citrus fruits fitted very well into and block the substrate binding site. In particular, hesperidin hits showed up multiple times, suggesting it has many modes of binding, while showing only mild and occasional adverse reactions [3].

Finally, another recent computational and experimental study found that multiple flavonoids abundant in citrus peels cooperate to prevent the SARS-CoV-2 infection [4]. In particular, simulated molecular docking shows that hesperidin, hesperetin and naringin have stronger binding affinity with the ACE2 receptor. Moreover, *in vitro* and *in vivo* experiments showed the potential of naringin for the inhibition of cytokine storms induced by the SARS-CoV-2, through multiple pathways, further adding to the functionality of integral flavonoids-rich extracts from citrus peels [4].

It is also relevant here that in 2006 hesperetin was found to exhibit *in vitro* antiviral activity against SARS-CoV (along with several other compounds including chloroquine) [5]. Similarly, in 2011 a large clinical study showed that hesperidin displayed a relevant role in the genomic effect of orange juice, resulting in

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significant anti-inflammatory and anti-atherogenic activities [4]. Moreover, in the same study, a daily dose of 292 mg of hesperidin, corresponding to 500 mL of orange, was sufficient to display the aforementioned effects [6].

Empirically known since 1876, when the beverage *Hesperidina* obtained from bitter and sweet orange peels was first introduced in Argentina [7], the numerous and different health beneficial effects of hesperidin led to a large pharmaceutical potential [8].

Among commercial products, the flavonoid vasoprotector and venotonic agent Daflon 500 mg, which has been commercialized since more than 25 years, contains 450 mg of diosmin and 50 mg of hesperidin (*i.e.*, 90% diosmin and 10% hesperidin), and has been proved effective and safe [9]. Many other products containing hesperidin (up to 500 mg per tablet) and other citrus flavonoids are today available and regularly consumed.

Today hesperidin is mostly extracted from the citrus peel as a flavonoid complex with 60-70 % hesperidin concentration via a time-consuming process, using large amounts of mineral acid and mineral base (treating the peel with a NaOH solution at pH 11.5 followed by acidification with mineral acid and heating the acid solution at pH 4.2 at 45 °C overnight) [10].

Greener production routes include hydroalcoholic extraction of hesperidin from lime peel and subsequent purification over polymeric adsorption resins to increase the recovery efficiency. This method has been demonstrated both on laboratory and on semi-industrial scale, even though requiring the addition of 10% dimethyl sulfoxide (DMSO) to the extract to improve the solubility of hesperidin [11]. Another state-of-the-art, greener hydro-distillation extraction method applied to orange peels allowed the extraction of total polyphenols in the aqueous phase with the yield of about 17% of the original content [12].

More recently, the integral extraction of waste citrus peel via hydrodynamic cavitation (HC) in water only was demonstrated directly on the semi-industrial scale using waste orange peels (WOP) [13] and waste lemon peels (WLP) [14] from citrus fruits organically grown in Sicily.

In this study, we call for the urgent uptake of the latter process for the efficient and green industrial production of pectin rich in hesperidin. No other process, to the best of our knowledge, allows to extract in just 10 minutes around 60% (in weight) of the overall polyphenol (flavonoid) content in fresh WOP [13]. The processing of 6.38 kg of wet WOP allowed the extraction of 36.26 g of hesperidin (0.6 wt%) in the aqueous phase (147 L of water). Along with hesperidin, a significant amount of naringin (16.39 g), other flavonoids (2.95 g) and essential oils (mainly *d*-limonene) were extracted.

In the same study, the feasibility of processing as much as 42 kg of fresh WOP in 120 L of water (35% wt%) was clearly proved,

pointing to a concentration of hesperidin in water as high as 0.2% (w/w), *i.e.*, 2000 mg/L. As well, all the flavonoids present in the aqueous phase, along with the water-soluble pectin, were isolated via lyophilization of the aqueous solution, affording a flavonoid-rich pectin dubbed "IntegroPectin" [13].

In the case of the HC-based processing of WLP, the IntegroPectin showed exceptional antioxidant properties and complete lack of cytotoxicity against pulmonary epithelial cells up to very high doses (1 mg/mL) [14], as well as strong antibacterial activity against *Staphylococcus aureus* [15].

Widely employed in the food industry as the natural hydrocolloid of choice [16], pectin exhibits a broad biological activity, including immunoregulatory, anti-inflammatory, and hypoglycemic activities, for which it is increasingly used in various pharmacological applications [17].

The orange IntegroPectin from *Citrus sinensis* WOP has a very low degree of esterification (17%), which makes it particularly appropriate for food, pharmaceutical, and nutraceutical applications [13]. In general, the antibacterial, antioxidant and the lack of cytotoxicity properties are all due to the high concentration of hesperidin and other flavonoids adsorbed and concentrated at the IntegroPectin surface during the freeze-drying process of the aqueous phase, as well as to essential oils, whose presence in the IntegroPectin was confirmed by its intense lemon scent.

It is worth noting that the well known antibacterial activity of *d*-limonene increases by many times when the substance is administered in form of nanoemulsion, which is a result of the HC-based processing [18].

Its low density and open, porous structure would allow the straightforward production of IntegroPectin tablets of different weight with the required dose of hesperidin for the tentative prevention and treatment of COVID-19, well beyond the 50 mg content in Daflon 500 mg pills. Human studies have shown since long that the substance is safe and well tolerated up to very high doses of administration. For example, in 1964 a study during which 94 menopausal women had a daily intake of 900 mg of hesperidin (in addition to 300 mg of hesperidin methyl chalcone and to 1200 mg of vitamin C) for 1 month demonstrated the safety of hesperidin even at such high dosage [19].

The practical application of HC-based extraction processing of citrus waste peels is immediately feasible in Italy, a country very heavily affected by the COVID-19 pandemic. In fact, a HC-based extraction plant with a 1,700 L nominal capacity is immediately available (Figure 1), which was developed for the industrialization in the field of beer brewing [20,21]. Such plant is readily applicable for the extraction of citrus waste peel.



Figure 1. Available HC-based extraction plant at the GBL S.r.l. mechanical workshop, Italy

The extraction process will take no longer than 15 min. However, including all necessary steps such as grinding the peels before the inlet to the processing unit, separating the solid residues, and discharging and packaging the aqueous extract, the overall process would require approximately 2 h. Implementing continuous production, a production output of at least 18,000 L per day can be obtained with this plant only.

Conflict of interest

The authors declare no conflict of interest.

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Undertaking the hydrodynamic cavitation of 500 kg waste citrus peel (as such) in 1500 L water, the process is able to extract 3 kg of hesperidin per cycle, hence at least 36 kg of hesperidin per day (in 12 cycles), corresponding to 36,000 daily doses of hesperidin 1000 mg. The only additional plant next to the industrial HC-based extractor would be an industrial lyophilizer, such as those commonly installed at pharmaceutical companies and used to remove solvent from a frozen product by sublimation.

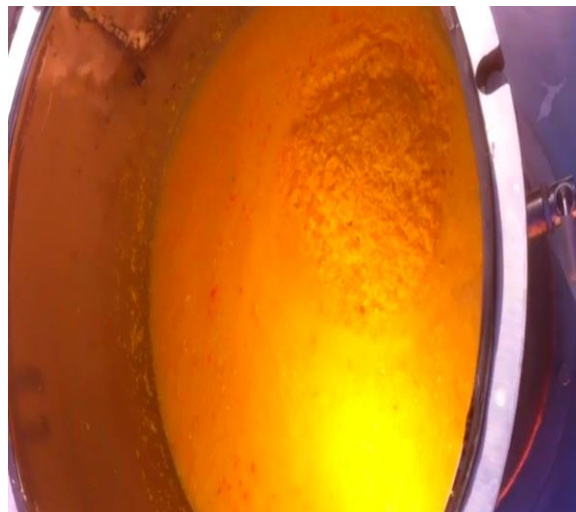
The HC-based extraction technology was described in detail in previous publications, and is immediately available for the production of hesperidin from citrus waste peels, as a component of integral aqueous extracts or IntegroPectin tablets. The product could undergo clinical trials aimed at establishing the prophylactic or therapeutic activity against COVID-19. Moreover, further plants could be quickly constructed in order to boost the production rate and, eventually, carry out the mass distribution of the product, aimed at boosting the resilience of the population to the infection. Trials in combination with other antiviral substances such as chloroquine or hydroxychloroquine are particularly recommended since a known side effect of the latter antimalarial drugs is retinopathy [22], which can be effectively counteracted by hesperidin [23].

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