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Hydrodynamic cavitation-based rapid expansion of hesperidin-rich products from waste citrus peel as a potential tool against COVID-19

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Abstract: Based on recent computational and experimental studies, hesperidin, a bioactive flavonoid abundant in citrus peel, stands out for its high binding affinity to the main cellular receptors of SARS-CoV-2, outperforming drugs already recommended for clinical trials. Thus, it is very promising for prophylaxis and treatment of COVID-19, along with other coexistent flavonoids such as naringin, which could help restraining the pro-inflammatory overreaction of the immune system. Controlled hydrodynamic cavitation processes showed the highest speed, effectiveness and efficiency in the integral and green aqueous extraction of flavonoids, essential oils and pectin from citrus peel waste. After freeze-drying, the extracted pectin showed high quality and excellent antioxidant and antibacterial activities, attributed to flavonoids and essential oils adsorbed and concentrated on its surface. This study reviews the recent evidence about hesperidin as a promising molecule, and proposes a feasible and affordable process based on hydrodynamic cavitation for the integral aqueous extraction of citrus peel waste resulting in hesperidin-rich products, either aqueous extracts or pectin tablets. The uptake of this process on a relevant scale is urged, in order to achieve large-scale production and distribution of hesperidin-rich products. Meanwhile, experimental and clinical studies could determine the effective doses either for therapeutic and preventive purposes.

Keywords: citrus fruits; coronavirus; COVID-19; flavonoids; hesperetin; hesperidin; hydrodynamic cavitation; pectin; SARS-CoV-2.

1. Introduction

The pandemic caused by the spreading of the Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the related disease named Coronavirus disease 2019 (COVID-19), from China since late 2019 to most of the world since January 2020, proved unprecedented under many aspects. A combination of high contagiousness rate even from asymptomatic infected people, long course of the disease, relatively high incidence of severe and lethal pneumonia especially for elderly and immunosuppressed subjects [1], lack of effective therapies and initial unpreparedness to cope with the pandemic, threatened to overwhelm local and national health care infrastructures and in particular intensive care units [2,3]. The consequent reaction concretized literally overnight in total or partial lockdowns and social distancing prescriptions, expanded to a remarkable fraction of the world population, which are still in force at the time of writing. While everyday chronicles proved the effectiveness of such measures in slowing down the infection rate, they are also likely to cause unpredictable economic havoc and endanger the livelihood of many people for a long time [4].

While similar to other coronaviruses responsible for past epidemics, especially SARS-CoV (early 2000s), with respect to targeted receptors of human type II lung cells [5,6], genetic mutations occurred in SARS-CoV-2 made it more infectious and likely more effective in slowing the response by the immune system until the virus has spread in the lung cells and start replicating [7]. In particular, long spike glycoproteins that protrude from the SARS-CoV-2 particle latch on to the Angiotensin Converting Enzyme-2 (ACE2), a protein located on the surface of type II lung cells [6].

As with SARS-CoV, most of the damage in COVID-19 is caused by the immune system carrying out an overreaction to stop the virus from spreading. Upon entry into alveolar epithelial cells, SARS-CoV2 replicates rapidly and triggers a strong immune response, resulting in cytokine storm syndromes, or hypercytokinemia, and pulmonary tissue damage. The uncontrolled production of pro-inflammatory cytokines (and chemokines) causes acute respiratory distress and multiple organ failure [8,9], even possibly affecting the male gonadal function [10]. Beyond the lethal cases, it is still unclear whether and at which extent these damages could be reversed in recovered subjects.

An unprecedented worldwide scramble is underway to search for effective vaccines [11]. However, their very feasibility and effectiveness is still uncertain, also due to recent preliminary results pointing to insufficient development, sometimes below the detection limit, of SARS-CoV-2-specific neutralizing antibodies in a fraction of recovered patients, especially the younger, or affected by common or mild symptoms [12]. The same finding, if confirmed, might reduce the expectancies about the perspective for herd immunity.

An intensive research is underway also to identify therapeutic drugs to be repurposed, few of which could already have shown preliminary positive results, however lacking large and randomized verifications and consideration of possible harmful side effects [13]. Natural bioactive compounds are also actively looked for, to assess their preventive or therapeutic activities, namely the ability to prevent the virus from binding to the ACE2 enzyme of the host cell, inhibit the virus replication after its penetration in the host cell, as well as restrain or counteract the pro-inflammatory overreaction of the immune system.

Past and recent studies proved that hesperidin, a citrus flavonoid abundant in citrus peel, which is a byproduct of the juice industry, as well as the major flavonoid in sweet orange and lemon, is endowed with plenty of beneficial biological activities, some of which shared with other citrus flavonoids. Thus, it is not surprising that many food supplements and drugs containing hesperidin and other citrus flavonoids have been available since long. Hesperidin and its aglycone hesperetin were attributed particularly strong binding affinity to the receptors of SARS-CoV-2, along with remarkable anti-inflammatory activity, making these molecules attractive ingredients for preventive and therapeutic drugs.

In Section 2, the significant bioactive properties of hesperidin and other citrus flavonoids are briefly reviewed, focusing on properties relevant to the contrast to COVID-19. Section 3 reviews the extraction methods of the same compounds, pointing to controlled hydrodynamic cavitation (HC) as the most effective, efficient and scalable in the perspective of large-scale production. The discussion in Section 4 highlights the immediate feasibility of mass production of hesperidin-rich products based on existing plants and their upscale and replication, also suggesting an affordable process line. Conclusions are set out in Section 5.

2. Bioactive properties of hesperidin and other citrus flavonoids

2.1. Safety and broad spectrum activities

Empirically known since 1876, when the beverage hesperidin obtained from bitter and sweet orange peels was first introduced in Argentina [14], the numerous and different health beneficial effects of hesperidin allowed the exploitation of its large pharmaceutical potential [15].

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

[16]. In murine studies, orally administered doses up to 5% (5 g per 100 g of body weight) showed no toxicity [17].

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 (90%) and 50 mg of hesperidin (10%), and has been proved effective and safe in the long term [18]. Many other products, mainly food supplements, containing hesperidin (up to 500 mg per tablet) and other citrus flavonoids are available on the market and regularly consumed since long, generally claiming to contrast the capillary fragility, and producing anti-edema and anti-inflammatory activity.

The broad spectrum of biological activities of hesperidin and other citrus bioflavonoids are known since the 1930s and were comprehensively reviewed two decades ago, also based on commercial products [17]. Hesperidin was attributed strong antioxidant activity, significant effects on the vascular system, in particular decreasing the capillary permeability and increasing the capillary resistance. Associated with naringin, another citrus flavonoid, hesperidin was shown to significantly lower levels of plasma and hepatic cholesterol, and hepatic triglycerides. Through various mechanisms, hesperidin and other flavanones were shown effective against hypertension. Associated with diosmin, another flavonoid glycoside, and through various mechanisms, hesperidin showed marked protective effect against inflammatory disorders. For example, the agent Daflon 500 mg (diosmin 90%, hesperidin 10%) was demonstrated to improve multiple histological aspects of the acute inflammatory reaction as well as of the chronic inflammation. The sulphonated and phosphorylated hesperidin compounds proved to be extremely potent inhibitors on the hyaluronidase enzyme, which causes a breakdown of hyaluronic acid, thereby increasing tissue permeability and favoring the penetration of certain harmful bacteria.

In a 2011 large clinical study, hesperidin displayed a relevant role in the genomic effect of orange juice, resulting in significant anti-inflammatory and anti-atherogenic activities [19]. A daily dose of 292 mg of hesperidin, corresponding to 500 mL of orange, was sufficient to display the aforementioned effects.

In a recent comprehensive review, all the above-mentioned effects were confirmed and updated [15]. A result was particularly relevant to this study: hesperidin and its aglycone hesperetin, the latter being relatively scarce in citrus fruits and also derived from hesperidin by means of intestinal bacteria following ingestion [17], were found effective to dwindle the release of pro-inflammatory cytokines from immune cells in several tissues, including cerebral, kidney, blood, and lungs. Hesperidin showed to be an effective antagonist of Th2 cytokine in the alveolar space, where localized inflammatory cytokine storms occur in the early phase of acute respiratory distress syndrome and are associated with profibrotic collagen synthesis, sometimes leading to the permanent replacement of the original tissue with scar tissue and eventually organ damage or failure [20,21].

In a study on cancer-induced cachexia (unintentional loss of body weight and skeletal muscle), an integral water extract of *Citrus unshiu* peel showed effective in reastraining the cachexia effects by means of the efficient suppression of the production of pro-cachectic cytokines in immune cells as well as cancer cells [22]. Hesperidin revealed the most effective molecule out of all the citrus flavonoids.

Also relevant to this study, hesperidin was found particularly effective against retinopathy induced by oxidative stress. This effect was attributed to its antioxidant activity and the suppression of excessive activation of calpain, a cysteine protease [23]. Hesperidin was also found very effective in the protection from diabetic retinopathy [24].

2.2. Antiviral activity

Specific antiviral activity of hesperidin and its aglycone hesperetin has long been known, based on in vitro studies, especially towards influenza virus and some herpes viruses [17]. Hesperetin was attributed inhibition activity against the replication of the same herpes viruses. Hesperidin showed also a potent inhibitory effect on the infectivity of rotavirus, both isolated and in integral extracts.

In murine experiments, hesperidin, at the dose of 100 mg per kg of body weight and with intragastric administration, was found to effectively inhibit influenza A virus replication and spread, by up-regulating certain cell-autonomous immune responses [25]. It is worth noting that, quite surprisingly, other flavonoids, such as kaempferol, induced down-regulation and promoted virus replication.

The flavonoid glycosides hesperidin and linarin, the latter derived from certain herbs, share common features such as rutinose at the A ring and methoxy (-OCH3) substitution at the B ring. A recent study showed that hesperidin and linarin were very effective, in a dose-dependent manner, in suppressing the replication of the R5-type of human immunodeficiency virus (HIV-1), which remain in the ileum of patients even after treatment with the most effective anti-retroviral drugs [26]. The mechanism of action was identified in the stimulation of peripheral blood mononuclear cells and the consequent secretion of certain cytokines. A notable result was that the rutinose-deficient analogs of hesperidin (its aglycone hesperetin) and linarin (acacetin), as well as other flavonoids lacking a methoxy substitution at the B ring, did not show similar effects, or only very attenuated ones.

In the aftermath of the SARS-CoV epidemic of the early 2000s, as early as 2005, hesperetin was found to be the most effective molecule, out of synthetic and other natural products (hesperidin was not included), in the inhibition of the SARS-CoV 3-chymotrypsin-like protease (3CL^{Pro}). It showed a level of the 50% inhibitory concentration (IC$_{50}$) of 8.3 μM in the cell-based assay, much smaller than the next natural molecule aloe emodin [27,28]. The 3CL^{Pro}, as a virus-encoded protease, mediates the proteolytic processing of certain replicase polypeptides into functional proteins, thus allowing the virus replication in the host cells and becoming an important target for the drug development. Chloroquine, a long known antimalarial drug, showed a slightly higher level of IC$_{50}$ of 8.8 μM [29]. Moreover, hesperetin turned out to be the most selective among the other considered molecules, thus showing the lowest level of cytotoxicity. Its showed a remarkably high selectivity index (the ratio of the concentration of the compound that reduced cell viability to 50%, or CC$_{50}$, to the concentration needed to inhibit the viral cytopathic effect to 50% of the control value), of about 300, which was tenfold the level for chloroquine (CC$_{50}$ = 30).

The spike protein of SARS-CoV was identified a general target for vaccines and therapeutic treatments [30]. A subunit (S1) of the spike protein contains a receptor-binding domain (RBD) that engages with the host cell receptor ACE2, while the other subunit (S2) mediates fusion between the viral and host cell membranes. However, the search for compounds effectively blocking the RBD-ACE2 binding and the spike protein-mediated infection, and/or the fusion of membranes of the virus and the host cell, for the SARS-CoV did not lead to conclusive results.

2.3. Early evidence of potential activity against SARS-CoV-2

According to recent studies, hesperidin, a citrus flavonoid abundant in citrus peel, showed remarkable binding affinity to the three main protein receptors of SARS-CoV-2, i.e., the SARS-CoV-2 protease domain, the receptor binding domain of the spike glycoprotein (RBD-S), and the receptor binding domain of the ACE2 at the protease domain (RBD-ACE2), responsible for cell infection and virus replication. The above-mentioned remarkable binding affinity to the three main targets was considered representative of the inhibitory activities of hesperidin against viral infection, by either inhibiting the latching of the virus to the ACE2, or inhibiting the virus replication in the cells. Thus, hesperidin could be a promising active substance for drugs potentially useful to prevent or treat COVID-19, possibly along with other citrus flavonoids.

In a molecular docking study, scholars in Indonesia found that hesperidin had the highest affinity to bind all three receptors (lowest docking score), thus inhibiting the proteins responsible for viral infection and virus development [31]. Hesperidin outperformed lopinavir, a repurposing drug involved in clinical trials for COVID-19, as well as nafamostat, a reference compound for RBD-S binding. Moreover, hesperidin outperformed several other natural molecules. In the same study, other citrus flavonoids also abundant in citrus peel, namely tangeretin, nobiletin and naringenin, as well as hesperetin that derives from hesperidin in the intestine after ingestion, showed excellent

affinity to the selected receptors, suggesting that all these citrus flavonoids might contribute to inhibit the viral infection and replication.

Hesperetin was the only citrus flavonoid, among the flavonoids investigated in an another study [32]. It showed high binding affinity to ACE2 enzyme, similar to the other flavonoids typical of Chinese Medicine, present in various herbs, roots, and soybean.

In another study, scholars in China reached similar conclusions [33]. 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. The method clearly showed that hesperidin was the only compound that could target the binding interface between Spike protein and human ACE2, so that by superimposing the RBD-ACE2 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 and prevent the virus from entering the cell.

In a further study, a molecular model was built of the 3-chymotrypsin-like protease ($M^{Pro}/3CL^{Pro}$) structure of the SARS-CoV-2, which is vital to virus replication (as it was for SARS-CoV) and is considered as a promising drug target [34]. The study carried out virtual screening to identify readily usable therapeutics derived from the previous progress about specific inhibitors for the corresponding SARS-CoV enzyme [27–29], which 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 blocked the substrate binding site, resulting as the top scorers. In particular, hesperidin hits showed up multiple times, suggesting it has many modes of binding. Both hesperidin and diosmin were attributed only mild, occasional and reversible adverse reactions.

Another computational and in vitro and in vivo study found that multiple flavonoids abundant in citrus peels have the potential to cooperate to prevent the SARS-CoV-2 infection and restrain its harmful consequences [35]. In particular, simulated molecular docking showed that naringin, hesperetin and naringenin, in descending order, have strong binding affinity with the RBD-ACE2 receptor, at a level similar to chloroquine and higher than hesperidin. Moreover, in vitro and in vivo experiments showed the potential of naringin for inhibiting or restraining the expression of the proinflammatory cytokines induced by different disorders through the overreaction of the human immune system, thereby suggesting that naringin could have a potential in preventing cytokine storms associated with severe forms of COVID-19. It appears that integral flavonoids-rich extracts from citrus peels could show simultaneously multiple activities against COVID-19.

In a further study, a library of phenolic natural compounds (80 flavonoids) was investigated by in silico based screening method against the crystallized form of SARS-CoV-2 main protease ($M^{Pro}/3CL^{Pro}$) [36]. The importance of $M^{Pro}/3CL^{Pro}$ derives from its key role in the self-maturation and processing of viral replicase enzymes, thus in virus replication. Hesperidin exhibited the highest binding energy at the active site of SARS-CoV-2, and revealed as the best potential inhibitor of $M^{Pro}/3CL^{Pro}$ by using a molecular docking approach, closely followed by rutin and diosmin (another citrus flavonoid). Moreover, both hesperidin and diosmin showed a better binding affinity to $M^{Pro}/3CL^{Pro}$ than nelfinavir, an antiviral widely used in the treatment of HIV, as well as one of the early candidates for the treatment of COVID-19 [37].

Two later studies, published in early April 2020, provided important confirmation to the potential role of hesperidin against COVID-19. The first study performed molecular docking study with about 7000 molecules from different classes such as flavonoids, glucosinolates, anti-tussive, anti-influenza, anti-viral, terpenes, terpenoids, alkaloids and other predicated anti-COVID-19 molecules [38]. The three docking targets were $M^{Pro}/3CL^{Pro}$, involved in virus replication, RNA-dependent RNA polymerase (RdRp), which carries out the synthesis of viral RNA from RNA

templates and is involved in the replication and transcription of viral genome, and human ACE2, which is the entry point of the virus. Based on the finding, in a previous study [33], that effective molecules should target multiple key proteins, out of all the considered molecules, only 11 were predicted as potentially effective against COVID-19 and, among these, several flavonoids showing better binding affinities to the three targets than existing synthetic anti-viral drugs. Out of the predicted molecules, hesperidin showed the highest average binding score across the targets, as well as by far the highest binding score with human ACE2, thus potentially representing one of the most promising molecules for any stage of the infection, as well as the most promising for prevention purposes.

In the second of the later studies, plant bioactive compounds were assessed based on their binding affinity with M^{pro}/3CL^{pro} and spike glycoprotein of SARS-CoV-2, by means of a molecular docking approach [39]. The well-known drugs, namely nelfinavir, chloroquine and hydroxychloroquine sulfate, which were widely recommended for clinical trials against COVID-19, were used as a comparison. Hesperidin turned out to have the highest binding score towards both targets, outperforming also the above-mentioned drugs and, especially, chloroquine and hydroxychloroquine. Other bioactive compounds from citrus fruits, such as rhoifolin, nobiletin, tangeretin, and chalcone, showed good binding affinity.

However, when it comes to bioavailability after oral administration, things get more complicated. Indeed hesperidin, with poor water solubility, showed also relatively poor bioavailability, which could negatively affect the performance of in vivo and clinical trials [39].

3. Extraction of hesperidin and other citrus flavonoids

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. In particular, the extraction foresees the treatment of 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 [40].

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 the laboratory and the semi-industrial scale, even though requiring the addition of 10% dimethyl sulfoxide (DMSO) to the extract in order to improve the solubility of hesperidin [41]. 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 [42].

HC methods invariably involve the creation of a periodic depression in a liquid mixture, either by means of the active circulation of the liquid through a nozzle of suitable geometric shape, or moving mechanical parts, such as rotor-stator arrangements, in a still liquid. Vapor filled nano- and micro-bubbles form whenever the liquid pressure falls below the vapor pressure, grow and subsequently implode under the external force produced by the recovered bulk liquid pressure. The implosion events release extraordinary intense energy pulses and, eventually, pressure shockwaves, hydraulic jets, extreme transient heating, and chemical dissociation reactions. Past studies and comprehensive reviews are available, which explain the above-mentioned mechanisms in great detail [43–45].

In terms of process yield, HC methods were found to outperform alternative long established and emerging methods, including acoustic cavitation, for most of applications including wastewater remediation, water disinfection, and especially extraction of natural products [43,46], showed compliance with the principles of green extraction [47], and demonstrated straightforward scalability [48]. In particular, fixed-reactor arrangements, such as based on orifice plates or Venturi tubes, are easy to construct and operate, reliable and can be easily optimized towards the desired effect [43]. Venturi-shaped reactors are particularly suitable for applications such as extraction of natural products, due to more diffuse bubble implosion events and avoidance of clogging, in comparison to orifice plates.

The HC-based integral extraction of waste citrus peel in water, without any other additive, was demonstrated directly on the semi-industrial scale using waste orange peels (WOP) [49], and waste lemon peels (WLP) [50], from citrus fruits organically grown in Sicily. No other processes allowed to extract in just 10 minutes around 60% (w/w) of the overall polyphenol content in fresh WOP [49]. 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.

The feasibility of processing as much as 42 kg of fresh WOP in 120 L of water (35% wt%) was proved, pointing to a concentration of hesperidin in water as high as 0.2% (w/v), i.e., 2000 mg/L. Moreover, 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" [49].

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) [50]. IntegroPectin also showed a strong antibacterial activity against the Gram-positive *Staphylococcus aureus* [51].

Widely employed in the food industry as the natural hydrocolloid of choice [52], pectin exhibited a broad biological activity, including immunoregulatory, anti-inflammatory, and hypoglycemic activities, for which it has been increasingly used in various pharmacological applications [53]. IntegroPectin from *Citrus sinensis* WOP had a very low degree of esterification (17%), making it particularly appropriate for food, pharmaceutical, and nutraceutical applications [49].

It was hypothesized that the antibacterial, antioxidant and the lack of cytotoxicity properties could be attributed 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. Likely, the anti-bacterial activity was boosted by the micronization of *d*-limonene, first in the form of cavitation-induced nanoemulsion, and then deposited onto the pectin surface. Indeed, it is known that the administration of *d*-limonene in form of nanoemulsion increases its anti-bacterial activity by many times [47].

Figure 1 shows the pilot device implementing the above-mentioned HC-based processes with WOP and WLP [49–51], including a closed hydraulic loop (total volume capacity around 230 L) and a centrifugal pump (7.5 kW nominal mechanical power, rotation speed 2900 rpm). The processes were carried out at atmospheric pressure.
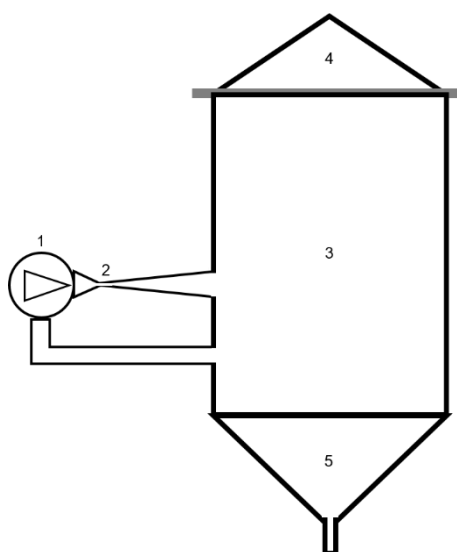

Figure 1. Scheme of the experimental Hydrodynamic cavitation-based installation. 1 – centrifugal pump, 2 – HC reactor, 3 – main vessel, 4 – cover, 5 – discharge.

Such device was used in past studies to carry out innovative beer-brewing [54–57], for which application an industrial-level plant (2,000 L) was developed and operated [48,58]. Among the other applications, the solvent-free extraction of bioactive compounds from the leaves of silver fir plants [47], and the enhancement of biochar properties [59]. The geometry of the Venturi-shaped cavitation reactor was defined in a previous study [60].

Venturi-shaped cavitation reactors were shown to outperform other reactors based on fixed constrictions, such as orifice plates, in the treatment of viscous food liquids [46]. This superiority holds especially with liquids containing solid particles, as well as for the inactivation of spoilage microorganisms [60], and for the creation of oil-in-water stable nanoemulsions [61], all these features being relevant to the processes under study.

4. Discussion and conclusions

Hesperidin, a flavonoid abundant in citrus peels, was identified as a potentially very interesting molecule in the fight against COVID-19. Its antiviral activity was proven for other viruses, in particular SARS-CoV, thus it could reveal useful also in case of further mutations of SARS-CoV-2.

In the therapeutic use, hesperidin has the advantage of strong binding affinity to all the main viral and cellular targets, outperforming not only other natural molecules, but also antiviral drugs recommended for clinical trials on COVID-19 inpatients. These targets correspond to different stages of the infection, from the entry of the virus into the host human cell, to the transcription of viral genome and virus replication.

The especially great binding affinity with the human ACE2, thus the potential to prevent the virus to spread into the cells, could suggest a special role of hesperidin in prophylaxis. On the other hand, the regular and prolonged administration of hesperidin for prophylaxis would be allowed by its safety, short lifetime in the body and the absence of cytotoxicity up to high doses.

Other flavonoids, coexistent with hesperidin in citrus peels, showed as well good binding affinity to one or more targets, especially hesperetin, which is the aglycone of hesperidin, and naringin. The latter flavonoid showed also the ability to restrain the pro-inflammatory overreaction of the immune system, which could help fighting the severe forms of COVID-19.

In this study, we call for the urgent uptake of HC-based processes, applied to citrus peels, for the efficient and green industrial production of aqueous extracts and pectin tablets rich in hesperidin. The extraction process takes no longer than 10-15 min, however, including all the necessary steps such as grinding the citrus peels before the inlet to the processing unit, separating the solid residues, and discharging and packaging the aqueous extract, the overall process for a plant with a nominal capacity of about 2000 L could require up to 2 hours. Based on the figures exposed in Section 3, undertaking the processing of 500 kg waste citrus peel (as such) in 1500 L water, the process would be able to extract 3 kg of hesperidin per cycle, hence at least 36 kg of hesperidin per day (in 12 cycles). The only additional technological components next to the industrial scale HC-based extractor would be a grinder, a filter/separator, and a lyophilizer, such as those commonly operated at pharmaceutical companies where they are used to remove solvent from a frozen product by sublimation. After the lyophilization, IntegroPectin tablets containing the required dose of hesperidin and other flavonoids could be readily produced, due to the low density and open, porous structure of the pectin.

Figure 2 shows the main technological components of the proposed process based on the experience gained by authors, although variants are easy to set up, such as replacing the centrifugal pump and the Venturi-shaped reactor with a rotor-stator arrangement, according to specific and local expertise or whatever preference. Moreover, the Venturi-shaped reactor could be realized in accordance with long established rules for circular-section ones [60], or in the form of generally more performing slit Venturi [43], as well as optimized by numerical simulations [62]. Other emerging setups could be used too, such as based on vortex diode [63]. The dosing pump is optional and could be useful for introducing any natural or technical additives. Minor components such as a thermometer and a pressure gauge can be applied to the working vessel and are not shown.
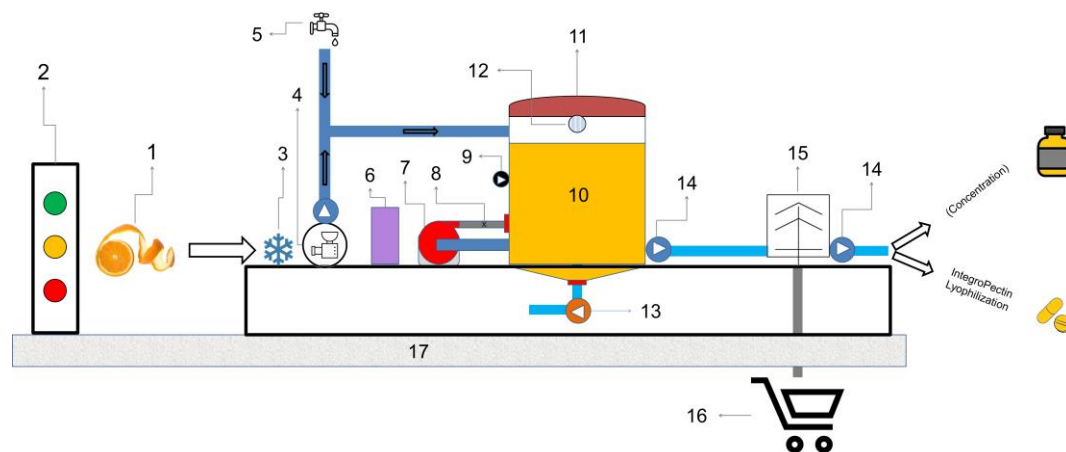
Figure 2. Main technological components of the proposed process. 1 – citrus waste peel; 2 – electronic control panel; 3 – ice machine; 4 – grinder; 5 – water supply; 6 – inverter; 7 – centrifugal pump; 8 – HC reactor, such as Venturi tube; 9 – dosing pump; 10 – working vessel; 11 – hatchway; 12 – washing sphere; 13 – lobe pump; 14 – multistage pump; 15 – filter/separator; 16 – discharge of residues; 17 – skid.

The product, either in the form of aqueous extracts of pectin tablets, could undergo in vivo, in vitro and clinical trials aimed at assessing the prophylactic or therapeutic activity against COVID-19 and the respective effective doses. As a reference, about one month after the outbreak of COVID-19, Chinese scholars were able to assess the specific antiviral activities of the well-known broad-spectrum antiviral drug remdesivir, and the long-known antimalarial drug chloroquine, against SARS-CoV-2 infecting Vero E6 cells in vitro, including the IC_{50} and the level of the 90% inhibitory concentration (IC_{90}) [64]. Remdesivir showed IC_{50} = 0.77 μM and IC_{90} = 1.76 μM, while chloroquine showed IC_{50} = 1.13 μM and IC_{90} = 6.90 μM, the latter level clinically achievable as demonstrated in the plasma of rheumatoid arthritis patients who received 500 mg administration. The selectivity index was also reasonably high, about 130 for remdesivir and 88 for chloroquine, suggesting a good safety level.

Since chloroquine is relatively toxic with high doses and prolonged use, as well as its production largely discontinued following the introduction of other antimalarial drugs, about two months later, the same authors proposed hydroxychloroquine as a further potential therapeutic agent against COVID-19 [65]. Hydroxychloroquine was synthesized long ago by introducing a hydroxyl group into chloroquine, and is still widely used in the treatment of inflammatory rheumatic diseases. It is about 40% less toxic than chloroquine, but shows a selectivity index about one third lower and requires higher doses to achieve comparable effectiveness, although still clinically achievable. Finally, hydroxychloroquine shares with chloroquine a good potential to attenuate the inflammatory response, thus potentially offering a broad-spectrum protection from COVID-19.

Despite lower toxicity, one of the main drawbacks of hydroxychloroquine is the well-known side effect of retinopathy, especially in case of prolonged use such as for treating rheumatic disorders and also due to its long half-life and accumulation in tissues and blood, leading to the recent widespread recommendation for lower doses [66,67]. While this could not be such a big issue for therapeutic use against COVID-19, it could jeopardize the use of hydroxychloroquine as a preventive drug. However, the neuroprotective activities attributed to hesperidin, mentioned in Section 2.1 [23,24], might suggest an integrated approach against COVID-19, with hesperidin-rich products and hydroxychloroquine administered together, at respective doses yet to be defined, for both therapy and prevention.

As recalled in Section 2.3, the bioavailability issue after oral administration could impair the performance of hesperidin-rich products during in vivo and clinical trials.

Based on the past experience with the HC processing of WOP and WLP [49–51], it could be hypothesized that the HC processing produces a conjugation of the polyphenols, including hesperidin, onto the pectin macromolecules, resulting in a product similar to that obtained by means of covalent conjugation via a proven preparation method involving epichlorohydrin chemistry [68]. Moreover, the exceptional antioxidant activity shown by the IntegroPectin, resulting from the HC processing [50], could suggest that, in comparison to standard covalent conjugation, hesperidin retains more of the original antioxidant activity. These topics are recommended for further research.

However, it is very likely that the IntegroPectin is endowed with a remarkably higher level of water solubility, thus with higher levels of bioavailability after oral administration. Indeed, pectin-based covalent conjugates obtained with the poorly water soluble hesperidin, showed faster dissolution rates (higher water solublity) than both neat pectin and hesperidin [68]. If this result could be verified, it would represent an additional incentive to consider IntegroPectin tablets for in vivo and clinical trials and, eventually, for mass production.

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