Extraction Methods, Stability and Biological Activities of Hesperidin

Subjects: Food Science & Technology
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Hesperidin is a bioflavonoid occurring in high concentrations in citrus fruits. Its use has been associated with a great number of health benefits, including antioxidant, antibacterial, antimicrobial, anti-inflammatory and anticarcinogenic properties. The food industry uses large quantities of citrus fruit, especially for the production of juice.

Keywords: hesperidin ; extraction ; stability ; biological activities

1. Extraction

Due to its biological activities, hesperidin is often used in the food, cosmetic and pharmaceutical industries. Optimal extraction processes from plant materials with high quality and purity are implied in these applications. Several procedures for the extraction of flavonoids, including hesperidin, have been explored, also taking into account those that are environmentally friendly [1][2][3][4][5][6][7][8][9][10][11][12][13][14][15]. Common extraction methods include dipping, percolation, reflux or continuous reflux. The quality of an extract and efficiency of a procedure are influenced by several factors such as solvent type, temperature, extraction time and liquid–solid ratio.

Methanol and ethanol, or their mixture with water at different proportions, as well as dimethyl sulfoxide (DMSO), are usually used. Maceration and Soxhlet extraction are increasingly being replaced by advanced techniques to increase efficiency and selectivity. They are generally faster, more environmentally friendly, and with higher automation levels. Several methodologies based on accelerated solvent extraction (ASE) [7], microwave-assisted extraction (MAE) [8][9], ultrasound-assisted extraction (USE) [2][8][9][10][11], subcritical water extraction (SWE) [12][13], pressurized liquid extraction (PLE) [14] and high hydrostatic pressure (HHP) [15] have been used for isolation of hesperidin from plant materials. Application of mathematical and statistical methods to the analysis of chemical data, like experimental design, response surface analysis and principal component analysis, have been often used for determining the optimum extraction conditions [2][6][7][8][9].

Two sequential extractions using 90% methanol for 20 min agitation at 55 °C were considered as optimal for isolation of hesperidin (24.77 mg/g dw) from sweet orange pulp (*Citrus sinensis* L.), while for 90% ethanol under the same conditions 17.9 mg/g of hesperidin was obtained (with significant differences at $p < 0.05$) [6]. Gómez-Mejia et al. [2] proposed extraction from orange peels with low concentration of ethanol (maximum 40%, *v*/*v*) run in an ultrasound bath for 10–15 min. It was stated that the use of a relatively high temperature (90 °C) could be compensated for by a very rapid and efficient extraction process. On the other side, a DMSO:methanol mixture (1:1) turned out to be a better medium during 10 min of ultrasound operation for the extraction of hesperidin from mandarin (*Citrus reticulata* Blanco) rinds [10]. The yield of hesperidin from peels of *Citrus unshiu* fruits after the MAE process (70% aqueous ethanol, heating 140 °C, 7 min) was comparable to the amount extracted at room temperature for 30 min using the DMSO:methanol (1:1) mixture [8]. The maximum yield of hesperidin from *Citrus unshiu* peel using SWE method was obtained at 160 °C for an extraction time of only 10 min [12]. It was 1.9-, 3.2- and 34.2-fold higher than those when 70% ethanol or methanol and hot water, respectively, were used.

2. Stability of Hesperidin

Hesperidin, as other flavonoids, during different extraction processes might degrade when exposed to light, air and elevated temperature. The presence of the oxidative enzymes and free radicals released during extraction could also promote a series of degradation reactions [8][14][16][17][18]. It was found that application of sonication caused degradation of hesperidin standard solution as well as other flavonoids [16]. The highest decomposition in the methanol solution was observed for myricetin (40% decrease), followed by hesperidin (30%). As a contrast, all studied compounds were stable during heating reflux in a water bath for 30 min and maceration for 24 h (recovery above 95%). Majumdar et al. reported

that aqueous solutions of hesperidin (5 µg/mL) did not demonstrate any decrease in its content up to 2 months in the pH range of 1–7.4 at 25 and 40 °C [17]. The only exception was pH 9, where the degradation rate constants were 0.03 and 23 at 25 and 40 °C, respectively, most likely by alkaline hydrolysis.

The effect of hesperidin degradation depends also on the type of food matrix [18][19]. Biesaga et al. [19] presented one concerning the stability of hesperidin and other flavonoids in some food samples (honey, apple and onion) during different extraction processes. After addition of 60% (*v*/*v*) aqueous acidified methanol (pH 2), the solutions were subjected to heating in a water bath for 15 min, sonication for 5 min (20 Hz) or microwave irradiation (90 W) for 1 min. The apple matrix seems to stabilise these compounds to a large extent, while the highest degradation was observed for the honey samples, particularly for heated reflux and MAE conditions. Low recovery (68%) of hesperidin from maize samples during USE extraction was reported, similar to its standard solution [17].

Zhang et al. investigated the effects of storage condition and heat treatment during pasteurization of juice from orange fruits (*Citrus sinensis* Osbeck cv. Newhall) on the hesperidin concentration [20]. They found that the hesperidin concentration decreased when the juice was stored at room temperature and even at 4 °C, but at a slower rate. After 6 and 20 h of storage at −18 °C, the hesperidin concentrations in the juice were 576 and 533 mg/L, respectively, compared to 658 mg/L determined in the freshly squeezed juice.

The concentration of hesperidin in juice subjected to heat treatment (80 °C for 10 min) was almost the same as that in freshly squeezed juice. However, when this heat-treated juice was kept at room temperature, its concentration reduced gradually from the initial value of 577 mg/L to 294 mg/L after 30 days of storage. Similar results were obtained for untreated juice. Additionally, it was confirmed that the reduction of hesperidin concentration was not caused by enzymatic degradation (heating at 100 °C for 10 min) [20]. It was suspected that the hesperidin had sedimented from the juice during storage. It was later confirmed after determination of the whole content of this compound (containing both soluble and precipitated hesperidin) using extraction with DMF. It was assumed that the precipitation of hesperidin might be due to the gradual decomposition of vitamin C.

3. Biological Activities

The human benefits of hesperidin taken from fruits and beverages or pharmaceuticals depend mainly on its bioavailability. The bioavailable fraction is commonly defined as the quantity of a given substance released from food that is absorbed through the intestinal barrier and enters the blood stream, reaching the systematic circulation, which is then distributed to organs and tissues and is transformed into a biochemically active form, which is effectively used by the organism [21]. The bioavailability of hesperidin is low due to its low aqueous solubility, absorption, and modification by microorganisms in the gastrointestinal tract and rapid excretion [22][23][24]. It is considered that the limiting step of the hydrolysis and absorption of hesperidin is the enzymatic activity α-rhamnosidase, which takes part in these processes. Although hesperidin is poorly absorbed and rapidly eliminated, it has a reasonable half-life of 6 h [25].

The biological and pharmacological properties of hesperidin have been extensively studied to reveal its antioxidant, anti-inflammatory, anticancer, antiviral effects, protective cardiovascular disorders and neurodegenerative properties, among others.

A number of researchers have examined the antioxidant activity of hesperidin using various assays [26][27][28][29][30]. Its antioxidant properties are expressed mainly by direct free radical scavenging or indirectly by inhibition of prooxidative enzymes that participate in the generation of these radicals as well as by chelation of transition metals which participate in reactive oxygen species, generating reactions. The results showed that hesperidin has more potential iron chelation activities compared to deferoxamine, a popular chelator for treatment of chronic iron overload [31]. The antioxidant activity of hesperidin exhibits also by reducing the production of reactive oxygen species and increasing the activities of antioxidant enzymes, catalase and superoxide dismutase [32][33]. It should be mentioned that hesperidin exhibits lower antioxidant activity in comparison to its aglycone form, alike other flavonoids [18]. Citrus peels showed higher antioxidant ability than pulp due to its high content of flavonoids, vitamin C and carotenoids [34]. Al-Ashaal and El-Sheltawy reported that hesperidin from orange peel extract was moderately active as an antioxidant agent; its capacity reached 36% against free radical DPPH· in comparison to 100% obtained for vitamin C [27].

Cardiovascular protective effects of hesperidin are expressed in decreasing diastolic blood pressure, glucose levels and various lipid profile parameters, reducing platelet aggregation and increasing in the expression of antioxidative enzymes [35][36][37][38]. It was stated that hesperidin also exhibits cardioprotective effect against doxorubicin cardiotoxicity, which is

widely used anticancer drug [35][39]. Razaee et al. found the protective effects of hesperidin against CO-induced cardiac injury in rat exposed to CO [40].

Administration of hesperidin decreased the expression of zinc finger E-box binding homeobox 2 (ZEB2, a transcription factor that binds to specific regions of DNA) by upregulating the expression of miRNA-132, which in turn promoted apoptosis and inhibited the proliferation of non-small cell lung cancer cells in mice [41]. Citrus peel extracts have been proven to be a promising therapeutic agent for diabetes mellitus, characterized by defects in insulin metabolism that can alter carbohydrate, protein and fat metabolism [42][43][44][45]. It was indicated that obesity is connected with insulin resistance and pancreatic β-cell dysfunction [46][47].

Mounting evidence has demonstrated that hesperidin possesses an inhibitory effect against the development of neurodegenerative conditions such as Alzheimer's and Parkinson's diseases [48][49][50][51]. It showed involvement of immunity in the development and progression of neurodegenerative disorders [52]. Hesperidin's neuroprotective potential is mediated by the improvement of neural growth factors and endogenous antioxidant defense functions, diminishing neuro-inflammatory and apoptotic pathways. Dietary supplements containing hesperidin can significantly improve cerebral blood flow, cognition, and memory performance [48]. Several investigators have dedicated their effort to explore neuropharmacological mechanisms and the molecular target of citrus flavonoids, including hesperidin [53][54].

The potential anti-inflammatory effects of hesperidin for its possible therapeutic application against diverse pathologies have been evaluated [55][56][57][58]. Xiao et al. used it to effectively enhance chondrogenesis (a process that leads to the establishment of cartilage and bone formation) of human mesenchymal stem cells to facilitate cartilage tissue repair [56]. The results presented by Homayouni et al. suggest that hesperidin supplementation may have anti-inflammatory and antihypertensive effects in type 2 diabetes [57].

The increasing antimicrobial resistance to synthetic antibiotics has attracted the interest of scientists in the direction of the use of naturally occurring compounds as effective antibacterial agents [59]. Several reports have demonstrated that hesperidin can also act against different pathogenic bacteria [38][60][61][62]. It can directly inhibit bacterial growth or act indirectly by modulating the expression of virulence factors, both of which reduce microbial pathogenicity. Hesperidin supplementation may be useful as a prophylactic agent against SARS-CoV-2 by blocking several mechanisms of viral infection and replication [63][64].

Hesperidin has also been associated with other, except those mentioned above, beneficial health effects, such as UV protection, wound healing and cutaneous functions [65] and radioprotective protection against ionizing radiation-induced damage [66]. Together with the flavone diosmin, under the trade name Daflon®, it decreases capillary fragility and is recommended for treating venous circulation disorders (swollen legs, pain, nocturnal cramps) and for treating symptoms due to acute hemorrhoidal attack [67]. Interested readers can find more specific information regarding biological activities of hesperidin as well as the results of the preclinical studies and clinical trials in these [26][34][36][48][60].

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