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Effect of naringin enzymatic hydrolysis towards naringenin on the anti-inflammatory activity of both compounds

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Abstract

The aim of this work was the hydrolysis of naringin towards naringenin with immobilized naringinase and the evaluation of their anti-inflammatory activity. An acute local inflammation model (rat paw edema induced by λ-carrageenan) was chosen to evaluate the contribution of antioxidant properties to a possible anti-inflammatory effect.

Grapefruit juice was processed with naringinase immobilized in k-carrageenan (2%) beads (≈3 mm). A 95% naringin conversion in grapefruit juice was obtained with immobilized naringinase (1000 mg L^{-1}), with an activity of 19.5 mg mL^{-1} min^{-1} and the formation of 215 mg L^{-1} of naringenin.

Ascorbic acid and indometacine were used as positive anti-inflammatory controls. All results were analysed using ANOVA with Dunnett's post-test. The results show that rats ($n = 9$) pre-treated with a solution of naringin, rats ($n = 9$) pre-treated with a solution of naringenin (in concentrations equal as in grapefruit juice) and rats ($n = 9$) treated with a solution of naringenin and naringin, revealed a significant reduction on edema formation, 6 h after λ-carrageenan injection. Naringenin demonstrated a high *in vivo* anti-inflammatory activity, only 8% of paw edema ($p < 0.001$) was observed in rats pre-treated with a solution of naringenin.

Comparability studies, in rats administered orally with grapefruit juice (before and after processing), showed that enzymatic processing did not affect the anti-inflammatory properties of the juice.

Keywords: Naringin; Naringenin; Naringinase; Anti-inflammatory; Comparability tests; Paw edema; Grapefruit juice

1. Introduction

Flavonoids, known as nature's tender drugs, possess various biological/pharmacological activities including antioxidant, anti-inflammatory, anticancer, antimicrobial, and antiviral.

The interest in bioactive compounds of fruits and vegetables has increased in recent years due to their health benefits, particularly, protection against a variety of diseases as cardiovascular and some types of cancer [1]. In citrus fruits the main bioactive compounds are ascorbic acid, carotenoids, flavonoids, limonoids and coumarins [2]. Ascorbic acid and carotenoids are well known for their antioxidant properties while flavonoids have demonstrated to act as free radical scavengers to modulate enzymatic activities and to inhibit cellular proliferation as well as to possess another several biological activities such as anti-ulcer, anti-allergenic, immunomodulatory, anti-diarrhea, analgesic, antibiotic and antithrombotic with inhibition of platelet aggregation [2–7].

Recently, attention has been given to isolated flavonoids, namely those from citrus, as potential anti-inflammatory agents. Acute inflammation is typically characterized by increased permeability of endothelial tissue and leucocyte leakage into the interstitium resulting in edema. Many different biological mediators influence the various steps of the inflammatory process, and typically, anti-inflammatory agents exhibit therapeutic properties by blocking the actions or synthesis of these mediators.

The antioxidant activity exhibited by several flavonoids seems to be related with the number of hydroxyl groups in the B ring (Fig. 1), responsible for part of the anti-inflammatory properties of these compounds [8]. Besides being related with free radicals scavenging and inhibition of lipid peroxidation, anti-inflammatory activity of flavonoids is also associated with the inhibition of cyclooxygenase and 5-lipooxygenase pathways involved in the arachidonate metabolism [8,9].

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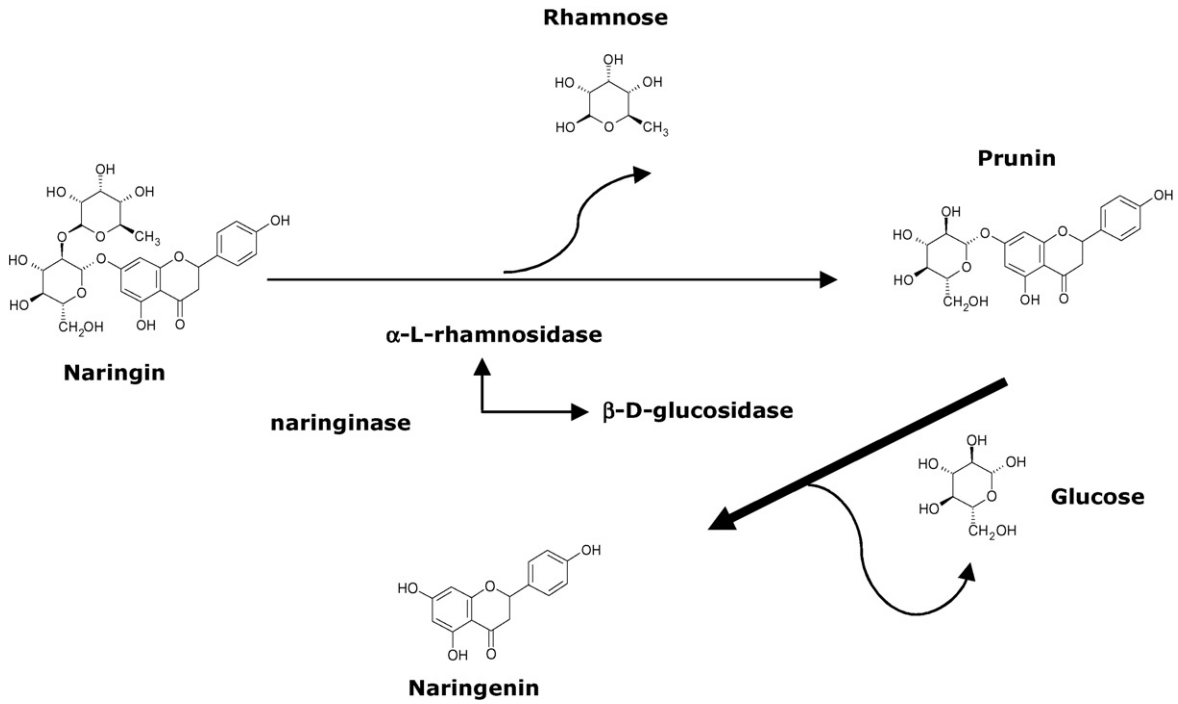

Fig. 1. Hydrolysis of naringin into prunin, rhamnose, naringenin and glucose by naringinase expressing α-rhamnosidase and β-glucosidase activities.

Citrus flavonoids and their metabolites, as potent antioxidants, are able to restrain many of the inflammatory and tumorigenic events through mechanisms mediated by reactive oxygen species [10]. Free radicals are well known to play an important part in the inflammatory process. They are involved in inflammation and tissue destruction and also implicated in the biosynthesis of prostaglandins therefore, the evaluation of anti-inflammatory properties of flavonoids is of great interest [11].

Naringin (4′,5,7-thrihydroxyflavanone-7-rhamnoglucoside) is the major flavonoid present in grapefruit juice and has been described to present antioxidant and anti-inflammatory activity, as well as, its aglycone naringenin (4′,5,7-thrihydroxyflavanone) [10,12–15]. This bioflavonoid can be obtained from naringin hydrolysis with naringinase.

The bitterness of citrus juices (e.g. orange and grapefruit) can restrain its consumption and naringin is the main compound responsible for this undesirable attribute in grapefruit juice.

Immobilization of biocatalysts has many advantages in large-scale processing, namely biocatalyst reuse, easy separation of biocatalyst from reaction media, continuous mode operation, prevention of contamination of the processed product, higher enzyme concentrations, higher superficial area to reaction, among others. Such systems using different immobilization supports have been evaluated in naringin hydrolysis, by several authors [16–19].

In order to reduce grapefruit juice bitterness, naringin hydrolysis was carried out by immobilized naringinase in k-carrageenan (2%) beads. This decrease in bitterness improves the commercial value of grapefruit and other citrus juices, increasing the acceptance by the consumer, controlling the quality and maintaining health properties.

Antioxidant activities of naringin and naringenin could have an important role *in vivo*. The potential of these compounds to act as antioxidants, and hence as anti-inflammatory, in animal experimental models of inflammation was investigated. The carrageenan-induced rat paw edema model, a model for acute local inflammation was used as *in vivo* model of inflammation. Model solutions containing the same amounts of naringin and naringenin as present in samples of grapefruit juice (before and after processing) were screened for anti-inflammatory activity.

Also comparability studies were carried out, with the purpose of evaluate the impact of naringin enzymatic hydrolysis in the juice quality. Eventual alteration of antioxidant properties related with ascorbic acid and carotenoids content were evaluated, as well as, juice anti-inflammatory properties by the experimental *in vivo* model (inhibition of induced paw edema in rats) before and after enzymatic hydrolysis.

2. Materials and methods

2.1. Materials and equipment

Naringin (naringenin-7-rhamnosidoglucosidose) 96.6%, naringenin (4′,5,7-trihydroxyflavanone) 99%, naringinase (CAS Number 9068-31-9), indometacin 99% and λ-carrageenan were purchased from Sigma (St. Louis, MO, USA). Ascorbic acid was purchased from Riedel-de-Haën (Hannover, Germany). Acetonitrile HPLC grade, sodium acetate trihydrate, 2,6-dichlorophenol indophenol, glacial acetic acid and absolute ethanol were from Merck (Darmstadt, Germany). k-Carrageenan from brown algae was obtained from Fluka (St. Louis, MO, USA). All other chemicals were analytical grade and obtained from various sources. Grapefruits were bought in local supermarkets.