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Rapid adsorptive separation of citrus polymethoxylated flavones in non-aqueous conditions

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Abstract

Flavanoids exist as secondary plant metabolites, which displays a wide variety of biological effects. The polymethoxylated flavones, such as nobiletin and tangeretin, present in *Citrus reticulata* peels are of great interest due to their pharmacological effects. Separation and isolation of these structurally very similar flavones has been achieved by using commercially available ion exchange resins. The strong cation exchange resin [H^+] selectively adsorbs the tangeretin and other phenolic compounds present in the matrix in comparison to the hexamethoxylated flavone, i.e. nobiletin. The cation exchange resin can be used successfully to isolate nobiletin and tangeretin from a plant extract. Purity of the isolated compounds was monitored by HPLC using a C-18 column with photometric detection at 280 nm. The structures of the isolated compounds have been confirmed by NMR and tandem mass spectrometry.

Keywords: Citrus; Adsorptive separation; Polymethoxylated flavones

1. Introduction

Diminishing the environmental impact of industrial wastes has been a subject of increasing concern in recent years. Many food industries have an adverse environmental impact because of the presence of residual organic compounds, especially in their wastes from the raw plant materials used. In industrial liquid effluents, these compounds considerably increase biochemical and chemical oxygen demands resulting in detrimental effects on the environment. In solid residues created upon the production of organic fertilizers, relatively high levels of phenolic compounds are a problem because of their inhibition of germination properties. Due to the large amounts of citrus being processed into juice, a considerable by-product has evolved to utilize the residual peels, membranes, seeds and other compounds. Residues of citrus juice production are a source of dried pulp and molasses, pectin, cold-pressed oils, essences, D-limonene, juice pulps and pulp wash, ethanol, seed oil, pectin, limonoids and flavonoids [1–4].

Flavones, which are widespread in the vegetable kingdom, generally occur as hydroxylated or glycosylated derivatives [5]. Polymethoxylated flavones (PMF's) constitute a special group that is present in certain citrus species. The peel of these fruits contains higher concentration of PMF's than their leaves or juices [6]. The PMF's are extremely important bioactive compounds (Fig. 1). Nobiletin is known to decrease both the rate of erythrocyte aggregation and the rate of blood cell sedimentation in humans [7]. Tangeretin inhibits the development of HL-60 cells, which are implicated in leukemia [7]. Tangeretin and nobiletin appear to be cytotoxic towards cancer cells and are believed to be potential anti-tumor promoting agents [8]. These compounds have also shown anti-mutagenic activity and anti-proliferative activity in several cell lines [9,10]. Nobiletin has also shown potential

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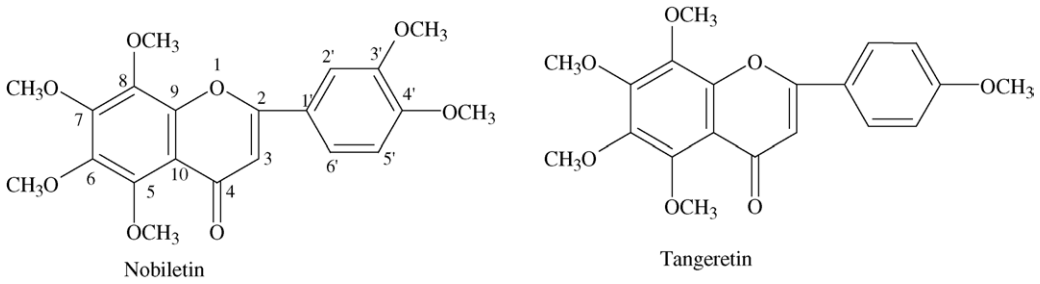

Fig. 1. Structures of polymethoxylated flavones.

as a de-pigmenting agent at the cellular level, which could be used again hyper-pigmentation [11]. Numerous flavonoids appear to have an anti-mutagenicity and carcinogenicity [12]. Nobiletin and tangeretin inhibited mutagenicity of B[*a*]P, 2-aminoanthracene, nitroquinoline N-oxide and quercetin in *Salmonella typhimurium* TA-1538 [13].

Nobiletin enhanced the mutagenicity of 2-acetylamino fluorine in *S. typhimurium* TA-100 [13]. Nobiletin and tangeretin increased the metabolic activation of B[*a*]P and aflatoxin B_1 to mutagens [14]. Because of the dual effect of the PMF's, it is very essential to predict their anti-mutagenic activity and the concentration of action. In nature, the flavonoids occur as a complex mixture and hence need to be isolated for systematic investigation of their activity. The mutagenicity of mixture of PMF's has been investigated, but since individual flavonoids have not been studied in detail, their differential effects cannot be clearly predicted. Another possible role of these compounds is related to defense mechanisms of the plant itself, since nobiletin and tangeretin have anti-viral and anti-microbial capacity, which, together with the other components of the essential oil, confer a certain degree of resistance against microbial infections in citrus [15]. Recently, anti-microbial activity of citrus peel extracts have been reported in vitro model system [16]. Tseng reported the isolation using a cold methanolic extract, but this was a rather tedious process [17]. The yield obtained in this process was quite low. Isolation of nobiletin and tangeretin from *Citrus deliciosa* has been reported by conventional solvent extraction with methanol followed by column chromatography using silica gel [7,18].

The present communication describes the adsorptive separation strategy for the isolation of two related polymethoxyflavones, nobiletin and tangeretin. Adsorptive separation has been studied for the separation of close boiling point alkyl phenols using zeolites [18]. Sorption on polymeric ion exchange resins has been accepted for aqueous media for ionic species as well as for ionizable organic molecules [19]. However, sorption on ion exchange resins using non-aqueous solvents as a strategy for the separation of naturally occurring polymethoxyflavones has not been studied. In this context, a study was carried out for the separation and purification of nobiletin and tangeretin from citrus peels, based on their structural difference and their interactions with ion exchange resins. The structures of purified compounds have been confirmed on the basis of NMR studies and tandem mass spectrometry.

2. Experimental

2.1. Material and reagents

HPLC grade methanol, hexane and other chemicals (AR grade) were obtained from Merck, Darmstadt, Germany. Dowex 50WX8 cation ion exchange resin, with mesh size 100–200, was obtained from Dow Chemical Company, (Midland, MI, USA).

2.2. Plant material

The peels of Mandarins (*Citrus reticulata*) were obtained from fully-grown ripe fruits (Texas A&M University—Kingsville Citrus Center, Weslaco). The fruits were collected in the month of February and March 2003.

2.3. Regeneration/activation of Dowex 50

Four resin volumes (400 mL) of 1N sodium hydroxide were percolated for 4 h and the resin was washed to neutral pH. Then, four resin volumes (400 mL) of 10% HCl were percolated for 3 h, and then displaced with four resin volumes (400 mL) of distilled water for 3 h. Finally, the resin was rinsed with distilled water to obtain a neutral pH.

2.4. Extraction and purification of flavonoids

Air dried citrus peel (1200 g) was pulverized and extracted exhaustively with hexane (5.0 L) at room temperature. The hexane extract was concentrated and treated with 100 mL of 10% sodium hydroxide solution and allowed to stand for 2.0 h with occasional stirring. The mixture was then extracted with diethyl ether (100 mL × 3) as long as appreciable components were extracted. The combined ether extracts were washed with water, and the solvent was concentrated. The mixture was dissolved in 125 mL hexane. Then the hexane extract was added to cation exchange resin Dowex 50WX2 (20.0 g).