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Hydrotropic extraction of bioactive limonin from sour orange (*Citrus aurantium* L.) seeds

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Abstract

Limonoids are potential bioactive compounds present only in citrus among fruits and vegetables. A new process for extraction of limonoid aglycones from sour orange (*Citrus aurantium* L.) seeds was investigated using aqueous hydrotropic solutions. The extraction efficiency was dependent on hydrotrope concentration, extraction temperature and percent of raw material loaded. Two hydrotropes such as sodium salicylate (Na-Sal) and sodium cumene sulphonate (Na-CuS) were studied using Box-Behnken experiment design. Response surface analysis (RSA) of data was performed to study the effect of parameters on extraction efficiency. Prominent limonoid aglycone such as limonin was extracted and quantified for process optimization. Both hydrotropes gave maximum limonin yield at 2 M concentration, extraction temperature of 45 °C and 10% solid loading. A maximum limonin yield of 0.65 mg/g seeds was obtained using Na-CuS whereas only 0.46 mg/g seed was obtained using Na-Sal. Using this process, the use of organic solvents can be reduced dramatically to keep the process environmental friendly for the extraction of bioactive compounds.
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Keywords: Sodium salicylate; Sodium cumene sulphonate; Limonin

1. Introduction

Limonoids are a unique class of bioactive compounds present in citrus fruits as a group of highly oxygenated tetracyclic triterpenoids. So far, 37 limonoid aglycones and 17 limonoid glucosides have been isolated from citrus and its closely related genera (Mandadi, Jayaprakasha, Bhat, & Patil, 2007). The aglycones occur as neutral dilactones, acidic monolactones or dicarboxylic acids whereas limonoid glucosides are 17-β-D-glucopyranoside esters of the acidic aglycones. Prominent aglycones are low to medium polarity compounds usually soluble in organic solvents.

Both aglycones and glucosides showed health maintaining properties and hence their isolation, identification and biological activity have been explored (Hasegawa, Berhow, & Manner, 2000; Hasegawa & Miyake, 1996; Rice Evans, Miller, & Paganga, 1997; Poulose, Jayaprakasha, Mayer, Girennavar, & Patil, 2007). Recently, we have observed that limonoid glucosides have the ability to induce caspase 3/7 activity, suggesting that limonoids were capable of inducing apoptosis (Poulose, Harris, & Patil, 2005). Furthermore, our studies also demonstrated that consumption of limonin or grapefruit suppresses colon cancer development (Vanamala et al., 2006). Studies on HepG2 cells indicated that citrus limonoids were partly responsible for lowering LDL cholesterol (Kurowska, Manthey, & Hasegawa, 2000). Furthermore, recent animal studies showed that citrus limonoids significantly decreased the LDL/HDL-cholesterol ratio, and prolonged LDL oxidation susceptibility, which will help reduce the risk of atherosclerosis (McGill & Green, 2001).

Despite increasing demand for limonoids with documented and potential health benefits, lack of well-defined

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environmentally friendly and economical extraction methods have precluded development of these bioactive compounds (Braddock & Cadwallader, 1992). Recently a method was developed for separation and purification of limonoid glucosides from citrus; this is the first method for the purification of limonoids on a multigram scale (Jayaprakasha, Bhat, & Patil, 2007). The major challenge in isolating and purifying limonoids from citrus raw material is their low abundance. Large amounts of raw material need to be processed using equivalent amounts of solvents to obtain sufficient yield of limonoids. Additionally different solvents are required for extraction and purification of limonoids with different polarities (Jayaprakasha, Brodbelt, Bhat, & Patil, 2006).

Limonoid aglycones are medium polarity compounds usually extracted using dichloromethane (DCM), ethyl acetate and acetone. Several methods were developed for the extraction of limonoid aglycones using organic solvents (Bennett & Hasegawa, 1982; Dreyer, 1966; Hasegawa, Bennett, & Verdon, 1980; Mandadi et al., 2007). Recently attempts were made to isolate limonoid aglycones and glucosides using supercritical fluid extraction (SFE) (Miyake et al., 2000; Yu, Dandekar, Toledo, Singh, & Patil, 2006; Yu, Dandekar, Toledo, Singh, & Patil, 2006, 2007; Patil et al., 2006). SFE methods are green environmentally friendly processes. However, very often organic solvents used to modify the supercritical CO_2 to improve yield and selectivity are at hazardous levels. Scaling up of SFE methods is also not easy as high pressure conditions used are difficult and not cost effective.

In recent years, hydrotropy phenomenon has been investigated for a number of potential process applications (Gaikar & Sharma, 1986; Sadvilkar, Samant, & Gaikar, 1995). Hydrotropes are highly water-soluble organic salts and hydrotropy is the phenomenon of increasing solubility of water insoluble or sparingly water-soluble organic compounds in aqueous solutions in the presence of hydrotropes. The increase in solubility of an organic substance is a function of the hydrotrope concentration and depends not only on the nature of hydrotrope, but also on the nature of solute (Balasubramanian, Srinivas, Gaikar, & Sharma, 1989). Previous research in hydrotropic solubilization was concentrated on its applications in drug and detergent formulations, enhancing the rates of heterogeneous reactions and extractive separations of close boiling substances. Recently Raman and Gaikar (2002) demonstrated that high solubilization capacity and selectivity in solubilization by hydrotropy could be used for extraction of water insoluble bioactive compound such as piperin from complex bio-matrices.

In order to reduce the use of organic solvents for the extraction of limonin, we have investigated the potential of hydrotropic extraction of limonoid aglycones from sour orange seeds. Two hydrotropes have been used for the isolation of citrus limonoids and the process has been successfully optimized for the extraction of limonin.

2. Materials and methods

2.1. Raw material and chemicals

Sour orange (*Citrus aurantium* L.) seeds were obtained from Texas A&M University-Kingsville-Citrus Center, Weslaco, TX. Kernels containing fatty material were manually removed, seed shells were collected and ground. All solvents used were of ACS/HPLC grade and obtained from Fisher Scientific (Atlanta, GA). Sodium salicylate (Na-Sal) was obtained from Aldrich (Milwaukee, WI). Aqueous solution of sodium cumene sulphonate (Na-CuS) (2 M) was obtained from Stepan Company (Romeoville, IL). Limonin was obtained from Aldrich Chemical Co. (Milwaukee, WI).

2.2. Design of experiment (DOE)

Box-Behnken design was used to achieve maximum information about the process from a minimum number of possible experiments. In current process, three variables namely concentration of hydrotrope, temperature of extraction, and solid loading were selected for each set of experiments while keeping the time of extraction (6 h) constant through all the experiments. The Box-Behnken design is an independent quadratic design in which the treatment combinations are at the midpoint of the edge of the process space and the center. This design has limited capability for orthogonal blocking compared to central composite design (CCD) but for three factors, Box-Behnken design requires fewer numbers of runs than CCD.

2.3. Method of extraction

Two hydrotropes of Na-Sal and Na-CuS were used for the extraction of limonoids. The extraction of limonoids from sour orange seed shell powder was conducted in a fully baffled cylindrical vessel of internal diameter 8 cm and height 8 cm, equipped with a four-blade turbine impeller of 3 cm diameter. The variables studied are described in design of experiment above. During the extraction, a known quantity of raw material was suspended in 200 ml of hydrotrope solution of known concentration, maintained at a predetermined temperature (Table 1). Extraction was conducted for 6 h and the extract was separated from the solid residue by filtration.

The 10 ml extract was diluted with 180 ml of deionised water to take hydrotrope concentration below minimum hydrotrope concentration (MHC). The MHC of Na-Sal is 0.65 M and that of Na-CuS is 0.1 M (Balasubramanian et al., 1989). The dilution was completed with or without pH adjustment to get limonoid aglycones as precipitate. The precipitated aglycones were recovered by centrifugation. The residue was washed with water and extracted with dichloromethane (DCM) and quantitatively analyzed for the limonin content.