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Efficient extraction of vicine from faba beans using reactive system of high-pressure CO_2/water

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ABSTRACT

Vicine from faba bean is a causative agent of favism, a genetic disease, which manifests as hemolytic anemia. Despite that low vicine varieties of faba bean exist, they are not widely cultivated. Several extraction methods of vicine from faba beans have been presented in the literature, however, their low efficiency associated to time-consuming and costly process limit the practical use. This work for the first time addresses the employment of high-pressure CO_2-assisted extraction of the pyrimidine glycosides from the faba bean. For this purpose, the effect of temperature, CO_2 pressure and time on vicine extraction was scrutinized using Box-Behnken design of experiments. Response surface methodology was used to determine the optimal extraction conditions. At 40.7 °C, 8.1 bar of CO_2 pressure and 5.1 min of extraction, 81% of total vicine should be extracted from faba bean.

1. Introduction

Recently, meat-based diet is becoming more frequently criticized not only from the health perspective but also due to its severe impact on environment. Thus, the alternative sources of proteins, other than of animal origin, are relevant to be considered. One of them are plant proteins, *e.g.* those obtained from *Fabaceae* family. A very promising legume but still underutilized is the faba bean. Seeds of faba bean, besides being a good source of proteins and carbohydrates, are very rich in biologically active compounds such as vitamins, minerals, fiber, polyphenols, L-3,4-dihydroxyphenyloalanine and γ-aminobutyric acid [1]. Bizarrely, despite its nutritional profile faba bean has not been extensively used in food industry or for breeding. A reason for this might be elevated content of condensed tannins, protease inhibitors, lectins and pyrimidine glycosides. Pyrimidine glycosides contained in faba bean *i.e.* vicine and convicine are hydrolyzed by a native β-glucosidase, forming the aglycones divicine and isouramil. They are causative agents of a genetic disease called favism. This disease is characterized by a hereditary deficiency of the erythrocyte located glucose-6-phosphate dehydrogenase manifesting in hemolytic anemia [2]. Moreover, these compounds were recognized as factors reducing productivity and viability in broiler chickens and laying hens [3,4]. The varieties with low levels of vicine and convicine have been already developed [5,6] and demonstrated improved energy metabolism values on chicken and adult cockerels and positive effects for a laying hen egg production. However, these vicine and convicine low-level varieties are still not widely cultivated. On the other hand, the aforementioned pyrimidine glycosides are responsible for protection of the plant against pests and have been reported to have anti-fungal activity [7,8]. Therefore, the low vicine and convicine content varieties of the faba bean may be less immune to the environmental factors and require specific treatment during cultivation [5]. Hence, there is a need for developing effective processing methods of removing pyrimidine glycosides from commonly cultivated varieties. In this work, high pressure CO_2-assisted extraction of vicine from the faba bean was tested. Many of the extraction methodologies applying CO_2, use its supercritical properties. However, in this study different approach was proposed. CO_2 in the hydrothermal processes forms unstable carbonic acid, which acts equally as a reaction catalyst and as a solvent. Such acidic conditions promote a hydrolysis of some constituents, *e.g.* polysaccharides [9–11]. Moreover, by decreasing pH of the extraction medium denaturation of membrane proteins occurs. This all together may contribute to a better water extraction of chemical compounds from plant material, however it also changes the macromolecular composition of the processed product. In many other processes, where the processed product is not primary interest, the use of CO_2 is beneficial because eliminates the necessity of mineral acids usage, increases efficiency of water extraction as well as makes the extraction more effective in lower

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