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Bioactive compounds from mustard flours for the control of patulin production in wheat tortillas

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ABSTRACT

Patulin (PAT) is a toxic fungal metabolite produced by *Penicillium*, *Aspergillus* and *Byssochlamys* growing especially in fruit and cereals. PAT exhibits a number of toxic effects in animals and its presence in food is undesirable. In this study the reduction of the mycotoxin PAT produced by a strain of *Penicillium expansum*, on wheat tortillas was studied using volatile bioactive compounds present in the oriental and yellow mustard flour and also using the standard solution of the antifungal compound allyl isothiocyanate (AIT), developing an active packaging with two different systems of release of those bioactive compounds. Also the kinetic of volatilization of the compounds used in the bioactive packaging was evaluated using the technique of the gas chromatography (GC) coupled to the flame ionization detector (FID). The PAT was extracted from the samples using the QUECHERS methodology and was determined using the technique of the liquid chromatography (LC) coupled to the mass spectrometry detector in tandem (MS/MS). The maximum of volatilization of the AIT in the bioactive packaging is produced between 1 and 24 h depending on the volatilization technique and is stable during two months, whereas the reduction of PAT evidenced in the samples treated ranged from 80 to 100%.

1. Introduction

Patulin (Fig. 1) (PAT) is a toxic secondary metabolite produced by a wide range of fungal species of the genera *Penicillium*, *Aspergillius* and *Byssochlamys*. Among the different genera, the most important PAT producer is *Penicillium expansum* (Moake, Padilla-Zakour, & Worobo, 2005). PAT has been found as a contaminant in many moldy fruits, vegetables, cereals and other foods. However, the major sources of contamination are apples and apple products, which are also the most important source of PAT in the human diet (Baert et al., 2007; Murillo-Arbizu, Amézqueta, González-Peñas, & de Cerain, 2009; Reddy et al., 2010).

PAT has been classified in Group 3 by IARC that means not classifiable as to its carcinogenicity to humans, although it has been shown to cause neurotoxic and mutagenic effects in animals (IARC, 2002). In 1995, the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA, 1995) recommended a provisional tolerable daily intake (pTDI) of 0.4 μg PAT/kg body weight/day based on long-term exposure (JECFA, 1995). As a result, the levels of PAT in fruits are subjected to legislative control. The Codex Alimentarius recommends levels of PAT in fruits and fruit juices to be lower than 0.05 mg/kg.

PAT causes gastrointestinal effects as distension, ulceration and hemorrhage in acute and short-term *in vivo* studies. Recent studies have also demonstrated that PAT alters the intestinal barrier function. PAT has electrophilic properties and high reactivity to cellular nucleophiles. At cellular level it can cause enzyme inhibition and chromosomal damage. PAT causes cytotoxic and chromosome-damaging effects mainly by forming covalent adducts with essential cellular thiols (Fliege & Metzler, 2000; Glaser & Stopper, 2012).

Vegetables like broccoli, cauliflower, cabbage, Brussels sprouts, belong to the Brassica genus and are widely consumed. A healthy diet should include Brassica vegetables because these vegetables are rich in health-promoting compounds like ascorbic acid, soluble fiber, selenium, glucosinolates (GLS), etc. Among these compounds,

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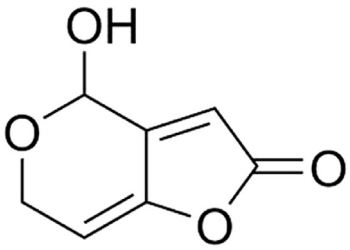

Fig. 1. Patulin chemical structure.

GLS have been extensively studied in the past decades. GLS are secondary metabolites that can be classified as aliphatic, aromatic or indolic depending on their side chain (Fahey, Zalcmann, & Talalay, 2001). GLS are hydrolyzed by a group of endogenous β-glucosidases termed myrosinase (Fig. 2). Myrosinase (MYR) is stored separately from GLS in the plants, but will mix with GLS upon tissue damage (Kissen, Rossiter, & Bones, 2009). Hydrolysis of the thioglucosidic bond by myrosinases releases an aglycone that can either rearrange into an isothiocyanate or be converted to other products such as nitriles, epithionitriles or organic thiocyanates depending on the presence of specific proteins and certain structural prerequisites.

Fungi growth inhibition by isothiocyanates has been reported since the late 1930's (Luciano & Holley, 2009). These compounds are very unique in comparison to other essential oils, since they are only formed when the plant cell suffers some kind of injury such as insect bite, grinding, milling or fungi contamination in the presence of water (Luciano & Holley, 2009). Then, the isothiocyanate precursors, called GLS, are transformed by the enzyme myrosinase. Therefore, isothiocyanates are not present in dry mustard flour, unlesswater is added to it.

ITCs exhibit biocidal activity against microorganisms including fungi (Nielsen & Rios, 2000) and bacteria (Luciano & Holley, 2011), as well as insects (Tsao, Yu, Potter, & Chiba, 2002) and nematodes (Flemming, Turner, & Hunt, 2006). In particular, it has been demonstrated that AIT effectively inhibits the growth of a variety of pathogenic microorganisms at low concentrations (Lin, Preston, & Wei, 2000; Luciano & Holley, 2009). The potential of AIT as a natural antimicrobial in different food matrices, including chicken breast (Shin, Harte, Ryser, & Selke, 2010), ground beef (Nadarajah, Han, & Holley, 2005), dry-cured ham (Graumann & Holley, 2007), fermented dry sausages (Chacon, Muthukumarasamy, & Holley, 2006), and tuna meet (Hasegawa, Matsumoto, Hoshino, & Iwashita, 1999) has been studied.

The aims of this study were to study a) the quantity of the GLS present in yellow and oriental mustard flours b) the kinetic of volatilization of the antimicrobial AIT present in two active packaging and c) the inhibition of the *P. expansum* growth and PAT production in wheat tortillas treated with AIT.

2. Materials and methods

2.1. Materials and chemicals

PAT, sinalbin and sinigrin (98% purity), formic acid (HCOOH), AIT (94% purity), para-hydroxybenzylisothiocyanate (PHBITC), tetrabutylammonium hydrogen sulfate (TBA), ammonium formate, and sodium chloride (NaCl) were obtained from Sigma–Aldrich (St. Louis, USA). Oriental (*Brassica juncea*) and yellow mustard (*Brassica alba*) flours were provided by G.S. Dumm dry mustard millers (Hamilton, Ontario, Ca). Methanol was purchased from Fisher Scientific (New Hempshire, USA). Deionized water (<18 MΩ cm resistivity) was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Chromatographic solvents and water were degassed for 20 min using a Branson 5200 (Branson Ultrasonic Corp., CT, USA) ultrasonic bath. The strain of *P. expansum* CECT 2278, was obtained from the Spanish Type Culture Collection (CECT, Valencia, Spain). The plastic trays used for the experiments were composed by multilayer polyethylene (13″ × 9.6″ clear, rectangular, with an oxigen trasmission of 6509 $cm^3/mil/m^2/24$ h) and were provided by Saplex (Barcelona, Spain).

2.2. GLS extraction and determination

GLS from oriental and yellow mustard flours were extracted using the method of Prestera et al. (1996) with modifications. Twenty grams of each flour were placed in a 50 mL glass tube and autoclaved at 115 °C during 15 min to inactivate the enzyme

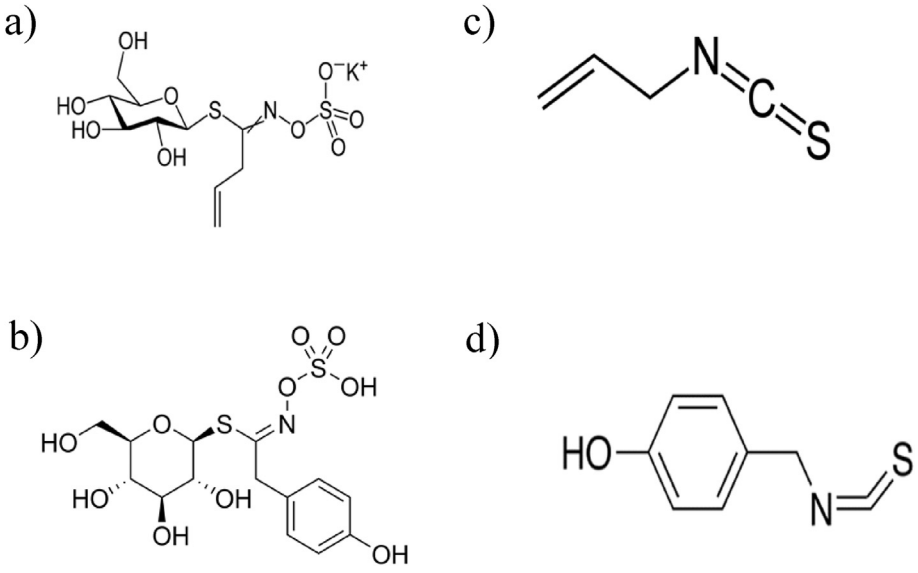

Fig. 2. Chemical structure of the bioactive GLCs a) sinigrin and b) sinalbin c) and of the ITCs c) AIT and d) PHBITC.