Available online at www.sciencedirect.com
SCIENCE DIRECT®

Food Research International 38 (2005) 701–708

FOOD RESEARCH INTERNATIONAL

www.elsevier.com/locate/foodres

Analysis of the isothiocyanates present in cabbage leaves extract and their potential application to control *Alternaria* rot in bell peppers

R. Troncoso [a,b,*], C. Espinoza [a], A. Sánchez-Estrada [a], M.E. Tiznado [a], Hugo S. García [b]

[a] *Centro de Investigación en Alimentación y Desarrollo, A.C. Dirección de Tecnología de Alimentos de Origen Vegetal, Apartado Postal 1735, Carretera a La Victorial km 0.6, Hermosillo, Sonora 83000, México*

[b] *UNIDA, Instituto Tecnológico de Veracruz, M.A. de Quevedo 2779, Veracruz 91897, México*

Received 20 January 2005; accepted 18 February 2005

Abstract

The potential use of cabbage isothiocyanates to control *Alternaria* rot in bell pepper was tested. Solid phase microextraction and gas chromatography–mass spectrometry found allyl, benzyl, 2-phenylethyl and phenyl isothiocyanates in a ratio of 1:3.5:5.3:9.6, respectively, in cabbage leaves. The same proportion was used to prepare an isothiocyanate mixture from reagent grade isothiocyanates (MCIT) to test the effect on *Alternaria alternata* growth in vitro. Application of 0.28 and 0.56 mg/ml of MITC, with or without packing in low density polyethylene bags (LDPE), were also tested on bell pepper fruit inoculated with *A. alternata*, using a commercial fungicide as positive control. A concentration of 0.03 mg/ml of MITC inhibited 100% *Alternaria* growth in vitro. A treatment with 0.56 mg/ml of MCIT with LDPE bags performed better than the commercial fungicide to control fungi rot on bell pepper with no adverse effects on fruit quality. MCIT combined with LDPE bag showed potential to control *Alternaria* rot on bell pepper.

Keywords: Isothiocyanates; Natural fungicide; Fungal disease; Postharvest quality; Bell pepper

1. Introduction

Bell pepper is an important commercial crop in the world. Sensory and nutritional characteristics make it a widely accepted vegetable by consumers. Peppers are grown in most countries of the world, being Asia the largest producer. The area devoted to pepper cultivation is estimated to be 3 million hectares in the world (Bosland & Votava, 2000). Nevertheless, bell pepper is a very perishable vegetable with a short shelf-life and high susceptibility to fungal disease (Hardenburg, Watada, & Wang, 1990). During prolonged storage, bell pepper is commonly infected by *Botrytis cinerea* Pers, ex Fr, and *Alternaria alternata* (Fr.) Keissler (Miller, Spalding, Risse, & Chew, 1984; Snowdon, 1991). Among these pathogens, *A. alternata* causing *Alternaria* rot is a fungus that not only affects bell pepper, but a wide variety of crops worldwide (Snowdon, 1991). Furthermore, this fungus represents a significant proportion of the aerospora and it has been shown to cause respiratory disorders and allergies (Green, Mitakakis, & Tovey, 2003). Its polyphagous nature and production of toxic, carcinogenic and even teratogenic metabolites (Woody & Chu, 1992) makes *Alternaria* a potentially dangerous food spoilage agent. Several methods have been reported to slow down the development of *Alternaria* rot, but most treatments provide only partial control (Fallik, Grinberg, Alkalai, & Lurie, 1996; Miller et al., 1984). The

* Corresponding author. Tel.: +52 6622 89 2400; fax: +52 6622 80 0422/662 280 0055.
E-mail address: rtroncoso@cascabel.ciad.mx (R. Troncoso).

0963-9969/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2005.02.004

main method to control *Alternaria* rot and other postharvest diseases, is based on synthetic chemical products. However, nowadays consumers demand less use of synthetic chemicals and still expect food to be free from microbial growth, toxins and other quality deteriorating factors.

One alternative could be the use of natural compounds with antimicrobial properties. Plants of the order *Capparales*, especially the agriculturally important *Brassica* spp. of the *Brassicaceae*, contain allelochemicals with antibiotic properties against certain soilborne pathogens (Rosa & Rodrigues, 1999). The inhibition of fungal growth displayed by the allelochemicals compounds produced by *Brassica* spp. is linked to the biologically active degradation products of glucosinolates, the isothiocyanates (ITCs), whose antifungal properties have been reported since 1937 (Walker, Morell, & Foster, 1937).

A wide spectrum of ITCs is possible given the variety of R-side groups on the parent glucosinolate. This R-side group can be an aliphatic, aromatic or heteroaromatic residues (Fahey, Zalcmann, & Talalay, 2001).

It had been found that isothiocyanates can be toxic to fungi (Charron & Sams, 1999; Harvey, Hannahan, & Sams, 2002; Smolinska, Morra, Knudsen, & James, 2003), bacteria (Delaquis & Mazza, 1995), nematodes and insects (Kermanshai et al., 2001).

The fungicidal effect of the ITCs against a given fungus varies depending of the specific ITC. Carter, Garraway, Spencer, and Wain (1963) reported that aromatic ITCs were more fungitoxic than those with aliphatic R groups. In contrast, Stahmann, Link, and Walker (1943), found no difference between the antifungal effect of the aromatic phenyl isothiocyanate (PITC) or the aliphatic allyl isothiocyanate (AITC). Studies have shown that ITC derived from the thioaliphatic glucosinolate glucoiberin (3-methylsulfinylpropyl), and the aromatic benzyl isothiocyanate (BITC) showed an elevated biocidal activity against *Diaporthe phaseolorum*, *Phytium irregulare*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani*, compared with ITCs derived from the aliphatic allyl, 3-butenyl and 2-hydroxy-3-butenyl glucosinolates and the aromatic p-hydroxybenzyl, and 2-hydroxy-2-phenylethyl glucosinolates (Manici, Lazzeri, & Palmieri, 1997). In those studies, the ITCs were evaluated in vitro one at a time, however, in most cases *Brassica* species contain more than one glucosinolate. No information is available in the literature about the antifungal effect of a combination of two or more ITC against *Alternaria* growth in vitro and in vivo conditions and whether they have or not an effect on postharvest quality of fruits and vegetables.

It has been proposed that the ITCs consumption can reduce the risk of some types of cancer (Stoner, Kresty, Carlton, Siglin, & Morse, 1999) and even some of them have been used as a therapeutic agents against bacterial infections of the respiratory and urinary tracts (Mennicke, Görler, Krumbiegel, Lorenz, & Hmann, 1988).

From the above, isothiocyanates are volatile compounds that bear a wide biocidal spectrum (Rosa & Rodrigues, 1999). Additionally, ITC do not pose a known human health hazard associated with their consumption. Hence, these compounds are good candidates to be used as an alternative treatment instead of the chemical products for controlling fungi rot during posharvest handling of fruits.

This paper reports analytical data of the isothiocyanates present in cabbage (*Brassica oleracea* var. *Capitata*) tissue and their antifungal activity on *A. alternata* growing in vitro and in inoculated bell pepper. Furthermore, it is also reported the effect of the treatment on the postharvest quality of bell pepper.

2. Materials and methods

2.1. Plant material

Freshly harvested leaves of cabbage were obtained from a commercial orchard located in Sonora, México. Youngest and full expanded leaves were collected from plants and stored in plastic bags at −10 °C until processed.

2.2. Isothiocyanates analysis in cabbage leaves

Glucosinolates were extracted from 100 g of leaf tissue that was a composite of 20 plants, as described previously by Mayton, Oliver, Vaughn, and Loria (1996) with some modifications. The plant tissue was microwaved for 3 min at full power (800 Watts) to destroy any endogenous thioglucosidase (EC 3.2.3.1) activity. The tissue was chopped with a food processor for about 1 min and then blended with 30% aqueous methanol for approximately one min. The slurry was filtered through organza fabric and the filtrate was rotary evaporated for 30 min at 40 °C to eliminate methanol. Barium and lead acetate buffer (0.5 M each; pH 6.5), were added to the remaining aqueous residue before centrifuging (BECKMAN J221) at room temperature for 15 min at 5110*g*. The supernatant was removed, frozen and freeze dried.

Isothiocyanates were obtained by enzymatic hydrolysis from glucosinolates according to the following protocol: freeze-dried samples (20 mg) were placed into 2 ml headspace vials capped with PTFE/silicone septa containing 0.5 ml of potassium-phosphate buffer (0.05 M; pH 6.5) and 3.5 U of thioglucosidase (ICN Biomedical Co., Ohio, USA) were added. The mixture was incubated at 37 °C for 3 h, to allow the enzymatic release of the isothiocyanates. Sampling of the headspace was done by