

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

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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.

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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

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main method to control *Alternaria* rot and other post-harvest 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 post-harvest 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 5110g. 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

a solid phase microextraction (SPME) device (Supelco Inc., Bellefonte, Pa), with a 100-m polydimethylsiloxane coating. The needle was inserted through the septum and the fiber was exposed to the vial headspace. After 10 min, the SPME device was removed and inserted into the injector of a gas chromatograph (VARIAN Star 3400 CX, Palo Alto, CA) connected to a mass spectrometry detector (SATURN). A fused silica DB-5 capillary column (30.0 m $\times$ 0.25 mm ID, 0.1 μ m film thickness; J&W Scientific, Folsom, Ca) was used. Analytical conditions were: Helium was the carrier gas; injector temperature, 180 °C; detector temperature, 200 °C; oven temperature program: 40 °C for 2 min, then increased at 25 °C/min to 96 °C, followed by 8 °C/min to 170 °C, and 25 °C/min to 250 °C. Compounds were identified comparing their mass spectra to those of authentic standards and previously published spectra (Kjaer, Ohashi, Wilson, & Djerassi, 1963). Quantification of ITCs was performed using methyl isothiocyanate (Sigma Chemical Co., St. Louis) as an internal standard.

2.3. *A. alternata* responses to ITCs in vitro

An strain of *A. alternata* was isolated from bell peppers harvested in the fall of 2002. The isolate was cultured on potato dextrose agar (PDA; Difco Laboratories, Detroit); the colony and sporulation characteristics were confirmed according to Simmons (1995).

Response of *A. alternata* to ITCs was tested using commercial reagents. Based in the isothiocyanates detected in cabbage leaves extract, allyl (AITC), phenyl (PITC), 2-phenylethyl (PEITC) (Sigma Chemical Co., St. Louis) and benzyl isothiocyanate (BITC, Alfa Aesar, Johnson Matthey Co.) were used in this experiment. Each compound and a mixture of commercial reagent grade ITCs (MCIT) were tested on mycelial growth of *A. alternata*. The MCIT was prepared according to the relative amounts of ITCs found in cabbage leaves.

Plugs (5 mm in diameter) were cut from margins of an actively growing *A. alternata* culture and placed in the center of a 9 cm Petri dish containing fresh PDA. Small and sterile circles of filter paper (Whatman # 1, with 2 cm in diameter) containing either AITC, BITC, PITC, PEITC or the MCIT, were soaked in the following ITC concentrations: 0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.08 and 0.1 mg/ml and was placed on the cover of the Petri dish. Inoculated dishes were sealed with parafilm and incubated at 26 °C. The diameter of mycelial fungi growth was recorded every 24 h. Control Petri dishes with Whatman paper circles soaked in sterile distilled water were included. When mycelial growth was asymmetrical, four diameters were determined and averaged. Inhibition of mycelial growth (IG) was calculated in percentage based on the difference between the mycelial growth of treated and control dishes.

Analysis of variance based on a completely randomized design and Duncan's multiple range test ($P < 0.05$) were performed. Three replicates were made for every ITC concentration. All statistical analyses were carried out using the SAS software package V. 8.2 (SAS, 2000).

2.4. Effects of the ITCs on bell pepper fruit inoculated with *A. alternata*

Freshly harvested green bell peppers (*Capsicum annuum* L.) were obtained from different local growers in Hermosillo, México. Bell peppers were sorted out to get pods with uniform size, firm-unripe color and free from bruising and rotting. Fruits were washed with chlorinated water (250 μ l/l), air dried at room temperature and divided into six groups of 28 fruits each. Inoculation was done by dipping the fruit (approximately 7 cm from the apical section) in a conidial suspension of *A. alternata* (1.8×10^5 conidia/ml) for 3 min (IN treatment). Preliminary experiments showed that a complete fruit immersion led to conidial suspension getting into the fruit internal cavity causing its complete decay in only a few days. Air dried pepper from four inoculated treatments were packaged in low density polyethylene bags (LDPE; 69 $\times$ 50 cm and 100 μ m thickness) 2 h after the inoculation step. ITCs exposure was performed using a mixture of commercial reagent grade ITCs (MCIT). A sterile filter paper (Whatman # 1) was soaked in the MCIT solution and placed inside the LDPE bag containing the peppers. MCIT was allowed to diffuse into the atmosphere surrounding the pods. The final concentrations of the MCIT were 0.28 mg/ml (MCIT-1 treatment) and 0.56 mg/ml (MCIT-2 treatment) of headspace. These concentrations of ITCs were chosen based on preliminary trials in which 100% of inhibition was attained in vitro. Two control groups were included: packaged-inoculated (LDPE) and unpackaged-untreated peppers (Control). A treatment in which a commercial fungicide (CF, Captan®, 300 μ g/l) was applied to a group of inoculated pepper was also included. All treatments were maintained for 18 h at room temperature (ranging between 24 and 27 °C). Bell peppers were removed from the LDPE bags after this time. Fruits from all the treatments were stored for 10 days at 20 °C with 90–92% relative humidity, to induce *A. alternata* disease development. The severity of infections was evaluated every day on 10 peppers from each treatment by measuring the wound size with a digital Vernier. Results are reported as damaged area in square centimeters (cm²). The whole experiment was replicated twice.

2.5. Postharvest quality of bell pepper

The effects of the different treatments on the postharvest quality of bell peppers during storage time at 20 °C (simulated shelf-life period), was determined by evaluating

general appearance, percentage of weight loss, firmness, titratable acidity, pH and total soluble solids every two days. General appearance (GA) was evaluated subjectively considering the fruit freshness, wilting and shriveling, according to the following scale: 1 = extremely poor, 2 = poor, 3 = fair, 4 = good and 5 = excellent. Peppers were weighed in a digital balance (Mettler Pe Mod. 2000), before and after the storage intervals to calculate percentage of weight loss. Firmness was measured in the pulp of four peppers using a Chatillon penetrometer (Mod DFG-50), equipped with a 10 mm plunger (John Chatillon and Sons, NY). Firmness was expressed as the force in Newtons (N) needed to penetrate the mesocarp tissue. Titratable acidity (TA%), pH and total soluble solids (TSS%), were measured according to the AOAC (1990) methods. The TA is reported as percentage of citric acid. The whole experiment was replicated twice.

Analysis of variance (ANOVA) based in a completely randomized design and Duncan's multiple range test ($P < 0.05$) were performed. General appearance data were analyzed after arctan transformation. All statistical analyses were done using the SAS software package V. 8.2.

3. Results and discussion

3.1. Isothiocyanates analysis in cabbage leaves

Various absorption and desorption times (1, 2, 3, 5 and 10 min and 1, 2 and 3 min, respectively) were evaluated using standard mixture of ITCs. Chromatographic resolution was improved by increasing the absorption and desorption time. The compounds were not observed with 1, 2 and 3 min of absorption time. Furthermore, although the uptake of most of the compounds in the standard mixture was attained at 5 min of absorption time, the best chromatographic resolution was observed at 10 min of absorption time. On the other hand, at 1 min of desorption time, the compounds were not observed, while the release of most of the compounds was attained at 2 min of desorption time. No differences were found by increasing the time up to 3 min. Therefore, the best chromatographic resolution was obtained at 10 min of absorption time and 2 min of desorption time.

Enzymatic hydrolysis of glucosinolates from *Brassica* tissue can result in the evolution of many different compounds (Fahey et al., 2001). Four isothiocyanates were detected in the macerated leaves of cabbage in this work. One aliphatic and three aromatic isothiocyanates: allyl isothiocyanate (AITC), phenyl isothiocyanate (PITC), benzyl isothiocyanate (BITC) and 2-phenylethyl isothiocyanate (PEITC), at 3.12, 4.14, 5.61 and 14.16 min of retention times, respectively (Fig. 1). The retention time and the mass spectra obtained from these compounds, agree well with those obtained using commercial standards as well as those reported in the literature (Kjaer et al., 1963).

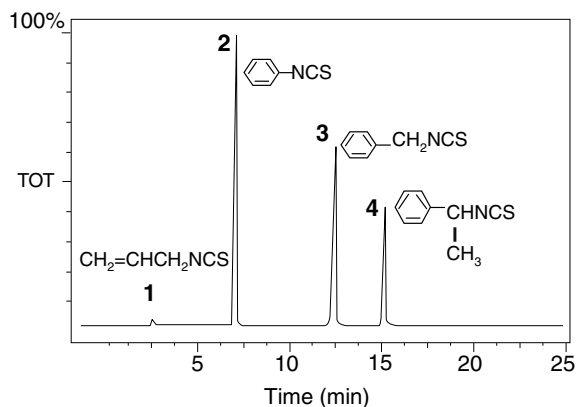


Fig. 1. Chromatogram of the isothiocyanates (ITCs) detected in cabbage tissue exposed at 37 °C for 3 h, by GC-MS and headspace solid phase microextraction: (1) allyl isothiocyanate; (2) phenyl isothiocyanate; (3) benzyl isothiocyanate, and (4) 2-phenylethyl isothiocyanate.

When the hydrolysis was carried out under optimum conditions for ITCs formation, 3 h at 37 °C, as reported by Manici et al. (1997), the amount of total ITCs detected in cabbage tissue was 117 mg/g dry weight (d.w.). The concentration of AITC, PITC, BITC and PEITC was 6, 58, 21 and 32 mg/g d.w., respectively.

Isothiocyanates were previously detected by GC in cabbage tissue by Buttery, Guadagni, Ling, Seifert, and Lipton (1976). The authors found allyl, 3-butenyl, 3-methylthiopropyl, 4-methylthiobutyl and 2-phenylethyl isothiocyanates in the volatile oil produced from boiled cabbage tissue by GC-MS analysis. VanEtten, Daxenbichler, Williams, and Kwolek (1976) detected 10 different ITCs in 22 varieties of cabbage, including AITC, PITC, BITC and PEITC. Recently, Charron and Sams (1999) studied ITCs from different *Brassica* species with solid phase microextraction followed by GC-MS and recorded the presence of allyl, 3-indolylmethyl, 2-hydroxy-3-butenyl and 3-indolylmethyl isothiocyanates. The profile and amounts of ITCs reported in those studies were different than the profile and amounts of ITCs found in this study. These differences observed on the type and quantity of ITC detected in those studies compared to our results, could be attributed to genetic variability that had been reported to occur among different varieties of cabbage (Kushad, Cloyd, & Babadoost, 2004; VanEtten et al., 1976).

3.2. *Alternaria* response to ITCs in vitro

Fig. 2 shows the effect of different concentrations of MCIT, AITC, BITC, PITC and PEITC on the *A. alternata* mycelial growth in vitro. It is fairly clear in the figure that *A. alternata* was more sensitive to MCIT than AITC, BITC, PITC and PEITC. It can be observed that the effective concentration of MCIT that inhibited 100% of the mycelial growth of *A. alternata* was 0.03 mg/ml, while 0.04, 0.05 and 0.08 mg/ml of AITC, PITC and

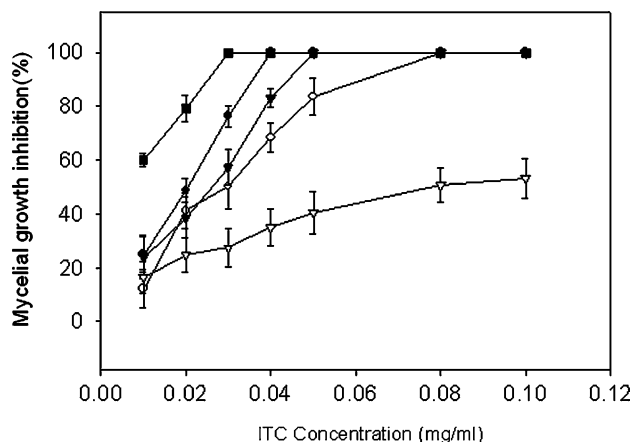


Fig. 2. Effect of different concentrations of isothiocyanates on mycelial growth of *Alternaria alternata* after 96 h of incubation at 26 °C. (■ MCIT, ● AITC, ○ BITC, ▼ PITC, ▽ PEITC). Data are reported as percentage of inhibition mycelial growth (IG%). Each value is the mean of three replications $\pm$ SE.

BITC, respectively, were needed to attain the same 100% inhibition. Furthermore, higher MCIT concentrations (0.04 to 0.1 mg/ml) had the same effect as 0.03 mg/ml on fungal growth; therefore, total fungal inhibition is reached at 0.03 mg/ml. Low antifungal activity was observed in the PEITC treatment. Even at 0.1 mg/ml which was the highest concentration of PEITC tested, only ca. 50% of inhibition was recorded. No greater concentrations of PEITC were tested for this particular ITC in the in vitro assay.

Several bioassays have demonstrated the antifungal activity of ITC applied one at a time. As an example, AITC is suppressive to many species of fungi (Charron & Sams, 1999; Harvey et al., 2002; Mayton et al., 1996). Benzyl ITC and 3-methylsulfinylpropyl ITC suppressed 100% of *A. alternata* growth at concentrations of 0.55 and 1.2 mg/ml, respectively (Manici et al., 1997). In this work, we did not test the effect of methylsulfinylpropyl ITC, so these results can not be compared. However, for BITC, we used 0.08 mg/ml to have 100% inhibition, whereas it was reported that a concentration of 0.55 mg/ml was enough to produce 100% inhibition. This concentration capable of producing 100% inhibition of fungi growth was 6.8 times greater than the one found in this study. It is possible that these differences observed could be related to differences in the method of antimicrobial assays that was used. In that report, BITC was applied using a traditional antimicrobial assay, such as the agar diffusion test, whereas in the present study, the ITC was allowed to evaporate into the headspace of the Petri dish. It is known that for small molecules, the antifungal activity decrease when applied in the agar directly instead of using the volatile method (Makarova, Chaikovskaia, Semenov, Vakulenko, & Levkovich, 1978; Suhr & Nielsen, 2003).

It is clear from the visual examination of Fig. 2 that the ITC mixture exhibited a synergistic effect, being more toxic than AITC alone, which has been reported to be highly toxic for fungi. To the best of our knowledge, this is the first experiment in which the synergistic effect of a group of ITCs on the inhibition of fungi growth is tested.

3.3. Effects of the ITCs on bell pepper fruit inoculated with *A. alternata*

The effect of commercial reagent grade mixtures of ITCs and a commercial fungicide on disease development caused by *A. alternata* in bell pepper during storage at 20 °C, is shown on Fig. 3. During the first three days of storage, a slight fungal growth was observed in the pods, particularly in LDPE and IN treatments. A complete lack of damage was observed for MCIT-2 after 10 days of storage, which was more effective than MCIT-1 and CF in controlling *Alternaria* rot. No significant differences ($P < 0.05$) were observed between MCIT-1, CF, LDPE and control treatments after 10 days at 20 °C.

The treatment that reduced fungal development to the greatest extent was MCIT-2 in conjunction with LDPE bag, although LDPE alone was not effective; therefore the effect of the MCIT was determinant in reducing fungi rot. This last statement agrees with Brackett (1990), Miller et al. (1984) and González-Aguilar et al. (1999) who found that the use of film packaging alone was not effective on controlling fungi decay in peppers. Furthermore, experimental evidence suggests that bulk or individual film packaging enhance decay development because the increase in relative humidity inside

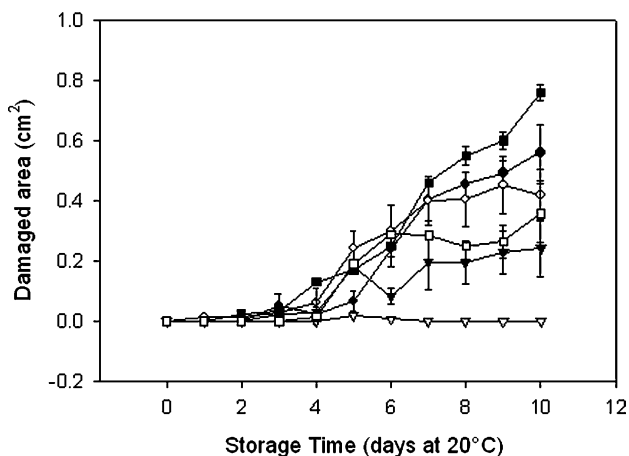


Fig. 3. Effect of different concentrations of an isothiocyanate mixture on damaged area (cm²) caused by *Alternaria alternata* in bell pepper fruit, during storage time at 20 °C for 10 days (● Control; ■ IN, bell pepper inoculated with *A. alternata*; ○ LDPE, pods inoculated and packaged in low density of polyethylene bags; ▼ MCIT-1, pods inoculated, packaged and exposed to 0.28 mg/ml of mixture of commercial ITCs; ▽ MCIT-2, pods inoculated, packaged and exposed to 0.56 mg/ml of mixture of commercial ITCs; □ CF, commercial fungicide). Each value is the mean of 10 replicates $\pm$ SE.

the package induces secondary infection (Brackett, 1990). Therefore, MCIT was able to control *A. alternata* growth in the fruit even with the enhancement effect on fungi growth of the LDPE bag.

The fungicidal effect observed with ITCs applied in vitro was greater than that on bell peppers inoculated with fungi. MCIT concentration of 0.03 mg/ml applied in the in vitro test produced complete inhibition of mycelial growth of *A. alternata*. In contrast, in the experiment with bell pepper, a concentration of 0.56 mg/ml of MCIT was needed to completely inhibit the disease caused by *A. alternata* in the pods. This is a concentration 18 times higher. The differences in fungicidal activity of ITCs observed in vitro and on the pepper experiments are consistent with other reports, in which a better effect in the in vitro test (Mari, Iori, Leoni, & Marchi, 1996) has been found. This behavior could be explained by several theories: (a) the more complex composition of the pepper fruit as compared with the PDA media used during the in vitro test, (b) the stability throughout time and volatility of the compounds studied, (c) the diffusive effect of the active compound inside the fruit, and (d) the possible leak of the volatile compounds through the low density polyethylene film used in the experiments.

There is no evidence in the literature regarding the effect of an ITC mixture in reducing fungal decay in horticultural products. Most of the studies regarding the antifungal activity of ITC deal with in vitro test of different ITCs individually; while in vivo studies, are limited (Mari et al., 1996). It has been found that the toxicity of

a particular ITC depends on both the species of fungi and the chemical nature of the ITC side chain (Manici et al., 1997). A better fungicidal effect was observed in this study with the MCIT treatment composed by allyl, benzyl, phenyl and phenylethyl isothiocyanate, compared to studies reported in literature using individual ITCs. Although the ITCs used in this part of the study were reagent grade chemicals, these results suggest that the type and relative amounts of ITCs produced by cabbage could be a good alternative to control *Alternaria* rot in bell pepper.

3.4. Postharvest quality of bell pepper

The effect of ITC treatments on general appearance, fresh weight loss and firmness of bell pepper during storage at 20 °C, is shown in Table 1. In the case of general appearance, after 10 days of storage, MCIT-2 treatment gave the best appearance and quality; while the appearance of the IN treatment was the worst. No significant differences were found among LDPE, MCIT-1 and CF treatments. Fresh weight loss and firmness were consistently reduced during storage at each evaluation in both pods, treated and untreated. There were not statistical differences between treatments ($P < 0.05$) after 10 days of storage at 20 °C.

Results of TA%, pH and TSS are shown in Table 2. No significant effects ($P > 0.05$) were observed due to the different treatments in TA, pH and TSS during the 10 days of storage.

Table 1

Effects of ITCs on general appearance (GA), fresh weight loss (%) and firmness (N) of bell peppers during storage days at 20 °C

Treatments	GA ^{CB}		Fresh weight loss (%) ^B		Firmness (N) ^A	
	0 ^D	10 ^D	10 ^D		0 ^D	10 ^D
Control	5.0	2.2 ± 0.47 ^c	9.05 ± 0.43		14.60 ± 2.1	16.45 ± 2.1
LDPE	5.0	3.1 ± 0.62 ^b	8.48 ± 0.54		16.52 ± 1.4	13.20 ± 1.8
MCIT-1	5.0	3.8 ± 0.58 ^b	8.60 ± 0.62		15.76 ± 1.9	10.47 ± 2.2
MCIT-2	5.0	4.3 ± 0.65 ^a	7.87 ± 0.86		17.55 ± 2.0	15.87 ± 2.4
IN	5.0	1.5 ± 0.80 ^c	9.21 ± 0.56		13.90 ± 2.3	10.62 ± 1.3
CF	5.0	3.4 ± 0.87 ^b	8.60 ± 0.68		14.02 ± 1.2	12.07 ± 2.3

^C General appearance evaluated as: 1 = extremely poor; 2 = poor; 3 = fair; 4 = good; 5 = excellent. Each value is the mean ± SE of ten^B and four^A determinations; means within a columns with different superscripts are significantly different ($P < 0.05$).

^D Days of Storage at 20 °C.

Table 2

Effects of ITCs on titratable acidity (TA%), pH and total soluble solids (TSS%) in bell pepper during storage days at 20 °C

Treatments	TA (%) ^A		pH ^A		TSS (%) ^A	
	0 ^B	10 ^B	0 ^B	10 ^B	0 ^B	10 ^B
Control	0.084 ± 0.003	0.09 ± 0.07	5.7 ± 0.18	5.4 ± 0.61	3.3 ± 0.30	3.5 ± 0.21
LDPE	0.061 ± 0.004	0.12 ± 0.13	5.9 ± 0.14	5.4 ± 0.12	3.6 ± 0.12	3.7 ± 0.33
MCIT-1	0.062 ± 0.002	0.10 ± 0.07	6.2 ± 0.28	5.5 ± 0.44	3.4 ± 0.31	3.5 ± 0.26
MCIT-2	0.081 ± 0.006	0.12 ± 0.16	5.8 ± 0.18	5.4 ± 0.61	3.3 ± 0.20	3.5 ± 0.41
IN	0.067 ± 0.010	0.13 ± 0.03	5.7 ± 0.50	5.3 ± 0.50	3.5 ± 0.18	3.6 ± 0.15
CF	0.095 ± 0.010	0.11 ± 0.09	5.7 ± 0.51	5.3 ± 0.21	3.4 ± 0.15	3.6 ± 0.20

^A Each value is the mean ± SE of four determinations; means within a columns with different superscripts are significantly different ($P < 0.05$).

^B Days of Storage at 20 °C.

The results in fresh weight loss, firmness, TA, pH and TSS, are consistent with other reports (Fallik et al., 1996; González-Aguilar, Cruz, & Baez, 1999; Miller et al., 1984), in which the postharvest quality of bell pepper of different storage conditions was evaluated. There are no previous studies on the effects of exposure of isothiocyanates on postharvest quality of bell pepper; nevertheless, our results suggest that the postharvest quality of the fruit was not affected by the mixture of ITCs applied and further storage at 20 °C.

4. Conclusion

Four isothiocyanates in cabbage tissue were identified and quantified by solid phase microextraction and GC-MS: phenyl, benzyl, 2-phenylethyl and allyl isothiocyanate.

An exposure of 18 h with a mixture of isothiocyanates, formulated according to the type and relative concentrations of ITCs produced by cabbage leaves, at 0.56 mg/ml, in combination with low density polyethylene bags showed a complete inhibition of *Alternaria* rot in bell pepper after 10 days of storage at 20 °C. This treatment was better than the commercial fungicide to control fungi rot on bell pepper without any effect on fruit quality.

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