Composition and antimicrobial activity of two Capsicum extracts

Combinación de dos extractos de Capsicum: composición y actividad antimicrobiana

Combinação de dois extratos de Capsicum: Composição e atividade antimicrobiana

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Resumen

Las bacterias patógenas son responsables de la mayoría de las epidemias alimentarias. Las bacterias se han vuelto cada vez más resistentes a los antibióticos a través de los años y ahora, se deben de considerar agentes nuevos y naturales para controlarlas. Los extractos de chile fueron obtenidos, secando, cortando y separando las diferentes partes de la fruta y colocándolos en sistema Soxhlet para su extracción con etanol. Se llevó a cabo la evaluación de su composición, la capacidad antioxidante y la evaluación de las concentraciones inhibitorias de la mezcla de los extractos de chile Serrano y Habanero contra *Escherichia coli* y *Listeria monocytogenes*. La ruta del chile habanero completa demostró tener el mayor contenido de capsaicinoides. La fruta completa de chile Serrano tuvo el mayor contenido de compuestos fenólicos y la mayor cantidad de flavonoides por sobre los demás extractos. Las semillas de Habanero demostraron tener la mayor capacidad antioxidante. Se observó que la fruta completa del chile Habanero y las semillas del mismo tienen el menor efecto

inhibitorio contra *E. coli* y *L. monocytoegenes* respectivamente. Se observó también efectos sinérgicos y aditivos cuando se combinaban los extractos en contra de ambas bacterias, lo que significó que se presente un mayor efecto antimicrobiano cuando se combinan los extractos que cuando se aplican individualmente. El efecto antimicrobiano depende de la especie de chile.

Palabras clave: *Capsicumm annuum, Capsicum chinense* concentración mínima inhibitoria, índice de concentración fraccional inhibitoria, fitoquímicos.

Abstract

Pathogenic bacteria, are responsible for most of the foodborne outbreaks. Bacteria became more resistant to antiobiotics throughout years and, nowadays, new and natural agents most be considered for controlling them. Pepper extracts were obtained by drying, cutting, and separating the different parts of the fruit and placing them into a Soxhlet system for further extraction with methanol. Evaluation of the composition, antioxidant activity, and inhibitory concentrations of a blend of Serrano and Habanero pepper extracts against *Escherichia coli* and *Listeria monocytogenes* was studied. Habanero whole fruit had the highest capsaicinoid content. Serrano whole fruit had the highest phenolic content and the most flavonoids of all extracts. Habanero seed had the highest antioxidant activity. It was observed that the Habanero whole fruit and its seeds had the lowest minimum inhibitory concentrations of *E. coli* and *L. monocytogenes*, respectively. It was also observed that there were synergistic and additive effects when extracts were combined against both bacteria; meaning that there is an increased antimicrobial effect when combined than when each extract was applied individually. The antimicrobial effect depends on the pepper species.

Key words: Capsicum annuum, Capsicum chinense, minimal inhibitory concentration, fractional inhibitory concentration index, phytochemicals.

Resumo

As bactérias patogénicas são responsáveis pela maioria das epidemias de alimentos. As bactérias têm se tornado cada vez mais resistentes aos antibióticos ao longo dos anos e agora devem ser considerados agentes novos e naturais para controlá-los. Os extractos foram obtidos de pimentão, secagem, corte e separação das diferentes partes do fruto e colocando no sistema de extracção de Soxhlet de etanol. avaliação da sua composição, foi realizada a capacidade antioxidante e de avaliação de concentrações inibitórias da mistura de extractos de pimentão e Serrano Habanero contra Escherichia coli e Listeria monocytogenes. O habanero rota completa provou ter o maior teor de capsaicinoids. O fruto cheio de Serrano Chile teve o maior teor de compostos fenólicos e flavonóides como muito acima dos outros extratos. sementes Habanero provou ter a maior capacidade antioxidante. Observou-se que a fruta e sementes completa habanero da mesma têm o efeito inibitório menos contra E. coli e L. monocytoegenes respectivamente. efeitos sinérgicos e aditivos também foram observados quando combinado extratos contra ambas as bactérias, o que significava que apresentam um maior efeito antimicrobiano quando os extratos quando aplicado combinados individualmente. O efeito antimicrobiano depende das espécies de pimentão.

Palavras-chave: Capsicumm annuum, concentração inibitória mínima Capsicum chinense, fracionário índice de concentração inibitória, fitoquímicos.

Practical Applications: the use of natural antimicrobial products against two well-known pathogenic bacteria with the advantage of its antioxidant properties and its pungent flavor.

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Introduction

Pathogenic bacteria, such as *Escherichia coli* and *Listeria monocytogenes* are very important to the food industry. They cause a significant number of gastric diseases worldwide every year (Centers for Disease Control and Prevention, 2013).

Escherichia coli is a Gram-negative, facultative anaerobic, rod-shaped bacterium found commonly in animal intestines (Pillai et al., 2005). Most strains of this specific kind of bacteria are harmless; however, some strains like O157:H7 may cause enterohemorrhagic

illness (Buchanan and Doyle, 1997). It can be found, among others, in beef, beef products, fermented sausages, raw vegetables, apples, and orange juice (Buchanan and Doyle, 1997; Pillai et al., 2005). *Listeria monocytogenes* is a Gram-positive, non-sporulating rod and ubiquious bacterium responsible for human listeriosis (Farber and Peterkin, 1991). Listeriosis is a foodborne disease with a 20 to 30% mortality rate. It is considered rare and only a serious disease when found in people with a weakened immune system, such as pregnant women and the elderly.

Today, consumer safety has acquired more importance due to major pathogenic bacteria foodborne outbreaks. In 2012, over 25 people were infected with *Listeria monocytogenes* from contaminated cheese products in the U.S; in the same year, approximately 33 people were hospitalized for *Escherichia coli* O157:H7 strain infection due to consumption of prepackaged leafy greens. Finally, in 2013, another outbreak of *Escherichia coli* O157:H7 in 19 states was due to contaminated frozen products (CDC, 2013).

The growing use of antibiotics has caused constant bacteria strain mutation and hence antibiotic resistance has been observed more and more throughout the years (Marinova et al., 2005).

Several plant species have been studied due to their effects against foodborne bacteria; therefore, plant extracts mixtures, and their combination with antimicrobials are recommended to inhibit growth of foodborne pathogenic bacteria. Synergic effects are observed when plant methanolic extracts are combined with antiobiotics against several bacterial strains.

Capsicum species possess bioactive compounds that have anti-oxidant, anti-cancerogenic, cardiovascular assistance, antiinflamatory and even as antimicrobial properties (Cichewicz and Thorpe, 1996). Research work about the combination of *Capsicum* phytochemicals by Acero-Ortega *et al* (2003) pointed out the synergy between isolated compounds from *Capsicum annuum* and *Capsicum chinense*.

The main objective of this work is to show the composition, antioxidant activity and antimicrobial effect of all six extracts under evaluation, and to obtain the Fractional Inhibitory Concentration index of nine permutations between extracts obtained from *Capsicum's* seeds, pericarp and, the whole fruit (*Capsicum annum* L. *acuminatum* and

Capsicum chinense) against *Escherichia coli* and *Listeria monocytogenes* by the checkerboard method.

Materials and methods

Two species of *Capsicum* fruits, Habanero pepper (*Capsicum chinense)* and Serrano pepper (*Capsicum annumm* L. *acuminatum*), were purchased from a local market of Puebla, Mexico.

The seeds and flesh (placenta included) were separated and placed in a food dehydrator (Excalibur, 4900, USA) at 65°C for 48 h and with an air flow of 2 m/s until 95% water content was removed; the dried material was stored in plastic bags at room temperature.

Extract preparation

Methanol (Merck, Mexico) was used as solvent to obtain extracts. Whole pepper, seeds and pericarp extracts were prepared with the method used by Dorantes *et al* (2000). The dried matter (3 g) was put in a flask with 70 mL of methanol and refluxed for 3 h. The mix was filtered through a No. 4 Whatman filter paper and subsequently added 15% (w/w) of activated carbon and filtered again. The solvent was evaporated and re-captured by simple distillation. Finally, the extracts were stored at -4°C until used.

Capsaicinoids quantification

Capsaicinoids determination was made using the method proposed by Gibbs y O´Garro (2004) in which capsaicin was quantified by spectrophotometry of yellow colored reaction. One milliliter of distilled water was added to 100 µL of methanolic extract. A 0.5 M solution of HCl (2 mL) was added to the extract solution and immediately added 1 mL of a solution containing 0.5 M of $NaNO_2$ (Omnichem, Mexico) and 0.025 M of $NaMoO_4$ (Omnichem, Mexico). After 15 min, a 0.1 M solution of NaOH (RBM, Mexico) was added and mixed. Absorbance was measured after 30 min at 430 nm with an UV-Visible spectrophotometer (UNICO, model 2800H USA). The curve was made with vanillin solution at the following concentrations 0.2, 0.4, 0.5, 1.0, 2.0, 3.0 and 4.0 mg vanillin $100mL^{-1}$ and used as a derivate of capsaicinoids.

Total Phenolic Compounds Determination

To determine total phenolic compounds, the method proposed by Ornelas-Paz *et al.* (2010) was employed. Half milliliter of 50% of Folin-Ciocalteu reagent (Sigma, Mexico) along with 8.5 mL of deionized water was added to 1 mL of the extract. The solution was incubated at room temperature for 10 min, and then mixed with 1.5 ml of 20% of sodium carbonate (Omnichem, Mexico) solution. The solution was incubated again at room temperature for 60 min. Absorbance readings were made at 750 nm with an UV-Visible Spectrofotometer using Gallic acid as a standard reference. Total phenolic content was expressed as gallic acid equivalents (mg g.a./L extract) and a five point calibration curve (20-100 mg L^{-1}) was made.

Antioxidant Capacity Determination

The method to determine antioxidant capacity used was the one proposed by Re *et al* (1999). ABTS+ (2,2'-azinobis-(3-ethylbenzothiazoline-6 sulfonic acid)) (Sigma Aldrich, St. Louis, E.U.) radical was formed by reacting 7 mM of ABTS+ radical with 2.45 mM of potassium persulfate and let stand at room temperature and in the dark for 12-16 h. Then, the solution was mixed with water and 95% ethanol (1:1), until an absorbance of 0.7 (+0.02) at 734 nm in a UV-Visible spectrophotometer. Extract (20 μL) was added and mixed with 6 mL of ABTS+ solution. The measurements were made at the beginning of the reaction and after 1 min. and the % inhibition (equation 1) was calculated.

$$I(\%) = A_i\text{-}A_f/A_i \qquad (1)$$

Standard curve was made using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (0.0125, 0.025, 0.0375, 0.05, 0.0625, 0.125, 0.15, and 0.175 mg Trolox mL^{-1})(Sigma Aldrich, St. Louis, MO, USA).

Carotenoids determination

To determine carotenoids in each extracts the method used by Aminifard *et al* (2012). A solution of acetone-hexane in a 4:6 ratio (16 mL) was added to a 1.0 g of pepper in a test tube. After homogenization, two phases were obtained and the upper phase was used for measurements at 663, 645, 505 and 453 nm in a UV-Vis spectrophotometer.

Lycopene and β-carotene were calculated using the equation 2 and 3 (Nagata & Yamashita, 1992).

Lycopene (mg/100 mL of extract) = $-0.0458*A_{663}+0.204*A_{645}+0.372*A_{505}-0.0808*A_{453}$ (2)

B-carotene (mg/100 mL of extract) = $0.216*A_{663}-1.22*A_{645}-0.304*A_{505}+0.452*A_{453}$ (3)

Red and Yellow fractions determination

Also, Red and Yellow fractions were determined using the method proposed by Hornero-Méndez & Mínguez-Mosquera (2001). Pepper samples (1 g) were extracted until exhaustion of color with 50 mL of acetone each time; sodium chloride (10%) solution was added to ensure phase separation; the solution was then filtered and transferred to a volumetric flask and made up to 100 mL. Readings were made using a UV-Vis spectrophotometer at 508 and 472 nm. Equations 4 and 5 were used to calculate red (content of capsanthin, capsorubin, β-cryptoxanthin, zeaxanthin and β-carotene) and yellow fractions (lutein and α-carotene) (CR and CY respectively).

$C^R(\mu g/Ml) = (A_{508}X*2144.0-A_{472}X*403.3)/270.9$ (4)

$C^Y(\mu g/Ml) = (A_{472}X*1724.3-A_{508}X*2450.1)/270.9$ (5)

Total flavonoid content

Flavonoid content in pepper was determined using aluminum chloride colorimetric method employed by Yoo *et al* (2008). One milliliter of the extract was mixed with 4 mL of distilled water. Then 0.3 mL of a 5% solution of $NaNO_3$ was added. After 5 minutes, the resultant solution was mixed with 0.6 mL of a 10% solution of $AlCl_3$. Sodium hydroxide (1M) solution (2mL) and 2.1 mL of distilled water were added. Absorbance at 510 was read. Quercetin was used as a standard and results were expressed as mg quercetin equivalents Kg^{-1} of dry weight. Quercetin calibration was made by preparing quercetin (0.2 a 2.0 mg mL^{-1}) in methanol.

Strains and growth conditions

Escherichia coli (ATCC 32218) and *Listeria monocytogenes* (ATCC 19115), were used and mantein at 4°C. They were inoculated in nutritive broth (BD Bioxon, Mexico) for 24 h at 37°C.

In order to standardize the cultures to obtain a 10^7 CFU/mL [1], the bacteria were cultured in trypticase soy broth (BD Bioxon, Mexico) for 18 h at 37°C. Using fresh broth, aliquots were taken until an absorbance of 0.05 at 600 nm was reached.

Determination of Minimum inhibitory concentration (MIC)

The microdilution assay method employed by Eloff (1998) was carried out to determine the minimum inhibitory concentration of the pepper extracts. A 96 well plate was used and 150 mL of previously prepared inoculums (10^7 CFU/mL) were placed in each well and then mixed with a known and variable volume of extract (100, 75, 50 and 25 µL of each extract). Fresh trypticase soy broth was used to complete a volume of 250 mL and thoroughly mixed. The plate was incubated for at 37°C and after 24 h., 50 µL of broth/extract solution were placed in Petri dish. Trypticase soy agar was poured in a dish and incubated at 37 °C and. After overnight incubation colonies quantification was made.

The minimum inhibitory concentration values of each extract were obtained as the minimum concentration of antimicrobial (extract) that inhibited the growth of microorganisms used after proper incubation (Andrews 2001).

Test for synergism

Combination of two Capsicum antimicrobials were tested against the aforementioned strains by the checkerboard method according to Pillai & Mcellering (2005). Concentration for both antimicrobials ranged from 50 to 300 µL/mL; each combination was tested in duplicates. The minimum inhibitory (MIC) for each extract in every combination is the concentration in which the microorganism was inhibited and the fractional inhibitory concentration (FIC) was calculated using equations 6 and 7:

$$FIC_a = MIC_{a/combination}/MIC_{a/alone} \quad (6)$$

$$FIC_b = MIC_{b/combination}/MIC_{b/alone} \quad (7)$$

To obtain the FIC for the whole combination or FIC index, the sum of the FIC values for each extract was calculated as follows (Equation 8):

$$FIC_{index} = FIC_a + FIC_b \quad (8)$$

According to Braga *et al*. (2005), the results can be defined in terms of FIC index values as synergism (≤ 0.5), additive (> 0.5 and ≤ 4.0) and antagonism (> 4.0).

Statistical analysis

The data obtained was analyzed with Minitab v. 15 (Minitab Inc., USA) by ANOVA with a 95% level of significance and with Tukey's comparison of means. A full factorial design of two factor; pepper (with two levels) and pepper part (with three levels) was applied to determine the influence of such factors into de Minimum Inhibitory Concentration (MIC) for each strain. Each experiment was conducted with three replicates and analyzed by an analysis of variance with a significance level of 95% with a general linear model.

Results and discussion

Capsaicinoids, carotenoids, total phenolic compounds and flavonoids determination

The data obtained for capsaicinoids, carotenoids, total phenolic compounds and flavonoids are presented in Table 1.

Table 1. Composition of methanolic extracts from two pepper cultivars; Serrano (*Capsicum annuum* L. *acuminatum*) and Habanero (*Capsicum chinense*) peppers.

			Carotenoids				
	Capsaicin	***Total phenolics***	***Lycopene***	***b-carotene***	***CR***	***CY***	***Flavonoids***
Pepper	mg 100 g^{-1}	mg 100 g^{-1} GAE	mg kg^{-1}				mg kg^{-1} QE
Serrano							
Seed	$1.90\pm0.09^{a,x}$	$46.25\pm10.20^{a,x}$	$43.95\pm 4.11^{a,x}$	$11.72\pm2.13^{a,x}$	$1.14\pm0.05^{a,x}$	$0.18\pm0.01^{a,x}$	$0.81\pm0.01^{a,x}$
Pericarp	$65.22\pm6.40^{b,x}$	$144.18\pm14.30^{b,x}$	$50.07\pm1.23^{b,x}$	$40.53\pm4.81^{b,x}$	$1.12\pm0.03^{a,x}$	$0.32\pm0.02^{b,x}$	$1.23\pm0.07^{b,x}$
Whole fruit	$323.32\pm15.70^{c,x}$	$336.42\pm15.41^{c,x}$	$42.37\pm3.6^{a,x}$	$46.32\pm2.63^{b,x}$	$1.11\pm0.03^{a,x}$	$0.59\pm0.01^{c,x}$	$1.44\pm0.12^{b,x}$
Habanero							
Seed	$2.30+0.07^{a,x}$	$3.18+0.20^{a,y}$	$4.32+0.82_{a,y}$	$2.01+0.13^{a,y}$	$0.12+0.01^{a,y}$	Traces	$0.11+0.05^{a,y}$
Pericarp	$63.70+9.61^{b,x}$	$34.07+.06^{b,y}$	$17.71+1.20^{b,y}$	$105.22+20.34^{b,y}$	$0.75+0.03^{b,y}$	$0.61+0.02^{a,y}$	$1.15+0.04^{a,y}$
Whole fruit	$742.50+20.12^{c,y}$	$232.51+13.71^{c,y}$	$19.55+3.52^{b,y}$	$102.03+11.89^{b,y}$	$0.64+0.03^{b,y}$	$0.70+0.07^{a,y}$	$0.26+0.9^{a,y}$

[a-c] Same letter show no significattive difference among pepper parts.

[x-z] Same letter show no significative difference among pepper species

Fuente: Elaboración propia

Capsaicin content was significantly higher in Habanero pepper (ranging from 2.30 to 742.50 mg $100g^{-1}$ dry weight), than in Serrano pepper (from 1.90 to 323.32 mg $100g^{-1}$ dry weight). This probably because Habanero pepper (*Capsicum chinense*) is considered as one of the hottest pepper in the world. Also, it was observed that whole pepper fruit were significantly higher ($p<0.05$) in capsaicin levels than in both seed and pericarp. This result is related to pepper parts were cleaned out from placental tissue; Broderick & Cooke (2009), stated that vesicles are responsible for the storage of capsaicin, which are present in plancental tissue.

Total phenolic content was higher in pepper seeds (34.07 and 144.18 mg galic acid equivalents 100 g^{-1}) than pepper pericarp (ranged from 3.18 to 46.25 mg galic acid 100 g^{-1}), with a significant difference ($p<0.05$). These results were in agreement with those obtained by Singh *et al*. (2008), who observed that phenolic contents, such as tannic, ferulic and cinnamic acids, were higher in seeds than in pulp of green and red peppers (*Capsicum annum*). Also, it was observed that amounts of phenolic compounds in Serrano pepper (from

46.25 to 336.42 mg of galic acid 100mg^{-1}) were higher than in Habanero pepper (from 3.18 to 232.51 mg of galic acid 100 g^{-1}). Hassimotto *et al.* (2005), found similar results where concentrations of phenolic compounds were significantly different between green and red peppers and the concentrations ranged from 1860 to 11220 mg kg^{-1}). However, Reis *et al*. (2013) found that in a Brazilian cultivar of *Capsicum chinense* concentrations of phenolic compounds were as high as 9000 mg GAE 100g^{-1}, being these in disagreement with the results presented above.

Carotenoid content (particularly b-carotene) were significantly higher ($p<0.05$) in Habanero pepper than in Serrano pepper. The aforementioned difference in β-carotenes (red, orange and yellow pigments) could possibly be due to the ripening stage (the ripening stage was chosen in an aleatory manner) of each cultivar used in this research. In a research by Wall *et al.* (2001), in which they compared the concentration of bioactive compounds (included carotenoids and β-carotene) of several pepper cultivars, found that Habanero pepper had a significantly higher amount of β-carotene than other cultivars, such as Serrano.

Both red and yellow fractions were different for the two cultivars studied. Serrano presented a red fraction (from 1.11 to 1.14 mg Kg^{-1}) higher than yellow (ranged from 0.18 to 0.59 mg Kg^{-1}) probably because it possesses an elevated amount of capsanthin and capsorubin. On the other hand, Habanero pepper contains similar quantities of both red and yellow fraction (from 0.12 to 0.75 mg Kg^{-1} and from traces to 0.70 mg Kg^{-1} for red and yellow fractions respectively), these results could probably be due to its similar content of capsanthin, capsorubin (red fraction) and zeasanthin, β-cryptoxanthin and b-carotene (yellow fraction) (Hornero-Méndez & Mínguez-Mosquera, 2001). The same could also explain the significantly higher quantity ($p<0.05$) of red fractions for Serrano pepper and the raised levels of the yellow fraction for Habanero than for Serrano pepper.

Regarding flavonoids content, Serrano pepper own a significantly ($p<0.05$) higher levels (0.81-1.44 mg Kg^{-1}) than Habanero (0.11-0.26 mg Kg^{-1}). Serrano was in the range of the data obtained by Marinova et al. (2005) were their values were from 4.1 to 27.4 mg 100 g^{-1} fresh weight. Butcher *et al*. (2013) observed and concluded that Habanero cultivars possess low quantities of flavonoids which was also what it was found in the present research comparing it with Serrano pepper. Also, as stablished by several research works, flavonoids

and other bioactive compounds depend on cultivation, ripeness, storage and other factors (Zhang & Hamauzu, 2003; Marinova *et al*., 2005; Navarro *et al*., 2006).

Antioxidant capacity determination

Antioxidant activity as Total Antioxidant Capacity (TAC) for both pepper cultivars is shown in Table 2.

Table 2. Antioxidant activity and percentage of inhibition of extracts obtained from Serrano (*Capsicum annuum* L. *acuminatum*) and Habanero (*Capsicum chinense*) peppers

	% inhibition	**μg Trolox 100 g^{-1} DW**
Serrano		
Seed	$89.06+1.56^{a,x}$	$185.04+9.65^{a,x}$
Pericarp	$82.61+2.15^{b,x}$	$168.47+10.54^{b,x}$
Whole fruit	$81.81+0.98^{b,x}$	$166.43+13.61^{b,x}$
Habanero		
Seed	$91.37+1.78^{a,x}$	$190.99+15.32^{a,x}$
Pericarp	$77.95+2.45^{b,x}$	$156.50+13.74^{b,y}$
Whole fruit	$79.71+2.78^{b,x}$	$161.02+12.61^{b,x}$
$^{a-c}$ Same letter show no significative difference among pepper parts $^{x-y}$ Same letter show no significative difference among pepper species		

Fuente: Elaboración propia

From the data obtained from TAC values, there was no significant difference ($p>0.05$) between the cultivars studied; although there was evidence that showed that both cultivars are significantly different regarding to its composition, combinations of all bioactive components could have similar antioxidant capacity. The latter is in agreement with the research made by Howard *et al. (*2000) were they compared different pepper cultivars and observed similar antioxidant capacity.

However, there is a significant difference in the antioxidant capacity obtained from the seed (185.04 mg Trolox 100 g^{-1} dw. For Serrano seeds and 91.37 mg Trolox 100g^{-1} dw for Habanero seeds) alone than from the other parts. It could be seen in previous work by Singh *et al*. (2008) that seed possesses great amounts of tannic acid (ranged from 50.97 to 105.79 μg g^{-1} fresh weight), ferulic acid (from 0.27 to 0.29 μg g^{-1} fresh weight) and

cinnamic acid (from 0.12 to 055 μg/g fresh weight). It is worth mentioned that these acids were no found in other pepper parts.

Determination of minimum inhibitory concentration

Minimum inhibitory concentration values are display in Table 3.

Table 3. Values of Minimum inhibitory concentration of two pepper cultivars; Serrano (*Capsicum annuum* L. *acuminatum*) and Habanero (*Capsicum chinense*) against *Escherichia coli* and *Listeria monocytogenes.*

	Escherichia coli	***Listeria monocytogenes***
	mg MI^{-1}	
Serrano		
Seed	$0.34+0.03^{a,x}$	$0.15+0.01^{a,x}$
Pericarp	$0.26+0.05^{b,x}$	$0.10+0.01^{b,x}$
Whole fruit	$0.13+0.01^{c,x}$	$0.11+0.01^{b,x}$
Habanero		
Seed	$0.15+0.01^{a,y}$	$0.14+0.01^{a,x}$
Pericarp	$0.13+0.01^{a,y}$	$0.08+0.01^{b,y}$
Whole fruit	$0.08+0.01^{c,y}$	$0.11+0.01^{c,y}$

Fuente: Elaboración propia

L. monocytogenes was significantly more sensitive than *E. coli* ($p<0.05$). This sensitivity was probably due to the large quantities of acids present in pepper extracts decreasing pH growth media, and it was previously established that *L. monocytogenes* growth at a pH between 4.4 and 9.4 (Marzocca *et al*., 2004).

Soumaya & Nair (2012) found higher MIC values against fungi species (ranged from 1.25 to 10 mg mL^{-1}), which are morphologically different than bacterial strains. The results presented in this paper disagree with the ones obtained by Salih (2006) were they showed, in oil extracts, a minimum inhibitory concentration of 2.5 μg mL^{-1} for *E. coli* and 5 μg mL^{-1} for *L. monocytogenes*, both being lower than the concentrations needed in this study.

Test for synergism

Based on the FIC index calculations (Table 4), only the combinations of Serrano seed/Habanero seed and Serrano whole fruit/Habanero pericarp show an additive effect against *Escherichia coli* with a FIC index value greater than 0.5 (0.55 and 1.044 respectively). Synergistic effects of the other seven combinations against the same bacteria are observed with a FIC index less than 0.5. These results can be attributed to the combination of bioactive compounds in both pepper species such as ortho-coumaric acid, capsaicin (present in Habanero pepper), meta-coumaric acid, ortho coumaric acid, trans-cinnamic acid, capsaicin and dihydrocapsaicin (present in Serrano pepper) (Acero-Ortega *et al*., 2003). Although there are few research works using Capsicum extracts in combination with another natural extracts (Morre and Morre, 2007; Lillehoj *et al*., 2011: Ilsley *et al*., 2002) in a study made by Gutierrez *et al*. (2009) they observed a synergistic and additive effects when two types of essential oils were combined (oregano, thyme, lemon and marjoram) against bacterial strains like *Enterobacter spp*., *Listeria spp*., *Lactobacillus spp.* and *Pseudoman spp*.

Table 4. Values of Fractional inhibitory concentration index of two pepper cultivars; Serrano (*Capsicum annuum* L. *acuminatum*) and Habanero (*Capsicum chinense*) against *Escherichia coli* and *Listeria monocytogenes.*

	FIC index	
	Escherichia coli	***Listeria monocytogenes***
S.P X H.P	0.23	0.56
S.P X H.S	0.38	1.04
S.P X H.WF	0.21	0.37
S.S X H.P	0.34	0.82
S.S X H.S.	0.55	0.69
S.S X H.WF	0.31	0.45
S.WF X H.P	1.04	0.43
S.WF X H.S	0.44	1.05
S.WF X H.WF	0.27	0.22
S.P. Serrano pericarp, S.S. Serrano seed, S.WF. Serrano whole fruit, H.S. Habanero seed, H.P. Habanero pericarp, H.WF. Habanero whole fruit.		

Fuente: Elaboración propia

In the same way, a synergistic effect against *Listeria monocytogenes* was observed when combined extracts obtained from Serrano pericarp/Habanero whole fruit; Serrano seed/ Habanero whole fruit; Serrano whole fruit/Habanero pericarp and Serrano whole fruit/Habanero whole fruit with FIC index values less than 0.5. An additive effect was found in the other combinations. Also Acero-Ortega *et al*. (2003), combined bioactive compounds of three pepper species (Habanero, Serrano and Bell peppers) and found that when they combined compounds from Serrano and Habanero peppers a synergistic effect was observed against *Erwinia carotovora* the latter is in agreement with the results observed in the present study.

Conclusions

Control of *Escherichia coli* and *Listeria monocytogenes* must be done in order to reduce the risk of food related outbreaks causing enterogastric diseases. Composition of the two Capsicum species studied in the present research depends upon the specie and the part studied. It was also proved that Capsicum extracts could be a useful way to inhibit growth of the aforementioned bacterial species. There were also synergistic and additive effects when two Capsicum extracts were combined, which resulted in improvement to the inhibitory effect of these extracts. These combinations could be used as a good alternative to food safety and food preservation and its applicability must be studied.

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