ANTIMICROBIAL EFFECTS OF CAPSAICINOIDS AND LECITHIN ON THE GROWTH OF FOOD BORNE PATHOGENIC BACTERIA

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Introduction

- Since the 16th century, the determination of the number of species of the Capsicum genus was often modified.
- In 1700, Tourneforte had identified 27 especies of *Capsicum*
- En 1753, Linnée subdivided (27 especies) in two: *C. annuum* et *C. frutescens.*
- In 1767, he added *C. baccatum* et *C. grossum.* /
- Nowadays, one identifies 5 domesticated species and more than 25 wild ones

Table 1: Different species of the *Capsicum genus*

TuCapsicum	***Pseudoacnistus***	***Capsicum***		
C. anomalum	*C. brevifolium*	***C. annuum***[1*]4 Var. *aviculare* Var. *annuum* *C. buforum* *C. cardenasii**2 ***C. chinense***[1*]4 *C. coccineum* *C. dimorphom* *C. eximium*[*2] Var. *tomentosum* *C. geminifolium*	*C. lanceolatum* *C. minutiflorum* *C. parvifolium* ***C. pubescens***[1*2] *C. scolnikianum* Var. *flexuosum* *C. villosum* ***C. baccatum***[1*]3 Var. *bacctum* Var. *pendulum* *C. campilopodium*	*C. chacoense**4 *C. ciliatum* *C. cornutum* *C. dusenii* ***C. frutescens***[1*]4 *C. galapagoense**4 *C. hookerianum* *C. leptopodum* *C. mirabile* *C. praetermissum**3 *C. schottianum* *C. tovarii**3

[1]Domesticated species; *Complexes; 2. *pubescens* Complex; 3. *baccatum* Complex; 4. *annuum* Complex

(Bosland et al., 1990; Bosland et Votava, 2000)

Table 2: Domesticated species of *Capsicum*

	C. annuum	*C. chinense*	*C. pubescens*	*C. baccatum*	*C. frutescens*
Varieties	**Bell** Pimiento Squash/tomato/cheese Yellow wax Cherry Paprika Chiltepin/chile piquin Ancho/ mulato/ pasilla Cayenne Chihuacle Cuban/Pepperoncini Costeño Mirasol Cascabel De Arbol **Jalapeño** Serrano New Mexican Santaka/Hontaka Piment ornemental	**Habanero** Scotch bonnet Datil Charapita/ Pimento de cherio Cheira Bell Cumario passarinho Fatalli Congo Rocotillo Aji Chombo Aji Panca Aji Limo Aji Pucomucho	Manzano Peron Siete caldos Caballo Locoto Rocoto	Aji Amarillo Aji Ayucllo	Bird pepper Tabasco Malagueta

(Bosland et Votava, 2000)

- Capsicum is widely cultivated in India, China and Africa. However, Asia is the greatest producer (FAOSTAT, 2004)

Table 3: Production of pepper in the world

Countries	Productions (1000 tons)	Pays	Productions (1000 tons)	Countries	Productions (1000 tons)	Countries	Productions (1000 tons)
South of Africa	10	Côte d'ivoire	14	Macedonia	0,10	Senegal	3,06
Algeria	7,60	Djibouti	0,28	Madagascar	3,20	Serbia-and Montenegro	5,40
Argentine	3,17	Egypt	45,60	Malaysia	1,80	Sierra Leone	2,50
Bangladesh	138	Spain	9	Malawi	1,80	Slovenia	1,30
Benin	14	United States	54,90	Maldives	0,01	Soudan	7,60
Bolivia	0,06	Ethiopia	116	Mali	3	Syria	0,50
Bosnia-Herzegovina	30	Ghana	22	Morocco	14	Tanzania	6,50
Brazil	10,94	Greece	0,30	Mexico	55	Czech, Republic	4,30
Bulgaria	0,70	Grenade	0,07	Myanmar	70	Thailand	38
Cambodia	10	Honduras	1,17	Nepal	14	Togo	2,50
Cameroon	6,30	Hungary	70	Nicaragua	0,05	Trinidad and Tobago	0,21
Cape Verde	1	India	1 100	Niger	0,40	Tunisia	7
Central-Africa	0,10	Iran	2,50	Nigeria	47,50	Turkey	20
Chile	11,35	Jamaica	10,40	Ouganda	3,80	Viet Nam	78,50
China	235	Kazakhstan	0,30	Uzbekistan	3	Zambia	0,90
Colombia	3,94	Kenya	5	Pakistan	90,40	Zimbabwe	13
Congo, RDC	33	Kyrghyzstan	0,10	Peru	16		
Costa Rica	2,74	Laos	4,70	Romania	30		

(FAOSTAT, 2004)

What led us to study the microbial effects of capsaicinoids and lecithin, two compounds found in pepper belonging to the Capsicum genus and the Solanaceae family?

- **1-**The pungent Teste, **2-**health benefit, **3-**antibacterial effect of Capsicum

1) What causes the pungent taste of pepper *Capsicum*?

- Capsaicin (Bosland et Votava, 2000) and dihydrocapsaicin (Garcia-Hirschfeld *et al.*,1995). are responsible for the pungent taste of pepper
- Pungent taste or the acridity level of *Capsicum* fruits depends on the genetic properties of the plant as well as environmental factors (Bosland et Votava (2000).
- Pepper with n = 13 chromosomes is less pungent than those with n =12 chromosomes (Tong et Bosland, 2000)
- Acridity level is expressed according to the Scoville scale (Nelson, 1919; Reilly et al., 2001). /
- higher the Scoville value, the more the pepper is pungent and rich in capsaicin and dihydrocapsaicin (Bosland et Votava, 2000).

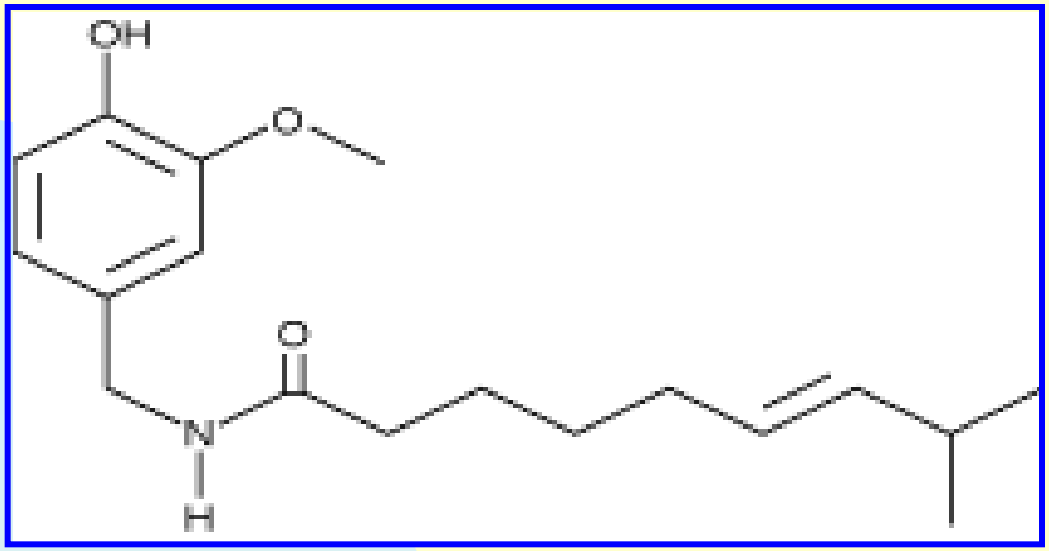


Figure 1: Chemical structure of capsaicin
(Bosland et Votava, 2000).

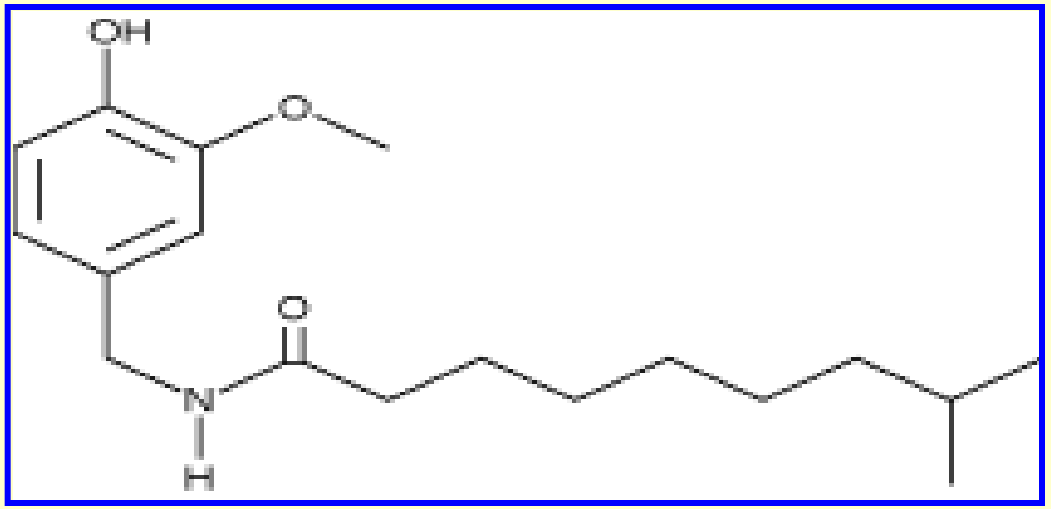


Figure 2: Chemical structure of dihydrocapsaicin
(Garcia-Hirschfeld *et al.*,1995).

Table 4: Level of pungency of different varieties of Capsicum in Scoville units (Bosland et Votava, 2000)

Names	Types de peppers	Species	Scoville Units
Orange Habanero	Habanero	*C. chinense*	210 000
Red Habanero	Habanero	*C. chinense*	150 000
Tabasco	Tabasco	*C. frutescens*	120 000
Tepin	Tepin	*C annuum*	75 000
Chiltepin	Tepin	*C. annuum*	70 000
Thai Hot	Asain	*C. annuum*	60 000
Jalapeño M	Jalapeño	*C. annuum*	25 000
Long Slim Cayenne	Cayenne	*C. annuum*	23 000
Mitla	Jalapeño	*C annuum*	22 000
Santa Fe Grande	Hungarian	*C. annuum*	21 000
Aji Escabeche	Aji	*C. baccatum*	17 000
Long Thick Cayenne	Cayenne	*C. annuum*	8 500
Cayenne	Cayenne	*C. annuum*	8 000
Pasilla	Pasilla	*C. annuum*	5 500
Primavera	Jalapeño	*C. annuum*	5 000
Sandia	New Mexican	*C. annuum*	5 000
NuMex Joe E. Parker	New Mexican	*C. annuum*	4 500
Serrano	Serrano	*C. annuum*	4 000
Mulato	Ancho	*C. annuum*	1 000
Bell	Bell	*C. annuum*	0

2) Health benefits of Capsicum

Based on data obtained, it has been widely reported that Capsicum is very beneficial to one's health, especially in the following areas :

- Capsicum tones up the cardiovascular system (Castle, 1992; Michael, 1995).
- Capsicum reduces cholesterol levels and fatty acids in the blood (Visudhiphan *et al.*, 1982).
- Capsicum helps obese persons to lose weight (Hobbs, 1994).
  - This small detail attracted my attention. Of course, it is not a question of offering a pepper-based diet to children. Rather, one needs to explore some more the therapeutic benefits of pepper
- A diet which is rich in Capsicum helps the body to fight against influenza and sore throat (Humbart, 1993). /
- Capsaicin promotes a better digestion as well as a proper absorption of nutriments (Horowitz et al., 1992).

Table 5: Nutritive value of 100 g of Cayenne pepper (*C. annuum*) (USDA, 2006)

Water : 8.05 g	Cendre totales : 6.04 g	Fibers : 27.2 g	Energetic values: 318 kcal
Proteins : 12.01 g	Lipids : 17.27 g	Carbohydrates: 56,63 g	
Trace elements			
Calcium : 148 mg	Iron : 7.80 mg	Magnesium : 152 mg	Phosphorus : 293 mg
Potassium : 2014 mg	Copper : 0.129 mg	Sodium : 30 mg	Zinc : 2.48 mg
Vitamins			
Vitamin C : 76.4 mg	Niacin : 8.701 mg	Folate, Food : 106 µg	Folate, EFD : 106 µg
Thiamine : 0.328 mg	Vitamin B_6 : 2.450 mg	Folic Acid : 0 µg	Vitamin B_{12} : 0.00 µg
Vitamin A : 41 610 UI	Retinol : 0 µg	Vitamin E : 29.83 µg	Vitamin K : 80.3 µg
Fatty acids			
Saturated fatty acids : 3.260 g	Monounsaturated : 2.750 g	Polyunsaturated fatty acids : 8.370 g	
Others			
β-carotene : 21 840 µg	Lycopene : 0 µg	Lutein + zeaxanthin :13 157 µg	β-cryptoxanthin : 6 252 µg

3) Antibacterial effects of *Capsicum*

- Extracts of certain varieties of Capsicum have an inhibitive effect on certain pathogenic microorganisms (Cichewicz et Thorpe, 1996; Careaga *et al*., 2003; Acero-Ortega *et al*., 2005a).
- Pepper fruits are rich in phenylpropanoids such as coumaric acids, trans-cinnamic acid and capsaicinoids.
- The antibacterial properties of peppers has largely been attributed to the presence of these phenylpropanoid compounds that are intermediates in the capsaicinoid pathway.
- Capsaicin is the main capsaicinoid and is the compound responsible for the pungency of chili peppers (Acero-Ortega et al., 2005).
- Capsaicin would be responsible for the antibacterial effect of Capsicum extracts (Jones *et al.*, 1997; Molina-Torres *et al.*, 1999; Snyman *et al*., 2001). /
- Capsaicin is not responsible for the antibacterial effect of Capsicum extracts (Dorantes *et al*., 2002)

Problematic

Table 6.1 Inhibition zone of growth produced by the extract of Morrón, Serrano and Habanero pepper

(Garcia *et al.*, 1995)

Bacteria	**Pimento Morrón (mm)** (0.12 g of Cap & dihycap/100 g of extract)	**Serrano (mm)** (0,40 g of Cap & dihycap/100 g of extract)	**Habanero (mm)** (1,95 g of **Cap** & **dihycap**/100 g of extract)
Bacillus cereus	12	9	8
Staphylococcus aureus	11	7	2
Listeria monocytogenes	7	6,5	5
Salmonella typhimurium	5	1,5	1,5

Cap: capsain; **Dihycap:** dihydrocapsaicin

Table 6.2 Inhibition zone of growth produced by some phenylpropanoids identified in Serrano pepper

(Dorantes *et al.*, 2000; 2002)

Bacteria	***o*-Coumaric acid (mm)**	***m*-coumaric acid (mm)**	***trans*-cinnamic acid (mm)**	**Capsaicin (mm)**	**Dihydrocapsaicin (mm)**
Bacillus cereus	Negative	10.0 ± 0.0	8.0 ± 0.8	Negative	Negative
Staphylococcus aureus	Negative	10.0 ± 0.8	6.0 ± 0.8	Negative	Negative
Listeria monocytogenes	Negative	6.0 ± 0.6	5.0 ± 0.8	Negative	Negative
Salmonella typhimurium	Negative	2.0 ± 0.8	2.0 ± 0.0	Negative	Negative

Objectives

- Evaluate the antibacterial effects of crude extracts from three Capsicum varieties.
- Verify the presence of m-coumaric acid, trans-cinnamic acid, lecithin and capsaicin in the three Capsicum varieties using TLC. Evaluate their antibacterial effects against 6 bacterial strains
- Extract lecithin and capsaicin from Capsicum varieties and evaluate their antibacterial effects, alone and in combination, against 6 bacterial strains./

Material and methods

- Extracts from three *Capsicum* varieties
  - *Capsicum annuum:* Bell Pepper; Jalapeno and
  - *Capsicum chinense:* Habanero
    (Atlantic Super Store,Moncton)

} were prepared using the methods of Dorantes *et al.* (2000) with modifications

- Bell pepper

- Jalapeno pepper

- Habanero pepper

- The direct drop plate method was used to evaluate, in triplicate, the antimicrobial effects of the three pepper extracts and synthetic and/or extracted molecules (trans-cinnamic acid, m-coumaric acid, capsaicin and lecithin).
- The six pathogenic bacteria (10^8 cfu/mL in TSA) used were:

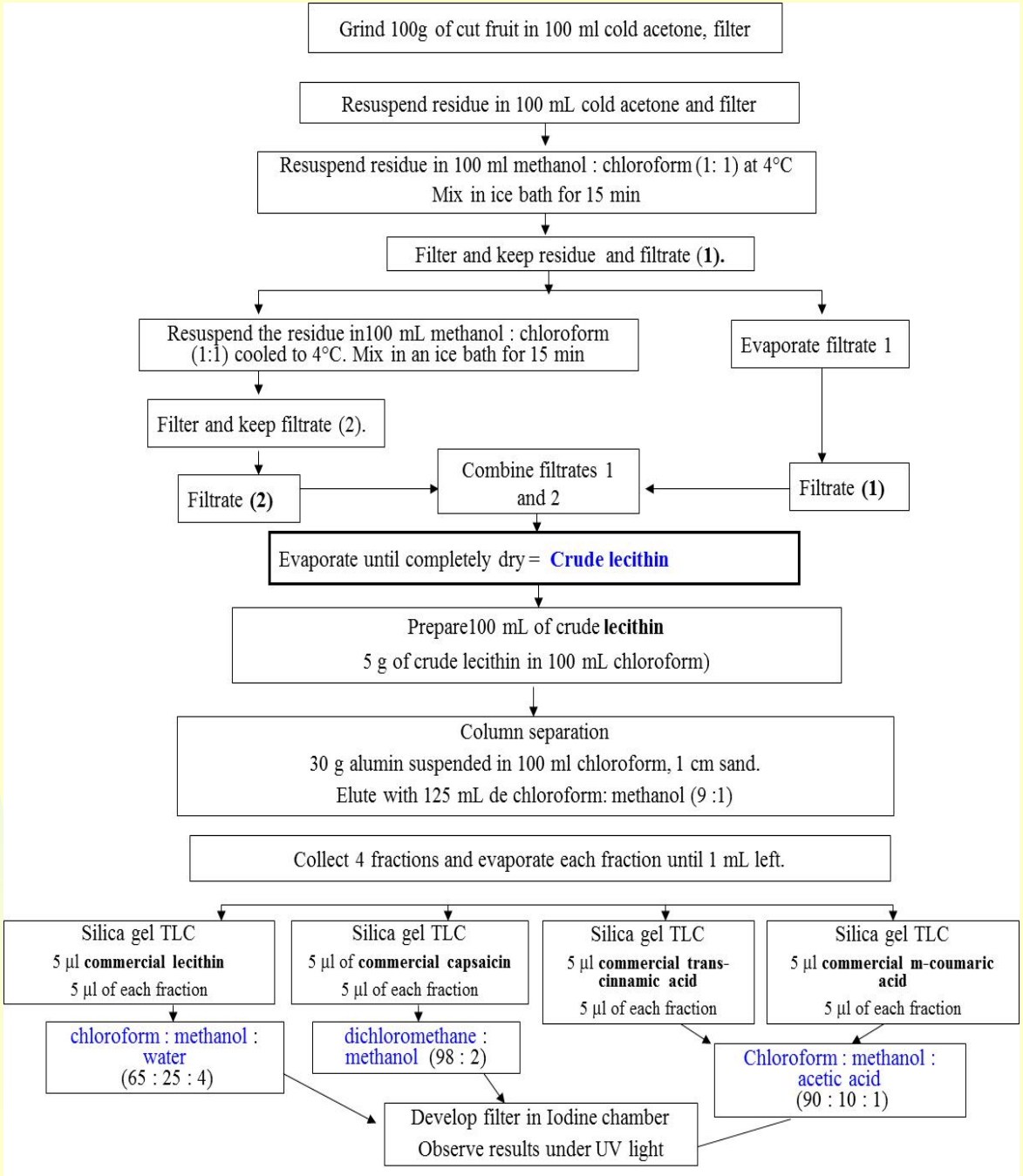
Material and methods

Microorganisms: CRA (Centre de recherche sur les aliments)
The six pathogenic bacteria (10^8 cfu/mL in TSA) used were:

- *Bacillus cereus* (ATCC 56926)
- *Enterobacter aerogenes* (ATCC 13048)
- *Escherichia coli* (ATCC 25922),
- *Listeria monocytogenes* (ATCC HPB#43)
- *Staphylococcus aureus* (ATCC 25923)
- *Salmonella typhimurium (CRA)*

Figure 4:

Summary of methodology used to prepare four pepper fractions containing phenylpropanoids and lecithin.



- Extraction of lecithin and the phenylpropanoids was performed as described by Dako *et al.(*5).
- Silica gel TLC was used to identify the phenylpropanoids and lecithin in the 4 fractions
- Statistical analysis was performed using SYSTAT (1992). The Fischer test was applied to compare pair-wise means.

Results

Bell, Jalapeño et Habanero Extracts

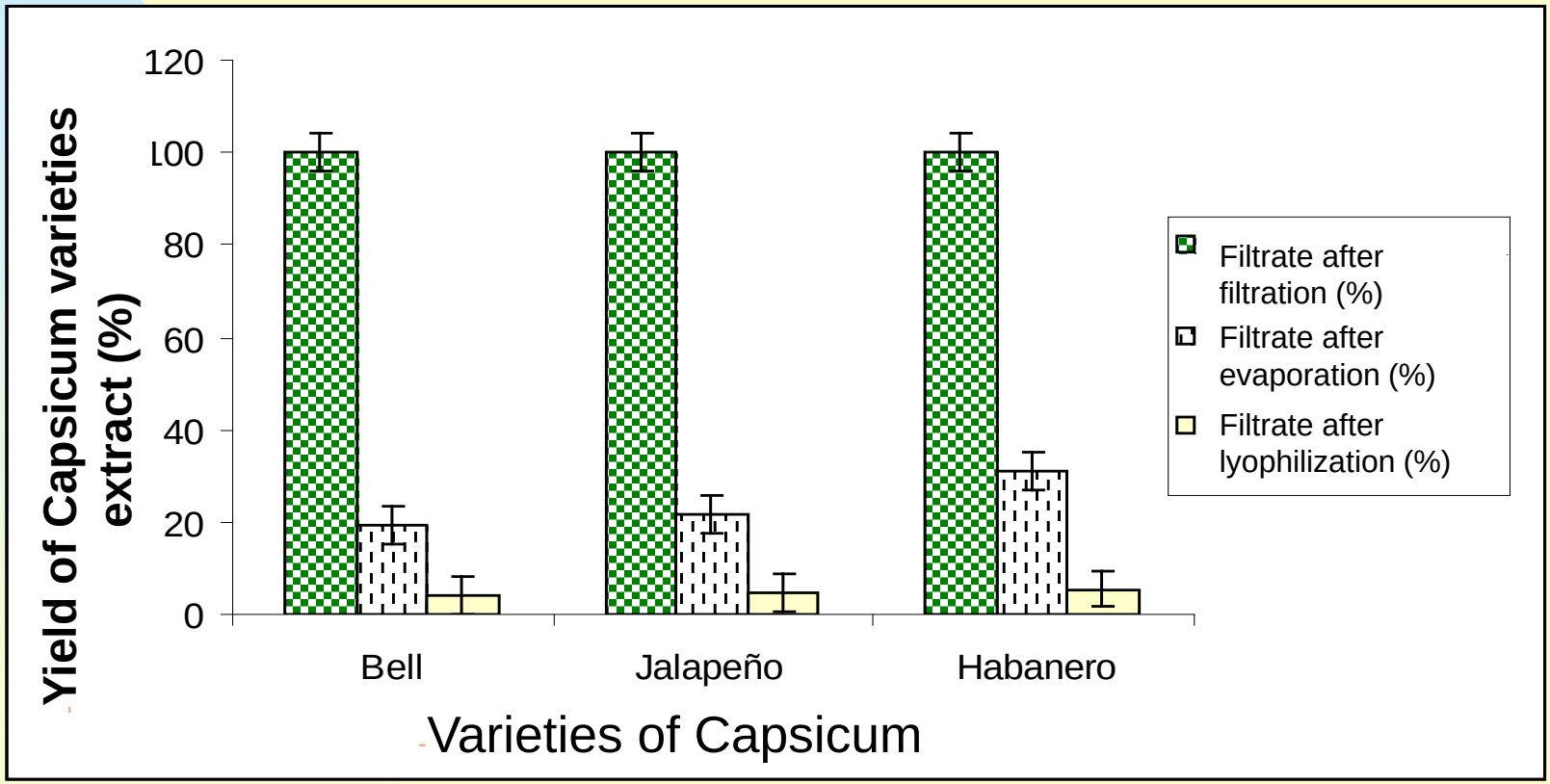


Figure 5: Yield of the extract of Bell, Jalapeño et Habanero pepper after techniques, filtration and lyophilisation.
Yields : Bell (4,27%), Jalapeño (4,71%) et Habanero (5,54%)

Results

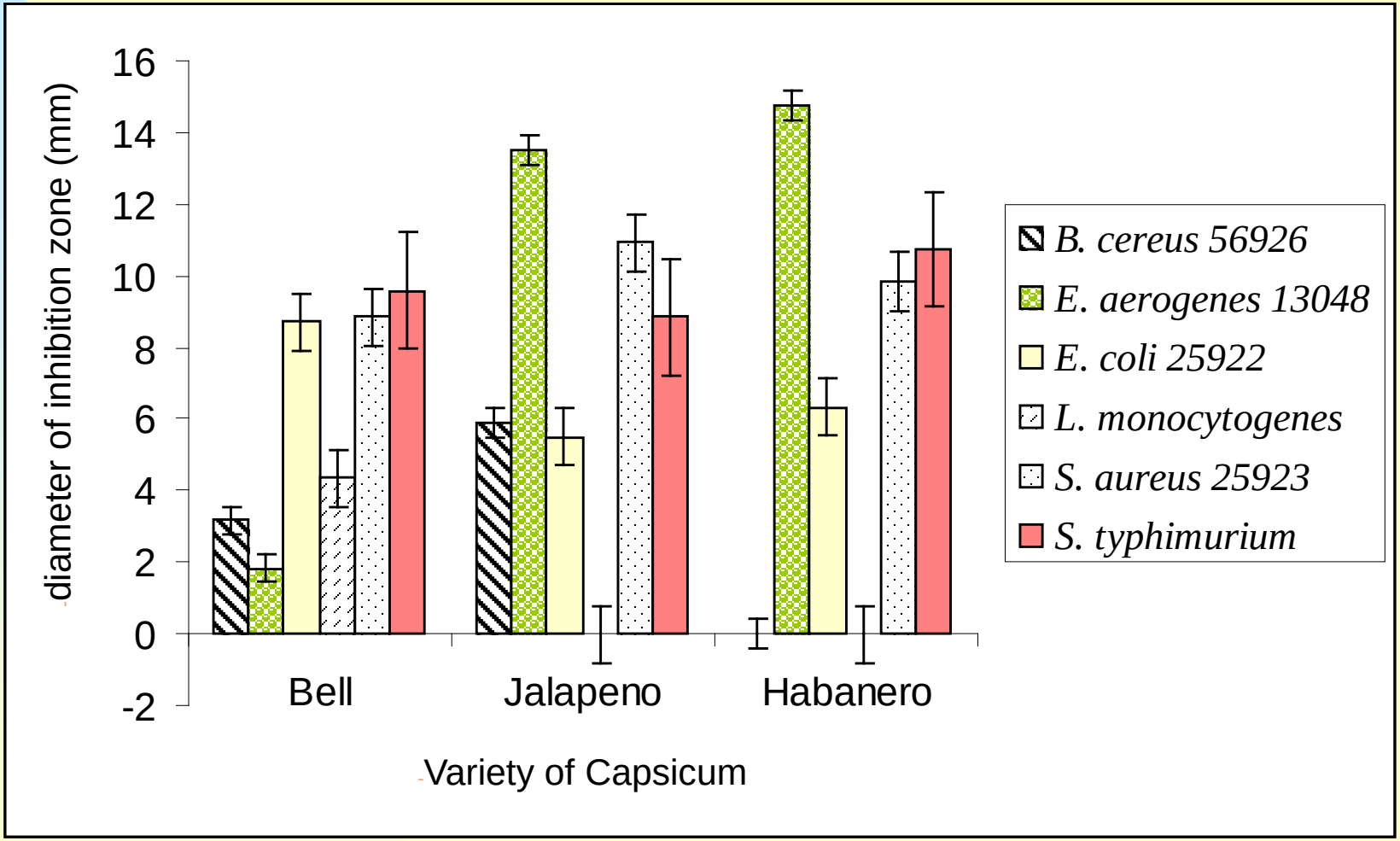


Figure 6: Inhibition zones (mm) for 20 µl crude extracts from three Capsicum varieties (Bell, Jalapeño, Habanero) on six bacterial species.

Separation and/or characterization of lecithin

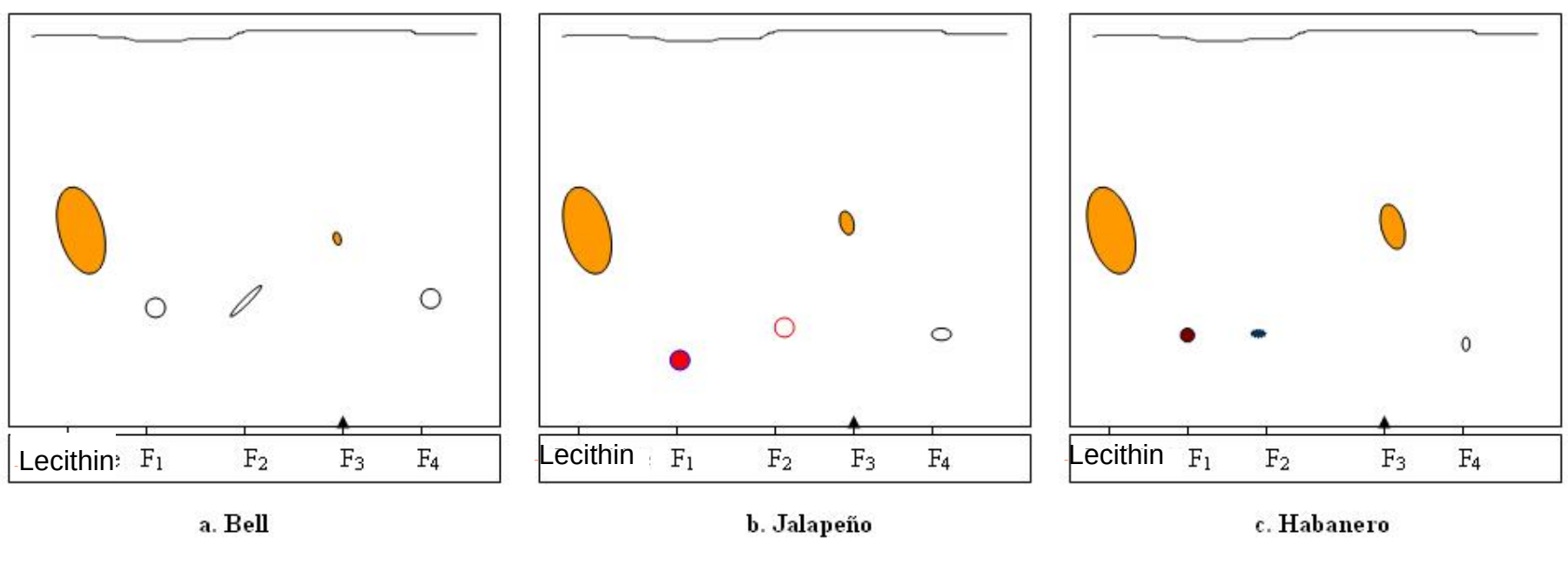


Figure 8.1 Characterization of lecithin on thin layers of silica gel using extracts from Bell, Jalapeño and Habanero after the migration of fractions lecithin commercial and extracted fractions F1, F2, F3, F4;

Separation and/or characterization of capsaicin

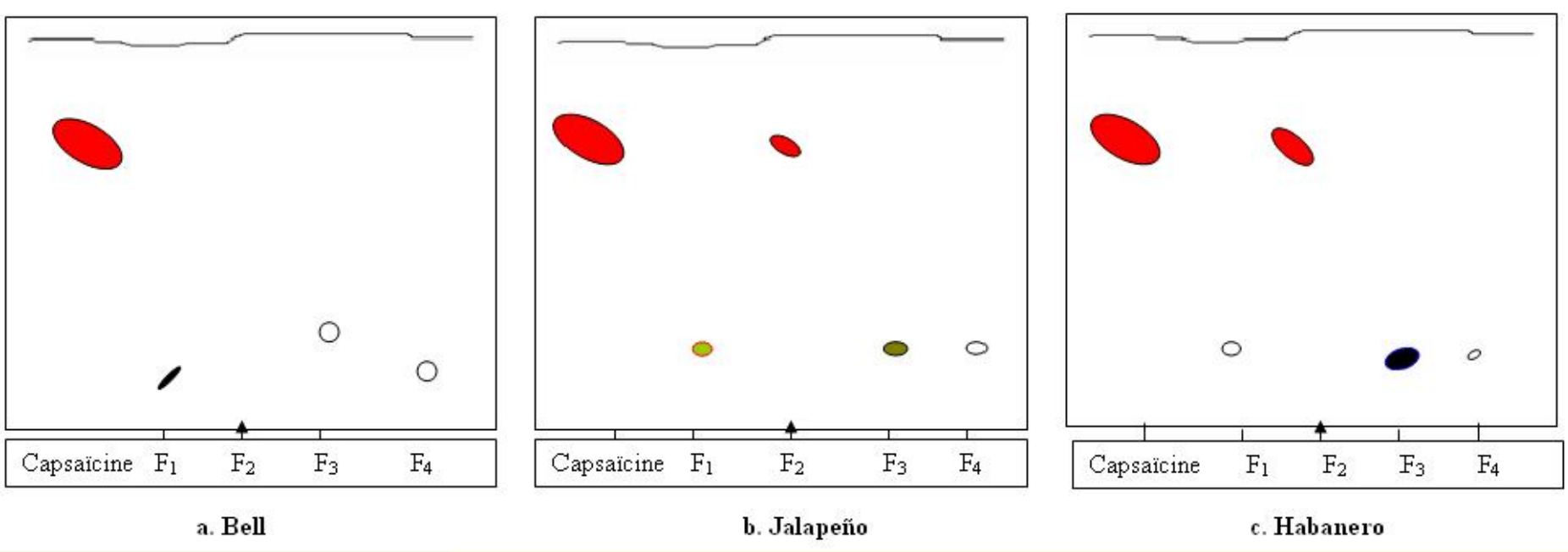


Figure 8.2: Characterization of capsaicin on thin layers of silica gel using extracts from Bell, Jalapeño and Habanero after the migration of fractions capsaicin commercial and extracted fractions F1, F2, F3, F4;

Separation and/or characterization of trans-cinnamic and m-coumaric acids

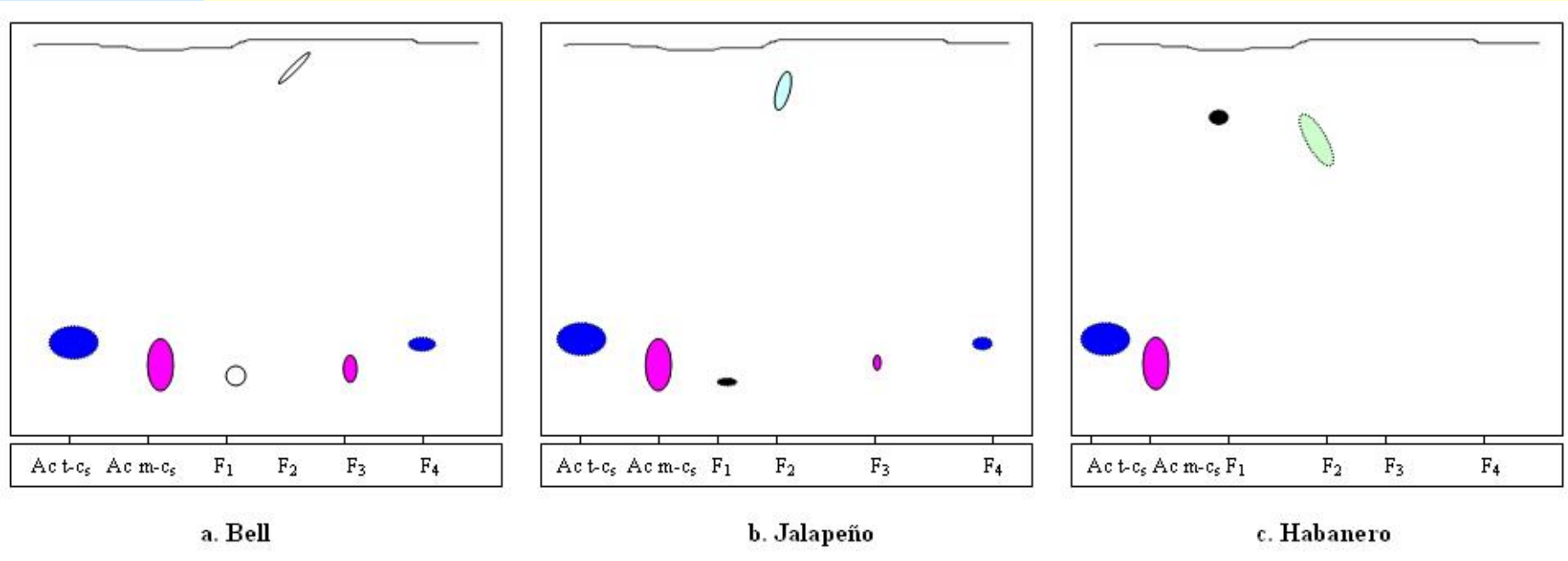


Figure 8.3 Characterization of trans-cinnamic and m-coumaric acids on thin layers of silica gel using extracts from Bell, Jalapeño and Habanero after the migration of fractions extracted fractions F1, F2, F3, F4; Commercial trans-cinnamic (Act-Cs) and m-coumaric acid (Acm-cs).

Table 7: Summary of results of growth inhibition by compound tested (mm of inhibition zone +/- standard error). 0.05 μg/ μl commercial compound, 5 μg/ul crude extract).

Bacteria	Lecithin Extracted	Lecithin Commercial	Capsaicin Extracted	Capsaicin Commercial	Trans-cinnamic Commercial	M-coumaric acid Commercial	Lecithin and capsaicin Commercial
***B. cereus* 56926**	1,3 ± 0,17	3,0 ± 0,7	-	-	5,3 ± 2,0	6,2± 1,0	-
***E. aerogenes* 13048**	1,8 ± 0,70	3,1 ± 0,5	-	-	7,1 ± 0,5	5,4± 0,9	-
***E. coli* 25922**	3,4 ± 1,80	4,8 ± 0,7	-	-	7,1 ± 0,5	5,7± 0,5	-
***L. monocytogenes* HPB**	2,0 ± 0,60	2,4 ± 0,8	-	-	5,8 ± 1,3	4,6± 0,4	-
***S. aureus* 25923**	1,9 ± 0,60	2,9 ± 0,6	-	-	7, 3± 1,8	5,8, ± 1,0	-
S. typhimurium	4,4 ± 0,8	0,34 ± 1,2	-	-	10,8 ± 1,2	8,1 ± 0,5	-

- : Absence of inhibition zone

Conclusions

- The results of this study are in agreement with those of Dorantes et al (2002). In fact, capsaicin has no inhibitory effect on bacterial growth, at least not on the microorganisms which were studied.
- This research showed that lecithin is found in the 3 varieties of Capsicum : Bell, Jalapeno and Habanero, but it is more concentrated in Habanero.
- The research also
  - reveals that lecithin is among the antibacterial compounds found in Habanero and confirmed that the Capsicum Bell fruit contains no capsaicin. This is found only in the Jalapeno and Habanero varieties.
  - suggests that trans-cinnamic acid and m-coumaric acid are essentially found in Bell and Jalapeno, and are not found in Habanero.
  - shows that the inhibitory effect of Capsicum (Bell, Jalapeno and Habanero) varies according to the amount of capsaicin present in its extract. The presence of capsaicin in the Capsicum extract inhibits or greatly reduces the inhibitory effect of Capsicum./
- Moreover, the presence of lecithin, as well as trans-cinnamic and m-coumaric acids increases the inhibitory effect of the extracts of these 3 varieties of Capsicum.

Table 8: Summary of results

Bell	Jalapeno	Habanero
	Capsaicin	Capsaicin
trans-cinnamic acid	trans-cinnamic acid	
m-coumaric acid	m-coumaric acid	
Lecithin	Lecithin	Lecithin
Inhibited bacteria		
Bacillus cereus *Enterobacter aerogenes* *Escherichia coli* *Listeria monocytogenes* *Staphylococcus aureus* *Salmonella typhimurium*	*Bacillus cereus* *Enterobacter aerogenes* *Escherichia coli* *Staphylococcus aureus* *Salmonella typhimurium*	 *Enterobacter aerogenes* *Escherichia coli* *Staphylococcus aureus* *Salmonella typhimurium*

- *L. monocytogenes* is the most resistant to the effect of Jalapeno and Habanero

THANK YOU !!