EFFECT OF ACRYLAMIDE, AMYGDALIN, CAPSICUM, FURFURAL AND VANILLIN ON SOME FOOD BORNE PATHOGENIC BACTERIA

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ABSTRACT

The growth of eleven food spoilage bacterial strains; four Gram negative (Enterobacter (Ent.) aerogenes, Escherichia (E.) coli, Pseudomons (Ps.) aeruginosa and Ps. fluorecsens) and seven Gram-positive strains (Bacillus (B.) cereus, B. firmus, B. pumilus, B. subtilis, Micrococcus (M.) luteus, M. varians and Staphylococcus (S.) aureus) was studied in liquid media in the presence of acrylamide, amygdalin, capsicum, furfural and vanillin. These compounds are naturally occurred in plant products or formed or added during food processing.

The Inhibitory effect of acrylamide, amygdalin capsicum, furfural and vanillin is concentration and strain dependent. The inhibitory action increased as the concentration increased. Furthermore, the presence of amygdalin, vanillin, furfural as well as capsicum inhibited Gram-negative bacterial strains, namely Ps. aeruginosa and Ps. fluorescens, while Gram-positive, namely B. pumilus, B. subtilis, M. luteus, and M. varians were inhibited by furfural (750 ppm) and vanillin (3000 ppm)

These findings indicate that vanillin and capsicum could be useful as preservatives for minimally processed products.

Keywords: Pathogenic bacteria, Gram-negative strains, Gram-positive strains, acrylamide, amygdalin, capsicum, furfural, vanillin, growth, inhibition

INTRODUCTION

The increase of international trade of food commodities has raised the risk of dispersion of pathogenic bacteria from the production sites to far places of consumption. High quality and safety of food are of major concern to the nowadays consumer, who prefers fresher, additive-free and minimally processed products. Hence, the use of natural antimicrobial agents for the preservation of foods could guarantee both high quality and safety. Natural antimicrobial compounds seem important not only to the preservation of food, but also to the control of human diseases of microbial origin (Gould, 1996; Baratta et al., 1998 and Quattara et al., 2000).

Earlier Kim and Ryeon (1979) reported the antibacterial effect of capsaicin from Korean hot peppers on *B. cereus, B. subtilis* and *Sarcina lutea*. Later on Serruti and Alzamora (1996) showed that vanillin (one of the capsaicin analogues) inhibits the growth of yeast. The trend of using natural antimicrobial compounds has led to growing interest in investigating a series of such compounds from the viewpoint of their antimicrobial activity (Alzamora and Guerrero, 2003).

Acrylamide occurs in plant material as potatoes, carrots, radish, lettuce, Chinese cabbage, parsley, onions, spinach and paddy rice (Arikawa and Shiga, 1980). Acrylamide does not occur naturally but it is found in

various fried, deep-fried and oven-baked foods. It concerns foods, which are regularly consumed throughout the year, like chips (French fries), crisps and bread, also biscuits, crackers and breakfast cereals (Tareke et al., 2002). So far no acrylamide has been found in foods that are boiled (Rosen and Hellenäs, 2002). Acrylamide has been shown to be neurotoxic in humans and laboratory animals. It has also been shown to induce tumors in experimental animals, and has been classified as 'probably carcinogenic for humans' (IARC, 1994; EC, 2002; FAO/WHO, 2002 and Vattem and Kalidas, 2003).

Amygdalin is a natural substance, known as cyanogenic glycosides; it was first isolated from bitter almond. It is found in the seeds of apricot, peach, plum, cherry, apple, sprouted alfalfa, mung, wheat, millet, lentil, watercress, lima beans, black eyed peas, clover, sorghum, broccoli, cabbage, flax seed, linseed...etc. (Moss, 1996 and Rosenberg, 1990). Herbert (1979) reviewed that intestinal bacteria contain enzymes (β -glucosidases) that activate the release of cyanide after amygdalin had been ingested. When amygdalin interacts with the enzyme β -glucosidase, or undergoes hydrolysis (breakdown upon reaction with water) in the absence of enzymes, hydrogen cyanide, benzaldehyde and glucose are produced (Newmark et al., 1981 and Rauws et al., 1982).

Red pepper (Capsicum annuum, Solanaceae), an important flavoring and coloring spice, is used as a natural flavoring. Its pungency is attributed to capsaicinoid, which is composed of 80 % to 90 % capsaicin and dehydrocapsaicincin (Pulseglove et al., 1988 and Perucka & Oleszek, 2000). Capsicum annuum extract has an inhibitory effect on B. cereus and S. aureus (Dorantes et al., 2000 a). Also, the same authors proposed the use of this extract as a natural antibacterial agent in a food such as raw beef, which is often contaminated (Dorantes et al., 2000 b). Also, Careaga et al. (2003) reported that the Capsicum annuum (bell pepper extract) exhibited an inhibitory effect against S. typhimurium and Ps. aeruginosa.

Furfural occurs naturally in many fruits, tea, coffee and cocoa and mostly is formed during the processing and domestic preparation of a broad range of foods (Monti et al., 2000 and Murata et al., 2002). It is also carried over into food from its use as an extraction solvent or as a component of flavor mixtures. Furfural and its derivatives from consumption of foods in which it occurs naturally is approximately 0.3 (Stofberg and Grundschober, 1987) to 0 - 0.5 mg /kg /day (IARC, 1994). The highest concentrations of furfural in food have been reported in cocoa (55 - 255 ppm), wholegrain-bread (26 ppm), heated skim milk (230 ppm) and coffee (90 - 881 ppm) (Adams et al., 1997).

Vanillin, a plant phenol, is the predominant phytochemical that occurs in vanilla beans and is a generally regarded as safe (GRAS). It is used widely as a food flavoring agent in confectioneries, chocolates, butter, toopings, icings, ice cream, beverages, biscuits, desserts, etc. (Beuchat & Golden, 1989; Hocking, 1997; Spillman et al., 1997 and Ramachandra Roa & Ravishankar, 2000). Its antioxidant and antimutagenic activity had been observed in both bacteria and mammalian cells (Tamai et al., 1992; Ohta, 1993; Prince & Gunson, 1994 and Santosh Kumar et al., 2000).

As information on the sensitivity of pathogenic and spoilage bacteria to some additives or compounds formed in foods are scanty, the aim of the present study was to test the effect of acrylamide, amygdalin, capsicum, furfural and vanillin on the behavior of some food bome pathogenic bacteria in Vitro.

MATERIALS AND METHODS

MATERIALS Additives

The compounds examined are acrylamide at 500, 1000 and 2000 ppm (SERVA, Heidelberg, Germany), amygdalin at 3.5, 50 and 100 ppm (Fluka Chemie, AG, CH-9470 Buchs, Switzerland), capsicum at 50, 100 and 200 ppm (Concentrated Capsicum, FLPT038-10, Ransom, William Ransom & Sonplc Hitchin, Herts, SG5, ILY, England), furfural at 250, 500 and 750 ppm (Merck, Darmstad, Germnay) and vanillin at 750, 1500 and 3000 ppm (Zhonghua Chemicals, Zhejiang, China). The minimal concentrations of these additives are naturally occurred or formed or added during food processing. Solutions of the additives were sterilized by filtration through a sterile 0.20 µm cellulose nitrate filter (Sartonus, AG. 37070 Goettingen, Germany) and then added at the concentrations mentioned above to the growth media.

Microorganisms and culture media

The following 11 Gram-positive and Gram-negative bacteria were used for antimicrobial activity studies. Gram-negative bacteria: Entrobacter (Ent.) aerogenes ATCC 15050, Escherichia (E.) coli ATCC 15130, Pseudomonas (Ps.) aeruginosa DSM 50071 and Ps. fluorescens DSM 50090. Gram-positive bacteria: Bacillus (B.) cereus DSM 31, Bacillus firmus ATCC 14575, Bacillus pumilus ATCC 14884, Bacillus subtilis DSM 10, Micrococcus (M.) luteus ATCC 15307, M. varians ATCC 15306 and Staphylococcus (S.) aureus ATCC 6538.

These strains were obtained from the American Type Culture Collection, Rockville, Maryland, USA (ATCC) and the German Collection of Micro-organisms, Braunschweig, Germany (DSM). All these strains were checked up and stored on Brain Heart Infusion (BHI) slants at 4 °C then subcultured twice in Brain Heart Infusion Broth (LAB M, Topley House, 52 Wash Lane, Bury, Lancashire, BL9 6AU, UK) and incubated at 22 °C (for Ps. aeruginosa and Ps. fluorescens) and 37 °C (for the rest of strains) for 24 h before use.

METHODS:

Determination of bacterial growth and inhibition activity

Flasks of BHI broth containing various concentrations of additives and control (without additives) were inoculated with apriori prepared cultures at 1% level (initial counts, 10^6 - 10^7 cfu ml $^{-1}$) and incubated at 22 and 37 °C. Triplicate flasks were treated for each additive at each concentration. The growth of each culture was monitored in two ways, by measuring its absorbance at 600 nm (OD₆₀₀) by a Spectronic 20D (Milton Roy Company, USA) at intervals for a total period of 72 hrs, and by plating on BHI agar (1.2% w/v) at 0, 3, 6, 9, 12, 24, 48, 72 hrs suitably diluted aliquots of the culture (viable counts). All experiments were repeated thrice.

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Growth analysis

The growth percentage of 12 h culture equals (ODt-ODto)test / (ODt-ODto)control × 100,

Where: OD is the optical density at 600 nm, *t* is time after 12 h, *to* is the initial time 0 h, *test* makes reference to the culture grown with additive(s) and *control* makes reference to the culture grown without additives (Nazer *et al.*, 2005). This variable indicates how much the growth is reduced in the presence of additives. A time of 12 h is chosen for the best discrimination of growth curves.

The inhibition percentage of the examined additives was calculated as follows:

Inhibition $\%= [Log N_2-Log N_1/Log N_2] \times 100$,

Where: Log N₁: Log cfu ml⁻¹ of the sample at the last hour (72nd hr)

Log N₂: Log cfu ml⁻¹ of control without additives at the last hour (72nd hr)

Statistical analysis

The results are presented as means \pm standard deviation from three replicates of each experiment. A P-value \leq 0.01 is used to denote significant differences among mean values determined by analysis of variance (ANOVA) (CoStat program ver. 3.03, 1986).

RESULTS AND DISCUSSION

The inhibitory effect of acryylamide, amygdalin, capsicum, furfural and vanillin on the growth of eleven food borne pathogenic bacteria was studied and the results are given in Tables (1-2) and Figures (1-2)

Acrylamide

Acrylamide showed slowest decrease of the growth percentage of Gram-negative (except Ps. aeruginosa) and Gram-positive bacteria. In case of B. pumilus it gave the highest growth percentage at different concentrations. In other words acrylamide seemed to be a promoter of the growth of this bacterium (Figs. 1 and 2).

As shown in Tables (1 and 2), acrylamide has no effect on *Ent. aerogenes, E. coli, Ps. fluorescens, B. firmus* and *B. pumilus,* whereas inhibits the growth of *Ps. aeruginosa* (by 60.08% at 2000 ppm). Grampositive bacteria, namely *B. cereus, B. subtilis, M. luteus, M. varians and S. aureus* are significantly affected by acrylamide. The inhibition percentages are 20.09, 32.12, 45.00, 49.06 and 42.43, respectively.

Amygdalin

Amygdalin leads a slowest decrease of the growth percentage of Gram-negative strains. The growth percentage of Gram-positive bacteria gives highest values at different concentrations. Amygdalin exhibited itself as a growth promoter for Gram-positive bacteria (Figs. 1 and 2).

Thus, from Table (1), it is seen that Gram-negative bacteria are significantly inhibited by amgydalin. *Ps. aeruginosa* and *Ps. fluorescens* are sensitive among the Gram negative or positive bacteria, at 3.5, 50 and 100 ppm. The inhibition percentages are 94.73, 96.51 and 96.68; 97.20, 97.38 and 98.48, respectively.

These results may be due to the assimilation of amygdalin by the bacteria, and the resulting product (HCN) in return affected them (Conn, 1978).

For some bacterial strains, these results may indicate that the bacteria do not ferment or assimilate amygdalin or hydrolyze it and release a free HCN (very toxic). Another assumption is that the bacteria secrete rhodanase, which possesses a disulphide bridge that can be broken down by the thiosulphate ion, with the release of non-toxic Na_2SO_4 and SCN (Rosenberg, 1990).

Capsicum

The same trend of amygdalin is observed. The growth percentage is higher for Gram-positive bacteria (Fig. 2).

The antibacterial activity of capsicum is considerably lower towards Gram-positive strains than towards Gram-negative, i.e. capsicum has no effect on the Gram-positive bacteria, whereas the growth of Gram-negative (*Ps. aeruginosa* and *Ps. fluorescens*) is significantly inhibited. The inhibition percentages are 95.27, 97.23 & 97.57; 98.11, 98.11 and 98.45% at 50, 100 and 200 ppm, respectively.

These results are in agreement with those of Dorantes *et al.* (2000 a) who reported that *Ps. aeruginosa* was sensible to *Capsicum annuum* extract. Also, Careaga *et al.* (2003) reported that the *Capsicum annuum* (bell pepper extract) exhibited an inhibitory effect against *S. typhimurium* and *Ps. aeruginosa*. The authors added that the minimum inhibitory concentration of the extract to prevent the growth of *Ps. aeruginosa* was 0.3 ml of the extract 100 g⁻¹ of meat as, bacteriostatic effect, while a concentreation of 3 ml of the extract 100 g⁻¹ of meat was bactericidal. On the contrary, Dorantes *et al.*, (2000 b) mentioned that *Capsicum annuum* extract had an inhibitory effect on *B. cereus* and *S. aureus* using an agar diffusion method

Furfural

The growth percentage after 12 h of Gram-positive bacteria has no linear relation with furfural concentration (Figs. 1 and 2). There is almost no inhibition below some threshold concentration, then the growth percentage quickly decreases as the concentration of furfural increases. For instances, at 250 ppm of furfural the growth percentage is 91.23 while at 500 ppm, 17.81 for Ps. aeruginosa. Furthermore, at 750 ppm the growth percentage is 1.84. Also, this attitude holds true for Ps. fluorescens, 80.74% and 54.15% at 250 and 500 ppm, whereas at 750 ppm the corresponding percentage is 20.40 (Fig. 1).

For Gram-negative bacteria Table (1) shows that furfural has an inhibitory effect on *Ps. aeruginosa* and *Ps. fluorescens*. The inhibition percentages are 96.40 and 68.96 at 750 ppm, respectively. However, the inhibitory effect is concentration-dependent. *B. cereus*, *B. firmus* and *Ent*.

aerogenes are not affected while the growth of *B. pumilus*, *B. subtilis*, *M. luteus*, *M. varians* and *S. aureus* is significantly inhibited. The inhibition percentages are 87.82, 69.77, 67.50, 59.06 and 43.64, respectively at 750 ppm.

Table (1): The effect of acrylamide, amygdalin, capsicum, furfural and vanillin on the growth of some Gram-negative food borne pathogenic bacteria

patriogeriic bacteria									
	Inhibition percent								
Item tested	Ent.	E.	Ps. aeruginosa	Ps. fluorescens					
	aerogenes_	coli	_						
Acrylamide,									
500 ppm	0.00 ± 0.01 ^a	0.00 ± 0.01^{a}	32.36 ± 4.97 ^b	0.00 ± 0.01^{b}					
1000 ppm	0.00 ± 0.01^a	0.00 ± 0.01^a	43.46 ± 4.76 ^b	0 00 ± 0 01 ^b					
2000 ppm	0.00 ± 0.01^{a}	0.00 ± 0.01^a	60.08 ± 3.38 ^a	5 56 ± 0 93°					
Amygdalin									
3.5 ppm	12.83 ± 0.33 ^b	14.77 ± 1.90 ^b	94 73 ± 0 35°	97 20 ± 0.61 ^a					
50 ppm	19.74 ± 1.98°	23.81 ± 1.91 ^a	96.51 ± 0 96°	97 38 ± 0 37°					
100 ppm	19.74 ± 1.98°	24.24 ± 1.14^{a}	96.68 ± 0.72^a	98.48 ± 0.25°					
Capsicum									
50 ppm	16.12 ± 2.96°	11.02 ± 0.42 ^b	95.27 ± 0.41 ^b	98.11 ± 0.55°					
100 ppm	17.11 ± 1.98 ^a	12.50 ± 3.41 ^b	$97.23 \pm 0.93^{\circ}$	98.11 ± 0.55 ^a					
200 ppm	17.76 ± 0.01 ^a	25.57 ± 3.22 ^a	97 57 ± 0.45°	98.45 ± 0 65°					
Furfural									
250 ppm	0.00 ± 0.01^{6}	0.00 ± 0.01^{5}	$0.00 \pm 0.01^{\circ}$	$4.88 \pm 0.01^{\circ}$					
500 ppm	0.00 ± 0.01^{b}	0.00 ± 0.01^{6}	22.95 ± 3 77 ^b	24 09 ± 0.92 ^b					
750 ppm	3.95 ± 1.14 ^a	23.67 ± 2.47 ^a	96.10 ± 0.21 ^a	68 96 ± 0.92ª					
Vanillin									
750 ppm	0.00 ± 0.01 ^b	0.00 ± 0.01^{6}	85.10 ± 0.04°	66 34 ± 0.52°					
1500 ppm	0.00 ± 0.01^{b}	0.00 ± 0.01 ⁶	98.32 ± 0.10 ⁶	70.67 ± 1.53°					
3000 ppm	29.93 ± 0.33^{a}	28.79 ± 0.38°	98.87 ± 0.03 ^a	85.40 ± 0.27^{a}					

Within each column and for each extract, means having the same superscripts are not significantly different at p≤ 0.01

Vanillin

Figs. 1 and 2 show that the growth percentage decreases as the vanillin concentration increases. However, this relation is not linear. The growth percentage of *B. firmus* at 750 ppm is 81.33%, while it falls to 26.35% at 3000 ppm. The same trend is observed in the case of *S. aureus*. In the case of *B. subtilis*, the growth percentage of the culture in the presence 750 ppm is almost equal to 74.35% and falls to 9.57% at 3000 ppm of vanillin.

From Tables (1 and 2), vanillin at low concentrations (750 ppm and 1500 ppm) is ineffective against *Ent. aerogenes, E. coli, M. luteus* and *S. aureus*. The inhibitory effect is against *Ps. aeruginosa* and *Ps. fluorescens*. The inhibition percent increases with the increase of vanillin concentration, being 85.10, 98.32 and 98.87 and 66.34, 70.67 and 85.40%, (at 750, 1500 and 3000 ppm) respectively. Notably, the inhibition percentages for *B. cereus, B. firmus, B. pumilus, B. subtilis, M. luteus, M. varians* and *S. aureus* are 59.64, 74.03, 87.39, 91.54, 22.02, 82.27 and 53.92 at 3000 ppm of vanillin, respectively. These results are in agreement with Fitzgerald *et al.*

(2004) who found the action of vanillin to be bacteriostatic and sometimes bactericidal (Friedman et al., 2002; Ultee, et al., 2002). Moreover, Cerrutti et al., (1997) reported that vanillin was used as an antimicrobial agent in fruit purées, where it inhibited yeast, molds and bacteria at 2000 – 3000 ppm. Fitzgerald et al., (2004) found that the inhibitory activity of vanillin resides primarily in its ability to detrimentally affect the integrity of the cytoplasmic membrane, with the resultant loss of ion gradient, pH homeostasis and inhibition of respiratory activity.

Table (2): The effect of acrylamide, amygdaline, capsicum, furfural and vanillin on the growth of some Gram-positive food borne pathogenic bacteria

<u>F</u>	pathogenic bacteria Inhibition percent							
Item	B.	B.	В.	B.	M.	М.	S.	
	cereus	firmus	pumilus	subtilis	luteus	varians	aureus	
Acrylamide								
500 ppm	12.85 ±	$0.00 \pm$	0.00 ±	28.46 ±	32.14 ±	39.17 ±	31.35 ±	
	1.22ª	0.01 ^a	0.01 ^a	1. 9 2ª	1 19ª	3.96 ^b	2.70 ^b	
1000 ppm	12.85 ±	$0.00 \pm$	0.00 ±	30.38 ±	35.86 ±	45 63 ±	33 24 ±	
	1.17ª	0.01 ^a	0.01 ^a	3.85°	0 01ª	1 25° b	1 35⁵	
2000 ppm	20.09 ±	$0.00 \pm$	0.00 ±	32.12 ±	45.00 ±	49 06 ±	42.43 ±	
, ,	3.74ª	0.01ª	0.01 ^a	0 <u>58</u> ª	5.78 ^a	_0 52ª	0.28ª	
Amygdalin								
3.5 ppm	$0.00 \pm$	$0.00 \pm$	0.00 ±	$0.00 \pm$	0 00 ±	7 5 6 ±	3 03 ±	
	0.01°	0.01 ^a	0.01 ^a	0. 01 ^a	0.01 ^b	3 91ª	0.01 ^a	
50 ppm	5. 51 ±	0.00 ±	0.00 ±	$0.00 \pm$	13.10 ±	9.90 ±	7 06 ±	
	1.30 ^b	0.01 ^a	0.01 ^a	0.01 ^a	1.20 ^a	3.13ª	0 01 ^a	
100 ppm	10.05 ±	0.00 ±	0.00 ±	0.00 ±	14.29 _±	15.63 __ ±	9 41 ±	
	2.11ª_	0.01 ^a	0.01ª	0.01 ^a	1.19 ^a _	2.09 ^a	1 18 ^a	
Capsicum								
50 ppm	0.00 ±	0.00 ±	0.00 <u>±</u>	0.00 ±	0 00 ±	0 0 0 ±	0 00 ±	
	0.01 ^b	0.01 ^a	0.01 ^b	0.01 ^a	0.01 ^b	0.01 ^b	0 01ª	
100 ppm	0.00 ±	0.00 ±	0.00 ±	0.00 ±	0.00 ±	0 00 ±	0 00 ±	
	0.01 ^b	0.01ª	0.01 ^b	0.01 ^a	0.01 ^b	0 01 ⁵	0.01 ^a	
200 ppm	4.21 ±	0.00 ±	11.76 ±	0.00 ±	11.31 ±	13.39 ±	0.00 ±	
_	1.87 ^a	0.01ª	0.14ª	0.01 ^a	1.79ª	1.31ª	0.01 ^a	
Furfural								
250 ppm	0.00 ±	0.00 ±	58.34 ±	10.00 ±	37.05 _±	52.40 ±	0 00 ±	
	0.01 ^b	0.01 ^b	1.24 ^c	0.77 ^c	0.75 ^b	5.31 ^a	0.01 ^b	
500 ppm	0.00 ±	0.00 ±	69.06 ±	21.54 ±	37. 65 ±	56 04 ±	0 00 ±	
	0.01 ^b	0.01 ^b	2. 8 3 ^b	0.39 ^b	0.45 ^b	0.21 ^a	0.01 ^b	
750 ppm	4.05 ±	5.70 ±	87.82 ±	69.77 ±	67 50 ±	59 06 ±	43 64 ±	
	0.45ª	1.32ª	0.01 ^a	5.31ª	3.15ª	1 56ª	0 0 1ª	
Vanillin								
750 ppm	36.40 ±	21.43 ±	69.06 ±	35.38 ±	0.00 ±	58.85 ±	23.35 ±	
	0.01°	1.08°	0.80 ^b	0.39 ^c	0.01 ^b	2.61 ^b	0.01 ^b	
1500 ppm	44.59 ±	54.22 ±	69.21 ±	62.69 ±	0.00 ±	59.43 ±	23.36 ±	
	0.64 ^b	0.82 ^b	1.09 ^b	0.23 ^b	0.01 ^b	1.72 ^b	1.20 ^b	
3000 ppm	59 64 ±	74.03 ±	87.39 ±	91.54 ±	22 02 ±	82.27 ±	53 92 ±	
	0.72 ^a	0.19ª	0.94°	0. 16 ª	0.60 ^a	2.46 ^a	0 14ª	

Within each column and for each extract, means having the same superscripts are not significantly different at $p \le 0.01$

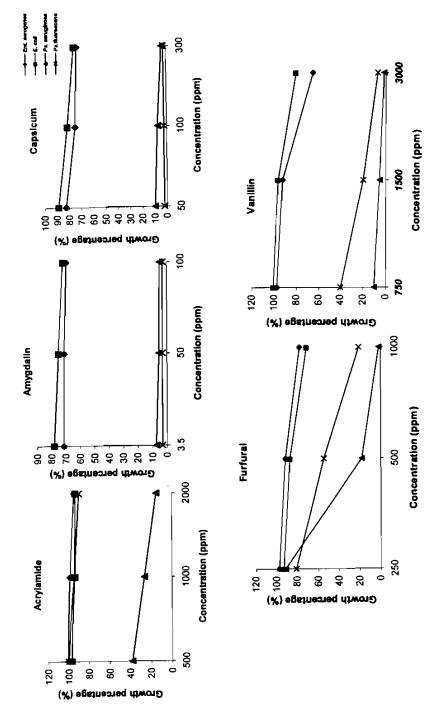


Fig (1): The effect of acrylamide, amygdalin, capsicum, furfural and vanillin on the growth percentage at 12 h of some Gram-negative food borne pathogenic bacteria.

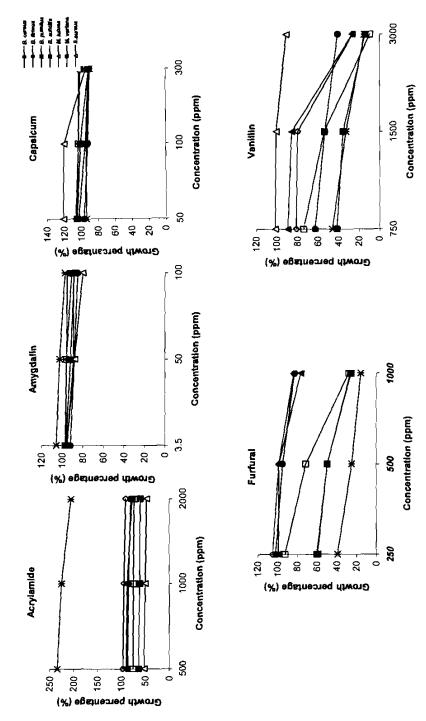


Fig (2): The effect of acrylamide, amygdalin, capsicum, furfural and vanillin on the growth percentage at 12 h of some Gram-positive food borne pathogenic bacteria.

In conclusion, capsicum and vanillin could be used as preservative agents for minimally processed foods.

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تأثير الاكريلاميد والاميجدالين و الكابسيكم و الفرفيورال والفانيلين علسى بعض البكتيريا الممرضة في الغذاء

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درس تاثير كل من الاكسريلاميد والاميجداليين و الكابسيكم و الغرفيورال والفانيلين على نشاط أحدى عشرة سلالـة بكتيرية ممرضة في الغذاء، أربع منها سالبة لجرام والفانيلين على نشاط أحدى عشرة سلالـة بكتيرية ممرضة في الغذاء، أربع منها سالبة لجرام وهي Beudomonas (Ps.) aeruginosa and Ps. fluorescens) وسبع سلالات مسوجبة لجرام وهي Beaillus (B.) cereus, B. firmus, B. pumilus, B. subtilis, Micrococcus (M.) luteus, M. varians and Staphylococcus (S.) وذلك في مرق Brain Heart Infusion محتوي على تركيزات مختلفة من المركبات السالفة الذكر. ومن المعروف أن هذه المركبات تتواجد طبيعيا في بعض المنتجات النباتية أو تتكون أو تضاف أثناء التصنيع الغذائي.

أعتمد تأثير المواد المضافة على التركيز المستخدم وكذا السلالة المختبرة. ثبط كل من الاميجدالين والفانيلين والفرفيورال والكابسيكم نمو السلالات البكتيرية السالبة لجرام (.Ps. fluorescens من أكثر aeruginosa and Ps. fluorescens من أكثر السلالات حساسية تجاه المركبات المختبرة. أما السلالات الموجبة لجرام ومنها ,Pymilus المسلالات الموجبة لجرام ومنها ,Ps. subtilis, M. luteus, M. varians فشبطت عند تركيز ٢٥٠ جزء في المليون فرفيورال و ٢٠٠٠ جزء في المليون فانيلين.

فى ضوء النتائج يمكن التوصية باستخدام الفانيلين و الكابسيكم كمواد حافظة فى الأغذية وخاصة فى الأغذية