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PROTEINASE AND PEPTIDASE SPECIFIC ACTIVITIES OF CRUDE CELL-FREE EXTRACTS OF SOME LACTIC ACID BACTERIAL STRAINS

[38]

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ABSTRACT

Characterization and enzyme specific activities were determined in crude cell-free extracts (CCFEs) acquired from *Str. thermophilus* ATCC 19987, *Lb.casei* ssp. *casei* ATCC 334, *Lb.delbrueckii* ssp. *bulgaricus* ATCC 7995 and *Lb.delbrueckii* ssp. *lactis* ATCC 12315, subjected to heat-shocking (69°C/15 sec), freeze-shocking (-20°C/1 week) and grinding with alumina. Ultrastructural observations by electron microscopy reveal that cell wall physical damage, concomitantly occur with cell autolysis. Treating *Lb. delbrueckii* ssp. *bulgaricus* and *Lb.delbrueckii* ssp. *lactis* with heat-shock resulted in CCFEs having the highest concentration (2.548 and 2.714 mg/ml, respectively) of intracellular protein as compared with those of the other treatments. Analysis of variance revealed significant differences ($p<0.05$) among strains with respect to their specific activities. Caseinolytic specific activities of CCFE produced from *Lb. casei* ssp. *casei* strain, as compared with the other strains. showed the highest values 0.254, 0.197 and 0.272 unit/mg protein, after being treated with heat-, freeze- shock and alumina, respectively. Heat-shocked CCFE of *Str. thermophilus* had the highest dipeptidase specific activity (47.546 unit/mg protein). whereas CCFE of *Lb. casei* ssp. *casei* gained the highest aminopeptidase specific activities (144.169 unit/mg protein) when was heat-shocked at 69°C for 15 sec. heat shocking CCFEs of those strains provides extra sources of ripening enzymes in cheese making technology.

Key words: Enzyme specific activities, Crude cell-free extracts, Lactic acid bacteria, Heat-shocking, Freeze-shocking, Alumina grinding

INTRODUCTION

Lactic acid bacteria (LAB) possess a complex proteolytic system, although they are less proteolytic than other microorganisms such as *Bacillus*, *Proteus*, *Pseudomonas* and coliforms (**Law and Kolstad, 1983; Kamaly and Marth, 1989; Khalid and Marth, 1990 a**). Proteolytic enzymes of the LAB may be localized in the cell wall, the cytoplasmatic membrane, or inside the cell (**Thomas and Pritchard, 1987**), however, this proteolytic activity is dependent on spe-

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cies and strain (**Rajagopal and Sandine, 1990**). The highest proteolytic activity was repated by **Thomas (1985)** for by *Lactobacillus helveticus, Lb.delbrueckii* ssp. *bulgaricus,* and *Lb. acidophilus* of the thermophilic lactobacilli group, followed by *Lb.casei* of the mesophilic lactobacilli group, and then the lactic streptococci of the lactococcus genus. He added that*Streptococcus thermophilus* is only weakly proteolytic compared with *Lactococcus lactis* ssp. *Cremoris.* Thus, in thermophilic starter cultures, *Str. thermophilus* is always combined with one or several members of aminopeptidase producing lactobacilli such as *Lb.helveticus, Lb.delbrueckii* ssp. *bulgaricus,* or *Lb.delbrueckii* ssp. *lactis.* Casein in milk is degraded outside the cell by proteinases, and the resulting peptides and amino acids are transported into the cell. Inside the cell, peptides are degraded to amino acids by aminopeptidases, dipeptidases and other peptide-hydrolysing enzymes (**Frey *et al* 1986 a**). Peptidases also participate in the modification of organoleptic qualities during cheese ripening and hydrolyze bitter peptides that are produced by bacterial proteases, thus decreasing bitterness. Peptidases obviously produce amino acids, which are used as aroma compound precursors. Little information is available about the specific characteristics of proteolytic enzymes of thermophilic lactic acid bacteria (**Tsakalidou and Kalantzopoulos, 1992**), and very few attempts were made to evaluate their enzymes in a cheese system (**Fox, 1993**).

As more studies confirm the proteolytic ability of LAB, it becomes important to study the specific characteristics of the proteolytic enzymes of thermophilic lactic acid bacteria and to evaluate these enzymes among a cheese system. Therefore, this study was implemented to study the effect of heat-, and freeze- shocking and grinding with alumina on dipeptidase, aminopeptidase, endopeptidase and the caseinolytic specific activity of crude cell-free extracts (CCFEs) obtained from some LAB strains.

MATERIAL AND METHODS

Lactic acid bacterial Strains

Lactobacillus delbrueckii ssp.*lactis* ATCC12315, *Lb.delbrueckii* ssp. *bulgaricus* ATCC7995, *Lb.casei* ssp. *casei* ATCC 334, and *Streptococcus thermophilus* ATCC 19987 were obtained lyophilized from the culture collection of Cairo Mircen (Microbiological Resources Center), Fac. of Agric., Ain Shams University. Each lyophilized strain was resuspended in 10% (w/v) sterile reconstituted skim milk powder at 37°C for 24 h. Cultures were individually maintained by subculturing in MRS broth (Oxoid) (**DE Man *et al* 1960**) containing 10% glycerol (**Khalid and Marth, 1990b**), and stored at -20°C until use. Frozen cultures were thawed and transferred in MRS broth before use.

Cultivation and harvesting

The four LAB strains were separately subcultured in MRS broth. After an appropriate incubation period of 40 h at 37°C, the cells were harvested by centrifugation at 6000 rpm for 15 min at 4°C and washed twice in a 0.01 M cold sterilized potassium phosphate buffer (pH 7.0 at 7°C). Different volumes of the washed cell pellets were resuspended in diluted buffer and monitored by measuring their

absorbency at 650 nm using a Spekoll 11 Colorimeter until the same optical density for all strains was obtained (**El-Soda *et al* 1978 a and b**).

Preparation of the crude cell-free extracts (CCFEs)

Cells crop of each strain was divided into three portions to extract the intracellular enzymes. The first and second portions were subjected to heat-(69°C/15 sec) and freeze- (-20°C/1 week) shocking, respectively as described by **Frey *et al* (1986 b)**. The third portion was ground with alumina powder (type F-20) (**El-Soda *et al* 1991**). The cells after treatment were examined by phase contrast microscopy to determine the efficiency of cells disruption. Each suspension was centrifuged at 6000 rpm for 30 min at 4°C to remove intact and debris cells and alumina. The supernatants (CCFEs) which contained the intracellular enzymes were collected, and stored at -20°C until use.

Morphological damage electron microscopy

The untreated cells and those subjected to heat- and freeze- shock and grinding with alumina were examined by a Jeol Scanning Electron Microscope (JSM-T 330 A) for morphological damage. Methods of fixing and staining of cells were applied according to **Hayat (1986)**. The treated and untreated cells were fixed with glutaraldehyde, then dehydrated using a series of various gradations of ethanol.

Examination of the CCFEs

The total protein concentrations were estimated according to the method of **Lowry *et al* (1951)**. Caseinolytic activity measured using the ninhydrin reaction (**Moore and Stein, 1954**) and Dipeptidase activity (**El Soda and Desmazeaud, 1982**). A unit of caseinolytic and dipeptidase activities were defined as that amount of enzyme producing 1 µ mole and 1 nmole of tyrosine/min, respectively. Aminopeptidase activity was assayed according to **El-Soda *et al* (1978 b)**. The concentration of P-nitroaniline was calculated as reported by **Pfleiderer (1970)**. Endopeptidase activity was assayed as mentioned by **El-Soda *et al* (1978 b)**. A unit of amiopeptidase and endopeptisase activities were defined as the amount of enzymes producing 1 µmole of P-nitroaniline/h. Specific activity was defined as the number of enzyme units per mg protein of the CCFE.

Statistical analysis:

All data were analyzed by the general linear models(GLM) procedures of SAS (**1989**). Least significant difference (LSD) was performed to determine differences in means at $p<0.05$.

RESULTS AND DISCUSSION

Electron microscopy

Figures (1 a, b and c) are electron micrographs of untreated cells and cells subjected to heat-, and freeze- shocking of *Str. thermophilus* ATCC 19987. Figure (1a) shows that untreated harvested cells appeared spherical with smooth surface. However, cells sublethally heat-shocked suffered severe damage of cell wall and cell membrane with conspicuous leakage of cell materials (Fig. 1b). Some cells appeared not to suffer complete rupture.

Moreover, cells of *Str. thermophilus* ATCC 19987 subjected to freeze-shocking were extremely wrinkled with obvious cell membrane damage showing naked cytoplasmic bodies. Some cells appeared to have been lysed (Fig. 1c).

Figures (2, 3 and 4) are electron micrographs of *Lb.casei* ssp. *casei* ATCC 334, *Lb.delbrueckii* ssp. *bulgaricus* ATCC 7995 and *Lb. delbrueckii* ssp. *lactis* ATCC 12315, respectively. The electron micrographs show that untreated whole cells appeared to be long rods with smooth membrans (Figs. 2a, 3a and 4a). However, heat-shocked (Figs. 2b, 3b and 4b) *Lb. casei* ssp. *casei*, *Lb. delbrueckii* ssp. *bulgaricus* and *Lb. delbrueckii* ssp. *lactis* strains appeared to have damaged cell walls as a results of the heat treatment. The optimum treatment varied within the temperature ranges from 56°C to 70°C, and heating times from 15 to 22 sec. Similar results were reproted by **Frey *et al* (1986 b); Kamaly and Marth (1988).** They found that sublethally heat-treated strains of lactic streptococci and lactobacilli (*Str. lactis* 25 SP, *Str. cremoris* KHA2 and *Lb.helveticus* (CNRZ32) at 69°C for 15 sec suffered severe damage of cell walls and cell membranes although not all cells showed complete rupture.

Autolysis of some cells appearantly occurred. Ultrastructural observations by electron microscopy revealed that cell wall physical damage occur concomitantly with cell autolysis. **El-Soda (1993 and 1996)** explained that the higher less autolysis of the heat-shocked cells was due to the denaturation of their autolytic system by heat. An increase in autolysis was observed during exponential growth phase reaching its maximum activity before transition to the stationary phase.

Cells of freeze-shocking at -20°C and then thawed at room temperature (20-25°C) appeared to has a cell wall not as smooth as those of untreated cells. The cells appeared as also wrinkled, irregular, with a damaged membrane (Figs. 2c, 3c and 4c). **Ray *et al* (1973)** reported similar results showing that freezing of bacterial cells of lactic streptococci at suboptimal temperature could injure the cell wall and membrane, inducing cell lysis. **Frey *et al* (1986 b)** reported that LAB were generally susceptible to damage during freezing and attributed the damage of cell walls to the slow growth of ice crystals.

Treated cells with alumina could not be detected by Scanning Electron Microscopy because of some difficulties of applying such technique.

Intracellular protein concentration

Table (1) shows the intracellular protein concentration in CCFEs obtained from different lactic acid bacterial strains subjected to different treatments. Statistical analysis reveals that both type of strain and treatment significantly ($p<0.05$) affected the intracellular protein concentration of CCFEs. The heat-shock of *Lb.delbrueckii* ssp. *lactis* resulted in a CCFE having the highest intracellular protein concentration (2.714 mg/ml) as compared with those of the other strains. The intracellular protein concentration of the different strains had the following decreasing order : *Lb.delbrueckii* ssp. *lactis*, *Lb. delbrueckii* ssp. *bulgaricus*, *Lb. casei* ssp. *casei*, and *Str. thermophilus*.

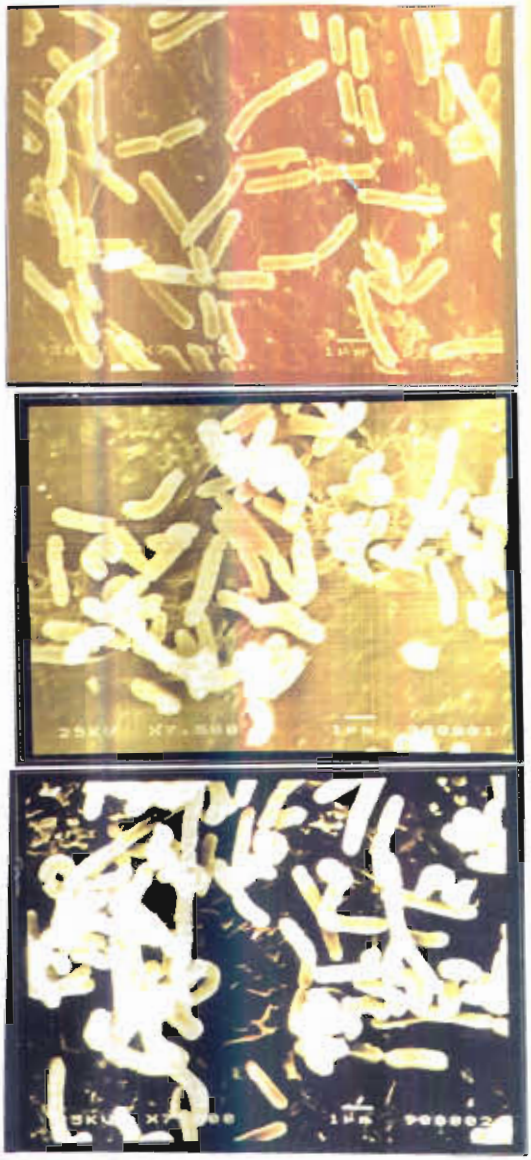
The freeze-shock of *Lb.delbrueckii* ssp. *bulgaricus* resulted in a CCFE contining the highest of intracellular protein concentration (2.197 mg/ml) as compared

(a)

(b)

(c)

Fig. 1. Electronmicrograph of *Str. thermophilus* ATCC 19987 (a) untreated cells, (b) heat-shocked cells, (c) freeze-shocked cells. X 7500.



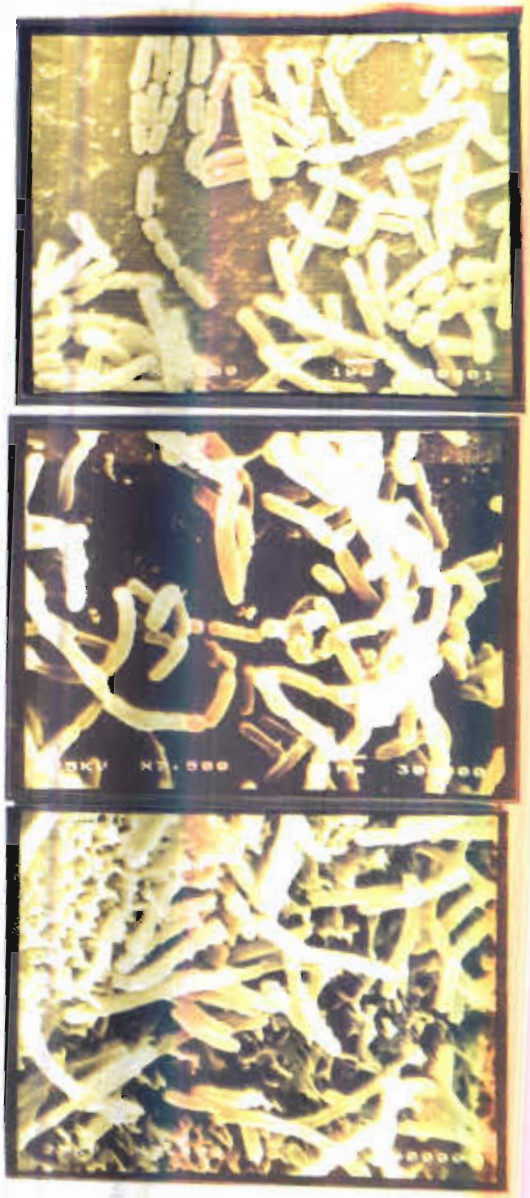
(a)

(b)

(c)

Fig. 2 Electronmicrogaph of *Lb. casei* ssp. *casei* ATCC 334. (a) untreated cells, (b) heat-shocked cells, (c) freeze-shocked cells X 7500.

Fig. 3. Electronmicrograph of *Lb. delbrueckii* spp. *bulgaricus* ATCC 7995. (a) untreated cells, (b) heat-shocked cells, (c) freeze-shocked cells. X 7500.



(a)

(b)

(c)

Fig 4 Electronmicrograph of *Lb. delbrueckii* spp *lactis* ATCC 12315. (a) untreated cells, (b) heat-shocked cells, (c) freeze-shocked cells X 7500.

Table 1. Intracellular protein concentration in crude cell-free extracts obtained from different lactic acid bacterial strains subjected to different treatments

Strains	Treatments		
	Heat-shock	Freeze-shock	Grinding with alumina
	(mg/ml)*		
Str.thermophilus	1.449^{Dc}	1.913^{Ca}	1.591^{Cb}
Lb.casei ssp. *casei*	1.772^{Cb}	2.108^{Ba}	1.562^{Dc}
Lb.delbrueckii ssp. *bulgaricus*	2.548^{BAa}	2.197^{Ab}	2.089^{Ac}
Lb.delbrueckii ssp. *lactis*	2.714^{Aa}	1.843^{Dc}	1.874^{Bb}

* Values are averages of 20 analyses.
- Means with the same capital letter in the same column are not significantly different at $p<0.05$.
- Means with the same small letter in the same row are not significantly different at $p<0.05$.

with other strains. The CCFE of freeze-shocked *Lb.delbrueckii* ssp. *lactis* showed the lowest intracellular protein concentration (1.843 mg/ml).

Crude cell-free extract produced from *Lb. delbrueckii* ssp. *bulgaricus* treated with alumina had the highest concentration (2.089 mg/ml) of intracellular protein compared with those of the other strains. Treating *Lb. delbrueckii* ssp. *bulgaricus* and *Lb.delbrueckii* ssp. *lactis* with heat-shock resulted in CCFEs containing the highest concentration of intracellular protein as compared with those of the other treatments (Table, 1). Treating *Lb. delbrueckii* ssp. *bulgaricus* and *Lb.casei* ssp. *casei* with freeze-shock resulted in CCFEs having the highest concentration of intracellular protein 2.197 a[illegible]2.108 mg/ml, respectively, as compared with those of the other treatments.

Similar observations have been reported for other lactobacillus strains by previous investigators (**Eggimann and Bachmann, 1980; Arora and Lee, 1990**).

Enzyme specific activities

Caseinolytic, dipeptidase, aminopeptidase and endopepti-dase activities of crude cell-free extracts (CCFEs) obtained

from *Str.thermophilus* ATCC 19987, *Lb.casei* ssp. *casei* ATCC 334, *Lb.delbrueckii* ssp. *bulgaricus* ATCC 7995 and *Lb.delbrueckii* ssp. *lactis* ATCC 12315 strains treated with heat-, freeze-shock and alumina were measured. Specific activities (unit/mg protein of ml CCFEs) were also calculated (Tables 2, 3, 4 and 5).

1. Caseinolytic specific activity

Table (2) shows the caseinolytic specific activity of crude cell-free extracts (CCFEs) obtained from *Str.thermophilus, Lb. casei* ssp. *casei, Lb.delbrueckii* ssp. *bulgaricus* and *Lb. delbrueckii* ssp. *lactis* strains treated with heat-, freeze- shock and alumina. The caseinolytic specific activities of CCFE produced from *Lb.casei* ssp. *casei* strain was significantly higher ($p<0.05$), compared with the other strains, after being treated with heat-, freeze- shock and alumina.

All alumina treated strains exhibited significantly higher ($p<0.05$) caseinolytic specific activities, being 0.149, 0.272, 0.198 and 0.188 for *Str.thermophilus, Lb.casei* ssp. *casei, Lb.delbrueckii* ssp. *bulgaricus,* and *Lb.delbrueckii* ssp. *Lactis,* respectively. Heat-shock showed to come next while freeze-shock had a little effect on the strains (Table, 2).

The recorded caseinolytic specific activities are in agreement with those reported by **Thomas (1985)** for *Lb. helveticus, Lb. bulgaricus* and *Lb.acidophilus* of the thermobacterial group, *Lb.casei* of the streptobacterial group, and then the lactic streptococci genius of the *Lactococcus.* Also, **El-Soda *et al* (1978 a)** found that *Lb.casei* NCDO151 strain had a higher caseinolytic specific activity as compared with *Lb.casei* ATCC 7469, CNRZ K6, CNRZ 57G and IAM 1043 strains.

2. Dipeptidase specific activity

Dipeptidase specific activities of CCFEs obtained from *Str. thermophilus, Lb. casei* ssp. *casei* , *Lb. delbrueckii* ssp. *bulgaricus* and *Lb.delbrueckii* ssp. *lactis* strains treated with heat-, freeze-shock and alumina are presented in Table (3).

The heat-shock of *Str.thermophilus* produced a CCFE having the highest dipeptidase specific activity ($p<0.05$) as compared with those of the other strains. However, CCFE of heat-shocked *Lb.delbrueckii* ssp. *lactis* had the lowest dipeptidase specific activity ($p<0.05$). As mentioned in Table (3), CCFE of *Lb.delbrueckii* ssp. *lactis* treated with freeze-shock had the highest value (31.273 unit/mg protein) of dipeptidase specific activity as compared with those of the other strains. While, the lowest (19.952 unit/mg protein) dipepetidase specific activity was observed in CCFE of freeze-shocked *Str.thermophilus. Lactobacillus casei* ssp. *casei* treated with alumina produced CCFE with the highest dipeptidase specific activity as compared with those obtained from the other strains. Furthermore, treating *Lb. delbrueckii* ssp. *bulgaricus* with alumina produced a CCFE with the lowest value of dipeptidase specific activity.

Dipeptidase specific activity was significantly higher ($p<0.05$) for heat-shocked CCFE produced from *Str. thermophilus* as compared with those of the other treatments. Moreover, *Lb.casei* ssp. *casei* treated with alumina resulted in CCFE having the highest dipeptidase specific activity than the other treatments.

Table 2. Caseinolytic specific activity of crude cell-free extracts obtained from different lactic acid bacterial strains subjected to different treatments

Strains	Treatments		
	Heat-shock	Freeze-shock	Grinding with alumina
	(unit/mg protein)*		
Str.thermophilus	0.142^{Cab}	0.110^{Dc}	0.149^{Ca}
Lb.casei ssp. *casei*	0.254^{Ab}	0.197^{Ac}	0.272^{Aa}
Lb.delbrueckii ssp. *bulgaricus*	0.189^{Bb}	0.180^{Bb}	0.198^{Ba}
Lb.delbrueckii ssp. *lactis*	0.179^{Bb}	0.168^{Cc}	0.188^{Ba}

* Values are averages of 20 analyses.
- Means with the same capital letter in the same column are not significantly different at $p<0.05$.
- Means with the same small letter in the same row are not significantly different at $p<0.05$.

Table 3. Dipeptidase specific activity of crude cell-free extracts obtained from different lactic acid bacterial strains subjected to different treatments

Strains	Treatments		
	Heat-shock	Freeze-shock	Grinding with alumina
	(unit/mg protein)*		
Str.thermophilus	47.546^{Aa}	19.952^{Cb}	17.177^{Cb}
Lb.casei ssp. *Casei*	37.021^{Bb}	25.648^{Bc}	46.341^{Aa}
Lb.delbrueckii ssp. *bulgaricus*	27.883^{Cab}	25.239^{Ba}	16.534^{Cc}
Lb.delbrueckii ssp. *lactis*	17.591^{Dc}	31.273^{Aa}	28.513^{Bb}

* Values are averages of 20 analyses.
- Means with the same capital letter in the same column are not significantly different at $p<0.05$.
- Means with the same small letter in the same row are not significantly different at $p<0.05$.

Treating *Lb. delbrueckii* ssp. *bulgaricus* and *Lb. delbrueckii* ssp. *lactis* with freeze-shock resulted in CCFEs having the highest dipeptidase specific activities as compared with those of the other treatments (heat-shock and grinding with alumina). As shown in Table (3), there are significant effects ($p<0.05$) of all strains and treatments on the dipeptidase specific activities of CCFEs.

Similar results were also reached by **Arora and Lee (1990)** when they noticed that CCFEs from *Lb.casei* ssp. *casei* ATCC 393 and LLG strains contained superior activities against all the dipeptidase compared with the other species of *Lb.casei* (*Lb. casei* ssp. *rhamnosus* ATCC 15820, S93 and *Lb.casei* ssp. *pseudoplantarum* L2F and 83.4). **Frey *et al* (1986)** mentioned that *Lb.bulgaricus*, *Lb.casei* and *Lb.lactis* strains possess dipeptidase activities. Also, **El-Soda *et al* (1978 b)** revealed that CCFE of *Lb.casei* NCDO 151 hydrolyzed various dipeptides and showed the presence of one or more dipeptidase-like enzymes. **Rabier and Desmazeaud (1973)** reported that dipeptidase of *Lb. casei* is similar to that of *Str. thermophilus* and attacks a great number of dipeptides.

3. Aminopeptidase specific activity

Aminopeptidase specific activity detected in the four CCFEs of lactic acid bacterial strains treated with heat-, freeze-shock and alumina are presented in (Table, 4). The heat-shocked *Lb.casei* ssp. *casei* resulted in a CCFE having the highest ($p<0.05$) aminopeptidase specific activity as compared with those of the other strain. While, CCFE of heat-shocked *Lb. delbrueckii* ssp. *lactis* had the lowest ($p<0.05$) aminopeptidase specific activity value. The freeze-shocked *Lb. delbrueckii* ssp. *bulgaricus* produced a CCFE with the highest value of aminopeptidase specific activity (151.494 unit/mg protein) as compared with those of the other strains. However, CCFE of freeze-shocked *Str.thermophilus* showed the lowest aminopeptidase specific activity (13.603 unit/mg protein). CCFE obtained from *Lb.casei* ssp. *casei* by treating with alumina showed the highest aminopeptidase specific activity as compared with those of the other strains. Furthermore, treating *Str. thermophilus* with alumina produced a CCFE with the lowest aminopeptidase specific activity.

Heat-shocked *Str. thermophilus* and *Lb. casei* ssp. *casei* resulted in CCFEs having the highest ($p<0.05$) aminopeptidase specific activities as compared with those of the other treatments (freeze-shock and grinding with alumina). Moreover, *Lb. delbrueckii* ssp. *bulgaricus* treated with freeze-shock resulted in CCFE having the highest ($p<0.05$) minopeptidase specific activity than the other treatments. Grinding *Lb. delbrueckii* ssp. *lactis* with alumina resulted in CCFE having the highest ($p<0.05$) aminopeptidase specific activity as compared with those of the other treatments.

The results of Aminopeptidase specific activity we studied are similar to those found by **Sienkiewicz *et al* (1996)** they revealed that the highest prolyl-aminopeptidase activity was detected in *Lb. casei* as compared with *Lb. delbrueckii* ssp. *bulgaricus*, *Lb. acidophilus*, *Lb. helveticus*, *Lb. delbrueckii* ssp. *lactis* and *Str. thermophilus*. **Lopez-Fandino and Ardo (1991)** also demonstrated high aminopeptidolytic activities of *Lb. delbrueckii* ssp. *bulgaricus* against peptides

Table 4. Aminopeptidase specific activity of crude cell-free extracts obtained from different lactic acid bacterial strains subjected to different treatments

Strains	Treatments		
	Heat-shock	Freeze-shock	Grinding with alumina
	(unit/mg protein)*		
Str.thermophilus	52.386^{Ca}	13.603^{Db}	7.869^{Dc}
Lb.casei ssp. *Casei*	144.169^{Aa}	83.593^{Bc}	134.502^{Ab}
Lb.delbrueckii ssp. *bulgaricus*	64.648^{Bb}	151.494^{Aa}	55.494^{Cc}
Lb.delbrueckii ssp. *lactis*	30.878^{Dc}	54.511^{Cb}	81.525^{Ba}

* Values are averages of 20 analyses.
- Means with the same capital letter in the same column are not significantly different at $p<0.05$.
- Means with the same small letter in the same row are not significantly different at $p<0.05$.

with terminal amino acids such as leucine, arginine or proline, which occur at high frequency in bitter peptides. Moreover, **Meyer and Spahni (1996)** revealed that *Lb. delbrueckii* ssp. *lactis* has a very high x-proline dipeptidyl- aminopeptidase (DPAP) activity.

4. Endopeptidase specific activity

Data presented in Table (5) show the endopeptidase specific activity of CCFEs obtained from the four lactic acid bacterial strains subjected to heat-, freeze-shock and alumina treatments. CCFEs of heat-, freeze- shocked and ground with alumina *Lb. delbrueckii* ssp. *bulgaricus* contained significantly the highest ($p<0.05$) endopeptidase specific activities compared with those of the other strains. However, CCFEs of heat-, freeze-shocked and ground with alumina *Lb. casei* ssp. *casei* showed significantly the lowest ($p<0.05$) endopeptidase specific activities.

Treating *Str. thermophilus* with heat-shock resulted in a CCFE having the highest endopeptidase specific activity as compared with those of the other treatments. Moreover, *Lb.casei* ssp. *casei*, *Lb.delbrueckii* ssp. *bulgaricus* and *Lb.delbrueckii* ssp. *lactis* treated with freeze-shock resulted in CCFEs having the highest endopeptidase specific activities than the other treatments (heat-shock and grinding with alumina). The effects of both type of strain and treatment were significant ($p<0.05$) on the endopeptidase specific activcity of CCFEs (Table 5).

Table 5. Endopeptidase specific activity of crude cell-free extracts obtained from different lactic acid bacterial strains subjected to different treatments

Strains	Treatments		
	Heat-shock	Freeze-shock	Grinding with alumina
	(unit/mg protein)*		
Str.thermophilus	172.475^{Ba}	108.660^{Cbc}	110.255^{Bb}
Lb.casei ssp. *Casei*	90.734^{Dab}	91.035^{Da}	74.210^{Dc}
Lb.delbrueckii ssp. *bulgaricus*	228.556^{Ab}	331.134^{Aa}	171.353^{Ac}
Lb.delbrueckii ssp. *lactis*	117.537^{Cb}	210.726^{Ba}	84.874^{Cc}

* Values are averages of 20 analyses.
- Means with the same capital letter in the same column are not significantly different at $p<0.05$.
- Means with the same small letter in the same row are not significantly different at $p<0.05$.

These results are in agreement with those reported by **Chandan *et al* (1982).** They found that *Lb. delbrueckii* ssp. *bulgaricus* had an endopeptidase activity to hdyrolyze β-casein. Moreover, an endopeptidase activity was detected in *Lb. casei* NCDO 151 strain by **EL-Soda *et al* (1978 b).**

Finally, these results reveal that type of strain and treatment applied, obviously had significant effect on the intracellular protein concentration; caseinolytic, dipeptidase and endopeptidase specific activities of CCFEs produced from *Str. thermophilus* ATCC 19987, *Lb. casei* ssp. *casei* ATCC 334, *Lb. delbrueckii* ssp. *bulgaricus* ATCC 7995 and *Lb. delbrueckii* ssp. *lactis* ATCC 12315. The obtained results will be helpful in selecting the best treatment and enzyme specific units to be used for accelerating Ras cheese ripening.

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مجلة حوليات العلوم الزراعية ، كلية الزراعة ، جامعة عين شمس ، القاهرة ، م٤٨ ، ع(٢)، ٥٤٣ - ٥٦٠ ، ٢٠٠٣

الأنشطه النوعيه لانزيمات البروتينيز والببتيديز فى المستخلصات الانزيميه لبعض سلالات بكتريا حمض اللاكتيك

[٣٨]

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تم دراسة الخواص المميزة والأنشطة النوعية للأنزيمات فى المستخلصات الأنزيمية المتحصل عليها من سلالات : *Str. thermophilus* ATCC 19987 ، *Lb. casei* ssp. *casei* ATCC 334 ، *Lb. delbrueckii* ssp. *bulgaricus* ATCC 7995 ، *Lb. delbrueckii* ssp. *eactis* ATCC 12315 المعامله بالصدمة الحرارية (٦٩°م /١٥ ث) ، صدمة التجميد (- ٢٠ °م / أسبوع) ، الطحن بألومنيا .

أوضحت الصور الفوتوغرافية المتحصل عليها بالميكروسكوب الإلكترونى الماسح حدوث تحطيم وتحلل لجدر الخلايا المعامله، وأن أعلى قيم لتركيز البروتين الداخلى للخلايا تم الحصول عليها فى المستخلصات الإنزيمية المتحصل عليها من سلالات *Lb. delbrueckii* ssp. *bulgaricus* , *Lb. brueckii* ssp. *lactis* المعامله بالصدمة الحرارية (٢,٥٤٨ ، ٢.٧١٤ ملجم /مل على التوالى) وذلك بالمقارنة بالمعاملات الأخرى . ايضا أوضحت التحليلات الإحصائية أن نوع السلالة قد أدى إلى حدوث أختلافات معنوية ($p<0.05$) فى النشاطات النوعية لها. كذلك تميزت المستخلصات الإنزيمية المتحصل عليها من السلالة *Lb casei* ssp. *casei* المعاملة بالصدمة الحرارية وصدمة التجميد والطحن بالألومينا بأعلى قيم للنشاط النوعى للأنزيمات المحلله للكازين حيث أعطت قيم ٢٥٤, ، ١٩٧, ، ٢٧٢, وحده/ملجم بروتين على التوالى وذلك بالمقارنة بالسلالات الأخرى. كما تم الحصول على أعلى قيم للنشاط النوعى لإنزيمات الداى ببتيديز (٤٧.٥٤٦ وحده/ملجم بروتين) ، والامينوببتيديز (١٦٩.١٤٤ وحده/ملجم بروتين) فى المستخلصات الإنزيمية المتحصل عليها من سلالات *Str. thermo-philus* , *Lb casei* ssp *lactis* المعاملة بالصدمة الحرارية على التوالى .

مما سبق يتضح أن أنسب طريقة للحصول على مصدر جيد للإنزيمات لاستخدامها من السلالات التى تمت دراستها فى تسوية الجبن هى تلك المستخلصات الإنزيمية الناتجة من سلالات معاملة بالصدمة الحرارية .

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