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Extraction of Serine Protease from *Balanites aegyptiaca* Pulp with Potential Applications in Cheesemaking

Robert Germain Beka[1,2*], Robert Adjia[1], François Krier[2], Marie-Christine Slomianny[3] and Dominique Vercaigne-Marko[2]

1Department of Food Science and Nutrition, National School of Agro-Industrial Sciences (ENSAI), Ngaoundere University, Ngaoundéré, Cameroon
[2]Laboratory of Biological Processes, Enzymatic and Microbial Engineering (ProBioGEM), IUT "A", Polytech'Lille, University of Science and Technology of Lille1, Cité Scientifique, Villeneuve d´Ascq, France
[3]Structural and Functional Glycobiology Laboratory, UGSF-UMR CNRS / USTL 8576, Building C9, University of Science and Technology of Lille1, 59650 Cité Scientifique, Villeneuve d´Ascq, France

***Corresponding author:** Robert Germain Beka, Department of Food Science and Nutrition, National School of Agro-Industrial Sciences (ENSAI), Ngaoundere University, Ngaoundéré, Cameroon, Tel: 00 237 677 08 55 08, E-mail: bekarobertger2004@yahoo.fr

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Abstract

The extract of *Balanites aegyptiaca* pulp possesses proteases. The present work has been carried out to extract serine proteases from *Balanites aegyptiaca* pulp. Crude extract was prepared by soaking *Balanites aegyptiaca* pulp in 50 mM Tris-HCl buffer (pH 8.1), containing 1 mM EDTA and 3 mM β-mercaptoethanol. Serine proteases were isolated using ion exchange chromatography. Their specific activity was determined using synthetic substrate, gelatin and caseins as well as remaining activity after inhibitors treatment. The SDS-PAGE of purified extract exhibited 28, 72 and 85 kDa bands. However, only 72 and 85 kDa were active on gelatin. α-, β- and κ-caseins were hydrolyzed by purified extract. It completely inhibited by specific inhibitors (phenylmethane sulphonyl fluoride and aprotinine), suggesting the presence of serine protease in the extract. Synthetic substrate (N-Suc-Ala2-Pro-Phe-pNA) has been hydrolyzed by purified extract. This may indicate that serine protease isolated was chymotrypsin-like. These results suggest that extract of *Balanites aegyptiaca* pulp contains serine protease potentially use for cheesemaking.

Keywords: *Balanites aegyptiaca*; Extraction; Chromatography; Serine Protease; Chymotrypsin-Like

Introduction

Proteases, hydrolase protein are used in dairy and meat technologies, in human health and pharmacology, detergent and textile industries. They are aspartic proteases (EC 3.4.23), cysteine proteases (EC 3.4.22), metalloproteases (EC 3.4.24) and serine proteases (EC 3.4.21). The sources of proteases are animal, microbial and plant. Patel, *et al.* [1] indicated that natural proteases from plant are suitable for pharmaceutical and food industries. This confirms the growing requirement of plant proteases. Milk-coagulating extract from several fruits with potential applications in cheesemaking has been identified [2-4]. Plant coagulants have a strong coagulant activity and are accessible for farmers [5,6]. Their use in cheese manufacturing is mainly driven by the chronic shortages in rennet, an enzyme extracted from the stomach of young calves [7-9]. Among them, plant serine proteases are the most stable and effective for industrial applications [10]. So, the extraction of plant serine proteases from novel sources is always of interest. Serine proteases are present in all parts of plant. However, they are more abundant in the fruits [11]. Serine proteases have been purified from fruits of *Cucumis trigonus Roxburghi*, *Cucumis melo L. var.* Prince, *Benincasa cerifera, Trichosantus kirrilowi* A., *Trichosantus cucumeroides* and *Solanum dubium* [12-17]. Previous study has shown that *Balanites aegyptiaca* fruits extract possess a coagulating properties [18]. *B. aegyptiaca* is a tropical plant which demands and interest is growing by the day. It is a woody tree growing in Northern Cameroon and is distributed in other arid and semi-arid areas. It is used for various needs such as food, fodder, medicines, charcoal and pesticides according to the part of tree used. The fruits of this plant, so-called "*desert date*" are the part which is mostly used. "Desert date" is described to be rather long in shape and narrow drupe. Unripe fruits are green, turning yellow and glabrous once ripe. The seed is light brown, fibrous and extremely hard. The almond is rich in saturated fatty acids, that fat matter is used as cooking oil. It also contains steroids (saponins, sapogenins, diosgenins) used as raw material for drug [19]. The pulp of ripe fruits is bitter-sweet and consumed raw as sweet by local population and cooked in mush. Extract of *Balanites aegyptiaca* pulp is also associated with proteolytic activity. Details about the class of *Balanites aegyptiaca* proteases remained unknown. Therefore, the present study aims to extract and characterize proteases from fruits of *Balanites aegyptiaca*.

Methods

Preparation of Crude Extracts

B. aegyptiaca fruits (180 g) were soaked in 500 mL of 50 mM Tris-HCl buffer (pH 8.1), containing 1 mM EDTA and 3 mM β-mercaptoethanol overnight at 4 °C. Extract were centrifuged at 4 °C for 30 min at 10,000xg to eliminate the insoluble materials. The supernatant was concentrated approximately three times through a 10,000 Da cut off membrane (Millipore). The obtained solution was crude extract which kept at -20 °C until analysis.

Purification of extract

Crude extract was treated by procedure described by Llorente, *et al.* [20], 10% (w/v) of activated charcoal were mixed with crude extract and kept on ice for 30 min and then mixture was centrifuged at 10,000xg for 60 min at 4 °C. Supernatant was submitted to strong ion exchange chromatography using the HI-LOAD 16/10 Q Sepharose Fast Flow column (Pharmacia). The column was equilibrated with 50 mM Tris-HCl buffer (pH 8.1), containing 1 mM EDTA and 3 mM β-mercaptoethanol. Elution was performed with the same buffer containing 1 M NaCl at a flow rate of 0.5 mL.min-1 under landing gradient from 0 to 1 M. The protein peaks were detected by the absorbance at 280 nm and fractions were collected at the outlet stream. The proteolytic and milk-clotting activities of each protein fraction were determined.

Milk clotting activity

The substrate was prepared by dissolving skimmed milk powder in 10 mM $CaCl_2$ to a final concentration of 12% (w/v). The substrate (1 mL) was pre-incubated for 5 min at 37 °C and 0.1 mL of extract was added according to Arima, *et al.* [21]. The milk-clotting unit (MCU) was defined as the amount of protein that coagulates reconstituted milk during 40 min at 37 °C. The calculation is $MCU = 2400 * V / t * v$, where V equals the volume of milk (mL), v the volume of extract (mL) and t the clotting time in seconds.

Protease assay

The proteolytic activity of extracts was assayed on azocasein following the method described by Sarath, *et al.* [22] with slight modifications. Aliquots (0.15 mL) of purified extracts were added to 0.25 mL of a 2% (w/v) solution of azocasein in 50 mM Tris-HCl buffer (pH 8.1). Each mixture was then incubated at 37°C for 60 min. The hydrolysis was stopped by adding 1.2 mL of 10% trichloracetic acid. After 15 min standing at room temperature to ensure complete precipitation of remaining azocasein, the samples were centrifuged at 8,000 g for 5 min. 1.2 mL of the supernatant were mixed with 1.4 mL of 1M NaOH and the absorbance was measured immediately at 440 nm. Protein concentration was determined according to the method described by Bradford [23] using bovine serum albumin as standard. Samples were incubated at room temperature, and absorbance was read at 595 nm.

SDS-polyacrylamide gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done, using the method of Laemmli [24], with 15% acrylamide separating gel and 3% acrylamide stacking gel containing 0.1% SDS. Samples were prepared in a Tris–glycine buffer (pH 8.8) containing 1% SDS. Electrophoresis was performed in a Miniprotean II cell (Bio-Rad) at a current of 100 V in electrophoretic Tris–glycine buffer containing 0.1% SDS. After electrophoresis, the gel sheets were stained for proteins with 0.25% (w/v) Coomassie brilliant blue-R250 in methanol/acetic acid/water (5:1:4). Protein stain was destained with methanol/acetic acid/water (2.5:1:6.5) solution.

Activity gel electrophoresis

The protease activity on gel was carried out according to Laemmli [24], activity was detected by adding heat denaturated gelatin 1% (w/v), final concentration to the acrylamide solution prior to polymerisation of the gel. After electrophoresis, the gel was washed three times, during 15 min in 50 mM Tris-HCl (pH 7) containing 2.5% (V/V) Triton 100X, and incubated overnight, at 37 °C in 50 mM Tris-HCl (pH 7). Gels were stained and destained as previously described.

Hydrolysis of caseins

Commercial bovine α-, β- and κ-caseins (2 mg) were dissolved in 1 mL of 100 mM sodium phosphate buffer (pH 6.2), and treated with purified extract (enzyme-substrate rate 1:100) at 30 °C for 1h, 2h, 4h and 24h. The reaction was quenched via addition of electrophoresis buffer containing β-mercaptoethanol [25]. The samples of hydrolysates were analyzed by SDS-PAGE as described previously.

Effect on synthetic peptides

Protease activity was determined by measuring p-nitroaniline liberation from the two chromogenic synthetic substrates: N-Suc-Ala-Ala-Ala-pNA which is the specific substrate for élastase and N-Suc-Ala-Ala-Pro-Phe-pNA specific substrate for chymotrypsin-like serine protease. Substrates were dissolved in dimethyl sulphoxide (DMSO) to give stock solutions of 20 mM. The assay was performed in a total reaction mixture of 1.5 mL. Two different substrates concentrations in different buffers 200 mM sodium acetate (pH 5), 50 mM Tris-HCl (pH 7 and 8), were pre-incubated at 25 °C for 5 min. The reaction was started when 150 µL of enzyme solution was added to 50 µL of substrate. The rate of hydrolysis for peptidyl-pNA substrates was measured at 410 nm.

Inhibitor Effects

The effects of various inhibitors: 50 mM iodoacetamide, 10 µM pepstatin A, 1 mM PMSF (phenylmethane sulphonyl fluoride), 3.8 $U.mL^{-1}$Aprotinin, 10 mM O-phenanthroline on proteolytic activity were evaluated by preincubating of purified extract for 30 min at 37 °C. The inhibition capacity was determined using azocasein as substrate. Controls were prepared by pre-incubating the enzymatic preparation with the solvent used to dissolve inhibitors.

Properties of purified extract

Temperature profile and thermal stability

Temperature profile of proteolytic activity was determined at 0, 20, 30, 40, 50, 60 and 70°C for 30 min on azocasein used as substrate dissolved in Tris-HCl buffer (pH 8.1). Thermal stability was determined by incubation sample for 10, 20, 40, 60, 80, 100, 120, 160, 200, 260, 340, 530 and 600 min at 4, 30, 37, 50, 60 et 70 °C. Residual activity was measured on azocasein.

pH Profile

pH profile was measured on azocasein in the pH range of 5 to 10 using different buffers: 50 mM phosphate buffer (pH 5, 5.5 and 6), 50 mM Tris-HCl (pH 7, 7.5, 8, 8.5, 9.5 and 10) at 37 °C for 1h.

Matrix assisted laser desorption ionization time-of-flight mass spectrometry

Samples (Gel pieces) were discolored using acetonitrile-ammonium bicarbonate (1:1), reduced with DTT solution and then alkylation was carried out using iodoacetamide solution. After digestion with trypsin (*MSG-Trypsine porcine Biosciences St Louis, Masspec Grade*), samples were desalted and purified on Zip Tip C18 P10. Sample was prepared by mixing a 1 µL aliquot (5-10 pmoles) with 1 µL of matrix α-cyano-4-hydroxy-trans-cinnamic acid (Aldrich) on the Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF) sample plate. MALDI-TOF experiments were performed on Voyager Elite DE-STR *Biospectrometry* equipped with a pulsed nitrogen laser (337 nm) and a gridless delayed extraction ion source. The photometer was operated in positive reflectron mode by delayed extraction with in accelerating voltage of 20 kV and a pulse delay time of 250 ns. All spectra shown represent accumulated spectra obtained by 100-300 laser shots.

LC/MS-MS coupling peptide sequences

LC / MS-MS coupling was performed on a Q-STAR Pulsar quadrupole TOF mass spectrometer (Applied Biosystems /

MDS Sciex, Toronto, Ontario, Canada) by nano-electrospray ionization source (Protana, Odense, Denmark). For MS/MS coupling analysis, the peptides were dissolved in equal volume methanol/water mixture (1/1) containing 0.1% formic acid. For recording conventional mass spectra, time-of-flight (TOF) data was acquired by accumulating 50 CMA (multiple channel acquisition) scans. The nano-HPLC-nano-ESI-MS/MS analyzes were carried out on a mass spectrometer (LCQ Deca XP+, Thermo-electron, San José, CA) equipped with a source of nano-electrospray ion coupled to high pressure nanoflow liquid chromatography (LC Packings Dionex, Amsterdam, Netherlands). The peptide sequences obtained were compared with NCBI and UniProtKB databases in order to obtain by blast, the match or the alignment of several sequences on the same protein of a sequenced organism whether the function is identified or not.

Results and Discussion

Extraction and purification of proteases

In order to extract the proteases from *B. aegyptiaca* berries in the minimum amount of duration, the procedure was involved: preparation of crude extract, discoloration using activated charcoal and ion exchange chromatography. The crude extract was treated with activated charcoal to remove some phenolic compounds which can interfere with enzymatic activity. These phenolic compounds color the crude extract in brown and render it viscous. After charcoal treatment, extract was become more clear; and ready to be submitted to Q-Sepharose fast flow column. The same column was used to purify cynarases from dried flowers of C. scolymus [26]. Four protein peaks (P1, P2, P3 and P4) were obtained (Figure 1). Among them, only the peak (P2) had milk-clotting activity and may be able to use as vegetable rennet for cheesemaking. This peak was used as sample for electrophoresis SDS-PAGE. The electrophoregram of P2 revealed three bands with a molecular weight approximately 28, 72 and 85 kDa (Figure 2). As to their molecular weight, the plant serine proteases known at present vary from 19 to 110 kDa, but the majority lies between 60 and 80 kDa [11].

Quantitative purification course of the proteases from *B. aegyptiaca* extract is presented in Table 1. Protein recovery was 35.5 and 3.8 % respectively for charcoal supernatant and Q-Sepharose peak 2. In addition, the purification fold increased from 0.45 to 1.02 respectively for first and second step. These results indicated that in alkaline medium, proteases from *B. aegyptiaca* can be isolated in two steps.

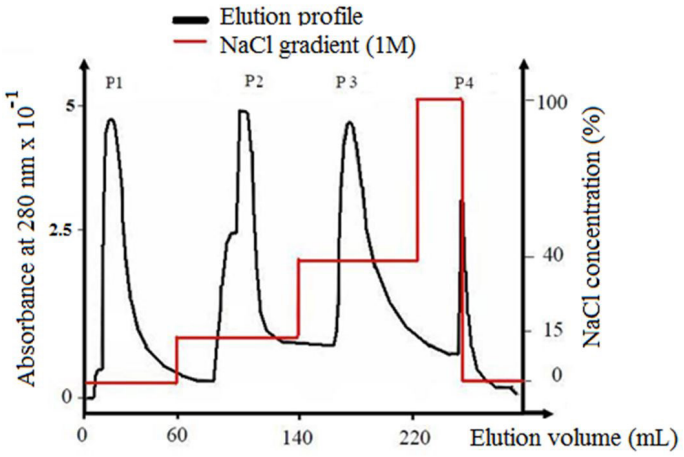


Figure 1: Strong anion exchange chromatography (Q-Sepharose Fast Flow) of *B. aegyptiaca* extract treated with 10% (w/v) charcoal. The Q-Sepharose column was pre-equilibrated with 50 mM Tris-HCl buffer (pH 8.1), containing 1 mM EDTA and 3 mM β-mercaptoethanol. Experiment was carried out with this buffer. Elution was performed using the same buffer containing 1 M NaCl at a flow rate of 0.5 mL/min under landing gradient of 0, 0.15, 0.4 and 1 M. (P corresponding to peak)

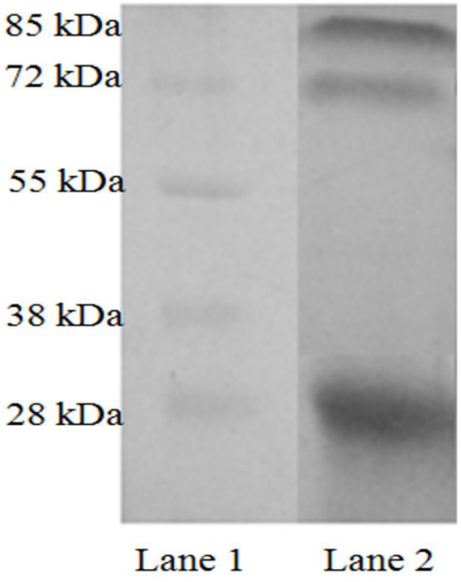


Figure 2: SDS-PAGE analysis of purified proteases extract from *B. aegyptiaca*. Samples were stained with Coomassie blue. The lane 1 corresponds to a molecular mass standards and the lane 2 to a purified protease using strong anion-exchange Q-Sepharose column (Peak 2)

Table 1: Purification of *B. aegyptiaca* extract

Parameters	Crude extract	Concentrated extract*	Peak1	Peak2	Peak3
Clotting activity ($U.mL^{-1}$)	0.25	0.41	_	0.20	_
Total protein (mg)	54	19.20	1.80	2.10	4.45

Specific activity ($U.mg^{-1}$)	0.46	0.21	_	0.47	_
Enzymatic activity $x10^{-3}$ ($\Delta A. min^{-1}.mg^{-1}$)	22	27	7.40	44	7.50
Purification fold	1	0.45		1.02	

*Concentrated extract was obtained after treatment of crude extract with activated charcoal.

Enzymatic activities

Enzymatic activities of peak 2 were performed using gel, bovine α-, β- and κ-caseins. Recent studies have shown that proteases from plant digested gelatin [27,10]. The activity gel of purified extract was detected in 72 and 85 kDa bands, corresponding to a white band appeared in electrophoregram showed in Figure 3. These results indicate the proteolytic nature of purified proteases from *B. aegyptiaca* extract by gelatin digestion.

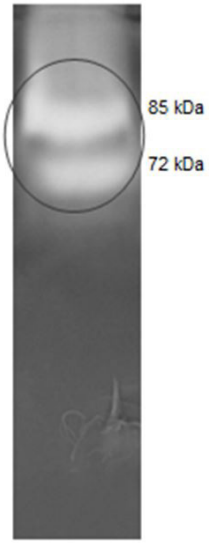


Figure 3: Activity gel of purified proteases (peak 2) from *B. aegyptiaca* after the elution on Q-Sepharose column. The gel was Gelatin stained with Coomassie Blue

If this extract is used as rennet substitute, it seems interesting to study the degradation of caseins because of their effect on final product (cheese) [28]. The caseinolytic activity of purified proteases is depicted in Figure 4. The electrophoretic patterns show that proteases from *B. aegyptiaca* extract cleave both κ-casein (Figure 4a) and α-, β-caseins (Figure 4b) as function of time. The results suggested that *B. aegyptiaca* extract might be used in dairy technology.

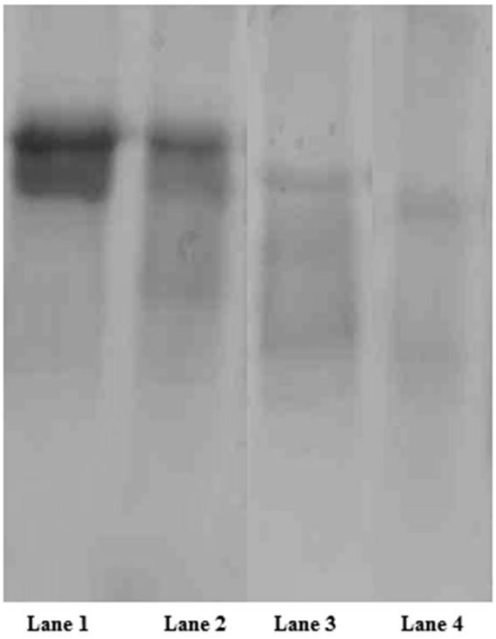


Figure 4a: SDS-PAGE electrophoregram illustrating the degradation patterns of bovine κ-casein by purified proteases extract (peak 2) from *B. aegyptiaca* after incubation for 1, 3 and 6h correspond to lanes 2, 3 and 4 respectively. Lane 1 represents control (κ-casein without enzyme). Commercial bovine κ-casein (2 mg) was dissolved in 1 mL of 100 mM sodium phosphate buffer (pH 6.2)

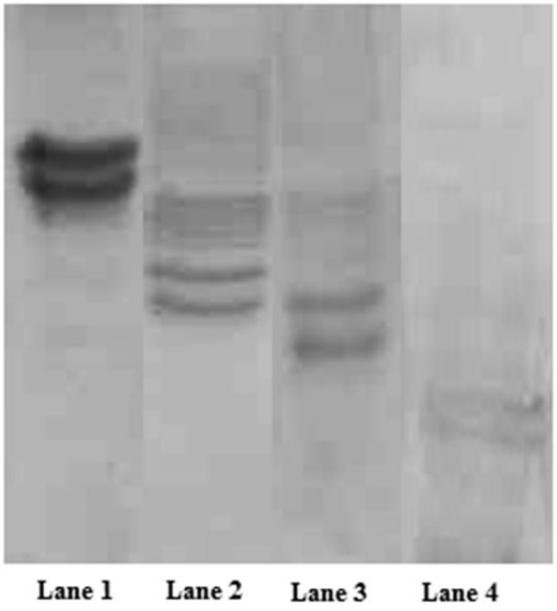


Figure 4b: SDS-PAGE electrophoregram illustrating the degradation patterns of bovine α- and β-caseins by purified proteases extract (peak 2) from *B. aegyptiaca* after incubation for 1, 3 and 6h correspond to lanes 2, 3 and 4 respectively. Lane 1 represents control (α, β-casein without enzyme). Commercial bovine α, β-caseins (2 mg) were dissolved in 1 mL of 100 mM sodium phosphate buffer (pH 6.2)

Specificity of purified extract

Specific inhibition

In order to study the proteases class, the purified extract from *B. aegyptiaca* was incubated in the presence of various inhibitors before caseinolytic reaction. The results show that, the purified extract from *B. aegyptiaca* was totally inhibited by PMSF and aprotinin, while it was not affected by other inhibitors used in this study (Table 2). The lack of effect of pepstatin A, iodoacetamide and O-phenantroline indicates that the purified extract is neither aspartic proteases nor cysteine proteases and metalloproteases. All proteases inactivated by PMSF are diagnostic for serine protease class [29]. Therefore, purified proteases from *B. aegyptiaca* extract belong to serine protease class.

Table 2: Effect of specific inhibitors on purified fraction

Inhibitors	Specificity	Concentration	Peak2 inhibition (%)
Pepstatin A	Aspartate protease	10 µmol.L^{-1}	0
PMSF	Serine protease	1 mmol.L^{-1}	100
Aprotinine	Serine protease	3.8 U.mL^{-1}	100
Iodoacetamide	Cysteine protease	50 mmol.L^{-1}	0

Substrate specificity

Generally, each type of proteases hydrolyzes a specific substrate. Synthetic peptides were often used to determine the type of proteases. In order to determine the specificity of proteases isolated from *B. aegyptiaca* in alkaline medium, N-Suc-Ala-Ala-Pro-Phe-pNA, N-Suc-Ala-Ala-Ala-pNA was used as substrate. N-Suc-Ala-Ala-Ala-pNA which is the specific substrate for elastase was not hydrolyzed by purified extract from *B. aegyptiaca*. As shown in Figure 5, N-Suc-Ala-Ala-Pro-Phe-pNA was more hydrolyzed at pH 7 than pH 8 as function of time, while the hydrolysis of this substrate was not significant at pH 5. The same substrate was hydrolyzed by Dubiumin [17]. Mohamed This substrate is specific to chymotrypsin-like serine proteases [30, 29]. Thus, these results suggested that proteases isolated from *B. aegyptiaca* in alkaline medium belong to chymotrypsin-like serine proteases class. According to Uchikoba, *et al.* [31] some serine proteases provided evidence for the broadness of its substrate specificity: peptidyl-p-nitroanilides and α-chymotrypsin were both hydrolyzed by alkaline enzyme.

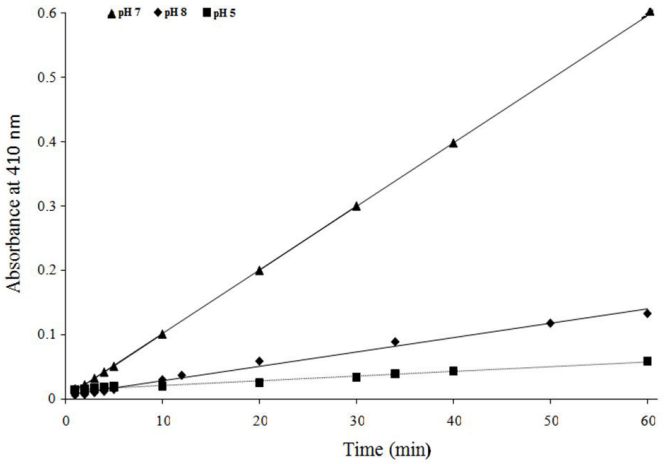


Figure 5: Activities of purified proteases extract from *B. aegyptiaca* on the synthetic substrate. N-Suc-Ala2-Pro-Phe-pNA was used as substrate and dissolved in dimethyl sulphoxide. The substrate was pre-incubated in different buffers at 25°C for 5 min: 200 mM sodium acetate (pH 5), 50 mM Tris-HCl (pH 7 and 8)

Properties of purified extract

Effect of pH

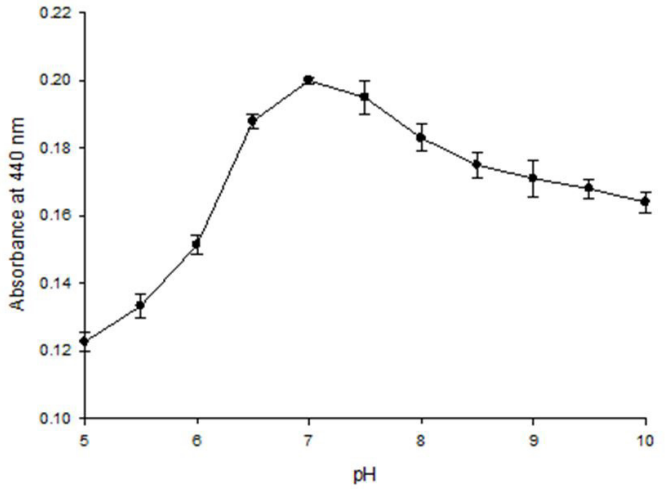


Figure 6: pH profile of purified proteases extract (peak 2) from *B. aegyptiaca*. Proteolytic activity was measured using azocasein as substrate. Blanks were done for each pH. The points are the means of triplicate determinations. Bars represent standard deviation

The pH activity profile of purified extract from *B. aegyptiaca* is shown in Figure 6. The activity of the proteases increased with pH until optimal value of pH 7. After this optimum, there is a weak decreasing of protease activity range from pH 7.5 to 10. These results are in consistence with those obtained on the serine protease (wrightin) extracted from plant *Wrightia tinctoria* (Roxb.) R. Br. [10]. As to their optimum pH activity, the plant serine proteases known at present vary from the alkaline range pH 7 to pH 11 [11]. The behavior of purified extract from *B. aegyptiaca* against pH is an important property for its utilization in food industry, especially in dairy technology.

Effect of temperature

As shown on Figure 7, the temperature profile of purified extract from *B. aegyptiaca* was performed at temperatures in the range of 0-70 °C. The optimal temperature was at 50 °C. The protease activity on azocasein increased linearly from 0 to 50 °C with a rapid decrease of activity afterward. These results are similar of that obtained with Benghalensin (serine protease from *Ficus benghalensis*) [32]. The optimum temperature is rather variable among alkaline enzymes, from 30 up to 80 °C, but most plant serine proteases usually act best in the range 20-50 °C [11].

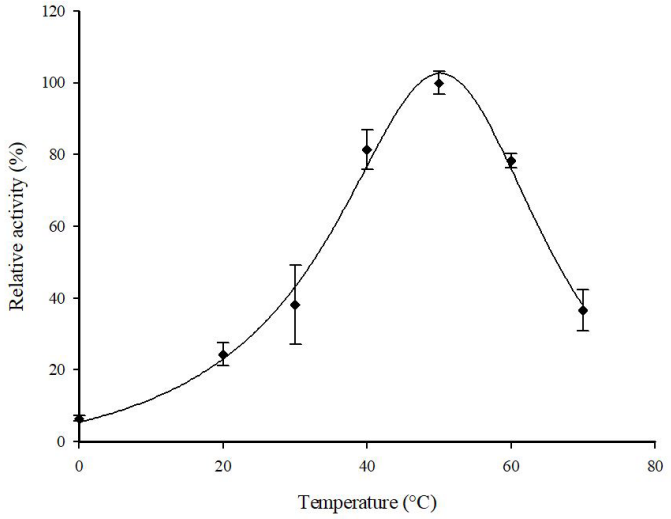


Figure 7: Temperature profile of purified proteases extract (peak 2) from *B. aegyptiaca*. Proteolytic activity was measured using azocasein as substrate. Blanks were done for each temperature. The points are the means of triplicate determinations. Bars represent standard deviation

The thermal stability of purified extract was monitored using temperatures in range of 4-70 °C for 0-10h (Figure 8). The results show that at 4 °C the activity was totally retained, while protease lost its activity at 70 °C after 100 min of incubation. For 60 °C of incubation, the activity was linearly decreased with time. The activity of protease decreased with the temperature in range 30-50 °C for 100 min, afterward the activity became constant. The study of thermal profile of purified proteases from *B. aegyptiaca* is very interesting feature in their use in technology.

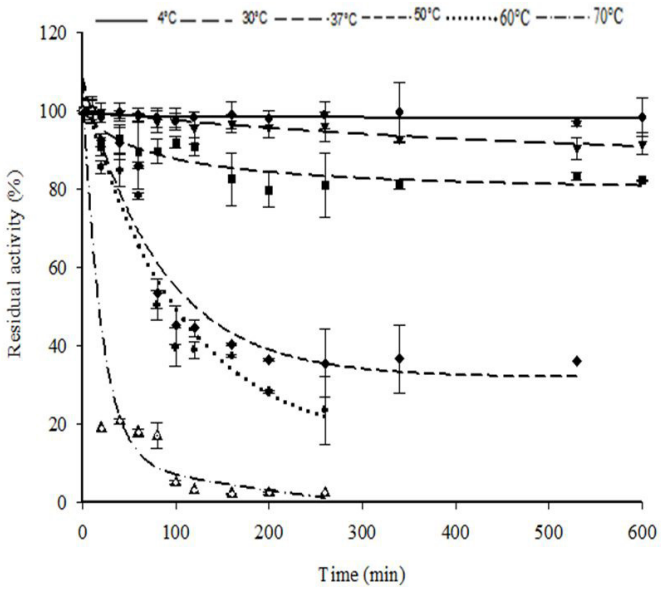
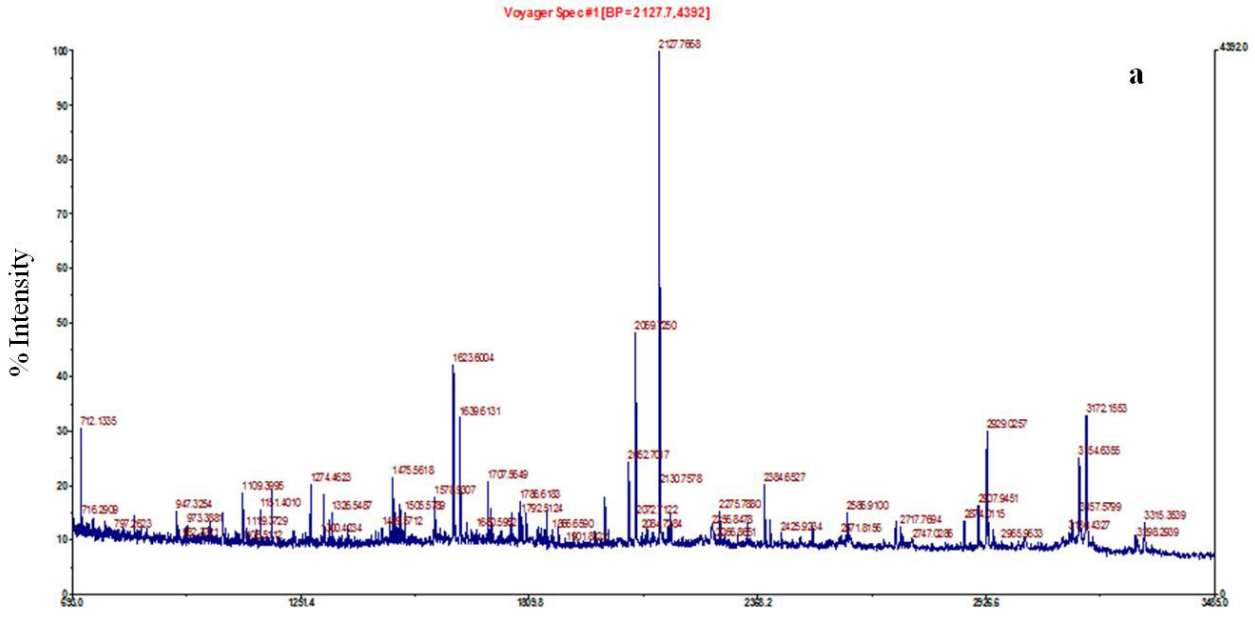


Figure 8: Thermal stability of purified proteases extract (peak 2) from *B. aegyptiaca*, during 10 h. Residual activity was measured using azocasein as substrate. Blanks were done for each temperature. The points are the means of triplicate determinations. Bars represent standard deviation

MALDI-TOF



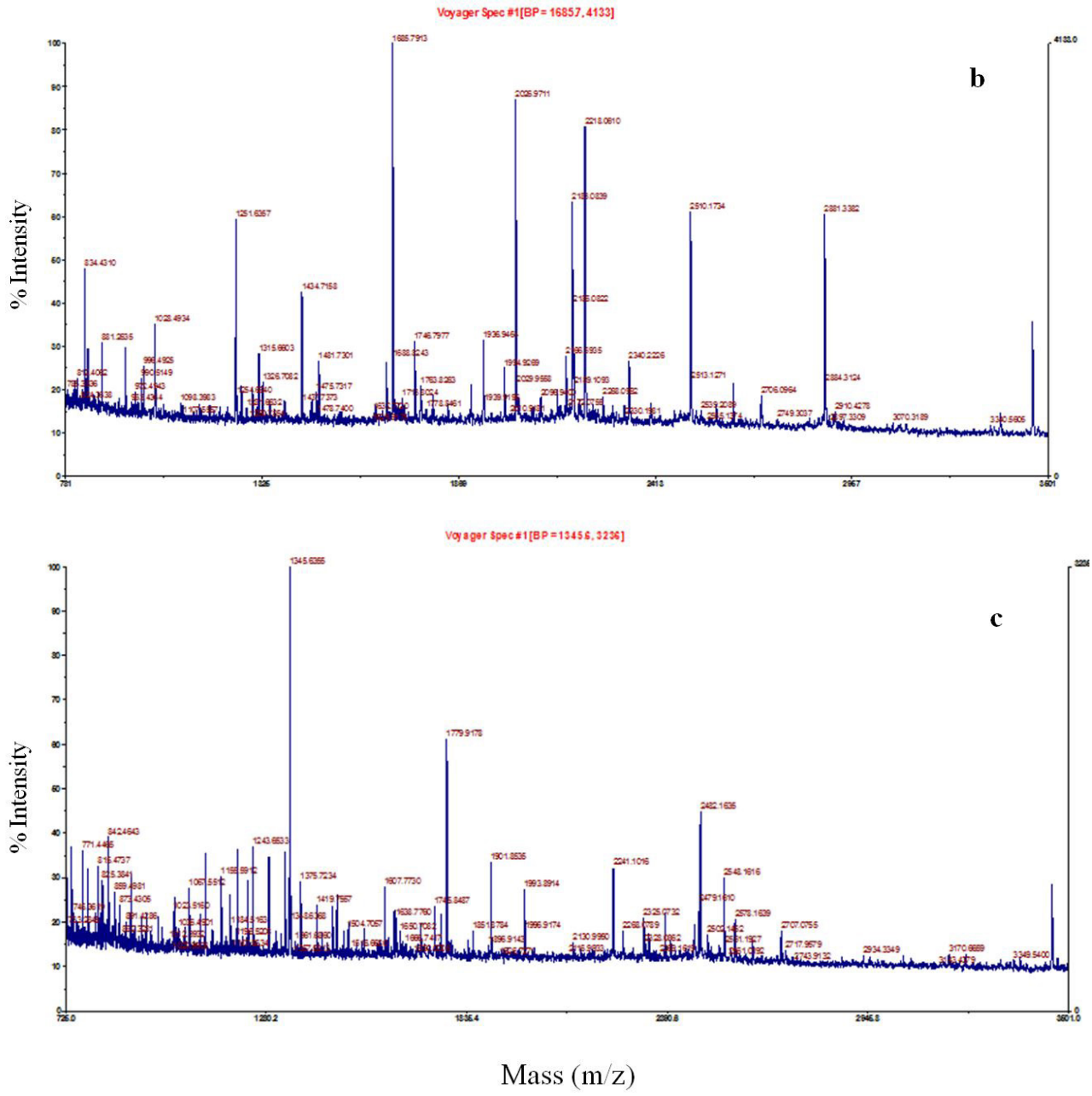


Figure 9: MALDI-TOF mass spectra of purified proteases extract from *B. aegyptiaca*. 28 kDa (a), 72 kDa (b) and 85 kDa (c) spectra were recorded in positive ion reflective mode using α-cyano-4-hydroxy-trans-cinnamic acid as matrix. The test gel pieces were discolored with acetonitrile-ammonium bicarbonate (1:1). The control and test gel pieces were further in gel digested with trypsin before analysis

The MALDI-TOF spectra of the purified proteases from *B. aegyptiaca* are shown in Figure 9. In order to check the homogeneity and purity of purified proteases, each band resulting to SDS-PAGE of peak 2 was submitted to MALDI-TOF mass spectrometry analysis. The high intensity in the mass spectra (Figures 9a-c) is due to the absence of contaminants such as keratin and polymers as well as the amount of protein in the sample [33], confirming the purity of proteases isolated from *B. aegyptiaca*. These results suggest that purified extract contains three serine proteases or one serine protease with three sub-units.

Determination of Peptide Sequences

After MALDI-TOF analysis, the samples were subjected to LC/MS-MS. The sequences of the peptides obtained are compared with the NCBI and UNIPROTKB databases. As the genome of the organism *B. aegyptiaca* was not yet sequenced, the sequences obtained were compared to databases by searching sequence homology by blast. The homologies sequences exhibited at a high percentage (> 76%) and show in Table 3. The peptide sequences of 28, 70 and 85 kDa SDS-gel bands are homologies

to serine proteases. 28 kDa Band contains 13 amino acid sequences homologous to *Oryza sativa* subsp. peptide sequence. 70 kDa band showed 15 amino acid sequences homologous to Arabidopsis thaliana serine protease. The 85 kDa band showed two sequences of 13 and 15 amino acids homologies with *Ricinus communis* and *Vitis vinifera* serine proteases.

Table 3: Peptide sequences and protease identification

SDS-gel Band (kDa)	**Peptide Sequences**	**Protease Name**	**Plant name**	**Access number**
85	LLKEFTSIASIDK	EC.3.4.19.1 Serine-type endopeptidase	*Ricinus communis*	B9RTG2 (UniprotKB) XM002516985.1 (NCBI)
85	TYIGSMPGRLIEGLK	EC.3.4.21 Serine-type endopeptidase	*Vitis vinifera*	D7TTJ0 (UniprotKB) CAN75012 (NCBI)
70	SFNLMPKYDVNVIPK	EC.3.4.16 Serine-type carboxypeptidase	Arabidopsis thaliana	Q9FFB0 (UniprotKB) NP197689 (NCBI)
28	AAAAADDGAVVAR	Serine protease	*Oryza sativa* subsp. japonica (Rice).	BAD37810 (NCBI)

Conclusions

This study was carried out to purify serine protease from *B. aegyptiaca* pulp. The purified extract was completely inhibited by PMSF and aprotinin, while other inhibitors were not affected its activity. It exhibited an activity on the specific substrate, especially to chymotrypsin-like serine proteases one. This study has developed a simple purification procedure to extract serine protease from *B. aegyptiaca* pulp in alkaline medium with potential applications in cheesemaking. Further work is on way to characterize serine protease from *B. aegyptiaca* pulp.

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