Characterization of general proteolytic, milk clotting and antifungal activity of *Ficus carica* latex during fruit ripening

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ABSTRACT

BACKGROUND: Fig latex's physiological role is protection from pathogens. Latex is a rich source of proteases, predominantly ficin. Fig latex contains collagenolytic protease and chitinolytic enzymes, too. Our aim was to investigate changes in protein composition, enzyme and antifungal activities of fig latex during fruit ripening.

RESULTS: Comparison of latex samples in different time periods showed uniform increase of protein concentration in chronological order. The content of collagenolytic protease did not differ significantly in the latex samples, while the content of ficin decreased. Ficin specific activity towards casein was the highest in the beginning of fruit development (about 80 U mg^{-1}). Specific milk clotting activity increased as well as the abundance of casein band in the clots. Specific chitinolytic activity at the beginning of flowering was 6.5 times higher than the activity in the period when fruits are ripe. Antifungal activity is the most extensive in spring.

CONCLUSION: Ficin forms with different casein specificities are present in different proportions during fruit ripening which is of importance for applications in dairy industry. Protection mechanism against insects and fungi which relies on chitinolytic activity is the most important in the early phases of flowering and is replaced with other strategies during time.

KEYWORDS

Ficus carica latex; ficin; ripening; chitinolytic activity; antifungal activity; milk clotting activity

INTRODUCTION

Ficus carica var. Brown Turkey (the common fig), is among earliest cultivated plant species because of their use as food and medicine. Curative effects of figs were noticed centuries ago, and today fig fruit is used worldwide not just as food, but also in various forms which are part of traditional medicine.[1]

Genus *Ficus* is one of forty members of *Moraceae* family which consists of large number of different species.[2] Common characteristic of all *Ficus* species is presence of latex fluid inside laticiferous cells. Latex is a complex sticky fluid, milky in appearance. Latex is produced by laticiferous cells and is excreted in the place of injury immediately after wound formation.[3]

One of the most important physiological roles of latex is protection from pathogen invasion throughout the injured tissue.[4] Younger parts of fig tree contain largest volumes of latex (young shoots, pedicles and leaves), since latex represents their only mechanism of protection. Older parts of plant have many additional protection strategies, *e.g*. high polyphenols concentration and toughness, and therefore are poorer in latex content.[3]

Defensive role of latex is achieved by mechanical and biochemical protection. Mechanical protection relies on latex's ability to coagulate on site of injury which is attributed to the presence of *cis*-1,4-polyisoprene commonly known as rubber. Biochemical protection relies on physiological action of active compounds. [4]

Latex is a complex mixture of many different kinds of secondary metabolites and proteins which differ between species and enable it to have specific protection strategies. Some of them are alkaloids; terpenoids; tannins; sterols;[5] polyphenols;[6] as well as proteins, *e.g*. proteases; oxidases; lectins; chitinases; glucosidases; and phosphatases, all of them often

found in high concentrations.[3] One of the main constituents of latex is rubber and fig tree is considered as an alternative rubber crop because it generates a large latex volume, has a fast growth habit and long life.[7]

Proteolytic fraction of latices of genus *Ficus* predominantly contains cysteine proteases, but there are also other classes of proteases, *e.g*. aspartic[8] and serine proteases.[9]

Ficin (EC 3.4.22.3) is a common name for endoproteolytic fraction of fig latex. It is known that there are multiple forms of this enzyme in fig latex, and all of them belong to cysteine protease class.[10] Ficin from fig latex is known as the enzyme with milk clotting activity (ancient writings such as the Iliad indicate that fig latex can be used for cheese-making), but also high proteolytic activity of broad range.[9, 11] Except ficin, fig latex contains newly described collagenolytic serine protease,[9] as well as few recently described chitinolytic enzymes.[12-14]

Fig latex has been extensively investigated due to its high proteolytic activity. However, time dependent changes in protein composition of fig latex have never been studied. We made a hypothesis that variations in protein composition, as well as in caseinolytic, milk clotting and antifungal activity of fig latex during fruit ripening could exist. Testing of such hypothesis would be of great importance for usage of latex to produce proteases for the dairy industry or to prepare a novel antifungal agent.

EXPERIMENTAL

Reagents

Bovine serum albumin (BSA), casein, Coomassie Brilliant Blue R-250 (CBB R-250), L-cysteine, chitin, *N*-acetyl-D-glucosamine (Glc*N*Ac), were purchased from Sigma–Aldrich (Steinheim, Germany). Unstained protein molecular weight markers were bought from

Thermo Scientific (Rockford, IL, USA). All other chemicals were commercial products of analytical grade and were used without further purification.

Collection and preparation of latex samples

Latex was collected from the private orchard in Bar, Montenegro by simple incision of green fruits of the same fig trees. It was sampled approximately every two weeks (starting from the 1st of May until the 15th of August). Each time 10 mL of the latex fluid was collected from the same fig trees (three in total, each time approximately 10 fruits were used). The latex fluid was poured into 1.5 mL micro-centrifuge tubes and was immediately stored at -20 °C until used. The frozen latex was thawed at 4 °C and was centrifuged at 10 000 x *g* at 4 °C for 60 min to remove insoluble gum (approximately 200 g kg^{-1} in weight) and other debris.[8] Obtained supernatants were frozen in liquid nitrogen and stored at -20 °C if not assayed immediately.

Determination of protein concentration and electrophoretic profiling of latex proteins

Total protein concentration in latex samples was determined in Bradford assay, using BSA as standard.[15] Before loading onto the gel, latex samples were diluted to 0.5 mg mL^{-1} and mixed with 5 times concentrated reducing sample buffer and heated at 95 °C for 5 min. The composition of 5 times concentrated reducing sample buffer was: 60 mmol L^{-1} Tris buffer pH 6.8 containing 250 g kg^{-1} glycerol, 20 g kg^{-1} SDS, 14.4 mmol L^{-1} 2-mercaptoethanol, and 1 g kg^{-1} bromphenol blue. Twenty microliters of samples were loaded onto the gel. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in 120 g kg^{-1} resolving gel and 40 g kg^{-1} stacking gel was performed in Protein electrophoresis unit GV202 (Bio-Step, Jahnsdorf Germany) according to Laemmli.[16]

Two gels were ran in parallel. The gels were stained with CBB-R250.

Densitometric analysis of each gel was done using *Gel-Pro* Analyzer 3.1 software (Media Cybernetics, MD, USA). The abundance of both, collagenolytic serine protease band and ficin band was compared between the samples (L1-L8) and also their relative abundances were calculated within the same lane and presented as a percent of all the bands within the lane.

General proteolytic activity

General proteolytic activity was determined using casein as a substrate at pH 7.0 which is pH optimum for ficin.[17] All the latex samples prepared as described, were diluted in 1:200 ratio in 100 mmol L^{-1} sodium phosphate buffer pH 7.0 containing 9 g L^{-1} NaCl (phosphate buffered saline (PBS)). Fifty microliters of samples were mixed with 250 µL substrate solution (6.5 g L^{-1} casein in PBS containing 10 mM L-cysteine). Reaction mixtures were incubated for 30 min at 37 °C. The reaction was stopped by adding 60 µL of 50 g L^{-1} trichloroacetic acid (TCA), followed with centrifugation for 15 min at 12 000 x *g*. Absorbance at 280 nm of supernatants was recorded. For every latex sample appropriate blanks were prepared. Enzyme blank was prepared by incubating 50 µL of latex sample for 30 min at 37 °C without casein as a substrate. After incubation, samples were mixed with 60 µL of TCA, and then 250 µL buffer was added. Substrate blank was prepared by incubating casein solution with buffer only (no enzyme added) and adding TCA solution after incubation of substrate alone for 30 min at 37 °C. Absorbance of both blanks was subtracted from the absorbance of the samples. All measurements were performed in triplicate. One unit of caseinolytic activity was defined as amount of enzyme which gives rise of absorbance at 280 nm for 1.0 for 1 minute.[9]

Specific activity was calculated as the ratio of the enzymatic activity to the total protein content of the sample, and expressed in U mg^{-1}.

Milk clotting assay

The Berridge coagulation assay was used to determine clotting time. The clotting time was defined as the time interval from the addition of the latex to the milk to the formation of the first white flakes of coagulated casein. The assay was done essentially as described.[18] Briefly, 0.5 mL of 100 g L^{-1} of commercial skimmed milk (containing 5 g kg^{-1} fat before 10 times dilution); containing 10 mmol L^{-1} $CaCl_2$ was preheated at 37 ºC. The pH value was previously adjusted at 6.5. Then, 200 µL of the preheated 1:10 diluted latex solution (containing 10 mmol L^{-1} L-cysteine) was added. The reaction was incubated at 37 ºC. The time was measured until the first flakes appeared.[18] The experiment was done in triplicates. Positive control (milk coagulated using chymosin in concentration 0.044 mg mL^{-1}) was also included. One unit of milk clotting activity was defined as amount of enzyme needed to coagulate 1 mL of 100 g L^{-1} milk in 1 min at 37 °C. Specific activity was calculated as the ratio of the enzymatic activity to the total protein content of the sample, and expressed in U mg^{-1}.

The ratio of milk clotting to general proteolytic activity against casein as a substrate (C/P value), was also calculated.

Electrophoretic analysis of milk clotting activity

The milk clot obtained using chymosin or using fig latices as coagulating agents was incubated for additional one hour at 37 °C to achieve total precipitation of milk coagulum.

After centrifugation at 12 000 x *g* for 20 min, whey was separated from the precipitate and prepared for SDS-PAGE analysis. SDS-PAGE was performed as previously described.

Chitinase activity

Chitinase activity was evaluated by Schales' procedure as described in Ferrari *et al.*[19] Latex samples were diluted in ratio 1:2 in PBS. The reaction mixtures were prepared by mixing 300 µL of samples with the same volume of substrate suspension (20 g kg^{-1} colloidal chitin in 50 mmol L^{-1} phosphate buffer pH 6.2). The reaction mixture was incubated for 20 h at 37 °C. After incubation, samples were centrifuged at 12 000 x *g* for 20 min at 4 °C. One volume of supernatant (250 µL) was added to the same volume of Schales' reagent (0.5 mol L^{-1} sodium carbonate and 0.5 g L^{-1} potassium ferricyanide in water). Reaction mixtures were incubated at 100 °C for 15 min and then centrifuged at 12 000 x *g* for 20 min. Supernatants were transferred to a 96 well microtitter plate and absorbance at 405 nm was measured using a LKB Micro plate reader 5060-006 (GDV, Roma, Italy). Blanks were prepared by mixing 300 µL of water with the same volume of substrate suspension and further prepared same way as latex samples. All measurements were performed in triplicate.

Different concentrations of *N*-acetyl-D-glucosamine (Glc*N*Ac) (1 mmol L^{-1} to 50 mmol L^{-1}) were used for construction of the standard curve. One unit of chitinase activity was defined as the amount of enzyme that produces 1 µmol of Glc*N*Ac per minute under described conditions.[7] Specific activity was calculated as the ratio of the enzymatic activity to the total protein content of the sample, and expressed in U mg^{-1}.

Antifungal activity

For the purpose of measurement of antifungal activity, strain of *Saccharomyces cerevisiae* was used. Inoculum was prepared from glycerol stock using a sterile tip and transferring *S. cerevisiae* cells to yeast tryptone dextrose (YTD) liquid medium and cultures were grown on 28 °C overnight. Inocula were diluted in YTD liquid medium to approximately 10^5 colony forming units (cfu) and then grown in the presence of latex samples. The incubation was carried out in sterile 96 well microplates and yeast growth was monitored by measuring the absorbance (optical density, OD) at 620 nm. Results were expressed as percentage of yeast growth compared to the control sample incubated in the appropriate buffer in the absence of latex (100 mmol L^{-1} Na–phosphate buffer pH 7.4 added to yeast culture). The mean growth values of triplicates were obtained and then converted to the inhibition percentage of cell growth as described in Prokopovic *et al*.[20]

RESULTS AND DISCUSSION

Collection of latex and determination of protein concentration

Period of latex collection and exact time intervals are presented in Table 1. This period includes complete phase of fruit ripening: from the beginning of flowering until the time fruit completely ripens. Great number of studies on *F. carica* latex and other plant latices, in which method of collection has been described, emphasized that latex has been exploited from the green parts of plant, while the fruit had still been immature.[10, 21] This prompted us to explore whether there are some variations of fig latex composition during time.

To explore the potential differences between protein profiles of latex samples in different time intervals, we first determined total protein concentrations (Table 1).

Quite uniform increase of protein concentration in chronological order can be noticed (Table 1). Gooding *et al*. discussed that the changes of latex protein content are the consequence of more pronounced dry conditions moving from spring to summer, leading to increased (macro)molecule concentration in latex.[22]

Despite fig latex proteins were investigated in many studies, starting from commercial crude ficin preparation rather than collected latex, there is not much information about *F. carica* latex available for comparison. However, in the study on *F. carica* latex of Chang *et al.* total protein concentration of the latex collected from the unripe fruit was estimated to be approximately 15 mg mL^{-1} [23] which is in good agreement with our result for latex collected on the 1st of May (16 mg mL^{-1}). In the study of Whitaker from 1958, protein concentration estimated by Lowry method was similar to our result from the second half of July and it was determined to be 33.6 mg mL^{-1}, although they did not point out the exact period of latex collection.[24]

Latices of other laticiferous plants are considered to be rich sources of proteins.[5, 25, 26] Taking in mind protein concentrations in fig latices determined during ripening, fig latex collected in the summer is one of the plant latices of highest protein content.

Electrophoretic profiles of latex samples

Then, we wanted to characterize and compare our latex samples in respect of protein pattern. To study protein composition in fig latex samples, SDS-PAGE analysis was performed. The results are shown in Fig. 1.

Fig latex protein profiles that we detected in this study contain the same protein bands as described previously.[7] Two main protein bands could be detected in each sample. Protein

band of around 25 kDa represents cysteine protease ficin, while band of around 48 kDa can be assigned to recently described collagenolytic serine protease.[9] Additionally, there are several components of molecular weight of 20 and 17 kDa and less than 14 kDa.

Taken into consideration that the protein profile of latex samples did not changed significantly during time regarding the presence of main protein bands (Fig. 1), we sought to examine contents of bands attributed to collagenolytic serine protease and ficin by more detailed densitometric analysis. The analysis showed that the content of collagenolytic protease did not differ significantly in the latex samples, while the content of ficin decreased from L6 to L8 (Supporting Fig. S1).

Total caseinolytic activity of latex samples

The changes in ficin specific activity during fruit ripening toward casein as a substrate are presented in Fig. 2. We had already shown in our previous study that other proteolytic component of fig latex (collagenolytic serine protease) do not have specificity for casein hydrolysis,[9] thus casein hydrolysis could be attributed to ficin only.

The results suggest that ficin specific activity decreased over time, being the highest in the beginning of fruit development (about 80 U mg^{-1}). At the moment of complete fruit ripening the activity declined to about one half of initial activity (about 40 U mg^{-1}). However, such a decrease in specific activity of latex samples attributed to ficin could be explained by the increase of other proteins' concentration (Table 1) and both, absolute (Supporting Fig. S1) and relative (Supporting Table S1) decrease of ficin band abundance. Our results confirm that ficin possesses relatively high proteolytic activity. Even the sample with the smallest proteolytic activity (L8 in Fig. 2) exhibited higher specific activity towards casein than latices

of other laticiferous plants, *e.g. Ficus religiosa*, *Ficus bengalensis, Calatropis procera, Carica papaya*).[27]

As latex defense mechanism is assigned (to a large extent) to proteolytic fraction, which major constituent is ficin, it seems that fig's major defense mechanism in the early stages of fruit development relies on ficin activity. This activity is most probably replaced over time with other protection strategies *e.g.* high phenol, tannins and lignin concentrations.[3]

Milk clotting activity of latex samples

It has been already reported that fig latex possesses enzymes that have appropriate milk clotting to caseinolytic activity ratio, which candidates it as a potential substitution for rennet in the production of cheese, since there is a constant need on identification and application of the enzymes from different sources in the cheese industry due to reduced supply in calf rennet and its high price.[28] Furthermore, there is a seeking demand on identification of plant derived milk clotting proteases, as the use of animal rennet may be limited for religious reasons (*e.g.*, Judaism and Islam), diet (vegetarianism), or consumer concern regarding genetically engineered foods (*e.g.*, Germany, Netherlands and France forbid the use of recombinant calf rennet).

Potential usage of fig latex in dairy industry could be of significant importance for commercial application of fig latex, which led us to explore if there is a dependency between latex samples collected in different period of time and milk clotting activity. The results are shown in Fig. 3A.

Fig. 3A shows that there is time dependent increase in milk clotting activity in latex samples. Specific activity of latex samples against κ-casein was around 10 times higher in latices

collected in July-August, when compared to samples collected in spring. Furthermore, when compared caseinolytic and milk clotting activities (Fig. 2 and Fig. 3A) it appeared that summer samples are better suited for application in cheese industry. When seeking for potential plant enzyme substitutes for rennet, the most important problem is that plant proteases usually have high proteolytic activity and cause extensive digestion of casein.[28] As an appropriate indicator of suitability for usage in diary industry, the ratio of milk clotting to general proteolytic activity (C/P value) is used,[29] since higher C/P value means less short peptides products that contribute to bitter taste of cheese.[30]

C/P values for fig latex samples are present in Fig. 3B. Steeper increase can be noticed compared to milk clotting activity as a consequence of opposite trends in caseinolytic and milk clotting activity during time. Determined C/P values for August samples of fig latex (0.6-0.7) suggested that fig latex collected in this period could be of use in cheese making.[31]

Interestingly, we found here that while caseinolytic activity decreased, milk clotting activity increased over time and both of them could be attributed to ficin.[17, 32] However, it is known that ficin represents a heterogeneous protein fraction of fig latex which includes at least five different isoforms.[33]

In an early study of Kramer and Whitaker it had been shown that different ficin forms partially purified from *F. carica* latex, had different milk clotting activities as well as total caseinolytic activities. Milk clotting activities and C/P ratios varied up to 50 times and 80 times, respectively among different ficin isoforms.[34] Alteration in specificity of fig latices toward total casein (as determined as caseinolytic activity) and κ-casein (as determined as milk clotting activity) presented in this work could be the consequence of differential expression of ficin isoforms in fig latex during flowering and fruit ripening. In the beginning of this period, it seems that ficin forms of general caseinolytic specificity are predominantly

present, enabling hydrolysis of α, β and κ-casein. As the fruit becomes more mature, it can be assumed that forms with general specificity for caseins are gradually replaced with forms that are more specific for κ-casein, giving the highest milk clotting activity per mg of protein.

Furthermore, we examined SDS-PAGE analysis of clots and whey of milk samples coagulated with different latices obtained from the previous experiment. Results of comparison of clot and whey content between differently coagulated milk samples are presented in Fig. 4. It can be noted that contents of milk clots differed by using latices collected in different period (Fig. 4). In fact, main fragment obtained after milk clotting had molecular weight of approximately 25 kDa in all samples, but its relative amount slightly differed between the samples. The casein fragment of 25 kDa was the most abundant if mid-August fig latex was used for milk coagulation in comparison to other latices.

Fig latex collected in the period when the fruits are completely ripe could be a promising candidate as an alternative coagulating agent in dairy industry because large volumes of fig latex could be collected at low price.[7] Furthermore, fig latex has been part of traditional medicine through the centuries even orally taken,[2] suggesting no need for purification of coagulating enzyme from other latex ingredients. However, it is known that the usage of the enzymatic preparations of high caseinolytic activity could produce bitter cheese.[30, 31] There is no literary information regarding optimal time for latex collection for the use in cheese production. However, our results strongly suggest that it is crucial to select appropriate season for latex collection, because of the significant difference in C/P ratio between the samples taken in spring, and the one taken in middle of August. Taking in concern that the highest C/P value is obtained for the last latex sample, it is best to perform latex collection during summer, *i.e.* from July to August.

Comparison of chitinolytic activity

One of the most important physiological roles of fig latex includes protection from herbivorous insects via chitin degradation. It has been already suggested that chitinases and chitinase like proteins are abundantly accumulated in plant latex, and that their existence is crucial in defense against insects. One of the possible mechanisms of defense could include chitin hydrolysis, as chitin represents main constituent of insects' shell.[35] This led us to also characterize fig latices in the term of chitinolytic activity.

Changes in specific chitinolytic activity of latex samples during time are presented in Fig. 5. It could be noticed that quite regular trend exists, and that there was a gradual decrease of specific activity towards chitin. The decrease was much more intensive compared to proteolytic activity. Level of specific chitinolytic activity at the beginning of fig plant flowering was almost 6.5 times higher than the activity in the period when fruits were completely ripe. The time dependent loss of chitinolytic activity, suggests that mechanism of protection of *F. carica,* which relies on chitin hydrolysis, is probably most important in early phases of fruit development.

Kim *et al*. reported that fruit hormone jasmonic acid is negative effector of expression of chitinase gene in *F. carica* latex.[36] It is also known that jasmonic acid is present in fruit in low concentration in the late phases of ripening. In fact, there are numerous reports which have shown that exogenously applied jasmonic acid delays ripening.[37, 38] Moreover, there is evidence that jasmonic acid has positive effect on expression of defense-related genes.[39] It can be concluded, taking our results into account, that chitinase dependent protection mechanism reduces over time.

Antifungal activity

Many papers dealing with antimicrobial activity of *F. carica* latex have been published. Most of them consider organic solvent extracts, e.g. methanol, hexanol, ethyl acetate extracts, for which it is shown to possess antibacterial and antifungal activities. These activities are assigned to low molecular weight compounds of fig latex.[40, 41]

Although interest for investigation of antifungal activity of *F. carica* latex has been present many years ago, antifungal activity of aqueous phase of fig latex has not been yet well understood. Modest number of studies revealed the existence of antifungal proteins in aqueous phase of latex of genus *Ficus*. However, few new antifungal proteins were recently discovered. Some of them include chitinase of *Ficus awkeotsang*, as well as two chitinases isolated from the latex of *Ficus microcarpa.*[42]

Results on antifungal activity of *F. carica* latex against *S. cerevisiae* are presented in Fig. 6. It can be noted that there was a steep decline in antifungal activity going from May to August, and that antifungal activity was most pronounced in spring, more precisely in May. Such result is not surprising since it is coherent with our result of chitinolytic activity. In fact, the great majority of recently described antifungal proteins of genus *Ficus* are chitinases, or chitinase like enzymes.[13, 14]

Identification of active compounds of *F. carica* latex and improved comprehension of mechanism of action against fungi induced diseases of crops may result in substitution of previously applied phytotoxic and environmentally harmful substances for nontoxic ones such as latex. Potential for utilizing this natural extract in organic agriculture should be further investigated.

Moreover, another interesting potential application of *F. carica* latex could be in food processing industry as a natural food preservative, especially when we consider that fig latex

has high content of minerals, vitamins, antioxidants, fibers and proteins, which makes it a valuable functional food.[43]

CONCLUSIONS

The overall protein concentration, ficin activity and specificity, as well as chitinase and antifungal activities differed in fig latices collected from the beginning of flowering until the complete fruit ripening. Protection mechanism against insects and fungi relies on chitinolytic activity which is the most important in the early phases of flowering and is replaced with other protection strategies during time. Ficin forms with different casein specificities are present in different proportions during fruit ripening which is of importance for applications in dairy industry.

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Table 1. Total protein concentration of *Ficus carica* latex samples collected in different periods of time. The values represent means ± standard deviation of data from three independent experiments.

Latex sample	Date of collection	Protein concentration ± SD ($mg\ mL^{-1}$)
L1	01.05.2013.	16 ± 3
L2	15.05.2013.	18 ± 1
L3	02.06.2013.	21 ± 8
L4	16.06.2013.	26 ± 9
L5	01.07.2013.	28 ± 3
L6	15.07.2013.	33 ± 1
L7	01.08.2013.	37 ± 9
L8	16.08.2013.	41 ± 2

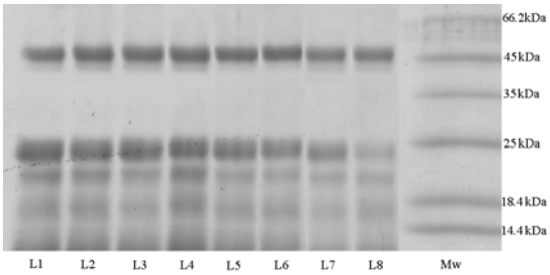

Figure 1. Electrophoretic profile of *Ficus carica* latex samples (collected in period May to August: L1-L8).

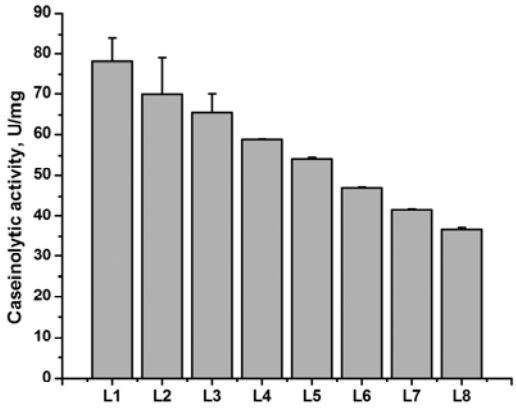

Figure 2. Caseinolytic activity of *Ficus carica* latex samples (collected in period May to August: L1-L8). The values represent means ± standard deviation of data from three independent experiments.

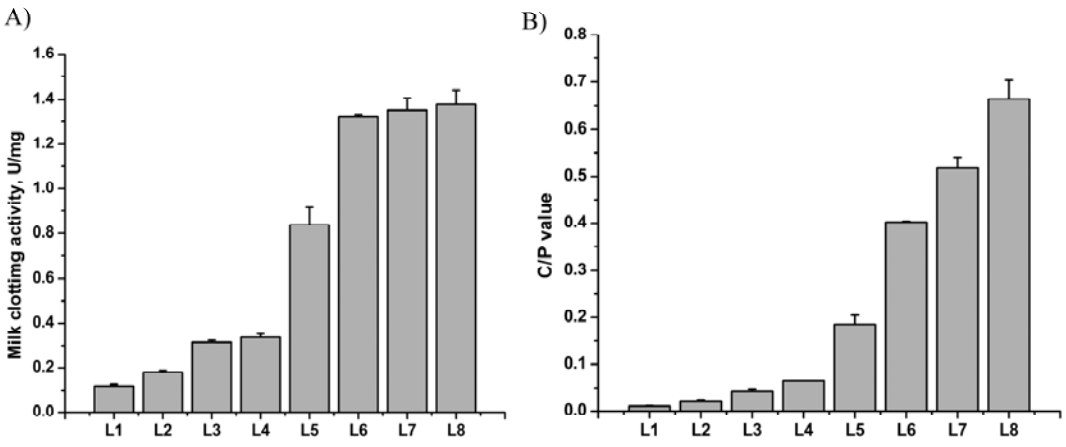

Figure 3. Milk clotting activity against milk with 5 g kg^{-1} fat as substrate (A) and milk clotting to general proteolytic activity ratio, C/P value (B) of *Ficus carica* latex samples (collected in period May to August: L1-L8). The values represent means ± standard deviation of data from three independent experiments.

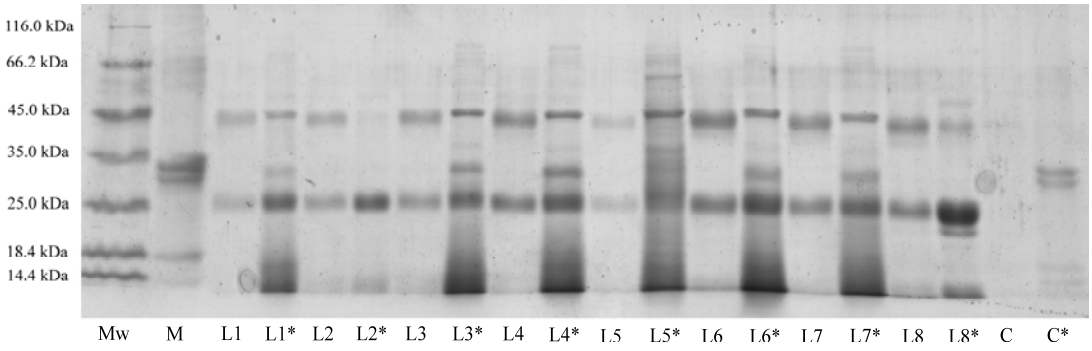

Figure 4. SDS-PAGE profiles of whey (L1-L8) and clots (L1*-L8*) of *Ficus carica* latex samples collected in period May to August against milk (M) with 5 g kg^{-1} fat as a substrate. Mw – molecular weight markers; C – chymosin whey; C* – chymosin clot.

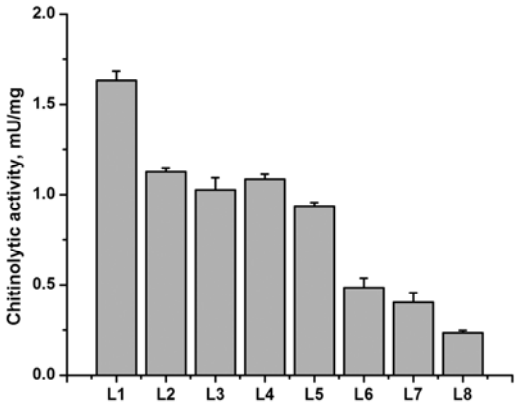

Figure 5. Chitinolytic activity of *Ficus carica* latex samples (collected in period May to August: L1-L8). The values represent means ± standard deviation of data from three independent experiments.

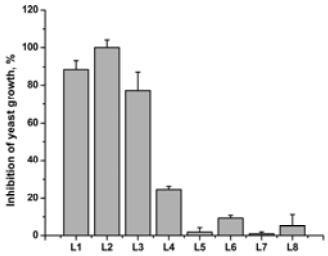

Figure 6. Antifungal activity of *Ficus carica* latex samples (collected in period May to August: L1-L8) against *Saccharomyces cerevisiae*. The values represent means ± standard deviation of data from three independent experiments.