Biochemical Characterisation of α- and β-Glucosidases and α- and β-Galactosidases from Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier) (Col.: Curculionide)

Nasir Saberi RISEH[1], Mohammad GHADAMYARI[1] and Behnam MOTAMEDINIYA[2]

[1]Department of Plant Protection, Faculty of Agriculture, University of Guilan, Rasht, Iran;
[2]Agricultural and Natural Resource Research Centre of Baluchestan, Baluchestan, Iran

Abstract

Riseh N.S., Ghadamyari M., Motamediniya B. (2012): **Biochemical characterisation of α- and β-glucosidases and α- and β-galactosidases from red palm weevil,** *Rhynchophorus ferrugineus* (Olivier) (Col.: Curculionide). Plant Protect. Sci., **48**: 85–93.

The red palm weevil, *Rhynchophorus ferrugineus* (Olivier), is one of the most destructive pests of palm trees in the southeast of Iran. Digestion in the alimentary canal of the red palm weevil is facilitated by some carbohydrases. Results of the *in vitro* studies indicated the presence of α- and β-glucosidases and α- and β-galactosidases in the digestive system and haemolymph of this pest. In the digestive system of females, the activities of α-glucosidase and β-galactosidase were higher than those of β-glucosidase and α-galactosidase. Also, the specific activity of α- and β-glucosidases and α- and β-galactosidases in the female digestive system was much higher than that in larvae. Results showed that the highest activities of α- and β-glucosidases were at pH 5 and of α- and β-galactosidases at pH 4. The *R. ferrugineus* α- and β-glucosidases and α- and β-galactosidases have an optimum temperature activity at 50, 50–60, 40–60, and 40°C, respectively. A zymogram pattern in the native gel revealed that *R. ferrugineus* α- and β-glucosidases and α- and β-galactosidases in the digestive system showed 2, 3, 1 and 1 major bands, respectively. The activity of α-glucosidase in the digestive system of larvae and female adults was higher than that of the other carbohydrases. Therefore, it is the most important subject for further study and design of a new approach to the control of this pest using carbohydrase inhibitors.

Keywords: carbohydrates; digestive system; palm trees

Most living organisms are able to exploit environmental polysaccharides. α-Glucosidase (EC 3.2.1.20) is an enzyme that catalyses the hydrolysis of 1,4-α-glucosidic linkages, releasing α-glucose. This enzyme strongly hydrolyses sucrose, maltose, maltodextrin, and pNP-α-D-glucopyranoside. It can be found in the alimentary canal, salivary secretions of insects (Lagadic & Chararas 1988; Ramzi & Hossininaveh 2010) and hypopharyngeal glands of some insects, such as *Apis mellifera* (Baker & Lehner 1972; Terra *et al*. 1996). So far, α-glucosidases have been isolated and characterised from many insects including *Dysdercus peruvianus* (Hemiptera: Pyrrhocoridae), *Pyrhocoridae zeamais* (Coleoptera: Curculionidae), *Apis mellifera* (Hymenoptera: Apidae), *Drosophila melanogaster (*Diptera: Drosophilidae), and *Glyphodes pyloalis* (Lep.: Pyralidae) (Huber & Mathison 1976; Tanimura *et al.* 1979; Baker 1991; Silva & Terra 1997; Ghadamyari *et al.* 2010). β-Glucosidase hydrolyses β 1–4 linkages between two glucoses or glucose-substituted molecules (such as cellobiose) (Terra *et al.* 1996). In addition to the important digestive role of enzymes, they can also act as elicitors (triggering agents of plant defence mechanisms) in plants when they are encountered with the feeding damage of the insect pests (Mattiacci *et al.* 1995).

α-D-Galactosidases (EC 3.2.1.22) are exo-acting glycoside hydrolases that cleave α-linked galactose residues from carbohydrates such as melibiose, raffinose, stachyose, and gluco- or galactomannans (Meier & Reid 1982). α-D-Galactosidases have been isolated from a variety of animal, plant and microbial sources, but there is little information about this enzyme in insects. β-D-Galactosidase (EC 3.2.1.23) is a hydrolase enzyme that catalyses the hydrolysis of β-galactosides into monosaccharides (Sezgintürk & Dinçkaya 2008). Also, our knowledge of this enzyme in insects is still rudimentary.

The damage caused by *R. ferrugineus* was reported for the first time in 1990 on traditional date palm groves in Saravan region (Sistan and Baluchestan province, Iran). This pest is a serious pest of various palm species including dates in Iran. The immature stages develop within the tree trunk, destroy its vascular system and eventually cause the collapse and death of the tree (Ju *et al.* 2011). The red palm weevil has been managed in Iran through employing an integrated pest management (IPM) strategy comprising several tactics such as mass trapping with pheromone-baited traps. Although these tactics have been widely used, controlling this pest has not been very effective yet when there is an outbreak of the red palm weevil. Currently, some of the major aspects in pest control are to achieve the selective inhibition of the digestive enzymes of many insect pests. Carbohydrases are active upon a large range of substrates and in some cases they are implicated in plant resistance to insect predation. Besides hydrolysing several carbohydrates, thus liberating monosaccharides that can be absorbed, they can cleave plant toxic glycosides. These, after being hydrolysed by β-glucosidase, can form cyanide or other toxic compounds such as inhibitors of glucose 6-phosphate dehydrogenase (Desroches *et al.* 1997) or trehalase (Nakano & Sacktor 1984). As the first step to achieving this it is necessary to characterise carbohydrate hydrolases of the digestive system. The study of insect digestive enzymes is important because the gut is the major interface between the insect and its environment. To understand how digestive enzymes act on their substrate in insects, it is essential to develop methods of insect control. So far, research has been done on proteases in the digestive system of the red palm weevil (Alarcon *et al.* 2002) but works on carbohydrases of *R. ferrugineus* have been missing. Also, there are some researches on α- and β-glucosidases in the digestive system of insects but our knowledge of α- and β-galactosidases in the digestive system of insects is still rudimentary. The aim of the present study was to determine biochemical characterisation of carbohydrate-hydrolysing enzymes in the digestive system of the red palm weevil in order to gain a better understanding of the digestive physiology of this insect.

MATERIAL AND METHODS

Insect. The insect was collected from palm trees in Sistan and Baluchestan province of Iran. The last larval instar and adults were randomly selected for measuring the enzyme activity.

Chemicals. *p*-Nitrophenol and bovine serum albumin were purchased from Merck (Darmstadt, Germany). *p*-Nitrophenyl-α-D-glucopyranoside (pNαG), *p*-nitrophenyl-β-D-glucopyranoside (pNβG), *p*-nitrophenyl-α-D-galactopyranoside (pNαGa), *p*-nitrophenyl-β-D-galactopyranoside (pNβGa), 4-methylumbelliferyl-β-D-glucopyranoside (4-MUβG), 4-methylumbelliferyl-α-D-glucopyranoside (4-MUαG), 4-methylumbelliferyl-β-D-galactopyranoside (4-MUβGa) and 4-methyl-umbelliferyl-β-D-galactopyranoside (4-MUαGa) were obtained from Sigma (St. Louis, USA).

Sample preparation. Larvae and adults were immobilised on ice and dissected under a stereo microscope in ice-cold saline buffer. Digestive systems were removed and their contents were eliminated. Digestive systems of the last larval instar were divided into three distinct divisions (V1, V2 and V3) (Figure 1). The samples were transferred to a freezer (–20°C). The sample was homogenised in cold double-distilled water using a hand-held glass homogeniser and centrifuged at 10 000 rpm for 10 min at 4°C. Also, the haemolymph was collected from larvae. Small incisions were made in the soft cuticle anterior to the second thoracic segment of larvae and the haemolymph was collected with a 75 µl glass capillary tube. Then the haemolymph was quickly added to a tube containing an anticoagulant solution (glucose 1.0 g; citrate 0.4 g; NaCl 0.21 g; double-distilled water was added to 50 ml). After collecting, the samples were transferred to a freezer (–20°C).

Determination of α- and β-glucosidase and α- and β-galactosidase activity, Activities of α- and β-glucosidases and α- and β-galactosidases activities were measured with pNαG, pNβG, pNαGa, and

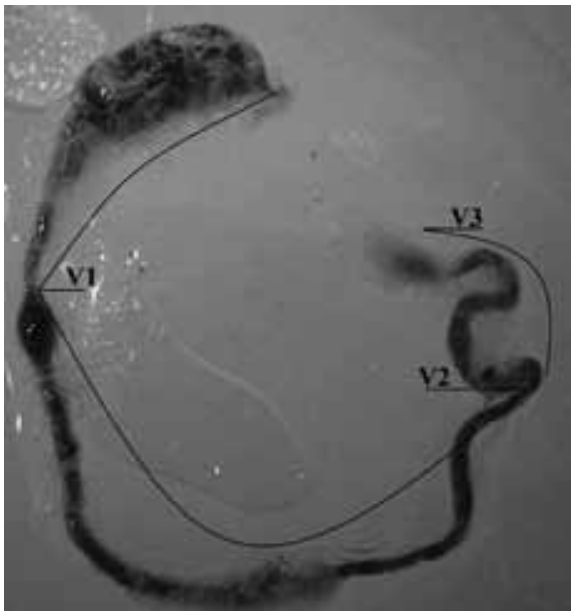

Figure 1. Different parts (V1, V2 and V3) of the alimentary canal in the larvae of *Rhynchophorus ferrugineus*

pNβGa as substrate, respectively. Homogenates were incubated for 20 min at 37°C with 45 µl of substrate (25mM) and 115 µl of 40mM glycine-phosphate-acetic-citric buffer. The reaction was stopped by addition of 600 µl of NaOH (0.25M). Optical density was measured at 405 nm using a microplate reader (Stat Fax 3200, Awareness Technology, Los Angeles, USA) after 10 minutes. Controls without enzyme or without substrate were included. A standard curve of absorbance against the amount of *p*-nitrophenol released was constructed to enable the calculation of the amount of *p*-nitrophenol released during the α- and β-glucosidase and α- and β-galactosidase assays.

Determination of pH optimum and effect of temperature on α- and β-glucosidase and α- and β-galactosidase activity. The activity of α- and β-glucosidases and α- and β-galactosidases was determined at several pH values using 40mM glycine-phosphate-acetic-citric buffer. The effect of temperature on α- and β-glucosidase and α- and β-galactosidase activities were measured using the homogenate of adults by incubating the reaction mixture at 20, 30, 40, 50, 60, and 70°C for 30 min, followed by measurement of the activity.

Protein concentration. Protein concentration was estimated by the Bradford method (Bradford 1976), using bovine serum albumin as standard.

Polyacrylamide gel electrophoresis and zymogram analysis. Non-denaturing polyacrylamide gel electrophoresis (PAGE) was carried out as described by Davis (1964). For a zymogram analysis of α- and β-glucosidases and α- and β-galactosidases, the samples were mixed with sample buffer and applied onto polyacrylamide gel (4% and 10% polyacrylamide for the stacking and resolving gels, respectively). Electrophoresis was performed with 100 V at 4°C. Afterwards, the gel was immersed in 3mM 4-MUβG, 4-MUαG, 4-MUβGa and 4-MUαGa in 0.1M sodium acetate (pH 5.0) for 10 min at room temperature to develop bands showing α- and β-glucosidase and α- and β-galactosidase activities, respectively. The blue-fluorescent bands appeared in a few minutes under UV.

Statistical analysis. The data were compared by one-way analysis of variance (ANOVA) followed by Tukey's test when significant differences were found at $P = 0.05$ using the SAS program, Version 8.0.

RESULTS

α- and β-glucosidase activity

The specific activities of α- and β-glucosidases in the digestive system of females were 6.09- and

Table 1. The specific activities (mean ± SE) of some digestive in haemolymph, adult and larvae of *Rhynchophorus ferrugineus*

Enzymes	Activity (µmol/min/mg protein)			
	larvae	female	male	haemolymph
α-Glucosidase	10.31 ± 0.3^{b*}	62.85 ± 0.3^{a}	3.03 ± 0.66^{c}	0.12 ± 0.001^{d}
β-Glucosidase	0.29 ± 0.005^{c}	0.74 ± 0.011^{b}	1.25 ± 0.015^{a}	0.067 ± 0.01^{d}
α-Galactosidase	0.24 ± 0.04^{b}	1.3 ± 0.02^{a}	1.47 ± 0.04^{a}	0.045 ± 0.01^{c}
β-Galactosidase	0.43 ± 0.02^{c}	11.09 ± 0.08^{a}	6.07 ± 0.04^{b}	0.14 ± 0.005^{d}

*different letters indicate that the specific activity of enzymes is significantly different from each other by Tukey's test ($P < 0.05$)

2.55-fold higher than those in the last larval instar, respectively. Whereas the α- and β-glucosidase activity in the digestive system of males was 0.29- and 4.31-fold higher than in the digestive system of the last larval instar, respectively (Table 1). Also, the α-glucosidase activity in V1, V2 and V3 of the last larval instar was 8.14 ± 0.92, 9.6 ± 0.38, and 2.52 ± 0.36 μmol/min/mg proteins, respectively. The β-glucosidase activity in V2 was 1.39- and 8.34-fold higher than that in V1 and V3 of the last larval instar, respectively (Figure 2).

α- and β-galactosidase activity

The specific activity of α-galactosidase in the digestive system of adults and the last larval instar was determined and results showed that the specific activity of α-galactosidase in the digestive system of females was 5.41-fold higher than that in the last larval instar. Whereas the activity of β-galactosidase in the digestive system of females was 25.7-fold higher than in the digestive system of the last larval instar (Table 1). The α-galactosidase activity in V2 section was higher than in V1 and V3 sections of the last larval instar. Also, the β-galactosidase activity in V1 was 2.19-fold and 8.6-fold higher than in V2 and V3 of the last larval instar, respectively (Figure 2).

Effect of pH and temperature on α- and β-glucosidase and α- and β-galactosidase activity

The effects of pH on the hydrolytic activity toward pNαG, pNβG, pNαGa, and pNβGa were tested using 40mM phosphate-acetic-citric buffer (pH 2–12). Maximum activity in the digestive system was observed at pH 5 for α- and β-glucosidases and at pH 4 for α- and β-galactosidase activity (Figure 3). The *R. ferrugineus* α- and β-glucosidases have an optimum temperature activity at 50°C and 50–60°C, respectively. Also, the optimal temperature for α- and β-galactosidases in the digestive system was 40–60°C and 40°C, respectively (Figure 4).

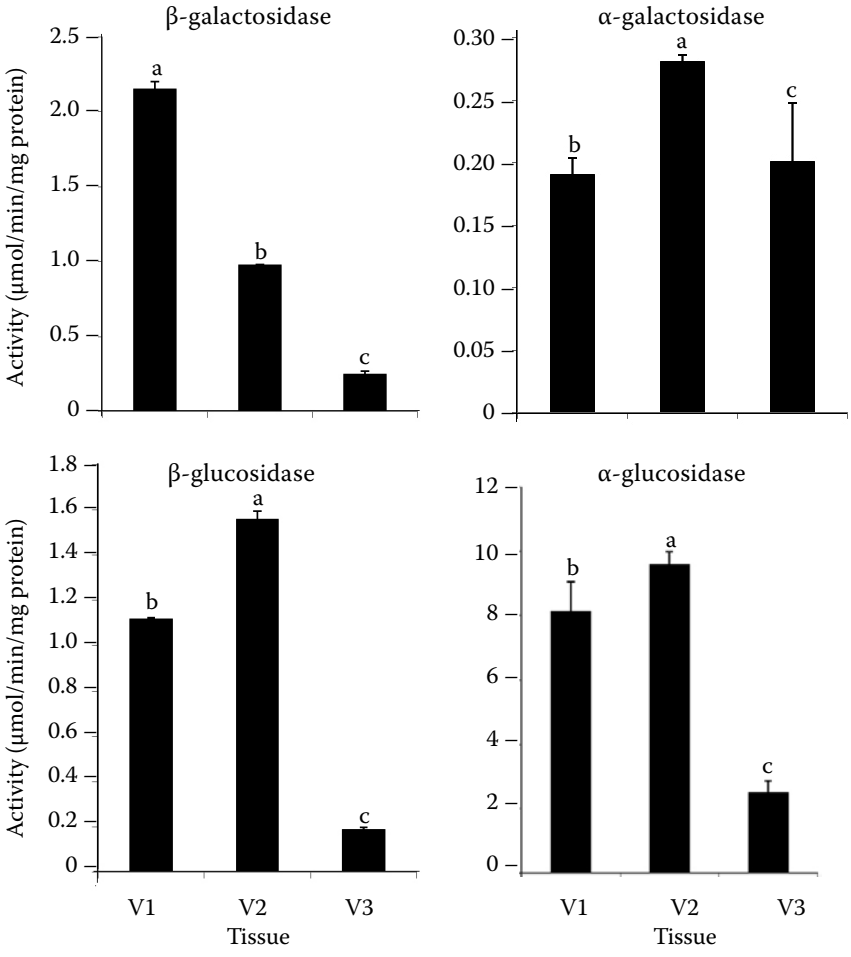

Figure 2. Comparison of the activity of α- and β-glucosidases and α- and β-galactosidases extracted from different parts of the digestive system of *Rhynchophorus ferrugineus* larvae. Different letters indicate that the activity of enzymes in different tissues is significantly different from each other by Tukey's test ($P < 0.05$)

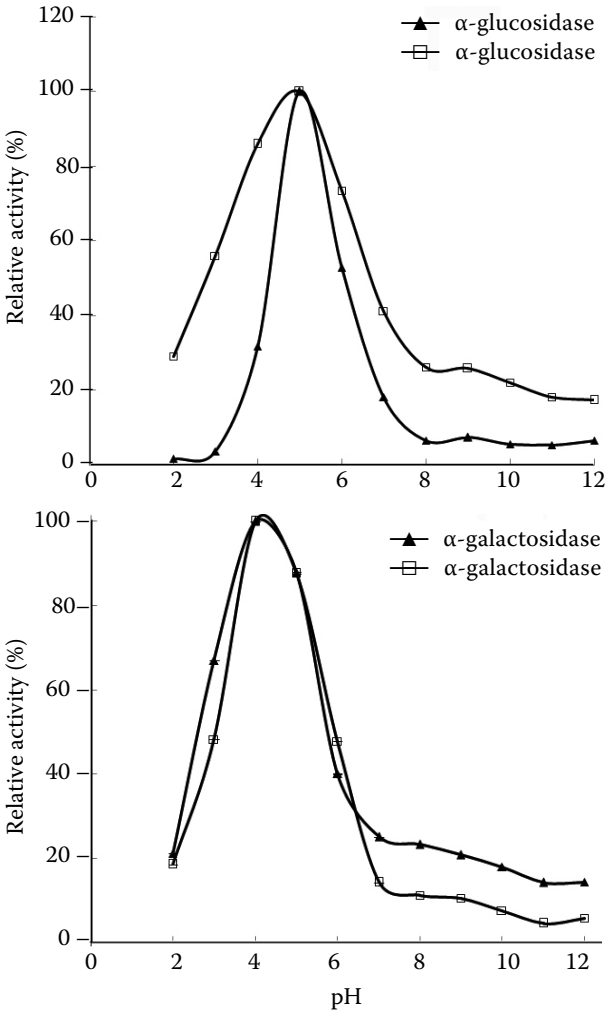

Figure 3. Effect of pH on the activity of α- and β-glucosidases, α- and β-galactosidases extracted from the digestive system of *Rhynchophorus ferrugineus*

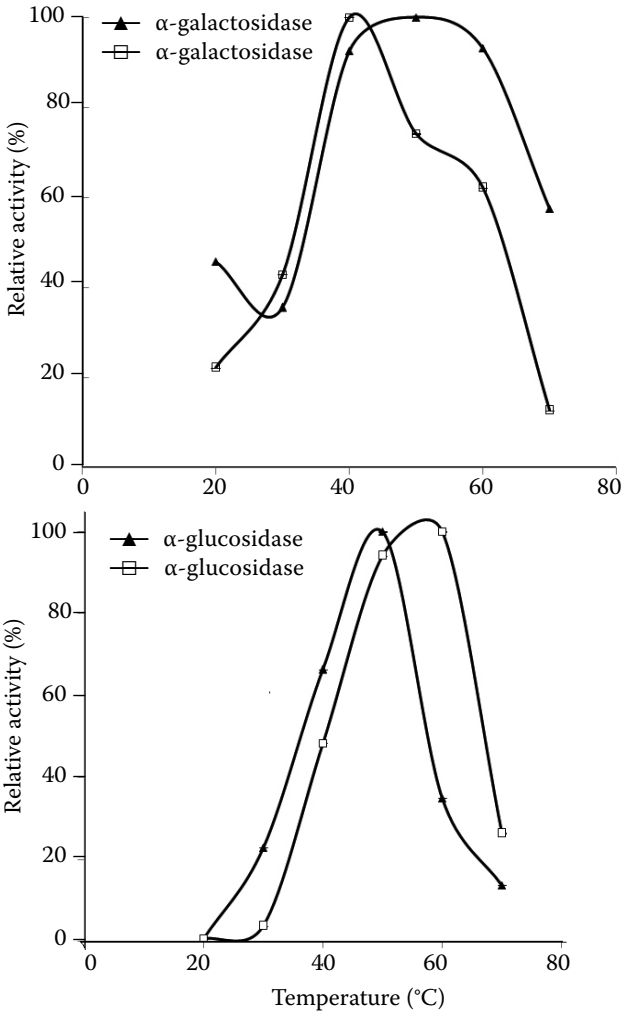

Figure 4. Effect of temperature on the activity of α- and β-glucosidases and α- and β-galactosidases extracted from the digestive system of *Rhynchophorus ferrugineus.* Highest activity at optimal temperature was taken as 100%; Relative activity (%) = (absorbance at respective temperature/ absorbance at optimal temperature) × 100

Zymogram analysis

The crude extracts of *R. ferrugineus* were analysed by native PAGE. After α- and β-glucosidase and α- and β-galactosidase activity staining, 4, 4, 2, and 1 isoforms of α- and β-glucosidases and α- and β-galactosidases in the digestive system were clearly detected, respectively (Figure 5). However, as depicted in Figure 5, the intensity of two, one and one bands was high for α- and β-glucosidases and β-galactosidase, respectively.

DISCUSSION

The data revealed that α- and β-glucosidases and α- and β-galactosidases are present in the digestive system of adults, the last larval instar and haemolymph of *R. ferrugineus*. The specific activities of α- and β-glucosidases and α- and β-galactosidases from the digestive system of females were higher than those of larvae, males and haemolymph of the last larval instar (Table 1). In the digestive system of females the activities of α-glucosidase and β-galactosidase were much higher than those of β-glucosidase and α- galactosidase (Table 1). Specific activities of α- and β-glucosidases and α- and β-galactosidases in the female digestive system were much higher than in larvae and males. The female needs a lot of energy for reproduction, which may be one of the reasons why this stage shows a high level of α- and β-glucosidase and

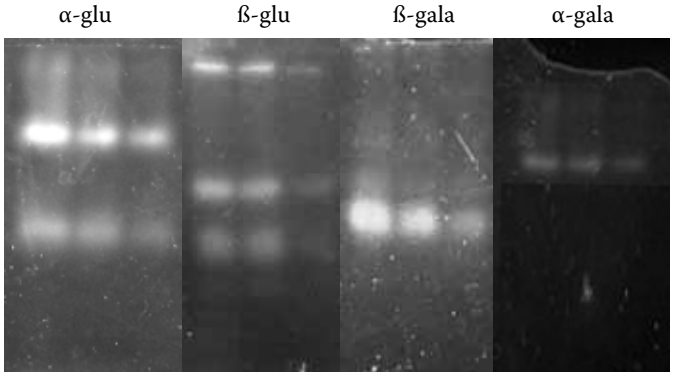

Figure 5. Zymogram of α- and β-glucosidases and α- and β-galactosidases in the digestive system of the last larval instar of *Rhynchophorus ferrugineus*

α-glu – α-glucosidase; β-glu – β-glucosidase; β-gala – β-galactosidase; α-gala – α-galactosidase
Two and three major bands for α- and β-glucosidases and one major band for α- and β-galactosidases were detected at different protein concentrations (1.83, 1.22, and 0.61 mg/ml)

α- and β-galactosidase activity compared with larvae and males. The activity of α- and β-glucosidases and α- and β-galactosidases was also characterized by a zymogram analysis after native PAGE which allowed the visualisation of the enzyme activity *in situ*. The results indicated 4, 4, 2, and 1 isoforms of α- and β-glucosidases and α- and β-galactosidases in the crude digestive system of the last larval instar, respectively (Figure 5). In other coleopteran insects, β-glucosidase in the digestive system of *Xanthogaleruca luteola* and *Osphranteria coerulescens* (unpublished data) has three and four isoforms, respectively. Also, β-galactosidase in the digestive system of *X. luteola* and *O. coerulescens* has one and six isoforms, respectively.

Results showed that the highest activities of α- and β-glucosidases were at pH 5 and of α- and β-galactosidases at pH 4 (Figure 3). Alpha- and β-glucosidases and α- and β-galactosidases in insects are generally the most active in neutral to slightly acid pH conditions (Lagadic 1994; Ghadamyari *et al.* 2010; Ramzi & Hosseininaveh 2010). Optimal pH values for glucosidases in larvae of several coleopterans were 3.0–6.5 (Chinnery 1971; Gatehouse & Anstee 1983; Lagadic & Chararas 1988; Lagadic 1994). Maximum activity in the digestive system of *X. luteola* was observed at pH 5 and 4 for α-glucosidase and α-galactosidase, respectively, whereas the optimal pH for β-glucosidase and β-galactosidase activity was 6 and 3, respectively (unpublished data). Similarly, α-glucosidase of *Callosobruchus maculatus* was the most active at pH 5.6 (Gatehouse & Anstee 1983). In other coleopterans, like in many insects, the optimal pH for α-glucosidase activity is generally in the slightly acid region. Maximal activities of sucrose hydrolysing *α*-glucosidase from the midgut of *Dermestes maculatus* adults occurred at pH 6.3 (Chinnery 1971). The optimal pH of the three major α-glucosidases found in the gut of *Bruchus afinis* (Coleoptera: Bruchidae) adults coincided well with the pH of the midgut where most of the digestive processes are known to occur (Lagadic & Chararas 1988). Similarly, digestive α-glucosidase from *D. maculatus* exhibited its optimal activity at pH values close to the midgut pH (Chinnery 1971). In *B. afinis* adults, alimentary carbohydrates are hydrolysed by highly active α-glucosidase (Lagadic & Chararas 1988) where pH would optimise their activities toward a number of various sugars present in the food of this bruchid (Luca 1966).

The pH optima of α-galactosidase in the midgut of *Odontotermes obesus* (Isoptera: Termitidae) were 5.2. Also, in the hindgut the pH optimum of this insect was 8.4 for α-galactosidase (Singh 1976). In non-coleopteran insects, Burton (1975) showed that the optimal pH was 5.5 for the α-glucosidase from both the ventriculus and the salivary glands of *Helicoverpa zea* (Lep.: Noctuidae). Also, Ghadamyari *et al.* (2010) showed that the optimal pH values for α- and β-glucosidases activity in the midgut and salivary glands of *G. pyloalis* were 7.5, 5.5, 8–9 and 8–9, respectively.

Comparing activities against *p*-nitrophenyl glycosides of glucose and galactose, the ratios of β-glucosidase/β-galactosidase were 0.67 and 0.066 for larvae and females of *R. ferrugineus*, respectively, when the activities were determined in the whole digestive system. Whereas the ratio of α-glucosidase/α-galactosidase was as 42.95 and 48.34 for larvae and females of *R. ferrugineus*, respectively. These results showed that the β-galactosidase and α-glucosidase activities in the digestive system of larvae and females were higher than β-glucosidase and α-galactosidase activities. Among the α- and β-glucosidase and α- and β-galactosidase activities tested, the activities

of β-glucosidase and α-galactosidase were very low in the digestive system of larvae, females and males. The ratio of β-glucosidase/β-galactosidase was reported as 88.5 in *Rhynchosciara americana* Wiedemann (Diptera: Sciaridae) (Terra *et al.* 1979), 105 in *Stomoxys calcitrans* (Deloach & Spotes 1984), 58 in the midgut tissue of *Rhodnius prolixus* (Terra *et al.* 1988), and 2.5 in *C. maculates* (Gatehouse *et al.* 1985). Also, in *D. peruvianus*, the β-glucosidase/β-galactosidase ratio in the midgut tissue was 28.7, but in the whole midgut (epithelium plus luminal contents) the ratio was 3.0, suggesting a major contribution of β-galactosidase activity by the seed meal present in the gut lumen (Silva & Terra 1997). The ratios of α-glucosidase/α-galactosidase found in adults and the last larval instar of *X. luteola* were 7.5 and 3.5, respectively (unpublished data). Ferreira *et al.* (1998) reported that high β-glucosidase activity was found in the foliage feeder *Abracris flavolineata* (Orthoptera: Acrididae), in the stored plant product feeder *Tenebrio molitor* (Coleoptera: Tenebrionidae) and in the pollen-feeder *Scaptotrigona bipunctata* (Hymenoptera: Apidae). Whereas our results showed that the β-glucosidase activity in the digestive system of larvae, males and females and in the haemolymph of larvae was very low. Our results coincided well with low β-glucosidase activity found among predaceous insects exemplified by *Pheropsophus aequinoctialis* (Coleoptera: Carabidae) and *Pyrearinus termitilluminans* (Coleoptera: Elateridae) (Ferreira *et al.* 1998). Also, β-glucosidase activity is low in the decaying plant-feeder *R. americana* and in feeders of plant aerial parts, exemplified by *Spodoptera frugiperda* (Lep.: Noctuidae), *Erinnyis ello* (Lep.: Sphingidae) and *Diatraea saccharalis* (Fabricius) (Ferreira *et al.* 1998). Our results showed that α-galactosidase activity is relatively low in the digestive system of larvae, males and females. The α- and β-glucosidase and α- and β-galactosidase activities were determined at different temperatures ranging from 20°C to 80°C. The maximum activity of α- and β-glucosidases and α- and β-galactosidases in *R. ferrugineus* incubated at different temperatures was observed at 50, 50–60, 40–60, and 40°C, respectively, which is consistent with α- and β-glucosidases activities in *G. pyloalis* (45°C) (Ghadamyari *et al.* 2010). Ramzi and Hosseininaveh (2010) showed that α- and β-galactosidase of *Brachynema germari* (Hemiptera: Pentatomidae) has an optimal activity at 30°C and 35°C for the midgut and salivary glands, respectively.

Results showed that glucosidase and galactosidase activity is present in all regions of the alimentary canal of the last larval instar of *R. ferrugineus* (Figure 2). Our results illustrated that there is a significant difference in the activity of α- and β-glucosidases and β-galactosidases in V1, V2, and V3 of the last larval instar (Figure 2). The rank order from the highest to the lowest β-galactosidase activity was V1 > V2 > V3 whereas the α- and β-glucosidase activities in V2 were much higher than in V1 and V3. Also, results showed that the ratio of α-glucosidase/α-galactosidase in V1 was higher than in V2 and V3. The alimentary canal of the pistachio green stink bug, *B. germari*, was divided into four distinct divisions (v1, v2, v3, and v4) by Ramzi and Hosseininaveh (2010) and activities of α-amylase and α- and β-glucosidase were measured in these parts. Their results showed that the highest α-amylase and α- and β-glucosidase activities were in section v3, whereas the lowest activities were in section v4. Also, results of the *in vitro* studies on certain digestive enzymes from the alimentary canal of *O. obesus* workers indicated the presence of α-galactosidase both in the mid- and hindgut of this termite (Singh 1976).

The activity of α- and β-glucosidases and α- and β-galactosidases was also characterised by a zymogram analysis after native PAGE which allowed visualisation of the enzyme activity *in situ* under UV. The results indicated the presence of at least 2, 3, 1 and 1 major bands for α- and β-glucosidases and α- and β-galactosidases in the digestive system, respectively. The activity of glucosidases from the alimentary canal of the last larval instar on the gel was much higher than that of galactosidases. Also, as depicted in Figure 5, the intensity of bands concerning α-galactosidase activity was lower than for α- and β-glucosidases. The zymogram pattern in the native gel revealed that *Xanthogaleruca luteola* (Col.: Chrysomelidae) β-glucosidase and β-galactosidase in the digestive system had three and one isoforms, respectively (Sharifi *et al.* 2011) and in this insect β-glucosidase activity was much higher than that of β-galactosidase in crude extract. PAGE analysis of α- and β-glucosidase activities in gel revealed the presence of one band in the salivary gland and midgut of *Lygaeus pandurus* (Hemiptera: Lygaeidae) (Vatanparast *et al.* 2011).

Glucosidases and galactosidases play an important role in insect digestion. Our results showed

that these enzymes are important in the initial and final phases of food digestion of *R. ferrugineus*. The glucosidase and galactosidase activity in females was higher than in larvae and males. Among the carbohydrases tested in this study, α-galactosidases and α-glucosidases showed the lowest and the highest specific activities in crude extracts and gel, respectively. Also, the carbohydrase specific activities in the haemolymph of red palm weevil were lower than in digestive systems.

Acknowledgments. We express our gratitude to the research council of the University of Guilan and Ministry of Science, Researches and Technology for financial support of this project.

References

Alarcon F. J., Martínez T.F., Barranco P., Cabello T., Diáz M., Moyano F. J. (2002): Digestive proteases during development of larvae of red palm weevil, *Rhynchophorus ferrugineus* (Olivier 1790) (Coleoptera: Curculionidae. Insect Biochemistry and Molecular Biology, **32**: 265–274.

Baker J.E. (1991): Properties of glycosidases from the maize weevil, *Sitophilus zeamais.* Insect Biochemistry, **21**: 615–621.

Baker R.J., Lehner Y. (1972): A look at honey bee gut functions. Bee Journal, **112**: 336–338.

Bradford M.M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, **72**: 248–254.

Burton R.L. (1975): Carbohydrate digestion in the adult moth *Heliothis zea.* Journal of Insect Physiology, **21**: 1855–1857.

Chinnery J.A.B. (1971): Carbohydrases of the midgut of the leather beetle *Dermestes maculatus.* Journal of Insect Physiology, **17**: 47–61.

Davis B.J. (1964): Disc electrophoresis II. Method and application to human serum proteins. Annals of the New York Academy of Sciences, **121**: 404–427.

Deloach J.R., Spates G.E. (1984): Glycosidase activity from midgut region of *Stomoxys calcitrans* (Diptera: Muscidae). Insect Biochemistry, **14**: 169–173.

Desroches P., Mandon N., Beaehr J.C., Huignard J. (1997): Mediation of host plant use by a glucoside in *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). Journal of Insect Physiology, **43**: 439–446.

Ferreira C., Torres B.B., Terra W.R. (1998): Substrate specificities of midgut β-glycosidases from insects of different orders. Comparative Biochemistry and Physiology, **119B**: 219–225.

Gatehouse A.M.R., Anstee J.H. (1983): The presence and partial characterization of carbohydrase enzymes in the gut of *Callosobruchus maculatus.* Cellular and Molecular Life Sciences, **39**: 1013–1015.

Gatehouse A.M.R., Fenton K.A., Anstee J.H. (1985): Carbohydrase and esterase activity in the gut of larval *Callosobruchus maculatus*. Experientia, **41**: 1202–1205.

Ghadamyari M., Hosseininaveh V., Sharifi M. (2010): Partial biochemical characterization of α- and β-glucosidases of lesser mulberry pyralid, *Glyphodes pyloalis* Walker (Lep.: Pyralidae). Comptes Rendus Biologies, **333**: 197–204.

Huber R.E., Mathison R.D. (1976): Physical, chemical and enzymatic studies on the major sucrose on honey bees (*Apis melifera*). Canadian Journal of Biochemistry, **54**: 153–164.

Ju R.T., Wang F., Wan F.-H., Bo L. (2011): Effect of host plants on development and reproduction of *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae). Pest Science, **84**: 33–39.

Lagadic L. (1994): Some characteristics of digestive α-glycosidases from adults of *Bruchus afinis* Friilich, in relation with intestinal pH. Comparative Biochemistry and Physiology, **108**: 249–253.

Lagadic L., Chararas C. (1988): Etude des activites osidasiques digestives d'adultes de *Bruchus afinis* en tours d'hivernation en Clevage artificial et en conditions seminaturelles. Entomologia Experimentalis et Applicata, **48**: 247–255.

Luca Y. (1966): Alimentation imaginale des bruchidts (Col.). Parasitica, **22**: 26–54.

Mattiacci L., Dicke M., Posthumus M.A. (1995): β-Glucosidase: an elicitor of herbivore induced plant odour that attracts host-searching parasitic wasps. Proceedings of the National Academy of Sciences USA, **92**: 2036–2040.

Meier H., Reid J.S.G. (1982): Reserve polysaccharides other than starch in higher plants. In: Loewus F.A, Tanner W. (eds): Encyclopedia of Plant Physiology. Springer Verlag. New York: 418–471.

Nakano M., Sacktor B. (1984): Renal trehalase: two subsites at the substrate binding site. Biochimica et Biophysica Acta, **791**: 45–49.

Ramzi S., Hosseininaveh V. (2010): Biochemical characterization of digestive α-amylase, α-glucosidase and β-glucosidase in pistachio green stink bug, *Brachynema germari* Kolenati (Hemiptera: Pentatomidae). Journal of Asia Pacific Entomology, **13**: 215–219.

Sezgintürk M.K., Dinçkaya E. (2008): β-Galactosidase monitoring by a biosensor based on Clark electrode: Its optimization, characterization and application. Biosens Bioelectron, **23**: 1799–1804.

Silva C.P., Terra W.R. (1997): α-Galactosidase activity in ingested seeds and in the midgut of *Dysdercus peru-*

vianus (Hemiptera: Pyrrhocoridae). Archives of Insect Biochemistry and Physiology, **34**: 443–460.

Singh N.B. (1976): Studies on certain digestive enzymes in the alimentary canal of *Odontotermes obesus* (Isoptera: Termitidae). Entomologia Experimentalis et Applicata, **20**: 113–122.

Sharifi M., Ghadamyari M., Saiidi F. (2011). Biochemical characterization of some digestive carbohydrases from elm leaf beetle, *Xanthogaleruca luteola* Mull. (Col.: Chrysomelidae). In: Global Conference on Entomology, March 5–9, 2011, Chiang Mai, Thailand.

Tanimura T., Kitamura K., Fukuda T., Kikuchi T. (1979): Purification and partial characterization of three forms of alpha-glucosidase from the fruit fly *Drosophila melanogaster*. Biochemical Journal, **85**: 123–130.

Terra W.R., Ferreir C., de Bianchi A.G. (1979): Distribution of digestive enzymes among the endo- and ectoperitrophic spaces andmidgut cells of *Rhynchosciara* and its physiological significance. Journal of Insect Physiology, **25**: 487–494.

Terra W.R., Ferreira C., Garcia E.S. (1988): Origin properties and functions of the major *Rhodnius prolixus* midgut hydrolases. Insect Biochemistry, **18**: 423–434.

Terra W.R., Ferreira C., Jordao B.P., Dillon R.J. (1996): Digestive enzymes. In: Lehane M.J, Billingsley P.F. (eds): Biology of the Insect Midgut. Chapman & Hall, London: 153–193.

Vatanparast M., Hosseininaveh V., Sajjadian S.M., Amiri S. (2011). Glucosidases in the midgut and salivary glands of the pistachio red seed bug *Lygaeus pandurus* (Hemiptera: Lygaeidae). In: Global Conference on Entomology, March 5–9, 2011, Chiang Mai, Thailand.

Recieved for publication April 14, 2011
Accepted after corrections September 19, 2011

Corresponding author:

Dr. Mohammad Ghadamyari, University of Guilan, Faculty of Agricultural Science, Department of Plant Protection, Rasht, Iran
tel.+ 98 1316 690 009; e-mail: ghadamyari@guilan.ac.ir or mghadamyari@gmail.com