Kinetic Properties and Metal Ion Stability of the Extracellular Naringinase Produced By Aspergillus Flavus Isolated From Decaying Citrus Maxima Fruits

Keerthini Srikantha¹, Ranganathan Kapilan²*, Vasanharuba Seevaratnam¹

¹Department of Agricultural Chemistry, Faculty of Agriculture, University of Jaffna, Sri Lanka
²Department of Botany, Faculty of Science, University of Jaffna, Sri Lanka

*Corresponding Author: Phone: +94212229645; Fax: +94212222685; Email: ranganat@ualberta.ca

Received 05 December 2016; Accepted 23 April 2017

Abstract. Important fungal species Aspergillus flavus has a worldwide attention for its industrial use and toxigenic potential. It is capable of producing diverse group of enzymes including lipase, xylanase, α amylase and naringinase. Naringinase is a biotechnologically important enzyme and has potential application in food and pharmaceutical industries. Naringinases that show better stability in acidic pH and low temperatures are highly preferred in food industries. This study was aimed to characterize and determine the kinetic properties of the crude naringinase enzyme produced by Aspergillus flavus isolated from Citrus maxima fruit. The crude naringinase enzyme from Aspergillus flavus was highly active at 45°C and it was very stable at 40°C and 45°C for at least 1 hour. Highest naringinase activity was obtained at pH 4.5 but the enzyme was stable at pH 4.0 for at least one hour. The enzyme showed zero order kinetics for 10 minutes. Vmax of the crude naringinase enzyme was 4.8076 umol/min and the Michaelis constant by Lineweaver-Burk Plot for naringin was 4.347 μmol/L under the conditions. Addition of 2mM of Hg²⁺, Cu²⁺ and Ba²⁺ decreased the naringinase enzyme activity, while addition of 2mM Na⁺, Mn²⁺, Ca²⁺ and Zn²⁺ increased the enzyme activity. The crude naringinase from Aspergillus flavus possesses the appropriate characteristics to be used in various industrial and biochemical applications. Acidic nature and optimum low temperature of enzyme, facilitates debittering of acidic juice without adjusting the pH and might be used to maintain nutritional and organoleptic nature of the lemon juice.

Keywords: Aspergillus flavus, Citrus maxima, debittering, naringinase, kinetic properties, Michaelis constant

1. INTRODUCTION

Commercial fruit juice production is facing serious challenges to minimize the bitterness of the fruit juices. This bitterness is due to the presence of a flavonone called naringin. Naringin concentration is linked with the maturity of the fruit. As the fruit ripens, concentration of the naringin tends to decrease (Yalim et al., 2004). Reduction of naringin content was done by using chemical applications that had several drawbacks resulting in the poor quality of fruit juice. Debittering of the juice using the enzyme naringinase is a promising approach, as it causes minimal damage to the nutritional quality and enhances organoleptic properties (Patil and Dhake, 2014).

The fungal species Aspergillus flavus can be easily isolated from decaying fruits and vegetables with low cost, limited facilities. The characterization of the naringinase enzyme produced by Aspergillus flavus isolated from Citrus fruit pulps is important to decide its application in food processing and to utilize the enzyme to its maximum.

Naringinase is a complex glycolytic enzyme that has both α-L rhamnosidase and β- D-glucosidase activity. Naringinase is an enzyme complex, containing a -L-rhamnosidase and b-glucosidase activity. During naringin hydrolysis there are two steps involved, where a -L-rhamnosidase first hydrolyzes the naringin to L-rhamnose and prunin and subsequently b-glucosidase hydrolyzes prunin into naringenin and D-glucose. The prunin can be hydrolyzed by the β-D glucosidase into aglyconenaringin and β-D-glucose (Puri, 2012). Naringinase is used in the production of the glycopeptide antibiotic chloropolysporin from Faenia interjecta, other than debittering process (Sankyo, 1988). Naringinase can function as a chiral intermediate and widely used as a pharmaceutical and plant protective agent (Daniels et al., 1990). Hence naringinase enzyme makes potential usefulness in pharmaceuticals and food industries (Ribeiro, 2011). With a wide range of applications, naringinase has
become one of the biotechnologically important enzymes. Naringinase has been reported in plants, yeasts, fungi, and bacteria (Ribeiro, 2011). Microbial enzymes are getting attractions due to cost-effective production with an economically viable process. Chemical methods of naringin reduction are preferred to replace the microbial naringinases in industries. Though the production of naringinase has been very well studied in fungal sources, very limited reports are available on bacterial naringinase (Mukund et al., 2014, Ni et al., 2011). Since Naringinase is an expensive enzyme there is scope for fermentation process development using new isolates, which would result in commercially viable processes.

*Aspergillus flavus* has a great attraction worldwide for its industrial usage and toxigenic potential (Venâncio et al., 2007). It is an efficient producer of α-amylase (Shafique et al., 2009), lipase (Pau & Omar, 2004), xylanase (Bhushan et al., 2012) and naringinase (Radhakrishnan et al., 2013). Naringinase enzyme production through microorganisms has great potential in the recent years due to low economic expenses. The organism facilitates the production of total requirement of enzyme by the change of culture medium and culture conditions. Enzyme production promotes utilization of the agricultural product waste as C source or N source to microorganism while production of enzyme. Naringinase is a complex of α-L-rhamnosidase activities and β-D-glucosidase activities, it hydrolysis naringin (4,5,7-trihydroxyflavonone 7-rhamnoglucose) into prunin, rhamnose, glucose and naringenin (Puri et al., 2012). Naringinase becomes one of the biotechnologically important enzymes, since it has been significantly used in debittering the citrus fruit juices and helps to improve stability and the properties of the juices (Angela, 2014). Apart from that, hydrolyzed products have potential application in food and pharmaceutical industries. Prunin, a hydrolyzed product of naringin, has antiviral and anti-inflammatory activities (Kaul et al., 1985) and may be used as a sweetening agent in diabetes therapy (Puri, 2011). Another hydrolyzed product naringenin could be used as an antioxidant, anti-cancers and anti-tumors and inhibitor of Hepatitis C virus (Angela, 2014). Therefore characterization of naringinase could be very useful in determining the conditions of the enzyme for the industrial applications. The objective of study was to determine the kinetic properties and the stability of the crude naringinase enzyme produced by *Aspergillus flavus* and to study the role of metal ions on its activity.

2. METHODS AND MATERIALS

2.1. Chemical

Naringin was obtained from Sigma, St. Louis, USA. All other reagents were in analytical grade. The chemicals used were from standard sources.

2.2. Microorganism for enzyme production

Naringinase from *Aspergillus flavus* that was previously isolated from decaying *Citrus maxima* fruit (Keerthini et al., 2016a).

2.3. Activation of *Aspergillus flavus* and fermentation process

The activation medium was prepared as liquid broth of 100ml in 500ml conical flask with (w/v) 0.2% naringin, 0.5% glucose, 0.7% peptone, 0.1% yeast extract, 0.05% KH₂PO₄, 0.01% MgSO₄.7H₂O and 0.07% ZnSO₄.7H₂O, 0.07% CuSO₄.5H₂O, 0.07% FeSO₄.7H₂O (Navaratnam et al., 2003). The fermentation medium containing (w/v) 0.2% naringin, 0.5% glucose, 0.7% peptone, 0.1%yeast extract, 0.05% KH₂PO₄,0.01% MgSO₄.7H₂O and 0.07% ZnSO₄.7H₂O, 0.07% CuSO₄.5H₂O, 0.07% FeSO₄.7H₂O was used. The pH was adjusted to 6.0 (Navaratnam et al. 2003). Twenty five mL of the sterilized liquid medium was taken into 100ml conical flask and 6day old cultures of each organism from potato dextrose agar (PDA) medium was inoculated in the liquid fermentation medium and allowed for fermentation at 200rpm in room temperature until 9thday. The spores were taken out using sterile 0.85%NaCl solution and spore size was adjusted as 10⁵ spores/mL.1ml of spore solution was in-oculated on duplicate fermentation medium of each fungal strain under aseptic condition. The fermented sample was withdrawn for the enzyme assay at 7th, 8th and 9th day. One unit of naringinase activity is defined as the amount of enzyme that produces 1μmol of reducing sugar in 1 minute at pH 5 and 60°C with 10 gL⁻¹ naringin (Keerthini et al., 2016a, 2016b and Karuppaija et al., 2016)

2.4. Extraction of enzyme

*Aspergillus flavus* spores was inoculated to solid fermentation medium having composition of (per L) 200g paddy husk, 5g NH₄NO₃, 5g of naringin, 5g citrus peel, 5g sucrose, 0.2g Kcl, 0.4g KH₂PO₄ and its initial pH was 6.0 and fermentation flasks were kept at room temperature for 8 days in dark room without shaking. Fermentation flasks were 100 ml conical flask with 1/4 part was occupied by liquid media with
the above composition. Optimum temperature, pH, substrate concentration, time, inducing metal ions and stability of temperature, pH and best 3 inducing minerals were calculated based on the β-D-glucosidase activity of naringinase with the help of DNSA assays method. All the experiments were done with triplicates.

2.5. Naringinase assay

Crude enzyme and substrate were preincubated at 35°C for 3 minutes. After that pre incubated 0.25ml of crude enzyme was added to 0.25ml pre incubated substrate that was naringin. For optimum temperature determination, the different reaction temperatures were maintained. Optimum pH and substrate concentration were calculated by prepare substrate at different pH and different concentration and allowed to react with enzyme. For optimum time determination, different incubation time was set. After predetermined reaction condition, the reaction was stopped by boil for 5 minutes after addition of DNS acid (Miller, 1959). Finally it was allowed to cool and final volume was made up to 6ml with distilled water. Absorbance of test was measured with the help of blank by spectrometer. Blank was prepared by add 0.5 ml DNS acid to the 0.25ml substrate and stirred well, then add the 0.25ml supernatant and followed the procedure same as test.

2.6. Effect of temperature on naringinase activity

The optimum temperature of naringinase was determined by incubating supernatant and substrate mixture (pH 5) at temperature ranging from 35°C - 75°C, with the interval of 10°C for 10 minutes, where substrate concentration and pH were 0.8% and 5. Optimum temperature was determined by calculating the resulted reducing sugar (glucose) from enzyme and substrate reaction, which is described above (Karuppaija et al, 2016).

2.7. Effect of pH on naringinase activity

The effect of pH on naringinase activity was determined by incubating supernatant with substrate at pH ranging from 4.0 – 7.5, with the interval of 1.0 for 10 minutes, where the substrate concentration and temperature were 0.8% and optimum temperature which was decided from above investigation. The optimum pH was decided under naringenase assay condition (Karuppaija et al, 2016). The different pH of substrate was obtained by Citrate-phosphate (pH 3.0, 4.0, 5.0 and 6.0), sodium phosphate (pH 7.0), Tris (pH 8.0) and Glycine NaOH buffer (pH 9.0).

2.8. Effect of incubation time on naringinase activity

Optimum time for naringinase enzyme activity was measured by incubating the crude enzyme with substrate at optimum pH and temperature for different time, ranging from 5-25 minutes, with the interval of 5 minutes. Optimum time was determined under standard assay condition.

2.9. Effect of substrate concentration on naringinase activity

The effect of various minerals were determined in the presence of 2mM of Ca²⁺, Cu²⁺, Mn²⁺, Ba²⁺, Hg²⁺, Zn²⁺, Mg²⁺, Na⁺ at pH 4.5 and 45°C using naringin as substrate at 10g/L concentration for 10 minutes. Activity of naringinase enzyme was determined by the assay method described above.

2.10. Effect of minerals on naringinase activity

The effect of various minerals were determined by incubating the crude enzyme with substrate at different concentration that ranging from 2g/L - 12g/L with the interval of 2g/L for optimum incubation time, where pH and temperature were maintained at optimum. The optimum substrate concentration was determined under naringinase assay condition.

2.11. Stability of Enzyme with Temperature

The crude enzyme was preincubated at different temperature ranging from 45°C ± 5°C for 1 hour. Then enzyme was allowed to react with substrate at 10g/L, pH 4.5 and reaction temperature was 45°C and incubation time was 5 minutes.

2.12. Stability of enzyme with pH

The pH stability of crude enzyme was determined by preincubating the enzyme at different pH for 1 hour at 45°C and residual activity was determined at 45°C for 5 minutes, with naringin as substrate at 10g/L.

2.13. Stability of enzyme with minerals

Stability of enzyme with 3 best minerals was determined by preincubating the enzyme with selected minerals for 1 hour at 45°C and residual activity was determined at pH 4.5 and 45°C for 5 minutes, with naringin as substrate at 10g/L. All the experiments were done with triplicates.
3. RESULTS AND DISCUSSIONS

3.1. Effect of temperature

The crude enzyme from *Aspergillus flavus* showed highest activity at 45°C, where different temperatures were assayed ranging from 35°C- 75°C (Figure 1). Enzyme activity was significantly higher between 45 and 65°C temperatures. After 65°C, the activity of crude naringinase declined with the increasing temperature.

![Fig. 1: Effect of temperature on crude naringinase enzyme produced by Aspergillus flavus.](image)

3.2. Effect of pH

Citrate-phosphate (pH 3.0, 4.0, 5.0 and 6.0), sodium phosphate (pH 7.0), Tris (pH 8.0), Glycine NaOH buffer (pH 9.0) buffers were used. The pH value of the reaction mixtures were measured and presented in the figure 2. When the pH was varied from 4.0-7.0, the activity of naringinase increased up to pH 4.5 and further increase of pH, decreased the enzyme activity (Figure 2). However naringinase showed remarkable activity between pH 4.0-6.0, which was more than 65% of its original activity. Since the highest activity obtained at pH 4.5 was selected for further studies.

![Fig. 2: Effect of pH on the activity of the crude Naringinase enzyme from Aspergillus flavus at 45°C.](image)
3.3. Effect of incubation time

The influence of incubation time on the production of glucose from the reaction of naringinase enzyme with naringin (8 g/L) was studied for 25 minutes at pH 4.5 and at 45°C. The enzyme showed a linear relationship between the time and product formation up to 10 minutes. Hence, it was decided to fix the reaction time for 10 min. Even though the enzyme activity was increased from 5 minutes to 10 minutes at 45°C and pH of 4.5, it showed decreased activity after 10 minutes.

![Graph showing effect of incubation time on crude naringinase activity at pH 4.5 and 45°C.](image)

**Fig. 3:** Effect of incubation time on crude naringinase activity at pH 4.5 and 45°C.

3.4. Effect of substrate concentration

Naringinase activity was increased with increasing concentration of naringin from 2 g/L to until the enzyme became saturated, that was 10 g/L naringin concentration. Then graph showed no increase in the enzyme activity with further increase in naringin concentration (Figure 4).

![Graph showing effect of substrate concentration on crude naringinase activity.](image)

**Fig. 4:** Effect of substrate concentration on crude naringinase activity at pH 4.5 and 45°C using different concentrations of substrate.
3.5. Determination of Kinetic Parameters

The Michaelis constant and Vmax were calculated for the preparations using Lineweaver Burk plot (Figure 5). Michaelis constant for the crude enzyme was 4.347 g/L and Vmax was 4.8076 at pH 4.5 and at 45ºC.

![Lineweaver-Burk plot of the crude naringinase activity at pH 4.5 and 45ºC using different concentrations of substrate.](image)

3.6. Effect of minerals on naringinase activity

The influence of various minerals at 2mM on crude enzyme is presented on table 1 showed, the activity was increased by presence of Na⁺, Mn²⁺, Ca²⁺ and Zn²⁺ and decreased by Cu²⁺ and Ba²⁺ and highly reduced by Hg²⁺. So, addition of inducing metal ions such as Na⁺, Mn²⁺, Ca²⁺ and Zn²⁺ to the fermentation medium may increase the naringinase activity.

| Table 1: Effect of metal ions on activity of naringinase from Aspergillus flavus at pH 4.5 and 45ºC |
|---|---|
| Relative activity of Naringinase (%) |
| Control | 100 |
| Ca²⁺ | 116.086 |
| Hg²⁺ | 45.217 |
| Cu²⁺ | 92.173 |
| Na⁺ | 133.913 |
| Zn²⁺ | 108.260 |
| Ba²⁺ | 98.695 |
| Mn²⁺ | 123.913 |
| Mg²⁺ | 100.869 |
3.7. Stability of Enzyme with temperature

The residual activities of crude naringinase incubated at different temperatures (40°C, 45°C and 50°C) for a period of 1 hour were estimated. Crude enzyme of naringinase showed 100% stability at 40°C, 45°C during 1 hour incubation. When the enzyme was preincubated at 50°C for 1 hour the residual activity left was 85.8%.

![Graph showing stability of enzyme with temperature](image)

**Fig. 6:** Relative activity of naringinase enzyme from *Aspergillus flavus* after 3 min and 1 hour preincubation at different temperatures. Naringinase activity was measured at pH 4.5, using 10 g/L naringin as substrate by incubating for 5 minutes.

3.8. Stability of enzyme with pH

The crude enzyme showed 100% residual activity at pH 4.0 when incubated for one hour. At pH 4.5 and 5.0, the residual activity of the enzyme was 95% and 89% after 1 hour preincubation.

![Graph showing stability of enzyme with pH](image)

**Fig. 7:** Relative activity of naringinase enzyme from *Aspergillus flavus* after 3 min and 1 hour preincubation at different pH. Naringinase activity was measured at pH 4.5 and 45°C, using 10 g/L naringin as substrate by incubating for 5 minutes.
3.9. Stability of enzyme with minerals

Stability studies of the enzyme with 2mM of Ca$^{2+}$, Na$^+$ and Mn$^{2+}$ for 1 hour showed a decreasing trend in the residual activity compared to the regular activity of the naringinase enzyme for 3 minutes of preincubation at 45ºC and pH 4.5 and 10 g/L substrate concentration.

![Fig. 8: Relative activity of naringinase enzyme from *Aspergillus flavus* after 3 min and 1 hour preincubation with 2mM of Ca$^{2+}$, Na$^+$ and Mn$^{2+}$. Naringinase activity was measured at pH 4.5 and 45ºC, using 10 g/L naringin as substrate by incubating for 5 minutes.](image)

Naringinase enzyme from *Aspergillus flavus* showed zero order kinetics similar to the commercial enzyme (that from *Aspergillus niger*). Hence the kinetic properties of *Aspergillus flavus* naringinase are comparable to that commercial naringinase. Initially naringinase enzyme activity showed an increased with increasing temperature, it could be due to the kinetic energy of the system was increasing trend with increasing temperature and conversion of kinetic energy to chemical energy will take place, when two molecules collide. If the chemical energy is greater enough, activation energy can be achieved and reaction would result in the final end product. Increasing temperature increases the collisions of enzyme and substrate per unit time at active site and more molecules would reach the level of active energy per unit time; therefore it increased the enzyme activity when temperature was increased. This was appropriate up to optimum temperature, which was 45ºC for naringinase enzyme. Study on debittering of grape juice at different temperatures ranging from 25ºC to 40ºC by naringinase from *Penicillus* sp showed that the higher naringin hydrolysis was obtained at 40ºC (Ceviker & Unal, 2005). Another study on debittering of citrus fruit juice by naringinase of *Penicillus purpurogenum* at a temperature range of 25ºC-40ºC revealed, that the maximum naringin hydrolysis was achieved at 40ºC (Patil & Dhake, 2014). From our study, naringinase from *Aspergillus flavus* showed highest activity at 45ºC. Optimum temperature for naringinase activity may vary with type of organisms used for the production of enzyme and the presence of components other than naringin. The citrus juice not only has naringin but also has some other components and this may affect the naringinase activity at different temperatures. Other thing is debittering study on grape juice and citrus juice only carried within range of 25ºC-40ºC by Ceviker Z, Unal MU., 2005 and Patil MB, Dhake AB,.2014, they did not check its debittering ability at 45ºC. Our experiment yielded a similar conclusion for optimum temperature for naringinase enzyme from *Aspergillus flavus* (Radhakrishnan et al., 2013) and *Aspergillus niger* (Ni et al., 2012) during different kinetic studies.

The crude naringinase showed remarkable naringinase activity between the pH range 4-6. In spite of the highest naringinase activity at pH 4.5, the activity declined very fast with increasing pH. The reduction of activity may be due to the higher sensitivity of naringinase enzyme to the alkaline pH. Enzyme activity is influenced by H$^+$ and OH$^-$.
concentrations. Above or below the optimum pH, the change of the shape of active site will not be complementary to the shape of substrate. Therefore it will reduce the enzyme activity. The remarkable activity over acidic pH and optimum activity at 4.5 revealed, this enzyme is acidic in nature, which makes suitable application in acidic environments.

The crude naringinase activity increased with the increasing concentration of the substrate from 2 g/L to 10 g/L; beyond 10 g/L, the activity remained same. The reason for these phenomena is during the enzyme substrate reaction, the initial velocity V_o will increase with the increasing substrate concentration. This would take place up to the substrate saturation level, that time the velocity is V_{max} (maximum velocity) and all available enzyme would have been converted to enzyme substrate complex. Further velocity increase will not be possible after saturation level of the substrate concentration. Therefore, at this saturated substrate concentration, the activity is maximum and this maximum activity remains same with increasing substrate concentration beyond the saturation level. The linear line obtained from Lineweaver-Burk plot for different naringin concentration indicated that the rate of reaction increases with the increase in substrate concentration. The low Km value of naringinase indicated that the enzyme has high affinity for substrate (Berg et al., 2002).

Previously, the effect of metal ions on the naringinase producing capacity of *Aspergillus flavus* revealed that Mg^{2+} and Ca^{2+} ions are required for the better activity of the naringinase enzyme by *Aspergillus flavus* where as Mn^{2+} showed an inhibitory action on the growth and enzyme production at 5-10mM concentration (Radhakrishnan et al., 2013). Any how our results suggests the 2mM of Mn^{2+} has an inducing enzyme producing ability of the *Aspergillus flavus* strain isolated from the Citrus fruit. The reason for increasing enzyme activity might be due to the catalytic action of the incorporated mineral that facilitates the catalysis of the reaction between enzyme and substrates. Another study on the effect of metal ions on naringinase production by *Clavispora lusitaniae* at 10 mM revealed that Hg^{2+}, Mn^{2+}, Zn^{2+} induced the enzyme activity, where as Fe^{2+}, Cu^{2+}, Ca^{2+} showed an inhibited enzyme action (Kaur & Sahota, 2015). Our study showed Hg^{2+} has an inhibitory action on naringinase produced by the *Aspergillus flavus* strain, where as Ca^{2+} shows an inducing trend at 2mM. Inducing or inhibitory ability of the metal ions may depend on their concentration and type of organism used to produce naringinase.

Naringinase from *Aspergillus aculeatus* JMUdb058 was stable between pH 3 and 6 and below 50ºC (Chen et al., 2013). The naringinase from *Aspergillus niger* 1344 had an optimum pH of 4.0 and temperature of 50ºC and the enzyme was stable at 37ºC for 72 hour, whereas at 40ºC the enzyme showed 50% inactivation after 96 hour of incubation (Puri & Kalra, 2005). Naringinase from *Aspergillus niger* showed stable pH range within 4.5-5.0 (Ni et al., 2011) and naringinase from *Penicillium decumbens* was most stable at a pH range of 4.5-5.0, while being sensitive at acidic pH less than 3.5 (Magario et al., 2008). From our study on the stability of crude enzyme naringinase from *Aspergillus flavus* strain isolated from Citrus revealed that the enzyme was stable at both 40ºC and 45ºC and showed highest activity at 45ºC. *Bacillus methyloptrophicus* showed significant increase in naringinase activity in Sucrose-yeast extract compared to the basal medium. Naringin was found to be the best inducer among naringin, naringenin, hesperidin, and L-rhamnose for the naringinase enzyme production at pH 6.0, by 48 hr old *Bacillus methyloptrophicus* with the inoculum size of 2% (v/v) (Mukund et al., 2014). From pH stability study it could be revealed that the enzyme was stable at pH 4.0. The difference in the results may be due to the difference in the strain of microorganism used for the naringinase production, purity of enzyme and condition maintained such as substrate concentration and reaction time.

4. CONCLUSION

This study suggests that the naringinase from *Aspergillus flavus* strain could be an appropriate enzyme for industrial and biotechnological applications. Naringinase from *Aspergillus flavus* showed optimum activity at pH 4.5 and stable for minimum for one hour at pH 4.0. The enzyme showed zero order kinetics for 10 minutes. V_{max} and K_m values of the crude naringinase enzyme were 4.8076 µmol/min and 4.347 g/L. Addition of 2mM of Hg^{2+}, Cu^{2+} and Ba^{2+} decreased the naringinase enzyme activity, while Na^+, Mn^{2+}, Ca^{2+} and Zn^{2+} increased the enzyme activity. Therefore the enzyme has potential application in acidic environment especially the debittering of acidic juices of diverse Citrus species, since at this low temperature there would be no loss of nutritional and sensory qualities of juice.

Acknowledgement

Authors express their sincere gratitude for Mr. PTJ.Jashothan of Department of Botany. Authors sincerely acknowledge the funding support from the University of Jaffna, through the University research grant 2015.
REFERENCES


Keerthini Srikantha - Obtained first class in her B.Sc Hons in Agricultural Chemistry from the University of Jaffna in 2016. After the completion of one year service as Lecturer in Agricultural Chemistry, she has now started to follow a fulltime research MPhil in Food Microbiology in the University of Jaffna.

Dr. Ranganathan Kapilan – Obtained B.Sc Hons in Botany and M.Phil in Biochemistry from the University of Jaffna and PhD in Plant and Molecular Biology from the University of Alberta, Canada. He is now attached to the University of Jaffna as a Senior Lecturer in Botany.

Dr. Seevaratnam Vasantharuba – Obtained B.Sc Hons in Agriculture from the University of Jaffna and M.Phil in Food Science & Technology from the University of Peradeniya and PhD in Food Science & Nutrition from Tamil Nadu Agricultural University, India. He is now attached to the University of Jaffna as a Senior Lecturer in Agricultural Chemistry.