International Journal of Advanced Engineering and Management Research
Vol. 3 Issue 3; 2018

www.ijaemr.com ISSN: 2456-3676

THE INTERACTIVE EFFECT OF THE ENZYMATIC HYDROLYSIS CONDITIONS ON THE TOTAL DRY MATTER AND THE ANTIOXIDANTS RECOVERY FROM THE PULP OF Limonia acidissima FRUITS

Pham Bao Nguyen[1*], Ma Thai Hoa[1], Vo Thi Diem Kieu[2]

[1]Tra Vinh University, 126 Nguyen Thien Thanh street, Ward 5, Tra Vinh city, Vietnam.

[2]Dong Nai Technology University, Nguyen Khuyen Street, Ward Trang Dai, Bien Hoa city, Vietnam

Abstract

In this study, the conditions of the enzymatic hydrolysis for Limonia acidissima (L. acidissima) pulp with the combination of cellulase and pectinase were identified. The interations of pH and temperature, the concentration of the combined enzymes and the hydrolysis time in the hydrolysis process effective affected on the total dry matter and the antioxidants recovery. The best hydrolysis conditions were established at pH of 4.2, temperature of 45oC, the concentration of the combined enzymes of 1.2 percent of volume per dry weight basic (% v/dwb) and the hydrolysis time of 90 min. As a result, the recovery efficiency of the total dry matter was highest, that increased 20% in comparison with the efficiency from the non-enzymatic extraction process. Besides, the antioxidants included total phenolic content (TPC), DPPH and ABTS radical scavenging activity increasing from 451.45±3.55 to 704.92±10.25 mg gallic acid equivalent in a liter (GAE/L), 0.93±0.01 to 1.52±0.05 and 1.08±0.06 to 2.02±0.07mM trolox equivalent antioxidant capacity (TEAC) respectively. That might promise producing many kind of foods from the extract of the the pulp, which provide many health benefits.

Key Words: Enzymatic hydrolysis, *Limonia acidissima*, Antioxidants

1. Introduction

L. acidissima (syn. *Wood apple*, *Feronia limonia*, *Feronia elephantum*, *Hesperethusa crenulat*, *Hesperethusa crenulata*, *Schinus limonia*) was a tropical fruit from the family of Rutaceae. This plant gave high fruit yield, they were planted popular in India, Sri Lanka, Pakistan, Bangladesh, Burma, Thailand, and most of the Southeast Asian countries [1]. The harvest season of the fruits in Vietnam was around later half of November to January. The fruits were still used for traditional medicine besides the use as a kind of food [2]. The fruits were rich in nutrient value compared with many other fruits [3] including a potential source of energy, protein and carbohydrate, minerals and many vitamins such as vitamin C, vitamin A, thiamine, riboflavin, niacin, Ca, P [4-5], Na, K, Mg, Zn and Cu, Fe, insoluble dietary and soluble dietary fiber (mucilage and pectin). [6]. The fruits had been widely used because of the multiple antioxidants

including phenolics, carotenoids, alkaloids, coumarins and other antioxidants. They may protect us against intercellular free radicals. The fruits were good for health and cheap source to develope nutraceuticals for diabetes [7-8]. Positive correlation was observed between polyphenol content and the antioxidant capacities with enormous health benefits. With these reasons, the fruits might be used in food and pharmaceutical applications [9]. Besides, the antioxidants in the pulp exhibited a good antibacterial activity against gram positive bacteria, antifungal, anti-inflammatory, astringent. The antimicrobial activity could be related to the presence of phenolic compounds, thymol [10]. Thymol had been shown to cause the inhibition of ATPase activity, release of intracellular ATP and other constituents [11]. That demonstrates that *L. acidissima* fruits might be used as nutraceuticals for disease prevention and health promoting benefits [4].

The pulp of *L. acidissima* fruits were also eaten raw or blended with coconut milk and palm-sugar syrup to drink as a beverage or nice cream [12]. Especially, the jam and jelly from wood apple were becoming popular in India and Sri Lanka. In India, the fruits were used as a "poor man's food" until processing techniques were developed in the mid-1950s. The demand for using of the fruits had increased remarkably in the last few decades [3].

Cell walls were efficiently degraded by endo-polygalacturonases and cellulases [13]. In particular, the combination use of cellulase and pectinase not only increased the recovery efficiency of the extract from the pulp but also ensuries the quality of the end juice [14-15]. These enzymes not only helped in softening the plant tissue but also leaded to release of cellular contents increasing the yield recovery of the juice [13].

Goals

- To identify the suitable conditions for the hydrolysis process (pH, temperature, concentration of the combined enzymes and incubation time of the hydrolysis) with using the combined enzymes.
- To evaluate the effect in the hydrolysis process with the chosen conditions to total phenolic compounds, DPPH and ABTS radical scavenging activities recovery in the extracted juice.

2.2 Materials and methods

2.2.1 Materials

Chhemicals:

1,1-Diphenyl-2-picryl-hydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) were provided by SIGMA-ALDRICH, USA; 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS) was obtained from BioBasic, Canada.; Potassium persulfate ($K_2S_2O_8$) was purchased from China.

Enzymes:

Commercial enzymes, Cellulase from *Trichoderma reeisei* (the endoglucanase activity of 700EGU/g) and pectinase from *Aspergillus aculeatus* (the polygalacturonase activity of 26000PG/ml), were obtained from Novozymes – Denmark and stored at 4^{o}C.

Plant material:

L. acidissima fruits were collected from Tra Vinh province, Vietnam. The mean weight of a fruit was about 400 g stored at -18^{o}C for further use.

2.2.2 Methods

The extraction of the L. acidissima juice

The *L. acidissima* pulp was removed from the fruits and diluted by pure water with a ratio of 1:1. A sample mass of 100g, with 50g the pulp and 50 ml of water, was used for each experiment. The volume ratios of cellulase to pectinase were adjusted of 0:1, 1:0, 1:1, 1:2, 2:1, 1:3, 3:1. From the chosen ratio, the suveys to find the best hydrolysis conditions were conducted with the interation of pH and temperature on the efficiency of the hydrolysis. pH was adjusted from 3.9 (the natural pH of *L. acidissima* pulp), 4.2 and 4.5 by adding citrate buffer and temperature was adjusted at 40, 45 and 50^{o}C. The best hydrolysis condition of pH and temperature was used to design the experiments of the interations between the concentrations of the combined enzymes (cellulase and pectinase) and the hydrolysis time. The concentrations of the enzymes were used in the range from 0.4, 0.8 and 1.2% (v/dwb), the hydrolysis times were stopped at 60, 90 and 120 min. The samples were incubated in a shaking water bath with a rate of 120 strokes in a minute. After the end of the incubation periods, the enzymes were inactivated at 85^{o}C for 10 min. The reaction mixtures were filtered by the vacuum filtration method through a Whatman filter paper. The extracted juice was weighed to calculate the efficiency of the total dry matter recovery. A volume of each the juice sample was diluted directly with distilled water to analyze the antioxidants: TPC, DPPH, ABTS. The best value of each variable was chosen by the statistical analysis (p<0.05) by using Stagraphics centurion XVI software. Experiments were repeated three times.

The preparation of samples to determine the contents of the antioxidants from the pulp

25 g of the *L. acidissima* pulp was dried over night at 45^{o}C. The dried power was grinded and mixed with 225 ml of methanol in a becher. They were then filtrated through a Whatman filter paper. The extracted juice was diluted with distilled water to a suitable concentration for the analysis tests [9].

The determination of total phenolic content

Total phenolic content (TPC) was determined by Foline-Ciocalteu method (Darsini DTP, 2013). 0.1 ml of the extracted juice was mixed with 3 ml of distilled water, then 0.5 ml of Foline-Ciocalteu reagent was added. After 3 min., 2 ml of sodium carbonate 20% was added and mixed thoroughly. The tubes were incubated in a boiling water bath (100^{o}C) for exactly 1 min. Then, these tubes were quickly cooled. 0.3ml of the solution from each tube was added to a cuvet to measure the absorbance at 650nm by spectrophotometer (Genesys 6, Thermo Spectroic, USA). The standard curve was linear between 10 and 60ppm of acid gallic. The results were expressed by milligram (mg) of gallic acid equivalent (GAE) in 100g of raw material (*L. acidissima pulp).* The values were done in triplicate.

Determine free radical-scavenging ability with using a stable DPPH radical cation

DPPH radical scavenging assay was determined by the method developed by Brand-Williams W *et al.* [15]. Here, 0.3ml of each test sample was mixed with 5.7ml of a DPPH-methanol solution (A_{515nm} of 1.1±0.02). The mixtures were then vortexed vigorously. Then, they were put in to the

dark box for 20 min. The absorbance was determined at 515nm. DPPH radical scavenging in the juice sample could be calculated by the formula as shown:

$$\text{The inhibition percent of DPPH by the juice sample} = [1 - \frac{\text{Asample}}{\text{Acontrol}}] * 100\ (\%) \quad (1)$$

From the equation (1) and the standard curve of the inhibition percent of DPPH by trolox = a_1 * trolox concentration + b_1, the molecular weight of trolox = 250.29 and the mass of the extracted juice from 100 g of the pulp, that deduced for DPPH radical cation by TEAC (mg) of the extracted juice from 100 g of the pulp.

Where, a_1 and b_1 are the coefficients of the standard curve of DPPH.

The standard curve was linear between 100 and 700µM trolox by using spectrophotometer (Genesys 6, Thermo spectroic, USA). The results were expressed by mg (TEAC) in 100g of the pulp. Three replicates of each sample were used for the statistical analysis and the chosen final values were reported as mean ± SD.

Determine free radical-scavenging ability with using a stable ABTS radical cation

Free radical scavenging activity by ABTS radical cation decolourisation assay was determined by the method described by Re R *et al*.. ABTS was dissolved in water to the concentration of 7µM. ABTS radical cation ($ABTS^+$) was produced by reacting ABTS stock solution with 2.45µM of potassium persulfate (the final concentration) and kept in the dark at room temperature for 12÷16 hours before use. The radical cation was stable in this form for more than two days in the dark at room temperature. The samples of $ABTS^+$ solution were then diluted with redistilled water to an absorbance of 0.70 (±0.02) at 734nm. 3.0 ml of diluted $ABTS^+$ solution (A_{734nm} = 0.700 ± 0.02) were added to a cuvet containg 30µL of the extract juice, the absorbance was then read exactly in 6 min by using spectrophotometer (Genesys 6, Thermo spectroic, USA). ABTS radical scavenging activity in the extracted juice sample could be calculated by the formula as shown:

$$\text{The inhibition percent of ABTS by the juice sample} = [1 - \frac{\text{Asample}}{\text{Acontrol}}] * 100\ (\%) \quad (2)$$

From the equation (2) and the standard curve of the inhibition percent of ABTS by trolox = a_2 * the concentration of trolox + b_2, the molecular weight of trolox = 250.29 and the mass of the extracted juice from 100 g of the pulp. That deduced for ABTS radical cation by TEAC (mg) of the extracted juice sample from 100 g of the pulp.

Where, a_2 and b_2 are the coefficients of the standard curve of ABTS. The standard curve was linear between 100 and 700 µM trolox. The results were expressed by mg TEAC in 100 g of the pulp. All of determinations were performed in triplicate.

The recovery efficiency of the total dry matter (E) from the hydrolysis was caculated by equation

$$E = \frac{\text{The mass of the total dry matter in the extracted juice}}{\text{The mass of the total dry matter in the pulp}} x100\ (\%) \quad (3)$$

The total dry matter content in the extracted juice = (100- the moisture mass in the extracted juice) (%),

The total dry matter content in the pulp = (100 – the moisture mass in the pulp) %,

The mass of the total dry matter in the extracted juice = The total dry matter content in the extracted juice * the weight of the extracted juice / 100,

The mass of the total dry matter in the pulp = The total dry matter content in the pulp * the weight of the pulp / 100.

Experimental design

The experiments were conducted with the variables combinations of pH – the hydrolysis temperature and the concentration of the combined enzymes - the hydrolysis time as follows:

Table 1. The ranges of pH and temperature in the hydrolysis process were designed as follows.

pH	Temperature		
	B_2 (40^{o}C)	B_2 (45^{o}C)	A_3 (50^{o}C)
A_1 (3.9)	A_1B_1	A_2B_1	A_3B_1
A_2 (4.2)	A_1B_2	A_2B_3	A_3B_2
A_3 (4.5)	A_1B_3	A_2B_3	A_3B_3

The best chosen pH and temperature conditions were used in following experiments (as shown in table 2)

Table 2. The ranges of the concentration of the combined enzymes and the hydrolysis time were designed as follows:

The concentration of the combined enzymess (% v/dwb)	The hydrolysis times (min.)		
	D_1 (60)	D_2 (90)	D_3 (120)
C_1 (0.4%)	C_1D_1	C_2D_1	C_3D_1
C_2 (0.8%)	C_1D_2	C_2D_3	C_3D_2
C_3 (1.2%)	C_1D_3	C_2D_3	C_3D_3

3. Results and discussion

The effect of the ratio of cellulase and pectinase on E of the hydrolysis process

The experience to identify the ratio of cellulase and pectinase was conducted in the conditions of pH = 4.2, temperature = 45^{o}C, the total of cellulase and pectinase concentration = 0.8% (v/dwb) and the hydrolysis time = 60 min. The control sample conducted with the enzymes concentration = 0 %. Results were shown in Fig. 1

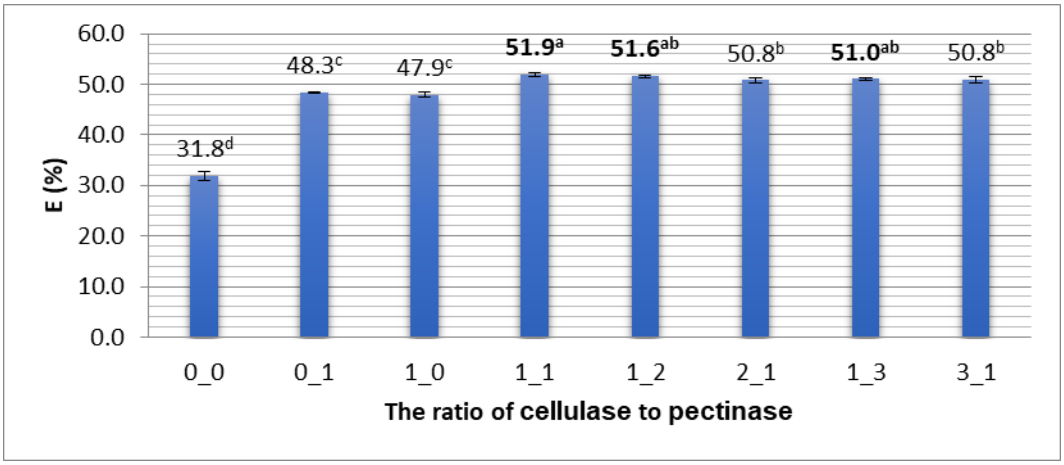

Figure 1. The effect of the ratio of cellulase to pectinase on E from the hydrolysis

Note: [a]highest significant value; [b, c, d]lower significant value.

The results in the Fig. 1 showed that E had a significant difference by the vary ratios of cellulase/pectinase. The best ratio of cellulose/pectinase achieved at 1/1 shown by the maximum value of E (51.9%), while the lowest value of E achieved at the control sample. This was explained that the combined enzymes helped to *reduce viscosity* of the sample rapidly and increase the reaction rate of the hydrolysis resulting in increasing the efficiency of the total dry matter recovery. We chose this best values to conduct the following experiments for the hydrolysis process.

The interactive effect of pH and temperature on E of the hydrolysis process

Table 3. The interative effect of pH and temperature on E in the hydrolysis process.

pH	Temperature			* Mean of E (%)
	B_1 (40°C)	B_2 (45°C)	B_3 (50°C)	
A_1 (3.9)	44.96	51.05	50.36	48.79±3.33[b]
A_2 (4.2)	48.18	53.04	50.36	51.32±2.43[a]
A_3 (4.5)	49.42	52.43	50.75	50.86±1.51[ab]
* Mean E (%)	47.52±2.30[b]	52.17±1.02[a]	51.28±0.22[a]	

*Note: *Means of triplicate determination ± SD; [a]highest value; [b]lower value.*

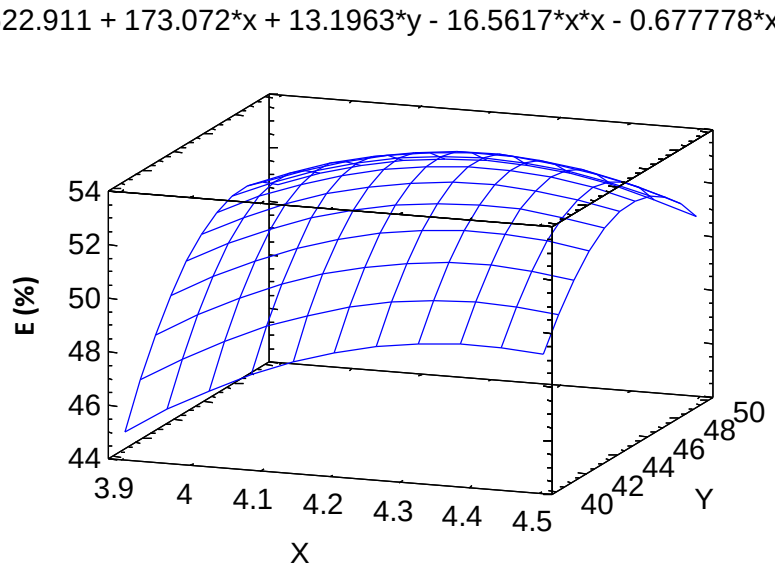

Figure 2. The interactive effect of pH and temperature of the hydrolysis on E.

Where X was pH in the hydrolysis process, Y was the hydrolysis teperature (^oC)

The results in Table 3 and Fig. 2 showed that at the pH from 4.2 to 4.5 and temperature from 45^{o}C to 50^{o}C, E reached the highest values ranging from 52.4 to 53%. In contrast, E would be reached significantly lower when pH conditions were higher or lower. The results could be explained that the optimum pH from 4.2 to 4.5 effected chemical bondings within of molecules that changed the shape of active sites and substrates. Accordingly, the reaction rate increased in the hydrolysis process. Besides, the unfavourable conditions of pH might reduce the activity of the enzymes or even damage structure of them. Therefore, the enzymes were deactivated [16]. This results were in good agreement with the earlier report by Esawy *et al*, (2013), Srivastava and Tyagi (2013) [17-18]. Besides, the hydrolysis temperature also effected significantly on the activity of the enzymes. The heat treatment of the enzymes might either enhance the enzymatic reaction at the suitable temperature or inhibit undesirable changes by the inactivation of the enzymes when the temperature was too low or high [19]. At the appropriate temperature for the hydrolysis process varying from 45^{o}C to 50^{o}C, the reaction of the enzymes reached the optimal rate shown by the highest values of E varying from 51.3 to 52.2% respectively. The value of pH = 4.2 and temperature = 45^{o}C was chosen to conduct the following experiments.

The effect of the concentration of the combined enzymes and the hydrolysis time on E from the hydrolysis process

Table 4. The interactive effect of the combined enzymes concentration and time on E.

The the combined enzymes concentration (% v/dwb)	The hydrolysis time			* Mean of E (%)
	D_1 (60 min.)	D_2 (90 min.)	D_3 (120 min.)	

C_1 (0.4%)	47.82	50.54	50.40	49.58±1.53[c]
C_2 (0.8%)	50.87	52.87	52.65	52.13±1.10[b]
C_3 (1.2%)	52.76	53.80	53.95	53.50±0.65[a]
* Mean E (%)	50.48±2.49[b]	52.40±1.68[a]	52.33±1.80[a]	

*Note: *Means of triplicate determination ± SD; [a]highest value; [b,c]lower value.*

$$32.6931 + 13.3743*x_1 + 0.253444*y_1 - 3.67014*x_1*x_1 - 0.0289583*x_1*y_1 - 0.00110802*y_1*y_1$$

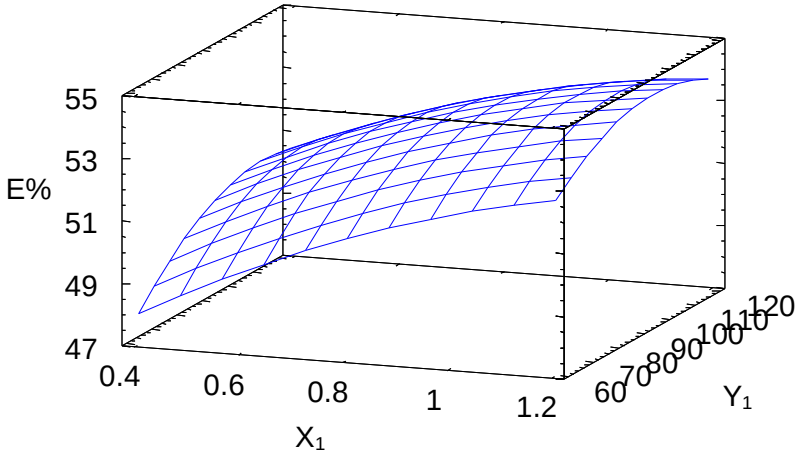

Figure 3. The interactive effect of the combined enzymes concentration and the hydrolysis time on E.

Where X_1 is the concentration of the combined cellulase and pectinase (v/dwb), Y_1 is the hydrolysis time (min.)

Table 5. The recovery yield of the antioxidants using the combined enzymes at the best chosen condition in compared with the control sample

	TPC (mgGAE/L)	DPPH (mM TEAC)	ABTS (mM TEAC)
The chosen hydrolysis conditions (pH of 4.2, temperature of 45°C, the enzymes concentration of 1.2 (v/dwb) and the hydrolysis time of 90 min.)	704.92±10.25	1.52±0.05	2.02±0.07
The control sample (without enzyme)	451.45±3.55	0.93±0.01	1.08±0.06

The results in Table 5 showed that the increase of the concentration of the combined enzymes played a role in increasing E, *TPC, DPPH and ABTS in L. acidissima juice. With the control*

sample (no adding enzyme), these values were the lowest that reached 33.8%, 451.5 mgGAE/L, 0.93 and 1.08 mM TEAC respectively. The increase of the concentration of the combined enzymes would promote the rate of reaction. *At 1.2% (v/dwb) of the combined enzymes and time of 90 min.,* E, *TPC, DPPH and ABTS* radical scavenging activities were highest, that reached 53.8%, 705 mg GAE/L, 1.52 and 2.02 mM TEAC *respectively as shown in Table 5. The results were explained due to the degradation of the pectin and partially due to the cellulose by hydrolysis. The enhanced extraction of phenolic compounds was key of antioxidant components responsible for higher antioxidant activity [20]. Besides, after 90 min., the increase of* E was no statistical significance. This results could be explained that the enzymes were broke down complex polysaccharides of plant tissues into simpler molecules as galacturonic acids, glucose, dextrin, maltose [21]. That made the extraction of the pulp easier and faster, and thereby increasing the extraction efficiency. However, in the case of the prolonged hydrolysis time, the substrates concentration became lower that was more difficultly to contract with enzyme leading to the stability maintain of E.

4. Conclusions

The effectiveness of the use of the combined enzymes to hydrolyze the fruit pulp was proved. The interation of pH and temperature, the concentration of the combined enzymes and the hydrolysis time had a positive effect on the increase in total dry matter mass and the antioxidants in the extracted juice. At the condition of pH of 4.2, temperature of 45^{o}C, the ratio of enzyme : raw material of 1.2% (v/dwb) and the hydrolysis time of 90 min., E was reached the highest values. Besides, The contents of TPC, DPPH and ABTS also reached high values in compared with the contents of them in the control samples. The enzymatic treatment could be used to produce many products from *L. acidissima* fruits for health benefits.

References

[1] Senthilkumar, A. and Venkatesalu, V. Chemical constituents, in vitro antioxidant and antimicrobial activities of essential oil from the fruit pulp of wood apple. Industrial Crops and Products 46 (2013) 66-72.

[2] Prathapan, A., Krishna, M.S., Nisha, V.M. Sundaresan A, Raghu KG - Polyphenol rich fruit pulp ofAegle marmelos(L.) Correa exhibits nutraceutical properties to down regulate diabetic complications - An in vitro study. Food Research International 48 (2012) 690-695.

[3] Ratnayake, R.M.R.N.K., Sumithra, H.J., Fernando, M.D., Palipane, K.B. Effect of GRAS compounds on Aspergillus rot of wood-apple (Feronia limonia). Phytopar-Asitica 37 (2009) 431–436.

[4] Pandey, S., Satpathy, G. and Gupta, R.K. Evaluation of nutritional, antioxidant and antibacterial activity of exotic fruit "*Limonia acidissima*". Journal of Pharmacognosy and Phytochemistry 3 (2014) 81-88.

[5] Vijayvargia, P., Choudhary, S. and Vijayvargia, R. Preliminary phytochemical screening of *Limonia acidissima* linn. International Journal of Pharmacy and Pharmaceutical Sciences 6 (2014) 134-136.

[6] Islam, M.M., Shams, B., Siraj, S., Hasan, M.K., Masum, S.M. and Chowdhury, J.U. Comparative Study of Minerals Content in Green and Ripe Bael (Wood Apple) Powder. International Journal of Basic & Applied Sciences 11 (2011) 133-136.

[7] Lambole, V.B., Murti, K., Bhatt, U.K., Kumar, S.P. and Gajera, V. Phytopharmacological

properties of Aegle marmelose as a potent medicinal tree. International Journal of Pharmaceutical Sciences Review and Research 5 (2010) 67-72.

[8] Charoensiddhi, S. and Anprung, P. Antioxidants and volatile compounds of Thai bael fruit (*Aegle marmelos* (L.) Correa) as a valuable source for functional food ingredients. International Food Research Journal 15 (2008) 287–295.

[9] Darsini, D.T.P., Maheshu, V., Vishnupriya, M., Nishaa, S., Sasikumar, J.M. Antioxidant potential and amino acid analysis of underutilized tropical fruit *Limonia acidissima* L.. Free Radicals and Antioxidants 3 (2013) 62-69

[10] Bapadopoulou, A. and Frazier, R.A. Characterization of Characterization of interactions. Trends in Food Science & Technology 15 (2004) 186–190.

[11] Oussalah, M., Caillet, S., Lacroix, M. Mechanism of action Spanish oregano, Chinese cinnamon, and savory essential oils against cell membrane and walls of E. coli O157:H7 and Listeria monocytogenes. J. Food Prot 69 (2006) 1046-1055.

[12] Panda, H. Medicinal Plants Cultivation and Their Uses. New Delhi: Asia pacific Business Press Inc (2000).

13] Sreenath, H.K. and Radola, B.J. The effect of removing cellulase(s) from a commercial pectinase on maceration and liquefaction of carrots. Journal of Biotechnology 4 (1986) 269-282.

[14] Kilara, A. Enzymes and their uses in the processed apple industry: a review. Process Biochem. 17 (1982) 35-41.

[15] Brand-Williams, W., Cuvelier, M.E. and Berset, C. Use of a free radical method to evaluate antio-xidant activity. Food Science and Technology 28 (1995) 25-30.

[16] Law, B.A. Enzymes in manufacture of daily products. In: Whitehurst RJ, Law BA (eds). Enzyme in food technology. CRC Press, Boca Raton (2002) 76-10

17] Esawy, M.A., HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/?term=Gamal%20AA%5BAuthor%5D&cauthor=true&cauthor_uid=23399177" Gamal AA, HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/?term=Kamel%20Z%5BAuthor%5D&cauthor=true&cauthor_uid=23399177" Kamel Z, HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/?term=Ismail%20AM%5BAuthor%5D&cauthor=true&cauthor_uid=23399177" Ismail AM and HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/?term=Abdel-Fattah%20AF%5BAuthor%5D&cauthor=true&cauthor_uid=23399177" Abdel-Fattah AF. Evaluation of free and immobilized Aspergillus niger NRC1ami pectinase. Carbohydrate Polymers 92 (2013) 1463-1469.

[18] Srivastava, S., Tyagi, S.K. Effect of Enzymatic Hydrolysis on the Juice Yield from. International Journal of Biotechnology and Bioengineering Research 4 (2013) 299-306.

[19] Belitz H.D., Grosch, W., Schieberle, P. Food chemistry (4 ed.). Berlin: Springer. (2009).

[20] Demir, N., Acar, J., Sarioglu, K., Mutlu, M. The use of commercial pectinase in fruit juice industry. Part 3: Immobilized pectinase for mash treatment. Journal of Food Engineering 47 (2001) 275-280.

[21] Dey, T.B. and Banerjee, R. Application of decolourized and partially purified polygalacturonase and anpha amylase in apple juice clarification. Brazilian Journal of Microbiology 45 (2014) 97-104.