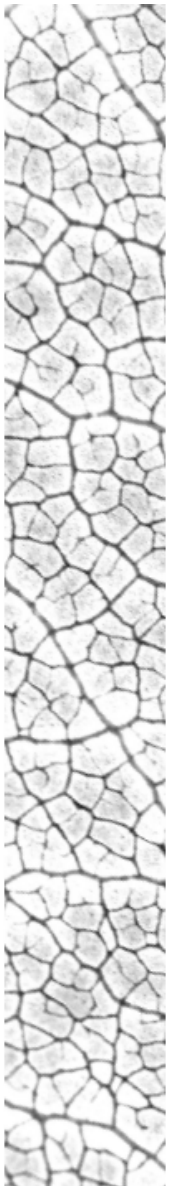

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Production of gallic and glutamic acid-rich extract from *Albertisia Papuana* Becc leaves using Tannase in various pH and temperature hydrolysis

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Abstract
The Dayak's Tribes in West Kalimantan of Indonesia uses the leaves of *san-sangk* (*Albertisia papuana* Becc.) as one of the seasoning ingredients. The leaves are known to contain gallic and glutamic acid, which are important chemical compounds for human health. Various extraction methods using solvents have been used to produce gallic and glutamic acid. Commercially, fermentation methods are preferred to use tannase from microbial for hydrolysis of tannins to produce gallic acid with glutamic acid as a by-product. Enzyme activity is strongly influenced by the condition of its enzymatic process conditions, especially pH and temperature. Therefore, the conditions of the research are carried out at certain pH and temperature conditions. The purpose of this research is to find conditions of hydrolysis to produce a crude extract which is rich in gallic acid and glutamic acid using commercial tannase. The research was applied factorial design, combination between the temperature (30; 35; 40°C) and pH (5; 5.5; 6). The observed variables: yield, total phenolic (as mg GAE/g) and total free amino acids (as glutamic acid). The results showed that the combination of temperature and pH affected the yield, total free amino acids and total phenolic. The highest yield and total phenolic, produced from incubation at 35°C and pH 5 and the highest total free amino acids which produced at 35°C and pH 6.0. This is important to explain that A. *papuana* Becc. leaves is very potential to be developed using enzyme hydrolysis to produce of gallic acid and glutamic acid.

Keywords: gallic acid, glutamic acid, *Albertisia papuana,* tannase

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INTRODUCTION

The *san-sangk* leaves (*Albertisia papuana* Becc.) is widely known as flavoring food (flavor enhancer) which is used as a seasoning in the Ethnic Dayak community in most of Borneo Island (West, Central and East Kalimantan of Indonesia). *Albertisia papuana* Becc. leaves have been known to contain gallic acid and glutamic acid (Purwayanti et al. 2013a, 2013b).

Gallic acid is a compound that has antioxidant, antimicrobial, antifungal, antibacterial (Gustavo et al. 2018, Oyagbemi et al. 2016, Rosman et al. 2018, Wang et al. 2017). Gallic acid in plants is available in free form and bound by glucose esters. The bound form of gallic acid is mostly in the form as tannin. Tannin compounds that are rich in gallic acid are called gallotanin. This type of tannin is generally in the form of gallic acid bound to glucose or with alcohol sugar (sorbitol) (Hagerman 2002). When gallotanin was hydrolyzed it will produce gallic acid and simple sugar (glucose) or alcohol sugar (Natarajan 2009).

Gallic acid production methods according to Banarjee et al. (2007), which became the most popular choice of many industries and used the microbial fermentation technique with tannase producer. This technique has been either used microbes that producing tannase or used tannase directly (Belur and Pallabhanvi 2011, Nallabilli 2016). Enzymes maximum catalytic activity is highly dependent on temperature and pH (Oueslati and Mounirhaouala 2014). Tannase activity depends on the operating conditions such as temperature and ph toas well as affected by source of microbes (Battestin and Macedo 2007, Iqbal and Kapoor 2012, Silvestre et al. 2012) and carbon source (El-Fouly et al. 2012, Gaur et al. 2017).

Glutamic acid is an amino acid that gives a major contribution to the taste of umami; the fifth basic taste (Ghirri and Bignetti 2012), which has the taste perception of savory and delicious. Foods that are rich in glutamic acid also have a strong umami flavor. In the body, the function of glutamic acid is as an energy

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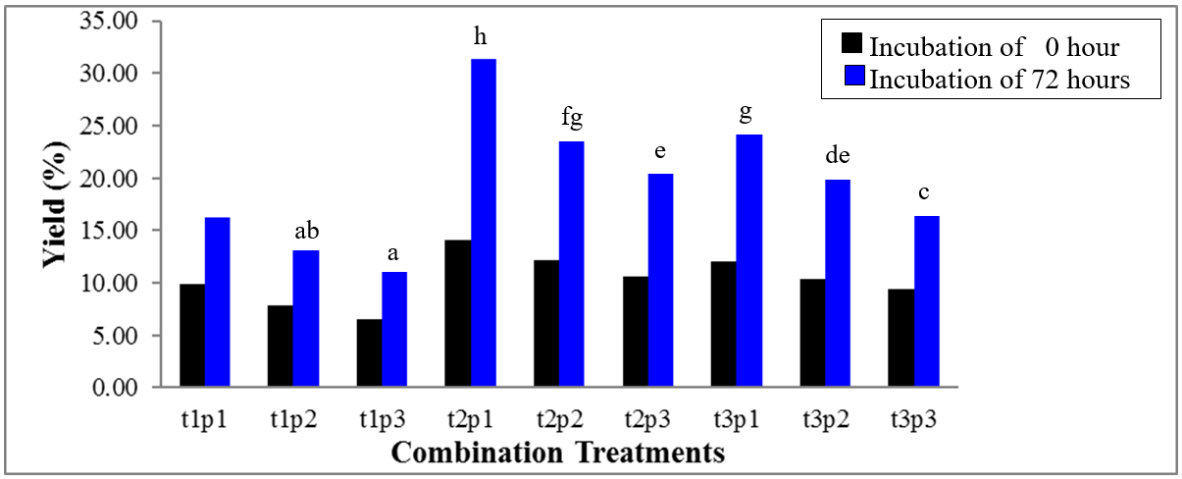


Notes: t = temperature; t1= 30°C; t2 = 35°C; t3 = 40°C
p = pH; p1 = 5; p2 = 5.5; p3 = 6

Fig. 1. The yields of different combination treatments of hydrolysis *A. papuana* Becc. leaves by using tannase. Mean (standard deviation); n =3. The Average value that is followed by same letter is insignificantly different in BNJ 0.05 test

producer in the intestine (Burrin and Stoll 2009), also plays an important role in neurotransmitter (Bauer and Robinson 2012).

The method of extracting gallic acid and glutamic acid from *A. papuana* Becc. leaves has never been using enzyme hydrolysis. It is necessary to be explored in order to produce the crude extract which rich in gallic acid and glutamic acid by using commercial tannase.

MATERIALS AND METHODS

Material and Chemicals

San-sangk leaves were bought from Sekais Village, Jelimpo, Landak District, West Kalimantan (Indonesia). Tannase (Aspergillus sp) was purchased from Dideu Industrial Group Limited, Shaanxi (China), 80% (v/v), meanwhile ethanol, Folin-Ciocalteu reagent, 0.1 N NaOH, $Na_2 CO_3$, standard gallic acid, citrate buffers (citric acid and sodium citrate), standard glutamic acid, ninhydrin reagent, were obtained from Sigma-Aldrich p.a. grade (Singapore).

Equipment

Stirrer, spectrophotometer, incubator, pH meters, analytical scales, a 100 mesh sieve.

Research Design

The study was conducted using factorial designs (2 factors); incubation temperature (t) at levels 30, 35, 40°C and pH (p) at level 5; 5.5; 6. So there were 9 experimental unit combinations and every combination is repeated 3 times.

Preparation of Hydrolysis of San-Sangk Leaves

The *san-sangk* leaves were dried for 1 month at room temperature, ground into powder and sieved with a size of 100 mesh. The hydrolysis of *san-sangk* leaves powder using modified of (Karamac et al. 2006) in which the ratio the material and citrate buffer was 1:20 (g/ml) while with tannase had been used as much as 5%. The mixture sample with tannase was incubated at the temperature conditions of 30, 35, 40°C and pH of 5; 5.5; 6 for 72 hours. The incubation results were filtered and stored in cold temperature for 24 hours to separate the solids and the supernatant and then to be analyzed using a spectrophotometre.

Analysis of Hydrolysis Products

The products of tannins hydrolysis were analyzed by spectrophotometric method, except the yield (Hartanti et al. 2003). The observed variables: total phenolics (Farhan et al. 2012), total free amino acids (Khokhani et al. 2011).

Data Analysis

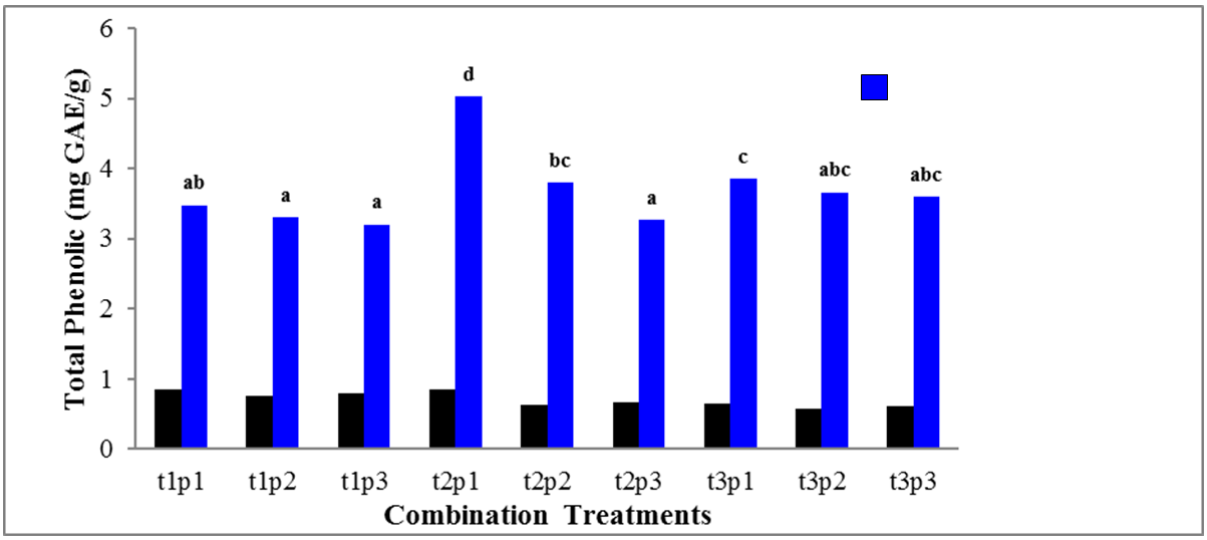
All experiments were carried out in triplicate. The data was analyzed using F test, continued with BNJ test with extent error 5%.

RESULTS AND DISCUSSION

Yield

Data analysis with ANOVA shows that there is strong effect of the combination of temperature and pH operating incubation on the yield of crude extract ($p>0.05$), continued to BNJ test. The presence of interaction between temperature and pH means that the yields changes for different pH depends on the temperature. Similarly, the yields changes for different temperatures depends on pH. The yield of the hydrolysis of *A. papuana* Becc. leaves using tannase can be seen from **Fig. 1**.

During 72 hours of incubation hydrolysis there was an increase in the yield compare than to the one without incubating. The level of the yield have been found to increase from 6 to 31%, it means the incubation has been able to fold 5 times. The trend of yield data shows that it is increasing at 35°C and decreasing again at



Notes: t = temperature; t1= 30°C; t2 = 35°C; t3 = 40°C
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Fig. 2. The total phenolics of different combination treatments of hydrolysis *A. papuana* Becc. leaves by using tannase. Mean (standard deviation); n =3. The Average value that is followed by same letter is insignificantly different in BNJ 0.05 test

40°C and getting decreasing along with increasing of pH. This is due to the incubation process with the addition of heat to help improve the extraction process, as the temperature is one factor that can affect the rate of extraction. The fastest reaction occurs at optimum temperature. The yield result ranged from 14.17% - 31.29%. The highest yield was obtained from the temperature conditions of 35ºC and pH of 5 with a yield of 31.29%.

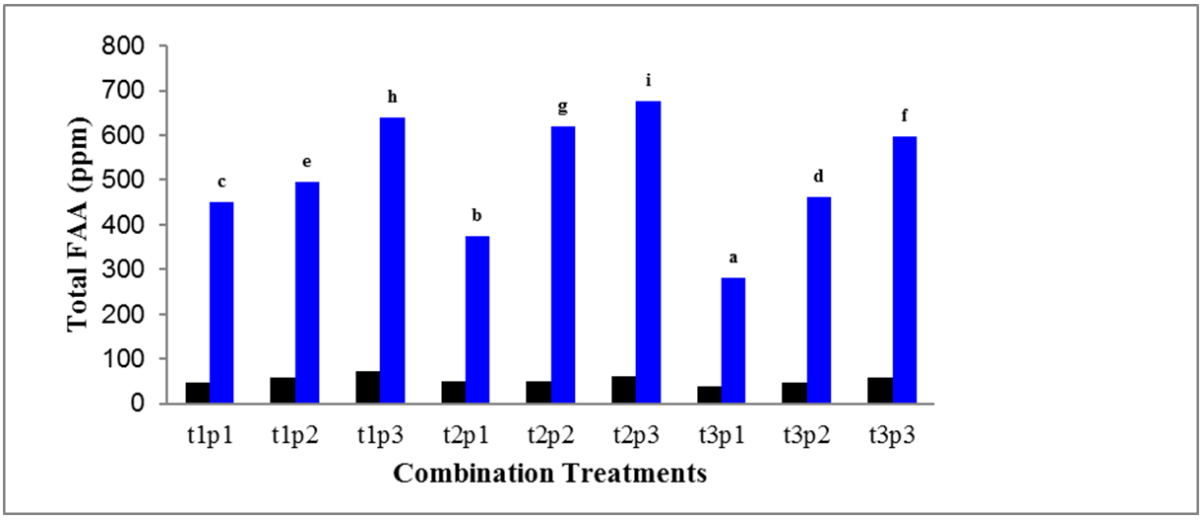
Total Phenolics

Total phenolics test was calculated as gallic acid equivalent which represents the mg GAE obtained from 1 g of dried leaves. Data analysis with ANOVA shows that there is strong evidence effect of the combination of temperature and pH during the incubation on the total phenolics of crude extract ($p>0.05$), continued with BNJ test. The presence of interaction between temperature and pH means that the total phenolics changes for different pH depends on the temperature. Similarly, the total phenolics changes for different temperatures depends on pH. The total phenolic content of the result of hydrolysis of *A. papuana* Becc. leaves can be seen from **Fig. 2**.

Trend data of total phenolic show that from 30°C there is increase in temperature at 35°C and decreased again at 40°C along with increasing of pH. During 72 hours of incubation hydrolysis there was an increase in total phenolics from *san-sangk* leaves extract compared to the without incubation. The average level of total phenolics has been found to increase from 0.5 to 5 mg GAE/g, which means 10 times fold. This explains that hydrolysis of tannins into gallic acid were ancountered. The total phenolics produced ranged from 3.2 to 5.02 mg GAE/g during 72 hours incubation. The higher the pH, the lower the total phenolics would be.

According to Yeni et al. (2014), the higher of phenolics levels are highly dependent on the ratio of ingredients and solvents, extraction time, also extraction method. The type of tannase used also greatly affected the results and in this research commercial tannase was used from Aspergillus sp. This can be seen also from the results of the study reported by El-Fouly et al. (2012). Gallic acid production using extracellular tannase (added) from *A. niger* AUMC 4301 in several types of food industry waste, showed that at 12 hours it had increased tamarind total phenolics from 0 to 3 mg/g, while in olive mill from 0 to 20 mg/g for the same 12 hours, meanwhile in peat moss that from 0 to 268 mg/g for 18 hours. In this research the total phenolic results were still low. While if we compared results of Hong et al. (2013) that using mixed commercial tannase and viscozyme (Aspergillus sp and *A. oryzae*) on green tea extract to release phenolic compounds, our total phenolic (3.2 to 5.02 mg GAE/g during 72 hours incubation) was not different than to that results (4.01± 0.13 mg/ml or ± 4mg GAE/ml) in successive 20 min treatments.

This might be due to the incubation did not being conducted by using other additives which were generally increasing tannase activity (El-Fouly et al. 2012, Selwal et al. 2011) explained that the degradation or hydrolysis of tannins by tannase is strongly influenced by additives (metal salts) which added in the substrate to increase tannase activity. Another factor that can also affect the low result during incubation is stirring. This technique can increase the reaction rate of enzyme. Stirring or agitation one of the key parameter in the effective use of



Notes: t = temperature; t1= 30°C; t2 = 35°C; t3 = 40°C
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Fig. 3. The total FAA of different combination treatments of hydrolysis *A. papuana* Becc. leaves by using tannase. Mean (standard deviation); n =3. The Average value that is followed by same letter is insignificantly different in BNJ 0.05 test

the enzyme (Oueslati and Mounirhaouala 2014, Mussatto et al. 2008). In this research, stirring was not carried out during incubation because the conditions of the incubator were closed and there was no direct equipment system with automatic stirrer from outside the incubator.

Total of Free Amino Acids (FAA)

The main contribution of umami flavor comes from glutamic acid, so that the standard used in the evaluation is glutamic acid. This is also done because *A. papuana* Becc. leaves has been known contains free glutamic acid based on Purwayanti et al. (2013b) and Mayasari (2013). Data analysis with ANOVA shows that all combination treatments show the significantly different average total FAA. So that there is strong evidence of the effect of the combination of temperature and pH on the operating conditions of incubation on the total FFA of crude extract ($p>0.05$), continued with BNJ test. The presence of interaction between temperature and pH means that the total FAA changes for different pH depends on the temperature. Similarly, the total FAA changes for different temperatures depends on pH. The total content of FAA resulting from the hydrolysis of *A. papuana* Becc. leaves using tannase can be seen from **Fig. 3**. During 72 hours of incubation hydrolysis there was an increase in total FFA compared to the one without incubation. The level of FFA has been found to increase from < 40 to > 600 ppm, it means 15 times fold. In general, the trend of total FAA shows a trend that is inversely proportional to total phenolics. The total amino acids produced ranged from 280.17 to 674.97 ppm. The highest total production of free amino acids occurs at pH of 6 temperature of 35°C with the value of 674.97 ppm.

Most research reports that one of sources of glutamic acid comes from fermented products especially from fermented beans (Jinap and Hajeb 2010) that break down proteins into free amino acids, mainly glutamic acid (Nout and Aidoo 2010). However, there are no reports that tannase from Aspergillus sp. can also produce amino acid as by products. Some tannase-producing microbes that can produce glutamic acids are from Brevibacterium (Nampoothiri and Pandey 1999), *Lactobacillus plantarum* (Zarein et al. 2012) and *Bacillus subtilis* and *B. lecheniformis* (Lawal et al. 2011). According to Purwayanti et al. (2013b), free glutamic acid of *A. papuana Becc*. leaves could be extracted in phosphate buffer solutions (pH 5-8) with the highest result at pH 5. The glutamic acid was not detected using HPLC in crude water extract (Purwayanti et al. 2013b) at pH 4-5, this may be caused by the small sampel size. In Tris HCl buffer solution (pH 8.0) amino acid polar was not detected too. It may be caused by the fouling occurs when the membrane filtration processes was done by using PES membrane material (Purwayanti et al. 2015). Besides that, Nofiyanti et al. (2012) reported that protease enzymes naturally available on *san-sangk* leaves. The nature of this enzyme is to hydrolyze proteins into amino acids, such as glutamic acid. That research concluded that optimal protease works at 50°C, while at 30, 40 and 60°C the activity is low. Because the temperature applied of this research were 30-40°C, hence glutamic acids formed was relatively low.

CONCLUSION

There was no report till date on gallic acid and glutamic acid-rich extract production by enzyme on *Albertisia papuana* Becc. Hence, in the present work has been taken up with a view of exploring the possibilities of using *A. papuana* Becc. leaves as a substrate for gallic acid and glutamic acid-rich extract production by

tannase commercial (industrially important enzyme). The result showed that temperature and pH combination affected the yield, total phenolic as a gallic acid and free amino acid as a glutamic acid. In the future, we are interested to scale up the production of gallic and glutamic acid from *A. papuana* Becc. leaves by using tannase combined with separation methods.

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REFERENCES

Banarjee D, Pati BR, Mahapatra S (2007) Gallic Acid Production by Submerged Fermentation of Aspergillus aculeatus DBF9. Research Journal of Microbiology, 2(5): 462-8. https://doi.org/10.3923/jm.2007.462.468

Battestin V, Macedo GA (2007) Effects of temperature, pH and additives on the activity of tannase produced by Paecilomyces variotii. Electronic Journal of Biotechnology, 16: 191-9. https://doi.org/10.2225/vol10-issue2-fulltext-9

Bauer D, Robinson M (2012) Glutamatergic Neurotransmission. In Primer on the Autonomic Nervous System. [Place unknown]: Academic Press: 103-7. https://doi.org/10.1016/B978-0-12-386525-0.00021-4

Belur PD, Pallabhanvi B (2011) Investigation on production of gallic acid from Terminalia chebula extract using cell associated tannase of Bacillus massiliensis. Proceeding of The International Conference on Advances in Biotechnology and Pharmaceutical Sciences (ICABPS), Bangkok.

Burrin DG, Stoll B (2009) Metabolic fate and function of dietary glutamate in the gut. American Journal Clinical Nutrition, 90(3): 850-6. https://doi.org/10.3945/ajcn.2009.27462Y

El-Fouly MZ, El-Awamry Z, Shahin AAM, El-Bialy HA, Naeem E, El-Saeed GE (2012) Gallic Acid Formation from Gallotannins-rich Agricultural Wastes Using Aspergillus niger AUMC 4301 or its Tannase Enzyme. Arab Journal of Nuclear Science and Applications, 45(2): 89-496.

Farhan H, Rammal H, Hijazi A, Hammad H, Daher A, Reda M, Badran B (2012) In Vitro Antioxidant Activity Ethanolic and Aqueous Extracts from Malva parviflora L., Grown in Lebanon. Asian Journal of Pharmaceutical and Clinical Research, 5(3): 234-8.

Gaur A, Nandini KE, Sundari SK (2017) Production of Tannase and Gallic Acid by Utilizing AgroIndustrial Wastes as Economical Raw Material and Purification of Tannase. Indo Global Journal of Pharmaceutical Sciences, 7(1): 45.

Ghirri A, Bignetti E (2012) Occurrence and role of umami molecules in foods. International Journal of Food Science Nutrition, 63(7): 871-81. https://doi.org/10.3109/09637486.2012.676028

Gustavo RV, Torres-Moreno H, Villegas-Ochoa MA, Ayala-Zavala JF, Robles-Zepeda RE, Wall-Medrano A, González-Aguilar GA (2018) Gallic Acid Content and an Antioxidant Mechanism Are Responsible for the Antiproliferative Activity of 'Ataulfo' Mango Peel on LS180 Cells. Molecules, 23: 695. https://doi.org/10.3390/molecules23030695

Hagerman AE (2002) Hydrolyzable Tannin Structural Chemistry. Retrieved from https://www.users.miamioh.edu/hagermae/

Hartanti S, Rohmah S, Tamtarini (2003) Kombinasi penambahan CMC dan Dekstrin pada Pengolahan Bubuk Buah Mangga dengan Pengeringan Surya. Proceedings of National Seminar and PATPI Meeting Yearly, Yogyakarta.

Hong Y, Jung EY, Park Y, Shin K, Kim TY, Yu KW, Chang UD, Suh HJ (2013) Enzymatic Improvement in the Polyphenol Extractability and Antioxidant Activity of Green Tea Extracts. Bioscience Biotechnology Biochemistry, 77: 22-9. https://doi.org/10.1271/bbb.120373

Iqbal H, Kapoor A (2012) Tannin Degradation Efficiency of Tannase Produced by Trichoderma harzianum MTCC 10841 dan Its Biochemical Properties. International Journal of Life Scence Biotechnology & Pharma Research, 1(4): 106-17.

Jinap S, Hajeb P (2010) Glutamate. Its applications in food and contribution to health. Apatite, 55: 1-10. https://doi.org/10.1016/j.appet.2010.05.002

Karamac MA, Kosiñska A, Pegg RP (2006) Content of Gallic acid in selected plant extracts. Polish Journal of Food Nutrition Sciences, 15/56(1): 51-8.

Khokhani K, Ram V, Bhaat J, Khatri T, Joshi H (2011) Spectrophotometric and Chromatogaphic analysis of Amino Acid Present in Leaves of Ailantus excels. International Journal Chem. Tech Research, 4(1): 389-93.

Lawal KA, Sanni AI, Oso AB, Olatunji OO (2011) L-glutamic acid production by bacillus spp. isolated from vegetable proteins. African Journal of Biotechnolgy, 10(2): 5337-45.

Mayasari E (2013) Pengaruh Kematangan Daun dan Suhu Curing Terhadap Komponen Kimia dan Profil Daun Sungkai Sebagai Flavor Enhancer [thesis]. Yogyakarta, Indonesia: University of Gadjah Mada.

Mussatto SI, Dragone G, Fernandes M, Milagres AMF, Roberto IC (2008) The effect of agitation speed, enzyme loading and substrate concentration on enzymatic hydrolysis of cellulose from brewer's spent grain. Cellulose, 15(5): 711-21. https://doi.org/10.1007/s10570-008-9215-7

Nallabilli L (2016) Utilization of Natural Tannins from Anacardium occidentalis Testa for Producing The Industrially Important Gallic Acid Through Submerged Fermentation. World Journal of Pharmaceutical Research, 5(8): 861-4.

Nampoothiri KM, Pandey A (1999) Fermentation and Recovery of L-Glutamic Acid from Cassava Starch Hydrolysate by Ion-Exchange Resin Column. Revista de Microbiologia, 30(3): 258-64. https://doi.org/10.1590/S0001-37141999000300013

Natarajan K (2009) Tannase: A tool for instantaneous tea. Current Biotica, 3(1): 0973-4031.

Nofiyanti T, Ardiningsih P, Rahmalia W (2012) Pengaruh Temperatur Terhadap Aktivitas Enzim Protoase Dari Daun San-sangk. Journal Kimia Khatulistiwa, 1(1): 31-4.

Nout MJR, Aidoo KE (2010) Asian Fungal Fermented Food. In: Hofrichter, M, editor. The Mycota, Industrial Applications X. Berlin: Springer: 21-58. https://doi.org/10.1007/978-3-642-11458-8_2

Oueslati A, Mounirhaouala (2014) Operating Conditions Effects on enzyme Activity: Case Enzyme Protease. Journal of Engineering Research and Applications, 4(9): 33-7.

Oyagbemi AA, Omobowale TO, Saba AB, Olowelu R, Dada RO, Akinrinde AS (2016) Gallic Acid Ameliorates Cyclophosphamide-Induced Neurotoxicity in Wistar Rats Through Free Radical Scavenging Activity and Improvement in Antioxidant Defense System. Journal of Dietary Supplements, 13: 402-19. https://doi.org/10.3109/19390211.2015.1103827

Purwayanti S, Santoso U, Supriyadi S, Garjito M (2013a) Taste Compounds from Crude Extract of Bekkai Lan (Albertisia papuana Becc.). Journal of Food Nutrition Sciences, 1(4): 33-7. https://doi.org/10.11648/j.jfns.20130104.11

Purwayanti S, Santoso U, Supriyadi S, Garjito M (2013b) Umami Potential from Crude Extract of Bekkai lan (Albertisia papuana Becc.) Leaves, an Indigenous Plant in East Kalimantan-Indonesia. International Food Research Journal, 20(2): 545-9.

Purwayanti S, Santoso U, Supriyadi S, Garjito M, Susanto H (2015) The Isolation of Taste Compounds in Bekkai Lan (Albertisia papuana Becc.) Leaves Extract Using Nanofiltrations. International Food Research Journal, 22(1): 225-32.

Rosman R, Saifullah B, Maniam S, Dorniani D, Hussei MZ, Fakurazi S (2018) Improved Anticancer Effect of Magnetite Nanocomposite Formulation of GALLIC Acid (Fe3O4-PEG-GA) Against Lung, Breast and Colon Cancer Cells. Nanomaterials (Basel), 8: 83. https://doi.org/10.3390/nano8020083

Selwal MK, Yadav A, Selwal KK, Aggarwal NK, Gupta R, Gautam SK (2011) Optimization of Cultural Conditions for Tannase Production by Pseudomonas aeruginosa IIIB 8914 Under Submerged Fermentation. World Journal of Microbiology and Biotechnology, 26(4): 599-605. https://doi.org/10.1007/s11274-009-0209-x

Silvestre M, Carreira R, Silva MR, Morais HA (2012) Effect of pH and Temperature on the Activity of Enzymatic Extracts from Pineapple Peel. Food and Bioprocess Technology, 5(5): 1824-31. https://doi.org/10.1007/s11947-011-0616-5

Wang Q, de Oliveira EF, Alborzi S, Bastarrachea LJ, Tikekar RV (2017) On mechanism behind UV-A light enhanced antibacterial activity of gallic acid and propyl gallate against Escherichia coli O157:H7. Scientific Reports, 7: 8325. https://doi.org/10.1038/s41598-017-08449-1

Yeni G, Gumbire-Sa'id E, Syamsu K, Mardliyati E (2014) Penentuan Kondisi Terbaik Ekstraksi Antioksidan dari Gambir Menggunakan Metode Permukaan Respon. Jurnal Litbang Industri, 4(1): 39-48. https://doi.org/10.24960/jli.v4i1.637.39-48

Zarein M, Ebrahimpour A, Bakar FA, Mohamed AKS, Forghani B, Ab-Kadir MSB, Saari N (2012) A Glutamat Acid-Producing Lactic Acid Bacteria Isolated from Malaysia Fermented Foods. International Journal of Molecular Sciences, 13: 5482-97. https://doi.org/10.3390/ijms13055482