Effects of Cellulase and Pectinase on Changes in Compounds of Flavor and Antioxidants in Roselle Extraction

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Abstract

Nowadays, enzymatic treatment is most frequently utilized for juice extraction. In some ways, several fruit pulps are said to benefit from the enzymatic procedure over mechanical-thermal comminution. This study aims to evaluate the role of enzymes for improving the quality of extraction result, yield, the content of phytochemical compounds such as antioxidant compounds, and for increasing flavor compounds. This research method uses the enzyme pectinase and cellulase as much as 1000 ppm, which is added to the extraction process of fresh roselle petals with water extracting ingredients with a ratio of (1:6) and 1% (w/v) citric acid and as a control for extraction of roselle petals without enzyme. Extraction was carried out at 50 ^{o}C for 1 hour. The types of oxidant compounds were identified using the LCMS ToF instrument and the analysis of flavor compounds was carried out the GCMS instrument. The results showed that roselle petal extract with the addition of pectinase and cellulase enzymes increased the number of antioxidants by 17 antioxidants produced from 56 antioxidant compounds detected. The addition of these enzymes also increased the number of flavor compounds, as many as two additional flavor compounds that were not previously detected in the roselle petal extract without enzymes.

Keywords: Roselle, Pectinase, Cellulase, Antioxidant, Flavor

1. Introduction

Hibiscus sabdariffa Linn, a Malvaceae family member, is s a medicinal plant that can grow in tropical and subtropical climates. It is also known as Roselle (English), I'Oiselle (French), Spanish (Jamaica), Karkade (Arabic), Bissap (Wolof), and Sour Tea (Farsi) (Mohagheghi *et al.,* 2011; Bedi *et al.,* 2020). One of the newest approaches to extracting plant foodstuffs is to use enzymes (Rafińska *et al*., 2022). The use of enzymes in the extraction is expected to increase the amount of yield and antioxidant content.

Cellulase, hemicellulase, and pectinase and their combinations have been used to pretest plant materials. The main action of cellulase and hemicellulase is in the cell wall. These enzymes act on cell wall components, hydrolyzing them, which can then increase cell wall permeability resulting in higher yields of flavor (Sowbhagya and Chitra, 2010). The application of pectinase enzymes includes food and non-food industries. Pectinase could be used in fruit juices and wines, extract oils, flavors, and pigments from plants, and for processing cellulose fibers, linen, jute, and hemp. Pectin enzymes also play a role in the degradation of pectin by releasing glycosidic bonds. Pectinase can soften cell walls and increase the yield of fruit juice extracts so that the pectinase enzyme becomes one of the essential enzymes in the food industry (Oyeleke *et al.,* 2012; Dalal *et al.,* 2007). Cellulase enzyme is one of the enzymes produced by microorganisms that functions to degrade cellulose into glucose. The results obtained using two enzyme preparations sequentially were similar to those obtained using a single enzyme preparation containing mainly the enzyme pectinase. Pretreatment with cellulolytic enzymes improves the separation of intracellular compounds (e.g., flavor compounds) from plant materials by solvent extraction (Sowbhagya and Chitra, 2010). Pectinase, cellulase,and hemicellulase for producing blueberries and blueberry peel extracts can increase anthocyanins and polyphenols (Lee and Wrolstad. 2004).

The extract roselle used pectinase enzyme and cellulase ratio (1:1) as much as 1000mg/L resulted in a significant yield of 45.9% (dry basis) (w/w) compared to control (without enzymes) of 26.5% (dry basis)(w/w). In addition, the use of these enzymes was able to increase the phenol content and antioxidant activity of the roselle extract produced (Barman *et al*., 2015). The phenol content in roselle extract with enzymes was 1854.7 g GAE/g sample and 1661.5 g GAE/g sample (without enzymes). The antioxidant activity of roselle extract added with enzymes was 589.5 mmol TE/g and 545.8 mmol TE/g (without enzymes). The addition of enzymes during the extraction process can have the functional food benefits of increasing acids, dyes, and aroma of the rosella petal extract (Da-Costa-Rocha *et al*., 2014). Iriani *et al.* (2005) found that adding pectinase could increase quinine juice yield and the distinctive flavour of quinine manga.

The antioxidant activity of the extract roselle is due to its solid scavenging effect on reactive oxygen and free radicals. Besides that, the antioxidant has ability inhibition of xanthine oxidase activity, protective action against tert-butyl hydroperoxide (t-BHP)-induced oxidative damage, protection of cell from damage by lipid peroxidation, inhibition in Cu2+-mediated oxidation of LDL and the formation of Thio barbituric acid reactive substances (TBARs), reduction of glutathione depletion, increase of the liver and decrease blood activity of superoxide dismutase and catalase (Da-Costa-Rocha *et al.*, 2014).

Roselle flower extract that has antioxidant effects contains quercetin, cyanidin, β-carotene, and vitamin C, compounds having the absorption ability and being quickly metabolized. Oxidative stress enhances the making of free radicals, which has a significant role in the pathogenesis and development of diabetes and decreases antioxidant protection (Bedi *et al.,* 2020). This study aimed to determine the effect of the addition of cellulase and pectinase enzymes in the roselle extraction process on the antioxidant content and flavour compounds of the roselle petal extract.

2. Materials and methods

2.1 Materials

The material used in this study was fresh purple roselle obtained from West Palimanan Cirebon, West Java. Pectinase and cellulase enzymes were purchased from Jiangsu Boli Bioproducts Co., Ltd. (Taizhou City, China). Other ingredients are aquadest and citric acid obtained from chemical stores.

2.2 Method

2.2.1 Roselle extraction stage

The extraction processes of fresh calyces were performed without and with added enzymes. For the experiments without adding an enzyme mixture, 500 g of new Roselle calyx sample and 1% (w/w) of citric acid were added to 3 L of distilled water. For the enzyme mixture, 500 g of fresh Roselle calyx sample, 1%(w/w) of citric acid, and 1000 mg/L of pectinase/cellulase (1:1) mixture were added to 3 L of distilled water. The solution containing fresh Roselle calyx was divided into two parts of 1.8 L (60%) and 1.2 L (40%) and then extracted at 50˚C for 60 min. The extracts of fresh Roselle calyces were filtered using the Whatman 0.45-µm filter paper.

2.2.2 Screening for antioxidants with LC-MS ToF

2.2.2.1 Sample preparation

The preparation of sample by weighing 1.0 ± 0.1 g of sample in a centrifugation tube of 50 mL and adding 3-5 mL of Hexane was firstly carried out by shaking the solution for ± 1 min (Calderon, 2009). The extraction of sample using 3 mL of methanol was performed by shaking the solution for ± 1 min and then transferred the methanol phase into a flask. Then the extraction of sample was repeated again for two times. The combined solution of methanol phase was ultrasonicated for 15 min and then filtered using a membrane filter of 0.22 µm GHP to produce an extract. Then the membrane filter was rinsed with 1 mL of methanol and then combined the rinse results with the existing extract. The extract was

evaporated using a TurboVap with the help of nitrogen gas and then reconstituted with 1 mL of methanol and then transferred into a 2 mL vial. After that, it was injected into the LC-MS ToF system with the mobile phase, lock mass solution, and Calibmass.

2.2.2.2 LC-MS ToF system settings

The instrumentation system of Xevo G2-S QToF from waters using the ACQUITY HSS T3 Column 2.1 x 100 mm 1.8 m was operated based on the pump gradient formation concept with the autosampler temperature of 15oC and the flow rate of 0.6 mL/min. ,The mobile phase A - (0.1% formic acid in in aquabidest) was prepared by conditioning 1% of formic acid in aquabidest at 0 to 0.5 min, increased to 35% at 16^{th} min and to 100% at 18^{th} min and then decreased to 1% at 20^{th} min of running the Xevo G2-S QToF. The mobile phase B - (0.1% formic acid in acetonitrile) was prepared by conditioning 99% of formic acid in acetonitrile at 0.5 min, decreased to 85% at 16^{th} min and to 0% at 18^{th} min and then increased again to 99% at 20^{th} min of running the Xevo G2-S QToF. The column temperature used was 40ºC with an injection volume of 2 l. The ionization used was ESI (+) and ESI (-) with a capillary voltage of 2.0 VK; Cone Voltage at 20 V; Low Collusion Energy at 5 V; High Collusion Energy using a gradual increase from 10 to 40 V. The source temperature used is 120°C with a desolvation gas flow of 1000 L/hour, a desolvation temperature of 550°C, and a gas cone flow rate of 50 L/h. The mass reading is carried out from 50 to 1200 Da.

2.2.2.3 Process data using UNIFI

The screening process for active substances from natural ingredients using LCMS/MS-QTOF is carried out using UNIFI software, which includes a mass spectrum library of active natural ingredients from the waters database. UNIFI software can identify the mass spectrum of the compound in the sample, which is then matched with the mass spectrum in the library.

2.2.3 Flavor test using GC-MS

2.2.3.1 Sample preparation

Sample preparation using Dynamic Headspace Sampling (DHS). Each sample was placed in a glass flask (300 mL, 7.5 cm diameter). A trap containing Tenax-TA (200 mg) was attached to the sealed flask. The samples' flasks were immersed in a water bath held at 40°C. Under magnetic stirring (200 rpm), the model was tempered for 10 minutes before purging with nitrogen (100 mL/min) for 30 mins. The traps were dry-purged with nitrogen (100 mL/min) for 10 mins to remove water (Juhari *et al*., 2015).

2.2.3.2 Flavor testing with GC-MS

Volatile compounds were identified by probability-based matching of their mass spectra with those of a commercial database. The software program MSD ChemStation was used for data analysis. Amounts are presented as peak areas. Shimadzu brand GCMS condition, 250^{o}C injector temperature, split less, hydrogen gas carrier, split speed 1.8 mL/min. Preheat oven to 35°C for 5 mins, then 6.0°C/mins to 250°C for 10 mins, using column HP-5MS (30m x 250m x 0.25 m), interface 280oC. Scan mass 29-550 m/z, MS source 230^{o}C, and MS Quad 150^{o}C.

The results are detected in the form of peaks. This peak indicates the presence of a component in the sample. Each rise, in this case, represents a specific component with different retention times, which can be seen from the Shimadzu GC-MS Post-run Analysis software by clicking on a peak and then seeing how much retention time it has. The way to identify volatile chemical components with GC-MS is (1) matching the MS (Mass Spectra) of the target chemical component of the sample injection with the MS contained in the software library; (2) confirming the match between the calculated LRI value of the component and the LRI from the reference. In the same column; (3) If a match is found in the two cases above, the volatile chemical component has been identified successfully; (4) If there is no match, the identification process needs to be carried out from the beginning again by starting to look for matches between the target component MS and other MS in software libraries.

3. Results and discussion

3.1 Screening of active antioxidant substances with LC-MS

Based on testing using the LC-MS ToF instrument, several antioxidant compounds in fresh roselle extract with the addition of enzyme were successfully detected according to the identification criteria. In Table 1, the results of the identification of compounds in each roselle extract are presented.

Adding pectinase and cellulase can increase 17 types of antioxidants but miss ten antioxidant compounds. Some missing antioxidant compounds include apocynin B, iridin, isocarthamidin, leucocyanidin, maltol, protocatechuic aldehyde, pyrogallic acid, spiraeoside, syringaldehyde, tran-ferulaldehyde. The decrease in total phenolic contents may be due to the endogenous enzymes

(polyphenol oxidases) and also to the oxidation of some phenolic compounds by hydrolytic enzymes. Some researchers reported that crude enzymes might contain small amounts of enzymes which can degrade phenolics, such as rutinase activity, laccase activity and rhamnosidase activity that can hydrolyze rutin to quercetin, oxidation of phenolic compounds and change rutin to quercetin-3-glucoside, respectively (Abbès et al., 2013)

The addition of 17 types of antioxidants with the addition of enzymes (in the treatment of roselle extract without enzymes, these compounds were not detected/absent) included 1,4-Dihydroxy-2-methoxybenzene, 1-Galloyl-β-D-glucose, 3-Hydroxy-1 -(4-hydroxy-3,5-dimethoxy phenyl)-2-propanone, 4,5-O-Dicaffeoylquinic acid, 5,7-Dihydroxychromone, 6-Gingerdione, Artemitin, Mirificin, Neocomplanoside, Neoisoastilbin, Onjixanthone Ⅱ, Patuletin, Pinocembrin- 7-neo hesperidoside, Polygoacetophenoside, p-Tolualdehyde, Sulfuretin and Viscidulin (Figure 1). In the treatment with enzymes, the compounds leucocyanidin, protocatechuic acid, apocynin, iridin, isocarthamidin, maltol, pyrogallic acid, spiraeoside, syringaldehyde, tran-ferulaldehyde, and trifolin were previously found in roselle extract without the addition of enzymes. According to Da-Costa-Rocha *et al.* (2014), protocatechuic acid is a phenolic compound widely found in dried roselle and assigned the structure of 3,4-dihydrobenzoic acid.

The addition of enzymes in the extraction can hydrolyze cell membranes and increase the extraction of active compounds. Fernandez *et al.* (2015) evaluated the effect of pectinase, cellulase, and tannase enzymes on the extraction of phenolic compounds from grape skins and seeds. The results showed that pectinase had the most effect on the extraction efficiency of phenolic compounds, thereby increasing the number of phenolic compounds by 2.5 times compared to the control sample. Increased enzyme dosage and maceration time increased maceration temperature and increased the total phenols yields (Sharma *et al.,* 2017). According to Ghandahari *et al.* (2019), cell walls consist of cellulose, hemicellulose, pectin and protein. Phenolic compounds are related to polysaccharides by hydrogen bonding and hydrophobicity. The enzymes such as cellulases, pectinases, and hemicellulases can be used as hydrolytic agents to destroy cell wall structures. This enzyme can also be used to increase the penetration potential of cell walls, which results in the release of phenolic compounds and increases the extraction yield of bioactive compounds. Enzymes can destroy cell walls; under acidic conditions, enzymes can release bound phenolic compounds. They increased the extraction yield of phenolic compounds by about, 25%, 63%, and 97% by using pectinase, cellulase and tannase, respectively. Ghandahari *et al*. (2019) using a combination of cellulase, pectinase, and tannase enzymes could increase the extraction yields and phenolic

compounds compared to using these enzymes alone. Phenolic compounds present in roselle extract (Table 1) include gallic acid, pyrogallic acid, isorhamnetin, protocatheuic aldehyde, catechins, coumaric acid, caffeic acid, and quercetagetin. Ghandahari *et al.* (2019) identified that cellulolytic enzymes had no effect on increasing gallic acid. While tannase, pectinolytic enzymes, and the combination of CPT (cellulose, pectinase, tannase) increased the amount of gallic acid up to 34%, 78%, and 98%, respectively.

Using cellulase and pectinase enzymes also improves the quality of olive oil with higher concentrations of natural antioxidants, volatiles, and tocopherols and high yields (Sowbhagya and Chitra, 2010).

Roselle flower extract (with enzymes and without enzymes) contains quercetin, dihydroquercetin, leucopelargonidin, malvidin and luteolin (flavonoid group) and caffeic acid and coumaric acid (phenolic acid group). The same antioxidant group was also found in ziziphus jujube fruit, which has an antidiabetic role because of inhibition amylase and glucosidase enzymes (Stoilova *et al*., 2017).

Roselle extract (Table 1) contains anthocyanin compounds such as leucopelargonidin, malvidin, and leucocyanidin. Anthocyanins are a group of flavonoid derivatives and natural pigments present in the dried flowers of roselle, and their color varies with pH. Pectinase treatment increased the release of anthocyanins more than the other enzyme treatments in white grape juice. Treatment of raspberry juices with pectolytic enzymes modified the level of the individual pigment, and the total anthocyanins content varied accordingly. The pectolytic enzymes showed a stationary high level of total anthocyanins over time (range: 289-306 mg l-1). On the other hand, a decrease in total anthocyanins after 6 h (Sharma *et al.,* 2017). Roselle has anthocyanins, flavonoids, and polyphenolic acids that function as antioxidants. The function of antioxidants can reduce free radical reactions as a trigger for degenerative diseases (Khan, 2017; Ojulari, *et al.,* 2019).

The results of the test of flavor compounds on the roselle extract without the addition of enzymes (control) compared to the roselle extract with the addition of pectinase and cellulase enzymes in Table 2 indicate the presence of additional types of flavors detected in the GCMS tool. From the test results, the GCMS method detected 5 hexanol compounds, silanediol, 3 octanols (alcohol group), 2 anthracenamines (polycyclic aromatic hydrocarbons), oxime, methoxy phenyl (ketone group), and hexane, 2,2,5,5, tetramethyl (ketone compound). alkanes) in both control and enzyme treatment. Table 2 shows the presence the 2 new compounds, 1,3,5-Cycloheptatriene (alkene group) and 2-Butene (alkene group), in the treatment of roselle extract with enzymes.

However, the diazene flavor compound dimethyl (nitrogen compound) disappeared in the roselle extract with enzymes. Aroma compounds are primarily present in low quantities in foods. They are primarily low molecular weight compounds, which are highly volatile. These aroma compounds can be classified, based on their functional groups, to be lactone, pyrazine, ester, terpene, ketone, aldehyde, alcohol, and fatty acid (Panakkal *et al*., 2021). Diazene and dimethyl groups are in Pyrazines, which are heterocyclic aromatic compounds with nitrogen atoms in the aromatic ring. It provides flavours of nuts or roasted nuts. The loss of diazene and dimethyl groups is probably due to the influence of the added enzymes. Enzymes are also used in various organic reactions for specific purposes such as the cleavage of ester bonds, separation of racemic mixtures to obtain a single optically active compound, and cleavage of double bonds (Sowbhagya & Chitra, 2010)

The results of GCMS showed that alcohol compounds were the most detected compounds. The headspace technique detected volatile compounds, including alcohol, acid, carbonyl ester, and other aromatic compounds (Jelen, 2012). According to Da-Costa-Rocha *et al.* (2014), volatile compounds in roselle are compounds including fatty acid derivatives (such as 2-ethylfuran and hexanal), sugar derivatives (furfural and 5-methyl-2-furaldehyde), phenolic derivatives (eugenol), terpenes (such as 1,4-cine-ole, limonene) and various compounds. A total of thirty-two compounds were identified and could be divided into five chemical groups: aldehydes (fourteen compounds), alcohols (ten compounds), ketones (five compounds), terpenes (two compounds), and acids (one compound). Compound 2 hexanol found in roselle extract also forms a fruit aroma in identifying the flavor in dragon fruit (Puspita *et al.,* 2011). It forms an olfactory description, such as grapefruit, also found in green and grass (Jelen, 2012).

Previous research on roselle extract found a total of 125 compounds identified, including terpenes (32), aldehydes (20), esters (16), ketones (14), alcohols and furans (13), acids (9), sulfurs (3), lactones (2) and others (3). Terpenes and aldehydes were the most represented classes by number, followed by esters, furans, ketones, alcohols, acids, sulfurs, lactones, and others. Limonene, -terpineol, and 1,8-cineole are three of the most abundant aroma compounds from the terpene group, and furfural is a type of flavor that is mainly found in dried roselle, while fresh roselle is slightly found. Furans (2-pentylfuran, 2-acetylfuran, and furfural) may be produced from the drying process, mainly the thermal processing and thermal decomposition of hydroperoxides or cyclic peroxides of linoleate (Juhari *et al.,* 2015). However, this study cannot provide data such as the research conducted by Juhari *et al*. (2015) even though it uses the same sampling techniqueof Dynamic Headspace Sampling.

In Table 2, it is shown that the sample of roselle extract with enzymes formed a compound 1,3,5 cycloheptariene. According to Dzhemilev *et al*. (1991), this compound is formed from the reaction of aromatic hydrocarbons with diazo compounds in the presence of transition metal complexes such as V, Fe, Co, Ni, Cu, Zr, Mo, Rh, Pd, Ag, W, Ir, Hg<CI, Pr, and Nd. Compound 2 anthracenamine is a polycyclic aromatic hydrocarbon compound.

According to Sowbhagya and Chitra (2010), the main action of cellulase and hemicellulase is in the cell wall. They act on cell wall components, hydrolyzing them, increasing cell wall permeability and resulting in a higher yield of flavor compounds. Treatment of cured vanilla beans with exogenous pectinase and glycosidase has an increase 14% in vanillin content.

4. Conclusion

The role of pectinase and cellulase enzymes during the extraction of fresh rosella petals can increase the number of antioxidants but eliminating the number of antioxidant compounds and increases the number of flavor compounds. The extraction of fresh rosella petals extracted using the pectinase and cellulase enzymes can result in the addition of 17 new antioxidant compounds belong to the group of phenolic compounds and the elimination of 10 antioxidant compounds. The extraction of fresh rosella petals extracted using the pectinase and cellulase enzymes can result in the addition of 2 new flavor compounds of 1,3,5-Cycloheptatriene and 2-Butene belong to alkene group) but eliminates the flavor compound of dimethyl diazene belong to the group of nitrogen compounds.

Conflict of interest

Authors declare no conflict of interest.

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385 **Tables and Figures**

386 Table 1. Results of LC-MS ToF identification for the roselle petal extract samples

Rosella petal extracted without enzyme		Rosella petal extracted with enzyme	
Result	antioxidant compounds	Result	antioxidant compounds
(+)	(+)-Catechin-pentaacetate	(+)	(+)-Catechin-pentaacetate
(-)	**1,4-Dihydroxy-2-methoxybenzene**	**(+)**	**1,4-Dihydroxy-2-methoxybenzene**
(-)	**1-Galloyl-β-D-glucose**	**(+)**	**1-Galloyl-β-D-glucose**
(+)	2,4,5-Trihydeoxybenzaldehyde	(+)	2,4,5-Trihydeoxybenzaldehyde
(+)	2,4,6-Trihydroxyacetophenone-2,4-di-O-β-D-glucopyranoside	(+)	2,4,6-Trihydroxyacetophenone-2,4-di-O-β-D-glucopyranoside
(+)	3,5,7-Trihydroxychromone	(+)	3,5,7-Trihydroxychromone
(-)	**3-Hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-2-propanone**	**(+)**	**3-Hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-2-propanone**
(-)	**4,5-O-Dicaffeoylquinic acid**	**(+)**	**4,5-O-Dicaffeoylquinic acid**
(+)	4-Hydroxyacetophenone	(+)	4-Hydroxyacetophenone
(-)	**5,7-Dihydroxychromone**	**(+)**	**5,7-Dihydroxychromone**
(-)	**6-Gingerdione_1**	**(+)**	**6-Gingerdione_1**
(+)	Apocynin B	**(-)**	**Apocynin B**
(-)	**Artemitin**	**(+)**	**Artemitin**
(+)	Cinchonain Ia	(+)	Cinchonain Ia
(+)	cis-Caffeic acid	(+)	cis-Caffeic acid
(+)	Cistanoside H	(+)	Cistanoside H
(+)	Coniferol	(+)	Coniferol
(+)	Dihydroquercetin	(+)	Dihydroquercetin
(+)	Gallic acid	(+)	Gallic acid
(+)	**Iridin**	**(-)**	**Iridin**
(+)	**Isocarthamidin**	**(-)**	**Isocarthamidin**
(+)	Isorhamnetin-3-gentiobioside-7-glucoside	(+)	Isorhamnetin-3-gentiobioside-7-glucoside
(+)	**Leucocyanidin**	**(-)**	**Leucocyanidin**
(+)	Leucopelargonidin	(+)	Leucopelargonidin
(+)	Luteolin-7-O-[β-D-apiofuranosyl(1→6)]β-D-glucopyranoside_1	(+)	Luteolin-7-O-[β-D-apiofuranosyl(1→6)]β-D-glucopyranoside_1
(+)	**Maltol**	**(-)**	**Maltol**
(+)	Malvidin-3-O-(6-O- acetyl-β-D-glucopyranoside)-5-O-β-D-glucopyranoside	(+)	Malvidin-3-O-(6-O- acetyl-β-D-glucopyranoside)-5-O-β-D-glucopyranoside
(+)	Methyl caffeate	(+)	Methyl caffeate
(+)	Methyl-5-O-caffeoylquinate	(+)	Methyl-5-O-caffeoylquinate
(-)	**Mirificin**	**(+)**	**Mirificin**
(+)	Moupinamide	(+)	Moupinamide

(+)	Mururin A	(+)	Mururin A
(-)	**Neocomplanoside**	**(+)**	**Neocomplanoside**
(-)	**Neoisoastilbin**	**(+)**	**Neoisoastilbin**
(+)	o-Coumaric acid	(+)	o-Coumaric acid
(-)	**Onjixanthone Ⅱ**	**(+)**	**Onjixanthone Ⅱ**
(-)	Paeonol	(+)	Paeonol
(-)	**Pinocembrin-7-neohesperidoside**	**(+)**	**Pinocembrin-7-neohesperidoside**
(-)	**Polygoacetophenoside**	**(+)**	**Polygoacetophenoside**
(+)	**Protocatechuic aldehyde**	**(-)**	**Protocatechuic aldehyde**
(-)	**p-Tolualdehyde**	**(+)**	**p-Tolualdehyde**
(+)	Puerarin	(+)	Puerarin
(+)	**Pyrogallic acid**	**(-)**	**Pyrogallic acid**
(+)	Quercetagetin	(+)	Quercetagetin
(+)	Quercetin-3-O-β-D-xylopyranosyl (1→2)-β-D-glucopyranoside_1	(+)	Quercetin-3-O-β-D-xylopyranosyl (1→2)-β-D-glucopyranoside_1
(+)	Quinic acid	(+)	Quinic acid
(+)	Scutellarein	(+)	Scutellarein
(+)	**Spiraeoside**	**(-)**	**Spiraeoside**
(-)	**Sulfuretin**	**(+)**	**Sulfuretin**
(+)	**Syringaldehyde**	**(-)**	**Syringaldehyde**
(+)	**tran-Ferulaldehyde**	**(-)**	**tran-Ferulaldehyde**
(+)	**Trifolin**	**(-)**	**Trifolin**
(+)	Undulatoside A	(+)	Undulatoside A
(-)	**Viscidulin Ⅰ**	**(+)**	**Viscidulin Ⅰ**
(+)	Viscumneoside Ⅱ	(+)	Viscumneoside Ⅱ

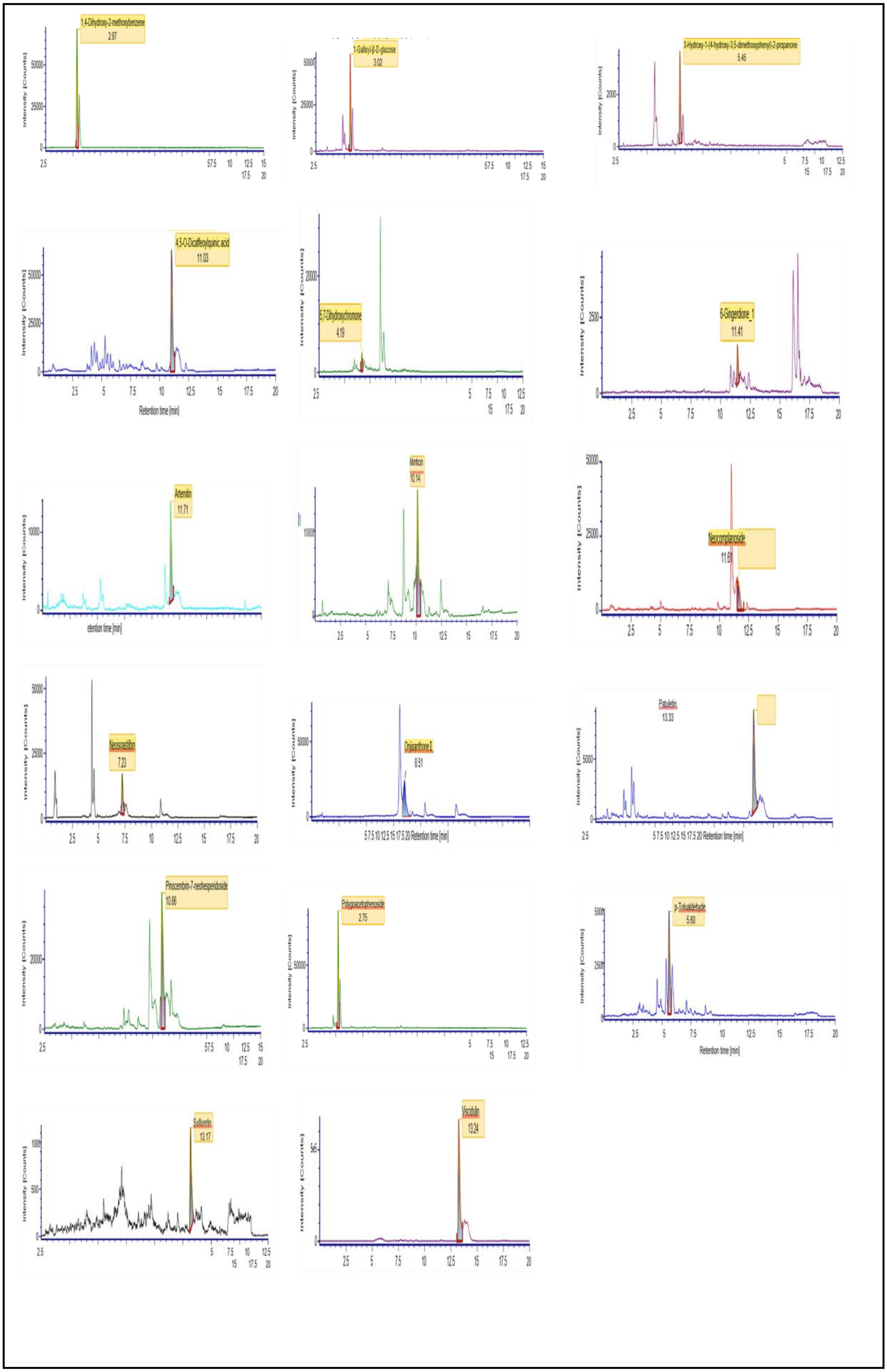
Note: the presence of antioxidant compounds is marked with '+' (present and '-' (not present)

Table 2. Results of roselle extract flavor compounds extracted without and with enzymes

Rosella petal extracted without enzyme		Rosella petal extracted with enzyme	
RT (min)	Flavor compound	RT (min)	Flavor compound
11.7357	Silanediol, dimethyl-	11.9438	Silanediol, dimethyl-
15.8325	1-Hexanol	**12.9368**	**1,3,5-Cycloheptatriene**
16.7898	Oxime-, methoxy-phenyl-	**13.8524**	**2-Butene, (E)-**
17.6282	Hexane, 2,2,5,5-tetramethyl-	15.9038	1-Hexanol
18.0563	2-Anthracenamine	16.8551	Oxime-, methoxy-phenyl-
18.2465	**Diazene, dimethyl-**	17.6698	Hexane, 2,2,5,5-tetramethyl-
21.8439	3-Octanol, 3,7-dimethyl-	18.0979	2-Anthracenamine
		21.8854	3-Octanol, 3,7-dimethyl-

Note: the compound marked in bold is the change in flavor compound without and with enzyme

Figure 1. Antioxidant compounds that appear in roselle extract with the addition of enzyme cellulase and pectinase



1,4-Dihydroxy-2-methoxybenzene
2.87
1-Galloyl-β-D-glucose
3.02
3-Hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-2-propanone
5.45
4,5-O-Dicaffeoylquinic acid
11.03
5,7-Dihydroxychromone
4.19
6-Gingerdione_1
11.41
Artemitin
11.71
10.14
Neocomplanoside
Neoisoastilbin
7.23
Onjixanthone I
8.51
Patuletin
13.33
Pinocembrin-7-neohesperidoside
10.86
Polygonacetophenoside
2.75
p-Tolualdehyde
5.60
Sulfuretin
13.17
Viscidulin
13.24
Intensity [Counts]
Retention time [min]