

18th October 2022

Authors: Mardiah, Fulazzaky, A., Djuanda, D., Rahmawati, S.I. and Amalia, L.

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

Manuscript ID: FR-2022-526

Dear Dr. Ir. Mardiah,

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18th October 2022

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To: Food Research <foodresearch.my@outlook.com>
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Dear Chief editor,
Sorry for the late reply to the email. I have revised as recommended. I hope this meets the requirements

best regards,
Dr.Ir.Mardiah,M.Si

Department of Food Technology and Nutrition
Faculty of Halal Food Science
Djuanda University, Indonesia
Mobile phone : (+62)85888118288

Pada tanggal Kam, 6 Okt 2022 pukul 23.35 Food Research <foodresearch.my@outlook.com> menulis:
Dear Dr.Ir.Mardiah

Please address all comments raised in the manuscript and revert to us at your earliest convenience. Ensure to make necessary changes only to the file attached as minor editing was done.

Best regards,
Son Radu PhD

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Sent: Wednesday, 5 October, 2022 11:09 AM
To: Food Research <foodresearch.my@outlook.com>
Subject: Re: Manuscript submission

Dear Chief editor,

I have revised according to the directions given. I've also checked for plagiarism. Henceforth I am waiting for his quick response for my manuscript. Thank you for your attention

best regards,
Dr.Ir.Mardiah,M.Si
Department of Food Technology and Nutrition
Faculty of Halal Food Science
Djuanda University, Indonesia
Mobile phone : (+62)85888118288

Pada tanggal Sel, 27 Sep 2022 pukul 21.47 Food Research <foodresearch.my@outlook.com> menulis:
Dear Dr.Ir.Mardiah,

Thank you for your submission to Food Research.
Before proceeding kindly revise the manuscript according to the comments attached and revert to us at your earliest

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From: Mardiah M <mardiah@unida.ac.id>
Sent: Friday, 23 September, 2022 8:10 PM
To: foodresearch.my@outlook.com <foodresearch.my@outlook.com>
Subject: Manuscript submission

23th September 2022

Professor Dr. Son Radu
Chief Editor Food Research
Foodresearch.my@outlook.com

Dear Sir/Madam,
We wish to submit a new original scientific paper entitled "Effects of Cellulase and Pectinase on Changes of Flavor Compounds and Antioxidants in Rosella Extraction" for your consideration for publication in food research journal (Please find through the attachment) We believe that this manuscript is appropriate for publication by Food Research Journal as the content is within the journal's aim and scope. This manuscript has not been published and is not under consideration for publication elsewhere.
Thank you for your consideration.

Sincerely,
Dr.Ir.Mardiah,M.Si

Department of Food Technology and Nutrition

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Dear Dr. Ir. Mardiah,

Manuscript FR-2022-526 entitled " Effects of Cellulase and Pectinase on Changes of Flavor Compounds and Antioxidants in Roselle Extraction " which you submitted to Food Research, has been reviewed. The comments of the reviewer(s) are included in the attached file.

The reviewer(s) have recommended publication, but also suggest some revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript. Once the revised manuscript is prepared, please send it back to me for further processing.

Because we are trying to facilitate timely publication of manuscripts submitted to Food Research, your revised manuscript should be submitted before or by 19th December 2022. If it is not possible for you to submit your revision by this date, please let us know.

Once again, thank you for submitting your manuscript to Food Research and I look forward to receiving your revised manuscript.

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Mardiah M <mardiah@unida.ac.id> kepada Food

28 Nov 2022, 12.46

Dear Chief Editor, Food Research

I attach my manuscript revision. I apologize in advance that there was an error in writing one of the names of the writing team, which was written by Ali Ulazzaky. It should have been written Mohamad Ali Fulazzaky . Thus additional information from me. I attach the actual name in the manuscript submission form/

Best regards,

[Dr.Ir.Mardiah,M.Si](#)

Department of Food Technology and Nutrition
Faculty of Halal Food Science
Djuanda University, Indonesia
Mobile phone : (+62)85888118288

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Food Research <foodresearch.my@outlook.com> kepada saya
Rab, 30 Nov 2022, 21:16

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Dear Dr. Ir. Mardiah,

Kindly address the comments raised in the manuscript and revert to us within 2 weeks. Adhering strictly to Food Research format is greatly appreciated.

Best regards,
Son Radu, PhD
Chief Editor

From: Mardiah M <mardiah@unida.ac.id>
Sent: Monday, 28 November, 2022 1:46 PM
To: Food Research <foodresearch.my@outlook.com>
Subject: Re: Manuscript ID: FR-2022-526

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Food Research <foodresearch.my@outlook.com> kepada saya 8 Des 2022, 22.46

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Dear Dr. Ir. Mardiah,

The comments raised in the previous email were not addressed, please ensure to refer to the previous attachment and leave track changes to facilitate the reviewing process.

Best regards,
Son Radu, PhD
Chief Editor

From: Mardiah M <mardiah@unida.ac.id>
Sent: Monday, 5 December, 2022 11:36 PM

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Mardiah M <mardiah@unida.ac.id> kepada Food | Jum, 9 Des 2022, 07.56

Dear Son Radu, PhD
Thankyou for the response. I have made revisions according to the reviewer's comments and I explained them in the table I have sent. Can I ask for clarification which part I have not responded to?

best regards,
Dr.Ir.Mardiah,M.Si

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Faculty of Halal Food Science
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Food Research | 9 Des 2022, 12.12

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Food Research <foodresearch.my@outlook.com> kepada saya | Jum, 9 Des 2022, 12.12 | Nonaktifkan untuk: Inggris

Dear Dr. Ir Mardiah,

Based on our previous attachment from the 30th of November, the comments were not revised accordingly. e.g., references were still missing, subheadings were still not revised, wrong symbols are still present etc. Please address all the comments raised and revert to us within 2 weeks.

Best regards,
Son Radu, PhD
Chief Editor

From: Mardiah M <mardiah@unida.ac.id>
Sent: Friday, 9 December, 2022 8:56 AM
To: Food Research <foodresearch.my@outlook.com>
Subject: Re: Manuscript ID: FR-2022-526

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Dear Dr Ir. Mardiah,

Please find additional comments from a reviewer. We strongly recommend considering their comments as well to improve the quality of the manuscript.
Therefore, we would extend the deadline for resubmission to the 30th of December 2022.
If you have any questions, please do not hesitate to reach out.

Best regards,
Son Radu, PhD
Chief Editor

From: Food Research <foodresearch.my@outlook.com>
Sent: Friday, 9 December, 2022 1:11 PM
To: Mardiah M <mardiah@unida.ac.id>

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Food Research <foodresearch.my@outlook.com> kepada saya
Sel, 20 Des 2022, 23.55

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Dear Dr.Ir.Mardiah,

Please find attached, the reason being that the comments from 30/11/2022 were clearly not applied to the new manuscript file [FR-2022-526(9)]. We have included the comments for your review on the New file.

In addition, we have no specific control over the time at which the reviewers reply to the invitation, as your manuscript is still under the 'reviewing stage', we would give authors the opportunity to improve their manuscript when comments are received slightly past the deadline of review invitation.

Hence, the additional comments given on 9/12/2022 are vital to the improvement of the manuscript.

Best regards,
Son Radu, PhD
Chief Editor

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Mardiah M <mardiah@unida.ac.id> kepada Food

Min, 25 Des 2022, 20.11

Dear Son Radu, PhD,
I have already revised all comments. I hope this manuscript already fulfill the criteria

sincerely
Dr.Ir.Mardiah,M.Si
Department of Food Technology and Nutrition
Faculty of Haial Food Science
Djuanda University, Indonesia
Mobile phone : (+62)85888118288

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FR-2022-526(9) r...

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Food Research <foodresearch.my@outlook.com> kepada saya
Sen, 26 Des 2022, 22.32

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Dear Dr.Ir.Mardiah,

No revision was made according to the second reviewer's comments given on the 9th of December, only the comments from the editor were addressed.
Please find attached, it is imperative that their comments are taken into consideration to further improve the quality of your manuscript.
Please revert to us before or by 17th January 2023.

Best regards,
Son Radu, PhD
Chief Editor

From: Mardiah M <mardiah@unida.ac.id>
Sent: Sunday, 25 December, 2022 9:11 PM

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Mardiah M <mardiah@unida.ac.id> kepada Food

Dear Son Radu, PhD


I have already reviewed this manuscript.

best regards,

Dr.Ir.Mardiah,M.Si

Department of Food Technology and Nutrition
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Jum, 13 Jan, 00.59

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Dear Dr.Ir.Mardiah,

Thank you for the revised copy of your manuscript. We will contact you again for further processing.

Best regards,
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Chief Editor

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Sent: Monday, 9 January, 2023 2:07 PM

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Mardiah M <mardiah@unida.ac.id> kepada Food

Dear Son Radu, PhD
What about the continuation of my manuscript? Can it be published in IFRJ? I wait to hear from you.

best regards,
Mardiah
Dr. Ir. Mardiah, M.Si

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Dear Dr.Ir.Mardiah,

It is currently in the queue for technical review.

Best regards,
Son Radu, PhD
Chief Editor

From: Mardiah M <mardiah@unida.ac.id>
Sent: Tuesday, 28 March, 2023 10:34 PM

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

^{1,*}Mardiah, ¹Fulazzaky, M. A., ²Djuanda, D., ¹Rahmawati, S.I. and ²Amalia, L.

¹Magister of Food Technology, Djuanda University, Bogor 16720, Indonesia

²Faculty of Halal Food Science, Department of Food Technology and Nutrition, Djuanda University, Bogor 16720, Indonesia

*Corresponding author: mardiah@unida.ac.id

Author No.1 : ORCID : <https://orcid.org/0000-0002-5936-9633>

Author No.2: ORCID : <https://orcid.org/0000-0002-4600-5742>

Author No.4 : ORCID : <https://orcid.org/0000-0001-5376-7860>

Author No.5 : ORCID : <https://orcid.org/0000-0002-1947-9248>

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 °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 a medicinal plant that can grow in tropical and subtropical climates. It is also known as Roselle (English), l'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.

41 Cellulase, hemicellulase, and pectinase and their combinations have been used to pretest plant
42 materials. The main action of cellulase and hemicellulase is in the cell wall. These enzymes act on cell
43 wall components, hydrolyzing them, which can then increase cell wall permeability resulting in higher
44 yields of flavor (Sowbhagya and Chitra, 2010). The application of pectinase enzymes includes food and
45 non-food industries. Pectinase could be used in fruit juices and wines, extract oils, flavors, and
46 pigments from plants, and for processing cellulose fibers, linen, jute, and hemp. Pectin enzymes also
47 play a role in the degradation of pectin by releasing glycosidic bonds. Pectinase can soften cell walls
48 and increase the yield of fruit juice extracts so that the pectinase enzyme becomes one of the essential
49 enzymes in the food industry (Oyeleke *et al.*, 2012; Dalal *et al.*, 2007). Cellulase enzyme is one of the
50 enzymes produced by microorganisms that functions to degrade cellulose into glucose. The results
51 obtained using two enzyme preparations sequentially were similar to those obtained using a single
52 enzyme preparation containing mainly the enzyme pectinase. Pretreatment with cellulolytic enzymes
53 improves the separation of intracellular compounds (e.g., flavor compounds) from plant materials by
54 solvent extraction (Sowbhagya and Chitra, 2010). Pectinase, cellulase, and hemicellulase for producing
55 blueberries and blueberry peel extracts can increase anthocyanins and polyphenols (Lee and Wrolstad.
56 2004).

57 The extract roselle used pectinase enzyme and cellulase ratio (1:1) as much as 1000mg/L
58 resulted in a significant yield of 45.9% (dry basis) (w/w) compared to control (without enzymes) of
59 26.5% (dry basis)(w/w). In addition, the use of these enzymes was able to increase the phenol content
60 and antioxidant activity of the roselle extract produced (Barman *et al.*, 2015). The phenol content in
61 roselle extract with enzymes was 1854.7 g GAE/g sample and 1661.5 g GAE/g sample (without
62 enzymes). The antioxidant activity of roselle extract added with enzymes was 589.5 mmol TE/g and
63 545.8 mmol TE/g (without enzymes). The addition of enzymes during the extraction process can have
64 the functional food benefits of increasing acids, dyes, and aroma of the rosella petal extract (Da-Costa-
65 Rocha *et al.*, 2014). Iriani *et al.* (2005) found that adding pectinase could increase quinine juice yield
66 and the distinctive flavour of quinine manga.

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

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

80 **2. Materials and methods**

81 *2.1 Materials*

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

85 *2.2 Method*

86 *2.2.1 Roselle extraction stage*

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

95 *2.2.2 Screening for antioxidants with LC-MS ToF*

96 *2.2.2.1 Sample preparation*

97 The preparation of sample by weighing 1.0 ± 0.1 g of sample in a centrifugation tube
98 of 50 mL and adding 3-5 mL of Hexane was firstly carried out by shaking the solution for \pm
99 1 min (Calderon, 2009). The extraction of sample using 3 mL of methanol was performed
100 by shaking the solution for \pm 1 min and then transferred the methanol phase into a flask.
101 Then the extraction of sample was repeated again for two times. The combined solution of
102 methanol phase was ultrasonicated for 15 min and then filtered using a membrane filter of
103 0.22 μ m GHP to produce an extract. Then the membrane filter was rinsed with 1 mL of
104 methanol and then combined the rinse results with the existing extract. The extract was

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

108 2.2.2.2 LC-MS ToF system settings

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

123 2.2.2.3 Process data using UNIFI

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

129

130 2.2.3 Flavor test using GC-MS

131 2.2.3.1 Sample preparation

132 Sample preparation using Dynamic Headspace Sampling (DHS). Each sample was
133 placed in a glass flask (300 mL, 7.5 cm diameter). A trap containing Tenax-TA (200 mg) was
134 attached to the sealed flask. The samples' flasks were immersed in a water bath held at
135 40°C. Under magnetic stirring (200 rpm), the model was tempered for 10 minutes before
136 purging with nitrogen (100 mL/min) for 30 mins. The traps were dry-purged with nitrogen
137 (100 mL/min) for 10 mins to remove water (Juhari *et al.*, 2015).

138 *2.2.3.2 Flavor testing with GC-MS*

139 Volatile compounds were identified by probability-based matching of their mass
140 spectra with those of a commercial database. The software program MSD ChemStation was
141 used for data analysis. Amounts are presented as peak areas. Shimadzu brand GCMS
142 condition, 250°C injector temperature, split less, hydrogen gas carrier, split speed 1.8
143 mL/min. Preheat oven to 35°C for 5 mins, then 6.0°C/mins to 250°C for 10 mins, using
144 column HP-5MS (30m x 250m x 0.25 m), interface 280oC. Scan mass 29-550 m/z, MS source
145 230°C, and MS Quad 150°C.

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

158
159 **3. Results and discussion**

160
161 *3.1 Screening of active antioxidant substances with LC-MS*

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

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

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

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

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

202 compounds compared to using these enzymes alone. Phenolic compounds present in roselle extract
203 (Table 1) include gallic acid, pyrogallol, isorhamnetin, protocatechuic aldehyde, catechins,
204 coumaric acid, caffeic acid, and quercetin. Ghandahari *et al.* (2019) identified that cellulolytic
205 enzymes had no effect on increasing gallic acid. While tannase, pectinolytic enzymes, and the
206 combination of CPT (cellulose, pectinase, tannase) increased the amount of gallic acid up to 34%,
207 78%, and 98%, respectively.

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

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

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

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

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

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

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

266 In Table 2, it is shown that the sample of roselle extract with enzymes formed a compound
267 1,3,5 cycloheptariene. According to Dzhemilev *et al.* (1991), this compound is formed from the
268 reaction of aromatic hydrocarbons with diazo compounds in the presence of transition metal
269 complexes such as V, Fe, Co, Ni, Cu, Zr, Mo, Rh, Pd, Ag, W, Ir, Hg<Cl, Pr, and Nd. Compound 2
270 anthracenamine is a polycyclic aromatic hydrocarbon compound.

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

275 **4. Conclusion**

276

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

285

286 **Conflict of interest**

287 Authors declare no conflict of interest.

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384

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-Trihydroxybenzaldehyde	(+)	2,4,5-Trihydroxybenzaldehyde
(+)	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
(+)	Isocarathamidin	(-)	Isocarathamidin
(+)	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-caffeoylquininate	(+)	Methyl-5-O-caffeoylquininate
(-)	Mirificin	(+)	Mirificin
(+)	Moupinamide	(+)	Moupinamide

(+)	Mururin A	(+)	Mururin A
(-)	Neocomplanoside	(+)	Neocomplanoside
(-)	Neoisostilbin	(+)	Neoisostilbin
(+)	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
(+)	Pyrogalllic acid	(-)	Pyrogalllic 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

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

388

389 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-Anthracenamamine	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-Anthracenamamine
		21.8854	3-Octanol, 3,7-dimethyl-

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

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395

396 Figure 1. Antioxidant compounds that appear in roselle extract with the addition of enzyme cellulase
397 and pectinase
398
399

