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Effect of Roasting Process on Antioxidant Activity of Carica Seeds Coffee (*Carica pubescens*)

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***Abstract*—**Carica seeds (*Carica pubescens*), one of the diversification of coffee drinks, served as antioxidants. The roasting process generated degradation of secondary metabolic compounds but produced Maillard reactions products (MRPs). The study aimed to determine the effect of the roasting process on the antioxidant activity of Carica coffee beans and its correlation to the Maillard reaction. The samples roasting temperatures were 100–115, 150–165, and 200–215 °C. The samples were analyzed for proximate, total phenolic, antioxidant, and FTIR. The moisture and ash content of dry seeds were according to SNI 01-2907-2008. The total phenolic content was 3.21–8.99 µgGAE/g, with the highest contents at 200–215 °C. The antioxidant activity was 83–459.67 ppm AEAC. The high phenolic content and antioxidant at 200–215 °C were thought to come from MRPs compounds. The FTIR spectra showed stretching of OH, C=C (aromatic), C=N, C=O, and C-OH (phenol). The spectrum intensity weakened at higher temperatures. However, only the nC–H stretching at 200–215 °C became stronger. The Cluster Ward analysis showed resemblance spectra at roasting temperature levels of 100–115 and 150–165 °C. The roasting process affected the antioxidant activity of Carica coffee beans, thereby increasing the roasting temperature level. This condition resulted in higher levels of antioxidants.

***Keywords*—**Antioxidant; Carica Dieng; Cluster; Phenolic content; Roasting

1. INTRODUCTION

Sources of antioxidant intake for the body can be obtained from coffee drinks. Processed coffee is increasingly diverse and diversified from ingredients other than coffee beans. The term diversified food product is always followed by the name of the ingredients. In this case, the coffee made from Carica beans is called Carica coffee beans. This diversification of coffee drinks is related to the need of people to avoid the effects of caffeine on coffee beans. The seeds from the Carica family are reported to have antioxidant potential due to their secondary metabolite content. The DPPH radical scavenging activity of Carica seeds has the highest value of 285.77 EC_{50} (µg/mL) [1]. The community of Dieng has not been able to process it because its structure is quite hard. So it is still a waste from the Carica fruit syrup industry. The utilization of Carica beans in coffee is considered prospective in optimizing the content of its secondary metabolites as antioxidants.

Processing of diversified coffee products includes several stages, such as making coffee in general. Roasting is included in the post-harvest treatment process for coffee products. Roasting is the process of forming taste and aroma in coffee by controlling the uniformity of size, specific gravity, texture, moisture content, and chemical structure [2]. Product quality factors in roasting to consider include roasting temperature and time. Roasting will affect the color and aroma due to the occurred chemical reaction. However, this process can cause secondary metabolite degradation due to the relative high-temperature heating. This allows a decrease in antioxidant activity in coffee drinks.

The roasting process also produces melanoidin compounds from the Maillard reaction, which are reported to have antioxidant activity. The roasting conducted at high temperatures (160–250 °C) can change the chemical components, such as polysaccharides, sugars, and amino acids which will be degraded to form CO_2, water vapor, and volatile components [3]. Research on the effect of the roasting

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process on antioxidant activity in Carica coffee beans has not been carried out. In fact, this is important in the development of Carica Dieng seed product innovation as an effort to support the added value of the carica processing industry. This study aims to determine the effect of the roasting process on the antioxidant activity of Carica bean coffee (*Carica pubescens*) and to discover its correlation to the Maillard reaction in the browning process.

2. EXPERIMENTAL SECTION

2.1. Materials

The materials used consisted of Carica seeds, distilled water, concentrated HCl 2N, absolute ethanol, acetic acid buffer, $FeCl_3$ solution, dichloromethane, methanol, and ascorbic acid obtained from Sigma Aldrich (St. Louis, MO, USA). Bouchardat reagent, Mayer reagent, Dragendorff reagent, Folin-Ciocalteu reagent, Na_2CO_3 solution 10%, gallic acid, acid buffer, DPPH reagent (2,2-diphenyl-1-picrylhydrazyl), KBr solid, and Mg/Zn powder were supplied from Merck (Darmstadt, Germany).

2.2. Instrumentations

The equipment used in this study included an oven, coffee roaster brand Shan Yen Machinery Co. Ltd (Taiwan), blender, aluminum foil, 50 mess sieve, hot plate, dark bottle, volumetric pipette, Mohr pipette, micropipette, freeze dryer, Shimadzu 1240 UV-Vis spectrophotometer, analytical balance, glassware, water bath, and FTIR (Bruker FTIR Tensor 37).

2.3. Procedure

2.3.1 Carica bean coffe sample preparation

Carica seeds were washed and dried under the sun. A total of 100 g of dry samples were analyzed proximately. Dry Carica beans were roasted at a roasting level of 100–115, 150–165, and 200–215 for 20 min, while other factors were considered constant. Each roast level consists of 7 roasting groups. The roasted seeds were ground into powder using a blender and finely sieved. Carica coffee powder was brewed with 250 mL of distilled water at 85–95 °C and filtered with special coffee filter paper. The extract was stored in a dark plastic bottle.

2.3.2 Phytochemical test of carica bean coffee extract

Phytochemical qualitative tests include alkaloids, flavonoids, saponins, phenols, and tannins as an initial screening for secondary metabolite content.

2.3.3 Determination of total phenol levels

Generating gallic acid standard series. A total of 5 mg of gallic acid was dissolved in 50 mL of 95% p.a methanol and then stirred until homogeneous to obtain a mother liquor with a concentration of 100 ppm. The mother liquor was diluted as standard series of gallic acid at concentrations of 10, 20, 30, 40, and 50 ppm in a 5 mL volumetric flask. Each standard solution was taken 2 mL for analysis and added with 500 L of Folin-Ciocalteu reagent 0.9 mol/L, 1 mL of 20% Na_2CO_3 solution, and 5 mL of distilled water. The solution was incubated in the dark for 60 min. The absorbance was determined at a wavelength of 725 nm using a UV-Vis spectrophotometer. Then a standard curve formula was obtained to determine the total phenol content of the sample expressed in gallic acid equivalents/gram of coffee grounds.

Total phenol level. About 0.5 mL of coffee extract from each sample was added with 1.5 mL of 95% p.a ethanol. Then added 500 L of Folin-Ciocalteu reagent 0.9 mol/L, 1 mL of 20% Na_2CO_3 solution, and 5 mL of distilled water. The solution was incubated in the dark room for 60 min. Then the absorbance was determined at a wavelength of 725 nm using a UV-Vis spectrophotometer.

Total phenolic content (TPC) was calculated by $TPC = C \times DF \times V/m$, with V = analyzed volume (mL); C = phenol content concentration from the standard curve (ppm); DF = dilution factor; dan m = weight of coffee sample (g).

2.3.4 Determination of antioxidant activity

Ascorbic acid standard series measurement for DPPH test. The DPPH stock solution was prepared from 24 mg DPPH crystals and dissolved in 100 mL p.a ethanol. Then 10 mL was taken and diluted with 45 mL of p.a ethanol. The solution was stored in aluminum-coated measuring flasks for further utilization. Furthermore, ascorbic acid stock was prepared from 0.1761 g of solid and dissolved in 1 mL of DMSO using sonication and homogenized with a vortex. A total of 1 mL of ascorbic acid solution was diluted in 10 mL of ethanol p.a and homogenized to obtain a solution of 100 mM. About 0.1 mL was taken from a 100 mM solution and diluted to 1 mM. Ascorbic acid standard series were prepared with a concentration of 1000; 500; 250; 125; 62.5; 31.25; 15.625; 7.8125 µM.

Measurement of antioxidant activity dpph method. Each sample was taken 150 µL in triples in a test tube and added with 2850 µL of DPPH reagent in replicates 1, 2, and 3. Replicate 3 acted as a negative control. Next, the standard solution was incubated for 24 h in the dark. Then the absorbance was determined at a wavelength of 515 nm.

2.3.5 Determination of functional groups with FTIR

The sample extract was freeze-dried. The functional groups of the sample were determined using FTIR to confirm the presence of functional groups of MPRs compounds in Carica bean coffee extract. A total of 10 mg of each powder sample was mixed with 90 mg of KBr and then analyzed in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1}.

2.3.6 Statistical analysis

Data in the form of total phenol levels and antioxidant activity obtained were analyzed with one-way analysis of variance (ANOVA) to determine the significance between groups at each roasting level. Multivariate cluster analysis using Ward's method was used to determine the proximity of the FTIR numbers between roasting levels. The data was presented in the form of a dendrogram to explain the grouping between levels.

3. RESULT AND DISCUSSION

3.1. Proximate analysis of dried carica seeds and qualitative phytochemicals

The moisture content in dried Carica seeds was 6.67%, and the ash content was 4.12% per 100 gr sample (**Table 1**). Moisture and ash contents are requirements for coffee quality before roasting because they affect the sensory and quality of the resulting coffee product. Referring to the SNI 01-2907-2008 standard regarding coffee, the maximum moisture and ash contents before roasting is 7% and 5%. This means that the Carica beans meet the coffee quality standards. The fat content of Carica seeds was 32.27% analyzed using the Soxhlet method to determine the total fat content in the sample. The protein content was tested using the Kjeldahl method to determine the crude protein content. Protein content was 25.67%, then carbohydrate by the difference in which stated the total content of digestible and undigested carbohydrates in Carica seeds was 31.26%.

Table 1. Proximate analysis of Carica seeds

No	Phytochemical test	Content (%)
1	Moisture content	6.67
2	Ash	4.12
3	Fat	32.27
4	Protein	25.67
5	Carbohydrate	31.26

Qualitative phytochemical tests were carried out to determine the presence of secondary metabolites in roasted Carica seeds at three temperature levels: 100–115, 150–165, and 200–215 °C, while other factors were held constant. Carica Dieng seeds were not detected when tested for phenol and tannin at all roasting temperature levels. Meanwhile, in Winarno's study [4], Carica Dieng dried seeds were positive for flavonoids, polyphenols, and tannins. This indicates that the roasting treatment affects the content of secondary metabolites.

The results of the flavonoid and saponin tests were detected up to a roasting temperature of 150–165 °C. The roasting process of Carica seeds at high temperatures causes the degradation of bioactive compounds. Yuliantari [5] specifically mentioned that the heating process above 50 °C can result in the loss of compound activities that are susceptible to temperature. Alkaloid tests using Wagner, Dragendorff, or Mayer reagents showed positive results at all levels of roasting temperature.

3.2. Total phenolic content

Phenolic compounds are divided into several subgroups: phenolic acids, flavonoids, tannins, and stilbenes based on the number of phenolic hydroxyl groups attached and the structural elements connecting the benzene ring. Phenolic compounds correlate with benefits as antioxidants. This is because phenolic compounds can modulate cellular oxidative status and prevent cellular oxidative damage to biological molecules, such as DNA, proteins, and lipid membranes. Phenol compounds act as reducing agents, singlet electron absorbers, and electron donors.

Determination of phenolic levels is related to the ability of phenol compounds to prevent oxidation reactions. So it is often correlated with antioxidant activity. Many of the group of phenolic compounds can exhibit antioxidant activity through the conjugate electron donor or hydrogen atom of the hydroxyl group. However, this depends on the structure that includes the number and position of hydroxyl groups on the ring or the extent to which the radical electrons can be delocalized throughout the molecule [6].

Table 2. Phenolic content and antioxidant capacity of Carica seeds coffee

T (°C)	Number of roasting	Total phenolic (µg GAE/g beens ground)	Antioxidant capacity (ppmAEAC)
100–115	1	$4.138^{b} \pm 0.3455$	$93.00^{a} \pm 4.71$
	2	$4.796^{b} \pm 0.7658$	$92.17^{a} \pm 3.54$
	3	$5.838^{b} \pm 0.9210$	$83.00^{a} \pm 2.89$
	4	$7.075^{b} \pm 0.4868$	$88.00^{a} \pm 4.71$
	5	$6.626^{b} \pm 0.5045$	$88.56^{a} \pm 2.55$
	6	$5.142^{b} \pm 0.2148$	$83.00^{a} \pm 2.89$
	7	$6.125^{b} \pm 0.2584$	$76.33^{a} \pm 2.36$
150–165	1	$5.330^{a} \pm 0.5051$	$211.33^{b} \pm 2.89$
	2	$3.530^{a} \pm 0.3472$	$139.67^{b} \pm 4.41$
	3	$3.210^{a} \pm 0.1212$	$170.78^{b} \pm 1.92$
	4	$3.401^{a} \pm 0.1194$	$158.56^{b} \pm 2.55$
	5	$3.753^{a} \pm 0.2363$	$151.33^{b} \pm 1.67$
	6	$3.967^{a} \pm 0.1401$	$146.89^{b} \pm 4.19$
	7	$4.953^{a} \pm 0.8778$	$210.22^{b} \pm 3.47$
200–215	1	$7.354^{c} \pm 0.2392$	$393.00^{c} \pm 3.33$
	2	$7.433^{c} \pm 0.8354$	$328.00^{c} \pm 3.33$
	3	$8.989^{c} \pm 0.1389$	$425.78^{c} \pm 5.85$
	4	$6.316^{c} \pm 0.1408$	$459.67^{c} \pm 5.00$
	5	$7.291^{c} \pm 0.3685$	$421.89^{c} \pm 3.85$
	6	$8.090^{c} \pm 0.3604$	$390.22^{c} \pm 2.55$
	7	$7.818^{c} \pm 0.3160$	$392.44^{c} \pm 1.92$

[a,b,c] Numbers followed by different letters showed a significant difference in the test level ($P<0.05$) Duncan's test

The total phenolic content in the sample is expressed in gallic acid equivalent (GAE). The total phenolic content using standard gallic acid with concentrations of 0, 10, 20, 30, 40, and 50 mg/L was represented in the standard curve of concentration to

absorbance, resulted in the equation y=0.018x–0.095. **Table 2** shows the total phenolic content of Carica coffee beans roasted at three temperature levels. The results of the ANOVA test for the total phenol content showed a significant difference at each roasting level with a 5% confidence level. This means that the temperature range at each level significantly affects the total phenolic content. Overall, the total phenol content in all roasting replicates was 3.21 g GAE/g to 8.989 g GAE/g Carica bean coffee grounds.

The total phenol content at roasting temperature of 150–165 °C was lower than 100–115 °C. This is because the thermal treatment of the sample can affect the stability of the phenolic compound content. Liazid et al. [7] stated that the phenol group is stable at 100–125 ℃ and degrades above 150 ℃. The high total phenol content of Carica coffee beans roasted at 200–215 ℃ is thought to be related to the formation of the product from the Maillard reaction in the form of melanoidin, which is allegedly to be able to increase its antioxidant activity [8].

3.3. Antioxidant capacity

According to Singh et al. [9], the antioxidant activity of Carica family plants comes from the secondary metabolite content in the form of salicylic acid, p–hydroxybenzoic acid, phenolic compounds in the form of hyperosids, antioxidant compounds of gentisyl alcohol, gallotannin ester compounds, and kaemferol hexoside which is a flavonol glucoside.

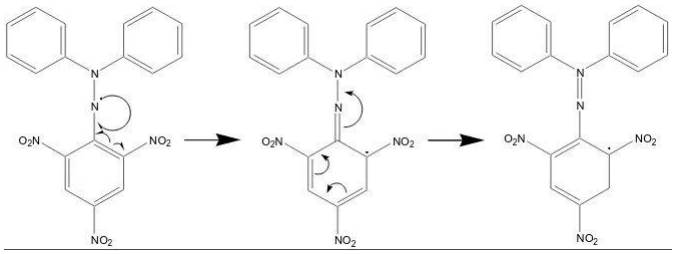

Fig. 1. DPPH radical scavenging reaction

The determination of the antioxidant activity of Carica Dieng coffee infusion was carried out in vitro using the DPPH method (2,2-diphenyl-1-picrihydrazil). The principle of this method is to measure the absorbance of an antioxidant solution whose value is proportional to the reduction of some DPPH radical electrons to non-radical diphenyl dipicrihydrazine. Antioxidant compounds can reduce the purple color of the DPPH solution to yellow. The antioxidant compounds donate H+ to DPPH, resulting in a structural resonance causing a reduction in the number of conjugated double bonds (**Fig. 1**). The higher the antioxidant activity, the yellower the color of the solution formed or the smaller the absorbance will be.

The DPPH method has the advantages, such as simple, fast, easy, and high sensitivity analysis [10]. The test was carried out at a wavelength of 517 nm in the form of absorbance values corrected by a negative control. The antioxidant activity value of the sample was compared with the standard of ascorbic acid in ascorbic acid equivalent ppm AEAC (ascorbic acid equivalent antioxidant capacity).

The measurement results of the antioxidant activity of Carica Dieng coffee bean extract can be seen in **Table 2.** The antioxidant capacity value shows the sample's activity in reducing radicals which is equivalent to the concentration of ascorbic acid. The standard regression formula for ascorbic acid was y=0.0006x–0.0138 with a determination of 99.86%. The antioxidant ANOVA test results showed a significant difference at each roasting level at the 5% confidence level, indicated by the difference in letter notation. This means that each treatment temperature level affects its antioxidant activity.

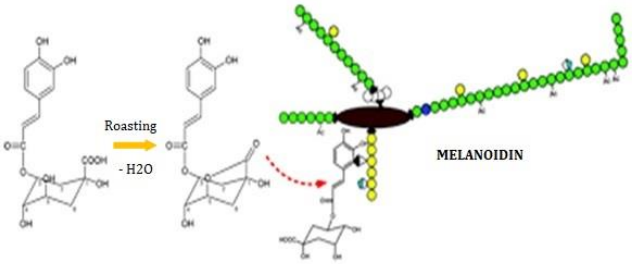

Fig. 2. Chemical structure of melanoidin

The antioxidant capacity of ascorbic acid equivalent in Carica coffee infusion ranged from 83 to 459.67 ppm AEAC. The antioxidant capacity increases with the roasting temperature. Antioxidant activity is often correlated with the secondary metabolite content. Data on the total phenolic content decreased at 150–165 °C, then increased at 200–215 °C. Its antioxidant activity was also high at 200–215 °C. The antioxidant activity of Carica bean coffee steeping does not only come from its phenolic compounds because it can be degraded at high temperatures but there are other reactions. Hustiany [11] and Kurniawan et al. [12] stated that roasting causes browning or the Maillard reaction produces compounds with antioxidant activity.

Maillard reaction products (MRPs) from the roasting process are also reported to have the ability to scavenge radicals. Further explanation from DeHond et al. [13] that the melanoidins in coffee are substantial contributors to antioxidant capacity. Melanoidins are thought to originate from sugar–amino acid interactions through the Maillard reaction mechanism (**Fig. 2**). MPRs as chromophoric substructures probably originated from cross-links between amino acid residues to form high molecular weight melanoidin structures [14]. Melanoidin compounds as MRPs contribute to antioxidant activity by reducing peroxyl radicals in the ORAC, ABTS, [15] and DPPH methods [16]. Phenolic compounds and MRPs also have antioxidant activity in vivo in the assay of mouse hepatocyte and macrophage models. They have been shown to protect cells from oxidative stress and inflammation by increasing glutathione and stimulating gene expression associated with cellular antioxidant systems [17].

3.4. Characterization of MPRs in Carica coffee bean extract

Carica Dieng coffee bean extract was characterized using FTIR to confirm the functional groups of MPRs in the sample. This technique is beneficial in identifying the functional groups of organic and organometallic compounds [18]. The IR spectra were measured in the mid-infrared region at a wavelength of 2.5–50 m or a wave number of 4000–200 cm^{-1}. The energy generated by electromagnetic radiation causes vibrations in molecules [19]. The energy involved depends on the bond length and the atom weight bonded together. Each atom will vibrate in such a way and with a different amount of energy.

Fig. 3. shows the combined three FTIR spectra of Carica bean coffee extract. The extract at the roasting level of 200–215 ℃ appeared to have the lowest transmittance intensity spectrum, followed by roasting at 150–165℃. While the lowest temperature roasting level also had the highest transmittance intensity. The high transmittance intensity indicates the number of molecules bound to the sample is less because the temperature treatment causes the bonds to break or deform into other compounds [20]. This is an initial assumption of the formation of MPRs as the roasting temperature level increases.

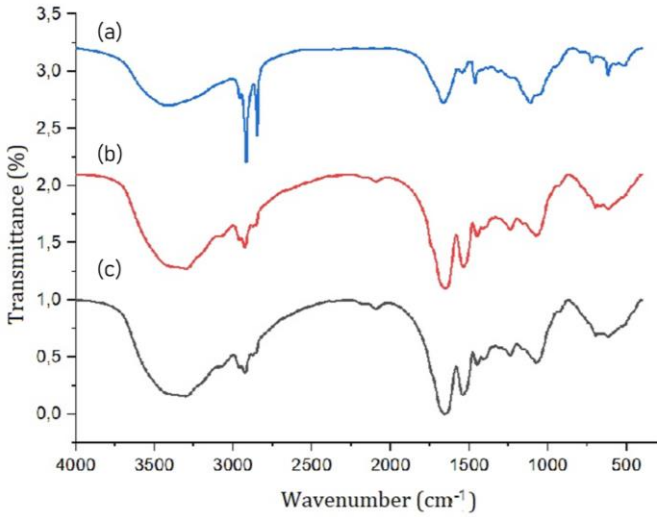

Fig. 3. FTIR spectra of Carica bean coffee brewed extract at (a) 200-215 °C, (b) 150-165 °C, and (c) 100-115 °C.

Based on the resulting spectra, there was a wide strain band of the OH functional groups at 3303.47 cm^{-1} for samples with a roasting level of 100–115 ℃; 3304.37 cm^{-1} at a level of 150–165 ℃; and 3413.84 cm^{-1} at the level of 200–215 ℃. This was also confirmed by the C–O strain in the 1100–1000 cm^{-1} fingerprint regions. The overall intensity of the OH strain dominates the spectra indicating the high content of hydroxyl groups in the melanoidin structure [21]. A significant difference was found at the peak of the hydroxyl strain at the roasting level of 200–215 ℃, which was sharper than the other levels. This is related to the hydroxyl group of phenolic origin bound to the melanoidin structure during roasting [22]. Furthermore, the C≡C or C≡N functional groups were found at the roasting level of 100–115 and 150–165 ℃, respectively, at 2092.06 cm^{-1} and 2091.81 cm^{-1}, while at the level of 200–215 ℃ were not found. This may be due to the higher temperature treatment of the sample [21].

Table 3. Functional groups in brewing Carica Dieng coffee beans

Wavenumber (cm^{-1})			Functional Group
100–115 ℃	150–165 ℃	200–215 ℃	
3303.47	3304.37	3413.84	nO-H
2924.05; 1459.72	2926.09; 1451.36	2956.79; 2917.79; 1462.72	nC-H (CH_2,CH_3)
2092.06	2091.81	-	nC≡C or nC≡N
1655.94	1655.09	1661.50	nC=O
1539.10	1538.44	1545.44	nC=C aromatic
1238.46	1237.31	-	C-O-H (phenol)
1073.95	1074.46	1109.18	nC-O, nC-C, nC-N

The C=O strain was visible with strong intensity in the three samples, 1655.94; 1655.09; and 1661.50 cm^{-1}, respectively. This was probably attributed to the COOH group. The aromatic C=C double bond strain was found at 1539.10; 1538.44; and 1545.44 cm^{-1}. The absorption bands decreased as the roasting level increased. The range of wave numbers 1700–1500 cm^{-1} indicates a change in the carbonyl structure due to the heating factors. This is assumed to be due to an aldol condensation reaction [23]. Slightly shifted to the right, the CH_2 strains were found at 1450.72; 1451.36; and 1545.44 cm^{-1}, respectively (**Table 3**).

3.5. Cluster analysis

Cluster analysis (Unscrambler® version X software) was used to group similar data elements into distinct and independent groups. The data with the closest similarity of characteristics based on a certain set of variables were in the same cluster. In contrast to other multivariate analysis, cluster analysis did not estimate the set of variables empirically but was determined by the researchers. The way cluster analysis works included three things, how to measure the similarity between objects, in terms of correlation, distance, and association; procedures for grouping objects with high similarity in one cluster; and the number of clusters formed, the fewer clusters the homogeneity would decrease [24].

Clustering in this study is to understand the effect of roasting temperature on the characteristics of the FTIR spectra of the sample. If each level is a different cluster, it is assumed that each temperature level has a different effect on the sample content seen from the

spectra. The Ward linkage method is used to group objects in the smallest possible internal variance, based on the distance between two clusters on the sum of squares. This method is effective and most often used compared to other methods because it has the most balanced structure to reduce groups in a systematic and hierarchical manner [25].

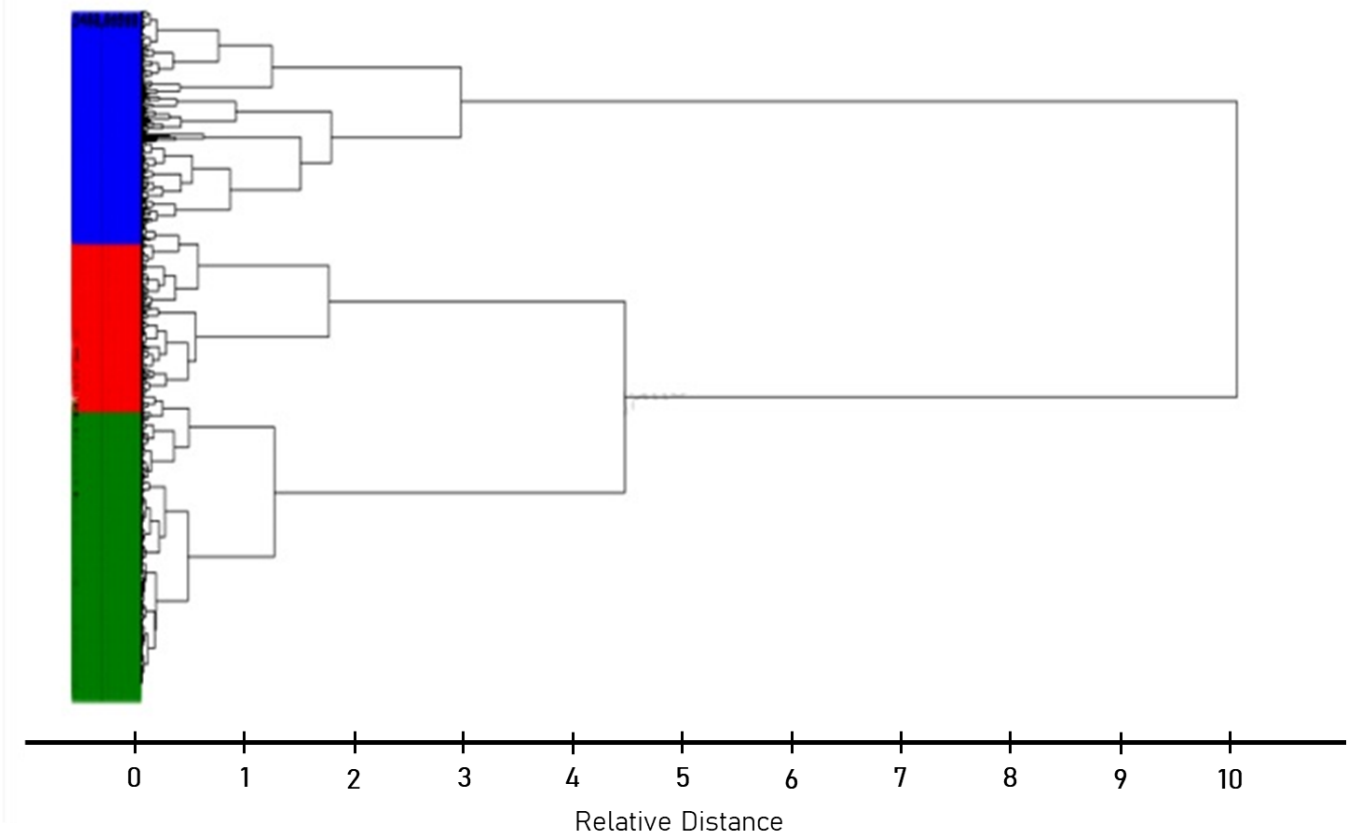

Fig. 4. Dendrogram of FTIR data clustering of Carica bean coffee extract by Ward's method using Squared elucidation distance. Wavenumber data roasting level 200–215 °C (green), 150–165 °C (red), and 100–115 °C (blue).

Fig. 4. shows the structure of the dendogram on the FTIR wave number data of Carica bean coffee extract. In the divisional clustering, initially, two clusters were formed, the roasting level clusters of 100–115 ℃ (green) and 150–165 °C (red) included in one cluster, and the roasting level of 200–215 °C (blue) to become their cluster. This means that there is a similarity in the wavenumber between the roasting samples at 100–115 and 150–165 °C. Furthermore, in the clustering of 3 groups, the roasting clusters of 100–115 °C (green) and 150–165 °C (red) were separated into different groups at a Euclidean distance of 3.23 and 2.75 units, respectively, while the roasting level was 200–215 °C separated by a distance of 7 units as big. Euclidean distance (ED) is the length of a line segment between two points in Euclidean space, so the closer the distance, the more similar the objects. The roasting level of 200–215 °C showed many different spectral characteristics than other levels, while levels 100–115 and 150–165 °C were resemblance. The roasting temperature level of 200–215 °C affected the compound content.

4. CONCLUSION

The roasting process in producing Carica bean coffee affected its antioxidant activity. The higher the roasting temperature level, the higher the potential for antioxidant activity. The antioxidant activity of the AEAC extract of the Carica Dieng coffee bean extract was derived allegedly from secondary metabolites of the phenolic group and Maillard reaction product compounds (MRPs) from the roasting process. The roasting process produced melanoidins as MPRs that contributed to the antioxidant activity. This was confirmed by the FTIR spectra of the dried extract of Carica coffee, which showed the presence of a marker of the melanoidin functional group. The analysis of the proximity of the FTIR spectra using the Ward method clustering showed a similarity between the roasting levels of 100–115 °C and 150–165 °C compared to the 200–215 °C level.

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CONFLICT OF INTEREST

No potential conflicts of interest were reported by the authors.

AUTHOR CONTRIBUTIONS

SAS conducted the experiment and wrote the manuscript. ZA and RH revised the manuscript. All authors agreed with the final version of this manuscript.

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