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FULL PAPER

Application of *Caulerpa racemosa* extract as a natural colourant in raw noodles

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Abstract

Caulerpa racemosa is a green macroalga that contains chlorophyll, a natural colorant, which has anti-inflammatory, antibacterial, antiparasitic, and antioxidant properties. This study aimed to determine the effect different concentrations of *C. racemosa* mixed with distilled water have on raw noodles and to discover the optimal concentration. Fresh *C. racemosa* was mixed with distilled water into 25%, 50%, and 75% w/v solutions. It was then added to the dough for making noodles, the latter of which were tested in triplicate for colour, total phenol, total chlorophyll, protein, tensile strength, and consumer preference. The *C. racemosa* extracts had a significant effect ($P<0.05$) on colour, total phenol, total chlorophyll, protein, and consumer preference, but did not significantly influence tensile strength. Noodles with a 75% *C. racemosa* concentration were preferred for their attractive green colour.

1. Introduction

Colour plays an important role in a consumer's preference for a product. The application of synthetic dyes tends to increase because it is cheaper, produces more attractive colour, and is more stable than natural ones (Saleem *et al.,* 2013). Fast Green FCF is a green synthetic dye. Continuous use or exceeding the standard (maximum 100 mg/kg) may be harmful to health (FAO, 2017). Synthetic colours are difficult for our intestines to absorb, have tumorigenic properties, and mutagenic effects in experimental animals and humans. It also irritates the eyes, skin, digestive tract, and respiratory tract (Okafor *et al.,* 2016; Sadar *et al.,* 2017). We have seen a shift back to sustainable and renewable natural products. One natural dye that can replace synthetic dyes comes from *Caulerpa racemosa*.

Caulerpa is a green algae group that includes feather seaweed (an edible macroalga) and contains bioactive substances (Tapotubun *et al.,* 2020). According to Merdekawati and Susanto (2009), green algae contain chlorophyll-a (26.82%), chlorophyll-b (12.91%), and are effective as an antioxidant. *Caulerpa racemosa*, also known as sea grape, is a grape-shaped green alga found widespread in Indonesian waters. *Caulerpa racemosa* in Indonesia still sees limited use, mostly in fresh vegetables or mixed with fruit.

Noodles are popular foods in Asia and are made from wheat flour into a long and thin shape. It is a staple Asian food that can be categorized as a substitute food because it acts as an alternative to rice, is affordable, and is easy to serve (Sikander *et al.,* 2017; Adejuwon *et al.,* 2019). Noodles are usually yellow but may come in a variety of colours to attract customers. Vegetables may be added to noodles to provide natural colouring and to make them healthier, such as noodles with spinach puree (Shere *et al.,* 2018), carrot and tomato (Yadav *et al.,* 2014), or celery (Minarovičová *et al.,* 2018). Frequent consumption of instant noodles remains unhealthy, making further innovation in healthier noodles is needed. This study aimed to determine the effect of various *C. racemosa* concentrations on the colour, total phenol, total chlorophyll, protein, and consumer preference for noodles.

2. Materials and methods

2.1 Materials

Fresh *C. racemosa* was obtained from the Centre of Brackish Water Research, Jepara, Indonesia. Distilled water was obtained from a chemical supplier in Semarang, Central Java, Indonesia. Wheat flour and eggs were bought from a local market in Semarang.

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2.2 Noodle preparation

Caulerpa racemosa was extracted by Oktiarni *et al.* (2012) in 25%, 50%, and 75% (w/v) solutions. The mixtures were then blended until homogenous, filtered, and mixed with the noodle dough. The preparation of raw noodles was referred to Koswara (2009) by mixing wheat flour 50%, eggs 5%, and the *C. racemosa* extracts 45% and then stirred until homogenous. The dough was left for 10 mins, then pressed with a roll press, stretched until it formed a thin dough sheet (1 mm), and cut into string-shapes (1.0–1.5 mm thick, 50 cm long).

2.3 Colour assay (L, a, b)

The colour assay was done with a Chroma Meter (Konica Minolta CR-410) connected to an electric current (Hruškqvá *et al.,* 2011). The measuring head was held horizontally over each sample, and measurements were made when the indicator light was on. Measurements for L, a*, and b* were taken three times and the values were noted from the Chroma Meter display each time.

2.4 Total phenol

The total phenol was measured using the method adapted from Priyadarisani *et al.* (2014). Approximately 5 g of noodles were dissolved in 100 mL of distilled water, the resulting homogenous solution was filtered, and 1 mL of the filtrate was taken and added to 0.5 mL of Folin-Ciocalteu reagent and 1 mL of saturated Na_2CO_3 solution, and left for 10 mins. Finally, the solution was added with up to 10 mL distilled water. The absorbance was measured with a spectrophotometer (Shimadzu, Japan) at 730 nm. The total phenol was then calculated with a phenol standard curve.

2.5 Total chlorophyll

A total chlorophyll assay was performed based on Hendriyani and Setiari (2009) for extracted solvents reference. A 5 g sample was extracted with 100 mL of distilled water using a blender (Maspion, Indonesia). The extract was then added to a cuvette and its absorbance was measured with a spectrophotometer (Shimadzu, Japan). The chlorophyll content was calculated using the formulae.

$$Chlorophyll\ a = 1.07\,(OD663) - 0.094\,(OD644) \quad (1)$$

$$Chlorophyll\ b = 1.77\,(OD644) - 0.28\,(OD663) \quad (2)$$

2.6 Protein content

The protein content was determined by the Micro-Kjeldahl method (AOAC, 2007). A 0.2 g sample was placed in a 30 mL Kjeldahl flask, then 2 g of K_2SO_4, 50 mg HgO, and 2.5 mL H_2SO_4 were added. The protein content was calculated using the formulae.

$$\%N = [(ml\ HCl - ml\ blank)/mg\ sample] \times 100\% \quad (3)$$

$$\%Protein\ (\%ww) = \%N \times 6.25\ (protein\ factor) \quad (4)$$

2.7 Tensile strength

A tensile strength analysis was performed according to the methods by Huh *et al.* (2019). The samples were cooked for 2 mins at 100°C. They were then cut into a string (10 cm) and attached to the sample holder TA-XT2i texture analyser (Universal Testing Machine, Brookfield, USA), set to TRAC mode with a tensile speed of 19.9 mm/s. This test was repeated three times for each treatment and the averages were taken. The tensile strength was measured with the formula.

$$TS = F/A = kgf/cm^2 = MPa = 1{,}000\ kPa \quad (5)$$

Where TS is tensile strength (kgf/cm^2), F is tensile load (N), and A is the cross-sectional area (cm^2).

2.8 Sensory analysis

The sensory analysis was performed following Anggraeni and Saputra (2018) on 30 panellists using five scales to determine their level of preference based on colour, texture, aroma, and flavour. The preference test consisted of values: 1 - dislike, 2 - somewhat dislike, 3 - neutral, 4 - somewhat like, and 5 - like.

3. Results and discussion

3.1 Colour (L, a, b)

The result of L*, a*, and b* colour assays using a chromameter on raw noodles with *C. racemosa* extracts are presented in Table 1. The L parameter indicates the level of brightness on a scale of 0 (dark/black) to 100 (bright) (Deskawi *et al.*, 2015). Our brightness measurements ranged from 47.52 to 66.70 with the lowest value of 47.52 found in the 75% *C. racemosa* noodles, and the highest value of 66.70 found in the control sample. Sanjaya *et al.* (2016) mentioned that L* values below 50 tend to be considered dark. The brightness (L) results indicated that the addition of *C. racemosa* extract significantly affects the brightness of the noodles. Noodles treated with *C. racemosa* were darker than the control. As the concentration increased, the brightness level decreased because of the green colour the *C. racemosa* extract gave the noodles.

The highest a* value of 17.79 was found in the 75% *C. racemosa* extract, and the lowest value of 0.72 was found in the control noodles. The higher the concentration, the greener the colour. A high a* value indicates the accumulation of chlorophyll from *C.*

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Table 1. Colour assay of noodle

Treatment	Colour			Degrees Hue
	L*	a*	b*	(°Hue)
Control	66.70±0.85[a]	0.72±0.07[a]	18.10±0.17[a]	87.71±0.20[a]
A	60.86±1.32[b]	-6.65±0.19[b]	19.84±0.18[b]	71.31±0.58[b]
B	55.08±1.37[c]	-14.89±0.20[c]	15.20±0.19[c]	45.56±0.66[c]
C	47.52±1.24[d]	-17.79±0.27[d]	12.87±0.23[d]	36.56±1.61[d]

Values are presented as mean±standard deviation. Values with different superscript in the same column are significant different (P<0.05). Control: Raw noodle without *C. racemosa* extract, A: Raw noodle with 25% *C. racemosa* extract, B: Raw noodle with 50% *C. racemosa* extract, and C: Raw noodle with 75% *C. racemosa* extract.

racemosa. According to Merdekawati and Susanto (2009), *Caulerpa* sp. contain chlorophyll derivatives (18.731%), chlorophyll-a (26.817%), and chlorophyll-b (12.906%). A low a* value is inversely proportional to L*, which means that the greener the product, the darker the colour. Putri *et al.* (2012), extracted chlorophyll from suji leaves and found that the higher the chlorophyll concentration, the darker the colour.

The b* value results showed that the noodles with a 25% extract were more yellow than the other samples. This is because of the carotenoid pigments derived from *C. racemosa,* which contain chlorophyll-a, chlorophyll-b, beta carotene, and caulerpin (Paul *et al.,* 2013; Sihono *et al.,* 2018). According to Hasbullah *et al.* (2014), the carotenoid content in *C. racemosa* is 0.0013 mg/kg, and Supriadi *et al.* (2016) emphasized that it contains 0.013 mg/kg carotenoid pigments. Furthermore, Agusman *et al.* (2020) confirmed that *Caulerpa* sp. extract may increase the a*(-) and b*(+) values that affect the incline of green and yellow colour intensity in noodles because of the chlorophyll and carotenoid it contains (Gaillande *et al.,* 2017; Sihono *et al.,* 2018).

3.2 Tensile strength

The tensile strength of noodles is influenced by gluten and amylose from flour. Gluten plays a role in forming elasticity, while amylose is forming the strength when the noodles are tensed. Tensile strength is also influenced by salt. Salt helps form the dough and inclines gluten texture to produce high stability and tensile strength. However, a high salt content could be harmful to the dough texture as excessive salt ions will ruin the gluten tissue (Hu *et al.,* 2017).

Table 2 shows the varied tensile strength of the raw and cooked noodles. The tensile strength of raw noodles ranged between 15.1–17.5 kPa, whereas the tensile strength of cooked noodles ranged between 55.4–58.1 kPa. Wheat flour in this study contained amylopectin, which provided tensile strength. The difference in tensile strength between raw and cooked noodles comes from the breakdown of amylopectin in starch. When starch heats up, some amylopectin double helix fractions are stretched and are released when a hydrogen bond is broken. The high temperatures resulted in more hydrogen being broken, resulting in water being absorbed in starch granules and forming a stronger bond (Li *et al.,* 2017).

Table 2. Tensile strength of noodle

Sample	Tensile Strength Value (kPa)	
	Raw Noodles	Cooked Noodles
Control	16.9±0.28[a]	55.4±2.12[a]
A	17.5±0.78[a]	57.9±1.51[a]
B	15.1±1.41[a]	55.4±0.94[a]
C	15.6±1.58[a]	58.1±1.12[a]

Values are presented as mean±standard deviation. Values with different superscript in the same column are significant different (P<0.05). Control: Raw noodle without *C. racemosa* extract, A: Raw noodle with 25% *C. racemosa* extract, B: Raw noodle with 50% *C. racemosa* extract, and C: Raw noodle with 75% *C. racemosa* extract.

The tensile strength in the noodles in this study was lower than reported by Kurniasari *et al.* (2015) who did a similar study on tapioca flour. The tensile strength of noodles without tapioca flour is 190 kPa because wheat flour contains less (89.77%) amylopectin than tapioca flour (91.94%) (Imanningsih, 2012). However, this study's noodle tensile strength was higher than Nurcahyo *et al.* (2014), which ranged from 1.87 to 2.58 kPa because of the use of breadfruit flour, which causes a decrease in the availability of gluten protein, thus reducing tensile strength.

Noodles with added *C. racemosa* extract showed no significantly different tensile strength. A high amylose results in a compact and robust texture (Herawati *et al.,* 2017; Huh *et al.,* 2019). Moreover, tensile strength is also influenced by salt content. Salt helps to form the dough and incline gluten texture to produce high stability and tensile strength. However, higher salt content could be harmful to the dough texture as the excessive salt ion will ruin the gluten tissue (Hu *et al.,* 2017). A different result was obtained by Agusman *et al.* (2020), where the *Caulerpa* sp. extract can increase the tensile strength. The higher salt content on *Caulerpa* sp. could affect the texture. The various salt content on *Caulerpa* sp. might

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cause by the dynamic of salinity in its habitat (Guo *et al.,* 2015).

3.3 Protein content

The protein content measurement results (Figure 1) of noodles after *C. racemosa* extract addition ranged from 6.20 to 10.18% (dw). The highest content was found in noodles with 75% *C. racemosa* extract and the lowest was in the control noodles. As the results reflect, protein in noodles comes directly from the protein-rich *C. racemosa* extract. Bhuiyan *et al.* (2016) explained that *C. racemosa* contains 19.72% protein, which is supported by the similar findings of Nagappan and Vairappan (2014) of 17.36%.

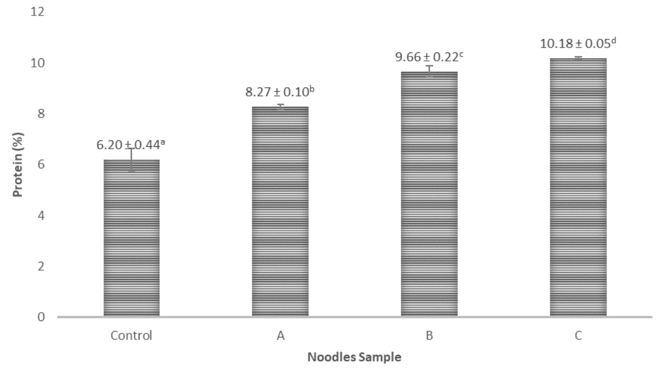

Figure 1. Protein of noodle. Values are presented as mean±standard deviation. Values with different superscript in the same column are significant different (P<0.05). Control: Raw noodle without *C. racemosa* extract, A: Raw noodle with 25% *C. racemosa* extract, B: Raw noodle with 50% *C. racemosa* extract, and C: Raw noodle with 75% *C. racemosa* extract.

Alkaloid compounds are indicative of the presence of alkaline groups. *C. racemosa* contains alkaloid compounds, as is indicated by protein element nitrogen (N), as well as triterpenoids and tannins (Kurniasari *et al.*, 2015). The composition of chemical elements in proteins is carbon (C) 50%, hydrogen (H) 75%, oxygen (O) 23%, and nitrogen (N) 16% (Raj *et al.,* 2015). The noodles without *C. racemosa* extract (control) and noodles with 25% *C. racemosa* extract had a protein content of 6.20% and 8.27% respectively, falling short of the 9% minimum stipulated by the National Standard of Indonesia (SNI) No. 2987-2015. However, the noodles with 50% and 75% *C. racemosa* extract did meet the requirements with 9.66% and 10.18% protein content respectively. These results concur with the findings of Kumar *et al*. (2017) who did a similar study on biscuits and found that the *C. racemosa* extract concentration is proportional to the protein content.

3.4 Total phenol

The total phenol assay aimed to determine the total phenolic compounds in the samples. Based on Figure 2, the total phenol ranged between 178.65-392.56 ppm, with the highest value was the noodles with the 75% extract of 392.56 ppm, and the lowest value was the control sample of 178.65 ppm. The higher the amount of extract added, the higher the total phenol value. It is related to the total phenol content on *C. racemosa*. According to Yap *et al*. (2019), *C. racemosa* contains between 10.33 and 13.41 mg GAE/g phenol. According to Nurjanah *et al*. (2019a), the total phenol in *C. racemosa* is 28.56 mg GAE/g and is influenced by external factors such as habitat, the availability of sunlight, and harvest time. This study had a linear relationship between the phenolic compounds and the sample's antioxidant activity. As shown in Figure 3, the total phenol ranged between 178.65 and 392.56 ppm, with the highest phenol value of 392.56 ppm measured from the noodles with the 75% extract, and the lowest value of 178.65 ppm from the control sample. The higher the extract concentration that was added, the higher the total phenol value. These results correspond to Kumar *et al*. (2017), who found that the total phenol content in biscuits increased parallel with the addition of *C. racemosa* concentration.

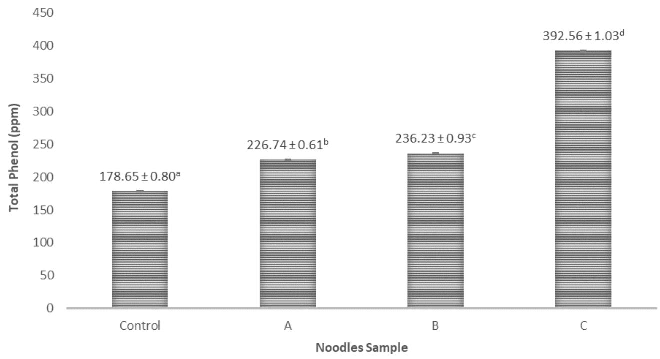

Figure 2. Total phenol of noodle. Values are presented as mean±standard deviation. Values with different superscript in the same column are significant different (P<0.05). Control: Raw noodle without *C. racemosa* extract, A: Raw noodle with 25% *C. racemosa* extract, B: Raw noodle with 50% *C. racemosa* extract, and C: Raw noodle with 75% *C. racemosa* extract

3.5 Chlorophyll

Caulerpa racemosa naturally contains chlorophyll-a and b (Tapotubun *et al.,* 2020) and chlorophyll-a is less polar and blue-green, while chlorophyll-b is polar and yellow-green (Indrasti *et al.,* 2018). The total chlorophyll assay aimed to determine the total chlorophyll-a and b content of the noodles (Figure 3). The chlorophyll contents ranged from 8.31 to 9.49 g/mL, with the highest value of 9.49 g/mL in noodles with the 75% *C. racemosa* extract, followed by the 8.89 g/mL from the 50% *C. racemosa* extract, and the lowest value of 8.31 g/mL in the noodles with the 25% *C. racemosa* extract.

The total chlorophyll content of the noodles was

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linearly proportional to the concentration of the *C. racemosa* extract. The higher the concentration extract, the higher the chlorophyll-a and chlorophyll-b content of the noodle. There tended to be more chlorophyll-b in noodles than chlorophyll-a. However, according to Sarojini *et al.* (2015), *C. racemosa* contains chlorophyll-a 10.08×10^{-6} – 17.4×10^{-6} g/mL and chlorophyll-b 15.2×10^{-6} - 31.2×10^{-6} g/mL. The chlorophyll contents in this study were higher, which increased in line with the green colour intensity (a*) from the colour analysis.

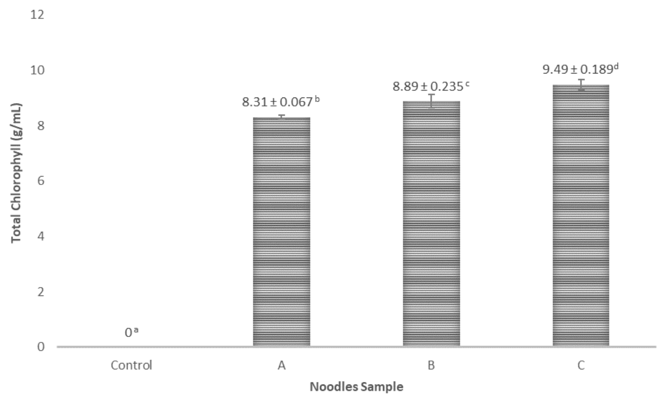

Figure 3. Total chlorophyll of noodle. Values are presented as mean±standard deviation. Values with different superscript in the same column are significant different (P<0.05). Control: Raw noodle without *C. racemosa* extract, A: Raw noodle with 25% *C. racemosa* extract, B: Raw noodle with 50% *C. racemosa* extract, and C: Raw noodle with 75% *C. racemosa* extract.

3.6 Sensory

3.6.1 Appearance

The panellists' preferences are presented in Table 3. The noodles in this study were identical in shape and size. The only visible difference was the green colour intensity (Figure 4). The appearance test results for noodles with 50% *C. racemosa* extract were significantly different from the 75% *C. racemosa*. All noodles have a dominant green colour, but none were greener than the 75% *C. racemosa* extract. The noodles with 50% *C. racemosa* extract were the most preferred by panellists. Noodles with 25% *C. racemosa* extract and the control noodles were less preferred for their appearance and colour. There was no green colour in the control noodles because there were no additions of *C. racemosa* extract. The higher addition of concentration of *C. racemosa* extract, the greener the appearance, and the more it was preferred by the panelists. The study on green extract *C. racemosa* addition was accorded to Shere *et al*. (2018), the higher the addition of spinach puree, the colour of the buns are dark green. The formation of green colour is the colour of chlorophyll pigments derived from spinach. On the other hand, Kumar *et al.* (2017) found that the consumer less preferred biscuits with a higher concentration of *C. racemosa*. It is caused by the decreasing colour intensity on L, a* dan b*.

Control **A** **B** **C**

Figure 4. The colour of noodles with the different concentration of *C. racemosa* extract. Control: Raw noodle without *C. racemosa* extract, A: Raw noodle with 25% *C. racemosa* extract, B: Raw noodle with 50% *C. racemosa* extract, and C: Raw noodle with 75% *C. racemosa* extract.

3.6.2 Aroma

The aroma was one of the sensory parameters, relying on the sense of smell (Table 3). The most preferred aroma was from the noodles with 75% *C. racemosa* extract, with a score of 4.23. However, the score of 4.13 for noodles with 50% *C. racemosa* extract came very close. Both noodles were well-liked by the panellists because both noodles had a distinctive, slightly fishy, aroma because of the *C. racemosa*. The aroma of *C. racemosa* is specific to seaweed and the fishy smell comes from the amino acid (Puspita *et al.,* 2019). Moreover, Vitriasari and Suyanto (2012) found that the addition of seaweed greatly affects a product's aroma because, during the processing, many components such as glutinous rice flour are mixed. The aroma in food is determined by one component and specific components that give a distinctive odour and the ratio of various ingredients used. The addition of *C. racemosa* extract to the 25% noodles appeared to have an indistinguishable aroma from the control noodles. However, there was a difference in the aroma that came from the control

Table 3. Sensory of noodles

Sample	Appearance	Aroma	Taste	Texture
Control	2.40±1.04^a	2.37±1.22^a	2.43±1.01^a	2.80±0.91^a
A	2.91±0.67^b	3.50±1.04^b	3.10±0.88^b	3.47±0.85^b
B	3.77±0.63^c	4.13±0.90^c	4.07±0.83^c	4.03±0.87^c
C	4.60±0.56^d	4.23±0.94^d	4.43±0.77^d	4.27±0.96^c

Values are presented as mean±standard deviation. Values with different superscript in the same column are significant different (P<0.05). Control: Raw noodle without *C. racemosa* extract, A: Raw noodle with 25% *C. racemosa* extract, B: Raw noodle with 50% *C. racemosa* extract, and C: Raw noodle with 75% *C. racemosa* extract.

noodles' dough.

3.6.3 Taste

Taste or flavour, is an assessment of food products that combine with the smell. The panellists reported sweet, bitter, sour, and salty tastes (Table 3). The most-liked noodles by the panellist were those with 75% *C. racemosa* extract. They had a salty taste thanks to the *C. racemosa* extract coming from a highly saline environment. In contrast, the control noodles were tasteless. Furthermore, *C. racemosa* noodles have a savoury taste that comes from amino acid derivatives, such as alanine, glutamic, and glycine (Ma'ruf *et al.,* 2013). Puspita *et al.* (2019) explained that cream soup with *C. racemosa* had a savoury taste, while other food additives did not have a significant effect.

3.6.4 Texture

The texture is a pressure sensation that is experienced when biting, chewing, swallowing, or touching the product. Ingredients influence the texture of a product (Table 3). The most preferred texture was of the noodles with 75% *C. racemosa* extract added, with an average score of 4.27, followed by the 50% noodles with 4.03 points. The 25% *C. racemosa* extract scored 3.47, and the lowest score of 2.8 was for the control noodle. The panellists indicated that they preferred the higher concentrations and a slightly chewy texture. The noodle elasticity or chewiness comes from gluten and amylopectin contained in the flour. Gluten plays a role in coagulation and starch gelatinization during heating, while amylopectin works like glue for the tissue and strength of the noodle structure (Nurjanah *et al.,* 2019b). This study's chewiness further influences by the salt contained in *C. racemosa*, as it strengthens the dough texture. *C. racemosa* is low in fibre, so it did not affect the noodles' hardness (Agusman *et al.,* 2020).

4. Conclusion

The addition of *C. racemosa* extracts affected the characteristics of noodles. The higher the concentration, the greener the colour intensity, and the phenol and chlorophyll content. While the addition of C. *racemosa* did not affect the tensile strength, it did positively influence consumer preference.

Conflict of interest

The authors have no conflicts of interest to declare.

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