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Physicochemical and Antioxidant Properties of Copper-Amaranth Leaf Purees Using Viscozyme-L Enzymatic Liquefaction

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ABSTRACT

This study evaluated the effect of cell wall-degrading enzyme (Viscozyme® L) on the physicochemical properties and antioxidant activity of copper-amaranth (*Amaranthus Viridis* Linn.) leaf purees. The purees were liquefied with varying concentrations of Viscozyme® L (0–3% v/w) over different incubation times (30 minutes to 24 hours). It was observed that treatment with 1% Viscozyme® L (v/w) at pH 5 for 3 hours, followed by incubation at 45 °C, resulted in a significant increase in total soluble solids (°Brix), acidity, and total chlorophyll content. The enzyme-treated purees exhibited higher DPPH (13.52 mM (TE)/g fresh weight), and FRAP (6.04 mM (TE)/g fresh weight) values, as well as in reducing sugar (118.43 mg/mL), soluble dietary fibre (4.32%) content and greener colour (-0.35) values, as compared to non-enzyme treated purees (0.13 mg/g, 9.47 mM (TE)/g and 3.72 mM (TE)/g fresh weight, and 12.75 mg/mL, 0.60%, and -0.28, respectively). These findings demonstrate that treatment with Viscozyme® L enzymes can effectively improve the nutritional and functional quality of vegetable-based purees, with potential applications in the processing of green vegetable juices.

Keywords: Amaranth; cell wall-degrading enzyme; chlorophyll content; Viscozyme® L

INTRODUCTION

Slender amaranth *(Amaranthus viridis* Linn.*)* is one of Malaysia's most popular green leafy vegetables (Amin *et al.*, 2006; Akbar *et al.*, 2017). Amaranth has nutritional values similar to those of spinach but significantly higher than those of other vegetables, such as cabbage (Akbar *et al.*, 2017). Amaranth is also high in vitamins, minerals, dietary fibre, polyphenols, flavonoids, anthocyanins, and chlorophylls (Wargovich, 2000; Dias and Ryder, 2011). Green amaranth leaves are higher in flavonoid content (1.5 mg CE/g FW) and antioxidant activity (61 µmol TE/g FW) than spinach (0.70 mg CE/g FW and 16 µmol TE/g FW, respectively) (Isabelle *et al.*, 2010; Mavhungu, 2011; Jiménez-Aguilar and Grusak, 2015). Despite all the health benefits of green leafy vegetables, their application in food processing is often limited due to chlorophyll degradation that occurs during thermal processing, acidification or enzymatic treatment, which causes discolouration (Östbring *et al*., 2014) and reduction in antioxidant activity. To address this issue, researchers explored to stabilise the magnesium ion in the porphyrin ring of chlorophyll by replacing the magnesium ion in its central porphyrin ring with divalent cations, such as copper (Cu), to form metallo-chlorophyll complexes (Humphrey, 2004). These metal-chlorophyll derivatives are more resistant to acid and heat and retain the green colour of the vegetable during processing (Leunda *et al*., 2000).

The enzymatic treatment approach offers an environmentally friendly alternative, with higher extraction efficiency and yield while minimising solvent and energy requirements compared to conventional methods (Puri *et al*., 2012). Furthermore cell wall membrane must be disrupted for chlorophyll extraction in vegetables, and the chlorophyll pigments can be separated from their associated proteins (Pocock *et al*., 2004) using enzymes. Consequently, enzymatic treatment of vegetables is a good technique for reducing the viscosity (Urlaub,

2002), facilitates easy pressing, and improves yield, clarity, filterability, flavour and colour in fruit and vegetable juice (Godfrey *et al*., 1996; Galante *et al*., 1998; Uhlig, 1998; Kashyap *et al*., 2001; Tochi *et al*., 2009). According to Chong and Wong (2015), enzyme liquefaction is more effective than adding water to the puree in reducing viscosity,, as it requires more energy to remove the additional water during spray drying (Grabowski *et al*., 2006). Several studies have reported the use of enzymatic liquefaction as a pre-treatment in juice processing and spray drying such as in carrots (Stoll *et al*., 2003), pineapple (Wong *et al*., 2015), mango (Sakhale *et al*., 2016) and cabbage (Buckenhüskes *et al*., 1990). However, there are scarce reports on the enzymatic liquefaction of green leafy vegetables.

Few studies have found that enzyme types and concentrations, temperature, and time influence the liquefaction process (Lee *et al*., 2006; Liew Abdullah *et al*., 2007) in several plants. According to Kotcharian *et al*. (2004), the cell wall structure and viscosity of carrot puree were altered by enzymatic cell wall degradation utilising the pectolytic (Rohapect AP) and cellulolytic (Rohament PL) enzymes. Chong and Wong (2015) demonstrated that liquefaction of sapodilla puree with 0.5% (v/w) Pectinex Ultra SP-L and Celluclast 1.5 L at 40 ºC for 1.5 h had a better reduction in viscosity before spray drying. Özkan and Bilek (2015) reported a similar observation that optimum conditions for chlorophyll extracted from spinach pulp were using 8% Pectinex Ultra SP-L at 45 ºC for 30 min. Meanwhile, Sun *et al*. (2007) required up to 8 hours using Viscozyme® L enzyme at 37 ºC to increase antioxidant activity, yield, soluble solid content, and colour of asparagus macerates.

In this research, multi-enzymatic complexes such as Viscozyme® L, which contain cellulases, arabinases, hemicellulases, glucanases and xylanases, cause the cell walls to rupture, facilitating the extraction of valuable compounds from vegetable tissues (Dueñas *et al*., 2007). Therefore, the range of all liquefaction parameters should be carefully selected to obtain the lowest viscosity of amaranth puree and increase the antioxidant activity and chlorophylls in the enzyme-treated puree. Therefore, this study aimed to determine the effects of Viscozyme® L concentrations and liquefaction time on the physicochemical properties of Cu-amaranth puree.

MATERIALS AND METHODS

Materials

Fresh green amaranth (characterised by slender leaves) was purchased from a local market in Kuala Lumpur, Malaysia. All reagents and solvents were analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA). Viscozyme® L was purchased from Novozymes Inc. (Copenhagen, Denmark). This commercial enzyme is a multi-enzyme complex containing cellulases, hemicellulases, pectinases, arabanase, β-glucanase and xylanase from *Aspergillus niger* with a declared activity of ≥100 FBG/g. The optimum conditions are pH 3.3 to 5.5 and temperature 25 to 55±1 °C.

Preparation of Enzyme-Treated Cu-amaranth Puree

Fresh amaranth leaves with stalks were washed, cleaned with running tap water, and chopped to remove the roots. It was then homogenised in a Waring blender for 5 min at high speed to form amaranth purees. The amaranth purees were further treated with 210 ppm of copper sulfate at pH 6 and heated at 80 ºC for 15 min, as described in our previous publication by Siti Faridah *et al*. (2021), to form Cu-amaranth puree. For enzymatic treatment, 300 g of Cu-amaranth puree was thawed at 4 °C and individually treated with different concentrations of Viscozyme® L (0-3.0% v/w) under fixed conditions (45 °C for 24 h at pH 5). The pH of the mixtures was adjusted with a 1% (v/v) citric acid solution and 1% NaOH solution (if necessary). The optimum enzyme concentrations were selected based on the purees with the highest chlorophyll content, the highest antioxidant activity, and the lowest viscosity. The same procedure was repeated for the effect of incubation time (0.5-24 h) using an optimised Viscozyme® L concentration at pH 5 and incubated at 45 °C. The non-enzyme-treated Cu-amaranth puree was also prepared as a control and set under the same conditions. At the end of the incubation, all samples were heated for 5 min at 90 °C to inactivate the enzyme. The samples were then strained through a plastic nylon strainer with a fine sieve of 0.4 mm mesh size, packaged in aluminium laminated polyethene

pouches, and stored at -20 °C until further analyses.

Apparent Viscosity

The RheolabQC rheometer (Anton Paar Physica, Gmbh, Germany) was used to evaluate the apparent viscosity of the enzyme-treated Cu-amaranth puree at room temperature (24 ± 1 °C) at 160 rpm using a concentric cylinder and spindle no. 3. A measuring cup with dimension of 52.6 mm in diameter, 75 mm in height, and 150 mL was filled with an aliquot of the enzyme-treated puree. The viscosity of the mixture was measured in millipascal-seconds (mPa.s).

Spectrophotometric Measurement of Total Chlorophyll Content

The chlorophyll content of the enzyme-treated Cu-amaranth puree was determined using the method described by Dere *et al*. (1998) and Siti Faridah *et al*. (2021). Briefly, 1 g of Cu-amaranth puree was ground with 100% acetone using a mortar and pestle until the residue was colourless. Then, the extract was transferred into a centrifuge tube covered with aluminium foil and repeatedly washed with 50 mL (100% acetone). The mixture was then centrifuged (3500 x *g*, 10 min), and the absorbances of the extracted chlorophylls in the supernatant were measured using a UV-Vis spectrophotometer (Shimadzu, Japan) at 662 and 645 nm against 100% acetone as a blank. The equation, Eq. (1) from Costache *et al*. (2012) was used to quantify the total chlorophyll content (mg/g fresh weight).

$$\text{Total chlorophyll content (TCC)} = (16.26A_{645} + 7.790A_{642}) \times \text{Dilution factor}/1000 \quad \text{Eq.(1)}$$

Antioxidant Activity Assay

The enzyme-treated Cu-amaranth purees were dried at 45 °C for 24 h in a convection oven (Venticell 111, MMM Group, Munich, Germany) and then ground into a fine powder using a Pulverisette 14 rotor mill (Fritsch, GmbH, Oberstein, Germany). Each powder (0.25 g) was extracted with 10 mL of 80% methanol at 40 °C for 24 h. The samples were then cooled to room temperature (27 ± 2 °C) and centrifuged at 3500 x *g* for 15 min. The supernatant was collected in an airtight glass vial for further analysis (Li *et al*., 2008).

Free Radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay

The antioxidant activities of the enzyme-treated Cu-amaranth extracts were measured using the DPPH assay as outlined by Brand-Williams *et al*. (1995). Briefly, 0.1 mL of amaranth puree extract was mixed with a 3.9 mL aliquot of 0.1 mM methanolic DPPH solution (prepared using 80% v/v methanol). The mixture was vortexed for 15 seconds and left in the dark for 15 min at room temperature. The absorbance was read at 515 nm using a UV-Vis spectrophotometer (Shimadzu, Japan) with 80% methanol (v/v) as the blank. The FRAP values were expressed as mM of Trolox Equivalents (TE) per gram fresh weight.

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was conducted as outlined by Benzie and Strain (1996) with slight modifications. In brief, 2.85 mL of FRAP reagent and 150 µL of Cu-amaranth puree extract were combined, and the mixture was kept in the dark at room temperature for 30 minutes. The absorbance of the reaction mixture was recorded at 593 nm. Trolox was used to construct a standard curve, and the results were represented as mM of Trolox Equivalents (TE) per gram of fresh weight.

Total Soluble Solids (TSS) and pH

The TSS content was measured using a portable hand refractometer (Atago®, Japan) with a 0-32 ºBrix scale. The pH value was measured using a digital pH meter (Jenway, model 3505, UK).

Colour Measurement

A HunterLab Ultra-Scan Colorimeter (Sphere Spectrocolorimeter, Hunter Association Inc., Reston, USA), in the reflectance mode and with illuminant D65, was used to determine a* (+a* = red, -a* = green) and b* (+b* = yellow, -b* = blue). The ratio - a*/b* was used as a measure of variation in green colour as described by Guzmán *et al*. (2002).

Reducing Sugar Measurement by 3,5–dinitrosalicylic Acid (DNS) Method

The activity of the cell wall-degrading enzymes on the enzyme-treated Cu-amaranth purees was determined by measuring reducing sugars released (as glucose equivalents) using a modified 3,5-dinitrosalicylic acid (DNS) method (Saqib and Whitney, 2011). The absorbance was measured at 540 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). Quantification was based on the standard curve (0.03 to 0.25 mg/mL) of D-(+)-glucose (≥99.5%, Sigma G8270) and the results were expressed as mg glucose equivalent in mL of sample (mg/mL).

Total Dietary Fibre Analysis

The soluble (SDF), insoluble (IDF) and total (TDF) dietary fibres were determined using the enzymatic-gravimetric method according to Lee *et al*. (1992). Dried samples (duplicate 1 g) were then subjected to sequential enzymatic digestion and protein digestion in three incubation steps: (i) heat-stable alpha-amylase (or termamyl) (1500 - 3000 units/mg protein; Sigma Chemical Co.) at 98 to 100 ºC for 30 min; (ii) amyloglucosidase (5000 - 8000 units/mL; Sigma Chemical Co.) at 60 ºC for 30 min, pH 4.0 - 4.7; and (iii) protease (7 - 15 units/mg protein; Sigma Chemical Co.) at 60 ºC for 30 min. Each sample was suspended in 40 mL MES/ TRIS buffer (pH 8.2). The enzyme digestate was then filtered using acid-washed celite on a Fibretec system E1023 filtration unit (Tecator, Sweden). After filtration, the remaining residue was the IDF, and the filtrate was the SDF. The IDF was washed with 10 mL of 95% ethanol and 10 mL of acetone. For SDF, the filtrate was precipitated with four volumes of 95% ethanol at 60 ºC before filtering. The SDF was then washed with two portions of 15 mL ethanol (78%, 95%) and 15 mL acetone. TDF, IDF and SDF residue values were all corrected for undigested protein, ash and blank.

Statistical Analyses

All the experiments were performed in triplicate. MINITAB (version 16) statistical software was used for one-way analysis of variance (ANOVA) and Tukey's test was carried out to determine the significant differences among means at the 5% level. Data were reported as mean ± standard deviation.

RESULTS AND DISCUSSION

Effect of Viscozyme® L Concentration

The viscosities of enzyme-treated Cu-amaranth purees with different concentrations (0-3%) of Viscozyme® L incubated at pH 5 and 45°C for 24 h are shown in Figure 1. The untreated Cu-amaranth puree (0% Viscozyme® L) exhibited the highest viscosity due to the presence of undigested compounds, including pulp fibre (hemicellulose and cellulose), protein, and pectin (Roslan *et al*., 2020). In contrast, as the viscosity of Cu-amaranth puree decreased, an increase in the enzyme concentration was observed. It was due to the synergistic effects of pectolytic, cellulolytic, and hemicellulolytic enzymes in the Viscozyme® L, which break down vegetable cell walls, reduce water retention, and release free water into the system (Schweiggert *et al*., 2008). According to Norjana and Noor Aziah (2012), the viscosity of Cu-amaranth puree is reduced due to the presence of free water. However, increasing the enzyme concentration to above 1% of the Viscozyme® L concentration showed no significant effect ($p > 0.05$) on reducing the viscosities of the purees. It may be due to the complete breakdown of complex polysaccharides into soluble substances, indicating that 1% Viscozyme® L is appropriate for cell wall hydrolysis of Cu-amaranth purees. Thus, 1% of Viscozyme® L was selected as a suitable concentration for cell wall hydrolysis of Cu-amaranth purees. Additionally, to prevent high manufacturing costs, the consumption of enzymes for enzymatic liquefaction should be maintained to a minimum (Chong and Wong, 2015).

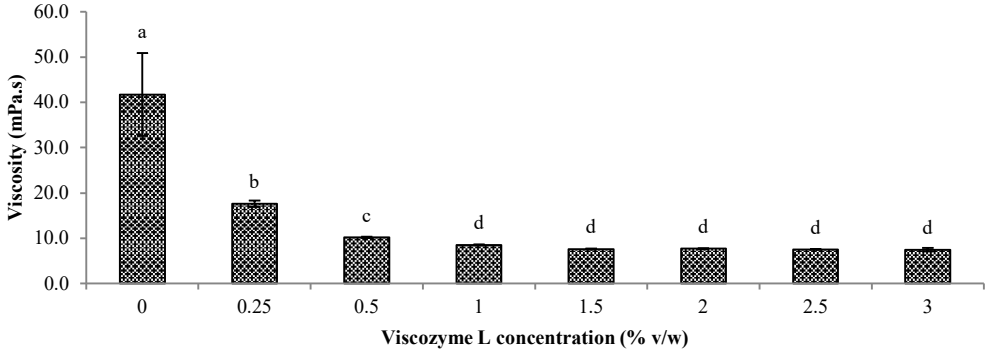

Figure 1. Viscosity of Cu-amaranth purees at pH 5 and 45 ºC for 24 h with different concentrations (0 - 3% v/w) of Viscozyme L. Each value is expressed as mean ± standard deviation (n = 3) of triplicate analysis. Bars with different lowercase letters indicate significant differences ($p<0.05$) by Tukey's HSD test.

The antioxidant activities (DPPH and FRAP) and total chlorophyll content (TCC) of Cu-amaranth purees after 24 h of enzymatic liquefaction at 45 °C and pH 5 were measured in response to the different concentrations of Viscozyme® L, as shown in Table 1. Cu-amaranth, enzymatically treated with 1% Viscozyme® L, significantly ($p<0.05$) contained the highest TCC (0.40 mg/g FW) as compared to other purees. A similar observation has been reported for the highest DPPH and FRAP values (10.65 and 5.83 mM (TE)/g FW, respectively) after liquefaction with 1% Viscozyme® L. However, DPPH and FRAP values were not significantly improved at concentrations above 1% Viscozyme® L. According to Puri *et al*. (2012), various enzymes, including cellulases, pectinases, and hemicellulases, interfere with the structural integrity of the plant cell wall. These enzymes hydrolysed the cell wall components, increasing their permeability and producing higher bioactive extraction yields. Furthermore, the formation of metallo-chlorophyll derivatives with metals such as copper has been reported to exhibit high antioxidant activity (Wrolstad *et al*., 2005).

A similar finding was reported by Sun *et al*. (2007) that liquefaction of asparagus with 1% Viscozyme® L (v/w) at 37 °C and 2 h incubation time gave higher antioxidant activity than control juice (1.4 and 1.2 mM (TE)/L juice, respectively). Koley *et al*. (2011) also showed that enzymatic processing using Viscozyme® L improved the antioxidant activity (FRAP) of Chinese apples (*Zizyphus mauritiana* Lamk).

Table 1. Effect of Viscozyme® L concentration on the antioxidant activities and total chlorophyll content of Cu-amaranth purees.

Viscozyme® L concentration (% v/w)	Antioxidant activity (mM (TE)/g fresh weight)		Total chlorophyll content (mg/g fresh weight)
	DPPH	FRAP	
0.00	9.47 ± 0.07^{b}	3.72 ± 0.04^{e}	0.13 ± 0.00^{e}
0.25	10.06 ± 0.06^{ab}	4.23 ± 0.10^{de}	0.24 ± 0.01^{d}
0.50	10.20 ± 0.59^{ab}	4.26 ± 0.09^{d}	0.21 ± 0.00^{d}
1.00	10.65 ± 0.64^{a}	5.83 ± 0.24^{a}	0.40 ± 0.00^{a}
1.50	10.26 ± 0.12^{ab}	5.49 ± 0.19^{ab}	0.34 ± 0.02^{b}
2.00	10.29 ± 0.48^{ab}	4.90 ± 0.27^{c}	0.25 ± 0.01^{cd}
2.50	10.03 ± 0.07^{ab}	5.48 ± 0.04^{ab}	0.29 ± 0.05^{bc}
3.00	10.27 ± 0.45^{ab}	5.06 ± 0.27^{bc}	0.31 ± 0.01^{b}

Values are the means ± standard deviations of triplicate analyses. $^{a-e}$ Different superscript lowercase letters indicate significant differences ($p<0.05$) within column.

Meanwhile, Hong *et al*. (2013) reported that DPPH radical scavenging activity and total polyphenols of green tea extract treated with Viscozyme® L were significantly higher than those treated with other commercial enzymes (Econase, Pectinex Ultra SP-L, Celluclast, and Rapidase PAC).

Effect of Incubation Time

Figure 2 represents the viscosities of Cu-amaranth purees treated with 1% (v/w) Viscozyme® L at pH 5 and 45 °C for 0.5 - 24 h. A rapid decline in viscosity was observed after 2 hours of incubation, but it gradually increased as the incubation time increased from 12 hours to 24 hours. A slight increase in the viscosity of Cu-amaranth purees might be due to the evaporation of free water in the puree as exposed for a longer time at 45 °C.

The TSS of the puree increased from 2 to 4 °Brix after liquefaction with 1% Viscozyme® L for 3 hours. Similarly, Yusof and Ibrahim (1994) reported that using an enzyme for soursop liquefaction significantly increased its total soluble solid content from 6.8 to 7.3 °Brix within the first hour of incubation. This may be due to the breakdown of cell wall polymers into oligomeric and monomeric soluble solids (Stoll *et al*., 2003), resulting in an increased TSS and decreased viscosity (Sharma *et al*., 2005). According to Sakhale *et al*. (2016), the enzymatic liquefaction process on Kesar mango pulp not only increases the overall yield of juice but also improves the quality features of the extracted juice. However, the pH values of the enzyme-treated Cu-amaranth purees were lower than those of the control (non-enzymatically treated) puree. Enzymatic degradation of polysaccharides and pectin, which releases carboxyl groups and galacturonic acid, may be caused by the slight decrease in pH (Gurrieri *et al*., 2000; Hesham and Manal, 2015).

Meanwhile, the effect of incubation time on the antioxidant activities and total chlorophyll content (TCC) of Cu-amaranth purees was examined (Table 2). An increase in the DPPH and FRAP values 13.52 and 6.04 mM (TE)/g FW, respectively and TCC (0.33 mg/g FW) were observed during the initial 3 hours of incubation with 1% Viscozyme® L. These results agreed with the findings of Li *et al*. (2006), who reported that cell wall-degrading enzymes disrupted the integrity of cell walls, thereby resulting in the efficient extraction of chlorophyll derivatives. Furthermore, degradation of cell-wall polysaccharides resulted in the release of phenolic compounds that contribute to higher antioxidant activity as measured by DPPH and FRAP (Shin & Lee, 2021).

Enzymatic treatment at 3 hours is selected because, according to Umsza-Guez *et al.* (2011), liquefying puree for an extended time at high temperatures is not recommended, as it would negatively affect the enzyme's activity. Additionally, sensitive components in the puree might be overexposed to heat, light, and oxygen. Furthermore, lower enzyme concentration and time are preferable for low-cost operation.

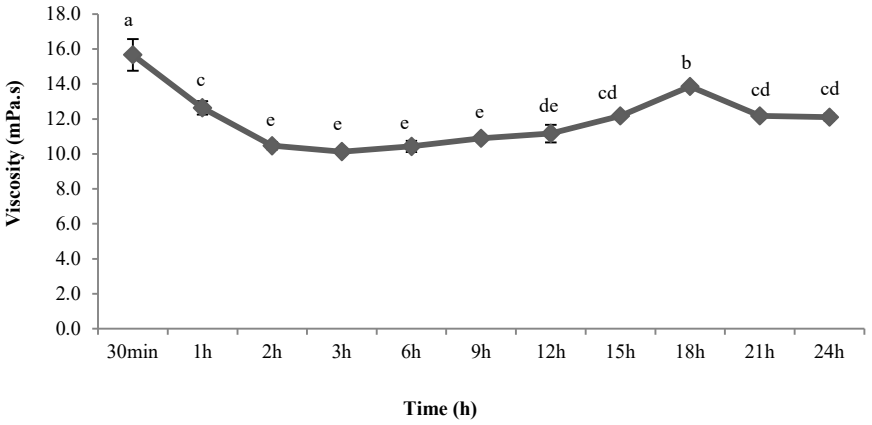

Figure 2. Effect of incubation time (h) on the viscosity (mPa.s) of Cu-amaranth purees with 1% (v/w) Viscozyme® L at pH 5 and 45 ºC. Each value is expressed as mean ± standard deviation (n=3) of triplicate analysis. Bars with different lowercase letters indicate significant differences ($p<0.05$) by Tukey's HSD test

Table 2. Effect of incubation time on the antioxidant activities and total chlorophyll contents of enzymatically treated Cu-amaranth purees.

Incubation time (h)	Antioxidant activity (mM (TE)/g fresh weight)		Total chlorophyll content (mg/g)
	DPPH	FRAP	
30min	10.38 ± 0.62^{c}	3.30 ± 0.04^{e}	0.23 ± 0.00^{d}
1h	10.75 ± 0.58^{bc}	5.51 ± 0.05^{ab}	0.30 ± 0.01^{b}
2h	10.54 ± 0.11^{c}	5.16 ± 0.24^{b}	0.30 ± 0.00^{b}
3h	13.52 ± 0.11^{a}	6.04 ± 0.24^{a}	0.33 ± 0.00^{a}
6h	13.63 ± 0.29^{a}	3.60 ± 0.14^{de}	0.33 ± 0.00^{a}
9h	12.61 ± 1.42^{ab}	3.56 ± 0.34^{de}	0.28 ± 0.00^{c}
12h	12.69 ± 1.40^{ab}	4.19 ± 0.27^{c}	0.30 ± 0.00^{b}
15h	9.86 ± 0.13^{c}	4.06 ± 0.16^{cd}	0.30 ± 0.00^{b}
18h	10.53 ± 0.31^{c}	4.22 ± 0.14^{c}	0.28 ± 0.00^{c}
21h	10.58 ± 0.16^{c}	4.10 ± 0.10^{cd}	0.28 ± 0.02^{c}
24h	10.39 ± 0.16^{c}	4.16 ± 0.08^{c}	0.28 ± 0.00^{c}

Values are the means ± standard deviations of triplicate analyses. $^{a-e}$ Different superscript lowercase letters indicate significant differences ($p<0.05$) within column. *with 1% (v/w) Viscozyme® L at pH 5 and 45 °C.

Physicochemical Characteristics of Non-Enzyme and Enzyme-Treated Puree

Enzyme-treated Cu-amaranth purees possessed higher total soluble solids (TSS), lightness (L*), a* and b* values, reducing sugar, and soluble dietary fibre (SDF) content as compared to the control (non-enzyme treated) puree as presented in Table 3. An increase in TSS might be due to the degradation of middle lamella and cell wall pectin present in the puree by cellulase, hemicellulase, and pectinase activity in Viscozyme® L, which forms soluble materials such as acid and neutral sugars (Chauhan *et al*., 2017). Similar findings on the physicochemical improvements in TSS, reducing sugar and lightness value are reported in sugar palm (Arsad *et al*., 2015), apricot (Bashir *et al*., 2021), and pear (Amobonye *et al*., 2022).

The a*/b* value of enzyme-treated puree was greener (-0.35) than the control (non-enzymatically treated) puree (-0.28). It may be due to cell wall-degrading enzymes, which facilitate the extraction of chlorophylls and impart green colour to the puree. Meanwhile, enzyme-treated Cu-amaranth purees have higher reducing sugar contents (118.43 mg/mL) than the control puree (12.75 mg/mL).

Table 3. Physicochemical characteristics of non-enzymatically and enzymatically treated Cu-amaranth purees

	Non enzyme-treated	Enzyme-treated *
TSS (°Brix)	2.00 ± 0.15^{b}	4.00 ± 0.20^{a}
pH	5.17 ± 1.78^{a}	4.56 ± 0.34^{b}
L*	18.20 ± 0.08^{a}	18.85 ± 0.06^{a}
a*/b*	-0.28 ± 0.01^{b}	-0.35 ± 0.01^{a}
b*	19.53 ± 0.22^{a}	19.69 ± 0.85^{a}
Reducing sugar (mg/mL)	12.75 ± 0.46^{b}	118.43 ± 0.73^{a}
Total dietary fibre (%)	28.55 ± 0.98^{a}	16.55 ± 0.83^{b}
Soluble dietary fibre (%)	0.60 ± 1.65^{b}	4.32 ± 1.38^{a}
Insoluble dietary fibre (%)	27.94 ± 1.72^{a}	12.23 ± 1.77^{b}

*at optimum conditions with 1% (v/w) Viscozyme® L at pH 5 and 45 °C for 3 hrs.

According to Weinberg *et al*. (1990), reducing sugars were mainly generated from the hydrolysis of cellulose, hemicellulose, pectin,

and other polysaccharides. The enzymatic hydrolysis of these polysaccharides improved the permeability of the cell wall, facilitating better recovery of cell content. The SDF content of Cu-amaranth purees also increased from an initial value of 0.60% to 4.32% after 3 hours of enzymatic liquefaction. Soluble dietary fibres possess prebiotic, hypoglycemic, and hypolipidemic effects (Chawla and Patil, 2010) and thereby provide additional beneficial values to these enzyme-treated purees.

CONCLUSION

The results of this study demonstrate that enzymatic treatment has significantly improved juice yield, total phenolic content, and DPPH scavenging activity. This study has revealed that the enzymatic treatment of Cu-amaranth purees with Viscozyme® L (1% v/w) at pH 5 and 45 °C for 3 hours significantly improved their physicochemical quality characteristics, including total chlorophyll content, antioxidant activity, green colour, reducing sugar, and reduced viscosity. These findings thus suggest the efficacy of cell wall-degrading enzymes in improving the potential application of vegetable juice in the food industry.

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