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Impact of juice extraction method on the physicochemical, functional, and sensory properties of Sabah snake grass (*Clinacanthus nutans*) juice mix

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Abstract

The present work investigated the impact of several juice extraction methods (blender, centrifugal juicer, and slow juicer) and thermal pasteurisation (72°C, 15 s) on the different properties [physicochemical, polyphenol oxidase (PPO) activity, and functional] of *Clinacanthus nutans* juice mix during storage (28 d, 4°C). Regardless of juicing technique, all juices had similar colour and antioxidants [tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) methods]. The juices also had similar PPO activity and sensory acceptance in terms of colour, aroma, flavour, mouthfeel, and overall acceptability. The blender yielded juice with higher pH, soluble solids, and relative viscosity than other methods. The slow juicer was the best at retaining ascorbic acid (39.33 ± 3.06 mg/100 mL), while the blender was best at retaining phenolic compounds (11.82 ± 0.12 mg gallic acid equivalents/100 mL) and chlorophyll (6.95 ± 0.31 µg/mL). Pasteurisation negatively affected the colour, functional properties, and sensory characteristics (colour, aroma, flavour, and mouthfeel) of the juice.

Introduction

Green juice consumption is on the rise nowadays. Green juice is seen as an alternative to healing and maintaining health. The functional compounds in green juice help improve a person's immune system, minimise the risk of cardiovascular diseases, and protect against cancers (Sánchez-Vega *et al.,* 2015). Moreover, various research works have revealed the benefits of vegetables such as kale (Korus and Lisiewska, 2011), bottle gourd (Bhat *et al.,* 2017), cabbage, spinach, broccoli, leek, celery (Boivin *et al.,* 2009), wheatgrass, and pennywort. Sabah snake grass (*Clinacanthus nutans*) is a herbal plant that is rich in phenolic compounds, vitamins, amino acids, and essential minerals (Moses *et al.,* 2015; Nor Hasni *et al.,* 2020). This plant is commonly found in Malaysia and Thailand. The active compounds in Sabah snake grass leaves have anticancer, antioxidant, and anti-inflammatory properties, and are good for treating diabetes and digestive illnesses (Farsi *et al.,* 2016). Many consumers, especially cancer and diabetic patients consume *C. nutans* juice or hot tea (Tiang, 2016).

Many studies have reported the effect of processing technologies on juice quality (Aadil *et al.,* 2015; Kim *et al.,* 2017; Roobab *et al.,* 2018). This effect depends on the type of juicing equipment used. In other words, the mechanism used to convert solid nutrient-dense plant material into juice is crucial in retaining the safety, quality, and sensorial properties of the end product (Gerson Institute, 2013). The Gerson Therapy for Cancer requires a two-step machine with grinder (for size reduction) and hydraulic press (for separation between pulp and juice). The grinder/press can produce juice that has 50 times the amount of certain essential nutrients, and 25 to 50% higher yield as compared to juice produced by other juicers using the same amount of raw material (Gerson Institute, 2013). Kim *et al.* (2017) investigated the effect of different juicing mechanisms on the quality of the end-product. They found that the total polyphenols and monomeric anthocyanin obtained from colloid mill-processed

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samples were approximately 46 and 68% higher, respectively, than gear juicer-processed mulberry juice (Li *et al.,* 2016). Additionally, colloid mill processing generated juice with smaller particle sizes. Studies have also shown that apple juice extracted via a low-speed masticated juicer had superior antioxidant activity and quality as compared to blended juice (Park *et al.,* 2018). Broccoli juice extracted with a juicer had the highest α-glucosidase inhibitory effects (Lee *et al.,* 2013). In blender juicers, solid plant materials are crushed by rotating metal blades (5,000 - 30,000 rpm); while in centrifugal juicers, extraction is achieved via rotating flat blade disks (8,000 - 12,000 rpm) (Kim *et al.,* 2015). In contrast, slow masticating juicers use a squeezing method via a rotating screw and at a lower speed (80 rpm). It then presses the juice through a fine screen (Park *et al.,* 2018).

The present work aimed to examine the impact of different types of juicers on the physicochemical, functional, and sensorial properties of *C. nutans* juice mix. Most studies on juicing methods studied juice extracted from a solid mass of fruit pulp or non-leafy vegetables such as carrots, celery, broccoli, or cauliflower. However, unlike these juices, *C. nutans* juice mix was extracted from soft leafy green *C. nutans,* then added with a small portion of green apple and lemon. The present work also aimed to assess the stability of this juice mix in refrigerated storage, with and without pasteurisation.

Materials and methods

Experimental design

The present work was carried out in three parts. In the first part, juice was prepared using three different equipment, namely a blender (BL), centrifugal juicer (CJ), and a slow juicer (SJ); with or without the thermal treatment of *C. nutans* juice mix. Next, a four-week storage study was conducted on thermally treated and untreated juices prepared using the three equipment. The physicochemical properties, antioxidant activity, chlorophyll content, and PPO content of all three juices were then evaluated. The final part involved assessing consumer acceptance of the samples.

Raw materials

The cuttings of *C. nutans* were obtained fresh from the University Agricultural Park (Serdang, Malaysia), while green apples (South Africa, size S, grade 1, light green) and lemons (Egypt, size M, grade 1, yellow) were purchased at commercial maturity from commercial fruit vendors (Putrajaya, Malaysia).

Preparation of juice mix

The juice was formulated according to Nor Hasni *et al.* (2020). The formulation was based on a common practice among cancer patients that seek alternative healing sources (Tiang, 2016). The ingredients for one serving were made up of 13 g of leaves (approximately 50 leaves), a medium-sized green apple, a quarter of lemon, and 250 mL of distilled water. Only the top stems were plucked from the mother plant. The leaves were hand-plucked from the stem and washed twice under running tap water. Soil, dead leaves, and excess stems from the *C. nutans* were removed using a stainless-steel knife, while green apples and lemons were washed using potable water. The apples were cored, and the peels as well as seeds in the lemon were removed.

Methods of juicing and pasteurisation

Three juicing methods (BL, CJ, and SJ) were applied to prepare the *C. nutans* juice mix. Six replicates were set for each treatment, and each replicate had two representative samples for analytical purposes. All representative samples were stored in clean and sterile glass bottles, and properly capped. The BL juice was produced using a blender (Blender MX-GM1011, Panasonic, Osaka, Japan), and centrifuged at 12,000 rpm for 90 s. The BL juice was filtered using a nylon filter with a mesh number of 60. The juice produced using CJ (Juice Extractor CJX-420W, Cornell Appliances, Selangor, Malaysia) was centrifuged at 15,000 rpm for 10 s. The juice produced using SJ (Kuvings C7000, NUC Electronics Co. Ltd., Daegu, South Korea) was centrifuged at 60 rpm for 30 s. Three replicates were taken from each juicing method, pasteurised in a water bath at 72 ± 1°C for 15 s, and labelled as BL*, CJ*, and SJ*, respectively (Amir, 2017). After these treatments, the juices were cooled (20 ± 1°C) under running tap water while the unpasteurised juices served as control.

Storage analysis

The raw and pasteurised juices were stored under refrigerated conditions (4 ± 1°C) for 4 w, and all the analyses were performed after every 7 d.

pH, total soluble solids (TSS), and titratable acidity

The pH of the juice samples was measured using a pH meter (Model No. 3505, Jenway, Essex, UK), and the °Brix was determined using a digital refractometer with a scale ranging from 0 - 45% (PR-101, Atago, Japan). The titratable acidity (TA) was determined following the method explained by Liu *et al.* (2016), i.e. via titration with standardised 0.1 mol/L NaOH (Classic Chemical Sdn. Bhd., Malaysia)

set to reach pH 8.1 by an automatic potentiometric titrator (751 GPD titrino, Metrohm, Switzerland). TA was analysed in triplicate, and expressed as malic acid. The TA of the samples was calculated using Eq. 1:

$$\text{TA (\%)} = \frac{C \times V_2 \times K}{V_1} \times \frac{V_0}{W} \times 100 \quad \text{(Eq. 1)}$$

where, C = standardised NaOH concentration (0.1 M); W = total weight of the sample (g); V_2 = volume of NaOH used (mL); V_1 = volume of the sample used (mL); V_0 = total volume of sample (mL); and K = conversion factor of malic acid.

Viscosity

The viscosity of the juice samples was measured using a Brookfield viscometer (Brookfield DV-II+ Pro, Brookfield Engineering Laboratories, Inc., MA, USA).

Colour

The colour of the juice samples was determined using a Hunter Lab colorimeter (UltraScan Pro, Hunter Associates Laboratory, Inc., Reston, VA, USA) with a D65 optical sensor. The colour of the juice samples was evaluated using the *L*, *a*, and *b* values; where *L* and the two colour-coordinated *a* and *b* values were measured as described by Granato *et al.* (2010). Chroma (C) and hue angle (h) were determined using Eqs. 2 and 3:

$$\text{chroma, } C = [(a^2 + b^2)^{1/2}] \quad \text{(Eq. 2)}$$

$$\text{hue angle, } h = [\tan^{-1}(b/a)] \quad \text{(Eq. 3)}$$

where, the *a*-axis represents the green-red component, and the *b*-axis represents the blue-yellow component.

Ascorbic acid content

The ascorbic acid content of the juice samples was determined following the 2,6-dichlorophenolindophenol visual titration method described by AOAC (2006). First, a standard indophenol solution was prepared by dissolving 0.05 g of 2, 6-dichloroindophenol in distilled water. This mixture was then made up to 100 mL, and filtered. A pure ascorbic acid standard solution was prepared by dissolving 0.05 g of pure ascorbic acid in 60 mL of 20% acetic acid. This mixture was then diluted with distilled water to exactly 250 mL. The dye solution was then standardised against the ascorbic acid solution via titration with 10 mL of ascorbic acid solution until a faint pink colour persisted for 15 s. The concentration was then expressed as mg ascorbic acid equivalent to mL of the dye solution. The ability of the ascorbic acid to reduce the oxidation-reduction indicator dye 2-6 dichloroindophenol indicated its concentration.

Total phenolic content

The total phenolic content was determined according to the Folin-Ciocalteu method with slight modifications (Ismail *et al.,* 2013). First, 0.2 mL of the supernatant sample obtained was added into 1 mL of 1:10 diluted Folin-Ciocalteu reagent (Merck KGaA, Germany). About 0.8 mL of 7% sodium carbonate (Merck KGaA, Germany) was added into the mixture after 5 min. The mixture was then incubated at room temperature for 90 min. The absorbance was measured at 765 nm using a UV-visible spectrophotometer (Genesys 10S UV-VIS; Thermo Scientific, Waltham, MA, USA). A calibration curve was plotted using a gallic acid standard solution of (0 - 250) mg/L. The result was expressed as mg gallic acid equivalent (mg GAE/100 mL).

DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity assay

First, 1 mL of 0.1 mM DPPH solution was added to 0.2 mL of the supernatant sample prepared as mentioned above (Kim, 2015). The absorbance was measured at 517 nm using a spectrophotometer. Methanol was used as a blank, and gallic acid as a standard solution (Ismail *et al.,* 2013). The antioxidant activity was calculated using Eq. 4:

$$\text{\% DPPH· scavenging activity} = \frac{A_0 - A_1}{A_0} \times 100 \quad \text{(Eq. 4)}$$

where, A_0 = absorbance of the control, and A_1 = absorbance of the sample.

Ferric reducing antioxidant power (FRAP) assay

A fresh FRAP reagent was prepared by mixing 25 mL of 300 mM acetate buffer and 2.5 mL of 10 nM TPTZ solution with 40 mM HCl and 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$ solution. This mixture was then warmed to 37°C before use. The supernatant (0.1 mL) sample was prepared as mentioned above (Kim, 2015). The sample was added to 1 mL of the FRAP solution, followed by 1 mL of DW. The absorbance of the reaction mixture was measured at 593 nm after incubation at 37°C for 4 min using a spectrophotometer. Standard solutions

of $FeSO_4 \cdot 7H_2O$ (50 - 300 mg/L) were used as the calibration curve. The result was expressed as mM Fe^{2+} per 100 mL sample (Sripakdee *et al.,* 2015).

Chlorophyll content

Chlorophyll content was determined using the Arnon method with slight modifications (Rajalakshmi and Banu, 2015). First, 10 mL of juice was extracted from a 40 mL mixture of acetone and water at a ratio of 80%:20% (v/v), and then poured into a centrifuge tube. This mixture was left for 30 min in the dark. Later, the juice was filtered via Whatman paper no. 1 and centrifuged at 10,000 rpm at 4°C for 10 min. The supernatant obtained was filled into a cuvette, and the absorption was measured at 663 and 645 nm using a spectrophotometer.

Polyphenol oxidase (PPO) enzyme

The extraction solution was made up with 0.2 mol/L sodium phosphate buffer of pH 6.5 (90 mL) consisting of 4% (w/v) polyvinylpyrrolidone, 1% Triton X-100, and 1 mol/L sodium chloride (5 mL). About 5 mL of the extraction solution in a test tube was added to 5 mL of *C. nutans* juice, and then thoroughly mixed in an ice pack. The mixture was centrifuged at 10,000 *g* for 20 min at 4°C. The supernatant was filtered using Whatman No. 1 filter paper to obtain crude enzyme extract.

The reaction mixture consisted of 0.1 mL of the enzyme extract and 3.0 mL of 0.07 mol/L catechol in 0.05 mol/L SPB (pH 6.5). The blank was prepared in the same way, but the crude enzyme extract was replaced with 0.2 mol/L SPB (pH 6.5). PPO enzymes were measured at an absorbance of 420 nm for 10 min using a spectrophotometer. The activity of the enzyme was expressed as the change of absorbance per min per 0.1 mL of *C. nutans* juice (Engmann *et al.,* 2014).

Consumer acceptability study

The sensory analysis of the juice samples was conducted among 30 untrained panellists who normally drink juice. The panellists were 10 - 58 years old. The sensory evaluation was carried out in monadic form at the university's Sensory Laboratory. The juice was prepared, pasteurised, and refrigerated overnight before evaluation. All samples were randomly coded with a three-digit number. Sensory attributes such as colour, aroma, flavour, mouthfeel, and overall acceptability were evaluated using a 9-point hedonic scale, with higher scales indicating a stronger acceptance of the specific attribute.

Statistical analysis

All samples were analysed in triplicate, and the values were expressed as mean ± standard deviation (SD). MINITAB 16 software was used to perform a one-way analysis of variance (ANOVA). Tukey's test was performed to determine significant differences among the means. The significance was set at $p < 0.05$, with a 95% confidence level.

Results and discussion

pH, TA, and TSS

The present work tested three juicing methods that produced juice with significantly different ($p < 0.05$) pH, TA, and TSS values (Table 1). The BL juice had the highest pH (3.46), while SJ and CJ produced juices with similar pH values (3.28). The juicing method did not affect acidity, but the juice extracted using BL had a significantly higher ($p < 0.05$) TSS value than CJ and SJ. When preparing BL juices, all solid and liquid materials were crushed, and the solid materials and juice were blended together. Meanwhile, the separation process in SJ and CJ removed most of the solid material from the juice. Thus, more solid materials were present in BL juices, as indicated by its TSS value.

The juice extracted using all three methods showed a significant decrease ($p < 0.05$) in pH after 2 w of storage. This decrease in pH seemed to correlate well with microbial growth (Koh *et al.,* 2016). Juices containing fruits with relatively high levels of sugar and low pH favour the growth of yeasts, moulds, and some acid-tolerant bacteria (Rawat, 2015). Although the reduced pH suggests the presence of microorganisms in unpasteurised juice, this reduction was only very slight as indicated by the acidity value, which remained the same throughout storage. After 1 w of storage, the TSS value of all the juices significantly decreased ($p < 0.05$), but all of them remained stable regardless of the juicing method. Pasteurisation did not affect the pH, acidity, or soluble solid content of all the juices prepared using the three juicing methods. Pasteurised juice had greater stability because less glucose fermentation occurred after pasteurisation since microorganisms were eliminated, and hence, the pH value did not change that much throughout storage. Pasteurisation delayed the decrease ($p < 0.05$) in the pH of the juices until the fourth week of storage. Apart from that, all pasteurised juices regardless of the juicing method did not show any difference ($p \geq 0.05$) in TA or TSS values throughout the four weeks of storage.

Viscosity

Table 1. Effects of juicing methods on pH, titratable acidity (TA), and total soluble solids (TSS) of *C. nutans* juice.

Parameter	Week	Method					
		BL	CJ	SJ	BL*	CJ*	SJ*
pH	0	3.46 ± 0.03^{Aa1}	3.27 ± 0.02^{Abc}	3.28 ± 0.02^{Abc}	3.39 ± 0.05^{Aab}	3.26 ± 0.06^{Ac}	3.24 ± 0.03^{Abc}
	1	3.43 ± 0.03^{ABa}	3.27 ± 0.02^{Ab}	3.26 ± 0.02^{Ab}	3.40 ± 0.06^{Aa}	3.19 ± 0.03^{Ab}	3.23 ± 0.03^{Ab}
	2	3.36 ± 0.01^{BCab}	3.23 ± 0.03^{Ac}	3.25 ± 0.05^{Ac}	3.45 ± 0.05^{Aa}	3.20 ± 0.05^{Ac}	3.29 ± 0.02^{Abc}
	3	3.33 ± 0.03^{Ca}	3.14 ± 0.03^{Bb}	3.17 ± 0.01^{Bb}	3.38 ± 0.06^{Aa}	3.19 ± 0.05^{Ab}	3.20 ± 0.03^{Ab}
	4	3.02 ± 0.03^{Da}	2.83 ± 0.03^{Cb}	2.86 ± 0.03^{Cb}	3.05 ± 0.07^{Ba}	2.79 ± 0.02^{Bb}	2.87 ± 0.04^{Bb}
TA (%)	0	0.51 ± 0.03^{Ab}	0.50 ± 0.04^{Ab}	0.65 ± 0.03^{Aa}	0.51 ± 0.07^{Ab}	0.44 ± 0.02^{Bb}	0.51 ± 0.02^{Bb}
	1	0.50 ± 0.02^{Aab}	0.45 ± 0.07^{Ab}	0.62 ± 0.03^{Aab}	0.58 ± 0.07^{Aab}	0.57 ± 0.06^{Aab}	0.65 ± 0.10^{Aa}
	2	0.50 ± 0.03^{Aab}	0.45 ± 0.03^{Ab}	0.58 ± 0.05^{Aa}	0.50 ± 0.07^{Aab}	0.46 ± 0.02^{Bab}	0.54 ± 0.02^{ABab}
	3	0.52 ± 0.04^{Aa}	0.55 ± 0.06^{Aa}	0.59 ± 0.06^{Aa}	0.53 ± 0.07^{Aa}	0.47 ± 0.02^{Ab}	0.56 ± 0.02^{Aab}
	4	0.51 ± 0.03^{Aa}	0.55 ± 0.05^{Aa}	0.60 ± 0.04^{Aa}	0.50 ± 0.06^{Aa}	0.51 ± 0.03^{ABa}	0.58 ± 0.02^{ABa}
TSS (%)	0	5.13 ± 0.11^{Aa}	4.10 ± 0.52^{Ab}	4.20 ± 0.36^{Ab}	4.60 ± 0.40^{Aab}	4.20 ± 0.20^{Ab}	4.33 ± 0.15^{Aab}
	1	4.96 ± 0.15^{Aa}	3.53 ± 0.50^{Ab}	3.86 ± 0.37^{Aab}	4.50 ± 0.36^{Aa}	4.16 ± 0.15^{Aab}	4.30 ± 0.17^{Aab}
	2	4.93 ± 0.15^{Ba}	3.40 ± 0.81^{Ab}	3.90 ± 0.20^{Aab}	4.60 ± 0.36^{Aa}	4.23 ± 0.25^{Aab}	4.43 ± 0.15^{Aab}
	3	4.83 ± 0.15^{Ba}	3.50 ± 0.62^{Ab}	3.83 ± 0.35^{Aab}	4.63 ± 0.32^{Aa}	4.33 ± 0.40^{Aab}	4.40 ± 0.26^{Aab}
	4	4.83 ± 0.11^{ABa}	3.60 ± 0.30^{Ab}	3.56 ± 0.37^{Ab}	4.63 ± 0.41^{Aa}	4.16 ± 0.20^{Aab}	4.46 ± 0.05^{Aa}

Data are means ± standard deviation of triplicates ($n = 3$). Means with different letters in the same column are significantly different ($p < 0.05$) from each other. BL: blender, CJ: centrifugal juicer, SL: slow juicer, BL*: blender and pasteurisation, CJ*: centrifugal juicer and pasteurisation, and SL*: slow juicer and pasteurisation.

The raw juice samples extracted using the various treatments showed a significant difference ($p < 0.05$) in viscosity. In specific, BL juice (4.81 ± 0.28 cP) was more viscous than CJ (3.86 ± 0.14 cP) and SJ (3.97 ± 0.09 cP). The difference in viscosity value was due to the different extraction methods to produce the juice. The BL juice had a higher solid content than the CJ and SL juices, as indicated by the higher soluble values in the former. Unlike the slow juicer and the centrifugal juicer, the blender did not separate the pulp or solid component of the plant materials from the juice. All parts were macerated and then filtered using a nylon filter, hence leading to a higher viscosity value. When comparing the pasteurised juice and the unpasteurised juice, pasteurised BL juice (5.67 ± 0.18 cP) had a higher viscosity than unpasteurised juice, while the pasteurised juice from SJ (3.90 ± 0.23 cP) and CJ (4.01 ± 0.18 cP) showed no significant ($p \geq 0.05$) difference as compared to the initial viscosity. It is possible that the solids in BL juice had swelled when exposed to heat, and the water components were trapped in the swollen matrix thus resulting in higher viscosity values. However, the CJ and SJ juice did not experience any swelling because both contained less soluble solids. During storage, the viscosity value of all the juices remained stable.

Ascorbic acid

There was a notable variation ($p < 0.05$) in the ascorbic acid content of the juices prepared using different methods. For instance, SJ had the highest ascorbic acid content (39.33 ± 3.06 mg/100 mL), followed by BL juice (30.33 ± 1.53 mg/100 mL), and CJ juice (30.33 ± 0.58 mg/100 mL). During the juice preparation, the slow juicer macerated and squeezed the juice out at a slow and continuous speed. Thus, the juice did not oxidise too much, and less heat was generated in the process. In contrast, a CJ or a blender promoted oxidation through the high-speed rotation of its metal blade. Both methods also generate a lot of heat during juicing (Kim *et al.,* 2015). In one study, BL (0.40 ± 0.03 mg/100 mL) and high-speed CJ (0.07 ± 0.01 mg/100 mL) produced juices with lower ascorbic acid content than the low-speed masticating juicer (0.78 ± 0.10 mg/100 mL) (Kim *et al.,* 2017). Pasteurisation reduced ($p < 0.05$) the ascorbic acid

content in all samples regardless of juicing method. Ascorbic acid is known to be a heat-sensitive bioactive compound; hence, the loss of ascorbic acid is greatly influenced by thermal processing through the aerobic pathway (Rabie *et al.,* 2015). Both the pasteurised and unpasteurised samples prepared via different juicing methods showed a significant decreasing trend ($p < 0.05$) in all sensory values throughout storage due to the oxidation of ascorbic acid (Kaddumukasa *et al.,* 2017).

Colour attributes

Table 2 shows no significant difference ($p \geq 0.05$) in *L* (lightness), *a* (redness), or *b* (yellowness) between the unpasteurised juices obtained via different juicing methods. Also, no significant difference ($p \geq 0.05$) was seen in the chroma and hue angle. Similar findings were reported by Lee *et al.* (2013), i.e., there was no significant difference ($p \geq 0.05$) in the *L, a,* and *b* values of broccoli juice made using SJ, CJ, and BL. Chroma values remained the same even after pasteurisation. However, the pasteurised juices showed significant differences ($p < 0.05$) in the *a* value as well as the hue angle. As the juice samples underwent thermal treatment (72 ± 1°C for 15 s), the juice samples changed from bright green to yellowish-green due to the browning effect of pasteurisation. This result correlated with an increase in the *a* value, indicating the loss of the green colour after thermal treatment. It may be possible that the chlorophyll was degraded to pheophytins (Armesto *et al.,* 2017). Chen *et al.*

Table 2. Effects of juicing methods on colour attributes of *C. nutans* juices.

Parameter	Week	Methods					
		BL	CJ	SJ	BL*	CJ*	SJ*
L	0	32.88 ± 1.58^{Aab}	31.37 ± 1.75^{Ab}	31.10 ± 0.44^{Ab}	35.93 ± 1.51^{Aa}	31.22 ± 1.31^{Ab}	32.19 ± 0.81^{Ab}
	1	32.28 ± 1.44^{Ab}	31.04 ± 1.33^{Ab}	31.39 ± 0.48^{Ab}	36.35 ± 1.66^{Aa}	32.07 ± 1.11^{Ab}	32.99 ± 0.37^{Ab}
	2	32.66 ± 1.43^{Ab}	32.83 ± 1.75^{Aab}	31.50 ± 0.34^{Ab}	36.14 ± 1.67^{Aa}	31.86 ± 1.11^{Ab}	32.72 ± 0.44^{Aab}
	3	32.08 ± 1.40^{Aa}	32.99 ± 1.65^{Aa}	33.90 ± 2.82^{Aa}	35.41 ± 1.44^{Aa}	33.23 ± 1.69^{Aa}	32.29 ± 0.36^{Aa}
	4	30.44 ± 1.63^{Ab}	29.46 ± 1.13^{Ab}	30.92 ± 0.54^{Ab}	35.99 ± 2.03^{Aa}	31.65 ± 0.71^{Ab}	30.92 ± 0.54^{Ab}
a	0	-4.19 ± 0.56^{Bb}	-3.53 ± 0.60^{Bb}	-3.81 ± 0.23^{Cb}	-1.48 ± 0.25^{Ca}	-1.28 ± 0.20^{Aa}	-1.41 ± 0.08^{Ba}
	1	-3.54 ± 0.06^{Bd}	-2.74 ± 0.10^{ABc}	-3.23 ± 0.10^{Bd}	-0.99 ± 0.15^{Ba}	-1.37 ± 0.10^{Ab}	-1.15 ± 0.14^{ABab}
	2	-2.50 ± 0.26^{Ad}	-1.96 ± 0.21^{Ac}	-2.61 ± 0.06^{Ad}	-0.65 ± 0.12^{ABa}	-1.32 ± 0.17^{Ab}	-0.93 ± 0.18^{Aab}
	3	-2.06 ± 0.40^{Ad}	-1.70 ± 0.13^{Acd}	-2.26 ± 0.19^{Ad}	-0.56 ± 0.05^{Aa}	-1.19 ± 0.10^{Abc}	-0.82 ± 0.20^{Aab}
	4	-2.36 ± 0.21^{Ac}	-1.95 ± 0.75^{Abc}	-2.72 ± 0.33^{ABc}	-0.88 ± 0.12^{Aab}	-1.32 ± 0.13^{Aab}	-1.07 ± 0.02^{ABab}
b	0	6.84 ± 1.24^{Aab}	5.45 ± 1.28^{Aab}	5.68 ± 0.54^{Aab}	8.17 ± 1.00^{Aa}	5.38 ± 0.13^{Ab}	6.05 ± 0.51^{Aab}
	1	6.81 ± 0.79^{Aab}	5.29 ± 0.98^{Abc}	5.43 ± 0.41^{Abc}	7.93 ± 1.04^{Aa}	4.71 ± 0.31^{ABc}	5.67 ± 0.32^{Abc}
	2	6.48 ± 0.93^{Aab}	5.30 ± 1.03^{Ab}	5.60 ± 0.51^{Ab}	7.85 ± 1.00^{Aa}	4.97 ± 0.39^{ABb}	5.78 ± 0.28^{Ab}
	3	6.18 ± 0.93^{Aab}	5.50 ± 1.21^{Aab}	5.60 ± 0.65^{Aab}	7.22 ± 0.76^{Aa}	4.41 ± 0.24^{Bb}	5.51 ± 0.37^{Aab}
	4	6.64 ± 0.66^{Aab}	4.43 ± 1.57^{Ab}	6.30 ± 0.67^{Aab}	8.51 ± 1.13^{Aa}	5.01 ± 1.03^{Ab}	6.06 ± 0.67^{Aab}
Chroma	0	8.02 ± 1.35^{Aab}	6.49 ± 1.40^{Aab}	6.84 ± 0.57^{Aab}	8.31 ± 1.02^{Aa}	5.54 ± 0.09^{Ab}	6.21 ± 0.49^{Aab}
	1	7.68 ± 0.72^{Aab}	5.97 ± 0.87^{Abc}	6.32 ± 0.40^{Aabc}	7.99 ± 1.05^{Aa}	4.91 ± 0.27^{Ac}	5.78 ± 0.30^{Ac}
	2	6.95 ± 0.92^{Aab}	5.66 ± 0.95^{Ab}	6.18 ± 0.45^{Aab}	7.88 ± 1.00^{Aa}	5.15 ± 0.33^{Ab}	5.85 ± 0.26^{Ab}
	3	6.52 ± 0.97^{Aab}	5.76 ± 1.19^{Aab}	6.04 ± 0.66^{Aab}	7.24 ± 0.76^{Aa}	4.57 ± 0.24^{Ab}	5.57 ± 0.34^{Aab}
	4	7.05 ± 0.68^{Aab}	4.84 ± 1.74^{Ab}	6.87 ± 0.74^{Aab}	8.55 ± 1.14^{Aa}	5.19 ± 1.01^{Ab}	6.15 ± 0.66^{Aab}
Hue angle	0	121.62 ± 1.30^{Aa}	123.20 ± 1.63^{Aa}	123.90 ± 1.20^{Aa}	100.29 ± 1.32^{Ac}	103.39 ± 2.37^{Ab}	103.20 ± 1.70^{Abc}
	1	117.60 ± 2.46^{Aa}	117.78 ± 4.16^{ABa}	120.82 ± 1.09^{Aa}	97.14 ± 0.63^{Bc}	112.24 ± 8.39^{Aab}	101.52 ± 1.95^{Abc}
	2	111.24 ± 2.17^{Ba}	110.75 ± 4.54^{BCa}	115.15 ± 2.25^{Ba}	94.77 ± 0.78^{Cb}	110.08 ± 7.24^{Aa}	99.24 ± 2.03^{Ab}
	3	108.46 ± 2.50^{Bab}	107.58 ± 2.70^{Cab}	112.04 ± 1.44^{Ba}	94.49 ± 0.31^{Cc}	105.18 ± 1.30^{Ab}	98.63 ± 2.59^{Ac}
	4	109.64 ± 1.22^{Ba}	113.55 ± 1.65^{BCa}	113.37 ± 1.01^{Ba}	95.95 ± 0.61^{BCc}	105.12 ± 2.88^{Ab}	100.12 ± 1.01^{Ac}

Data are means ± standard deviation of triplicates ($n = 3$). Means with different letters in the same column are significantly different ($p < 0.05$) from each other. BL: blender, CJ: centrifugal juicer, SL: slow juicer, BL*: blender and pasteurisation, CJ*: centrifugal juicer and pasteurisation, and SL*: slow juicer and pasteurisation.

(2015) conducted similar research on the colour parameters of green asparagus juice that had undergone thermal processing. The results showed that the *a* value of the control (1.57 ± 0.04) decreased to 0.44 ± 0.02 after the juice was heated. The *a* value also increased significantly ($p < 0.05$) during storage. Because the hue angle is inversely proportional to the *a* value, the increase in the a value resulted in a decline in the hue angle. On the other hand, pasteurised juices were stable during storage showing no significant difference ($p \geq 0.05$) in the *L, a,* and *b* values or chroma and hue angle throughout storage. The changes in the unpasteurised juice colour during storage may be due to the enzyme browning by active PPO naturally present in the juice.

Total phenolic content and antioxidant activity

Figure 1A shows similar total phenolic content of the juice regardless of the juicing method. In fact, raw juice extracted using BL had slightly higher phenolic content than the SJ and CJ samples due to a higher fibre and pulp content (Pyo *et al.,* 2014). The BL samples contained many parts of the apple peel as compared to SJ or CJ, and apple peels also contain phenolics. Pasteurisation did not affect the total phenolic content of all the juices. The duration of storage negatively affected the total phenolic content of the juice mix as shown by the downward trend in Figure 1A. Oxygen from the surroundings induced the oxidation of the phenolic compounds in the juice resulting in the formation of melanin pigment, which in turn, caused a reduction in the polyphenol content and browning of the juices (Queiroz *et al.,* 2008). The polyphenol structure might also be degraded by oxidation and

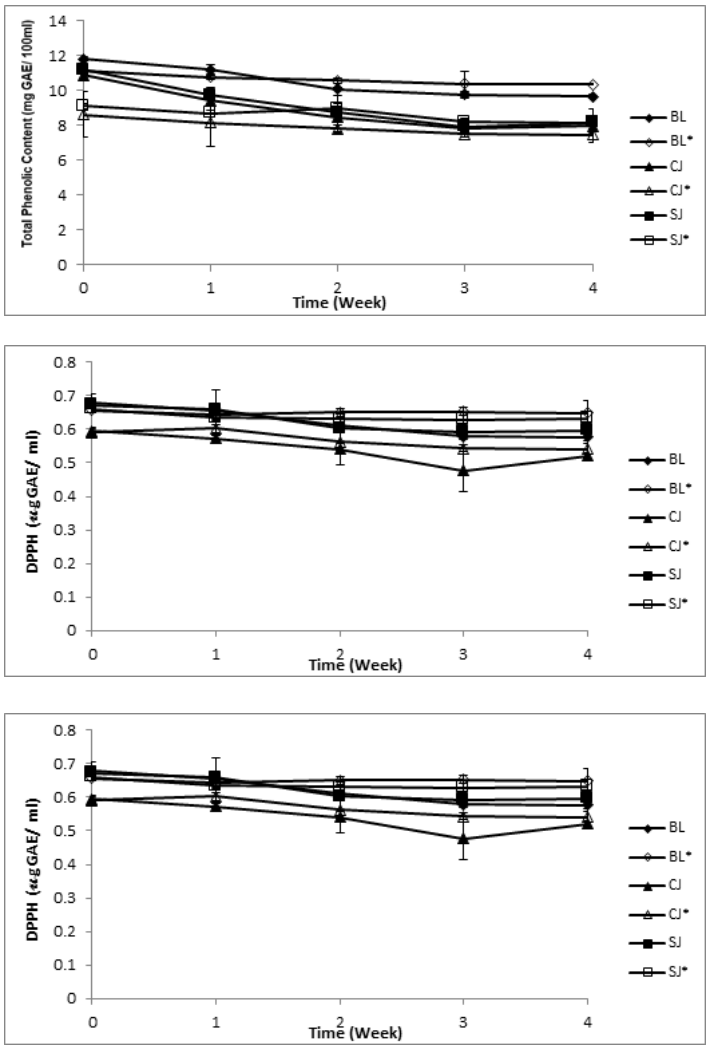

Figure 1. Effect of different juicing methods and pasteurisation on total phenolic content and antioxidant activity of *C. nutans* juice stored at 4°C. (A) total phenolic content, (B) DPPH scavenging activity, and (C) ferric reducing antioxidant power. BL: blender, CJ: centrifugal juicer, SL: slow juicer, BL*: blender and pasteurisation, CJ*: centrifugal juicer and pasteurisation, and SL*: slow juicer and pasteurisation.

polymerisation when exposed to light if the juices are stored in transparent glass bottles (Ali *et al.,* 2018). There is also the possibility of PPO enzymes that are naturally present in the juice and act on the phenolic compounds (Queiroz *et al.,* 2008).

The DPPH radical scavenging activity of unpasteurised BL juice (0.68 ± 0.027 µg GAE/mL) was not significantly ($p \geq 0.05$) different from SJ (0.67 ± 0.003 µg GAE/mL) and CJ (0.59 ± 0.011 µg GAE/mL). Figure 1B shows that the DPPH scavenging activity of CJ was slightly lower, possibly due to the instant heat produced by the friction of the high-speed spinning, which degrades the antioxidant content (Kim *et al.,* 2015). Pasteurisation did not affect the DPPH scavenging activity of all juices. Flavonoid is a heat-stable compound present in *C. nutans* juice (Alam *et al.,* 2016). This compound was not affected by the 72°C pasteurisation treatment. The pasteurised juice also displayed better stability than unpasteurised juice during storage.

Figure 1C shows that the FRAP values of unpasteurised juice were not significantly ($p < 0.05$) affected by the juicing method. The FRAP values of BL juice (24.54 ± 0.28 mM Fe^{2+}/100 mL) were similar to SJ (24.42 ± 0.17 mM Fe^{2+}/100 mL) and CJ (23.90 ± 0.23 mM Fe^{2+}/100 mL). Overall, the FRAP values obtained from all the juices decreased over storage time. This is because the antioxidant content in the juices was reduced due to the enzyme oxidation of the PPO enzyme (Queiroz *et al.,* 2008) and light oxidation (Ali *et al.,* 2018).

Chlorophyll and PPO enzyme activity

Figure 2A shows that the highest value of chlorophyll content was in unpasteurised BL (6.95 ± 0.31 µg/mL), followed by SJ (5.26 ± 0.50 µg/mL) and CJ (3.19 ± 0.36 µg/mL). This result might be due to the juice produced by the blenders that had higher fibre and pulp content as compared to CJ. Additionally, the high-speed spinning in CJ could result in more oxygen in the juice and could cause oxidative degradation of the chlorophyll content (Vila *et al.,* 2015). In SJ, most of the chlorophyll from the leafy materials was discarded during pressing. The pasteurised juice had a lower chlorophyll content than unpasteurised juice possibly due to the cellular acid released during heat treatment in the former, which can cause a change in pH and lead to the conversion of chlorophyll into pheophytin, such as in the case of peas. Chlorophyllase enzymes in juices can hydrolyse pheophytins to form pheophorbides when the temperature ranges from 60 to 82.2°C (Erge *et al.,* 2008). The chlorophyll content of all juices decreased during storage. During storage, glucose fermentation caused a decrease in the pH value of the juices and resulted in the conversion of chlorophyll to pheophytin (Erge *et al.,* 2008). The chlorophyll

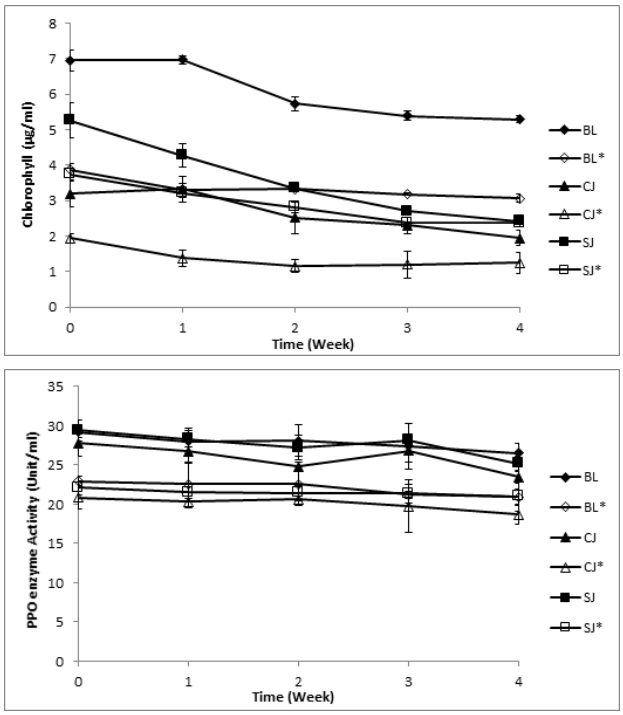

Figure 2. Effect of different juicing methods and pasteurisation on chlorophyll and polyphenol oxidase enzyme content in *C. nutans* juice stored at 4°C. BL: blender, CJ: centrifugal juicer, SL: slow juicer, BL*: blender and pasteurisation, CJ*: centrifugal juicer and pasteurisation, and SL*: slow juicer and pasteurisation.

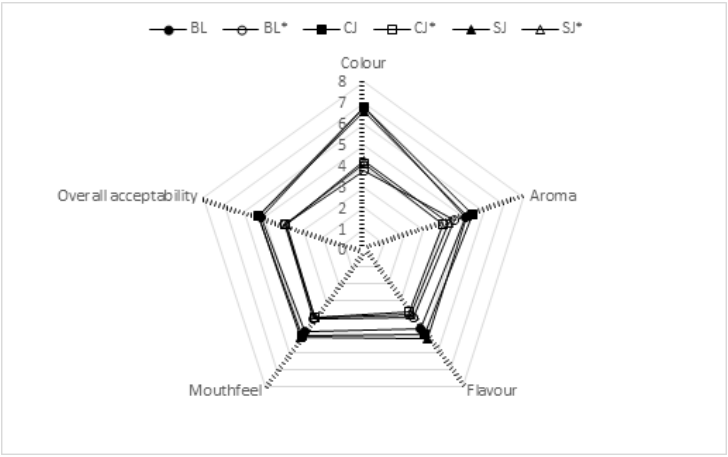

Figure 3. Sensory analysis of *C. nutans* juices. BL: blender, CJ: centrifugal juicer, SL: slow juicer, BL*: blender and pasteurisation, CJ*: centrifugal juicer and pasteurisation, and SL*: slow juicer and pasteurisation.

content in juice could also oxidatively degrade when the juice is exposed to light and air (Su *et al.,* 2010).

PPO is a copper-containing enzyme that is present in all fruit and vegetable products (Aadil *et al.,* 2015). Although the juicing method (unpasteurised) did not show any significant differences ($p \geq 0.05$) in PPO enzyme activity, some enzyme activity was still detected (Figure 2B). Unfortunately, the pasteurisation conditions used in the present work were not sufficient to completely inactivate the enzymes. Pasteurisation, on the other hand, reduced the PPO activity in all juices regardless of the juicing method. PPO activity did not result in any colour change in the unpasteurised juices as indicated by the colour values. It is important to note that the present work pasteurised the juice in a water bath, introducing a significant limitation. If a heat exchanger were used, the results could have been different.

Sensory analysis

Figure 3 shows the average scores of the different sensory attributes of *C. nutans* juice mix extracted using various juicing methods. The result indicated no significant difference ($p \geq 0.05$) in colour, aroma, flavour, or mouthfeel for the juice prepared from different juicing methods. However, the pasteurised juice scored significantly lower ($p < 0.05$) colour, aroma, flavour, and mouthfeel values than unpasteurised juice since pasteurised juice presents a "cooked aroma". According to Cullen *et al.* (2012), when applying thermal treatment, the volatile compounds in watermelon juice significantly decreased, while new compounds such as those representing a cooked odour were formed. Moreover, the flavour of pasteurised juices is less pleasant as compared to unpasteurised juices. Bitter and less sweet characteristics were also noted in the pasteurised juice. The panellists found the unpasteurised juice more acceptable. In other words, freshly produced juice had better sensory qualities than thermally treated juice regardless of the juicing method employed.

Conclusion

Sabah snake grass (*C. nutans*) juice mix produced via three different juicing methods (blender, slow juicer, and centrifugal juicer) shared similar colour, DPPH and FRAP values, PPO activity, and sensory acceptance. The slow juicer was the best method to retain ascorbic acid in the juice, whereas the blender produced a less acidic, more viscous juice, and higher in chlorophyll and phenolic contents. Although the pasteurised juices were more stable during storage, the thermal process caused negative changes in colour, functional properties, and sensory attributes of the juice.

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