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Simultaneous development of cloud stability and antioxidant preservation in cloudy guava juice using hydrocolloid combinations

^{1,2}*Mousa, R. M. A.

¹Department of Home Economic, Faculty of Specific Education, Assiut University, 71516 Assiut, Egypt ²Department of Biochemistry, College of Science, University of Jeddah, P.O. 80327, 21589 Jeddah, Saudi Arabia

<u>Article history</u>

<u>Abstract</u>

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Keywords

cloud stability, antioxidant preservation, guava juice quality, hydrocolloid additives, sensory Cloudy guava juice (CGJ) is one of the most popular rich sources of natural antioxidants, such as vitamin C (L-ascorbic acid, AA). The most challenging issue facing CGJ is the severe loss of antioxidants following the mitigation of cloud stability during juice processing or storage. The effects of food hydrocolloids, e.g. gum arabic (GA), pectin, and sodium carboxymethyl cellulose (CMC), and their binary combinations as natural additives in CGJs on the simultaneous development of cloud stability and AA preservation during juice processing and during 14 months of storage at 4 and 25°C were firstly investigated. Among the studied hydrocolloids, the binary incorporation of GA (0.05 g/100 mL juice) and CMC (0.3 g/100 mL juice) showed the largest improvement in CGJ (G2C2 sample) quality. The cloud stability of the G2C2 sample was approximately 97%, which was 4.4 and 6.3 times higher than the control sample without hydrocolloids at 4 and 25°C after 14 months of storage, respectively. Consequently, the G2C2 sample significantly inhibited AA degradation, and yielded an AA content of approximately 130 mg AA/100 mL juice, which was 2.6 and 6.6 times higher than control sample at 4 and 25°C, respectively. Zeta-potential (ζ) and viscosity (η) were used to investigate the potential effect of hydrocolloid combinations on the cloud stability. The G2C2 sample had a ζ potential of -31 mV and a viscosity of 17 cP providing better cloud stability when compared with other samples. The physicochemical properties of G2C2 sample were: pH, 4.2 ± 0.5 ; total titratable acidity, 0.7 ± 0.1 g citric acid/100 mL; °Brix, 9.5 ± 0.1 ; % scavenging activity, 540.3 ± 0.9 %; total phenolic compounds, 63.6 ± 1.0 mg GAE/L; and colour coordinates, 70.8 ± 0.6 L*, 0.5 ± 0.1 a*, and 14.5 ± 0.2 b*. Consumer acceptability of the G2C2 sample was also investigated, giving overall acceptability of more than 6.8 hedonic score without any deterioration in juice safety. The proposed simple hydrocolloids composite could increase the health benefits of cloudy juices.

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Introduction

The increasing awareness in the public about nutritious juices with bio-functional ingredients has made the fruit juice industry as one of the largest agribusinesses in the world (Al-juhaimi et al., 2018). The consumption of fresh fruit juice, such as guava juice, has grown due to the natural and rich nutrient contents, such as vitamin C (Ninga et al., 2018). Fresh guava juice contains as much as three to six times higher vitamin C than orange juice, and provides a unique aroma and mouthfeel (Vijaya Kumar Reddy et al., 2010). Vitamin C, or L-ascorbic acid (AA), is a water-soluble vitamin that plays an important role in many physiological processes, such as immune response, iron absorption, collagen synthesis, wound healing, cardiovascular disease reduction, and cellular protection against free radicals (Paciolla et al., 2019). However, due to the lack of L-gulonolactone oxidase

in the final step of the AA synthesis, vitamin C cannot be synthesised or stored in the human body, thereby requiring its daily intake (Mandl *et al.*, 2009). The most significant challenge facing guava juice production is the severe loss of vitamin C during processing or storage under various temperatures; these processes are normally accompanied by changes in colour, flavour, and appearance (Aishah *et al.*, 2016).

Cloudy juices are attractive due to their nutritional features and simple preparations (Beveridge, 2002). Oszmianski *et al.* (2007) found that cloudy juices are rich sources of natural antioxidants when compared with clear juices. However, all nutrients in cloudy juices, such as vitamin C, remain unchanged in their colloidal suspensions only for a very short time (Will *et al.*, 2008). Although these juices lose cloud stability during shelf-life storage, this could be addressed by thermal treatment (Kathiravan *et al.*, 2014; Shaheer *et al.*, 2014). Unfortunately, thermal treatment severely degrades natural antioxidants in the fruit juices (Raybaudi-Massilia et al., 2009). Thus, there is a need for a non-thermal procedure to develop cloud stability without any significant deterioration in natural antioxidants (Yang et al., 2017; Al-juhaimi et al., 2018). The cloud stability of juices could be enhanced by adding minimal amounts of hydrophilic colloids (food hydrocolloids), thereby foregoing thermal processing (Genovese and Lozano, 2001; Ibrahim et al., 2011). In this case, negatively charged hydrocolloids (dispersion medium) could increase electrostatic repulsive forces among negatively charged dispersed juice particles (Ibrahim et al., 2011); furthermore, hydrophobic forces could anchor hydrocolloids to the cloud particles. However, only few studies have explored the subsequent effects of developed cloud stability by food hydrocolloids on natural antioxidant preservation in fruit juices.

Gum arabic (GA), pectin, and sodium carboxymethyl cellulose (CMC) are commonly used food hydrocolloids (negatively charged water soluble polymers) (Genovese and Lozano, 2001; Ibrahim et al., 2011; Mousa, 2018). GA has a unique combination of excellent emulsifying properties and low solution viscosity. The bulky hydrophilic polysaccharide part of GA provides steric repulsion, whereas the hydrophobic protein anchors the hydrocolloid to cloud particles (Patel and Goyal, 2015). Pectin provides a stabilising effect by increasing juice viscosity and through its hydrophobic protein and/or negatively charged repulsive forces with juice dispersed particles (Endreß and Rentschler, 1999). CMC stabilises cloudy juices by electrostatic repulsion and forms protective coatings that prevent juice particles from aggregating, flocculating, and coalescing (Yasar et al., 2007). Several studies have used GA, pectin, and CMC hydrocolloids as natural antimicrobial agents in food products (Sayanjali et al., 2011; Gutierrez-Pacheco, 2016; Ali and EL Said, 2020).

The present work aimed to study the influences of GA, pectin, and CMC hydrocolloids at variable concentrations and their binary mixtures on vitamin C preservation in CGJs, followed by developing cloud stability during 14 months of storage at 4°C refrigeration temperature, and 25°C ambient temperature. The zeta-potential (ζ) and viscosity (η) studies of the proposed CGJs blended with hydrocolloids were used to investigate the cloud stability mechanisms. Physicochemical properties, such as colour, pH, total titratable acidity, °Brix, total phenolic compounds, and scavenging activity were measured. Furthermore, the proposed CGJs were subjected to consumer acceptability using the hedonic test. Since there are no studies available on the effects of adding hydrocolloids (GA, pectin, and CMC) singly or in binary combinations on the quality of CGJs, the results of the present work would be of benefit to consumers, researchers, and the food industry.

Materials and methods

Chemicals and reagents

White-fleshed guavas at commercial maturity were purchased from a local market. Gum arabic (GA, *Acacia senegal*, 8.33% polysaccharide and 2.41% protein), pectin (24% degree of esterification (DE) and MW 112 kDa), sodium carboxymethyl cellulose (CMC, MW 771 kDa), ascorbic acid (AA \geq 99.8%), formic acid, EDTA, and phosphoric acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, Folin-Ciocalteu reagent, and methanol were purchased from Aldrich (Steinheim, Germany). Pure water was prepared using a Millipore water system (Millipore, Billerica, MA).

Fresh cloudy guava juice preparation and storage with/without hydrocolloids

White-fleshed guavas were carefully washed with running tap water, and cut into $35 \times 25 \times 5$ mm slices. Then, 100 g guava slices were homogenised with 100 mL water (control sample) or with 100 mL hydrocolloid solutions. The hydrocolloid solutions were prepared from different concentrations of GA, pectin, or CMC, singly or in binary combination, as described in Table 1. The obtained mixtures were blended in a mixer for 5 min, and filtered through a juice strainer with gentle shaking to separate seeds and pulp. To avoid microbial spoilage, treated and control CGJ samples were sonicated using a digital Cavitek Ultrasonic at 40 kHz power at $25 \pm 2^{\circ}$ C for 15 min. Then, all samples were immediately placed in sealed 150-mL pre-sterilised glass bottles. A portion of the CGJ samples were immediately analysed, and the remaining portions were stored for 14 months at 4°C (refrigeration temperature) and at 25°C (ambient temperature). The temperatures were selected because they are the most commonly used temperatures at retail outlets.

Measurement of vitamin C in cloudy guava juices

Vitamin C was extracted from the CGJ samples by dilution (1:40) with 1% phosphoric acid, and centrifuged at 10,000 rpm for 10 min at 4°C to remove insoluble fibre and protein. The supernatant was filtered through a 0.2-µm filter, and transferred to an amber vial to protect AA from light. Then, analysis was performed by HPLC/MS in triplicate, following

	Usage level (g/ 100 mL juice)					
Sample	Gum Arabic	Pectin	СМС			
Control	0.00	0.00	0.00			
G1	0.01					
G2	0.05					
G3	0.10					
P1		0.10				
P2		0.30				
Р3		0.50				
C1			0.10			
C2			0.30			
C3			0.50			
G2P2	0.05	0.30				
G2C2	0.05		0.30			
P2C2		0.30	0.30			

Table 1. The studied CGJs samples blended with individual and binary hydrocolloids.

previously published work of Boonpangrak et al. (2016). A Dionex Ultimate 3000 liquid chromatography system (Dionex Softron GmbH, Germany) consisting of a pump, an autosampler, and a column compartment was used. The mobile phase was a 0.1% formic acid under isocratic conditions of 0.1 mL/min flow rate, and 10°C column temperature. A linear ion trap mass spectrometer (Amazon SL) equipped with an electrospray ionisation (ESI) source was operated in negative ion mode (Bruker, Billerica MA, USA). Chromatographic separation was achieved using a C₁₈ column (2.1 i.d. \times 150 mm, 3 μ m; Thermo Scientific) with a C_{18} guard cartridge (2.0 i.d. \times 10 mm, 5 μ m; Thermo Scientific). HPLC-MS system was controlled by HyStar software version 3.2 (KNAUR, Berlin, Germany). All data were acquired and analysed by QuantAnalysis version 2.0 (Bruker, Billerica MA, USA). A calibration curve (y = 250,601x + 134,117) over a concentration range (2.0 to 50.0 μ g/mL) with a correlation coefficient (R^2) of 0.998 was obtained. Limit of quantification (LOQ) and limit of detection (LOD) values were 2.0 and 0.8 μ g/mL, respectively.

Measurement of cloud stability in guava juices

During storage, aliquots (10 mL) of the proposed CGJs were drawn from the upper portion of the bottles. CGJ turbidity was measured before and after 15 min centrifugation at 4,200 g using a UV-Vis Shimadzu Spectrophotometer (UV-1601 PC) at 660 nm (Yemenicioglu *et al.*, 2000). The cloud stability was deduced from the relative turbidity equation (Eq. 1):

$$\Gamma\% = T_a/T_b \times 100 \tag{Eq. 1}$$

where, T_b and T_a = juice turbidities before and after centrifugation, respectively. All measurements were repeated in triplicate.

Properties of cloudy guava juices Measurement of apparent viscosity (η)

The apparent viscosity was measured by rotational viscometer (Rheotest 2, Germany). The investigated samples were introduced into the "S2" cylinder of the viscometer. The apparent viscosity was calculated using Eq. 2 (Ibrahim *et al.*, 2011):

$$\eta = \tau / \gamma \times 100 \tag{Eq. 2}$$

where, τ = shear stress (dyn/cm²), and γ = shear rate (S⁻¹). τ was calculated from the obtained torque value (α) and the cylinder (z) using Eq. 3:

$$\tau = z x \alpha$$
 (Eq. 3)

The measurements of apparent viscosity (η) were performed in diluted CGJ samples with concentrations as cited in Table 1, at a constant shear rate of 5.3 s⁻¹, and were reported in triplicate.

Measurement of zeta potential (ζ)

The zeta potential (ζ) was determined using a Zetasizer Nano ZS, ZEN 3600 (Malvern Instruments, Worcestershire, UK). Measurements were taken at room temperature (25°C) after diluting the CGJ sample 100 times in acetate buffer at pH 4.0 ± 0.05. The zeta potential (ζ) was determined by measuring the direction and velocity of droplet movement in a well-defined electric field; and measurements were reported in triplicate (Genovese and Lozano, 2001).

Colour analysis

Sample colour was measured with a digital colorimeter (CR-400, Minolta-Konica Sensing, Inc., Osaka, Japan) using the CIE Lab colour scale: L* (lightness), a* (redness), and b* (yellowness). Triplicate readings were carried out at 25°C on three equidistant locations of cloudy juice, and the mean value was recorded.

pH, total titratable acidity (TA), and degree Brix

The pH of the samples was determined by a pH meter with a glass electrode (Model H198130, Hanna Instruments, Italy). Readings were taken in triplicate, and mean values were recorded. The total titratable acidity was determined according to AOAC method (AOAC, 2000). A 10 mL volume of each

sample at ambient or refrigeration temperature was pipetted into a conical flask, and three drops of 1% phenolphthalein indicator were added. The mixture was titrated against 0.1 mol/L NaOH in a burette while swirling gently; a permanent faint pink colour was considered the end point. Mean values were used in calculating the titratable acidity (TA) expressed as grams of anhydrous citric acid per 100 mL of sample. Degrees Brix (°Brix) of soluble solids was measured using an RFM 80 digital refractometer (Bellingham Stanley, Ltd., England) with automatic temperature correction.

Measurement of antioxidant capacity

2,2-diphenyl-1-picrylhydrazyl (DPPH) is a stable radical that exhibits strong absorbance at 517 nm. The absorbance at 517 nm decreases proportionately with the loss of the radical in exchange for a proton, resulting in a colour change from purple to yellow. DPPH is commonly used to estimate the antioxidant capacity of juices (Mousa, 2018). Aliquots (0.2 mL) of trolox (as the reference standard) and juice extraction samples were reacted with 1.0 mL of DPPH (0.1 mmol/L) in methanol and in the dark for 30 min at room temperature. Absorbance changes were monitored using a VersaMax plate reader (Molecular Devices, Sunnyvale, CA) and compared with a control containing 0.2 mL of 80% methanol and 1.0 mL DPPH. The scavenging activity (%) was calculated using Eq. 4:

Scavenging
activity (%) =
$$[1 - A/B)] \times 100$$
 (Eq. 4)

where, A = absorbance of sample at 517 nm, and B = absorbance of control at 517 nm.

Analysis of total phenolic compounds

Total soluble phenolics were analysed using the Folin-Ciocalteu metal reduction assay previously described by Talcott *et al.* (2005) using gallic acid as a standard. For analysis, 100 μ L of CGJs were reacted with a commercial preparation of 0.25 N Folin-Ciocalteu reagent; after a 3 min reaction, the solution was neutralised with 1 N sodium carbonate. After 7 min, water was added to bring the total volume to 10 mL, and the sample was mixed using a vortex. Approximately 30 min later, the samples and standard were pipetted into a microtiter plate, and the absorbance at 726 nm was measured by a VersaMax plate reader (Molecular Devices, Sunnyvale, CA). Total phenolic compounds were reported in mg/L equivalents of gallic acid (mg GAE/L).

Hedonic evaluation test

The proposed CGJs blended with/without hydrocolloids were subjected to consumer acceptability using the hedonic test. The panel consisted of panellists that included collaborators and students that like or usually consume cloudy juices (total number of collaborators was 78; 35 females aged 20 - 30 years old and 43 males aged 20 - 35 years old). All collaborators were free from cold or sinus problems during the evaluation period. Thirteen panellists were distributed in six separate sections. Sensory valuations were required for 13 different samples coded by three digits, and distributed in random arrangements in each section. Evaluations were performed under "daylight" illumination, at 25°C ambient temperature, and in isolated booths. At the beginning of the evaluation, a control sample was presented to each panellist. Panellists were asked to assess their degree of liking of the samples on paper ballots with a 9-point hedonic rating scale (Mousa, 2018), where 9 = like extremely, and 1 = dislike extremely. The panellists evaluated the samples in terms of colour, appearance, aroma, taste, and overall acceptability. They were instructed to clean their palate with water between samples.

Statistical analysis

Treatments of CJGs were repeated three times with controls. Values were expressed as means \pm standard deviations. Analysis of variance (ANOVA) was carried out using Minitab statistical software (USA). Differences were evaluated for significance based on mean values, and were calculated at a significance level of p < 0.05 using Tukey's range test.

Results and discussion

Properties of cloudy guava juice (CGJ) without hydrocolloids

The CGJ sample without hydrocolloids (control sample) was prepared and stored at 4 and 25°C for 14 months. At the beginning of storage, the pH value, total titratable acidity (TA), and °Brix of soluble solids were 4.0 ± 0.1 , 0.8 ± 0.1 g citric acid/100 mL, and 8.9±0.1, respectively. The initial apparent viscosity (η) and zeta-potential (ζ) at 25°C were 14.4 ± 0.3 cP and -20.8 ± 1.2 mV, respectively. The initial colour coordinates, L*, a*, and b* were 70.9 ± 1.8 , 0.3 ± 0.1 , and 14.5 ± 0.7 , respectively. The initial cloud stability of the CGJ control sample was a T% of 96.9 ± 1.2 as shown in Table 2. The AA amount was determined by HPLC/MS. Figure 1 shows the HPLC and MS graph for the detection of AA in CGJ; $128.2 \pm 0.8 \text{ mg}/100$ mL was measured for AA (Table 3). The antioxidant activity of control CGJ was also evaluated using DPPH radical-scavenging, which free has been

recommended as an easy and accurate assay for measuring the scavenging activity of fruit juices (Mousa, 2018). The % scavenging activity and total phenolic compounds of control CGJs immediately after processing were $518.1 \pm 0.5\%$ and 54.2 ± 1.3 mg GAE/L, respectively.

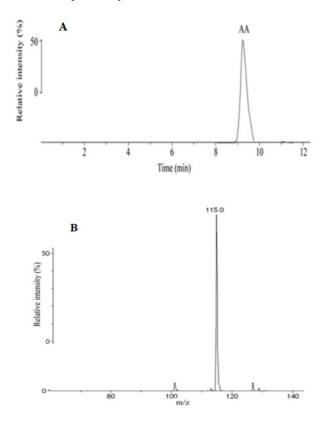


Figure 1. HPLC (A) and MS (B) graphs for the analysis of ascorbic acid (AA) in CGJ.

The effects of storage time (14 months) and temperature (4 and 25°C) on the properties of the CGJ control sample were further investigated (not shown). The pH, TA, and °Brix were 4.0 ± 0.2 to 4.1 ± 0.1 , 0.8 ± 0.3 to 0.9 ± 0.2 g citric acid/100 mL, and 8.8 ± 0.2 to 8.9 ± 0.1 , respectively (no significant differences were determined; p > 0.05). However, the colour coordinates, L*, a*, and b* significantly changed (p < 0.05) over the storage period at 25°C to 35.6 ± 0.7 , $7.1 \pm$ 0.5, and 32.3 ± 0.9 , respectively. This could be attributed to ambient temperature and exposure to atmospheric oxygen and light. These conditions accelerated the oxidation of AA and the reaction of AA with amino groups of protein fractions producing dark pigments through polymerisation, and therefore causing colour loss (Silva et al., 2016). Furthermore, cloud stability dramatically changed during storage at 4 and 25°C, as shown in Table 2. The cloud stability significantly decreased (p < 0.05) to 63.9 ± 0.9 (T%) at 4°C and to 63.1 ± 1.8 (T%) at 25°C within two months of storage. Cloud stability of the control sample further decreased to 22.0 ± 0.9 (T%) at 4°C and 15.4 ± 1.4 (T%) at 25°C after 14 months of storage. The loss of cloud stability could be attributed to the formation of oxidised polyphenol compounds (Beveridge, 2002). The % scavenging activity and total phenolic compounds also markedly (p < 0.05) decreased by approximately 11 and 15% after two months of storage, respectively, and continued to decrease, but not significantly. Furthermore, the amount of AA decreased by 9.9% after two months of storage, as indicated in Table 3. AA levels decreased to $49.7 \pm 1.0 \text{ mg}/100 \text{ mL}$ (an approximate 61.2% drop) at 4°C after 14 months of storage. CGJ control sample at 25°C rapidly lost AA content from 128.2 ± 0.8 mg/100 mL at the beginning of storage to $69.5 \pm 0.1 \text{ mg}/100 \text{ mL}$ (a 45.7% drop) after two months of storage. The AA amount sharply dropped to $19.7 \pm 0.5 \text{ mg}/100 \text{ mL}$ (a 84.6% drop) at 25°C after 14 months of storage, as shown in Table 3. These results agree with past works of Oszmianski et al. (2007) and Will et al. (2008).

Cloudy guava juices blended with single hydrocolloids Cloud stability

Table 2 presents the effects of single hydrocolloids (GA, pectin, and CMC) with variable concentrations (Table 1) on the cloud stability (T%) of CGJs stored for a period of 14 months at 4 and 25°C. The cloud stability in the CGJs improved significantly (p < 0.05) with different concentrations of pectin and CMC up to 0.3 g/100 mL juice for a period of two and four months of storage, respectively, as compared to control sample. Further increases in pectin and CMC concentrations beyond 0.3 g/100 mL juice did not show any significant additional stabilisation. The stabilising effects of the hydrocolloids could be due to their electronegativity rather than their viscosity-producing effects as predicted by Genovese and Lozano (2001). Beveridge (2002) explained that hydrocolloids stabilised juices by electrostatic repulsion with juice particles. We confirmed this explanation by measuring the zeta-potential (ζ) and viscosity (η). Figure 2A shows the variations of ζ with concentrations (g/100 mL juice) of CMC and pectin at 25°C after 14 months of storage. ζ potential decreased to -38 and -34 mV, respectively, when CMC and pectin concentrations increased to 0.3 g/100 mL juice as compared to -21 mV of control sample (p < 0.05). Since CGJ particles are negatively charged, the addition of CMC and pectin molecules with negative charges was expected to increase the electrostatic repulsive forces between particles. The magnitude of the zeta-potential (ζ) provides an indication of the potential stability of the colloidal system. When particles have large ζ values, i.e. generally above \pm 30 mV, particles repel each other and maintain the

				Cloud stab	Cloud stability (T%) during storage period (month)	ing storage per	iod (month)			
Sample			Temperature 4°C	C				Temperature 25°C	5°C	
	0	2	4	8	14	0	2	4	8	14
Control	$96.9 \pm 1.2^{\mathrm{a}}$	$63.9\pm0.9^{\mathrm{ed}}$	43.1 ± 1.0^{d}	32.5 ± 1.9^{e}	$22.0\pm0.9^{\mathrm{ef}}$	96.9 ± 1.2^{a}	63.1 ± 1.8^{ed}	$38.2 \pm 1.2^{\circ}$	$29.8 \pm 1.0^{\mathrm{ef}}$	$15.4 \pm 1.4^{\mathrm{abc}}$
GI	96.9 ± 1.0^{a}	$85.5\pm0.8^{\circ}$	$55.5\pm0.9^{\mathrm{f}}$	42.1 ± 1.2^{d}	$35.0\pm0.9^{\circ}$	96.9 ± 1.0^{a}	73.1 ± 1.3^{cd}	$51.7 \pm 1.4^{\mathrm{f}}$	39.1 ± 1.5^{e}	$29.7\pm1.0^{\rm ef}$
G2	99.6 ± 1.2^{b}	$98.9 \pm 1.0^{\mathrm{b}}$	$96.3\pm1.2^{\mathrm{a}}$	95.5 ± 1.1^{a}	94.2 ± 1.5^{a}	$99.6\pm1.2^{\rm b}$	97.6 ± 1.1^{a}	$95.3\pm1.7^{\rm a}$	94.7 ± 1.4^{a}	92.0 ± 1.0^{ab}
G3	$99.5 \pm 1.1^{\mathrm{b}}$	94.2 ± 1.5^{a}	$89.2\pm1.2^{\circ}$	$85.8\pm1.0^{\circ}$	$81.2 \pm 1.2^{\circ}$	$99.5 \pm 1.1^{\mathrm{b}}$	90.3 ± 1.0^{ab}	$84.8\pm1.0^{\rm c}$	$81.9 \pm 1.2^{\circ}$	79.0 ± 1.6^{cd}
P1	96.9 ± 1.1^{a}	$85.1 \pm 1.2^{\circ}$	45.4 ± 1.2^{d}	$33.9\pm1.0^{\circ}$	28.6 ± 0.8^{ef}	96.9 ± 1.0^{a}	80.5 ± 1.0^{cd}	$44.0\pm1.5^{\rm d}$	$31.7\pm0.8^{\rm e}$	$22.1\pm1.0^{\rm ef}$
P2	97.1 ± 1.0^{a}	$88.4\pm1.5^{\rm c}$	$57.0\pm1.6^{\rm f}$	48.9 ± 0.9^{d}	$39.4\pm1.0^{\circ}$	97.1 ± 1.2^{a}	$87.9\pm1.2^{\circ}$	$56.5 \pm 1.3^{\mathrm{f}}$	43.1 ± 0.9^{d}	$37.8\pm1.7^{\mathrm{e}}$
P3	$95.1\pm1.2^{\rm a}$	80.1 ± 1.5^{cd}	$53.2\pm1.0^{\rm f}$	44.2 ± 1.3^{d}	35.7 ± 1.3^{e}	95.1 ± 1.3^{a}	78.6 ± 1.2^{cd}	$51.8\pm1.2^{\rm f}$	$43.0\pm1.0^{\rm d}$	$33.1\pm1.7^{\rm e}$
C1	$96.6\pm1.2^{\mathrm{a}}$	$88.0\pm1.4^{\rm c}$	75.5 ± 1.3^{cd}	45.3 ± 1.2^{d}	$39.9 \pm 1.2^{\circ}$	96.6 ± 1.0^{a}	$84.3\pm1.0^{\circ}$	$73.9 \pm 1.5^{\rm cd}$	42.9 ± 1.0^{d}	$36.8\pm1.2^{\circ}$
C2	$97.5 \pm 1.4^{\rm b}$	93.3 ± 1.5^{ab}	$81.2\pm1.0^{\circ}$	$46.4\pm1.0^{\text{d}}$	$33.8 \pm 1.3^{\circ}$	97.5 ± 1.2^{b}	91.4 ± 1.8^{ab}	$81.0\pm1.0^{\circ}$	44.8 ± 1.1^{d}	$31.4 \pm 1.6^{\circ}$
C3	96.4 ± 1.0^{a}	$85.2 \pm 1.3^{\circ}$	$55.1\pm1.2^{\rm f}$	$43.8\pm1.0^{\text{d}}$	32.0 ± 1.2^{e}	96.4 ± 1.0^{a}	$84.5 \pm 1.0^{\circ}$	$54.0\pm1.2^{\rm f}$	42.9 ± 1.3^{d}	$30.8\pm1.2^{\circ}$
G2P2	$99.6 \pm 1.0^{\mathrm{b}}$	$98.9 \pm 1.4^{\mathrm{b}}$	$97.0 \pm 1.3^{\mathrm{b}}$	$96.2 \pm 1.2^{\mathrm{b}}$	95.2 ± 1.3^{b}	$99.6 \pm 1.3^{\mathrm{b}}$	$98.5 \pm 1.0^{\mathrm{b}}$	$96.8\pm1.2^{\mathrm{b}}$	$96.0 \pm 1.1^{\mathrm{b}}$	$95.0 \pm 1.0^{\mathrm{b}}$
G2C2	99.8 ± 1.0^{b}	$99.5 \pm 1.5^{\mathrm{b}}$	$98.0 \pm 1.5^{\mathrm{b}}$	$97.7 \pm 1.4^{\rm b}$	97.3 ± 1.3^{b}	$99.8 \pm 1.0^{\mathrm{b}}$	$99.0 \pm 1.3^{\mathrm{b}}$	$97.8 \pm 1.2^{\rm b}$	97.4 ± 1.2^{b}	97.0 ± 1.6^{b}
P2C2	$98.3 \pm 1.4^{\mathrm{b}}$	$92.3\pm1.0^{\circ}$	85.0 ± 1.1^{cd}	60.6 ± 1.5^{ed}	45.1 ± 1.0^{d}	$98.3 \pm 1.0^{\mathrm{b}}$	$91.2 \pm 1.3^{\circ}$	83.6 ± 1.0^{cd}	65.6 ± 1.4^{ed}	42.5 ± 1.0^{d}
Values are	Values are mean \pm S.D. of three determinations	f three determi		(n = 3). Means with different letters indicate significant difference $(p < 0.05)$	lifferent letters	indicate signi	ficant differen	ice $(p < 0.05)$.		

Table 2. Changes in the cloud stability (T%) of CGJ samples with/without hydrocolloids stored at 4°C (refrigerator) and 25°C (ambient temperature).

						c) uuring storag	minimi morrad a	_		
Sample			Temperature 4°C	7.)			L	Temperature 25°C	7.)	
	0	2	4	8	14	0	2	4	8	14
Control	128.2 ± 0.8^{a}	115.5 ± 0.3^{gh}	$89.3\pm0.5^{\circ}$	$68.5\pm0.8^{\circ}$	$49.7 \pm 1.0^{\rm ef}$	128.2 ± 0.8^{a}	$69.5\pm0.1^{\rm e}$	$49.3\pm0.3^{\mathrm{ef}}$	38.5 ± 1.1^{cd}	19.7 ± 0.5^{ab}
Gl	$138.4\pm0.5^{\rm h}$	$118.3\pm0.8^{\rm g}$	111.6 ± 0.8^{g}	$98.5\pm0.9^{\mathrm{b}}$	$80.5\pm0.3^{\circ}$	$138.4\pm0.5^{\rm h}$	111.1 ± 1.0^{g}	$100.2\pm0.6^{\text{gh}}$	$88.1\pm0.7^{\rm c}$	$75.8\pm0.9^{\rm d}$
G2	$143.2\pm0.7^{\rm i}$	$139.4 \pm 1.1^{\rm h}$	$135.5\pm0.9^{\rm h}$	129.7 ± 0.8^{a}	120.3 ± 0.9^{a}	$143.2\pm0.7^{\rm i}$	$135.7\pm1.0^{\rm h}$	$131.3\pm0.8^{\rm h}$	127.6 ± 0.9^{a}	$118.9\pm1.3^{\rm g}$
G3	$141.1\pm0.9^{\rm i}$	128.8 ± 1.0^{a}	120.7 ± 1.1^{a}	110.3 ± 0.9^{g}	101.1 ± 0.7^{gh}	$141.1\pm0.9^{\rm i}$	121.3 ± 0.7^{a}	$118.3\pm1.0^{\rm g}$	105.5 ± 0.8^{gh}	$98.0\pm0.9^{\rm b}$
P1	$130.1\pm0.7^{\rm h}$	110.7 ± 0.5^{g}	$99.8\pm0.4^{\rm b}$	78.8 ± 0.9^{d}	$59.8\pm1.1^{\rm ih}$	$130.1\pm0.7^{\rm h}$	105.9 ± 0.8^{gh}	$95.9\pm0.7^{\mathrm{b}}$	$72.7\pm0.9^{\rm d}$	55.9 ± 0.9^{ih}
P2	134.6 ± 0.9^{h}	130.5 ± 0.8^{g}	109.6 ± 0.7^{gh}	$90.8\pm0.7^{\rm b}$	79.8 ± 1.1^{d}	$134.6\pm0.9^{\rm h}$	128.8 ± 0.9^{a}	105.8 ± 0.6^{gh}	$88.6\pm0.9^{\rm c}$	$75.6\pm1.0^{\rm d}$
P3	$134.5 \pm 1.0^{\text{h}}$	128.4 ± 0.9^{a}	104.7 ± 0.8^{gh}	$84.9\pm0.8^{\rm c}$	$75.6 \pm 1.0^{\text{d}}$	$134.5\pm1.0^{\rm h}$	125.9 ± 0.8^{a}	$100.9\pm0.9^{\rm gh}$	$81.8\pm0.8^{\rm c}$	$72.8\pm0.9^{\rm d}$
C1	135.6 ± 0.9^{h}	120.8 ± 0.7^{a}	100.6 ± 0.8^{gh}	$81.9\pm0.9^{\circ}$	$60.9\pm1.0^{\rm e}$	$135.6\pm0.9^{\rm h}$	118.5 ± 0.9^{g}	$98.9\pm0.3^{\mathrm{b}}$	$79.8\pm0.1^{\rm d}$	58.96 ± 0.5^{ih}
C2	$139.4\pm0.9^{\rm h}$	134.1 ± 0.9^{h}	$130.8\pm0.9^{\rm h}$	115.7 ± 0.8^{g}	$85.6\pm0.9^{\circ}$	$139.4\pm0.9^{\rm h}$	$133.0\pm0.8^{\rm h}$	129.9 ± 0.8^{a}	113.9 ± 0.9^{g}	$83.9\pm1.0^{\rm c}$
C3	$138.3\pm0.7^{\rm h}$	128.5 ± 1.0^{a}	113.9 ± 0.8^{g}	$95.8\pm0.9^{\mathrm{b}}$	$80.7\pm0.5^{\rm c}$	$138.3\pm0.7^{\rm h}$	122.1 ± 1.1^{a}	110.6 ± 0.9^{g}	$92.9\pm0.4^{\rm b}$	$^{\rm p}6.0 \pm 0.77$
G2P2	144.5 ± 0.9^{i}	$140.1\pm1.0^{\rm i}$	$136.8\pm1.0^{\rm h}$	129.7 ± 0.8^{a}	122.5 ± 0.9^{a}	144.5 ± 0.9^{i}	$139.6\pm1.1^{\rm h}$	$134.3\pm1.0^{\rm h}$	126.1 ± 0.9^{a}	$119.3\pm1.0^{\rm g}$
G2C2	$146.9\pm0.7^{\rm i}$	143.2 ± 1.1^{i}	140.9 ± 1.0^{i}	$135.2\pm0.9^{\rm h}$	$130.2\pm0.9^{\rm h}$	$146.9\pm0.7^{\rm i}$	142.8 ± 1.0^{1}	$138.7\pm1.1^{\rm h}$	132.1 ± 0.9^{h}	$130.0\pm1.0^{\rm h}$
P2C2	140.3 ± 0.9^{i}	$135.5\pm0.7^{\rm h}$	$131.6\pm0.6^{\rm h}$	$118.4\pm0.3^{\rm g}$	$89.7\pm0.8^{\circ}$	140.3 ± 0.9^{i}	$133.8\pm0.9^{\rm h}$	$130.8\pm0.5^{\rm h}$	112.1 ± 0.7^{g}	$85.9\pm0.9^{\circ}$

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stability of the solution. When the ζ value is between \pm 30 mV, particles agglomerate due to lower potential difference between particles and dissolution medium (Prabhuswamy et al., 2019). Moreover, for juices treated with CMC and pectin concentrations beyond 0.3 g/100 mL juice, there appeared to be a slight change in the ζ value due to the slow mobility of large particles sizes (Genovese and Lozano, 2001). The values of apparent viscosity were also measured as functions of CMC and pectin concentrations at a constant shear rate of 5.3 s⁻¹. The low shear viscosity would be most relevant to the mobility of very small particles. The viscosity of CGJs significantly (p < 0.05) increased after increasing the concentrations of CMC and pectin, as indicated in Figure 3A. The higher viscosity of CMC-CGJs samples (up to 55 cP) as compared to pectin-CGJ samples (up to 30 cP) could be due to the higher molecular weight of CMC. A gel network formed through the entwining and crosslinking of the polymer chains present in hydrocolloids to form a three-dimensional network (Prabhuswamy et al., 2019).

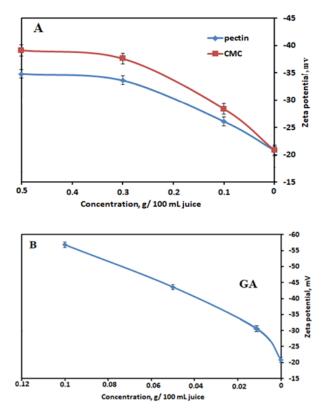


Figure 2. Zeta-potential (ζ) in the CGJs at 25°C after 14 months as a function of the hydrocolloid content [CMC and pectin (A) and GA (B)].

Gum arabic (GA) exhibits promising features, such as low viscosity Newtonian flow due to a highly branched molecular structure and relatively low molecular weight (Mousa, 2018). In the present work, Figures 1B and 2B show that the values of the ζ

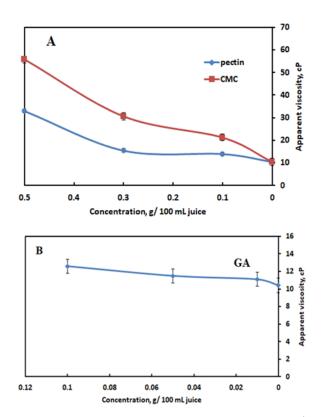


Figure 3. Apparent viscosity (η) at shear rate 5.3 s⁻¹ in CGJs at 25°C after 14 months as a function of the hydrocolloid content [CMC and pectin (A) and GA (B)].

potential (from -30.1 to -56.8 mV) and y viscosity (from 11.1 to 12.6 cP) significantly increased (p <0.05) with GA concentration from 0.01 to 0.1 g/100 mL juice as compared to the control sample without hydrocolloids ($\zeta = -20.8 \text{ mV}$ and $\eta = 10.4 \text{ cP}$) at 25°C after 14 months of storage. Further increases in GA concentration beyond 0.05 g/100 mL juice should be avoided because GA molecules begin to come into contact with one another in a concentrated system (ζ values become more negative with higher concentrations of GA, Figure 2B). Under these high concentrations, the movement of molecules becomes restricted (n values increase with GA concentration, Figure 3B) (Patel and Goyal, 2015). High cloud stability was observed due to GA at 0.05 g/100 mL juice (G2 sample) within 14 months of storage at 4 and 25°C as indicated in Table 2. The major stabilising effect of GA in CGJ was likely due to its greater electronegativity, which leads to high particle repulsion in addition to the steric repulsion coming from its adsorption on the CGJ particles (Genovese and Lozano, 2001; Prabhuswamy et al., 2019).

Vitamin C (ascorbic acid) stability

The effects of adding GA, pectin, and CMC single hydrocolloids on vitamin C retention in CGJs stored for 14 months at 4°C and 25°C are indicated in Table 3. Generally, the retention of AA in the presence

of hydrocolloids was better than in the control sample. By adding pectin at 0.3 g/100 mL juice (sample P2), the AA amount decreased by 40.7% at 4°C and 43.8% at 25°C after 14 months, as compared to those in the control sample (61.2% at 4°C and 84.6% at 25°C). The addition of 0.3 g/100 mL juice CMC (sample C2) significantly improved (p < 0.05) the stability of AA by lowering the percentage loss to 38.6% at 4°C and 39.8% at 25°C. These results indicated that CMC could retain AA better than pectin. Further interesting observations were achieved after adding GA (samples G1 \rightarrow G3). The amount of AA in GA samples ranged from 138.4 ± 0.5 to 143.2 ± 0.7 mg/100 mL juice immediately after juice processing, which was approximately 10.5% higher than that in the control sample (128.2 ± 0.8 mg/100 mL juice). Furthermore, the addition of 0.05 g/100 mL juice GA (sample G2) significantly improved (p < 0.05) the stability of AA after 14 months of storage giving the lowest percentage drops of 16% at 4°C and 17% at 25°C, as indicated in Table 3. These observations of GA could be attributed to the reaction of carboxylic groups in AA with amino groups of proteins or hydroxyl groups of polysaccharides in GA via hydrogen bonding (Ali and El Said, 2020).

Cloudy guava juices blended with binary hydrocolloids

Cloud stability

The effects of binary combination of hydrocolloids at their optimal levels (0.05 g/100 mL juice of GA, 0.3 g/100 mL juice of pectin, and 0.3 g/100 mL juice of CMC) on cloud stability (T%) are shown in Table 2. Generally, the cloud stability of CGJs significantly improved (p < 0.05) in all studied binary incorporations at the beginning of storage giving % enhancements of 2.3, 2.9, and 1.4% in the G2P2, G2C2, and P2C2 samples, respectively, as compared to the control sample. However, the cloud stability of the P2C2 sample significantly decreased (p < 0.05) by 54.1 and 56.8% within 14 months of storage at 4 and 25°C, respectively. The cloud stability values of the G2P2 and G2C2 samples markedly improved (p <0.05) to 4.3 and 4.4 times higher than that of the control sample after 14 months of storage at 4°C, respectively. The cloud stability values of G2P2 and G2C2 samples at 25°C were 6.1 and 6.3 times higher than the control sample after 14 months of storage, respectively. These observations were confirmed by zeta-potential (ζ) and viscosity (η) measurements of the binary hydrocolloid samples (G2P2, G2C2, and P2C2) as shown in Figure 4 (A and B). Figure 4A shows that the G2C2 and G2P2 samples had ζ potentials of -31 and -40 mV, respectively, when compared with a control sample value of -20.8 mV. The viscosity values of the G2C2 and G2P2 samples were 17 and 15 cP, respectively, when compared with a control sample value of 10.4 cP (Figure 4B). These results agree with prior studies which indicated the positive effects of hydrocolloid binary combinations on the quality of fruit juices (Turkyılmaz *et al.*, 2020; Yemenicioglu *et al.*, 2020).

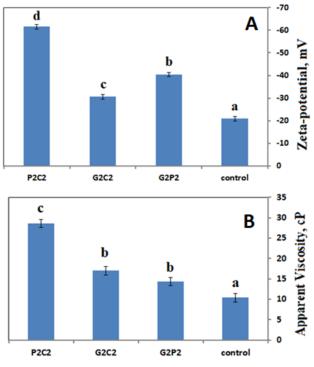


Figure 4. The comparison of zeta-potential (ζ) (A) and of apparent viscosity (η) at shear rate 5.3 s⁻¹ (B), between the CGJ control and the studied CGJs blended with the binary hydrocolloids at 25°C after 14 months.

Vitamin C stability

The effects of binary combination of hydrocolloids on AA retention in CGJs stored for 14 months of storage at 4 and 25°C are presented in Table 3. High AA amounts $(146.9 \pm 0.7 \text{ and } 144.5 \pm 0.9 \text{ mg}/100 \text{ mL})$ juice) were detected in the G2C2 and G2P2 samples, respectively, immediately after juice processing, which are approximately 12.7 and 11.3% higher than the control sample ($128.2 \pm 0.8 \text{ mg}/100 \text{ mL}$ juice). After 14 months of storage, the AA stability in the G2C2 sample was highest among all other treatments (Table 3). The G2C2 sample had the highest AA content at 25°C (130.0 \pm 1.0 mg AA/100 mL juice) and at 4° C (130.2 ± 0.9 mg AA/100 mL juice) when compared with the control sample (49.7 ± 1.0 and 19.7 ± 0.5 mg AA/100 mL juice, respectively). This result could be attributed to the presence of large numbers of free carboxylic and hydroxyl groups on GA and CMC that interact via hydrogen bonds with carboxylic groups in AA molecules (Turkyılmaz et al., 2020). Improved cloud stability also aided the stability of AA by facilitating the impact of these interactions in the

colloidal state (Ali and El Said, 2020). Therefore, the binary combination of GA (0.05 g/100 mL juice) and CMC (0.3 g/100 mL juice) helped prevent AA oxidation in cloudy guava juice. This combination (G2C2 sample) could be used as a simple solution to preserve the quality of cloudy juices during ambient long-term storage.

Physicochemical properties of cloudy guava juices with GA and CMC binary hydrocolloids

The pH, TA, and °Brix for the G2C2 sample were 4.2 ± 0.1 , 0.6 ± 0.3 g citric acid/100 mL, and 9.4 \pm 0.2 at the beginning of storage, respectively. The increased pH and decreased TA when compared with the control sample could be due to the presence of sodium ions from CMC. For the colour coordinates, L*, a*, and b* were 70.7 \pm 1.4, 0.4 \pm 0.2, and 14.3 \pm 0.3, respectively, at the beginning of storage, which were not significantly different from those of the control sample. Moreover, the % scavenging activity and total phenolic compounds of G2C2 sample at the beginning of storage were $540.3 \pm 0.9\%$ and $63.6 \pm$ 1.0 mg GAE/L, respectively. These values were significantly higher than those of the control sample (p <0.05). After 14 months of storage, the physicochemical properties of the G2C2 sample were further investigated. The pH (4.2 ± 0.5), TA (0.7 ± 0.1 g citric acid/100 mL), °Brix (9.5 ± 0.1), and colour coordinates (70.8 $\pm 0.6, L^*; 0.5 \pm 0.1, a^*; and 14.5 \pm 0.2, b^*)$ did not significantly differ when compared with the values at the beginning of storage. Interestingly, the colour stability of the G2C2 sample was completely different from the control sample $(35.6 \pm 0.7, L^*; 7.1 \pm 0.5, a^*; and$ 32.3 ± 0.9 , b*). This agree with a past study on the effects of hydrocolloids on colour parameters (Prabhuswamy et al., 2019). Furthermore, the values of %

Table 4. Sensory characteristics of the studied CGJs blended with/without hydrocolloids, and stored at ambient temperature (25°C).

Sample	Colour	Appearance	Aroma	Taste	Overall Acceptability
At the beg	ginning of stor	rage			1 0
Control	8.4 ± 1.2^{ab}	7.6 ± 0.5^{a}	7.7 ± 0.6^{ab}	$8.0 \pm 1.3^{\text{ac}}$	7.9 ± 1.0^{ac}
G1	$6.1 \pm 1.0^{\circ}$	6.5 ± 1.4^{b}	6.3 ± 0.4^{a}	6.1 ± 1.7^{d}	6.2 ± 1.8^{b}
G2	5.7 ± 1.5^{b}	$6.6\pm0.9^{\rm b}$	6.4 ± 1.3^{a}	6.6 ± 1.2^{d}	6.7 ± 1.1^{b}
G3	$7.4 \pm 1.0^{\mathrm{a}}$	$7.5\pm1.4^{\rm a}$	6.7 ± 1.0^{a}	7.0 ± 1.3^{a}	$6.9\pm1.4^{\text{bc}}$
P1	$6.6 \pm 1.2^{\circ}$	$6.5 \pm 1.3^{\text{b}}$	5.0 ± 1.1^{b}	$5.0 \pm 1.3^{\mathrm{b}}$	$5.5 \pm 1.5^{\circ}$
P2	$5.3 \pm 1.0^{\mathrm{b}}$	6.6 ± 1.9^{b}	6.0 ± 1.9^{a}	$6.0\pm1.5^{\text{d}}$	6.2 ± 1.9^{b}
P3	5.4 ± 1.8^{b}	$6.8 \pm 1.3^{\mathrm{b}}$	6.2 ± 1.3^{a}	$6.6 \pm 1.0^{\text{d}}$	$6.4\pm0.8^{\rm b}$
C1	$6.5 \pm 1.0^{\circ}$	6.5 ± 1.1^{b}	$5.4\pm1.0^{\rm b}$	5.2 ± 1.2^{b}	$5.7 \pm 1.1^{\circ}$
C2	$6.7 \pm 1.0^{\circ}$	6.9 ± 1.1^{b}	6.2 ± 1.2^{a}	$6.3\pm1.0^{\text{d}}$	6.3 ± 1.5^{b}
C3	$5.5 \pm 1.6^{\text{b}}$	6.7 ± 1.7^{b}	6.3 ± 1.5^{a}	6.5 ± 1.5^{d}	6.7 ± 1.3^{b}
G2P2	5.6 ± 1.1^{b}	6.9 ± 1.0^{b}	6.4 ± 1.3^{a}	6.4 ± 1.2^{d}	6.7 ± 1.9^{b}
G2C2	7.4 ± 1.2^{a}	$7.5 \pm 1.0^{\mathrm{a}}$	$6.5 \pm 1.0^{\mathrm{a}}$	$7.0 \pm 1.0^{\mathrm{a}}$	7.2 ± 1.0^{bc}
P2C2	5.7 ± 1.6^{b}	$6.5\pm1.5^{\text{b}}$	$5.4\pm1.0^{\text{b}}$	$5.9 \pm 1.5^{\mathrm{b}}$	$5.9 \pm 1.7^{\circ}$
After 14 r	nonths of stor	age			
Control	$1.1 \pm 0.5^{\circ}$	$1.6\pm0.6^{\rm b}$	$3.4 \pm 1.0^{\circ}$	$1.7 \pm 1.8^{\circ}$	$2.2 \pm 1.0^{\circ}$
G1	$1.8\pm0.8^{\rm c}$	$1.9\pm0.8^{\rm b}$	$3.2 \pm 1.2^{\circ}$	$1.5 \pm 1.0^{\circ}$	$2.0 \pm 1.5^{\circ}$
G2	5.5 ± 1.2^{b}	$5.9\pm1.0^{\rm c}$	5.1 ± 1.2^{b}	$5.0 \pm 1.0^{\mathrm{b}}$	5.2 ± 1.0^{b}
G3	$5.8\pm1.2^{\rm b}$	6.7 ± 1.3^{d}	6.3 ± 1.0^{a}	6.6 ± 1.0^{d}	$6.6\pm1.4^{\text{d}}$
P1	$1.0\pm0.9^{\circ}$	$1.3\pm0.8^{\rm b}$	$2.7 \pm 1.5^{\circ}$	$1.5 \pm 1.3^{\circ}$	$2.0\pm0.6^{\rm c}$
P2	$1.7\pm0.8^{\circ}$	$1.8\pm0.9^{\rm b}$	$3.5 \pm 1.1^{\circ}$	$1.9 \pm 1.1^{\circ}$	$2.2 \pm 1.3^{\circ}$
P3	$1.0\pm0.8^{\circ}$	$1.2\pm0.5^{\rm b}$	$2.6 \pm 1.4^{\circ}$	$1.5 \pm 1.7^{\circ}$	$2.1 \pm 0.3^{\circ}$
C1	$1.6\pm0.9^{\circ}$	$1.7\pm0.8^{\rm b}$	$3.6 \pm 1.0^{\circ}$	$1.8 \pm 1.0^{\circ}$	$2.3 \pm 1.2^{\circ}$
C2	$1.0\pm0.8^{\rm c}$	$1.2\pm0.5^{\rm b}$	$2.6 \pm 1.4^{\circ}$	1.5 ± 1.7^{c}	$2.1\pm0.3^{\circ}$
C3	$1.0\pm0.5^{\circ}$	$1.4\pm0.6^{\rm b}$	$2.5 \pm 1.0^{\circ}$	$1.7 \pm 1.2^{\circ}$	$2.0\pm0.8^{\rm c}$
G2P2	$6.0\pm0.9^{\text{d}}$	$6.3\pm0.9^{\rm c}$	6.0 ± 1.5^{a}	6.0 ± 1.5^{d}	$6.2\pm0.8^{\text{d}}$
G2C2	$6.5\pm1.6^{\text{d}}$	$6.8 \pm 1.4^{\rm d}$	6.9 ± 1.4^{a}	6.8 ± 1.3^{d}	$6.8\pm1.0^{\rm d}$
P2C2	$2.9 \pm 1.3^{\circ}$	4.6 ± 1.5^{a}	$3.9\pm1.0^{\text{b}}$	3.7 ± 1.3^{b}	4.1 ± 1.3^{bc}

Values are mean \pm S.D. of three determinations (n = 3). Means with different letters within a column indicate significant difference (p < 0.05).

scavenging activity and total phenolic compounds of the G2C2 sample remained the same when compared with the beginning of storage. This reflected the ability of binary hydrocolloids for stabilising natural antioxidants in CGJs for long periods (Turkyılmaz *et al.*, 2020).

Consumer acceptability

The results of the consumer acceptability surveys at the beginning of the CGJ sample preparation and after storage at ambient temperature for 14 months are presented in Table 4. The different samples of CGJs at the beginning of preparation exhibited acceptable scores for all sensory attributes; all values were appreciably higher than the minimum acceptability score, i.e. 5.0 (Mousa and Al-Khateeb, 2017). The CGJ control sample had the highest scores in most sensory attributes and overall acceptance at the beginning of storage, but after 14 months of storage, the panellists did not accept the sensory attributes of the CGJ control sample (overall acceptability = 2.2 ± 1.0). However, the binary mixtures of food hydrocolloids (the G2C2 and G2P2 samples) showed significantly improved scores (p < 0.05) after 14 months of storage. The highest perceived scores of sensory attributes were in the G2C2 sample (overall acceptability = 6.8 ± 1.0). The use of hurdle technology consisting of the combination of natural antimicrobial hydrocolloids (GA and CMC) with ultrasound at the beginning of juice preparation could be the predominant factor in preserving the quality and safety of unpasteurised CGJs for long periods without adversely affecting sensory characteristics (Knorr et al., 2004; Sayanjali et al., 2011; Tomadoni et al., 2016; Ali and El Said, 2020). Moreover, the other storage conditions of acidic pH, storage temperature, and sealed pre-pasteurised storage bottle could also help maintain the shelf-life stability of CGJs. The effects of hydrocolloids mixtures combined with several non-thermal treatments on the safety of cloudy juices should be investigated in future studies.

Conclusion

The present work found that the addition of food hydrocolloids significantly (p < 0.05) improved cloud stability and vitamin C retention in cloudy guava juice (CGJ) within 14 months of storage at refrigerated and ambient temperatures. The use of GA alone at 0.05 g/100 mL juice improved the cloud stability and AA retention at 4 and 25°C. However, the combination of GA with pectin (the G2P2 sample) and with CMC (the G2C2 sample) yielded cloud stabilities of 4.3 and 4.4 times at 4°C, and 6.1 and 6.3 times at 25°C, higher than those of the control sample. AA stability in the G2C2 sample was highest among all other treatments giving 130.0 ± 1.0 mg AA/100 mL juice at 25°C and 130.2 ± 0.9 mg AA/100 mL juice at 4°C. These properties of the G2C2 sample are likely attributed to its higher particle-to-particle repulsion in addition to steric repulsion. These intermolecular interactions in the stable colloidal matrix improved the preservation of natural antioxidants in CGJ. Furthermore, this binary mixture of GA and CMC improved the physicochemical properties and overall consumer acceptability of CGJ after long storage periods at ambient temperature. These observations could improve the preparation of CGJs at home or at industry scales. The proposed hydrocolloid composite methodology could increase the health benefits of cloudy juices preserving original natural antioxidant levels over long storage times.

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