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Full Length Research Paper

Effect of storage temperature on the physicochemical, nutritional and microbiological quality of pasteurised soursop (*Annona muricata* L.) Juice

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This work was aimed at investigating the effect of storage temperature on the physicochemical, nutritional, and microbial quality of soursop juice. Physicochemical quality was determined by measuring changes in pH, titratable acidity, total soluble solids and colour (L*a*b* values). Ascorbic acid levels, total phenolic content and total antioxidant capacity were analysed to determine the effect of storage temperature on nutritional quality. The changes in aerobic mesophilic and psychrophilic bacteria, lactic acid bacteria, *Enterobacteriaceae*, as well as yeast and moulds were enumerated to determine the effect of storage temperature on microbial quality. Soursop juice was pasteurised at 85°C for 5 min and stored at varying temperatures of 4, 10 and 25°C. Storage was carried out for 12 weeks at both 4 and 10°C, while at 25°C the juice was stored for 3.5 weeks when visible signs of spoilage was detected. Storing soursop juice at 4ºC did not affect quality, however, at 25°C, decreases in pH and total soluble solids with an increase in titratable acidity was observed. In addition, a faster rate of ascorbic acid degradation was observed at 25°C. The main group of microorganisms that were responsible for the spoilage of soursop juice stored at 25°C were lactic acid bacteria, and yeasts and moulds. The results of this work show that pasteurised soursop juice can be stored at refrigeration temperatures without changes in quality.

Key words: Soursop juice, pasteurization, storage temperature, lactic acid bacteria, ascorbic acid.

INTRODUCTION

Extraction of juice from fruits can be used to improve the usage of fruits as the extracted juice can be easily transported and stored (Bhardwaj and Pandey, 2011). The extraction process, however, can lead to microbial contamination, which can limit the shelf life of the extracted juice. To improve the shelf life of the extracted juice, thermal processing methods such as pasteurization can be carried out to reduce the level of microbial contamination and other reactions (enzymatic and non-enzymatic) which can lead to spoilage (Rivas et al., 2006). However, due to the fact that pasteurization do not completely destroy all microorganisms and enzymes, changes in quality may occur in the juice during storage (Chia et al., 2012; Polydera et al., 2003; Touati et al.,

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2016; Umme et al., 2001; Wibowo et al., 2015). Some of these changes may affect colour and flavor which in the end may reduce the sensory acceptability of the juice (Umme et al., 2001). The degradation of some bioactive compounds such as ascorbic acid may also occur during storage, thus, reducing the nutritional composition of the juice (Touati et al., 2016). The resuscitation and growth of microorganisms is also possible during storage. The temperature of storage usually influences the changes that occur during storage. It is, therefore, important to investigate the effect of storage temperature on the changes in fruit juice after pasteurization.

This study sought to specifically pasteurise soursop juice and determine how storage temperature impacted on physicochemical, nutritional and microbial safety. Soursop juice was pasteurised at 83°C for 5 min and stored at temperatures of 4, 10 and 25°C. The effect of storage temperature on the physicochemical quality of the juice was determined by measuring the changes in pH, total soluble solids, titratable acidity and colour (L*a*b*). The effect of storage temperature on the changes in nutritional components such as ascorbic acid levels, total phenolic content and total antioxidant capacity were determined. Additionally, a kinetic model was developed to explain the effect of storage temeprature on the changes in ascorbic acid levels. The microbial quality of the juice during storage was assessed by enumerating the changes in aerobic mesophilic and psychrophilic bacteria, lactic acid bacteria, *Enterobacteriaceae*, and yeast and moulds.

MATERIALS AND METHODS

Pasteurization of soursop juice and shelf life studies

Mature and uniformly ripe soursop fruits (*Annona muricata* L.) were cleaned, disinfected and peeled. The seeds were removed from the pulp and the juice extracted. The extracted juice were pasteurised in sterilised glass tubes at and rapidly cooled in ice-cold water. Pasteurization was carried out on a water bath at 83°C for 5 min. To determine the initial microbial load immediately after pasteurization, some soursop juice samples were store at 4°C for 24 h to allow for the resuscitation of stressed microorganisms.

The pasteurised soursop juice were stored in incubators at different temperatures (4, 10 and 25°C) and changes in quality monitored. Storage was carried out for 12 weeks at both 4 and 10°C, while at 25°C the juice was stored for 3.5 weeks until spoilage was detected. Periodically, the soursop juice were sampled and physicochemical and microbial analyses carried out on the same day. The sampled juice was, however, stored at -80°C for subsequent nutritional analyses. The quality of both the pasteurised and unpasteurised juices were analysed before storage, and the pasteurised juice was used as control for comparative purposes.

Microbial analyses

The effect of pasteurization on the survival of microorganisms in soursop juice was determined by enumerating the changes in aerobic mesophiles and psychrophiles, lactic acid bacteria, *Enterobacteriaceae*, and yeast and moulds. After performing the appropriate decimal dilutions in peptone water, agar plates were inoculated with the juice samples. Both aerobic mesophilic and psychrophilic bacteria were enumerated on plate count agar (Oxoid Ltd., UK). The plates for aerobic mesophiles were incubated at 30°C for 48 h while the plates for aerobic psychrophiles were incubated at 10°C for 5 days. Lactic acid bacteria were enumerated on de Man-Rogosa-Sharpe agar (Oxoid Ltd., UK) were incubated at 30°C for 48 h. Yeast and moulds were enumerated on Sabouraud Dextrose Agar (Oxoid Ltd., UK) that were incubated at 35°C for 24 h while *Enterobacteriaceae* were enumerated on Violet Red Bile Glucose agar (Oxoid Ltd., UK) incubated at 37°C for 24 h (Suárez-Jacobo et al., 2010).

Physicochemical analyses

The effect of storage temperature on physicochemical quality was assessed by determining the changes in pH, total soluble solids, titratable acidity and colour. A pH meter and digital refractometer (MA871, Milwaukee Instruments USA) were used to determine the pH and the total soluble solids, respectively. The titratable acidity was determined by titrating the soursop juice against NaOH (AOAC, 2010), while a colour meter (CS-10, CHN Spec, China) was used to measure the changes in L*a*b values.

Nutritional analyses

Ascorbic acid was extracted with metaphosphoric-acetic acid solution and determined using the 2,4- dinitrophenylhydrazine based assay (Kapur et al., 2012). L-ascorbic acid was used as a standard and the results expressed as mg ascorbic acid per 100 g of soursop juice. Both the total phenolic content and total antioxidant capacity were analysed after extracting the juice with equal volumes of methanol. The Folin-Ciocalteu based assay was used to determine the total phenolic content (Meda et al., 2005) while the total antioxidant capacity was determined based on the scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (Sánchez-Moreno et al., 1999). Gallic acid was used as the standard for both the total phenolic content and total antioxidant capacity.

Kinetic modelling of the effect of storage temperature on ascorbic acid levels

A first-order kinetic model (Equation 1) was developed to explain the effect of storage temperature on ascorbic acid levels.

$$\frac{d[AA]}{dt} = k_{AA} \cdot [AA] \quad (1)$$

The rate of change of ascorbic acid with time was denoted as $\frac{d[AA]}{dt}$, whereas the measured ascorbic acid levels and first-order rate constant were denoted as [AA] and k_{AA}, respectively. The Arrhenius equation (Eq. 2) was used to model the effect of temperature on the first-order rate constant;

$$k_{AA} = k_{AA,ref} \cdot e^{\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)} \quad (2)$$

$k_{AA,ref}$ was the reference first-order rate constant at a chosen

Table 1. Physicochemical, nutritional and microbial quality of unpasteurised and pasteurised (83 °C for 5 min) soursop juice.

Variable	Unpasteurised	Pasteurised
pH	3.86±0.10	3.79±0.09
Total soluble solids (°brix)	15.51±0.48	16.10±0.73
Colour		
L*	63.71±1.46	62.29±64.22
a*	-3.20±0.19	-3.05±0.20
b*	4.33±0.35	4.29±0.39
Titratable acidity (mg/100 g)	27.85±2.95	25.19±3.19
Ascorbic acid (mg/100 g)	44.76±5.15	41.98±3.16
Total phenolic content (mg/100 g)	187.95±12.47	179.61±12.81
Total antioxidant capacity (mg/100 g)	326.69±15.27	298.94±16.04
Aerobic mesophiles	3.77±0.31	2.10±0.26
Aerobic psychrophiles	2.68±0.19	2.12±0.18
Lactic acid bacteria	3.90±0.35	2.83±0.17
Yeast and moulds	3.27±0.21	2.40±0.27
Enterobacteriaceae	ND	ND

D*, not detected.

reference temperature(T_{ref}) of 20°C while E_a was the activation energy in J/mol and R is the universal gas constant (8.314 J/mol/K). The kinetic model was implemented in Optipa (Hertog et al., 2007).

Statistical analyses

Statistical analyses to determine the effect of storage temperature on the quality of soursop juice was carried out using analysis of variance (ANOVA). The effect of storage temperature was assessed by comparing the quality of the stored juice to the control (freshly pasteurised juice). When a significant effect was observed, the Tukey test was performed to determine which juice samples were different from the control. The same procedure was performed to determine the effect of storage temperature at a specific storage time. The difference among means were identified at a significance level of 0.05. All statistical analyses were performed at a significance level of 0.05 using SPSS (IBM, SPSS Statistics 20).
The statistical analyses of the obtained model parameters were estimated using an error based bootstrap resampling technique. By bootstrapping the complete dataset multiple times, the model was fitted to the obtained bootstrap datasets several times, hence obtaining the distribution around individual parameters from which the standard deviations were estimated.

RESULTS AND DISCUSSION

Effect of pasteurization on the quality of soursop juice

The effect of pasteurization on the quality of soursop juice is shown in Table 1. The pH, total soluble solids, titratable acidity and colour of the juice were not significantly affected by pasteurization. Pasteurization resulted in reductions in ascorbic acid levels, total phenolic content and total antioxidant capacity; however, these reductions were not significantly different compared to the unpasteurised juice. The levels of aerobic mesophiles, aerobic psychrophiles, lactic acid bacteria, and yeast and moulds were significantly lower in the pasteurised compared to the unpasteurised juice. *Enterobacteriaceae* was not detected in both the unpasteurised and pasteurised soursop juice.

Ascorbic acid levels have been observed to decrease due to the effects of pasteurization (Ranu and Uma, 2012; Vikram et al., 2005). Pasteurization offers the possibility of extending the usage of fruit juices by reducing the action of microorganisms. Temperatures between 80-95°C for time durations between 1-10 min are usually employed in the pasteurization of fruit juices (Chia et al., 2012). In this study, pasteurization at 85°C reduced the microbial load of soursop juice. Fruit juice are usually expected to have between 3-5 $\log_{10}$cfu/ml of yeast (Vasavada and Heperkan, 2002) with the limit of microbial shelf life around 6 $\log_{10}$cfu/ml. The levels of microorganisms in both the unpasteurised and pasteurised juice were within acceptable limits.

Effect of storage temperature on the microbial of soursop juice

Figure 1 shows the effect of storage temperature on the growth of aerobic mesophiles. At 4°C, no significant changes in aerobic mesophiles was observed between the control and the stored samples. A similar observation was made in the changes in yeast and moulds, and lactic acid bacteria. However, storage at 4°C resulted in a gradual but not significant increase in aerobic

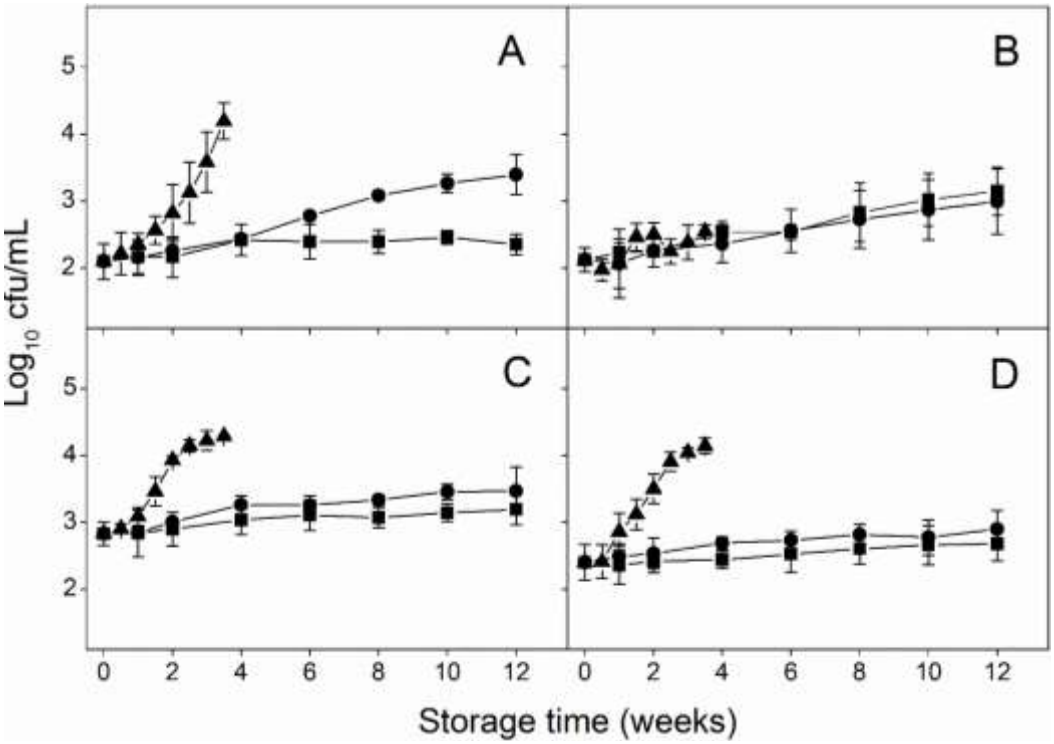

Figure 1. Changes in aerobic mesophiles (A), psychrophilic mesophiles (B), lactic acid bacteria (C), and yeasts and moulds of soursop juice stored at 4 (■), 10 (●) and 25 °C (▲). The error bars represent standard deviation.

psychrophiles.

Storage at 10 and 25°C resulted in growth of aerobic mesophiles with significant differences between the control and the stored sample observed after 6 and 1.5 weeks of storage, respectively (Figure 1). In addition, growth of aerobic psychrophiles, lactic acid bacteria (Figure 1), and yeasts and moulds (Figure 1) was observed. Lactic acid bacteria, and yeast and moulds were the predominant group of microorganisms that were able to grow in the soursop juice. The growth of these two groups of microorganisms might be due to low pH of soursop juice, which favors the growth of acidophilic microorganisms. Indeed the spoilage of most juices has been attributed to the presence and growth of yeasts (Alwazeer et al., 2002; Elez-Martínez et al., 2005). The inhibition of microbial growth at 4ºC means that the storage of soursop juice can be extended beyond 12 weeks. In pasteurised pineapple juice, the microbial population remained unchanged during refrigerated temperature storage for 12 weeks (Chia et al., 2012).

Effect of storage temperature on pH, titratable acidity and total soluble solids of soursop juice

The effect of storage temperature on the pH of soursop juice is shown in Figure 2. Compared to the control (freshly pasteurised juice before storage), storage at refrigeration temperature (4°C) did not have a significant effect on the pH of soursop juice. A similar effect have been observed in other fruit juices stored a refrigeration temperatures. In thermo-sonicated soursop nectar, the pH did not change significantly during storage at 4°C for 45 days (Anaya-Esparza et al., 2017). Also, no significant changes in pH were observed during refrigerated storage of thermally pasteurised pineapple juice (Chia et al., 2012), thermally treated juice blend of orange and carrot (Rivas et al., 2006) and heated orange juice (Bull et al., 2004; Yeom et al., 2000). Higher temperatures of storage have been observed to affect the quality of fruit juices. At 10°C and 25°C, significant decreases in pH were observed between the control and the stored samples after 10 and 1.5 weeks of storage, respectively (Figure 2). This decrease is similar to the observation made by Touati et al. (2016) in thermally treated grape, orange and pear nectars and in soursop juice (Abbo et al., 2006).

Figure 3 shows the effect of storage temperature on the titratable acidity of soursop juice. There was a general increase in titratable acidity during storage, however, no significant differences were observed between the control and the juice stored at 4°C. During storage at 10 and 25°C, significant differences in titratable acidity were observed between the control and the stored juice after10

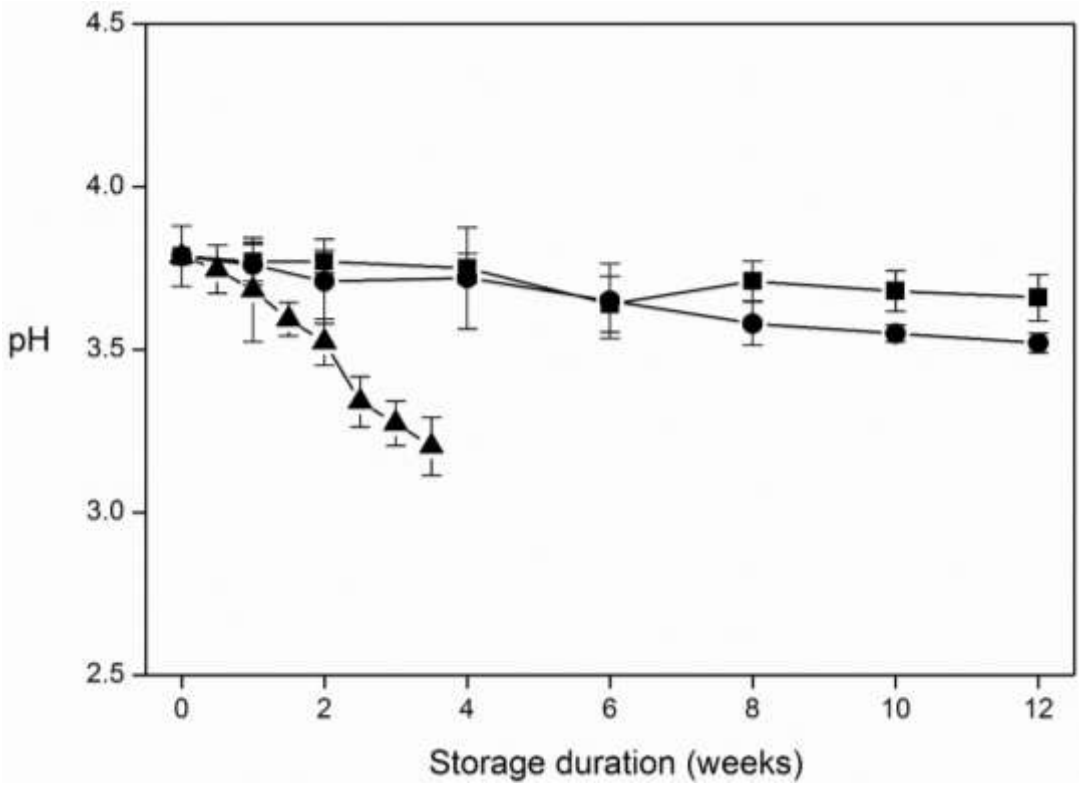

Figure 2. Changes in the pH of pasteurised soursop juice stored at 4 (■), 10 (●) and 25 °C (▲). The measured data points are the average of 4 replicates and the error bars represent standard deviations.

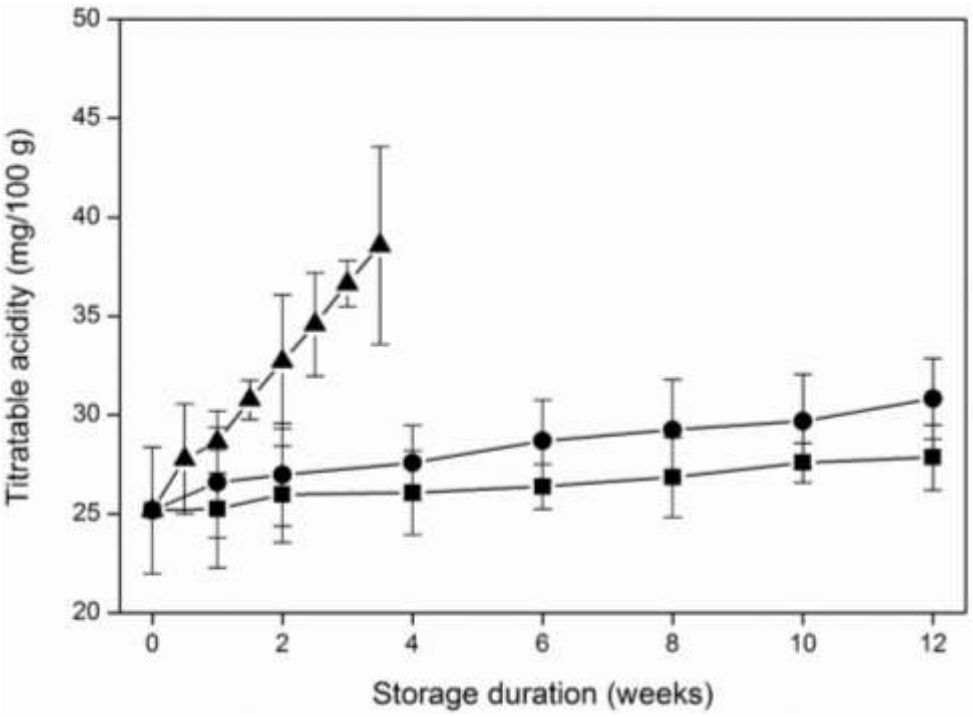

Figure 3. Changes in the titratable acidity of pasteurised soursop juice stored at 4 (■), 10 (●) and 25 °C (▲). The measured data points are the average of 4 replicates and the error bars represent standard deviations.

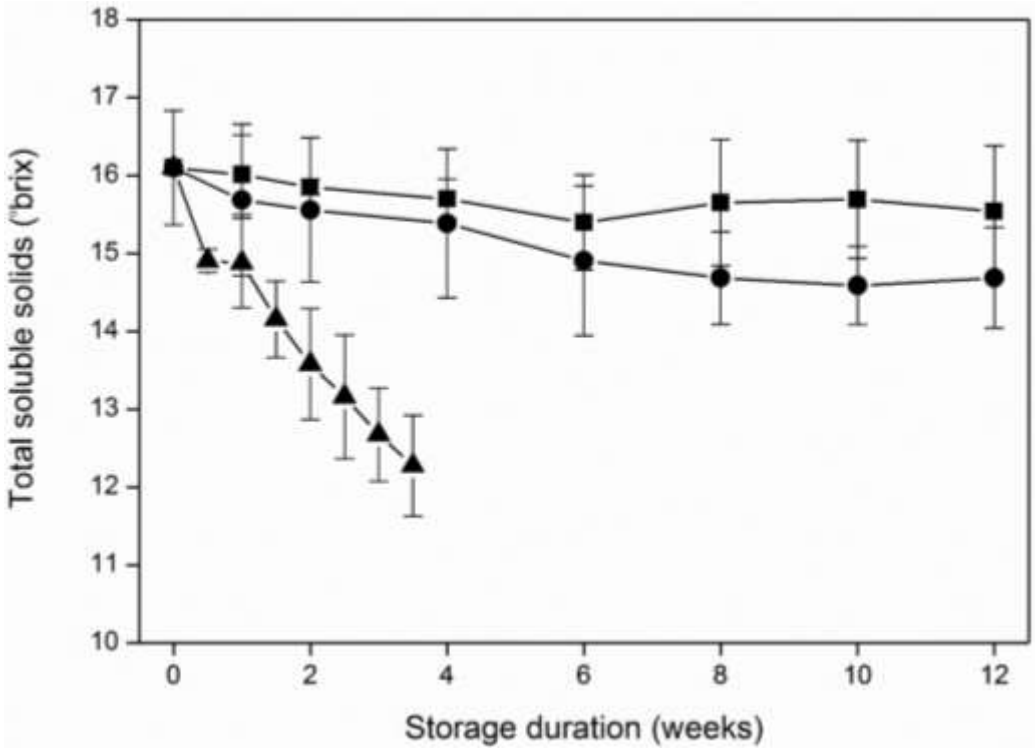

Figure 4. Changes in the total soluble solids of pasteurised soursop juice stored at 4 (■), 10 (●) and 25 °C (▲). The measured data points are the average of 4 replicates and the error bars represent standard deviations.

and 2 weeks, respectively. An increase in titratable acidity has also been observed in other fruit juices during storage (Anaya-Esparza et al., 2017; Bull et al., 2004; Chia et al., 2012). The changes in total soluble solids of soursop juice during storage is shown in Figure 4. Storage at 4°C did not have a significant effect on the total soluble solids of the juice. Anaya-Esparza et al. (2017) made a similar observation. According Bhardwaj and Pandey (2011), the retention or slight increase in total soluble solids of fruit juices during storage is desired for quality preservation. Storage at low temperature, therefore, can help retain the total soluble solids content of soursop juice. At 10 and 25°C, however, significant decreases in total soluble solids were observed after 8 and 1.5 weeks of storage, respectively, compared to the control. Comparing the three storage temperatures, significant differences in total soluble solids were observed on weeks 3 and 3.5, where the juice samples stored at 25°C recorded significantly lower total soluble solids content. The changes in pH, titratable acidity and total soluble solids of soursop juice during storage, especially at the higher temperatures, can be attributed to the growth of microorganisms. The utilisation of sugars by microorganisms can led to the production of organic acids, which can lead to reduction in pH and total soluble solids, and an increase in titratable acidity (Rivas et al., 2006).

Effect of storage temperature on the colour of soursop juice

Figure 5 shows the changes in colour (L*a*b*) of soursop juice store at different temperatures. There was a general decrease in L* values. No significant difference in L* value were observed between the control and soursop juice stored at 4 and 10°C after one week. However, the soursop juice stored at 25°C recorded a significantly lower L* value compared to the control after one week. At 4°C, significant differences in L* values were observed between the control and stored samples on weeks 6, 8, 10 and 12. Similarly, at 10°C, significant differences in L* values were observed between the control and stored samples on weeks 4, 6, 8, 10 and 12. During storage at 25°C, significant differences in L* values between the control and the stored samples were observed after 0.5 weeks of storage. Comparing the different storage temperatures, significant difference in L* values were observed between the juice samples stored at 4 and 10°C on weeks 10 and 12. Significant differences were also observed between the samples stored at 25 °C and the lower storage temperatures (4 and 10°C) on all sample weeks except on week 0.5 (Figure 2). There was a slight increase in a* values of soursop juice during storage, however, no significant differences in a* values were observed between the control and all the stored

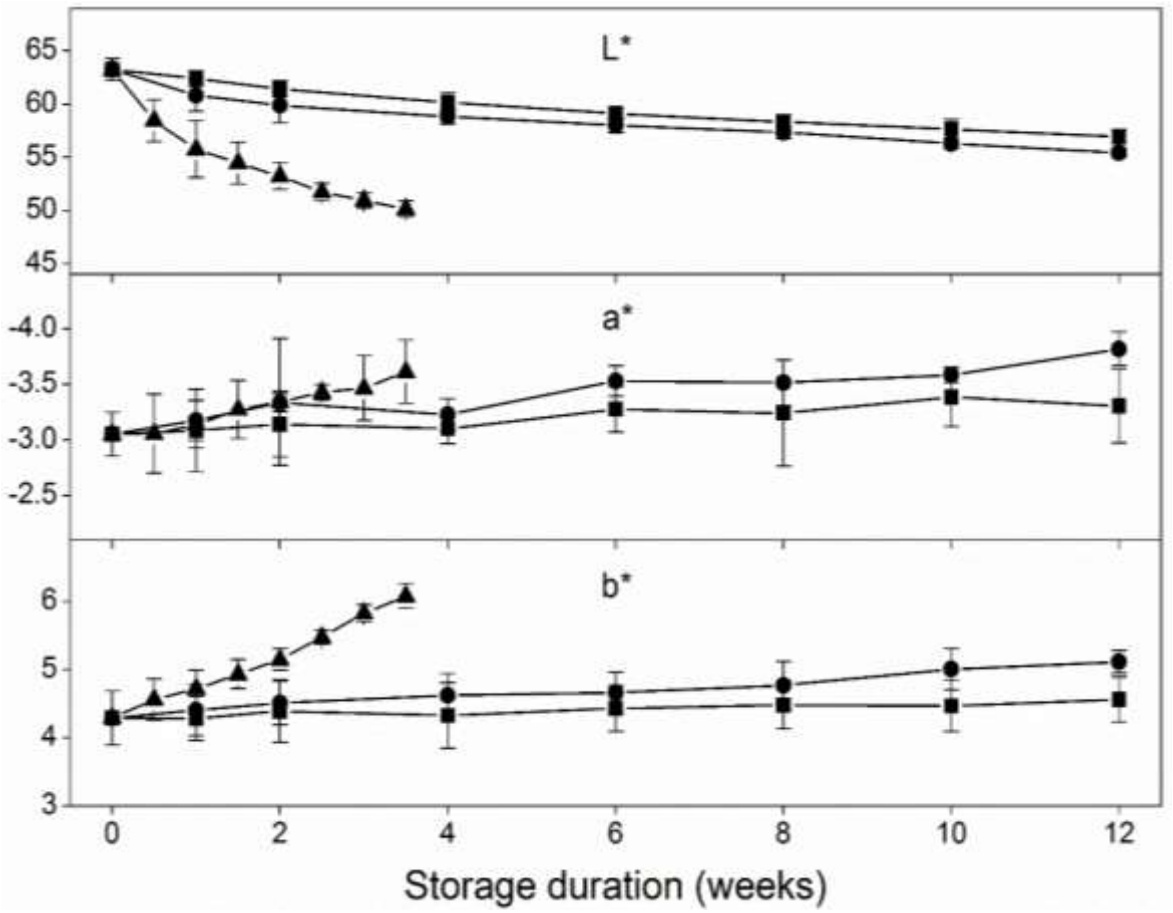

Figure 5. Change in the colour (L*a*b*) values of soursop juice during storage at 4 (■), 10 (●) and 25 °C (▲). The measured data points are the average of 4 replicates and the error bars represent standard deviations.

samples. There was a general increase in b* values of soursop juice during storage (Figure 2B). At 4°C, no significant differences in b* values were observed between the control and the stored samples. At 10°C, however, significant differences were observed compared to the control on weeks 10 and 12. During storage at 25°C, significant differences in b* values were observed after 1.5 weeks of storage. Comparing the different storage temperatures, a significant difference in b* values were observed between the juice samples stored at 4 and 10°C only on week 10. Significant differences were, however, observed between the juice samples stored at 25°C and the lower storage temperatures (4 and 10°C) after 2 weeks of storage (Figure 2B).

Though extracted soursop juice is white in color, it can gradually change to creamy white and eventually yellowish depending on the storage condition. The L*a*b* values of soursop juice in this study did not change significantly at the storage temperature of 4°C. This observation is supported by the observations in other studies where soursop nectar retained its color at 4°C for 45 days and again after pasteurization and storage at 4°C and evaluated during sensory analysis. The retention of color at the low storage temperature can be attributed to a reduction in browning reactions. At higher storage temperatures, browning reactions can occur thus reducing the whiteness of soursop juice. This is reflected in the decreasing L* values and the changes in a* and b* values.

Effect of storage temperature on nutritional quality of soursop juice

The effect of storage temperature on the total phenolic content and total antioxidant capacity of soursop juice is shown in Table 2. There was a general reduction in the total phenolic content of soursop juice within the first few weeks of storage, irrespective of the storage temperature. Afterwards, the total phenolic content remained relatively constant throughout the storage period. The total phenolic content of the stored soursop juice were no significantly different compared to the control. Storage at 4 and 10°C resulted in a reduction in total

Table 2. Total phenolic content and total antioxidant capacity of pasteurised soursop juice stored at different temperatures.

Temperature (ºC)	Time (min)	Total phenolic content (mg GAE /100 g)	Total antioxidant capacity (mg GAE /100 g)
4	0	179.61±12.81	298.94±16.04
	1	167.52±17.40	284.89±12.74
	2	169.89±6.38	280.41±14.60
	4	161.76±7.35	298.42±8.03
	6	169.85±17.54	293.21±16.29
	8	171.26±14.26	285.99±6.35
	10	166.19±7.68	280.32±21.23
	12	168.79±3.95	282.09±15.37
10	0	179.61±12.81	298.94±16.04
	1	177.01±9.07	287.63±3.20
	2	167.98±8.02	275.09±6.31
	4	166.34±13.11	278.10±16.55
	6	167.37±12.27	274.48±19.47
	8	168.38±16.32	286.60±13.06
	10	169.05±11.82	276.54±17.83
	12	169.24±13.56	290.37±14.30
25	0	179.61±12.81	298.94±16.04
	0.5	176.23±6.38	296.71±17.85
	1	155.02±4.62	278.42±13.15
	1.5	164.89±14.64	277.72±16.48
	2	163.07±8.46	286.05±18.64
	2.5	164.82±4.84	281.72±17.75
	3	162.71±9.09	280.61±14.14
	3.5	159.58±9.16	288.42±11.89

antioxidant capacity of soursop juice until week 4. However, at week 6 the total antioxidant capacity increased and generally decreased again until the end of the storage period. At 25°C storage, the reduction in total antioxidant capacity occurred until week 1.5, increased at week 2 and remained constant until the end of storage (Table 2). Similar to the total phenolic content, the total antioxidant capacity of the stored soursop juice were no significantly different compared to the control.

Storage temperature have been observed to have a variable effect on the total phenolic content and total antioxidant capacity of fruit juices. The phenolic content of pineapple juice remained relatively unchanged during 13 weeks of storage (Chia et al., 2012); however, decreases were observed in soursop nectar (Anaya-Esparza et al., 2017). It is possible that the total phenolic content of the soursop juice remained relatively unchanged due to the inactivation of peroxidase during pasteurization (Odriozola-Serrano et al., 2008). In other fruit juices, no changes in antioxidant capacity have been observed during storage (Anaya-Esparza et al., 2017; Mgaya-Kilima et al., 2014).

Effect of storage temperature on ascorbic acid level

Figure 6 shows the changes in ascorbic acid levels of soursop juice as well as the modelled first-order degradation kinetics of ascorbic acid during storage. There was a general decrease in ascorbic acid levels during storage. No significant differences in ascorbic acid levels were observed after one week of storage at the different temperatures. During storage at 4 and 10°C, significant differences in ascorbic acid levels were observed between the control and stored samples after 2 weeks. During storage at 25°C, however, significant differences in ascorbic acid levels were observed after 1.5 weeks of storage. Comparing the different storage temperatures, significant differences in ascorbic acid levels were observed between the juice samples stored at 4 and 10°C only on week 12. The estimated model parameters for the degradation of ascorbic acid in soursop juice gave a degradation rate constant and activation energy of 0.087 min^{-1} and 43.98 kJ/mol with standard deviations of 0.004 and 2.99, respectively. The degradation of ascorbic acid during storage of fruit juices

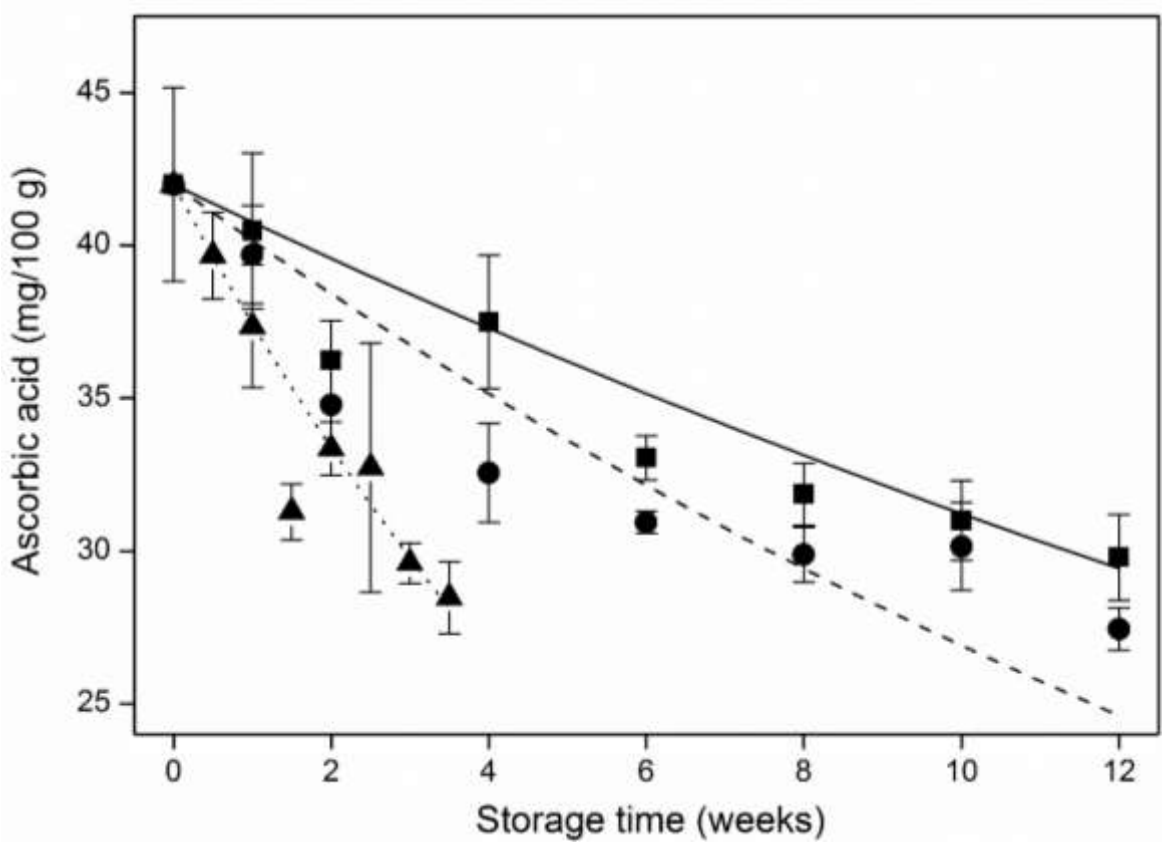

Figure 6. Changes in ascorbic acid levels of soursop juice stored at 4 (■), 10 (●) and 25 °C (▲). The measured data points (average of 4 replicates) are plotted along with the first-order degradation modelled (dash lines) of ascorbic acid during storage. The error bars represent standard deviation.

is one of the most important factors affecting quality (Davey et al., 2000). In most fruit juices, ascorbic acid is the most important factor influencing nutritional quality (Franke et al., 2004) The degradation of ascorbic acid have been observed in other fruit juices (Ajibola et al., 2009; Polydera et al., 2003; Roig et al., 1995). Different levels of degradation of ascorbic acid have been reported in the literature. In the soursop juice, ascorbic acid levels reduced by 29.04 and 34.65% during storage at 4 and 10°C, respectively. However, during storage a 25°C, ascorbic acid levels decreased by 32.93% within 3.5 weeks. This shows that the degradation of ascorbic acid in soursop juice is temperature dependent. In several fruit juices, the temperature dependence of ascorbic acid degradation during storage have been confirmed (Burdurlu et al., 2006; Polydera et al., 2003; Roig et al., 1995; Sapei and Hwa, 2014; Uddin et al., 2002). The first-order model was adequate to explain the degradation of ascorbic acid in soursop juice. Similar rate constant and activation energy has been reported in the literature for the degradation of ascorbic acid in other fruit juices (Zheng and Lu, 2011; Wibowo et al., 2015; Polydera et al., 2003; Sapei and Hwa, 2014).

Conclusions

The physicochemical, nutritional and microbial quality of soursop juice is affected by the temperature of storage. Storage at 4°C can be used to achieve shelf life in excess of 12 weeks without changing the quality of soursop juice. At higher storage temperatures, the growth of acidophilic microorganisms such as lactic acid bacteria, and yeasts and moulds enhances the spoilage of soursop juice resulting in changes in the quality of soursop quality. Loss of ascorbic acid occurs during storage of soursop juice. However, the temperature of storage influences this loss with higher losses occurring at the high storage temperatures.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Biochemical composition of Ethiopian coffees (*Coffea arabica* L.) as influenced by variety and postharvest processing methods

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This study investigated the influence of variety, processing methods and their possible interactions on the biochemical composition of green coffee beans. The results revealed that the biochemical composition was significantly affected by the interaction effect of variety and processing methods. As compared to coffees processed in semi-washed and dry method, the highest total chlorogenic acid (TCGA) content was obtained in coffee varieties 74-112, 74-110 and 74-165, processed by washed method. The washed processing method also enhanced the caffeine content of the coffee beans. The highest value was observed in the coffee varieties 74-4, 74-112 and 74-165. The dry method, on the other hand, increased the sucrose content and the highest value was obtained in the varieties 74-110, 74-140 and 74-148. In general, the present study indicated the existence of variation in biochemical composition of Ethiopian Arabica coffee varieties and is influenced by the different processing methods. This study, however, was carried out with coffee varieties grown under same growing conditions and did not consider season variation. Therefore, it is advisable to further evaluate with more number of coffee varieties grown in different growing environments and seasons.

Key words: Processing methods, green beans, caffeine, chlorogenic acid, sucrose.

INTRODUCTION

Coffee is one of the most consumed beverage by more than one-third of the world's population (DaMatta et al., 2018; Samper et al., 2017). Awareness of quality and health benefits of coffee among consumers is increasing the demand for coffee consumption in both developed and developing countries (Cheng et al., 2016; Ryota, 2018). Coffee is grown in more than 80 countries in tropical and subtropical regions of the

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world and is exported in green or roasted beans to more than 165 countries (Abreu et al., 2012). The crop accounts for 75% of export revenue and provides livelihoods for smallholder coffee producers around the world (WIPR, 2017). According to the statistical report of ICO (2018), 158.9 million bags of green coffee beans have been produced in 2017/2018.

Ethiopia is the largest coffee producer in Africa and is the 10th exporter in the world (ICO, 2018). Arabica coffee is known as backbone of the country's economy, accounting for 22% of the export (Bart, 2018). The country is naturally gifted with a suitable climate and has the potential to produce single origin specialty Arabica coffee beans with a wide range of flavours (Coste et al., 1992; Labouisse et al., 2008). On global markets, Ethiopia's Arabica coffee is valued for its unique taste and aroma. For the country itself, this makes Arabica coffee one of the most traded commodities with a significant social and economic impact for more than 25 million farmers (WIPR, 2017). Furthermore, premium price (ca. $7-8 per kg of coffee beans) is being paid for specialty coffee of some specific origin such as Harar, Yirgacheffe and Sidama. Thus, an increased specialty coffee market is beneficial for Ethiopia to remain competitive on the international market, provided that the country produces high quality coffee and the supply remains stable.

The inherent quality and compositional characteristics of coffee beans are, however, among others, controlled by interacting effects of genetic trait (Bertrand et al., 2006; Leroy et al., 2006), growing altitude (Avelino et al., 2005; Tolessa et al., 2017; Worku et al., 2018), shade management (Avelino et al., 2007; Tolessa et al., 2017; Worku et al., 2018), harvest periods (Guyot et al., 1995; Guyot et al., 1996; Tolessa et al., 2017) and postharvest processing technique (Duarte et al., 2010; Joët et al., 2010; Selmar et al., 2002; Tolessa et al., 2018).

The chemistry of coffee quality is highly complex with a wide range of compounds that are found in green coffee beans. Caffeine (0.9 to 3%), chlorogenic acids (CGA, 5 to 8%) and sucrose (7 to 11%) are the key compounds found in coffee beans (De Castro and Marraccini, 2006; Ky et al., 2001). These chemical constitutes are considered as precursors for coffee cup quality attributes e.g. aroma and flavour (Cheng et al., 2016; Joët et al., 2010). For example, sucrose and trigonelline give rise to a very good flavour. Sucrose also acts as aroma precursor, originating several substances (e.g. furans, aldehydes and carboxylic acids) affecting both flavour and aroma of the beverage (Farah et al., 2006b). It reacts with amino acids to produce pyrazines and carbonyl compounds, which are involved in flavour formation (Feldman et al., 1969). Furthermore, sucrose to amino acids ratio in green beans determines also profile of volatile compounds, explaining coffee quality attributes (Tressl et al., 1982).

The species and genotype of the coffee plant can also play an important role in the biochemical compositional content of green coffee beans (Ky et al., 2001; Leroy et al., 2006). In the past four decades, Jimma Agricultural Research Center has developed and distributed improved Ethiopian Arabica coffee varieties which are high yielding, with disease resistance and adaptability to different agro-ecological zones (Tirfe et al., 2015). The existence of a wide diversity for quality trait has also been reported elsewhere (Abeyot et al., 2011; Kitila et al., 2011). Van der Vossen (1985) also reported significant differences in cup quality attributes among different Arabica coffee cultivars and their crosses.

However, studies exploring the combination of biochemical compounds present in these varieties still lack more detailed analysis. The metabolic reactions in coffee seeds occurring during different processing methods also affect the chemical composition of the green beans. Thus, the changes in chemical composition of coffee beans in response to different postharvest processing methods are also not fully understood. Therefore, the present study has been initiated to determine the biochemical composition of ten coffee berry disease (CBD) resistant Ethiopian Arabica coffee varieties in relation to different postharvest processing methods.

MATERIALS AND METHODS

Study site

The experiment was conducted at Suntu coffee farm, under Limu coffee plantation, Oromia, Ethiopia, during the 2012/2013 cropping season. It is located in the Oromia regional state, Jimma Zone, Limmu Kossa district, at 410 km distance from Addis Ababa and 75 km from Jimma town. The total area coverage of the farm was 1602 ha and the coffee was produced for both the local and international market. This study area was selected based on the availability of the top ten leading CBD coffee varieties. The description of the study site is shown in Table 1.

Study material and experimental design

Ten coffee berry diseases (CBD) resistant Arabica coffee varieties (Table 2) were used for the study. These varieties were the top ten leading coffee varieties producing a large number seeds. They were supplied to the farmer by the Jimma Agriculture Research Center over the last four decades (1979-2010) (Kufa et al., 2011). Some differences were observed among these coffee varieties with regard to their popularity, adaptability, as well as preference and demand by the users (Kufa et al., 2011). Three hundred randomly selected shade grown coffee trees with similar age range (5 to 6 years old after stumped) were used as sources of red ripen cherries for each variety (Table 2).

About 21 kg fully ripen, red coloured coffee cherries were collected per variety and subjected to three (each of 7 kg coffee cherries) processing methods: dry (without washing), semi-washed (partial washing) and washed (fully washed). The experiment was arranged in a split plot design with ten levels of varieties as main plot and three levels of processing methods as

Table 1. Description of the study site.

Description of the Suntu coffee farm	
Geographical location	
Latitude	8°05'N
Longitude	36°57'E
Meteorological data	
Altitude (masl)	1600 -1800
Rainfall (mm)	1720
Temperature (°C)	
Minimum	12
Maximum	28
Soil	
Classes	Eutric Nitosol
pH	4.5 - 5.8
Coffee area (ha)	1602
Coffee type	Arabica pure line selections

Table 2. Description of Arabica coffee varieties grown at Suntu coffee farm.

Variety	Yield (kg ha^{-1})			Growing altitude		
	On station	On Farm	Special feature	High altitude (1750-2100 m)	Mid altitude (1550-750 m)	Low altitude (1000-1550 m)
74-110	1910	900-1000	Compact	HS	S	US
74-112	1810	900-1000	Compact	HS	S	US
7-41	1220	600-700	Open	S	S	US
74-140	1970	900-1000	Compact	HS	HS	US
74-158	1910	900-1000	Compact	HS	HS	US
75-227	1790	800-900	Open	HS	S	US
74-148	1800	600-700	Compact	HS	HS	US
74-4	1660	800-900	Open	S	HS	S
74-40	1620	800-900	Mid Open	S	S	S
74-165	1730	800-900	Compact	HS	S	US

HS: Highly suitable, S: suitable and US: unsuitable.

sub-plot with three replications (farms). Moreover, the detailed coffee cherry processing procedures are presented in Tolessa et al. (2016).

Analyses of caffeine and chlorogenic acid

Caffeine and chlorogenic acid content were analyzed as described by Alonso-Salces et al. (2009a). Ground green coffee beans (100 mg) were submitted to direct solvent extraction with 10 mL of methanol/water/acetic acid (30:67.5:2.5, v/v/v) containing 2 mg mL^{-1} ascorbic acid in an ultrasonic bath for 15 min. Then, the solvent extract was filtered through a 0.45 µm PTFE filter prior to injection into the HPLC having a prevail C18 (250 × 4.6 mm, i.d. 5 µm, 25°C) column. The mobile phase composed of 0.2% acetic acid in water (v/v) (solvent A) and methanol (solvent B). The following elution conditions were applied one after the other: the first 0-30 min, linear gradient from 10 to 30% B; 30-40 min, linear gradient from 30 to 40% B; 40-45 min, 40% B isocratic; 45-50 min, linear gradient from 40 to 50% B; 50-55 min, 50% B isocratic; 55-65 min, linear gradient from 50 to 70% B. Flow rate was 1 mL min^{-1} and the injection volume was 50 µL. Caffeine and chlorogenic acid were detected by a DAD detector at 280 and 320 nm wavelength, respectively.

Quantification and identification of the chlorogenic acids subclasses: caffeoylquinic acid (CQA), feruloylquinic acid (FQA), dicaffeoylquinic acid (diCQA) and feruloyl-caffeoylquinic acid (FCQA) were done using an already established liquid chromatography-time of flight-mass spectrometry (LC-TOF-MS) method (Alonso-Salces et al., 2009b). Total chlorogenic acid concentration is reported as the sum of all the mentioned individual chlorogenic acids. For these variables, data acquisition and processing were done using ChromQuest 4.1 SP2 software.

Sucrose analysis

Sucrose content was determined according to Knapp (1979). First,

Table 3. Caffeoylquinic acid (CQA) content of ten Arabica coffee varieties processed by the dry processing (DP), semi- washed processing (SW) and washed processing (WP) method, grown under similar conditions at Suntu coffee plantation, Jimma Zone.

Variety	3-CQA			4-CQA			5-CQA			TCQA		
	Processing method			Processing method			Processing method			Processing method		
	DP	SW	WP	DP	SW	WP	DP	SW	WP	DP	SW	WP
74-1	0.37^{h}	0.29^{op}	0.34^{k}	0.53^{i}	0.47^{p}	0.54^{k}	3.83g^{h}	2.84^{o}	4.09^{f}	4.74^{h}	3.59^{o}	4.97^{g}
74-4	0.37^{h}	0.28^{r}	0.28^{qr}	0.53^{i}	0.47^{p}	0.49^{n}	3.84^{gh}	3.91^{g}	3.57^{j}	4.74^{h}	4.66^{i}	4.35^{j}
74-40	0.39^{def}	0.31^{m}	0.38^{h}	0.55^{j}	0.48^{o}	0.59^{d}	3.82^{h}	3.48^{k}	4.21^{de}	4.76^{h}	4.27^{jk}	5.18^{e}
74-110	0.41^{bc}	0.34^{jk}	0.32^{m}	0.58^{e}	0.52^{L}	0.54^{k}	3.7^{i}	2.76^{op}	4.62^{b}	4.72^{hi}	3.62^{o}	5.47^{c}
74-112	0.41^{b}	0.31^{m}	0.39^{de}	0.58^{e}	0.51^{m}	0.62^{ab}	2.22^{rs}	3.37^{L}	5.00^{a}	3.21^{qr}	4.19^{kL}	6.01^{a}
74-140	0.43^{a}	0.35^{ij}	0.39^{fg}	0.61^{bc}	0.57^{fgh}	0.61^{c}	2.71^{p}	4.25^{d}	2.99^{n}	3.75^{n}	5.16^{e}	3.99^{m}
74-148	0.39^{g}	0.29^{o}	0.38^{g}	0.50^{ghi}	0.51^{m}	0.53^{L}	3.38^{L}	4.54^{b}	3.73^{i}	4.33^{j}	5.35^{d}	4.64^{i}
74-158	0.39^{de}	0.33^{L}	0.35^{i}	0.57^{fgh}	0.53^{L}	0.57^{efg}	2.23^{r}	4.35^{c}	3.22^{m}	3.23^{pq}	5.21^{e}	4.15l
74-165	0.39^{cd}	0.31^{mn}	0.32^{m}	0.58^{ef}	0.49^{no}	0.63^{a}	3.83^{h}	2.50^{q}	4.98^{a}	4.79^{h}	3.30^{p}	5.92^{b}
75-227	0.41^{b}	0.30^{no}	0.34^{ij}	0.56^{hi}	0.45^{q}	0.55^{i6}	3.19^{m}	2.09^{t}	4.16^{ef}	4.17^{L}	2.85^{s}	5.06^{f}
LSD$_{0.05}$	0.008	-	-	-	0.009	-	0.007	-	-	0.02	-	-
CV (%)	1.332	-	-	-	1.119	-	1.353	-	-	1.135	-	-

Mean values followed by the same letter(s) per variable are not significantly different from each other at (*p>0.05*). 3-CQA = 3-caffeoylquinic acid, 4-CQA = 4-caffeoylquinic acid, 5-CQA = 5-caffeoylquinic acid, TCQA = total caffeoylquinic acids. Results are shown as mean values, expressed as g/100 g of green coffee beans on a dry weight basis.

an internal standard solution was prepared using 600 mg fenyl-beta-D-glucopyranoside and 100 mL distilled water. Ground green coffee beans (0.5 g) were extracted with 25 mL distilled water containing 30 mg of fenyl-beta D-glucopyranoside in a hot water bath at 60°C for 30 min. Carrez I, potassium hexacyanoferrate II $K_4Fe(CN)_6$ in water (15 g L^{-1}), and Carrez II, zinc sulfate $ZnSO_4$ in water (30 g L^{-1}), were added to the extract as well as distilled water to obtain a total volume of 50 mL. The extract was filtered over a 0.45 µm PTFE filter and 0.5 mL of the filtrate was dried using nitrogen gas in a HPLC-vial. Then 0.5 mL of "STOX" reagent (hydroxylamine hydrochloride 25 g L^{-1} in dry pyridine) was added to the dried extract and the vials were placed in an oven at 60°C for 30 min. After cooling the extract to room temperature, 0.5 mL of hexamethyldisilazane (HMDS) and 0.05 mL trifluoroacetic acid (TFA) were added and kept for 60 min to allow phase separation. Finally, 1 µL was taken from the upper layer and injected into a gas chromatograph (GC-3380, Varian, USA) using an automatic injector. The detection was done using a flame ionization detector (FID).

Data analysis

Data were analysed with statistical analysis system software (v. 9.2, SAS Institute Inc., Cary, NC USA), using a mixed model procedure for a split plot design with variety as the main plot and processing methods as sub-plot. Significant differences between treatment means were determined using the Tukey's honest significant difference (HSD) test.

RESULTS

Biochemical composition of green beans

In this study, six sub-classes of chlorogenic acid (CGA): 3-caffeoylquinic acid (3-CQA),4- caffeoylquinic acid (4-CQA), 5-caffeoylquinic acid (5-CQA), total caffeoylquinic acids (TCQA) feruloylquinic acids (FQA),dicaffeoylquinic acids (diCQA) and feruoyl-caffeoylquinic acid (FCQA), caffeine and sucrose were analyzed and compared in ten Ethiopian Arabica coffee varieties, processed by the dry, semi-washed and washed method. The CQA, FQA, diCQA and FCQA content of the ten CBD resistant coffee varieties, processed by dry, semi-washed and washed postharvest processing methods, are presented in Tables 3, 4 and 5.

CQA, FQA, diCQA, FCQA and total chlorogenic acid (TCGA) contents were significantly (*P < 0.05*) affected by the interactions between variety and postharvest processing methods (Tables 3, 4 and 5). TCQA, FQA, diCQA, FCQA and TCGA contents ranged from 2.85 - 6.01, 0.31 - 0.42, 0.58 - 0.93, 0.11- 0.26 and 3.95 - 7.42 g/100 g, respectively. The highest TCQA content was observed in variety 74-112 processed by the washed method (6.1 g/100 g) while the lowest value was observed in variety 75-227 processed by the semi-washed method (Table 3). In addition, the highest FQA (0.42 g/100 g) and diCQA (0.93 g/100 g) were obtained from variety 74-40 processed by the washed method (Table 4). Higher FCQA (0.25 g/100 g) and TCGA (7.42 g/100 g) contents were also observed in variety 74 to 112, processed by the washed method (Table 5).

Caffeine and sucrose contents were also significantly (*P < 0.05*) influenced by the interaction effects of variety and postharvest processing methods. The highest caffeine content (1.67 g/100 g) was observed in the varieties 74-4 and 74-165, which were processed by the washed method, while the lowest (1.22 g/100 g)

Table 4. Feruloylquinic acid (FQA) and dicaffeoylquinic acid (diCQA) content of ten Arabica coffee varieties processed by the washed (WP), semi-washed (SW) and dry (DP) method, grown under similar conditions at Suntu coffee plantation, Jimma Zone.

Variety	FQA			di-CQA		
	Processing method			Processing method		
	DP	SW	WP	DP	SW	WP
74-1	0.31^{n}	0.31^{n}	0.36^{gh}	0.64^{n}	0.70^{j}	0.80^{ef}
74-4	0.35^{ij}	0.36^{gh}	0.41^{b}	0.62^{o}	0.79^{f}	0.88^{b}
74-40	0.36^{g}	0.37^{f}	0.42^{a}	0.68^{m}	0.77^{h}	0.93^{a}
74-110	0.35^{ij}	0.33^{Lm}	0.39^{cd}	0.68^{m}	0.68^{k}	0.88^{ab}
74-112	0.34^{ij}	0.33^{klm}	0.39^{d}	0.62^{o}	0.75^{h}	0.85^{c}
74-140	0.34^{ij}	0.35^{ij}	0.36^{fg}	0.61^{o}	0.68^{l}	0.73^{i}
74-148	0.37^{f}	0.38^{e}	0.39^{d}	0.58^{p}	0.84^{c}	0.82^{d}
74-158	0.35^{ij}	0.35^{hi}	0.38^{e}	0.59^{p}	0.69^{j}	0.77^{g}
74-165	0.35^{ij}	0.33^{kl}	0.40^{c}	0.65^{n}	0.73^{i}	0.90^{b}
75-227	0.32^{m}	0.31^{n}	0.34^{j}	0.63^{o}	0.68^{j}	0.80^{e}
$LSD_{0.05}$		0.007			0.004	
CV (%)		1.231			1.401	

Mean values followed by the same letter(s) per variable are not significantly different from each other at (*p>0.05*). Results are shown as mean values, expressed as g/100 g of green coffee beans on a dry weight basis.

content was obtained from variety 74-1 processed via the dry method. Coffee beans of variety 74-110 treated with the dry processing method gave the highest sucrose content (10.28 g/100 g), followed by variety 74-140 (10.01 g/100 g). Coffee beans of variety 74-4 processed by the semi-washed method, on the other hand, gave the lowest sucrose content (7.45 g/100 g) (Table 6).

DISCUSSION

Biochemical composition of green beans

There are six subclasses of chlorogenic acid (3-CQA, 4-CQA, 5-CQA, FQA, diCQA, FCQA), TCGA, caffeine and sucrose were compared in Ethiopian Arabica coffee varieties processed by washed, semi-washed and dry methods. Several studies have also shown that the mode of coffee processing strongly influences and determines the chemical composition of green coffee beans and thereby the coffee flavour and aroma (Duarte et al., 2010; Joët et al., 2010). The present finding also revealed that variety differences were observed in their chlorogenic acids, caffeine and sucrose contents processed via washed, semi-washed and dry methods. The contents of TCQA, FQA, di-CQA, FCQA and TCGA varied from 2.85-6.01, 0.31-0.42, 0.58-0.93, 0.11-0.26 and 3.95-7.42 g/100 g, respectively. The CGA contents obtained are consistent with previous data for coffee in general (Duarte et al., 2010; Farah and Donangelo, 2006a). Coffee beans processed via the washed method gave a higher CGA and caffeine content than those processed by the semi-washed and dry methods for all varieties. This difference was observed in all subclasses of chlorogenic acids, except for the 3-CQA.

Moreover, the varieties responded differently to different postharvest processing methods. For instance, coffee variety 74-112, 74-165, 74-110 and 74-40 had higher TCGA values (7.42, 7.36, 6.99 and 6.79 g/100 g, respectively) with the washed processing method compared to beans processed with the semi-washed and dry methods. The coffee bean variety 74-227, on the other hand, showed the lowest TCGA content (3.95 g/100 g) when processed with the semi-washed method (Table 5). These results are in agreement with a previous study (Duarte et al., 2010) who reported higher contents of chlorogenic acids for coffee processed by the washed method compared to the semi-washed and dry methods. Balyaya and Clifford (1995) also reported a significant effect of postharvest processing method on total chlorogenic acids and its subclasses. Joët et al. (2010), on the other hand, reported no influence of postharvest processing method on the green coffee beans chlorogenic acid content.

Similar to the CGA content, the washed processing method increased the caffeine content of all coffee varieties. The highest caffeine content, mean value of 1.67 g/100 g, was obtained in varieties 74-165 and 74-4, processed by the washed method followed by the coffee varieties 74-148, 75-227 and 74-112 with mean values of 1.64, 1.64, and 1.64 g/100 g, respectively (Table 6). Compared with the other coffee varieties processed by the semi-washed or washed method, coffee beans harvested from variety 74-1, 74-40, 74-158

Table 5. Feruoyl-caffeoylquinic acid (FCQA) and total chlorogenic acids (TCGA) contents of ten Arabica coffee varieties processed by the dry (DP), semi- washed (SW) and washed (WP) method, grown under similar conditions at Suntu coffee plantation, Jimma Zone.

Variety	FCQA			TCGA		
	Processing method			Processing method		
	DP	SW	WP	DP	SW	WP
74-1	0.14^{bc}	0.14^{kl}	0.19^{ij}	5.83^{h}	4.74^{o}	6.31^{e}
74-4	0.12^{a}	0.13^{l}	0.24^{o}	5.80^{gh}	5.93^{fgh}	5.87^{i}
74-40	0.12^{a}	0.15^{g}	0.26^{h}	5.90^{f}	5.56^{j}	6.79^{c}
74-110	0.12^{a}	0.12^{no}	0.25^{mn}	5.84^{fgh}	4.75^{o}	6.99^{b}
74-112	0.19^{d}	0.13^{m}	0.15^{g}	4.36^{q}	5.41^{l}	7.42^{a}
74-140	0.19^{cd}	0.15^{a}	0.15^{h}	4.89^{n}	6.33^{de}	5.23^{m}
74-148	0.18^{f}	0.13^{mn}	0.14^{jk}	5.45^{kl}	6.69^{c}	5.99^{fg}
74-158	0.19^{b}	0.13^{kl}	0.12^{p}	4.35^{q}	6.38^{d}	5.41^{l}
74-165	0.19^{e}	0.12^{p}	0.14^{jk}	5.98^{fg}	4.47^{p}	7.36^{a}
75-227	0.18^{ef}	0.11^{q}	0.14^{hi}	5.27^{m}	3.95^{r}	6.35^{de}
$LSD_{0.05}$		0.004			0.02	
CV (%)		1.473			0.919	

Mean values followed by the same letter(s) per variable are not significantly different from each other at (*p>0.05*). Results are shown as mean values, expressed as g/100 g of green coffee beans on a dry weight basis.

Table 6. Caffeine and sucrose contents of ten Arabica coffee varieties processed by the dry (DP), semi-washed (SW) and washed (WP) method, grown under similar conditions at Suntu coffee plantation, Jimma Zone.

Variety	Caffeine			Sucrose		
	Processing method			Processing method		
	DP	SW	WP	DP	SW	WP
741	1.22^{n}	1.42^{kl}	1.46^{hij}	8.67^{op}	7.60^{g}	7.63^{mno}
744	1.44^{jkl}	1.48^{hi}	1.67^{ab}	8.70^{op}	7.45^{fg}	8.18^{j}
7440	1.25^{n}	1.42^{kl}	1.62^{cde}	9.45^{e}	8.92^{c}	8.63^{ghi}
74110	1.44^{jk}	1.53^{g}	1.58^{ef}	10.28^{ad}	9.21^{d}	7.63^{mno}
74112	1.40^{l}	1.52^{g}	1.64^{bcd}	9.19^{d}	8.44^{i}	7.47^{op}
74140	1.34^{m}	1.46^{n}	1.44^{jk}	10.01^{b}	8.87^{ef}	7.98^{kl}
74148	1.48^{h}	1.61^{de}	1.64^{abc}	9.88^{ghi}	8.56^{b}	7.98^{kl}
74158	1.33^{m}	1.40^{l}	1.57^{f}	8.75^{hi}	8.63^{gh}	7.48^{op}
74165	1.34^{m}	1.45^{ijk}	1.67^{a}	8.80^{lm}	8.14^{jk}	7.71^{mn}
75227	1.45^{hij}	1.42^{m}	1.64^{abc}	8.13^{jk}	7.62^{ghi}	8.16^{jk}
$LSD_{0.05}$		0.035			0.188	
CV (%)		1.456			1.373	

Mean values followed by the same letter(s) per variable are not significantly different from each other at (*p>0.05*). Results are shown as mean values, expressed as g/100 g of green coffee beans on a dry weight basis.

and 74-140 and processed via the dry method, respectively, gave a lower 1.22, 1.25, 1.33 and 1.34 g/100 g caffeine content. The differences in caffeine and CGA content between varieties could be attributed to differences in their genetic trait of each variety. A similar finding was also reported by Link et al. (2014). These authors found a significant difference in caffeine content among different genotypes.

In contrast, e.g. Guyot et al. (1995) and others, observed a reduction of caffeine (3%) in coffee beans processed by the washed method as compared to beans processed by the dry method. However, other studies reported that the caffeine content remained unchanged in response to different postharvest processing methods (Duarte et al., 2010; Joët et al., 2010; Leloup et al., 2005). This discrepancy can be explained by differences in

growing conditions, varieties, coffee cherry type and postharvest processing conditions (e.g. fermentation time during the washed processing procedure, water quality status and drying conditions). It shows that the biochemical composition of green coffee beans is highly affected and controlled, among others, by interaction effects of growing conditions, genetic traits, harvesting time and postharvest processing techniques. Worku et al. (2018), also recently confirmed the complex interaction effects of growing conditions (e.g. altitude, shade) and postharvest processing on biochemical composition of green Arabica coffee beans.

The relative increase in TCGA and caffeine contents of coffee beans processed by the washed method compared to the semi-washed and dry method could be the loss of other chemical components mainly due to their water solubility nature and effect of thermal degradation (Duarte et al., 2010). Moreover, this might be reinforced by intense metabolic reconversion that occurs during the washing procedure (Joët et al., 2010). Bytof et al. (2005) also reported that various metabolic processes occur inside the coffee seeds leading to changes in chemical composition of green coffee beans e.g. conversion of glutamic acid into γ-aminobutyric acid. These authors also related the metabolic reaction to a physiological stress condition and to the specific type of processing method applied.

Sucrose accounts for up to 11% on dry matter basis of green Arabica beans and acts as aroma precursor (De Castro and Marraccini, 2006; Farah et al., 2006b). The content of sucrose in coffee beans processed by the dry, semi-washed and washed methods ranged from 8.13 to 10.28 g/100 g, 7.45 to 9.21 g/100 g and 7.47 to 8.63 g/100 g, respectively (Table 6). For all Arabica varieties tested in this study, the sucrose content in the beans processed by the dry method was significantly higher than in the beans processed by the other methods. A similar finding was reported earlier for a hybrid coffee variety (Duarte et al., 2010). A higher content of sucrose in dry processed coffee beans was reported compared with washed processed coffees. The reduction of the sucrose content observed in washed processed coffee beans is possibly due to the high water solubility of this compound during washing and soaking. Additionally, sucrose may be depleted as a result of anaerobic fermentation during washed processing methods.

Furthermore, the present study also shows that the sucrose content significantly varied among varieties. A higher concentration was observed for some varieties (74-110, 74-140, 74-148, 74- 40 and 74-112) processed by the dry method (Table 6). The similar response of these varieties might be due to the fact that they have the same genetic background, interacting in the same way in this processing method. In contrast to the present study, a higher sucrose content was found for coffee beans harvested from higher altitude and processed by the semi-washed (Tolessa, 2017) and washed method (Worku et al., 2018) than by dry method. Other studies, on the other hand, demonstrated that sucrose is not significantly affected by the mode of postharvest processing method tested, neither for Arabica nor for Robusta coffee (Bucheli et al., 1996; Knopp et al., 2006; Leloup et al., 2005). Similarly, in our previous study (Tolessa, 2017), the postharvest processing method did not show significant differences in sucrose content at mid and low altitude regions. This implies that there is still no convincing information in the literature with regard to the effect of postharvest processing methods on sucrose content of green coffee beans. The reason for these discrepancies could be due to the variability of factors considered during the studies rather than to processing methods (e.g. varietal differences, growing conditions: altitude and shade).

Conclusions

In general, the present study shows that the bio-chemical composition of green coffee was significantly influenced by the interactions between variety and postharvest processing methods. The coffee varieties 74-112, 74-110 and 74-165, processed by the washed method gave the highest TCGA content compared to the semi-washed and dry methods. The washed processing method also enhanced the caffeine content and the highest value was obtained with the coffee varieties 74-4, 74-112 and 74-165. However, the dry processing method increased the sucrose content of green coffee beans particularly for coffee variety 74-110, 74-140 and 74-148. Therefore, the study highlighted the influence of postharvest processing method on chemical composition of green coffee beans. The chemical composition is also affected by the coffee bean varieties. Moreover, the present study also confirmed that the biochemical composition of green coffee beans is highly variable depending on genetic traits, growing conditions and postharvest processing conditions. However, further study is needed in order to correlate the biochemical composition differences observed among the processing methods with cup quality. Furthermore, this study has been done using limited coffee varieties grown at similar conditions. Therefore, it would be advisable to further evaluate more varieties under different growing environments.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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