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Table of Content

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Full Length Research Paper

Comparative analysis of nutritional contents in the leaf, pulp and seed of *Adansonia digitata* L. consumed in Adamawa State, Nigeria

Enoch B. B.[1], Abubakar I. M.[2], Sakiyo D. C.[3*] and Bashiloni N.[1]

[1]Department of Forestry Technology, Adamawa State Polytechnic Yola, Adamawa State, Nigeria.
[2]Department Agricultural Education, Federal College of Education, Yola. Adamawa State Nigeria.
[3]Department of Forestry Technology, Adamawa State College of Agriculture Ganye, Adamawa State, Nigeria.

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Adansonia digitata L. **is a tree commonly called Baoba tree which is a native of African savannah widely distributed in arid zones of Sahara. This study was carried out to investigate the native uses and nutritional content (peoximate composition and minerals profile) of the leaf, pulp and seed of *A. digitata L.* (Baoba). The methods adopted for data collection included well-structured questionnaire, field and laboratory methods. Results from the survey revealed that different ethnic groups in Adamawa used parts of *A. digitata* L. for medicine, food, spices and special drinks. The results of proximate composition showed protein 38.18, 17.57 and 48.49% in the leaf, pulp and seed of Baoba tree, respectively. The carbohydrate content showed 37.30, 63.71 and 22.95%, respectively. The moisture and ash contents in the three samples ranged between 6.30 and 11%, while the crude fiber ranged from 1 to 3%. The minerals content in the pulp samples revealed that Mg and Fe significantly recorded the highest. There was significant difference in both minerals and nutritional profiles in the three parts of *A. digitata* L. In conclusion, the three parts of the plants studied contained important nutrients and minerals that are good for human consumption and therefore conservation strategies should be employed to ensure sustainability in utilization of the plant products.**

Key words: Savannah, *Adansonia digitata* L, Baoba, minerals, leaf, pulp.

INTRODUCTION

Adansonia digitata L., is a tree commonly called Baoba tree which is a native of African savannah, Madagascan, Australia and Arabia which belonged to genus *Adansonia* and to the family Malvaceae (Wickens and Lowe, 2008). The tree is widely distributed in arid zones of most countries of Sahara. The trees are commonly found in the thorn woodlands of African savannahs which tended to be at low altitudes with 4 to 10 dry months in a year (Wickens and Lowe, 2008). The tree usually grows in solitary manner, though it can be found in small groups depending on the soil types. The plant is not found in places that are deeply sandy and very sensitive to frost and water logged areas.

All locations where *A. digitata* is found are in the arid or

*Corresponding author. E-mail: dsakiyo1@gmail.com. Tel: 08033276866.

semi-arid regions of the world (Shuaibu and Rabi, 2014). Baoba tree is visible in both residential area and wild. In Nigeria, the Baoba trees are widely distributed in Sodano-Sahelian part of the country engulfing Kano, Katsina, Sokoto, Zamfara, Kebi and Jigawa states in the Northwestern part and in the Northeastern part covering Yobe, Borno, Gombe, Bauchi and Adamawa states (Shuaibu and Rabi, 2014).

A. digitata L., being a massive deciduous tree, it can grow up to 20 to 30 m in height with a diameter of 2 to 10 m at adult age. It has trunk with vast girth, smooth bark, and reddish brown to grey, soft and possesses longitudinal fibers. *A. digitata* is highly branched which produces extensive lateral roots system up to 50 m from the trunk. The roots' tips are often tubular while the tap root of the tree is shallowed rarely extend beyond 2 m depth which makes them easily fell down by storms (Sidibi and Williams, 2002). The adult tree begins each season by producing simple leaves with 2 to 3 leaflets. The flowers are white large, pendulous, solitary or paired in the leaf axils. Flowering begins about the end of dry or just before the first rains often when the first leaves appear after annual shedding (Jitin et al., 2015). Baoba trees have life span ranging from 200 to 300 years and others can live up to 1000 years (Jitin et al., 2015). *A. digitata* L. is a multi-purpose tree and is called tree of life and small pharmacy because it offers goods and services that include protection, food, clothing materials, medicinal, fiber materials derived from barks, seeds, leaves, and roots (Jitin et al., 2015). According to traditional sources, leaves of Baoba have been reported to cure malaria fever, diaphoretic fever remedy, toothache (gingivitis), diarrhea, fever, inflammation of kidney and bladder diseases, blood clearing and asthma (Wickens and Lowe, 2008). The bark cures anemia and wound healing while seeds are used as therapy for fever, diarrhea and cough (Van Wyk and Gericke, 2000; Brendler et al., 2003; Tapsoba and Deschamps, 2006; De Caluwe et al., 2009; Nguta et al., 2011).

From economic point of view, *A. digitata L.* make great socio-economic impact on the livelihoods of people living in the arid zones. The leaves of Baoba trees are staple for populations in Africa especially in the Sudano-Sahelian and central regions of the continent (Gebauer et al., 2002). During rainy season when the Baoba leaves are tender, people harvest the fresh batch of leaves for domestic consumption (Abelrumand, 2011). The fresh young leaves are used widely cooked as spinach, ground into powder and used over porridge, thick gruels of grains or boiled rice. Toward the end of rainy seasons, leaves of Baoba tree are harvested in abundance and are dried for domestic consumption and sold in the markets for income generation that sustains local livelihoods (Sidibi and Williams, 2002). This is evident from a report carried out in six local government areas in Katsina State in Nigeria where the results revealed that out of 240 people considered as sample population for the research, 23.33% use *A. digitata* as staple food, 32.58% as means of superstitious beliefs, 21.25% for protection, 13.33% for income generation and 9.25% for medicinal purposes (Shuaibu and Rabi, 2014).

The pulp of Baoba fruit is another new discovery which revealed that the fruit contained high percentage of vitamin C which is almost ten times that of *Citrus* (sweet orange) (Nguta et al., 2011). Another source revealed that vitamin content in the pulp of Baoba is very high ranging from 280 to 300 mg/100 g while orange is 51 mg/100 g (Manfredini et al., 2002). The consumption of 40 g of Baoba pulp provided 100% of the recommended daily intake of vitamin C in pregnant women (Chadare et al., 2009). It contained sugar but not starch and rich in pectin. It can be dissolved in water or milk and used as a drink, a sauce for food, a fermenting agent in production of local brewing or as a substitute for cream of tartar in baking (Juliani et al., 2009). The seeds of Baoba are used as thickening agent in soups, and can also be fermented to be used as a flavoring agent (daddawa) or roasted and eaten as snacks (Kabore et al., 2011). This study with available cultural or traditional information on multipurpose uses *of A. digitata* L. intended to scientifically carry out comparative laboratory analysis of nutritional content in the leaf, pulp and seed of Baoba tree.

MATERIALS AND METHODS

Questionnaire for survey study, field and laboratory studies were adopted for collection of data during the study.

Survey method

A well-structured questionnaire was adopted to extract information on demographic data and local consumption of *A. digitata* L. parts by different ethnics groups in Adamawa State.

Sample collection

The samples (leaves, pulp and seeds) were obtained from Jimeta modern market in Yola, Adamawa State from traders who sale wild plant products harvested from savannah lands in Northern region of Nigeria.

Sample preparation

The dried leaf samples obtained were prepared by pounding the leaves into powdery form using laboratory motor and pestle. The powdered samples were then sieved to fine texture using 0.005 sieves as described by Munthali and Mkunda (2002). The dried white pulp was also made ready for laboratory analysis by removing the pericarp (hard cover) of the fruit from the inner mesocarp (white pulp) by using stone on the hard ripe pericarp. This was followed by gently pounding the solid pulp to separate it from the seeds without destroying the seeds or avoiding mixing it with the pulp using motor and pestle. The seeds were separated from the powdered pulp by hand picking the seeds and the pulp powder was sieved using

0.005 to remove fibrous particles and fine powder texture was obtained as described in the treatment of leaf samples earlier stated. The separated seeds were soaked to easily remove the husks then washed and sun dried. The seeds were then pounded as described earlier and made ready for laboratory determination.

Laboratory analysis

The laboratory analysis was restricted into two analyses involving proximate and minerals profiles of nutrient groups and essential elements contained in the leaf, pulp and seed samples of *A. digitata* L.

Proximate analysis

Moisture content determination

The moisture content of each sample was determined using moisture analyzer model LSC 60D (Country origin). The analyzer was heated at 120°C and 5 g of each sample was put into the analyzer. After 10 min the samples were removed from the analyzer. Each of the samples was weighed and new weight values were subtracted from the old values (5 g) as described by AOAC (2005).

Ash content determination

The ash content of the samples was determined by the use of mechanical conventional oven and desiccator. The crucibles containing the samples were put inside the oven heated at 105^0C for 15 minutes to remove the water content in the samples. The hot samples were then placed in a desiccator to cool. The charred samples were then transferred into muffle furnace for 3 to 4 h at 550°C at constant temperature. After heating, the ash in each crucible were carefully removed and put in the desiccator to cool and after which the ash content in each of the samples were weighed on weighing balance and finally determined (Onwuka, 2005).

Fat content determination

The fat content was determined using Soxhlet apparatus, the weight of 2 g of each samples were put on a filter paper and folded and tightened. This was then transferred into an extractor and an empty weighed extraction flask for each sample was mounted, respectively. Few drops of hexane were added to each sample in the extractor which was trapped with a liquid condenser to allow water to flow freely while the condenser was covered with a cotton wool. The oil was then extracted from each sample in the extraction flask which was weighed after cooling but the hexane was recovered in each samples. The readings of oil in extractor flask were recorded and fat content was determined (AOAC, 2005).

Crude fiber determination

The crude fiber content was determined in the samples adopting the method described by A OAC (2005). Two grams of each samples were put into three beakers, respectively and 2.5 ml of sodium hydroxide (NaOH) per 200 ml of water was added and boiled for 30 min. The solution of each sample was filtered through linen on a fluted funnel and washed with boiling water. The residue of each sample was then put into respective crucibles and dried in a mechanical oven and incinerated for minutes and cooled in a desiccator. The crucibles were reweighed and readings were taken appropriately for crude fiber determination.

Protein content determination

The protein content was determined adopting method described by Onwuka (2005). Two grams of each samples were digested in 25 ml of sulphuric acid with two Kjeldahi tablets put into digestion tube and mounted on digestion apparatus. The mixture was heated to the temperature of 450°C till black to green cooler was observed. This was followed by steaming the mixture for 15 min in distillation apparatus under a condenser where 98 ml of water containing 2% of boric acid in such way that the condenser tip is under the liquid. This was followed by pipetting of 2 g of each sample via a funnel. This was washed down with distilled water, followed by adding 5 ml of 2% sodium hydroxide. Each sample was steamed for 5 to 7 min to collect enough ammonium sulphate. Each solution of the sample was collected in receiving flask for titration, respectively. For blank titration, 0.1N was used in 150 ml of water and 2 to 3 drops of methyl red indicator were used. The solution of each sample in their respective receiving flasks were titrated using 0.1N per 150 ml against 5 ml of boric acid and the readings were taken for final protein determination.

Minerals analysis

Determination of calcium, magnesium, manganese, iron, aluminium and copper

Determination of the minerals was done using atomic absorption spectrometer (Model, company, country origin) (Onwuka, 2005). About 1.0 g of the sample was first digested with 20 ml of concentrated HNO_3; per chloric acid, 20 ml concentrated H_2SO_4 and aliquots of the diluted clear digest were used for atomic absorption spectrophotometer using filters that match the different elements. This was followed by standard solutions preparation for elements under study. The concentrations of the elements were determined using calibration curves.

Determination of potassium and sodium

The two elements were determined using flame photometer as described by AOAC (2005) as cited by Onwuka (2005). The samples were prepared for atomic absorption spectrometry (Model, company, country origin); this was followed by an appropriate dilutions prepared from chloric acid digest for each sample. Each sample was analyzed through the instrument following the instructions provided in the manual. The determination of the elements was done by taking the absorption of Na at 767 nm while K was at 589 nm in line with their concentration standards.

Determination of phosphorus content

Phosphorus was determined in each samples by molybdate method using hydroquinone as a reducing agent as described by Onwuka (2005), followed by addition of 10.05 ml mineral digest, 1.0 ml of ammonium molybdate was allowed to stand for 30 min. Blue color was observed which was used for quantification at calorimeter reading at 660 nm against a standard curve.

Determination of nitrogen content

The Kjeldahl method as described by Onwuka (2005), 2 g of each

Table 1. Demographic data of the respondents.

Tribe	Sex		Educational Level					Age			
	M	F	Informal	FSLC	SSCE	ND/NCE	BSC	30-40	41-50	51-60	61-70
21 (00%)	80.95% (14)	19.05% (4)	19.05% (4)	9.52% (2)	14.39% (3)	14.39% (3)	42.86% (9)	33.33% (7)	28.57% (6)	28.57% (6)	9.52 (9)

Table 2. Survey on local consumption of Baoba tree parts among ethnic groups in Adamawa State

Part	Spice (%)	Medicinal (%)	Food (%)	Water Source (%)	Special Drinks (%)
Seed	66.67	0.00	33.33	0.00	0.00
Pulp	0.00	9.52	14.29	0.00	76.19
Root	0.00	19.05	8.33	8.33	0.00
Leaf	0.00	8.33	85.71	0.00	0.00
Stem	19.05	23.81	0.00	0.00	0.00
Flower	8.33	0.00	28.57	0.00	0.00

samples was weighed into a heating substance with sulphuric acid, which decomposed the organic substance by oxidation to liberate and reduce nitrogen as ammonium sulphate, followed by addition of potassium sulphate to increase boiling point of the medium from 337 to 373°C. This was followed by chemical decomposition of each sample where dark colored medium which gradually became clear and colorless. This was followed by distillation of each sample solution with small quantity of ammonia for blank titration.

Statistical analysis

The methods of data analysis adopted include use of simple descriptive statistics and two-way analysis of variance (ANOVA). The simple descriptive statistic was used to analyze demographic data and local consumption of *A. digitata* parts by different prominent ethnic groups in Adamawa State (Yelwa et al., 2014). The following expression was used to calculate percentage of the ethnic groups' consumption for each parameter under consideration.

$$n/N \times 100\%$$

where n= response on each parameter by all twenty one tribal group representatives and N=total number of tribal group representative (21) which is consonant.

RESULTS AND DISCUSSION

The demographic data in Table 1 unveiled that 21 ethnic groups in Adamawa State were used for the survey where 14 males and 4 females formed the respondents. The gender imbalance was as a result of poor access to women due to lockdown created by Covid-19 pandemic. Educational levels showed that four of the respondents had informal education, four primary school certificate holders, four senior secondary school certificate holders, four ND/NCE holders and nine with degree certificates and above.

The data in Table 2 indicated that 66.67 and 33.33% of the ethnic groups in Adamawa State used seed as spices popularly called dawadawa, and fried as food, respectively while 19.05 and 8.33% used stem and flower to prepare local potash, respectively. 14.29 and 76.19% used pulp as food and special yoghurt drink, 8.33% eats the young roots as food, especially during famine period, while 19.05% root and 23.81% stems are used as medicinal. 85.71 and 8.33% used fresh leaves as food and medicine, respectively. 28.27% eat fresh flower of Baoba though reported only among children. This result agreed with the findings of Shuaibu and Rabi (2014), who opined that 56 out of 76 respondents obtained from six local government areas of Katsina State, Nigeria were reported to use Baoba as food.

Field survey reported during the interview revealed that pulp powder is applied on open wounds for faster healing and leaked for treatment of cough. While the stem and root barks are boiled and used for treatment of gastro-intestinal fungal infection among children below the age of three years and are also used for treatment of tooth infections. This followed the assertion that *A. digitata* L. is a small pharmacy because its parts (fruits and leaf) are used for treatment of numerous diseases in Africa which include asthma, allergic skin, paralysis conditions, mosquito repellent, diarrhea, malaria and cough (De Caluwe et al., 2009). In preparation of special drinks, 76.19% of the respondents established that local drinks like yoghurt can be prepared from pulp of Baoba fruit and Fulani ethnic group supplement cow milk with Baoba pulp in times of scarcity (Yaro et al., 2014).

The results of laboratory analysis for both proximate and minerals profile revealed that the parts of Baoba tree under study contained some amount of nutritional elements and other key classes of food that can support

Table 3. Proximate composition of the leaf, pulp and seed of *Adansonia digitata* L. (baoba tree).

Nutrient	Leaf (%)	Pulp (%)	Seed (%)
Moisture	9.02	10.12	8.06
Ash	11.00	6.30	7.00
Crude fiber	1.00	1.30	3.00
Fat	3.50	1.00	10.50
Crude protein	38.18	17.57	48.49
Carbohydrate	37.30	63.71	22.95

Table 4. Minerals profile in the leaf, pulp and seed of *Adansonia digitata* L. (Baoba tree).

Nutrient	Leaf (mg/l)	Pulp (mg /l)	Seed (mg/l)
Na	0.870	0.879	1.029
K	4.118	3.748	7.250
Ca	0.780	5.425	4.823
Mg	1.260	126.620	50.300
Fe	3.640	54.120	43.880
Mn	0.409	0.298	0.105
Al	0.006	0.006	0.004
N	0.278	0.366	0.454
P	0.162	2.944	2.494

nutrient requirement of the human body. This is evident in Tables 3 and 4. Table 3 shows that proximate composition results of the leaf, pulp and seed of *A. digitata* L. which showed higher content of protein amounting to 38.18, 17.57 and 48.49, respectively. The result obtained on protein in the three samples fell almost within same range (17.22 - 46.24%) with leaves of *Allium porrun* and *Spilanthes acimellia, Catasetum cernuum*, *Moringa oleifera* and cashew nut for crude protein (Abelrumand, 2011; Tapans et al., 2013). These figures are more than average daily body protein requirement for human beings because average sedentary man and woman need 56 to 91 g (5. 6 - 9.1%) and 46 to 75 g (4.6 - 7.5%) per day, respectively. People like athletes, who engaged in vigorous activities need more proteins than the one required for average sedentary persons (Kris, 2018). The carbohydrate content showed similar results indicating 37.30, 63.71 and 22.95% in the leaf, pulp and seed samples, respectively. The figures are in agreement with results obtained on proximate composition of the leaves of *Hmyriatha* (60.02%), *Elaeis guineensis* (59.70%), *Abelmoschus esculentus* (55.23%), *Talinum triangulare* (50.59%), *Vernonia amygdalina* (62.1%) (Dike, 2010; Igwe et al., 2015). The moisture and ash contents in the three samples under study ranges between 6.30 and 11% but the crude fiber recorded the least in quantity ranging from 1 to 3%. The figures mentioned though lower but showed higher nutrients content as compared to the findings of Simon et al. (2015) on four leafy vegetables (*Ficus thoninngii, Emillia coccinia, Hibiscus sabdariffa* and *Annona senegalensis*) commonly consumed in Benue State, Nigeria. The figures obtained for all the four plants indicated closed relation to crude fat content (0.05 - 0.716%), crude fiber (1.30 - 20.15%), and ash (1.06 - 2.37%) except for the values of carbohydrate (4.16 - 15.12%) and crude protein (0.03 - 0.33%) that are very low while moisture content (62.32 - 93.38%) appeared to be very high in the samples of Baoba under study. The ANOVA Table 5 indicated that there was significant difference in the content of nutrients in the three parts of *Adansonia digitata* L. and in each nutrient groups showed significant difference ($p<0.05$).

The results of minerals profile in Table 4 revealed that leaf sample of potassium and iron recorded the highest with 4.118 and 3.640 mg/l00 g, respectively, followed by magnesium 1.260 mg/l00 g, sodium 0.870 mg/l and calcium 0.780 mg/l00 g. These figures fell within normal range (0.11 - 3.00 mg/l00 g) of standard values for leafy fruit tree analysis of pear, plum, apple, peach, cherry sour and cherry sweet for macro-minerals Na, K, Ca, Mg P and N (Joseph, 2004). The contents of manganese, aluminium, nitrogen and phosphorus ranged from 0.006 to 0.409 mg/l. The elements content in the pulp samples showed great variation from records observed in the leaf content. Magnesium and iron significantly recorded highest, amounting to 126.620 and 54.120 mg/100 g, respectively, followed by calcium 5.425, potassium 3.748 and phosphorus 2.944 mg/l00 g while contents of

Table 5. ANOVA for hypotheses tests of between-subjects effects.

Dependent Variable: Nutrient		Type I Sum of Square	Df	Mean Square	F	Sig.
Source						
Treatment	Hypothesis	4990.015	3	1663.338	4.379	0.040
	Error	3189.644	8.397	379.838[a]	-	-
Factor	Hypothesis	4280.791	5	856.158	6.043	0.008
	Error	1416.776	10	141.678[b]	-	-
Treatment * Factor	Hypothesis	1416.776	10	141.678	-	-
	Error	0.000	0	.[c]	-	-

a. 0.333 MS(Factor) + 0.667 MS (Treatment × Factor); b. MS (Treatment × Factor); c. MS(Error).

Table 6. ANOVA table for hypothesis tests of between-subjects effects.

Source		Type I Sum of Squares	Df	Mean Square	F	Sig.
Treatment	Hypothesis	5566.796	3	1855.599	2.471	0.093
	Error	14146.516	18.841	750.843[a]	-	-
Factor	Hypothesis	10451.972	8	1306.497	2.762	0.040
	Error	7568.267	16	473.017[b]	-	-
Treatment × Factor	Hypothesis	7568.267	16	473.017	-	-
	Error	0.000	0	.c		

a. 0.333 MS(Factor) + 0.667 MS (Treatment × Factor); b. MS (Treatment × Factor); c. MS(Error).

manganese, aluminium, nitrogen and sodium ranged between 0.006 mg/l and 0.879 mg/l00 g. The seed sample also recorded significant changes in the quantity of elements in the pulp and leaf *A. digitata* L. where magnesium and iron recorded 50.300 and 43.880 mg/l00 g, respectively. The high figures of Mg and Fe in the leaf and pulp samples agreed with this results (Mg=27.51 - 288.65 and Fe=16.43 - 39.04 mg/100 g) in the fluted pumpkin (*Oleifera occidental*), roselle plant (*Hibiscus sabdaiffa),* smooth amaranths (*Amaranthus hybridus*), biter leaf (*Veronia amygdalina*), India spinach (*Bosella alba*) and bush buck (*Gongronema latifolia*) (Asaolu et al., 2012), followed by potassium 7.25 mg/l, calcium 4.823 mg/l, and phosphorus 2.494 mg/l. The elements in the seed of *A. digitata* L. recorded with lower content included aluminium 0.004 mg/g, nitrogen 0.454 mg/g, and manganese 0.105 mg/l. The ANOVA Table 6 indicated that there is no significant difference ($p>0.05$) in the minerals distribution in the three parts of *A. digitata* L. but in between the elements, there is significant difference ($p < 0.05$).

Conclusion

The scientific and survey studies carried out on *A. digitata L.,* showed it can be established that the three parts (leaf, pulp and seeds) of the plants studied contained important nutrient groups and minerals that are good for human consumption and can also be supplements for improving human dietary intake.

RECOMMENDATIONS

Considering the huge potentials discovered in Baoba tree, there is need to harness these resources for sustainable livelihoods through adopting appropriate policies and laws that will ensure both *in situ* and *ex situ* conservation of *A. digitata* L. in savannah regions of the world. The samples studied can be harvested by food production industries for better standard processing methods that will guarantee better markets for income generation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Quality assessment of *Borassus aethiopum* Mart fruit pulp pectin precipitated with various solvents

Sylvie Assoi[1]* and Louise Wicker[2]

[1]Laboratory of Biotechnology, Agroresources Valorization and Natural Substances, UFR of Biological Sciences, University of Peleforo Gon Coulibaly, P. O. Box 1328 Korhogo, Ivory Coast.
[2]School of Nutrition and Food Science, LSU College of Agriculture, 297B Knapp Hall Baton Rouge, LA 70803, USA.

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Borassus aethiopum **Mart fruits are underutilized in Africa and most of them are left to rot in the field. These fruits have great potential as an alternative and commercially viable pectin source for the pectin industry. Physicochemical and rheological characteristics of *B. aethiopum* pectins are significantly affected by the extraction process which permits the isolation of tailored pectin of specific applications in the food industry. One important step in pectin recovery is its precipitation from the liquid extract and this can considerably impact the quality attributes of the final product. In this study the effects of precipitating solvents (isopropanol, acetone and 50/50 isopropanol-acetone) on the physicochemical and functionalities of pectin recently extracted from *B. aethiopum* fruit were investigated. Most of the results for the physicochemical characteristics were not statistically different however great variability was noticed in the functional and rheological properties of pectins precipitated with isopropanol (IPA), acetone (ACTN) and the 50/50 IPA-ACTN solvents. Isopropanol precipitated pectin exhibited statistically higher ($p<0.05$) emulsifying activity and a better gel sensorial property than the ACTN and IPA-ACTN precipitated pectins. Moreover, regardless of the precipitating solvent, high purity pectin with high viscosifying, emulsifying and gelling properties were obtained. Therefore, production of pectin from *B. aethiopum* fruit pulp must be governed by its intended use since extraction and precipitation processes isolate pectin samples with various functionalities.**

Key words: *Borassus aethiopum* Mart, isopropanol, acetone, precipitating solvent, solvent mixture.

INTRODUCTION

The palm *Borassus* is a dioecious plant that belongs to the Arecaceae family. The species *Borassus aethiopum* Mart is mostly found in the savannah region of Côte d'Ivoire where it grows wild. A single tree of *B. aethiopum* can bear around 6 to 12 bunches of about 50 fruits per year. Ripe and mature fruits are large ovoid drupes with diameter between 15 to 20 cm and one fruit weighs about 1.5 kg. Because of its high water content and lack of effective conservation methods, more than 60% of the fruits are lost during the harvesting period (Ali et al., 2010).

Recently, studies aiming at valorizing the fruits and

*Corresponding author. E-mail: assoisylvie@gmail.com.

therefore reducing the post-harvest losses have been reported. The fresh fruit of *B. aethiopum* has 80% water and contains 42.6 mg/100 g, 12 mg/100 g and 33 mg/100 g of potassium, sodium and calcium respectively. The energy calorie is 72.1 Kcal/100 g and the pulp is rich in vitamin C (17.68 mg/100 g), vitamin A (445.8 µg/100 g) and carotenoid (5350 µg/100 g) (Oryema and Oryem-Origa, 2016). By using soft drying conditions, it is possible to produce flour with acceptable technological attributes and economically exploitable (Ali et al., 2010). Oven, solar or freeze-dried flours of *B. aethiopum* fruit pulp present good flowability and can potentially be used as ingredients in the manufacture of pasta, puddings, cakes, biscuits, breads, crackers, and doughnuts (Abe-Inge et al., 2018a). Moreover, the antioxidant properties as well as the mineral, anti-nutrient and phytochemical compositions of these flours were reported by Abe-Inge et al. (2018b). Juice with acceptable sensory attributes can be produced by enzymatic hydrolysis of *B. aethiopum* fresh fruit pulp puree at 35°C for 2 h (Koffi et al., 2010)

B. aethiopum fruit is described as a good source of pectin. Yield and galacturonic acid content of pectin extracted from the fruit pulp at various pH (natural pH of 5.2-5.5, 2.5, 7), temperature (70, 80 and 90°C) and time (30-120 min) were 47-149 g kg^{-1} and 808-852 g kg^{-1} respectively. This high content of galacturonic acid makes *B. aethiopum* pectin an excellent raw material for industrial production of pectin (Assoi et al., 2014). *B. aethiopum* pectin forms gel in presence of acid and sucrose despite its high degree of acetylation (~5%) (Assoi et al., 2016). The gels produced were very stable thus indicating strong intermolecular associations between *B. aethiopum* pectin molecules during network formation (Ibănescu et al., 2010). When compared to commercial pectin Fluka (76280, Sigma-Aldrich), gel with equivalent characteristics was noticed. Furthermore, *B. aethiopum* pectin exhibits goods emulsifying properties probably because of its high protein (80 g kg^{-1}) and galacturonic acid content (Assoi et al., 2016)

Pectin is used in the manufacture of jam, jellies, and marmalade; low sugar and calorie foods; bakery; meat packing; acidified milk drinks; and is also used as emulsifier and stabilizer (Voragen et al., 2009, 1995). Commercial production of pectin is typically derived from apple pomace and citrus peels. After extraction, pectin precipitation from the mother liquor is generally done with alcohol. However, at the laboratory level, various organic solvents as well as salts of polyvalent metals and physical techniques are used to evaluate the appropriate precipitation conditions of pectin from a specific plant material. Several studies aiming at exploring the impact of precipitating solvents on the yield and physicochemical indices of pectin are available in the literature. The work of Hunt (1918) reported comparable yield when apple pectin was precipitated with ammonium sulfate and alcohol. Saulnier and Thibault (1987) recovered pectin from the pulp of grape berries through cupric ion precipitation. Muminov (1997) used organic solvents and salts of polyvalent metals to determine the best precipitating solvents for pectin extracted from the valves of cotton bolls. Kravtchenko et al. (1992b) reported the recovery of more purified commercial apple and lemon pectins after precipitation with copper acetate. Alcohol and aluminum-chloride precipitation of galgal (*Citrus pseudolium*) pectin affected the physicochemical characteristics and functional properties of the purified pectin (Attri and Maini, 1996). The quality, composition and physicochemical properties of yellow passion fruit ring pectin precipitated by dialysis or with alcohol and metal ion were also reported by Yapo (2009). The influence of pH on sugar beet pectin precipitation was mentioned by Guo et al. (2016a). Moreover, coexistence of five pectin fractions of different chemical and molecular characteristics have been identified when a stepwise ethanoic-precipitation was used during the recovery of sugar beet pectin from the water soluble extract (Guo et al., 2016b). Nagel et al. (2017) used selective precipitation steps to recover mango peel pectin from the liquid extract. Also, to establish an alternative method for pectin recovery from pomelo albedo residues, Vega et al. (2018) evaluated the effect of methanol, ethanol and 1-propanol on the quality of the precipitated pectin.

Therefore, since pectin was extracted for the first time from Palmyra palm (*B. aethiopum* Mart) (Assoi et al., 2016) the need to examine the impact of precipitating solvents on the quality attributes of that pectin must be determine to complement the work started on pectin extraction from this potential raw material. In this study pectin isolated at room temperature and under the natural pH of the fruit (5.2-5.5) was precipitated with isopropanol, acetone and the 50/50 mixture of the two solvents; and their effects on the physicochemical, functional, and rheological properties of *B. aethiopum* pectins were analyzed.

MATERIALS AND METHODS

Sample preparation, *B. aethiopum* pectin extraction and recovery

Mature, ripe fruits of Palmyra palm *(B. aethiopum* Mart), that weighed between 1.4 and 2 kg, were purchased at a local market in Dimbokro (Côte d'Ivoire). After cleaning, the fruit pulp was separated from the skin and kernel using a knife. An aliquot of 77 fruits was randomly selected which had a total weight of 100.4 kg and a yield of 39.10 kg of pulp was obtained. The pulp was cut into small pieces and dried in a Memmert laboratory oven (ULE 500, Memmert, Schwabach, Germany) at 60°C for 48 h (Agbo and Simard, 1992) and packed in plastic storage bags before being shipped to the Food Biotechnology Laboratory at Alabama A & M Huntsville, AL, USA where it was ground, alcohol washed and stored at room temperature until use. Pectin extraction was done as described by Assoi et al. (2016). Briefly, the alcohol insoluble solids (1 g) was dispersed in water (25 ml) and pectin was extracted using a shaking water bath (model 50, Thermo Fisher Scientific Inc. Waltham, MA, USA) maintained at room temperature. After

extraction and centrifugation, the pH of the pectin extract was adjusted to pH 4 with 0.1 mol/L HNO_3 and the precipitating solvent, namely isopropanol, acetone or the 50/50 mixture of the two solvents, was added and the mixture was kept overnight at 4°C for pectin precipitation. After centrifugation, pectin gel was successively washed with 70 and 95% isopropanol, freeze-dried and ground into a fine powder. Yield of pectin was calculated as follows:

Yield (g kg^{-1}) = (weight of dried pectin / weight of alcohol insoluble solids) × 100 (1)

Proximate analysis and physicochemical characterization of *B. aethiopum* pectin

Pectin moisture was determined after drying in a Precision Telco laboratory oven (Winchester, VA, USA) according to the method of Kalapathy and Proctor (2001). Ash content was determined by incinerating pectin sample (1 g) in a muffle furnace (Lindberg Blue M, Ashville, NC, USA) at 660°C overnight. Pierce BCA (bicinchoninic acid) protein assay kit (Pierce Biotechnology, Rockford, IL, USA) was used for protein analysis. Galacturonic acid content (GalA) was estimated colorimetrically using *m*-phenyl phenol; degree of methylation (DM) and degree of acetylation (Dac) were estimated by methanol released after saponification; individual neutral sugars (arabinose, rhamnose, xylose, mannose, galactose and glucose) were estimated from per-*O*-trimethylsilyl derivatives by gas chromatography–mass spectrometry. Molecular weight (Number average molecular weight, Mn; Weight average molecular weight, Mw) and polydispersity were estimated by laser light scattering (Dawn Heleos® II) and refractive index Optilab-T-Rex detectors (Wyatt Technology, Santa Barbara, CA) using a PL Aquagel-OH size exclusion columns (Agilent Technologies, Santa clara, CA) as described by Corredig and Wicker (2001). Intrinsic viscosity of diluted pectin samples (10-50 g kg^{-1}) prepared in 0.1 mol/L sodium phosphate buffer (pH 7) was determined according to a modified protocol described by Constenla et al. (2002). Samples were analyzed at 25°C and constant shear stress of 0.1 Pa using a controlled stress dynamic rheometer Rheometric Scientific model SR-500 (Rheometrics, Piscataway, New Jersey, USA). A concentric cylinder geometry with a cup diameter of 32 mm; bob diameter of 29.5 mm, and bob length of 44.5 mm was used during the test. Zeta potential, which provides information on the surface charge of polymers in solution, was measured using a Particle Size Analyzer coupled with the BI-Zeta option (90 Plus, Brookhaven Instruments Corporation, Holtsville, NY, USA), and equipped with a 50 mV diode laser (90 angle) and a BI-9000 AT correlator. Refractive index and laser beam were set at 1.330 and 659.0 nm and the temperature at 25°C (Kim and Wicker, 2011). Before running the analysis, 3 ml of the aqueous dispersion of pectin (5 g kg^{-1}) containing 0.2 g kg^{-1} of sodium azide was filtered through a 5.0 µm filter (Millipore, Bedford, MA) after pH adjustment to 4.

Functional and rheological properties of *B. aethiopum* pectin

Emulsifying activity was determined according to the modified method of Dalev and Simoneonova (1995). Aliquot (3 ml) of aqueous pectin solutions (5 g kg^{-1}) containing 0.2 g kg^{-1} of sodium azide was homogenized for 3 min with 3 ml of vegetable oil. The oil-in-water emulsion was centrifuged at 600 × g for 5 min using Allegra X-22R centrifuge (Brea, CA, USA). The whole volume (Wv) of the emulsion and the volume of the emulsified layer (ELv) were used to calculate the emulsifying activity (EA) as follows:

EA (%) = (ELv / Wv) × 100 (2)

All rheological analyses were performed with a ARG-2000 controlled stress rheometer (TA instrument, New Castle, Delaware, USA) mounted with a truncated cone (diam. 40 mm; angle 2°; truncation 54 µm) and peltier geometry. Solvent trap was used to prevent drying of the sample during the test. Steady shear flow behavior test was performed to determine the apparent viscosity of 20 g kg^{-1} *B. aethiopum* pectin solution prepared by dissolving pectin in distilled water at room temperature for 1 h by magnetic stirring. Data was collected over a shear rate range of 10^{-3} to 10000 s^{-1} at 25°C. Viscoelastic behavior of *B. aethiopum* aqueous solution was analyzed through small oscillatory deformation test. Stress sweep test conducted at 25°C and constant frequency of 1 Hz was performed to estimate the maximum deformation attainable by a sample in the linear viscoelastic region (LVR). Frequency sweep test was carried out in the frequency range of 0.1 to 10 Hz at constant stress (0.35 Pa) selected within the LVR. The mechanical spectra of the solution was obtained by reporting the elastic modulus (G') and viscous modulus (G'') as a function of frequency. *B. aethiopum* gels properties were determined on 1 g/ 100 g pectin gels prepared in a sealed vial. Pectin samples and 65 g/100 g of dried sucrose were dissolved in citrate buffer (pH 3). The mixture was heated for 30 min in boiling water under continuous stirring and kept overnight at room temperature for gel formation. Textural properties of *B. aethiopum* pectin gels were assess through oscillatory measurements performed at 25°C and 0.35 Pa selected in the LVR. The magnitude of tan delta, elastic (G') modulus and viscous modulus (G'') were recorded and analyzed to describe the quality attributes of the gels (strength, softness).

Statistical analysis

Data were analyzed for comparison of the means using SAS 9.2. Differences in the means were obtained using the Tukey test at α = 5%, values are expressed as means ± standards deviation of the means.

RESULTS AND DISCUSSION

Effect of precipitating solvents on yield and physicochemical characteristics of *B. aethiopum* pectins

In this study, water soluble pectin was extracted from *B. aethiopum* fruit pulp for 30 min at room temperature and natural pH (5.2-5.5) of the fruit. To improve the yield and purity of *B. aethiopum* pectin, isopropanol (IPA), acetone (ACTN), and the 50/50 mixture of the pure solvents were used to recover pectin from the liquid extract. At industrial level, pectin precipitation from the mother liquor is generally done using organic solvents such as IPA or ethanol (May, 1990). The use of ACTN as pectin precipitant was mostly reported in the literature (Suhaila and Zahariah, 1995; Muminov, 1997; Aina et al., 2012). Good yield, color and gelling characteristics were reported for pectins extracted from various tropical agro-wastes when acetone was used as precipitating solvent (Suhaila and Zahariah, 1995). Therefore, IPA and ACTN were selected to assess their effects on the physicochemical and functional properties of *B. aethiopum* pectin. In addition, mixture of the pure solvents (50/50) for pectin precipitation was also investigated.

Table 1. Yields, proximate composition and physicochemical characteristics of *B. aethiopum* pectin precipitated by the 3 solvents.

Parameter	Precipitating solvents		
	Isopropanol	**IPA-ACTN (50/50)**	**Acetone**
Yield (g kg^{-1})	$102.3^{a} \pm 0.04$	$96.2^{a} \pm 0.03$	$105.8^{a} \pm 0.11$
Moisture (g kg^{-1})	$76.1^{a} \pm 0.02$	$64.3^{a} \pm 0.28$	$67.2^{a} \pm 0.31$
Ash (g kg^{-1})	$35.0^{a} \pm 0.03$	$35.3^{a} \pm 0.01$	$41.9^{a} \pm 0.01$
Protein (g kg^{-1})	$87.4^{a} \pm 0.11$	$87.2^{a} \pm 0.27$	$90.5^{a} \pm 0.05$
GalA (g kg^{-1})	$845.2^{b} \pm 2.17$	$889.1^{a} \pm 3.16$	$909.0^{a} \pm 1.62$
DM (%)	$71.92^{a} \pm 2.61$	$71.35^{a} \pm 2.46$	$68.31^{a} \pm 1.56$
Dac (%)	$5.25^{a} \pm 0.54$	$4.14^{a} \pm 0.18$	$5.07^{a} \pm 0.64$
Molecular weight			
Firstly eluted pectin molecules			
Polydispersity	1.48	1.24	1.41
Mn $\times 10^{5}$ (g mol^{-1})	2.02^{a}	1.61^{b}	2.15^{a}
Mw $\times 10^{5}$ (g mol^{-1})	2.92^{a}	1.97^{b}	3.00^{a}
Secondly eluted pectin molecules			
Polydispersity	1.0	1.03	1.0
Mn $\times 10^{5}$ (g mol^{-1})	1.44^{a}	1.07^{a}	1.51^{a}
Mw $\times 10^{5}$ (g mol^{-1})	1.46^{a}	1.11^{a}	1.52^{a}
Intrinsic viscosity (ml g^{-1})	291.7^{b}	316.56^{a}	295.67^{b}
Zeta potential (mV)	$-25^{a} \pm 0.12$	$-22^{b} \pm 0.95$	$-24^{a} \pm 0.83$

GalA: Galacturonic acid, DM: Degree of methylation; Dac: Degree of acetylation; Mn: Number average molecular weight; Mw: Weight average molecular weight; IPA-ACTN: Isopropanol- Acetone. Values are mean±SD; ^{abc}Means in a row with the same superscript are not significantly different ($p > 0.05$).

Pectin purity is mostly based on GalA content and lack of ash. In this study, statistical analysis showed no significant difference ($p>0.05$) in the yield (96-106 g kg^{-1}), moisture (64 -76 g kg^{-1}), ash (35-42 g kg^{-1}) and protein (87-91 g kg^{-1}) of the *B. aethiopum* pectin precipitated with the different solvents (Table 1). It is noted that despite the cold extraction process *B. aethiopum* pectin contained high level of protein and this could suggest the presence of elevated amount of protein into the ripe and mature fruit. This protein may exist either in free form or bound to the arabinogalactan moiety (Oosterveld et al., 2002) probably liberated during the ripening process (Sirisomboon et al., 2000, Steele et al., 1997). Lower ash content in pectin sample is desired (Ceylan et al., 2017) because of its ability to negatively affect gel formation. Ash maximum limit to produce good quality gel is 100 g kg^{-1} (Huda, 2016). El-Nawawi and Fadia (1988) reported a decrease in the jelly grade of Egyptian orange peel pectin due to an increased amount of ash in the pectin. Ash content of *B. aethiopum* pectins was below the maximum limit regardless of the solvents used.

GalA content of *B. aethiopum* ranged from 845 g kg^{-1} (IPA) to 909 g kg^{-1} (ACTN). Acetone and the 50/50 solvent mixture provided pectin with significantly ($p<0.05$) higher GalA content as compared to the IPA solvent (Table 1). High GalA is known to improve the functional properties of the biopolymer. GalA content of *B. aethiopum* pectin was higher than the maximum limit (65%) defined for commercial pectin (May, 1990). Pectin with high purity was extracted from *B. aethiopum* fruit pulp as indicated by the high GalA content and low ash.

B. aethiopum fruit pulp pectins were highly methylated (HM) and acetylated, and values ranged from 68.31% (acetone) to 71.92% (isopropanol) and from 4.14% (50/50 IPA-ACTN) to 5.25% (IPA) respectively. The degree of methylation (DM) and the degree of acetylation (Dac) were not significantly affected ($p>0.05$) by the precipitating methods (Table 1). Based on their DM, ACTN pectin can be classified as medium rapid set pectin and both IPA and 50/50 IPA-ACTN pectins as rapid set pectins (Thibault and Ralet, 2003).

Neutral sugar (NS) content of precipitated *B. aethiopum* pectin is presented in Figure 1. NS contributes to the functional (gelling and rheological) properties of pectin in the food system (Hwang et al., 1993). Acetone precipitated *B. aethiopum* pectin was richer (7.95 g/100 g) in neutral sugars than isopropanol precipitated (5.18 g/100 g) pectin. The lowest neutral sugar content (3.8 g/100 g) was observed for *B. aethiopum* pectin precipitated with the 50/50 mixed solvent. These data showed a partial purification of *B. aethiopum* pectin by the pure solvents as compared to the 50/50 mixed solvent (Kravtchenko et al., 1992b). Acetone solvent probably allowed the co-precipitation of higher amount of free neutral polysaccharides; such as xyloglucans, arabinans, arabinogalactans, and mannans as compared

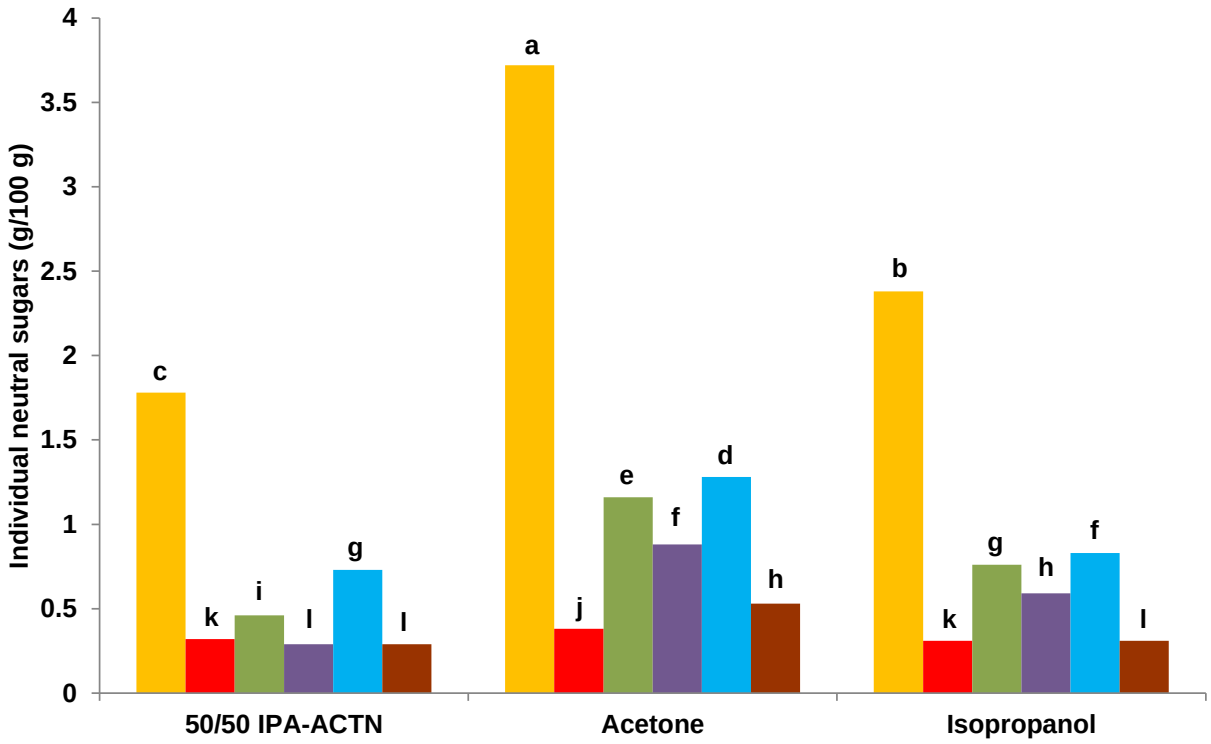

Figure 1. Individual neutral sugar (g/100 g) of *B. aethiopum* pectin extracted at room temperature and pH natural pH of the fruit and precipitated with Isopropanol, Acetone or 50/50 isopropanol/acetone. Symbols: Arabinose ( ), Rhamnose ( ), Xylose ( ), , Mannose ( ), Galactose ( ), , Glucose ( ).

to the 70% isopropanol solvent. A co-precipitation of salts, free proteins and sugars has also been reported (Brigand et al., 1990; Garna et al., 2007). The efficiency of isopropanol solvent at reducing co-precipitation of impurities in *B. aethiopum* pectin was about 1.3 times improved when isopropanol was mixed with acetone rather than water. Consequently, the mixture of acetone and isopropanol solvent, in the ratio of 50:50, which helped to obtain *B. aethiopum* pectin with low amount of impurities, could be better choice.

A bimodal distribution of the molar mass was observed, regardless of the precipitating solvent, thus revealing two distinct populations of the *B. aethiopum* pectin polymers. First eluted pectin population was composed of high molecular weight polymer chains (1.97×10^5 - 3.0×10^5 g mol^{-1}) while the second eluted population contained polymers chains of much lower molecular weight (1.11×10^5 - 1.52×10^5 g mol^{-1}) (Table 1). Molecular weights of pectin precipitated by the pure solvents were statistically higher ($p<0.05$) than the molar mass of the pectin precipitated with the solvent mixture.

Polydispersity (PD) provides information on the uniformity of the polymer chain lengths, and it is calculated as the ratio of Mw and Mn. For PD closers to 1 all polymers chains approach uniform chain length. When PD falls between 1.01 and 1.2, low variation in chain length is observed. Higher variability in chain length is reported when PD > 1.4 (Whitfield et al., 2019). PD of *B. aethiopum* pectin varied from 1.24 (IPA-ACTN) to 1.48 (IPA). Pectin precipitated with the 50/50 solvent mixture was less polydisperse than the others, and it also displayed the lowest Mw probably due to the removal of the covalently unlinked substances to the pectin molecules. These impurities may have contributed, to a certain extent, to the relatively high molar mass of pectin precipitated with acetone and isopropanol (Berth, 1988; Kravtchenko et al., 1992a). This result was in a close agreement with observations previously reported for the neutral sugars, intrinsic viscosity and molar mass analysis. Comparable results were also reported by Kravtchenko et al. (1992b) when attributes of the aqueous copper acetate purified commercial pectin was compared to that of the alcoholic purified one.

Intrinsic viscosity, which specifies the hydrodynamic volume of the individual polymer molecule in solution, is reported by using the Solomon-Ciuta equation (Pamies et al., 2008) because conditions for a linear extrapolation to zero polymer concentration could not be met (Evageliou et al., 2005). The 50/50 IPA-ACTN precipitated *B. aethiopum* pectin (Mw of 1.97×10^5 g mol^{-1}) exhibited the highest intrinsic viscosity (316.56 ml g^{-1}) as compared to isopropanol (291.7 ml g^{-1}, Mw of 2.92×10^5 g mol^{-1}) and

Table 2. Functional properties of *B. aethiopum* pectin precipitated with various solvents.

Parameter	Precipitating Solvents		
	Isopropanol	IPA-ACTN (50/50)	Acetone
Emulsifying activity (%)	62.1[a] ± 0.59	56.3[b] ± 0.05	52.1[b] ± 2.95
Apparent viscosity (Pa.s) of 20 g/100 kg^{-1} pectin solution at near zero shear rates	3.57[b]	3.08[b]	4.71[a]
Elastic modulus (Pa) of 1 g/100 g gel (obtained at 1 Hz)	1634[a]	1497[b]	1611[a]
Tan delta of 1 g/100 g gel	0.18-0.23	0.2-0.25	0.18-0.23
Sensorial property (G'/G") of 1 g/100 g gel	5-6	4-5	4-5

IPA / ACTN: Isopropanol/Acetone; Pa: Pascal; G': Elastic modulus; G": viscous modulus; Hz: herz. Values are mean±SD; [abc]Means in a row with the same superscript are not significantly different ($p > 0.05$).

acetone (295.67 ml g^{-1}, Mw of 3.00×10^5 g mol^{-1}) precipitated ones (Table 1). Since the intrinsic viscosity of polymer solutions is mostly related to the volume of the polymers in solution (Round et al., 2010); it could be inferred that the lower intrinsic viscosity exhibited by the higher Mw pectin samples may be due to the presence of compact polymers in the solution (Koliandris et al., 2010).

Zeta potential, which illustrates the magnitude of charges at a specific pH on the surface of a particle in colloidal dispersion, derives mostly from the chemical ionization of functional groups in solution (Alkan et al., 2005). All aqueous solution of *B. aethiopum* pectin exhibited negative zeta potential values at pH 4. IPA (-25 mV) and ACTN (-24 mV) precipitated pectins exhibited more negatively charged surface ($p<0.05$) than the 50/50 IPA-ACTN precipitated pectin (-22 mV). At pH of 4, carboxyl groups are dissociated and pectin molecules carry a negative surface charge. The density of charge depends both on the DM of the pectin and the pH used (Schmidt et al., 2016). DM of *B. aethiopum* precipitated pectins were not statistically different but IPA and ACTN precipitated pectin exhibited statistically higher negative surface charge than the 50/50 IPA-ACTN precipitated pectin (Table 1). This difference could be related to a different distribution of the negatively charged carboxyl groups within the pectin molecule (Gawkowska et al., 2018). Indeed, a blockwise charge distribution generates a more negatively surfaced charge than a randomly distributed charged carboxyl groups (Lutz et al., 2009). A high zeta potential is a sign of greater stability of the aqueous dispersion of pectin (Pacheco et al., 2019). Therefore, more stable aqueous dispersions will be obtained with the IPA and ACTN precipitated pectins than the 50/50 IPA-ACTN precipitated one.

Effect of precipitating solvent on functional and rheological properties of *B. aethiopum* pectins

Emulsifying activity (EA) of the *B. aethiopum* pectin ranged from 52.1% (acetone) to 62.1% (isopropanol). *B. aethiopum* pectin with statistically ($p<0.05$) higher EA was precipitated by the isopropanol solvent. Precipitation with acetone and the 50/50 IPA-ACTN solvent led to the isolation of *B. aethiopum* pectin with reduced EA (Table 2). All of the pectin samples, at concentration of 5 g kg^{-1}, exhibited high emulsifying activities (EA> 50%) probably due to their high protein content (≈ 90 g kg^{-1}). *B. aethiopum* pectins can be considered as true emulsifiers as they were able to adsorb and form at the surface of the newly formed oil droplets a protecting layer which prevents them from coalescing with neighboring oil droplets (Ngouémazong et al., 2015).

Precipitating solvents significantly affected ($p<0.05$) the rheological properties of the *B. aethiopum* pectins. Apparent viscosity of *B. aethiopum* pectin solutions decreased with increasing shear rates (Figure 2), and this is an indicative of a shear thinning flow behavior. Similar flow behavior was reported by Hwang and Kokini (1992) for commercial apple pectin prepared by precipitation with Cu(II) followed by 1.0% acid alcohol washing or EDTA treatment. Thinning of a polymer solution during shear is the result of the disruption of network and alignment of the polymers in the direction of the flow (Dangi and Yadav, 2020; Pacheco et al., 2019). For all pectin samples, the Newtonian plateau (NP) was not observed probably because the concentration used was high enough to shift the NP toward much lower values than the lowest shear rate available. The same trend was reported by Razavi et al. (2011) for sage seed gum. At very low shear rate, apparent viscosity of acetone precipitated pectin solution was statistically higher (4.71 Pa.s) than that of IPA (3.57 Pa.s) and 50/50 IPA-ACTN (3.08 Pa.s) precipitated pectins. This higher viscosity, at similar concentration (20 g Kg^{-1}), suggests the presence of higher intermolecular interactions between ACTN precipitated pectin chains at near-zero shear rates (Chen et al., 2020). When the shear rate was increased, a rapid decrease of the apparent viscosity was observed for the acetone precipitated pectin solution. This might reflect a much weaker association of the acetone precipitated polymers in solution (Sato et al., 2004). The overlapping of the graphs obtained with isopropanol and 50/50 IPA-ACTN precipitated pectin may reveal the formation of

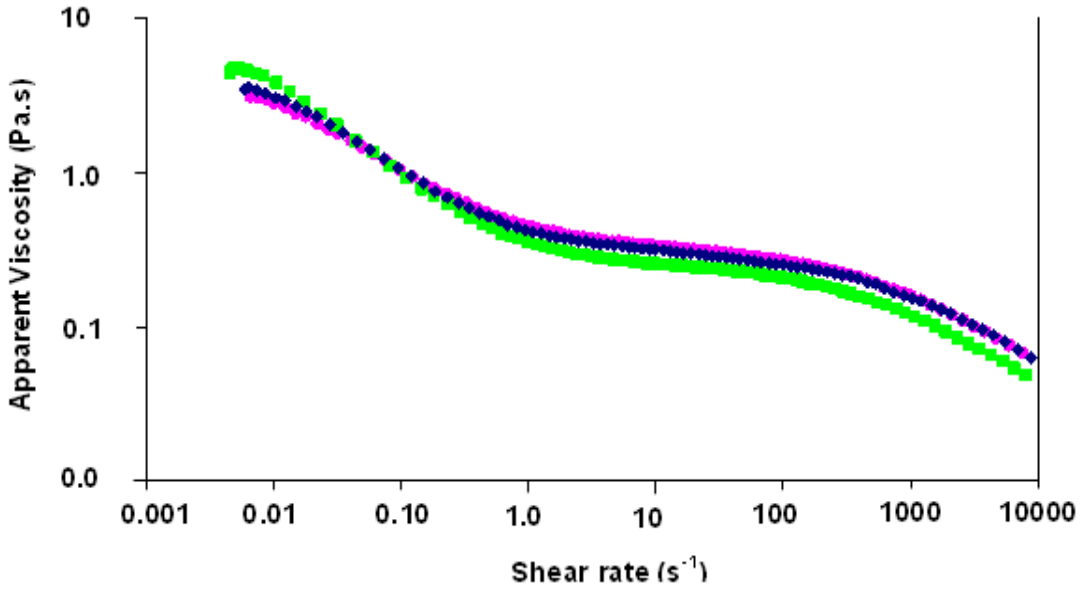

Figure 2. Steady state flow behavior of 20 g kg^{-1} solution *B. aethiopum* pectin extracted at pH natural, room temperature and precipitated with isopropanol (◆), acetone (■), and 50/50 isopropanol/acetone mixed solvent (●). Measurements were conducted at 25°C.

network structures of comparable characteristics (Assoi et al., 2016).

Results of the oscillatory tests revealed that viscoelastic properties of *B. aethiopum* pectin solutions were affected by the precipitating solvents. Stress sweep results (Figure 3) exposed viscoelastic liquid-like characteristics (G" > G') for *B. aethiopum* pectin solutions (20 g kg^{-1}). Moreover, at very low shear stress (0.01 - 1 Pa), acetone precipitated pectin solution exhibited higher elastic modulus (G') than IPA and 50/50 IPA-ACTN precipitated pectin solutions. Since network strength is measured by the magnitude of G' (Ström et al., 2014), it could be inferred that sufficient entanglements were formed between acetone precipitated pectin polymers chains thus leading to the formation of a continuous network (Tam et al., 1999). At shear stress beyond 1 Pa (critical stress), G' of acetone precipitated pectin decreased faster than that of the others, thus revealing weak associations of the pectins polymers. Mechanical spectra displayed in Figure 4 is showing changes in elastic moduli (G') and viscous moduli (G") with frequency variation. All pectin solutions exhibited an increase in the magnitude of G' and G" with increasing frequency which is a characteristic of fluid-like behavior typical of random-coil entanglement networks. Moreover, G' and G" tend to converge at higher frequency thus indicating network formation between polymers.

Variability in the properties of the gel (1 g/100 g) prepared with the precipitated *B. aethiopum* pectins was also noticed (Table 2 and Figure 5). All the precipitated pectins formed a gel in presence of 65 g/100 g of sucrose at pH of 3. Under Stress sweep test, the gel elastic modulus (G") varied from 1497 Pa (50/50 IPA-ACTN) to 1634 Pa (IPA). Pectin precipitated with the pure solvents exhibited greater G' than the 50/50 IPA-ACTN precipitated pectin, thus revealing the formation of denser network with more interconnected polymers chains. Gelling and thickening aptitude of pectin is generally assessed by its ability to form interconnected network in solution and the occurrence of these entanglements depends greatly on several intrinsic and extrinsic factors (Dangi and Yadav, 2020). The differences observed in the gel properties could be attributed to the low Mw and neutral sugar content of the pectin precipitated by the mixed solvents (Abid et al., 2017). Therefore it could be assumed that compounds not precipitated by the 50/50 solvent mixture may have contributed to the increase in the elastic modulus of the gel prepared with *B. aethiopum* pectin precipitated with each of the pure solvent.

For all *B. aethiopum* pectin gels, the frequency sweep results displayed the characteristic spectrum of gel-like structure (G' > G") (Figure 5) with tan delta around 0.2 (Table 2). Over the entire available frequency range, a minimal dependency to frequency was observed for G' and G", thus proving the formation of stable three-dimensional networks (Abid, 2017; Rasidek et al., 2018). IPA precipitated pectin formed stronger gel than the acetone and 50/50 IPA-ACTN pectins as indicated by its higher G'. HM pectin gel is stabilized by intermolecular hydrogen bounds and hydrophobic interactions between the methyl ester of the carboxyl groups (Chan et al., 2017). Since the Degree of Methylation of all precipitated

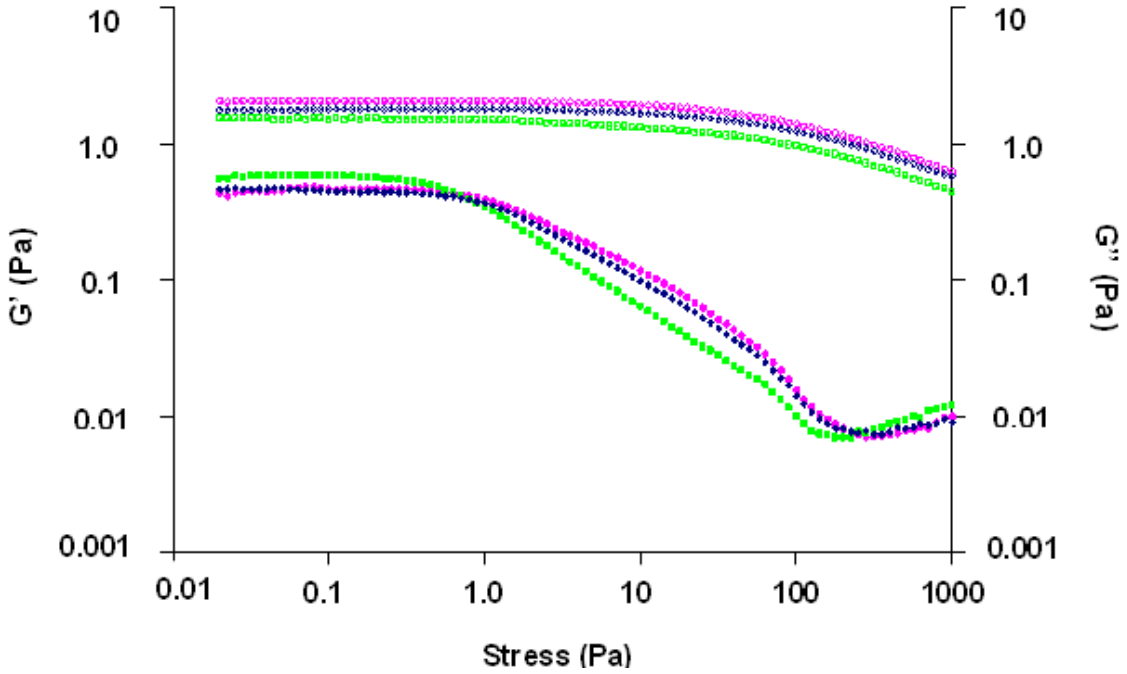

Figure 3. Stress weep graph of *B. aethiopum* pectin extracted at pH natural and room extracted at pH natural and room temperature and precipitated with isopropanol (◆◇) acetone (■□)and 50/50 isopropanol/acetone mixed solvent (●○). Measurements were done at 25°C and 1 Hz. Filled symbols represent the elastic modulus (G') and the open symbols represent le viscous modulus (G"). Pa: Pascal.

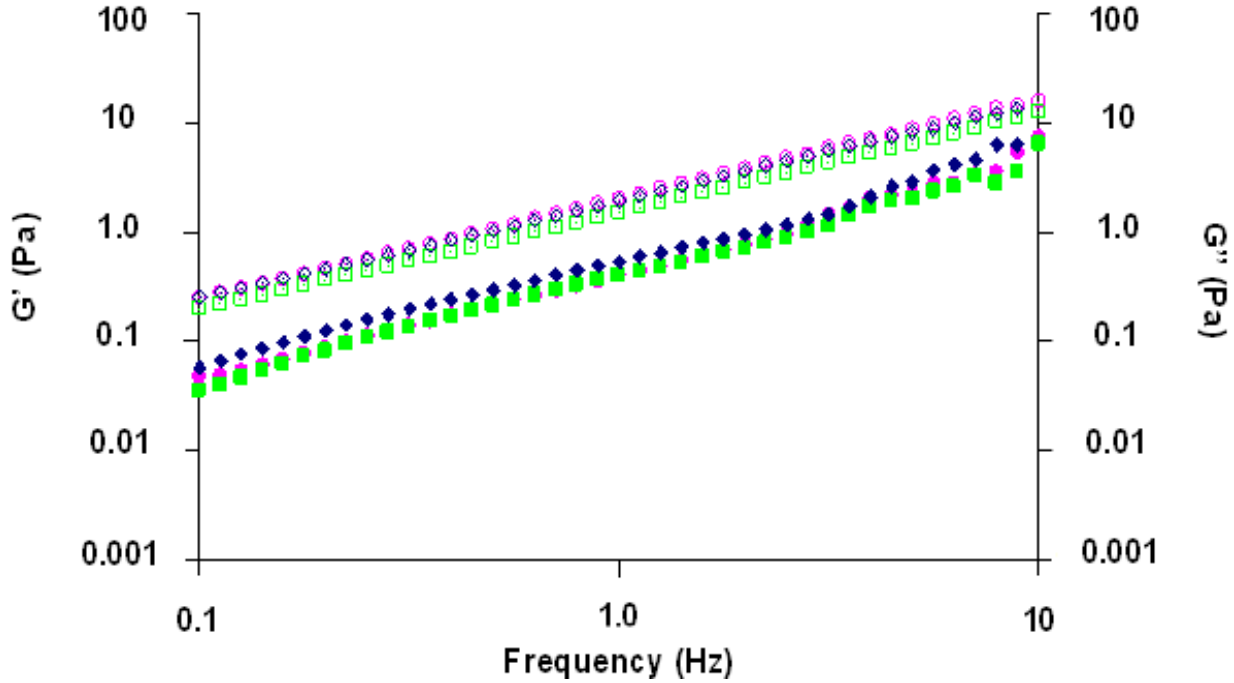

Figure 4. Dynamic mechanical spectra of 20 g kg^{-1} of *B. aethiopum* pectin solution extracted at pH natural and room temperature and precipitated with isopropanol (◆◇) acetone (■□)and 50/50 isopropanol/acetone mixed solvent (●○) done at 25°C using a constant stress of 0.35 Pa selected in the LVR. Filled symbols represent the elastic modulus (G') and the open symbols represent le viscous modulus (G"). Pa: Pascal; Hz: hertz.

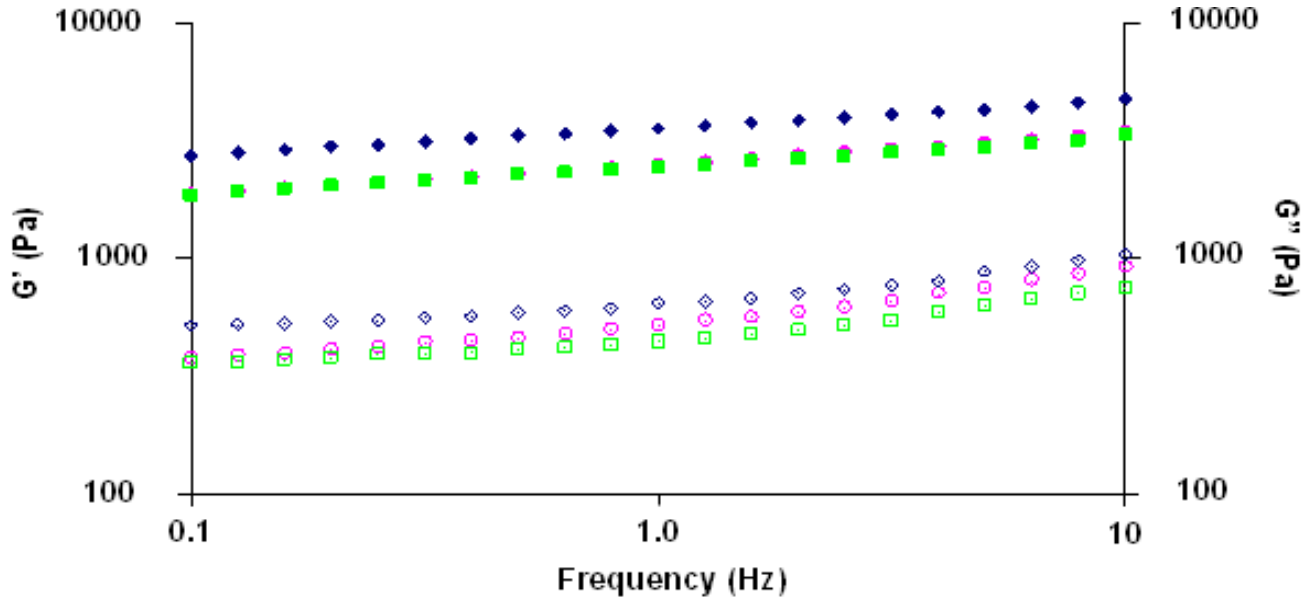

Figure 5. Dynamic mechanical spectra of 1 g/100 g gel of *B. aethiopum* pectin extracted at pH natural and room temperature and precipitated with isopropanol (◆◇) acetone (■□)and 50/50 isopropanol/acetone mixed solvent (●○). Measurements were done at 25°C using a constant stress of 0.35 Pa selected in the LVR. Filled symbols represent the elastic modulus (G') and the open symbols represent le viscous modulus (G"). Pa: Pascal; Hz: hertz.

pectins showed a difference that was not statistically significant (Table 1) it could be assumed that hydrogen bonds were solely responsible for gel formation and stabilization. Hydrogen bounds are weak and easily broken but a large number of them can confer great stability to a network (Chan et al., 2017). Sensorial properties of gels are usually described by the ratio of the elastic modulus to the viscous modulus (G'/G") (Iglesias and Lozano, 2004). Table 2 shows the ratio (between 4 and 6) obtained for *B. aethiopum* pectins precipitated with the different solvents. These small values demonstrated that gels with soft texture were produced with *B. aethiopum* pectins samples (Iglesias and Lozano, 2004).

Conclusions

Palmyra palm (*Borassus aethiopum* Mart.) fruits offer an inexpensive raw material to extract pectin. In this study polar solvents (isopropanol and acetone) and their mixture (50/50) were used to precipitate pectin extracted at room temperature and natural pH (5.2-5.5) of the fruit. Results showed that precipitating solvents affected the physicochemical and functional properties of *B. aethiopum* pectins. Additionally, regardless of precipitating solvent, Palmyra palm pectin showed high viscosifying, emulsifying and gelling properties. Therefore, the choice of a specific extraction condition (Assoi et al., 2016) and precipitating method for the isolation of pectin from *B. aethiopum* fruit pulp should depend on the intended use of that pectin.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Nutritional physico-chemical composition of pumpkin pulp for value addition: Case of selected cultivars grown in Uganda

Immaculate Nakazibwe*, Eunice Apio Olet and Grace Kagoro-Rugunda

Department of Biology, Mbarara University of Science and Technology, P.O. Box 1410, Mbarara, Uganda.

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Pumpkin is one of the underutilized fruit-vegetables in Uganda although it is one of the crops believed to have carotenoids with pro-vitamin A activity that are not common to many other crops. While other crops are being bred or fortified to increase their nutritional benefits of β-carotene and other micro nutrients required for healthy growth and care for immunosuppressed people, the value of pumpkins in this regard remains under researched. This study sought to determine the nutritional physico-chemical parameters of selected pumpkin cultivars grown in Uganda. The seeds of 14 cultivars of pumpkin from four agro-ecological zones of Uganda were grown under similar conditions. Their mature pulp was then analyzed for their proximate, mineral and carotenoid composition including glucose, starch, crude fibre, crude fat, ash, crude protein, moisture, iron, zinc, calcium, potassium, lutein, α-carotene, *trans*-β-carotene, *cis*-β-carotene, total carotenoid and total pro-vitamin A carotenoid content using standard procedures. The proximate, calcium, potassium and the carotenoid content varied significantly across all the accessions ($P<0.05$) while the iron and zinc content did not vary significantly across all the accessions ($P>0.05$) respectively. The results from the study show that pumpkin pulp is a good source of dietary fibre, protein, calcium, potassium, iron and carotenoids with pro-vitamin A activity. Therefore, pumpkin can be utilized in the food processing industry as a food supplement and for several other value addition avenues such as wine and flour production.

Key words: Accession, carotenoid, cultivar, proximate, pumpkin, value addition.

INTRODUCTION

Pumpkins are fruits of several species belonging to the genus *Cucurbita* and family Cucurbitaceae. *Cucurbita* is one of the most economically important genera of vegetable crops (Paris, 2010). In much of Uganda, pumpkins are mainly cultivated as a marginal crop often on the edges of field crops or scantly scattered between staple crops such as maize or sorghum (Hamisy et al., 2002). However, it is known that cultivation of any crop is

*Corresponding author. E-mail: nimmaculate@must.ac.ug. Tel: +256779024816.

necessary in order to know important characters considered by farmers in selecting crop varieties for improvement and commercialization (Ondigi et al., 2008).

Pumpkins are cultivated in different parts of the world for their pulp and seeds for human nutrition, either for direct consumption or for formulation of other foodstuffs such as syrups, jellies, jams, and purees (Provesi et al., 2011; Kim et al., 2012). Pumpkin pulp has both nutritional and health protective value of proteins, polysaccharides such as pectin, carotene, mineral salts, vitamins and other substances such as phenolic compounds and terpenoids (Jun et al., 2006; Kampuse et al., 2015). Pumpkin can be processed into flour which has a longer shelf-life. Pumpkin flour is used for wheat fortification and preparation of porridge because of its highly-desirable flavour, sweetness and deep yellow-orange colour (Nakazibwe et al., 2019). It has also been reported to be used to make soups, sauces, instant noodle and spice as well as a natural colouring agent in pasta and flour mixes (See et al., 2007; Kulaitienė et al., 2014).

Even though various studies about determining the physico-chemical composition of pumpkins have been conducted in other parts of the world, very few studies have been conducted to determine the physico-chemical parameters of the several pumpkin cultivars grown in Uganda since the pumpkin is one of the underutilized crops in the country. Consequently, this has created a knowledge gap about the nutritional composition of the pumpkin cultivars grown in Uganda to the processors and also the consumers, and this has continued to lead to the under exploitation of the pumpkin crop in the region. Therefore, this study was carried out to establish the status of some of the important nutritional physico-chemical parameters of pumpkin cultivars grown in Uganda. Need to say, these parameters are dependent on the species and the environment in which the pumpkins are grown (Achu et al., 2005; Applequist et al., 2006). Relating to this study, nutritional profiling of pumpkin cultivars is vital for the recommended increase in productivity of such traditional crops that are nutritionally rich (Sanchez and Swaminathan, 2005). The information from this study will also enable the consumers and processors to have an overview of the pumpkin cultivars that have better nutritional values, so that pumpkin consumption, processing and general value addition respectively is carried out based on factual information. In addition, the results will provide a basis for the processors to select specific cultivars for appropriate value addition avenues.

METHODOLOGY

Pumpkin accession collection and planting

Cross sectional surveys were used to look for pumpkin farmers. Pumpkin seeds were collected from farmers in six districts from four agro-ecological zones of Uganda. These were Southwestern highlands (Kabale and Kanungu districts), Western mid-altitude farmlands and Semiliki flats (Masindi and Mubende districts), Western medium-high farmlands (Kabarole district) and Lake Victoria and Mbale farmlands (Mityana district) as represented in Figure 1. Villages namely, Mwangyale and Nyakibande in Kabale, Rugyeyo in Kanungu, Kihubba and Bugyenje in Masindi, Butologo in Mubende, Nyamiseke in Kabarole, Nkokonjeru and Kalangalo in Mityana were visited. The planted accessions were randomly assigned numbers basing on the districts from where they were collected. The accessions from Kabale were given ‘Kab’ with a corresponding number, those from Kanungu were given ‘Kan’ with a corresponding number, those from Masindi were given ‘Mas’ with a corresponding number, those for Fort portal, were given ‘Fort’ with a corresponding number and those from Mityana were given ‘Mit’ with a corresponding number.

The seeds were grown in a randomized complete block design experiment at the Mbarara Zonal Agricultural Research and Development Institute (MbaZARDI) located 0° 60′ 20″ S and 30° 60′ 97″ N. The garden was on an area of dimensions (32 x 72 m). The experiment had four complete blocks, assigned letters a, b, c and d for easy identification. Each block had 24 plots therefore, the garden had a total of 96 plots. Each plot had dimensions of 4 x 2 m, a modified spacing from that stated by (Balkaya et al., 2010). At the start, three seeds were planted per hole on a given plot. However, only one vine from one seed was maintained in each hole per plot. Therefore, two vines were planted in each plot per accession, with a spade full of well decomposed goats dung added to each of the holes where the seeds were planted. The accessions were randomly distributed in all the four blocks. The accessions acted as the different treatments, while the four blocks acted as the replicates in the experiment. The experiment ran from 30th March, 2017 to 22nd December, 2017 and two seasons where involved. However, the yields for the first season were so poor due to the low amounts of rainfall received in the region during the first season of the year hence yields from the second season were considered for analysis since they were reasonably high. Soil pH and soil texture were determined by use of a pH meter and a soil textural triangle respectively following the procedures described by Okalebo et al. (2002).

Pumpkin cultivar sampling and pulp sample preparation

At maturity, averagely after four months it was observed that out of the 24 accessions planted, 10 accessions were not consistent in morphological appearance throughout in all the four blocks while 14 accessions were consistent. Therefore, for the pumpkin accessions considered for analysis in the laboratory, a given accession in block one, had to bear fruits with similar morphological appearance with those of the same accession in the rest of the blocks. The identification of fruit morphological appearance at maturity was guided by pumpkin descriptors as described by Srivastava et al. (2001), with modifications. So, from the experiment, 14 accessions were observed to be consistent in at least two blocks where they had successfully grown to maturity. Eventually, three fruits of only one block for each consistent accession were randomly selected and further prepared for nutrient analysis. For each accession, the three fruits from the same vine were cut and the seeds removed to retain the pulp. A representative sample from each of the three pumpkin heads of a given accession was picked following the protocol described by Maria et al. (2015). The pulp was sliced into small pieces that were freeze dried for further analysis. The dried sample was ground into powder to form a homogenized sample using universal high-speed grinder model ID008760 and then

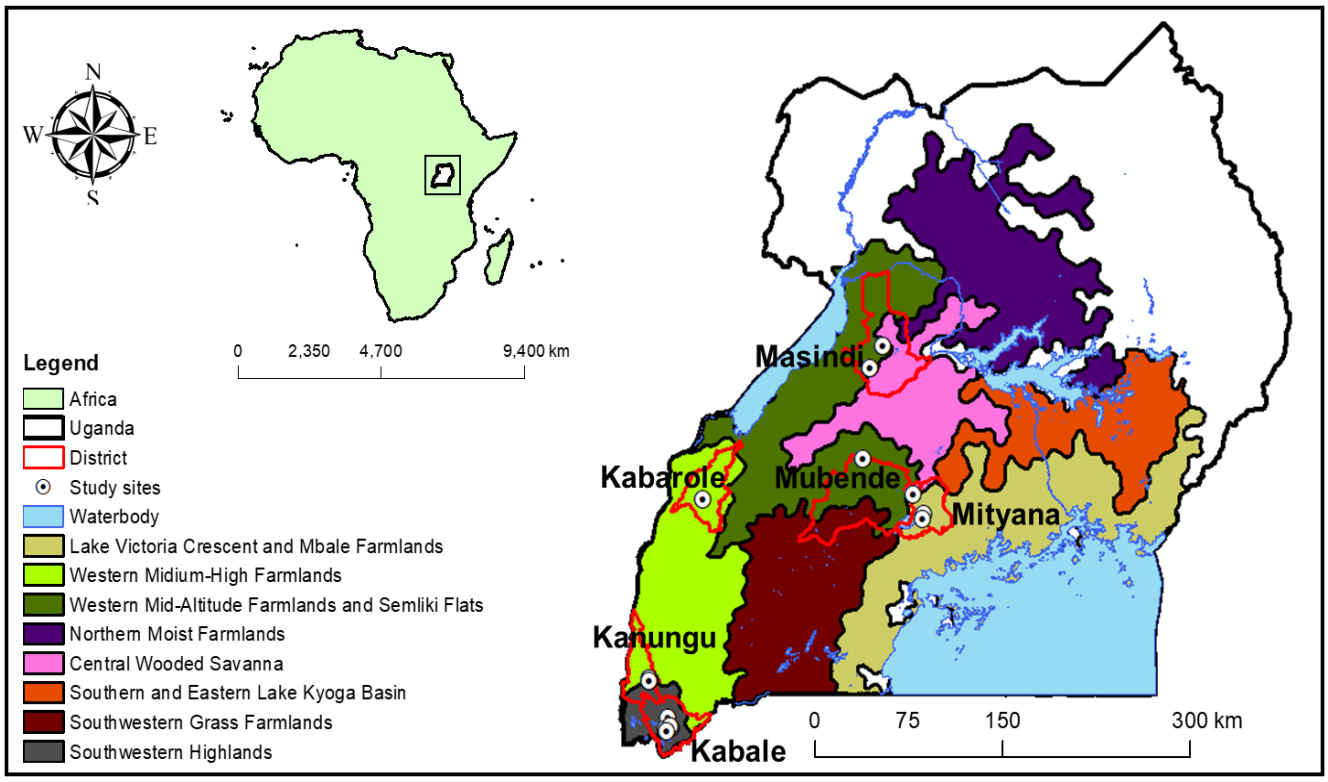

Figure 1. Map showing sites from which data was collected.
Source: Drawn using Arc GIS software version 10.5 (Agro-ecological Zones described by (Wortmann and Eledu, 1999).

stored in well labelled and sterilized sample bottles. Some parameters such as moisture, ash, starch and reducing sugars were determined using fresh samples.

Pumpkin pulp physico-chemical determination

Moisture and ash content

The moisture content was determined by using a moisture analyzer (Mettler Toledo model MJ33) as described by Nielsen (2010). 2 g per sample of fresh pumpkin pulp were used. The moisture content per sample was determined in triplicate.

Ash content

Ash content was determined by using the dry ashing method as described by Nielsen (2010). 2 g per sample of each sample were used for the analysis. The ash content per sample was determined in triplicate.

Crude fat content

Crude fat content was determined by using the AOAC Method 960.39, described by Nielsen (2010). 5 g of each sample were used for the analysis. The crude fat content per sample was determined in triplicate.

Crude fibre content

The crude fibre content was determined by using the A.O.A.C. method 930.10, which was modified as follows; 5 g per sample of ground material were extracted with petroleum ether to remove fat (Initial boiling temperature 35 - 38°C and final temperature 52°C). 5 g of dried material were boiled with 200 ml of sulphuric acid for 30 min with bumping chips. The mixture was filtered through muslin and washed with boiling water until the residue was no longer acidic. The mixture was boiled with 200 ml of sodium hydroxide solution for 30 min, followed by filtering through muslin cloth again and washing with 25 ml of boiling 1.25 % sulphuric acid, three 50 ml portions of water and 25 ml alcohol. The residue was removed and transferred to an ashing dish (pre-weighed dish W_1). The residue was dried for 2 h at 130°C, to a constant weight, and the dish cooled in a desiccator, then weighed to obtain (W_2). The mixture was ignited for 30 min at 600°C, followed by cooling in a desiccator and reweighed to obtain (W_3). The fibre content per sample was determined in triplicate according to the formula below:

$$\% \text{ crude fibre in ground sample} = \frac{\text{Loss in weight on ignition } (W2-W1)-(W3-W1)}{\text{Weight of sample}} \times 100$$

Crude protein content

Crude protein content was estimated by using Kjeldahl's method described by Okalebo et al. (2002). 2 g per sample was used and the analysis was done in triplicate.

Starch content

The starch content was determined with slight modification of the method reported by Mistry and Eckhoff (1992) as described. The fresh mature pumpkin was thoroughly washed with distilled water. It was peeled, and the pulp cut into small pieces. 150 g of small pieces of the pulp per sample were transferred to the blending motor (ID008760) and then blended. The blended suspension was filtered and washed with 0.1 N sodium hydroxide. The suspension was washed with excess distilled water and the filtrate left to stand for about 30 min. Then the excess alkali was removed by washing several times with distilled water; adding excess distilled water to the filtrate and leaving it to stand. The alkali and water were decanted off and left to stand for 10 minafter which, the supernatant fluid a non-starch layer was decanted off. The tightly packed starch was then collected and dried in an oven at 60°C for 6 h. The starch was then ground to a fine powder and the weight of the dry starch was determined by use of the analytical balance (Mettler Toledo, MS304S/01). The analysis per sample was done in triplicate. The % of starch in the sample was calculated by using the formula below:

$$\%\ \text{starch in sample} = \frac{\text{Weight of starch in sample}}{\text{Weight of sample}} \times 100$$

Glucose content

The amount of glucose in the pulp was determined using the Somogyi-Nelson assay as described by Nielsen (2010). The pumpkin samples were washed thoroughly with distilled water, then peeled and sliced into small pieces. 100 g of small pieces of the pulp per sample were transferred to the blending motor (ID008760) and then blended. The carbohydrate standards (glucosamine) were prepared with the concentrations 0, 2.5, 5, 7.5, 10, and 15 ml l^{-1}. 1 ml of each standard was added to separate tubes, and to the tube used as blanks 1 ml of distilled water was added. The samples whose concentration was unknown were prepared in appropriate dilution in separate tubes. Then to each tube, 1 ml of the copper reagent was added and mixed very well. The tubes were heated in a boiling water bath for 10 min. The tubes were then removed and allowed to cool to room temperature. 1 ml of arsenomolybdate reagent-Nelson reagent was added to each tube. The content in the tubes was mixed, diluted and remixed. Then the absorbance was measured at 520 nm (UV-spectrophotometer). The standard calibration curve was plotted for absorbance against the concentration of glucose in standard series. The concentration of the glucose in the sample solutions was read from the graph. Then the actual concentration of glucose in the sample was determined by subtracting the concentration of the blank from the determined concentration of glucose in the sample. The analysis per sample was done in triplicate.

Mineral content determination

The mineral cations in the samples were determined by first carrying out complete oxidation of the samples using Kjeldahl procedures then followed by spectrophotometric analysis. Flame photometer used for K and Ca analysis while atomic absorption spectrometer used to analyze iron (Fe) and zinc (Zn). All the procedures followed were extracted from (Okalebo et al., 2002). The analysis per sample was done in triplicate.

Carotenoid content determination

The leutin, α-carotene, trans-β-carotene, cis-β-carotene, total carotenoids and total pro-vitamin A content analysis and quantification was done with High Performance Liquid Chromatograhy (HPLC), following the procedure described in a previous study by Buah et al. (2016). However, instead of using 200 mg of sample, 30 ± 0.1 mg per sample were used since the amount of carotenoids were predicted to be in very high concentrations compared to those in banana tissue basing on the intense colour of the pulps of the samples. The analysis per sample was done in duplicate and on dry basis.

Data analysis

The data was presented as mean ± standard deviation in tables. The proximate, mineral and carotenoid measurements were subjected to one-way analysis of variance (ANOVA) to establish the means with significant differences using IBM SPSS 20.0 software (SPSS, 2011) at 5% level of significance. A p-value < 0.05 was considered significant.

RESULTS AND DISCUSSION

Pumpkin cultivars selected

There is a wide range of pumpkin cultivars grown in Uganda. From the grown accessions obtained from farmers, the consistent cultivars that were used for analysis and their morphological descriptors are as summarized in Table 1. These were Kan 3, Kab 10, Kan 2, Kan 4, Kab 5, Kan 6, Mit 9, Mit 11, Mas 14, Mas 16, Fort 18, Fort 19, Kan 22 and Kab 23. The common names used by farmers to refer to the various accessions are also indicated in Table 1, although one needs to know that the local names vary from one ethnic group to another or from one agro-ecological zone to another.

Pumpkin pulp proximate composition

The chemical analysis of the pulp of the selected accessions for proximate composition, revealed a significant difference ($p< 0.05$) in the glucose, starch, moisture, ash, crude fat, crude fibre and crude protein content (Table 2). Accession Kan2 (Anderina) had the highest glucose concentration of 18.96 mg/ml while Mas14 had the lowest concentration of 4.24 mg/ml. However, the value of glucose content in this study was higher than that reported from the study by Muzzaffar et al. (2016). This difference could be due to the variation in pumpkin cultivars considered in the two studies and also difference in maturity stage at which the analysis was done since the glucose levels increase as the fruit ripens (Sharma and Rao, 2013). Determination of the glucose content of the pumpkin pulp gives an insight to a processor the accessions with relatively high sugar levels. The pumpkins with high sugar levels could be used for making sweet wine since glucose is broken down during fermentation to produce alcohol. However,

Table 1. Samples of pumpkin cultivars considered for nutritional physico-chemical analysis.

Accession (local name)	Picture of mature pumpkin	Morphological descriptor used
Kan22 (Anderina)		1. A flared base of peduncle. 2. Cylindrical shape and a soft epicarp. 3. Fruit skin is mottled with dark green and creamish patches as primary and secondary mature skin colours respectively.
Kan3 (Kihaza/Wujju)		1. Non-flared base of peduncle. 2. Globular shape and soft epicarp. 3. Fruit skin is stripped with a grey primary skin colour but without a secondary skin colour.
Kan4 (Enkogotte/Dulu)		1. A flared base of peduncle. 2. Globular shape and hard epicarp. 3. Fruit skin is mottled with a dark green and whitish patches as primary and secondary skin colours respectively.
Kan2 (Anderina)		1. A flared base of peduncle. 2 .Cylindrical shape and intermediate epicarp. 3. Fruit skin is mottled with dark green and creamish patches as primary and secondary skin colours respectively.
Kan6 (Enkogotte/Dulu)		1. A flared base of peduncle. 2. Pyriform shape and hard epicarp. 3. Fruit skin is mottled with dark green and creamish patches as primary and secondary skin colours respectively.
Kab10 (Ekihaza/Wujju)		1. Non-flared base of peduncle. 2. Globular shape and soft epicarp. 3. Fruit skin is stripped with a grey primary skin colour but without a secondary skin colour.
Kab5 (Rwamabondo/Sweety pumpkin)		1. A flared base of peduncle. 2. Flattened shape and soft epicarp. 3. Fruit skin is uniform with a dark green and yellowish-green as primary and secondary skin colours respectively.

Table 1. Contd.

Kab23 (Mulembe/Dulu)		1. A flared base of peduncle. 2. Cylindrical shape and hard epicarp. 3. Fruit skin is mottled with a dark green and creamish patches as primary and secondary skin colours respectively.
Mas16 (Oziga)		1. A flared base of peduncle. 2. Globular shape and soft epicarp. 3. Fruit skin is mottled with a dark green and creamish patches as primary and secondary skin colours respectively.
Mas14		1. A flared base of peduncle. 2. Globular shape and hard epicarp. 3. Fruit skin is uniform with an orange and yellowish-green as primary and secondary skin colours respectively.
Mit11 (Oziga)		1. A flared base of peduncle. 2. Globular shape and soft epicarp. 3. Fruit skin is mottled with a dark green and creamish patches as primary and secondary skin colours respectively.
Mit 9 (Rwamabondo/Sweety pumpkin)		1. A flared base of peduncle. 2. Flattened shape and soft epicarp. 3. Fruit skin uniform with a dark green and yellowish-green as primary and secondary skin colours respectively.
Fort18 (Anderina)		1. A flared base of peduncle. 2. Pyriform shape and soft epicarp. 3. Skin mottled with a dark green and creamish patches as primary and secondary skin colours respectively
Fort19 (Bala)		1. A flared base of peduncle. 2. Globular shape and intermediate epicarp. 3. Skin color uniform with a dark green primary skin colour without a secondary skin colour.

this suggestion can be affirmed by conducting further research about the amount of phenolics, organics, mineral salts and pectins which are the major constituent of the wine extract and vary from wine to wine.

The starch content was highest in accession Fort19 (0.88%) while Kan4 (0.01%) had the lowest starch content. The relatively low starch content observed across all the varieties could be due to the reduction of starch content in a fruit as it ripens both on and off the vine. The loss of pumpkin starch seems to be highly

Table 2. The proximate composition of fourteen pumpkin accessions; Values are given as means of three replicates ± Standard deviation.

Accession	Glucose (mg/ml)	Starch (%)	Moisture (%)	Ash (%)	Crude fat (%)	Crude fibre (%)	Crude protein (%)
Kan22	9.42±0.40	0.59±0.01	82.34±1.46	**15.21±1.38**	0.53±0.01	60.09±0.31	9.22±0.17
Kan3	6.38 ±0.13	0.53±0.00	88.49±0.28	0.75±0.03	0.55±0.12	22.12±0.10	7.41 ± 0.12
Kan4	14.85±0.10	0.01±0.00	89.61±0.57	1.08±0.01	**1.58±0.09**	40.15±0.03	10.28±0.21
Kan2	**18.96±0.02**	0.40±0.00	81.26±0.07	2.07±0.07	1.55±0.10	31.26±0.01	8.88± .24
Kan6	7.77±0.05	0.05±0.00	92.35±3.57	4.07±0.01	0.61±0.04	20.16±0.01	6.57±0.20
Kab10	4.33±0.03	0.06±0.00	82.54±1.71	1.62±0.01	1.24±0.00	37.77±0.12	7.61±0.24
Kab5	7.69±0.11	0.26±0.00	77.22±1.22	2.55±0.11	0.46±0.01	39.63±0.48	15.54±0.96
Kab23	10.55±0.15	0.48±0.00	80.60±0.36	4.43±0.17	0.63±0.01	70.55±0.06	**16.55±0.37**
Mas16	12.11±0.05	0.85±0.04	87.85±2.39	3.43±0.32	0.66±0.02	38.41±1.23	12.94±0.32
Mas14	4.24±0.14	0.41±0.00	90.22±0.03	1.27±0.00	0.83±0.11	22.26±0.01	11.12±0.35
Mit11	15. 86±0.08	0.50±0.00	79.51±0.94	3.25±0.03	0.54±0.01	36.80±0.07	9.79±0.21
Mit9	8.67±0.10	0.05±0.00	83.59±8.39	6.11±0.15	0.78±0.10	50.95±0.11	9.91±0.22
Fort18	9.63±0.03	0.06±0.00	**90.66±0.67**	2.33±0.15	1.00±0.06	**73.98±0.54**	10.40±0.35
Fort19	5.88±0.04	**0.88±0.00**	82.22±0.67	2.46±0.22	1.20±0.04	52.73±0.23	12.37±0.17
P-value	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Highest values are bolded.

related to fruit climacteric and physiological changes such as increasing ethylene production and respiration rate (Thammawong and Arakawa, 2007).

The moisture content ranged from 77.22% for Kab 5 to 92.35% for Kan 6. The moisture content observed among the accessions in this study was similar to those reported in other studies by Karanja et al. (2014). Drying the pumpkins is one of the stages carried out during value addition process and also a way of reducing losses during surplus production seasons to ensure continuous availability of pumpkin products during the off peak season (Ravani and Joshi, 2014). However, the moisture content of a fruit influences the time taken for it to dry. Therefore, it would be better to use the cultivars such as Kab5 that have a relatively low moisture content for flour production as one of the pumpkin value addition avenues but also rich in other nutritional components as revealed by this study.

On the other hand, the ash content was highest in accession Kan 22 (15.21%) and lowest in Kan 3 (0.75 %). The ash content observed among some of the accessions analyzed in this study was far higher than that reported by some similar studies on pumpkin. The difference could be attributed to the dissimilarity in genetic constitution of the cultivars and soil characteristics in which the pumpkins considered for the studies were cultivated since environment has a significant influence on the inorganic composition of a crop. Furthermore, the difference could also be due to the maturity stage at which the fruits were picked for analysis (Shewfelt, 1990). This study reveals that accessions such as Kan22 could be a rich source of some important minerals for human health since ash content of a food stuff represents the total mineral content in that food.

The crude fat content ranged from 0.46% in Kab5 to 1.58% in Kan4. Generally, the fat content in the pulp across all the accessions was very low. This is because fat is not the primary energy reserve in the pumpkin pulp but rather in the pumpkin seeds (Loy, 2004). It may be important to study the oil content in pumpkin pulp flour since fats/oil are important for gluten development during dough kneading for baking. This would also enable one to establish the status of the oil groups present in pumpkin pulp flour if it is to be used in wheat fortification since according to Figoni (2008), oils in wheat flour easily oxidize and turn rancid which in turn limits the shelf life of the flour.

The crude fibre content ranged from 22.12% in Kan3 to 73.98% in Fort 18. This study revealed that pumpkin cultivars grown in Uganda are a very good source of dietary fibre. Dietary fibre is good for human health since it is believed that they ease digestion and rate of food passage through the alimentary canal. Fiber-rich diet hence reduce the risk of certain alimentary canal cancers, coronary heart diseases (Černiauskienė et al., 2014). Therefore, pumpkin flour could be used to enrich other foods as fibre enhancement.

Crude protein content was recorded highest in accession Kab23 (16.55%) and lowest in Kan3 (7.41%). The protein values from this study were far higher than those reported by Karanja et al., 2014). The difference in results could mainly be attributed to the variation in the cultivars used in the two studies. In comparison, the protein compositions of pumpkin pulp are similar to those of staple food grains, such as maize (9.4%), rice (7.1%), wheat (12.6%), and sorghum (11.6%). In contrast,

Table 3. Mineral composition of the pulp of fourteen pumpkin cultivars; Values are given as means of three replicates ± Standard deviation.

Accession	Calcium (g/100 g)	Potassium (g/100 g)	Iron (mg/100 g)	Zinc (mg/100 g)
Kan22	0.82±0.10	4.02±0.65	10.37±2.27	1.67±0.18
Kan3	0.86±0.07	4.51±0.29	11.45±1.64	**5.17±5.02**
Kan4	0.68±0.15	3.40±0.60	10.09±5.97	2.52±0.20
Kan2	0.62±0.06	3.14±0.37	8.61±3.62	2.12±0.51
Kan6	0.90±0.20	3.87±0.27	8.13±3.18	1.95±0.31
Kab10	0.71±0.18	2.57±0.43	9.94±3.33	2.56±0.96
Kab5	0.95±0.37	4.36±1.08	11.00±2.93	3.26±1.40
Kab23	**1.33±0.52**	**4.81±0.26**	**18.34±7.83**	3.11±1.25
Mas16	0.65±0.24	3.63±1.28	7.52±5.25	2.46±1.34
Mas14	0.74±0.07	3.42±0.59	10.12±3.06	2.56±0.19
Mit11	0.65±0.08	2.86±0.04	8.82±1.94	1.72±0.52
Mit9	0.78±0.11	4.03±0.34	12.93±5.66	2.40±0.46
Fort18	0.81±0.11	4.21±0.65	9.84±2.09	2.22±0.32
Fort19	0.68±0.05	3.93±0.40	9.10±1.46	2.65±0.69
P-value	0.042	0.004	0.263	0.515

Highest values are bolded.

pumpkin pulps' protein content is much higher than other staple root tubers such as irish potato (2 %), cassava (1.4%), white flesh sweet potato (1.6%), and yam (1.5%) (Neela and Fanta, 2019). In addition, according to Hui (2006), the nitrogenated compounds in fruits are generally low as the crude protein ranges from 0.1 to 1.5%, implying fruits are not an adequate source of proteins. However, fruits such as pumpkins, cherimoya and avocado are good sources of protein hence pumpkins may be a good alternative for value addition or bio-fortification to avail more protein. Generally, the significant difference between proximate compositions of different cultivars could be attributed to the genetic diversity within the accessions and the populations of the genus *Cucurbita* as also observed in the studies by Ferriol et al. (2004) and Martins et al. (2015).

Mineral composition of pumpkin pulp

The mineral composition analysis for this study revealed that there was a significant difference in the distribution of the selected macro-elements in the pulp across all the pumpkin accessions ($P<0.05$) while the distribution of the selected micro-elements in the pulp did not differ significantly across the accessions ($P>0.05$) as shown in Table 3. The calcium content ranged from 0.62 g/100 g in Kan2 to 1.33 g/100 g in Kab23. The calcium content of some of the common foods used to prepare baked products and baby weaning recipes are 15 – 25, 6, 4.2, 140, 13 and 6.4 mg/100 g for wheat flour, rice flour, edible mushroom, chicken egg York, butter and potatoes respectively. The calcium content of the pumpkin pulp as per this study was higher than that of the mentioned foodstuffs, therefore, pumpkin pulp can also be used for preparing weaning food, porridge for children and wheat enrichment.

The potassium content was highest in Kab23 (4.81 g/100 g), but lowest in Kab10 (2.57 g/100 g). Potassium is involved in cell membrane transport, muscle building, protein and carbohydrate metabolization. Potassium deficiency is associated with a number of symptoms such as slow reflexes, fatigue, dry skin and muscle weakness (Vahčić et al., 2010). The minimum daily requirement for potassium by the human body is about 782 mg. The results from this study show that the pumpkin pulp could be a good source of potassium. This means that if pumpkin is used to enrich flour, it would increase the potassium content of the flour since the potassium content of wheat flour was only 96.35 mg/100 g according to Finnie and Atwell (2016). Furthermore, foods used to make baby weaning recipes, such as potatoes, rice flour, edible mushrooms, chicken egg yolk, butter contain 418, 103, 341, 138 and 16 mg/100 g respectively of potassium (Belitz et al., 2009). Pumpkin pulp can be a good source of potassium for baby weaning food since it contains a relatively higher amount compared to the foods mentioned above.

The iron content ranged from 7.52 mg/100 g in Mas16 to 18.34 mg/100 g in Kab23. Iron is vital for hemoglobin synthesis and whose deficiency can result into anemia and cognitive impairment. The Recommended Dietary Allowance (RDA) for iron for pre-menopausal women is 18 mg per day while post-menopausal women and men

Table 4. Lutein, α-carotene, *trans*-β-carotene, *cis*-β-carotene, total carotenoid and total pro-vitamin A carotenoid content of pulp of the 14 pumpkin accessions; Values are given as means of three replicates ± Standard deviation.

Accession	Lutein (µg/g dW)	α-carotene (µg/g dW)	*Trans*-β-carotene (µg/g dW)	*Cis*-β-carotene (µg/g dW)	Total carotenoids (µg/g dW)	Total PVA carotenoids (µg/g dW)
Kan 22	230.52±9.02	88.15±2.50	666.38±16.31	0.00±0.00	985.04±27.82	754.53±18.80
Kan 3	1.89±0.28	54.7±7.27	27.00±2.07	10.50±0.74	94.07±10.35	92.19±10.08
Kan 4	32.92±7.01	18.88±3.71	175.74±33.41	0.00±0.00	227.53±44.13	194.61±37.12
Kan 2	156.01±4.96	76.39±3.05	424.87±20.39	0.00±0.00	657.27±28.40	501.26±23.43
Kan 6	44.98±4.63	72.85±8.50	323.33±31.20	0.00±0.00	441.16±43.34	396.18±39.70
Kab10	0.97±0.31	46.5±16.83	61.14±21.31	**19.31±6.85**	127.92±45.30	126.95±45.00
Kab 5	**1157.56±76.48**	665.03±26.13	**1215.49±70.20**	0.00±0.00	**3038.08±172.80**	1880.52±96.32
Kab 23	43.43±7.10	48.83±8.18	129.14±21.04	0.00±0.00	221.42±36.32	177.98±29.22
Mas16	308.15±16.21	326.5±22.96	802.46±53.44	0.00±0.00	1437.12±92.61	1128.96±76.39
Mas14	7.75±0.41	17.97±0.27	98.28±2.58	0.00±0.00	124.00±3.26	116.25±2.84
Mit 11	361.48±10.80	90.79±2.79	544.65±17.97	0.00±0.00	996.93±31.56	635.44±20.75
Mit 9	901.53±23.02	**889.39±8.70**	1212.09±0.30	0.00±0.00	3003.01±14.02	**2101.48±9.00**
Fort 18	213.24±18.10	68.14±6.03	472.76±44.11	0.00±0.00	754.14±68.25	540.90±50.14
Fort 19	483.71±32.11	96.02±12.39	569.05±40.06	0.00±0.00	1148.78±84.56	665.07±52.45
P value	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**

Analysis carried out on dry weight basis. Highest values are bolded.

is 8 mg/day respectively. However, the RDA for iron for expectant mothers is 27 mg/day (Russell et al., 2001). The pumpkin pulp could be a good source of iron to enrich diets that have low iron content. However, the pregnant mothers need to consume more iron rich foodstuffs such as meat, poultry, fish and non-animal sources such as green leafy vegetables, cereals and oilseeds (Gautam et al., 2008) in order to meet their daily iron requirements.

On the other hand, the zinc content was highest in Kan 3 (5.17 mg/100 g) and lowest in Kan22 (1.67 mg/100 g). Zinc is an essential component of a large number of enzymes, and plays a central role in cellular growth and differentiation in tissues that have a rapid differentiation and turnover including those of the immune system and those in the gastrointestinal tract (Allen et al., 2006). The RDAs for zinc are 8 and 11 mg/day for women and men, respectively (Russell et al., 2001). The zinc content was relatively low in pulp. Therefore, other sources such as egg yolk, read meat, milk, legumes, grains and cereals (Harris, 2014) can be consumed to enhance zinc intake to meet the recommended values for proper body functioning. The calcium, potassium, iron and zinc content recorded in this study was higher than that reported by Adubofuor et al. (2016). These differences could be as a result of the variation in the soils in which the pumpkins were grown and other environmental factors such as precipitation. Furthermore, from the studies conducted by Karanja et al., (2014); Adubofuor et al. (2016), the pumpkins were collected from different gardens but yet the pumpkins assessed in this study, were grown in the same garden which could be another source of the differences observed. However, the limitation of this study was that the mineral content of the soil was not assessed after amending the soil with goats' dung. Nonetheless the soil pH was 6.41 and the soil texture was sandy loam. The soil pH 6.4 was just in the recommended range for good pumpkin yields as reported by Kemble et al. (2000).

Carotenoid composition

There was a significant variation in the lutein content, α-carotene content, *trans*-β-carotene content, *cis*-β-carotene content, total carotenoid content and total pro-vitamin A carotenoid content of pulp across the different pumpkin accessions analyzed ($p < 0.05$) as shown in Table 4. The lutein content ranged from 0.97 µg/g dW in Kab10 to 1157.76 µg/g dW in Kab5. The α-carotene content was highest in Mit9 (889.39 µg/g dW) and lowest in Mas14 (17.97 µg/g dW). The *trans*-β-carotene content was highest in Kab5 (1215.49 µg/g dW) and lowest in Kan3 (27.00 µg/g dW). *Cis*-β-carotene content was detected in only two accession Kab10 (19.31 µg/g dW) and Kan3 (10.50 µg/g dW). The total carotenoids were highest in Kab5 (3038.08 µg/g dW) and lowest in Kan3 (94.07 µg/g dW). The total pro-vitamin A carotenoid content was highest in Kab5 (1880.52 µg/gdW) and lowest in Kan3 (92.19 µg/g dW). The α-carotene, *trans*-β-

carotene, and the total carotenoid content observed in this study, for some accessions such as Kab5, Mit9, Mas16, Mit11, Fort19, Kan22 was higher than that for the cultivars studied by Maria et al. (2012), but the *cis*-β-carotene content was very low just like what was observed in this study. The difference in the composition could mainly be due to the genetic diversity among the *Cucurbita* species and the environmental conditions under which the pumpkins were grown. The α-carotene, *trans*-β-carotene, and the total carotenoid content observed in the pumpkin is by far higher than that reported in fruits like banana (Mbabazi et al., 2020). Therefore, considering that *trans*-β-carotene has 100% pro-vitamin A activity (Carvalho et al., 2014), accessions like Kab5, Kan22, Mit9, Mas16, Mit11 and Fort19 are promising sources of vitamin A. Pro-vitamin A carotenoid availability is of particular importance in developing countries where vitamin A deficiency (VAD) is a significant public health concern. The main underlying cause of VAD in low-income countries is a poor diet that is consistently insufficient in vitamin A, eventually leading to depleted stores that fail to achieve physiological needs in the human body. Persistent and severe deficiency can lead to xerophthalmia, a form of preventable, but irreversible, blindness in young children, and facilitates infectious diseases such as measles, diarrhea, and intestinal parasites, which increase infant mortality risks (Chen, 2015; NIH, 2016). Kab 5 and Mit 9 accessions can be given first priority to be used for bio-fortification of foodstuffs commonly consumed by people especially children but yet have low quantities of carotenoids such as lutein, β-carotene and α-carotene. These accessions can also be used in the preparation of baby foods and immune-depressed patients such as those with Acquired Immune Deficiency Syndrome (AIDS). The limitation of this study was that the colour of the pulp of the analyzed accessions was not scientifically assessed yet it can help one to have a rough estimate of the type and concentration of the total carotenoids present in a given cultivar. In addition, genetic finger printing was not performed to conclude if some of the accessions included in this study were not genotypically the same though their seeds had been picked from different agro-ecological zones.

Conclusion

The different pumpkin accessions analyzed can be used for various value addition avenues for example, Kan2, Kan4 and Mas16 can be used for production of pumpkin pulp juice and also pumpkin pulp wine because they contain a high moisture and glucose content. Accessions Kan22, Kab23 and Fort18 can be processed to produce dietary fibre supplements since this study revealed that they contain a very high crude fibre content. On the other hand, accessions Kab5, Mit11 and Mit9 can be used for production of pumpkin pulp flour for wheat fortification and preparation of baby foods such as porridge since they have relatively lower moisture content yet rich in mineral content, lutein, *trans*-β-carotene and α-carotene. However, the pumpkin pulp generally has a very high moisture content thus, the processors should dry the pumpkin pulp with appropriate methods to prevent microbial and aflatoxin attack to the produce but yet not destroying the other nutraceutical components of the pumpkin fruit during processing. In general pumpkin pulp is recommended for consumption by people of all ages to meet their daily nutritional and nutraceutical needs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Consumer acceptability and nutrient content of Westwood (*Cirina forda*) larva-enriched *Amaranthus hybridus* vegetable soups

Adepoju Oladejo Thomas* and Daboh Oladele Olatunji

Department of Human Nutrition and Dietetics, Faculty of Public Health, College of Medicine, University of Ibadan, Ibadan, Oyo state Nigeria.

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Insects account for the greatest amount of biodiversity in forests with over 1,400 species reportedly eaten as human food, but are the least studied of all fauna. Studies have shown that they may contribute significantly to livelihoods in both rural and urban areas. This study was carried out to assess the consumer acceptability and nutrient content of *Cirina forda* larva-enriched vegetable soups. Dry *C. forda* (CF) larva was purchased from a market in Burkina Faso and refrigerated at -4°C. Four vegetable soup samples (plain vegetable soup; *Egusi* soup; Vegetable soup+CF; and *Egusi* soup+CF) were prepared traditionally. Dry CF larva and the four vegetable samples were analysed using standard AOAC methods, while acceptability of the soup samples was carried out using 9-point Hedonic scale. Moisture content of CF was 3.98 g while that of soups ranged from 59.78 to 77.14 g /100 g. *C. forda* larva contained 54.38 g protein and 16.81 g fat which were rich in essential amino acids and unsaturated fatty acids respectively; and high in macro-minerals. Nutrient content of vegetable soups enriched with CF larva were significantly higher ($p<0.05$), and more acceptable than un-enriched ones; with *Egusi* soup+CF larva being the most acceptable. *C. forda* larva is rich in both macro and micronutrients and generally acceptable to consumers. *C. forda* larva consumption should be popularized as means of improving dietary diversity, nutrient intake and overall health of humans.

Key words: *Cirina forda* larva, edible insects, enriched vegetable soups, consumer acceptability, nutrient content.

INTRODUCTION

Protein-energy malnutrition is still an important public health issue in the developing countries of the world, especially in Africa, with its attendant problem of morbidity, mortality, stunted growth, and impaired neurobehavioral development in children (Iombor et al., 2017). The common sources of animal protein such as meat and eggs are becoming more and more costly and out of reach of the common man (Headey et al., 2018;

*Corresponding author. E-mail: tadepoju@com.ui.edu.ng.

Ebenebe et al., 2020), hence, alternative cheap sources of protein of high biological value need to be explored in order to meet human body protein requirements. Insects remain a vital and preferred food and essential source of protein, fat, minerals and vitamins in many developing countries and various cultures throughout the world (Durst and Shono, 2010).

Research studies have documented that some edible insects have nutritional value that is comparable with that of meat and fish (Braide et al., 2010); and are often a welcome source of protein in the absence of meat from vertebrates (Sponheimer et al., 2005). Edible insects are important dietary components in many cultures where they contribute significantly to protein, fats, and micronutrient intake of consumers (Akinnawo and Ketiku, 2000; Anvo et al., 2016); and are not used as emergency food to ward off starvation, but included as normal part of the diet whenever available (Adeoye et al., 2014).

Insects have played important part in the history of human nutrition in Africa, Australia, Asia and the Americas (Jongema, 2015; Dossey et al., 2016; Koko and Mariod, 2020).

Several studies have shown that edible insects contain appreciable amount of proteins of good quality and high digestibility, with beneficial amino acid and fatty acid profile comparable to conventional livestock and fish (Braide et al., 2010; Igwe et al., 2011; Iombor et al., 2017). In some African countries such as Malawi, Zambia and Tanzania, malnutrition in children have been fought through flour made out of dried caterpillars. Pregnant and nursing women as well as anaemic patients also eat caterpillar species high in protein, calcium and iron (Igbabul et al., 2014), hence, the potential of insects needs to be considered in malnutrition alleviation strategies in Nigeria.

Insects of nutritional importance include (but not limited to) grasshoppers, caterpillars, beetle grubs and (sometimes) adults, winged termites, *Cirina forda* larva, and a variety of aquatic insects (Adeoye et al., 2014). Among the edible insect species, *C. forda* (Westwood) larva has been reported to have the potential to provide substantial amount of protein, minerals and polyunsaturated fatty acids to the diets which are usually deficient in animal protein (Akinnawo and Ketiku, 2000; Adepoju and Daboh, 2013). The larva of this insect is widely consumed in the Northern, Central and South-Western parts of Nigeria (Ogunleye, 2006). The insect larva is processed into dry form and consumed as a delicacy served as snack or as an essential ingredient in vegetable soups along with carbohydrate food in Southern part of Nigeria and many homes in Africa (Omotoso, 2006). Animal sourced foods (ASFs) are often costly and remain out of reach for many low-income households (Headey et al., 2018). Due to changes in environmental factors, dietary changes are urgently required (Springmann et al., 2018); and since insects form a part of traditional food system with high levels of energy, quality protein with good amino and fatty acid profile, and variety of essential minerals (Igwe et al., 2011), they should be considered as important food source.

Our previous study on consumption pattern of *C. forda* larva as important source of nutrients confirmed the findings of Omotoso (2006), and revealed that the most preferred mode of consumption of the insect larva is as an additive to vegetable soups (Daboh and Adepoju, 2020). This study was therefore carried out to assess the consumer acceptability and nutrient content of *C. forda* larva-enriched vegetable soups.

MATERIALS AND METHODS

Sample collection and preparation

Dry *C. forda* (CF) larva sample was purchased from a local market in Burkina Faso and kept under refrigeration at –4°C. Fresh *Amaranthus hybridus* vegetable, '*Egusi*' (melon seed), pepper, palm oil, onion, maggi cubes and salt used for the study were purchased from Bodija market in Ibadan, Nigeria.

A portion of the dry *C. forda* larvae was ground and labelled as Sample A.

Soup preparation

The vegetable soups were prepared traditionally (by engaging the service of a local food vendor) at the Dietetic kitchen of the Department of Human Nutrition and Dietetics, University of Ibadan, Ibadan, Nigeria. The vegetable leaves were rinsed with distilled water to eliminate soil and pebbles and then sliced. A total of 350 g *C. forda* larvae was weighed, rinsed and soaked in hot water to soften a little and was divided into two portions of 175 g each.

Sample B: Efo riro soup

About 250 ml of palm oil was added to the cooking pot placed on a heater to warm. Ground pepper (10 g) and onions (4 g) were added and fried for 5 min, followed by addition of 4.5 g of salt and two bouillon cubes. Water (250 ml) was then added, stirred together and allowed to simmer for 20 min. Then, 25 g of sliced vegetable leaf was added and allowed to cook for 5 min (Adepoju and Ugochukwu, 2019).

Sample C: Efo riro + C. forda larvae enriched soup

About 250 ml of palm oil was added to the cooking pot placed on a heater to warm. Ground pepper (10 g) and onions (4 g) were added and fried for 5 min, followed by addition of 4.5 g of salt, two bouillon cubes, and 175 g of *C. forda* larvae. Water (250 ml) was then added, stirred together and allowed to simmer for 20 min. Then, 25 g of sliced vegetable leaf was added and allowed to cook for 5 min.

Sample D: Egusi soup

About 250 ml of palm oil was added to the cooking pot placed on a heater to warm. Ground pepper (10 g) and onions (4 g) were added and fried for 5 min, followed by addition of 4.5 g of salt and two

bouillon cubes. Water (250 ml) was added, stirred together and allowed to simmer for 20 min. Then, 15 g of ground *Egusi* and 25 g of sliced vegetable leaf were added and allowed to cook for 5 min.

Sample E: Egusi + C. forda larvae enriched soup

About 250 ml of palm oil was added to the cooking pot and allowed to warm on a gas cooker. Ground pepper (10 g) and onions (4 g) were added and fried for 5 min, followed by addition of 4.5 g of salt, two bouillon cubes, and 175 g of *C. forda* larvae. Water (250 ml) was then added, stirred together and allowed to simmer for 20 min. Then, 15 g of ground *Egusi* and 25 g of sliced vegetable leaf were added and allowed to cook for 5 min.

Chemical analyses

The dry CF larva sample and the four soups were analysed for their nutrient content using the standard methods of Association of Official Analytical Chemists (AOAC, 2005).

Proximate composition determination

The moisture content of the samples was determined by hot-air oven method (Plus 11 Sanyo Gallenkamp PLC, UK) at 105°C for 4 h. The crude protein was determined using micro-Kjeldahl method (Method No. 978.04); crude lipid was determined by Soxhlet extraction method (Method No. 930.09). The ash content was determined through incineration in muffle furnace at 550°C for 4 h (Method No. 930.05). Total carbohydrate content was obtained by difference. Gross energy of the samples was determined using ballistic bomb calorimeter.

Mineral content determination

Potassium and sodium content of the samples were determined by digesting the ash of the samples with perchloric and nitric acids, and then taking the readings on Jenway digital flame photometer/spectronic20 (AOAC, 2005: [975.11]). Phosphorus was determined by vanado-molybdate colorimetric method (AOAC, 2005: [975.16]). Calcium, magnesium, iron, zinc, manganese, and copper were determined by atomic absorption spectrophotometric method (Buck Scientific, Norwalk, UK) and compared with absorption of standards of these minerals (AOAC, 2005: [975.23].

Vitamin content determination

Vitamin A determination

Vitamin A was determined through ultraviolet absorption measurement at 328 nm after extraction with chloroform (AOAC Method 960.5 & 974.29, 2005). Calibration curve of vitamin A acetate was made and sample vitamin A concentration estimated as microgram (µg) of vitamin A acetate.

Thiamine (vitamin B_1) determination

Thiamine content of the samples was determined by weighing 1 g of sample into 100 ml volumetric flask with addition of 50 ml of 0.1 M H_2SO_4 and boiled in a boiling water bath with frequent shaking for 30 min. Five milliliter (5 ml) of 2.5 M sodium acetate solution was added and flask set in cold water to cool contents below 50°C. The flask was stoppered and kept at 45-50°C for 2 h and thereafter made up to 100 ml. The mixture was filtered through a No. 42 Whatman filter paper, discarding the first 10 ml. Ten milliliters (10 ml) was pipetted from remaining filtrate into a 50 ml volumetric flask, and 5 ml of acid potassium chloride solution was added with thorough shaking. Standard thiamine solutions were prepared and treated same way. The absorbance of the samples as well as that of standards was read on a fluorescent UV Spectrophotometer (Cecil A20 Model, USA) at a wavelength of 285 nm.

Riboflavin (vitamin B_2) determination

One gram (1 g) of each sample was weighed into a 250 ml volumetric flask; 5 ml of 1 M HCl was added, followed by the addition of 5 ml of dichloroethene. The mixture was shaken and 90 ml of de-ionized water was added. The whole mixture was thoroughly shaken and was heated on a steam bath for 30 min to extract all the riboflavin. The mixture was then cooled and made up to volume with de-ionized water. It was then filtered, discarding the first 20 ml of the aliquot. Two milliliters (2 ml) of the filtrate obtained was pipetted into another 250 ml volumetric flask and made up to mark with de-ionized water. Samples were read on the fluorescent spectrophotometer at 460 nm. Standard solutions of riboflavin were prepared and readings taken at 460 nm. The sample riboflavin was obtained through calculation.

Niacin (vitamin B_3) determination

Five grams (5 g) of sample was extracted with 100 ml of distilled water and 5 ml of this solution was drawn into 100 ml volumetric flask and made up to the mark with distilled water. Standard solutions of niacin were prepared and absorbance of sample and standard solutions was measured at of 385 nm on a spectrophotometer, and niacin concentration of the sample estimated.

Pyridoxine (vitamin B_6) determination

The vitamin B_6 content of the samples was determined by extracting 1 g of sample with 0.5 g of ammonium chloride, 45 ml of chloroform and 5 ml of absolute ethanol. The mixture was thoroughly mixed in a separating funnel by shaking for 30 min, and 5 ml of distilled water added. The chloroform layer containing the pyridoxine was filtered into a 100 ml volumetric flask and made up to the mark with chloroform. Standard solutions of 0-10 ppm of vitamin B_6 were prepared and treated in a similar way as samples; and their absorbance measured on Cecil 505E spectrophotometer at 415 nm. The amount of vitamin B_6 in the sample was then calculated.

Cyanocobalamin (vitamin B_{12}) determination

Cyanocobalamin content of the samples was determined by extracting 1 g of sample with distilled water with shaking for 45 min, followed by filtering the mixture. The first 20 ml of the filtrate was rejected, and another 20 ml filtrate collected. To the collected filtrate, 5 ml of 1% sodium dithionite solution was added. Standard cyanocobalamin solutions (0-10 µg/ml) were prepared, and absorbance of sample as well as standards was read on spectronic21D spectrophotometer at 445 nm. Amount of sample

Table 1. Proximate composition of *C. forda* and vegetable soup samples (g*/*100g as consumed)*.

Parameter	Sample A	Sample B	Sample C	Sample D	Sample E
Moisture	3.98±0.03^{a}	77.14±0.02^{b}	68.90±0.05^{c}	73.04±0.03^{d}	59.78±0.03^{e}
Crude Protein	54.38±0.10^{a}	12.11±0.20^{b}	18.68±0.10 ^{c}	14.88±0.10^{d}	23.68±0.10^{e}
Crude Fat	16.81±0.03^{a}	2.45±0.02^{b}	3.26±0.02^{c}	2.45±0.03^{b}	3.79±0.04^{d}
Ash	1.78±0.03^{e}	1.35±0.03^{d}	1.46±0.02^{c}	1.42±0.03^{b}	1.61±0.03^{a}
Dietary Fibre	8.86±0.02^{a}	11.62±0.03^{b}	14.57±0.34^{c}	12.89±0.03^{d}	15.81±0.02^{e}
Carbohydrate	22.50±0.05^{a}	4.53±0.22^{b}	5.51±0.14^{c}	5.64±0.16^{d}	8.79±0.09^{e}
Gross Energy (kcal/)	492.05±0.35^{a}	132.89±2.52^{b}	173.74±3.00^{c}	153.05±1.52^{d}	216.23±3.51^{e}

Values with the same superscript along the same row are not significantly different (p>0.05). Sample B, Vegetable soup; Sample C, Vegetable soup + *C. forda* larva; Sample D, Vegetable + Egusi soup; Sample E, Vegetable + Egusi + *C. forda* larva soup.

cyanocobalamin was then estimated through calculation.

Ascorbic acid determination

Ascorbic acid in the samples was determined by titrating the aqueous extract of each sample with solution of 2, 6 – dichlorophenol-indophenol dye to a faint pink end point.

Anti-nutrients determination

Oxalate content of the samples was determined by extraction of the samples with water for about 3 h and standard solutions of oxalic acid prepared and read on spectrophotometer (Spectronic20) at 420 nm, and amount of oxalate estimated.

Phytate was determined by titration with ferric chloride solution (Sudarmadji and Markakis, 1977), while trypsin inhibitory activity was determined on casein and comparing the absorbance with that of trypsin standard solutions read at 280 nm (Makkar and Becker, 1996). The tannin content of the samples was determined by extracting the samples with a mixture of acetone and acetic acid for 5 h, measuring their absorbance and comparing the absorbance of the sample extracts with the absorbance of standard solutions of tannic acid at 500 nm on spectronic20 (Griffiths and Jones, 1977). Saponin was also determined by comparing the absorbance of the sample extracts with that of the standard at 380 nm (Makkar and Becker, 1996). All determinations were carried out in triplicate.

Sensory evaluation of vegetable soups

The vegetable soups acceptability study was carried out at the Department of Human Nutrition and Dietetics sensory evaluation laboratory, University of Ibadan, Nigeria. The soup samples were assessed for their acceptability using 30 untrained panelists drawn within the University community, who had eaten or known *C. forda* larva before and were willing to participate. The samples were rated on a 9-point hedonic scale in which the degree to which a product is relished was expressed as: like extremely (9), like very much (8), like moderately (7), like slightly (6), neither like nor dislike (5), dislike slightly (4), dislike moderately (3), dislike very much (2), dislike extremely (1). The panelists were required to observe the sample, taste and score based on colour, taste, aroma, texture and overall acceptability. The panelists were provided with water to rinse their mouths in between sample evaluation, and were instructed to rinse their mouth with water before tasting another sample.

Statistical analysis

The data obtained from the chemical analyses were expressed as mean and standard deviation of triplicate determinations and subjected to independent t-test using Statistical Package for Social Sciences (SPSS) version 20 (SPSS Inc., USA); and the data obtained from sensory evaluation presented as means of 30 panelists' assessment which was analysed using one – way ANOVA. The level of significance was set at $p < 0.05$.

RESULTS

Proximate composition of *C. forda* and vegetable soups

The proximate composition of *C. forda* larva and the four vegetable samples is shown in Table 1. The dry *C. forda* sample (sample A) was high in crude protein (54%) and gross energy content (492.05 Kcal), high in fat, ash, and dietary fibre, moderate in carbohydrate, but low in moisture content (3.98%).

Vegetable soup (*Efo riro*) (sample B) was high in moisture (77.14%) and ash (1.35%) contents, high in dietary fibre (11.62%), moderate in crude protein (12.4%), but low in carbohydrate content (4.53%). Addition of ground melon seed (*Egusi*) to the vegetable soup (sample D) lowered the moisture content (73.04%) and increased the crude protein (14.88%), dietary fibre (12.89%), ash (1.42%), carbohydrate (5.364%), and gross energy content (153.05 Kcal.) of the vegetable soup significantly ($p<0.05$). Likewise, addition of *C. forda* larva to the vegetable soup (sample B) and Egusi soup (sample D) led to significant increase in most parameters with significant reduction in the moisture content of enriched vegetable soup samples (samples C and E) ($p<0.05$).

Mineral content of *C. forda* and vegetable soups

The *C. forda* larva contained substantial amount of sodium, potassium, calcium, magnesium and phosphorus, with potassium being the most abundant, followed by

Table 2. Mineral content of *C. forda* and vegetable soup samples (mg/100g as consumed)*.

Parameter	Sample A	Sample B	Sample C	Sample D	Sample E
Sodium	290.00±2.00^a	166.67±2.52^b	195.33±3.01^c	182.0±3.61^d	220.67±2.52^e
Potassium	623.67±3.06^a	195.33±3.06^b	309.67±2.08^c	208.67±2.51^d	327.67±3.06^e
Calcium	262.0±3.00^a	260.67±2.52^a	261.33±3.06^b	325.0±3.00^c	330.67±3.06^d
Magnesium	278.67±3.51^a	359.33±2.52^b	340.67±2.52^c	336.67±2.52^d	323.67±3.06^e
Phosphorus	146.67±1.53^a	286.67±2.52^b	261.0±3.61^c	301.33±3.06^d	278.67±3.05^e
Iron	8.94±0.02^a	2.80±0.01^b	3.36±0.01^c	3.13±0.01^d	3.96±0.01^e
Zinc	1.39±0.03^a	0.27±0.01^b	0.43±0.00^c	0.28±0.00^b	0.46±0.00^c
Copper	0.33±0.02^a	0.13±0.00^b	0.17±0.01^c	0.15±0.01^c	0.19±0.00^d
Manganese	0.001±0.00^a	0.001±0.00^a	0.001±0.00^a	0.001±0.00^a	0.001±0.00^a

*Values are mean ± standard deviation of three determinations. Values with the same superscript along the same row are not significantly different (p>0.05).

Table 3. Vitamin content of *C. forda* and vegetable soup samples (mg/100g as consumed)*.

Parameter	Sample A	Sample B	Sample C	Sample D	Sample E
Vitamin A (µg/)	0.33±0.02^a	0.87±0.02^b	1.25±0.02^c	1.07±0.03^d	1.39±0.03^e
Vitamin B_1	0.19±0.02^a	0.04±0.01^b	0.13±0.02^c	0.11±0.03^d	0.19±0.04^a
Vitamin B_2	0.37±0.01^a	0.09±0.02^b	0.15±0.01^c	0.14±0.02^c	0.21±0.02^d
Vitamin B_3	0.62±0.03^a	0.71±0.02^b	0.91±0.03^c	0.91±0.03^c	1.08±0.03^d
Vitamin B_6	0.46±0.02^a	2.11±0.03^b	2.29±0.03^c	2.26±0.02^c	2.45±0.03^d
Vitamin B_{12} (µg/)	3.53±0.02^a	17.01±0.03^b	13.52±0.04^c	14.57±0.05^d	11.94±0.03^e
Vitamin C	19.24±0.03^a	15.23±0.03^b	28.75±0.02^c	18.62±0.04^d	31.81±0.03^e
Vitamin E (µg/)	1.89±0.03^a	3.01±0.04^b	4.66±0.41^c	3.79±0.05^d	5.27±0.02^e

*Values are mean ± standard deviation of three determinations. Values with the same superscript along the same row are not significantly different (p>0.05).

sodium, magnesium, calcium and phosphorus. It is also high in iron content compared with other animal sources. However, the larva was low in zinc (1.39 mg $100g^{-1}$) and copper (0.33 mg $100g^{-1}$), and low in manganese (0.001 $mg100g^{-1}$) (sample A) (Table 2). Addition of *Egusi* to vegetable soup (sample B) significantly increased its mineral content (sample D), (p<0.05). Addition of CF larva to the vegetable and *Egusi* soups (samples B and D) increased the sodium, potassium, iron zinc and copper significantly (p<0.05), while it decreased the calcium, magnesium and phosphorus content significantly (p<0.05).

The vegetable soup (sample B) was rich in magnesium, phosphorus, and calcium, and high in potassium and sodium content. Addition of *Egusi* to the vegetable (sample D) increased the nutrient content of the product significantly. Also, addition of the insect larva to both vegetable (sample B) and *Egusi* (sample D) soups significantly increased the sodium, potassium, iron, zinc and copper of the larva-enriched vegetable soups (Samples C and E) (p<0.05).

In Table 3, the vitamin composition of *C. forda* larva revealed that it contains substantial amounts of vitamins, especially vitamins B_{12} and C. The larva was low in water soluble vitamins (sample A). The vegetable soup (sample B) was low in vitamins A (0.87), B_1 (0.04), B_2 (0.09), and B_3 (0.71 mg 100 g^{-1}), but high in vitamins B_6 (2.11), B_{12} (17.01), C (15.73) and E (3.01 mg 100 g^{-1}). Addition of ground melon to the vegetable soup significantly improved all the vitamin content of the product (sample D) except vitamin B_{12} (p<0.05). Enriching both vegetable and *Egusi* soups (samples B and D) with CF significantly increased the vitamin content of the products (p<0.05) except vitamin B_{12} (samples C and E).

Anti-nutrients level of *C. forda* larva and vegetable soups

The phytate and trypsin inhibitor content of *C. forda* larva were negligible, while oxalate, tannin, and saponin were not detectable (Table 4). However, there was a significant difference (p<0.05) in the trypsin inhibitor content of the samples (p<0.05), with the larva – enriched vegetable

Table 4. Anti-nutrient content of *C. forda* and vegetable soup samples (mg/100g as consumed)*.

Parameter	Sample A	Sample B	Sample C	Sample D	Sample E
Phytate	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]
Oxalate	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.01±0.00[a]	0.00±0.00[a]
Tannin	0.00±0.00[a]	0.01± 0.00[a]	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]
Saponin	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.01±0.00[a]	0.00±0.00[a]
Trypsin Inhibitor	0.70±0.03[e]	0.91±0.03[d]	1.30±0.02[c]	1.08±0.03[b]	1.45±0.02[a]

*Values are mean ± standard deviation of three determinations. Values with the same superscript along the same row are not significantly different (p>0.05).

Table 5. Amino Acid profile of *Cirina forda* larva.

Amino acids	Content (%)
Alanine	3.72
Arginine	2.85
Aspartic acid	7.65
Cysteine	1.87
Glutamic acid	13.33
Glycine	5.77
Histidine	3.54
Isoleucine	5.37
Leucine	5.77
Lysine	19.96
Methionine	2.19
Phenylalanine	4.47
Proline	3.36
Threonine	8.09
Tryptophan	1.64
Tyrosine	5.10
Cystine	0.67
Serine	0.24
Valine	2.56

samples having higher values. Enriched vegetable with *Egusi* had the highest value of trypsin inhibitors. Addition of *C. forda* larva to enrich the vegetable soups did not increase their antinutrient content, as their values were negligible except for trypsin inhibitor. The level of trypsin inhibitors in the enriched vegetable soups (samples C and E) was very low, and cannot cause any significant reduction in the bioavailability of protein in the soups.

Amino acid and fatty acid profiles of *C. forda* larva

The CF larva has good amount of essential amino acids such as lysine, threonine, phenylalanine, leucine, isoleucine, valine, tryptophan and cysteine as well as dispensable amino acids such as glutamic acid, histidine, arginine, aspartic acid and glycine among others, hence, its protein content is of high quality (Table 5).

The *C. forda* larva is rich in linolenic acid (poly-unsaturated), and high in oleic acid (monounsaturated), stearic and palmitic acids among others (Table 6). Linolenic, stearic, palmitic, and oleic acids were very much abundant in fat of the larva. Unsaturated fatty acids constitute 52.27% of the total fatty acids in the larva and 12.15% of this was monounsaturated fatty acid (MUFA).

Sensory evaluation of vegetable soups

In Table 7a, all the soups were acceptable to the panelists, as none of them was rejected or scored below average. Overall, the control vegetable soup (sample B)

Table 6. Fatty acid profile of *Cirina forda* larva.

Fatty Acids	Content (%)
Arachidonic	0.33
Lauric	3.38
Linoleic	7.07
Linolenic	33.06
Margaric	0.03
Myristic	0.63
Oleic	11.35
Palmitic	16.38
Palmitoleic	0.43
Stearic	27.16

Table 7a. Sensory characteristics of vegetable soup samples.

Parameter	Sample B	Sample C	Sample D	Sample E
Taste	6.40±2.53^{a}	8.03±1.07^{b}	7.70±1.32^{b}	8.60±0.67^{b}
Colour	6.13±2.73^{a}	7.77±1.43^{b}	7.43±1.57^{b}	8.13±0.87^{b}
Aroma	6.03±2.63^{a}	7.73±1.51^{b}	7.40±1.50^{b}	8.13±0.82^{b}
Texture	6.33±2.45^{a}	7.60±1.19^{b}	7.17±1.62^{b}	7.47±1.46^{b}
Overall Acceptability	6.13±2.78^{a}	7.87±1.07^{b}	7.67±1.40^{b}	8.20±1.24^{b}

*Values are mean ± standard deviation of 30 panelists (n = 30). Values with the same superscript along the same row are not significantly different (p>0.05). Sample B, Vegetable soup; Sample C, Vegetable soup + *C. forda* larva; Sample D, Vegetable + Egusi soup; Sample E, Vegetable + Egusi + *C. forda* larva soup.

was the least accepted for colour, taste, texture, aroma and overall acceptability. The two *C. forda* larva-enriched vegetable soups (samples C and E) were more relished compared with the plain vegetable soup (sample B) and vegetable + *Egusi* soup (sample D). The vegetable + *Egusi* + CF larva soup (sample E) was the most acceptable for colour, taste, aroma and general acceptability; while vegetable soup + CF larva (sample C) was the most accepted for texture.

Significant differences existed between plain vegetable soup (sample B) and vegetable soup enriched with CF larva (sample C) in all the parameters assessed, the CF larva-enriched vegetable soup scoring higher ($p<0.05$) (Table 7b). *Egusi* soup enriched with CF larva soup (sample E) significantly scored higher than *Egusi* soup (sample D) in taste, colour and aroma, ($p<0.05$); while there was no significant difference in their texture and overall acceptability ($p>0.05$). There was no significant difference in overall acceptability of CF larva – enriched vegetable soups and *Egusi* vegetable soup (samples C, D, and E) ($p>0.05$). The *Egusi* soup enriched with CF larva (sample E) scored highest in all parameters assessed.

DISCUSSION

Proximate composition of *C. forda* and vegetable soups

In Table 1, the dry *C. forda* (CF) larva was low in moisture content (3.98%). The low moisture content obtained for the CF larva is in line with the reports of Banjo et al. (2006) and Osasona and Olaofe (2010). The low moisture content is suggestive of its high keeping quality (that is long shelf life), and supportive of the assertion of respondents in our consumption survey carried out earlier that the insect larva can be kept for a long period of time (Daboh and Adepoju, 2020). The low moisture content will prevent microbial contamination.

The insect larva contained high amount of protein. The value obtained for the larva protein falls within the range of values reported by Igbabul et al. (2014), similar to 55.41% obtained by Yapo et al. (2017) but lower than 63% reported by Anvo et al. (2016) for *Cirina butyrospermi*. The variation observed in the biochemical composition of the insect larva may be due to the host tree because the amount of protein varies between insect

Table 7b. Statistical sensory evaluation of the soup samples.

Parameter	Sample B	Sample C	Sig.	Sample D	Sample E	Sig.
Taste	6.40±2.53[a]	8.03±1.07[b]	**0.002**	7.70±1.32[b]	8.60±0.67[b]	**0.02**
Colour	6.13±2.73[a]	7.77±1.43[b]	**0.005**	7.43±1.57[b]	8.13±0.87[b]	**0.03**
Aroma	6.03±2.63[a]	7.73±1.51[b]	**0.003**	7.40±1.50[b]	8.13±0.82[b]	**0.02**
Texture	6.33±2.45[a]	7.60±1.19[b]	**0.014**	7.17±1.62[b]	7.47±1.46[b]	**0.45**
Acceptability	6.13±2.78[a]	7.87±1.07[b]	**0.002**	7.67±1.40[b]	8.20±1.24[b]	**0.12**

*Values are mean ± standard deviation of 30 panelists (n = 30). Values with the same superscript along the same row are not significantly different (p>0.05). Sample B, Vegetable soup; Sample C, Vegetable soup + *C. forda* larva; Sample D, Vegetable + Egusi soup; Sample E, Vegetable + Egusi + *C. forda* larva soup.

species and within the same species depending on the nutritional quality of the leaves of the host tree (Banjo et al., 2006; Yapo et al., 2017), the difference in the geographical location where the CF larva was obtained, as well as method of harvesting/processing of the insect larva (Ekpo, 2011; Womeni et al., 2012; Adepoju, 2013).

The crude fat content of the insect larva obtained in this study is within the range of values quoted for insects (10 – 30%) (Durst et al., 2010). The value is higher than the values reported by Osasona and Olaofe (2010) and Igbabul et al. (2014) and 15% reported by Anvo et al. (2016), but lower than the 22.21% reported by Ogunleye (2006). The difference in the crude fat content of *C. forda* in this study compared with other studies could be due to the difference in the geographical location where the samples were obtained and also, the method of processing (Ekpo, 2011; Womeni et al., 2012; Adepoju, 2013). The moderate fat content of the *C. forda* larva is beneficial because it may reduce its rate of susceptibility to rancidity.

However, the *C. forda* larva contained a moderate amount of dietary fibre. Dietary fibre helps to regulate the digestive system, aid bowel health and weight management (Rolfe et al., 2009). The value of ash of *C. forda* larva sample was less than the range of values (2.91 – 3.97%) reported by Igbabul et al. (2014) but comparable to that of Akinnawo and Ketiku (2000), Omotoso (2006) and Osasona and Olaofe (2010). The carbohydrate content of the *C. forda* larva was higher than the range reported by Igbabul et al. (2014). The gross energy content of *C. forda* sample was very high. This may be due to the fact that at larval stage, the insect feeds much to derive and store the nutrient and energy needed for growth and development during the pupal stage to the adult stage (Chen et al., 2009). This finding is in line with the report of FAO (2004) that reported high energy content in feeding caterpillar flours. The high energy content could also be as a result of its high protein, fat and carbohydrate content. The gross energy content in this study is very similar to those of 492.31 kcal. reported by Yapo et al. (2017) for *Cirina butyrospermi*.

The moisture content of vegetable soup was very high (sample B). The vegetable soup was moderately high in protein content compared with other plant protein (Adepoju and Ugochukwu, 2019). This amount of protein is believed to have been contributed partly by other ingredients used in the preparation of the vegetable soup. The soup was low in fat and carbohydrate content despite the addition of palm oil. Leafy vegetables are generally poor source of lipids and carbohydrates (Adepoju and Ugochukwu, 2019). However, the soup had moderate value of ash and gross energy, and high in dietary fibre. Vegetables are generally good source of dietary fibres. The increase in the dietary fibre of the soups is very beneficial, as dietary fibre promotes a healthy bowel function, helps to control blood sugar level, lowers blood cholesterol levels, and helps in weight management (Rolfe et al., 2009). The significant increase in the ash content of the *C. forda* larva-enriched soups is an indication of their higher mineral content compared with plain and *Egusi* vegetable soups.

Mineral composition of *C. forda* larva and vegetable soups

The sodium content of the larva is within the range reported for other edible insects (Ajai et al., 2013). The recommended daily dietary allowance of sodium is 2400 mg per day (FAO, 2010). Sodium assists in maintaining the proper acid-balance and in controlling osmotic pressure that develops between the blood and cells due to ionic concentration differences (Paiko et al., 2014).

Potassium was the most abundant of all the minerals in *C. forda* larva. This corroborated the findings of Osasona and Olaofe (2010) who reported potassium to be the most abundant mineral in the flour of CF. The high potassium content is highly beneficial because a high intake of potassium has been reported to protect against increasing blood pressure and other cardiovascular risk (Insel et al., 2007), and it is essential for the functioning of the brain and nerve (Iombor et al., 2017). The potassium-sodium ratio (K:Na) has frequently been used as a diagnostic tool to identify adrenal insufficiency (Iombor et al., 2017). The potassium to sodium ratio in

this study is 2.1:1, hence, the larva could be incorporated into diets for the management of hypertension as high potassium intake has been found to lower blood pressure by antagonizing the effect of sodium (Iombor et al., 2017).

The calcium content of CF larva was found to be high. The calcium value for the larva in this study was higher than those reported by Akinnawo and Ketiku (2000), Omotoso (2006) and Osasona and Olaofe (2010). The observed difference could be due to the differences in the source of the larva coupled with methods of processing. Calcium is an integral component of the skeleton, about 99% of total body calcium is found in bones and teeth, where it plays a structural role (Insel et al., 2007; Yapo et al., 2017). Calcium is important in the development and maintenance of bones and teeth, blood clotting, nerve impulse transmission, muscle contraction and cell metabolism (Heaney, 2006; Insel et al., 2007).

The magnesium content of *C. forda* larva was higher than those reported by Akinnawo and Ketiku (2000), Omotoso (2006) and Osasona and Olaofe (2010). Magnesium is a co-factor participating in more than 300 enzyme reactions, making it an essential element for the synthesis of carbohydrates, lipids, nucleic acids and proteins, as well as for other actions in different organs of the cardiovascular and neuromuscular systems (Chen and Feng, 2002; EFSA, 2015). The phosphorus content of *C. forda* larva in this study is higher than the value reported by Paiko et al. (2014) but lower than the 215.54 mg/100 g reported by Omotoso (2006). Phosphorus like calcium is also involved in calcification of bones and teeth. It plays a vital part in the oxidation of nutrients in the form of phosphate groups in ATP (Paiko et al., 2014).

C. forda larva contained substantial amount of heme iron. Iron plays an important role as a heme molecule in red blood cells, as it permits oxygen transport (WHO, 2006). It can serve as an antioxidant and can prevent cardiomyopathy and growth retardation (Paiko et al., 2014). Since *C. forda* larva contained substantial amount of iron, its inclusion in human diets will be beneficial, and as blood building element in anaemic conditions (Rolfe et al., 2009).

Micro-minerals such as copper, zinc, and manganese are also contained in substantial amount in *C. forda* larva. The copper content of *C. forda* larva is similar to the value reported by Ande (2003). Zinc is important because its deficiency can lead to growth retardation, delayed sexual and bone maturation, skin lesions, diarrhoea, alopecia, impaired appetite, increased susceptibility to infection mediated via defects in the immune system (FAO/WHO, 2001).

Vitamin content of *Cirina forda* larva and vegetable soups

Generally, insects have been reported to contain varying degree of both water-soluble and fat-soluble vitamins. *C. forda* larva was found to contain substantial amounts of B-vitamins, vitamins C and E. Among these, vitamin C was the most abundant. Vitamin C helps in maintaining blood vessels flexibility and improves circulation in the arteries (Alamu et al., 2013). One of the most important benefits derivable from vitamins A, C and E is their role as antioxidants, oxygen free radical scavengers, while that of the B-vitamins is their role as co-enzymes in several enzyme systems of the body (Insel et al., 2007; Alamu et al., 2013). Addition of *Egusi* and the CF larva led to significant increase ($p<0.05$) in the vitamin content of the products (samples C, D and E).

Anti-nutrients level of *C. forda* larva and vegetable soups

The absence of oxalates, tannins and saponins and negligible phytate content in *C. forda* is believed to be due to full conversion of the leaf consumed by the larva during the process of its entering the soil before being harvested, as its name implies in Yoruba language (*Kanni wole* – meaning the larva has entered the soil) (Osasona and Olaofe, 2010). The phytate, oxalate, tannin, and saponin content of *C. forda* larva in this study was much lower than those reported by Alamu et al. (2013) and Omotoso and Adesola (2018).

The difference in the antinutrients content of the larva in this study and past studies might have resulted from the differences in the source of the insect larva. Anti-nutrients are generally known to reduce the bioavailability of nutrients in the body. High phytate level in human nutrition decreased the availability of some minerals such as calcium, magnesium and iron by formation of insoluble compounds with the minerals (Anuonye et al., 2012), while tannins reduced protein bioavailability when bound to protein by inducing a decrease in solubility and functionality of the protein. Tannins are capable of lowering available protein by antagonistic competition, and can therefore elicit protein deficiency syndrome (Ekop, 2004).

Amino acid profile of *C. forda* larva

C. forda larva contained a wide array of both essential and non-essential amino acids (Table 5). The quality of a food protein depends largely on its amino acid component. *C. forda* larva was rich in essential amino acids. The most predominant amino acids of the larva are lysine, glutamic acid, threonine, aspartic acid, leucine, glycine, isoleucine, tyrosine and phenylalanine. This is similar to the work of Yapo et al. (2017) who also reported most of these amino acids to be the most abundant in *C. butyrospermii*. Amino acids are important components for healing and protein synthesis processes;

any deficiency in these essential amino acids will hinder the recovery process (Zuraini et al., 2006). Glycine together with other essential amino acids such as alanine, arginine and phenylalanine form a polypeptide that promotes growth and tissue healing (Witte et al., 2002; Adeoti et al., 2013). Also, glycine is involved in the transmission of impulses in the nervous system, and alanine strengthens the immune system and prevents buildup of toxic substances in the body (Paiko et al., 2014). The high content of essential amino acid in CF larva is very beneficial, as this implied that its protein is of a high biological value. Addition of CF larva to enrich the vegetables (samples C and E) significantly increased their protein content ($p<0.05$).

Fatty acid profile of *C. forda* larva

The larva of CF contained a high amount of polyunsaturated fatty acid (PUFA), (33.05% linolenic acid and 7.07% linoleic acid). The ratio of polyunsaturated to saturated fatty acids (P/S) has been used widely to indicate the cholesterol lowering potential of a food (Akinnawo and Ketiku, 2000). Akinnawo and Ketiku (2000) reported that a P/S ratio of 0.2 has been associated with high cholesterol level with high risk of coronary heart disorders. The P/S ratio of *Cirina forda* larva in this study is 0.8, and a ratio as high as 0.8 has been reported to be associated with desirable levels of cholesterol and reduction of coronary heart disease [Akinnawo and Ketiku, (2000)]. Thus, regular consumption of *Cirina forda* larva could help in intake of healthy fat that can prevent onset of cardiovascular diseases.

Sensory evaluation of vegetable soups

Although, no significant difference was observed in the scoring of sensory attributes of samples C, D and E ($p>0.05$), the enriched samples (Samples C and E) had the highest sensory scores. The exoskeleton of insects has a great influence on the texture. Insects are crunchy and the sounds accompanying their eating resemble the sounds of crackers or pretzels (Kouřimská and Adámková, 2016). This may explain why CF-enriched vegetable soups were more preferred and scored higher than either plain or *Egusi* vegetable soups. The result of sensory evaluation obtained in which the vegetable soups rated higher in all the parameters is similar to the report of Adejoju and Ugochukwu (2019) in which the vegetable sauce was rated higher than ordinary vegetable soup.

Conclusion

C. forda larva was very rich in protein, high in fat, and essential minerals, and very low in anti-nutrients. The amino acid composition of the larva showed that it contained all the essential amino acids needed for human growth in good proportion, hence, the protein content can be considered as a complete protein of high biological value, more so, it is of animal origin. Fat content of the insect larva contained higher amount of unsaturated fatty acid compared with the saturated fatty acid component, showing that it can serve as a good source of healthy fat that is fit for human consumption for promotion of good health.

The insect larva and the enriched vegetable soups contained negligible level of antinutrients which cannot pose any threat to nutrient bioavailability from the larva and the vegetable soups, hence, it is believed that the consumption of the insect larva, either as snack or in vegetable soups or sauces is very safe and will promote quality nutrient intake by consumers.

The soups enriched with *C. forda* larva were more acceptable than the plain vegetable or vegetable with *Egusi* soups. *C. forda* larva-enriched *Egusi* soup was the most acceptable soup. Likewise, in terms of nutrient content it was the most nutrient-dense of the soups. Inclusion of *C. forda* larva in vegetable soups improved both its palatability and nutrient content; therefore, its inclusion in soups and sauces should be encouraged. Also, popularizing the consumption of this insect larva should be encouraged as a means of increasing dietary diversity of the population of the people where the insect larva is readily available. This will help to promote the intake of quality protein and essential minerals among the populace of the host community, and assist in combating protein and micronutrient malnutrition; thereby improving the general health of the people. Also, its consumption could serve as a means of conserving the host tree from going into extinction due to human activities.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effect of heat treatment on yeast inactivation, vitamin C and physicochemical quality of fresh pineapple juice

Mênouwesso H. Hounhouigan[1]*, Anita R. Linnemann[2], Mohamed M. Soumanou[3] and Martinus A. J. S. Van Boekel[1]

[1]Ecole des Sciences et Techniques de Conservation et de Transformation des Produits Agricoles, Université Nationale d'Agriculture, Sakété, Bénin.
[2]Food Quality and Design, Wageningen University, the Netherlands.
[3]Ecole polytechnique d'Abomey-Calavi, Département de Génie de Technologie alimentaire, Université d'Abomey-Calavi, Cotonou, Bénin.

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The effect of pasteurization-range heat treatment on yeast inactivation, vitamin C and the physicochemical characteristics of fresh pineapple juice were assessed. Yeast inactivation could be described by the Weibull model. The desired 6 log reduction was achieved at 63 and 65°C for 8 and 2 min, respectively. The pH, degree brix and organic acids did not change from 55 to 95°C. A significant change in fructose and glucose contents started to occur at 85°C, while sucrose hydrolysis was observed from 95°C. Likewise, hydroxymethylfurfural, one of the intermediate products of the Maillard reaction, was detected at 95°C. Little degradation of ascorbic acid, the most important nutrient in pineapple juice- was observed. Hence, a mild heat treatment of 2 min at 65°C was sufficient to inactivate yeast and to preserve the nutritional and physicochemical quality of the pineapple juice.

Key words: Pineapple juice, heat treatment, yeast inactivation, physicochemical and nutritional quality.

INTRODUCTION

Pineapple is one of the most appreciated tropical fruit due to its exotic aroma and flavour (Rattanathanalerk et al., 2005). The fruit can be consumed fresh or processed into many products including canned slices, juice concentrate, pulp, dried parts and pasteurized juice. The latter is the most popular due to its pleasant sensorial attributes.

Heat treatments are generally applied to extend the shelf life of fruit products. Regarding pineapple juice, they mostly target yeast because lactic acid bacteria are less heat resistant (Aneja et al., 2014; Hounhouigan et al., 2014a). However, heat treatment can affect nutritional and sensory quality attribute: vitamin C losses and nonenzymatic browning are reported as the main consequences of thermal treatment on pineapple juice (Rattanathanalerk et al., 2005; Wurlitzer et al., 2019). Nowadays, new technologies are being developed to avoid nutritional and sensorial losses, such as ultrasound, high pressure, ultraviolet radiation, pulsed

*Corresponding author. E-mail: harold.hounhouigan@gmail.com. Tel: 0022996869393.

light and pulsed electric fields (Gopal et al., 2017; Zhang et al., 2018; Indriani et al., 2018). However, thermal pasteurization remains important because it is a simple and inexpensive technique for small agro-industries. Therefore, it is relevant to assess how pineapple juice can be processed through pasteurization to avoid spoilage while preserving its nutritional and physicochemical quality.

The effect of heat treatment on hydroxymethylfurfural (HMF) and brown pigment accumulation in pineapple juice was investigated in the literature (Rattanathanalerk et al., 2005). However, the authors used a spectrophotometric method, which is not very specific and could have been disturbed by other compounds (Zappala et al., 2005). As for the sensory properties of pineapple juice, organic acids such as citric and malic acids are known to provide the basic acid taste that characterizes the juice (De Vasconcelos Facundo et al., 2010). High concentrations of organic acids and low pH in most fruits are known to be critical for the preservation of fruit juice (Igual et al., 2010). They also help to stabilize ascorbic acid and anthocyanins (Wang et al., 2007).

Kinetic studies are helpful to predict quality loss resulting from different process conditions. For instance, the application of the Weibull model to describe thermal inactivation of microbial vegetative cells has been investigated (Dementavicius et al., 2016) and kinetic models have been used to evaluate vitamin C degradation in fruit products such as orange juice (Vikram et al., 2005), strawberry juice (Odriozola-Serrano et al., 2008) and citrus juice (Burdurlu et al., 2006). Despite the multitude of research on fruit juice preservation (Aleman et al., 1996; Linton et al., 1999; Nguyen et al., 2019), information regarding yeast inactivation, kinetics of vitamin C degradation, the change of sugars, organic acids and HMF using a more specific method such as High Performance Liquid Chromatography (HPLC) are lacking for pasteurized pineapple juice. The aim of this work is to investigate the effect of heat treatment in the pasteurization range on yeast inactivation and the physicochemical and nutritional quality-mainly ascorbic acid- of pineapple juice.

MATERIALS AND METHODS

Yeast inactivation

Raw material

Three batches of fresh and mature pineapples, variety ‘Kona Sugarloaf’, from different production areas in Benin, West Africa were used to perform this experiment. The pineapples were processed immediately after purchase.

Juice preparation

After rinsing the fruits in tap water, the shell was removed using a stainless steel knife. The flesh was cut into small pieces and the juice was extracted using a hydraulic machine (Compact Health Stream Juice press, UK) at ambient room temperature (28°C).

Heat treatment

A series of glass tubes was filled with 15 mL of fresh pineapple juice and subjected to heat treatments in a heating block (Liebisch Labortechnik, the Netherlands) at 57, 59, 61, 63 and 65°C, respectively for different time periods. The juice was treated at 57°C for 5, 10, 15, 20 and 25 min; at 59°C for 1, 2, 4, 6, 8 and 10 min; at 61°C for 1, 2, 3, 4 and 5 min; at 63°C for 25, 40, 60 80, and 100 s; and finally at 65°C for 10, 20 and 30 s. After the treatment time the tubes were immediately cooled in an ice-water bath.

Yeast counts

Ten millilitres of fresh pineapple juice was transferred aseptically into 90 mL sterile peptone salt (Merck Millipore) (5 g of peptone, 8.5 g of NaCl, 1000 mL of distilled water, pH = 7.2 ± 0.2) and homogenized for about one minute using a stomacher (Stomacher 400 circulator Seward, England). Yeasts were enumerated by the pour plate method. Yeasts and moulds were grown on Malt Extract Agar (MEA) (Merck Millipore) incubated at 25°C for 72 h (Heard and Fleet, 1985; Skaar and Stenwig, 1996).

Chemical changes in pasteurized juice

Juice preparation

Three batches of ‘Kona Sugarloaf’, purchased in Benin from three different farmers to perform this experiment in triplicate were stored at a temperature between 12 and 16°C. Batch (A) was processed into juice on the first day after the arrival in the Netherlands (day 1). On day 2, batch (B) was processed and on day 3, batch (C). After juice preparation, the samples were stored frozen at –20 °C, except for the samples in which vitamin C was determined.

Heat treatment

Triplicate experiments were conducted at five temperatures (55, 65, 75, 85 and 95°C) and seven time periods (0, 10, 20, 30, 40, 50 and 60 min), using the three batches of pineapple (A, B and C). A 5 x 7 factorial design was used in the scheduling of each experiment. For each experiment, five series of seven glass tubes were filled with 8 mL of fresh pineapple juice, sealed with tube caps and subjected to heat treatment in a heating block (Liebisch Labortechnik, the Netherlands) at 55, 65, 75, 85 and 95°C, respectively, from 10 to 60 min. After treatment, the tubes were immediately cooled in an ice-water bath. Vitamin C, pH, degrees brix, malic acid, citric acid, HMF, fructose, glucose and sucrose were measured.

pH, degree brix, citric and malic acids determination

The pH was measured using a pH meter (Inolab PH 720) at room temperature. The degree brix was measured with a refractometer (Bellingham-Stanley Eclipse equipment). Organic acids (citric and malic acids) were determined by HPLC. The prepared pineapple juice samples were first centrifuged for 10 min at 1000 rpm and then supernatants were filtered with a 0.2 µm cellulose acetate (CA) filter. The solution was poured in a vial and analysed by HPLC equipped with a Prevail Organic Acids Column 250 × 4.6 mm Grace Alltech 88645 and a Dionex Ultimate 3000 RS Diode-Array Detector(DAD) monitored at 210 nm. The analytical conditions were : flow 1 ml min^{-1}, isocratic mobile phase, a column temperature of

30°C, eluent 0.1 M KH_2PO_4 in Milli-Q water of which the pH was adjusted to pH 2.5 using phosphoric acid.

Vitamin C determination

Vitamin C consists of ascorbic acid (AA) and its oxidized form, dehydroascorbic acid (DHA). Fresh pineapple juice (1 mL) was collected into a 1.5 ml Greiner tube and then centrifuged for 10 min at 10,500 rpm and 4°C. The supernatant was collected and filtered with a 0.2 µm CA filter; 0.5 ml of the filtered supernatant was transferred into a glass tube that contained 7.5 ml of 3% metaphosphoric acid (MPA) (Merck Millipore) and 1 mM of tert-butylhydroquinone (Merck Millipore) (THBQ). The vitamin C concentration was determined by measuring ascorbic acid (AA) using an HPLC system equipped with a C18a Polaris Column 150 × 4.6 mm and an UV detector monitored at 245 nm. The analytical conditions were flow 1 ml min^{-1}, isocratic mobile phase, eluent 0.2% (v/v) orthophosphoric acid in Milli-Q water, and a column temperature of 20°C. Then, DHA was reduced back to AA; for that purpose, 1.5 ml of filtered fruit juice was mixed with 15 µl 1M Tris-2-carboxyethylphosphine (TCEP) (Merck Millipore) solution in amber HPLC vials and stored in the dark for at least 20 min in ice (Hounhouigan et al., 2014b)

Sugar determination

Pineapple juice was diluted 400 times with Milli-Q water. These solutions were filtered using a 0.2 µm CA filter. The filtrate's contents of glucose, fructose and sucrose were determined by HPLC using an Alltech Prevail Carbohydrates ES 5 µm 250 × 4.6 mm column equipped with an Evaporative Light Scattering Detector (ELSD). The temperature of the evaporator was set at 80°C, the temperature of the nebulizer at 60°C, and the carrier flow rate was 1.3 ml min^{-1}. The analytical conditions were: flow 1 ml min^{-1}, isocratic mobile phase, a column temperature of 25°C, and an eluent 75% (v/v) acetonitrile in demineralized water.

Hydroxymethylfurfural determination

The juice samples were centrifuged for 10 min at 1000 rpm; the supernatant was filtered with a 0.2 µm CA filter and transferred into HPLC vials. The HMF contents of the filtrates were determined by HPLC equipped with a Varian C18a Polaris Column 150 × 4.6 mm and UV/VIS detector monitored at 284 nm. The analytical conditions were: flow 1 ml min^{-1}, isocratic mobile phase, eluent 5% (v/v) acetonitrile in Milli-Q water, and a column temperature of 20°C.

Kinetic modelling of yeast inactivation

The kinetics of yeast inactivation in the pineapple juice during heat treatment was described according to the Weibull model (Van Boekel, 2009). The survival curves were obtained by plotting the log (N/No) (with N the number of survivors and No, the initial number) versus time (min) for each temperature. The parameters in the Weibull model were obtained via nonlinear least-square regression using the solver option in Excel (Microsoft). The error analysis on the parameters was done using the Excel macro solverAid (De Levie, 2004). The cumulative function of the survival curve used is:

$$S(t) = \exp(-(\frac{t}{\alpha})^{\beta}) \quad (1)$$

$$\log S(t) = -\frac{1}{2.303}(\frac{t}{\alpha})^{\beta} \quad (2)$$

The time needed to achieve a 6 log reduction was calculated according to Equation 3: (Van Boekel, 2009)

$$t_d = \alpha(-\ln(10^{-d})^{1/\beta}) \quad (3)$$

in which *d* is the number of decimal reductions.

Statistical analysis

Results are given as mean ± SD of three independent determinations. A one-way Anova was used to test the effect of heat treatment on the different physicochemical and nutritional parameters of the pasteurized pineapple juice.

RESULTS AND DISCUSSION

Effect of heat treatment on the inactivation of yeasts

The Weibull model has been proposed to describe non-linear inactivation curves (Dementavicius et al., 2016; Li et al., 2018) The model is sufficiently robust to describe a concave upward survival curve if β < 1 and a concave downward if β > 1 (Dementavicius et al., 2016). Figure 1 shows the data and the fit of the Weibull model to yeast inactivation at 57, 59, 61, 63 and 65°C. Two curves resulted in β < 1 and three in β > 1. At 57 and 59°C, at which β < 1 (Figure 1a to b); the results suggest that the remaining cells of the yeast seem to resist the heat stress. However, at the higher temperatures 61, 63 and 65°C, at which β > 1, the opposite is the case (Figure 1c to e); the remaining cells seem to become increasingly damaged at increasing heating time. Visual inspection of the curves indicates that the fits obtained are very reasonable; the residuals are, by and large, randomly distributed. It is also obvious that first order kinetics does not apply to the data in Figure 1a to e. The calculated Weibull model parameters with corresponding correlation coefficients between the parameters are listed in Table 1.

The results show a transition in heat resistance at 61°C with a shift from $\beta < 1$ to $\beta > 1$. This is an interesting phenomenon because it shows that something critical happens to the cells at temperatures above 61°C. Unfortunately, it makes the temperature dependence of the Weibull parameters complicated. The parameters α and β are correlated: if β changes, α does as well. If the parameter β remains constant, the temperature dependence of α can be modelled in a straightforward way: there is a logarithmic relation between α and temperature (Van Boekel, 2002; Oliveira et al., 2018). However, because of the correlation between α and β and the fact that β is not constant, this relation is disturbed, as shown in Figure 2.

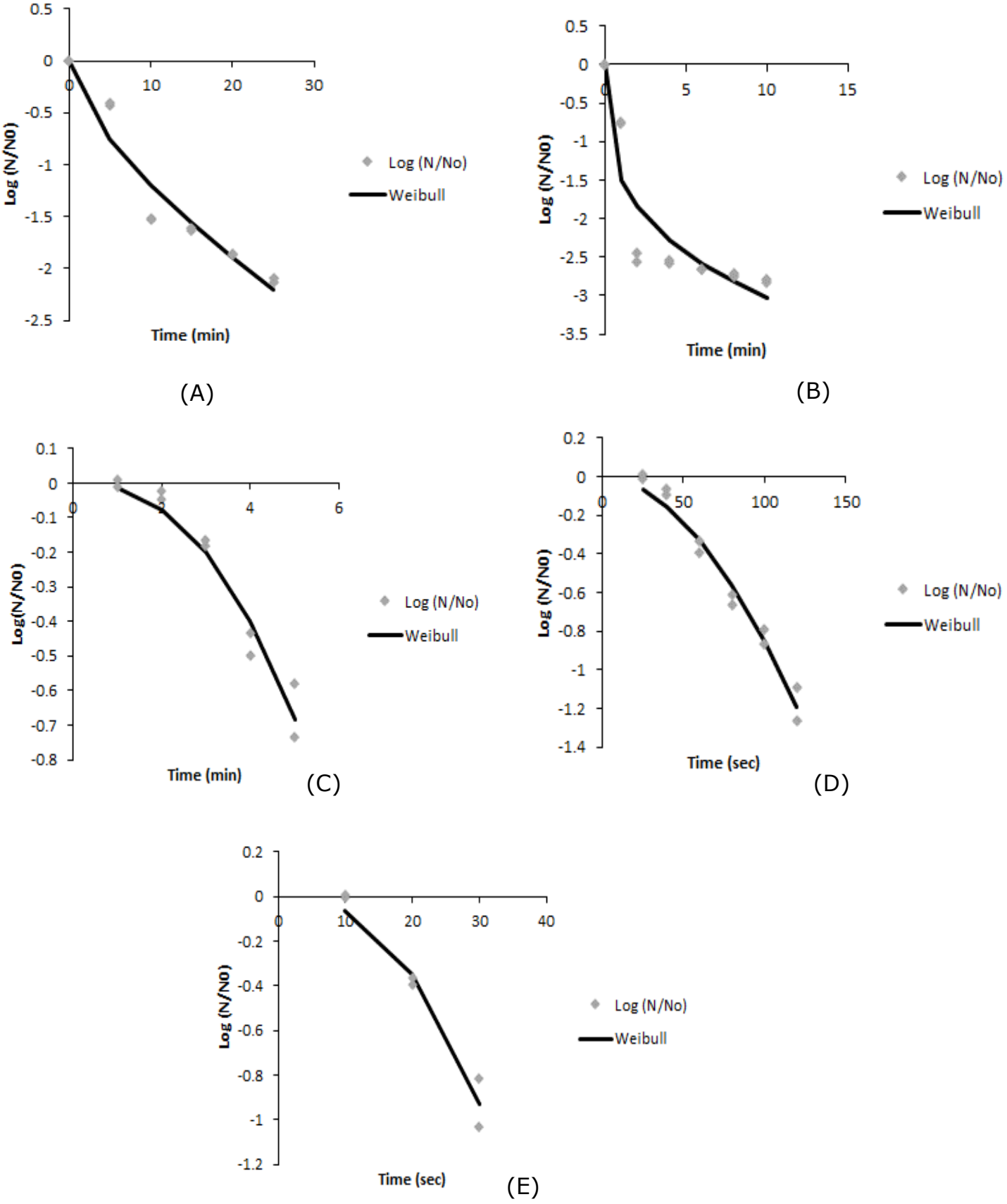

Figure 1. Fit of the Weibull model for inactivation of yeasts at 57 (a), 59 (b), 61 (c), 63 (d) and 65°C (e).

Table 1. Effect of temperature on the Weibull model parameters α and β and their standard deviations.

Temperature (°C)	α (min)	β	*r**
57	2.20 ± 0.75	0.66 ± 0.10	0.98
59	0.01 ± 0.02	0.30 ± 0.07	0.99
61	4.13 ± 0.10	2.39 ± 0.32	0.51
63	1.15 ± 0.04	1.84 ± 0.15	0.89
65	0.36 ± 0.02	2.42 ± 0.45	0.88

**r* is the correlation coefficient between the parameters.

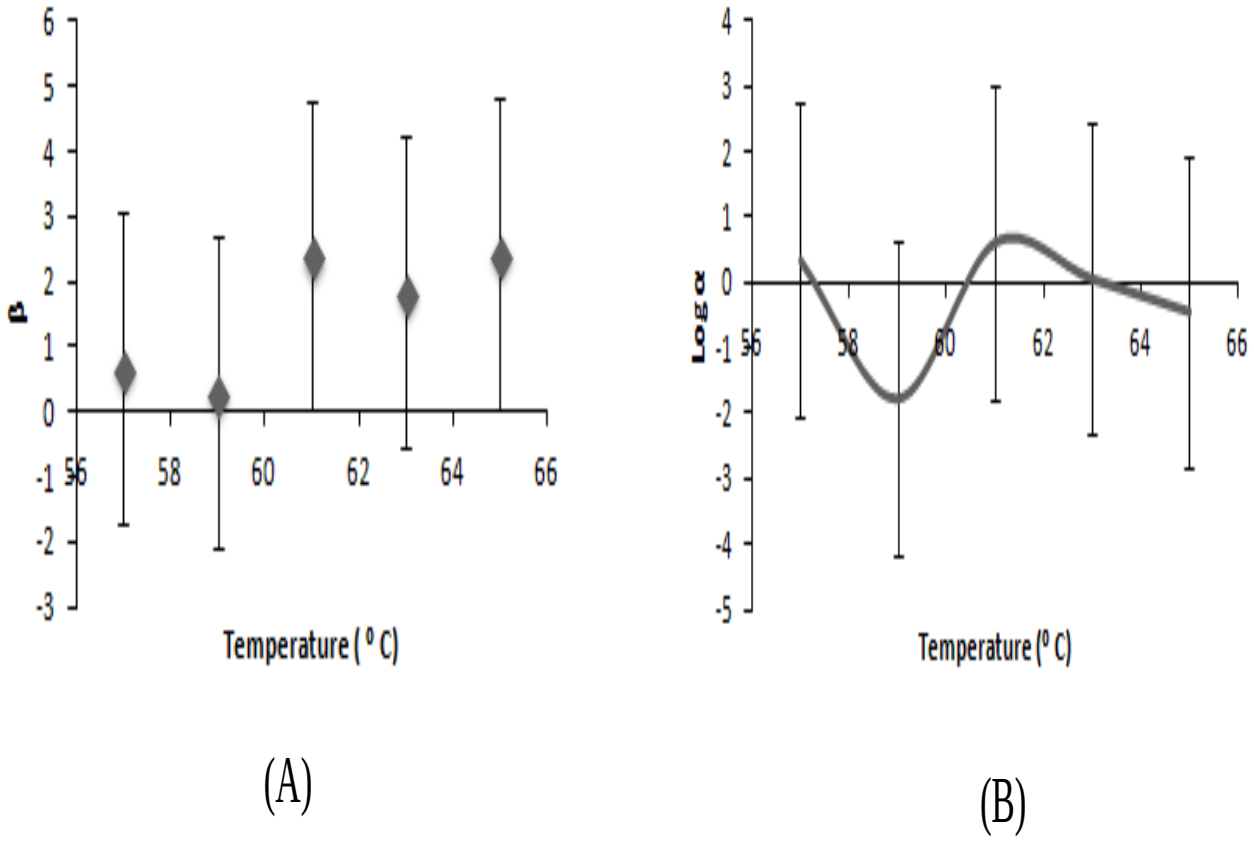

Figure 2. Weibull shape parameter B (a) and scale parameter A (b) as a function of temperature for inactivation of yeasts in pineapple juice; error bars indicate 95% confidence intervals.

The time needed to achieve a 6 log reduction of yeast in the heated pineapple juice was 37 min at 57°C, 8 min at 63°C and 2 min at 65°C. D-values from literature are $D_{60\,°C} < 1$ min for yeast in general and $D_{60°C}$ = 7-22 min for the strain *Saccharomyces cerevisiae* (Hocking and Jensen, 2001). Despite the complications in kinetics, the results suggest that a heat treatment of 65°C for 2 min is sufficient to inactivate yeast in pineapple juice and therefore to give juice a proper microbial shelf life by pasteurization. The remaining question is what happens to the non-microbial characteristics of the juice. This is discussed in the following sections.

Effect of heat treatment on physicochemical characteristics

PH, degree Brix, citric and malic acids

The initial pH of the juice was 3.86 ± 0.05 and the degree brix was 11.0 ± 1.5. The pH and the degree brix did not

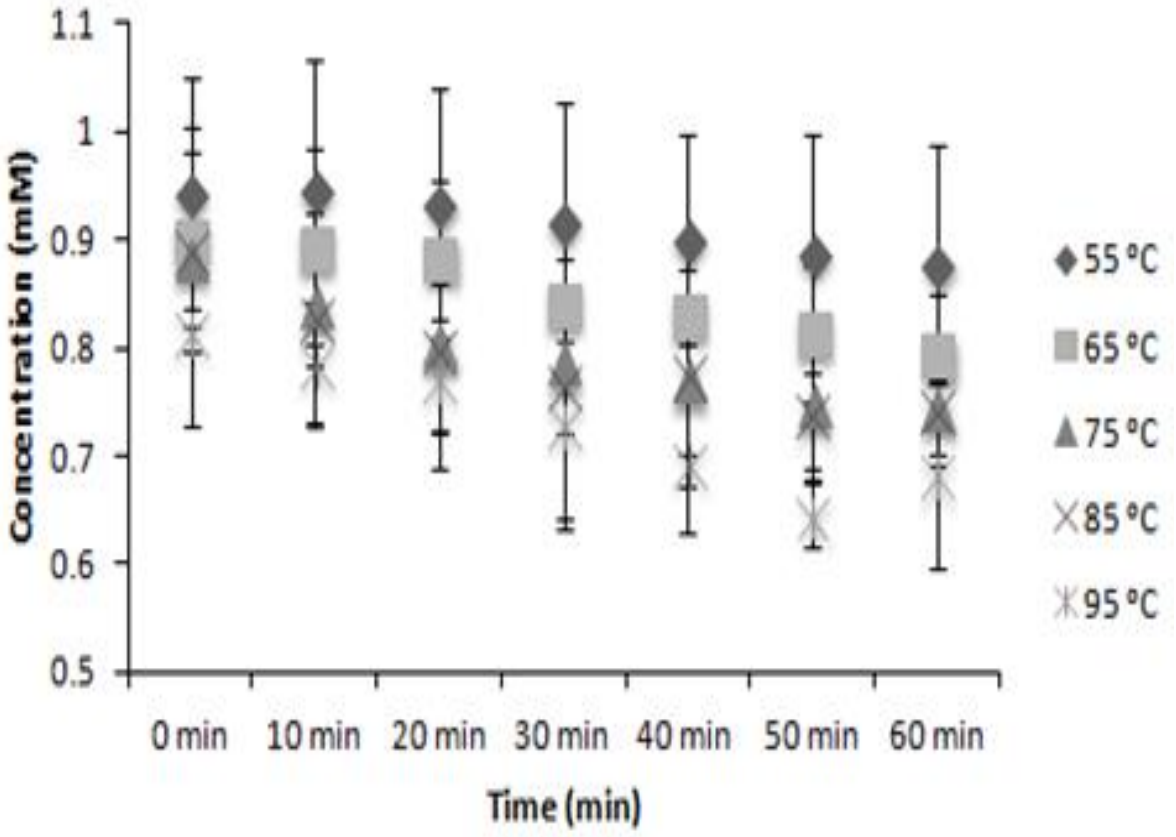

Figure 3. Thermal degradation of ascorbic acid in pineapple juice at different temperatures. Error bars indicate standard deviation for 3 replicates.

change significantly ($P > 0.05$) due to the heat treatments of the pineapple juice, which is in line with other studies (Yeom et al., 2000).These quality parameters are important as they are closely related to the stability of bioactive compounds in fruit products (Kaddumukasa et al., 2017). There were no changes in the malic and citric acids contents after the heat treatments; on average these were 0.02 ± 0.002 M and 0.031 ± 0.005 M, respectively.

Vitamin C

Figure 3 shows the change in the AA concentration during heat treatments at 55, 65, 75, 85 and 95°C in pineapple juice. AA significantly ($P < 0.05$) decreased from 0.91 ± 0.32 mM at 55°C to 0.73 ± 0.26 mM at 95°C. In addition, a significant difference ($P < 0.05$) was observed in the AA content when juice was heated at 65, 75 and 85°C (Figure 3). This result suggests that the increase in temperature affects the AA in pineapple juice to some extent. AA is thermolabile and several studies have reported the effect of temperature on AA content (Vikram et al., 2005). Even though the total loss of vitamin C between the highest and the lowest temperatures studied was around 20%, the losses in AA from a temperature to another were very low (< 8%). The low degradation rates made it impossible to do a proper kinetic modelling; generally, degradation must be at least 30-40% before a model can be applied (Van Boekel, 2008). A very rough calculation of activation energy from our data shows this to be < 20 $kJ.mol^{-1}$. However, this only indicates that the temperature sensitivity of AA degradation in pineapple juice is very low; because of the low degradation rates, a precise calculation of the activation energy is not possible. This is a remarkable result because AA is usually susceptible to thermal degradation. Probably, the low pH conditions, as is the case in pineapple juice (3.86 ± 0.05), might stabilize it. A similar behaviour of ascorbic acid was reported in orange juice (Plaza et al., 2006) and marula juice (Hiwilepo-Van Hal et al., 2012). It is also reported that the degradation of vitamin C is very low in citrus juice, in contrast to their expectation (Dhuique-Mayer et al., 2007). Perhaps it is time to reconsider the general perception that vitamin C is always very thermolabile.

The AA content of the pineapple juice treated at 55°C was initially 0.82 mM in batch A, 1.02 mM in batch B and

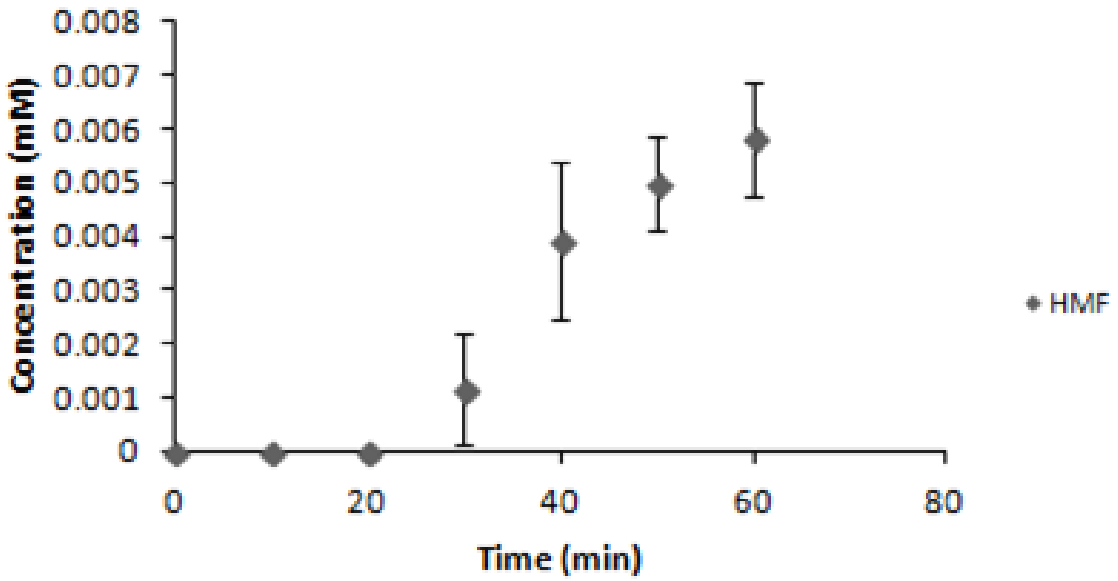

Figure 4. Effect of heating at 95°C on the HMF content in pineapple juice. Error bars indicate standard deviation for 3 replicates.

0.99 mM in batch C. The standard deviation appeared to be very large (± 0.10 mM), but this is not due to the treatment or the analysis method but to the natural variation in content. This high variation in the initial vitamin C was also found in the juice treated at 65, 75, 85 and 95°C. This indicates that the variation between the three batches was inherent in the pineapple juice.

Hydroxymethylfurfural (HMF)

As non-enzymatic browning (Maillard reaction) is one of the major causes of colour change in fruit products, the effect of heating time on the accumulation of HMF was investigated. It was observed that HMF formation was initiated only at 95°C with a minimum time of 30 min. HMF generation can only occur at temperatures above 95°C in orange juice (Marcotte et al., 1998). However, this result deviates from the findings in the literature (Rattanathanalerk et al., 2005) that detected, by spectrophotometry, HMF in pineapple juice treated at 55°C. This could be due to the HMF detection method. Spectrophotometry may suffer from interferences from other compounds that absorb light at the same wavelength as HMF. Figure 4 shows the effect of heating at 95°C on the HMF content (with standard deviation for 3 replicates) in pineapple juice.

Fructose, glucose and sucrose

Sucrose, glucose and fructose were studied as they are the major sugar components of pineapple juice (Khalid et al., 2016). The concentrations of fructose (123 ± 5.1 mM), glucose (100.5 ± 4.7 mM) and sucrose (320.8 ± 8.1 mM) in pineapple juice did not change after treatments at 55, 65 and 75°C (Figure 5). At 85°C, sucrose showed a significant ($P < 0.05$) decrease from 320 to 292.4 mM. Overall, the glucose and fructose significantly increased at 95°C (20, 30, 40, 50 and 60 min) (Figure 5), whereas the sucrose content significantly decreased at 85°C (30, 40 and 50 min) and 95°C (20, 30, 40, 50 and 60 min) (Figure 5). The decrease of sucrose with a simultaneous increase of fructose and glucose is explained by the hydrolysis of sucrose. Obviously, reducing sugars, glucose and fructose are also subject to degradation.

However, our results show that this is very limited at the heat treatments applied here, in line with the fact that HMF formation is very limited. Since fructose is the sweetest of all naturally occurring carbohydrates (Hanover and White, 1993), pasteurization of pineapple juice starting from 85°C might affect the taste of the fresh pineapple juice, making it sweeter than its initial taste. Figure 5 shows the changes in sugars in pineapple juice during heat treatment (with the standard deviation for three replicates).

Conclusion

A heat treatment of 65°C for 2 min appeared to be sufficient to achieve a 6 log reduction of yeast in pineapple juice. Chemical characteristics such as pH, degree brix, malic and citric acids concentrations were

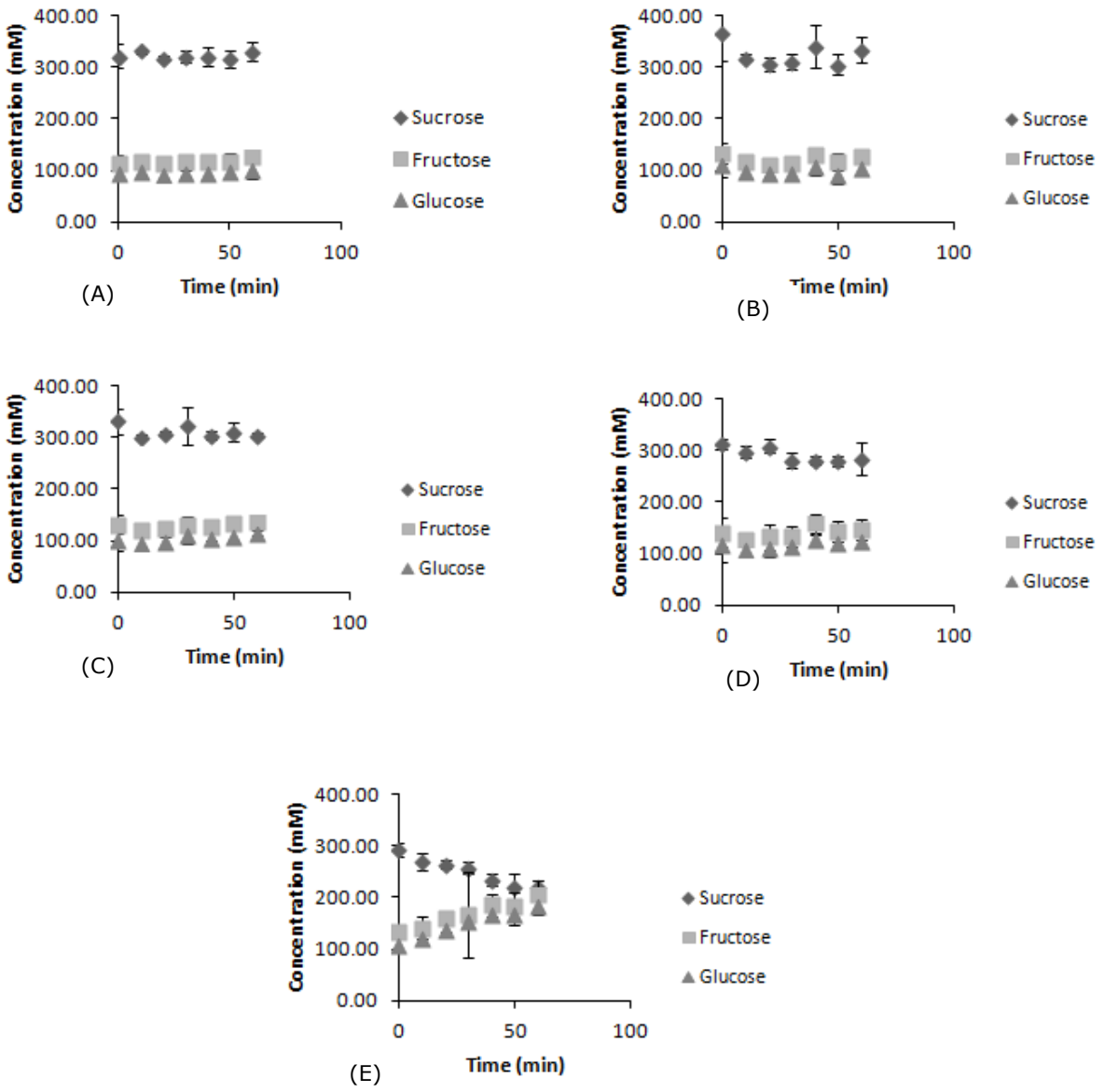

Figure 5. Changes in sugar composition in pineapple juice after heat treatments at 55 (a), 65 (b), 75 (c), 85 (d) and 95 °c (e). Error bars indicate standard deviations for 3 replicates.

not affected by the heat treatments in the range studied. Hydrolysis of sucrose and formation of HMF was observed at 85 and 95°C respectively, which could indicate the start of the Maillard reaction. The vitamin C content decreased with heating temperature but the effect of temperature on vitamin C degradation was low in the temperature range studied. Therefore, a pasteurization treatment at a relatively low temperature could be used to prevent pineapple juice from yeast spoilage while preserving its nutritional and physicochemical quality. These results are of importance for pineapple juice processors in small and large-scale juice industries. Most likely, pineapple juice is over-processed. Our results show that pasteurization does not need to be severe in order to obtain a reasonable shelf life. We did not study the organoleptic quality of the juice, but since pasteurization can be done at a rather low temperature, we anticipate that damage to organoleptic quality will be limited.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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