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Table of Content

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Full Length Research Paper

The tomato paste quality attributes along the industrial processing chain

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Tomato is widely consumed in the world and its concentrated paste represents the main product from industrial tomato cultivars. One of the industry's biggest challenges is to produce in a sustainable and effective chain to simultaneously fulfill the cost reduction and quality improvement demands. This study aimed to show the loss of tomato paste quality attributes along its industrial processing chain, focusing on three parameters: (1) ascorbic acid content; (2) color and (3) consistency. Samples of tomato paste (29 °Brix) were subjected to thermal treatment at 60, 70 and 80°C, cooled down and analyzed for the mentioned attributes by kinetic parameters. Both ascorbic acid (z = 62.8°C, R^2 = 0.9499) and instrumental color (z = 19.0°C, R^2 = 0.9924 for a*/b*, and z = 16.3°C, R^2 = 0.9897 for TPS) parameters degradation were well described by first-order kinetic models. Consistency attributes were not affected by the thermal treatment temperature. The temperature effect at each stage of processing allowed identifying the sterilization and storage steps as responsible for the greater degradation of color and ascorbic acid, respectively. The industry must control sterilization and increase sun protection in the offseason to preserve color and ascorbic acid, ensure lower losses of sensorial quality without reducing its productive flexibility, always prioritizing food safety.

Key words: Color, storage, shelf life, vitamin C, consistency.

INTRODUCTION

Tomatoes are widely consumed in the world and as a fruit of limited durability and short agricultural season, much of the tomato produced for industrial purposes is transformed into concentrated pulp, which is reconstituted throughout the year, mainly during the off-season (Ochida et al., 2019). In Brazil, the Support Program for the Production and Marketing of Horticultural Products (PROHORT) was implemented in 1992-2012, when it was noted although only garlic, onions, potatoes, and tomatoes were priorities for PROHORT, the modernization of production and commercialization of these four vegetables resulted in positive impacts in the chains of many others (Camargo Filho and Camargo, 2017).

The quality of the processed tomato product is dependent upon processing conditions (Xu et al., 2018)

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and the essential quality parameters for the classification and marketing of processed tomato products are color and consistency. The desired color pattern, both in fresh and concentrated tomatoes products, is a red-intense and uniform pulp, by the presence of carotenoids, which lycopene represents about 80%. The color maintenance of tomato products depends directly on the control of process factors that trigger oxidation or thermal isomerization of carotenoids. Throughout the processing chain of concentrated pulp, the presence of oxygen must be limited, making heating the main cause of color changes (Qiu et al., 2019; Motamedzadegan and Tabarestani, 2011; Shi and Maguer, 2000; Barreiro et al., 1997). Remini et al. (2015) showed the effect of storage temperature and deaeration was the most influent factors on kinetics degradation of color and ascorbic acid in pasteurized blood orange juice.

Consistency in tomato products is closely linked to cost, as well as sensory acceptance. The main factors affecting the tomato pulp consistency are the differences in composition between cultivars, different maturation stages of the fruit, the content of total solids and insoluble solids, the particle size distribution and the shape of the insoluble particles, and initial heat processing parameters (hot break or cold break) (Page et al., 2019; Xu et al., 2018; Sánchez et al., 2002).

Tomato is also considered an important source of ascorbic acid (AA) due to its high consumption, both fresh and processed products. AA is relatively thermolabile and it is considered one of the most sensitive nutrients to adverse conditions, so its content is often taken as an indicator of general quality changes in processed foods (Tchuenchieu et al., 2018; Koh et al., 2011).

The loss of quality of the concentrated tomato pulp generates a direct impact on derived products like ready sauces, extracts, and ketchup, whose quality is perceived and evaluated directly by the final consumers. Based on California, the US state responsible for significant 35% of world production, other producers in the world opt for the less expensive storage system under uncontrolled conditions, that is, the ambient temperature in open courtyards. But the American product remains exposed to the mild winter conditions of the southern United States, averages from 9.1 to 20.2°C (Rssweather, 2019); while in other tropical counties the product is subjected to warmer climate conditions. For this reason, this storage system, although cost efficient, can contribute to the deterioration of the concentrated pulp throughout its shelf life, exposed to heat and direct sunlight. Sometimes, the maximum storage time in the mentioned conditions is established around 24 months, but it can also be added to the shelf life of the concentrated pulp for another 12 to 18 months, considering the cases in which it is used in the tomato processed products, such as sauces and extracts, during the off-season. In these cases, the pulp quality loss is perceived directly in the products that arrive at the consumer's table, reflecting in great variability in the quality parameters of processed tomato products throughout the year.

Since even the quality attributes of homemade tomato sauce can vary according to different storage temperatures (Nkhata and Ayua, 2018), many authors have shown the applicability of a kinetics-based testing approach to obtain faster insight into food product changes, useful for shelf-life investigations (Peleg et al. 2018), for example for strawberry juice (Buvé et al., 2018), or tomato hot pot sauce (Li et al., 2018), among others.

This study can, therefore, help the industry to identify the critical stages for the degradation of the main attributes of quality along the industrial processing chain of concentrated tomato pulp: color, consistency and AA content. The study of the kinetics of degradation must provide data to direct the application of improvements in the processing, manipulation, and storage of concentrated tomato pulp, combining food safety and sensorial quality.

MATERIALS AND METHODS

In the kinetic studies, concentrated tomato pulp samples, produced from the varieties N-901 (80%), H-9553, H-9992, U-2006, IT-761, IT-619 were used (quantity in descending order). The samples were collected, aseptically packed in 4 kg bags, at an industrial tomato processing plant and stored at -15°C until physicochemical analysis.

Physicochemical analysis

The pH was measured in Digimed DM-70 pH meter and the total soluble solids (ºBrix) determined by direct reading in Abee Mark II, Reichert Technologies refractometer (AOAC, 2010).

Titratable acidity was determined by titration (AOAC, 2010) and the wet basis moisture was determined by thermogravimetric, with halogen drying, using the HB43-S Mettler Toledo® moisture analyzer.

The determination of AA was done by iodometry (IAL, 2008 - 3664/IV method). Aliquots of pulp added with 20% sulfuric acid solution, 10% potassium iodide solution and 1% starch indicator solution were titrated in triplicate with potassium iodate solution (0.2 mM) to blue coloration.

Determination of kinetic parameters

The Bigelow model (Singh and Heldman, 2013) was applied to determine the kinetic parameters: decimal reduction time, *D* (hour) and thermal resistance constant, *z* (°C), for color and AA degradation, and loss of consistency, in 29 °Brix tomato concentrate pulp. The kinetic parameters were determined after the data analysis of the experiment planning: 60 and 70°C for 12, 24, 46, 48, 60, 72, 84 and 96 min; and 80°C for 6, 12, 24, 30, 36 and 48 min.

Samples were packed in 120 ml screw cap polystyrene bottles which were submitted to thermal treatment, immersed in a

thermostatic Lauda® bath, kept closed to minimize temperature fluctuations. The temperature in the center of the vials was measured with a thermometer and at each set time interval; the vials were transferred to a cold thermostatic bath set at 2°C in order to interrupt the heat treatment. As suggested by Barreiro et al. (1997), the samples were preheated in the microwave to accelerate the heat transfer, thus reducing the come up time.

Instrumental color analysis

This analysis was carried out in a colorimeter (Hunterlab® model LabScan XE), with a 64 mm quartz cuvette (Hunterlab, 2015). The apparatus was calibrated for reflectance measurement (RSIN), CIELAB color system (L*, a*, and b*), C-type illuminant and 2° observer angle, according to the equipment manual, to obtain the colorimetric coordinates L*, a* and b*. The equipment was also calibrated with standard black and white tiles prior to measurements. Checks with red standard tile (L* = 26.18; a* = 27.3 and b* = 12.7) were performed periodically. The Concentrated Tomato Paste Score (TPS) was calculated according to Equation (1):

$$TPS = -40.926 + 1.061a^* + 9.473b^* - 0.346b^{*2} \quad (1)$$

At each reading, the cuvette was filled with the same sample volume and an opaque black cover was placed over it to prevent interference of the ambient light in the measurement.

Consistency and shear stress measurements

After the heat treatment, the samples were cooled down for consistency and shear stress measurements. Consistency test was carried out at 25°C according to Garcia and Barrett (2005), using a Marconi® Bostwick device MA441, 75 mL, 0.5 cm scale intervals. The concentration of the tomato pulp samples was standardized by dilution to 12% NTSS (natural tomato soluble solids) or 12 °Brix with deionized water.

Shear stress measurement was carried out by back extrusion using the Universal Testing Machine, model TA XT Plus, Stable Micro Systems. In the back-extrusion test, a piston is forced through the sample and the product is expelled around the disc, and the maximum force (gf) is measured.

The readings were made directly in the polystyrene pot, inner 40 mm in diameter, and 70 mm in height, where the samples were before conditioned for the heat treatment. The geometry (probe) used for the back-extrusion test was the 35 mm in diameter acrylic disc, code A/BE-d35.

Correlating theoretical analysis and real process conditions

The kinetic parameters of color and AA degradation were correlated to the industrial tomato processing conditions observing time and temperature parameters from the tomato reception to storage in the yard or shelves, up to industrial steps, taking into account how much each stage influenced these characteristics.

Equation 2 represents the linear form of the first order model, where (C_o) and final (C_t) represent respectively the initial and final concentration of the analyzed parameter after a process time *t* (hour), based on its *D* (hour) value:

$$\log(C_t) = \log(C_0) - \frac{t}{D} \quad (2)$$

Knowing the thermal resistance constant *z, D* values can be calculated for other temperatures *T* (°C) (Equation 3):

$$\log(D_2) = \log(D_1) - \frac{(T_2 - T_1)}{z} \quad (3)$$

From *D* (hours) values, the dimensionless degradation rate (*DR*) at each step of the concentrated tomato pulp process was calculated (Equation 4):

$$DR = 10^{\frac{(T_p - T_R)}{z}} \quad (4)$$

Where, *DR* represents the degradation rate; T_p (°C) is the step process temperature; T_R (°C) is the average temperature of the kinetics study, 70°C, adopted as the reference temperature.

The integration of *DR* over time allows us to obtain an equivalent process, called *F* (hours), at the reference temperature (70°C). In practice, the integration took into account the processing time *Δt* (hours) and its *DR* at the corresponding T_p along *i* to *n* process steps (Equation 5):

$$F = \sum_i^n \mathrm{DR}_i \Delta t_i \quad (5)$$

Then it is possible to determine the process Effect, *E* (%) in each step (Equations 6):

$$E(\%) = \left(1 - \frac{C_t}{C_0}\right) 100\% = \left(1 - 10^{-\frac{F_T}{D_T}}\right) * 100\% \quad (6)$$

Result analysis

All analyses were performed in triplicate, and the mean result and its respective standard deviation were considered. When applicable, the results of the determinations were statistically evaluated using ANOVA Variance Analysis and Tukey's Test at a significance level of 5% for the comparison between means.

RESULTS AND DISCUSSION

The physical-chemical characterization of the aseptic pulp used in the kinetic study showed 67.35±0.23% wet basis moisture, 29.64±0.10 °Brix; pH of 4.31±0.01; 2.11±0.06% citric acid; and 75.3±0.9 mg of AA/100 g. Xu et al. (2018) found similar results (55.9±0.05 mg of AA/100 g) to high temperature short time 21.6 °Brix processed tomato pulp.

The come up time of the samples in the microwave was of 5 min to reach 60°C; 7.5 min to reach 70°C, and 9.5 min to reach 80°C and may be considered negligible in relation to the applied heat treatments.

Kinetics of ascorbic acid (AA) degradation

The data obtained from the degradation of AA in the

Table 1. Concentrations of AA during heating at different temperatures, and respective kinetic parameters calculated by the Bigelow's model. Values expressed as average ± standard deviation.

Time (h)	AA (mg/100 g)		
	60°C	**70°C**	**80°C**
0	75.3±0.9	75.3±0.9	75.3±0.9
6	-	-	45.9±1.0
12	53.8±1.1	46.1±1.6	33.4±0.5
24	51.7±4.4	33.2±1.0	21.9±1.8
30	-	-	23.5±1.1
36	42.4±1.4	27.4±1.1	20.8±1.4
48	38.1±1.5	22.0±0.7	24.0±3.4
60	37.2±0.4	20.2±0.7	-
72	33.6±1.9	19.4±1.0	-
84	26.7±0.9	19.3±1.2	-
96	21.2±1.2	16.1±0.8	-
D (h)	203.22	163.18	97.65
z °C		62.84	
R^2		0.9499	

three studied temperatures (Table 1) were analyzed based on the reaction order and then the kinetic parameters were calculated by the Bigelow model.

In general, the values of *D* and *z* obtained in the present study demonstrate thermal dependence within the ranges reported in the literature for similar products. The AA concentration decreased with heating and 50% of this reduction occurred after 48 min at 60°C, 20 min at 70°C and 10 min at 80°C. The determination coefficients (R^2) suggest that the first order model satisfactorily describes the degradation reaction of AA in tomato pulp only at 60°C. The z values obtained are in accordance to other published kinetic parameters, as 64°C for guava pure and 36.7°C for cupuaçu nectar, among other cited by Ling et al. (2015).

Many authors have mentioned that most of the reactions studied in foods are characterized as zero order or first order kinetics; and the most frequent first-order reactions in foods are microbial development, degradation of vitamins and pigments during processing and storage, enzymatic inactivation and destruction of toxins by heating. Sarkar et al. (2015) reported a deleterious effect of the temperature when studying storage conditions on the nutritional quality of tomato pulp. Sonar et al. (2019) showed the combined effect of thermal treatment and oxygen transmission rate on quality and nutritional attributes of carrot puree processed in pouches, obtaining zero and/or first-order kinetics for AA degradation, depending on the permeability of the pouch.

For Ruiz et al. (2018), the kinetic modelling of AA in a model solution at different temperatures and oxygen contents showed that AA degradation increased with temperature and oxygen concentration; a kinetic model based on first order reactions was developed to simulate the AA degradation as a function of temperature and concentration in dissolved oxygen.

For Robertson and Samaniego (1986), the first-order reaction is valid only when oxygen is present in abundance (aerobic degradation) or when it is completely absent from the medium. When oxygen is contained in limited concentrations, as in the present study in which the air incorporation in the sample was not controlled, the authors report that the second order kinetics may represent that the reaction depends on both the oxygen concentration and the AA.

As the focus of this work was to compare the changes of three important quality parameters during processing and storage, the anaerobic degradation of AA best represents the behavior of the tomato pulp during the storage period after concentration and aseptic packaging. For this reason, the first order model was selected for this work. The first order model was also used to describe the kinetics of AA degradation in pasteurized mango juice (Wibowo et al., 2015), in lyophilized plums (Gabas et al., 2003), in concentrated citrus juices (Burdurlu et al., 2006), in ketchup (Rajchl et al., 2010), in tomato extract (Pedro, 2009), and in concentrated tomato juice during heating and drying (Qiu et al., 2018).

For comparison purposes, the kinetic results of

Table 2. Results obtained for the color parameters L*, a*, b*, TPS, and a*/b* during the heating of the tomato pulp at different temperatures. Values expressed as average ± standard deviation.

T(°C)		Time (h)										
	Parameter	**0**	**6**	**12**	**24**	**30**	**36**	**48**	**60**	**72**	**84**	**96**
60	L*	23.93±0.01	-	23.91±0.03	23.81±0.00	-	23.61±0.05	23.6±0.02	23.38±0.00	22.86±0.04	22.99±0.02	22.64±0.01
	a*	22.98±0.04	-	23.33±0.07	23.06±0.03	-	22.76±0.02	22.28±0.02	22.05±0.02	21.49±0.01	21.56±0.01	21.23±0.02
	b*	13.79±0.01	-	13.87±0.02	13.79±0.00	-	13.69±0.01	13.65±0.01	13.55±0.01	13.22±0.02	13.35±0.01	13.16±0.01
	TPS	48.27±0.06	-	48.63±0.06	48.37±0.06	-	48.07±0.06	47.57±0.06	47.3±0.00	46.6±0.00	46.77±0.06	46.33±0.06
	a*/b*	1.67±0.00	-	1.68±0.00	1.67±0.00	-	1.66±0.00	1.63±0.00	1.63±0.00	1.63±0.00	1.62±0.00	1.61±0.00
70	L*	23.93±0.01	-	23.41±0.04	23.07±0.01	-	22.37±0.01	21.56±0.01	20.99±0.02	20.16±0.02	19.55±0.01	19.14±0.01
	a*	22.98±0.04	-	22.29±0.07	21.79±0.02	-	20.54±0.00	19.08±0.03	18.32±0.02	16.97±0.03	16.09±0.02	15.48±0.01
	b*	13.79±0.01	-	13.5±0.06	13.41±0.01	-	12.93±0.01	12.43±0.01	12.1±0.02	11.57±0.02	11.21±0.02	10.94±0.01
	TPS	48.27±0.06	-	47.53±0.06	47.±0.00	-	45.5±0.00	43.6±0.00	42.5±0.00	40.37±0.06	38.87±0.06	37.7±0.00
	a*/b*	1.67±0.00	-	1.65±0.00	1.63±0.00	-	1.59±0.00	1.54±0.00	1.51±0.00	1.47±0.00	1.44±0.00	1.41±0.00
80	L*	23.93±0.01	22.43±0.01	21.340±0.03	19.58±0.01	18.82±0.00	18.31±0.02	17.04±0.01	-	-	-	-
	a*	22.98±0.04	20.81±0.02	19.04±0.02	16.13±0.02	15.19±0.00	14.22±0.02	12.61±0.05	-	-	-	-
	b*	13.79±0.01	12.97±0.01	12.33±0.02	11.19±0.01	10.82±0.01	10.42±0.01	9.66±0.01	-	-	-	-
	TPS	48.27±0.06	45.81±0.02	43.48±0.02	38.86±0.02	37.19±0.01	35.29±0.05	31.67±0.07	-	-	-	-
	a*/b*	1.67	1.6	1.54	1.44	1.40	1.36	1.31	-	-	-	-

Bigelow's model when converted to the Arrhenius model resulted in $k_{60°C}$ = 1.13E10^{-}2/h; $k_{70°C}$ = 1.41E10^{-}2/h; and $k_{80°C}$ = 2.36E10^{-}2/h, and *Ea* = 35.70 kJ/mol, in accordance to 51 to 135 kJ/mol obtained by Remini et al. (2015) for AA degradation in pasteurized blood Orange.

The values of *D* and *z* found in the literature for the degradation of AA in fruit products do not establish a common standard, and this study exemplifies how the complexity of food matrices, as well as differences in processing and study conditions, interferes with the observed kinetic results.

Color degradation kinetics

The results obtained for the color parameters L*, a*, b*, TPS, and a*/b* during the heating of the tomato pulp at the three temperatures are presented in Table 2. For the determination of the reaction order, the values of each parameter versus the heating time at each temperature were adjusted for both the zero and first order models. The first order model showed coefficients of determination (R^2) slightly higher or equal to those found through the zero order model for all the parameters and, therefore, it was adopted for the calculation of kinetic parameters of color degradation. The first order of behavior has already been observed for color degradation in tomato products (Barreiro et al., 1997; Rajchl et al., 2010). Similarly, Lee and Chen (2002) described first-order kinetic behavior for the degradation of lycopene in model systems at different temperatures. As expected, all color parameters (L*, a*, b*, a*/b* and TPS) decrease with temperature increment. To evaluate the intensity of the binomial time and temperature in the color degradation, the kinetic constants of the color degradation attributes were determined by the Bigelow model (Table 3).

The values found in the present study are closer to those obtained by Rajchl et al. (2010) with ketchup, Ganje et al. (2018) with tomato paste, and even by Kale and Nath (2018) who worked with storage of tomatoes.

Some divergences may be related to the conditions of the study (warming time, evaluated temperatures, and aeration) and composition characteristics of the food matrix used. Barreiro et al. (1997), in a similar study with tomato pulp, although they reported the same degradation profile for all the color attributes, found kinetic

Table 3. Kinetic parameters for color degradation (L*, a*, b*, TPS, and a*/b*) in concentrated tomato pulp, and R^2 for *z* determination.

Parameter	$D_{60\,°C}$ (h)	$D_{70\,°C}$ (h)	$D_{80\,°C}$ (h)	*z* (°C)	R^2
L*	3850	939	331	18.8	0.9925
a*	2284	523	184	18.3	0.9904
b*	4177	900	184	14.8	0.9999
TPS	4463	844	263	16.3	0.9897
a*/b*	5037	1248	446	19.0	0.9924

Table 4. Results of the maximum force, measured by back extrusion, after heat treatment at 60 and 70 °C for different time intervals. Values expressed as mean ± standard deviation.

Time (h)	The maximum force (gf)*	
	60 °C	70 °C
0	1070±62^{a}	1070±62^{a}
12	1000±13^{ab}	1050±40^{a}
24	1029±48^{ab}	1014±22^{ab}
36	1002±38^{ab}	835±27^{c}
48	900±17^{b}	845±20^{c}
60	976±17^{ab}	915±34^{bc}
72	925±55^{b}	925±34^{bc}
84	941±20^{ab}	923±21^{bc}
96	929±25^{b}	927±8^{bc}

*Values in the same column with the same letter do not differ significantly from each other at the significance level of 5% by the Tukey test.

parameters demonstrating greater color sensitivity to temperature.

Consistency and shear stress measurements

Consistency analysis using the Bostwick device showed no significant variation between the measurements carried out at 60°C (4.0±0.1 cm/30s) and 70°C (4.1±0.1 cm/30 s). At 80°C, the degradation of the consistency was observed only after 6 h of heating. However, there was no statistically significant difference between samples heated for 6 to 48 h (from 4.3 to 4.5 cm/30 s). In this way, it can be inferred that in a closed environment and admitting insignificant moisture loss, the heat treatment temperature exerts a very small effect on the consistency of the concentrated tomato pulp.

For the shear stress measurement only the results of the maximum force for samples submitted to treatment at 60 and 70°C were presented, because at 80°C the polystyrene flasks deformed, preventing the comparison of the readings by the initial height difference between the (Table 4).

The firmness degradation was observed after 48 h of heating at 60°C and after 36 h of heating at 70°C. However, the samples collected in the following times, until the end of the study, did not present statistical difference. The shear stress measured by back extrusion showed similar behavior to that observed for the Bostwick consistency, showing little influence of the temperature. It is possible to affirm that the Bostwick method is satisfactory for the evaluation of consistency of tomato pulp. Although not very precise, this simple and low-cost equipment was able to demonstrate results comparable to those obtained in the back extrusion system, a more precise and sophisticated equipment.

Storage correlation

Based on the *z* values of each attribute measured, color showed to be more temperature dependent than AA. In this way, greater color degradation is expected during thermal processing, when higher temperatures are

Table 5. Color (TPS) extracted from the analytical reports in industrial concentrated tomato pulp production.

Parameter	Crop A - 27 months			Crop B - 16 months			Crop C - 3 months		
	Initial*	Final**	%	Initial	Final	%	Initial	Final	%
Color (TPS)	46.9	18.7±0.0	-60.1	46.2	41.5±0.0	-10.2	47.3	48.3±0.1	+2.1

*Average value referring to the factory sampling plan for quality assurance of the concentrated tomato pulp. **Measured values.

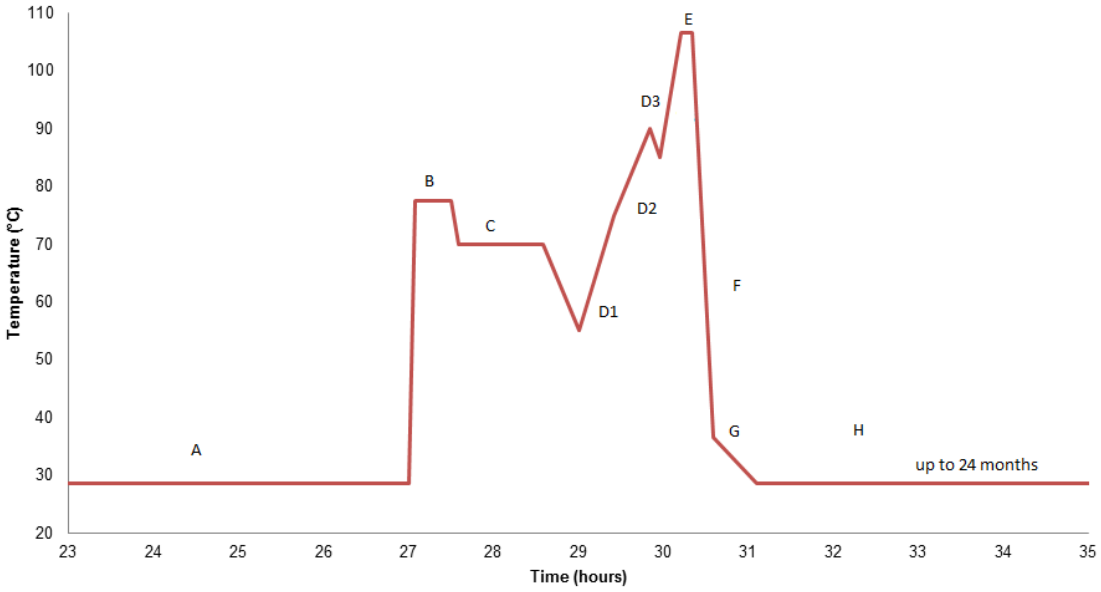

Figure 1. Thermal map of the productive process of concentrated tomato pulp, where A = harvest, transport, reception, classification, washing and selection; B = crushing and enzymatic inactivation; C = refining and storage; D_1, D_2 and D_3 concentrations of first, second and third effects, respectively; E = sterilization; F = cooling; G = container; H = storage.

achieved, with some loss along with the storage. With respect to AA, the reverse is expected, that is a higher resistance to processing and a more time-dependent degradation, throughout the store.

Kinetic models are restricted to variations within the studied temperature range. The extrapolation of the results obtained for temperatures outside the studied range (60 to 80°C) is commonly used in the food industry in accelerated stability tests, with the objective of reducing the time required to determine the product shelf life. The results obtained must be interpreted with caution and, where possible, validated under the actual storage conditions.

Table 5 shows the TPS results extracted from the analytical reports issued in industrial concentrated pulp production. For AA content there is no data available since this parameter does not usually take part in the manufacturer's routine controls.

A 60 and 10% drop were observed in the TPS color measurements, respectively after 27 and 16 months storage. Considering a storage temperature of 28.7°C, based on the annual average of the daily maximum temperatures of the factory (Agrometeorological Information Center), it is possible to mathematically predict TPS color drop during storage. Using the $D_{70°C}$ for TPS degradation (844 h) and z = 16.3°C, equation 3 shows $D_{28.7°C}$ = 288460 h. Them, using equation 1, after 27 months (19440 h), 16 months (11520 h) and 3 months (2160 h) of storage, the TPS value should be respectively 40.2 (crop A), 42.1 (crop B) and 46.5 (crop C). With the exception of Crop A, the other predicted values were satisfactory.

Figure 1 shows the thermal map of an industrial process of concentrated tomato pulp, built from industrial temperatures and processing times. The time in each process step may vary according to the flow of the lines, which in turn is regulated according to the classification of the tomato received (initial solids concentration, color,

Table 6. Effect of the temperature of each stage of the processing of concentrated tomato pulp on the degradation of AA and TPS color.

Step			AA			Color TPS		
	T(°C)	Δt(h)	*DR**	*F*(h)**	*E*(%)***	*DR*	*F*(h)	*E*(%)
Harvest	28.7	0	0.22	0.00	0.00	0.00	0.00	0.00
Field/factory transport	28.7	2	0.22	0.44	0.14	0.00	0.01	0.00
Receiving/Drainage	28.7	6	0.22	1.32	0.41	0.00	0.02	0.01
Sampling/Selection	28.7	18	0.22	3.96	1.22	0.00	0.05	0.02
Water transport, washing, selection	28.7	1	0.22	0.22	0.07	0.00	0.00	0.00
Crushing, enzymatic inactivation	77.5	0.08	1.32	0.11	0.20	2.88	0.23	0.43
Enzymatic inactivation (holding)	77.5	0.42	1.32	0.55	1.02	2.88	1.21	2.23
Refining	70.0	0.08	1.00	0.08	0.11	1.00	0.08	0.11
Storage of juice (tank)	70.0	1	1.00	1.00	1.40	1.00	1.00	1.40
3° effect Concentration	55.0	0.42	0.58	0.24	0.20	0.12	0.05	0.04
2° effect Concentration	75.0	0.42	1.20	0.50	0.85	2.03	0.85	1.43
1° effect Concentration	90.0	0.42	2.08	0.87	2.53	16.87	7.08	18.78
Flash Cooler Tank	85.0	0.13	1.73	0.23	0.55	8.32	1.08	2.61
Heating for sterilization	106.5	0.25	3.81	0.95	4.99	173.49	43.37	90.28
Sterilization Flash Cooler (holding)	106.5	0.13	3.81	0.50	2.63	173.49	22.55	70.25
Cooling and aseptic filling	36.5	0.25	0.29	0.07	0.03	0.01	0.00	0.00
Equilibrium at room temperature	28.7	0.50	0.22	0.11	0.03	0.00	0.00	0.00
Storage (24 months)	28.7	17280	0.22	3805	99.999	0.00	50.56	14.54

**DR* = dimensionless rate of degradation (Equation 4). ***F(h)* = Equivalent process time (hours) at the adopted reference temperature of 70 °C (Equation 5). ****E(%)* = Corresponding degradation Effect (%) for each phase of the process (Equation 6).

and consistency).

From the thermal map and kinetic parameters of AA and TPS degradation, the *DR* at each stage of processing of the concentrated tomato pulp (Equation 4) was calculated. Then, it was possible to compare how much the heat treatment at each stage of the process affected the quality. The integration of *DR* over time allowed to obtain equivalent processes in the reference temperature (Equation 5), or the *F* value (hours). Table 6 presents these results for AA and TPS color.

AA loss occurs almost exclusively during the storage period of the concentrated pulp due to its high *z* value (62.84 °C). Probably the decrease in vitamin C over time helps to explain the sharp drop in the industrial storage, according to results presented by the crop A industrial pulp (Table 6), because AA works as an antioxidant, preventing other alterations.

The TPS index used to classify the tomato pulp in terms of color also shows its dependence on the process parameters. Although the store has an important influence on color degradation, the intensity of the sterilization step is also relevant. Improvements in the sterilization stage would allow improvement in the TPS index, as better-conditioned storage for prolonged storage.

In order to minimize losses, different scenarios of sterilization and storage conditions for concentrated tomato pulp can be simulated. In theory, the product would be conditioned in better storage conditions. A second planned change would be the optimization of the sterilization step, that is the heat treatment time/ temperature binomial, always considering the effectiveness in reducing microbial load. In this sense, considering the operation temperature range of the heat exchanger (104 to 108°C) and assuming 5 logarithmic cycles reductions of *Bacillus coagulans* spores, whose $D_{121.1\ °C}$ = 0.0347 min and z = 10°C - mean value according to Peng et al. (2012) - equivalent process can be proposed.

Table 7 shows the effect of heating treatment and sterilization flash cooler, changed from 106.5 to 107.5°C, and storage temperature (24 and 12 months), changed from 28.7 to 20°C, on AA degradation and color TPS retention.

As noted, comparing Tables 6 and 7, changes in the sterilization temperature (from 106.5 to 107.5°C) were more significant for vitamin C retention, while the storage temperature (from 28.7 to 20.0°C) helped color retention.

Conclusion

The consistency was not significantly affected by the heat

Table 7. Simulation of different heating treatments and storage conditions.

Step			AA			Color TPS		
	T(°C)	Δt(h)	*DR**	*F*(h)**	*E*(%)***	*DR*	*F*(h)	*E*(%)
Heating for sterilization	107.5	0.1986	3.95	0.78	4.3	199.81	39.68	89.06
Sterilization Flash Cooler (holding)	107.5	0.1033	3.95	0.41	2.3	199.81	20.64	68.36
Storage (24 months)	20	17280	0.16	2766	99.8	0.00	17.79	3.29
Storage (12 months)	20	8640	0.16	1383	95.6	0.00	7.40	1.66

**DR* = dimensionless rate of degradation (Equation 4). ***F(h)* = Equivalent process time (hours) at the adopted reference temperature of 70 °C (Equation 5). ****E(%)* = Corresponding degradation Effect (%) for each phase of the process (Equation 6).

treatment temperature and the Bostwick method (widely used in the industry) was satisfactory for this evaluation and quality control in tomato pulp. The Bostwick results were in practice comparable to the maximum force obtained by a back extrusion system.

The effect of temperature at each stage of the concentrated tomato pulp processing allows identifying the steps of sterilization and storage as those responsible for the greater part of the degradation of color and AA, respectively. Tomato industry must strictly control sterilization and increase the sun protection in the off-season in order to preserve AA and color, respectively, ensuring lower losses of sensorial quality without reducing flexibility, always prioritizing food safety

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Phytochemical components of beverages from African star apple (*Chrysophyllum albidum*) tissue fractions under ambient storage

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There is increasing awareness of functional potentials of African star apple (ASA) as an underutilized tropical fruit, yet little is known of alterations to its product properties during storage. Changes in antioxidant and antinutritional composition of beverages derived from ASA peel, pulp and seed coat fractions during 8-week storage at 26°C were investigated. Drinks derived from the peel were registered and retained significantly higher concentration of vitamin C (0.53 mg/ml, 45%) than pulp (0.17 mg/ml, 6%) and seed coat (0.35 mg/ml, 9%) drinks. Pulp fraction had higher initial flavonoid and total phenolic contents (6.44 mg/100 ml and 13.37 mg/ml) than peel (3.41 mg/100 ml and 3.05 mg/ml) and seed coat fractions (3.65 mg/100 ml and 3.04 mg/ml) but decreased significantly at 92 and 93%, 90 and 67% and 81 and 67% respectively. Beverages derived from the peel, pulp and seed coat showed 98.67, 75.30 and 76.97% DPPH and FRAP of 22.20, 10.90 and 11.87 µmol/ml respectively. A continuous decrease in oxalates and saponin, and slight increase in tannin and phytate levels were observed in all samples throughout the storage period. Findings confirmed the functional potential of ASA tissue beverages as rich sources of natural antioxidants. Effect of ambient storage on stability of studied bioactive compounds was found to be significant and may be a possible indication of product shelf life.

Key words: Bioactive components, antioxidant activity, anti-nutritional factors, *Chrysophyllum albidum*, storage stability.

INTRODUCTION

A rich diversity of edible fruit species make up part of the daily diet and provide nourishment to millions of people. Some indigenous tropical fruits especially those of Sapotaceae family (*Chrysophyllum albidum, Synsepalum dulcificum, Vitellaria paradoxa*) are rich sources of natural dietary bioactive compounds such as vitamin C, anthocyanin, flavonoids, phenolic acids etc. The protective effect of fruits in inhibiting or delaying oxidative stress in ageing and the reduced risk of chronic diseases have been attributed to the antioxidant potentials of their bioactive components (Arueya and Ugwu, 2017).

However, they are largely underutilized due to little or

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no value addition to the fresh fruits resulting in high post-harvest loss and accompanying environmental pollution at their annual peak season periods. Processing into drinks and juices for consumption are practical ways of extending shelf-life of fruits at household and commercial levels while conserving their inherent micronutrient components (Aguilar et al., 2017). The production processes however may involve heat treatments which accelerate undesirable degradation of inherent bioactive compounds (Andrés et al., 2016). Sensitive essential micronutrients in fruit juices may be degraded under process and storage conditions and consequently may be an indicator of their nutritional quality during storage.

African star apple (ASA) also known as 'Agbalumo' or 'Udara' (Nigeria), 'Azongogwe' (Benin) and 'Alaso' (Ghana) is a non-climacteric fruit widely distributed in the rain forest zones of Africa. The use of its parts in ethno-traditional medicine have been corroborated by research evidences and found to possess hepatoprotective, anti-plasmodial and pathological activities (Abiodun et al., 2011; Adewoye et al., 2010). Aqueous extract of ASA pulp inhibited resistant *Staphylococcus aureus* strains at higher concentrations than ciprofloxacin, a synthetic antibiotic (George et al., 2018). Nutritionally, ASA pulp possesses higher vitamin C content at 446 mg/100 g than mango, pineapple, pawpaw and hog plum at 98.0, 38.3, 39.3 and 10.1 mg/100 g respectively (Edem et al., 1984; Ellong et al., 2015). Proximate composition and antioxidant activity of ASA follow a tissue distribution with a higher radical scavenging potential than common tropical fruits like pawpaw, pineapple and hog plum (Abiodun and Oladapo, 2011; Bello and Henry, 2015). Variations have been documented with anti-nutrients in ASA tissue fractions (Ibrahim et al., 2017). Acceptable single strength juice has been produced from ASA (Dauda et al., 2017).

During juice processing, peels and seed pericarp of the fruit are discarded as waste products but have been shown to contain higher nutritive constituents than pulp fractions (Abiodun and Oladapo, 2011). To the best of our knowledge, there are no reported investigations into the systematic comparison of antioxidant and anti-nutritional activities of beverages from parts of ASA fruit during ambient storage. Accordingly, this study aimed to elucidate influence of storage on antioxidant and anti-nutritional properties of drinks made from peels, pulp and seed coat fractions of ASA.

MATERIALS AND METHODS

Sample preparation

Ripe, firm ASA fruits (150 pieces) were collected from Oje fruit market in Ibadan, Nigeria during 2017/2018 harvest season. Fruits were sorted, washed with 0.15 g/L chlorinated water, rinsed with tap water and blanched at 80°C for 3 min to aid separation of peel from the pulp. Seed coats were removed from each seed.

Juice was produced from pulp according to the method of Dauda et al. (2017). Peel and seed coat fractions were pulverized separately (Marlex CM/L7962804 food liquidizer, KIL, DAMAN) with activated carbon filtered tap water (30% w/v). The mixtures were filtered using muslin cloth, pasteurized at 80°C for 5 min and bottled for further analysis. Triplicate sample analyses were carried out on all fractions after 0, 14, 28, 42 and 56 days of ambient storage (26.5±2°C).

Antioxidant analysis

Determination of ascorbic acid content (AA)

2, 6 dichlorophenolindophenol (DCPIP) assay with spectrophotometric analysis was used to determine ascorbic acid concentration in the samples according to a method described by Bungau et al. (2011) with modifications. Samples were diluted in 0.1 M citric acid and 0.1 M sodium citrate buffers at ratio 1:40 and 1:20. The spectrophotometer (T70 UV-VIS spectrophotometer, PG instruments, Alma Park, UK) absorbance was read at 520 nm. Two milliliters of DCPIP was added to 10 ml of each sample and the tube immediately capped. Absorbance was read within 5 min of adding DCPIP solution. Values for AA were expressed as mg/ml of sample

Determination of total flavonoid content (TFC)

Aluminum chloride colorimetric method ($AlCl_3$) was used for the determination of the TFC of samples. About 1.5 ml of each sample was mixed with 5 ml distilled H_2O and 0.3 ml 5% $NaNO_2$ and 1.5 ml of 2% methanolic $AlCl_3$ solution was added after 5 min. Double distilled water (ddH_2O) was used instead of sample as blank. Two millilitre of 1 mol/L NaOH was added after 5 min and volume made up to 10 ml with ddH2O. Mixture was shaken on orbital shaker for 5 min at 200 rpm. Absorbance was read at 367 nm after 10 min incubation period. Total flavonoid contents were calculated using a standard calibration curve prepared for quercetin and expressed as mg quercetin/100 mL of sample (Jagadish et al., 2009).

Determination of total phenolic compounds (TPC)

The Folin–Ciocalteau method was used to quantify the total phenolic compounds by spectrophotometry. About 0.5 ml of sample was introduced into test tubes followed by 2.5 ml of 10% Folin Ciocalteu reagent (Sigma Chemical, St. Louis, Missouri, USA) and 2 ml of 7.5% Na_2CO_3. Mixture was allowed to stand for 30 min at 37°C and absorbance was read at 765 nm. Total phenolic content was expressed as milligram of gallic acid equivalent (GAE) per ml of sample (mg GAE/mL) (Chan et al., 2009).

Determination of ferric reducing antioxidant power (FRAP)

Reducing power was determined using the method of Benzie and Strain (1999) as modified by Adeboyejo et al. (2016). FRAP reagent was prepared by mixing 5 ml of 2,4,6-tris (2-pyridyl)-1,3,5triazine (TPTZ) solution (10 mM) in 40 mM hydrochloric acid solution with 5 ml $FeCl_3.6H_20$ solution (20 mM) and 50 mM acetate buffer solution (0.3 M, pH 3.6) and incubated at 37°C after mixing. Extract 150 µl was mixed with 2850 µl of FRAP reagent for 30 min under dark conditions. The absorbance at 593 nm of 200 µl of the mixture was determined (T70 UV-VIS spectrophotometer, PG instruments, Alma Park, UK). FRAP values were compared with quercetin (µmol/ml).

DPPH Radical-scavenging Activity

Free radical scavenging activities of samples were determined using the 1, 1-diphenyl-1, 2-picrylhydrazl (DPPH) method by Blois (1958) and Botchway et al. (2007) with modifications. A solution of 0.002% µM DPPH was prepared by dissolving 0.002 g of DPPH in 80% ethanol. 400 µl of sample was added to 7.6 ml of 0.002% DPPH and left in the dark for 30 min. Concentrations of 0.2, 0.1, 0.05, 0.025, 0.020 and 0.01 mg/ml gallic acid were used to plot the standard curve. The reduction ability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants after 30 min incubation in the dark. Ethanol was employed as a blank and absorbance read. Activities of test samples were determined as percentage reduction or inhibition of DPPH.

Determination of total antioxidant activity (TAC)

Total antioxidant activity of samples were determined by the method of Prieto et al. (1999). 0.1 ml sample (100 µg/ml) was added to 1.9 ml of reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate). The blank solution contained only 2 mL of reagent solution. The absorbance was measured at 695 nm after 60 min. Ascorbic acid was used as standard and total antioxidant capacity was expressed as milligrams of ascorbic acid equivalents (AAE) per 100 ml of sample.

Anti-nutritional factors assay

Determination of tannin

Tannin was quantitatively determined as reported in the manual of food quality control (AOAC, 1984). 0.5 ml of each sample was mixed with 10 ml of 80% ethanol. This was shaken and allowed to stand for 1 h and 1 ml of extract was pipetted into another test tube. 5 ml distilled water, 2 drops of $FeCl_2$ in 0.1M HCl was added, shaken and 4 drops of potassium ferrocyanide was added. Absorbance of mixture was read at 620 nm

Determination of saponin

Saponin content was determined as described by Brunner (1984).

Oxalate content determination

Oxalate content was determined according to the method described by Oke (1966).

Determination of phytate

Phytate analysis was done following a modified procedure of Sivakumaran et al. (2017). Sample (2.0 ml) was extracted with 40 ml of 2.4% HCl (68.6 ml of 35% hydrochloric acid in total volume of 1 L of D_2O) while constantly shaking at 25°C for 3 h. All extracts were filtered and phytate content determined at 640nm in a spectrophotometer. Amount of phytic acid was calculated from organic phosphorus.

Statistical analysis

Data obtained were analysed using Statistical Package for Social Sciences (SPSS) version 21. One-way ANOVA was used to determine the means expressed as mean ± standard deviation (SD) followed by Duncan post-hoc test. Test of significance was done at 5% probability level ($P<0.05$).

RESULTS AND DISCUSSION

Antioxidant assay

Ascorbic acid content (AA)

Data obtained for fresh and stored beverages from ASA fractions are summarized in Figure 1. Evidently, peel fraction contained significantly higher vitamin C level (0.53 mg/ml) than pulp (0.17 mg/ml) and seed coat (0.35 mg/ml) fractions. A steady, rapid ($p<0.05$) loss of vitamin C was observed in the pulp juice while peel drink retained over 45% of its initial vitamin C content after 56 days of storage. Del Caro et al. (2004) reported similar loss of vitamin C in stored citrus fruit juice while variations have been reported by methods of extraction and maturity stage of fruits (Hernández et al., 2005). However, ascorbic acid content was stabilized by sonication and thermosonication treatments during storage (Aguilar et al., 2017). Intense irreversible oxidation of L-ascorbic acid, the biologically active form of vitamin C into diketogluconic acid in the presence of air may explain loss of vitamin C in samples. Previous studies showed that free radicals are largely generated in fruit juices produced under aerobic systems, causing oxidation of vitamin C, which thus may explain the result obtained in this study.

Flavonoid

Mean concentrations of flavonoids obtained for ASA peel, pulp and seed coat drinks during storage are shown in Figure 2. Total flavonoid content was highest in pulp sample before storage (6.44 mg/100 ml) but significantly decreased ($P<0.05$) by 73.3, 84.6 and 93.3% after two (1.72 mg/100 ml), four (0.99 mg/100 ml) and six (0.43 mg/100 ml) weeks respectively with slight increase at eight weeks (0.49 mg/100 ml). This indicates a 7.61% retention of original flavonoid content in pulp drink. The seed coat drink retained highest amount at 18.90% while peel drink retained only 10.26% showing a significant variation due to tissue fraction and storage. Hoffmann et al. (2017) similarly observed a trend of total flavonoid degradation during a 3 months storage of *Butia odorata* fruit pulp. 86% loss of initial flavonoid in rowanberry fruit stored at 22^{o}C after 20 weeks of storage has also been reported (Baltacioğlu et al., 2011). Ibrahim et al. (2017) reported flavonoid contents of fresh peel, pulp and seed coats fractions of ASA fruit at 17.23, 11.24 and 15.11% respectively. These results are in reverse order to those observed for drinks produced from similar fractions. Degradation of flavonoids in fruit tissues

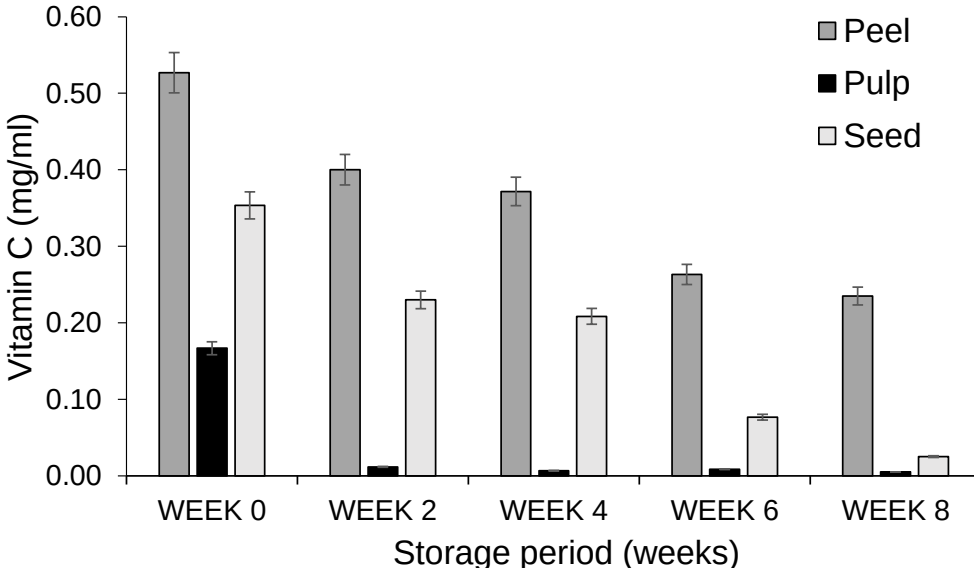

Figure 1. Changes in Vitamin C content of drinks from African star apple peel, pulp and seed coat fractions during storage.

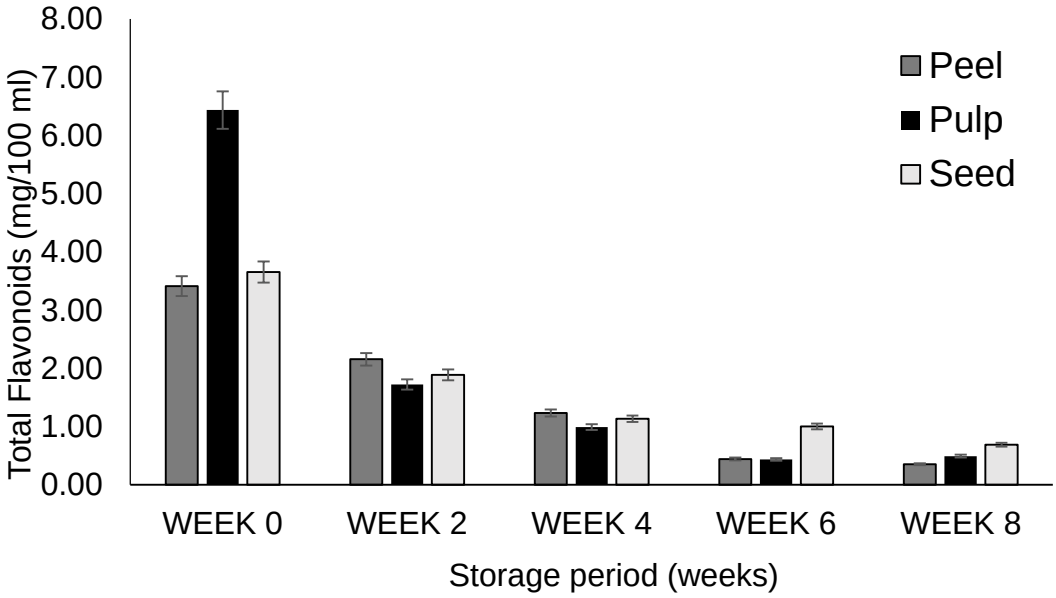

Figure 2. Changes in total flavonoid content of drinks from African star apple peel, pulp and seed coat fractions during storage.

and their products may be attributed to activity of enzymes polyphenol oxidase and peroxidase, initiated and sustained by temperature, light, pH and reaction of other components in the product matrix.

Total phenolic content (TPC)

The phenol concentrations in pulp sample analyzed at week 0 differed significantly from those of peel and seed drink samples as shown in Figure 3. Values ranged from 3.04 and 3.05 mg/ml in seed and peel drinks to 13.37 mg/ml in pulp juice. A continuous rapid decrease in TPC was observed during the 56 days storage period for pulp juice with only 6.43% of original content retained at the end. Peel and seed drinks however retained 33.12 and 32.24% of phenol after storage duration respectively. This indicates a higher rate of

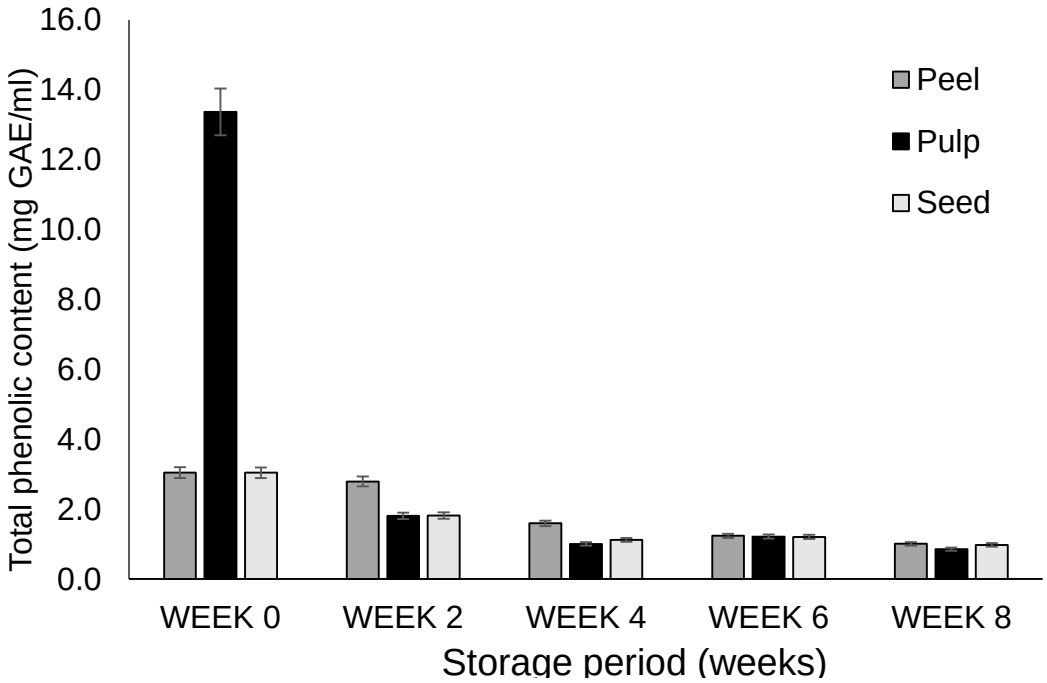

Figure 3. Changes in total phenolic content of drinks from African star apple peel, pulp and seed coat fractions during storage.

phenolic content degradation in the pulp fraction beginning from the 2nd week of storage after which 86.46% of phenolic content had been lost. Structural features of major phenolic hydroxyl groups found in plant material may influence the variation of total phenolic content in fruit tissue fractions. The marked reduction of total phenolic content could also result partly from increased oxidation of phenolic substrate to quinone occasioned by high average ambient temperature (26.5±2°C) of storage. Decreased synthesis of phenolic compounds in fruits and juices as a result of storage temperature fluctuations have been reported (Galani et al., 2017). Phenolic compounds act as antioxidants by forming stable radical intermediates, preventing further oxidative processes in food products. Thus, amount of these compounds detected by analysis may be determined by the bound-status of different tissue fractions of fruit.

Ferric reducing antioxidant power (FRAP)

FRAP assay quantifies the total reducing capability of antioxidants as a measure of the total antioxidant power in which the antioxidants act as reductants in a redox colorimetric reaction, releasing hydrogen atom to the ferric complex produced to discontinue the radical chain reaction (Singh and Rajini, 2004).

The reducing power of the beverages from peel, pulp and seed coat fraction of ASA in storage is presented in Figure 4. The peel fraction exhibited the highest reducing power (22.20 µmol/ml) followed by the seed (11.87 µmol/ml) and pulp (10.90 µmol/ml) fractions. This agrees well with the trend obtained by Prasad et al. (2010) for peel, seed and pulp fractions of *Canarium odontophyllum* fruit extracts. Guo et al. (2003) earlier confirmed a general trend of similar tissue variation of FRAP values for some fruits. Significant losses of 6.44, 18.11, 51.49 and 63.20% in reducing power of the peel fraction were however observed after 14, 28, 42 and 56 days of ambient storage respectively. Although, initially lower than values recorded for peel fraction (at 53.47 and 49.1% respectively), rate of FRAP loss in seed and pulp fractions were stabilized over the storage period with a total loss of 25.27 and 27.80% compared to 63.20% in the peel fraction after 56 days.

DPPH radical scavenging activity

2, 2-di (4-tertoctylphenyl)-1-picrylhydrazyl (DPPH) method is important in quantifying the antioxidant capacity of fruit and vegetable juices or extracts. The DPPH radical scavenging activity of beverages from all fractions of ASA fruit decreased as storage days increased (Figure 5). The peel fraction exhibited the highest scavenging activity at 98.67% compared to pulp (75.30%) and seed coat fractions (76.97%). The seed coat fraction however retained the most activity during storage (71.27%). The scavenging activity loss was in the order pulp>peel>seed

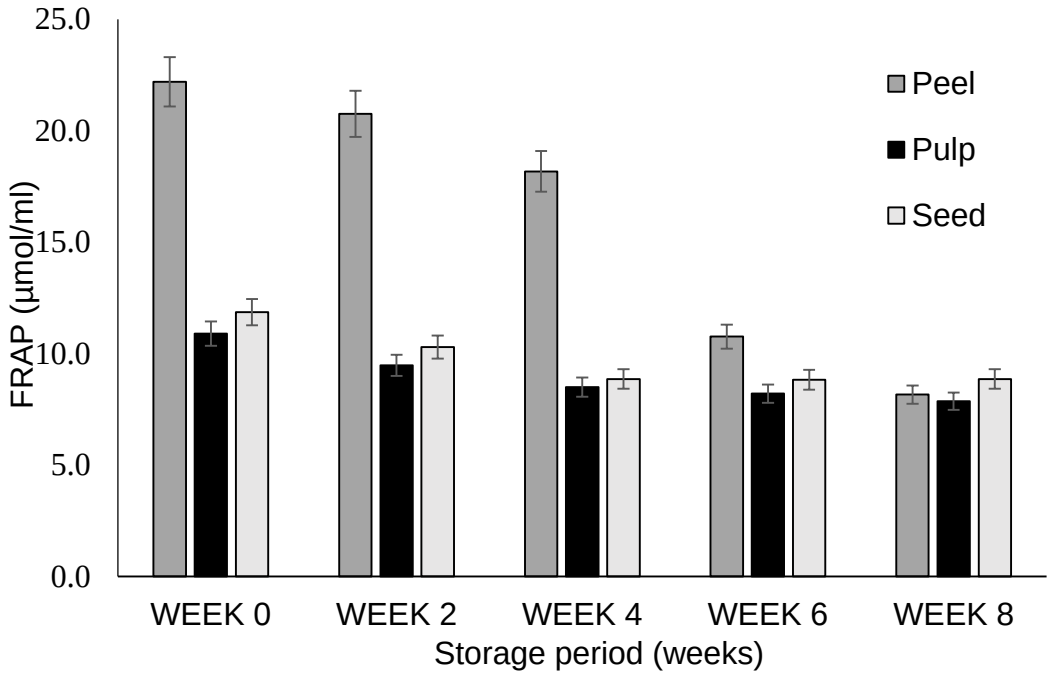

Figure 4. Changes in ferric reducing antioxidant power of drinks from African star apple peel, pulp and seed coat fractions during storage.

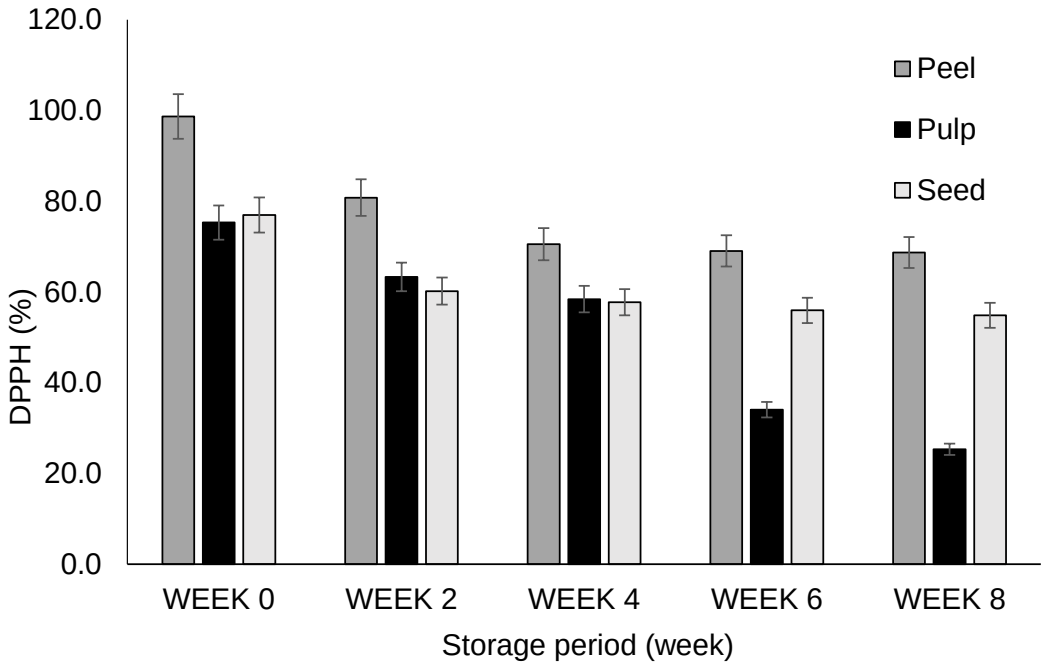

Figure 5. DPPH free radical scavenging activity of drinks from African star apple peel, pulp and seed coat fractions during storage.

at 66.35, 30.4 and 28.73% respectively after 56 day storage. The pulp juice significantly ($p<0.005$) lost activity of 32.34% between 28 and 42 days alone compared to the loss of only 6.5% between the 14th and 28th days and 11.61% between 42 and 56 days of storage. This implies that the product life cycle of pulp juice till it reach

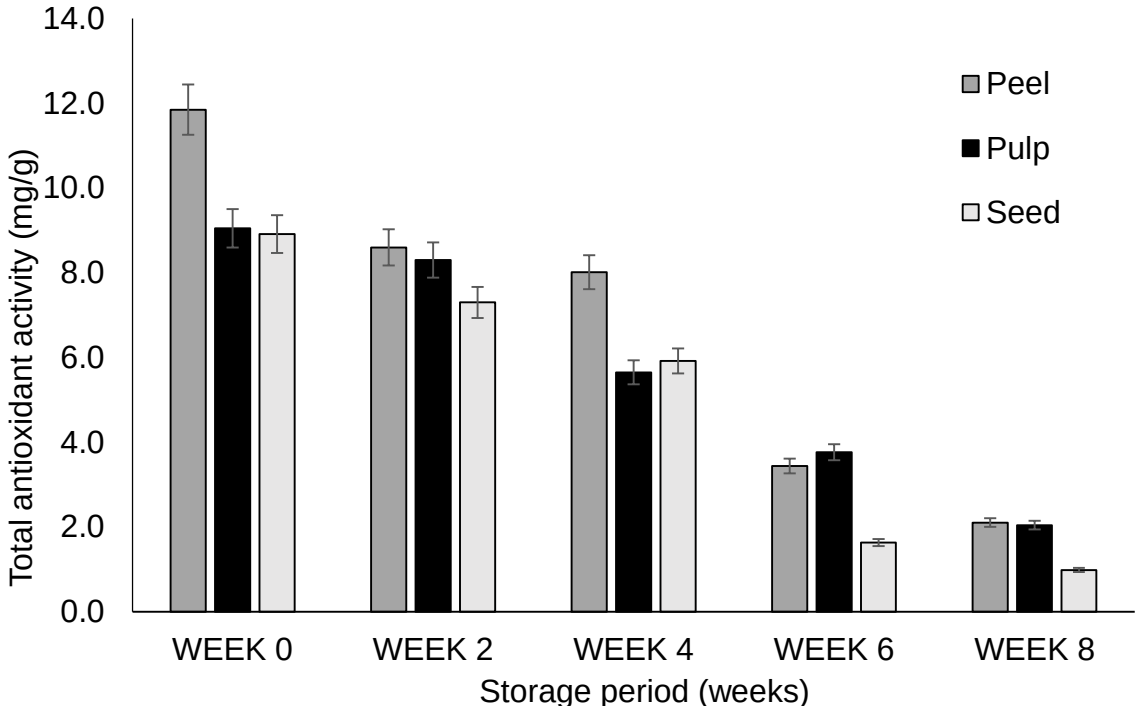

Figure 6. Changes in total antioxidant activity of drinks from African star apple peel, pulp and seed coat fractions during storage.

consumers may be set within 28 days of production during which the product maintains over 77% of its capacity to inhibit the DPPH radical.

Total antioxidant activity

Antioxidant activity of beverages made from fruit tissues as measured using phosphomolybdate solution is presented in Figure 6. During storage, a significant ($p<0.05$) decrease in antioxidant activity was noted. Initial values ranged from 11.85 mg AAE/100 ml (peel) to 9.05 mg AAE/100 ml (pulp) and 8.92 mg AAE/100 ml (seed). Over 56 days of ambient storage, retention of antioxidant activity of pulp juice was significantly higher (22.54%) than peel (17.72%) and seed coat drinks (10.99%), although peel drink had initial higher values. It seems oxidative reactions in the beverages during storage were initiated by light and heat conditions encountered during processing. Reduction in antioxidant activity has been postulated to be due to oxygen induced degradation of vitamin C and other polyphenolic constituents in fruits (Galani et al*.,* 2017). Oxidative stability in fruit beverages may thus be monitored by assessing stability of its vitamin C content during storage. The different tissue fractions, and consequently their products are composed of diverse types and quantity of bioactive compounds which may have influence on their antioxidant activity.

Anti-nutritional factors

Tannin

Tannins are heat-stable, non-nutritive secondary metabolites and polyphenolic compounds known to have bitter, astringent tastes. Table 1 shows tannin contents of beverages from peel, pulp and seed coat fractions of ASA fruit. Tannin contents increased steadily from 1.79 to 2.77%, 0.17 to 1.23% and 1.83 to 2.61% in peel, pulp and seed coat fractions respectively over the storage period. The pulp juice has a significantly ($p<0.05$) lower content of tannin than others. Omoboyowa et al. (2016) reported similar values of 127.77 mg tannin in 100 g of fresh *C.albidum* seed cotyledon as observed for drinks from the seed cotyledon in this study. However, our values are lower than those reported for tropical fruits like banana (3.4 mg/g), apple (8.5 mg/g), guava (20.36 mg/g), pawpaw (10.16 mg/g) and sweet orange (48.16 mg/g) (Onibon et al., 2007). The increase in tannin content on storage could be as a result of increased bioaccesibility of tannins in the chloroplast structure released through mechanical homogenization and pasteurization during processing. Furthermore, irreversible oxidative transformation of proanthocyanidins, some flavonoids monomers and polyphenols to form new tannin-like compounds in the presence of polyphenol oxidase as catalyst may explain the increase in tannin content on storage.

Table 1. Variation of anti-nutritional factors in African star apple peel, pulp and seed coat beverages during ambient storage.

Samples	Duration of storage (weeks)	Tannin	Phytate	Oxalate	Saponin
Peel drink	0	1.79±0.08^{b}	0.95±0.01^{ab}	0.06±0.01^{a}	0.05±0.04^{a}
	2	1.94±0.05^{b}	1.04±0.05^{a}	0.05±0.01^{ab}	0.01±0.00^{b}
	4	2.54±0.10^{a}	1.05±0.05^{a}	0.03±0.01^{ab}	0.01±0.00^{b}
	6	2.64±0.09^{a}	1.03±0.02^{a}	0.03±0.01^{ab}	0.01±0.00^{b}
	8	2.77±0.04^{a}	1.02±0.02^{a}	0.03±0.01^{ab}	0.01±0.00^{b}
Pulp juice	0	0.17±0.15^{e}	0.64±0.02^{d}	0.03±0.01^{ab}	0.00±0.00^{b}
	2	0.44±0.11^{e}	0.71±0.02^{cd}	0.03±0.01^{ab}	0.03±0.00^{ab}
	4	0.92±0.02^{d}	0.88±0.01^{b}	0.03±0.01^{ab}	0.02±0.01^{ab}
	6	1.17±0.07^{cd}	0.89±0.01^{b}	0.03±0.01^{ab}	0.01±0.00^{b}
	8	1.45±0.39^{c}	0.82±0.13^{bc}	0.02±0.00^{b}	0.01±0.01^{b}
Seed coat drink	0	2.05±0.44^{b}	0.68±0.03^{d}	0.06±0.03^{a}	0.00±0.00^{b}
	2	2.48±0.11^{a}	0.73±0.01^{c}	0.04±0.02^{ab}	0.01±0.00^{b}
	4	2.56±0.10^{a}	0.78±0.04^{c}	0.03±0.01^{ab}	0.01±0.00^{b}
	6	2.63±0.02^{a}	0.76±0.03^{c}	0.03±0.01^{ab}	0.01±0.00^{b}
	8	2.60±0.05^{a}	0.77±0.03^{c}	0.03±0.01^{ab}	0.01±0.00^{b}

*Means ± Standard Deviation superscripted by different alphabets within the same column are significantly different (n=3) ($p<0.05$).

Saponin

Saponin contents of *C. albidum* beverages are shown in Table 1. Data obtained for all samples are significantly different with peel drink having the highest content at 0.049%. The pulp juice had increased saponin content after 14 days (0.03%) which gradually reduced till end of storage period. After 28 days, all saponin contents stabilized at <0.01%. Ibrahim et al. (2017) established that freeze-dried peel fraction of *C. albidum* contained higher saponin content (0.41%) than pulp (0.007%) and seed coat (0.09%) fractions, which corresponds well with our findings in this study. Although saponins are regarded as anti-nutrients in food, research evidences show that they have beneficial hypocholesterolemic effects in human diets because they form insoluble complexes with cholesterol, thereby inhibiting their absorption (Aletor, 1993).

Oxalate

The oxalate levels in ASA beverages are shown in Table 1. The peel and seed coat drinks had 0.06 mg/g oxalate contents while pulp juice recorded 0.03 mg/g. These values reduced by half after 4 weeks of storage and stabilized till the end of storage period, although these changes were not significant ($p<0.05$). Oxidative degradation of phenolic compounds as a result of change in pattern of enzyme activity, particularly chloroplast phenolase may be responsible for oxalate degradation in the products under study. Oxalate contents in three varieties of ASA were reported in the range of 0.528-0.538 mg/100 g (Adepoju and Adeniji, 2012). Oxalate nephropathy with acute renal failure has been reported for ingestion of large amount of sour and *Averrhoa bilimbi* fruit juices (Miah et al., 2018).

Phytate

The phytate content in the beverage made from peel increased slightly but insignificantly ($p<0.05$) from 0.95 mg/100 g at week 0 to 1.02 mg/100 g at week 8 (Table 1). Pulp juice phytate level increased steadily but not significantly after 4 weeks of storage till end of storage period from 0.64 mg/100 g to 0.90 mg/100 g. Phytate content in three varieties of *C. albidum* was found to range from 0.037 to 0.062 mg/100 g in fresh pulp (Adepoju and Adeniji, 2012), values markedly lower than those observed in this study. These values are significantly lower than 2.88-10.71 mg/g range of phytate content reported for common tropical fruits (Onibon et al., 2007). The slight decrease of phytate levels from 6 weeks of storage is probably related to the capacity of endogenous phytates to be metabolized on incubation. Al Hassan et al. (2016) in their study of phytate in diets of pregnant women concluded that phytate is the strongest inhibitory predictor of mineral bioavailability as it is significantly associated with bioavailability of calcium, iron

and zinc from diet.

Conclusion

This study reported the influence of storage time on antioxidant and anti-nutritional properties of beverages from peel, pulp and seed coat fractions of African star apple. During the storage period, all studied bioactive components decreased significantly ($p<0.05$) as storage time increased except tannins and phytates which increased slightly. Highest FRAP and DPPH free radical scavenging activities were retained in the pulp and seed drinks while the peel drink retained highest Vitamin C contents after storage. The results indicated that impact of storage time at ambient conditions on retention of bioactive components of ASA tissue fraction beverages is significant and may be a major deciding factor in setting product shelf life. This study therefore provides invaluable information for guiding bioavailability and oxidative stress biomarker assays for future studies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Development and characterization of biodegradable films from fermented yam (*Dioscorea trifida* L. f.)

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The objective of this work was to develop and characterize biodegradable starch films at 14 and 21 days of fermentation, aiming to develop packaging with added value. For the extraction of starch, yam tubers were washed, crushed, filtered and fermented for 14 and 21 days, and then filtered and dehydrated. Water-starch-glycerol mixtures were prepared in a randomized design using a factorial scheme (5x2), with five concentrations of glycerol (0, 0.5, 1.0, 1.5 and 2.0 mL) using two durations (14 and 21 days) and 6 replicates. The analyzed parameters were thickness, density, solubility, sorption kinetics, humidity, scanning electron microscopy characteristics and color. Analysis of variance revealed significant difference at the 1% level for the interaction of fermentation time and glycerol concentration for the studied variables. Overall, a 21 day fermentation period gave better characteristics of density, humidity and color and the addition of plasticizer positively influenced the parameters studied.

Key words: Gelatinization, packaging, fermentation, starch.

INTRODUCTION

Growing demand for both higher quality food and shelf life extension, along with improving environmental management policies, has intensified the search for new methods and technologies to improve food conservation (Pereda et al., 2011; Almeida, 2014). Among these, packaging plays an important role for the food industry, as it must contain the product, and preserve and maintain its quality and safety, while acting as a barrier to factors responsible for deterioration (Coles, 2003). Growing concern about food safety, shelf life extension, cost-effectiveness, consumer convenience and environmental problems has driven the development of both new packaging forms and new raw materials for its production (Coles, 2003). Currently, there is much interest in the

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development of biodegradable packaging, which is designed to interact with the food, both prolonging shelf life and/or conferring desirable sensory and nutritional characteristics (Almeida et al., 2013).

Biodegradable packaging is a sustainable development alternative to petrochemical-based products, being generated from renewable resources it can also increase income for local farmers (Arenas, 2012). Developing the use of natural products in biodegradable packaging is of great interest to both industry and society in general, since it brings benefits for food-based industries and the environment (Dantas et al., 2015).

In addition, the use of packaging based on nationally-source materials in packaging manufacture enhances the profitability of Brazilian agroindustry (Reis, 2011). Among the raw materials used in the preparation of biopolymers, the potential of starch, either as pulp or granules, as a source biodegradable films has been widely studied, as it is renewable, cheap and widely available (Cano et al., 2014; Santos, 2015). It can be obtained from a variety of plant sources, including cereals, roots and tubers, as well as seeds and fruit pulp, with the derived materials having different physical, chemical and functional characteristics depending on their origin (Mali el al., 2010).

Among the various sources of starch is the yam, a member of the family Dioscoreaceae and a producer of starch-rich, high-energy and nutritious food tubers (Oliveira, 2002). These contain between 28.1 and 29.5% dry matter, of which 70.3 to 79.5% is starch, 1.7 to 4.3% is reducing sugars, 0.6 to 2.9% fibers, and 4.6 to 7.1% proteins (Leonel and Cereda, 2002). In addition, the plant has known anti-microbial, diuretic and energizing properties (Ramos-Escuredo et al., 2010).

Dioscorea yams are the fourth most important tuber/root crop in the world, lying only behind potato (*Solanum tuberosum* L.), cassava (*Manihot esculenta* Crantz) and sweet potato (*Ipomoea batatas* L.). However, because it is not included in the list of "noble" crops, the genus is rarely included in agricultural policies, government projects, economic and financial plans for exportable monocultures, and even if the area cultivated is more than one million hectares worldwide. In Brazil, this yam is typically a smallholder crop used for direct consumption or local sale (Leonel and Cereda, 2002). Thus, with the aim of adding value to the by-products of yam culture, the objective of this study was to develop and characterize biodegradable films of starch derived from this yam after 14 and 21 days of fermentation.

MATERIALS AND METHODS

Study area

Experiments were conducted at the Food Chemistry and Physics Laboratory, National Amazon Research Institute (3°5'29"S, 59°59'37"W), and at the Vegetable-Origin Products Technology Laboratory at the Amazonas Federal University (3°5'28"S, 59°57'57"W) in Manaus, Amazonas State, Brazil.

Sample preparation

Artisanal techniques adapted from the methods described by Leonel and Cereda (2002), and Nunes et al. (2010) was used in the preparation of the mash to be fermented. The tubers were cleaned to remove soil and foreign bodies, peeled, washed, and sectioned into smaller pieces. The filtrate was fermented for 14 and 21 days, respectively, with water changed every two days at a temperature of 40°C. At the end of the fermentation period, the supernatant was discarded and the fermented material dehydrated at room temperature, then sieved, crushed and stored in plastic pots.

Physico-chemical analysis of yam tuber filtrate

Physico-chemical analysis was conducted in triplicate, following the methods of the Instituto Adolfo Lutz- IAL (2008) and the Associação de Químicos Analíticos Oficiais– AOAC (1980).

Moisture content

This was determined following IAL (2008) protocol 012/IV. Samples were dried in a vacuum kiln at 105°C for 24 h.

Ash content

This was determined by incineration, following protocol 923.03 of AOAC (1980) in a crucible at 550°C until calcination was complete.

Mineral content

This was determined by atomic absorption spectrometry, following protocol 394/IV of IAL (2008), and the guidelines in Varian (2000). Samples were prepared in a MARS-Xpress microwave digester (CEM Corporation, MD-2591), with organic material mineralized with concentrated nitric acid sequentially diluted with deionized water. Readings were made directly from diluted solutions in an atomic absorption photo spectrometer (Spectra AA, model 220, FS, Varian 2000), using manufacturer-specified light sources. The analyzed elements were: Ca, K, Na, Mg, Fe, Zn, Mn and Cu.

Lipids

Lipid content was determined following IAL (2008) protocol 032/IV, with samples obtained via a Soxhlet extractor with petroleum ether.

Protein

Protein content was determined by the micro-Kjedhal method, following AOAC (1980) protocol 926.86, using a 6.25 conversion factor.

Crude fibre

For the quantification of soluble and insoluble digestible fibre, the enzymatic-gravimetric method given in IAL (2008) protocol 046/IV was used. The proportion of total fibre was determined by adding the values obtained for the soluble and insoluble fractions.

Carbohydrates

Carbohydrate content was determined following IAL (2008) for

Table 1. Formulation of films from fermented yam mash filtrate.

Sample	Starch (g)	Distilled water (mL)	Glycerol (mL)
I	1.125	30.0	0.00
II	1.125	30.0	0.50
III	1.125	30.0	1.00
IV	1.125	30.0	1.50
V	1.125	30.0	2.00

cereals, starches and soya extracts, using centesimal differences of the sum of moisture, ash, lipids and dietary fiber.

Starch

Starch content was determined following protocol AOAC 996.11 modified by Water et al. (2005). Total starch was determined by subtracting the sum of the available starch and all other non-starch residuals from total weight.

pH

Hydrogenation potential was determined following protocol 017/IV of IAL (2008) by direct reading of the supernatant liquid.

Soluble achohol acidity

This was determined following IAL (2008) protocol 415/IV. Samples were titrated with 0.1 mol NaOH until rose coloration appeared.

Biodegradable film production

Films were made following a method described by Dantas et al. (2015) and Lorotonda (2002). Water-starch-glycerol mixtures were prepared by varying the glycerol concentration in relation to the starch-water mixture (Table 1). A randomized factorial scheme (5x2) design was used, with five concentrations of glycerol (0.0, 0.5, 1.0, 1.5 and 2.0 mL), two fermentation durations (14 and 21 days) and 6 replicates, a total of 60 experimental units.

The mixtures were heated to boiling, and the temperature maintained at about 185°C until, under constant stirring, a gel formed. This was then spread on 8 cm^2 petri dishes, and oven cooled at 40°C for 48 h.

Characterizing the films

The starch films were tested for thickness, solubility, moisture, density, sorption kinetics, scanning electron microscopy characteristics and color.

Thickness

Thickness was measured with digital calipers at 10 randomized points across the surface of each film (Batista, 2005).

Solubility in water

The solubility tests were by difference of the initial and final mass of the sample (Matta Júnior et al., 2011).

Scanning electron microscope analysis of biofilm structure

The films were plated with gold and surface structure visualized with a scanning electron microscope (FEI, model QUANTA 250).

Density

Density was determined by the difference between the final and the initial mass in a desiccator (Müller et al., 2008).

Water sporption and humidity kinetics

Moisture sorption kinetics was determined by weighing at successive time intervals until a constant weight was obtained (Mali et al., 2005).

Optical properties: Color

Color was determined using a portable color photospectrometer (Miniscan XE, Hunter Lab, Reston, Virginia, USA)(Hunterlab, 1997).

Statistical analysis

Analyzes of variance (ANOVA) and Tukey's test were performed to compare means, with significance level at 5%. Calculations were performed using Assitat version 7.7.

RESULTS

Physico-chemical analysis of yam tuber filtrate

The final product was a fine, odorless powder with color varying according to the number of days of fermentation: At 14 days it had a pink coloration, while samples from 21 days fermentation were whitish (Figure 1). Table 2 gives mean values and standard deviations of the physico-chemical composition of the fermentates studied.

At the 1% probability level, Tukey tests showed that ash, crude protein, lipids and pH had statistically different values ($p \leq 0.01$) for studied fermentates in relation to the fermentation period. Highest average values were found from the 21 days fermentation sample (Table 2).

The variation in pH between the samples is worthy of comment: After 14 days of fermentation, the starch-rich fermentate was slightly acidic, however, after 21 days the pH has risen dramatically, so that the samples were

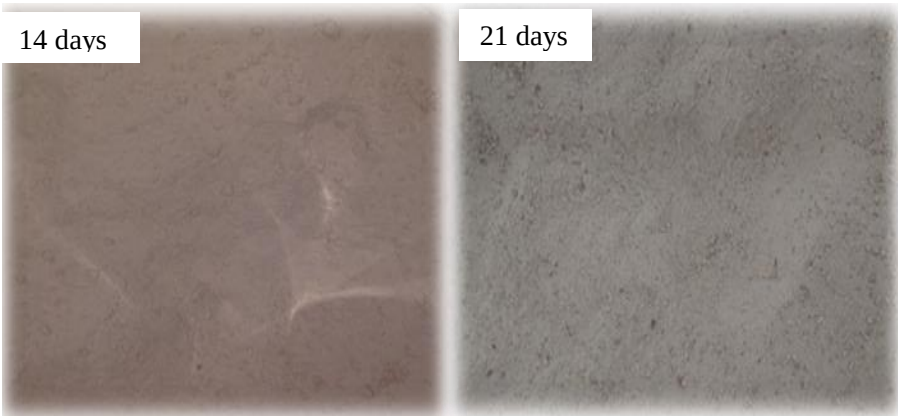

Figure 1. Character starch at 14 days and 21 days of fermentation.

Table 2. Mean values and standard deviations of the physico-chemical parameters analysed for cush-cush yam fermentates for two different fermentation durations.

Parameter*	Sample	
	14 days fermemtation	21 days fermentation
Dry material	77.61 ± 3.62[a]	81.38 ± 2.88[a]
Moisture (%)	22.39 ± 3.62[a]	18.62 ± 2.88[a]
Ash (%)	0.05 ± 0.01[b]	0.31 ± 0.01[a]
Lipídeos (%)	0.21 ± 0.06[b]	0.55 ± 0.22[a]
Crude Protein (%)	0.79 ± 0.01[b]	0.99 ± 0.10[a]
Total Fibre (%)	1.02 ± 0.07[a]	1.16 ± 0.06[a]
Carbohydrates (%)	75.55 ±3.66[a]	78.38 ± 2.99[a]
pH	6.00 ± 0.30[b]	8.73 ± 1.56[a]
Soluble Alcohol Acidity (%)	1.13 ± 0.12[a]	1.27 ± 0.12[a]
Starch	87.35 ± 0.11[a]	87.32 ± 0.16[a]

*Means of 3 samples ± standard deviations. *CV% = 15.96* (Moisture)*; DMS = 7.42399* (Moisture); *CV% = 6.86* (Ash); *DMS = 0.02743* (Ash); *CV% = 29.77* (Lipids)*; DMS = 0.2549* (Lipids); *CV% = 5.62* (Crude Proteín)*; DMS = 0.11336* (Crude Proteín); *CV% = 6.19* (Total Fibre)*; DMS = 0.15305* (Total Fibre); *CV% = 4.34* (Carbohydrate)*; DMS = 7.58009* (Carbohydrate); *CV% = 3.07* (pH)*; DMS = 0.51266* (pH); CV% = 9.69 (Titratable acidity); DMS = 0.2636 (Titratable acidity); *CV% = 0.17* (Starch); *DMS = 0.3368* (Starch). Means followed by the same letters do not differ statistically from each other (Tukey test, 1% significance).

becoming alkaline. In relation to mineralogical analysis, fermentates had high mineral contents, of which potassium, iron and magnesium were present in greatest quantity (Table 3).

Film extraction (drying and removal)

Films of glycerol plastified starch, were homogeneous, continuous, and without fractures or ruptures. Handling qualities of the films were excellent or good in most treatments, except for films with 1.5 and 2.0 mL of glycerol (Treatments 4 and 5), which were difficult to detach from the petri dish surfaces without tearing. However, after removal, all films could all be manipulated without any risk of rupture.

Analysis of density, solubility, moisture content, thickness and kinetics

There was a significant difference in the interaction between the fermentation time and the glycerol concentration at 1% for the parameters thickness, solubility, density and humidity (Table 4).

Thickness data showed differences in thickness between films for all glycerol concentrations for mash derived from 14-day fermentate. However, for 21-day fermentate, only treatment 1 (0 mL of glycerol) differed significantly in thickness from the other four treatments, these being indistinguishable.

For films derived from 14-day fermentate, regression analysis was significant ($p<0.01$), indicating that when glycerol concentration increases, the yam-based film

Table 3. Mean concentration of macro- and micro-minerals (mg/100 g).

Mean mineral concentration (mg/100 g)*	Sample	
	14 days fermemtation	21 days fermentation
Ca	0.10 ± 0.01	0.10 ± 0.01
Mg	1.87 ± 0.04	3.37 ± 0.07
K	64.99 ± 2.64	87.49 ± 0.86
Na	0.00 ± 0.00	0.00 ± 0.00
P	0.00 ± 0.00	0.00 ± 0.00
Mn	0.00 ± 0.00	0.02 ± 0.01
Cu	0.00 ± 0.00	0.55 ± 0.74
Zn	1.00± 0.21	1.12 ± 0.07
Fe	1.04 ± 0.02	1.74 ± 0.11

*Means of 3 samples ± standard deviations.

Table 4. Values for thickness, solubility, density and moisture content of yam-derived starch biofilms following 14 and 21 days fermentation with the addition of either 0 mL(T1), 0.5 mL (T2), 1.0 mL (T3), 1.5 mL (T4) and 2.0 mL (T5) of glycerol.

Parameter	Fermentation duration (days)	Glycerol concentration (mL)				
		0.0 (T1)	0.5 (T2)	1.0 (T3)	1.5 (T4)	2.0 (T5)
Thicknes (mm)	14	0.23^{aE}	0.29^{bD}	0.35^{aC}	0.41^{aB}	0.46^{aA}
	21	0.22^{aB}	0.34^{aA}	0.35^{aA}	0.34^{bA}	0.34^{bA}
Solubility (%)	14	12.98^{bE}	46.98^{bD}	61.71^{aC}	70.7^{aB}	74.29^{bA}
	21	49.84^{aE}	52.70^{aD}	63.31^{aC}	69.70^{aB}	76.92^{aA}
Density (g/cm^2)	14	0.10^{aC}	0.12^{aB}	0.18^{aA}	0.17^{aA}	0.18^{bA}
	21	0.097^{aD}	0.12^{aC}	0.15^{bB}	0.17^{aB}	0.26^{aA}
Moisture (%)	14	13.53^{aC}	24.17^{bB}	42.72^{aA}	43.49^{aA}	42.53^{bA}
	21	11.80^{bE}	26.74^{aD}	41.86^{aC}	43.84^{aB}	45.83^{aA}

* Distinct letters in the columns (lower case letters) and rows (upper case letters) differ significantly from each other by the Tukey test

becomes thicker (Figure 2).

For solubillity, the studied yam-derived starch films were after 24 h water immersion, semi-intergal in shape, and very flexible and foldable. Films derived from both 14 and 21-day fermentate showed gradual increases in solubility as a function of glycerol addition. Biofilms lacking any glycerol had low solubility, due to the loss of water during the drying process, which made them more rigid and brittle. However, as glycerol was added, the films became more soluble gradually reaching an average of 75.57% water.

The humidity of the films showed a significant difference in the interaction between the fermentation time factors and glycerol concentration at 1% level. For films derived from 14 days of fermentation there were statistical differences between treatments 1 and 2 and all other treatments (which were statistically identical to each other). However for 21 day-derived films there were differences between films at concentrations (Table 4).

Regression analysis was significant ($p < 0.05$) for both 14 and 21 day-derived films, indicating a tendency for films to increase in humidity as a function of the addition of glycerol. As shown in Figure 2, the addition of 1 mL of glycerol results in an increase of 33.29% (14 days) and 34.02% (21 days) in film moisture content (Figure 3).

The density of the starch films differed statistically ($p < 0.01$) between the formulations with 0 mL (T1) and 0.5 mL (T2) of glycerol in film derived from 14-day and 21-day fermentates. The regression in Figure 4 indicates a clear trend for progressive density increase to occur as a function of glycerol addition.

There were no significant differences between the fermentation time and the interaction for water sorption kinetics. However, glycerol content influenced the water adsorption process, so that treatment 3 showed the highest water adsorption value (Table 5).

Scanning electron microscope

The films shown in the photomicrographs in Figures 5 and 6 appear as an extensive and amorphous mass, with

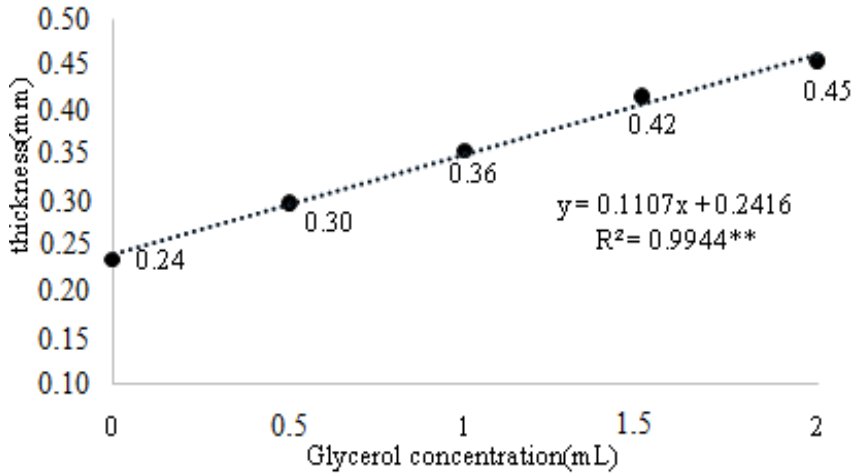

Figure 2. Yam-starch film thickness as a function of glycerol concentrations after 14 days of fermentation.

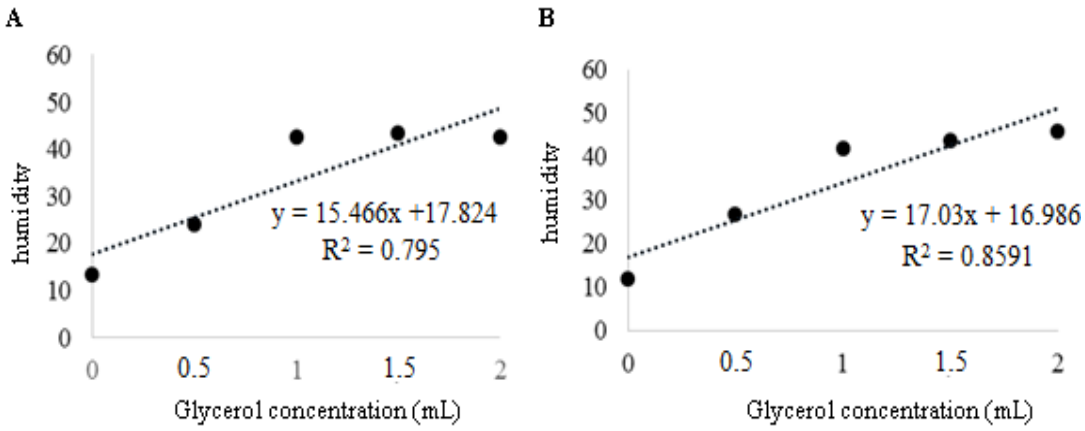

Figure 3. Moisture content of films as a function of glycerol concentration for films derived from (A) 14- and (B) 21-day fermentation.

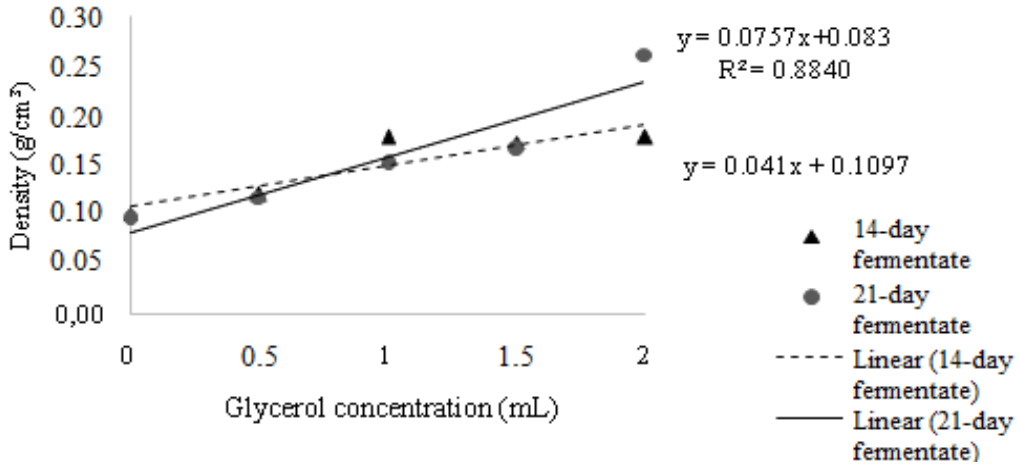

Figure 4. Density of films as a function of glycerol concentration for films derived from 14- and 21-day fermentation.

Table 5. Means of sorption kinetics values as a function addition of glycerol to biodegradable films.

Glycerol concentration (mL)	Mean
0.0 (T1)	27.83[c]
0.5 (T2)	17.23[d]
1.0 (T3)	34.58[a]
1.5 (T4)	33.38[b]
2.0 (T5)	32.98[b]

CV% = 3.23; dms = 1.08873. The means followed by different letters differ statistically between each other.

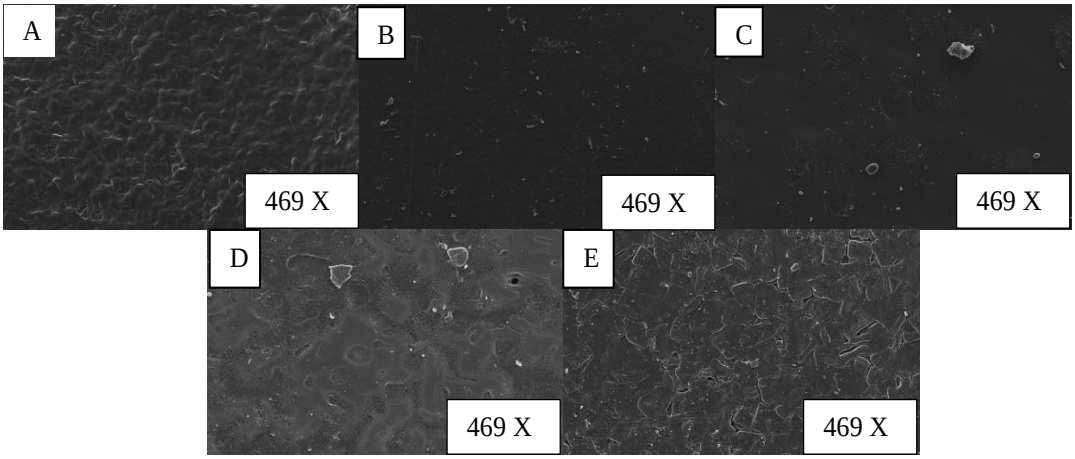

Figure 5. Scanning electron micrograph (SEM) at 300 µm of films from 14-day fermentate: A (0% glycerol), B (0.5% glycerol), C (1.5% glycerol), D (2.0% glycerol), E (2.5% glycerol).

the presence of rounded depressions.

Color

Evaluating the luminosity parameters for the glycerol concentration and the fermentation time, in which L_0* represents the results of standard films made from cassava starch and L* the results of yam starch films, a proximity among the values indicate that there were variations between cassava-derived (standard) and yam-derived films. However, when comparing the yam-derived samples with each other, there was little variations between them, except for films from treatment 1 made from 14-day fermentate (Table 6).

The intensity of these variations (ΔE) however varies between films. For films derived from 14-day fermentate those with pure starch and with addition of 1.5 mL of glycerol showed more intense variation in color. However, for films derived from 21-day fermentate, those to which glycerol had been added showed more intense variations than films without glycerol.

DISCUSSION

Physical-chemical analysis of yam starch

No legal classification exists for yam fermentate, and no references were found in the literature for its physical and chemical characteristics. Consequently, the data was compared with existing studies on cassava. Moisture levels for both the 14-day and 21-day fermentation samples were higher than those reported by Aquino et al. (2011) and de Reis et al. (2010) for yam tuber starch (11.9 and 7.61%, respectively). These values would not comply with regulations for moisture content operating in Brazil (ANVISA RDC n° 263 of 2005 September 22, which ratified the Technical Regulation for cereals, starches, flours and vegetable-powders).

Variation in moisture levels could be related to the artesanal methods used to prepare the base material, as well as the drying time and the conditions under which drying occurred. Maieves (2010), studying the composition of different tubers as a function of part of the year in which they were collected, posited that moisture

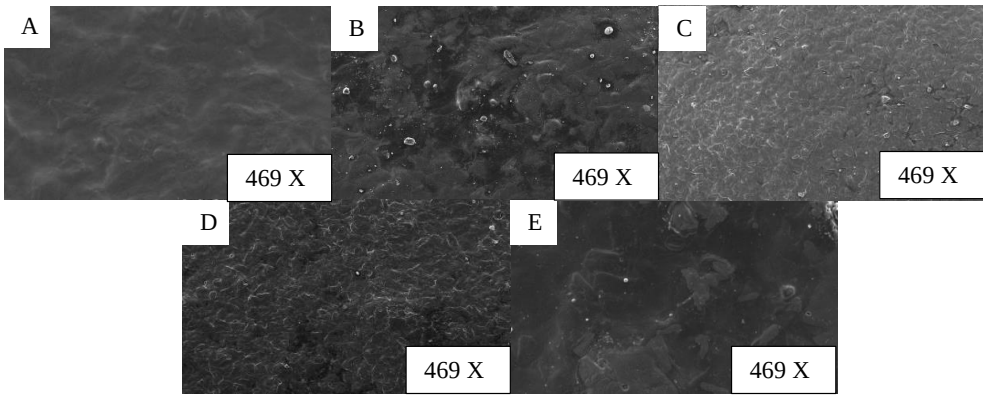

Figure 6. Scanning electron micrograph (SEM) at 300 µm of films from 14-day fermentate: A (0% glycerol), B (0.5% glycerol), C (1.5% glycerol), D (2.0% de glycerol), E (2.5% glycerol).

Table 6. Photospectometric analysis of yam-derived starch filmes as a function of fermentation time and glycerol concentration.

Fermentation time (Days)	Glycerol concentration (mL)	L_0^*	L^*	a_0^*	a^*	b_0^*	b^*	ΔE
14	0.0	34.07	29.72	0.04	-0.27	- 0.46	-1.6	4.51
14	0.5	34.07	32.73	0.04	0.33	- 0.46	0.26	1.57
14	1.0	34.07	32.61	0.04	0.7	- 0.46	0.63	1.97
14	1.5	34.07	32.25	0.04	0.51	- 0.46	0.26	2.03
14	2.0	34.07	33.94	0.04	0.9	- 0.46	1.2	1.92
21	0.0	34.07	33.48	0.04	0.18	- 0.46	0.37	1.05
21	0.5	34.07	31.96	0.04	0.03	- 0.46	0.57	2.35
21	1.0	34.07	32.79	0.04	0.52	- 0.46	1.55	2.45
21	1.5	34.07	31.92	0.04	0.1	- 0.46	0.69	2.44
21	2.0	34.07	31.76	0.04	-0.05	- 0.46	-0.06	2.35

Initial (L_0*) and final (L*) luminosity, chromatic parameters control a* and b* initial (a_0* and b_0*) and final (a* and b*) and colour alteration (ΔE).

content might vary in line with soil water content, since the months with highest water availability were also those with greatest tuber moisture content (43.12%). Similar values were reported by Dantas et al. (2010) (45.89%), and by Alves et al. (2005) (55.54%). Accordign to Diniz (2006), there are no published studies to show that extreme moisture contents negatively influence the technological properties of bitter manioc powder. There may be some exaggeration about the risks that moisture contents above 18% could cause (Cereda and Vilpoux, 2002). According to Maeda (1999), the water content fermented cassava starch ranges from 0.40 to 0.60, so it should be possible to change the limits required by the legislation.

Ash levels from 21-day fermentate were higher than those reported both for yam by Vilpoux et al. (2002) (0.22%), and for cassava by Cereda et al. (2001) and Aquino et al. (2016) (both 0.13 to 0.25%). However, the proportions encountered here were lower than those reported by Reis et al. (2010) for yam (0.92%). Ash values reported by Silva et al. (2012) for cassava flour (0.08%) were similar to those found in the current study for material derived from 14 day fermentate.

Lipid values (Table 2) were lower than those reported by Reis et al. (2010) (0.64%), but greater than those of Silva et al. (2006) (0.06%) for yam, and resembling those for cassava from the studies of Pereira et al. (1999) (0.26%) and Marcon et al. (2006) (0.18 to 0.21%). Ladeira and Pena (2011) found that low lipid levels in fermentation-derived flours were caused by their elimination during product processing, so that the repeated washings in the preparation methodology of the current study may explain the low values reported here.

Obtained crude protein values (Table 2) were lower than those reported by Aquino et al. (2011) (4.88 to 4.99%) and Daiuto and Cereda (2003) (4.19%). According to these authors, elevated crude protein values may be associated with nitrogen residues resulting from the breakdown of mucopolysaccharides (mucilage). An analysis of proteins in flour from three varieties of bitter manioc by Ladeira and Pena (2011) found values similar to those in the current study (0.20 to 1.06%), and much

higher than those reported by Cereda and Vilpoux (2002), for sweet yam (*Dioscorea* sp.) flour (0.09%). According to Vieira et al. (2010) and Pereira et al. (1999) a reduction in the protein content can be attributed to the loss of water-soluble proteins water soluble during raw material processing.

Brazilian legislation has no established guidelines for percentage of total fibre. However, it is notable that, with the exception of the study by Dias and Leonel (2006) (0.57 to 2.75%), the values obtained by the current study were greater than any of bitter manioc flour (Reis et al., 2010: 0.17%; 0.02%; Vieira et al., 2010: 0.28%; Leonel et al., 2004: 0.74%; Fiorda et al., 2013: 0.61%; Trombini et al., 2013: 0.39%; Maciel et al., 2013: 0.30%) (Table 3). Fiorda et al. (2013) and Tromboni et al. (2013) found that different crude fibre levels can depend on the species studied, as well as the time of planting, the soil type, and the climatic conditions under which the plant grew, and the methods by which the sample was extracted. However, although the values found here were higher than those of the authors cited, the analyzed fermentates had low fiber contents based on the classification of Mattos and Martins (2000) (below 2.4 g fibers/100 g).

During the fermentation process, it was noted that a reduction in the level of carbohydrates was associated with increased moisture content (Table 2), while centesimal analysis revealed high carbohydrate levels in the yam fermentate. Mean carbohydrate values were similar to those reported by de Ferreira (2014) for saffron starch (79%), thus agreeing with the data of de Luna et al. (2013) for cassava (78.55), these being greater than those reported by Nascimento et al. (2013) for sweet potato (65.18%) and Holland and Oliveira (2015) cassava-derived gum (70.02%). However, these values are lower than those reported by Rocha et al. (2012) wolf-tomato flour (*Solanum lycocarpum*) (84.99%) and Fiorda et al. (2013) for cassava starch (85.53%).

The pH values from samples classified as slightly acid (pH > 4.5) (Tupinamba and Souza, 2010). Similar values have been reported by Silva et al. (2012) (6.03 to 6.21), Dias and Leonel (2006) (4.24 to 6.10) and Ladeira and Pena (2011) (7.09) for cassava flour. The variation in pH values encountered in the current study is explained by Cereda (1987) and Ascheri and Vilela (1995) who observed that natural fermentation process result in a lack of product uniformity, even in the same species in the same environment, due to the different phases of the microbial growth and acid production, that occur when there is no quality control. Contrary to expectations no rapid falls occurred after the first two days of fermentation, after which low values were reached which continued until the end of the fermentation period. pH at the end of 21 days was alkaline, which can be explained by the species used, for which both the fermentation processes and the microorganisms involved in fermentation are not known, so requiring further specific studies for effective culture.

Brazilian legislation classifies vegetable-derived flours as sweet and bitter, based on their titratable acidity (BRASIL, 1978), as a result all the material in this study is classified as “bitter” (Table 3). The acidity results are similar to those reported by Leonel et al. (2004) physical-chemical characterization of starches (1.05%), and with those of Luna et al. (2013) for cassava (1.4%), and higher than those reported by Ladeira and Pena (2011) (0.89 to 0.96%). The increase in acidity between 14 and 21 days of fermentation most likely occurred as a result of prolonged exposure of the material to high ambient temperatures and the extension of the fermentation period (Tupinamba and Souza, 2010). It is interesting to note that the values found in our study were lower than those of Machado et al. (2010) (3.12%), Garcia et al. (2014) (2.16 to 6.36%), Machado et al. (2013) (3.12%), Aquino et al. (2016) (1.66 to 7.05%), Dias and Leonel (2006) with values between 2.08 and 7.4% and Reginatto et al. (2009) means between 2.5 and 4.0% for cassava.

Silva et al. (2006) reported that elevated acidity levels are related to increased availability of carboxil groups, which are probably the result of acid residues derived from the degradation of the macromolecules that make up the starch. Variation in acidity is explained by Cereda and Lima (1981), who noted that a level of titratable acidity is characteristic of the natural fermentation used to create such acid flours. The authors encountered a great variety of values, which were explained not only by the percentage of acidic compounds present, but also by their nature, since acidic character may vary depending on the size of the chain and the number of carboxylates.

The starch values encountered here meet those stipulated by ANVISA RDC n° 263 of 2005 September 22. Such results are similar to those of Reis et al. (2010) and Cereda and Vilpoux (2002) also from yam (88.58 and 83.06%, respectively). However, values are lower than those for cassava, where starch values of over 90% have been reported by Ladeira and Pena (2011) (94.79%), Silva et al. (2012) (93.47 to 97.46%), Oliveira (2011) (90.48 to 94.7%) and Peroni (2003) (98.94 to 99.58%). The purity of such starches is related to their chemical composition, and the low levels of proteins, lipids and ash, and absence of those protein that adhere to the starch granules is desirable (Oliveira, 2011). The quantity of constituents depends on the composition of the plants used and the methods of extraction, which is pertinent as the physical-chemical composition of yam is, following the classification of Peroni (2003), considered one of the lowest for non-starch substances, and one of the highest for starch, both in terms of quantity and quality of starch, which is classified as having the highest grade of purity.

Minerals are inorganic substances present in all tissues. Their presence is essential for the functioning of various key biological processes (Anavi et al., 2013). According to Underwood (1999), potassium is the principle intercellular ion in tissues, essential for muscle tone and activity, a contributor to acid-alkaline

homeostasis and respiration, via chloride exchange. Comparing Potassium values in the fermentate and tuber (Table 4), the observed significant reduction can be attributed to the extensive washing process that occurred during the starch extraction process.

Film extraction (drying and removal)

Films of glycerol plastified starch, were homogeneous, continuous, and without fractures or ruptures. On the other hand, cassava-derived films without added glycerol were shown by Shimazu et al. (2007) to be more brittle than those containing glycerol. For films with 1.5 and 2.0 mL of glycerol (Treatments 4 and 5), which were difficult to detach from the petri dish surfaces without tearing. Mali et al. (2005) consider that the greater adhesion of films is caused by the greater proportion of starch and glycerol in the formulation, thus conferring greater adhesiveness.

Analysis of density, solubility, moisture content, thickness and kinetics

For 21-day fermentate, only treatment 1 (0 mL of glycerol) differed significantly in thickness from the other four treatments, these being indistinguishable. Such variation in film thickness (Table 3) is explained by Ratnayake et al. (2002), who found that glycerol acts by interrupting the formation of an amylose double helix, resulting in shrinkage of the resulting gels and a consequent increase in their thickness. Such results parallel the findings of Liu and Kerry (2005), Matta Júnior et al. (2011), Leyva et al. (2008) and Laohakunjit and Noomhorm (2004) for starch films derived from pinus, pea, wheat and rice respectively. As the filmogenic solution dries, the water evaporates allowing the concentration of the starch to locally increase, so forming an inter-linked network.

For films derived from 14-day fermentate, regression analysis was significant ($p < 0.01$), indicating that when glycerol concentration is increases, the yam-based film becomes thicker (Figure 1). Such results are similar to that of Matta Júnior et al. (2011) on pea-starch films, which showed a linear relationship between glycerol concentration and thickness increase.

For solubility, the studied the biofilms that did not contain glycerol were found to have low solubility due to the loss of water during the drying process, which made them more rigid and brittle. However, as glycerol was added, the films gradually became more soluble reaching an average of 75.57%. This fact is explained by Matta Júnior et al. (2011), Mehyar and Han (2004), Zhang and Han (2006), Leyva et al. (2008), Laohakunji and Noomhorm (2004) and Garcia et al. (2006), who observed that glycerol interacts with the film matrix, increasing the free space between the chains, facilitating the water entry into the film and consequently increasing the solubility.

The humidity of the films showed a significant difference in the interaction between the fermentation time factors and glycerol concentration at 1% level. According to Chivrac et al. (2010), the relative humidity of storage conditions is an influential factor, since starch tends to absorb large amounts of water in conditions of high relative humidity, due to its hydrophilic nature. This strongly influences its physical and barrier properties (Mali et al., 2005). Regression analysis indicates a tendency for films to increase in humidity as a function of the addition of glycerol. According to Arenas (2012), glycerol content influences the film moisture content because it is hygroscopic, so increasing water content as plasticizer proportion increases.

Similar data have been reported by Shimazu et al. (2007) and Mali et al. (2010) who also found that glycerol favours water absorption. Soares et al. (2016) recorded variations from 9.20 to 23.79 as a function of storage time and pH concentration, noting that the addition of glycerol increases the absorption of moisture from the environment. Fernandes et al. (2015) reported variations in moisture content of between 42.20 and 84.60%, lower than those found here for yam, for films derived from milk whey.

The difference likely occurs because starch with added plasticizer produces films with high density due to non-complete gelatinization of the starch at higher glycerol concentrations (Dias, 2008). The density values recorded here are lower than those reported by Müller et al. (2008) (2.41 g/cm^3) for manioc starch films, those of Moore et al. (2006) (0.92 to 1.10 g/cm^3) for keratin-derived films or by Pelissari et al. (2013) (1.34 g/cm^3) for films derived from green banana starch.

Higher sorption kinetics values indicate that a smaller amount of water is being adsorbed, at a reduced rate (Araújo-Farro et al., 2010). The adsorption of moisture was faster in treatments 1 and 2. That smaller amounts of water were adsorbed under treatment 3, corroborates the data of Mali et al. (2005). According to Galdeano et al. (2014), the glycerol is incorporated into the polymer matrix as it slowly adsorbs the water.

Scanning electron microscope

The films shown in the photomicrographs in Figures 4 and 5 appear as an extensive and amorphous mass, with the presence of rounded depressions. In this they resemble films studied by de Matta Junior et al. (2011) who stated that such topology may be due to differential drying which results in the presence of non-fully gelatinized and non-fragmented starch granules in the matrix.

The cross-sectional aspect of the film was similar for all treatments, each having a more homogeneous and a less homogeneous phase, similar to that reported by Batista et al. (2005) and Yang and Paulson (2000) who observed that as glycerol was added the matrix structure became more discontinuous affecting the consistency of the film. Unlike Dantas et al. (2015) who, in starch biofilmes derived from the yellow Guinea yam (*Dioscorea cayenensis)* reported that the presence of glycerol increased film plasticity by making them more uniform.

The structure of biofilms of treatments 2 and 3 for starch derived from 14-day fermentate (Figure 4B and C) and those of treatments 1 and 3 (Figure 5A and C) from 21-day fermentate is similar to those found by Araujo-Farro et al. (2010) and Pagno et al. (2015) for quinoa-derived biofilms. The compact and uniform structure of these treatments suggests a good interaction between amylose, amylopectin, glycerol and water in the biofilm.

Color

For the chromatic parameters a* and b*, which express the degree of variation between green (-a) and red (+a) and between blue (-b) and yellow (+b), for samples from both 14 and 21 days was a function of the glycerol content of the films. This difference is explained by Silva et al. (2007), who stated that during the gelatinization process of the starch coloration changes occurs due to loss of the initial crystaline structure of the starch granules, so giving the film certain opacity.

Conclusion

The results of the physico-chemical analysis showed that the samples showed no significant difference in moisture, lipids, titratable acid, fibre, starch and total carbohydrates. Differences were found for ash, protein and pH. Values for ash, starch and titratable acid for the flours manufactured lay within existing legal limits. However, it should be possible to restructure processing methodology so that moisture content is reduced to levels that ensure product stability. For pH, studies are required on the alkaline nature of the samples derived from 21 day fermentate, which differed from the expected. The 21-day fermentate had the best physical-chemical and nutritional profile, and both show an encouraging potential for this product and increased profile for this currently under-valued crop.

The glycerol-free films were more brittle, with the best concentrations being of treatment 2 (0.5 mL glycerol) and 3 (1.0 mL glycerol) in both fermentation periods due to their adherence to the petri dish surface. The thickness and solubility values were best using 14 day fermentation at concentrations of 2.0 and 0.0 mL of glycerol respectively. However for density, color and water content, the best treatments came from 21-day fermentate. There were no significant differences for water sorption kinetics by fermentation period. However, adding plasticizer influenced this parameter significantly. Scanning electron microscopy showed that the starch without added glycerol has the most homogeneous matrix; as the glycerol is added, some starch granules will be present that have not been fully dissolved.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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