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Bioactive compounds and shelf life of clarified umbu juice

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Abstract

This study aimed at evaluating the chemical, physical and microbiological stability of clarified umbu juice. The microfiltered juice was filled in sanitized glass bottles in a clean chamber system and stored in incubator at 6°C for 90 days. Juice samples were collected immediately after processing and each 30 days during storage period for evaluation of the product main quality parameters. Clarified juice showed clear appearance, being slightly yellowish and free of suspended solids. The juice presented antioxidant activity, probably related to their vitamin C and phenolic compounds contents. The storage of clarified umbu juice in glass bottles at 6°C allowed good preservation of the product characteristics, which remained proper for consumption during 90 days.

Introduction

Brazil is a great world producer of fruits (Reetz *et al*., 2015), presenting a big diversity of tropical fruits, some of which are few explored such as umbu, a native fruit of the Brazilian northeastern semiarid region. The umbu fruit is characterized by its juiciness when ripe, and it has a delightful bittersweet taste. It is composed by 93% water and 6% sugar and contains bioactive substances such as vitamin C, carotenoids and phenolic compounds (Ribeiro, Godoy, Freitas *et al*., 2015).

The umbu represents a source of incoming for the families' farmers in the semiarid Northeast region. However, it is a fruit of short seasonal period (December-March), sold in the region mainly as frozen pulp and fresh fruit, which generates many losses due to its high perishability. Some studies were developed to evaluate the potential of umbu fruit to obtain stable products with higher added value such as jelly and fermented beverage (Folegatti *et al*., 2003; Paula *et al*., 2012).

Currently, it has been observed an increase in the consumption of fruit beverages like whole juices, blends and ready-to-drink products, mainly due to the demand for healthier and convenient foods. The application of membrane separation processes allows the achievement of more stable products for both direct consumption as well as raw material or ingredient for new drinks products.

As compared to conventional filtration, the use of microfiltration, one of the membrane separation processes, for fruit juices clarification has several advantages, among which can be highlighted: elimination of diatomaceous earth that reduces costs and environmental impacts, increase in the quality of product by reducing microbial load, decrease of process time and yield increase (Cheryan, 1998).

Several works have used microfiltration for fruit juices clarification (Vaillant *et al*., 2005; Cassano *et al*., 2010; Mirsaeedghazi *et al*., 2010; Razi *et al*., 2011; Oliveira *et al*., 2012) although few works had been developed with umbu fruit. Ushikubo *et al.* (2006) studied umbu juice clarification by polymeric membranes using diluted umbu pulp. The authors reported that the best conditions for umbu juice microfiltration were observed at low transmembrane pressure, high crossflow velocity and using enzyme pretreatment. However, the procedure to extend its storage life is still a technical challenge. Therefore, the aim of this study was to evaluate the effect of microfiltration processing on bioactive compounds of umbu juice and the physical, chemical and microbiological stability of clarified umbu juice during storage.

Material and Methods

Umbu pulp

The raw material used in this work was frozen commercial umbu pulp, supplied by a small enterprise (Itiúba, Bahia-Brazil). The pulp was transported and

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storage under freezing at -18°C until its use in the experimental assays.

Chemicals and reagents

Rapidase Smart Color enzymatic complex (DSM FoodSpecialties®, Germany); sodium hydroxide (Vetec, Brazil); 2,6-dichlorophenol indophenol (Vetec, Brazil); 2,2'-azino-bis-[3-ethylbenzthiazoline-6-sulphonic acid] diammonium salt ($ABTS^{\bullet+}$) (Sigma-Aldrich®, Brazil); Folin-Ciocalteu reagent (Merck®, Germany); gallic acid (Sigma-Aldrich®, Brazil).

Pre-treatments and microfiltration

Enzymatic hydrolysis of umbu pulp was performed as a pretreatment for clarification process. It was carried out in a mixture tank using the enzymatic complex Rapidase Smart Color (DSM FoodSpecialties®), in a concentration of 0.1 $mL.kg^{-1}$, at 35°C, for 40 minutes. Rapidase Smart Color is a liquid pectinase obtained from *Aspergillus niger* strain and its best enzyme activity is in acid medium and temperature up to 60°C. Ribeiro, Gouvea, Penha *et al.* (2015) previously defined these conditions as ideal for the umbu juice enzymatic hydrolysis. The hydrolyzed juice was centrifuged at 2500 g in a basket centrifuge (Centrifugal IEC - Model K7165, USA) with a 100 µm nylon filter aiming at decreasing the pulp suspended solids content.

Clarification of umbu juice was carried out in a microfiltration unit (Model L, GEA Filtration, USA) composed by four ceramic tubular membranes with 0.2 µm pore size and a permeation area of 0.022 m², at 35°C and 3.5 bar of transmembrane pressure (RIBEIRO, 2014). The permeate stream was continuously collected, while the retentate stream was recirculated. The permeation flux (J) was determined by the ratio between the permeate flow and the membrane area as shown in Equation (1):

$$J\ (\mathrm{kg}.h^{-1}m^{-2}) = \frac{m}{t \times A} \qquad (1)$$

In which m is the permeated mass (kg) collected during a determined time (t) and A is the membrane surface area (m²).

Samples of the different input and output streams, pulp (P), hydrolyzed juice (HJ), centrifuged juice (CJ), clarified juice (CL) and retentate from microfiltration process (RE), were collected for analysis.

Stability of clarified umbu juice

Clarified juice was filled in glass bottles previously sanitized and stored at 6°C for 90 days in incubator. Samples were taken each 15 days for microbiological and instrumental color analysis and at each 30 days for physico-chemical analysis.

Physico-chemical analysis

The total soluble solids were measured in digital pocket refractometer. The values were expressed in degrees Brix (ATAGO, Japan) (AOAC, 2005).

The pH values and acidity were measured using an automatic titrator (Metrohm, 785 DMP). Acidity was titrated using 0.1 N NaOH (Vetec, Brazil) (AOAC, 2005).

Vitamin C was determined by the 2,6-dichlorophenol indophenol (DCPI, Vetec) titrimetric method with modification (Silva, 1999). By this method, a 0.01% DCPI solution was calibrated with a 0.03% L-ascorbic acid solution. DCPI solution was used to titrate 2.0 g of samples diluted with 25 mL of oxalic acid at 1% concentration, up to a slight pink color. Results were expressed as mg of ascorbic acid equivalents per 100 g of sample.

The in vitro antioxidant capacity was quantified according to Re *et al.* (1999) using the acetonic extracts. This spectrophotometric method is based on the decoloration of the free radical 2,2'-azino-bis-[3-ethylbenzthiazoline-6-sulphonic acid] diammonium salt ($ABTS^{\bullet+}$) (Sigma-Aldrich®, Brazil). The results were expressed as Trolox equivalent.

The concentration of total phenolic compounds was determined by colorimetry in the acetonic extracts using the Folin-Ciocalteu reagent (Merck®, Germany). The quantification was done at 760 nm with gallic acid (Sigma-Aldrich®, Brazil) as standard (Singleton and Rossi, 1965; Georgé *et al.,* 2005). Results were expressed in mg gallic acid per 100 g samples.

Microbiological quality

The microbiological determination (mesophilic and psycrotrophic bacteria total counting, mold and yeast, coliforms at 45°C and Salmonella) was performed according to American Public Health Association (2001).

Physical analysis

Instrumental color was measured by transmittance in colorimeter (ColorQuest XE, Hunterlab) and results were expressed in the CIELab/CIELCH scales as L^* (brightness, from 0 black to 100 white), b^* (blue chromaticity (-) to yellow (+)) and Hue Angle (H°) (Ferreira, 1981).

Pulp content of the samples was determined by centrifugation (Fanem, 215, Brazil) at 1400 g by 25 minutes according to Redd *et al*. (1986). This determination was performed to evaluate the suspend solids content on samples of microfiltration

processing. The values were expressed in mass percentage.

Steady-shear experiments were performed at 20°C, in a ARG2 rheometer (TA Instrument, USA), fitted with a coaxial cylinders geometry. Steady state flow was performed from 1.0 to 6.10^2 s^{-1} shear rates.

Statistical analysis

All assays were carried out in triplicate and results were expressed as mean ± standart deviation. All statistical analyses were performed with Statistica software v. 7.0. Differences at $p<0.05$ were considered to be significant. The Tukey and Pearson Correlation tests were used to evaluate the data.

Results and discussion

Microfiltration process

The yield of clarified juice in relation to the pulp used as raw material was equal to 60%, which is in the range observed by Viana *et al*. (2012), between 54 and 74%, for the clarification of organic acid lime juice by microfiltration. A sharp reduction of permeate flux in the first 50 minutes can be observed, which is associated to the formation of a solid layer on the membrane surface (Figure 1). After this step the permeate flux remained relatively stable, with a slight drop at the end of the process. The average permeate flux was around 70 $kg.h^{-1}m^{-2}$ and concentration mass factor and process time were slightly superior to 2.5 and 150 minutes, respectively.

Ushikubo *et al.* (2007), working with diluted umbu pulp (1:2), reported permeate fluxes between 50 and 80 $kg.h^{-1}m^{-2}$ at 100 minutes of process, which were dependent on operational conditions used as transmembrane pressure, crossflow velocity and enzyme treatment. Regarding the microfiltration of pineapple juice, using a 0.2 µm membrane, Laorko *et al.* (2010) reported a permeate flux of 24.2 $kg.h^{-1}m^{-2}$ at the end of the process. These data ratify that permeate flux is dependent on the raw material, pretreatment, membrane material and pore size, besides of the operational conditions.

In Table 1 it is presented the microbiological and instrumental color data of the microfiltration process streams, where it is possible to see that both clarified and centrifuged juice attended the Brazilian requirements (Brazil, 2001) as they did not present Salmonella sp and coliforms at 45°C. This shows that the Good Manufacturing Practices were followed during processing steps. Furthermore, microfiltration was able to retain microorganisms, reducing the presence of mesophilic aerobic bacteria (Table 1).

Microfiltration process produced an umbu juice free of suspended solids, clear and slightly yellowish. Color differences between umbu pulp and clarified juice were visible and instrumental color parameters data corroborates it (Table 1). The results show that the processing for obtaining clarified umbu juice affected the original pulp color. Microfiltration in ceramic membranes intensified both brightness and Hue angle of clarified umbu juice, in accordance with the clearness of the product. The parameter b^*, which is related to the yellow color, was reduced significantly due to the decrease of the carotenoids. On the other side, the yellow color was intensified in the retentate, clearly showing the concentration of these bioactive compounds in this fraction. Matta *et al*. (2004) reported a similar behavior in acerola juice clarified by microfiltration, where there was an increase in the brightness of clarified juice, due to the removal of suspended solids.

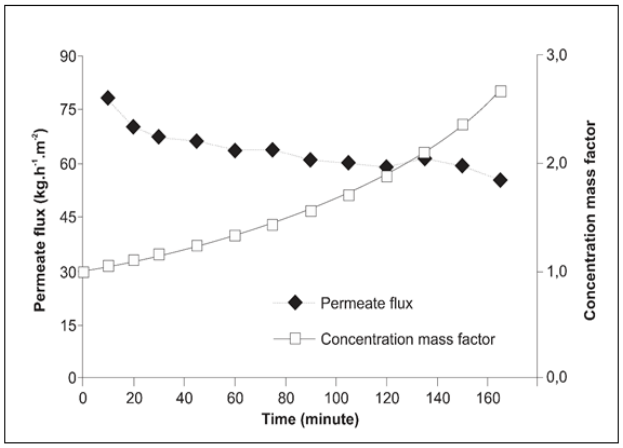

Figure 1. Permeate flux and concentration mass factor behavior during the microfiltration of umbu juice in ceramic membranes at 35°C

The main effect of the enzymatic treatment was the reduction of both the viscosity at $100s^{-1}$, shear rate recorded in the liquid food processing and the pulp content of umbu juice (Table 2). The results confirm the efficiency of enzymatic hydrolysis for breaking pectin molecules and other substances such as starch, cellulose and hemicellulose that can promote the concentration polarisation phenomena and the formation of the gel layer during membrane processes, reducing permeate flux.

Results also show retention of soluble solids and organic acids by microfiltration membrane, a similar behavior of that reported by Ushikubo *et al.* (2007) in the microfiltration of umbu juice using polymeric membranes. Reduction of soluble solids content was also observed by Amirasgari and Mirsaeedghazi (2014) in microfiltration of red beet juice. According to the authors, it is due to removal of large particles by the membrane. Data presented in Table 2 show that there was an increase in the phenolic compounds

Table 1. Microbiological and instrumental color parameters of umbu juice in microfiltration streams

Sample	Mold and yeast (CFU g^{-1})	Plate count* (CFU g^{-1})	*Salmonella* sp. (in 25 g)	Coliforms at 45°C (MPN g^{-1})	L*	b*	Hue Angle (H°)
CJ	<1.0 x 10^1	2.2 x 10^3	Absence	<3	63.14 ± 0.37^a	36.14 ± 0.30^a	83.18 ± 0.04^a
CL	<1.0 x 10^1	<1.0 x 10^1	Absence	<3	93.54 ± 0.01^b	10.41 ± 0.03^b	93.64 ± 0.06^b
RE	<1.0 x 10^1	2.1 x 10^3	Absence	<3	42.16 ± 0.28^c	48.08 ± 0.21^c	79.66 ± 0.07^c

CJ: centrifuged juice, CL: clarified juice, RE: retentate, *mesophilic bacteria. Different letters in the same column show significant difference among values according to Tukey test ($p<0.05$).

Table 2. Physical-chemical characterization of umbu juice in all process streams

Sample	pH	Soluble solids (°Brix)	Acidity (g ac. citric.100 g^{-1})	Pulp content (percent)	Viscosity 100 s^{-1} (Pa.s)	Phenolic Compounds (mg ac. galic.100 g^{-1})	Vitamin C (mg.100 g^{-1})	Antioxidant Activity (µmol Trolox.100 g^{-1})
P	2.58 ± 0.02^b	5.1 ± 0.0^b	1.66 ± 0.02^b	52.20 ± 0.44^a	0.38 ± 0.06^a	207.81 ± 6.10^a	6.78 ± 0.18^a	9.84 ± 0.42^a
HJ	2.56 ± 0.00^{a,b}	5.4 ± 0.1^a	1.80 ± 0.00^a	30.98 ± 0.15^b	0.14 ± 0.03^b	220.54 ± 5.41^b	4.64 ± 0.33^b	10.32 ± 0.23^a
CJ	2.56 ± 0.00^a	5.4 ± 0.1^a	1.77 ± 0.00^c	4.18 ± 0.19^c	np	102.92 ± 0.61^c	2.83 ± 0.03^c	5.74 ± 0.12^b
CL	2.57 ± 0.01^b	4.6 ± 0.1^c	1.59 ± 0.00^d	0.00 ± 0.00^d	np	76.68 ± 1.18^d	2.38 ± 0.07^c	4.37 ± 0.09^c
RE	2.55 ± 0.01^a	6.1 ± 0.1^d	1.78 ± 0.00^{a,c}	7.78 ±0.03^e	np	124.93 ± 3.49^e	4.29 ± 0.20^b	6.36 ± 0.05^d

P: pulp, HJ: hydrolyzed juice, CJ: centrifuged juice, CL: clarified juice, RE: Retentate, np: not performed. Different letters in the same column show significant difference among values according to Tukey test ($p<0.05$).

content after the enzymatic hydrolysis, probably due to the cell wall rupture that causes the release of these compounds, increasing their concentration in the bulk. In subsequent steps of centrifugation and microfiltration occurred a significant reduction ($p<0.05$) of these compounds and, consequently, of the antioxidant activity. The phenolic compounds are directly related to the antioxidant activity of the umbu juice and they are also present in the suspension matter. Therefore, when there is the suspended solids removal by centrifugation and microfiltration, the phenolic content is reduced in the juice. At the microfiltration step the reduction in both phenolic and antioxidant activity was about 25% in relation to the centrifuged juice. Therefore, the bioactive compounds content and antioxidant activity increased in the retentate fraction. Viana *et al.* (2012) observed a significant reduction, of 20%, in concentration of phenolic compounds in the organic acid lime juice clarified by microfiltration. This result corroborates with the data observed in this work for reduction of the antioxidant activity of umbu clarified juice.

Vitamin C content (Table 2) decreased significantly ($p<0.05$) after hydrolysis, possibly due to the temperature (35°C) and stirring during enzymatic hydrolysis, allowing umbu juice oxidation. Regarding the microfiltration step, it was not observed significant difference ($p<0.05$) between centrifuged (feed) and clarified juice (product). Matta *et al.* (2004) also observed no significant reduction in vitamin C content of acerola juice clarified by microfiltration as well as Barreto *et al.* (2013) for camu-camu juice, also clarified by microfiltration in ceramic membranes with pore size of 0.1 µm.

Stability of clarified umbu juice

Microbiological parameters of clarified umbu juice during storage are presented in Table 3. Although the low microorganism counting observed at 60 and 75 days for mold and yeast and psycrotrophic bacteria, respectively, there was no increase in microorganisms counting at the 90 days, showing that this fact should, probably, be related to sampling, filling and/or bottle conditions. The product stability may be also associated with the cold storage (6°C), which is a barrier to microbiological growth.

Laorko *et al.* (2013) observed no growth of mold and yeast, mesophilic aerobic bacteria and coliforms in pineapple juice clarified by microfiltration and stored at 4, 27 and 37°C for six months. Campos *et al*. (2002) did not also report microorganism growth in clarified cashew apple juice stored at 4 and 30°C during two months. These results show the contribution of the microfiltration process for obtaining stable products, acting like a cold pasteurization.

Clarified umbu juice stored at 6°C presented significant changes ($p<0.05$) in the parameters of instrumental color. In Table 3 it is possible to observe brightness and Hue angle behavior. These data presented positive correlation among them ($r=0.98$ and $p<0.05$) according to Pearson's correlation test. The loss in instrumental brightness as compared to time zero was less than 1.5% and although very low, it was significant ($p<0.05$). Despite this, storage had no visual effect on the juice brightness. Laorko *et al*. (2013) also observed darkening (L^*) of pineapple

Table 3. Microbiological evaluation and color parameters behavior (L^* and H°) of clarified umbu juice stored at 6°C

	Storage time						
	0	15	30	45	60	75	90
Coliforms at 45°C (MPN.g^{-1})	<3	np	np	np	np	np	<3
Salmonella (in 25 g)	Absence	np	np	np	np	np	Absence
Mold and yeast (CFU.g^{-1})	<10[1]	<10[1]	<10[1]	<10[1]	1.5x10[2]	<10[1]	<10[1]
Plate count[1] (CFU.g^{-1})	<10[1]	<10[1]	<10[1]	<10[1]	<10[1]	5.0x10[1]	<10[1]
Hue angle (H°)	93.64 ± 0.06^a	91.62 ± 0.08^b	91.32 ± 0.06^c	90.44 ± 0.06^d	88.96 ± 0.05^e	88.05 ± 0.06^f	87.98 ± 0.03^f
Brightness (L*)	93.54 ± 0.01^a	93.23 ± 0.01^b	93.08 ± 0.08^c	92.68 ± 0.04^d	92.51 ± 0.01^e	92.25 ± 0.04^f	92.32 ± 0.01^f

[1]psycrotrophic bacteria, np: not performed. Different letters in the same line show significant difference among values according to Tukey test ($p<0.05$).

Table 4. pH, acidity, phenolic compounds and antioxidant activity behavior of clarified umbu juice during storage at 6°C

Storage time (days)	pH	Acidity (g ac. citric.100 g^{-1})	Phenolic Compounds (mg ac. galic.100 g^{-1})	Antioxidant Activity (µmol Trolox.100 g^{-1})
0	2.57 ± 0.01^a	1.59 ± 0.00^a	76.68 ± 1.18^a	4.37 ± 0.09^a
30	2.48 ± 0.01^b	1.72 ± 0.01^b	70.68 ± 1.03^b	4.09 ± 0.13^{a,b}
60	2.48 ± 0.01^b	1.71 ± 0.00^b	71.23 ± 0.23^b	4.08 ± 0.07^{a,b}
90	2.41 ± 0.01^c	1.75 ± 0.01^c	68.67 ± 1.31^b	4.00 ± 0.18^b

Different letters in the same column show significant difference among values according to Tukey test ($p<0.05$).

juice clarified by microfiltration after six months stored at 4, 27 and 37°C, which was lower for the lowest temperature. According to the authors, it was due to non-enzymatic reactions, possibly, chemical oxidation of compounds as vitamin C and phenolics.

Phenolic compounds content and antioxidant activity of clarified umbu juice decreased during storage (Table 4), presenting high correlation with storage time ($r=0.82$ and $p<0.05$) according to Pearson's test. Tukey test for the phenolic compounds content showed that all results differed as compared to zero storage time, but there were no significant changes between 30 and 90 days. These results show a certain stability of these compounds after one month. According to Laorko *et al.* (2013), the reduction of the phenolic compounds during storage is due, probably, to oxidation and polymerization reactions. On the other hand, antioxidant activity remained stable during storage, showing a significant difference ($p<0.05$) only at 90th day.

Physical-chemical stability of the clarified umbu juice was observed during this study and its behavior is showed in Table 4. There was a decrease in the pH of clarified juice after 30 days of storage, and a relative increase in acidity. Both pH and acidity had changed ($p<0.05$) during storage. However, between 30 and 60 days, these parameters remained stable ($p<0.05$). There was negative correlation ($r=-0.94$ and $p<0.05$) among the results according to the Pearson's test. This behavior was expected due to inverse relation between pH and acidity.

Soluble solids from the clarified juice remained unchanged during storage (4.7±0.1°Brix), presenting no significant difference ($p<0.05$) in the evaluated period. In general, the results of the evaluated parameters suggest good stability of the clarified umbu juice, which remained suitable for consumption until 90 days, when stored at 6°C.

Conclusion

Clarified umbu juice obtained by microfiltration process in ceramic membranes at 35°C showed clear appearance, transparency and a slightly yellowish color. It presented antioxidant activity, probably related to its vitamin C and phenolic compounds contents. Furthermore, microfiltration using ceramic membranes was able to retain microorganisms, making it to be considered a cold pasteurization. Clarified umbu juice remained chemical, physical and microbiologically stable during 90 days stored at 6°C, attending the current Brazilian legislation.

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