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Some Quality attributes of Four Sudanese Forest Fruits Nectars

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A B S T R A C T

Fruit juices industry is becoming widely spread in Sudan, but, these products are usually expensive. The aim of this study is to substitute fruit juice by nectars made from four indigenous forest fruits. Nectars were prepared from doum (*Hyphaene thebaica* L), kirkir (*Randia geipaeflora*), and a mixture of karmadoda (*Naucleae latifolia*) and godeim (*Grewia tenax*). The prepared nectars were subjected to a physico-chemical and microbiological studies. The physico-chemical attributes included total soluble solids, pH, titratable acidity, ascorbic acid, tannins and colour (optical density). The microbiological parameters studied were total bacterial count, lactic acid bacteria, *Salmonella spp*, *Staphylococcus aureus*, spore former bacteria and yeasts and moulds. The processed nectars were stored at ambient (30 $\pm$ 5 ºC) and refrigeration (4 -6 ºC) temperatures. The physico-chemical analyses were carried out at zero time and at the end of the storage, while the microbial analyses were carried out monthly for six months. Results obtained indicated that the highest and the lowest level of pH-values were recorded for doum and karmadoda fruits 5.30 and 2.00, respectively. Karmadoda forest fruit contains the highest value of vitamin C (389.82 mg/100g) and tannins (2.76 %), while, doum fruits contain the lowest value of these components, 31.74 mg/100 g and 1.84 %, respectively. Nectars were stored for six months without deterioration in the physico-chemical properties, as well as, they were free of microbial growth. **Need to the more details**

Keywords: *Characteristics; forest fruits; storage temperature; storage period; microorganisms; nectar.*

INTRODUCTION

The world fruit juices and beverages market has reached a dynamic growth (Bermudez-Aguirre , 2011; Lemanowics and Krukowski, 2011); which is expected to prevail also in the 20th century. Statistics has shown that a beverage fruit blend has called the attention of fruit growers and processors to meet the increasing demand (Costa , 2003). Forest fruit nectars are very popular Sudan throughout Sudan mainly Western (Abdel Muti, 2002), but proper product development and quality assessment is lacking except for a few products e. g tamarind (Abdel Rahman, 2011). As a result, the socio-economic and nutritional importance of such products is over looked when formulating relevant policies and programs (Ismail, 2010). Fruit juices contain variable levels of macro and micronutrients (Esitken , 2010; Pimentel and Maia, 2001), as well as antioxidant compounds. The antioxidant components are providing protection against harmful free radicals and have been strongly associated with reducing the risk of chronic diseases (Ramos, 2008; Rossi , 2008). The total phenolics and flavonoids are the major contributors to the antioxidant activity of fourteen wild edible fruits from Burkina Faso (Meda , 2008). Doum, kirkir, karmadoda and godeim have many antioxidant compounds e.g. polyphenols in term of taninns (Abdel-Rahman, 2011; Abdel-Rahman , 2014).

The first goal of the thermal treatment of food industry is to increase the shelf-life of the food products by controlling the microorganism's growth. FDA (2006; 2004) has mandated principles for the safe processing of vegetable and fruit juices, which included a 5-log pathogen reduction treatment. Being rich in many nutrients, fruit juices are considered a good medium for

microbial growth (Tsiga , 2008 and Al-Jedah and Robinson, 2002). The most contaminants in juices and nectars are yeasts, moulds and nonspore-forming bacteria as micro flora. Walls and Chuyate (1998) mentioned that microorganisms made many complaints in juices and nectars that include off aroma and colour loss. Vieira (2002) reported that the acid fruit extracts and pasteurization process is enough to destroy this flora except spore former bacteria could survive. Contamination of juices with pathogenic microorganisms such as E-coli and salmonella has caused numerous illnesses and some fatalities (Bremer , 2004; FAO, 2001). Microorganisms have multiple requirements for amino acids and vitamins and have the ability to survive pasteurization processes (Adler , 2013; Sant´Ana and Tribst; 2008; Chan and Kong, 2004). This study looks at some physico-chemical composition and stability of some Sudanese forest fruits nectars against microorganism during storage.

MATERIALS AND METHODS

Fruits preparation

Fruits nectars were prepared according to the method described by Abdel-Rahman (2011). Doum fruits were cleaned, peeled and crushed using a mortar and a pestle to separate the seeds, then sieved through 4 meshes, weighed, washed and soaked in water in a ratio of 1: 2 for2 hours to obtain a fruit juice. Then the juice was fine-filtered through one layer of muslin cloth.

kirkir fruits were cleaned, sorted (according to the size), washed and soaked in water in a ratio of 1: 4 for 8 hours. The soaked fruits were mixed well using an electric stirrer (model: Lightnin, mixer, N. C.–2, USA) and the juice was fine-filtered as described above. karmadoda fruits were cleaned, washed, peeled, and cut using sharp clean stainless steel knives. Then they were weighed, blanched with water in a ratio of 1: 2 for 10 minutes and pulped using an electric blender. Godeim fruits were prepared according to the method of Saeed and Ali (1976). The fruits were sorted, washed and soaked overnight in water in a ratio of 1:8. The soaked fruits were mixed well using an electric stirrer (model: Lightnin, mixer, N. C.–2, USA). All the fruits extract were fine-filtered through one layer of muslin cloth.

Nectars processing

Three nectars were made, doum nectar, kirkir nectar and a mixture of Karmadoda and godeim pulps in a ratio of 1: 2 (v: v). The nectars were processed by heating as described by to Kotecha and Kadam (2003) as 1: 1; Fruit extract: water. The total soluble solids and total titratable acidity (as citric acid) of the three nectars were adjusted from initial to a range of 13.0 - 15.0 % and 0.30 to 0.40 %, respectively, according to CODEX/FAO/WHO (2000). The nectars were pasteurized at 85 ºC for 10 minutes under atmospheric pressure. 0.10 % sodium benzoate was added as a chemical preservative (Mehmood , 2008). The products were filled in a previously washed tin-plate can containers (45 g), tightly sealed and cooled under running water. Then, they were stored at ambient temperature (30 ± 5º C) and refrigeration (4 - 5º C) for six months.

Physico-chemical analyses

The raw materials used for nectars processing and the prepared nectars were analyzed for total soluble solids (TSS%) useing a hand-type refractometer (0-50% Brix) at 20º C (AOAC, 2000).The pH-values were determined with a glass electrode pH meter (model: HANNA instrument 8521 Portugal) at ambient temperature (Egan, , 1981). Total titratable acidity (as citric acid) was carried by sodium hydroxide (NaOH) titration to 8.1 endpoint according to Ranganna (2001). Ascorbic acid was determined according to Pearson (1982), while the quantitative determination of tannins was carried out using the modified vanillin-HCl colorimetric method as described by Price (1978). Soluble colour (optical density at 420 nm) was determined using a spectrophotometer (Askar and Treptow, 1993). These attributes were carried out at zero time and at the end of storage the period.

Microbiological analyses

Plate count agar (PCA) was used for the enumeration of total viable bacterial count (TVBC) according to Harrigan and McCance (1976). De Man, Rogosa and Sharp (MRS) medium was used for counting lactic acid bacteria (Kiss, 1984). The coliform test was carried out in Mac Conkey broth using the Most Probable Number (MPN) method and recorded using the MPN Table (Andrew, 1992). *Salmonella spp*. was determined using the method of Harrrigan (1998). Baird-Parker (BP) agar medium was used for the enumeration of *Staphylococcus aureus* according to Flowers (1993). Finally, Yeast and moulds were counted using malt extract agar (MEA) and spore-former bacteria were counted using starch milk agar (Harrigan and McCance, 1976). The microbiological analyses were carried out monthly for six months.

RESULTS AND DISCUSSION

Physico-chemical characteristics of raw materials

The physico-chemical characteristics of the raw materials are given in Table 1. The moisture content of doum forest fruits were 5.47 %, this level was higher than the findings of FA0 (2006) for African doum (4.00 %). Kirkir, karmadoda and godeim fruits reported values of 7.92, 60.52 and 16.63 %, respectively. These values were within the range (13.2 – 85.2 %) of some wild edible fruits from Malawi (Saka , 1994). These differences might be due to the cultivation and post-harvest conditions. The pH values ranged from 2.00 to 5.30; these values are approximately similar to the values observed by Amarteifio and Mosase (2006) and Saka (1994). The titratable acidity values ranged between 0.17 and 1.17 %, these results are within the values suggested by Bates (2001), and higher than the values given by Anila and Radha (2003) and Jayaraman (1982). The samples under experiment were found rich in vitamin C (ascorbic acid); 389.82, 156.25, 76.33 and 31.74 mg/100 g for karmadoda, kirkir, godeim and doum, respectively. The content of vitamin C of forest fruits are higher than the results mentioned by Fasoyiro (2005) and Manfredini (2002). The tannins content of fruits are 2.76 % (karmadoda and godeim), 2.22 % (kirkir) and 1.84 % (doum), these percentages are higher than those obtained by Nwosu (2008). Fruits and vegetables are containing many different antioxidant components (including polyphenols) that provide protection against harmful free radicals and have been strongly associated with reduced risk of chronic diseases (Amin , 2004 and Sahlin , 2004). The colour density (soluble colour) of forest fruits measured as optical density, ranged from 0.7750 (kirkir) to 1.8815 (doum).

Physico-chemical characteristics of the processed nectars

The findings of fruit nectars characteristics are presented in Tables 2, 3 and 4. Statistically there are significant differences ($P \leq 0.05$) in TSS values of doum and kirkir nectars stored at ambient temperature. The results showed a decrease from 13.00 to 12.75 % and from 15.00 to 14.50 % for doum and kirkir nectars, respectively. While the nectars stored at the refrigeration temperature showed stable reading for TSS%. The behavior of TSS % of apple cashew juice during storage recorded the same result (Costa 2003). Results showed that the values of TSS of mixed nectar are stable at both storage temperatures. The pH values of nectars stored at ambient and refrigeration temperatures significantly ($P \leq 0.05$) decreased to 3.14 and 4.05 (doum) and to 3.20 and 3.37 (mixed), respectively. These results agree with those obtained by Bajwa (2003). The pH values of kirkir nectar significantly ($P \leq 0.05$) increased to 4.13 (ambient) and 3.90 (refrigeration) at the end of storage period. The results obtained in this study are in accordance to those of Hussain (2005). A significant ($P \leq 0.05$) decrease in ascorbic acid content of nectars was noted throughout the storage time. The loss reached 84.34 % and 50.25 % (doum), 56.34 % and 50.25 %(kirkir), and 70.73 % and 62.00 % (mixed) for nectars stored at ambient and refrigeration temperatures, respectively. Iversen (2006) observed similar deterioration of ascorbic acid content during storage of black currant nectar. The loss of ascorbic acid is mostly oxidative reactions which occurs in fruit juice during storage and is highly dependent on the presence of oxygen in the head space or dissolved in the juice as well as processing temperature (Costa , 2003). The tannins of doum, kirkir and mixed nectars significantly ($P \leq 0.05$) decreased during storage from 0.443, 0.315 and 0.172 % (initial) to 0.165, 0.071 and 0.082 % (ambient) and 0.278, 0.241 and 0.139 % (refrigeration), respectively. The decrease in tannins content implies that the browning can also be caused due to the oxidation of phenolic compounds and/or caramelization of sugars (Chang , 1998). Browning reactions in food are widespread phenomena that take place during processing and storage. The optical density of nectars throughout the storage period significantly ($P \leq 0.05$) increased at the two storage temperatures. The data of optical density at the end of storage were reported as 0.218, 0.176, 0.267 (at ambient) and 0.146, 0.144, 0.232 (at refrigeration) for doum, kirkir and mixed nectars, respectively. Yuksel and Koca (2008); Duangmal (2004) and Burdurlu and Karadeniz, 2003) recorded similar behavior for different common fruits nectar.

Microbiological results

1. Total viable bacterial count (TVBC)

Doum and kirkir nectars did not show any viable bacteria during the storage period at the ambient and refrigeration temperatures. However, the mixed fruit nectar showed a TVBC of 1.9×10^2 and 2.2×10^2 cfu/ ml at the 5th and last month of storage when stored at ambient (30 $\pm$5 °C) and a TVBC of 1.5x10 cfu / ml at the 6th month of storage at refrigeration (4 – 6 °C). The total viable bacterial count of the mixed nectar is still in the acceptable range according to the standards of the Sudanese Standards and Metrology Organization (SSMO, 2001) for microbial levels in formulated fruit juices (10^2 to 10^3 cfu /ml). These results obtained were better than those (4.2×10^4 cfu / ml) reported by Mehmood (2008) for apple juice similarly processed and stored at ambient temperature. Hashmi (2007) recorded a TVBC of 7.8x10 cfu/ g for mango pulp treated with 0.20 % sodium benzoate and stored for three months at ambient temperature (30 $\pm$ 5 °C).

2. Lactic acid bacteria

Doum, kirkir and mixed fruit nectars were found free of Lactic acid bacteria throughout the storage period, at both ambient and refrigeration temperatures. This could be attributed to the sodium benzoate (0.1 %) added to the fruit nectars.

CODEX/FAO/WHO (2004) claimed that, beverages with a relatively high pH (3.5 – 4.0) and benzoate concentrations in the range 0.06 – 0.10 % often prevent growth of fermentive organisms. Barbosa-Cánovas (2003) reported that lactic acid bacterium is not naturally present in food; it is formed during the fermentation of foods such as sauerkraut, pickles, olives, and cheese.

3. Coliform bacteria

Results obtained, show that coliform bacteria was not detected in forest fruit nectars during the storage temperature investigated. These results comply with the SSMO (2001) for formulated fruit juices, which specifies that the coliform bacterial range should not exceed one colony/ 100 ml. These results obtained were better than those recorded by Oyarzabal (2003) for apple, pineapple, white grapefruit juice concentrates and banana purée through 12 weeks of storage at 23ºC.

4. Salmonella spp.

Salmonella spp was not detected in the stored forest fruit nectars. These findings comply with those reported by SSMO (2001) for formulated fruit juices. This observation is confirmed with those reported by Addo (2008) for some common fruit juices in Ghana. Sandi , (2004) recorded the same results for passion fruit juice stored at refrigeration temperature. However, Aea and Bushnell (1962) found Salmonella growth (100 cfu/ml) in passion fruit nectar stored at ambient temperature. Bates (2001) reported that contamination of juices with Salmonella spp has caused numerous illnesses and some fatalities. It caused several outbreaks associated with unpasteurized juices (Nogueira , 2003).

5. Staphylococcus aureus

Contamination with Staphylococcus aureus was not detected in fruit nectars throughout the storage period. CODEX/FAO/WHO (2004) reported that the benzoates inhibit the growth of bacterial pathogens, such as Staphylococcus aureus.

6. Yeasts and moulds

The mixed fruit nectar was contaminated with moulds of 0.4 x10 and 0.9x10 cfu/ ml at the last two months of storage at the ambient temperature. In addition, moulds of 0.2x10 cfu/ ml at the 6^{th} month of storage were detected at the refrigeration temperature (4 – 6 °C). These results were within the standard limit of 1 – 10 colonies / 100 ml, recorded by SSMO (2001). Corrèa de Souza (2004) stated that the yeast count in fruit juices increased over time. However, Barbosa-Cánovas (2003) reported that the use of sodium benzoate as a chemical preservative has limited the microbial growth. It is mainly used as an antimycotic agent; most yeasts and moulds are inhibited by 0.05 – 0.10 % (FAO, 2003). Bachceci and Acar (2007) reported that the spores may survive in fruit juices and nectars after pasteurization treatment commonly applied in food industry.

7. Spore-former bacteria

Spore-former bacteria were not detected in doum fruit nectar for the whole period of storage at refrigeration temperature. At ambient temperature spore-former bacteria count of 1.5x10 cfu/ml was recorded at the last month of storage. Kirkir nectar showed a spore-former bacteria count of 0.4x10 and $1.7x10^2$ cfu/ml at the 5^{th} and 6^{th} months, respectively, when stored under ambient temperature. Similarly, the mixed fruits nectar as well recorded spore-former bacteria count of 1.3x10 and 4.8x10 cfu /ml at the 5^{th} and 6^{th} months, respectively during storage at ambient temperature. It was noted that no spore-former bacteria was detected in the mixed fruit nectars under study during the storage period at refrigeration temperature (4-6 °C). It is well known that spore-former bacteria can withstand pasteurization temperature and whenever the conditions are suitable for growth they start to multiply. Walker and Phillips (2007) reported that the spore-former bacteria is a surviving bacterium and may be observed in fruit juice and fruit juice products. Spinelli (2009) stated that the spore-former bacteria was typically present in hot-filled orange juice which should be stored at or below 20°C to avoid spoilage by this microorganism. But, it was observed in pasteurized orange juice stored at or below 20°C for six months. Moreover, Vieira, (2002) recommended high temperature short time (HTST) principle processing to achieve a 5D reduction of acidophilus sporformer bacteria in cupuacu (Theobroma grandiflorm) nectar. Desse and Taye (2001) observed spore-former bacteria in cassava (Manihot esculenta Crantz) juice of $2.9\ x10^2$ cfu/ ml after processing at zero time.

The prevention of spoilage by spore-former bacteria is a current challenge for fruit juice and beverage industries worldwide due to the bacterium's acidothermophilic growth capability, heat resistance, and spoilage potential (Spinelli , 2009).

CONCLUSION

The forest fruits nectars under study were found rich in some vitamins and antioxidants (in tannins term). Due to the adequate physico-chemical and microbiological quality of the products, nectars were stored up to the six months without deterioration, either at ambient or refrigeration temperature.

Table 1. Physico-chemical characteristics of forest fruits (on dry weight basis)

Component	Doum	Kirkir	Kamadoda	Godeim
Moisture (%)	5.47	7.92	60.52	16.63
pH-value	5.30	3.60	2.00	4.12
Titratable acidity (%)	0.85	0.591	1.17	0.17
Vitamin C (mg/100g)	31.74	156.25	389.82	76.33
Tannins (%)	1.84	2.22	2.76	2.76
Colour (O.D at 420 nm)	1.8815	0.7750	0.9390	0.9390

Table 2. Changes in Physico-chemical characteristics of doum nectar after six months storage*

Component	Zero time	Ambient	Refrig.	$Lsd_{0.05}$	SE±
TSS (%)	13.00^{a}	12.75^{b}	13.00^{a}	0.00066	0.00022
pH-value	4.47^{a}	3.14^{c}	4.05^{b}	0.06782	0.02236
Titratable acidity (%)	0.30^{c}	0.520^{a}	0.339^{b}	0.00068	0.00022
Vitamin C (mg/100g)	10.60^{a}	1.66^{c}	5.04^{b}	0.11750	0.03873
Tannins (%)	0.443^{a}	0.165^{c}	0.278^{b}	0.00067	0.00020
Colour (O.D at 420 nm)	0.103^{c}	0.218^{a}	0.146^{b}	0.00082	0.00036

* Means±SD bearing different superscript letters within columns and rows are significantly different (P≤0.05).

Table 3. Changes in Physico-chemical characteristics of kirkir nectar after six months storage*

Component	Zero time	Ambient	Refrig.	$Lsd_{0.05}$	SE±
TSS (%)	15.00^{b}	14.50^{a}	15.00^{b}	0.5254	0.1732
pH-value	3.58^{c}	4.13^{a}	3.92^{b}	0.0006782	0.0002236
Titratable acidity (%)	0.400^{a}	0.335^{b}	0.273^{c}	0.06782	0.02236
Vitamin C (mg/100g)	24.46^{a}	10.68^{c}	12.17^{b}	0.06782	0.02236
Tannins (%)	0.315^{a}	0.071^{c}	0.241^{b}	0.0006782	0.0002236
Colour (O.D at 420 nm)	0.117^{c}	0.176^{a}	0.144^{b}	0.0006782	0.0002236

* Means±SD bearing different superscript letters within columns and rows are significantly different (P≤0.05).

Table 4. Changes in Physico-chemical characteristics of mixed nectar after six months storage*

Component	Zero time	Ambient	Refrig.	$Lsd_{0.05}$	SE±
TSS (%)	15.00^{a}	14.50^{a}	15.00^{a}	1.008	0.3324
pH-value	4.03^{a}	3.20^{c}	3.37^{b}	0.09592	0.03162
Titratable acidity (%)	0.381^{c}	0.513^{a}	0.485^{b}	0.0006782	0.0002236
Vitamin C (mg/100g)	24.84^{a}	7.27^{c}	9.44^{b}	2.164	0.7134
Tannins (%)	0.172^{a}	0.082^{c}	0.139^{b}	0.0006782	0.0002236
Colour (O.D at 420 nm)	0.107^{c}	0.267^{a}	0.232^{b}	0.0006782	0.2236

* Means±SD bearing different superscript letters within columns and rows are significantly different (P≤0.05).

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