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Full Length Research Paper

Evaluation of the microbiological and physico-chemical characteristics of local tomato 'Solanum lycopersicum' puree produced on a small scale in Togo

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Tomatoes are produced, processed and widely consumed by the Togolese population. However, the local production is not developed enough to be able to provide locally processed tomatoes. In order to promote local development, stimulate local processing and reduce post-harvest losses, the processing into puree was undertaken with three locally grown varieties of tomatoes in Togo (Aklikonvi, Tohounvi, and Pomvi). A method developed at the LAMICODA laboratory was used and the process was adapted to be mastered by any local producers. Microbiological and physico-chemical analysis of crushed tomatoes and tomato purees of the three different varieties were performed in order to validate product stability and to determine final product physico-chemical and nutritional qualities. Results showed that the hygienic quality of these tested products was validated according to the criteria considered by the European Union. Also, the results indicated that purees produced contained lycopene (3.94 mg to 7.36 mg/100 g), vitamin E (0.38 mg to 1.14 mg/100 g), β -carotene (0.27 mg to 0.56 mg/100 g), and sugars (such as fructose: 0.75 mg to 1.56 mg/100 g; glucose: 0.78 mg to 1.52 mg/100 g) whatever the variety. The total sugar content is significantly different (p<0.05) for crushed tomatoes and purees. A deterioration of the color and an increase of the acidity were observed in the obtained tomato purees. These preliminary results obtained on these processed products are helpful for further processing and promotion of these different varieties of locally grown tomatoes in Togo.

Key words: Local tomato, crushed tomato, tomato puree, hygienic quality, physicochemical quality, Togo.

INTRODUCTION

Tomatoes are widely consumed around the world and in 2017 its production is estimated at 182 million tons worldwide, of which 21 million tons in Africa and 13 328.2 tons in Togo (FAO, 2018). In Togo, tomato production

remains seasonal, artisanal and unorganized (Dossou et al., 2007). Nethertheless, Togolese tomato production remains the priority activity of 65% of the population in the Savanna region in the north of the country and one

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> of the most important income-generating activities for local producers in Togo (Anonyme 1). Three main varieties are cultivated: Aklikonvi, Tohounvi, and Pomvi. The production period includes a period of shortage from October to May and a period of abundance from June to September. Due to this production period, fresh tomatoes and processed tomatoes are imported from bordering countries such as Ghana and Burkina-Faso (Malet, 2017) but not only. Some Togolese companies are specialized in tomato processing into puree; however despite their production, a postharvest loss of tomatoes is observed in Togo (Anonyme 2), due to a lack of processing method that could be done directly by local producers. Also, the tomatoes are of interest due to its rich content in mineral elements such as potassium, vitamins (A, C, E) (Sadok and Zedak, 2016; Dossou et al., 2007) and different antioxidants compounds such as phenolic compounds rutin, naringenin, and chlorogenic (mainly acid), carotenoids (mainly lycopene and \beta-carotene) (Toor and Savage, 2005; Chanforan, 2010). These antioxidants make it a formidable bulwark against diseases (Sadok and Zedak, 2016). This composition of fresh tomatoes can significantly, especially depending vary on the varieties but also growing conditions (agricultural techniques and environmental factors) or post-harvest preservation that can lead to compositional variability within the same cultivar (Hernandez et al., 2008). It is obvious that heat treatments have an effect on the biochemical and nutritional composition of food. This effect can be positive on certain compounds such as lycopene improving their absorption by the body (Boumendiel et al., 2012). However to our knowledge, no studies were assessed on Aklikonvi, Tohounvi, and Pomvi varieties composition and its processed product.

Also, our previously carried out investigations have revealed that currently these varieties tend to disappear and to be replaced by hybrid varieties, more resistant to damage and extreme weather conditions. This study therefore aims (1) to propose a method to preserve tomatoes using a process adapted to be mastered by any local producers. Some microbiological analysis will be performed to validate the quality of the process applied to obtained tomato puree. (2) To obtain information about characteristics physico-chemical and nutritional composition of the main compounds (sugar content, fibers, antioxidant, vitamins) of Aklikonvi, Tohounvi, and Pomvi tomatoes varieties before and after processing into puree.

MATERIALS AND METHODS

Varieties of tomatoes

The three main varieties of tomatoes grown in Togo were studied: The variety "Pomvi" is a smooth round fruit grown in the north in the Savannah region of northern Togo, the varieties "Aklikonvi" elongated fruit and "Tohounvi" slightly rounded fruit flattened and lobed grown in the south in the Maritime region in the prefectures of the Lakes, Bas-Mono and Vo. Twenty-five kilograms of each tomatoes were bought at the Lomé Grand Market in August and October, 2018. These raw tomatoes were then stored for a week at room temperature (28-32°C), out of the indirect sunlight, to reach optimum maturity before processing.

Process applied for tomato puree production

Tomato purees were obtained using a process easily transferable to local producers, at the laboratory LAMICODA in Lomé (TOGO). The raw tomatoes (25 kg) were sorted to remove rotten tomatoes and then washed three times with clean water. Raw tomatoes were crushed using a manual tomato crusher (TRE SPADE, Ptoclamm and Ptoclaii, Grecostore, Italy) to separate the skin and seeds from the pulp. At this stage, 21±1 kg of crushed tomatoes was obtained. For each variety, samples of 200 g of crushed tomatoes (CT) were directly packed into a double-closure freezer bag (Ultra-Zip, Alba Melitta Group) and stored at -20°C before analysis. They will be used as control sample. Crushed tomatoes were cooked during 30 min at 90°C in a saucepan under manual stirring, then concentrate using a clean linen for filtration. Tomato puree obtained were packaged in 25 cL sterilized glass bottles and were pasteurized at 75°C during 30 min in order to guarantee the hygienic quality of the finished product. The pasteurized tomato purees (TP) were stored at room temperature. For each variety (Aklikonvi, Tohounvi, and Pomvi) and each period of harvest (August and October), samples of crushed tomatoes (CT) and samples of pasteurized tomato purees (TP) were obtained. Microbiological analysis was done the next day after total cooling of the pasteurized purees. All samples were analyzed at least in triplicate at LAMICODA, Lomé, Togo for microbiological analysis and the USC GRAPPE in Angers-France for physico-chemical analyses.

Evaluation of the microbiological quality of tomato purees

Microbiological analysis was carried out on tomato purees in order to evaluate their hygienic quality and validate their preservation at room temperature. Each variety, for each period of harvest was analyzed following microbiological requirement recommended by European Union (Regulation 178/2002/EC; updated the 24/05/2007). It includes total mesophilic aerobic flora (PCA, 30°C, 24-48 h), total and thermotolerant coliforms (VRBL agar, 30°C or 44°C, 24-48 h), sulfite-reducing anaerobic bacteria (TSN agar, 37°C, 24-48 h), yeasts and molds (Sabouraud + Chloramphenicol agar, 30°C, 48-72 h). Each analysis was done in triplicate.

Evaluation of the conventional physico-chemical parameters

pH, titratable acidity and soluble solids content

pH and titratable acidity were determined using respectively a pH meter (Orion Star A111-Thermo Scientific) and an automatic titrator (877 Titrino Plus, Metrohm). For titratable acidity, diluted mixture of 10 g of sample and 25 ml of deionized water was titrated with sodium hydroxide (0.1N NaOH) to reach pH 8.1 (NFV05-101). The results are expressed as citric acid equivalent per 100 g (conversion factor of 0.07). Soluble Solids Content (°Brix) was measured using a refractometer (Refracto 30PX- Mettler Toledo). Each analysis was done in triplicate.

Color measurement

Color analyses were performed using the spectrocolorimeter (CM-

700, Konica-Minolta) with a D 65 illuminant and CIE (10°) observer as technical characteristics. Color change was described as L*, a* b* and five repetitions were made for each sample. The redness of crushed tomatoes and tomato puree was determined by a*/b* ratio. The color difference (ΔE^*) between two samples was determined using Equation 1.

$$\Delta E^* = \sqrt{((L^*_{e} - L^*_{ref})^2 + (a^*_{e} - a^*_{ref})^2 + (b^*_{e} - b^*_{ref})^2)}$$
(1)

Where L^*_{ref} , a^*_{ref} , b^*_{ref} are the reference values and in this case, it is the values of the crushed tomatoes taken as reference and L^*_e , a^*_e , b^*_e being the values of the tomato purees.

Evaluation of nutritional compounds

Sample preparation before analysis

Crushed tomatoes samples and tomato purees samples were frozen at -80°C during 12 h before using the freeze dryer (VirTis *SP* SCIENTIFIC *Sentry* 2.0). 200 g of samples were freeze dried during 7 days in order to remove water content and to obtain a dry powder.

Chemicals solution for analysis

The standards used in this study were: D-(+)-Glucose, D-(-)-Fructose and Sucrose (\geq 99%, Sigma Aldrich), Lycopene (analytical standard, Sigma Aldrich), Beta-carotene (>97%, Sigma Aldrich) and α -Tocopherol (analytical standard, Sigma Aldrich), Naringenin (>98%, Sigma Aldrich), Chlorogenic acid (\geq 98%, Fluka), Coumaric acid (>98%, Acros Organics), and Rutin (>99%, ExtraSynthese). The HPLC grade solvents used were purchased from Fischer Scientific: Acetone, Acetonitrile, Methanol, Hexane, Tetrahydrofuran, and Trifluoroacetic acid.

Sugar content (Glucose, fructose)

The main sugars (fructose and glucose) were quantified by Agilent Model 1200 HPLC (Agilent Technologies, USA) coupled to an Evaporative Light Scattering Detector (ELSD) (Sedere, France). The extraction method was developed by the USC GRAPPE. 50 mg of crushed tomatoes or tomato puree were mixed with 1.5 ml of distilled water and vortexed for 1 min. Samples were then placed on stirring plate for 20 min and were centrifuged at 15000 rpm, 20°C, and 15 min. The supernatant was collected and filtered using nylon filter (0.45 µm/13 mm, Interchim) before injection of 20 µl on HPLC/ELSD. The column Rezex RCM-Monosaccharide Ca²⁺ (300x7.8 mm, Phenomenex) was used at 70°C with 100% demineralized water at a flow rate of 0.5 ml/min. ELSD parameters were a nebulization temperature of 40°C and 2 bars of pressure. Analysis was done in triplicate.

Fiber content

Fiber content or alcohol insoluble matter (AIM) was determined by the gravimetric method according to gravimetric method according to Renard (2005a). 1 g of freeze-dried tomato puree was suspended in 20 mL of 70% ethanol. The mixture was stirred for 10 min, transferred to a 75 mL separating column (Sep-pak, Interchim) equipped with a 20 μ m filter and filtered under vacuum. The pellet obtained was recovered and the operation was repeated 12 to 15 times. The first two washing were performed with 70° ethanol heated to 80°C in order to inactivate the endogenous enzymes of the fruit. The following 13 washing were carried out with unheated 70° ethanol. The absence of soluble sugars in the filtrate was measured by the sulfuric phenol method described by Dubois et al. (1956). The pellet was dried after 3 washing with 30 mL of 96% ethanol followed by 3 washes with 10 mL of acetone, then placed overnight in an oven at 40°C. The net mass of alcohol-insoluble material gives an estimate of the total fiber content of the sample (expressed as a percentage of the total content of the sample expressed as % of fresh tomato).

Polyphenol content

The analysis of rutin, chlorogenic acid, naringenin content and the evaluation of total polyphenol content was carried out by an Agilent Model 1200 HPLC (Agilent Technologies, USA) according to the method developed at USC GRAPPE laboratory. 3 solvents were used for extraction: solvent 1 (100% methanol), solvent 2 (30% distilled water, 70% acetone, 0.05% trifluoroacetic acid), solvent 3 (89% distilled water / 10% methanol / 0.1% hydrochloric acid 37%). 500 mg of the lyophilizate sample was mixed with 20 ml solvent 1 and then vortexed during 1 min.

After 10 min, the supernatant was collected and placed in a centrifugal evaporator (miVac, Genevac) at 30°C during 1 to 2 h. This step was done twice, adding 20 ml of solvent 2. Then 1.5 ml of solvent 3 was added. The mixture was placed on a stirring plate during 10 min then centrifuged at 10000 rpm, 15°C, 10 min. Supernatant was filtered (PTFE filter of 0.45 µm/13 mm) before injection of 5 µL. Column Kinetex C18 (150×4.6 mm, Phenomenex) was used at 30°C at a flow rate of 1 ml/min. A gradient elution using solvent A (95% distilled water / 5% formic acid) and solvent B (80% Acetonitrile / 15% distilled water / 5% formic acid) was applied as followed: 3 min 97% solvent A / 3% solvent B, 3 to 34 min to reach 20% solvent A / 80% solvent B. Measurements were done at 280 nm for total polyphenol content and naringenin, 320 nm for chlorogenic acid and 360 nm for rutin. Analysis was done in triplicate.

Carotenoid content (beta-carotene, lycopene) and a-tocopherol

Analysis of carotenoid (beta-carotene and lycopene) and atocopherol (or vitamin E) was carried out using an Agilent Model 1200 HPLC (Agilent Technologies, USA). 1 mL of hexane was added to 50 mg of freeze-dried sample. The mixture was vortexed 30 s, placed on the stirring table (10 min, maximum speed) then centrifuged at 4°C, 10 min, and 15000 rpm. The supernatant was placed in a centrifugal evaporator (miVac, Genevac) at 30°C during 30 min to obtain the total evaporation of the hexane. Once evaporated, 1 ml of the solvent (20% Acetonitrile + 80%Tetrahydrofuran) was added. The mixture was vortexed 30 s and filtered using PTFE filter (0.45 µm) before injection of 5 µL. Column Kinetex C8 (150×4.5 mm with pre-column C8, Phenomenex) was used at 60°C at a flow rate of 1 ml/min. A gradient of elution was used with 2 mobile phase: A (70% Methanol / 30% Ammonium Acetate 1 M) and B (60% distilled water, 40% Methanol). Gradient analysis was applied as followed: 0 min 60% solvent A / 40% solvent B, 0 to 40 min to reach 15% solvent A / 85% solvent B. Measurements were done at 295 nm for αtocopherol, 450 nm for lycopene and 470 nm for beta-carotene. Analysis was done in triplicate.

Statistical analysis

A statistical analysis was performed on the different samples using the Statgraphics Centurion 18 software at a p < 0.05 threshold with

 Table 1. Microbiological analysis of tomato puree.

Germs	Number of g	germs in pasteu purees	rized tomato	European Union (EU) criteria, Regulatic		
	Aklikonvi	Tohounvi	Pomvi	- 178/2002/EC; updated on 24 May 2007		
Mesophilic aerobic flora	<10	<10	<10	10 ⁴ /1 g of puree		
Total and thermotolerant Coliforms	Absent	Absent	Absent	Absent /1 g of puree		
Sulfite-reducing anaerobic bacteria	Absent	Absent	Absent	Absent /1 g of puree		
Yeasts and Molds	Absent	Absent	Absent	10 ⁴ /1 g of puree		

The analyzed tomato puree is of satisfactory hygienic quality.

multi-variance ANOVA. The differences among treatments were verified by their least significant difference. Experiments were conducted in triplicate, mean values and standard deviation with different exponent letters are significantly at p < 0.05.

RESULTS

Microbiological characteristic of tomato purees

Pasteurization process was necessary to obtain products that could be preserved at room temperature for a long period. This heat treatment could permit to propose a simple process, easily transferable to local producers, to valorize local tomato varieties and to reduce losses during abundance period. With this objective, it is of high importance to evaluate the sanitary quality of the tomato puree obtained. The microbiological analyses made on the tomato purees produced in August and October revealed that the tomato purees after pasteurization were germ-free (Table 1, results obtained after 2 days of storage at 22 ± 1°C) and does not exceed the threshold values indicated by the regulation related of microbiological criteria of European Union. The analysis of total and thermotolerant coliforms did not reveal any colonies, whereas the standard tolerates 10 per gram of puree. As for the mesophilic aerobic flora, yeasts and molds, the analyses showed respectively less than 10 germs/g of puree. Puree tested did not contain any colonies of sulfite-reducing anaerobes (SRA) which are the germs responsible for deterioration of canned food at pH below 4.5. These results showed a good level of control of the parameters applied for pasteurization treatment. The process applied is effective and sufficient to stabilize the product during its duration of conservation.

Evaluation of conventional physicochemical parameters and nutritional compounds of fresh tomatoes and tomato puree

Local varieties of tomatoes (Aklikonvi, Tohounvi, and Pomvi) are not well characterized yet and the following results will help to bring knowledge related to physicochemical characteristics and the analysis of some specific compounds (two periods of harvest, August and October). Also, this study will help to understand the losses or evolution of the composition of the products related with the process applied in order to validate the final nutritional quality of the product.

First, different physico-chemical parameters (pH, °brix, titratable acidity, color) of the crushed tomato and tomato puree were done. Results are shown in Tables 2 to 4. pH values range between 4.10 and 4.42 for all varieties tested, pH<4.5. Whatever, the treatment and the month of production, there is little significant difference due to the general maturity of the tomatoes before processing into puree. Crushed tomatoes produced in August have a pH between 4.18 and 4.40, and their puree a pH ranging from 4.18 to 4.42. For October crushed tomatoes, we obtain a pH of 4.13 to 4.32 and for their puree a pH ranging from 4.12 to 4.31.

Soluble solids content values for all varieties range from 2.70 to 5.73%. Whatever the month of production, we observe an increase in the Brix degree during the transformation of the fresh tomato to the puree produced. Crushed tomatoes produced in August have a soluble solid content between 3.27 and 5.20, and their puree a pH ranging from 3.67 to 5.73. For October crushed tomatoes, we obtain a degree brix of 2.70 to 3.97 and for their puree a pH ranging from 4.40 to 5.53. The process of tomatoes into puree increases the brix degree of the tomatoes as revealed by the heat treatment. The total acidity of the tomatoes and purees produced expressed in citric acid equivalent (g/100 g) from our three varieties reveal a content of around 0.20 to 0.60%. The total acidity of the tomatoes and purees produced expressed in citric acid equivalent (g/100 g) from our three varieties reveal a content of around 0.20 to 0.60%.

Crushed tomatoes acidity produced in August range from 0.22 to 0.60, and their puree pH ranges from 0.24 to 0.55. For October crushed tomatoes, we obtain 0.21 to 0.37 for acidity value and for their titratable acidity it ranges from 0.31 to 0.62. The heat treatment increases the acidity of the purées whatever the variety.

As for color, the illuminant D65 in combination with the 10° CIE 1964 reference observer is commonly used. This

Variety (p<0.05, IC=95%)	Month of production	Treatment	рН	Brix Degree (°B)	Total acidity (equivalent in citric acid g/100 g)	Glucose (mg/100 g)	Fructose (mg/100 g)
	A	Crushed tomato	4.40 ± 0.02^{a}	3.67 ± 0.06^{d}	0.22 ± 0.01f	0.65 ±0.10 ^f	0.88 ± 0.13^{e}
Aklikonvi	August	Puree	4.42 ± 0.03^{a}	4.00 ± 0.26^{d}	$0.24 \pm 0.02 f$	0.78 ± 0.09^{e}	1.07 ± 0.12^{d}
ARIKOTT	October	Crushed tomato	$4.28 \pm 0.05^{\circ}$	3.97 ± 0.21^{d}	0.33 ± 0.13^{e}	0.93 ± 0.04^{cd}	1.07 ± 0.04^{d}
	October	Puree	4.30 ± 0.01^{bc}	5.40 ± 0.30^{ab}	$0.41 \pm 0.05^{\circ}$	1.21 ± 0.01 ^b	1.38 ± 0.01 ^{bc}
	August	Crushed tomato	4.20 ± 0.01^{d}	3.27 ± 0.06^{e}	$0.42 \pm 0.01^{\circ}$	$1.00 \pm 0.03^{\circ}$	$1.33 \pm 0.03^{\circ}$
Tobounvi	August	Puree	4.30 ± 0.05^{bc}	3.67 ± 0.15 ^d	$0.43 \pm 0.20^{\circ}$	$1.01 \pm 0.02^{\circ}$	1.38 ± 0.02^{bc}
Tonounvi	October	Crushed tomato	4.13 ± 0.01^{e}	3.20 ± 0.44^{e}	0.37 ± 0.63^{d}	0.58 ± 0.02^{fg}	0.75 ± 0.03^{f}
	October	Puree	4.12 ± 0.01^{e}	5.53 ± 0.15^{ab}	0.62 ± 0.17^{a}	$1.01 \pm 0.01^{\circ}$	$1.29 \pm 0.01^{\circ}$
	August	Crushed tomato	4.18 ± 0.01^{d}	5.20 ± 0.01^{b}	0.60 ± 0.02^{a}	1.22 ± 0.07^{b}	1.45 ± 0.01^{b}
Domi	August	Puree	4.18 ± 0.01^{d}	5.73 ± 0.06^{a}	0.55 ± 0.01^{b}	1.52 ± 0.10^{a}	1.56 ± 0.10 ^a
	Octobor	Crushed tomato	4.32 ± 0.01^{b}	2.70 ± 0.10f	0.21 ± 0.03f	0.54 ± 0.01 ^g	0.68 ± 0.02^{f}
	OCIODEI	Puree	4.31 ± 0.01 ^{bc}	$4.40 \pm 0.26^{\circ}$	0.31 ± 0.05^{e}	0.87 ± 0.02 ^{de}	1.07 ± 0.02 ^d

Table 2. Composition of acids and sugars of tomato varieties studied and purees.

a^{-e}Different classes of crushed tomatoes and purees, the figures bearing different letters in the column are significantly different at the threshold of 0.05%.

Table 3. Color and Carotenoids content of tomato varieties studied and puree	s.
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Variety (p<0.05, IC =95%)	Month of Production	Treatment	L	а	b	Ratio a/b	Lycopene (mg/100 g)	Beta-Carotene (mg/100 g)
Aklikonvi	August	Crushed tomato Puree	25.33 ^d 33.08 ^c	12.37 ^{gh} 24.30 ^{de}	9.00 ^d 21.17 ^{bc}	1.39 ± 0.14 ^c 1.15 ± 0.05 ^{def}	2.46 ± 0.08^{e} 3.94 ± 0.21^{d}	0.15 ± 0.01^{f} 0.27 ± 0.04^{de}
	October	Crushed tomato Puree	28.57 ^d 35.42 ^{bc}	16.56 ^{fg} 30.92 ^{bc}	10.58 ^d 22.94 ^{ab}	1.56 ± 0.04^{b} 1.34 ± 0.01^{c}	4.44 ± 0.85^{cd} 7.37 ± 0.45 ^a	0.18 ± 0.06^{ef} 0.49 ± 0.06^{ab}
Tohounvi	August	Crushed tomato Puree	27.82 ^d 33.75 ^c	7.33 ^h 20.62 ^{ef}	6.21 ^d 19.69 ^{bc}	1.18 ± 0.02^{dc} 1.05 ± 0.03^{f}	$4.89 \pm 0.04^{\circ}$ 5.96 ± 0.37 ^b	0.51 ± 0.02^{ab} 0.51 ± 0.03^{ab}
	October	Crushed tomato Puree	32.99 ^c 39.62 ^a	28.37 ^{bcd} 38.35 ^a	16.59 ^c 27.09 ^a	1.72 ± 0.06^{a} 1.42 ± 0.02^{c}	4.36 ± 0.61^{cd} 7.09 ± 0.85 ^a	0.27 ± 0.05^{d} 0.56 ± 0.08^{a}
Pomvi	August	Crushed tomato Puree	27.94 ^d 34.67 ^c	12.03 ^{gh} 21.33 ^{ef}	11.17 ^d 19.89 ^{bc}	1.09 ± 0.11 ^{ef} 1.07 ± 0.01 ^{ef}	2.33 ± 0.10^{e} 4.61 ± 0.54 ^{cd}	0.23 ± 0.01^{de} 0.39 ± 0.09^{c}
	October	Crushed tomato Puree	35.71 ^{abc} 39.53 ^{ab}	26.18 ^{cde} 33.34 ^{ab}	19.22 ^{bc} 27.04 ^a	1.35 ± 0.09 ^c 1.23 ± 0.04 ^d	3.02 ± 0.51^{e} 6.85 ± 0.40^{a}	0.13 ± 0.03^{f} 0.45 ± 0.02^{bc}

a^{-e}Different classes of crushed tomatoes and purees, the figures bearing different letters in the column are significantly different at the threshold of 0.05%.

was determined by ratio a/b determining the redness of our sample. The L fraction expresses the luminosity of

the product; it remains related to the non-enzymatic browning reactions. The coordinates a*(red/green),

Variety (p<0.05, IC =95%)	Month of production	Treatment	Vitamin E (α-tocopherol; mg/100 g)	Fibers (g/100 g)
	August	Crushed tomato	0.20 ± 0.01^{h}	1.63 ± 0.25^{cd}
	August	Puree	0.38 ± 0.02^{fg}	2.27 ± 0.10^{ab}
Aklikonvi	October	Crushed tomato	0.45 ± 0.11^{ef}	0.97 ± 0.03^{9}
		Puree	1.08 ± 0.13^{ab}	1.76 ± 0.11^{19}
	August	Crushed tomato	0.62 ± 0.05^{de}	1.52 ± 0.46^{a}
	August	Puree	0.79 ± 0.07^{cd}	1.58 ± 0.55 ^{de}
Tohounvi		Crushed tomato	0.40 ± 0.09^{fg}	1.53 ± 0.16^{def}
	October	Puree	0.93 ± 0.15^{bc}	2.09 ± 0.03^{bc}
	August	Crushed tomato	0.64 ± 0.06^{d}	1.11 ± 0.17 ^{efg}
	August	Puree	1.14 ± 0.22^{a}	1.94 ± 0.63^{bcd}
Pomvi			$a a a a a a a a a^{ab}$	
	October	Crushed tomato	0.23 ± 0.09^{9}	1.07 ± 0.08^{19}
		Puree	0.93 ± 0.15^{20}	1.94 ± 0.04^{300}

Table 4. Fibers content and Vitamin of tomato varieties studied and puree.

^{a-e}Different classes of crushed tomatoes and purees, the figures bearing different letters in the column are significantly different at the threshold of 0.05%.

b*(yellow/green) are directly correlated to the color values. As for color, the ratio a/b of crushed tomatoes produced in August varies between 1.09 and 1.39, and their puree has a ratio between 1.05 and 1.15. For crushed tomatoes produced in October, we obtain a ratio of 1.35 to 1.72 and for their puree a ratio ranging from 1.23 to 1.42 for all varieties. The ratio showed us that the heat treatment acts on the color of our tomatoes during cooking. The result in a low a/b value from crushed tomato to puree represented an orange to brown color due to the formation of Maillard reaction products by the intensive heat treatment.

The fiber content varies according to the month of cultivation. Crushed tomatoes produced in August have a fiber content of between 1.11 and 1.63 g/100 g, and their puree between 1.58 and 2.27 g/100 g. The crushed tomatoes produced in October have a fibers value of 0.97 to 1.53 g/100 g and for their puree values ranging from 1.76 to 2.09 g/100 g whatever the variety. Heat treatment of tomato puree increases the fiber content of our samples due to the concentration the product undergoes after cooking. For global analyses of all varieties, there was no statistically significant difference in the pH parameters, acidity; on the other hand, the effect of heat treatment (cooking) showed a significant difference in Brix degree, color (Table 2).

Then specific nutritional compounds were analyzed. All results obtained are in Tables 2 to 4 and are parallel with the results of the conventional analysis for further

comparison.

Glucose and fructose and are the most common main sugars of the fruit. The values of crushed tomatoes in August showed a sugar content of 1.52 and 2.73%, and 1.85 to 3.30% for their puree. Crushed tomatoes produced in October have a sugar content of between 1.21 and 2.12 mg/100 g and their puree value of between 1.86 and 2.60 mg/100 g whatever the variety. Under the action of too much heating, fructose and glucose can be degraded in our purees. The sugar content of tomatoes varies due to the climatic and soil characteristics of each tomato variety and the presence or degradation of sucrose. This carbohydrate content may vary depending on different factors: light, temperature, irrigation and fertilizer.

The carotenoid composition of the tomato varieties studied reveals compounds with different levels of lycopene, carotenoids and vitamin E in Tables 3 and 4. Each variety vitamin E content values for all varieties range from 2.70 to 5.73%. Vitamin E content of crushed tomatoes produced in August is 0.20 to 0.64 mg/100 g and 0.38 to 1.14 mg/100 g for their puree. Vitamin E content values for crushed tomatoes produced in October range from 0.23 to 0.45 mg/100 g and pureed tomatoes range from 0.93 to 1.08 mg/100 g.

Beta-carotene results vary from 0.15 to 0.56 mg/100 g whatever the variety. The crushed tomatoes in August had a content of 0.15 to 0.51 mg/100 g and 0.38 to 1.14 mg/100 g for their puree.

Variety (p<0.05, IC=95%)	Month of production	Treatment	Naringenin (µg/100 g)	Chlorogenic acid (µg/100 g)	Coumaric acid (µg/100 g)	Rutin (µg/100 g)
Aklikonvi	August	Crushed tomato Puree	$556.45 \pm 68.88^{\circ}$ 980.80 ± 142.22 ^b	466.76 ± 42.45 ^e 1098.34 ± 43.35 ^c	19.84 ± 2.98 ^{ef} 33.97 ± 3.93 ^c	1420.85 ± 136.08 ^{bc} 1548.60 ± 45.90 ^b
	October	Crushed tomato Puree	101.18 ± 14.32 ^{de} 134.39 ± 7.41 ^d	287.24 ± 10.30^{f} 472.03 ± 74.40 ^e	9.13 ± 1.20 ^{hi} 10.31 ± 1.11 ^{ghi}	1283.07 ± 46.76 ^{cd} 1460.76 ± 197.90 ^b
Tohounvi	August	Crushed tomato Puree	924.50 ± 25.27 ^b 1022.22 ± 50.80 ^b	2100.95 ± 53.71 ^ª 2146.54 ± 117.81 ^ª	67.86 ± 0.47^{a} 54.81 ± 0.87 ^b	1157.41 ± 22.36 ^d 1316.18 ± 31.98 ^c
	October	Crushed tomato Puree	82.23 ± 18.50 ^e 98.49 ± 3.38 de	542.08 ± 150.28 ^b 1671.60 ± 42.14 ^b	16.78 ± 5.25 ^{efg} 20.64 ± 1.81 ^{de}	208.05 ± 3.12^{9} 447.71 ± 6.42 ^{fg}
Pomvi	August	Crushed tomato Puree	1305 ± 109.61 ^a 173.99 ± 14.39 ^d	940.40 ± 35.35 ^d 512.83 ± 150.52 ^e	25.77 ± 7.25 ^d 15.85 ± 5.53 ^{ef}	2404.27 ± 79.24 ^a 621.70 ± 15.17 ^e
	October	Crushed tomato Puree	10.33 ± 3.18 ^f 111.03 ± 6.89 ^{de}	82.44 ± 0.41 ⁹ 342.05 ± 3.91 ^f	$14.37 \pm 0.41^{\text{fgh}}$ $6.60 \pm 0.67^{\text{i}}$	453.27 ± 9.30f ^g 537.46 ± 7.82 ^f

Table 5. Phenols' composition of the tomato varieties and purees.

^{a-e}Different classes of crushed tomatoes and purees, the figures bearing different letters in the column are significantly different at the threshold of 0.05%.

Lycopene, the most abundant antioxidant in tomatoes, reveals its different levels in the three varieties studied. The month of August produced crushed tomatoes with a lycopene content of 2.33 to 5.01 mg/100 g and 3.94 to 4.60 mg/100 g of puree. Crushed tomatoes produced in October have a lycopene content of 3.02 to 4.44 mg/100 g and a value of 6.85 to 7.36 mg/100 g for their puree for each variety. There is a significant difference in carotenoids due to the effect of the heat treatment (cooking). As a result, purees have higher carotenoids content than crushed tomatoes and extended thermal heating can degrade carotenoids.

The results for phenolic compounds are shown in Table 5. For all varieties, values of 0.55 to 4.66 mg/100 g of total polyphenol content measured are obtained.

In August, crushed tomatoes gave us a total phenolic compound content of 2.47 to 4.66 mg/100 g and 1.29 to 3.66 mg/100 g for purees. Crushed tomatoes produced in October have a polyphenol content of 0.55 to 1.68 mg/100 g and a value of 0.99 to 2.10 mg/100 g for their puree. Differences found between tomato varieties depend on the month of cultivation of the tomatoes. This can be explained by the strong dependence of various factors, such as maturity, variety and agronomic conditions of the nutritional content of our tomatoes.

DISCUSSION

The evaluation of the hygienic quality of the different

products showed a satisfactory result in relation to the European Union's microbiological criteria (2007). This can be explained by the respect of good manufacturing practices during our production with a pasteurization scale (75°C at 30 min) applied to canned products with a pH lower than 4.5. Dossou et al. (2007) obtained similar results. In their studies, the analysis of fecal coliforms and total coliforms on their purees revealed no germ, whereas the standard tolerates 10 g⁻¹ of puree.

As for their total germs and the yeasts and molds, the analysis revealed respectively less than 30 microorganisms/g of puree, against 300 g⁻¹ of product, tolerated by the standard. This indicates a good level of hygiene in the production of the puree. The pH of the puree between 4 and 4.5 significantly reduces the rate and range of microorganisms that can grow on the product. Only acidophilic microorganisms, including yeasts and moulds and lactobacilli, can grow. This result corroborates those of studies conducted in Benin which also revealed an acidic pH (4.01 - 4.17) in processed tomatoes (Dossou et al., 2007).

A pH of 4 to 4.4 was found for our crushed tomatoes and purees studied. Oboulbiga et al. (2017) found values slightly lower than ours, ranging from 3.71 to 4.08 and 3.70 to 4.1 for Fagbohoun and Kiki (2000). As for the titratable acidity of the products, the significant variations observed between varieties could be due to soil and climate characteristics (Fagbohoun and Kiki, 1999). Adsule (2006) found in his studies that round tomato varieties have an acidity rate between 0.42 and 0.75% and those with elongated shape have a rate of 0.36 to 0.45%. The titratable acidity of our crushed tomatoes and purees reveals semilar results to those found by Adsule. Our round ribbed tomato varieties (Tohounvi) have an acidity of 0.42 to 0.62 citric acid equivalent and 0.22 to 0.42 citric acid equivalent for our elongated tomatoes (Aklikonvi). Brix results of purees being higher than those of crushed tomatoes could be explained by the heat treatment of tomato during cooking but production in different seasons does not affect the three varieties.

Brix degree values found ranging from 2.70 to 5.73% corroborate the results of the work carried out by Helyes et al. (2006) which obtained values of 4 to 5.5% and 4.5 to 5% from Dossou et al. (2007). The variation of the Brix value goes in the downward direction. Brix is more affected as the temperature of the treatment is higher. Brix being a key parameter of the quality of the concentrated, its decline is interpreted as a decrease quality of the concentrate according to Boumendjel et al. (2012). Brix being a key parameter of concentrate quality, its increase would be interpreted as good product quality in our case.

Aklikonvi purees have a better color compared to the other two varieties. Moreover, the results showed that a/b ratio is the appropriate parameter to characterize the degree of ripeness of tomato fruits. Helyes et al. (2006) obtained a value for a/b ratio between 0.5 and 1.5; Diantom et al. (2017) obtained values between 1.07 and 1.10. Jacob et al. (2010) obtained values between 0.68 and 1.11.

Results found for a/b ratio fall within the range of values obtained by the authors cited. The difference in color between the crushed tomato and the puree is due to the heat treatment that crushed tomatoes undergo when cooked for 20 min at 100°C. Boumendjel et al. (2012) had noticed that the color is more affected by the time of exposure to heat than the temperature of the treatment. The color being a technological parameter, its variation does not affect the commercial quality. The values obtained for total fiber ranging from 0.97 to 2.73 g/100 g for crushed tomatoes and purees are in the same range as the fiber content measured in Diantom et al. (2017) who found a content of 1.5 g of fiber in their puree.

The total sugar content of our different samples is 1.21 to 3.30 g/100 g. These results corroborate with those found by some authors who have worked in Benin, Nigeria and Italy. Akinboye et al. (2018) obtained values between 2.48 and 3.05 g/100 g. Helyes et al. (2013) also found a value of 2 to 3 g/100 g for the sugar content. Fagbohoun and Kiki (2000) found 1.97 and 3.1 g/100 g.

Lycopene is a particularly effective antioxidant capable of combating free radicals much more effectively than beta-carotene. Crushed tomatoes and tomato puree are high in lycopene, which is an advantage for the consumer because it is the main pigment in tomatoes that indicates the maturity of the fruit and contributes to the prevention of various forms of cancer (Agarwal et al., 2000). The analysis on crushed tomatoes and puree reveals a lycopene content of 2.33 to 7.37 mg/100 g and a betacarotene content of 0.13 to 0.56 mg/100 g of product. Georgé et al. (2011) obtained a lycopene concentration of 3.7 mg/100 g and a beta-carotene concentration of 1.1 mg/100 g. Akinboye et al. (2018) obtained for the studied tomato varieties a content of 2.61 to 2.75 mg/100 g lycopene and 0.43 to 0.44 mg/100 g beta carotene. The vitamin E or α -tocopherol content on the crushed tomatoes and puree allowed us to obtain a content of 0.20 to 1.18 mg/100 g of fresh fruit. These results are in line with values found by Grasselly et al. (2000) ranging from 0.04 to 1.20 mg/100 g of vitamin E.

The main phenolic compounds in tomatoes are rutin, chlorogenic acid and naringenin, which are considered antioxidants with a beneficial effect on human health. The amount of phenolic antioxidants in processed tomatoes is higher than in raw tomatoes (Chanforan, 2010).

Our results reveal rutin contents of 0.20 to 2.40 mg/100 g, chlorogenic acid of 0.08 to 1.67 mg/100 g, naringenin of 0.009 to 1.30 mg/100 g and 0.006 to 0.06 mg/100 g of coumaric acid. Values are close to those of Jacob et al. (2010) who obtained a content of 0.09 to 1.73 mg/100 g chlorogenic acid, 0.08 to 2.12 mg/100 g rutin and 0.13 to 0.33 mg/100 g coumaric acid.

Conclusion

At the end of this study, tomato purees were made from three tomato varieties produced in Togo: Pomvi, Aklikonvi and Tohounvi. These tomatoes have proven to be good sources of nutritional content. The physico-chemical characteristics as well as the microbiological quality of these purées were determined. The purees show microbiological stability. The results of the various analyses show the usefulness of the transformation of crushed tomatoes into puree in a small-scale local context.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Physico-chemical properties of kernel from coconut (Cocos nucifera L.) varieties grown at the Kenyan Coast

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The coconut tree (*Cocos nucifera*) is widely referred as the "tree of life" for its important role in the livelihoods of 10 million persons from over 90 countries globally. In Kenya, the coconut sub-sector is valued at KES 25 billion, yet only 65% is utilised, which is partly contributed by insufficient knowledge of the coconut's physico-chemical features and investment in the sector. Physico-chemical analysis of four coconut varieties' kernel grown in two counties (Kilifi and Kwale) of Kenya was carried out. Based on the de-husked coconut fruit, coconut kernel was the highest component (46.58 - 48.70%) in comparison to shell ($25.93\pm0.72\% - 28.46\pm0.29\%$) and water ($23.75\pm1.07\% - 27.11\pm1.49\%$) across the varieties. The colour of the coconut kernel was marked by generally high L* values (85.34 - 93.35) and low a* (0.51 - 0.81) and b* (1.53 - 2.20) values among the varieties confirming the milky- white colouration of the kernel. All the varieties contained high crude fat ($35.01\pm1.0 - 38.28\pm1.09\%$) content. Fatty acid analysis profile revealed that lauric acid (45.91 - 50.72%) was the predominant fatty acid. Most of the oil extract was saturated (91%) but comprising of middle chain fatty acids. This indicates stability for use in ketogenic diets.

Key words: coconut kernel, coconut variety, colour, proximate analysis, fatty acid profile.

INTRODUCTION

Coconut (*Cocos nucifera* L.), as a member of the *Palmacea* family, grows in the tropics and is generally referred to as a coconut palm (Patil and Benjakul, 2018). The coconut palm is commonly termed as the "tree of life," "heavenly tree," "tree of abundance," or "nature's supermarket" due to its important role as a direct source of materials, nutrition, and income to over 10 million households in about 90 countries worldwide (Omar and Fatah, 2020). Asia tops in the world as the major coconut

producer with 90% of the total production emanating from Indonesia, India, Philippines, Sri-Lanka, and Thailand (Patil and Benjakul, 2018). Generally, the entire coconut fruit takes a year to mature with various developmental stages: the husk and shell develop first, followed by enlargement of embryo sac cavity, which is then filled with liquid; the husk and shell becomes thicker after 4 months; the meat starts to form against the inner wall of the cavity after 6 months with the first layer being thin and

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> gelatinous; after 8 months, the soft white endocarp turns dark brown and becomes hard; and lastly the fruit becomes mature within 1 year (12 months) (Patil and Benjakul, 2018).

The mature coconut fruit contains 25% water, 28% meat, 12% shell, and 35% husk and weighs between 3 and 4 kg (DebMandal and Mandal, 2011). On a global scale, approximately 70% of coconuts are consumed locally with more than half being eaten fresh (Patil and Benjakul, 2018). The edible coconut products are predominantly obtained from the meat (solid endosperm) and water (liquid endosperm) (DebMandal and Mandal, 2011). In obtaining the edible portion, the shell of the coconut is removed after eliminating the husk, followed by pairing and draining water. The meat/kernel is then manually collected and grated to yield other products. The coconut kernel is a good source of protein, fat, fibre and carbohydrate (Appaiah et al., 2014; Wynn, 2017; Patil et al., 2017). Lauric acid, a saturated, medium-chain fatty acid is its predominant fatty acid (Laureles et al., 2002; Azeez, 2007; Ghosh et al., 2014; Pham, 2016; Boateng et al., 2016). The composition of the mature coconut kernel, however, is dependent on variety, nut maturity, geographical location, and cultural practices (Senphan and Benjakul, 2015).

Globally, studies on the composition of coconut kernel are either old or incomplete in their scope (that is, lacking some constituents). For instance, proximate analysis is "saturated" with old studies (Dendy and Timmins, 1973; Grimwood and Ashman, 1975; Balachandran et al., 1985; Chakraborty, 1985; Kwon et al., 1996). Recent studies lack analysis of fibre, carbohydrate, and protein (Appaiah et al., 2014; Patil et al., 2017). Moreover, in relation to fatty acid profile, majority of studies omit analysis of either caproic acid (C6:0) or arachidic acid (C20:0) (Laureles et al., 2002; Azeez, 2007; Ghosh et al., 2014; Pham, 2016; Boateng et al., 2016) all of which are important in determining the nutritional and health benefits of the oil.

In Kenya, the coconut sub-sector is valued at KES 25 billion (\$231 million), yet only 65% of the market is exploited, which is partly contributed by insufficient research (AFA-NOCD, 2015). The Kenyan coconut sub-sector "is depicted as the sleeping giant" (Muhammed et al., 2012). By applying strategic interventions, more than 100,000 farmers can be supported to contribute to over 1.5% of the agricultural GDP and 0.4% of the national GDP (KCDA, 2013). This study was carried out to analyse the physicochemical parameters of kernel from different coconut varieties grown at the Kenyan coast targeting enhancing the utilization window of coconut.

MATERIALS AND METHODS

Study design

Coconut fruit of four varieties (East African Tall-Green, East African Tall-Yellow, East African Short-Green, and Dwarf-Yellow) were

obtained from farmers at Msambweni (Kwale County). However, only two of the varieties were obtained in Kilifi County (that is, only East African Tall- Green and East African Short-Green) at the time of research. It was assumed that all the fruits were mature based on the solid brown colouration of the husk. The fruits, with their husks intact, were then stacked in bags and transported in a wellventilated van to Jomo-Kenyatta University of Agriculture and Technology post-harvest laboratory for subsequent analysis as shown in Figure 1.

Determination of coconut fruit composition

The various coconut fruits were first de-husked. The de-husked fruits were weighed, deshelled, and the various coconut components (shell, kernel, and water) separated, and subjected to physical and chemical analysis (Figure 1). An illustration of the various components is shown in Figure 2. After weighing, the weight of individual component was divided by the total weight of the coconut fruit and expressed as a percentage.

Determination of physico-chemical properties of coconut Kernel

Colour of coconut kernel

Colour measurement for the kernels of different coconut varieties were determined using a handheld HunterLab colour difference meter (Minolta, Chroma Meter CR-200; Minolta Camera Co., Ltd., Osaka, Japan), which uses the principle of Opponent-Colour Theory assuming that the human eye receptors perceive colour as pairs of opposites: L*-scale (light vs. dark); a*-scale (red vs. green); and b*scale (yellow-blue) (Patil et al., 2017). Calibration of the instrument was done prior to colour measurement of samples with the aid of a white and black ceramic plate. The measurements were done in triplicate while putting into consideration three sections of the sample. The results were expressed using the Lab* colour system. Difference in colour change between the samples and the standard (Δ E*) and the chromaticity difference (Δ C*) were also determined using the following equations (Patil et al., 2017):

$$\Delta \mathsf{E}^* = \sqrt{[(\Delta \mathsf{L}^*)^2 + (\Delta \mathsf{a}^*)^2 + (\Delta \mathsf{b}^*)^2]} \mathsf{C}^* = \sqrt{[(\Delta \mathsf{a}^*)^2 + (\Delta \mathsf{b}^*)^2]}$$
$$\Delta \mathsf{C}^* = \mathsf{C}^* \mathsf{sample} - \mathsf{C}^* \mathsf{standard}$$

Where,

 ΔL^* , Δa^* , and Δb^* are the differences between the corresponding colour element of the sample and the standard colour of coconut kernel (L^{*} = 93.55, a^{*} = 0.84, and b^{*} = 0.37).

Proximate analysis

First, the coconut kernel was grated followed by proximate analyses of the samples in triplicates (AOAC, 2000). The moisture and ash content were determined using volatilization gravimetry (that is, thermal decomposition of the sample with the aim of measuring the resulting change in mass), crude protein by semi micro-Kjedahl method, crude fat by Soxhlet method, crude fibre by acid and alkali digestion method, and carbohydrates by difference.

Moisture content

About 5 g of the sample was weighed and also the weight of the moisture dish taken. The sample while in the moisture dish was placed in the moisture oven and the temperature upscaled to 105°C. The sample was then dried at this temperature until constant



Figure 1. Experimental design on physico-chemical properties of coconut varieties grown in Kenya.



Figure 2. A visual illustration of the various parts of coconut fruit.

weight was achieved, removed, cooled, and ultimately weighed. The amount of moisture in the sample(s) was calculated using the below formula:

Ash

About 5 g of the sample was weighed into a clean crucible (weight also taken), and then charred in a fume hood by heating until smoking stopped. The charred sample(s) was transferred into a muffle furnace and temperature adjusted to 550°C. The sample (s) was then ashed for about 5 h until all the organic matter was pylorized. The sample was removed using a tong and placed in desiccator to cool, and finally weighed in calculating the amount of ash aided by the following formula:

Weight of the ash
Percentage (%) ash =

Weight of sample × 100

Crude fibre

About 2 g of the sample was weighed and placed into 200 ml of 1.25% sulphuric acid and boiled for 1 h. Then, the solution and content were poured into Buchner funnel equipped with glass wool, allowed to cool, and filtered. Consequently, the residue was boiled in 200ml of sodium hydroxide for 1 hour, transferred again onto the Buchner funnel for filtering. The resulting residue was washed thrice with alcohol and petroleum either. The final residue was placed onto a clean crucible (weight already taken), dried in the moisture oven for 1 hour (at constant weight), removed, cooled, and weighed. The crucible(s) containing the sample(s) was placed in a muffle furnace (550°C) for 1 hour, removed, cooled, and weighed. The difference in weight (loss in ignition) was recorded as crucible fibre and expressed in crude fibre as shown in the formula:

Percentage (%) crude fibre = $\frac{\text{Weight of the extract}}{\text{Weight of sample}} \times 100$ (3)

Crude protein

About 2 g of the sample(s) was weighed into a digestion flask and a combined catalyst comprising of 5 g of potassium sulphate and 0.5 g of copper sulphate added as well as 15 ml of sulphuric acid. The mixture was then heated in a fume hood until the digest colour became blue, signifying the end of the digestion process. After cooling the digest, it was transferred into 100 ml volumetric flask and topped up to the mark with deionised water. Meanwhile a blank digestion was also prepared. Approximately 10ml of the diluted digest was then transferred into the distilling flask and washed with distilled water. This was followed by addition of 15 ml of 40% sodium hydroxide, and also washed with distilled water. Distillation was done to a volume of 60ml distillate, and finally the distillate was titrated using 0.02 N hydrochloric acid (HCL) to orange colour (end point signification) of the mixed indicator. The percentage nitrogen was calculated as follows:

%N = (V1- V2) * N * F * 100/ (V * 100/S)

Where, V1 is the titre for sample in ml, V2 is titre for blank in mL; N= normality of standard HCL; f= factor of standard HCL solution; V= volume of diluted digest taken for distillation; S= weight of sample taken for distillation.

The percentage protein was then determined as follows:

Percentage (%) protein= Nitrogen * Protein factor (6.25) (4)

Crude fat

About 5 g of the sample was weighed into an extraction thimble and stoppered with defatted cotton wool, and placed in the extraction apparatus. A clean flask was weighed and filled with petroleum ether up to two-thirds. The Soxhlet apparatus was set by fixing the extraction apparatus on the flask and connecting them to a condenser to start fat extraction for about over eight hours. The extraction solvent was then rota-evaporated and the extracted fat dried in hot air oven at 70C for 15 minutes and final weight of the flask taken.

Weight of the extracted fat

Percentage (%) crude fat =

Weight of sample

Carbohydrate

Carbohydrate was determined by difference [that is, 100 – (crude fat +crude protein+ash + moisture + crude fibre)].

Fatty acid profile

The NaOCH₃-MeOH methylation process as described by Wang et al. (2015) was used. In this process, 0.5 g of dry ground coconut kernel was measured and placed in the test tube and 2 ml of 0.5 M sodium methoxide solution added. The test tube was then placed in the oven to enhance reaction for 1 h 50 min at 55°C with mixing for 5 s after every 20 min. Then, 2 ml of saturated sodium bicarbonate and 3 ml of n-hexane were added followed by wellmixing of the tubes. Finally, the extracts (organic layer containing the fatty caid methyl esters) were removed and used for gas chromatography (GC) analysis. Agilent 7890B gas chromatograph (Agilent Technologies, Stevens Creek Blvd, Santa Clara, CA, United States) equipped with DB-FATWAX UI, 30 m x 0.25 mm, 0.25 µm column attached to mass spectrometry (MS) detector was used. Conditions set for analysis included: split mode of injection (split ratio 50:1) at 250°C; oven temperature 50°C (2 min), 50°C/min to 174°C (14 min), 2°C/min to 215°C (25 min); hydrogen as the carrier gas at constant flow, 40 cm/s at 50°C, and injection volume (1 µl). Fatty acid identification was done using a standard fatty acid methyl ester (FAME) mix. The composition of fatty acid was qualitative and quantitatively analysed using Agikent MassHunter Software (Agilent Technologies, Stevens Creek Blvd, Santa Clara, CA, United States) and reported by the normalisation method and expression done in terms of percentage relative composition of individual fatty acids.

Statistical analysis

The results were subjected to analysis of variance (ANOVA) using Stata software version 13 (Stata Corp, College Station, TX, USA), and expressed as means \pm standard deviations and separation of means carried out by the Bonferroni adjustment at p<0.05.

RESULTS AND DISCUSSION

Coconut composition

Among the three de-husked coconut fruit constituents, coconut kernel had the hiahest composition (45.10±1.64%-50.32±0.16%), followed by coconut shell (25.93±0.72%) 28.46±0.29%), and lastly water -(23.75±1.07%) -27.11±1.49%) with no significant difference among the varieties except the coconut kernel of varieties from Kilifi county (Table 1). A similar order in the composition of kernel, shell, and water is consistent with the literature (DebMandal and Mandal, 2011; Wynn, 2017). Among the three, coconut kernel is the most used as far as food- based products are concerned (Sangamithra et al., 2013). Accordingly, the East African Tall - Green (EAT-G) from Kilify county had the highest kernel (50.32±0.16%) as compared to its counterpart from Kwale county making it ideal for processing of coconut-food products. In terms of kernel weight, it ranged between 0.20±0.01 kg - 0.27±0.01 kg

County	Variety	Kernel (%)	Water (%)	Shell (%)
	EAT-G	47.08±1.80 ^a	25.34±0.92 ^a	27.58±0.72 ^a
	EAS-G	45.10±1.64 ^a	27.11±1.49 ^a	28.46±0.29 ^a
Kwale	EAT-Y	47.26±0.40 ^a	24.48±0.47 ^a	28.27±0.38 ^a
	D-Y	48.05±0.15 ^a	25.58±0.51 ^a	26.37±0.57 ^a
	<i>p</i> -value	0.13	0.17	0.07
		50.00×0.40 ^a	00 75 4 07 ^a	05 00 0 7 0 ^a
Kilifi	EAT-G	50.32±0.16	23.75±1.07	25.93±0.72
NIIII	EAS-G	48.07±0.27 ^b	24.50±0.53 ^a	27.10±0.54 ^ª
	<i>p</i> -value	0.001	0.1	0.09

Table 1. % Composition (weight) of coconut kernel, water, and shell.

EAT-G: East African Tall Green; EAS-G: East African Short Green; EAT - Y: East African Tall Yellow; and D - Y: Dwarf Yellow. Values are means \pm standard deviation (n=18). Means with different superscript letters in the same row are significantly different at p<0.05.



Figure 3. Weight of coconut kernel. EAT-G: East African Tall Green; EAS-G: East African Short Green; EAT - Y: East African Tall Yellow; D - Y: Dwarf Yellow.

(Figure 3), which, however, is low as compared to kernel weight of coconut grown in major producing countries such as Indonesia, ranging between 0.32and 0.6 kg (Tuhumuri et al., 2016). Such a difference is associated with differences in coconut variety, stage of maturity, geographical location, and cultural practices (Senphan and Benjakul, 2015; Patil et al., 2017). The kernel weight, which is related to its overall composition, is a critical parameter in terms of production with higher weight leading to higher production efficiency due to generation of more kernel-based products as compared to lower weight (Mpagalile, 2005; Sangamithra et al., 2013).

Colour of coconut kernel

Generally, L* values were high ($85.23\pm0.76 - 93.35\pm0.30$), followed by b* values ($1.50\pm0.03 - 2.20\pm0.05$), and lastly a* values ($0.50\pm0.14 - 0.81\pm0.02$) as evident in Table 2. These values are agreement with those generated by other studies (Ghosh et al., 2014; Patil et al., 2017). Coconut kernel has a milky white colouration, and indication of lightness as manifested by high L* values (Patil et al., 2017). The L* values were significantly different among the coconut varieties from Kwale county with the highest being observed in Dwarf Yellow (D-Y) – 93.35\pm0.30 and the lowest in East African Tall-Green

County	Variety	L*	a*	b*	ΔE*	∆C*
Kwale	EAT-G	85.34±0.15 ^a	0.56±0.07 ^a	1.78±0.29 ^a	7.80±0.12 ^a	1.44±0.27 ^a
	EAS-G	86.15±0.15 ^a	0.67±0.18 ^a	1.53±0.04 ^ª	6.95±0.14 ^ª	1.18±0.03 ^ª
	EAT-Y	92.08±1.87 ^b	0.81±0.02 ^a	2.20±0.05 ^a	2.43±0.97 ^b	1.83±0.05 ^ª
	D-Y	93.35±0.30 ^b	0.51±0.01 ^a	1.79±0.03 ^a	1.51±0.11 [°]	1.46±0.03 ^a
	<i>p</i> -value	<0.001	0.08	0.13	<0.001	0.1
Kilifi	EAT-G	85.23±0.76 ^a	0.50±0.14 ^a	1.52±0.05 ^a	7.86±0.89 ^a	1.20±0.21 ^a
	EAS-G	86.22±0.54 ^a	0.50±0.02 ^a	1.50±0.03 ^a	6.89±0.86 ^a	1.18±0.16 ^ª
	<i>p</i> -value	0.09	0.1	0.3	0.08	0.12

Table 2. Colour of coconut kernel.

EAT-G: East African Tall Green; EAS-G: East African Short Green; EAT – Y: East African Tall Yellow; and D – Y: Dwarf Yellow. Values are means \pm standard deviation (n=54). Means with different superscript letters in the same row are significantly different at p<0.05.

Table 3. Proximate composition of coconut kernel.

County	Variety	%Moisture	%Ash	%Fat	%Protein	%Fibre	%Carbohydrate
Kwale	EAT-G	48.23±0.79 ^a	1.14±0.34 ^a	37.28±1.06 ^a	3.95±0.24 ^a	2.53±0.76 ^a	7.41±0.56 ^a
	EAS-G	45.31±0.40 ^b	1.02±0.40 ^a	35.01±1.04 ^a	3.63±0.28 ^a	2.14±0.25 ^a	13.30±0.37 ^a
	EAT-Y	47.28±0.47 ^a	1.15±0.85 ^ª	38.28±1.09 ^a	4.57±0.14 ^a	3.26±0.60 ^a	6.15±0.25 ^{ab}
	D-Y	43.17±0.70 ^c	1.30±0.34 ^ª	36.14±0.39 ^a	5.81±0.21a	3.75±0.09 ^a	10.20±0.12 ^c
	<i>p</i> -value	<0.001	0.17	0.21	0.07	0.15	<0.001
Kilifi	EAT-G	46.00±0.37 ^a	1.15±0.40 ^a	36.43±0.98 ^a	4.49±0.39 ^a	2.92±0.46 ^a	9.27±0.48 ^a
	EAS-G	46.77±0.19 ^a	1.08±0.30 ^a	35.65±0.85 ^ª	3.79±0.49 ^a	2.34±0.52 ^a	10.39±0.20 ^a
	<i>p</i> -value	0.97	0.76	0.16	0.85	0.61	0.12

EAT-G: East African Tall Green; EAS-G: East African Short Green; EAT – Y: East African Tall Yellow; and D – Y: Dwarf Yellow. Values are means \pm standard deviation (n=54). Means with different superscript letters in the same row are significantly different at p<0.05.

(EAT-G) - 85.34±0.15. Also, ΔE^* (the change in colour - L^{*}, a^{*} and b^{*} values – as compared to the standard - L^{*} = 93.55, a^{*} = 0.84, and b^{*} = 0.37) varied among the coconut varieties from Kwale county: the highest ΔE^* was observable in EAT-G (7.80±0.12), whereas the lowest in D-Y (1.51±0.11). Such differences can be attributed to genotypic variation (Patil et al., 2017). Nonetheless, colour comparison between the two counties, generally, did not show much difference at p<0.05.

Proximate analysis

As evident in Table 3, the proximate analysis revealed high moisture content $(43.17\pm0.70-48.23\pm0.79\%)$, followed by crude fat $(35.01\pm1.0-38.28\pm1.09\%)$, then carbohydrate $(6.15\pm0.25-13.30\pm0.37\%)$, crude protein $(3.63\pm0.28-5.81\pm0.21\%)$, crude fibre $(2.14\pm0.25-3.75\pm0.09\%)$, and lastly ash content $(1.02\pm0.40-1.30\pm0.34\%)$. A similar order is corroborated by existing studies (Dendy and Timmins, 1973; Grimwood and

Ashman, 1975; Balachandran et al., 1985; Chakraborty, 1985; Kwon et al., 1996; Patil et al., 2017; Wynn, 2017).

Crude fat content did not vary significantly among the varieties in both counties. East African Tall- Yellow (EAT-Y) had the highest crude fat content (38.28±1.09%), while East African Short-Green (EAS-G) had the lowest crude fat content (35.01±1.0), all of which came from Kwale county. These values compared well with the range reported by Appaiah et al. (2014) and Patil et al. (2017). Crude fat content of coconut is directly proportional to the extractable oil content (Sangamithra et al., 2013; Ghosh et al., 2014), and therefore EAT-Y from Kwale county would be preferable for the production of oil-based coconut products like virgin coconut oil. For carbohydrate, the results indicated a significant difference across the varieties from Kwale county and not from Kilifi. EAS-G had the highest content (13.30±0.37%) and EAT-Y the least content (6.15±0.25%), all from the same county (i.e., Kwale). This variation may be attributed to genotypic difference between the varieties. Nonetheless, comparison based on the two counties show variation in carbohydrate content, for

Table 4. Fatty acid profile of coconut kernel.

County	Variety	Caproic	Capryllic	Capric	Lauric	Myristic	Palmitic	Stearic	Oleic	Linoleic	Arachidic
	EAT-G	0.72±0.21 ^a	6.99 ^a ±0.64 ^a	4.87±0.60 ^a	50.72±0.73 ^a	16.53±0.44 ^a	11.18±0.86 ^a	2.64±0.64 ^a	4.57±0.47 ^a	1.94±0.12 ^ª	0.17±0.03 ^a
	EAS-G	0.90±0.21 ^a	7.43±0.39 ^a	5.87±0.70 ^a	47.13±0.90 ^b	18.06±1.08 ^b	9.22±0.83 ^a	3.20±0.53 ^ª	6.08±0.63 ^a	2.17±0.40 ^a	0.19±0.23 ^ª
Kwale	EAT-Y	0.64±0.23 ^a	8.35±0.29 ^a	7.22±0.19 ^a	47.47±0.45 ^b	17.02±0.14 ^b	8.71±0.41 ^a	3.09±0.08 ^a	5.14±0.20 ^a	2.19±0.27 ^a	0.18±0.02 ^a
	D-Y	0.73±0.30 ^a	8.65±0.24 ^a	5.46±0.33 ^a	45.91±0.32 ^{ab}	20.47±0.40c	8.53±0.43 ^a	2.84±0.16 ^a	5.31±0.33 ^a	2.20±0.22 ^a	0.18±0.06 ^a
	<i>p</i> -value	0.12	0.11	0.21	0.001	<0.001	0.1	0.06	0.31	0.13	0.81
IZ:1:4:	EAT-G	0.76±0.25 ^a	7.74±0.93 ^a	5.84±0.87 ^a	47.90±1.65 ^ª	17.79±1.64 ^a	9.77±1.44 ^a	2.96±0.55 ^a	5.09±0.59 ^a	2.15±0.34 ^a	0.18±0.03 ^a
Kilifi	EAS-G	0.77±0.24 ^a	7.44±0.33 ^a	5.41±1.14 ^a	48.73±2.58 ^a	17.76±1.35 ^ª	9.48±1.06 ^a	2.89±0.51 ^ª	5.68±0.99 ^a	2.00±0.09 ^a	0.17±0.03 ^a
	<i>p</i> -value	0.98	0.3	0.22	0.25	0.95	0.53	0.7	0.08	0.15	0.41

EAT-G: East African Tall Green; EAS-G: East African Short Green; EAT – Y: East African Tall Yellow; and D - Y: Dwarf Yellow. Values are means ± standard deviation (n=54). Means with different superscript letters in the same row are significantly different at p<0.05.

example, EAS-G from Kwale recorded carbohydrate content of $13.30\pm0.37\%$, whereas EAS-G from Kilifi had carbohydrate content of $10.39\pm0.20\%$, and such variation may be due to difference in coconut variety, stage of maturity, and geographical location (Senphan and Benjakul, 2015). These values are comparable with the existing studies (Chakraborty, 1985; Kwon et al., 1996; Ghosh et al., 2014).

Crude protein did not vary significantly across the coconut varieties in both counties. D-Y had the highest crude protein content ($5.81\pm0.21\%$), while EAS-G had the lowest content ($3.63\pm0.28\%$) with the variation being attributed to genotypic difference since they were sourced from the same county (Kwale). These findings are corroborated by other studies (Chakraborty, 1985; Kwon et al., 1996; Pham, 2016).

Crude fibre, just like crude protein, did not differ significantly across the varieties in each County. The highest crude fibre content was recorded by D-Y ($3.75\pm0.09\%$) whereas the least crude fibre content was observed in EAS-G ($2.14\pm0.25\%$) with genotypical variation being the likely cause. A similar range is supported by other studies (Kwon et al., 1996; Pham, 2016). The fibre content is important in diet decreasing chances of constipation and maintaining bowel health (Barber et al., 2020).

Similarly, the total ash did not vary significantly among the varieties in both counties. D-Y had a relatively high ash content $(1.30\pm0.34\%)$ compared to EAS-G, which had the lowest ash content $(1.02\pm0.40\%)$ with the possible cause of this variation being varietal difference and soil composition. This range is an agreement with the findings of most studies (Chakraborty, 1985; Kwon et al., 1996; Patil et al., 2017; Wynn, 2017). Coconut kernel has relatively high minerals like iron, magnesium, phosphorous, zinc, and sodium (Wynn, 2017) signifying that varieties such as EAS-G may be preferable.

Finally, moisture content differed significantly across varieties grown in Kwale county as opposed to Kilifi county. The highest moisture content was observed in EAT-G ($48.23\pm0.79\%$) while the lowest moisture content was realised by D-Y ($43.17\pm0.70\%$). This range is corroborated by a plethora of studies (Chakraborty, 1985; Kwon et al., 1996; Patil et al., 2017; Wynn, 2017). Low

moisture, unlike high moisture, is preferable since water content in indirectly proportional to the dry matter required for the crispiness of coconut flakes, durability of coconut flour, and increased production of virgin coconut oil and coconut protein powder (Sangamithra et al., 2013) and as such, D-Y would be preferable (Table 3).

Fatty acid profile

Table 4 shows the fatty acid profiles of coconut kernel, and revealed that the highest component was lauric- C12:0 ($45.91\pm0.32\%$ - $50.72\pm0.73\%$) and the least was arachidic - C20:0 ($0.17\pm0.03\%$ - $0.19\pm0.23\%$). These values are supported by other studies (Laureles et al., 2002; Azeez, 2007; Ghosh et al., 2014; Pham, 2016; Boateng et al., 2016). A significant difference was only observed in the values of lauric and myristic across the varieties from Kwale county. From the results, over 90% of the coconut oil was saturated (caproic - C6:0, caprylic - C8:0, capric - C10:0, lauric - C12:0, myristic - C14:0, palmitic- C16:0,

stearic- C18:0, and arachidic - C20:0). Additionally, the ratio of saturated fatty acid (SFA - C6:0,C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, and C20:0): monounsaturated fatty acid (MUFA - C18:1): polyunsaturated fatty acid (PUFA - C18:2) was found to be 1: 0.06: 0.02 confirming the 91% saturation, which does not meet the recommendation by the American Heart Organisation of 1:1:1 (SFA: MUFA: PUFA) in edible fats and oils (Gulla and Waghray, 2011). Among the coconut varieties, EAT-G from Kwale county seemed to relatively have high level of saturation with a lauric content of 50.72±0.73% in comparison to D-Y, which had the lowest lauric content (45.91±0.32%), from the same county and, therefore, the variation is attributable to genotypic difference. This high level of saturation was attributed to high atherogenicity index (18.03±0.71- 19.57±0.68) and thrombogenicity index (8.48±0.56 - 9.32±0.87) of the oil extracted from the kernels of various coconut verities. High atherogenicity and thrombogenicity indices are indicators of susceptibility of the consumers to developing cardiovascular diseases. Accordingly, Boateng et al. (2016) argue that since coconut oil is saturated, it is not highly preferred by a section of health-conscious consumers. Nonetheless, the presence of high-level saturated acids is an indication of shelf stability of the coconut oil (Ghosh et al., 2014). Also, it should be noted that palmitic acid, which is the most abundant and highly lipotoxic dietary fatty acid (Martinez et al., 2015; Carta et al., 2017), is relatively low in coconut oil (Table 4).

Moreover, almost half coconut oil comprises of lauric acid (commercially, coconut oil is referred to as lauric acid), a medium chain fatty acid which upon absorption is transported directly to the liver (unlike the long chain fatty acids), where it is metabolized to produce energy and ketone bodies, rather than being stored as fat (Dayrit, 2015). A recent study found that supplementation of a high fat diet with 3% palmitic acid led to a significant increase in visceral fat, insulin resistance and both visceral adipose and hepatic inflammation; while supplementation with 3% lauric acid led to an even higher increase in visceral fat, but without insulin resistance, and with less inflammation (Saraswathi et al., 2020). The laurate-associated higher visceral fat increase in this case may be considered to be due to adipose tissue expansion through increased adipocyte numbers (hyperplasia) in order to safely store the excess fat, rather than the pro-inflammatory, hypertrophic expansion caused by palmitic acid, where preadipocyte differentiation is impaired, and the existing adipocytes end up storing excess fat (Caputo et al., 2020). Moreover, from the mentioned study of Saraswathi et al. (2020) it cannot be concluded that lauric acid is obesogenic, because it was supplemented to a high fat diet. In addition, the palmitate- but not the lauratesupplemented high fat diet caused increased liver enzymes aspartate amino-transferase and alanine aminotransferase (Saraswathi et al., 2020), which predict the development of (pre)diabetes (Oberlinner et al., 2010). The potential

benefits of coconut oil against diabetes and other cardiometabolic disorders have indeed been reported, although the results have been mixed (Malaeb and Spoke, 2020). Apart from the medium chain fatty acids, coconut contains the beneficial monoglyceride, monolaurin, which is antimicrobial (antibacterial, antiviral, and antifungal), antitoxic, immune-modulating, and metabolic-enhancing, (Azeez, 2007; Ghosh et al., 2014; Pham, 2016). The main limitation of coconut oil usage as a dietary fat, however, is its low level of essential fatty acid, linoleic acid (Azeez, 2007). Nevertheless, linoleic acid is abundant in many readily available dietary oils such as corn, sunflower and soybean oils.

Conclusion

Generally, results of the physico-chemical analysis of coconut kernel grown at the Kenyan Coast did not differ significantly across the varieties in the two counties. Among the components of de- husked coconut fruit, coconut kernel was averagely the highest $(47.67\pm0.90\%)$, followed by shell $(27.20\pm0.51\%)$, and finally water $(25.43\pm1.28\%)$. In addition, high L* values and low a* and b* confirmed the milky white colouration of coconut kernel. All the varieties had a relatively high crude fat content which might indicate high calorific value of the kernel. Although the oil from coconut is largely saturated, it is low in the highly lipotoxic palmitic acid, and high in medium chain fatty acids, especially lauric acid, which may be more beneficial than harmful.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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