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Abdullahi, M., Zainab, F. A., Pedavoah, M. M., Sumayya, U. B., & Ibrahim, A. D. (2014). Evaluating the suitability of *Adansonia digitata* fruit pulp for the production of yoghurt. *International Journal of Biological and Chemical Sciences*, 8(2), 508-516. <https://doi.org/10.4314/ijbcs.v8i2.10>

Published in:

International Journal of Biological and Chemical Sciences

Document Version:

Publisher's PDF, also known as Version of record

Queen's University Belfast - Research Portal:

[Link to publication record in Queen's University Belfast Research Portal](#)

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Evaluating the suitability of *Adansonia digitata* fruit pulp for the production of yoghurt

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ABSTRACT

The potentials of neglected and under-utilized plant species (NUS) to enhance food security and safety has been highlighted in recent years. NUS have the potential to fight malnutrition and improve human health particularly in Africa. Despite their potentials, there is still a huge knowledge gap as to their potential effect when used to fortify foods. This research was conducted to evaluate the suitability of *Adansonia digitata* fruit pulp for yoghurt production using different mixtures of milk and *A. digitata* fruit pulp powder in ratios of 4:1, 3:2, 2:3, 1:4 and 5:0. Proximate and mineral content analysis was conducted using the AOAC method. Sensorial analysis was done and the outcome informed the choice of samples analysed for volatile compounds profile by GC-MS analysis of the chloroform extract. The proximate composition of the yoghurt samples increased with the addition of *A. digitata* pulp powder and the results showed that the ratio of 2:3 had highest lipid content (5.5%) and fiber, 1:4 had highest protein content (5.65%) while commercial yoghurt had trace ash and no fiber. Calcium content was highest in the mixture; 2:3 and 4:1 (0.5 mg/kg), 2:3 had highest magnesium content (0.8 mg/kg) and potassium content was highest in 4:1 (1250 mg/kg) respectively. Gas chromatography and mass spectroscopy (GC-MS) analysis revealed that 2:3 mixture had eleven (11) volatile metabolites, 1:4 had (9) while plain powder also had (9) volatile metabolites. This study shows that incorporation of *A. digitata* fruit pulp increased the bioavailability of nutrients, minerals and a volatile metabolite with medicinal properties. The fortification of yoghurt in the ratio of 2:3 *A. digitata* milk is suitable and could lead to reduction in yoghurt price and create job.

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Keywords: *Adansonia*, GC-MS, volatile metabolites, yoghurt.

INTRODUCTION

In many African countries, all parts of *Adansonia digitata* are used as ingredients in the preparation of traditional foods (Lockett et al., 2000). The fruit is exceptionally

nutritional containing high level of antioxidants, essential minerals including calcium, potassium, iron, magnesium and vitamin C (Chadare et al., 2009). There is strong evidence that people in Africa, India,

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DOI : <http://dx.doi.org/10.4314/ijbcs.v8i2.10>

Sri Lanka and the West Indies suffering from malaria utilize a mash containing dried baobab bark to treat the fever associated with this illness (Wickens and Lowe, 2008).

The fruit pulp of baobab has been used as an analgesic, anti-inflammatory, antipyretic, pesticide and as astringent in the treatment of diarrhoea and dysentery (Al-qarawi et al., 2003). Furthermore, there are some degenerative diseases that the fruit pulp has been discovered to cure which may be due to the presence of certain compounds. *A. digitata* fruit pulp contains. Outside Africa, baobab dried fruit pulp has been acknowledged as a novel food by the European Union (The Commission of the European Communities, 2008). Lockett et al. (2000) examined the nutritional composition of baobab fruit pulp (as %) and results documented were as follows: moisture (0%), energy 0 kJ/100 g, protein 2.19%, fat 0.37%, carbohydrate 70.03%, fibre 11.15%, ash (5.71%) and dry matter (89.4%). Baobab is also believed to contain high amount of antioxidants (Chadare et al., 2009), which can also prevent oxidative related diseases (Besco et al., 2007). In addition, Murray et al. (2001), found that baobab fruit pulp supplies both soluble and insoluble fibre up to about 45 grams per 100 grams of product. All parts of the baobab tree can be used for the treatment of fever, diarrhoea, dysentery, haemoptysis and small pox (Van Wick and Gericke, 2000).

Beside health and food uses, baobab also has huge commercial potentials according to the report by the British's Natural Resources Institute estimated that harvesting baobab could generate more than \$960 million worth of trade a year and employ over 2.6 million households in Southern Africa alone (Bennett, 2006).

In recent times, the cost of milk and other ingredients have always been the justification of yoghurt producers to put-up the price of yoghurt. This cost can be cut-down by replacing some portion of the milk used with an underutilized plant product such

as baobab fruit pulp. The cost of milk is four times the cost of *A. digitata* fruit pulp as being sold in main market, Sokoto town, Nigeria. The objectives of this research was to determine the proximate and mineral composition of the yoghurt samples using different mixtures of *Adansonia digitata* fruit pulp, carry out sensorial analysis of the yoghurt samples and determine the volatile compounds profile of the yoghurt samples by GC-MS analysis.

MATERIALS AND METHODS

Sample collection and preparation of raw material

Powder form of baobab fruit pulp (*Adansonia digitata*) and of milk were purchased from Sokoto main market in new polythene bags. The pulp powder was passed through a sieve to remove unwanted particles that were present in it. The plain yoghurt used as inocula (starter culture) was purchased from minimart, Usmanu Danfodiyo University Sokoto, Nigeria and plain yoghurt used for sensorial analysis was purchased from Habash Yoghurt Enterprises, Sokoto. The sieved pulp powder was stored in an air tight container for further use.

Fermentation

This was done as described by Tamime and Robinson (2000), with little modification. Different ratios of milk to *Adansonia digitata* fruit pulp was prepared in five different concentrations (1:4, 2:3, 3:2, 4:1 and plain pulp powder (5:0) by mixing the mixture with water. Each preparation was pasteurized to 85 °C for two minutes, cooled to 44 °C, inoculated with the culture, mixed thoroughly and allowed to ferment for six hours to produce the yoghurt which was refrigerated.

Sensorial analysis

Sensorial analysis for the yoghurt samples was done as described by Ogunjobi et al. (2005). Panellists of twenty four individuals were educated about the analysis

and scored based on their satisfaction at a total of nine.

Proximate composition

The yoghurt samples were analysed in triplicate for proximate composition in accordance with the Official Methods of the Association of Official Analytical Chemists (AOAC, 2005). Ash was determined by incinerating two grams (2 g) of the dried pulp powder at 550 °C in lenton furnaces (England) over night. Fibre content was determined by drying two grams (2 g) of the fermented yoghurt samples overnight at 105 °C in the oven (Gallenhamp oven BS) and incinerated at 550 °C for 90 minutes in lenton furnaces (England). Moisture content was determined by drying two grams (2 g) of the fermented yoghurt samples overnight at 105 °C in the oven (Gallenhamp oven BS). Crude lipid was determined using saturated method. Two gram (2 g) of the yoghurt samples were weighed into 50 ml conical flask and n-hexane was added and allowed to stand at room temperature overnight. The mixture was decanted into a pre-weighed empty flask and designated W_1 . It was placed in an oven to allow the n-hexane evaporate in the oven (Gallenhamp oven BS). Protein (%N x 6.25) was determined by the Micro-Kjeldahl and soluble carbohydrate was determined as the difference between crude protein and sum of ash, protein, crude lipid and crude fiber.

Mineral analysis

Analysis of minerals was done according to methods described in (Hack, 2000). The investigated minerals include: phosphorus, sodium, potassium, calcium and magnesium. Phosphorus was determined using spectrophotometer (JENWAY 6100) at 660 γ (wavelength), potassium and sodium were determined using flame photometer (Corning 400 Essex, England), while that of magnesium and calcium was done using ethylene diamine tetra acetic acid (EDTA) titration method.

Extraction of volatile compounds

Volatile compounds were extracted using general purpose solvent as described by Ibrahim et al. (2011a). Two mills (2 ml) of each of the three best yoghurt samples from sensorial analysis i.e. sample A, C and D, was weighed into different bottles, saturated with 20 ml of chloroform and allowed to stand at room temperature overnight. Each bottle was filtered using Whatman filter paper and the filtrate was collected in a sterile bottle, closed tightly before the GC-MS analysis.

Gas chromatography - mass spectroscopy (GC-MS) analysis

GC-MS analysis was performed using GC-MS-QP2010 plus (Shimadzu, Japan), at 60 °C for 5 min in the oven and finally at 10 °C/min to 280 °C (held for 10 min). Chromatography separations were performed using DB-WAX analytical column 30 m, 0.25 mm (J&W scientific, Folsom C.A) with helium as carrier gas at a constant flow rate of 0.8 ml/Min.

Identification and quantification of volatile compounds

The chromatography peak identification was carried out by comparing their mass spectra with those of the bibliography data of unknown compounds from the NIST library (Hewlett-Packard co., Palo alto, CA). Approximate quantification of volatile compounds was estimated according to methods of Hobart et al. (2007), by the integration of peaks on the total ion chromatogram using Xcalibur software (Vienna, VA). The results are presented as the peak area normalized (%).

RESULTS

The result of proximate composition of yoghurt samples is presented in Table 1. The result revealed that sample F and B had higher carbohydrate content (96.12%), (95.36%) and the least, sample E (87.85%). Sample A (plain yoghurt) and F had higher moisture content

(77.5%), (74.5%) respectively and the least, sample E (51.0%). Sample E had the highest value of crude protein (5.65%) followed by sample D (4.40%) and the least, sample F (1.88%).

The mineral content of the yoghurt samples are shown in Table 2. The result shows that sample A and B had higher sodium content (230 mg/kg), (170 mg/kg) and the least, sample F (55 mg/kg). The content of potassium and calcium was higher in Sample B and D (1250 mg/kg), (1100 mg/kg) with similar calcium content of (0.50 mg/kg) respectively.

Table 3 presents the sensory analysis of the six samples. The result shows that sample C, D and E had the highest value 127, 124, and 123 respectively. From the analysis of variance, no significant difference in terms of taste was observed among the yoghurt samples at $p > 0.05$.

Volatile metabolite profile was conducted on samples (A, C and D) and the results are presented in Table 4. The result revealed that nine (9) metabolites were detected in sample A, sample C had ten (10), while eight (8) was also detected in sample D. Two volatile metabolites were common to sample C and D which are: benzyl alcohol; sample C (17%), sample D (12.61%) and an unknown compound; sample C (2.47%), sample D (1.3 %). The three samples had palmitic acid common to them in different concentration: (12.59%), (9.20%) and (5.57%) respectively. The results also show that Stearic acid (10.03%) and glycerol-2-monooleate (1.3%) were unique to sample A and 2, 5- dimethyl 1, 6-heptadiene (0.51%) was unique to sample D, other compounds determined were not known.

Table 1: Proximate composition of *A. digitata* yoghurt samples.

Parameters (%)	Samples					
	A	B	C	D	E	F
Ash	0.50(0.26)	0.50(0.14)	1.00(0.68)	1.00(0.90)	1.00(0.14)	0.50(0.40)
Fiber	0.00*(0.00)	0.50*(0.90)	0.50*(0.52)	1.00*(0.68)	0.50*(0.68)	1.00*(0.68)
Crude protein	3.77(0.26)	3.14(0.68)	3.77(0.50)	4.40*(0.68)	5.65*(0.40)	1.88*(1.40)
Carbohydrate	95.23(0.68)	95.36*(0.68)	94.23(0.14)	88.10(0.14)	87.85*(1.30)	96.1*(0.90)
Moisture	77.50*(0.68)	73.50(0.26)	69.50(0.68)	64.00(0.14)	51.00(0.90)	74.5*(0.14)
Lipid	0.50(0.26)	0.50(0.68)	0.50(0.90)	5.50(0.50)	5.00(0.26)	0.5 (0.90)

(SD=standard deviation, in parenthesis);

Values are means of three replicate and significant* at 5%

Key:

- A- Plain yoghurt (100% milk)
- B- 4: 1 (milk: *A. digitata*)
- C- 3: 2 (milk: *A. digitata*)
- D- 2: 3 (milk: *A. digitata*)
- E- 1: 4 (milk: *A. digitata*)
- F- 5:0 (*A. digitata*).

Table 2: Mineral content of the six yoghurt samples.

Parameters (mg/kg)	Samples					
	A	B	C	D	E	F
Na	230(2.20)	171(1.40)	150(2.20)	70(1.40)	140(1.40)	54(1.40)
K	600(2.20)	1250(2.90)	1000(2.90)	1100(2.20)	1000(2.20)	950(2.20)
Ca	0.35(0.69)	0.5(0.69)	0.3(0.25)	0.5(0.14)	0.35(0.14)	0.25(0.25)
Mg	0.55(0.10)	0.62(0.50)	0.45(0.68)	0.8(0.14)	0.5(0.20)	1.1(0.14)
P	2.82(1.3)	3.53(1.40)	3.24(0.68)	3.1(0.68)	2.78(0.80)	3.36(0.68)
Nitrogen	0.6(0.1)	0.5(0.14)	0.6(0.50)	0.7(0.90)	2.78(0.68)	0.30(0.9)

Table 3: Sensorial analysis of different yoghurt samples made by *Adansonia digitata* fruit pulp and a plain yoghurt.

Number of Scores	Samples					
	A	B	C	D	E	F
1-3	0	23	10	10	12	16
4-6	60	34	51	59	58	36
7-9	59	53	46	55	53	60
Total	179	119	127	124	123	112

(SD=standard deviation, in parenthesis);

Values are means of three replicate and significant* at 5%.

Key:

- A-Plain yoghurt (100% milk)
- B-4: 1 (milk: *A. digitata*)
- C-C-3: 2 (milk: *A. digitata*)
- D-D-2: 3 (milk: *A. digitata*)
- E-E-1: 4 (milk: *A. digitata*)
- F-5:0 (*A. digitata*)

Table 4: Gas chromatography and mass spectroscopy of the three best yoghurt samples from the result of sensorial analysis.

RT-1 (min)	Compounds	Area Normalized (%)		
		A	C	D
5.90	Benzyl Alcohol	-	17.00	12.61
7.58	Unknown	-	1.24	-
7.58	2, 5-dimethyl-1, 6-heptadiene	-	-	0.51
15.08	Unknown	-	1.51	-
16.82	Unknown	-	2.47	1.30
17.84	Unknown	0.96	3.22	1.84
18.89	Unknown	-	0.89	-
20.12	Palmitic acid	12.59	5.57	9.20
22.85	Unknown	46.22	41.20	47.22
23.21	Stearic acid	10.03	-	-
25.24	Unknown	0.82	-	-
26.98	Glycerol-2-monooleate	1.30	-	-
27.52	Unknown	6.40	6.83	7.88
29.55	Unknown	13.71	-	-
29.84	Unknown	7.97	5.90	-
29.85	Unknown	-	-	4.73

R/T=Retention time in minutes (Mins)

P/A=Peak area in percentage (%)

Sample A = Plain yoghurt (only milk) purchased in retail outlet

Sample C= 3:2(milk: *A. digitata*)Sample D= 2:3(milk: *A. digitata*).

DISCUSSION

The result from the proximate analysis shows that sample F and B had higher carbohydrate content 96.10% and 95.36%, the least was sample E, 87.85% and a significant variation was observed. A significant variation was also observed in the moisture content between sample A and F. Crude protein content shows a significant variation between sample E and D which had higher value 5.65% and 4.40% and sample F, 1.88% which was the least. Significant variation was observed in the fiber content of the yoghurt samples as it was absent in plain yoghurt (milk with no fiber). These results indicate that the presence of *A. digitata* contributes to the increase in fiber content of the yoghurt. The highest fibre content was sample D and F (1.0%) and the rest had trace (0.5%) in contrast to the earlier report (Murray et al., 2001), baobab fruit pulp supplies a quantity of soluble (22.54%) and insoluble (22.04%)

fibres which can reach up to about 45 g per 100 grams of product. Variation to this result may be due to the fermentation and the sieving process that ensure the smoothness of the yoghurt production which reduce the fiber content of the samples. A significant variation was observed in the ash, lipid and carbohydrate content of the fermented yoghurt samples. Reduction in lipid and protein could be as result of their breakdown to long chain fatty acid and peptides that contribute to the characteristic flavor of yoghurt. Ibrahim et al. (2011a) made similar observation during production of a soup condiment.

The result from the mineral analysis shows that sodium and potassium are the major minerals in the fermented yoghurt samples, 230 mg/kg and 171 mg/kg in sample A and B, while the content of potassium was 1250 mg/kg and 1100 mg/kg in samples B and D respectively. Osman (2004) reported that the fruit pulp of baobab tree has a high

calcium and potassium content. The phosphorous content shows a significant increase in the yoghurt samples with the highest in sample B, 3.53 mg/kg and lowest in E, 2.78 mg/kg. Considerable amount of potassium and calcium is believed to help maintain a healthy blood pressure and strong bones in humans. Variations in the nutritional composition of baobab fruit pulp may be due to environmental factors including different soil and climate conditions (Magdi, 2004). Ibrahim et al. (2011b) observed similar result during the fermentation of *H. sabdariffa* for 'dawadawan botso' production.

The sensorial analysis of the sample revealed that sample C had highest (127) next to sample D (124) and the least sample F (112). Sample F being the least may be due to the sour taste and sample C being the highest may be due to the sweetness with little sour taste. Sibibe and Williams (2002) reported that *Adansonia digitata* fruit pulp has a nutty, acidic taste. This may have been the reason most panelist score some of the yoghurt low even though analysis of variance, showed no significant difference in terms of taste was observed among the yoghurt samples at $p > 0.05$.

The GCMS analysis of the two best samples and one other based on sensory analysis shows the presence of some compounds which are beneficial to the human health. The results shows several compounds were unique to each sample in which most of the compounds are unknown. Stearic acid (10.03%) and glycerol-2-monooleate (1.3%) are unique to sample A. The presence of Glycerol-2-monooleate may be due to the catalytic activity of transferin, lysozyme, and lactoperoxidases component of the milk, which are known to have antibacterial and other antimicrobial effects (Masola et al., 2009). Stearic acid helps removing undesirable saturated fatty acid and trans - fatty acids from the human diet and help

promote cardiovascular health (Al qarawi et al., 2002).

Curcumin (2, 5-dimethyl-1, 6-heptadiene) was unique to sample D. This compound is known to have antioxidant properties. This compound together with Vitamin C content of baobab fruit pulp may be responsible for the effective antioxidant properties (Arrigoni and De tullio, 2002). Antioxidants could help preventing oxidative stress related diseases such as cancer, aging, inflammation and cardiovascular diseases as they may eliminate free radicals which contribute to these chronic diseases (Chadare et al., 2009; Besco et al., 2007).

Palmitic acid was common to the three samples, A, C and D. The presence of this compound could be as a result of the presence of the acids triterpenoids, β -sitosterol, β -amyryn palmitate, terpenoids, and ursolic acid present in the fruit. Palmitic acid has been shown (in rats fed on a 20% fat (palmitic acid) 80% (carbohydrate diet) to alter aspects of the central nervous system responsible for the secretion of insulin and to suppress the body's natural appetite-suppressing signals from leptin and insulin - the key hormones involved in weight regulation (Benoit et al., 2009). Three unknown compounds were common to the three samples, while one unknown compound was common to sample A and C, and two were common to sample C and D. The inability to find a perfect match for these unknown compounds may be attributed to the shortfalls of a GC-MS in that it detects mass spectra and the library used is not that great in the identification of compounds that may have interfered compounds.

Conclusion

The study shows that incorporation of *Adansonia digitata* fruit pulp caused an increase in proximate and mineral content of yoghurt samples. From the sensorial analysis, majority of people like sample A (control), C and D. It could be deduce from the experiment

that sample D have higher lipid , fibre, protein and mineral content compared to sample C and the control and also a beneficial unique compound (2,5-dimethyl-1,6- heptadiene). This study suggests that sample D ratio (40:60), could lead to the production of yoghurt with high quality and increased nutritional value, and thus reinforce its use as a beverage to meet the nutritional needs of the poor in Africa.

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