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Research Article

Phytochemical content, fatty acid characterization and antioxidant activity of Yoghurt prepared from extracts of coconut, tigernut and dates.

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Abstract

Blends of coconut, tigernut and dates extracts were used in the preparation of Plant based yoghurt. The formulated yoghurts were evaluated for their phytochemical content, anti-oxidant properties and fatty acid characterization using GC-MS chromatography. Results of phytochemical content showed the presence of phenols at 2.31mg/g and flavonoids 0.9mg/g. The absence of alkaloids, saponins, terpenoids and phlobatannins were observed in the yoghurt samples. Analysis of antioxidant scavenging activity (DPPH assay) reveals the anti-oxidant activity ranged from 7–32%. Results of fatty acid profile showed palmitic acid (53.12%) as the most dominant fatty acid followed by lauric acid (7.2%) and capric acid 5.35%.

1. Introduction

Yoghurt is a fermented milk product with probiotic functionality, widely consumed around the world. As a result of the health benefits associated with the consumption of yoghurt, demand for the product has been on a steady increase over the years. Tiger nut (*Cyperus esculentus* L.) is a crop that is widely grown in sub-Saharan Africa for its nutritional and economic value. A number of health benefits have been reported with the consumption of Tiger nuts. The nut was found to be ideal for children, older persons and sportsmen. Tiger nut is regarded as a digestive tonic and also helps in the treatment of indigestion, colic diarrhoea, diabetes, dysentery and excessive thirst. It was reported that tiger nut helps in reducing the risk of colon cancer [1].

Coconut (*Cocos nucifera*) is one of the important economic crops, especially in Southeast Asian countries [2]. Recently, much attention has been paid to coconut milk as a milk substitute. Investigations have revealed that coconut contains 31–35% fat and 3.5–4.0% protein, high amount of essential amino acids, calcium, phosphorus, potassium, vitamin C, E

and B6 and is easily digested [3]. Coconut oils are rich in medium chain fatty acids, which are clinically proven to have preventive effects against hyperlipidemia, fatty liver, and diabetes [4]. Coconut milk is an oil-in-water emulsion in nature. Coconut proteins (globulin and albumin) and phospholipids help stabilize the emulsion by adhering to the surface of coconut oil droplets as emulsifiers, preventing phase separation [5].

Antioxidant compounds in foods play a significant role as a health-protecting factor. They are capable of deactivating free radicals which can cause cells and tissue damages. These damages cause malfunctioning of cells or cell death. Epidemiological studies have shown that antioxidants can prevent development of degenerative diseases such as cancer, coronary heart diseases, obesity, type 2 diabetes, hypertension, premature ageing and inflammatory diseases [6].

However, there are very scanty reports concerning this subject [7, 8]. However, some recent studies were performed mainly on cow milk and its dairy products [9, 10] and little or none for plant yoghurts. In this study, extracts of different plants Coconut, Tiger-nut, and Dates were used in yoghurt preparation and the phytochemical, anti-oxidant properties and fatty acid composition of the yoghurt were examined. The market value of the plant based yoghurt lies in its potential to be used as functional food a product with health benefits beyond basic nutrition.

2. Materials and methods

2.1 Production of the probiotic yoghurt from the optimized milk blend

The production of the yoghurt was carried out according to the procedure of Tamime and Robinson [11] (Fig 1). The optimized milk blend with the ratio 0.167 (Coconut), 0.667 (Tigernut), 0.167 (Date) was used in yoghurt production. The milk was freshly prepared and pasteurized at 70^{o}C for 30 min, it was thereafter transferred into the biosafety cabinet and allowed to cool to 45^{o}C before inoculating it with the isolated strains (*Lactobacillus bulgaricus* and *Streptococcus thermophiles*) confirmed during the preliminary investigation of this study at the chosen concentration, then transferred into the fermentation jar and incubated anaerobically at the corresponding temperature and time The yoghurt produced was stored in a refrigerator at 4^{o}C.

2.2 Chemical Analysis

The method described by Debela [12] was used in the determination of Phytochemical content of the various samples.

2.3 DPPH Radical Scavenging Assay

The free radical scavenging ability of the extracts was tested by DPPH radical scavenging assay as described by Desmarchelier, [13]. A solution of 0.1mm DPPH in methanol was prepared, and 2.4 mL of this solution was mixed with 1.6mL of extract in methanol at different concentrations (12.5–150 µg/mL). The reaction mixture was vortexed thoroughly and left in the dark for 30 min. The absorbance of the mixture was measured spectrophotometrically (Jenway model 7315) at 517 nm. BHT was used as reference. The percentage of DPPH radical scavenging activity was calculated by the following equation:

% of DPPH radical scavenging activity =

$$\{(A_0 - A_1)/A_0\} \times 100\%$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the extractives/standard. Then % of inhibition was plotted against concentration. The experiment was repeated two times at each concentration.

2.4 GC-MS Characterization of Fatty acids of oil extracted from the yoghurt

2.4.1 Sample Preparation

Sample preparation was carried out in accordance with the method described by Hara and Radin [14] using an aliquot of plant extract fractions. Two hundred milliliter (200 mL) of the yoghurt sample was saturated with hexane and allowed to stand for 24h. A clear separation of oil was obtained, decanted and used for Fatty Acid Methy Esterase (FAME) analysis using GC-MS.

2.4.2 GC-MS Characterization

Fat from plant and cow yoghurt were extracted and hydrolyzed by fatty acids methyl esterase or FAME. Fatty acids profiles were determined using a gas chromatography (Agilent model 6475) and the individual fatty acid contents were expressed as weight percentages (g/100 g of fat). Qualitative Analysis as described by Dimitra, [15] was used and the qualitative characterization was carried out using GC-MS scan mode. The carrier gas (Helium) was used at constant flow of 1.49 mL/min at an initial nominal

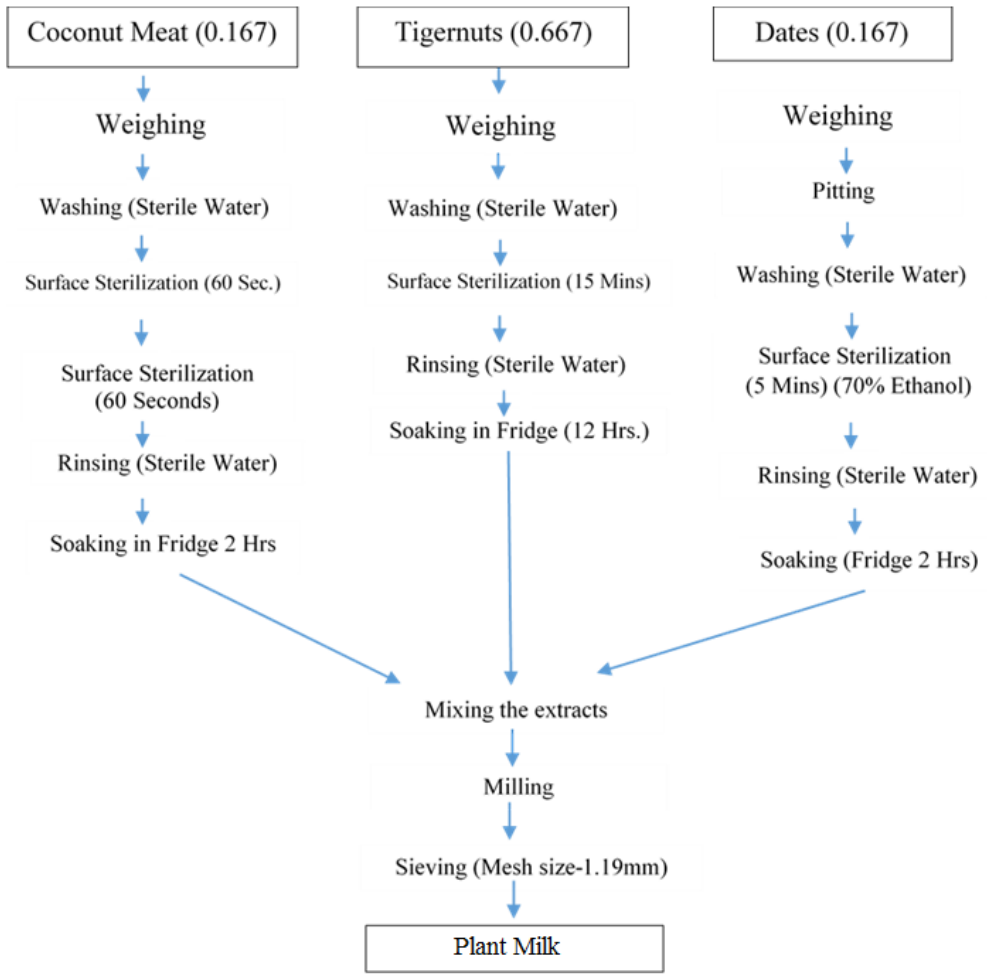

Figure 1. Flowchart for plant based yoghurt product

pressure of 1.49 psi and average velocity of 44.22 cm/sec. 1µL of the samples were injected in splitless mode at an injection temperature of 300 °C.

The mass spectrometer was operated in electron-impact ionization mode at 70eV with ion source temperature of 230°C, quadrupole temperature of 150°C and transfer line temperature of 300°C. Scanning of possible fatty acid compounds were from *m/z* 45 to 550 amu at 2.00s/scan rate and were identified by comparing measured mass spectral data with those in NIST 14 Mass Spectral Library and literature. Analysis validation was conducted by running replicate samples in order to see the consistency of the constituent compound name, respective retention time, molecular weight (amu), Quality ion (Q-Ion) and %Total.

$$\%\text{Total} = \frac{\text{Abundance of Individual Constituents}}{\text{Total Abundance of all Constituents in Extract}} \times \frac{100}{1}$$

These abundances were outputs from the NIST 14 Library search report of the extract constituents. Each compound identified has a corresponding mass spectrum showing the abundance of the possible numerous *m/z* peaks per compound.

3. Results

Table 1 represents the phytochemical composition of ethanol extracts from the optimized plant milk and yoghurt. The results indicated the presence of phenols and flavonoids in both samples. Phenols were found at the level of 2.320mg/g for milk and 2.314mg/g for yoghurt. Flavonoids were measured at 1.066mg/g for milk and 0.9mg/g for yoghurt respectively. The quantitative phytochemical assessment revealed a higher concentration of both phyto-constituent in the milk sample over that of yoghurt. Other phytochemicals (Alkaloids, Saponins, Phyto-steroids, Terpenes, and Phlobatanins) were not detected in both samples.

Table 1. Phytochemicals contents of the optimized milk and yoghurt samples.

Phyto-constituents	Qualitative		Quantitative (mg/g)	
	Milk	Yoghurt	Milk	Yoghurt
Alkaloids	-	-		
Saponins	-	-		
Phyto-steroids	-	-		
Phenols	±ve	±ve	2.320[a]	2.314[a]
Terpenes	-	-		
Xanthoproteic	-	-		
Flavonoids	±ve	±ve	1.066[a]	0.900[a]
Phlobatanins	-	-		

Key: ±ve = Present, -ve = Absent

3.1 Fatty acid profile of the plant and cow yoghurt oil

Results of the fatty acid profile of plant and dairy yoghurt are shown in Table 2. The yoghurt oil showed the presence of essential fatty acids. The groupings of fatty acids were as follows: (1) saturated fatty acids (SFA), (2) short-chain saturated fatty acids (C4 to C10, SCFA), (3) medium-chain saturated fatty), (4) long-chain saturated fatty acids (C16 to C24, LCFA), (5) monounsaturated fatty acids (MUFA) and (6) polyunsaturated fatty acids (PUFA). Lauric acid (MCFA) in the plant yoghurt is 7.2% while in the dairy yoghurt is 2.2%. Palmitic acid (LCFA) was the most dominant fatty acid in the plant yoghurt, 53.12% while in the dairy yoghurt, it was 16.54%. Capric acid (SCFA) was 5.35% in dairy yoghurt oil and 1.93% in the plant yoghurt oil Fig 2. shows that the levels of inhibition of DPPH for antioxidant activity of the plant products were 32.7%, 7.85%, 8.63%, and 7.00% inhibition at a DPPH concentration of 0.1, 0.3, 0.5 and 0.7mg/ml for the plant yoghurt. The plant milk only showed 2.02% inhibition at 0.9mg/ml concentration and no inhibition at other concentrations.

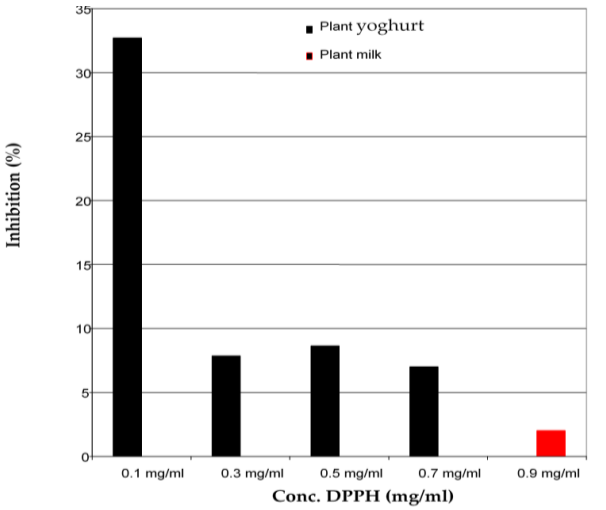

Figure 2. Anti-oxidant assay of the plant milk and yoghurt.

4. Discussion

The results indicated the presence of phenols and flavonoids in both samples at the level of 2.320mg/g for milk and 2.314mg/g for yoghurt; and 1.066mg/g for milk and 0.9mg/g for yoghurt, respectively. Flavonoids and Phenols are very powerful antioxidants and help regulate cellular activity and mop up free radicals responsible for oxidative stress in the body. A quantitative phytochemical assessment revealed a higher concentration of both phytoconstituents in the milk sample over yoghurt. Other phytochemicals assessed (Alkaloids, Saponins, Phyto-steroids, Terpenes, and Phlobatanins) were not detected in both samples. Martin-Sánchez [16] reported the abundance of phytochemicals such as carotenoids, polyphenols, tannins, and sterols in the date palm.

Processing affects the fatty acid stability of dairy and plant milk, and it is essential to study the stability after processing. The presence of saturated fatty acids limits dairy consumption, and contrary to common belief, they may have some beneficial effects on man. Palmitic acid (PA) has been for a long time negatively depicted for its putative detrimental health effects, shadowing its multiple crucial physiological activities.

A balanced ratio of consumption of Palmitic acid and other polyunsaturated fatty acids (PUFA) is all one requires to harness the benefits of most fatty acids (FA). This requires that Palmitic Acid must be consumed in a certain ratio to avoid imbalance, predominantly since it can also be synthesized endogenously via *de novo* lipogenesis (DNL).

In this study, the plant yoghurt yielded 53.12% Palmitic Acid, and the cow yoghurt yielded 16.54%. From fasting studies in certain patients, a high intake of Fatty Acid in the liver could be attributed to insulin resistance in adipose tissue [17]. The tight homeostatic control of Palmitic Acid tissue concentration is likely related to its fundamental physiological role in several biological functions. It has been recently reviewed that palmitic acid plays a crucial role in developing Infants [18].

According to Senyilmaz, [19], the maintenance and regulation of mitochondria morphology and function

Table 2. Fatty acid profile of the plant yoghurt

Sl. No.	Compound Name	Group	Fatty acid (%) plant Yoghurt	Fatty acid (%) dairy Yoghurt
1	Caproic (Hexanoic) acid, methyl ester	SCFA	1.05	1.93
2	Caprylic (Octanoic) acid, methyl ester	SCFA	4.49	1.8
3	Capric (Decanoic) acid, methyl ester	SCFA	1.93	5.35
4	Undecanoic acid, methyl ester	MCFA	1.06	0.06
5	Lauric (Dodecanoic) acid, methyl ester	MCFA	7.20	2.2
6	Tridecanoic acid, methyl ester	MCFA	11.14	0.05
7	Cyclopropanenonanoic acid, methyl ester		3.45	ND
8	Tridecanoic acid, 12-methyl-, methyl est	MCFA	6.15	5.23
9	Cyclopropanenonanoic acid, methyl ester		1.66	ND
10	Pentadecanoic acid, methyl ester	MCFA	0.42	0.8
11	Palmitoleic (7-Hexadecenoic) acid, methy	MCFA	0.46	0.67
12	Palmitic (Hexadecanoic) acid, methyl est	LCFA	53.12	16.54
13	Hexadecenoic acid, methyl ester, (Z)-		0.35	ND
14	Heptadecanoic acid, methyl ester	LCFA	0.58	0.58
15	Stearic (10-Octadecenoic) acid, methyl e	LCFA	0.54	11.73
16	trans-13-Octadecenoic acid, methyl ester		0.63	ND
17	Stearic acid methyl ester	LCFA	0.56	ND
18	cis-10-Heptadecenoic acid	MUFA	1.80	ND
19	Arachidic (Eicosanoic) acid, methyl este	LCFA	0.34	0.21
20	Heneicosanoic acid, methyl ester	LCFA	0.63	0.12
21	11-Hexadecenoic acid, methyl ester	LCFA	0.84	ND
22	Behenic (Docosanoic) acid, methyl ester	LCFA	0.94	0.09
23	Tricosanoic acid, methyl ester		0.66	ND

ND: No Data, SCFA: short-chain saturated fatty acids, MCFA: medium-chain saturated fatty acids, LCFA: long-chain saturated fatty acids, MUFA: monounsaturated fatty acids, and PUFA: polyunsaturated fatty acids (PUFA). Data source for dairy: Sumarmono, [7].

According to Senyilmaz, [19], the maintenance and regulation of mitochondria morphology and function can be attributed to stearic acid. It also plays a role in the lowering of bad cholesterol LDL in the body. The plant yoghurt revealed a rich dose of saturated fatty acid which plays various roles in the biological functions of the body. During storage, it is believed that Fatty Acid is solely responsible for off-flavours. However, the undesired flavours of plant-based milk have been studied by different research teams who have deduced that the presence or absence of lactic acid bacteria, which would produce compounds like exopolysaccharides is primarily responsible for the undesired flavour, aroma, etc. [20-22].

The antioxidant analysis of the plant products showed activity at varying concentrations. Percentage inhibition (32.7%) was seen in the plant yoghurt at (0.1) concentration. DPPH assay is used to predict free radical scavenging capacity in a product, and this assay revealed that the yoghurt has high antioxidant activity. The hydrogen atom donating ability of the plant extractives was determined by the decolorization of methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). DPPH produces violet/purple color in methanol solution and fades to shades of yellow color in the presence of antioxidants However, the plant milk showed inhibition (2.02%) at only 0.9 concentration. It can be deduced that fermentation increased the antioxidant activity of the blend. The DPPH assay provides an easy and rapid approach to evaluating potential antioxidants. Coconut meat contains phenolic compounds: caffeic acid, salicylic acid, gallic acid, and p-coumaric acid; these are antioxidants that help to protect cells from oxidative damage as a result of free-radicals scavenging activity [23], which we also hope to harness in the blend. The plant yoghurt contains antioxidants and could be regarded as a functional food.

5. Conclusions

The prepared yoghurts showed an anti-oxidant activity of 32.7%. The antioxidant activity could be

attributed to the presence of flavonoids in the plant based yoghurt. This indicates the capacity of the product to inhibit the formation of free radicals which accelerate the rate of oxidation in the body.

Authors' contributions

Concept and methodology, A.N.L. and E.E.J.; Investigation and Resources, A.N.L.; Preparation of draft, A.N.L. and W.V.; Writing, review and editing, W.V.; Supervision I.C. and E.E.J.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

Authors have declared that no competing interests exist.

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