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Research article

Metabolite profile of Bambara groundnut (*Vigna subterranea*) and *dawadawa* (an African fermented condiment) investigation using gas chromatography high resolution time-of-flight mass spectrometry (GC-HRTOF-MS)

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ABSTRACT

Metabolite profile provides an overview and avenue for the detection of a vast number of metabolites in food sample at a particular time. Gas chromatography high resolution time-of-flight mass spectrometry (GC-HRTOF-MS) is one of such techniques that can be utilized for profiling known and unknown compounds in a food sample. In this study, the metabolite profiles of Bambara groundnut and *dawadawa* (unhulled and dehulled) were investigated using GC-HRTOF-MS. The presence of varying groups of metabolites, including aldehydes, sterols, ketones, alcohols, nitrogen-containing compounds, furans, pyridines, acids, vitamins, fatty acids, sulphur-related compounds, esters, terpenes and terpenoids were reported. Bambara groundnut fermented into derived *dawadawa* products induced either an increase or decrease as well as the formation of some metabolites. The major compounds (with their peak area percentages) identified in Bambara groundnut were furfuryl ether (9.31%), bis (2-(dimethylamino)ethyl) ether (7.95%), 2-monopalmitin (7.88%), hexadecanoic acid, methyl ester (6.98%), 9,12-octadecadienoic acid (Z,Z) and 2-hydroxy-1-(hydroxymethyl)ethyl ester (5.82%). For dehulled *dawadawa*, the significant compounds were palmitic acid, ethyl ester (17.7%), lauric acid, ethyl ester (10.2%), carbonic acid, 2-dimethylaminoethyl 2-methoxyethyl ester (7.3%), 9,12-octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester (5.13%) and maltol (4%), while for undehulled *dawadawa*, it was indoline, 2-(hydroxydiphenylmethyl) (26.1%), benzoic acid, 4-amino-4-hydroximino-2,2,6,6-tetramethyl-1-piperidinyl ester (8.2%), 2-undecen-4-ol (4.7%), 2-methylbutyl propanoate (4.7%) and ë-tocopherol (4.3%). These observed metabolites reported herein provides an overview of the metabolites in these investigated foods, some of which could be related to nutrition, bioactivity as well as sensory properties. It is important to emphasize that based on some of the metabolites detected, it could be suggested that Bambara groundnut and derived *dawadawa* might serve as functional foods that are beneficial to health.

1. Introduction

Fermentation is a biochemical process that results in modifications (increase/decrease) as well as formation (synthesis) of metabolites. These metabolites contribute to the nutritional qualities, taste, shelf life, safety, aroma, health promoting properties and overall composition of fermented foods. Traditionally, these fermented foods are produced from legumes or cereals and undergo various forms of fermentation, such as alkali fermentation, lactic acid fermentation, acetic acid fermentation and alcoholic fermentation (Oliveira et al., 2014). The fermentation process whereby the pH of a legume increases to alkaline levels and possibly pH values of 9 and above is known as alkaline fermentation (Omafuvbe et al., 2002) and such is due to breakdown of proteins to ammonia, peptides and amino acids (Wang and Fung, 1996; Kiers et al., 2000).

In African and Asian countries, several alkaline fermented food condiments, such as *dawadawa, thua-nao*, *iru*, *natto* and *soumbala,* are mostly produced from legumes and from Bambara groundnut (Fadahunsi and

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Olubunmi, 2009; Parkouda et al., 2009; Ademiluyi and Oboh, 2011; Akanni et al., 2018a; Muhammad et al., 2018). These fermented condiments are processed mostly through natural fermentation; however, this is not always the case, as some undergo controlled fermentation. During the production of *dawadawa*, raw legume seeds are soaked, manually dehulled and boiled to soften the seeds. The boiled softened raw seeds are wrapped with leaves (such as banana leaves), kept in sacks or bags and incubated in a plastic bowl/calabash/earthen pot for three to five days (the fermentation period is usually based on human discretion) (Onawola et al., 2012). The most important and major processing step for this product is fermentation and has been proven to enhance the organoleptic and beneficial health properties of fermented legumes (Oboh et al., 2009; Ademiluyi and Oboh, 2011; Chinma et al., 2020).

Monitoring of these metabolic, biochemical, physicochemical and structural changes occurring during the fermentation process may be somewhat difficult, necessitating the utilization of techniques and robust equipment, which can provide a better overview of these metabolites. Gas chromatography–mass spectrometry is a non-biased, comprehensive and sensitive technological system used for the detection of diverse volatile and semi-volatile metabolites (Adebo et al., 2021). It has advantages of better resolution, high sensitivity, good reproducibility and, with the necessary databases, makes identification of compounds relatively easier (Hu et al., 2018). Particularly for gas chromatography coupled with high-resolution time of flight mass spectrometry, it is a powerful and highly effective analytical tool with excellent capabilities including a better chromatographic separating capability over a wide mass range with an accurate mass measurement (Brits et al., 2018; Kewuyemi et al., 2020). The exact mass information and mass resolution provided by high-resolution time-of-flight mass spectrometry (HR-TOFMS) can enhance target identification of compound and also assist in the identification of unknown compounds (Ubukata et al., 2015).

While few authors have studied the composition of *dawadawa* (Akanni et al., 2018a; Onyenekwe et al., 2012; Adebiyi et al., 2019), there is still no study providing a comparison of the metabolite profile of two types of *dawadawa* (dehulled and unhulled) from Bambara groundnut (BGN) obtained through natural fermentation. Thus, this study was aimed to profile metabolites in BGN and *dawadawa* (dehulled and unhulled) using GC-HRTOF-MS, envisaging that the metabolites would be beneficial to consumers of these products.

2. Materials and methods

2.1. Raw materials and sample preparation

Bambara groundnuts (mixed varieties) (i.e. brown, cream and red) used in this study were procured from a local farmer in Limpopo Province, South Africa. These were subsequently sorted to remove extraneous material or debris and cleaned with water.

2.2. Fermentation of Bambara groundnut into dawadawa

The production of both dehulled and unhulled *dawadawa* has been previously described in our earlier study Adebiyi et al. (2019). Briefly, the BGN was soaked for 24 h in water and dehulled manually, the dehulled raw seeds were rinsed with water and boiled for 1 h. Later, the boiled and cooled BGN seeds were spread and covered with a sterile banana leaves, wrapped in jute bags and incubated at 35 °C for 84 h. For the unhulled *dawadawa* (UHD), the hulls or seed coats were not removed after soaking and were incubated at 35 °C for 120 h. The fermentation time and temperature conditions (35 °C for 84 h and 35 °C for 120 h, for DD and UHD, respectively) that were used for producing the *dawadawa* samples was guided by the optimized results earlier achieved and reported in Adebiyi et al. (2020). The samples were freeze-dried at −55 °C for 24 h (LyoQuest Telstar Technologies, Spain) and stored at 4 °C prior to analysis.

2.3. Sample preparation for metabolite profiling

At the initial stage of sample preparation, different extraction solvents (all analytical grade) [100% acetonitrile (ACN), 100% methanol (MeOH), 100% water (H_2O), 80% ACN in H_2O, 80% MeOH in H_2O, 50% ACN:MeOH (v/v), 1% HCl in MeOH, ACN:MeOH:H_2O (4,4,2, v/v/v) and isopropanol:ACN:H2O (4,4,2, v/v/v)] were used to investigate for a possible range of available metabolites in the samples.

An informed compromise was reached and extraction using 80% MeOH in H_2O was finally adopted based on a wider range of relevant metabolites detected on the GC-HRTOF-MS system. Briefly, 10 mL of 80% MeOH was added to 1 g of freeze-dried sample, agitated and the mixture sonicated in an ultrasonic bath (Integral Systems Ultrasonic Bath UMC 5, Labotec, South Africa) at 4 °C for 1 h. The mixture was then centrifuged at 3 500 rpm for 5 min at 4 °C (Eppendorf 5702R, Merck South Africa), transferred into a 250 mL round bottom flask and concentrated at 30 °C, under pressure using a rotary evaporator (Buchi, Switzerland). The extract was reconstituted with 1 mL chromatographic grade MeOH (Sigma Aldrich, Germany) and filtered into a dark amber vial for analysis. The extraction was done in triplicates for each sample.

2.4. GC-HRTOF-MS analysis

A mass calibration of the instrument was performed prior to analysis on the LECO Pegasus GC-HRTOF-MS system (LECO Corporation, St Joseph, MI, USA) and subsequent sample analyses done using the method of Adebo et al. (2019). The samples were then analyzed on the GC-HRTOF-MS system equipped with an Agilent 7890A (Agilent Technologies, Inc., Wilmington, DE, USA) gas chromatograph running in a high-resolution. This was coupled to a Gerstel MPS multipurpose autosampler (Gerstel Inc. Germany) and analytical column was a Rxi®-5ms (30 m × 0.25 mm ID × 0.25 μm) (Restek, Bellefonte, USA). One microlitre (μL) of each sample was injected (in a splitless mode) with helium as the carrier gas at a constant flow rate (1 mL/min). The transfer line and inlet temperatures were 225 °C and 250 °C respectively. The oven temperature was initially set at 70 °C, held for 0.5 min, ramped at 10 °C/min to 150 °C and held for 2 min. The oven temperature was later ramped at 10 °C/min to 330 °C and held for 3 min Triplicate extraction for each sample were respectively injected once into the GC-HR-TOF-MS equipment as well as solvent blanks to observe impurities and possible contamination.

2.5. Data analysis

From the data obtained, peak picking, retention time alignment, peak matching and detection were done on the ChromaTOF-HRT® software (LECO Corporation, St Joseph, MI, USA). Other data processing parameters adopted included a signal to noise ratio of 100 and a minimum match similarity of >70% prior to when compound name is assigned, using the Mainlib, Feihn and NIST metabolomics database by comparing the molecular formula, retention time and mass spectra data. Percentage peak areas were subsequently calculated, and the respective observed *m/z* fragments obtained from the ChromaTOF-HRT® data station were recorded after which the metabolite class was annotated with corresponding *m/z* fragments and molecular formula.

3. Results and discussion

3.1. Metabolites of Bambara groundnut and dawadawa profiled using GC-HRTOF-MS

The metabolic compounds of BGN and the two *dawadawa* produced (unhulled and dehulled) were analyzed using GC-HRTOF-MS. This is the first report to the best of our knowledge to profile and investigate the

Table 1. Metabolites identified in Bambara groundnut and *dawadawa* (dehulled and unhulled) samples.

t_R (min)	Compound name and metabolite class	Observed *m/z*	*m/z* fragments	MF	Percentage peak areas		
					BGN	DD	UHD
	Acids						
04:47	6-Methylbicyclo[2.2.1]hept-2-ene-5-carboxylic acid	122.4808	65.9583, 105.1135	$C_9H_{12}O_2$	ND	ND	4.69
06:27	1-Hydroxycyclohexanecarboxylic acid	131.6358	68.0502, 98.9424	$C_7H_{12}O_3$	ND	ND	3.46
	Alcohols						
05:03	2-undecen-4-ol	192.9805	71.0490, 131.0703	$C_{11}H_{22}O$	ND	1.98	4.70
06:02	Maltol	126.0312	55.0180, 71.0128	$C_6H_6O_3$	4.85	4.00	ND
06:03	Phenylethyl alcohol	122.0728	91.0544, 122.0728	$C_8H_{10}O$	ND	1.36	ND
17:00	1-Hexadecanol	196.2187	55.0544, 83.0856	$C_{16}H_{34}O$	0.10	ND	ND
	Aldehyde						
08:11	à-Ethylidenebenzeneacetaldehyde	146.0728	115.0544, 138.0913	$C_{10}H_{10}O$	ND	0.06	ND
	Amines						
14:13	1-Naphthalenamine, N-ethyl-	171.1045	129.0702, 156.0810	$C_{12}H_{13}N$	ND	0.12	ND
12:12	N-acetylphenethylamine	163.0994	30.0342, 104.0623	$C_{10}H_{13}NO$	ND	0.62	ND
13:20	p-Aminobiphenyl	169.0888	141.0700, 167.0733	$C_{12}H_{11}N$	ND	0.12	ND
	Benzenes						
07:53	Benzene, 1,3-bis(1,1-dimethylethyl)-	190.1711	124.0756, 175.1482	$C_{14}H_{22}$	ND	0.04	ND
10:13	Benzeneethanol, à-(phenylmethyl)-	208.2062	92.0622, 103.0544	$C_{15}H_{16}O$	ND	0.49	ND
24:55	Benzeneethanamine, 2-fluoro-á,3,4-trihydroxy-N-isopropyl-	226.2167	59.0367, 72.0445	$C_{11}H_{16}FNO_3$	0.66	0.21	ND
	Esters						
03:55	Benzoic acid, 4-amino-, 4-hydroximino-2,2,6,6-tetramethyl-1-piperidinyl ester	120.4566	80.4620, 83.5630	$C_{16}H_{23}N_3O_3$	ND	ND	8.23
06:56	Benzofenac methyl ester	174.1069	61.0106, 91.0211	$C_{16}H_{15}ClO_3$	0.05	ND	ND
07:08	Benzoic acid, 4-amino-, 4-acetoxy-2,2,6,6-tetramethyl-1-piperidinyl ester	153.0501	107.1252, 120.4566	$C_{18}H_{26}N_2O_4$	0.17	ND	1.23
08:27	Cyclobutanecarboxylic acid, 2-dimethylaminoethyl ester	151.1098	58.0653, 71.0730	$C_9H_{17}NO_2$	ND	0.38	ND
09:18	Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(1-methylethyl)-1,3-propanediyl ester	329.0325	43.0543, 71.0492	$C_{16}H_{30}O_4$	0.34	0.25	ND
09:37	Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester	174.1206	71.0492, 89.0598	$C_{12}H_{24}O_3$	0.33	ND	ND
10:19	Fumaric acid, ethyl 2,3,5-trichlorophenyl ester	167.1065	99.0442, 127.0390	$C_{12}H_9Cl_3O_4$	0.07	ND	0.57
10:34	Fumaric acid, monoamide, N,N-dimethyl-, 3-chlorophenyl ester	185.0676	98.0602, 126.0552	$C_{12}H_{12}ClNO_3$	ND	0.16	ND
11:04	Phthalic acid, 3,4-dichlorophenyl methyl ester	194.0571	77.0386, 163.0392	$C_{15}H_{10}Cl_2O_4$	0.22	ND	ND
11:04	Phthalic acid, methyl 4-(2-phenylprop-2-yl)phenyl ester	283.0486	103.0139, 163.0307	$C_{24}H_{22}O_4$	ND	0.10	0.33
11:08	4-Butylbenzoic acid, 2-dimethylaminoethyl ester	161.1200	58.0653, 71.0731	$C_{15}H_{23}NO_2$	ND	0.19	ND
12:02	3,4-Dimethyl-2-(3-methyl-butyryl)-benzoic acid, methyl ester	208.5497	54.5083, 191.4042	$C_{15}H_{20}O_3$	ND	ND	3.42
12:33	Ethyl 2-cyano-3-methylbutanoate	153.9684	68.0387, 82.5285	$C_8H_{13}NO_2$	0.10	ND	0.61
12:57	6-Methoxythymyl 2-methylbutyrate	180.2455	121.4904, 165.0691	$C_{16}H_{24}O_3$	ND	ND	0.42
13:23	Phthalic acid, monoamide, N-ethyl-N-(3-methylphenyl)-, ethyl ester	194.0570	149.0235, 177.0545	$C_{19}H_{21}NO_3$	0.04	ND	ND
13:25	Butyric acid, thio-, S-hexyl ester	194.1546	73.0543, 71.0492	$C_{10}H_{20}OS$	ND	0.23	ND
15:42	Fumaric acid, butyl 2-phenylethyl ester	267.9997	104.0623, 203.0943	$C_{16}H_{20}O_4$	ND	0.33	ND
16:43	Ethyl 13-methyl-tetradecanoate	270.2554	88.0520, 101.0599	$C_{17}H_{34}O_2$	ND	0.17	ND
16:56	Phthalic acid, heptyl tridec-2-yn-1-yl ester	460.9532	57.0701, 149.0236	$C_{28}H_{42}O_4$	0.42	ND	ND
17:45	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	292.2035	147.0808, 277.1799	$C_{18}H_{28}O_3$	0.02	ND	ND
17:55	DL-Alanine, N-methyl-N-(byt-3-yn-1-yloxycarbonyl)-, tridecyl ester	224.1825	86.0966, 154.0738	$C_{22}H_{39}NO_4$	ND	1.67	ND

(*continued on next page*)

Table 1 (*continued*)

t_R (min)	Compound name and metabolite class	Observed *m/z*	*m/z* fragments	MF	Percentage peak areas		
					BGN	DD	UHD
17:57	Phthalic acid, 8-chlorooctyl nonyl ester	236.2140	148.8379, 205.4445	$C_{25}H_{39}ClO_4$	0.56	0.94	0.30
17:57	Phthalic acid, 2-chloropropyl heptyl ester	224.0991	149.0235, 205.0860	$C_{18}H_{25}ClO_4$	0.53	ND	0.28
17:58	Phthalic acid, 8-chlorooctyl decyl ester	224.1005	103.0392, 149.0235	$C_{26}H_{41}ClO_4$	0.57	0.33	0.60
18:20	Fumaric acid, 2,6-dimethoxyphenyl dodec-2-en-1-yl ester	213.1026	68.0386, 153.9559	$C_{24}H_{34}O_6$	ND	1.10	2.60
18:30	L-Proline, N-valeryl-, decyl ester	219.0079	55.1733, 84.0285	$C_{20}H_{37}NO_3$	ND	0.86	ND
20:10	2-Methylbutyl propanoate	142.8595	56.5643, 70.0906	$C_8H_{16}O_2$	ND	ND	4.68
21:00	Octanoic acid, 2-dimethylaminoethyl ester	218.0598	58.0652, 72.0808	$C_{12}H_{25}NO_2$	2.48	1.98	ND
22:28	Carbonic acid, 2-dimethylaminoethyl 2-methoxyethyl ester	194.1912	58.0652, 71.0729	$C_8H_{17}NO_4$	4.65	7.33	ND
23:11	Phthalic acid, dicyclohexyl ester	300.2082	149.0236, 167.0342	$C_{20}H_{26}O_4$	0.47	0.11	0.82
24:06	Isophthalic acid, phenylethyl undecyl ester	267.0185	104.0825, 131.6355	$C_{27}H_{36}O_4$	ND	0.02	0.46
24:19	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	348.0901	67.0543, 262.2299	$C_{21}H_{38}O_4$	5.82	5.14	ND
24:29	Octadecanoic acid, 2,3-dihydroxypropyl ester	359.3167	74.0362, 98.0728	$C_{21}H_{42}O_4$	2.32	ND	ND
25:30	Butylphosphonic acid, decyl 4-(2-phenylprop-2-yl)phenyl ester	472.3099	221.1319, 457.2876	$C_{29}H_{45}O_3P$	0.06	ND	ND
25:30	Succinic acid, 2-chloro-6-fluorophenyl phenethyl ester	400.9841	105.0699, 279.2308	$C_{18}H_{16}ClFO_4$	ND	0.32	ND
25:37	Succinic acid, 3,4-dimethylphenyl 2-(dimethylamino)ethyl ester	312.3026	58.0135, 71.7611	$C_{16}H_{23}NO_4$	0.37	0.16	ND
25:37	Carbonic acid, 2-dimethylaminoethyl isobutyl ester	186.1471	58.0652, 71.0729	$C_9H_{19}NO_3$	0.21	0.27	ND
29:25	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	427.3893	189.1643, 218.2032	$C_{31}H_{48}O_3$	0.14	ND	ND
30:39	Olean-12-en-28-oic acid, 3-oxo-, methyl ester	452.3664	203.1796, 262.1931	$C_{31}H_{48}O_3$	0.74	ND	ND
	Fatty acid ethyl esters						
17:08	Pentadecanoic acid, ethyl ester	270.2552	88.0520, 101.0599	$C_{17}H_{34}O_2$	ND	0.25	ND
18:00	9-hexadecenoic acid, ethyl ester	282.2556	69.0699, 88.0521	$C_{18}H_{34}O_2$	ND	0.26	ND
18:14	Lauric acid, ethyl ester	228.2055	88.0521, 101.0600	$C_{14}H_{28}O_2$	ND	10.15	ND
18:19	Palmitic acid, ethyl ester	285.2786	88.0522, 101.0601	$C_{18}H_{36}O_2$	ND	17.74	ND
23:29	Stearic acid, ethyl ester	312.2990	88.0520, 101.0599	$C_{20}H_{40}O_2$	ND	1.41	ND
	Fatty acid methyl esters						
15:59	Myristic acid, methyl ester	256.2399	88.0521, 101.0600	$C_{16}H_{32}O_2$	ND	0.75	ND
17:30	Hexadecanoic acid, methyl ester	270.2556	74.0363, 87.0442	$C_{17}H_{34}O_2$	6.98	ND	ND
19:15	9,12-Octadecadienoic acid, methyl ester	294.2561	81.0699, 95.0858	$C_{19}H_{34}O_2$	2.55	ND	ND
19:19	trans-13-Octadecenoic acid, methyl ester	296.2714	55.0543, 74.0363	$C_{19}H_{36}O_2$	0.73	ND	ND
19:31	Octadecanoic acid methyl ester	298.2871	74.0363, 143.1070	$C_{19}H_{38}O_2$	1.61	ND	ND
23:00	Cerotic acid, methyl ester	356.3559	74.0363, 87.0442	$C_{27}H_{54}O_2$	ND	1.41	ND
	Fatty acid						
18:05	Palmitic acid	256.2404	60.0207, 73.0284	$C_{16}H_{32}O_2$	4.03	ND	ND
	Fatty acid derivatives						
20:29	Myristic acid amide	227.2204	59.0367, 72.0445	$C_{14}H_{29}NO$	ND	1.04	ND
22:55	2-monopalmitin	331.2852	104.0738, 128.5062	$C_{19}H_{38}O_4$	7.88	3.65	ND
	Furans						
03:35	Furanoeudesma-1,4-diene	108.0683	47.0327, 64.0181	$C_{15}H_{18}O$	ND	1.50	ND
07:46	3-Butene-1,2-diol, 1-(2-furanyl)-	128.0357	49.0073, 97.0286	$C_8H_{10}O_3$	2.39	1.90	0.32
19:45	Furfuryl ether	176.0922	81.0335, 143.0342	$C_{10}H_{10}O_3$	9.31	ND	ND
	Ketones						
04:09	Hex-4-yn-3-one	95.8902	67.0060, 68.0471	C_6H_8O	ND	ND	1.02
06:02	3-Acetoxy-2-methyl-pyran-4-one	129.0913	71.0128, 126.0312	$C_8H_8O_4$	3.57	3.67	ND

(*continued on next page*)

Table 1 (*continued*)

t_R (min)	Compound name and metabolite class	Observed *m/z*	*m/z* fragments	MF	Percentage peak areas		
					BGN	DD	UHD
07:41	2-Coumaranone	134.0364	78.0464, 106.0414	$C_8H_6O_2$	ND	0.12	ND
08:44	Ethanone, 1-(2-hydroxy-5-methylphenyl)-	150.0677	107.0493, 135.0442	$C_9H_{10}O_2$	0.44	0.30	ND
09:47	7-Chloro-1,3,4,10-tetrahydro-10-hydroxy-1-[[2-[1-pyrrolidinyl]ethyl]imino]-3-[3-(trifluoromethyl)phenyl]-9(2H)-acridinone	268.9973	84.0809, 132.0548	$C_{26}H_{25}ClF_3N_3O_2$	0.91	0.15	3.75
11:04	2-(6-Chloro-3-nitro-4-phenyl-quinolin-2-ylsulfanyl)-1-(2,3-dihydro-benzo[1,4]dioxin-6-yl)- ethanone	194.0574	132.5996, 163.0307	$C_{25}H_{17}ClN_2O_5S$	ND	0.12	0.36
17:32	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	276.1718	175.119, 205.0861	$C_{17}H_{24}O_3$	0.11	ND	ND
23:20	2-methoxy-,2-octen-4-one,	152.0474	99.0443, 114.0677	$C_9H_{16}O_2$	0.19	0.08	ND
28:04	3,6,13,16-tetraoxatricyclo[16.2.2.2(8,11)]tetracosa-8,10,18,20,21,23-hexaene-2,7,12,17-tetrone	380.0489	208.0519, 341.0657	$C_{20}H_{16}O_8$	0.08	ND	ND
	Nitrogenous compounds						
04:48	Indoline, 2-(hydroxydiphenylmethyl)-	314.5135	103.0505, 118.4013	$C_{21}H_{19}NO$	ND	ND	26.11
07:34	Indole, 3-(2-(diethylamino)ethyl)-	130.6049	85.5811, 129.5840	$C_{14}H_{20}N_2$	ND	ND	0.16
	Others (Miscellaneous compounds)						
03:45	N-[3,3′-dimethoxy-4'-(2-piperidin-1-yl-acetylamino)-biphenyl-4-yl]-2-piperidin-1- yl-acetamide	128.0471	93.0701, 98.0364	$C_{28}H_{38}N_4O_4$	0.55	5.14	ND
05:50	Succinic anhydride	102.0283	36.5607, 55.5498	$C_4H_4O_3$	ND	ND	6.71
06:22	Decamethylcyclopentasiloxane	358.0680	73.0469, 266.9992	$C_{10}H_{30}O_5Si_5$	0.12	0.05	ND
06:27	N,N-Dimethylglycine	103.0631	42.0338, 58.0653	$C_4H_9NO_2$	ND	0.62	ND
06:39	1H-Imidazole-4-methanol	98.0364	69.0335, 97.0286	$C_4H_6N_2O$	ND	2.31	ND
06:52	Thiourea, N-(3-methyl-2-pyridinyl)-N'-[(tetrahydro-2-furanyl)methyl]-	332.0663	44.0733, 150.0677	$C_{12}H_{17}N_3OS$	ND	0.12	ND
07:32	Catecholborane	120.0570	100.0759, 148.0994	$C_6H_5BO_2$	ND	0.71	ND
07:42	2-Benzoxazolamine, N-(1,1-dimethylethyl)-	153.9813	105.0764, 133.6238	$C_{11}H_{14}N_2O$	ND	ND	0.32
08:43	Dodecamethylcyclohexasiloxane	434.0840	73.0468, 341.0179	$C_{12}H_{36}O_6Si_6$	0.76	0.91	1.69
08:44	Phenyl-1,2-diamine, N,4,5-trimethyl-	149.8967	106.1084, 134.6487	$C_9H_{14}N_2$	ND	ND	0.36
09:53	Benzaldehyde, 3-methoxy-4-[(2-methylphenyl)methoxy]-	195.1248	105.0701, 132.0810	$C_{16}H_{16}O_3$	ND	1.00	ND
11:15	4,4′-Dichlorodibutyl ether	158.0202	91.0312, 93.0280	$C_8H_{16}Cl_2O$	0.03	ND	ND
11:40	Tetradecamethylcycloheptasiloxane	508.1064	73.0467, 281.0513	$C_{14}H_{42}O_7Si_7$	2.04	1.72	ND
11:50	Tetradonium Bromide	165.0703	58.0653	$C_{17}H_{38}BrN$	0.13	ND	ND
12:58	2,3,5,6-Tetrafluoroanisole	180.0782	137.0569, 165.0548	$C_7H_4F_4O$	ND	0.23	ND
13:14	3-Methyl-4-phenyl-1H-pyrrole	157.0886	127.5468, 155.9884	$C_{11}H_{11}N$	ND	1.19	1.12
13:24	2-propynenitrile, 3-fluoro-	69.0591	53.4495, 81.5012	C_3FN	ND	ND	1.05
14:20	Hexadecamethyl-cyclooctasiloxane	580.1260	73.0468, 355.0702	$C_{16}H_{48}O_8Si_8$	1.71	1.41	1.53
17:09	2,7-Dimethylcarbazole	195.1039	140.0702, 167.0726	$C_{14}H_{13}N$	ND	0.03	ND
17:50	1-Methyl-2,5-dipropyldecahydroquinoline	195.4010	86.6185, 166.1360	$C_{16}H_{31}N$	ND	ND	0.15
19:41	2-(1-Pyrrolidinyl)ethyl 4-propoxysalicylate	238.7279	83.5285, 96.8999	$C_{16}H_{23}NO_4$	ND	ND	0.43
19:42	Monoethanolamine stearic acid amide	282.2785	85.0523, 98.0602	$C_{20}H_{41}NO_2$	3.21	ND	ND
20:47	3-Cyclopentylpropionamide, N,N-dimethyl-	170.1547	45.0574, 87.0680	$C_{10}H_{19}NO$	0.72	0.12	ND
22:07	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	244.1207	125.0709, 153.0660	$C_{14}H_{16}N_2O_2$	ND	0.22	ND
22:25	Bis(2-(Dimethylamino)ethyl) ether	156.1014	58.0652, 71.0729	$C_8H_{20}N_2O$	7.95	0.25	ND
24:55	Acetaldehyde, diethylhydrazone	114.0678	71.0492, 99.0443	$C_6H_{14}N_2$	ND	0.09	ND
26:59	S-[2-[N,N-Dimethylamino]ethyl]N,N-dimethylcarbamoyl thiocarbohydroximate	218.0808	58.0652, 71.0729	$C_8H_{17}N_3O_2S$	0.20	ND	ND
	Phenols						
09:14	2-methyl-6-tert-butylphenol	164.1196	121.0649, 149.0963	$C_{11}H_{16}O$	0.02	ND	ND

(*continued on next page*)

Table 1 (*continued*)

t_R (min)	Compound name and metabolite class	Observed *m/z*	*m/z* fragments	MF	Percentage peak areas		
					BGN	DD	UHD
12:02	2,4-Di-tert-butylphenol	206.1665	57.0700, 191.1431	$C_{14}H_{22}O$	2.00	ND	ND
12:04	Phenol, 2,4,6-tris(1-methylethyl)-	220.1824	177.1274, 205.1588	$C_{15}H_{24}O$	0.13	0.13	0.56
12:05	Butylated Hydroxytoluene	220.1824	43.0179, 205.1589	$C_{15}H_{24}O$	0.19	0.12	0.65
12:57	2-tert-Butyl-4-methoxyphenol	180.0781	137.0596, 165.0548	$C_{11}H_{16}O_2$	0.35	ND	ND
16:48	Resorcinol/3-Hydroxyphenol	110.0602	82.0289, 201.1147	$C_6H_6O_2$	ND	0.15	ND
16:52	Taxicatigenin	153.9559	68.0387, 124.5019	$C_8H_{10}O_3$	ND	ND	0.58
22:14	Phenol, 2,2′-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	340.2401	161.0964, 177.1277	$C_{23}H_{32}O_2$	3.52	0.76	5.21
	Pyridines						
11:18	o-phenylpyridine	154.9679	126.5207, 155.9841	$C_{11}H_9N$	ND	ND	0.29
11:19	m-Phenylpyridine	155.0730	127.0544, 156.0764	$C_{11}H_9N$	ND	0.46	0.23
15:05	4-pyridinamine, N-[(4-methoxyphenyl)methylene]-	212.1306	91.0544, 197.1073	$C_{13}H_{12}N_2O$	ND	0.14	ND
20:56	2,6-diphenyl-pyridine,	231.1044	58.0653, 202.0777	$C_{17}H_{13}N$	ND	0.16	ND
	Sterols						
28:08	Ergosta-5,24-dien-3-ol, (3á)-	384.3342	281.2269, 314.2608	$C_{28}H_{46}O$	0.04	ND	ND
28:11	Campesterol	400.3701	145.1014, 213.1642	$C_{28}H_{48}O$	0.49	0.12	ND
28:24	Stigmasterol	412.3710	83.0856, 159.1172	$C_{29}H_{48}O$	1.39	0.41	ND
28:53	Stigmasta-5,24(28)-dien-3-ol, (3á,24Z)-	412.3703	314.2609, 281.2269	$C_{29}H_{48}O$	0.51	0.15	ND
29:02	Cycloeucalenol	412.3684	95.0857, 107.0858	$C_{30}H_{50}O$	0.17	ND	ND
29:06	Olean-12-en-3-ol	426.3862	203.1797, 218.2032	$C_{30}H_{50}O$	0.29	ND	ND
	Sulphur related compounds						
04:13	Dimethyl trisulfide	125.9627	78.9671, 127.9585	$C_2H_6S_3$	ND	1.88	ND
07:16	2,5-dihydrothiopene	86.0364	45.0336, 57.0336	C_4H_6S	ND	1.52	ND
08:18	Hemineurine	143.0401	85.0108, 112.0217	C_6H_9NOS	ND	0.10	ND
20:45	O-Ethyl S-2-diethylaminoethyl ethylphosphonothiolate	257.7809	85.6057, 98.9677	$C_{10}H_{24}NO_2PS$	ND	ND	1.21
21:12	1H-Indole-3-carbonitrile, 2-(4-chlorobenzenesulfonylmethyl)-1-methyl-	225.1118	169.1224, 201.1485	$C_{17}H_{13}ClN_2O_2S$	ND	0.08	ND
	Terpenes and Terpenoid						
04:56	Eucalyptol	154.1354	81.0700, 93.0701	$C_{10}H_{18}O$	0.26	ND	ND
07:02	Naphthalene	128.0622	76.0307, 99.0442	$C_{10}H_8$	0.34	0.27	ND
28:47	Clionasterol	414.3864	145.1015, 213.1643	$C_{29}H_{50}O$	0.61	0.15	ND
	Vitamins						
26:08	ë-Tocopherol	402.3499	137.0599, 177.0914	$C_{27}H_{46}O_2$	3.43	1.05	4.30
26:51	ç-Tocopherol	416.3654	151.0755, 191.1069	$C_{28}H_{48}O_2$	1.77	0.41	3.28
23:58	dl-7-azatryptophan	204.0760	88.0336, 131.0524	$C_{10}H_{11}N_3O_2$	ND	0.28	1.22

t_R – Retention time; *m/z* – mass-to-charge ratio; MF – Molecular formula; ND- Not detected; BGN – Bambara groundnut, DD – Dehulled *dawadawa*; UHD – Unhulled *dawadawa*

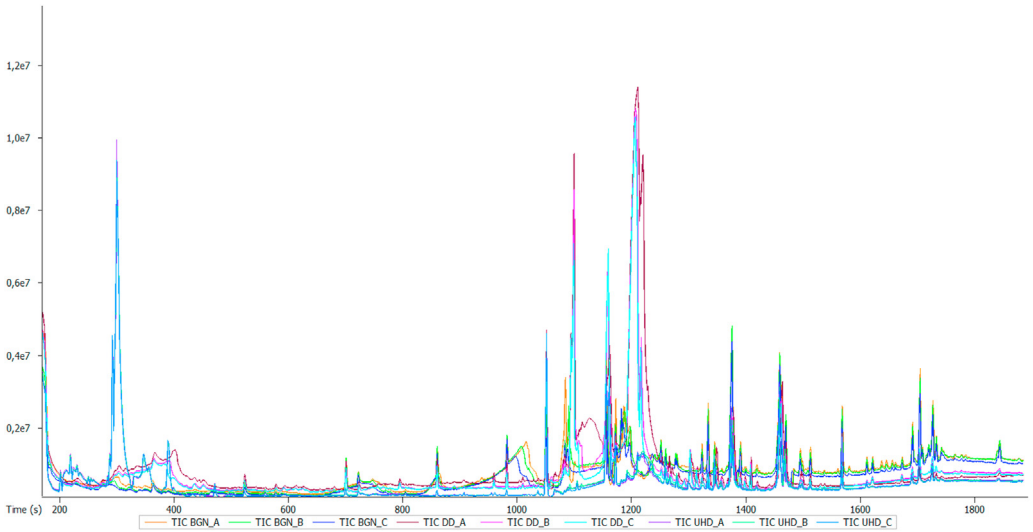

Figure 1. GC-HRTOF-MS chromatogram of the BGN (Bambara groundnut), DD (Dehulled *dawadawa*) and UHD (Unhulled *dawadawa*) samples.

metabolites of BGN, unhulled (UHD) and dehulled (DD) *dawadawa* using GC-HRTOF-MS. In total, 134 metabolites were identified, and their identities presented in Table 1. Figure 1 represents the GC-HRTOF-MS chromatogram of BGN, DD and UHD samples. The group of compounds detected were terpenes and terpenoids (2%), amines (2%), sulphur related compounds (4%), ketones (7%), pyridines (3%), vitamins (2%), esters including fatty acid methyl and ethyl esters (37%), alcohols including sterols (7%), phenols (6%) and other miscellaneous compounds (20%). Eight compounds were identified in both *dawadawa* products, 29 in BGN, 42 in only DD, 17 in only UHD and 12 in all the samples analyzed (Figure 2A). Generally, more metabolites were detected in DD samples as compared to UHD, which might be attributed to increased microbial activity enhancing metabolic activities and better breakdown and/or formation of compounds. This was also the observation in an earlier study (Adebiyi et al., 2019; Adebiyi, 2020) and can be related to higher antioxidant activities and antinutritional factors (ANFs) in UHD as compared to DD samples, which might influence microbial activities. Some of the compounds identified in Table 1, were not detected in the raw BGN, but observed in DD and UHD samples. It can thus be speculated that these compounds were presumably produced during fermentation. The major metabolites that were only found in the fermented condiments include 9,12-octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester (5.13%), carbonic acid, 2-dimethylaminoethyl 2-methoxyethyl ester (7.3%), lauric acid, ethyl ester (10.2%), maltol (4%) and palmitic acid, ethyl ester (17.7%) for dehulled *dawadawa* and 2-methylbutyl propanoate (4.7%), 2-undecen-4-ol (4.7%), benzoic acid,4-amino-4-hydroximino-2,2,6,6-tetramethyl-1-piperidinyl ester (8.2%), ë-tocopherol (4.3%) and indoline, 2-(hydroxydiphenylmethyl)- (26.1%) for unhulled *dawadawa* samples (Table 1).

Esters were the principal compounds reported in this study. Other similar studies on fermented condiments contradict this observation, with pyrazines being the major constituent in *sonru*, *afitin*, *iru* (Azokpota et al., 2008), acids the dominant group in castor oil bean fermented condiment (Ojinnaka and Ojimelukwe, 2013), aldehydes in locust bean *daddawa*, soybean and melon seed *ogiri* (Onyenekwe et al., 2012), while aldehydes, acids and ketones were reported to dominate *dawadawa* from BGN using *Bacillus* species (Akanni et al., 2018a). Nevertheless, esters are major metabolite groups common to several fermented condiments in Africa and are mostly formed during fermentation by esterification of alcohols with fatty acids (Fan and Qian, 2005). Chemical reactions between alcoholic metabolites as well as microbial acidic metabolites could also lead to the formation of esters during fermentation. Their contributions to food aroma/odour are important, combined with the fact that esters at ambient temperatures are highly volatile and their perception thresholds are much lower compared to their alcohol precursors (Nogueira et al., 2005). Compounds belonging to the esters group constitutes 29% (Figure 2B) of the total metabolites recorded and were more prominent in the DD as compared to UHD. Phthalic acid 8-chlorooctyl decyl ester, phthalic acid dicyclohexyl ester and phthalic acid 8-chlorooctyl nonyl ester were the major esters detected in BGN, DD and UHD (Table 1).

The identified compounds in this study could be as a result of the breakdown and constituents in BGN such as proteins, lipids and other bioavailable compounds through the activities of the microbial enzymes. As reflected in the GC-HRTOF-MS data presented in Table 1, fermentation of BGN into derived *dawadawa* led to the formation, increase, as well as decrease of some compounds. Formation of these constituents could be attributed to the presence of microorganisms involved in the fermentation process and other processing factors as well as operations involved during *dawadawa* preparation (Azokpota et al., 2010). Compounds belonging to an acid group were only present in UHD samples, which can be attributed to the relatively longer fermentation period for the UHD samples. Acids are sometimes considered as undesirable compounds that confer unpleasant characteristics such as rancid, sweaty and pungent flavors (Frauendorfer and Schieberle, 2008), although they have been reported to confer some acidic, fruity and sour notes in fermented foods (Park et al., 2013).

Compounds belonging to the sulphur related group were mostly present in the DD with none detected in the BGN, in agreement with the study of Akanni et al. (2018b), in which sulphur-related compounds were equally not detected in the raw BGN. Dimethyl trisulfide (sulphur related compound) is known to confer meaty, sulfureous, eggy, alliaceous, cooked, savory, and onion note (Liu et al., 2012). It can also be identified as a possible product of amino acid metabolism (Tamman et al., 2000). Speculated possible amino acid degradation and significantly ($p \leq 0.05$) different values in the amino acid of BGN and derived *dawadawa* (Adebiyi et al., 2019) could also explain the detected amine-related and nitrogenous compounds (Table 1).

Both ketones and aldehydes are formed by fatty acids beta-oxidation as well as oxidation catalyzed by lipoxygenase and hyper-oxidase enzymes, yielding important flavor compounds (Nzigamasabo, 2012). Aldehydes are not only flavor components, but also known as vital reactants associated with heterocyclic compounds formation (Ziegleder, 2009). Ketones are generally derived from amino acid and lipid degradation with the presence of these compounds having an impact on food flavor (Adebo et al., 2018). Nine ketones were detected, *i.e.* six from DD and three from UHD. The ketones in UHD decreased, as compared to the

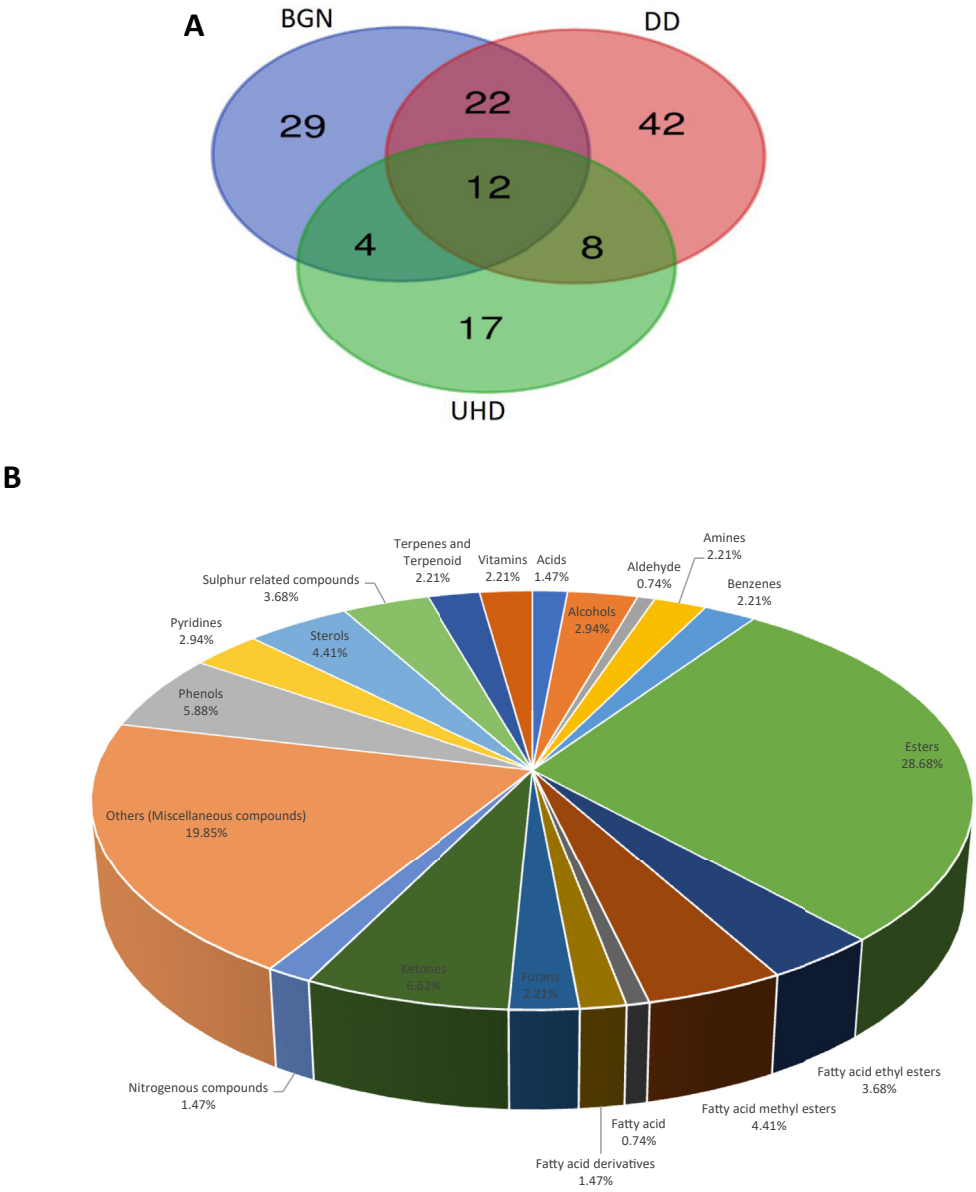

Figure 2. (A) Venn diagram showing the relationship between the metabolites in BGN (Bambara groundnut), DD (Dehulled *dawadawa*) and UHD (Unhulled *dawadawa*) samples, (B) Pie chart showing percentage distribution of the compounds.

raw BGN, whereas there was a slight increase of the ketone group in DD (relative to the percentage peak areas). Dehulling of the seedcoats exposed fats related components to more oxygen coupled with removal of available antioxidants in the hull. This thus suggests that fat oxidation would likely be higher in the dehulled samples as compared to the unhulled samples (Akkad et al., 2019). Therefore, an increased level of ketones in the dehulled samples might be due to partial oxidation of the alcohols as well as synthesis through several metabolic pathways, particularly reduction of methyl-ketone (Curioni et al., 2002; Akkad et al., 2019). This may be associated with the disappearance and/or reduction of some ketones in BGN and *dawadawa*.

Alcohols constituted 3% of metabolites in Figure 2B and are generated by reduction reaction of corresponding aldehydes and oxidation of acids (Pham et al., 2008). According to Estrella et al. (2004), aldehydes and ketones are relatively unstable intermediate compounds and can easily be reduced to alcohols. In total, four alcohols were detected in this study, *i.e.* 2-undecen-4-ol at high levels in two fermented samples, maltol in BGN and DD, phenylethyl alcohol in DD, with 1-hexadecanol in BGN. The phenylethyl alcohol compound has a rose-like odor and is known as one of the major Korean fermented soy sauce odor-active compounds (Lee et al., 2006), suggesting that these alcohol-related compounds might contribute to the flavor of these *dawadawa* samples. A decrease in the number of alcohols in the fermented samples might be due to the heat treatment (i.e. cooking) applied during processing (Cho et al., 2017; Wang et al., 2019).

The pyridines group was not detected in the BGN except in the *dawadawa* samples, indicative of a formation of these compounds. Pyridines are usually formed during cooking of food (Gupta et al., 2019) or meat, probably due to the reaction of amino acids with alkanals (Hui, 2012). They are classified as the flavor component of beer and as important organoleptic compounds of foods from cocoa, peanuts, cheeses, beans and barley (Maga, 1981). Due to the physical properties of BGN (hard to cook phenomenon), the seeds are usually cooked briefly then dehulled (depending on the product) prior to fermentation, which might explain the occurrence of pyridines in this study.

Three vitamin-related compounds (Table 1) were detected in *dawadawa* samples except for dl-7-azatryptophan, which was completely absent in BGN. Other notable vitamins observed were ç-Tocopherol and

ë-Tocopherol, which are forms of vitamin E. Not only is vitamin E of nutritional and dietary importance, but it also functions as an antioxidant by preventing the propagation of lipid peroxidation (Frei, 2004). It was observed that in dl-7-azatryptophan, the peak area of UHD (1.22%) was higher than that of DD (0.28%), while UHD has the highest percentage peak area in all the vitamins reported. Furans are heterocyclic compounds, known to possess sweet, roasted, burnt, caramel and sugar notes as previously reported in *dawadawa* (Akanni et al., 2018a; Azokpota et al., 2008).

Phenols are a major group of antioxidants and of great significance due to their biological and free radical scavenging activities (Koleva et al., 2018). Compounds belonging to the phenol group were also identified in this study. There was formation of taxicatigenin, which is also known as 3,5-dimethoxyphenol in UHD sample. 3,5-dimethoxyphenol belongs to methoxyphenols (a class of compounds comprising of a methoxy group) and connected to the benzene ring of a phenol moiety. The occurrence of this compound and its presence in only UHD could further explain its higher antioxidant activity in a previous study (Adebiyi, 2020), as taxicatigenin is a bioactive compound with potential antioxidant activity (Nithya et al., 2018). Bioactive compounds are also known to inhibit microbial growth that might have contributed to lesser microbial activity in UHD samples, resulting in reduced pH values (Adebiyi et al., 2020). Compounds belonging to the sterols group were common in raw BGN but none of these sterols were detected in UHD. There are claims that naturally occurring plant cholesterols may promote the health of animals and humans once consumed regularly either as food supplements or naturally in foods for a reasonable amount of time (Ogbe et al., 2015).

Fatty acid methyl esters were common in raw BGN, with the formation of methyl esters (myristic acid and cerotic acid methyl esters) in DD, while none of the fatty acid methyl esters were detected in UHD. This difference might have been due to the cooking process adopted (i.e., boiling), as heat treatment is known to affect fatty acid constituents of foods (Ouazib et al., 2015). Hexadecanoic acid methyl ester and octadecanoic acid methyl ester were detected only in raw BGN and are both known as the most abundant saturated fatty acids in nature, reported in plants, animals, lower organisms and functions in cells as specific proteolipids (*i.e.* connected to internal cysteine residues through thioester bonds) (Anonymous, 2013).

4. Conclusion

A total of 134 metabolites were detected in Bambara groundnuts and derived *dawadawa* samples using GC-HRTOF-MS. From the two fermented samples, dehulled samples had the highest number of metabolites as compared to their unhulled counterparts. Compounds identified included esters, ketones, phenols, flavor related compounds and constituents that could confer organoleptic properties, nutritional and functional benefits of BGN and derived *dawadawa*. The BGN seeds and dehulled *dawadawa* possess beneficial components that can potentially be incorporated into human diets for health benefits. Further investigations into the quantification of the metabolites in this study are still needed, particularly for those significant metabolites obtained in all three samples. These would not only provide a better understanding of legume fermentation, but also assist in providing an insight into these significant metabolites that could potentially be biomarkers of *dawadawa*.

Declarations

Author contribution statement

Janet Adeyinka Adebiyi: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Patrick Berka Njobeh, Eugenie Kayitesi: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Oluwafemi Ayodeji Adebo: Performed the experiments; Analyzed and interpreted the data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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