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Metabolite signatures and distribution patterns of processed pasta from fractionated whole wheat and Bambara groundnut using gas chromatography high-resolution time-of-flight mass spectrometry

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ABSTRACT

This study investigated the metabolite signatures and distribution in cooked whole wheat pasta enriched with Bambara groundnut using gas chromatography high-resolution time-of-flight mass spectrometry (GC-HRTOF-MS). Before pasta production, whole wheat grains were fractionated using mechanical sieves of different aperture sizes (112, 300, 350, and 500 μm) and each fraction was enriched with Bambara flour. A total of 45 volatile metabolites were found in the cooked pasta and classified into different metabolite groups of esters (18%), miscellaneous compounds (13%), fatty acids (9%), amides and amines (7%), aromatic compounds (7%), and pharmaceuticals (7%). Other metabolites found included ketones (4%), furans (4%), methyl ester (4%), phthalates and plasticizers (4%), phenolic compounds (4%), terpenes and triterpene (4%), alcohols (4%), benzene-related compounds (2%), monoacylglycerols (2%), phthalic acids (2%), surfactants (2%), and vitamins (2%). Similar (8) metabolites were observed across the four pasta samples using the Venn diagram to show the relationship between the samples, while pasta from sieve of particle size 350 and 300 μm showed higher numbers of unique metabolites, 8 and 7, respectively compared to pasta from sieve of particle size 112 μm (4) and 500 μm (3). The information from this study can be used as biomarkers for pasta enriched with pulses.

1. Introduction

The application of gas chromatography-mass spectrometry (GC–MS) as a comprehensive and sensitive technological approach for the detection of various volatile and semi-volatile metabolites has continued to attract much interest in food science and technology research (Adebo et al., 2021). Other methods such as capillary electrophoresis–mass spectrometry (CE-MS), liquid chromatography–mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) are also promising techniques that have been used in metabolomics studies (Ten-Doménech et al., 2020). More recently, to acquire rapid and comprehensive information on classes of metabolites present in food samples, different techniques have evolved such as Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) (Onzo et al., 2021) and GC-HRTOF-MS (Adebo et al., 2018). These techniques are known to provide accurate mass measurements and offer several advantages compared to low-resolution MS (Acquavia et al., 2021). According to Brits et al. (2018), GC coupled with high-resolution time of flight mass spectrometry (HRTOF-MS) is a highly effective and powerful analytical tool that could be applied for accurate mass measurement of metabolites. GC-HRTOF-MS has been used to analyse various food products such as fermented condiments (Adebiyi et al., 2021), whole grain ting (a Southern African fermented product) (Adebo et al., 2019; Kewuyemi et al., 2020), germinated Bambara groundnut (Oyedeji et al., 2021), fresh wheat grains (Feng et al., 2022) and pasta products (Geng et al., 2022; Oyeyinka et al., 2022).

Pasta remains an important staple across the globe. Its consumption is increasing due to the ease of preparation, low cost, long shelf life and sensory properties (Wójtowicz & Mościcki, 2014). Previous studies reported that pasta is generally not considered an aromatic product, how-

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ever, the cereal flour from which they are produced, generates volatiles which may influence the aroma and contribute to the overall composition of the pasta products (Beleggia et al., 2009; Bredie et al., 2002; Feng et al., 2022; Parker et al., 2000). Feng et al. (2022), for instance, reported eight hundred and sixty-six (866) metabolites in fresh wheat using non-targeted metabolomics. Likewise, Beleggia et al. (2011) also found seventy (76) metabolites during the processing of semolina to dried pasta (Beleggia et al., 2011).

Consumption of foods made from whole wheat grain including pasta is being encouraged because it may contribute to an adequate intake of bioactive compounds, including dietary fibre and antioxidants, which are known to reduce the risk of several chronic diseases (Ciccoritti et al., 2017). However, whole wheat pasta possesses some techno-functional challenges which may affect consumer acceptability and profitability from the food manufacturers' standpoint. The presence of bran in pasta products, for example, has been found to result in weak dough properties (Kaur et al., 2012), poor pasta cooking properties (Aravind et al., 2012) and inferior organoleptic quality (Steglich et al., 2015; Vignola et al., 2018). To address the challenge of poor dough properties and inferior sensory quality of pasta from whole wheat pasta, fractionation of whole wheat flour before pasta production was reported to improve the sensory attributes of pasta (Oyeyinka et al., 2021). These authors reported that before fractionation, the addition of Bambara groundnut (*Vigna subterranea*) enhanced the protein content, antioxidant properties and reduction in the cooking time of the pasta. In a recent study, untargeted metabolite profiling of pasta made from whole wheat flour enriched with Bambara grains showed the presence of forty-nine (49) different metabolites including aldehydes, amines, amides, alcohols, ketones, aromatic compounds, phenolic compounds, fatty acid and their esters (Oyeyinka et al., 2022). According to the authors, cooking of the pasta samples resulted in a greater diversity of metabolites (21) compared with the uncooked pasta sample (10). During fractionation of whole wheat and Bambara grains for pasta production, it is hypothesised that further modifications and or distributions of the metabolites before cooking may occur and data from this study could provide information for future optimisation studies. Therefore, this study investigated the metabolite signatures and distribution patterns of processed pasta from fractionated whole wheat and Bambara groundnut using GC-HRTOF-MS.

2. Materials and methods

2.1. Materials

Whole-grain wheat (*Triticum turgidum* ssp. durum) and Bambara groundnut (*Vigna subterranea*) were obtained from Ilorin, Nigeria. All other reagents used in the study were laboratory grade.

2.2. Fractionation of whole-wheat flour and compositing with Bambara flour

Cleaned wheat grains were milled into flour using a Warring blender (HGBTWTS3, Torrington USA). The flour sample (100 g) was separated into fractions using a mechanical sieve shaker with varying aperture sizes (112, 300, 350, and 500 μm). After 10 mins of shaking, the flour retained on each sieve was collected and kept in Ziploc bags. Each flour fraction was composited with 20% Bambara groundnut flour and used to make pasta as previously reported (Oyeyinka et al., 2021).

2.3. Pasta production

Pasta (spaghetti), was made following the modified method of Aranibar et al. (2018) described by Oyeyinka et al. (2021). The pasta was produced by mixing flour (50 g), with water (22.5 g) and salt (1.0 g) in a bench-top mixer till a consistent dough was formed. The dough was divided and extruded using a metal clay extruder (YG-21, China) with a diameter of 1.3 mm into trays laid with aluminium foils. Extruded samples were dried at 80 ± 5 °C for 2 h in a hot air oven (D-37,520, Thermo Fischer Scientific, Germiston, South Africa). Dried pasta was packaged in Ziploc bags and stored at room temperature (25 ± 2 °C) until needed for further analyses.

2.4. Sample preparation, GC-HRTOF-MS and data analysis

The method described by Oyeyinka et al. (2022) was used in the sample preparation, GC-HRTOF-MS analysis and data analysis of the pasta samples. A mass calibration of the instrument was performed before analysis on the LECO Pegasus GC-HRTOF-MS system (LECO Corporation). The pasta samples were analysed on the GC-HRTOF-MS system equipped with an Agilent 7890A (Agilent Technologies, Inc.) gas chromatograph and a high-resolution LECO MS. This was coupled to a Gerstel MPS multipurpose autosampler (Gerstel Inc.). The GC analytical column was a Rxi®−5 ms (30 m × 0.25 mm ID × 0.25 μm) (Restek). Sample (1 μl) was injected (in a split-less mode) using helium as the carrier gas at a constant flow rate of 1 ml/min. The transfer line temperature was 225 °C, while the inlet temperature was 250 °C. 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 from 10 °C/min to 330 °C and held for 3 min. Samples from triplicate extraction were injected singly into the GC-HR-TOF-MS at a constant flow rate of 1 mL/min. The transfer line and inlet temperatures were set to 225 and 250 °C, respectively. In the MS, the ion source temperature was set at 250 °C, the electronspray ionization (ESI) voltage was set at 70 eV, the data (spectra) acquisition rate was set to 13 spectra/s, and the mass range (i.e., *m/z* range) was set at 30–1000.

For a metabolite to be confirmed present in a sample, it should appear in at least two of the triplicate extractions. Following the acquisition of spectra data, retention time alignment, peak picking, metabolite detection, and matching to the library was achieved using the ChromaTOF-HRT® software (LECO Corporation, St Joseph, MI, USA). Metabolites were positively identified at a signal-to-noise ratio (S/N) of 100 and a minimum similarity match of 70% (Kewuyemi et al., 2020).

3. Results and discussion

The result of the pasta samples produced from the different flour fractions of a mechanical sieve (112, 300, 350, and 500 μm) of whole wheat and Bambara groundnut obtained using the LECO GC-HRTOF-MS Folded Flight Path (FFP) technology showed the presence of forty-five (45) compounds (Table 1). GC-HRTOF-MS enables the generation of high-quality spectra data with a full mass range acquisition, accurate mass determination (with an accuracy root mean square (RMS) of less than 1 ppm), ultra-high resolution (50,000 FWHM), and capture rates of up to 200 spectra/second. This implicates the equipment in the generation of precise mass and high-resolution data required to differentiate between analytes with identical nominal masses at low concentrations. It was possible to positively identify these metabolites because of the unique advantages of high mass accuracy and mass fragment information provided by high-resolution mass spectrometry equipment (Adebo et al., 2019; Kewuyemi et al., 2020). Metabolite identification was achieved by accurate mass determination, retention time evaluation, and matching of sample spectra information (ions fragmentation "fingerprints") for each peak against standard metabolite libraries (Mainlib, NIST, and Feihn metabolomics libraries). Esters (18%) were the major metabolites found in the pasta samples (Fig. 1). Other metabolites found in relatively high quantities were fatty acids (9%), amides and amines (7%) and aromatic compounds (7%). Miscellaneous compounds also constituted 13% of the compounds found. Alcohols, aldehydes, benzene-related compounds, furans, ketones, monoacylglycerols, phenolic compounds and vitamins were other notable metabolites found in the pasta samples (Fig 1). Previous studies by Beleggia et al. (2009) found thirty-five (35) volatile compounds including aldehydes, ketones, alcohols, esters, and

Table 1
Metabolites in cooked pasta from fractionated whole wheat grain enriched with Bambara groundnut.

Retention time (min)	Observed ion *m/z*	*m/z* fragments	Metabolite name	Molecular formula	Average peak area			
					500 μm[a]	350 μm[c]	300 μm[c]	112 μm[b]
Alcohols								
3.438	77.0385	31.0181; 43.0180	Ethylene glycol	C_2H_5ClO	ND	ND	5,251,813	8,477,256
24.507	264.2436	67.0542; 55.0543	9,12-Octadecadien-1-ol, (Z,Z)-	$C_{18}H_{34}O$	ND	ND	404,190	ND
Aldehydes								
19.974	262.2303	41.0387; 81.0699	9,12-Octadecadienal	$C_{18}H_{32}O$	4,859,524	3,335,839	ND	1,341,696
Amides and amines								
5.946	87.0439	44.0259; 57.0337	Methylpent-4-enylamine	$C_6H_{13}N$	2,461,032	1,942,833	1,464,588	ND
21.512	192.1384	98.0601; 85.0523	Caprylic acid monoethanol amide	$C_{1n}H_{21}NO_2$	ND	659,028	ND	966,919
25.142	86.0600	59.0366; 72.0443	Palmitic amide	$C_{16}H_{33}NO$	ND	ND	39,886	ND
Aromatic compound								
8.973	150.0676	150.0675; 135.0441	2-Methoxy-4-vinylphenol	$C_9H_{1n}O_2$	71,208	37,608	37,067	ND
13.234	180.0781	180.0780; 165.0544	3-tert-Butyl-4-hydroxyanisole	$C_{11}H_{16}O_2$	54,675	39,006	31,113	ND
19.252	116.0704	98.0601; 116.0704	p-Xylene-d10	C_8D_{1n}	ND	25,610	25,756	ND
Benzene related compounds								
25.150	72.0444	59.0367; 72.0444	Benzene ethanamine, 2-fluoro-ß,3,4-trihydroxy-N-isopropyl-	$C_{11}H_{16}FNO_3$	ND	28,818	ND	ND
Fatty acids								
18.193	256.2394	43.0543; 60.0206	Palmitic acid	$C_{16}H_{32}O_2$	3,054,747	1,538,624	618,873	682,290
19.498	164.1204	67.0543; 95.0856	Linoleic acid	$C_{19}H_{34}O_2$	ND	ND	ND	148,580
19.938	252.2317	55.0543; 67.0542	17-Octadecynoic acid	$C_{18}H_{32}O_2$	ND	ND	1,177,811	ND
24.517	265.2509	67.0542; 81.0699	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	565,802	624,918	ND	526,150
Esters								
5.094	86.0362	85.0285; 59.0127	Tetrahydropyran Z-10-dodecenoate	$C_{17}H_{3n}O_3$	519,531	630,757	253,262	115,164
6.467	102.0312	43.0180; 102.0312	Butanoic acid, 2-methyl-3-oxo-, ethyl ester	$C_7H_{12}O_3$	517,404	228,977	ND	ND
9.902	85.0481	84.0445; 28.0184	L-Proline, 5-oxo-, methyl ester	$C_6H_9NO_3$	ND	98,722	ND	ND
17.762	227.2001	74.0363; 87.0441	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	210,042	ND	ND	165,813
17.897	227.2006	74.0363; 87.0441	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	182,152	209,032	139,966	ND
19.502	151.1113	67.0542; 81.0699	11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	ND	225,068	124,638	136,338
23.100	257.2465	43.0544; 57.0700	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	ND	ND	249,238	ND
24.680	285.2775	43.0543; 98.0727	Pentadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{18}H_{36}O_4$	274,209	277,116	166,866	237,057
Furans								
7.715	126.0311	41.0388; 97.0285	5-Hydroxymethylfurfural	$C_6H_6O_3$	ND	ND	ND	332,544
19.444	115.0390	81.0334; 53.0387	Difurfuryl ether	$C_{1n}H_{1n}O_3$	ND	ND	161,406	ND
Ketones								
6.629	144.0417	43.0180; 144.0418	Pyranone	$C_6H_8O_4$	862,196	444,560	764,976	343,310
15.122	106.0405	99.0805; 81.0700	Methanone, (1-hydroxycyclohexyl) phenyl-	$C_{13}H_{16}O_2$	77,976	55,628	76,684	128,821

(*continued on next page*)

Table 1 (*continued*)

Retention time (min)	Observed ion *m/z*	*m/z* fragments	Metabolite name	Molecular formula	Average peak area			
					500 μm[a]	350 μm[c]	300 μm[c]	112 μm[b]
Miscellaneous								
6.460	102.0312	43.0181; 102.0312	Ethyl acetoxycarbamate	$C_5H_9NO_4$	ND	ND	313,899	ND
9.934	110.0359	84.0445; 56.0496	N-Ethyl-2-isopropoxycarbonylazetidine	$C_9H_{17}NO_2$	ND	ND	138,778	191,165
21.219	72.0807	58.0652; 71.0730	Carbonic acid, 2-dimethylaminoethyl isobutyl ester	$C_9H_{19}NO_3$	181,711	138,500	91,216	114,492
21.943	72.0809	58.0652; 72.0808	Fumaric acid, 2-dimethylaminoethyl hexadecyl ester	$C_{24}H_{45}NO_4$	ND	276,876	ND	ND
22.381	177.0890	176.0832; 149.0596	Piperonyl butoxide	$C_{19}H_{3n}O_5$	26,118	ND	ND	ND
22.666	77.0384	58.0652; 71.0730	Bis(2-(Dimethylamino)ethyl) ether	$C_8H_{2n}N_2O$	495,122	ND	275,146	315,801
Monoacylglycerols								
23.113	299.2576	43.0544; 98.0727	Glycerol 1-palmitate	$C_{19}H_{38}O_4$	688,643	633,668	440,666	505,854
Pharmaceuticals								
3.062	98.0363	98.0364; 69.0335	Furfurol	$C_5H_6O_2$	706,244	ND	ND	342,921
9.950	85.0475	84.0444; 56.0495	Mefloquine	$C_{17}H_{16}F_6N_2O$	ND	87,331	ND	ND
6.704	117.0366	44.0259; 74.0362	Meglumine	$C_7H_{17}NO_5$	ND	530,116	206,282	ND
Phenolic compounds								
7.316	110.0362	110.0363; 64.0309	Catechol	$C_6H_6O_2$	90,945	46,200	ND	64,081
12.326	206.1662	191.1431; 57.0700	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	73,269	83,743	78,195	85,638
12.334	206.1666	191.1432; 57.0701	Phenol, 2,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	52,280	ND	ND	ND
Phthalates/Plasticizer								
23.428	167.0337	149.0236; 57.0700	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	ND	ND	ND	57,282
18.222	150.0270	149.0235; 76.0307	Palatinol C	$C_{16}H_{22}O_4$	ND	ND	ND	35,603
Phthalic acids								
18.216	150.0259	149.0237; 150.0271	Phthalic acid, hex-2-yn-4-yl nonyl ester	$C_{23}H_{32}O_4$	ND	ND	42,263	ND
Surfactants								
19.945	238.2168	85.0522; 98.0600	Lauric acid monoethanolamine	$C_{14}H_{29}NO_2$	ND	268,468	114,574	ND
Terpenes and triterpene								
29.057	414.3855	43.0543; 81.0700	ß-Sitosterol acetate	$C_{31}H_{52}O_2$	ND	224,979	127,218	196,647
29.070	415.3885	43.0543; 95.0857	9,19-Cyclolanostan-3-ol, acetate, (3ß)-	$C_{32}H_{54}O_2$	353,640	ND	ND	ND
Vitamin								
26.389	402.3489	137.0597; 177.0910	Vitamin E	$C_{27}H_{46}O_2$	ND	30,538	ND	ND

ND: Not detected. Superscripted alphabets on the pasta samples indicate the level of statistical difference in samples based on ANOVA post hoc analysis. Sample names with the same alphabet are not statistically different ($p>0.05$) whereas sample with different alphabets are statistically different ($p \leq 0.05$).
Metabolites in cooked pasta from fractionated whole wheat grain enriched with Bambara groundnut.

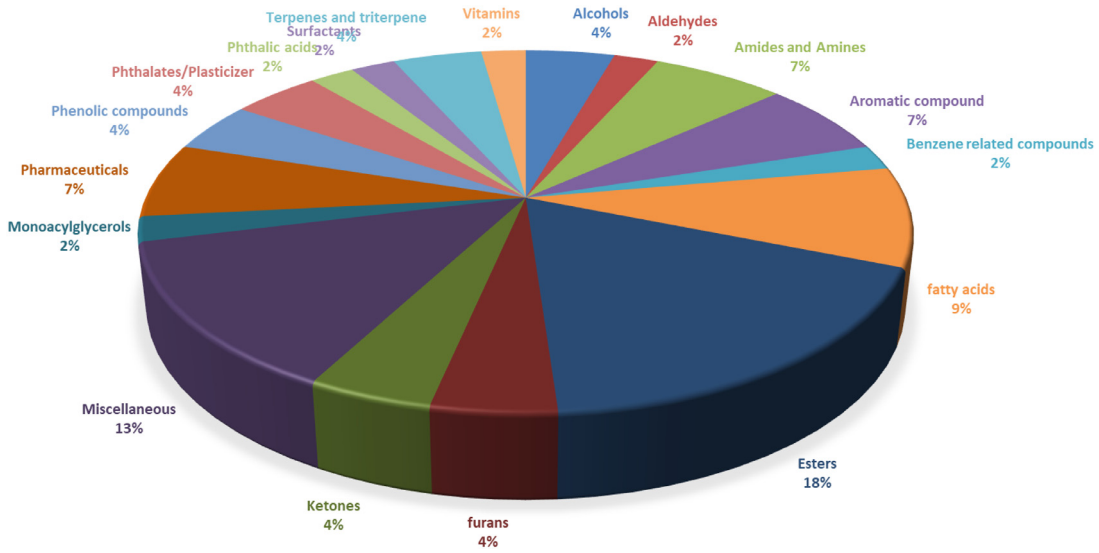

Fig. 1. Pie chart showing the percentage distribution of the constituents in raw and cooked pasta from whole wheat grain enriched with Bambara groundnut. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

hydrocarbons in wheat semolina and pasta samples, while some other authors found a higher number of metabolites (79) such as phytosterols, policosanols, unsaturated fatty acids, amino acids, carotenoids and minerals in pasta samples (Beleggia et al., 2011). In a recent study, forty-nine (49) metabolites were identified in pasta made from whole wheat flour and Bambara groundnut but without fractionation (Oyeyinka et al., 2022). The variation in the quantities of metabolites found in the current study compared with other authors may depend on the source of the wheat grain, the cultivar as well as processing conditions such as fractionation, as was the case in this study. Fractionation seems to have substantially altered the metabolite composition of the pasta samples after cooking.

A factorial analysis of variance (ANOVA) performed on the data ($R^2 = 0.967$, Adjusted $R^2 = 0.954$) revealed that there were significant differences ($p \leq 0.05$) in the metabolite distribution patterns between the pasta samples produced from the various flour fractions (Tables S1-S5). The interaction effect between the different pasta samples and individual identified metabolites was significant ($p = 0.00$). This implies that the different pasta samples can be differentiated by the type/quantity of individual metabolites they contain. Zooming in on the different pasta samples, all the samples statistically differed from one another except the pasta produced from the 300 and 350 μm sieve size flour ($p = 0.310$) (Tables S2 & S3). Quantitatively, pasta produced with flour samples from the 500 μm sieve size was the richest, containing huge amounts of metabolites, however, qualitatively, the same pasta sample was the least diverse (in terms of the different types of metabolites contained therein). Pasta produced with 300 μm sieve size flour was the most diverse in terms of the types of compounds it contained. In terms of the quantitative distribution of the metabolite classes, there were significant differences ($p \leq 0.05$) in the amounts of the different metabolite classes in the samples (Tables S4 & S5). Quantitatively, Alcohols were the most abundant class of metabolites followed by Aldehydes, Amides and Amines, and Fatty acids. Benzene-related compounds and Vitamins were the least abundant metabolite classes in the samples overall (Tables S4 & S5).

A dendrogram from hierarchical cluster analysis classified the pasta into groups and revealed their similarity to each other in terms of metabolite profile and distribution (Fig. 2). Pasta sample from flour of 500 μm was similar to pasta produced using flour from sieve size of 350 μm and these two samples (500 and 350 μm) were distinctly separated from those of 300 and 112 μm, which were closely linked. The relatedness of pasta samples made from 300 to 112 μm could be due to the smaller particle size of the flour compared with those of 500 and 350 μm, which are bigger. The pasta produced from fractionated flours with particle size of 300 and 500 μm had the highest and lowest number of metabolites, respectively (Fig. 3). The number of metabolites found in each pasta fraction was in the order 300 μm (8) > 350 μm (7) > 112 μm (4) > 500 (3) μm. Generally, pasta from smaller particle size sieve (112–350 μm) had higher number of metabolites than pasta made from flour with a bigger particle size (500 μm), though the occurrence of metabolite in the pasta samples did not follow a particular order. The lower number of metabolites in the pasta from coarse flour samples suggest that more metabolites passed through the sieve with aperture size of 500 μm leaving fewer amounts in the flour. This suggests that the degree of milling (fine or coarse) influences the distribution of the metabolites, with finer milling increasing the passage of more metabolites which otherwise would have been retained in the flour with bigger particle size. The distribution pattern of the metabolites is an indication that fractionation is a promising way to redistribute nutrients and bioactive compounds in pasta. This seems plausible since pasta prepared from the different fractions (112, 300, 350, and 500 μm) also showed varying levels of proteins, total phenol and antioxidant properties with a decrease in particle size (Oyeyinka et al., 2021).

In the current study, eight (8) metabolites including palmitic acid, 2,4-di-tert-butylphenol, tetrahydropyran Z-10-dodecenoate, carbonic acid, 2-dimethylaminoethyl isobutyl ester, pentadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, pyranone, methanone, (1-hydroxycyclohexyl) phenyl-, glycerol 1-palmitate were identified amongst the four pasta fractions (112, 300, 350, and 500 μm). In our earlier study (Oyeyinka et al., 2022), cooked samples that were not fractionated similarly showed the presence of these compounds suggesting that with or without fractionation, these compounds would be present in the pasta samples. Some of these compounds, for example, the ketones (pyranone and methanone, (1-hydroxycyclohexyl) phenyl-) and pentadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester are important metabolites with various pharmacological properties such as strong antioxidant properties and cholesterol-lowering effect (Al-Marzoqi et al., 2015; Zayed & Samling, 2016). Ketones can be formed by fatty acids beta-oxidation as well as oxidation catalysed by lipases (Nzigamasabo, 2012). They can also be derived from amino acid and lipolysis as well as contribute to food flavour (Adebo et al., 2018). Beleggia et al. (2009) reported an increase in aldehyde content and the appearance of ketones which were absent in semolina during pasta making and cooking. These authors found that

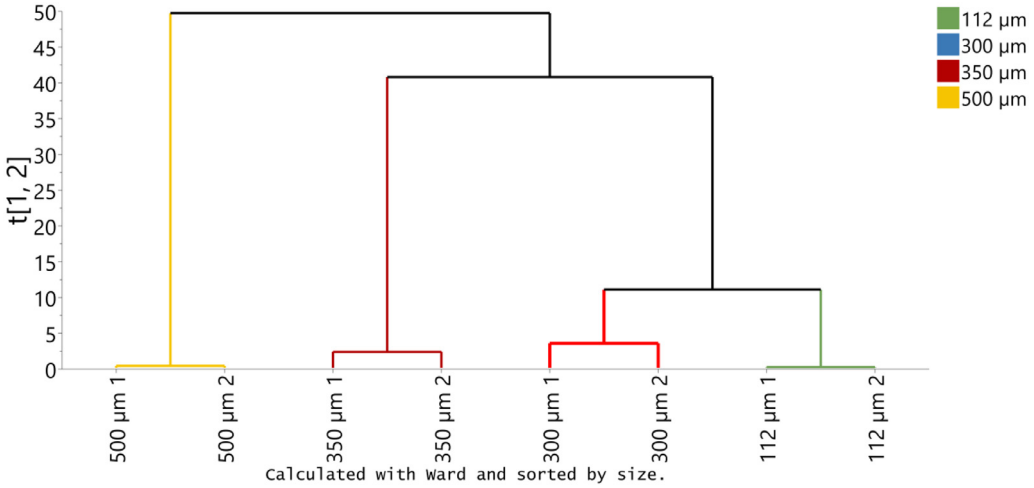

Fig. 2. Dendrogram of hierarchical cluster analysis showing sample relatedness. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

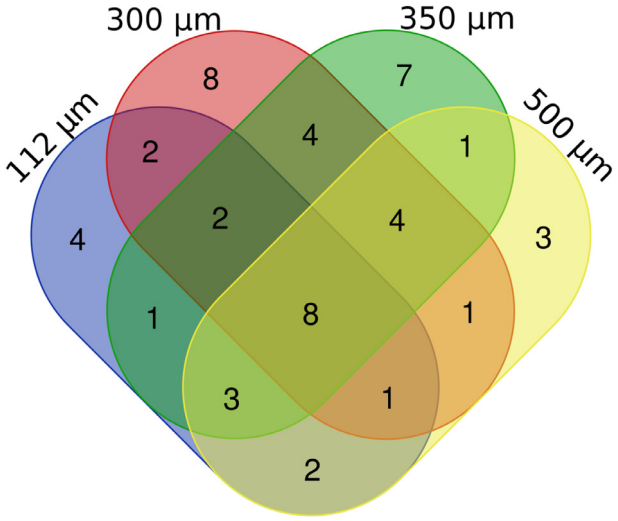

Fig. 3. Venn diagram showing the relationship between pasta from fractionated whole wheat grain enriched with Bambara groundnut. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ketones such as 2-heptanone, 6-Methyl-2-heptanone, 2-Octanone and 2-Nonanone in cooked pasta contributed to the flavour of cooked pasta. Oyeyinka et al. (2022) also reported the presence of three ketones, 7-Chloro-1,3,4,10-tetrahydro-10-hydroxy-1-[[2-[1-pyrrolidinyl] ethyl] imino]−3-[3-(*trifluoromethyl*) *phenyl*]−9(2H)-acridinone, Pyranone and Methanone, (1-hydroxycyclohexyl) phenyl- as possible flavour compounds in whole wheat pasta enriched with Bambara groundnut but without fractionation.

The antioxidant potential of 2,4-di-tert-butylphenol in preventing oxidative stress and their potentials as a valuable food additive for improving food safety and enhancing good health have been previously demonstrated by various researchers (Choi et al., 2013; Dharni et al., 2014; Song et al., 2018; Varsha et al., 2015).

Using the molecular docking approach, Venkatramanan et al. (2020) suggested that hexadecenoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester could be potentially used for treating *Chromobacterium violaceum* infections. This ester is similar in structure to pentadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, which was found in the pasta samples, and it may be worth investigating the role of these esters and other similar metabolites in preventing diseases in future research. Esters are not only important pharmacologically, but their functional role in food aroma and flavour has equally been documented (Kewuyemi et al., 2020; Xiong et al., 2019). They contribute to volatiles and flavour compounds that enhance the aroma of the pasta (Beleggia et al., 2009; Bredie et al., 2002; Feng et al., 2022; Parker et al., 2000). Other aromatic compounds found in the pasta samples (300, 350, and 500 μm) were 2-methoxy-4-vinylphenol and 3-tert-butyl-4-hydroxyanisole (Table 1). The 3-tert-butyl-4-hydroxyanisole in addition to contributing to food flavour, for example, in buckwheat (Janeš et al., 2009), may also exhibit antioxidant properties. Earlier studies reported that this metabolite can inhibit the action of a broad range of carcinogens, mutagens and tumour promoters (Wattenberg, 1986).

In general, four (4) fatty acids were identified from the pasta samples (Table 1). These include palmitic acid, linoleic acid, 17-Octadecynoic acid and 9,12-Octadecadienoic acid. Except for palmitic acid, a saturated fatty acid, other fatty acids present in the pasta samples are polyunsaturated fatty acids (PUFAs). PUFAs are essential constituents of cell membranes and are well-known to play a significant role in various chronic illnesses and regulation of human health (Lee et al., 2016). It is important to note that while palmitic acid was distributed in varying quantities in the four fractions, linoleic acid was present only in pasta made from flour with particle size of 150 μm. Furthermore, 17-Octadecynoic acid was present only in pasta made from flour with particle size of 300 μm while 9,12-Octadecadienoic acid was found in all the pasta samples except in pasta made from 300 μm. Adeoye-Isijola et al. (2018) reported that 9,12-Octadecadienoic acid possesses several pharmacological properties such as anti-inflammatory, antiandrogenic, antiarthritic, anti-coronary and hypocholesterolemic effect, while the antihypertensive properties of 17-Octadecynoic acid have also been documented (Bhattacharyya et al., 2019; Fadeyi et al., 2015; Jerez et al., 2012). The varying levels and distribution of these fatty acids amongst the pasta samples from different flour fractions could be attributed to the location of these compounds within the wheat and Bambara grains as well as the relative sizes of these metabolites. This could have influenced their presence in the respective flour fractions used in pasta production. Another plausible reason could be due to the transformations of metabolites in the raw (uncooked and unfractionated) pasta samples into new compounds after cooking. A previous study showed significant modifications in the metabolite composition of pasta made from unfractionated whole wheat flour and Bambara groundnut after cooking (Oyeyinka et al., 2022). Furthermore, Beleggia et al. (2011) reported that metabolite compositions not only change during the pasta-making process but may also vary with the processing conditions in-

cluding cooking. The palmitic acid levels in the cooked pasta showed variation in the average peak areas. The average peak area seems to decrease with a reduction in particle size. The same trend was observed for pentadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, pyranone, carbonic acid, 2-dimethylaminoethyl isobutyl ester and glycerol 1-palmitate. The differences in the average peak areas observed were likely due to the distribution of these four metabolites in the fibrous portion of the composite flours used in making the pasta. The fibre content (5.61%) for the composite flour sieved through the 500 μm aperture size was significantly higher than those from 112, 300 and 350 μm (average of 3.51%) (Oyeyinka et al., 2021). Since these flours were used in making pasta in this study, the higher levels of these metabolites in the pasta made from 500 μm flour could be attributed to the higher fibre contents in the flour. Prabhasankar et al. (2000) for example, showed that palmitic acid (29.3%) was the highest fatty acid in milled wheat.

Another important metabolite found in one of the pasta fractions (112 μm) is vitamin E, a powerful antioxidant which has been reported to have a regulatory role in inflammation (Lewis et al., 2019), protection against infection (Lewis et al., 2019; Wu & Meydani, 2019) as well as in cancer prevention (Yang et al., 2020). It remains unclear why the vitamin was only found in this fraction. The presence of this vitamin suggests that this pasta fraction may be promoted as a good vehicle to encourage the consumption of vitamin E-rich pasta. Furthermore, the pasta samples made from fractions 112, 300 and 350 μm showed the presence of β-sitosterol acetate, a plant sterol (Table 1) which has also been reported for whole wheat pasta (Beleggia et al., 2016, 2011). β-sitosterol and its derivatives are well known to exhibit anticancer activity against some types of cancers (Zmysłowski et al., 2021).

4. Conclusion

The current study investigated the metabolite signatures and distribution patterns of processed pasta from fractionated whole wheat and Bambara groundnut using GC-HRTOF-MS. A total number of 45 metabolite compounds with eight (8) similar metabolites across the pasta types were identified. Esters were the major metabolites found in the pasta samples. These compounds as well as the minor ketones present are important metabolites that could confer sensory properties to the pasta. The results further show that fractionation before pasta making is a promising step to distributing metabolites within the different pasta fractions. This study further provides information that could be used as biomarkers for the enrichment of pasta with pulses. Further studies on the vitamins, and amino acid profile as well as the digestibility of the pasta samples are still needed.

CRediT authorship contribution statement

Samson A. Oyeyinka: Conceptualization, Methodology, Data Curation, Formal Analysis, Writing – original draft, Sefater Gbashi: Methodology, Data Curation and Formal Analysis, Oluseyi Ajayi: Validation and Reviewing and Editing, Chiemela E. Chinma: Methodology, Data Curation and Formal Analysis, Janet A. Adebo: Conceptualization, Methodology and Formal Analysis, Oluwafemi A. Adebo: Funding Acquisition, Methodology, Validation, Supervision and Reviewing of Manuscript. Patrick Njobeh: Formal Analysis, Funding Acquisition, Validation and Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2023.100357.

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