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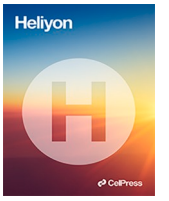

Influence of traditional dehulling on mycotoxin reduction and GC-HRTOF-MS metabolites profile of fermented maize products

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ABSTRACT

Contamination with mycotoxins has been a worldwide food safety concern for several decades, and food processing has been suggested as a potential method to mitigate their presence. In this study, the influence of traditional dehulling (TD) on the mycotoxin reduction and metabolites profile of fermented white maize products obtained via natural and three controlled fermentation methods (involving Lactobacillus fermentum, Lactobacillus plantarum, and their mixed cultures) was examined. Gas chromatography coupled with high resolution time-of-flight mass spectrometry (GC-HRTOF-MS) and ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) were employed. TD brought the levels of fumonisin B1 (FB1) and B2 (FB2) in the white maize below the regulatory limit set by the European Union (EU) for maize consumed by humans. While TD increased the concentration of several mycotoxins in the fermented maize products obtained from other studied fermentation methods, it primarily reduced aflatoxin B1 (AFB1), FB1, deoxynivalenol, and 15-acetyldeoxynivalenol in the L. plantarum-fermented products. By tempering the dehulled maize, a solid-state fermentation process began. This was used in TD to make it easier to remove the pericarp. GC-HR-TOF-MS metabolomics revealed that TD brought about the generation of 12 additional compounds in the dehulled maize though some metabolites in the whole maize were lost/biotransformed. The fermented dehulled maize products obtained from the four studied fermentation procedures contained fewer compounds than the fermented whole maize products. Overall, the analysis

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showed that all fermented maize (whole and dehulled) produced had varied nutritional metabolites and mycotoxin concentrations below the EU maximum level, except for fermented maize obtained from mixed strains (AFB1 + AFB2 > 4.0 g/kg).

1. Introduction

According to Chaves-López et al. [1], one of the most significant cereals in the world is maize (Zea mays). It is a staple food that contains important nutrients like carbohydrates, minerals, and vitamins, as well as considerable amounts of bioactive substances with positive effects on human health. While being a significant source of metabolic energy [1], it is prone to contamination by mycotoxins, particularly AFs and FBs [2], which when combined, increase the risk of liver cancer [3].

Adebiyi et al. [4] indicated that food processing can be a useful approach for the reduction of mycotoxins in raw materials and pinpointed chemical transformation that produces metabolites of reduced or increased toxicity as one of the forms via which the mycotoxins reduction occurs. While [5] had earlier mentioned dehulling and fermentation as components of African traditional processing techniques that have drawn research interest for lowering the level of mycotoxins in food. Dehulling has been proven to be efficient for reducing aflatoxins in maize grains by Siwela et al. [6]. The outer layer of maize kernels is removed by abrasion during the dehulling process because it is the layer most likely to become contaminated by fungus [6]. According to Ref. [7], maize dehulling is a prevalent procedure in Africa and is accomplished either manually (using the mortar-and-pestle method) or mechanically (using dehullers). Traditional dehulling (TD), which involves wetting the pericarp with water to soften it, may cause the grain to ferment, giving it a distinctive flavour [8].

An age-old method of food processing and preservation known as fermentation is used to preserve and enhance the physicochemical and nutritional qualities of food [4]. Through the use of microorganisms, substrate is transformed in this process [9]. The production of fermented maize has been documented in literature using both natural also known as spontaneous, uninoculated, or traditional [10] and controlled fermentation techniques which is regarded as starter culture or inoculated fermentation. According to Chaves-López et al. [1], maize can be changed through natural fermentation to enhance its health advantages. Lactic acid bacteria (LAB), one of the often used groups of microorganisms in the manufacture of fermented foods in various regions of the world, play a role in both of these types of fermentation (natural and controlled), as noted in Ref. [11].

Following the promotion of dehulling for decrease in mycotoxins in corn [12]. The goal of this study was to evaluate the impact of TD on the reduction of mycotoxins and the metabolite profile of fermented maize products obtained via natural and controlled (involving L. fermentum, L. plantarum, and L. fermentum + L. plantarum) fermentation methods. It was known that processing methods/unit operations prior to fermentation, such as dehulling, can result in a reduction in mycotoxins levels [13]. Furthermore, nothing is known about the dynamic changes in metabolite composition during fermentation of whole and dehulled maize, which makes this research necessary.

2. Material and methods

2.1. Chemicals and reagents

The following standards were purchased from Sigma-Aldrich Ltd. (Belgium): AFB1, AFB2, FB1, FB2, DON, 15-acetyldeoxynivalenol (15-ADON), deepoxy-deoxynivalenol (DOM), sterigmatocystin (STERIG), ZEN, and zearalanone (ZAN), while FB3 was obtained from Promec Unit (South Africa). The following chemicals were used: LC-MS grade methanol, analytical-grade acetonitrile, glacial acetic acid, formic acid, ammonium acetate (Merck, Darmstadt, Germany), dichloromethane, ethyl acetate (Acros Organics, Geel, Belgium), and n-hexane (VWR International, Zaventem, Belgium), as well as ultra-pure water from the Arium® pro Ultra (Goettingen, Germany). Grace Discovery Sciences provided GracePure aminopropyl (NH2) solid phase extraction (SPE) 1000 mg/6 mL cartridges (Lokeren, Belgium).

2.2. Preparation of mycotoxin standard solution

AFB1, AFB2, FB1, FB2, FB3, DON, 15-DON, STERIG, ZEN, and ZAN stock solutions were made in methanol at a concentration of 1 mg/mL. In acetonitrile, DOM (50 μg/mL) was obtained as a solution. The standard stock solutions were diluted in MeOH to create the working standard solutions, which were then stored at −18 °C. The working standard solutions were then combined to create standard solution mixtures of AFB1 (2 μg/mL), AFB2 (1 μg/mL), FB1 (5 μg/mL), FB2, FB3, DON (each 10 μg/mL), 15-DON (25 μg/mL), STERIG (1 μg/mL), ZEN (2.5 μg/mL), and ZAN, 2.5 μg/mL.

2.3. Preparation of starter cultures and estimation of viable microbial cell counts

The National Collection of Industrial, Food and Marine Bacteria (NCIMB; Scotland, United Kingdom) and the Belgian Co-ordinated Collection of Micro-organisms embedded in the Laboratory of Microbiology (BCCM/LMG), respectively, provided the lactic acid bacteria strains Lactobacillus fermentum (NCIMB 12116) and Lactobacillus plantarum (LMG 9205) as freeze-dried stock. The isolates were identical to those previously recovered from fermented maize product and were generally recognized as safe (GRAS). The

inoculum were made in accordance with the method outlined by Nyamete & Nyamete [14].

2.4. Processing of whole and dehulled fermented maize

2.4.1. Physical processing of the maize grains

Within a week after shipment, natural mycotoxin-contaminated white maize grains were acquired from a retail outlet in Makueni county, Eastern Kenya. Prior to sampling, the uncooked maize grains were carefully mixed to provide for even dispersion. In the TD of half of the whole maize grains, a slightly modified version of the Matumba et al. [7] technique was used. To soften the grain pericarp, water was added, and a porcelain mortar and pestle was used. By hand winnowing, the dehulled maize was removed from the seed coat and other leftover tissue. Prior to mycotoxin and metabolite analysis of the resultant fermented products, the whole and TD maize samples were fermented.

2.4.2. Natural fermentation

Each batch of maize (150 g) was placed in a Termaks incubator (Bergen, Norway) and incubated at 25 °C for 72 h. After discarding the steeped water, the whole and dehulled maize grains were wet-milled in a blender at Gurgaon, Haryana, India, and then sieved. The slurry was soured for 72 h, then freeze-dried with a Ruckwand VaCo 5 standard freeze drier (Zirbus Technology, Germany) (Addendum E), then stored at −20 °C for additional analyses.

2.4.3. Controlled fermentation

According to the modified method of Teniola & Odunfa [15], L. fermentum and L. plantarum were used separately and together to control the fermentation of whole and dehulled maize samples (2001). Separately, 300 mL of sterile distilled water and 150 g of sterile whole and dehulled maize flour were combined with the prepared substrates. Afterwards, 500 L of a controlled inoculum of L. fermentum, L. plantarum, or a combination of the two, i.e., L. fermentum and L. plantarum, were added to each of these individually (at equal ratio).

2.5. Determination of mycotoxins contents

2.5.1. Sample extraction and clean-up

The entire, dehulled, and fermented maize samples were extracted and cleaned up using the method described by Njumbe Ediage et al. [16]. Internal standards (ZAN, 2.5 g/mL, and DOM, 50 g/mL) were introduced to 3 g of each of the powdered samples at volumes of 60 and 20 L, respectively, and left to equilibrate for 15 min in the dark. Each sample received 20 mL of the extraction solvent, which is composed of methanol, ethyl acetate, and water (70/20/10, v/v/v). An overhead shaker (Agitelec, France) was used to vortex the mixture for 40 min, and it was then centrifuged at 4000 g for 10 min. A fresh tube was used to receive the supernatant. Following the addition of 10 mL of n-hexane to the supernatant, defatting was carried out by centrifugation and shaking. The upper phase of the solution, n-hexane, was discarded while the lower phase underwent solid phase extraction (SPE). The fumonisins' carboxylic acid functional groups had a strong affinity for the resin in the GracePure amino SPE cartridges, thus the defatted extract was split into two sections and put through two distinct cleaning processes. The defatted extract (2.5 mL) was added to 10 mL solution of (5/95, v/v) formic acid/dichloromethane, vortexed and centrifuged at 4000 g for 10 min. The defatted extract was then allowed to run through a 1000 mg amino SPE column (GracePure) that was fixed to a hoover elution manifold and pre-conditioned with 10 mL of the extraction solvent. Glass test tubes were used to collect the SPE's eluate. Together, the cleaned extracts from the amino SPE cleanup and the dichloromethane/formic acid solution were evaporated at 40 °C to dryness under a mild nitrogen flow. The residue was reconstituted in 300 L of mobile phase that was made up of 200 L of n-hexane and mobile phases 1 and 2 combined in an equal ratio of acetic acid/methanol/water (1/5/94, v/v/v) in 5 mM ammonium acetate and acetic acid/methanol/water (1/97/2, v/v/v) in 5 mM ammonium acetate. The reconstituted extract was centrifuged for 10 min at 10,000 g and then filtered before injection into the LC-MS/MS.

2.5.2. UHPLC-MS/MS analysis

With the use of a Waters Acquity UPLC system connected to a Quattro Premier Tandem mass spectrometer, mycotoxins could be identified and quantified (Waters, Milford, MA, USA). The instrument has a C18 column (internal diameter −5 m, 150 mm 2.1 mm) that is preceded by a guard column (2.1 mm 10 mm) with comparable physical characteristics (Waters, Zellik, Belgium). 10 L of injection volume was applied. The flow rate for mobile phases 1 and 2 was set at 0.3 mL/min, and the total run time for each sample was 28 min. Both Masslynx and Quanlynx version 4.1 were used to operate the instrument and process the data (Manchester, UK). The signal/noise ratio was used to determine the limits of detection (LOD) and quantitation (LOQ), which were set at 3.33 and 10 times, respectively.

2.6. Determination of metabolites profile

2.6.1. Sample preparation

Each of the freeze-dried samples weighed 1 g, and 10 mL of the extraction solvent acetonitrile/methanol/chloroform/distilled water (40/40/10/10, v/v/v/v) was added to the mixture. After 1 h of agitation and sonication in an ultrasonic bath (Scientech 704, Labotech, South Africa), centrifugation at 3500 rpm for 5 min at 4 °C was performed (Eppendorf 5702R, Merck South Africa). A

concentrator was used to concentrate the supernatant at 40 °C for 6 h after being moved into a fresh centrifuge tube. After being reconstituted with chromatographic-grade methanol, the dry extract was filtered into amber vials for examination.

2.6.2. GC-HRTOF-MS analysis

Using an Agilent 7890A gas chromatograph (Agilent Technologies, Inc, Wilmington, DE, USA) connected to a high-resolution time of flight-mass spectrometer (LECO Corporation, USA), the metabolites profile of the whole, dehulled, and fermented maize samples was assessed (GC-HRTOF-MS). To assure mass accuracy, the instrument underwent mass calibration. The utilized GC-HRTOF-MS was outfitted with a Rxi 5 ms (0.25 m 0.25 mm ID 30 m; Restek, USA) column and a Gerstel MPS multifunctional autosampler (Gerstel Inc Germany). As the carrier gas, helium gas was pumped in splitless mode at a constant flow rate of 1 mL/min. The instrument's oven temperature was originally set at 70 °C for 30 s, ramped up to 150 °C for 180 s, and then held at 330 °C for 270 s. The mass spectrometer's 13 spectra/s, 30–1000, 70 eV, and 250 °C settings for the data acquisition rate, *m/z* range, electron ionisation, and ion source temperature, respectively. 1.25 kHz was the recommended extraction frequency. For a total of nine analytical injections for each sample, sample extract from each duplicate sample was analyzed and injected three times (sample injection volume: 1 mL) in order to increase the accuracy of the discovered metabolites.

2.6.3. Data processing and statistical analysis

Using LECO ChromaTOF-HRT software, a data set from the GC-HRTOF-MS analysis of the sample extracts was converted to mzML (Markup language) format. The XCMS open-source tool was used to process this by selecting the peaks and aligning them. Less than a 70 % similarity match was used to exclude compounds retrieved from sample data. The metabolites profile of the examined samples was compared using pie charts and Venn diagrams (http://bioinformatics.psb.ugent.be/webtools/Venn/), with appropriate independent-sample t-tests (p 0.05) run using SPSS® (version 26, IBM Statistics for Windows, New York, NY, USA).

3. Results and discussion

3.1. Effect of traditional dehulling on mycotoxins reduction in natural and controlled fermented maize

Mycotoxins analysis of the raw maize sample showed the presence of nine toxins (AFB1, AFB2, FB1, FB2, FB3, DON, 15-ADON, STERIG, and ZEN). The initial mycotoxin concentration of the raw maize sample (published elsewhere [10]) (Supplementary Table 1) showed a higher level of FB_1 and FB_2 in the raw maize above the regulatory limit. In natural and controlled fermentations using *L. fermentum, L. plantarum*, and mixed strains of the two (i.e., *L. fermentum* + *L. plantarum*), results of the influence of TD in maize fermented products are shown in Table 1. In general, it was shown that fermentation either resulted in a decrease in the parent mycotoxins' concentration in the maize (whole and dehulled) samples or in their biotransformation into breakdown products. According to Adebiyi et al. [4], physical absorption has been shown to be the mechanism by which LAB strains detoxify mycotoxins. These authors also noted that the bacteria type/state, substrate type, initial mycotoxin level, bacteria count, pH of the growth substrate, and incubation time all affect the LAB's ability to bind mycotoxins. Interestingly, natural fermentation increased the dehulled maize samples' FB1, FB2, and FB3 concentrations. The ineffective binding relationship of the LAB can be connected to the process by which these toxins were raised during fermentation. According to Adebiyi et al. [4], attachment of fumonisins to the components of the LAB cell wall is the primary process involved in fumonisin elimination. The decreased binding interaction and efficiency may be the cause of the increased fumonisin concentration found in the dehulled maize samples by these LAB. Although L. *fermentum* and mixed cultures (*L. fermentum* + *L. plantarum*) fermentation of conventionally dehulled maize resulted in a rise in AFB1 and AFB2 content. As indicated by Adebiyi et al. [4], rise in AFs after fermentation may be ascribed to the reformation and closing of their lactone ring during the fermentation process.

Table 1
Effect of traditional dehulling on mycotoxins reduction in the fermented maize products.

Mycotoxins	Natural fermentation		Controlled fermentation					
			L. fermentum		L. plantarum		*L. fermentum* + *L. plantarum*	
	WM	DM	WM	DM	WM	DM	WM	DM
AFB_1	< LOD	< LOD	< LOD	1.86 ± 0.13	1.73 ± 0.26	< LOD	< LOD	8.93 ± 0.80
AFB_2	< LOD	< LOD	< LOD	0.46 ± 0.03	< LOD	< LOD	< LOD	4.00 ± 0.50
FB_1	0.56^{a} ± 0.28	170^{b} ± 17.9	19.2^{a} ± 4.03	39.6^{b} ± 6.48	13.1 ± 1.65	< LOD	85.0^{b} ± 2.87	40.9^{a} ± 9.25
FB_2	< LOD	36.23 ± 1.23	< LOD	7.36 ± 1.03	< LOD	< LOD	< LOD	7.70 ± 0.20
FB_3	< LOD	7.93 ± 0.93	< LOD	3.60 ± 0.60	< LOD	< LOD	< LOD	< LOD
DON	< LOD	< LOD	< LOD	1.90 ± 0.09	1.17 ± 0.17	< LOD	< LOD	2.37 ± 0.37
15-ADON	< LOD	< LOD	< LOD	< LOD	3.17 ± 0.17	< LOD	< LOD	< LOD
STERIG	< LOD	< LOD	< LOD	1.63 ± 0.13	< LOD	< LOD	< LOD	< LOD
ZEN	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD

Values = means ± standard error. Means showing same letters are not significantly different ($p < 0.05$). AFB_1: Aflatoxin B_1; AFB_2: Aflatoxin B_2; FB_1: fumonisin B_1; FB_2: fumonisin B_2; FB_3: fumonisin B_3; DON: deoxynivalenol; 15-ADON: 15- acetyldeoxynivalenol; STERIG: Sterigmatocystin and ZEN: zearalenone; LOD: limit of detection; WM: whole maize; DW: dehulled maize; *L. fermentum*: *Lactobacillus fermentum; L. plantarum*: *Lactobacillus plantarum*; *L. fermentum* + *L. plantarum*: Mixed culture of *Lactobacillus fermentum and Lactobacillus plantarum* strains.

3.2. Effect of traditional dehulling on maize metabolites profile

Due to their reputation as excellent providers of dietary fibre, antioxidants, and bioactive substances, whole grains have been accepted for inclusion in healthy diets. According to Shahidi & Ambigaipalan [17], the endosperm of cereal grains has a lower concentration of total phenolics than the outer layers made up of the husk, pericarp, testa, and aleurone cells. According to certain other researchers, phenolic acids are primarily found in the endosperm and aleurone layer [17]. Overall, the findings of Odukoya et al. [18] who discovered that sorghum had higher levels of micronutrients than maize are supported by the low number of metabolites (19) observed in the raw whole maize (Supplementary Fig. 1) in their study. In their research, Kewuyemi et al. [19] also noted a higher number of metabolites in sorghum. The raw whole maize samples used in this investigation may have had a reduced influence on the maize composition due to the presence of some mycotoxins. The TD method used in the current study, which involves tempering the grain to make the pericarp easier to remove [20], resulted in the loss or biotransformation of six compounds (Fig. 1[A,B]; Supplementary Table 2), the retention of 13 compounds found in the raw whole grain of maize (Fig. 1A,B; Supplementary Table 3), and the production of 12 new compounds (Fig. 1A,B; Supplementary Table 4) that were not present in the original whole grain of maize.

The six substances that were either lost or biotransformed as a result of TD in the maize sample were 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (a ketone), carbonic acid, 2-dimethylaminoethyl Two phenols (2-methoxy-4-vinylphenol and phenol, 2,6-dimethoxy), two phytosterols (stigmasterol and stigmasta-5,24(28)-dien-3-ol, (3β,24Z)), and 2-methoxyethyl ester (an ester) are also present (Supplementary Fig. 1 and Supplementary Table 2). Whereas 2,6-dimethoxyphenol possesses potent antioxidant and antibacterial activities, 2-methoxy-4-vinylphenol has a wide variety of biological (antioxidant, antimicrobial, anti-inflammatory, and analgesic) effects [21]. This shows that TD, which involved pericarp removal, resulted in the loss of some metabolites or compounds with favourable biological activity that were present in the raw whole maize. The findings of this study concur with those of Dapevi-Hadnaev et al. [22], who found that the components of cereals with the highest concentration of phytochemicals include the pericarp (hull and bran) and cereal germ, which may be lost during grain processing. Even though both raw whole and dehulled maize samples contained 13 metabolites that were similar to one another, TD significantly increased (p 0.05) the concentration of certain compounds in the dehulled maize sample, including 2,4-di-*tert*-butylphenol, 2,4-dimethoxyacetophenone, hexadecanoic acid, methyl ester, and 9,12-octadecadienoic acid, methyl (Supplementary Table 3). This illustrates how tempering affected the dehulled maize sample and led to a type of solid-state fermentation. Only 2,4-di-*tert*-butylphenol, one of the three phenolic compounds present in the raw whole maize, was discovered in the dehulled maize sample, in agreement with Shahidi & Ambigaipalan's [17] findings that the majority of the total phenolics in cereal grains are present in the outer layer while the endosperm has a lower concentration (Supplementary Tables 2 and 3). Natural substance 2,4-Di-*tert*-butylphenol has been shown to have some biological properties, such as anticancer and antifungal actions [23]. The level of dl-a-tocopherol, a food additive and synthetic phenolic antioxidant, was not significantly different between the whole and dehulled maize samples ($p = 0.817$) but did reveal the compound's dominating character (Supplementary Table 3). In the meantime, the discovery of stigmasterol, stigmasta-5,24(28)-dien-3-ol, (3β,24Z), and campesterol in raw whole and dehulled maize samples (Supplementary Tables 2 and 3) supports the assertion made by authors that maize and its products are significant sources of phytosterols. According to research by Dapevi-Hadnaev et al. [22], these maize phytosterols are an important component of the family of chemicals that contribute to cereals' health advantages and help reduce blood cholesterol levels in people [24]. According to Hossain & Jayadeep [24], campesterol is the major free phytosterol present in maize with similar

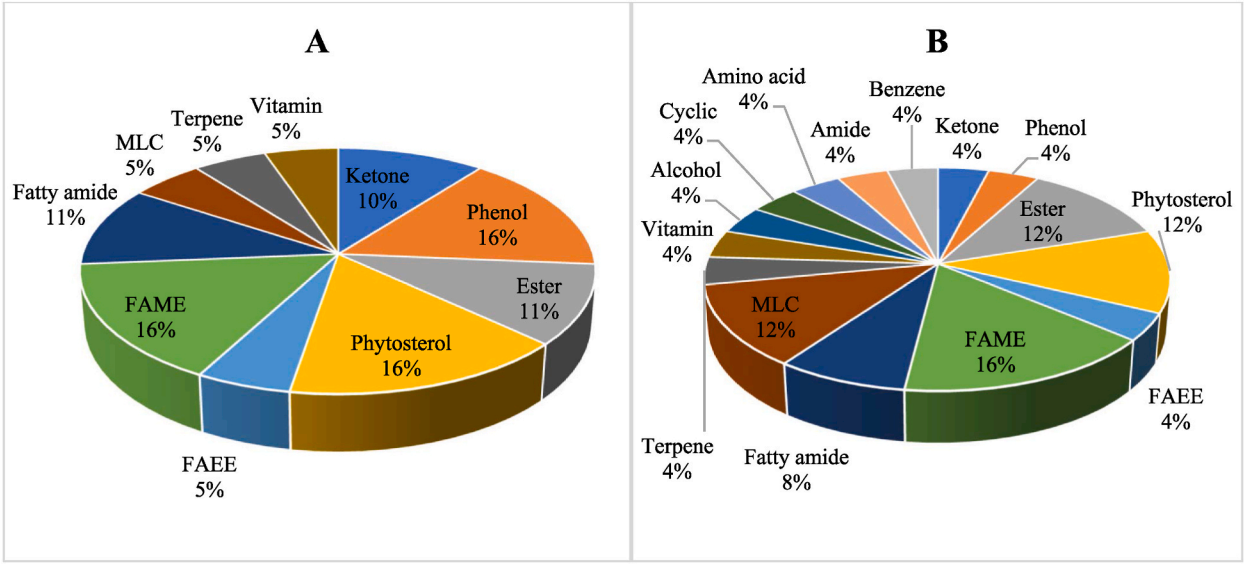

Fig. 1. Percentage of the different metabolites in [A] whole maize, and [B] dehulled maize. FAME: Fatty acid methyl ester; FAEE: Fatty acid ethyl ester; MLC = Miscellaneous compounds.

structural characteristics to cholesterol. It can be identified in both raw, whole, and dehulled maize samples. Nevertheless, it, or campesterol, hinders dietary cholesterol absorption in a number of ways [24].

The fermentation process brought on by TD using water can be blamed for the discovery of 12 new metabolites (grouped into alcohol, esters, fatty acid methyl ester (FAME), amide, benzene, cyclic, amino acid, phytosterols, and other compounds) in traditional dehulled maize but not in raw whole maize (Supplementary Fig. 1; Supplementary Table 4) [8]. This explains the higher number of compounds classes identified in the dehulled maize sample (Fig. 1B) and supports the assertion made by Manfe et al. [25] that solid state fermentation produces higher product yields. Ergosterol, a provitamin D, was found in the dehulled maize sample, which indicated fungal contamination of the maize germ that was exposed because of the dehulling process [26]. Ergosterol, a phytosterol that only occurs in fungi, is typically employed as a biological marker to track how quickly oil is degrading due to fungal growth [26]. This substance has typically been utilized to identify fungal invasion in grain [26], confirming that the study's use of maize involved fungi attack that resulted in mycotoxins contamination.

3.3. Impact of traditional dehulling on the metabolites profile of fermented maize products obtained via natural and controlled fermentation methods

Several types of compounds were found in the fermented whole and dehulled maize products, supporting the findings of Adebo et al. [27], who claimed that fermented meals have a varied composition. Controlled fermentation cannot match the strength of natural fermentation with consortia of microorganisms (such as various species of bacteria or a combination of bacteria, fungus, or yeast) [28]. These bioactive substances have been shown to prevent mutation, cancer, diabetes, obesity, and hypertension among other disorders [29]. According to the results of the fermentation of maize and dehulled samples of maize, TD (including pericarp removal) resulted in a lower number of metabolites (Fig. 2A-D), whereas controlled fermentation using L. plantarum produced the largest total number of compounds.

It is indeed interesting to note that utilizing a single strain of L. fermentum or L. plantarum allowed for the detection of more important metabolites in both fermented whole and dehulled maize products than using a mixed culture of these two LAB strains (18). (Fig. 2). According to the One Strain Many Compounds (OSMC) phenomenon, where a single strain produces a large number of secondary metabolites, this is the case [30]. As some of the synthesised metabolites are frequently used to generate chemical signals for competing communities, the lowest number of metabolites (37) obtained through natural fermentation (Fig. 2) can be attributed to the

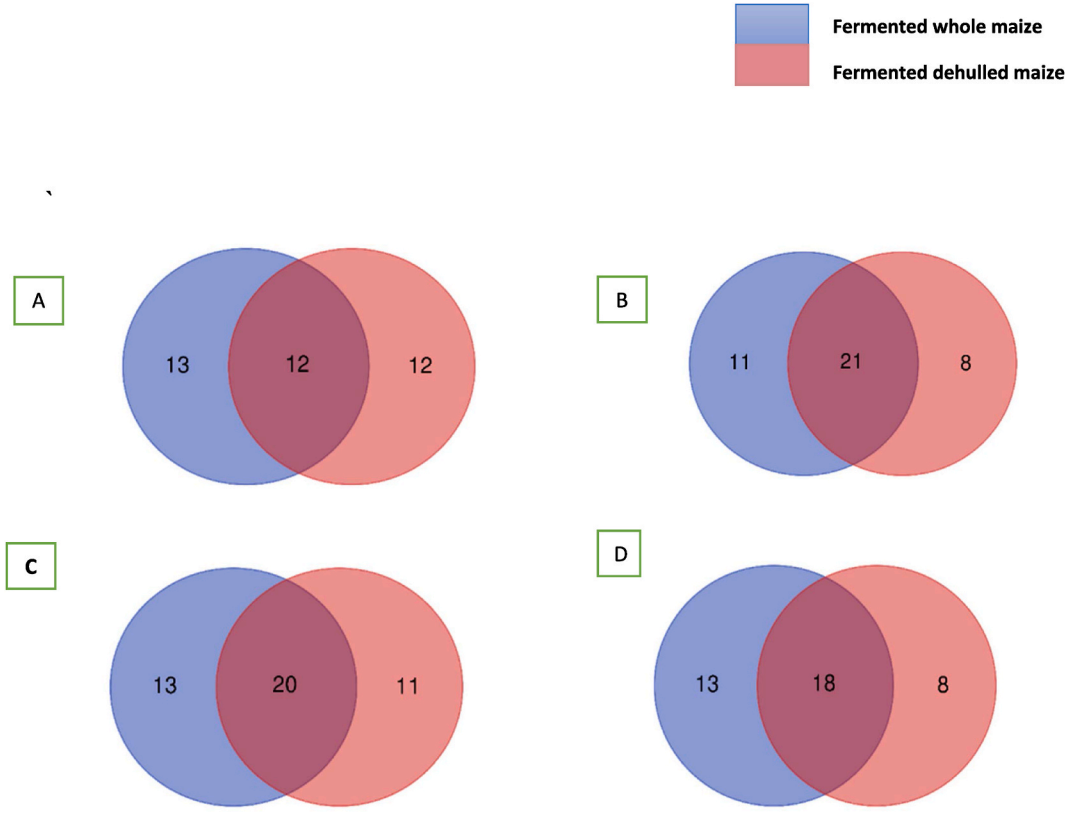

Fig. 2. Distribution of metabolites in the fermented whole and dehulled maize product obtained via [A] – Natural fermentation, [B] Fermentation with L. *fermentum* strain, [C] Fermentation with L. *plantarum* strain, and [D] Fermentation with mixed cultures of *L. fermentum* + *L. plantarum* strains.

action of concurrent microorganisms in the natural fermenting medium, which decreases growth, protein, and secondary metabolites production (Nai & Meyer, 2018). Information about further metabolites in the fermented whole maize (Table 2), noteworthy compounds in the fermented whole and dehulled maize (Table 3), and additional metabolites in the fermented traditionally dehulled maize (Table 4) is also provided.

Fig. 2 demonstrated that 13 metabolites (from natural fermentation), 11 (from fermentation involving L. fermentum strain), 13 (from fermentation involving L. plantarum strain), and 13 (from fermentation involving mixed cultures of L. fermentum and L. plantarum strains) were not present in the corresponding fermented dehulled maize. The elimination or biotransformation of these metabolites present in the fermented maize products was therefore facilitated by TD. A-tocopherol, d-tocopherol, dl-a-tocopherol, stigmasterol, and -sitosterol are a few of these metabolites (Table 2) found in naturally fermented whole maize, mixed cultured fermented whole maize, and L. fermentum strain fermented whole maize. In general, tocopherols fight against free radical reactions that might result in gene alterations and slow the growth of precancerous lesions and tumours [17]. The single fatty acid identified in the

Table 2
Distinct metabolites in the fermented whole maize products.

R_t (mins)	*m/z*	MF	Metabolites	MC	Natural ($\overline{X}$ PA)	Controlled ($\overline{X}$ PA)		
						L. fermentum	L. plantarum	*L. fermentum* + L. plantarum
4.25	710.729	C_4H_9N	Prolamine/Pyrrolidine	Amine	n.d.	697207	n.d.	n.d.
4.96	240.0912	$C_{31}H_{57}N$	Cholestan-3-amine, N,N,4,4-tetramethyl-, (3β,5a)-	MLC	n.d.	n.d.	2209180	n.d.
5.28	120.0569	C_8H_8O	Bicyclo[4.2.0]octa-1,3,5-trien-7-ol	Benzene	n.d.	n.d.	434458	n.d.
5.61	85.0523	F_2H_2NP	Phosphoramidous difluoride	Amine	n.d.	n.d.	2078797	n.d.
6.16	144.0417	$C_6H_8O_4$	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Ketone	4322500	n.d.	2697828	n.d.
6.87	268.9973	$C_{14}H_{24}N_2O_2$	252A Spiropyrrolizidine	MLC	n.d.	n.d.	n.d.	242113
7.29	120.057	$C_6H_5BO_2$	Catecholborane	MLC	n.d.	n.d.	n.d.	1434540
7.84	224.1151	$C_{16}H_{34}$	Hexadecane	HC	n.d.	n.d.	141061	n.d.
8.04	143.0401	C_6H_9NOS	5-Thiazoleethanol, 4-methyl-	Alcohol	n.d.	162072	n.d.	n.d.
8.09	158.0393	$C_3H_4Cl_2F_2O$	Methoxyflurane	MLC	n.d.	n.d.	367402	n.d.
11.23	505.1056	$C_{18}H_{52}O_7Si_7$	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy)tetrasiloxane	MLC	583552	668777	n.d.	n.d.
11.50	220.2651	C_3F_9P	Phosphine, tris(trifluoromethyl)-	MLC	16735	n.d.	n.d.	n.d.
12.31	125.0835	$C_7H_{11}NO$	Hexahydropyrrolizin-3-one	Ketone	n.d.	n.d.	n.d.	2745689
12.53	180.078	$C_{11}H_{16}O_2$	3-*tert*-Butyl-4-hydroxyanisole	Phenol	n.d.	n.d.	n.d.	1595296
12.54	180.0779	$C_{10}H_{12}O_3$	2′,4′-Dimethoxyacetophenone	Ketone	n.d.	n.d.	2630473	n.d.
13.93	417.033	$C_{16}H_{48}O_8Si_8$	Cyclooctasiloxane, hexadecamethyl-	MLC	n.d.	n.d.	1102193	n.d.
14.55	224.0406	$C_{19}H_{40}$	Nonadecane	HC	291215	n.d.	211675	323521
14.56	194.0936	$C_{11}H_{14}O_3$	(E)-2,6-Dimethoxy-4-(prop-1-en-1-yl) phenol	Phenol	n.d.	70348	n.d.	58758
14.75	219.1743	$C_{14}H_{28}O_2$	Tridecanoic acid, methyl ester	FAME	997049	389765	n.d.	n.d.
14.92	207.1375	$C_{10}H_{14}N_2O_3$	3-Methyl-1,4-diazabicyclo[4.3.0]nonan-2,5-dione, N-acetyl-	MLC	n.d.	277305	3450568	n.d.
17.75	256.2402	$C_{16}H_{32}O_2$	Palmitic acid	Fatty acid	23072665	24450475	n.d.	n.d.
17.77	210.2369	$C_{11}H_{18}N_2O_2$	Pyrrolo [1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	MLC	672174	n.d.	n.d.	n.d.
17.86	200.0637	$C_{12}H_{25}NO$	Dodecanamide	Amide	2426598	n.d.	1079395	3753520
17.88	157.178	$C_9H_{19}NO$	Nonanamide	Amide	n.d.	n.d.	506406	n.d.
18.90	265.2155	$C_{17}H_{30}O_2$	9,12-Hexadecadienoic acid, methyl ester	FAME	6422996	n.d.	n.d.	n.d.
20.47	169.1927	$C_{12}H_{25}NO$	Decanamide	Amide	1446071	1492510	n.d.	n.d.
20.49	226.2165	$C_{16}H_{33}NO$	Hexadecanamide	Amide	557610	n.d.	n.d.	276641
20.63	239.2375	$C_{16}H_{23}NO_4$	Succinic acid, 3,4-dimethylphenyl 2-(dimethylamino)ethyl ester	Ester	n.d.	n.d.	1204493	n.d.
22.03	191.0552	$C_9H_{19}NO_3$	Carbonic acid, 2 dimethyl aminoethyl isobutyl ester	Ester	n.d.	n.d.	n.d.	706188
22.11	144.1021	$C_8H_{20}N_2O$	Bis(2-(Dimethylamino)ethyl) ether	MLC	703457	n.d.	n.d.	n.d.
24.64	160.0973	$C_7H_{15}NO_3$	Carbonic acid, 2-dimethyl aminoethyl ethyl ester	Ester	n.d.	n.d.	5217851	2684214
25.79	402.3495	$C_{27}H_{46}O_2$	d-Tocopherol	Vitamin	n.d.	n.d.	n.d.	54109
27.06	430.3797	$C_{29}H_{50}O_2$	dl-a-Tocopherol	Vitamin	n.d.	n.d.	n.d.	1182756
27.07	430.3805	$C_{29}H_{50}O_2$	Vitamin E/a-Tocopherol	Vitamin	711834	n.d.	n.d.	n.d.
28.03	412.3695	$C_{29}H_{48}O$	Stigmasterol	Phytosterol	n.d.	n.d.	n.d.	241008
28.40	414.3852	$C_{29}H_{50}O$	β-Sitosterol	Phytosterol	n.d.	1441495	n.d.	n.d.

R_t: retention time (min); *m/z*:mass/charge ratio; MC: metabolite class; $\overline{X}$ PA: average peak area; FAME: fatty acid methyl ester; HC: hydrocarbon; FAME: fatty acid methyl ester; MLC: miscellaneous compounds; *L. fermentum*: *Lactobacillus fermentum; L. plantarum*: *Lactobacillus plantarum*; *L. fermentum* + *L. plantarum*: mixed culture of *Lactobacillus fermentum and Lactobacillus plantarum* strains; n.d.: not detected.

Table 3
Significant metabolites in the fermented whole and dehulled maize products.

R_t (mins)	*m/z*	Molecular formula	Metabolites	MC	*p*-value	FC
Natural						
7.84	224.1151	$C_{16}H_{34}$	Hexadecane	HC	0.246	1.78
8.45	150.0675	$C_9H_{10}O_2$	2-Methoxy-4-vinylphenol	Phenol	0.0001	0.50
8.93	154.0624	$C_8H_{10}O_3$	Phenol, 2,6-dimethoxy-	Phenol	0.497	0.65
11.64	206.1664	$C_{14}H_{22}O$	2,4-Di-*tert*-butylphenol	Phenol	0.157	0.62
12.54	180.0779	$C_{10}H_{12}O_3$	2′,4′-Dimethoxyacetophenone	Ketone	0.025	0.41
17.14	270.2551	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	FAME	0.012	0.55
19.76	224.2028	$C_{14}H_{29}NO$	Tetradecanamide	Amide	0.898	0.96
21.40	281.2713	$C_{18}H_{35}NO$	9-Octadecenamide, (Z)-	Amide	0.338	0.83
24.89	410.3888	$C_{30}H_{50}$	Squalene	Terpene	0.246	0.60
27.82	400.3696	$C_{28}H_{48}O$	Campesterol	Phytosterol	0.666	0.71
28.40	414.3852	$C_{29}H_{50}O$	β-Sitosterol	Phytosterol	0.436	0.67
28.52	412.3685	$C_{29}H_{48}O$	Stigmasta-5,24(28)-dien-3-ol, (3β,24Z)-	Phytosterol	0.435	0.72
L. fermentum						
8.45	150.0675	$C_9H_{10}O_2$	2-Methoxy-4-vinylphenol	Phenol	0.065	0.51
8.93	154.0624	$C_8H_{10}O_3$	Phenol, 2,6-dimethoxy-	Phenol	0.966	0.98
9.08	256.0162	$C_{17}H_{30}O_3$	Tetrahydropyran Z-10-dodecenoate	Ester	0.893	1.11
9.47	164.0612	$C_{26}H_{25}ClF_3N_3O_2$	7-Chloro-1,3,4,10-tetrahydro-10-hydroxy-1-[[2-[1-pyrrolidinyl] ethyl]imino]-3-[3-(trifluoromethyl)phenyl]-9(2H)-acridinone	MLC	0.365	1.18
11.64	206.1664	$C_{14}H_{22}O$	2,4-Di-*tert*-butylphenol	Phenol	0.172	0.71
12.54	180.0779	$C_{10}H_{12}O_3$	2′,4′-Dimethoxyacetophenone	Ketone	0.002	0.50
13.93	417.033	$C_{16}H_{48}O_8Si_8$	Cyclooctasiloxane, hexadecamethyl-	MLC	0.003	0.40
14.55	224.0406	$C_{19}H_{40}$	Nonadecane	HC	0.660	0.95
17.14	270.2551	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	FAME	0.033	0.64
17.86	200.0637	$C_{12}H_{25}NO$	Dodecanamide	Amide	0.639	0.74
18.89	294.2553	$C_{19}H_{34}O_2$	9,12-Octadecadienoic acid, methyl ester	FAME	0.361	0.81
18.94	296.2707	$C_{19}H_{36}O_2$	*Trans*-13-Octadecenoic acid, methyl ester	FAME	0.801	0.93
19.76	224.2028	$C_{14}H_{29}NO$	Tetradecanamide	Amide	0.326	1.89
21.40	281.2713	$C_{18}H_{35}NO$	9-Octadecenamide, (Z)-	Amide	0.412	1.10
22.03	191.0552	$C_9H_{19}NO_3$	Carbonic acid, 2-dimethylaminoethyl isobutyl ester	Ester	0.237	0.73
22.11	144.1021	$C_8H_{20}N_2O$	Bis(2-(Dimethylamino)ethyl) ether	MLC	0.251	1.55
24.89	410.3888	$C_{30}H_{50}$	Squalene	Terpene	0.269	0.79
27.06	430.3797	$C_{29}H_{50}O_2$	dl-a-Tocopherol	Vitamin	0.001	0.51
27.82	400.3696	$C_{28}H_{48}O$	Campesterol	Phytosterol	0.056	0.83
28.03	412.3695	$C_{29}H_{48}O$	Stigmasterol	Phytosterol	0.148	0.71
28.52	412.3685	$C_{29}H_{48}O$	Stigmasta-5,24(28)-dien-3-ol, (3β,24Z)-	Phytosterol	0.776	0.94
L. plantarum						
4.07	219.0566	$C_{10}H_{17}N_3O_2$	1-(2-Dimethylamino-ethyl)-3,6-dimethyl-1H-pyrimidine-2,4-dione	Cyclic	0.040	0.54
4.25	710.729	C_4H_9N	Prolamine/Pyrrolidine	Amine	0.835	0.94
4.39	850.887	$C_5H_{11}N$	Piperidine	Amine	0.212	0.83
8.45	150.0675	$C_9H_{10}O_2$	2-Methoxy-4-vinylphenol	Phenol	0.040	0.39
9.08	256.0162	$C_{17}H_{30}O_3$	Tetrahydropyran Z-10-dodecenoate	Ester	0.482	1.88
11.64	206.1664	$C_{14}H_{22}O$	2,4-Di-*tert*-butylphenol	Phenol	0.047	0.52
17.14	270.2551	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	FAME	0.002	0.50
17.75	256.2402	$C_{16}H_{32}O_2$	Palmitic acid	Fatty acid	1×10^{-4}	0.15
18.89	294.2553	$C_{19}H_{34}O_2$	9,12-Octadecadienoic acid, methyl ester	FAME	0.02	0.73
18.94	296.2707	$C_{19}H_{36}O_2$	*Trans*-13-Octadecenoic acid, methyl ester	FAEE	0.005	0.51
19.65	225.2051	$C_{11}H_{16}FNO_3$	Benzeneethanamine, 2-fluoro-ß,3,4-trihydroxy-N-isopropyl-	Benzene	1×10^{-4}	0.16
19.76	224.2028	$C_{14}H_{29}NO$	Tetradecanamide	Amide	0.710	0.82
21.40	281.2713	$C_{18}H_{35}NO$	9-Octadecenamide, (Z)-	Amide	0.049	0.74
22.11	144.1021	$C_8H_{20}N_2O$	Bis(2-(Dimethylamino)ethyl) ether	MLC	0.028	0.29
24.89	410.3888	$C_{30}H_{50}$	Squalene	Terpene	0.131	0.63
27.06	430.3797	$C_{29}H_{50}O_2$	dl-a-Tocopherol	Vitamin	0.001	0.52
27.82	400.3696	$C_{28}H_{48}O$	Campesterol	Phytosterol	0.004	0.69
28.03	412.3695	$C_{29}H_{48}O$	Stigmasterol	Phytosterol	0.036	0.75
28.40	414.3852	$C_{29}H_{50}O$	β-Sitosterol	Phytosterol	1×10^{-4}	0.12
28.52	412.3685	$C_{29}H_{48}O$	Stigmasta-5,24(28)-dien-3-ol, (3β,24Z)-	Phytosterol	0.005	0.64
L. fermentum + L. plantarum						
4.25	710.729	C_4H_9N	Prolamine/Pyrrolidine	Amine	0.546	1.21
8.08	152.1194	$C_{10}H_{16}O$	2,4-Decadienal, (E,E)-	Aldehydes	0.424	1.68
8.45	150.0675	$C_9H_{10}O_2$	2-Methoxy-4-vinylphenol	Phenol	0.659	0.80
8.93	154.0624	$C_8H_{10}O_3$	Phenol, 2,6-dimethoxy-	Phenol	0.557	0.81
9.08	256.0162	$C_{17}H_{30}O_3$	Tetrahydropyran Z-10-dodecenoate	Ester	0.005	0.40
9.47	164.0612	$C_{26}H_{25}ClF_3N_3O_2$	7-Chloro-1,3,4,10-tetrahydro-10-hydroxy-1-[[2-[1-pyrrolidinyl] ethyl]imino]-3-[3-(trifluoromethyl)phenyl]-9(2H)-acridinone	MLC	0.006	0.65
11.64	206.1664	$C_{14}H_{22}O$	2,4-Di-*tert*-butylphenol	Phenol	0.242	0.75
17.14	270.2551	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	FAME	0.002	0.56
17.75	256.2402	$C_{16}H_{32}O_2$	Palmitic acid	Fatty acid	0.246	0.60

(*continued on next page*)

Table 3 (*continued*)

R_t (mins)	*m/z*	Molecular formula	Metabolites	MC	*p*-value	FC
18.89	294.2553	$C_{19}H_{34}O_2$	9,12-Octadecadienoic acid, methyl ester	FAME	0.0001	0.59
18.94	296.2707	$C_{19}H_{36}O_2$	*Trans*-13-Octadecenoic acid, methyl ester	FAME	0.001	0.73
19.76	224.2028	$C_{14}H_{29}NO$	Tetradecanamide	Amide	0.709	0.81
21.40	281.2713	$C_{18}H_{35}NO$	9-Octadecenamide, (Z)-	Amide	0.026	0.63
22.11	144.1021	$C_8H_{20}N_2O$	Bis(2-(Dimethylamino)ethyl) ether	MLC	0.275	0.60
24.89	410.3888	$C_{30}H_{50}$	Squalene	Terpene	0.693	0.94
27.82	400.3696	$C_{28}H_{48}O$	Campesterol	Phytosterol	0.001	0.73
28.40	414.3852	$C_{29}H_{50}O$	β-Sitosterol	Phytosterol	0.020	0.77
28.52	412.3685	$C_{29}H_{48}O$	Stigmasta-5,24(28)-dien-3-ol, (3β,24Z)-	Phytosterol	0.018	0.73

R_t: retention time (min); *m/z*: mass/charge ratio; MC: metabolite class; FC: fold change (value of the average peak area of dehulled maize/raw whole maize); HC: hydrocarbon; FAME: fatty acid methyl ester; MLC: miscellaneous compounds; *L. fermentum*: *Lactobacillus fermentum; L. plantarum*: *Lactobacillus plantarum*; *L. fermentum* + *L. plantarum*: mixed culture of *Lactobacillus fermentum and Lactobacillus plantarum* strains.

study, palmitic acid, was found in the fermented whole maize derived by natural and L. fermentum fermentation methods (Table 2).

Additionally, Fig. 2 demonstrated that some metabolites, notably those from the L. fermentum strain, can be shared by fermented whole and dehulled maize. The content of certain metabolites in the fermented whole maize derived from the four studied fermentation procedures was considerably reduced ($p < 0.05$) by TD, as shown in Table 3. Hexadecanoic acid, methyl ester (from the four fermentation methods), *trans*-13-octadecenoic acid, methyl ester, and 9,12-octadecadienoic acid, methyl ester (from fermentation involving L. plantarum and mixed culture of L. fermentum and L. plantarum), as well as three phytosterols (campesterol, -sitosterol, stigmasta-5,24(28)- (from fermentation involving L. plantarum and mixed culture of L. fermentum and L. plantarum). Additional significant metabolites that underwent TD reduction in the fermented maize produced by L. plantarum fermentation were stigmasterol, palmitic acid, and two phenols (2-methoxy-4-vinylphenol and 2,4-di-*tert*-butylphenol). In the meantime, stigmasterol, β-sitosterol, and campesterol were described by Schaller [31] as functional precursors of growth hormones. According to Lozano-Grande et al. [32], they are biosynthesized in plant cells via the mevalonate pathway.

Moreover, the study revealed that raw, whole, and dehulled maize contained squalene, a natural lipid that is a precursor to the manufacture of cholesterol (Bates, 2015) [33]. This is in line with Lozano-Grande et al. [32]. observation that maize oil contains a negligible amount of squalene, a natural compound that is highly relevant to human health. It has been found to have a variety of bioactive qualities, including antioxidant, antibacterial, antifungal, antitumor, and anticancer activity. It is a carbon source in the anaerobic fermentation of microorganisms [32]. As shown in Table 3, its concentration in the fermented dehulled maize products from the four fermentation processes did not differ substantially ($p > 0.05$) from that in the fermented whole maize. As seen in Fig. 2, TD enabled the discovery of 12 compounds (from natural fermentation), 8 compounds (from fermentation involving L. fermentum strain), 11 compounds (from fermentation involving L. plantarum strain), and 8 compounds (from fermentation involving mixed cultures of L. fermentum and L. plantarum strains) that were absent from the fermented whole maize obtained using the four different fermentation techniques. These substances include dl-a-tocopherol, a synthetic phenolic antioxidant, which was only detected in the fermented whole maize products from the controlled fermentation using a blend of L. fermentum and L. plantarum strains (Table 2), the fermented whole and dehulled maize products derived from L. fermentum and L. plantarum (Table 3), as well as the naturally fermented dehulled maize product (Table 3). (Table 4). According to Annan et al. [34], the distinctive aroma of nsiho (a dehulled fermented maize) can be partially explained by the discovery of benzeneacetaldehyde, an aldehyde, exclusively in the naturally fermented dehulled maize. This aldehyde was produced with the help of the natural fermentation of dehulled maize after the pericarp was removed and a consortium of microorganisms. Aldehydes are generally recognized as flavouring agents in food [19,35], but Welke et al. [36] found benzeneacetaldehyde to be a significant volatile metabolite of wine with a rose and floral scent. Kewuyemi et al. [19] speculate that Strecker degradation, which converts amino acids into aldehydes, may be the cause of the creation of this molecule, benzeneacetaldehyde.

According to Shahidi & Ambigaipalan [17], the discovery of 3-*tert*-butyl-4-hydroxyanisole (BHA) in fermented whole maize from the use of a mixed culture of L. plantarum + L fermentum (Table 2) and fermented dehulled maize from fermentation with L. plantarum (Table 4) suggests that this artificial phenolic antioxidant was purposefully added to the raw maize used for this study during storage to increase its shelf life. According to the literature, BHA is added to food to stop lipid oxidation and to preserve freshness, flavour, and nutritional value [17]. Remarkably, despite the bulk maize being properly mixed before sampling, BHA was not found in the studied raw whole maize sample (see Supplementary Tables 2 and 3). According to Nesci et al. [37], the challenge of irregular distribution linked with the use of fungistatic preservatives like BHA, this can be related to the unequal dispersion of BHA in the maize during its application by the maize processor(s). The fungistatic properties of this synthetic polyphenolic compound, i.e. BHA, are used as a means of regulating mycotoxin production, as was shown in a study by Nesci et al. [37]. According to Nesci et al. [37], BHA may be able to prevent Aspergillus flavus insect vectors from producing aflatoxin when maize is being stored.

Ogunremi et al. [29] noted that esters are frequently present in fermented cereals, contributing to their pleasant flavour and fruity aroma. In general, the increased number of esters after fermentation of the whole and dehulled maize samples, as well as the production of some new esters in the fermented dehulled maize (Table 4), are consistent with this observation. According to Kewuyemi et al. [19], the production of these esters also results from the chemical reaction between alcoholic metabolites and acidic microorganisms as well as the esterification of alcohols with fatty acids during fermentation.

Table 4
Distinct metabolites in the fermented traditionally dehulled maize products.

R_t (mins)	*m/z*	Molecular formula	Metabolites	MC	Natural ($\overline{X}$ PA)	Controlled ($\overline{X}$ PA)		
						L. fermentum	*L. plantarum*	*L. fermentum* + *L. plantarum*
3.48	182.1086	$C_{28}H_{38}N_4O_4$	N-[3,3′-Dimethoxy-4'-(2-piperidin-1-yl-acetylamino)-biphenyl-4-yl]-2-piperidin-1-yl-acetamide	MLC	n.d.	n.d.	3527156	n.d.
3.72	233.1314	$C_{21}H_{32}O_4$	Androst-5-en-17-one, 3-hydroxy-16,16-dimethoxy-, (3β)-	MLC	293720	n.d.	n.d.	n.d.
4.04	142.0795	$C_3H_6Cl_2O$	2,2-Dichloroethyl methyl ether	MLC	n.d.	n.d.	30795829	n.d.
4.70	156.995	$C_{11}H_{22}O$	2-Undecen-4-ol	Alcohol	n.d.	n.d.	n.d.	748940
4.80	120.0570	C_8H_8O	Benzeneacetaldehyde	Aldehyde	1191266	n.d.	n.d.	n.d.
4.96	240.0912	$C_{31}H_{57}N$	Cholestan-3-amine, N,N,4,4-tetramethyl-, (3β,5a)-	MLC	n.d.	275368	n.d.	n.d.
6.16	144.0417	$C_6H_8O_4$	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Ketone	n.d.	3340903	4138229	1265084
8.08	152.1194	$C_{10}H_{16}O$	2,4-Decadienal, (E,E)-	Aldehyde	986992	293551	n.d.	n.d.
8.51		$C_9H_{17}NO_2$	Ethyl 1-methylpipecolinate	MLC	n.d.	n.d.	149420	n.d.
8.93	154.0624	$C_8H_{10}O_3$	Phenol, 2,6-dimethoxy-	Phenol	n.d.	n.d.	375183	n.d.
9.14	219.0275	$C_9H_{20}FO_2P$	Heptyl ethyl phosphonofluoridate	MLC	n.d.	85995	n.d.	n.d.
9.47	164.0612	$C_{26}H_{25}ClF_3N_3O_2$	7-Chloro-1,3,4,10-tetrahydro-10 hydroxy-1-[[2-[1-pyrrolidinyl] ethyl]imino]-3-[3-(tri fluoromethyl)phenyl]-9(2H)-acridinone	MLC	3185631	n.d.	4279576	n.d.
11.19	503.1073	$C_{18}H_{52}O_7Si_7$	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	MLC	n.d.	n.d.	n.d.	593025
11.20	505.1045	$C_{19}H_{54}O_7Si_7$	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy)tetrasiloxane	MLC	468296	n.d.	n.d.	n.d.
12.30	142.0795	$C_7H_{13}NO_2$	3-Pyrrolidin-2-yl-propionic acid	MLC	n.d.	n.d.	409794	n.d.
12.31	125.0835	$C_7H_{11}NO$	Hexahydropyrrolizin-3-one	Ketone	n.d.	456400.3	544437	n.d.
12.53	180.078	$C_{11}H_{16}O_2$	3-*tert*-Butyl-4-hydroxyanisole	Phenol	n.d.	n.d.	1366332	n.d.
12.54	180.0779	$C_{10}H_{12}O_3$	2′,4′-Dimethoxyacetophenone	Ketone	n.d.	n.d.	n.d.	1083504
13.93	417.033	$C_{16}H_{48}O_8Si_8$	Cyclooctasiloxane, hexadecamethyl-	MLC	n.d.	n.d.	n.d.	881747
17.57	222.1159		Phthalic acid, 8-chlorooctyl decyl ester	MLC	n.d.	40089	n.d.	n.d.
17.84	237.2207	$C_{10}H_{19}FO_5$	Methyl-6-deoxy-6-fluoro-2,3,4-tri-*O*-methylßd-galactopyranoside	MLC	56088	n.d.	n.d.	n.d.
17.88	157.178	$C_9H_{19}NO$	Nonanamide	Amide	482091	n.d.	n.d.	n.d.
18.89	294.2553	$C_{19}H_{34}O_2$	9,12-Octadecadienoic acid, methyl ester	FAME	3855939	n.d.	n.d.	n.d.
19.65	225.2051	$C_{11}H_{16}FNO_3$	Benzeneethanamine, 2-fluoro-ß,3,4-trihydroxy-N-isopropyl-	Benzene	791180	n.d.	n.d.	n.d.
20.49	226.2165	$C_{16}H_{33}NO$	Hexadecanamide	Amide	n.d.	n.d.	405268	n.d.
20.63	239.2375	$C_{16}H_{23}NO_4$	Succinic acid, 3,4-dimethylphenyl 2-(dimethylamino)ethyl ester	Ester	n.d.	n.d.	n.d.	514741
22.81	250.985	$C_{22}H_{34}O_4$	Phthalic acid, di(hept-4-yl) ester	Ester	172773	165814	88531	128324
22.83	279.158	$C_{20}H_{26}O_4$	Dicyclohexyl phthalate	Ester	n.d.	135527	n.d.	142237
27.06	430.3797	$C_{29}H_{50}O_2$	dl-a-Tocopherol	Vitamin	99812	n.d.	n.d.	n.d.

R_t: retention time (min); *m/z*: mass/charge ratio; MC: metabolite class; $\overline{X}$ PA: Average peak area; FAME: fatty acid methyl ester; MLC: miscellaneous compounds; *L. fermentum*: *Lactobacillus fermentum; L. plantarum*: *Lactobacillus plantarum*; *L. fermentum* + *L. plantarum*: mixed culture of *Lactobacillus fermentum and Lactobacillus plantarum* strains; n.d: not detected.

4. Conclusion

The current study evaluated the effects of TD on the decrease of mycotoxins and the metabolite profile of raw and fermented maize products. The study's conclusions showed that TD, which involves tempering for pericarp removal, can both cause a type of solid-state fermentation in the dehulled maize and lower the level of several mycotoxins in raw and fermented maize. The use of GC-HRTOF-MS metabolomics technology revealed that TD increased the amount of some metabolites in maize while dehulled maize fermentation produced some unique compounds. Ergosterol, a phytosterol, was found in the dehulled maize sample with the use of metabolomics, suggesting that the maize germ may have been contaminated by fungi.

Principally, the investigation validated that TD reduces the level of several mycotoxins and can bring about the loss/biotransformation or creation of some metabolites with reported biological activities in the dehulled (raw and fermented) maize products. Also, it describes how SO_2 is used in the food industry when it is essential to stop any type of fermentation that can happen during corn conditioning. The produced fermented whole and dehulled corn needs to be examined for potential mycotoxin degradation products to

ensure food safety.

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Data availability

Data will be made available on request.

CRediT authorship contribution statement

Julianah Olayemi Odukoya: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation. **Sarah De Saeger:** Supervision, Methodology, Investigation, Conceptualization. **Marthe De Boevre:** Supervision, Methodology, Conceptualization. **Gabriel Olaniran Adegoke:** Supervision, Methodology, Funding acquisition, Conceptualization. **Frank Devlieghere:** Resources, Methodology, Conceptualization. **Siska Croubels:** Project administration, Investigation, Conceptualization. **Gunther Antonissen:** Project administration, Investigation, Conceptualization. **Johnson Oluwaseun Odukoya:** Software, Resources, Methodology, Formal analysis. **Patrick Berka Njobeh:** Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Appendix A. Supplementary data

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