Spoilage volatiles and sensory properties of a grilled stick meat product inoculated with *Pediococcus acidilactici* FLE07 as starter culture

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Abstract

Introduction. Grilled meat products are normally eaten as snacks in many developed and developing countries usually during leisure. The use of biological agents such as lactic acid bacteria may help improve the associated volatiles and sensory appeal of such products.

Materials and methods. A grilled meat product (*Tsire*) was inoculated with 6 log CFU/g of *Pediococcus acidilactici* FLE07 as starter culture (IS), with the objective of improving the associated spoilage volatiles during a 4 day storage at 30 °C; while uninoculated sample of *Tsire* served as control (UC). Solid phase mass extraction gas chromatography-mass spectrometry (SPME-GCMS) and taste panel using hedonic scaling were used to evaluate the volatiles and sensory quality respectively during storage.

Results and discussion. In preliminary experiments, ten *Pediococcus* strains were evaluated for production of organic acids; among these, 34 g_{acid} /10^7CFU was recorded as highest lactic acid production by *P. acidilactici* FLE07 and was chosen as starter culture for inoculation of *Tsire*. The strain was shown to produce acetic acid of concentration lower than 12 g_{acid} /10^7 CFU. SPME-GCMS analysis of the grilled meat product showed that a total of fourty eight (48) volatiles, belonging to ketones (35.42%), acids (8.33%), alcohols (25%), aromatic/cyclic (14.58%) and nitrogenous compounds (16.67%), were detected during storage. Volatiles including acetone, 2-butanone, 2,3-butanedione, 3-hydroxy-2-butanone, 2-hexanone, 2-heptanone and 1-hydroxy-2-propanone were among the prominent ketone compounds observed in the *Tsire* samples, and their total are units (TAUs) showed significant difference ($p<0.05$) between the IS and UC samples. There was reduction in spoilage volatile indicators; concentrations (μg/g) of 0.57, 1.98, 0.93 and 1.39 were recorded for heptanal, 1-octen-3-ol, 3-methyl-butanoic acid and nonanal respectively in inoculated samples (IS) compared to 2.43, 3.21, 2.94 and 2.94 obtained for uninoculated control (UC) on day 3. Sensory study with the use of 50 panelists to provide data showed that higher scores ($p<0.05$) were recorded in the aroma, appearance, tenderness, taste and general acceptability properties of IS than UC.

Conclusion. It was concluded that *Pediococcus acidilactici* FLE07 and other suitable starter culture(s) may be used in promoting sensory quality development and availability of the product beyond the day of production. This is the first report of this type on the grilled meat product.

Introduction

Grilled meat products are normally prepared by grilling of shredded meat cuts hanged on sharpened edges of sticks, and *Tsire* is common example in Nigeria. The meat cuts are normally spiced with peanut cake, spices, vegetable oil, salt or other flavorings and then cooked by grilling. They are sprinkled with groundnut oil during the grilling process to simulate the traditional technique of avoiding burning or charring [1]; grilling is usually carried out around glowing charcoals. *Tsire* could be prepared from muscles of beef, goat, mutton or chicken, though preparation from beef is common [2]. It is a popular traditional meat product in Nigeria commonly eaten as delicacies, served or sold along streets or in club houses.

Cooking of meat involves a complex series of thermally induced reactions occurring between non-volatile components of lean and fatty tissues, resulting in a large number of reaction products (Lorenzo *et al.*, 2016); the volatile compounds formed in these reactions are largely responsible for the characteristic flavour of cooked meat [1]. Such products include aldehydes, carboxylic acids, alcohols, ketones, esters, sulfur compounds, nitrogenous compounds, terpenes, alkanes and alkenes, aromatic and cyclic hydrocarbons, which have been noted to contribute to flavour development in meat products [3].

Pediococcus is a genus under the group of lactic acid bacteria (LAB) which play positive role in fermentation and preservation of many foods, especially by their abilities to produce considerable amounts of volatile and organic compounds [2]. LAB have a generally regarded as safe (GRAS) status and have been widely used as starters in the industrial preservation of meats [4]. For instance, Olaoye *et al.* [5] reported the application of LAB cultures, *P. pentosaceus* GOAT 01 and *Lactobacillus plantarum* GOAT012, in the preservation of goat meat during storage at 30^{o}C. According to the authors, the LAB cultures were able to extend the shelf life of the meat for days beyond the uninoculated control samples. In another study, the use of *Pediococcus* cultures in the generation of antioxidant nitrogen compounds in Iberian dry-fermented sausages was reported by Fernández *et al.* [6]. Moreover, Lorenzo *et al.* [7] investigated the effect of commercial cultures of *Pediococcus* spp. on volatile compound profile and sensory characteristics of dry‐cured foal sausage during ripening. Olaoye [1] and Olaoye [3] reported studies on the effect of storage period on volatiles and consumers' acceptability of *Tsire* and pork *balangu* (a grilled meat product) respectively; the authors recommended that inoculation of the meat product with LAB cultures should be carried out in future studies for possible improvement in volatile characteristics.

In spite of the available reports on meat products, no study is currently available on the effect of LAB cultures on volatiles of *Tsire* during storage. Hence, the present study focused on evaluating the influence of *P. acidilactici* FLE07 culture on the volatile compounds, especially those that may be spoilage indicators, and sensory characteristics of the traditional meat product during storage.

Materials and methods

Source of meat and ingredients. The beef used in this study was purchased from a butcher's shop in the city of Nottingham, UK, and conveyed to the laboratory over ice crystals for immediate use. The ingredients used included ground red pepper (*Capsicum* sp.), onions (*Alium cepa*), ginger (*Zingiber officinalis*), groundnut (*Arachis hypogaea*) and salt, obtained from a Nigerian shop in the same city.

Sources of LAB used and growth conditions. Ten presumptive LAB isolates, previously isolated and phenotypically identified from meat in a preliminary study were used in the present study [8]. They included *Pediococcus pentosaceus* LIV01, *P. acidilactici* FLE07, *P. acidilactici* FLE02, *P. pentosaceus* INT01, *P. pentosaceus* INT02, *Lactobacillus plantarum* FLE04, *P. acidilactici* INT04, *P. acidilactici* FLE07, *Leuconostoc mesenteroides* FLE03 and *P. pentosaceus* INT04. The optimum growth temperature of the isolates in MRS medium (Oxoid, UK) was 30 °C. The isolates were routinely maintained in MRS broth medium containing 20% glycerol at –20 °C as working cultures, and at –80 °C for long-term storage.

Production of organic acids. Prior to selection of one of the LAB isolates as starter culture, the isolates were evaluated for their abilities to produce organic acids (lactic and acetic acids), using the method of Olaoye *et al*. [8]. One of the isolates, *P. acidilactici* FLE07, showed considerable production of organic acids, and was hence chosen as starter culture for inoculation of the traditional meat product prior to storage. Concentrations of the organic acids were expressed in g_{acid} /10^7 CFU (i.e grams of lactic/acetic acid per 10^7 colony forming units)

Confirmation of identity of presumptive *P. acidilactici* FLE07 used as starter culture. The LAB isolate *P. acidilactici* FLE07 used as starter culture was presumptively identified in a previous study [8]. Full identity of the isolate was obtained in the present study using 16S rDNA nucleotide sequencing after successful amplification by PCR. Deoxyribonucleic acid (DNA) was extracted using a boiling method [9]. PCR amplification was performed using the following set of primers [10]: Forward, 5′-CCTACGGGAGGCAGCAG-3′ and Reverse, 5′-ATTACCGCGGCTGCTGG-3′, targeting approximately 200 bp of 16S rDNA gene (V3 region). PCR conditions used were as described by Olaoye *et al.* [5]. Electrophoresis of the 16S rDNA-PCR products, purification and sequencing were carried out as previously described [5]. The specific nucleotide sequences were subjected to BLAST programme of NCBI (website; http://www.ncbi.nlm.nih.gov/blast/) to determine the homology of the *Pediococcus* isolate with related genera and species [11].

Preparation of *Tsire* and inoculation with *P. acidilactici* FLE07. *Tsire* samples were prepared according to the method described by Olaoye [1]. Some of the *Tsire* samples were inoculated with 6 log CFU/g of *P. acidilactici* FLE07 culture according to the method of Olaoye and Dodd [2] while uninoculated samples served as control.

Storage of *Tsire.* The *Tsire* samples were wrapped in aluminium foils and stored for four days in a storage cabinet at 30^{o}C to represent ambient temperature in Nigeria. Samples were taken daily, in three replicates, for analysis of thiobarbituric acids and volatiles compounds.

Analysis of thiobarbituric acid in *Tsire.* Thiobarbituric acid (TBA) values were determined for the *Tsire* samples as described by Olaoye and Onilude [12].

Analysis of volatile compounds in *Tsire* using solid phase mass extraction-gas chromatography mass spectrometry (SPME-GCMS)

The volatiles in the *Tsire* samples taken daily during storage were analyzed using SPME-GCMS. This was performed by placing 2 g of samples in 20 ml headspace vials (22.5 mm x 75.5 mm, Grace Alltech, UK). The vials were sealed with a magnetic cap (20mm diameter, 5mm centre, PTFE/Silicone Liner; Grace Alltech) using a Crimper (Part no 60045, Alltech Associates Inc., USA) and allowed to equilibrate at room temperature (22 °C) for 30 min before commencement of analysis.

A Stableflex fibre coated with 50/30 μm divinylbenzenecarboxen on polydimethylsiloxane bonded to a flexible fused silica core (Supelco, Bellefonte, PA, 16823–0048 USA) was used for the extraction of the flavour volatiles in the headspace of the vials. For volatile sampling, an extraction time of 15 min at room temperature was used, while desorption time was set to 4 min at 230 °C.

GCMS was carried out using a Trace GC Ultra gas chromatograph (Thermo Electron Corporation, UK) and a DSQ mass spectrometer (1.4.1 SP3 Thermo Electron Corporation, USA). Samples were injected in splitless mode into the GC with a PAL auto-sampler. Chromatography was carried out with a TRACE GC 2000 series gas chromatograph using a ZB-WAX capillary column (Serial no 162147, Order no 7HG-G007-22, L 30m x I.D. 0.25mm x df 1μm, USA). Helium was employed as the carrier gas, at a constant pressure of 15 psi and splitless time of 1 min. The oven temperature programme was as follows: an initial temperature of 40°C was maintained for 1 min, with ramps 8 °C/min to 200 °C and 10 °C/min to a final temperature of 230 °C. Mass spectrometry was performed with a DSQ mass spectrometer. The mass spectrometer was operated in positive ionisation electron impact mode (EI+) at electron energy of 70 eV. The scan time was 0.29 s. Samples for injections into the GC were prepared in three replicates. The detector was operated in scan mode, scanning from m/z 20 to 210. Source temperature was 200 °C. The data generated were processed with Xcalibur ™ 1.4 SR1 (Thermo Electron Corporation) software.

Volatile compounds were identified by comparing their mass spectra with those in the National Institute of Standards and Technology (NIST) mass spectral library and/or by calculation of the retention indices relative to a series of n-alkanes (C5–C19; Sigma-Aldrich, UK) and matching them with standard compounds and data reported in the literature [13,14]. The results were reported as relative abundance expressed as total area counts, TAUs ($\times 10^4$).

Sensory study. Sensory study was conducted on the *Tsire* samples that depended on inoculation with or without *P. acidilactici* FLE07 and storage time. Samples were evaluated for the sensory properties of aroma, appearance, tenderness, taste and general acceptability using a 50 member panel (n=50), composing of Nigerians who were already familiar with the product. Freshly prepared *Tsire* samples were used as reference for comparison of sensory properties of other samples during storage. Panelists were asked to allocate scores to three coded replicates of samples, using a 9-point hedonic scale, from 1-dislike extremely to 9-like extremely. Data obtained were subjected to statistical analysis to determine significant differences among samples.

Statistical data analysis. Results which depended on starter culture and storage time were analyzed according to a completely randomized design with three replicates. The data obtained were subjected to one way analysis of variance (ANOVA) to evaluate the effect of starter culture on the samples. Differences between means were evaluated by Duncan's multiple range test and significant difference was expressed at $p < 0.05$. The SPSS statistic programme (version 10.01) was used.

The relationship between the inoculated and uninoculated control samples, storage time and their volatile compounds was evaluated by principal component analysis (PCA) using Xlstat software (ver. 17.3.01.19703; Addinsoft, NY).

Results and discussion

In this study, ten LAB isolates previously isolated and presumptively identified from meat in a preliminary study [8], were screened for production of organic acids (lactic and acetic acids), with the objective of selecting suitable candidate(s) as starter culture for possible improvement of volatiles and sensory properties of a Nigerian traditional meat product (*Tsire*) during storage. Among the ten isolates, *Pediococcus pentosaceus* LIV01, *P. acidilactici* FLE01, *P. acidilactici* FLE02 and *P. acidilactici* FLE07 produced lactic acid higher than 25 g_{acid} /10^7 CFU (Figure 1); however, *P. acidilactici* FLE07 had the highest production of the acid (34 g_{acid} /10^7 CFU) and was chosen as starter culture for inoculation of *Tsire*. Acetic acid production by the LAB isolates was generally lower than 12 g_{acid} /10^7CFU.

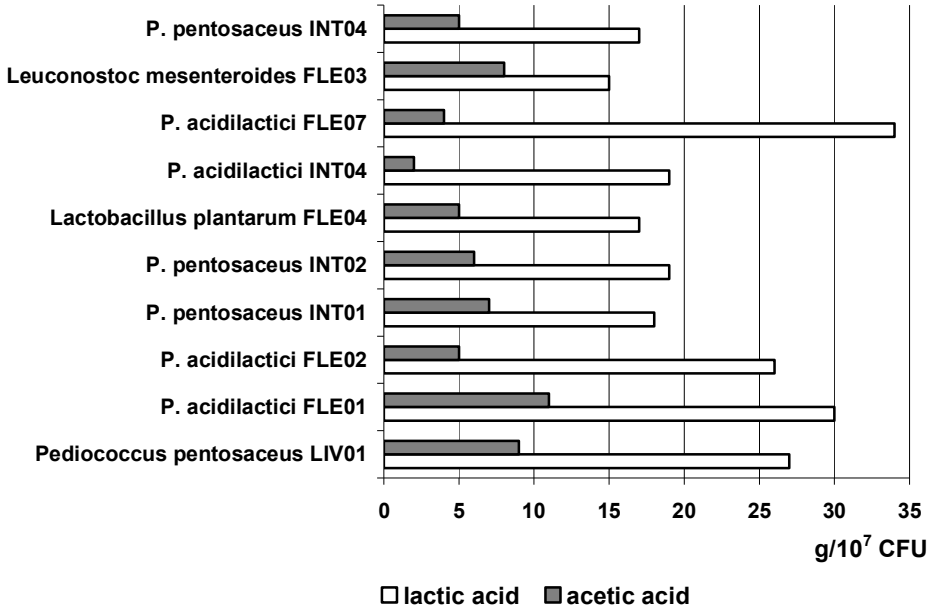

Figure 1. Concentrations of lactic and acetic acids (g_{acid}/10^7 CFU/g) produced by the LAB isolates

Sixty four (48) volatile compounds were detected in the meat product during storage, and they were categorized into different classes including ketones (class 1; 35.42%), acids (class 2; 8.33%), aromatic/cyclic hydrocarbons (class 3; 14.58%), nitrogenous compounds (class 4; 16.67%) and alcohols (class 5; 25.0%); these are presented in Tables 1 and 2.

Similar reports of detection of these classes of volatiles in meat products have been made by other research investigators [3,15].

(In the Table 1: Each value is mean of three replicates of samples; SD1, storage day 1; SD2, storage day 2; SD3, storage day 3; SD4, storage day 4; StdD, standard deviation; P-value, probability value; RI, retention index; MI, method of identification; 1, Identification using authentic standards; 2, Identification using retention indices from the literature and their mass spectra with the NIST mass spectral library)

Table 1. Total area units (x10^4) of ketone and acid compounds in *Tsire*

Compounds		*Tsire* inoculated with *P. acidilactici* FLE07							
	RI	SD1	StdD	SD2	StdD	SD3	StdD	SD4	StdD
Class 1 – Ketones									
Acetone	<600	5862	134	4102	129	2981	172	2410	74
3-methyl-2-butanone	<600	25	4	17	5	25	3	53	12
Methyl-isobutyl-ketone	612	0.79	0	2	1	6	2	46	13
2-butanone	618	540	20	520	112	605	47	725	129
2,3-butanedione	621	541	64	323	32	275	12	172	17
3-methyl-2-pentanone	634	0.11	0.01	6	2	13	3	68	6
5-methyl–2-hexanone	687	81	19	76	7	69	14	58	10
2-hexanone	699	24	4	20	8	16	2	13	3
2,5-hexanedione	712	92	12	178	36	471	86	1290	128
2-heptanone	719	2	0.8	11	1	19	4	50	3
2-octanone	720	5	3	11	2	24	17	18	5
6-methyl-2-heptanone	723	1	-	1.2	0.3	4	1	20	3
5-methyl-2-heptanone	725	1.3	0.5	2	1	9	3	52	4
3-hydroxy-2-butanone	727	251	89	264	101	272	86	281	27
1-hydroxy-2-propanone	733	21	12	45	15	85	12	31	5
6-methyl-5-hepten-2-one	742	21	2	25	7	29	12	35	8
cyclopentanone	748	32	2	39	13	47	9	64	21
Class 2 – acids									
2-methyl-propanoic acid	752	2	0	20	4	18	2	13	3
2-methyl-hexanoic acid	768	6	2	42	7	46	7	71	16
3-methyl-butanoic acid	756	156	25	87	8	34	8	50	13
Hexanoic acid	772	12	3	20	6	21	12	10	2

Compounds	Uninoculated *Tsire*								*P*-value	MI
	SD1	StdD	SD2	StdD	SD3	StdD	SD4	StdD		
Class 1 – Ketones										
Acetone	5852	43	5574	116	5574	321	5404	373	0.002	1
3-methyl-2-butanone	9	3	35	13	50	10	53	9	0.0004	2
Methyl-isobutyl-ketone	0.93	0	10	3	29	3	47	9	0.0004	2
2-butanone	539	12	732	103	842	101	1431	108	0.035	2
2,3-butanedione	514	98	451	75	351	45	367	43	0.003	1
3-methyl-2-pentanone	9.37	0.3	20	9	66	17	77	9	0.0016	2
5-methyl-2-hexanone	80	13	89	23	93	18	126	16	0.019	2
2-hexanone	24	7	22	5	21	3	21	2	0.012	1,2
2,5-Hexanedione	91	27	167	31	251	65	452	98	0.0021	2
2-heptanone	3	1	38	18	108	33	120	12	0.0013	2
2-octanone	6	1	12	5	24	9	37	4	0.002	1
6-methyl-2-heptanone	0.38	0	5	1	24	14	34	6	0.001	2
5-methyl-2-heptanone	2	1	15	4	50	21	76	10	0.0005	2
3-hydroxy-2-butanone	262	92	321	103	461	122	519	97	0.007	2
1-hydroxy-2-propanone	7	2	16	4	55	23	31	9	0.031	2
6-methyl-5-hepten-2-one	22	5	21	7	40	13	62	11	0.0014	2
Cyclopentanone	33	10	41	5	54	11	71	14	0.073	2
Class 2 – Acids										
2-methyl-propanoic acid	0.8	0	16	2	18	3	12	2	0.01	2
2-methyl-hexanoic acid	4	3	52	5	112	28	62	12	0.003	2
3-methyl-butanoic acid	156	12	136	18	112	28	63	13	0.036	2
Hexanoic acid	6	1	7	2	9	1	12	5	0.281	1,2

Table 2

Total area units (x10^4) of aromatic/cyclic hydrocarbon, nitrogenous and alcohol compounds in *Tsire*

Compounds	RI	*Tsire* inoculated with *P. acidilactici* FLE07							
		SD1	StdD	SD2	StdD	SD3	StdD	SD4	StdD
Class 3 – Aromatic/cyclic									
Cyclopentene	<600	15	2	24	3	27	13	125	20
1-pentyl-propyl-Cyclopropane	742	7	1	4	2	3	0	4	1
Ethylbenzene	747	13	3	8	2	3	0.64	3	0.33
2-methoxy-2-propenyl-Benzene	754	41	5	35	7	32	9	24	5
O-xylene	756	8	1	5	1	3	0.79	3	0.57
Tetramethyltricyclo-Undec-2-ene	>1000	10	2	10	2	8	1	9	0
Hexamethyl-cyclotrisiloxane	>1000	7	2	8	2	6	3	7	0
Class 4 – nitrogenous									
1-methyl-1h-pyrrole	761	99	23	86	7	75	8	58	15
3-methyl-butanenitrile	873	14	4	12	6	11	3	9	1
2-methyl-pyrazine	881	7	2	11	1	14	2	37	2
Trimethyl-pyrazine	903	1017	19	879	89	724	92	584	198
Tetramethyl-pyrazine	910	175	23	138	37	52	8	31	8
3-ethyl-2,5-dimethyl-Pyrazine	921	4	1	5	0	5	2	12	2
2,5-dimethyl-pyrazine	954	48	9	149	62	225	34	1760	143
2,3-dimethyl-pyrazine	962	34	5	27	4	16	2	16	5
Class 5 – alcohols									
1-propanol	641	2	0	3	0	4	0	8	4
2,6-dimethyl-4-heptanol	645	3	1	6	1	10	2	13	4
1-pentanol	676	27	4	16	2	6	2	5	2
3-methyl-1-butanol	701	14	2	21	4	41	10	325	28
4-methyl-2-hexanol	729	15	5	29	8	27	3	46	13
3-methyl-3-buten-1-ol	732	1	0	4	1	13	3	13	1
1-octen-3-ol	741	53	7	57	18	48	9	39	11
3-methyl-2-butanol	748	3	1	4	1	9	4	33	12
P-menth–1-en–8-ol	749	10	1	8	1	7	1	7	1
Tricyclo(1,5)dec-5-en-8-ol	759	25	6	15	2	10	2	6	1
Phenylethyl-alcohol	792	2	0	3	1	3	0	33	9
Ursane-3,16-diol	812	17	7	11	4	10	6	23	5

Table 2
(continuation)

Compounds	Uninoculated *Tsire*								*P*-value	MI
	SD1	StdD	SD2	StdD	SD3	StdD	SD4	StdD		
Class 3 – Aromatic/cyclic										
Cyclopentene	77	12	79	9	76	22	174	17	0.005	2
1-pentyl-propyl-Cyclopropane	7	2	3	1	7	2	9	2	0.0061	2
Ethylbenzene	12	2	9	3	6	0.63	6	1	<0.001	2
2-methoxy–2-propenyl-Benzene	40	12	48	9	50	11	71	5	0.004	2
O-xylene	8	3	6	2	4	0.87	4	0.63	0.005	2
Tetramethyltricyclo-Undec–2-ene	8	2	8	3	9	2	9	3	0.2719	2
Hexamethyl-cyclotrisiloxane	3	1	5	2	6	2	12	3	0.0331	2
Class 4 – nitrogenous										
1-methyl-1h-pyrrole	98	21	103	31	113	14	144	28	0.004	2
3-methyl-butanenitrile	14	2	13	5	16	8	21	3	0.0017	1
2-methyl-pyrazine	11	2	17	5	51	11	73	3	0.0004	2
Trimethyl-pyrazine	1020	37	987	29	975	184	760	35	0.03	2
Tetramethyl-pyrazine	176	18	156	39	129	10	46	9	0.024	2
3-ethyl-2,5-dimethyl-pyrazine	4	0	9	4	39	9	33	3	0.0728	2
2,5-dimethyl-pyrazine	68	12	820	123	2462	128	3783	210	0.0003	2
2,3-dimethyl-pyrazine	31	13	29	5	22	2	21	0	0.013	2
Class 5 – alcohols										
1-propanol	2	0	5	2	13	6	18	10	0.0009	1
2,6-dimethyl-4-heptanol	4	1	10	2	19	4	27	7	0.004	2
1-pentanol	27	6	19	2	10	2	8	2	0.005	1
3-methyl-1-butanol	19	4	33	11	910	40	1455	300	0.0035	2
4-methyl-2-hexanol	14	2	22	12	42	4	59	6	<0.0001	2
3-methyl-3-buten-1-ol	1	0	6	1	13	1	20	2	0.0001	2
1-octen-3-ol	53	8	59	11	64	10	97	19	0.019	2
3-methyl-2-butanol	2	0	16	7	27	6	35	10	0.0005	2
P-menth–1-en-8-ol	9	1	7	1	9	3	11	3	0.0189	2
Tricyclo(1,5)dec-5-en-8-ol	25	3	21	6	14	2	9	1	0.017	2
Phenylethyl-alcohol	2	1	3	1	19	4	36	5	0.0004	2
Ursane-3,16-diol	6	1	17	3	13	4	20	3	0.009	2

Each value is mean of three replicates of samples; SD1, storage day 1; SD2, storage day 2; SD3, storage day 3; SD4, storage day 4; StdD, standard deviation; *P*-value, probability value; RI, retention index; MI, method of identification; 1, Identification using authentic standards; 2, Identification using retention indices from the literature and their mass spectra with the NIST mass spectral library

The total area units (TAUs) of the class of ketone compounds identified in the meat product are shown in Table 1. The volatiles acetone, 2-butanone, 2,3-butanedione, 3-hydroxy–2-butanone, 2-hexanone, 2-heptanone and 1-hydroxy–2-propanone were among the prominent ketone compounds which recorded significant differences ($p<0.05$) in their TAUs between the IS and UC samples. Some of the ketone volatiles have been noted to play important roles in sensory characteristics of meat products [7]. One of the important ketone compound was 3-hydroxy–2-butanone (acetoin), a product of degradation due to maillard reaction; its identification from meat products has been reported [3,16]. Presence of this compound especially in relatively high concentration may cause spoilage of food [17]. Lower values of TAUs of acetoin ($p=0.0004$) were recorded in IS than UC samples, indicating that inoculation of the meat product with starter culture had significant effect on the compound; this observation may help enhance shelf life of the product. The reduced TAU of acetoin in the IS sample may be attributed to possible antioxidative property of *P. acidilactici* FLE07 used as starter culture [18,19,20], which may bring about reduction of undesirable volatile compounds in meat products. Acetoin has been reported as a spoilage molecule associated with in meat products during storage [21].

The compounds, 2-hexanone, 2-heptanone and 2-butanone were among the ketone compounds identified in the present study, and they have been noted as contributors to off flavour development in meat products [22]; however their TAUs were lower ($p<0.05$) in the IS samples than UC. This may therefore translate that they are present in reduced concentration in IS than UC samples, indicating that there may be reduction of off flavour development in the meat product inoculated with the starter culture. Another ketone compound identified in this study was 2,3-butanedione (diacetyl), which has been reported as a product of lactose and citrate metabolism by the action of bacteria, especially LAB [23]. The occurrence of the compound in *Tsire* is in support of a similar report by Huan *et al*. [24] in a research investigation during storage of a Chinese meat product – *Jinhua ham*. Diacetyl may be of technological importance as it possesses anti-microbial properties against many unwanted microorganisms, especially the spoilage types, in foods [25].

The volatile compounds in class 2 comprised of 2-methyl-propanoic acid, hexanoic acid, 3-methyl-butanoic acid and 2-methyl-hexanoic acid, all of which had higher TAUs in IS than UC (Table 1). Significant difference ($p<0.05$) was recorded in the acids of IS and UC, with the exception of hexanoic acid. Lower values of TAUs were recorded for the acids ($p<0.05$) in IS than UC, and this may be of significance as a result of possible association of certain acids, especially 3-methyl-butanoic acid, butanoic acid (and some of its derivatives) with meat spoilage [17]

The seven volatile compounds of aromatic/cyclic hydrocarbons belonging to class 3 compounds in IS were significantly different ($p<0.05$) from those of UC (Table 2), with the only exception of tetramethyltricyclo-undec-2-ene. They have reduced TAUs in the IS samples compared to UC, indicating possible influence of the starter culture. One of the compounds belonging to class 3 was ethylbenzene, which presence in the meat product may be very significant as it may be associated with spoilage of meat and fish [17,26]. The reduced values of TAUs of the compound in the IS samples is therefore desirable towards possible reduction of spoilage in the meat product. The nitrogenous compounds (class 4) consisted mostly of pyrazines, which are regarded as products of maillard reactions; their formation in meat products could be attributed to application of heat and salting during processing [26]. Contribution of pyrazines to development of desirable sensory characteristics of grilled and roasted meat has been reported [16]. The identification of nitrogenous compounds, 2,3-dimethyl-pyrazine and tetramethyl-pyrazine in meat products was observed by Gianelli *et al.* [16], thus supporting their occurrence in *Tsire* in the present study. The class 5 volatile compounds consisted of twelve alcohol compounds (Table 2), most of which recorded lower values of TAUs ($p<0.02$) in IS samples than UC. Among

these compounds, 1-octen-3-ol and 3-methyl-1-butanol have been noted as spoilage molecules in meat products [21]. The lower values of TAUs of these compounds in IS samples than UC may thus indicate possible extension in shelf life of the former over the latter.

The chart of PCA carried out on the volatile compounds during storage of *Tsire* samples inoculated with starter culture is presented in Figure 2. At the upper portion in the left hand side of the chart are represented the principal components associated with the meat samples on storage day 1 (Sd1). The principal components on Sd1 were composed of 2,3-dimethyl-pyrazine, o-xylene, p-menth-1-en-8-ol, ethylbenzene, hexamethyl-cyclotrisiloxane, 3-methyl-butanoic acid, 1,2-diepoxyhexadecane, 1-pentyl-2-propyl-cyclopropane, tetramethyltricyclo-undec-2-ene, and 3-methyl-butanenitrile, which represent 15.6% of the total volatile compounds (TVCs) in the meat product. On Sd2 (lower portion of the left hand side), the principal components included octane, 2-hexanone, 1-methyl-1h-pyrrole, 2,3-butanedione, acetone, 1-pentanol, (e)-3-dodecene, heptane, trimethyl-pyrazine, 2-pentyl-furan, tricycle(1,5)dec-5-en-8-ol, tetramethyl-pyrazine, 2-methoxy-2-propenyl-benzene and 5-methyl-2-hexanone, representing 21.9% of TVCs. A major cluster was formed on Sd2 consisting mainly of ketones, alcohols and pyrazines, suggesting that these compounds were the major ones associated with the product on second day of storage. On Sd3 (lower portion in the right hand side of chart), the principal components identified were 3-methyl-3-buten-1-ol, 2-octanone, 2-methyl-propanoic acid, 1-hydroxy-2-propanone, nonanal and hexanoic acid, forming 9.4% of TVCs. Twenty two principal components (representing 34.4% of TVCs) occurred on Sd4 (upper portion in the right hand side of chart), belonging mainly to alcohols, ketones, nitrogenous compounds, acids and pyrazines.

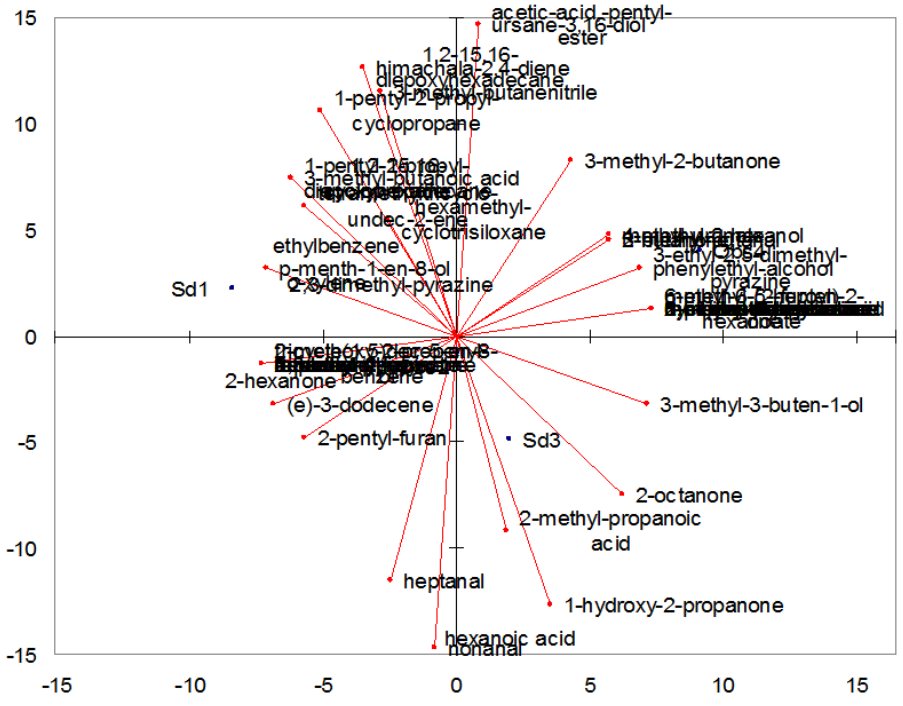

Figure 2. Principal component analysis of volatiles and SPME-GCMS peak area data from the headspace of inoculated *Tsire*

Sd1, Storage day 1; Sd2, Storage day 2; Sd3, Storage day 3; Sd4, Storage day 4.

The mean quantities (μg/g) of some volatile molecules identified in the meat product are shown in Table 3. Result indicated that compounds which have been regarded as spoilage indicators of meat had higher TAUs in UC than IS samples ($p<0.05$), suggesting the influence of the starter culture used. The compounds included 3-hydroxy-2-butanone, heptanal, nonanal, 2-butanone, 1-octen-3-ol and 3-methyl-butanoic acid, some of which had been noted earlier.

Table 3

Mean quantities (μg/g) of some head space compounds in *Tsire*

Compounds	RI	*Tsire* inoculated with *P. acidilactici* FLE07							
		SD1	StdD	SD2	StdD	SD3	StdD	SD4	StdD
2-butanone	618	38.52	2.78	38.47	4.84	42.17	9.26	47.39	11.93
2,3-butanedione	621	37.23	11.02	29.83	5.4	30.03	5.82	16.73	3.29
2-hexanone	699	1.9	0.51	1.76	0.25	1.7	0.42	1.21	3
3-methy-1-butanol	701	1.26	0.54	1.82	0.37	3.37	0.09	29.93	7.26
2-heptanone	719	0.23	0.01	1.16	0.09	1.65	0.39	2.17	0.82
Heptanal	725	1.62	0.08	0.58	0.03	0.57	0.07	0.49	0.11
3-hydroxy-2-butanone (acetoin)	727	23.26	9.18	25.01	4.19	25.92	7.02	27.28	5.4
1-octen-3-ol	741	2.36	0.19	2.41	0.24	1.98	0.08	2.13	0.17
Ethylbenzene	747	1.22	0.18	0.69	0.04	0.31	0.07	0.32	0.01
3-methyl-butanoic acid	756	2.31	0.77	1.29	0.14	0.93	0.04	0.77	0.12
Nonanal	982	1.59	0.09	1.62	0.42	1.39	0.41	0.89	0.02

Compounds	Uninoculated *Tsire*								*P*-value	MI
	SD1	StdD	SD2	StdD	SD3	StdD	SD4	StdD		
2-butanone	38.51	9.28	47.83	7.31	49.15	8.97	59.53	9.28	0.035	2
2,3-butanedione	37.19	5.47	33.89	10.29	30.95	12.4	31.28	8.35	0.013	1
2-hexanone	1.87	0.24	1.85	0.71	1.83	0.61	1.84	0.12	0.007	1,2
3-methyl-1-butanol	1.28	0.08	1.97	0.28	50.17	10.3	61.29	14.29	0.005	2
2-heptanone	0.22	0.03	2.27	0.93	10.41	2.19	12.86	0.84	0.009	2
Heptanal	1.6	0.18	1.89	0.55	2.43	0.82	5.86	0.93	0.008	1
3-hydroxy-2-butanone (acetoin)	23.75	4.5	29.72	10.2	34.12	2.93	37.84	4.72	0.005	2
1-octen-3-ol	2.42	0.26	2.97	0.36	3.21	1.02	4.32	0.63	0.008	2
Ethylbenzene	0.93	0.03	2.92	0.07	3.1	0.74	3.96	1.01	0.01	2
3-methyl-butanoic acid	2.32	0.09	2.83	1.29	2.94	0.91	2.89	0.72	0.027	2
Nonanal	1.6	0.16	2.43	0.34	2.94	1.04	3.72	0.65	0.005	1

Each value is mean of three replicates of samples; SD1, storage day 1; SD2, storage day 2; SD3, storage day 3; SD4, storage day 4; StdD, standard deviation; *P*-value, probability value; RI, retention index; MI, method of identification; 1, Identification using authentic standards; 2, Identification using retention indices from the literature and their mass spectra with the NIST mass spectral library

Result of sensory study carried out on the meat samples during storage is presented in Table 4. It was observed that IS samples recorded higher mean scores by the panelists than UC ($p<0.05$) from 24 h of storage in the sensory properties of aroma, appearance, tenderness and taste. The IS samples also recorded higher preference than UC in term of general acceptability ($p<0.05$). The result of sensory study corroborates the report of Calo-Mata *et al.* [27] who noted that LAB cultures may be used to develop desirable sensory characteristic properties in food products. The number of panelist who allocated scores higher than 5 to the meat samples decreased gradually as storage period progressed, the decrease was however more pronounced in UC than IS samples. Result further indicated that IS samples recorded higher acceptability by consumers than UC ($p<0.05$).

Table 4

Result of sensory study on the *Tsire* samples during storage

SP (h)	Samples	Aroma	Appearance	Tenderness	Taste	G.acceptability	% Acceptability (% n who scored > 5)
0	F	7.5 ± 1.29	6.9 ± 0.18	7.8 ± 0.76	7.6 ± 2.01	8.1 ± 1.29	100
24	IS	7.3 ± 1.20	7.0 ± 0.92	7.4 ± 0.87	7.1 ± 2.30	7.5 ± 2.17	90
	US	6.9 ± 0.98	6.1 ± 1.28	6.2 ± 1.07	6.7 ± 0.29	6.9 ± 1.55	75
48	IS	7.0 ± 2.08	6.5 ± 1.33	6.9 ± 0.94	6.8 ± 0.67	6.4 ± 1.36	87
	US	5.8 ± 0.93	5.3 ± 0.77	5.1 ± 1.25	5.3 ± 1.33	5.2 ± 0.55	59
72	IS	6.2 ± 1.20	6.0 ± 0.88	6.3 ± 1.29	6.1 ± 0.82	5.9 ± 0.72	74
	US	4.7 ± 0.73	4.2 ± 0.15	4.3 ± 1.08	3.9 ± 0.73	3.8 ± 0.88	36

Each value is mean of three replicates of samples; SP, Storage period; *F*, freshly prepared *Tsire*; *IS*, *Tsire* samples inoculated with starter culture; *US*, uninoculated *Tsire* samples; G.acceptability, General acceptability; *n*, number of panelists

From the results of this study, it was concluded that inoculation of *Tsire* with *P. acidilactici* FLE07 as starter culture had significant and desirable influence on the associated volatile compounds and sensory properties. This observation is in support of previous studies which reported impact of LAB cultures on volatile compounds of meat products. It was further concluded that the use of *P. acidilactici* FLE07 may contribute to extended shelf life of *Tsire* during storage as a result of reduction recorded in some of the known spoilage molecules. Suitable LAB starter cultures may therefore be applied towards promoting sensory quality development and availability of the traditional meat product beyond the day of production.

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