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Volatile compounds produced by *Lactobacillus fermentum*, *Saccharomyces cerevisiae* and *Candida krusei* in single starter culture fermentations of Ghanaian maize dough

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ABSTRACT

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Aims: To identify and compare the volatile compounds associated with maize dough samples prepared by spontaneous fermentation and by the use of added starter cultures in Ghana.

Methods and Results: The starter cultures examined were *Lactobacillus fermentum*, *Saccharomyces cerevisiae* and *Candida krusei*. For identification of aroma volatiles, extracts by the Likens-Nickerson simultaneous distillation and extraction technique were analysed by gas chromatography–mass spectrometry (GC–MS) and using a trained panel of four judges by GC-Olfactometry (GC-sniffing). Compounds identified by GC–MS in maize dough samples after 72 h of fermentation included 20 alcohols, 22 carbonyls, 11 esters, seven acids, a furan and three phenolic compounds. Of the total 64 volatile compounds, 51 were detected by GC-sniffing as contributing to the aroma of the different fermented dough samples. Spontaneously fermented maize dough was characterized by higher levels of carbonyl compounds while fermentations with added *L. fermentum* recorded the highest concentration of acetic acid. *S. cerevisiae* produced higher amounts of fusel alcohols and increasing levels of esters with fermentation time and *C. krusei* showed similarity to *L. fermentum* with lower levels of most volatiles identified.

Conclusion: The present study has given a detailed picture of the aroma compounds in fermented maize and demonstrated that the predominant micro-organisms in fermented maize dough can be used as starter cultures to modify the aroma of fermented maize dough.

Significance and Impact of the Study: The study has documented the advantage of using starter cultures in African traditional food processing and provided a scientific background for introducing better controlled fermentations.

Keywords: Maize, dough, aroma, fermentation, starter cultures.

INTRODUCTION

Work by several authors over the years has shown a consistency in the microflora of Ghanaian fermented maize dough produced under ambient temperatures (Akinrele 1970; Halm *et al.* 1993, 1996; Jespersen *et al.* 1994). Halm *et al.* (1993) demonstrated that a very uniform microflora occurred in 15 samples of maize dough from different large commercial production sites in Ghana. The aroma volatiles of dough samples from two commercial producing sites were also found to be similar. Attempts to improve the organoleptic acceptability of fermented maize dough as well as control the quality of fermented maize products has led to studies into the use of starter cultures in the fermentation of maize. Akinrele (1970) showed that 'Ogi' produced from a mixed culture of *Lactobacillus* spp. and *Acetobacter* spp. enriched nutrient quality by increasing

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the concentrations of riboflavin and niacin above that found in both the unfermented grain and the 'Ogi' produced by traditional spontaneous fermentation. Using a combined inoculum of *Saccharomyces cerevisiae* and *Candida kefyr*, Nyako and Danso (1991) found that the acceptability of fermented maize dough was significantly improved over the non-inoculated naturally fermented dough. Halm *et al.* (1996) carried out controlled fermentation experiments using six strains of *Lactobacillus fermentum* and one strain of *Saccharomyces cerevisiae*. They found that for most of the inoculated samples the required pH of 3·7 was attained within 24 h of dough fermentation instead of 48 h as observed with spontaneous dough fermentations. An evaluation of fermented maize products showed that 'Koko', a traditional maize porridge, prepared from dough fermented with starter culture for 24 h was found acceptable by a trained panel of judges. A cooked, stiff maize dumpling, 'Kenkey', also prepared from dough with added starter culture was reported as comparable by a trained taste panel of judges, in terms of appearance, odour, taste, sourness, texture and overall acceptability, to that produced from spontaneously fermented dough (Halm *et al.* 1996).

Previous work by Hayford and Jakobsen (1999), Hayford and Jespersen (1999) and Hayford *et al.* (1999) have confirmed to species and strain level, the dominant microflora in Ghanaian fermented maize dough to be *L. fermentum*, *S. cerevisiae* and *C. krusei*. The primary fermentation products of heterofermentative lactic acid bacteria include lactic acid, acetic acid and diacetyl (Lindsay 1985). Homofermentative lactic acid bacteria (e.g. *Streptococcus*, *Pediococcus*, *Lactococcus* and some lactobacilli) produce twice as much lactic acid as heterofermentors from the fermentation of glucose using the Embden-Meyerhof-Parnas pathway (Axelsson 1998). Major aroma groups produced by action of metabolic active yeasts in cereals include fatty acids, esters, aldehydes and alcohols (Stam *et al.* 1998; Labuda *et al.* 1997).

The main organic acids in Ghanaian fermented maize dough were reported to be lactic, acetic, butyric and propionic acids (Banigo and Muller 1972; Plahar and Leung 1982). Halm *et al.* (1993) found other acids like pentanoic, hexanoic, heptanoic, octanoic, benzoic and dodecanoic acids as well as alcohols and a few carbonyls in Ghanaian fermented maize dough. The aroma profile of 'Uji' (a fermented maize and millet slurry) fermented with starter cultures of lactic acid bacteria was characterized by Masha *et al.* (1998) as having high concentrations of hexanoic, octanoic and nonanoic acids and some alcohols including 1-propanol, 1-hexanol, 1-nonanol and 2-undecenol. Sanni *et al.* (1994) also found higher levels of ethanol in spontaneously fermented Nigerian maize 'Ogi' compared with samples using inocula of lactic acid bacteria.

Knowledge about the influence of added starter cultures on the aroma profile of fermented maize dough products forms an important contribution to establishing the overall acceptability of these dough. A detailed aroma analysis and odour description to clarify the effect of the predominant bacteria and yeasts on these factors has, however, not been performed in previous studies.

The purpose of this study is to compare the volatile compounds produced during spontaneous fermentation of maize dough with compounds produced from the use of single starter culture inoculations of *L. fermentum*, *S. cerevisiae* and *C. krusei* and to define the role of these predominant micro-organisms in maize fermentation.

MATERIALS AND METHODS

Maize

The 'Local' variety of normal dent white maize (*Zea mays*) grains were purchased from a retail outlet in Accra, Ghana. They were cleaned, sealed in polyethylene bags and stored at room temperature (28–30°C) until ready for use.

Cultures

The lactobacilli and yeasts to be used for inoculation and fermentation of the maize had been previously isolated from fermented maize dough and identified (Halm *et al.* 1993; Jespersen *et al.* 1994). They were preserved as freeze-dried cultures and stored at −18°C. They had also been further characterized by molecular biological methods by Hayford *et al.* (1999), Hayford and Jespersen (1999) and Hayford and Jakobsen (1999). One culture of lactic acid bacteria, *L. fermentum*, *coded* LB-11 and two cultures of yeasts, *S. cerevisiae* and *C. krusei*, coded 26-1-11 and 18A-3 respectively, were used in inoculation trials.

Preparation of inocula

Stock cultures of *L. fermentum*, *S. cerevisiae* and *C. krusei* were subcultured in duplicate at 30°C for 24 h in de Man, Rogosa and Sharpe medium (MRS broth, MERCK) in the case of *L. fermentum*, and in malt yeast peptone broth (MYPG, Difco) in the case of the yeasts. The cultures were successively subcultured to final volumes of 1 l with concentration of at least 10^8 cells per ml for *L. fermentum* and at least 10^7 cells per ml in the case of the yeasts. Cell concentrations were estimated by microscopy. The cultures were centrifuged at 4500 × *g* for 15 min, washed in distilled water and centrifuged again. The pelleted cells were added to steep water to give the required concentration of 10^7 cells per ml for *L. fermentum* and 10^6 cells per ml for each of *S. cerevisiae* and *C. krusei*.

Experimental design

Fermentation experiments were conducted in duplicate on two separate occasions. Results of all analysis represent the mean values of four replicate trial fermentations with duplicate determinations.

Fermentation of maize dough

Four 5-kg batches of cleaned whole maize kernels were steeped in 7·5-l water in 15-l capacity aluminium bowls. For three of the batches the steep water had been inoculated to attain concentrations of 10^7 cells per ml for *L. fermentum* and 10^6 cells per ml for each of *S. cerevisiae* and *C. krusei*, respectively. One batch of maize was not inoculated and served as a control (i.e. spontaneous fermentation). The batches of maize were left to steep at room temperature (28–30°C) for 24 h. The steep water was then decanted and the maize milled in the Food Research Institute, pilot plant corn mill (Disc Attrition Mill, Rajan Universal, Chennai, India) to an average particle size of about 0·3 mm. The mill had previously been cleaned and disinfected with ethanol and distilled water before and after use. It was similarly cleaned and dried in between milling of different fermented samples. The meal was mixed with water and kneaded into a dough of 50 ± 2% moisture content. The doughs were smoothed at the surface by hand and allowed to ferment at room temperature (28–30°C) for 72 h. Surface layers of the fermented dough samples were removed at the end of the required fermentation period, as is the practice in food use, before analysis. A final pH of 3·7 ± 0·1 after 72 h in the control spontaneous fermentation was used as an index of adequate fermentation for all experiments. Traditional spontaneously produced Ghanaian fermented maize dough has a typical pH of 3·7 and a moisture content of 50 ± 2% (Plahar and Leung 1982).

Determination of dough acidity

Titratable acidity and pH measurements were determined in 10% (w/v) slurries of dough samples in distilled water. After stirring for 30 min, 10-ml aliquots of filtrate obtained using Whatman No. 1 filter paper (Whatman International Ltd, Maidstone, England) were titrated against 0·1 N NaOH to determine acidity while pH was measured with a pH meter (H35010, HANNA Instruments, Ronchidivillafranca, Italy) calibrated with known buffers. Acidity was expressed as lactic acid based on the conversion of 1-ml 0·1 N NaOH being equivalent to $9{\cdot}008 \times 10^{-3}$ g lactic acid.

Microbiological analysis

Microbiological analysis was undertaken using methods described by Halm *et al.* (1996). Surface layers were asceptically removed before sampling. Ten grams of dough samples were homogenized in 90-ml sterile diluent (0·1% peptone water, 0·8% NaCl, pH 7·2) using a Stomacher (Lab Blender, Model 4001, Seward Medical, London, England) for 30 s at 'normal speed'. Ten-fold dilutions were then prepared and pour plate technique carried out using the following media and incubation conditions: Lactic acid bacteria were enumerated in universal beer agar (UBA, CM 651 Oxoid, Hampshire, England) incubated anaerobically (Anaerocult^R A, Merck) at 30°C for 7 days. Yeasts were enumerated in malt agar (Merck 5398) containing 100 mg l^{-1} chloramphenicol (Chloramphenicol Selective Supplement, Oxoid) and 50 mg l^{-1} chlortetracycline (Sigma Chemical Co., St Louis, MO, USA) incubated at 25°C for 7 days.

Determination of volatile components

Extraction of aroma components in maize dough samples was performed by the Likens-Nickerson method (Nickerson and Likens 1966) using a micro-scale steam distillation low-density solvent extraction device (micro-SDE, Chrompack, Middelburg, the Netherlands). The extraction procedure was conducted using 18 g maize dough diluted with 400 g distilled water to obtain 25% slurries of samples (w/v) based on 50% moisture content of dough. One millilitre internal standard solution (50 ppm, 4-methyl-1-pentanol in H_2O) was added to the fermented sample slurry in a 1-l Erlenmeyer flask. Six millilitres of a mixture of pentane and diethyl ether (1 : 1) were placed in a 9-ml pear-shaped solvent flask. Both flasks was appropriately connected to the distillation apparatus and the solutions brought to the boil. Extraction of volatiles was carried out for 30 min, from the beginning of condensation of vapours on the walls of the condenser. The Likens-Nickerson procedure can be used as the Ghanaian fermented maize dough is boiled in the preparation of traditional foods. Freezing out water present at −18°C purified the collected solvent phase. The solvent extract was poured off, dried over *ca*. 2g of Na_2SO_4 and concentrated to about 100 mg by gently blowing N_2 gas over the surface. The concentrated extract was analysed for volatile compounds using the gas chromatography–mass spectrometry (GC-MS).

Separation and identification of volatiles in extracts of fermented maize dough samples were carried out on a Hewlett–Packard G1800A GCD System (GC-MS, Hewlett–Packard, Palo Alto, CA, USA). The instrument was equipped with a Hewlett–Packard DB-WAX column (30 m × 0·25 μm i.d., × 025 mm film thickness). Two-microlitre extracts were injected (split ratio 1 : 20) using the temperature programme: 10 min at 40°C, increased to 240°C at 6°C min^{-1} and held constant at 240°C for 30 min. Identification of aroma compounds was determined in the

Total Ion mode scanning a mass to charge ratio (m/z) of range between 25 and 550. Further identification was obtained by probability-based matching with mass spectra in the G1033A NIST PBM Library (Hewlett–Packard) containing 75 000 reference spectra as well as by matching with the mass spectra and retention indices of the standard reference compounds used.

Quantification of aroma compounds was carried out by spiking separate slurries of 72-h fermented maize dough samples (25% w/v) with different concentrations of standard reference compounds. The standard reference compounds were purchased as pure compounds with purities $\geq 97\%$ (Fluka Chemie, Steinheim, Germany; Sigma Chemical Co. St Louis, MO, USA; Merck, Darmstdt, Germany and Aldrich Chemie, Steinheim, Germany). The concentrations prepared were 0·001, 0·01, 0·1, 1·0, 10·0 and 1000 p.p.m in the case of acetic acid. Extracts of the standard reference aroma compounds were obtained by the Likens-Nickerson distillation method for both the spiked and unspiked slurries and analysed by GC-MS. The unspiked slurries served as blanks in the calculation of peak areas in spiked samples. Plots of relative peak areas (peak area of compound/peak area of internal standard) vs concentrations in p.p.m were generated and used to calculate the concentrations of aroma compounds in fermented maize dough samples. Concentrations were then expressed in mg kg^{-1} of dough.

Gas chromatography-olfactometry – 'GC-sniffing'

Extracts of maize dough samples fermented spontaneously and with starter cultures for 48 h were prepared by the Likens-Nickerson simultaneous distillation and extraction technique, as described above. The extracts were sniffed on a Hewlett–Packard 5890 GC (Hewlett–Packard GmbH, Hewlett–Packard-Strasse 8, D-76337 Waldronn, Germany) equipped with an SGE Olfactory Detector Outlet ODO-1 (SGE Ltd, 7, Argent Place, Ringwood, Australia). The instrument was equipped with the DB-WAX column (J & W Scientific, Folson, CA, USA; 30 m $\times$ 0·25 mm i.d., 0·25 μm film thickness). Two microlitre extracts were injected using the temperature programme: 10 min at 40°C, increased to 240°C at 6°C min^{-1} and held constant at 240°C for 30 min. The GC was operated using the splitless injection method. The extracts were sniffed by four trained judges. They were instructed to note the start time of each odour, description of the odour quality (using expressions of their choice) and to evaluate the intensity of the odour on a scale from 1 to 5. Each sniffing session continued for 40 min. A model solution of 12 aroma compounds, diacetyl, ethyl butanoate, hexanal, 2-heptanone, octanal, 2-methoxy-3,5-isopropylpyrazine, linalool, phenylacetaldehyde, β-demascenone, 2-phenylethanol, octanoic acid and eugenol, (Sigma) was previously sniffed and analysed by GC-MS. From the GC-MS runs, retention indices were calculated and the 12 compounds used as references for calculation (linear interpolation) of retention indices of the odour signals detected by the judges.

Statistical analysis

Statistical difference between mean values were determined by analysis of variance (ANOVA) and least significance difference using the SAS Statistical Software package (SAS Institute Inc. Release 8.1, Cary, NC, USA). Multivariate data analysis using the partial least squares regression method was carried out on data of relative concentrations of aroma volatiles. The Unscrambler Software 7·51 package was used (CAMO ASA, Oslo, Norway).

RESULTS

Acidity and microbial growth during spontaneous and starter culture fermentation of maize dough

Results of acidity development and microbial growth patterns in maize dough fermented spontaneously and with starter cultures are shown in Table 1. As seen from the table a reduction in fermentation time based on a decrease in pH was attained with starter cultures of *L. fermentum* and *C. krusei*. Addition of these starter cultures to the already existing microflora in spontaneous maize fermentation achieved in 24 h similar pH levels as maize dough fermented for 48 h without added starter culture. The decline in pH in dough with added *S. cerevisiae* starter culture was not significantly different ($P < 0·05$) from dough fermented spontaneously, although a lower pH was attained with *S. cerevisiae* after 72 h. The concentration of lactic acid was highest in dough fermented with *L. fermentum* and lowest in spontaneously fermented dough. Levels of lactic acid in fermentations with *S. cerevisiae* and *C. krusei* were not significantly different ($P < 0·05$) from each other (Table 1).

The population of lactic acid bacteria reached final counts of 10^{10} CFU g^{-1} in fermentations with *L. fermentum* and 10^{9} CFU g^{-1} in the yeast and spontaneously fermented dough samples (Table 1). Yeast counts in fermentations with added *S. cerevisiae* and *C. krusei* increased in both cases from initial concentrations of 10^{6} CFU g^{-1} to 10^{7} CFU g^{-1} after 24 h of fermentation while maximum counts were 10^{6} CFU g^{-1} in doughs fermented spontaneously and with *L. fermentum* starter culture. The effects of added starter culture on the microbial population were not observed during the steeping stage i.e. 0 h of fermentation but increases occurred after 24 h of dough fermentation. Higher counts of lactic acid

Table 1 Acidity values and microbial counts during spontaneous and starter culture fermentation of maize dough*

	Fermentation time (h)	Type of fermentation			
		Spontaneous	*Lactobacillus fermentum*	*Saccharomyces cerevisiae*	Candida krusei
pH	0	$6{\cdot}13 \pm 0{\cdot}09^{a}$	$5{\cdot}91 \pm 0{\cdot}04^{a}$	$6{\cdot}10 \pm 0{\cdot}02^{a}$	$6{\cdot}01 \pm 0{\cdot}02^{a}$
	24	$3{\cdot}90 \pm 0{\cdot}04^{a}$	$3{\cdot}80 \pm 0{\cdot}01^{b}$	$3{\cdot}88 \pm 0{\cdot}01^{a}$	$3{\cdot}83 \pm 0{\cdot}01^{c}$
	48	$3{\cdot}80 \pm 0{\cdot}01^{a}$	$3{\cdot}66 \pm 0{\cdot}07^{b}$	$3{\cdot}77 \pm 0{\cdot}02^{a}$	$3{\cdot}78 \pm 0{\cdot}01^{a}$
	72	$3{\cdot}79 \pm 0{\cdot}01^{a}$	$3{\cdot}53 \pm 0{\cdot}02^{b}$	$3{\cdot}73 \pm 0{\cdot}01^{c}$	$3{\cdot}72 \pm 0{\cdot}01^{c}$
Total acidity	0	$0{\cdot}22 \pm 0{\cdot}07^{a}$	$0{\cdot}27 \pm 0{\cdot}02^{a}$	$0{\cdot}25 \pm 0{\cdot}01^{a}$	$0{\cdot}25 \pm 0{\cdot}01^{a}$
(% as lactic acid)	24	$0{\cdot}61 \pm 0{\cdot}07^{a}$	$0{\cdot}84 \pm 0{\cdot}02^{b}$	$0{\cdot}76 \pm 0{\cdot}02^{c}$	$0{\cdot}88 \pm 0{\cdot}04^{b}$
	48	$0{\cdot}85 \pm 0{\cdot}01^{a}$	$0{\cdot}98 \pm 0{\cdot}01^{b}$	$0{\cdot}88 \pm 0{\cdot}01^{c}$	$0{\cdot}91 \pm 0{\cdot}01^{d}$
	72	$0{\cdot}86 \pm 0{\cdot}01^{a}$	$1{\cdot}04 \pm 0{\cdot}04^{b}$	$0{\cdot}92 \pm 0{\cdot}04^{c}$	$0{\cdot}94 \pm 0{\cdot}04^{c}$
Lactic acid bacteria (CFU g^{-1})	0	$4{\cdot}5 \times 10^{7} \pm 1{\cdot}3^{a}$	$5{\cdot}3 \times 10^{7} \pm 1{\cdot}8^{a}$	$6{\cdot}0 \times 10^{6} \pm 1{\cdot}2^{b}$	$4{\cdot}6 \times 10^{6} \pm 2{\cdot}5^{b}$
	24	$6{\cdot}1 \times 10^{8} \pm 3{\cdot}2^{a}$	$9{\cdot}0 \times 10^{9} \pm 2{\cdot}6^{b}$	$3{\cdot}4 \times 10^{8} \pm 0{\cdot}4^{a}$	$1{\cdot}6 \times 10^{9} \pm 0{\cdot}6^{b}$
	48	$2{\cdot}2 \times 10^{9} \pm 1{\cdot}6^{a}$	$1{\cdot}3 \times 10^{10} \pm 2{\cdot}5^{b}$	$7{\cdot}4 \times 10^{8} \pm 2{\cdot}6^{c}$	$2{\cdot}0 \times 10^{9} \pm 1{\cdot}0^{a}$
	72	$3{\cdot}0 \times 10^{9} \pm 1{\cdot}5^{a}$	$1{\cdot}1 \times 10^{10} \pm 0{\cdot}1^{b}$	$9{\cdot}0 \times 10^{9} \pm 5{\cdot}3^{a}$	$1{\cdot}2 \times 10^{9} \pm 2{\cdot}8^{a}$
Yeasts (CFU g^{-1})	0	$9{\cdot}0 \times 10^{4} \pm 1{\cdot}0^{a}$	$1{\cdot}0 \times 10^{5} \pm 0{\cdot}5^{a}$	$1{\cdot}4 \times 10^{6} \pm 3{\cdot}6^{b}$	$1{\cdot}0 \times 10^{6} \pm 0{\cdot}1^{b}$
	24	$1{\cdot}5 \times 10^{6} \pm 2{\cdot}7^{a}$	$2{\cdot}6 \times 10^{6} \pm 1{\cdot}5^{a}$	$1{\cdot}9 \times 10^{7} \pm 0{\cdot}3^{b}$	$4{\cdot}0 \times 10^{7} \pm 3{\cdot}0^{b}$
	48	$9{\cdot}8 \times 10^{6} \pm 4{\cdot}8^{a}$	$4{\cdot}1 \times 10^{6} \pm 1{\cdot}5^{a}$	$1{\cdot}8 \times 10^{7} \pm 0{\cdot}8^{b}$	$1{\cdot}0 \times 10^{7} \pm 1{\cdot}7^{b}$
	72	$7{\cdot}2 \times 10^{6} \pm 3{\cdot}2^{a}$	$3{\cdot}5 \times 10^{6} \pm 2{\cdot}5^{a}$	$2{\cdot}2 \times 10^{7} \pm 0{\cdot}7^{b}$	$1{\cdot}5 \times 10^{7} \pm 0{\cdot}2^{b}$

*Values are mean of determinations from two separate fermentation trials. Mean values with same letter in a row are not significantly different ($P < 0{\cdot}05$).

bacteria were observed in the presence of added *C. krusei* than in the presence of *S. cerevisiae* after 24 and 48 h (Table 1).

Volatile compound development in maize dough fermented spontaneously and with starter cultures

The maximum concentrations of volatile compounds attained during spontaneous fermentations of maize dough and fermentations with starter cultures are shown in Table 2. A total of 64 compounds were identified, excluding long chain (C > 12) fatty acids and esters. The compounds shown in Table 2 comprised 20 alcohols, 22 carbonyls, 11 esters, seven acids, a furan and three phenolic compounds. Ethanol, which was the alcohol produced in highest amounts, was higher in dough fermented spontaneously and with *S. cerevisiae* than with *C. krusei* or *L. fermentum*. The fusel alcohols, 1-propanol, 2-methyl-1-propanol and 3-methyl-butanol were found in highest amounts in fermentations with *S. cerevisiae* while phenylethyl alcohol was found in highest amounts in fermentations with *C. krusei*. Among esters, ethyl acetate, the most abundant ester formed, was highest in fermentations with *S. cerevisiae* while levels in the other fermentations were not significantly different from each other (Table 2). Acetic acid was highest in fermentations with *L. fermentum* reaching levels that were double that in spontaneous fermentations. Higher levels of acetic acid were formed in fermentations with *C. krusei* than with *S. cerevisiae*, which produced amounts that were not significantly different ($P < 0{\cdot}05$) from those in spontaneously fermented maize dough (Table 2).

The development of groups of volatile compounds is shown in Fig. 1. Alcohols, excluding ethanol, showed increasing concentrations with time in fermentations with *S. cerevisiae* and *C. krusei* while levels in spontaneously fermented maize dough and fermentations with *L. fermentum* tended to reach maximum levels after 1 day. With the exception of *L. fermentum*, the fermentations indicated a decrease in level of carbonyls with time of fermentation. Highest amounts of carbonyls were observed in spontaneous fermentations attaining a peak after 2 days. As seen from Fig. 1, the concentration of total esters were high for fermentations with *S. cerevisiae* and a pronounced increase was observed from day 2 to day 3. Organic acids (Fig. 1), with the exception of acetic acid, were low in fermentations with *L. fermentum*. Although fermentations with *C. krusei* attained the highest level of organic acids after day 1, a pronounced drop occurred throughout the rest of the fermentation period. The levels of organic acids were similar in fermentations with spontaneously fermented dough and *S. cerevisiae* both reaching maximum levels after 2 days (Fig. 1).

Partial least regression analysis (Fig. 2) of the data from aroma analyses conducted on days 1, 2 and 3 showed that the data separate into three groups relative to two principal components (PC1 vs PC2). Group 1: Sp, spontaneously fermented; group 2: Sc, fermented by *S. cerevisiae*; and group 3: Lf, Ck, fermented by *L. fermentum* or *C. krusei*. The trend of increasing relative concentration of volatiles,

Table 2 Comparison of maximum concentrations attained for volatile compounds (mg kg^{-1}) during starter culture fermentations of maize dough*

Compound	Types of fermentation			
	Spontaneous	*Lactobacillus fermentum*	*Saccharomyces cerevisiae*	*Candida krusei*
Alcohols				
Ethanol	612 (2)^{a}	494 (3)^{b}	584 (1)^{a}	480 (2)^{b}
Propanol	2·50 (2)^{a}	1·35 (2)^{b}	4·53 (3)^{c}	1·39 (3)^{b}
2-Methyl-1-propanol	4·93 (2)^{a}	2·67 (2)^{b}	30·1 (3)^{c}	3·31 (2)^{b}
2-Pentanol	0·19 (2)^{a}	0·19 (2)^{a}	0·18 (1)^{a}	0·15 (2)^{a}
1-Butanol	0·30 (3)^{a}	0·42 (1)^{b}	0·19 (0)^{c}	0·73 (0)^{d}
1-Penten-3-ol	0·65 (3)^{a}	1·81 (1)^{b}	0·51 (2)^{c}	0·51 (1)^{c}
3-Methyl-butanol	23·6 (1)^{a}	50·8 (3)^{b}	418 (3)^{c}	162 (3)^{d}
1-Pentanol	1·02 (1)^{a}	0·78 (1)^{a}	0·86 (1)^{a}	0·80 (1)^{a}
1-Hexanol	37·4 (1)^{a}	28·2 (3)^{b}	33·1 (1)^{a}	32·3 (1)^{a}
1-Octen-3-ol	0·20 (2)^{a}	0·21 (1)^{a}	0·21 (2)^{a}	0·23 (1)^{a}
Heptanol	0·38 (3)^{a}	0·26 (1)^{b}	0·31 (1)^{b}	0·31 (1)^{b}
1-Octanol	14·0 (1)^{a}	17·9 (3)^{a}	36·1 (3)^{b}	16·8 (2)^{a}
2-Undecen-1-ol	1·42 (2)^{a}	1·45 (1)^{a}	0·77 (2)^{a}	1·34 (1)^{a}
Nonanol	27·6 (1)^{a}	73·8 (2)^{bc}	83·9 (2)^{b}	59·8 (2)^{c}
3-Nonenol	0·18 (3)^{a}	0·03 (3)^{b}	nd	nd
2-Nonenol	0·45 (2)^{a}	nd	nd	0·20 (1)^{b}
Phenylethyl alcohol	0·31 (3)^{a}	0·97 (2)^{b}	4·35 (3)^{c}	13·1 (3)^{d}
2,4-Decadienol	0·54 (2)^{a}	0·36 (2)^{b}	0·74 (3)^{a}	nd
Nerolidol	0·33 (3)^{a}	0·45 (2)^{b}	0·39 (3)^{a}	0·33 (0)^{a}
Carbonyls				
3-methyl-butanal	1·26 (2)^{a}	1·29 (1)^{a}	1·54 (0)^{a}	0·85 (0)^{b}
Diacetyl	0·51 (3)^{a}	0·28 (1)^{b}	0·32 (1)^{b}	0·33 (1)^{b}
Pentanal	1·97 (2)^{a}	0·84 (1)^{b}	1·08 (1)^{b}	1·14 (3)^{b}
Hexanal	6·61 (1)^{a}	2·67 (0)^{b}	2·28 (0)^{b}	4·39 (1)^{c}
2-Pentenal	nd	0·33 (1)^{ab}	0·23 (2)^{a}	0·38 (1)^{b}
2-Heptanone	3·42 (0)^{a}	1·33 (1)^{b}	0·80 (0)^{b}	1·79 (3)^{b}
Heptanal	8·71 (0)^{a}	6·50 (0)^{b}	6·47 (0)^{b}	8·29 (0)^{a}
1-Octen-3-one	0·11 (2)^{a}	nd	0·09 (1)^{a}	0·04 (1)^{b}
Nonanal	84·4 (0)^{ac}	46·0 (0)^{b}	122 (0)^{b}	109 (0)^{bc}
Furfural	9·66 (2)^{a}	7·66 (2)^{b}	10·3 (3)^{a}	7·25 (2)^{b}
2-Octenal	28·1 (2)^{a}	19·9 (1)^{b}	16·1 (1)^{b}	20·2 (1)^{b}
2,4-Heptadienal	0·09 (2)^{a}	0·05 (1)^{ab}	0·03 (2)^{b}	0·04 (1)^{b}
Benzaldehyde	0·08 (1)^{a}	0·08 (2)^{a}	0·24 (1)^{b}	0·16 (2)^{b}
2-Nonenal	0·14 (2)^{a}	0·10 (1)^{a}	0·10 (2)^{a}	0·10 (1)^{a}
Benzeneacetaldehyde	0·10 (3)^{a}	0·03 (3)^{b}	0·12 (3)^{a}	0·24 (3)^{c}
2-Decenal	0·68 (2)^{a}	0·40 (1)^{b}	0·35 (2)^{b}	0·38 (1)^{b}
2,4-Nonadienal	0·21 (2)^{a}	0·05 (1)^{b}	0·03 (1)^{b}	0·06 (1)^{b}
2-Undecenal	1·42 (2)^{a}	0·62 (1)^{b}	0·39 (1)^{b}	0·54 (1)^{b}
2,4-Decadienal, (E,E)-	1·73 (2)^{a}	1·30 (1)^{ab}	0·99 (1)^{b}	1·28 (1)^{ab}
2,4-Decadienal, (E,Z)-	7·49 (2)^{a}	4·78 (1)^{b}	3·60 (1)^{b}	4·34 (1)^{b}
.gamma.-nonalactone	0·75 (3)^{a}	1·23 (1)^{a}	0·77 (3)^{a}	0·67 (3)^{a}
Pentadecanl	0·21 (3)^{a}	nd	0·24 (3)^{a}	nd
Esters				
Ethyl acetate	16·5 (2)^{a}	15·3 (1)^{a}	38·8 (3)^{b}	16·8 (1)^{a}
Ethyl propionate	0·15 (3)^{a}	0·61 (2)^{b}	7·15 (3)^{c}	0·74 (2)^{b}
Isoamylacetate	0·24 (3)^{a}	1·35 (1)^{b}	1·71 (3)^{b}	nd
Ethyl hexanoate	0·07 (3)^{a}	0·01 (3)^{b}	0·02 (3)^{b}	0·01 (3)^{b}
Hexyl acetate	0·11 (3)^{a}	0·10 (2)^{a}	0·15 (3)^{a}	1·84 (2)^{b}
Ethyl lactate	2·12 (2)^{a}	1·65 (2)^{a}	6·39 (3)^{b}	2·00 (2)^{a}
Ethyl heptanoate	0·02 (2)^{a}	0·01 (2)^{a}	0·01 (2)^{a}	nd

Table 2 (*Contd.*)

Compound	Types of fermentation			
	Spontaneous	*Lactobacillus fermentum*	*Saccharomyces cerevisiae*	*Candida krusei*
Heptyl acetate	nd	0·06 (2)[a]	0·11 (3)[a]	nd
Ethyl octanoate	0·03 (2)[a]	0·05 (3)[a]	0·14 (3)[b]	nd
Ethyl nonanoate	nd	0·01 (2)[a]	0·02 (2)[a]	nd
Ethylphenyl acetate	nd	0·09 (2)[a]	0·13 (3)[a]	0·08 (3)[a]
Ethyl dodecanoate	nd	0·01 (2)[a]	1·24 (3)[b]	0·31 (2)[c]
Ethylphenyl propionate	nd	nd	0·16 (3)[a]	0·11 (3)[a]
Acids				
Acetic acid	1102 (2)[a]	2348 (1)[b]	1036 (1)[a]	1616 (1)[c]
Propionic acid	0·87 (3)[a]	0·80 (2)[a]	0·93 (2)[a]	0·99 (1)[a]
Pentanoic acid	1·17 (2)[a]	0·94 (2)[a]	0·83 (1)[a]	0·14 (2)[b]
Hexanoic acid	7·35 (3)[a]	5·77 (1)[b]	6·51 (2)[b]	6·25 (1)[b]
Heptanoic acid	1·88 (2)[a]	1·08 (2)[a]	1·30 (2)[a]	1·18 (1)[a]
Octanoic acid	3·12 (2)[a]	1·98 (3)[b]	4·36 (2)[c]	2·93 (2)[a]
Nonanoic acid	0·52 (2)[a]	0·08 (2)[b]	0·39 (2)[a]	3·74 (1)[c]
Furan, phenols				
2-Pentyl furan	5·04 (0)[a]	1·77 (1)[b]	1·80 (2)[b]	3·82 (3)[a]
Guaiacol	1·57 (1)	nd	nd	nd
4-Ethyl-guaiacol	0·01 (2)[a]	nd	0·03 (1)[a]	nd
4-Vinyl-guaiacol	0·02 (2)[a]	0·64 (2)[b]	0·77 (0)[b]	nd

*Values in parenthesis indicate fermentation time in days. Mean values with the same letter in a row are not significantly different ($P < 0·05$). Results are mean from two separate fermentation trials. nd = not detected.

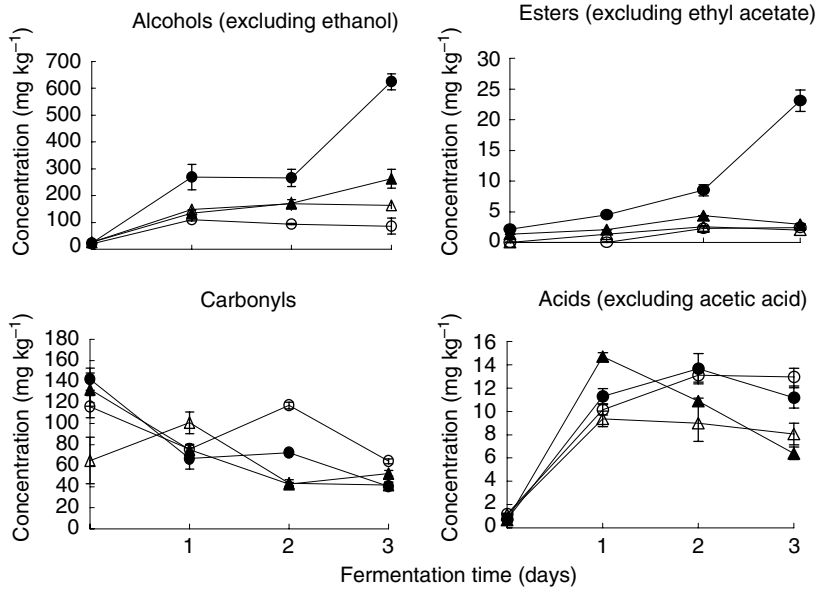

Fig. 1 Development of groups of volatile compounds during fermentation of maize dough without starter cultures (spontaneous) and with addition of starter cultures of *Lactobacillus fermentum*, *Saccharomyces cerevisiae* and *Candida krusei*, respectively. Symbol for starter cultures: (○) no starter culture (spontaneous), (Δ) *L. fermentum*, (●) *S. cerevisiae* and (▲) *C. krusei*. Bars indicate standard deviations of mean values from two separate fermentation trials

particularly esters and alcohols, with fermentation time could also be observed in the bi-plot for doughs with added *S. cerevisiae*. As shown by their closeness to the centre of the plot samples fermented by *S. cerevisiae* for day 1 and day 2 are poorly described in the analysis. However, fermentation after day 3 shows a distinct character attributable to a high concentration of esters. These esters included ethyl acetate, ethyl propionate, isoamyl acetate, hexyl acetate, ethyl lactate, heptyl acetate, ethyl octanoate, ethyl dodecanoate and ethylphenyl propionate. The fusel alcohols, 1-propanol, 2-methyl-propanol and 3-methyl-butanol, were also found in higher concentrations in maize dough samples with added

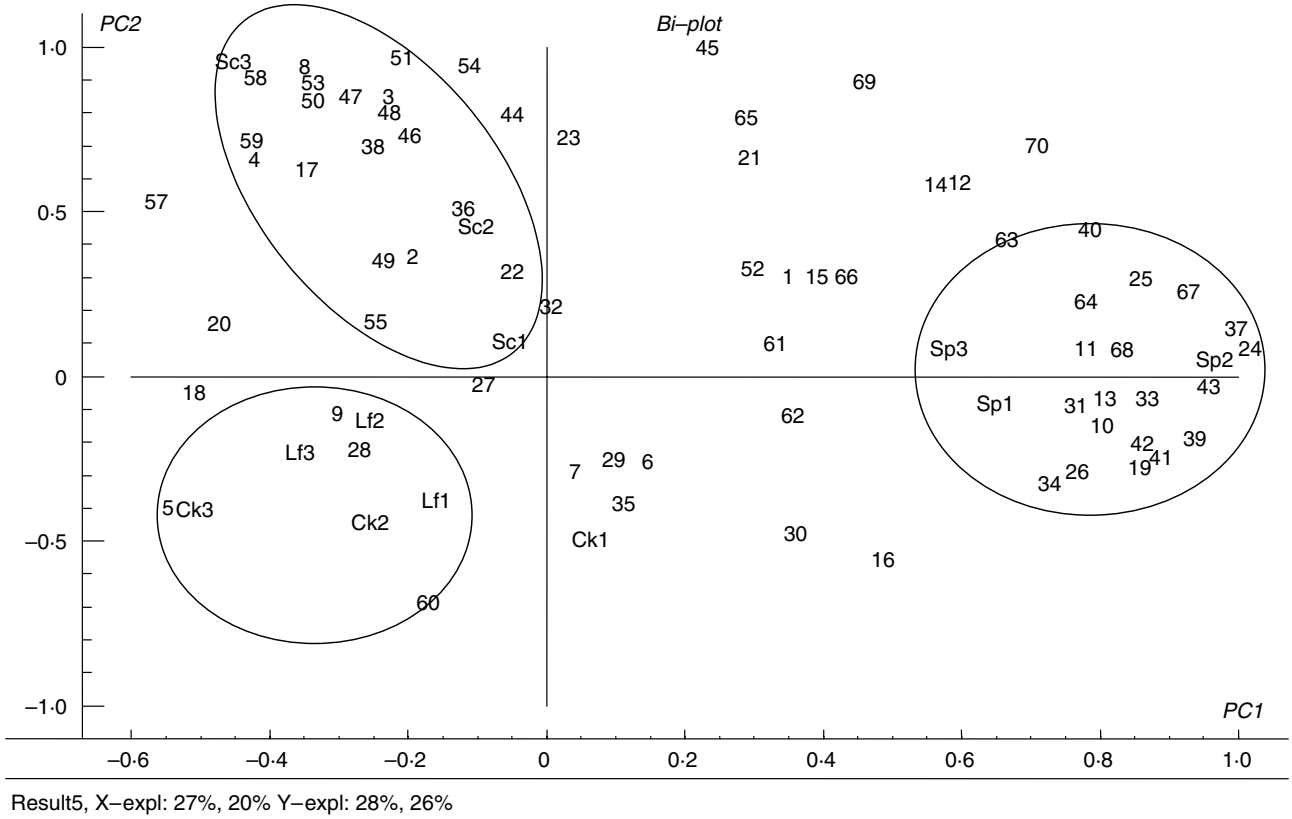

Fig. 2 Bi-plot (PC1 vs PC2) from partial least squares regression analysis of relative concentrations of volatiles in fermented maize dough samples, Sp = spontaneously fermented, Lf, Sc, and Ck = fermented by *Lactobacillus fermentum*, *Saccharomyces cerevisiae* and *Candida krusei*, respectively. The assigned numbers 1, 2 and 3 respresent duration of fermentation in days. Volatiles are described by numbers: 1, Ethanol; 2, Propanol; 3, 2-methylpropanol; 4, 3-pentanol; 5, 2-pentanol; 6, 1-Butanol; 7, Heptanal; 8, 3-methylbutanol; 9, 3-methyl-3-butenol; 10, 1-pentanol; 11, 2-pentenol; 12, 1-hexanol; 13, 1-octen-3-ol; 14, Heptanol; 15, 1-octanol; 16, 2-undecenol; 17, Nonanol; 18, 3-nonenol; 19, 2-nonenol; 20, Phenylethyl alcohol; 21, 2,4-decadienol; 22, Nerolidol; 23, 3-methylbutanal; 24, Diacetyl; 25, Pentanal; 26, Hexanal; 27, 2-pentenal; 28, 2-Heptanone; 29, Heptanol; 30, 1-octen-3-one; 31, Nonanal; 32, Furfural; 33, 2-octenal; 34, 2,4heptadienal; 35, Decanal; 36, Benzaldehyde; 37, 2-nonenal; 38, Phenylacetaldehyde; 39, 2-Decenal; 40, 2,4-nonadienal; 41, 2-undecenal; 42, 2,4-decadienal; 43, trans,trans-decadienal; 44, furanone; 45, Pentadecanal; 46, Ethyl acetate; 47, Ethyl propionate; 48, Isoamylacetate; 49, Ethyl hexanoate; 50, Hexyl acetate; 51, Ethyl lactate; 52, Ethyl heptanoate; 53, Heptyl acetate; 54, Ethyl octanoate; 55, Ethyl nonanoate; 56, Ethyl decanoate; 57, Ethylphenyl acetate; 58, Ethyl dodecanoate; 59, Ethylphenyl propionate; 60, Acetic acid; 61, Propionic acid; 62, Pentanoic acid; 63, Hexanoic acid; 64, Heptanoic acid; 65, Octanoic acid; 66, Nonanoic acid; 67, 2-pentylfuran; 68, Guaiacol; 69, 4-ethyl guaiacol; 70, 4-vinyl guaiacol

S. cerevisiae. Fermentations with *L. fermentum* and *C. krusei* were characterized by higher concentrations of acetic acid and low concentrations of most volatiles produced. In comparison, spontaneously fermented maize dough samples showed higher concentrations of carbonyl compounds and alcohols, particularly, diacetyl, which was characteristic of samples fermented for 2 days. The alcohols in spontaneously fermented samples included 1-pentanol, 2-penten-1-ol, hexanol, 1-octen-3-ol, 1-octanol, heptanol and 2,4-decadienol. A few acids, namely, pentanoic, hexanoic and heptanoic acids, were also found to be characteristic of spontaneously fermented samples as well as the phenolic compounds guaiacol and 4-ethyl guaiacol.

Identification of aroma compounds by GC-olfactometry

A total of 51 aroma compounds identified by GC-MS could also be detected by sniffing the different dough samples (Table 3). Some odours detected by sniffing could not be related with compounds found by GC-MS or corresponded to regions where no compounds were found by GC-MS. In general, concentrations of aroma compounds corresponded well with intensity of aroma notes perceived by the judges. Samples of maize dough fermented spontaneously recorded the highest number of alcohols and aldehydes detected by sniffing whilst fermentations with *S. cerevisiae* recorded the

Table 3 Identification of aroma volatiles during spontaneous and starter culture fermentation of maize dough for 48 h by method of gas chromatography olfactometry (GC-sniffing)*

Compound	No. of judges†				Odour description	Average Intensity ‡§			
	Sp	Lf	Sc	Ck		Sp	Lf	Sc	Ck
Alcohols									
Ethanol	2	2	3	2	Alcohol, fruity	2·0[a]	0·7[b]	2·0[a]	1·0[b]
1-Propanol	2	3	2	4	Alcoholic, fruity, gum	2·0[a]	1·0[b]	2·0[a]	1·5[c]
2-Methyl-propanol	1	2	2	2	Sweet smell, fruity	1·0[a]	0·5[b]	2·0[c]	1·5[d]
2-Pentanol	–	3	–	–	Gum, fruity	nd	1·0	nd	nd
1-Butanol	2	1	3	2	Pungent, rubber	1·0[a]	0·5[b]	1·5[c]	1·0[a]
1-Penten-3-ol	1	–	–	1	Boiled potatoes	2·0[a]	nd	nd	1·0[b]
3-Methyl-butanol	2	2	4	4	Vegetables, green	0·5[a]	1·7[b]	2·3[c]	1·6[b]
Hexanol	2	2	2	3	Sweet, flower	1·0[a]	0·5[b]	1·5[c]	1·7[c]
Heptanol	1	–	–	–	Alcohol	2·0	nd	nd	nd
1-Octanol	2	3	–	2	Orange, sweet, fruit	2·0[a]	1·0[b]	nd	1·0[b]
Nonanol	4	4	–	3	Popcorn, vitamin pill	3·0[a]	2·6[b]	nd	2·6[b]
Trans-2-undecenol	2	–	–	–	Mouldy, wet soil	3·0	nd	nd	nd
Phenylethyl alcohol	3	3	4	3	Flowers, rose	2·0[a]	1·2[b]	2·2[a]	2·6[c]
Nerolidol	4	2	1	–	Dill, dried, green	2·5[a]	1·0[b]	1·0[b]	nd
Carbonyls									
3-Methyl-butanal	3	2	2	2	Bread, sweet, flower	2·0[a]	0·7[b]	1·0[b]	1·7[c]
Hexanal	4	4	4	4	Green, grass, pine	2·0[a]	1·4[b]	1·5[b]	1·5[b]
2-Heptanone	1	3	1	2	Bad, unpleasant	2·0[a]	0·7[b]	1·0[b]	1·0[b]
Heptanal	2	1	4	2	Green, sourgreen	2·0[a]	1·0[b]	2·0[a]	1·0[b]
Octanal	–	3	–	–	Green, fresh fruit	nd	2·1	nd	nd
1-Octene-3-one	4	3	4	4	Mushrooms	3·3[a]	2·0[b]	2·5[b]	2·0[b]
Nonanal	4	2	4	4	Aquarium, green, fatty	2·5[a]	1·7[b]	2·0[b]	1·3[c]
Trans-2-octenal	4	3	4	4	Bad, peas, cucumber	3·0[a]	2·7[a]	2·8[a]	2·5[a]
2,4-heptadienal	3	2	4	4	Hot, potato, silage	1·5[a]	2·0[b]	2·5[b]	2·0[b]
Benzaldehyde	3	–	–	–	Vegetables, green	2·0	nd	nd	nd
2-Nonenal	4	2	–	4	Old, musty, onions	2·5[a]	2·7[a]	nd	2·0[b]
Phenylacetaldehyde	3	3	3	3	Hyacinth, tulip, rose	3·0[a]	2·2[b]	1·7[c]	1·3[d]
2,4-Nonadienal	4	–	4	3	Fermented, deep frying	3·0[a]	nd	2·7[b]	2·2[c]
2-Undecenal	4	–	3	4	Rice, fruity, cooked	2·0[a]	nd	3·0[b]	1·9[a]
2,4-Decadienal (E,Z)	3	4	3	4	Soup, fatty, dough	2·0[a]	2·2[a]	3·0[b]	1·8[ac]
2.4-Decadienal (E,E)	4	3	–	4	Bacon, lemon, chips	2·0[a]	1·5[b]	nd	2·4[c]
Furanone	–	3	3	3	Oatmeal, cooked peas	nd	1·0[a]	1·7[b]	2·0[c]
Pentadecanal	3	–	–	–	Sweet, spicy	2·0	nd	nd	nd
Esters									
Ethyl acetate	–	4	4	3	Flowery, ester	nd	1·1a	2·0[b]	2·0[b]
Ethyl propionate	3	2	3	3	Fruity, sweet, gum	2·5[a]	2·0[b]	3·0[c]	2·0[b]
Isoamylacetate	–	1	3	–	Fruity	nd	1·0[a]	1·2[a]	nd
Ethyl hexanoate	2	3	4	2	Pear, plant, fruity	3·5[a]	1·8[b]	1·9[b]	2·0[b]
Hexyl acetate	–	3	3	3	Green, onion	nd	0·7[a]	2·0[b]	1·0[a]
Ethyl heptanoate	–	–	2	–	Flowery	nd	nd	2·0	nd
Ethyl lactate	–	1	2	–	Fruity	nd	0·5[a]	2·0[b]	nd
Ethyl laurate	–	–	3	–	Licorice	nd	nd	1·7	nd
Ethylphenylpropionate	–	–	3	–	Flower	nd	nd	2·3	nd
Acids									
Acetic acid	3	4	4	3	Sour, vinegar	2·7[a]	3·5[b]	1·5[c]	1·0[c]
Pentanoic acid	2	–	3	–	Burnt rubber	4·0[a]	nd	2·7[b]	nd
Hexanoic acid	4	2	1	3	Strong, urine-like, hay	4·5[a]	2·3[b]	1·0[c]	2·0[b]
Heptanoic acid	–	2	–	–	Rubber	nd	0·7	nd	nd
Octanoic acid	3	–	–	–	Urine-like	2·0	nd	nd	nd
Nonanoic acid	–	2	2	1	Roast nut, chemical	nd	0·5[a]	2·0[b]	3·0[c]

Table 3 (*Contd.*)

Compound	No. of judges†				Odour description	Average Intensity ‡§			
	Sp	Lf	Sc	Ck		Sp	Lf	Sc	Ck
Others									
2-Pentyl-furan	3	2	4	4	Sweet, liquorice, hay	$2{\cdot}0^{a}$	$2{\cdot}1^{a}$	$2{\cdot}0^{a}$	$1{\cdot}6^{b}$
Heptadecane	2	–	–	–	Perfume	2·0	nd	nd	nd
Guaiacol	3	–	–	–	Chemical, hospital	3·5	nd	nd	nd
4-vinylguiacol	3	–	–	–	Old, hospital, raw bacon	3·0	nd	nd	nd
Unidentified peak	2	2	4	2	Eraser, hospital, chemical	$3{\cdot}5^{a}$	$0{\cdot}7^{b}$	$2{\cdot}5^{c}$	$1{\cdot}0^{b}$
Unidentified	3	3	–	–	Linseed oil, insecticide	$3{\cdot}0^{a}$	$2{\cdot}5^{b}$	Nd	nd
Unidentified peak	3	3	–	–	Library, insecticide	$3{\cdot}0^{a}$	$1{\cdot}0^{b}$	Nd	nd
No peak	3	3	4	2	Sour, spoiled fruit	$3{\cdot}0^{a}$	$3{\cdot}0^{a}$	$3{\cdot}0^{a}$	$2{\cdot}0^{b}$
Unidentified	3	3	–	–	Synthetic, smelly	$2{\cdot}1^{a}$	$3{\cdot}0^{b}$	Nd	nd
Unidentified	2	1	–	4	Fruity, candy	$2{\cdot}0^{a}$	$1{\cdot}0^{b}$	nd	$1{\cdot}5^{b}$
No peak	4	3	3	–	Cooked food, meaty	$2{\cdot}0^{a}$	$0{\cdot}5^{b}$	$1{\cdot}3^{c}$	nd

*Sp = spontaneous fermentation, Lf, Sc, Ck = fermentations with *L. fermentum*, *S. cerevisiae*, *C. krusei*, respectively.
†number of judges that could perceive odour.
‡mean odour intensity perception scores recorded by judges (on a scale of 1–5 in increasing order).
§nd = not detected. Mean values with the same letter in a row are not significantly different ($P < 0{\cdot}05$).

lowest number of these compounds. In contrast, the highest number of esters was detected in fermentations with *S. cerevisiae* while spontaneous fermentations recorded the least. Among the alcohols, however, the highest scores for aroma intensity were detected for 2-methyl-propanol and 3-methyl-butanol in fermentations with added *S. cerevisiae*. Acetic acid had the highest intensity score in fermentations with added *L. fermentum* while scores for acetic acid recorded for *S. Cerevisiae* and *C. krusei* were not significantly different ($P < 0{\cdot}05$) from each other. Fewer odours attributable to esters and volatile acids were detected in fermentations with *C. krusei* by GC-sniffing.

DISCUSSION

Changes in dough acidity and microbial growth

The decline in levels of pH with the corresponding rise in amounts of titratable acids in maize dough fermentations with and without starter cultures observed in the present study have been similarly reported by other authors (Plahar and Leung 1982; Jespersen *et al.* 1994; Halm *et al.* 1996; Masha *et al.* 1998; Nche *et al.* 1994; Sanni *et al.* 1994; Hounhouigan *et al.* 1999). Fermentations involving starter cultures of lactic acid bacteria have typically been characterized by drastic drops in pH. Masha *et al.* (1998) reported a drastic decrease in pH from over 5·0 in the unfermented sample to final pH levels of 3·5 in 'Uji', a maize and millet gruel, fermented with pure cultures of lactic acid bacteria and 4·1 in spontaneously fermented 'Uji'. In their studies using six strains of *L. fermentum* and one strain of *S. cerevisiae*, as starter cultures in maize dough fermentations, Halm *et al.* (1996) also observed rapid decreases in pH from over 5·0 at the start of steeping maize kernels to values between 3·65 and 3·81 within 24 h of dough fermentation compared with 3·90 in the spontaneously fermented dough. In the present study, fermentations with *S. cerevisiae* and *C. krusei* had similar pH levels as spontaneously fermented samples with slightly lower levels occurring after 72 h. In maize dough samples inoculated with yeast starter cultures, Nyako and Danso (1991) reported that acidity patterns were not significantly different from spontaneous fermentations. Hounhouigan *et al.* (1999) observed little activity in acid production when *C. krusei* and *S. cerevisiae* were used singly in the fermentation of 'mawe', an African maize product.

The increase in counts of lactic acid bacteria from 10^{7} to 10^{10} CFU g^{-1} in fermentations with *L. fermentum* in the present study was slightly higher than that observed by Halm *et al.* (1996) in studies using different strains of *L. fermentum* in maize dough fermentations. In the present study, the higher counts of lactic acid bacteria recorded in inoculated dough could only be achieved after 48–72 h in spontaneously fermented dough in contrast to 24 h reported by Halm *et al.* (1996). In the presence of yeast starter cultures, the growth of lactic acid bacteria seemed not to be affected and yeast numbers increased from 10^{6} to 10^{7} CFU g^{-1} (Table 1). Studies have shown that growth of lactobacilli are stimulated by yeast species through the release of amino acids, peptides or vitamins (Spicher and Schroeder 1978; Berg *et al.* 1981; Wood and Hodges 1985; Gobetti *et al.* 1994), but such an effect was not observed in the present study.

Volatile compound composition in maize dough fermented spontaneously and with starter cultures

Fermentations of maize dough with single starters of yeast or lactic acid bacteria were significantly different ($P < 0{\cdot}05$) from spontaneous fermentations. Fermented maize dough made from *S. cerevisiae* contained compounds typically reported in the literature as attributable to metabolic active yeasts (Stam *et al.* 1998). These included several esters and the fusel alcohols, 1-propanol, 2-methyl-1-propanol and 3-methyl-butanol. In their studies, Damiani *et al.* (1996) found that fusel alcohols, 2-methyl-1-propanol and 2/3-methyl-1-butanol, with their respective aldehydes and ethyl acetate, were characteristic volatile compounds of sourdough started with fermentative yeasts belonging to the genera *Saccharomyces* and *Hansenula*. Ethyl acetate was also produced in highest amounts with *S. cerevisiae* in the present fermentation study. In general, fermentations with added *L. fermentum* and *C. krusei* produced lower concentrations of most volatile compounds than fermentations with added *S. cerevisiae* (Fig. 1). In sourdough made from single starters, the lactic acid bacteria, *L. brevis*, produced less aroma compounds compared with *S. cerevisiae* (Meignen *et al.* 2001). Hansen and Hansen (1994) also observed lower concentrations of volatile compounds in sourdough bread made with lactic acid bacteria compared with sourdough bread made with yeast.

Concentrations of most volatile organic acids, with the exception of acetic acid, were found to be lowest in fermentations with added *L. fermentum* (Fig. 1 and Table 2). In studies on wheat sourdough, Martinez-Anaya *et al.* (1990) found that lactic acid bacteria did not produce high amounts of C_3–C_6 volatile organic acids when used individually. Higher concentrations were, however, observed when mixed inoculations of *S. cerevisiae* and lactic acid bacteria were used due to a synergistic effect (Martinez-Anaya *et al.* 1990). They attributed this effect to a mutual growth stimulation of lactobacilli and yeast on the basis of their amino acids and carbohydrate metabolisms. Acetic acid, which was highest in fermentations of dough with added *L. fermentum* (Table 2), has been reported as the dominant volatile organic acid in many fermentations with heterofermentative lactobacilli (Martinez-Anaya *et al.* 1990; Barber *et al.* 1991; Lund *et al.* 1989; Halm *et al.* 1993; Gobbetti *et al.* 1995). Wheat sourdough started with single cultures of *L. brevis* produced more acetic acid than *S. cerevisiae* when fermented for 20 h at 30°C (Meignen *et al.* 2001).

Maize dough fermented with *S. cerevisiae* had higher concentrations of yeast fermentation products such as fusel alcohols, esters and ethanol than *C. krusei* (Table 2). Headspace composition of wheat sourdough fermented with yeast alone showed that *S. cerevisiae* produced larger amounts of all compounds than did *C. boidini* (Martinez-Anaya *et al.* 1990). The addition of sourdough yeasts in sourdough fermentations was found to increase considerably the numbers of alcohols and esters (Hansen and Hansen 1994).

Carbonyl compounds, alcohols, a few acids and phenolic compounds were observed to be characteristic of spontaneously fermented maize dough with less numbers and lower concentrations of esters produced than in starter culture, in particular *S. cerevisiae*, fermented dough (Fig. 2 and Table 2). Halm *et al.* (1993) detected only a few carbonyls, alcohols, acids and phenols but no esters in Ghanaian fermented maize dough sampled from commercial producing sites. In cereal fermentations high levels of carbonyl compounds have often been reported for the raw material, unfermented samples, or samples at earlier stages of fermentation (Frasse *et al.* 1993; Masha *et al.* 1998; Hansen and Lund 1987). The presence of more carbonyls in the spontaneously fermented maize dough samples in the present study indicates that inoculations with starter cultures result in a faster reduction of these initial fermentation products to corresponding alcohols, esters and acids.

Comparison of aroma profiles determined by GC-olfactometry (GC-sniffing) for maize dough fermented spontaneously and with starter cultures

Results of GC-sniffing of aroma volatiles in maize dough fermented spontaneously and with starter cultures for 48 h showed some differences in the contributions from aroma compound groups, particularly esters (Table 3). Six esters in fermentations with added *L. fermentum*, nine with *S. cerevisiae* and four with *C. krusei* were detected by sniffing in the aroma profile of maize dough samples in contrast to two in spontaneously fermented samples. These esters were described as flowery, fruity, green or plant-like which is typical (Labuda *et al.* 1997; Petersen *et al.* 1998; Yoshiharu *et al.* 1997). The fermentations with added *L. fermentum* were characterized by an increased sour, vinegar-like aroma impression caused by an increased concentration of acetic acid.

High aroma intensities attributable to yeast fermentation products were also recorded for fermentations with *S. cerevisiae* in the present study. They included a sweet, fruity note for 2-methyl-propanol and a green aroma for 3-methyl-butanol. A malty, sweet, aroma is described in the literature for 3-methyl-butanol (Frasse *et al.* 1993; Hansen and Hansen 2000). An average intensity score of 2·0 was recorded for all nine esters found in fermentations with *S. cerevisiae*. The odour thresholds of ethyl acetate, ethyl propionate, hexyl acetate and ethyl hexanoate have been

reported to be 5, 10, 2 and 1 ng ml^{-1} water, respectively (Takeoka *et al.* 1989). Based on the taste and flavour, Nyako and Danso (1991), reported a better acceptability of maize dough inoculated with *S. cerevisiae* than with *C. krusei* or spontaneously fermented dough when tested by sensory panellists familiar with the Ghanaian fermented maize dough.

Fermentations with added *C. krusei* showed lower odour intensity scores for the yeast fermentation products, 1-propanol, 2-methyl-propanol and 3-methyl-butanol, and lower number of esters than *S. cervisiae*, which is in agreement with reports of similar studies with sourdough wheat bread (Martinez-Anaya *et al.* 1990).

In general, spontaneous fermentations tended to have higher intensity scores for most carbonyls and alcohols. These gave spontaneous maize dough fermentations after 48 h with green, fatty, fruity, flowery and mushroom-like odours. Significant contributions were also made by acids, particularly pentanoic and hexanoic acids, resulting in strong and somewhat unpleasant flavours.

CONCLUSIONS

The present study has, for the first time, given a detailed picture of aroma compounds produced in fermented maize dough with starter cultures and described the corresponding flavour impressions of these compounds. It has also been shown how the predominant micro-organisms, *L. fermentum*, *S. cerevisiae* and *C. krusei*, influence the aroma of fermented maize. A distinct and significant effect has been demonstrated for the three types of micro-organisms indicating how they can be used as starter cultures to modify and select the aroma of fermented maize. The role of the micro-organisms appears to be closely related to the roles of lactobacilli and yeasts reported for aroma formation in wheat sourdough fermentations.

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