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Aroma profile of gowe, a traditional malted fermented sorghum beverage from Benin

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Spontaneous fermented gowé was characterized by specific flavor produced by different enzymatic activities of microorganisms involved. Gowé was also produced by controlled fermentation using strains of *Lactobacillus fermentum* and *Weissella confusa* singly or in combination with *Kluyveromyces marxianus* and *Pichia anomala* after an accelerated saccharification. Investigation using Likens-Nikerson extraction method and gas chromatography-mass spectrometry (GC-MS) analysis revealed that the volatile compounds identified in the product obtained by spontaneous and controlled fermentation were composed of alcohols, aldehydes, acids, esters, hydrocarbons, furan, phenol and piperidine. The use of the starter cultures preceded by an accelerated saccharification led to a drastic reduction in the volatile components concentration for the inoculated samples. A principal component analysis performed revealed an important concentration of volatile acids in the inoculated samples.

Key words: Sorghum, fermentation, starter culture, volatile compound.

INTRODUCTION

Gowé is a fermented cereals beverage mostly made of a blend of malted and non-malted red sorghum flour. Previous studies had reported the various changes observed in the physico-chemical, proximate and microbiological characteristics during the production process (Vieira-Dalodé et al., 2007). A decrease of the pH with a concomitant increase of the titratable acidity and organic acid content were observed. The identification of the microbiota involved in *gowé* fermentation showed that the dominant microorganisms were mainly lactic acid bacteria (LAB) and yeasts. Several functions have been attributed to these microorganisms involved in the fermentation. The LAB and yeasts identified in kenkey, a Ghanaian fermented maize dough product, play an important role in the distinct aroma profile (Halm et al., 1993). Flavor and

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texture improvement has often been mentioned as beneficial effects for fermented foods and beverages (Jespersen, 2002). Beside the demand for safe food, there is always the need to eat foods that taste good. Consumers' behavior and perception of food quality are some parameters which must be taken into account (Cayot, 2007). Aroma characteristics are of most importance for the consumer perception of food quality and acceptability.

Several studies have been done to characterize the aroma profile of fermented and non-fermented products (Halm et al., 1993; Lasekan et al., 1997; Annan et al., 2003a, b, c; Gran et al., 2003; Mugula et al., 2003; Mamede et al., 2005; Cayot, 2007; Ho et al., 2006; Komthong et al., 2007). Volatile compounds identified were produced during various steps of the production process as a result of fermentation by lactic bacteria and yeasts, enzymatic activities, lipid oxidation or maillard reactions (Feillet, 2000). Malting and fermentation involved in the production of gowé contribute to its specific taste and aroma enhancing consumer's acceptance (Adinsi et al., 2014). The organoleptic acceptance, such as aroma improvement of fermented product had led to the use of starter cultures in the fermentation of maize dough for kenkey production (Annan et al., 2003a, b). These authors reported how *Lactobacillus fermentum, Saccharomyces cerevisiae* and *Candida krusei* can be used as starter cultures to modify the aroma profiles of fermented maize dough. The use of *Lactobacillus fermentum* and *Weissella confusa* previously identified as the predominant lactic acid bacteria during the natural fermentation process of gowé in association with *Kluyveromyces marxianus* and *Pichia anomala* the predominant yeasts (Vieira-Dalodé et al., 2007) as starter cultures for a controlled fermentation, may have an effect on the volatile compounds of the product. An accelerated saccharification process was performed before the use of the starter cultures for *gowé* fermentation. The main objective of the present work is to identify the different volatile compounds characterizing gowé aroma and to evaluate the combined effect of saccharification and use of starter cultures on the volatile compounds, during gowé fermentation.

MATERIALS AND METHODS

The red variety of sorghum [*Sorghum bicolor* (L) Moench] for the production of gowé was purchased from a local market in Cotonou. The strains of lactic acid bacteria and yeasts used as starter cultures were isolated from gowé obtained by spontanaous fermentation and identified as the predominant species during the production process.

Samples preparation

Two different processing methods were used. The first one followed the spontaneous fermentation process described by Vieira-Dalodé et al. (2007). Samples of product were taken at 0, 4, 8 and 12 h during primary fermentation and at 12 h of the secondary fermentation. A modified technology was used during the second process. The modification involved the production of a gruel (23 to 25% D.W.) made from the malted sorghum flour diluted in water. The gruel was kept at 40°C for 2 h in a water bath. This treatment initiated an accelerated saccharification. The saccharified product was mixed with a non-malted sorghum flour. The LAB (*L. fermentum* and *W. confusa*) and yeast (*K. marxianus* and *P. anomala)* selected were used singly or in combination (one LAB + one yeast) as inoculum enrichment to inoculate the mixture as described by Vieira-Dalodé et al. (2008). Sampling was done at 0, 12 and 24 h of fermentation. At each sampling time, samples (100 g) were taken, freeze dried and kept at -20°C for further determination of volatile compounds.

Determination of volatile compounds

Volatile compounds extraction

The Likens-Nickerson simultaneous distillation and extraction was used to determine the volatile organic compounds in gowé according to the method used by Annan et al. (2003a). Extraction was done using a micro-scale steam distillation low-density solvent extraction device (micro-SDE, Chrompack, Middelburg, the Netherlands). The extraction procedure was conducted using 10 g of freeze dried sample diluted in 400 ml distilled water to obtain 2.5% slurries of samples (w/v) in a 1 L conical flask. One (1 ml) of internal standard (50 ppm, 4-methyl-1-pentanol in H_2O) was added to the slurry in an Erlenmeyer flask. Six (6 ml) of a mixture of pentane and diethyl ether (1:1) were placed in 9 ml pear-shaped solvent flask. The conical and the pear shaped flasks were connected to the Likens-Nickerson distillation extraction apparatus and the solutions were brought to boil. Extraction of volatile compounds was carried out for 30 min, from the beginning of condensation of vapors on the walls of the condenser. The pear shaped flask containing entrapped volatiles in solvent was removed and placed in freezer to freeze out the aqueous portion in solution. The solvent extract was poured off, dried over in 2 g of Na_2SO_4 and concentrated to about 100 mg by gently blowing N_2 gas over the surface. The concentrated extract was analyzed for volatile compounds using the gas chromatograph-mass spectrophotometer (GC-MS). Separation and identification of volatile compounds in extracts of *gowé* samples were carried out on Hewlett-Packard G1800 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., × 0.25 mm film thickness).

Identification of volatile compounds

Two microlitres 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). The quantity of an individual compound was estimated by comparing its total ion relative peak area with that of the 4-methyl-1-pentanol internal standard. The volatile organic compounds taken into account were those having a quality identification mark or quality Index (QI: degree of agreement between mass spectrum of sample and mass spectrum in database on scale of 0 to 100) above 70%.

Statistical analysis

Principal component analysis (PCA) was performed using statistica

7 (StatSoft, Tulsa, Uk, USA).

RESULTS

Aroma compounds during *gowé* production by natural fermentation

Gowé was characterized by 61 volatile compounds, composed of 9 alcohols, 17 aldehydes, 5 acids, 14 esters, 12 hydrocarbons, 1 furan, 2 phenol and 1 piperidine (Table 1).

Alcohols

Nine types of alcohol were identified. Ethanol was the most abundant alcohol found throughout the process. Its concentration increased from 0.94 g kg^{-1} at 0 h of the primary fermentation to a peak of 2.2 g kg^{-1} at 12 h of the secondary fermentation. It decreased below the initial value after 24 h of the secondary fermentation. The concentrations of the other alcohols identified were lower and varied between 0.88 and 19.12 mg kg^{-1}. 1-Pentanol was found from 0 h of the primary fermentation to 24 h of the secondary fermentation. Its concentration was very low at the end of the process. With the exception of heptanol, all the identified alcohols could be found at 24 h of the secondary fermentation.

Aldehydes

Seventeen aldehydes were found through the fermentation process. Hexanal could be identified during primary fermentation and at 12 h of secondary fermentation but not at 24 h. The concentration of hexanal decreased from 76.18 mg kg^{-1} at 0 h of the primary fermentation to 15.93 mg kg^{-1} at 12 h of the secondary fermentation. Most of the aldehydes were identified during the primary fermentation. During the secondary fermentation only 3 aldehydes could be found namely hexanal, furfural and 2-4- Decadienal, which was the only one found at the end of the second fermentation.

Esters

Fourteen esters were found and several could be identified from the primary fermentation to 12 h of the secondary fermentation. Ethyl linoleate was the ester with the highest concentration increasing from 39.09 at 0 h to 1664.70 mg kg^{-1} at 12 h of the secondary fermentation. The second abundant ester was ethyl linoleate which increased from 18.42 to 702.85 mg kg^{-1}. At 12 h of the secondary fermentation, ethyl linoleate was the volatile compound in highest concentration after ethanol. No ester was detected at the end of the fermentation process.

Hydrocarbons

A total of 12 hydrocarbons were identified with p-xylene and 1-3 dimethyl benzene being found during the primary and the secondary fermentation. They were the two compounds found in high concentration after 12 h of the secondary fermentation.

Acids

Five acids were detected during the fermentation process. Hexadecanoic acid, oleic acid and 9-12 octadecadienoic acids were found in concentrations that increased from 0 h of the primary fermentation to 12 h of the secondary fermentation. Acetic acid was identified in increasing amount from 12 h of the primary fermentation to the end of the process. Butanoic acid was identified only at the end of the process after 24 h of the secondary fermentation.

Other compounds

Phenols, furans and piperidine were the other volatiles compound identified but they were not detected at the end of the process.

Aroma compounds during gowé production after saccharification and use of starter cultures

A total of 94 compounds were identified during the fermentation process after saccharification and the use of the starter cultures. The volatile compounds identified in the saccharified and inoculated samples were composed of alcohols, aldehydes, acids and esters, hydrocarbons, furan and phenol as in the spontaneous fermentation. The concentrations of the compounds were drastically reduced compared to the spontaneous fermentation (Table 2).

DISCUSSION

Flavors and taste are important quality characteristics of traditional foods. Fermented foods are particularly appreciated for their pleasant flavor and taste (Holzapfel, 2002). Traditional gowé is a naturally fermented product characterized by specific taste and flavors well known by the consumers. Several groups of volatile compounds could be identified during the production of gowé including alcohols, aldehydes, esters, hydrocarbon and acids. Most of the volatiles could be identified from 0 h of fermentation and may have been initiated during the steps preceding fermentation such as steeping and germination of sorghum kernels. Most of the volatile compounds of the different groups were observed during

Table 1. Volatiles compounds (mg kg^{-1}) during *Gowé* production by spontaneous fermentation.

Compounds	Quality index	Primary fermentation			Secondary fermentation	
		0 h	8 h	12 h	12 h	24 h
Alcohols						
Ethanol	***	940.50	1056.70	1483.08	2196.75	906.42
2-methylpropan-1-ol	**	Nd	Nd	5.69	Nd	5.37
3-methyl butan-1-ol	**	Nd	Nd	Nd	Nd	15.66
1-pentanol	**	13.16	15.12	19.12	3.19	1.71
Hexanol	**	Nd	Nd	5.45	9.94	5.04
Heptanol	**	Nd	Nd	5.18	Nd	Nd
1-octanol	**	2.96	3.32	5.31	Nd	2.73
Mequinol	**	Nd	Nd	Nd	Nd	1.45
Benzyl alcohol	***	Nd	Nd	Nd	Nd	0.88
Total		**956.62**	**1075.14**	**1523.83**	**2209.88**	**939.26**
Aldehydes						
3-methyl butanal	**	Nd	7.61	20.78	Nd	Nd
Hexanal	***	76.18	56.86	55.36	15.93	Nd
Heptanal	***	20.50	14.64	8.11	Nd	Nd
Trans2-heptanal	***	Nd	ND	13.68	Nd	Nd
Nonanal	***	9.30	8.08	8.81	Nd	Nd
Octanal	**	11.54	Nd	Nd	Nd	Nd
2-octenal	***	Nd	7.87	14.60	Nd	Nd
3- propanal	**	1.39	ND	Nd	Nd	Nd
Furfural	***	Nd	Nd	Nd	4.22	Nd
Benzaldehyde	***	1.82	0.78	2.16	Nd	Nd
2-nonenal	***	13.45	11.76	10.48	Nd	Nd
2-decenal	***	6.87	8.56	12.09	Nd	Nd
2,4-nonadienal	***	4.03	5.54	6.04	Nd	Nd
2-undecenal	***	Nd	ND	13.44	Nd	Nd
2,4-decadienal	***	23.21	32.50	47.24	Nd	6.54
Octadecanal	***	Nd	Nd	2.39	Nd	Nd
Total		**168. 92**	**154.2**	**215.18**	**20.15**	**7.94**
Esters						
Ethyl lactate	**	Nd	Nd	Nd	9.61	Nd
Ethyl octanoate	***	Nd	Nd	3.96	5.93	Nd
Ethyl tetradecanoate	***	5.11	3.24	4.36	Nd	Nd
Ethyl palmitate	**	Nd	Nd	0.87	Nd	Nd
Methyl hexadecanoate	***	Nd	Nd	1.75	Nd	Nd
Ethyl hexadecanoate	***	Nd	23.46	56.66	111.04	Nd
9-Ethyl hexadecanoate	***	Nd	ND	Nd	43.14	Nd
9-methyl octadecenoate	***	10.39	ND	Nd	Nd	Nd
Ethyl oleate	***	18.42	65.555	397.27	702.85	Nd
9-12-methyl octadecenoate	***	19.90	21.10	29.26	Nd	Nd
Ethyl linoleate	***	39.09	567.68	787.72	1664.70	Nd
9-12-15ethyl octadecatrienoate	***	Nd	Nd	30.96	85.87	Nd
Dibutyl phthalate	***	10.51	Nd	Nd	Nd	Nd
9-methyl octadecenoate	***	39.33	Nd	Nd	Nd	Nd
Total	***	**142.75**	**681.03**	**1312.81**	**2623.14**	-
Hydrocarbons						
P-xylene	***	14.34	8.15	7.78	14.45	8.09

Table 1. Contd.

1,3 dimethyl benzene	***	7.97	10.20	15.98	20.84	Nd
2-heptacene	**	12.50	6.30	Nd	Nd	Nd
3-ethyl 2 methyl 1,3 hexadiene	***	28.97	22.65	23.99	Nd	Nd
Octyl cyclopropane	**	Nd	Nd	Nd	Nd	4.41
1-methylene	**	Nd	Nd	Nd	Nd	4.15
Trans trans-2-4-decadiene	***	Nd	12.70	47.24	Nd	Nd
Cyclododecane	***	Nd	Nd	Nd	4.69	Nd
1-dodecene	***	Nd	Nd	Nd	Nd	2.92
1-octadecene	***	Nd	Nd	2.30	Nd	Nd
Heptacosane	***	Nd	Nd	4.87	Nd	Nd
Docosane	***	Nd	Nd	1.95	Nd	Nd
Total		**63.78**	**60.0**	**104.11**	**39.98**	**19.57**
Acids						
Acetic acid	**	Nd	Nd	1.57	2.84	4.53
Butanoic acid	***	Nd	Nd	Nd	Nd	21.44
Hexadecanoic acid	***	144.20	97.45	Nd	1036.59	Nd
Oleic acid	***	Nd	Nd	185.01	327.27	Nd
9-12 octadecadienoic acid	***	39.33	154.6	423.94	845.79	Nd
Total		**183.53**	**252.05**	**608.95**	**2209.65**	**25.97**
Phenol, Furans						
2 pentyl furan	***	23.22	14.32	Nd	Nd	Nd
2 methoxy phenol	***	Nd	ND	10.72	Nd	Nd
Phenol	***	2.77	9.56	42.20	20.82	Nd
Total		**25.99**	**23.88**	**52.92**	**20.82**	
Others						
Piperidine	***	Nd	Nd	3.04	Nd	Nd

QI (Quality index): degree of agreement between mass spectrum of sample and mass spectrum in database on scale of 0 to 100; ***: quality index > 90, **: quality index between 80 and 90. N: analysis was not done. Nd: compound was not detected.

Table 2. Volatiles compounds (Total amount mgkg^{-1}) of Gowé produced after saccharification using starter cultures.

Compounds	C	Lf	Wc	Km	Pa	WK	WP	LK	LP
Alcohols	1.4	0.47	0.82	1.55	2.58	0.96	1.51	0.88	0.85
Aldehydes	1.97	0.37	0.30	0.99	0.66	0.52	1.02	0.96	0.69
Esters	15.25	7.01	5.57	15.61	11.21	12.17	12.32	10.13	11.88
Hydrocarbons	0.59	0.27	0.18	0.47	1.58	1.11	0.88	0.74	0.52
Acids	10.43	9.52	8.07	6.89	6.43	8.15	10.48	8.78	9.21
Phenol/furans	0.24	0.19	Nd	0.06	0.83	Nd	0.13	Nd	Nd

L: *lactobacillus fermentum ;* W *: Weissella confusa ;* C: control ; K *: Kluyveromyces marxianus ;* P *: Pichia anomala; ;* WK , WP: Starters composed of *W. confusa* and *K. marxianus* or *P. anomala.* LK, LP: starters composed of *L. fermentum* and *K. marxianus* or *P. anomala.*

the primary fermentation stage where an increase of the lactic acid bacteria and yeast counts could be observed. After 12 h of the primary fermentation a hot gruel of non-malted sorghum flour was added resulting in a decrease in the number of the compounds identified. Alcohols were produced throughout the fermentation process and were identified from 0h of the primary fermentation. Alcohols are produced from fermentation by yeasts which had their numbers increasing during the primary fermentation. Ethanol, the main alcohol produced was found to occur in increasing amounts as fermentation progressed. During spontaneous fermentation of maize dough for Kenkey production in Ghana, ethanol was the most abundant alcohol found (Annan et al., 2003a). Ethanol was a flavor compound found in Amasi, a Zimbabwean naturally fermented raw milk product (Gran et al., 2003).

Most of the aldehydes were found during the primary fermentation. Several steps such as milling into flour and kneading carried out during gowé processing could lead to aldehyde production. During milling into flour, lipoxygenase enzymes in plant cell lysosomes react with linolenic and linoleic acids in the presence of oxygen to produce aldehydes such as hexanal (Gray et al., 1999). The intensity of kneading may have an effect on the concentration of some aldehydes (Cayot, 2007). Hexanal was the aldehyde found in the highest amount at 0h of fermentation when the malted sorghum flour was kneaded and left to ferment. Hexanal has also been identified in several cereal based fermented products such as kenkey (Annan et al., 2003a) and sorghum malt beverage (Lasekan et al., 1997). Ethanol was the alcohol found in the highest concentration during gowé production. This corresponded with the formation of several ethyl esters. Esters are mainly produced by yeast during alcoholic fermentation in reactions between alcohol and acetyl-CoA catalysed by enzymes (Mamede et al., 2005). Ethyl oleate and ethyl linoleate were observed to be in increasing amounts from 0 to12 h of the secondary fermentation when gowé is ready to be cooked. The different volatile acids identified were mainly hexadecanoic acid, oleic acid and 9 to 12 octadecadienoic acid. Their concentrations increased considerably after 12 h of the secondary fermentation.

Gowé is generally cooked after 24 h of fermentation (12 h of the secondary fermentation). The characteristic volatiles at this stage were mainly ethanol, ethyl linoleate and hexadecanoic acid.

The use of starter cultures for gowé fermentation produced volatile compounds similar to what was observed in the spontaneously fermented gowé but in significantly decreased amounts. This may have been due to the accelerated saccharification process where the product was kept at 40°C for 2 h. Most of the volatile compounds increased with the fermentation time when the yeasts were used in single or in association with the LAB. The ability of selected microorganisms to modify taste, flavors and other characteristics of fermented products has been studied by several authors (Annan et al., 2003b, c; Mugula et al., 2003; Zorba et al., 2003; Gran et al., 2003; Ouoba et al., 2005; Azokpota, 2005). Fermentation of maize dough involving yeasts induced higher concentrations of compounds related to yeasts fermentation products such as alcohols and esters (Annan et al., 2003c). The fermentation of gowé with *K. marxianus* and *P. anomala* used singly led to a relatively higher concentration of alcohol. The use of *L. fermentum* singly in the fermentation of maize dough gave lower concentration of alcohol than when used in combination with yeasts (Annan et al., 2003). Lower concentrations of aldehydes, esters and hydrocarbons were observed when the LAB were used singly in the saccharified and inoculated product. Lower concentrations of volatile compounds were observed when LAB were used to ferment sourdough bread compared to sourdough fermented with yeasts (Hansen and Hansen, 1994). Annan et al. (2003b, c) found that the concentrations of most volatile organic acids were the lowest in fermentation of maize dough with only *L. fermentum* added as a starter culture.

A principal component analysis was performed on the volatile compounds produced by traditional and starter fermented gowe. The aroma compounds obtained after 24 h of fermentation exhibited two volatile profiles groups. The first two principal components accounted for 94.89% (PCI) and 5.04% (PC2) of the variation in the data, respectively. The PC1 reflects the content of acids on the left of the plot (Figure 1). The high concentrations of volatile acids (6.43 to 10.48 mg/kg) are in correlation with the starters used, lactic acid bacteria used singly or in association with a yeast. Fermentations of Ghanaian maize dough with *L. fermentum* with *C. krusei* were characterized by similar trend, higher concentration of acetic acid and low concentrations of most volatiles produced (Annan et al., 2003b). This high production of acids was not observed when the yeasts were used singly. The other volatile compounds were found on the right and were composed by compounds mainly identified in the traditional product. Gowe obtained by natural flora fermentation (control) was characterized by particularly high levels of phenol, furans (20.82 mg/kg), esters (255.54 mg/kg) and aldehyde (20.15 mg/kg) and low level of acid (2.84 mg/kg). These compounds were also detected in the inoculated samples but in low concentration. Using principal component analysis for comparison, the aroma profiles of gowe fermented with the yeasts *K. marxianus* and *P. anomala* and lactic acid bacteria *L. fermentum* used singly or in association with one of the yeasts can be separated from that of naturally fermented gowe.

Conclusion

Gowé obtained by spontaneous fermentation of sorghum flour is characterised by several volatile compounds developed with the activities of the different lactic acid bacteria and yeasts involved. The use of *L. fermentum* and *W. confusa* in association with *K. marxianus* and *P. anomala* as inoculum enrichment for a controlled fermentation of gowé modified the profile of volatile compounds and the volatiles acids were the best component detected in the inoculated products. The observed reduction in the concentration of the volatile compounds compared to the spontaneous fermentation

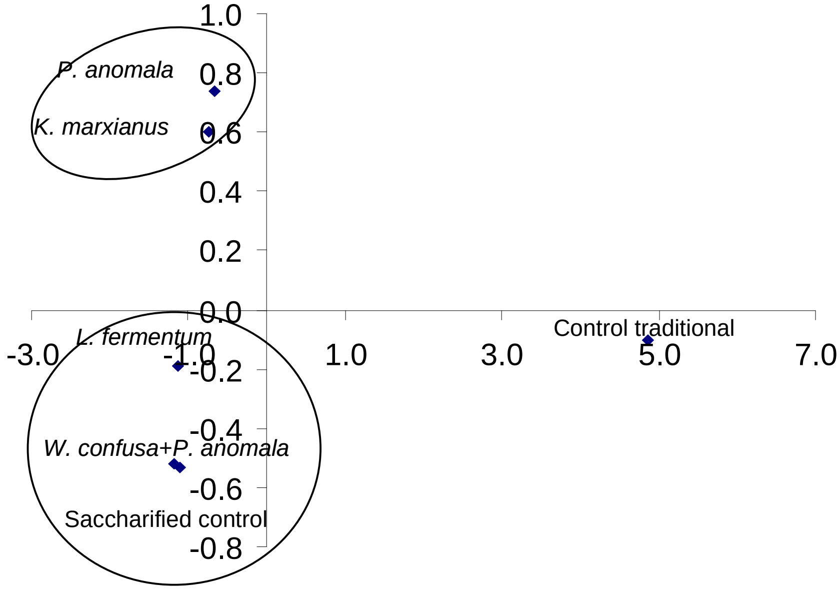

Figure 1a. Grouping of types of inoculated gowé related to the starter used.

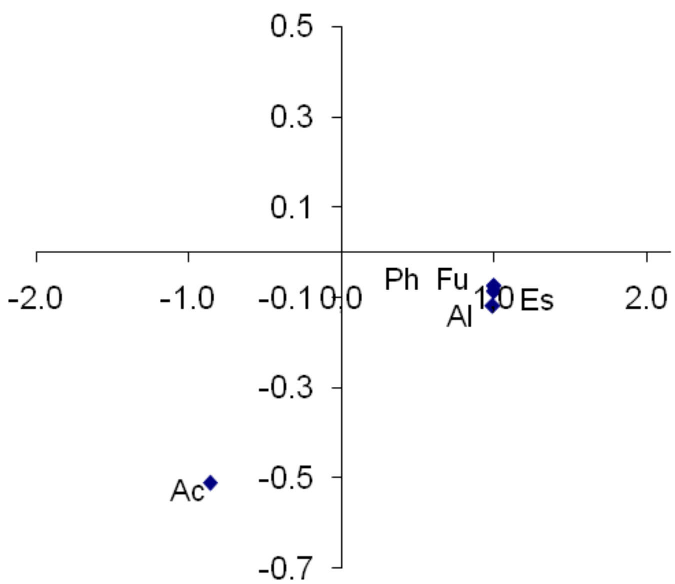

Figure 1b. Volatile compounds identified in different groups of inoculated gowé. Ac: acids, Al: aldehydes, Es: esters, Fu: furan, Ph: Phenols.

may be due to the saccharification process realised before inoculation. A standardized production process was aimed with the use of starter cultures. The products inoculated with lactic acid bacteria showed a high concentration of acids. These volatile acids may confer to the gowé obtained some qualities and specific flavors sought by the consumers. Analysis by GC-sniffing can provide further knowledge about the contribution of the identified compounds to the characteristic aroma of cooked *gowé*.

Conflict of interests

The authors have not declared any conflict of interest.

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