Contribution of autochthonous yeasts with probiotic potential to the aroma profile of fermented Guajillo pepper sauce

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Abstract

BACKGROUND

Three yeast strains with probiotic potential, *Hanseniaspora opuntiae*, *Pichia kudriavzevii*, and *Wickerhamomyces anomalus* were inoculated in the fermentation of Guajillo chilli pepper (*Capsicum annuum* L.) sauce, and the different aroma profiles were investigated. Using headspace solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) analysis and gas chromatography-olfactometry (GCO), flavour compounds production was evaluated during the fermentation of the Guajillo chilli pepper sauces.

RESULTS

A total of 78 volatile compounds were identified during the yeast fermentation of the sauce. Most aldehydes and terpenes detected were present since the beginning of the fermentation, indicating a Guajillo chilli pepper origin. Among the 34 active aroma compounds detected by GCO, propanoic acid (cheesy), 3-methylbutanoic acid (sharp, cheese), ethyl 2-methylbutanoate (fruity), and 6-methyl-5-hepten-2-one (strong, citrus) were identified as key aroma contributors produced by the inoculation of the yeasts. Different aroma profile was produced by probiotic yeast. *H. opuntiae* produced an

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aroma profile with herbal and green notes based on high production of aldehydes, ketones, and acetic acid. *P. kudriavzevii* and *W. anomalus* produced fruity, green-herbal, and cheesy notes based on ester compounds, alcohol and branched-chain acids production although, the production of propanoic acid by *W. anomalus* increased the cheesy character in the sauces.

CONCLUSION

The aroma profile of fermented chilli pepper sauces depends not only on the chili pepper varieties used but also on the fermentation process as a source of aroma compounds. The use of probiotic yeast can be used to improve and diversify the aroma profile of fermented chilli pepper sauces.

Keywords: Chili pepper; aroma; yeast; *Hanseniaspora opuntiae*; *Pichia kudriavzevii*; *Wickerhamomyces anomalus*.

INTRODUCTION

Chilli peppers are used in the Mexican daily diet. Within the *Capsicum* genus, the *Capsicum annuum* L. specie is the most widely consumed in fresh or processed food, in sauces, powder, and pickled.¹

Among sauces, the “borracha sauce”, elaborated with Guajillo chilli peppers, a variety of *C. annuum* L., is characterised by its alcoholic content produced during a spontaneous fermentation.²

Nowadays, the fermentation of vegetables with autochthonous starters is a method that maintains or improves the nutritional value, sensory properties, and increased shelf life.³ Aroma is one of the most important indicators to determine the quality of fermented foods and consumers acceptance, and is based on the production of volatile compounds with aroma properties.⁴ There are several studies about the aroma profile of *Capsicum* fruits, and its changes according to the variety, ripening stages, and storage method.⁵ The most abundant compounds reported are esters, alcohols, aldehydes, ketones, terpenes, acids, furans, pyrazines, and sulphur compounds, which are responsible for the aroma characteristics.⁵⁻⁷ The production of volatile compounds in the spontaneous fermentation of red hot chilli peppers by lactic acid bacteria and yeasts affected the characteristic product flavour.⁸ Moreover, the fermentation of tomato and red pepper pomaces by *Kluyveromyces marxianus* and *Debaryomyces hansenii* resulted in different aroma notes produced by acids, esters, and alcohols.⁹ Therefore, not only chilli pepper varieties may affect the aroma profile but also the fermentation process may provide a different source for aroma compounds.^{7,10} Still, few studies have focused on the effect of yeast inoculation on aroma fermentation.

Among the beneficial microorganisms known as probiotics in fermented vegetables, many yeasts have been isolated from vegetables.¹¹ However, the use of probiotics in fermented foods can produce an impact on sensory properties.¹² There is still a limitation of probiotic yeasts contribution to aroma compounds production. In spontaneous fermented Guajillo pepper, three yeast strains, *Hanseniaspora opuntiae*, *Pichia kudriavzevii*, and *Wickerhamomyces anomalus*, with different probiotic characteristics were isolated.¹³ The antioxidant activity of these yeasts inoculated in Guajillo chilli pepper fermentations could impart a positive effect on Guajillo sauces' chemical composition during and after product manufacturing. However, the role of these probiotic yeast strains in the

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fermentation of Guajillo sauce is not known and it can affect the aroma profile and sauce quality. Therefore, the aim of this study was to investigate the different aroma profile produced by the inoculation of yeast strains with probiotic potential, *H. opuntiae*, *P. kudriavzevii* and *W. anomalus*, on the fermentation of Guajillo chilli pepper sauce.

MATERIALS AND METHODS

Yeast strains and preparation of yeast suspensions

Three yeast strains, *Hanseniaspora opuntiae* IPNFG2, *Pichia kudriavzevii* IPNFG5 and *Wickerhamomyces anomalus* IPNFG3 previously isolated during the fermentation process of Guajillo chilli peppers¹³ were used in this study. Yeast strains were maintained on GPYA medium (20 g/L glucose, 5 g L⁻¹ peptone, 5 g L⁻¹ yeast extract, and 20 g L⁻¹ agar; pH 6.5). Fresh cells were collected by centrifugation at 4000 rpm at 10 min from GPYA medium and suspended in physiological saline solution. Yeast suspensions at OD 655 nm (Biophotometer AG Eppendorf 22331, Hamburg, Germany) were prepared and adjusted to reach a final concentration of 10⁶ cells mL⁻¹ in Guajillo sauce (GS).

Fermentation conditions for production of Guajillo pepper sauce

Guajillo chilli peppers (*C. annuum* L.) were washed with sterile, distilled water and sliced in hygienic conditions. A brine solution was prepared with 75 g L⁻¹ glucose and 15 g L⁻¹ sodium chloride and sterilised in an autoclave at 121 °C for 20 min. Guajillo sauce was prepared mixing the brine solution and sliced Guajillo chilli peppers (300 g L⁻¹) in a blender until fully homogenised. Flasks of 250 mL containing 80 mL of GS were inoculated with the yeast strains (10⁶ CFU per ml in each flask) and a non-inoculated flask was used as control. A total of five batches were obtained: C (control non-inoculated), Ho (*H. opuntiae*), Pk (*P. kudriavzevii*), Wa (*W. anomalus*), and T (inoculated with a mix of the three yeasts in the same proportion). Incubation of Guajillo sauce fermentation flasks was at 30 °C for 4 days without agitation. Each experiment was conducted in triplicate. During incubation, yeast count and volatile analyses of samples from each batch took place at 0, 1, 2, 3 and 4 days,

with olfactometry aroma analysis at the end of the fermentation (4 d) in the T batch. pH was measured at the beginning and the end of the fermentation with a pH metre HI 99163 (Hanna Instruments Inc., Hoonsocket, USA).

Yeast counts

Yeast counts were done on 25 g of GS. Samples were mixed with 225 mL of buffered peptone water and homogenised in a Pulsifier (Microgen Biotech, Spain). Homogenates were used to prepare decimal dilutions which were spread on Rose Bengal Agar with chloramphenicol (100 g L⁻¹ dextrose, 50 g L⁻¹ bacteriological peptone, 10 g L⁻¹ potassium phosphate, 5 g L⁻¹ magnesium sulphate, 1 g L⁻¹ chloramphenicol, 0.5 g L⁻¹ rose Bengal, 15 g L⁻¹ bacteriological agar) and incubated at 30 °C for 48 h.

Profile and Quantification of Volatile Compounds

An Agilent HP 7890 series II GC (Hewlett-Packard, Palo Alto, CA, USA) with an HP 5975C mass selective detector (Hewlett-Packard) equipped with Gerstel MPS2 multipurpose sampler (Gerstel, Mülheim an der Ruhr, Germany) was used in all experiments. Extraction of headspace (HS) volatile compounds were performed using solid phase microextraction (SPME) with an 85 µm Carboxen®/Polydimethylsiloxane (CAR/PDMS) fibre (Supelco, Bellefonte, PA, USA). For each experiment 3 g of GS and 1 g of sodium chloride were weighed into a 20 mL vial sealed with a PTFE faced silicone septum and analysed. The vial was maintained at 50 °C for 15 min to equilibrate the headspace, and the fibre was exposed to the HS for 10 min at the same temperature. Previously, volatile extraction conditions were assayed at different extraction times (5, 10, 20, and 30 min) to select the appropriate one. The compounds adsorbed by the fibre were desorbed in the gas chromatograph injection port for 5 min at 240 °C in splitless mode. The compounds were separated using a DB-624 capillary column (30 m, 0.25 mm i.d., film thickness 1.4µm, (J&W Scientific, Agilent Technologies, Santa Clara, CA, USA)). Helium was used as the carrier gas with a linear velocity of 34.337 cm/s. The GC oven temperature programme began at time 0 min and 40 °C, ramped to 140

°C at 3 °C/min, then 4 °C/min to 220 °C and maintained for 5 min. The total run time was 58.33 min. Mass spectra were obtained by electron impact at 70 eV, and data were acquired across the range 29 - 400 amu.

Compounds were identified by comparison with mass spectra from the library database (NIST 05), Linear Retention Index (LRI)¹⁴ and by comparison with authentic standards. Identified volatile compounds were quantified in SCAN mode using either a total or extracted ion chromatogram (TIC or EIC) on an arbitrary scale. Abundance of volatile compounds is expressed in TIC $\times 10^5$. Each GS batch was analysed per duplicate.

Analysis of aroma compounds by Gas chromatography-olfactometry (GCO).

A gas chromatograph with flame ionization detector (GC-FID) (Agilent 6890, Santa Clara, CA, USA) equipped with a sniffing port (ODP3, Gerstel, Mülheim an der Ruhr, Germany) was used to analyse aroma compounds as described by Corral *et al.*¹⁵ For aroma extraction, 2 g of GS and 0.66 g of sodium chloride were added to a 10 mL HS vial, and equilibrated at 50 °C for 30 min in a thermo block (J.P. Selecta, Barcelona, Spain) and the same SPME fibre (CAR/PDMS) as above was used and exposed to the HS for 1 h at 50 °C. The highest extraction time was used to help identify and describe the aroma compounds by the panellists. Fibre was desorbed in the injection port for 15 min at 240 °C in splitless mode. Then compounds were separated using a DB-624 capillary column (60 m, 0.32 mm i.d., film thickness 1.8 μ m, (J&W Scientific, Agilent Technologies, Santa Clara, CA, USA)). Helium was used as the carrier gas at a flow rate of 47 cm/s. The oven temperature program began at 40 °C for 5 min, ramped to 140 °C at 3 °C/min, then ramped to 210 °C at 4 °C/min and maintained for 5 min, the total run time was 60.83 min and the detector temperature was set at 240 °C. The sniffing port ODP3 equipped with a humidified air make up and a computer voice recorder interacted in the ChemStation software (Agilent, USA). The detection frequency method was used to estimate the aroma impact of each volatile compound.¹⁶ Four trained assessors evaluated the odour from the GC-effluent. A total of 9 assessments were conducted on the inoculated T batch of GS. The final detection frequency value (DF) for each compound was obtained by summation of the

9 “sniffings”. The detection of an odour by less than two assessors was considered noise. For each assessment, evaluation of the odour took place over two different time intervals of 30 min. Aroma compounds were identified by three different methods: comparison with mass spectra and Linear Retention Indices obtained for the same samples in the GC-MS, comparison with the Linear Retention Indices of authentic standards, and by coincidence of the assessors’ descriptors with those in the Fenaroli’s handbook of flavour ingredients.¹⁷

Statistical analysis

Data were analysed using generalised linear model (GML) procedure of statistical software XLSTAT 2018 (Addinsoft, Paris, France). The model includes the effect of yeast inoculation and time of fermentation as a fixed effect and replicates as random effects. When significant effect of the treatment group was detected ($P < 0.05$), least square means (LSM) were compared using Tukey test. At the end of the fermentation (4 d), principal component analysis (PCA) and a heat map were done. PCA evaluated the relationships among different inoculated yeasts and all volatile compounds produced, while the heatmap was calculated based on the relative concentration of identified odour-active compounds produced by yeast strains.

RESULTS AND DISCUSSION

Yeast growth on Guajillo sauce fermentation

Yeast growth increased up to 2×10^8 CFU mL⁻¹ after 1 d of fermentation (Fig. 1) except for Ho fermentation, which reached its maximum growth at 2 d. Yeast growth was similar in Pk, Wa, and T fermentations, whereas Ho population decreased after 3 d of fermentation. No yeasts were found in the control (non-inoculated) batch sauce (C). The high yeast counts reached at the end of GS fermentation by Pk and Wa yeasts are comparable with the standards for commercially available probiotic formulations.¹⁸

The Sauces’ pH was 5.31 ± 0.16 at the initial fermentation time and decreased in all batch sauces at the end of fermentation. Control and Ho sauces reached the lowest pH at 3.9 ± 0.1 and 3.8 ± 0.2

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respectively, while Pk, Wa, and T sauces were always above pH 4 (4.6 ± 0.1 , 4.6 ± 0.2 , and 4.4 ± 0.1 , respectively). Decrease of pH occurs commonly during vegetable fermentation and has been reported during the acidification of red and yellow peppers by lactic acid bacteria.¹⁹ The presence of lactic acid bacteria was evaluated on MRS media, but no growth was detected (data not shown). In comparison, our results show a final pH consistent with the lowest acidification power of yeast versus lactic acid bacteria.²⁰ Moreover, *H. opuntiae* population decreased significantly after 3 d (Fig. 1), due to acid accumulation and decrease of pH in the GS.

Evolution of volatile compounds during fermentation

Table 1 shows the identification of volatile compounds by GC-MS in the control and fermented GS. A total of 78 volatile compounds were found, including aldehydes (10), alcohols (15), sulphides (2), acids (7), esters (19), ketones (5), nitrogen compounds (2), furans (3), terpenes (13), and miscellaneous compounds (2). Among them 18 were tentatively identified by mass spectrum (Table 1). Forty-six volatile compounds were detected at the beginning of the fermentation, therefore, they must be naturally present in the Guajillo chilli peppers. Most of them have been reported previously in different fresh varieties of chilli peppers.^{6,7,21}

The production of volatile compounds in each GS depended on fermentation time and inoculated yeast as shown in Table S1 (supplementary material). While, the evaluation of the ability of the yeasts to generate volatile compounds at the end of the fermentation (4 d) was done by PCA analysis (Fig. 2 (A,B)). A projection of variables (grouped by chemical group) obtained for the four yeast batches explained 81.99% of the total variance. Principal component 1 (PC1) accounted for 65.5% of the variability and distinguishes GS by fermenting yeast. Sauce Ho appears in the left quadrant, Wa and T are in the centre and Pk appears in the right quadrant. In contrast, Principal component 2 (PC2) explained 16.5% of the variability and was because of the experimental replicates. It is worth noting that sauce Ho seems to be different from the other sauces, as all replicates are in the negative part of the x axis in Fig. 2A. The most relevant characteristic of Ho sauce seems to be the high abundance

of acetic acid (3 to 5 times more than the other sauces, Table S1), which would contribute to the decrease of *H. opuntiae* population at the end of the fermentation.

Thirty-eight active aroma zones were identified in fermented GS by mass spectra, linear retention indices, and odour description, although 4 of them were not identified (Table 2). The highest detection frequency (DF) values mean the highest aroma impact. The most potent odorants detected were propanoic acid (cheesy), 3-methylbutanoic acid (sharp, cheese), ethyl 2-methylbutanoate (fruity, fresh, banana, strawberry), 6-methyl-5-hepten-2-one (strong, citrus, green), benzaldehyde (strange mushrooms), 3-methylbutyl propanoate (sweet, fruity, fresh, strawberry), ethyl octanoate (sweet, green, herbaceous), and 2-acetylpyrrole (musty, sweet, vegetable). The effect of incubation time was significant in all aroma compounds seen in Fig. 3 and Table S1.

To show the aroma profile of odour-active compounds generated by each yeast strain, a heatmap with hierarchical clustering was represented (Fig. 4). The dendrogram at the top shows that the four yeast fermentations divided into two groups, one including all replicates of Ho-t4 and the second group all replicates of Pk-t4, Wa-t4, and T-t4. This second group is further divided in two groups, differentiating Pk-t4 and Wa-t4 from T-t4. The heatmap shows the relative lowest production of aroma ester compounds and alcohols by Ho-t4 batches, except for ethyl 2-methylbutanoate. Moreover, Ho-t4 batches showed the highest relative production of 6-methyl-3,5-heptadiene-2-one and 2,2,6-trimethyl cyclohexanone.

Acids described as potent aroma contributors, such as 2-methylpropanoic and 3-methylbutanoic acids were produced in Wa-t4 batches, while propanoic acid appeared in Wa and T batches. This confirms that vegetable fermentations produced by these yeasts differ from other characteristic foods. This is like traditional pickled peppers fermented by lactic acid bacteria, where acetic acid is an aroma contributor.²² but not in GS sauces (Table 2), although it was produced in Ho sauces. In other pepper fermentations, like Korean fermented chilli pepper paste (Gochujang) fermented by *Lactobacillus parabuchneri*, several ester compounds (ethyl 2-methylpropanoate and ethyl butyrate, among others) were reported as aroma contributors.²³ Mixed fermentations of red hot chilli pepper mash, by *Lactobacillus lactis* and *Saccharomyces cerevisiae*, produced the intensification of volatile

compounds such as alcohols and esters.⁸ Still, this fermentation is different from the GS, which are characterised by the low production of aldehydes (Fig. 3a).

Our study shows the highest abundance of aromatic esters in Pk, Wa, and T sauces (Fig. 3d). Among the aromatic esters, the most potent compounds were branched-chain esters and medium-chain fatty acid (MCFA) ethyl esters contributing with fruity and sweet notes (Table 2). Among all the alcohols identified in the fermented sauces (Table 1), five of them (2-methyl-1-butanol, 3-methyl-2-buten-1-ol, 3-ethoxy-1-propanol, 6-methyl-5-hepten-2-ol and benzylalcohol) have not been reported in the fermentation of chilli peppers before.^{8,21–23} Moreover, 6-methyl-5-hepten-2-ol and benzylalcohol have been detected as odour-active compounds in our study, although benzylalcohol does not appear to be produced by the inoculated yeasts, since the highest abundance was found in the non-inoculated batch (Table S1).

Regarding aldehydes, Ho sauce contained the highest abundance followed by C, Pk, Wa, and T sauces (Table S1). Lara-Hidalgo *et al.*¹³ reported the lower oxidant power of *H. opuntiae* compared to the other two yeast strains. Therefore, it might be possible that other microorganisms in the control sauce prevented the oxidation of alcohols for the formation of aldehydes. Among aromatic aldehydes (Fig. 3a), benzaldehyde and 3-methylbutanal have been reported as components in the aroma profile of fermented pickled peppers²² and Gochujang.²³ These compounds usually contribute to herby and malty flavours, respectively; however, in this study descriptors as strange mushrooms and fruity were reported for these compounds and other aldehydes contributed the green and herbal notes (Table 2). The corresponding alcohols or acids derived from 3-methylbutanal (3-methyl-1-butanol/3-methyl butanoic acid), hexanal (hexanol/hexanoic acid), and benzene acetaldehyde (phenylethyl alcohol) were too detected (Table 1).

The ketones identified in our study (Table 1) have already been reported previously in *C. annuum* fresh fruits.^{7,24} In our study, the abundance of 6-methyl-5-hepten-2-one increased significantly at 24 h of fermentation, most probably due to *P. kudriavzevii* and *W. anomalus* inoculation and decreased until the third day of fermentation, increasing again later (Table S1). Mo *et al.*²⁵ have described these compounds as intermediates for the production of other volatile compounds. Yan *et al.*²⁶ detected an

increment of ketones in the fermentation of *Daqu*, a Chinese liquor produced from cereals, carried out by non-*Saccharomyces*, of which yeasts *P. kudriavzevii* and *W. anomalus* were among them.

The presence of furans and nitrogen compounds identified in GS (Table 1) has been reported in fresh ripened *C. annuum*, paprika, and Gochujang,^{6,26,27} but their contribution to the aroma has not been explained. Our results show a contribution of these compounds to toasted and sweet notes, which are associated to Maillard reactions.²⁸

Terpene production increased irregularly during GS fermentation (Fig. 3h and Table S1). These changes could be attributed to yeast or microbial metabolism that could break the terpene-sugar bond and enhance terpene release, and subsequent bioconversions in other terpenes or aromatic compounds.²⁹ These volatiles contributed to fresh, green, and spice aroma notes (Table 2), and have not been reported before as aroma contributors in this variety of chilli pepper.²³

In summary, this is the first time that the contribution of potential probiotic yeasts to the aroma profile of Guajillo pepper (*Capsicum annuum* L.) sauce has been described. The use of different yeast in GS fermentation provided different aroma notes and profiles. *P. kudriavzevii* and *W. anomalus* produced a similar aroma profile in GS with fruity, green-herbal, and cheesy odour notes based on ester compounds, alcohol, and branched-chain acids production. However, *W. anomalus* inoculation increased the cheesy character in GS because of its ability to produce propanoic acid. In contrast, inoculation of *H. opuntiae* produced a completely different aroma profile in GS with herbal and green notes based on high production of aldehydes, ketones, and acetic acid but with low fruity character. Moreover, the contribution of *H. opuntiae* inoculation in the T sauce aroma seems to be insignificant. The use of yeast mixtures (T) affected positively the aroma of GS sauces with the production of key aroma compounds. Among these, propanoic acid (cheesy), 3-methylbutanoic acid (sharp, cheese), ethyl 2-methylbutanoate (fruity), and 6-methyl-5-hepten-2-one (strong, citrus), in conjunction with additional 31 compounds, rounded off the aroma of the fermented sauce. In conclusion, these results provide a new perspective for improvement and diversification of the aroma quality of fermented traditional foods and establish a basis for further research on the properties of Guajillo sauce such as processing conditions, stability, consumer acceptance, and purchase intention.

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FIGURE LEGENDS

Figure 1. Yeast growth during the fermentation of Guajillo sauce (Control (■); Ho, *H. opuntiae* (◆); Pk, *P. kudriavzevii* (◇); Wa, *W. anomalus* (●); T, mixed culture (○)).

Figure 2. Loadings of the first two principal components (PC1 – PC2) of Guajillo fermented sauce at the end of the fermentation (4 d) (A), and volatile compounds produced (B). PCA shows the replicates of each yeast fermented sauce. Ho, *H. opuntiae*; Pk, *P. kudriavzevii*; Wa, *W. anomalus*; T, mixed culture.

Figure 3. Abundance of aroma compounds (Table 2), expressed as Total Ion Current, TIC per 10⁵, produced during the fermentation of Guajillo sauce and classified according to chemical group. (a) aldehydes, (b) alcohols, (c) acids, (d) esters, (e) ketones, (f) nitrogen, (g) furans, and (h) terpenes compounds. Symbols represents the different batches; Control (■), Ho, *H. opuntiae* (◆); Pk, *P. kudriavzevii* (◇); Wa, *W. anomalus* (●); T, mixed culture (○).

Figure 4. Heatmap representing aroma profile including only odour-active compounds at 4 d of fermentation of GS (control C and yeast fermented: Ho, *H. opuntiae*; Pk, *P. kudriavzevii*; Wa, *W. anomalus*; T: mixed culture). The colours in the map represent the following: red colour indicates relatively high abundance; blue colour indicates relatively low abundance of each aroma compound; white colour indicates no difference.

Table 1. Identification of volatile compounds detected in the headspace of fermented Guajillo sauce with and without inoculated yeast strains

Compounds	LRI ²	RI ³	C ⁴	C-t4	Yeasts			
					Ho-t4	Pk-t4	Wa-t4	T-t4
Aldehydes								
Acetaldehyde	460	a	+ ⁵	+	+	+	+	+
2-Methylpropanal	593	a	+	-	-	-	-	-
3-Methylbutanal	692	a	+	+	-	-	-	-
Diethyl acetal	753	a	-	+	+	+	+	+
Hexanal	844	a	+	-	-	-	-	-
Furfural	899	a	+	+	+	-	-	-
Benzaldehyde	1018	a	+	+	+	+	+	+
(E,E)-2,4-Heptadienal	1077	a	+	-	-	-	-	-
Benzene acetaldehyde	1108	a	+	+	+	+	+	+
2,5-Dimethylbenzaldehyde	1256	a	+	-	-	-	-	-
Alcohols								
Ethanol	509	a	+	+	+	+	+	+
1-Propanol	612	a	-	+	+	+	+	+
2-Methyl-1-propanol	679	a	-	+	+	+	+	+
1-Butanol	720	b	-	+	-	+	+	+
3-Methyl-1-butanol	790	a	+	+	+	+	+	+
2-Methyl-1-butanol	795	a	-	+	+	+	+	+
3-Methyl-2-buten-1-ol	829	a	-	+	-	-	-	-
2,3-Butanediol [R-(R*,R*)]-	878	a	+	+	+	+	+	+
2,3-Butanediol [R-(R*,R*)]-	886	a	+	+	+	+	+	+
3-Ethoxy-1-propanol	896	a	-	-	+	+	+	+
1-Hexanol	921	a	-	+	+	+	+	+
6-Methyl-5-hepten-2-ol (95) ¹	1042	a	-	+	+	+	+	+
2-Ethyl-1-hexanol	1080	a	+	+	+	+	+	+
Benzylalcohol	1121	a	-	+	+	+	+	+
Phenylethyl alcohol	1196	a	+	+	+	+	+	+
Sulfide compounds								
Dimethylsulfide (62)	534	a	+	-	-	-	-	-
3-Methylthio-1-propanol	1060	a	-	-	+	-	+	+
Acids								
Acetic acid	702	a	+	+	+	+	+	+
Propanoic acid	793	a	+	-	+	+	-	-
2-Methylpropanoic acid	851	a	+	+	+	+	+	+
Butanoic acid	881	a	-	-	-	+	+	-
3-Methylbutanoic acid	935	a	+	+	+	+	+	+
2-Methylbutanoic acid	941	a	+	+	+	+	+	+
Hexanoic acid	1069	a	+	-	+	+	+	+
Esters								
Ethyl formate	540	b	-	+	+	+	+	+
Methyl acetate	553	a	-	+	+	+	+	+
Ethyl acetate	638	a	+	+	+	+	+	+
Ethyl propanoate	739	a	-	-	+	-	+	+
Propyl acetate	743	a	-	-	+	+	+	+
Ethyl 2-methylpropanoate	783	a	-	-	-	+	+	+
2-Methylpropyl acetate	804	a	-	-	+	+	+	+
Ethyl butyrate	827	a	-	+	-	+	+	+
Ethyl 2-Methylbutanoate	875	a	-	+	+	+	+	+

3-Methylbutyl acetate	906	a	-	+	+	+	+	+
2-Methylbutyl acetate	909	a	-	-	+	+	+	+
3-Methylbutyl propanoate	997	a	-	-	-	+	+	+
Ethyl hexanoate	1026	a	-	+	+	+	+	+
Ethyl heptanoate	1126	a	-	-	+	+	+	+
Ethyl octanoate	1226	a	-	+	+	+	+	+
Methyl salicylate	1256	b	-	-	-	+	+	-
2-Phenylethyl acetate	1315	a	-	-	+	+	+	+
Ethyl nonanoate	1326	a	-	-	+	+	+	+
Ethyl dodecanoate	1626	b	-	+	+	+	+	+
Ketones								
2,3-Butanedione	626	a	-	+	+	+	+	+
3-Hydroxy-2-butanone	779	a	-	+	+	+	+	+
6-Methyl-5-hepten-2-one	1034	a	+	+	+	+	+	+
2,2,6-Trimethyl cyclohexanone	1083	b	+	+	-	+	+	-
6-Methyl-3,5-heptadiene-2-one	1173	b	+	+	+	+	+	+
Nitrogen compounds								
Tetramethylpyrazine	1118	a	+	+	+	+	+	+
2-Acetylpyrrole	1155	a	+	-	+	+	+	+
Furans								
2-Methylfuran	619	a	+	-	-	-	-	-
Acetylfuran (95)	974	a	+	+	+	+	+	+
2-Pentylfuran	1011	a	+	-	-	+	+	-
Terpenes								
D-Limonene (93)	1044	a	+	+	+	+	+	+
α -Isophorone	1109	b	+	+	+	+	+	+
4-Oxoisophorone	1225	a	+	-	+	-	-	-
β -Cyclocitral	1285	a	+	+	+	+	+	+
α -Cubebene	1404	b	+	+	+	+	+	+
β -Elemene	1426	b	+	+	+	+	+	+
Longifolene	1489	b	+	+	+	+	+	+
Terpene	1507	c	+	+	+	+	+	+
Dihydropseudoionone	1510	b	+	+	+	+	+	+
Longifolene	1527	b	+	+	+	+	+	+
Eremophilane	1533	b	+	+	+	+	+	+
Selinene	1539	b	+	+	+	+	+	+
β -Ionone	1567	a	+	+	+	+	+	+
Miscellaneous								
p-Xylene	894	a	+	+	+	+	+	+
Safranal	1264	b	+	+	+	+	+	+

¹ Target ion used to quantify the compound when the peak was not completely resolved.

² Linear retention indices (LRI) of the compounds eluted from the GC-MS using a DB-624 capillary column (J&W Scientific 30 m×0.25 mm i.d.×1.4 μ m film thickness).

³ Reliability of identification: a, identification by mass spectrum and by coincidence with the LRI of an authentic standard; b, tentative identification by mass spectrum; c: unidentified compound.

⁴ C: Control batch without inoculated yeast and yeast inoculated batches at the end of the fermentation (4 d); Ho-t4, *H. opuntiae*; Pk-t4, *P. kudriavzevii*; Wa-t4, *W. anomalus*; T-t4, mixed culture.

⁵ presence (+) or absence (-) of compound in the headspace.

Table 2. Odor active compounds identified in the headspace of Guajillo sauce at 4 days of fermentation

Compound	GC-MS ^a		GCO ^b		RI ^c	Descriptor	DF ^d
	LRI	LRI std	LRI Initial	LRI Final			
Aldehydes							
3-Methylbutanal	692	687	689	692	a	Fruity	3
Hexanal	844	839	834	841	a	Green, grassy	3
Furfural	899	898	901	904	a	Sweet, fruity	4
Benzaldehyde	1018	1013	1017	1021	a	Strange mushrooms	8
(E,E)-2,4-Heptadienal	1077	1076	1075	1077	a	Herbaceous, green	3
Benzene acetaldehyde	1108	1104	1108	1113	a	Unpleasant, harsh	5
2,5-Dimethylbenzaldehyde	1256	1257	1236	1252	a	Spicy, brown odor	7
Alcohols							
2-Methyl-1-propanol	679	681	682	683	a	Sweet, fruity	3
6-Methyl-5-hepten-2-ol	1042	1040	1045	1049	a	Green, fresh	3
Benzyl alcohol	1121	1120	1116	1119	a	Green, herbal	3
Phenylethyl alcohol	1196	1191	1196	1205	a	Chemical, camphor	7
Acids							
Propanoic acid	793	806	784	787	a	Cheesy	9
2-Methylpropanoic acid	851	864	867	869	a	Cheese, unpleasant	4
3-Methylbutanoic acid	941	947	920	927	a	Sharp, cheese	9
Esters							
Ethyl 2-methylpropanoate	783	786	781	784	a	Sweet, fruity, strawberry, banana	5
Ethyl butyrate	827	829	824	827	a	Fruity, sweet, peach odor	4
Ethyl 2-methylbutanoate	875	876	871	873	a	Fruity, fresh, banana, strawberry	9
3-Methylbutyl acetate	906	905	906	910	a	Sweet, citrus, green	3
3-Methylbutyl propanoate	997	996	987	990	a	Sweet, fruity, fresh, strawberry	8
Ethyl hexanoate	1026	1026	1021	1024	a	Fruity, sweet	6
Ethyl octanoate	1226	1226	1221	1224	a	Sweet, green, herbaceous	8
Ethyl nonanoate	1326	1327	1324	1327	a	Alcohol, wine	5
Ketones							
6-Methyl-5-hepten-2-one	1034	1031	1028	1032	a	Strong, citrus, green	9
2,2,6-Trimethylcyclohexanone	1083		1087	1089	b	Sweet, caramel	5
6-Methyl-3,5-heptadiene-2-one	1173		1159	1165	b	Spicy, green	5
Nitrogen compounds							
2-Acetylpyrrole	1155	1158	1144	1150	a	Musty, sweet, vegetable	8
Furans							
Acetylfuran	974	968	962	965	a	Toasted, grilled	4
2-Pentylfuran	1011	1009	1001	1006	a	Sweet, fresh	4
Terpenes							
α-Isophorone	1109		1104	1110	b	Fruity, fermented	4
4-Oxoisophorone	1225	1221	1205	1211	a	Pepper, spicy, green, herbal	7
β-Cyclocitral	1285	1286	1281	1293	a	Fresh, green, vegetable	5
α-Cubebene	1404		1395	1402	b	Green, waxy	4
β-Elemene	1426		1467	1477	b	Fresh, spicy	7
Longifolene	1489		1471	1474	b	Sweet, fresh, herbaceous	4
Others							
Unknown			707	714	c	Sulphurous, unpleasant	4
Unknown			955	957	c	Sweet, caramellic	5
Unknown			967	970	c	Mushrooms, fermented vegetable	6
Unknown			1185	1189	c	Herbaceous	5

^aLinear retention indices (LRI) of the compounds eluted from the GC-MS and LRI of standard compound using a DB-624 capillary column (J&W Scientific 30 m×0.25 mm i.d.×1.4 μm film thickness).

^bInitial and end linear retention index (LRI) of the compounds eluted from the GC-FID-O using a DB-624 column capillary column (60 m x 0.32 mm i.d. x 1.8 μm film thickness).

^cRI: reliability of identification: a, identification by mass spectrum and by coincidence with the LRI of an authentic standard and by coincidence of the assessors' descriptors with those in the Fenaroli's handbook of flavour ingredients (Burdock, 2002), b, tentative identification by mass spectrum, c: not identified.

^dDF: Detection frequency value.

Figure 1.

Observations (F1 and F2: 81.99 %)