Screening of yeast strains for flavour potential in meat products under reduced concentration of preservative nitrifying agents

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Abstract

Aroma characteristics of fermented sausages depends on the processing factors such as ingredients and preservatives as well as on the starters used during the fermentative process. Consumers' demand for healthier products is leading to a reduction in the preservative curing agents (nitrate and nitrite) used in the processing of meat products while preventing detriment to sausage aroma. *D. hansenii* yeasts are known contributors to sausage flavour, however little is known about their potential to produce volatiles under reduced concentration of curing agents or its consequences for amino acid metabolism. *D. hansenii* strains isolated from sausages manufactured with different raw materials (meat from pork or llama) were evaluated in a model system resembling the sausage formulation containing free amino acid and additives (salt and glucose) and variable concentrations of nitrite and nitrate. The different ability of the yeast strains to produce volatile compounds from different amino acids and the changes in aroma profile due to nitrifying agents' reduction were evaluated.

Introduction

The conversion of amino acids, generated through proteolysis during sausage manufacturing [1], into aroma compounds depends largely on microbial metabolism during fermentation where yeasts play an important role [2]. The occurrence of *D. hansenii* as the dominant yeast in a large number of fermentation and ripening processes for production of dry meat products has led to its utilization as starter culture for meat fermentation. Aroma characteristics of the sausages depend not only on the yeast strain used for fermentation but also on the processing factors (raw materials, meat ingredients, preservatives, technological parameters, presence of starter cultures) that can affect the metabolic activity of the yeasts.

Actual trends to reduce the use of preservatives (nitrite and nitrate) in meat products, despite their role in safety and technological properties, has led the industry to look for strategies to maintain safety and quality. However, it is unknown the effect of a reduction in concentration of nitrite and nitrate used as preservatives in fermented sausages on yeast amino acid metabolism and its contribution to generation of volatile compounds. The objective of this study is to search for yeast with flavour production potential in meat products and determine the effect of a reduction in nitrate and nitrite concentration on their amino acid metabolism and volatiles production.

Experimental

Yeast strains

Debaryomyces hansenii strains: L1-L9 were isolated from naturally fermented sausages manufactured with pork meat (L1-L6) [3-5] and llama meat (L7-L9) [6].

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Meat model system

The meat model system was prepared with a similar composition of dry fermented sausages in terms of additives and amino acid content [7]. The model was prepared using 0.67 % YNB (Yeast Nitrogen Base, Difco Inc.), 30 g/L NaCl, 10 g/L glucose, amino acids in concentration reported by Corral et al. [7] and variable concentrations of $NaNO_2$ and KNO_3 as follows: 0.150 g/L each in control medium (C) and 0.128 and 0.113 g/L in media RN15 and RN25, respectively. A total of 11 experiments (50 mL media in 100 mL Erlenmeyer flasks) were carried out using each media C, RN15 and RN25. Nine experiments were inoculated with *D. hansenii* strains and two not inoculated and used as controls before and after incubation [8]. Incubation was at 25°C for 16 d. Experiments were performed in triplicate. After incubations all media were centrifuged and the supernatant recovered for volatile and amino acid content analyses [8].

The free amino acids content was determined by reverse phase HPLC using phenylthiocarbamyl amino acid derivatives according to Aristoy & Toldrá [9] using norleucine (65.6 µg) as internal standard. Quantification of amino acids was done relative to the internal standard and expressed as a percentage of concentration present in the control media before incubation [8].

The volatile analysis was done by SPME-GC-MS using an automatic injector Gerstel MPS2 multipurpose sampler (Gerstel, Germany) and an 85µm CAR/PDMS fibre [8]. Compounds were identified by comparison with mass spectra from the NIST/EPA/NIH Mass Spectral Database, linear retention index and by comparison with authentic standards. Identified volatile compounds were quantified and the abundance expressed as the increase respect to control media after incubation [8].

Statistical analysis

Data were analysed using Generalized Linear Model (GML) procedure of statistical software (XLSTAT 2011, v5.01, Addinsoft, Barcelona, Spain). The model included the effect of yeast inoculation as fixed effects and replicates as random effects. Principal component analysis (PCA) was used to evaluate the relationships among aroma compounds, free amino acids and model inoculated media. The inoculated model media represented the difference experiments (L1-L9) carried out using each media C, RN15 and RN25.

Results and discussion

Nine yeast strains isolated from pork or llama sausages and pertaining to the species *D. hansenii* were screened for their ability to produce volatiles on amino acid rich media containing nitrifying agents used as preservatives in meat products. The most usual nitrite/nitrate concentration used for the elaboration of meat products around Europe were added to the medium (150 ppm) although specific regulations for other traditional European meat products exist [10]. The volatile compounds produced by yeast strains from the degradation of val, ile, leu, met and phe are summarized in Table 1. Statistical analysis revealed a clear difference among yeast strains to produce these volatile compounds from the selected amino acids (Figure 1). Yeasts isolated from llama sausages were characterized by the production of propanoate ester compounds and branched alcohols, while those from pork sausages produced branched aldehydes and acids. Yeast L5, isolated from pork sausages displayed a distinct volatile profile characterized by the presence of ethyl esters derived from methyl branched acids.

Table 1. Volatile compounds identified in yeast inoculated model systems after incubation.

Volatile Compounds	*LRI*[1]	*RI*[2]	*Volatile Compounds*	*LRI*	*RI*
Valine derived comp			*Leucine derived compounds*		
2-mehylpropanal	592	a	3-methylbutanal	689	a
2-methyl-1-propanol	682	a	3-methylbutanol	794	a
Ethyl 2-methylpropanoate	788	a	Ethyl 3-methylbutanoate	881	a
2-methylpropyl acetate	805	a	3-methylbutanol acetate	906	a
2-methylpropanoic acid	862	a	3-methylbutanoic acid	937	a
Propyl 2-methylpropanoate	896	a	3-methylbutanol propanoate	996	a
Isoleucine derived compounds			*Phenylalanine derived compounds*		
2-methylbutanal (58)[3]	700	a	Benzaldehyde	1017	a
2-methylbutanol	797	a	Phenylethyl alcohol	1194	a
Ethyl 2-methylbutanoate	878	a	2,3-dimethylbenzaldehyde	1292	b
2-methylbutanol acetate	909	a	2-Phenylethyl acetate	1315	a
2-methylbutanoic acid	943	a	2-Phenylethyl propanoate	1405	b
2-methylbutanol propanoate	999	a			
Methionine derived compounds					
Dimethyl disulfide	772	a			

[1]LRI: Linear Retention Index calculated for DB-624 column. [2]RI: Reliability of identification: (a) mass spectra and LRI in agreement to standard compound, (b) tentatively identified by mass spectra. [3]Target ion in brackets used to quantify the compound when the peak was not completely resolved.

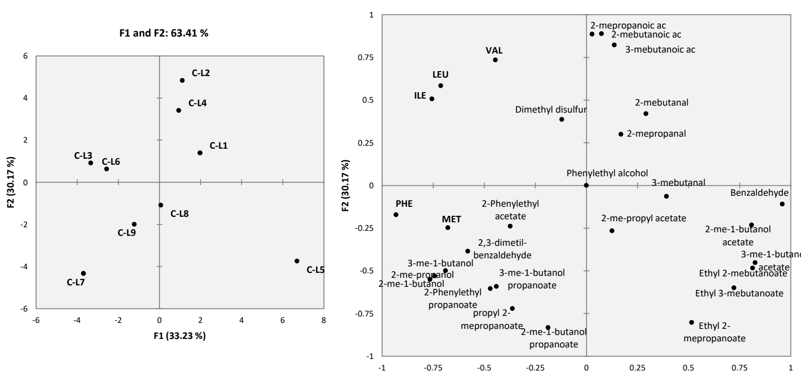

Figure 1: Loadings of the first two principal components F1 and F2 of volatile compounds derived from the degradation of amino acids Ile, Leu, Val, Phe and Met in media inoculated with yeast strains (pork strains L1-L6; llama strains L7-L9).

Among the yeast studied, those isolated form llama sausages (L7-L9) and L5 were the highest producers of ester compounds and could be useful to impart specific flavour notes in dry meat products. Ester compounds have been identified in fermented sausages providing fruity aromas and contributing to mask rancid and vegetable cooked odours [11]. In contrast, branched aldehydes, identified in fermented meats as contributors to the overall flavour [12], have been found in sausages inoculated with *D. hansenii* strains [13]. The main branched aldehydes producers were among the pork isolated yeasts (L1-L4) which may also be suitable for fermentative processes.

Regarding the effect of preservative reduction on yeast metabolism, nitrate-nitrite reduction affected the yeast ability to produce volatiles. Particularly, yeasts L1 and L5, isolated from pork sausages, increased the production of branched acids (L1) and ethyl ester compounds (L5), as can be seen in the PCA graph (Figure 2). Until now, there are no reports regarding the effect of nitrate and nitrite reduction on amino acid catabolism of *D. hansenii* strains from meat products. Previous research on other meat starters reported a reduction in leucine catabolism when nitrate and nitrite were added to an experimental model system containing a staphylococcus starter [14]. Under these premises, the current trend to reduce the use of nitrites and adjust their levels in meat products makes necessary an evaluation of its impact on microbial starters in terms of volatile compounds generation [8].

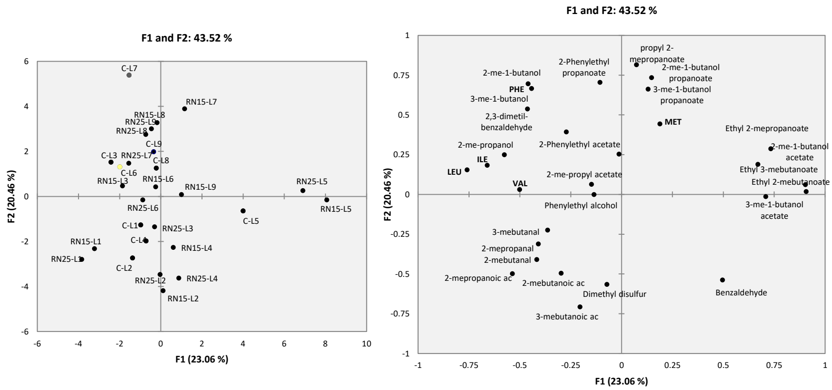

Figure 2: Loadings of the first two principal components F1 and F2 of volatile compounds derived from the degradation of amino acids Ile, Leu, Val, Phe and Met in control (C) and nitrifying reduced media RN15 and RN25 media, produced from yeast inoculation (L1-L9).

Conclusions

Yeast strains isolated from sausages manufactured with different raw materials (meat from pork or llama) have different ability to produce volatile compounds. Yeast amino acid metabolism and production of volatiles are significantly affected by the presence of variable nitrate/nitrite concentrations. The inoculation of selected yeast strains during manufacturing of dry sausages may produce a significant effect on the overall sausage flavour.

Acknowledgements

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