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Book of Abstracts

Volatile composition of experimental beers fermented with selected yeasts to emphasize their aromatic expression.

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Summary: *The aromatic profile of some experimental beers was characterized, focusing on the yeast-matrix interaction and on the formation of volatile thiols of varietal origin. Aromatic profiles were analysed by GC-MS after extraction by SPME, while a new preparatory approach was adopted for polyfunctional thiols that includes derivatization with ethylpropiolate.*

Keywords: *Hop, Yeast, volatile compounds*

1 Introduction

The composition in volatile compounds of beers is the result of several complex processes including malting, fermentation and hopping, able to shape the aromatic uniqueness of the final product. From this point of view a relevant importance is played by the interaction yeast-hop. Different yeast strains have variable ability to release aromatic substances from precursors present in the hops or to transform VOCs, already present in free form, into odorous compounds. This work presents some results the use of different *Saccharomyces cerevisiae* strains having β-liasic activity which characterize the aromatic profile of beers. These yeasts, belonging to the collection of CREA-VE (CREA-CMVE collection), are potentially able to release polyfunctional thiols from their respective precursors into solution. The aim of the work is to provide new competencies in the production of beers with high aromatic impact, particularly required by the market.

2 Experimental

To identify yeasts with highest β-lyase activity a preliminary qualitative test was carried out [1]. Basically, the production of methylsulfide and dimethyldisulfide was measured in a growth medium containing s-methylcysteine, an amino acid specifically degraded by Saccharomyces β-lyases. The culture medium, inoculated at 1% with the selected yeast, was analyzed after 7 days by SPME/GC-MS (Solid Phase Micro Extraction) according to the method proposed by Dziekońska-Kubczak et al. [2].
The yeast ISE60 presented a high β-lyase activity, and it was chosen as starter for the subsequent fermentation.

Following a complete factorial experimental plan, 4 fermentation tests were carried out, each of them repeated in triplicate. The yeast from the collection was compared with a commercial yeast (Rock, Lamothe Abiet Italia, Via Della Scienza, 11, 15067 Novi ligure AL). The beers were flavored with two different types of hops: Mosaic, characterized by a high content of terpenes (in particular myricene) and Hallertau Mittelfruh which has a lower content. The beer aromatic profile was obtained directly analyzing the samples by SPME/GC-MS. The thiols were instead analyzed by SPE/GC-MS after derivatization adapting the method proposed by Herbst-Johnstone and Piano [3]. Briefly, polyfunctional thiols (3-mercaptohexanol, 4-mercapto-4-methyl-2-pentanone and 3-mercaptohexylacetate) were derivatized using ethylpropiolate in an alkaline medium (pH 10), extracted on a reversed phase SPE cartridge (C18 EC, 1g, Biotage, Sweden) and separated on a non-polar column.

3 Results

The major aromatic differences between the experimental beers are related to the use of different hops. Mosaic hops yield abundant amounts of terpenes, both linear and cyclic, to beers. Myricene represents the main compound of the aromatic fraction, in accordance with what is reported in the literature [4]. Terpenols, particularly linalol, are in higher concentration in Mosaic theses than those with Hallertau Mittelfruh
There are also significant concentrations of sesquiterpenes, for example, caryophyllene and humulene in all fermented products. It should be emphasized that the concentration of these compounds appears dependent, at a first analysis,

also on the yeast used, being more abundant in beers fermented with ISE60.

Beers obtained from different yeasts are also distinguished for the different concentrations of fermentation compounds. Those obtained using ISE60 are characterized by a higher presence of acetates of higher alcohols (isoamyl acetate and 2-phenylethyl acetate). While beers fermented with the commercial yeast show a higher concentration of ethyl esters of medium-chain fatty acids (ethylhexanoate and ethyloctanoate) characterized by intense and pleasant fruity or floral notes. On the other hand, ISE60 beers are richer in ethyl decanoate and ethyl dodecanoate, slightly aromatic. Finally, the ISE60 tests show the strong presence of vinylguaiacol (2-methoxy-4-vinylphenol) a degradation compound of cinnamic acids, characterized by intense spicy and pharmaceutical notes.

Polyfunctional thiol compounds, with pleasant olfactory notes, are present in high concentrations, well beyond the respective perception thresholds in all the theses. 3-mercaptohexylacetate shows differences in concentration between theses fermented with the yeast ISE60 and commercial yeast, presumably for a greater efficiency in the acetylation reactions of the collection yeast ISE60.

4 Conclusions

Gas chromatography coupled with mass spectrometry is nowadays an indispensable analytical tool for evaluating the aromatic profile of agri-food products. This exploratory work in brewing microbiology highlights the flexibility of this analytical approach which allows wide applications starting from the selection phase of brewer's yeasts up to the evaluation of odorous compounds present in ultratraces in experimental beers. The results emphasize that the final beer aroma profile is directly linked to the yeast strain selection and interaction yeast-hop.

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