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Torulaspora delbrueckii in the brewing process: A new approach to enhance bioflavour and to reduce ethanol content

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ABSTRACT

Nowadays, consumers require fermented alcoholic beverages with particular and enhanced flavour profiles while avoiding the health concerns due to high ethanol content. Here, the use of *Torulaspora delbrueckii* was evaluated for beer production, in both pure and in mixed cultures with a *Saccharomyces cerevisiae* starter strain (US-05). The yeast interactions were also evaluated. In mixed fermentations with *S. cerevisiae*, the main analytical characters from *T. delbrueckii* were comparable with those of the *S. cerevisiae* starter strain, but the beers were characterized by a distinctive overall analytical and aromatic profile. Indeed, there were interactions between *S. cerevisiae* and *T. delbrueckii*, with enhanced ethyl hexanoate (0.048 mg l^{-1}) and ethyl octaonate (0.014 mg l^{-1}) levels at the 1:20 and 1:10 inoculation ratios, respectively; while phenyl ethyl acetate increased in all mix combinations. The presence of *T. delbrueckii* resulted in reduced β-phenyl ethanol and isoamyl acetate levels, which are responsible for floral and fruity aromas, respectively. Beer produced with *T. delbrueckii* pure cultures had a low alcohol content (2.66%; v/v), while also showing a particularly analytical and aromatic profile.

1. Introduction

Over the years, brewers have always tried to find yeast strains that can improve the quality of their beer and provide it with specific sensory notes. The various aroma compounds that characterize different beers styles come from the raw materials of barley, malt and hops. However, yeast has a central role in the brewing process, metabolizing of sugars in the beer wort into ethanol, carbon dioxide, and several aroma compounds, including esters, higher alcohols, aldehydes and organic acids (Kyselová and Brányik, 2014; Lodolo et al., 2008; Pires et al., 2014). In particular, in the beer industry, the goal of the use of inoculated yeast is to increase the fermentation efficiency, to develop new beers, and especially to enhance the sensory complexity of the final beer produced (Harrison, 2009). The production of aroma compounds through biological methods exploits the metabolic pathways of the yeast, for the promotion of the so-called bioflavour (Cheetham, 1993; Vanderhaegen et al., 2003). This approach can include microbial bioconversion of the flavour precursors, use of strains that produce the required compounds, and genetic modification of the yeast (Dequin, 2001; Priefert et al., 2001; Ramachandra Rao and Ravishankar, 2000; Mertens et al., 2015).

In winemaking, there has been a re-evaluation of the role of non-*Saccharomyces* yeast and their use in mixed fermentations, with the aim to enhance the analytical and aromatic profile of the final wine and to reduce the alcohol content (Benito et al., 2011; Ciani et al., 2010; Comitini et al., 2011; Contreras et al., 2014; Morata et al., 2012; Quirós et al., 2014; Sadoudi et al., 2012). Within the non-*Saccharomyces* yeast species, attention has been focused on *Torulaspora delbrueckii*, as this yeast has shown a positive impact in terms of low production of undesirable compounds, such as acetaldehyde, acetoin and acetic acid, and concomitant enhancement of other desired compounds (Azzolini et al., 2015; Bely et al., 2008; Comitini et al., 2011; Jolly et al., 2014; Loira et al., 2012). The use of non-*Saccharomyces* yeast has been less investigated in the brewing industry, where most beers are brewed with the use of a single yeast strain. However, the Belgian lambic beers are obtained from the spontaneous fermentation of *Saccharomyces* and *Brettanomyces* yeasts, with the contribution of lactic acid bacteria and acetic acid bacteria (Bokulich et al., 2012; Spitaels et al., 2014; Vanderhaegen et al., 2003). These mixed fermentations were also used in the production of some weissbier German style beer (Vriesekoop et al., 2012). During the maturation of acidic ale beers, different yeasts belonging to *Candida*, *Torulopsis*, *Pichia*,

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Hansenula and *Cryptococcus* genera were isolated, but their contribution on the aroma composition was not investigated (Vanderhaegen et al., 2003). Other non conventional beers such as Tchapalo, are brewed using *Candida tropicalis* and *Saccharomyces cerevisiae* cultures selected for their ability to ferment sorghum wort (N'Guessan et al., 2010). Regarding to the use of *T. delbrueckii* strains in brewing process, only a few studies have been conducted. King and Dickinson (2000, 2003) reported that *T. delbrueckii* has the ability to transform hop aroma terpenoids, influencing the aroma profile of the final beer. More recently, Tataridis et al. (2013) carried out a preliminary study on the use of *T. delbrueckii* strains in the production of "wheat" style beers. These authors found that this species was able to consume maltose more slowly than *S. cerevisiae* commercial starter strain, giving more intensity and complexity to the product. In the present study, after a preliminary screening, we evaluated the use of a selected strain of *T. delbrueckii* in wort fermentation in pure and mixed cultures. The influence on the analytical and aromatic profile of beer, as well as the potential of producing a low-alcohol beer with *T. delbrueckii* was evaluated.

2. Materials and methods

2.1. Yeast strains

The 28 yeast strains used in this study belong to the species *T. delbrueckii* and were obtained from the Yeast Collection of the Department of Life and Environmental Sciences (DiSVA) of the Polytechnic University of Marche (Italy). These had been isolated from natural matrices from different environments and in different geographical areas (i.e., Italy, Cameroons) (Table 1). All of the *T. delbrueckii* strains were identified through 5.8S internal transcribed spacer rDNA polymerase chain reaction restriction fragment length polymorphism analysis, and sequencing of the D1/D2 domains of the 26S rDNA gene, as reported by Comitini et al. (2011) and Solieri et al. (2006). The *S. cerevisiae* commercial strain US-05 (Fermentis, Lesaffre, France) was used as the control.

For short-term storage, all of the yeast strains were maintained on YPD medium (1% yeast extract, 2% peptone, 2% glucose, 1.8% agar; all w/v) (Oxoid, Basingstoke, UK) at 4 °C, and for long-term storage, in YPD broth supplemented with 80% (w/v) glycerol at −80 °C.

2.2. Preliminary screening

The fermentation of glucose, maltose and sucrose by these 28 *T. delbrueckii* strains was assessed using the Durham test, according to Kurtzman and Fell (1998). The fermentative performance of eight of these *T. delbrueckii* strains that fermented maltose were determined at 20 °C in flasks that contained 500 ml malted barley wort under sterile conditions. The main parameters of the fermentation kinetics (fermentation rate, total CO_2 evolved) of these *T. delbrueckii* strains and of the S. *cerevisiae* starter strain on the wort were assayed.

2.3. Fermentation trials

From preliminary screening *T. delbrueckii* DiSVA 254 was selected and used in the pure and mixed fermentations with the *S. cerevisiae* US-05 starter strain at different *S. cerevisiae* to *T. delbrueckii* ratios (i.e., 1:1, 1:10, 1:20, respectively). A batch of 1500 l of malted barley wort for the production of American Amber Ale was used in this study. Its main analytical characters were: pH 5.47; specific gravity 12.7 °Plato. The fermentation potential of the selected strain was evaluated in fermentation trials carried out at 20 °C in flasks containing 500 ml wort under sterile conditions. The flasks were locked with a Müller valve containing sulphuric acid, to allow only CO_2 to escape from the system.

Pre-cultures were grown in 10% malt extract at 20 °C for 48 h. The fermentation kinetics were monitored by measuring the weight loss of the flasks due to the CO_2 evolution, which was followed to the end of the fermentation (i.e., constant weight for 3 consecutive days). The growth kinetics were monitored using viable cell counts on WL Nutrient Agar (Oxoid, Hampshire, UK) and Lysine Agar (Oxoid, Hampshire, UK) a selective medium unable to support the growth of *S. cerevisiae* (Lin, 1975), for differentiation of the *T. delbrueckii* yeast from the *S. cerevisiae* starter strain. The fermentations were carried out in duplicate trials under static conditions.

2.4. Analytical determinations

The specific gravity was measured using a DA-300 specific gravity meter (Kyoto Instruments). The volatile acidity and pH determinations were performed according to the Official European Union Methods (EC, 2000). Ethanol was measured according to the Association of Official Analytical Chemists (1990). Acetaldehyde, ethyl acetate, higher alcohols, and volatile compounds were determined by direct injection into a gas–liquid chromatography system, as reported by Canonico et al. (2014). The free amino nitrogen was determined following a procedure described previously by Dukes and Butzke (1998). Specific enzymatic kits (Megazyme, Ireland) were used to determine the concentrations of glucose sucrose, maltose (kit k-masug) and ammonia (kit k-amiar) according to the manufacturer instructions.

2.5. Sensory analysis

At the end of the fermentation process, the beers obtained were transferred into 500-ml bottles, adding 5 g l^{-1}sucrose. The secondary fermentation in the bottle was carried out at 18–20 °C for 7–10 days. After this period, the beers underwent sensory analysis (Analytica EBC, 1997) on the basis of a list of descriptors related to

Table 1
Torulaspora delbrueckii strains used in this study.

Source of isolation	Geographic origin	Strain code[a]
Winery environment	Sardinia, Italy	315, 130
Soil	Italy	55
Papaya leaves	Cameroon	254, 419, 343, 413, 255
Sugar cane juice	Cameroon	426, 430, 431, 432, 363, 603
Grapes	Italy	258, 259, 260, 261, 313, 606, 607, 608, 609
Fig fruit	Italy	604, 605
Coconut palm	Cameroon	445
Corossol fruit	Cameroon	602, 399

[a] Accession number of DiSVA Collection (Department of Life and Environmental Sciences).