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Brewer´s Spent Yeasts for Oenology: from Characterisation to Must Fermentation

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Abstract

In order to follow the principles of the circular economy, the spent brewer´s yeasts were in this work evaluated to be considered as a raw material for production of yeast derivatives for oenology. They provide natural source of organic nutrition, improve organoleptic properties and physico-chemical stability of wines. In this work, the spent yeasts from different beer styles were evaluated for their primary characteristics. Three types of yeast derivatives were prepared, inactive yeasts used for active dry yeast rehydration, lysates for organic nitrogen supply during fermentation and yeast hulls for adsorption of fermentation inhibitors. The chemical properties, fermentation kinetics and aromatic compounds formation of spent yeast derivatives were comparable to the well-recognized commercial products. However, the sensory analysis of final wines revealed bread-like and yeast autolysis off-flavors.

Keywords: yeast derivatives; spent brewer´s yeasts; oenology

1 Introduction

Due to its increasing popularity yeast derivatives in winemaking represented the worldwide market size of $1,15 billion in 2021 and are expected to grow at a compound annual growth rate 9.9% CARG to 2029 (Data Bridge Market Research). Commonly, for each hl of fermented must, in between 20 to 120 g of yeast derivatives is used, that count for around 90 tons of yeast derivatives used every year only to cover wine production in Czechia (internal evaluation). According to the International Organization for Vine and Wine) codex (OIV codex), yeast derivatives for winemaking must originate from yeasts of the genus *Saccharomyces*. An untapped resource of this valuable raw material is found in large quantities in brewing industry. The year-round production of beer brings advantage, in contrast to the seasonal production of wine, where the indigenous microflora including non-saccharomyces strains, fungi and bacteria is usually present.

Inactive yeasts and autolysates mainly serve as a source of nutrition for active microorganisms, facilitate the rehydration of dried yeasts and lactic acid bacteria, support the successful course of fermentation, stabilize the colour, and reduce the astringency of red wines (OIV codex, 2021). Yeasts can be characterized by a high content of glutathione and sulphur-containing amino acids (cysteine, methionine), which help to reveal and preserve thiol aromas in wine (OIV codex, 2021; Gabrielli et al., 2017). Yeasts with a high content of glycosidase (beta-glucosidase) support the release of wine-based aromatic terpenes from the bound odourless form, and yeasts with strong antioxidant activity help maintain the wine's reduction potential (Zhang et al., 2021). Yeasts with a higher content of vitamins and especially sterols support the production of terpenoids and maintain the membrane permeability. Further, the yeast derivatives help to prevent the production of undesirable compounds such as volatile acids, volatile phenols and H_2S and reduce the amount of SO_2 used to sulphurate the wine. The yeast hulls are used to detoxify musts from pesticides, moulds and fermentation inhibitors (medium-chain fatty acids produced by active yeasts) and thus help to restart stuck fermentation. Mannoproteins, unlike the previous products, are not used for fermenting must, but for finished wines before bottling. Mannoproteins inhibit tartaric acid crystals forma-

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tion and stabilize proteins to prevent unwanted cloudiness, increasing the sensation of sweetness and fullness in the mouth ("mouth-feel effect").

In this work, the brewer´s yeasts were characterized according to technological properties as glutathione level, antioxidant capacity, beta-glucosidase activity and total mannose content to meet the specific requirements for oenological yeast derivatives with different function. The brewer's spent yeast biomass was further transformed into oenological preparations: inactive yeasts, lysates and yeast hulls. Different batches of the products were characterized for chemical properties, tested in laboratory scale vinifications on synthetic must and in real vinifications to access the fermentation kinetics, aroma compound profile and sensory analysis.

2 Materials and methods

2.1 Microorganisms

The microorganisms used in this study make part of microbial collection of EPS biotechnology, s.r.o. The spent yeasts from *Saccharomyces pastorianus* (EPS1160, White Labs) and two strains of *Saccharomyces cerevisiae* (EPS1153, EPS1161) were used for oenological preparations. The indigenous wine yeasts *Saccharomyces cerevisiae* (EPS851, EPS152) previously selected in typical vineyards in the region of Moravia and Bohemia were used for must fermentation. The brewer´s spent yeasts biomass was kindly supplied from a minibrewery ROTOR between October 2020 and January 2021.

2.2 Yeast collection and debittering

The collection of spent brewer´s biomass is carried out immediately after the completion of worth fermentation. Prolonged times of resting yeasts in beer cause cell autolysis. Ideally, a medium stream of waste yeast, which contains the smallest amount of ballast substances as hops and wort, is collected. The biomass is stored in disinfected containers under refrigerated conditions for max. 14 days before further processing.

The biomass was debittered as described elsewhere (Simard and Bouksaim, 1998; Nand, 1987). Basically, iso-α-bitter acids readily dissolve in organic solvents or react with Na^+ to form water-soluble salts. The simple method of washing the yeasts with a strong base of 2M NaOH, pH 10 at 50 °C was applied with subsequent washes in softened water.

2.3 Preparation of yeast derivatives

The debittered and washed spent yeast biomass was diluted in 10% dry weight content. The inactivation was carried out at 57 °C for 15 min. The autolysis was induced by thermal treatment at 50 °C for 28 hours. The yeast hulls were produced by a mechanical cell breakage in a homogenizator (Panter 3006, GEA) and the insoluble fraction was separated by centrifugation (Rousselet). After the treatment the product was dried by freeze-drying (LZ30, Regucon).

2.4 Bitterness measurement

Bitter substances, essentially iso-α-bitter acids, were extracted from acidified yeast suspension with isooctane. The absorbance of isooctane fraction was measured at 275 nm. Bitterness Units (IBU) were calculated according to the formula:

$$IBU = A_{275} .50$$

2.5 Yeast biomass characterisation

The samples of pure culture biomass or rehydrated oenological preparations were subjected to lysis treatment through 3 freeze-thaw cycles, cleared by sonification and centrifuged. The lysates were used for glutathione, antioxidant and beta-glucosidase determination.

Glutathione

The protocol of Rahman et al. (2006) was adapted for a reduced form of glutathione determination. The spectrophotometric method involves oxidation of GSH by the sulfhydryl reagent 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) to form the yellow derivative 5'-thio-2-nitrobenzoic acid (TNB), measurable at 412 nm.

Antioxidant capacity

The determination of antioxidant capacity by the phosphomolybdenum method was adapted from Prieto et al. (1999) and is based on the reduction of Mo (VI) to Mo (V) by yeast lysate and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH measurable spectrophotometrically at 695 nm. The antioxidant capacity is expressed as the equivalent concentration of the standard substance – ascorbic acid.

Beta-glukosidase activity

Beta-glucosidase activity in live cells was determined spectrophotometrically as the amount of released yellow-coloured product p-NP (4-nitrophenol) at 405 nm from model substrate (Strahsburger et al., 2017).

Mannoproteins

The amount of mannoproteins was determined as the amount of the mannan fraction from total polysaccharides, i.e. acid hydrolysis of soluble polysaccharides fol-

lowed by the determination of the mannan fraction using a D-mannose assay kit (Megazyme, Ireland) and measurement of absorbance in the UV spectrum at 340 nm.

2.6 Microvinifications

The synthetic must YPD19 (4.5 g pepton, 2.5 g yeast extract, 3 g tartaric acid, 5 g malic acid and 0.5 g citric acid per 1 l; pH 3.5) with 260 g/l of fermentable sugars was inoculated with the same number of cells (10^6/ml) and the oenological products were provided in the dose of 20 g/hl. The fermentation was carried out at 18 °C and the weight loss was monitored gravimetrically. Each test was carried out in triplicate. The results are presented as an arithmetic mean with relative standard deviations ≤3%.

2.7 Vinification in real conditions

The grapes were processed in accordance with common practises. The must was inoculated by rehydrated active dry yeasts in dose of 20 g/hl. The yeast derivatives were supplied in two consecutive doses of 25 g/hl.

2.8 HPLC, GC analysis

The residual sugars, alcohol content and acetic acid were determined by High Performance Liquid Chromatography on Agilent 1260 Infinity using Hi-Plex H (300 × 7.7 mm) column with RID detector and VWD 210 nm. The software OpenLab CDS was used for chromatogram analysis. higher alcohols and the ester content were measured by Gas Chromatography GC-450 Bruker using Rxi 624 Sil MS (30 m × 0.25 mm × 1.4 µm) column with FID detector. The ester score expresses the sensory activity of total measured esters by multiplication of their measured concentration by sensory threshold concentration. Unlike the real concentration this value demonstrates actual contribution of the substance to sensory profile. Each analysis was carried out in triplicate from tree independent tests. The results are presented as an arithmetic mean with relative standard deviations ≤3%.

3 Results and discussion

3.1 Characterisation of brewer's yeasts

Brewer's yeasts characteristics depends on the yeast strain and growth conditions. Pure yeast strains in comparison to oenological spent brewer´s yeast derivatives were characterized (Table 1, Table 2). Each of the characteristics is discussed individually in the paragraphs bellow.

Glutathione

According to the OIV codex, inactive yeasts containing more than 1% glutathione in dry matter can be classified as inactive yeasts with glutathione. All tested strains as pure culture contained less than 1% of glutathione (Table 1). The glutathione, small peptide with antioxidant properties is synthetized in higher amounts under stress conditions. After processing, the brewer´s spent yeasts may contain up to 5% of dry weight of glutathione (Podpora and Swiderski, 2016). Gijs et al. (2007) measured 0.3–1.1% dry weight of glutathione in brewing yeasts. From our results can be seen that glutathione was consumed by cells during the autolysis (0.4–0.8% dry weight). The short treatment at a higher temperature for cell inactivation preserved the glutathione level at 1.1–2.2 % of dry weight (Table 2).

Beta-glucosidase activity

Beta-glucosidase activity of brewer´s strain was high compared to wine yeast strains. All tested brewer´s strains fell into the category of very high enzymatic activity with p-NP >55 µM. However, the activity of this important enzyme releasing aromatic terpenes from glycosidic bonds dropped down significantly at exposure to higher temperatures during debittering, cells inactivation and autolysis. Indeed, exposure to inactivation conditions led to enzyme inactivation with very low concentration measured in a final product (1.2–5.0 µM eq. p-NP).

Table 1 *Characteristics of pure brewer´s cultures.*

Strain/characteristics	EPS 1160	EPS 1161	EPS 1153
Glutathione (g/100 g dry weight)	< 1	< 1	< 1
Beta-glucosidase activity eq. p-NP (µM)	99.3 (high)	101.5 (high)	89.9 (high)
Antioxidant capacity (ascorbic acid mg/g dry weight)	3.6	–	–
Mannose (g/100 g dry weight)	10.3	7.1	6.3

Table 2 *Characteristics of brewer´s spent yeast preparations in comparison to commercial products.*

Preparation type	Source	Glutathione[1]	Antioxidant activity[2]	Beta-glucosidase[3]
Inactive Yeasts	EPS	1.1–2.2*	4.9–9.2*	1.2–5,0*
	Com.	2.3	3.5	3.1
Lysate	EPS	0.4–0.8*	8.7–9.2*	8.9–13.4*
	Com.	1.3	9.8	6.5

*[1] (g/100 g dry weight); [2] eq. ascorbic acid (mg/g dry weight); [3] eq. p-NP (µM); * Results from 3 independent batches*

Antioxidant capacity

The spent brewer's yeasts have a strong antioxidant activity due to the great number of phenolic compounds adsorbed from malt and hops that might be free or bounded, such as: gallic acid, protocatechuic acid, (+)-catechin, p-coumaric, ferulic and cinnamic acid (Fărcaş et al., 2017). The antioxidant capacity measured as ascorbic acid equivalent of the preparations varied within the batches in the range 4.7–9.2 mg/g and was in accordance with the values measured for commercial products.

Mannoproteins

Only *Saccharomyces cerevisiae* strains can be used for the production of mannoproteins as stated in OIV codex. The mannan content in the dry matter varies between 7–14% (Kwiatkowski and Edgar, 2012) which corresponds with our results (Table 1). However, the mannose content in EPS1161 strain after malt fermentation decreased to 5.3 % of dry weight. At the end of the fermentation, the number of mannans decreases and the number of proteins increases, which leads to yeast flocculation and their rapid sedimentation (Verstrepen et al., 2003). For this reason, the spent brewer's biomass is not a good source of mannoprotein production.

Debittering

Although, the common techniques for spent yeast debittering were applied, the bounded iso-α-bitter acids were not completely removed and the bitterness of yeast cream ranged from 30–185 IBU depending on the type of beer produced (lower values for pilsner type, higher values for special-type beers).

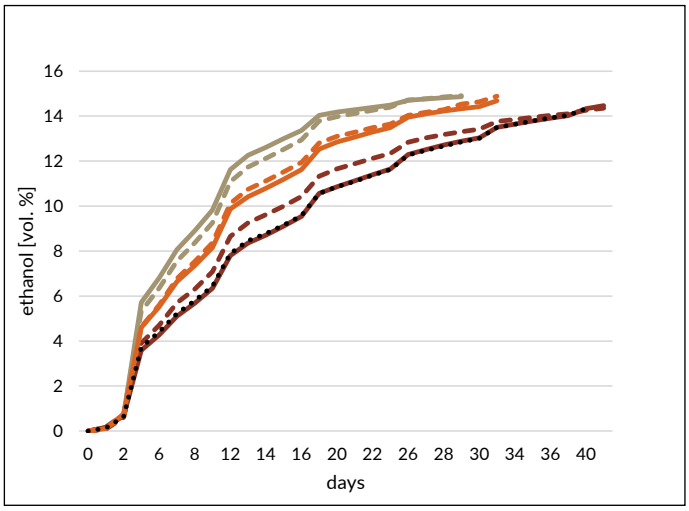

Figure 1 *Microvinification on synthetic must with an addition of oenological brewer´s spent yeast derivatives (solid line) and commercial oenological preparations (dashed line). Inactive yeasts (grey); yeast hulls (orange); lysates (dark red), Control with no addition (black dotted line).*

Table 3 *Chemical analysis of synthetic must after vinifications using spent brewer´s yeast derivatives in comparison to commercial products.*

Preparation type	Source	Alcohol (% vol.)	Total sugars (g/l)	Acetic acid (g/l)	Ester score
Inactive yeasts	EPS	15.0	2.5	1.170	79.0
	Com.	14.9	3.1	1.173	75.4
Lysate	EPS	13.1	30.4	1.403	61.6
	Com.	14.7	8.9	1.440	65.2
Yeast hulls	EPS	14.9	6.3	1.343	51.5
	Com.	15.0	4.0	1.247	59.9
Non	Control	13.7	22.7	1.530	55.7

3.2 Microvinification on synthetic must

Batches of yeast derivatives produced from spent brewer´s biomass were subjected to vinification on synthetic medium YPD19 with a reduced nitrogen content in order to determine the effect of added oenological products on the overall kinetics of fermentation. None of the yeast derivatives showed an inhibitory effect on active fermenting yeasts, although the preparations still contained α-bitter acids from hops with resulting IBU ranging between 45–55. Commercially purchased products from leading world manufacturers served as a positive control. The addition of preparations accelerated the course of fermentation and increased the amount of ethanol formed in the saccharomycetic strain EPS851 (Figure 1) and in the non-saccharomycetic strain EPS115 *Metschnikowia pulcherima* (data not shown). All inactive yeasts and yeast hulls had the greatest influence on the fermentation rate. The yeast strain EPS851 fermented all sugars within 30 days with addition of inactive yeasts in dose of 20 g/hl at the beginning of fermentation in comparison to control with no addition of yeast derivatives. The inactive yeasts supply the active yeasts with growth factors, ergosterol and gradually released organic nitrogen. The yeast hulls adsorb the fermentation inhibitors and thus have beneficial effect on overall fermentation kinetics. The lysates represent an organic source of nitrogen and the fermentation was completed within 45 days as in the case of negative control with no addition of yeast derivatives. The production of unwanted acetic acid was also reduced by up to 23 % in the case of use of inactive yeasts (Table 3). The ester score increased with an addition of preparation with an organic nitrogen content as the amino acids can serve as precursors (Rigou et al., 2021).

3.3 Yeast rehydration

The addition of inactive yeasts to the starter protocol for active dry yeast rehydration in dose of 20 g/hl was investigated in laboratory conditions on synthetic must YPD19 with 260 g/l of fermentable sugars at 18 °C. The appearance and smell of the preparations themselves varied considerably. As the Figure 2 shows the commercial yeast derivative for rehydration (D) had a cream colour and pleasant smell of yeasts, the inactive brewer's yeasts (E) bore the typical beer smell and had a grey-brown colour. The addition of inactive yeasts led to a slightly prolonged lag phase, still the fermentation was completed without residual sugar. Yeasts EPS152 with no nutrition supplementation was unable to use all the sugar in the medium to convert to alcohol, leaving 17.9 g/l of residual sugar after fermentation. The addition of inactive brewer's yeasts had the greatest effect on the total sugar consumption with a balance of only 2 g/l of sugar.

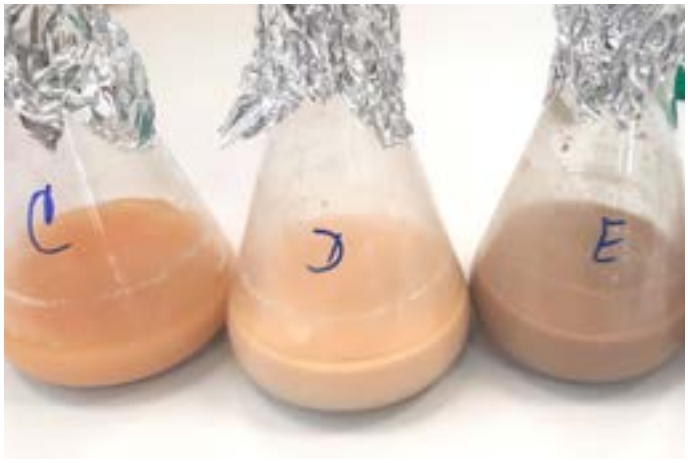

Figure 2 *Rehydration of active dry yeasts C) without the addition of preparation; D) with the addition of commercial preparation; E) with the addition of inactive brewer´s spent yeasts IN_P3.*

3.4 Vinifications in real conditions

Similarly to synthetic must, the addition of spent yeast derivatives had a beneficial effect on fermentation kinetics of Moravian Muscat with <0.5 g/l of residual sugars compared to commercial control with 6 g/l of remaining sugars. The ester score was also higher using spent yeast derivatives. However, the degustation committee recognized bread-type or yeast autolysis off-flavours in young wines. According to the OIV guidelines, yeast preparations must not add foreign flavours to the wine.

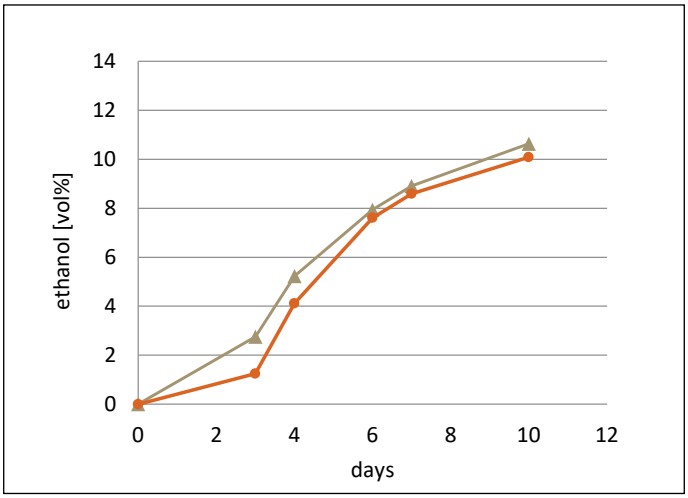

Figure 3 *Vinification of must Moravian Muscat using commercial organic nutrients (orange circles) and oenological product made out of spent brewer´s biomass (grey triangles).*

4 Conclusion

With an emphasis on the principle of circular economy, spent brewer's yeasts were selected as a suitable raw material for the preparation of oenological yeast derivatives in accordance with the OIV. Brewer's yeasts are characterized by high nutritive value, high beta-glucosidase activity and great reduction potential, which is a good prerequisite for the preparation of quality oenological yeast derivatives aimed at supporting and maintaining the aroma in wine.

Even though the yeast derivatives had a comparable effect on a kinetics rate and final physico-chemical properties as the commercially available oenological products, the bread-like and yeast autolysate aroma have been detected in the mature wine.

5 Acknowledgement

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