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BIO-INGREDIENTS BASED ON SPENT INDUSTRIAL YEAST BIOMASS

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Abstract

The paper relates to obtaining new bio-ingredients based on wine/brewing yeast biomass rich in polyphenols, which can be used for feed purpose. The drying process by lyophilization of yeast biomass was performed in the pilot phase in order to obtain active yeast and the content of total polyphenol and protein was also determined. Inactivation of yeast by drum dryers with rolled drums was also achieved and the content of total polyphenol and protein from spent brewing yeast and mixture of this with new bio-ingredients based on wine/beer yeast biomass was performed. A content of 580-930 mg/100 g (referred to gallic acid) was obtained for yeast that accumulates total polyphenol at pilot level, 320 mg/100 g (referred to gallic acid) dry spent brewing yeast and 310 mg/100 g (referred to gallic acid) for mixture (the yeast obtained at pilot level and spent brewing yeast). It was also noticed that the content of protein at pilot level for yeast that accumulates protein at pilot level was 41.24 g/100 g, 45.73 g/100 g for spent brewing yeast and 42.85- 43.39 g/100 g of protein from mixed yeasts.

Key words*: bioingredient, new materials, polyphenols, protein, yeast.*

INTRODUCTION

Yeast has been used for thousands of years to produce food and beverages such as beer, wine, sake and bread. Despite the fact that *S. cerevisiae* remains by far the most commonly used species of yeast, other unconventional yeasts such as the *Scheffersomyces jamb, Yarrowia lipolytica, Kluyveromyces lactis* and *Dekkera bruxellensis* have also supported the contributors to industrial fermentation processes (Steensels et al., 2014). *Dekkera* species are also important in the bread and brewing industry. When high levels occur, food spoilage occurs. However, small amounts of *Dekkera* contribute to the desired metabolites in bread, lambic beer, ale and kombucha tea (Schifferdecker, 2014).

Beer production inevitably leads to the generation of different wastes and by-products. The most common by-products are spent grains, spent hops and spent yeast, which are generated from the main raw materials used to produce beer.

From environmental point of view, the disposal of industrial by-products is a solution to pollution problems (Mussatto, 2009).
For example, yeast can be used for accumulation of organic minerals such as selenium, zinc, copper in biomass. By optimizing media factors such as sucrose, yeast extract, $MnSO_4$, $ZnSO_4$, $CuSO_4$, vitamins, $FeCl_3$, (Barbulescu et al, 2010) yeast biomass enriched with 1145.8 $mg \times l^{-1}$ copper, 1143.4 $mg \times l^{-1}$ zinc and 33.9 $mg \times l^{-1}$ manganese has been obtained.
Most of the spent brewer's yeast, a waste product, is used as a source of protein, B vitamins, and minerals in animal feed production. Spent brewer's yeast is a good source of inexpensive protein (45-60%), B vitamins, minerals (York & Ingram, 1996; Ferreira et al., 2010; Jarmołowicz et al., 2013; Waszkiewicz R., 2013; Bartłomiej et al., 2016).
Spent brewing yeast has been studied as source of polyphenols as well. Total analysis of polyphenols and total flavonoids was performed using the spectrophotometric method described by (Vijayalaxmi et al., 2015), with some changes, and the polyphenols were isolated from the spent brewer's yeast by applying various aqueous extraction solutions, water or ethanol/water (20/80) at different extraction temperatures (León-González et al, 2017).

MATERIALS AND METHODS

The preinoculum, inoculum and fermentation processes were static; the wine and brewing selected yeasts used for pitching are obtained from a maintenance culture that is harvested on medium based on yeast extract. The method for preparation of preinoculum, inoculum and the fermentation process were described by Barbulescu et al (2018) in Figures 1a-1d.

Figure 1a. Selection of yeast colonies

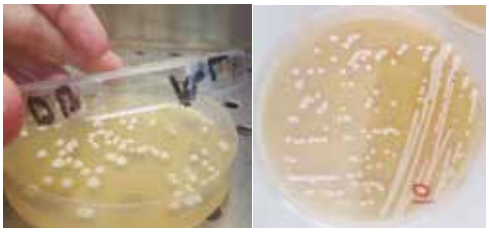

Figure 1b. Selected yeast colonies

Figure 1c. Preinoculum obtainment - Yeast cultures developed at agar slant

Figure 1d. Inoculum development at orbital shaker

The mixture between liquid spent brewing yeast and consortium wine and brewing yeast obtained at pilot level was done by a specific technique and a protocol for homogenization.
The culture fermented media was a subject of the post-fermentation process comprising the following steps (Figures 2 and 2a-2d):

Figure 2. Fermented media

1) Separation of biomass by centrifugation.

Figure 2a. Centrifugation of fermented media

Figure 2b. Biomass of separated yeast by centrifugation

2) Washing the biomass.

Figure 2c. Washing with distilled water of yeast biomass

3) Drying the biomass by lyophilisation/drier drum.

Method for separation of wet cell yeast by culture medium

Post-fermentation processing:

- *Separation of fermented media*: The easiest and fastest way to perform yeast biomass separation from the fermentation medium is to apply centrifugation, by which separation of the yeast cells from the culture medium and metabolites occurs;
- *Washing of biomass*: To remove the culture medium retained between yeast cells, it is necessary to wash the separated biomass two or three times with distilled water.

The washing is done by vigorous stirring followed by centrifugation and discharging the wash water. The volume of the wet cell biomass was used to estimate the cell concentration in the broth, taking into account that the mass of the dry cells (g) is about 25% of the volume of the wet cells (ml) (Vieira et al., 2013).

The centrifugal cells were washed with distilled water and centrifuged twice after being transferred to pre-weighed dishes. The biomass was dried at 60 ± 5°C at a constant weight (Vieira et al., 2013)

Figure 2d. Wet yeast biomass

During the drying process, the cells that are excessively dry are inactive, unlike those where drying is not so advanced and, therefore, remain active. This is primarily due to the drying conditions and is related to the homogeneity of the material to be dried.

Drying the wet biomass by lyophilisation

The yeast biomass was freeze-dried using a Delta 2-24 LSC lyophilizer (Martin Christ, Germany) as following: the yeast biomass were

put into Petri dishes and initially frozen at -40°C for 8 hours followed by main freeze-drying at -40°C and 0.1 mbar for 10 hours. Then the temperature increased in steps to 10°C during 10 hours, to 20°C during other 8 hours and to 30°C for 8 hours at same pressure, 0.1 mbar. The final freeze-drying lasted 3 hours: 1 hour at 30°C and 0.001 mbar followed by 2 hours until temperature reached 35°C. After 48 hours of freeze-drying (Table 1 and Figure 3) the obtained dried yeast biomass was sent to analysis.

Table 1. Yeast biomass lyophilization program

No. phase	Stage of the lyophilization process	Time, hours	Shelf temperature, °C	Pressure mbar
1	Freezing *	8	-40	1000
2	Main freeze-drying	10	-40	0.1
3	Main freeze drying	10	10	0.1
4	Main freeze drying	8	20	0.1
5	Main freeze drying	8	30	0.1
6	Final freeze drying	1	30	0.001
7	Final freeze drying	3	35	0.001

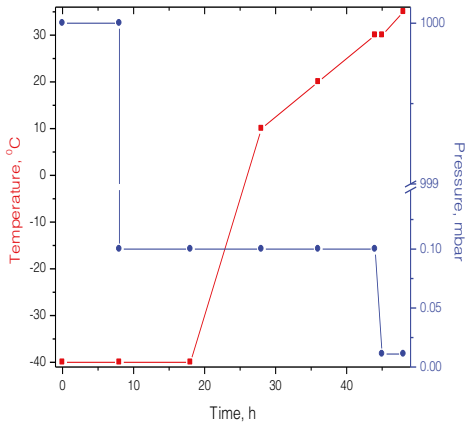

Figure 3. Lyophilization program

Drying the wet biomass using drum dryers (drying of spent brewing yeast and mixture of yeast biomass to obtain inactive yeast) (Figure 4).

The raw material to be subjected to the drying process, in order to obtain dried yeast biomass, is the spent yeast resulted from the brewing process in the form of yeast milk. It was used using a dryer with drum dryers. Drum dryers are heat-transfer conduction.

A heating medium (generally steam) is sent to the rotating drum (cylinder) and the liquid material is fed to the heated drum to evaporate and concentrate and at the same time the liquid material is blocked on the surface of the drum in the form of a film, evaporated and dried.

Dry materials are continuously scraped with a knife. The mixture of yeast biomass was dried by inactivation with a specific technology. The dry yeast biomass is presented in the form of a brownish-brown powder with a bitter-sweet taste, having the specific smell and aroma of yeast.

The time for drying is shorter when the liquid spent yeast or the mixtures of yeast biomass are not exposed to high temperatures for a long period.

Figure 4. Dry yeasts biomass samples

Methods of determination of moisture (%) and crude protein (CP) for yeast biomass

Standardized methods were used, in accordance with Regulation (EC) No. 152 (2009). The moisture and crude protein (CP) were determined from the biomass in accordance with recognized standardized methods (SR 8613-1: 2009, SR 8613-6: 2009).

Method for total polyphenols determination

Total polyphenols were analyzed by Folin-Ciocalteau method.

About 250 mg of samples were weighted in a centrifuge tube, and 10 ml of water were added. After that, the samples were centrifuged at 6000 rps for 20 minutes.

The supernatant was separated and centrifuged again at 12000 rps for 15 minutes.

Gallic acid was used to prepare the standard curve. The working reagent was prepared by diluting a stock solution of Folin-Ciocalteu's

phenol reagent with distilled water (1:10, v/v). Sodium carbonate solution was prepared by mixing 7.42 g Na_2CO_3 with 100 ml distilled water (0.7 M).
Methanol solution was prepared in ration 4:1 with distilled water. Samples (the supernatant resulted from the second centrifugation) (50 µl) were aliquoted into test cuvettes, 200 µl of methanol solution and 1250 µl of prepared Folin-Ciocalteu's phenol reagent was added.
After a few minutes, 1000 µl of saturated sodium carbonate solution-Na_2CO_3 (7.5% w/v in water) was added. The mixture was then incubated at 50^0C for 5 min.
Afterwards, the absorbance of the reaction mixture was measured at 760 nm using a Spectronic Helios Gamma UV Visible spectrophotometer (Thermo Fisher Scientific). The results were expressed as mg GA/100 g of dry yeast sample.

RESULTS AND DISCUSSIONS

Three pilot biomass samples based on consortium of wine and brewing yeast and dried by lyophilisation (S1, S2, S3) were taken in the study for the assessment of protein and total polyphenols.
The difference between these samples is based on the inoculum ratio (results not presented in this paper).
The biomass samples S1 and S2 have been further mixed with spent brewing yeast (as wet biomass), and the bioingredients S1 mixture and S2 mixture have been obtained.
Table 2 illustrates the characterisation of biomass samples as regards the content in polyphenols, and the content of crude protein and moisture is presented in Table 3.
It was observed the higher content of total polyphenols in the sample S1 (Table 2).

Table 2. Content of total polyphenol from different samples of yeasts biomass (average values)

Samples	Moisture, %	Crude protein, g/100g
S1	11.5	41.24
S2	11.61	35.76
S1 mixture	9.62	43.39
S2 mixture	7.32	42.85
Spent brewing yeast	5.69	45.73

Table 3. Content of protein and moisture from yeast biomass

Samples	Total polyphenol, mg/100g (referred to gallic acid)
S1	930
S2	760
S3	580
Control dry spent brewing yeast	310
S1 mixture	340
S2 mixture	330

The concentration of total polyphenols in the mixture samples is decreased because of the mixture with spent brewing yeast, in order to be valorised as a polyphenol source for the feed receipts for laying hens.
The polyphenol content in the spent brewer's yeast was found between 0.23 and 2.9 mg of gallic acid/g by León-González et al. (2017), these results being comparable with those presented in this paper.
The content of polyphenols was highly dependent on the treatment applied to the spent brewing yeast (lyophilized and air-dried yeast).
The content of crude protein for mixture samples was higher in comparison with the content of crude protein for the spent brewing yeast.

CONCLUSIONS

This study demonstrated the possibility of utilisation of spent brewing yeast for feed receipts. The new bioingredients are good source of polyphenols and protein.
The higher content of protein was noticed for the S2 mixture.
The drying using drum dryers is preferable because the method is economic and efficient.
The mixture obtained was taken into study in order to set up a new feed receipt formula that has been tested on laying hens (results not presented).
The yeast biomass is a good and cheap source of protein and polyphenols.

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