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POST-FERMENTATION BREWING YEAST AS A FEED SOLUTION FOR LAYING HENS

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Abstract

The paper reveals the potential nutritive impact of the spent brewing yeast as component of feeding supplements for laying hens. The post-fermentation brewing yeast biomass was analyzed for total phenolic content, by applying extraction with methanol as solvent for samples of dried yeast biomass derived from an industrial brewing process. The obtained results suggest that drying process is reproducible and residual brewing yeast could be a source of polyphenols in order to be used for the development of bio-based nutritional feed solutions.

Key words*: brewing spent yeast biomass, total polyphenols, laying hens, feed.*

INTRODUCTION

The valorization of food waste represents the fundament of the sustainable material management and there are sustained efforts in food industry to develop feasible circular economy models that can be multiplied and adapted to the size and diversity of food business operators. According to the EU Commission Council Directive 2018/851 that modifies the EU Commission Council Directive 2008/98/EC, the efficiency of resource use should be improved in order to value the waste in the bioeconomy, including food industry. Therefore, the development and implementation of viable recovery solutions that are able to support the translation from food waste to food by-products are urgently required (Directive (EU) 2018/851; Galanakis, 2015; Rodino et al., 2019; Watanabe et al., 1980).

At EU level, the brewing industry represents a major player in waste generation. The EU countries represent globally the second largest beer producer, after China. Taking into consideration the increasing beer production in EU (i.e. the beer production volumes over 400 million hectoliters in 2018), the volumes of wastes generated by the brewing industry are significantly increasing (link 1; link 2).

In the context of circular economy, the interest for the valorization of agri-food wastes such as the spent brewing yeast (SBY) is increasing. The SBY represents the post-

fermentation yeast that results from the brewing process. It contains a large amount of carbohydrates, vitamins, minerals and proteins, and, therefore, it is used as an ingredient in feed and food supplements (León-González et al., 2018; Mathias et al., 2014; Olaru et al., 2016; Pallag et al., 2018; Podpora et al., 2015; Pogurschi et al., 2019).

Beer is produced from malted cereals and grains (mainly from barley and wheat), along with water, hops and a yeast strain (Humia et al., 2019). Rodino et al. (2019) highlighted two types of use for the application of SBY, namely by using yeast cells that have incorporated various essential elements as feed, as well as by using cell ingredients, especially intracellular components of yeast, as antitumor agents. The study performed by Podpora et al. (2016) proved that a high content of essential amino acids can be found in the yeast extracts and the extracts also have high antioxidant activities in comparison to tea. The yeast strain (commercial *Saccharomyces* and alternative non-*Saccharomyces* yeast strains) together with the original wort content and the spent yeast cropping time have a direct impact over the different components of the yeast extract (Jacob et al., 2019).

The study on the SBY demonstrates that it can be safely used as animal feed for poultry and as protein source in order to replace soya bean (Chollom et al., 2017). Due to the relatively high protein and essential amino acids content, SBY can be considered as an ingredient of feeding supplement for laying hens. The diets rich in antioxidant compounds can greatly improve the performance of hens, but also the quality of eggs.

The oxidative reactions are known to be the basis of numerous biochemical pathways and cellular functions. The imbalance of pro-oxidants and endogenous antioxidant mechanisms in living tissues lead to oxidative stress (Kohen et al., 2002). In animals, the oxidative stress is an important mechanism that leads to pathological disorders and affects the growth of birds (Fellenberg and Speisky, 2006).

The SBY is considered as a valuable source of bioactive polyphenols that can bring added value through its use in the cosmetic, food and pharmaceutical industries (León-González et al., 2018) In addition, some studies have shown the ability of yeast (such as *Saccharomyces cerevisiae*) to act as a delivery system for bioactive molecules based on their capacity to absorb polyphenols during the fermentation process (Jilani et al., 2015, 2016).

The paper introduces the characterization of SBY biomass regarding the content in total polyphenols in order to demonstrate the reproductibility of the drying process, and releves the potential nutritive importance of the SBY as component of feeding supplements for laying hens.

MATERIALS AND METHODS

Spent brewing yeast and chemicals

The SBY samples analyzed in this study, represent inactivated brewing yeast in form of powder resulted from the drying of slurry SBY. These five samples of SBY are collected from the industrial brewing process of different batches of the same brand of beer from the Bergenbier S.A. brewery (Ploiești, Romania), provided by Agsira S.R.L. The brewing yeast employed was a bottom-fermenting yeast.

The liquid (slurry) SBY resulted from the brewing process is the raw material that was subjected to the dying process with the purpose of obtaining dried and inactivated yeast biomass. The spent brewing yeast biomass was dried using a drum dryer (Marinescu et al., 2019) with a drying time applied in order to protect the liquid spent yeast from high temperature exposure (Bărbulescu et al., 2018). The dried yeast is presented in the form of a brownish-brown powder with a bitter-sweet taste, having the specific smell and aroma of yeast.

Five batch samples from five drying processes were tested for dry meter and polyphenols in order to demonstrate the reproducibility of the drying process of liquid spent yeast.

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 (Milli-Q®

Reference) were added. The extraction procedure was carried out by using ultrasonic bath (VWR® Ultrasonic Cleaner). The ultrasonic treatment took 60 minutes at 18°C.
After that, the samples were centrifuged (Hettich® MICRO 220/220R) at 6000 rpm for 20 minutes.
The supernatant was separated and centrifuged again at 12000 rpm for 15 minutes. The supernatant was separated again, and this was used to measurements.
As polyphenol content is referred to gallic acid, gallic acid standard solutions were used to calibration. These solutions were prepared diluting stock solution of gallic acid reagent (Sigma-Aldrich, Merck KGaA).
The working reagent of Folin-Ciocalteu's phenol was prepared by diluting a stock solution (Sigma-Aldrich, Merck KGaA) with distilled water (1:10, v/v).
Sodium carbonate solution was prepared by mixing 7.42 g Na_2CO_3 (Suprapur®, Sigma-Aldrich, Merck KGaA) with 100 ml distilled water (0.7 M).
Methanol working solution was prepared in ration 4:1 using methanol stock solution (Reanal, analytical grad) and 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 50ºC 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 polyphenol content of samples was expressed referred to gallic acid.

Moisture determination

Total moisture determination analysis was performed by the method described by León-González et al., (2018). The determination of the moisture content of SBY was performed after heating at 130^0C during 2h using a WTB Binder drying oven (Tuttlingen Germany).

Estimation of total polyphenols

The determination of total phenolic content in SBY biomass was performed using the Folin-Ciocalteu method, which is the mostly used analytical tool for the determination of polyphenols (Ignat et al., 2011; Krumpe et al., 2019; Pallag et al., 2018).

RESULTS AND DISCUSSIONS

Moisture content

The sixth sample is a mix sample was prepared from the 5 yeast sample.
The determined moisture content (%) in the SBY was between 8.73 and 9.31.
By the same drying technology, 5 batches of dry brewer's yeast were obtained.
For each batch of brewer's yeast, the polyphenol content (TPP), expressed in mg, relative to 100 g of gallic acid (TTP mg / 100 g referred to gallic acid), was determined in sextuplicate. The calibration curve for total polyphenols is presented in Figure 1.

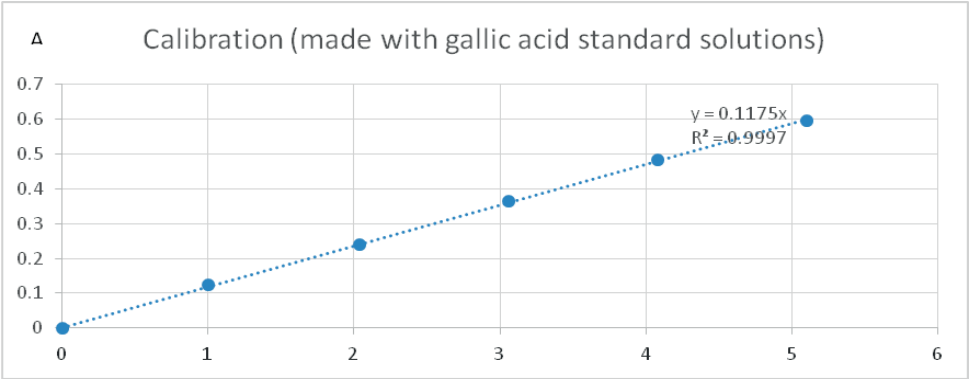

Figure 1. Total polyphenols content in spent brewing yeast extracts under different extraction conditions

Table 1. Results of polyphenol content analysis for each batch and brewer's yeast mixture, respectively

Batch	TTP mg/100 g referred to gallic acid						Average±SD	RSD (%)
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6		
1	408	400	387	409	402	441	**408±18.0**	**4.4**
2	409	419	394	435	440	456	**425±22.9**	**5.4**
3	406	415	438	397	440	445	**423± 20.0**	**4.7**
4	385	378	417	396	389	391	**393±13.4**	**3.4**
5	403	420	416	403	425	414	**413±9.0**	**2.2**
mix (1-5)	397	416	407	389	438	404	**409±17.0**	**4.2**

The analysis of the results listed in Table 1 shows a very good reproducibility of the results for each batch (1-6), supported by the values obtained for TTP mg/100 g referred to gallic acid and the corresponding standard deviation and relative standard deviation respectively. Thus, the results obtained reflect a reduced intralot variability in polyphenol content (TPP), expressed in mg, relative to 100 g of gallic acid (TTP mg/100 g referred to gallic acid) (Figure 2).

TTP mg / 100 g referred to gallic acid vary between 393 ± 13.4 and 425 ± 22.9.

In addition, the results on TTP mg/100 g referred to gallic acid of the mixture of the 5 batches of dry brewer's yeast demonstrate the homogeneity of each batch, but also of the mixture. The value obtained for TTP mg / 100 g referred to gallic acid for the mixture of the 5 batches is 409 ± 17.0 and is within the above range (393 ± 13.4; 425 ± 22.9. These results reveal a low interlot variability of polyphenol content (TPP), expressed in mg, relative to 100 g of gallic acid (TTP mg/100 g referred to gallic acid) (Figure 2).

Practically, both intra- and interbatch variability is reduced, which shows that the method / technology of drying the brewer's yeast samples, respectively the drying conditions were well chosen.

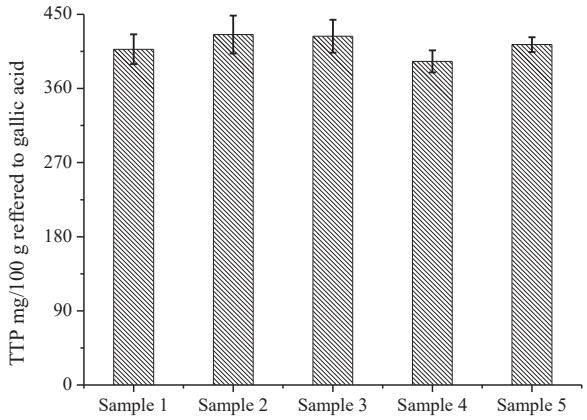

Figure2. Intra- and interbatch variability of polyphenol content per 100 g of gallic acid

The results of this study are in accordance with the study performed by (Amorim et al., 2016) as regarding the physical-chemical characterization and nutritional value of all fractions of yeast biomass. In addition, the results are only partially in accordance with the conclusions of the study performed by León-González et al. (2018) regarding the relationship between the polyphenol content and the type of sample, since in our study the total polyphenol content in the liquid SBY is lower that the values for the dried SBY biomass.

Similarly to the conclusions of León-González et al. (2018) and Rizzo et al. (2006), the different biosorption of polyphenols into yeast could be the explanation for these disparities as well as for the content in total polyphenols vs. the behavior of yeast biomass to separation, drying and extraction procedures.

CONCLUSIONS

The batch-to-batch quality of the spent brewing yeast biomass was demonstrated through the reproductibility of the results obtained for the content of polyphenols.
Based on the performed studies, it is shown that the spent brewing yeast has a relevant content of total polyphenols.
In addition, the different behavior related to biosorption of polyphenols depends on the yeast strain as well as on the brewing fermentation process and the post-fermentation treatments applied to yeast biomass. The reproducibility of drying process was proved.
Our aim in the future is to evaluate different brewing yeast biomasses based on total polyphenol content in order to obtain valuable bio-based ingredient with potential to be used as nutritional supplements for laying hens.

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