

## Spent brewer's yeast as an ingredient on the elaboration of cookies

### ABSTRACT

Several studies have been conducted to find ways to use the spent yeast from brewery due to their high nutritional content (e.g., proteins, vitamins and minerals). Therefore, this study aimed to characterize the spent yeast from brewery and add it to cookies as an ingredient to valorize the waste from beer production. Spent yeast in its natural and autolysed form were characterized by physical-chemical analysis. Subsequently, formulations of cookies were developed with autolysed biomass (10% and 20%). These cookies were under physical-chemical and microbiological analysis. Finally, the cookies were submitted to the evaluation of acceptability as well. Brewer's yeast is a potential source of protein, resulting in cookies with less carbohydrate content. The cookies without biomass has better acceptability, therefore, it is necessary to improve the formulations with spent yeast. However, it was noticed that brewer's yeast has potential for its application in food and, its use as an ingredient on food formulations can be a way to valorize the residue from beer production.

**KEYWORDS:** spent yeast; brewery; cookies; sensorial analysis.

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## INTRODUCTION

Among the products from bakery, cookies are the most consumed product in the world. The high consumption and popularity of cookies are attributed to their price, wide variety of flavors, convenience and long shelf-life (JAN; SAXENA; SINGH, 2016; ROCCHETTI *et al.*, 2019; YANG; GUO; ZHAO, 2020). Several studies have been conducted to nutritionally enrich cookies, including the addition of vegetables, fruits and even by-products from the food industry (INFANTE *et al.*, 2017; BARREIRA *et al.*, 2019; YANG; GUO; ZHAO, 2020; KADERIDES; MOURTZINOS; GOULA, 2020).

The yeast used in the fermentation stage in beer production is the second-largest residue of the entire process (PODPORA *et al.*, 2015; LÉON-GONZÁLEZ *et al.*, 2018). It is considered a cheap source of nitrogen, an excellent source of proteins, vitamins (such as B1, B2, B3, B5, B6, B7, and B9), and it has a good amino acid profile. Also, they are rich in minerals (ÖZTÜRK *et al.*, 2017; MARTINS; PINHO; FERREIRA, 2018), macro and microelements, particularly selenium and dietary fiber (mainly beta-glucans) (ÖZTÜRK *et al.*, 2017; MARTINS; PINHO; FERREIRA, 2018). Besides, spent yeast from brewery is recognized as GRAS (generally recognized as safe) (VIEIRA *et al.*, 2016; BERLOWSKA *et al.*, 2017). Due to that, yeast and its derivatives, for example, autolysed yeast and extracts are widely used as a nutritional and flavoring complement (AMORIM *et al.*, 2016).

The components of interest that compose the yeast are inside the cell, therefore, it is necessary to break them to obtain the maximum nutritional value (AMORIM; PINHEIRO; PINTADO, 2019). This process occurs naturally when the cell completes its growth cycle and enters to the death phase by endogenous enzymes which will hydrolyze the cellular components (PÉREZ-BIBBINS *et al.*, 2015). Also, the autolysis process can be induced by chemical agents, temperature, ultrasound, and high pressure. The method directly influences the release of the compounds and the composition of the extract to be obtained (MEULLEMIESTRE *et al.*, 2016; JACOB; HUTZLER; METHNER, 2019).

Therefore, the present study aims to characterize the spent yeast from brewery in its natural form and after the autolysis process. Also, add it to cookies to find a way to valorize the waste from brewery.

## MATERIALS AND METHODS

The yeast biomass used was kindly provided by the Dalla Brewery (Chapecó, SC). The process to obtain natural biomass (NB) was adapted from Sgarbieri *et al.* (1999): samples were centrifuged (Centribio, Cienlab, Brazil) at 2500 rpm for 10 minutes for recovery of the cells; biomass was resuspended at 0.2% (v/v) NaOH solution at 1:1 ratio. Homogenization was carried out for 30 minutes on a magnetic stirrer (Tecnal, TE-0851) at room temperature. Then, all the samples were centrifuged (Centribio, Cienlab, Brazil) to eliminate NaOH. Biomass samples were washed with distilled water (v/v) at 1:1 ratio of biomass/distilled water and then centrifuged (Centribio, Cienlab, Brazil) at 2500 rpm for 10 minutes to obtain a clean biomass: the natural biomass (NB). After all centrifugations, the supernatant was discarded. NB was placed in Petri dishes and taken to the ultra-freezer (IULT 335 D, Indrel, Brazil) for 24 hours at a temperature of -86 °C and freeze-dried (TFD5503, Ilshin Lab. Co. Ltd., Korea) for 24 hours at -61 °C and 55 mbar. At the end of the

freeze-drying process, NB was crushed with pistil and mortar and sieved (mesh 32 - 500  $\mu\text{m}$ ) to obtain a powder of uniform granulometry.

To obtain the autolysed biomass (AB), part of NB was used. The autolysis process was adapted from Sgarbieri *et al.* (1999): samples of NB were resuspended in distilled water 1:1 (w/v), with ethanol (7% v/m) and NaCl (2% v/m). The mixture was taken to the incubator (Luca-223, Lucadema, Brazil) for 24 hours at 55 °C and 150 rpm for shaking and homogenization. After 24 hours the autolysis was interrupted with pasteurization at 85 °C for 15 minutes in a water bath (Dubnoff Luca 157/28, Lucadema, Brazil). Autolysed biomass (AB) was placed in Petri dishes and taken to the ultra-freezer (IULT 335 D, Indrel, Brazil) for 24 hours at a temperature of -86 °C and freeze-dried (TFD5503, Ilshin Lab. Co. Ltd., Korea) for 24 hours at - 61 °C and 55 mbar. At the end of the freeze-drying process, AB was crushed with pistil and mortar and sieved (mesh 32 - 500  $\mu\text{m}$ ) to obtain a powder of uniform granulometry.

Three formulations of cookies were proposed: standard sample (0% of AB) and another two with different concentrations of AB, 10% and 20%, according to the ingredients described in Table 1, adapted by Baptista *et al.* (2012). The percentages of autolysed biomass were calculated in relation to the amount of flour used.

**Table 1.** Cookie's formulations. SF (0% AB), F1 (10% AB) and F2 (20% AB).

Ingredients	SF	F1	F2
Flour (g)	36.76	36.76	36.76
Refined sugar (g)	17.67	17.67	17.67
Brown sugar (g)	11.78	11.78	11.78
Chocolate powder (g)	11.78	11.78	11.78
Butter (g)	8.98	8.98	8.98
Water (mL)	8.00	8.00	8.00
Dehydrated egg (g)	3.86	3.86	3.86
Baking powder (g)	0.69	0.69	0.69
Cinnamon powder (g)	0.44	0.44	0.44
Salt (g)	0.03	0.03	0.03
Vanilla essence (g)	0.01	0.01	0.01
Autolysed biomass (g)	0.00	3.68	7.35

All ingredients were weighed and added to a vessel, where the homogenization was carried out. After, cookies were configured in a circular shape. Then, they were placed in a rectangular aluminum recipient. Finally, cookies were baked in an electric oven (Nardelli) at 180 °C for about 10 minutes.

The physical-chemical characterization of NB, AB and cookies were carried out by determining: moisture content by gravimetric method using drying oven (Centribio, Cienlab, Brazil) at 105 °C, until constant weight; ash by muffle incineration (Q-318M24, Quimis, Brazil) at 550 °C; lipids content by Soxhlet extractor (LUCA-145/6, Lucadema, Brazil); nitrogen content by micro Kjeldahl (LUCA-341/02, Lucadema, Brazil) and protein was determined using a conversion factor of 5.8. Water activity (*aw*) was measured in a *aw* determiner (Pre Water Activity, Decagon, Brazil) at 25 °C. All analyzes were performed according to the procedures described by the Adolfo Lutz Institute (2008). Total carbohydrates were obtained by difference.

Color analysis of NB, AB and, cookies (SF, F1, F2) was made by colorimetry (EZ 0374 4500 L, Hunter Lab MiniScan, Brazil), operating in the CIELAB ( $L^*$ ,  $a^*$  e  $b^*$ ) system. Total Color Difference ( $\Delta E^*$ ) and Chroma ( $C^*$ ) were calculated using the equations 1 and 2 respectively (WROLSTAD; SMITH, 2010).

$$\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2} \quad \text{(Equation 1)}$$

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad \text{(Equation 2)}$$

Microbiological analyzes on the cookies were: Coliforms at 45 °C by Most Probable Number (MPN), Coagulase Positive *Staphylococcus* Count, and *Salmonella* sp. Analysis were carried out according to the methodology of American Public Health Association (DOWNES; ITÖ, 2001).

Sensorial analysis of cookies was conducted at the laboratory of Sensory Analysis (Universidade do Estado de Santa Catarina). The analyses were performed in individual cabins, with white light. Sixty-one people over the age of 18 participated, according to the project approved by the Research Ethics Committee under CAAE No. 45180015.1.0000.0118. Each taster received one cookie from each formulation. Cookies were coded with 3 random digits, served at room temperature, and with water in a disposable cup. To evaluate the acceptability of cookie, the judges analyzed the global acceptance by using the hedonic scale consisting of 9 points (9 = extremely liked, 5 = not liked or disliked, 1 = extremely disliked).

The analyses were carried out in triplicate. A completely randomized statistical design was used, with the results expressed as the mean  $\pm$  standard deviation. Statistical analysis was performed by Assistat 10 software, using Analysis of Variance (Anova), and the Tukey comparison test at 5% significance level.

## RESULTS AND DISCUSSION

To valorize the residues from the brewing industry, spent yeasts in its natural form and autolysed form were characterized by physical-chemical analysis before being added to cookies. The results are found below, in Table 2.

Moisture content between NB and AB showed a significant difference ( $p < 0.05$ ). The AB moisture was higher than NB probably due to the autolysis process. Moreover, the AB moisture content should be less than 10 g/100 g to produce a shelf stable product (AWOLU *et al.*, 2017), therefore, the freeze-drying method should be evaluated. The ash content also showed a difference between the samples. This difference can be justified by the fact that, in autolysis, NaCl is used as a plasmolizing agent, increasing the amount of ash in relation to natural biomass (BERTOLO *et al.*, 2019). Regarding autolysed biomass, the value was lower than reported by Bertolo *et al.* (2019) who studied spent brewer's yeast (13.14%); on the other hand, for whole cells (2.36%), the result was similar to the one found by this study.

**Table 2.** Centesimal composition g/100 g (%) in dry basis and water activity of NB and AB.

	NB	AB
Moisture	4.96±0.54 <sup>b</sup>	14.55±0.62 <sup>a</sup>
Ash	2.45±0.05 <sup>b</sup>	9.83±0.27 <sup>a</sup>
Proteins	48.39±1.91 <sup>a</sup>	45.30±1.76 <sup>a</sup>
Lipids	0.68±0.05 <sup>b</sup>	0.97±0.01 <sup>a</sup>
Carbohydrate	43.52±1.80 <sup>a</sup>	29.36±1.51 <sup>b</sup>
Aw	0.336±0.009 <sup>a</sup>	0.315±0.001 <sup>b</sup>

NOTE: Values expressed as average (n=3) ± standard deviation. Different letters in the same line indicate significant differences ( $p \leq 0.05$ ) by Tukey test.

The largest component of spent yeast biomass is protein. The protein content of spent yeast biomass is around 46.5% (SHURSON, 2018), in its natural form. But protein content may increase when the cell wall ruptures. However, in this study, protein contents for NB and AB are similar, it means that the results showed no significant difference and it can be explained, for example, by the use of NaCl. NaCl can act as a dehydrating agent, resulting in protein retention in the cell wall. Also, coagulation of proteins may occur (ROZMIERSKA *et al.*, 2019). An example of this situation can be observed in a study made by Biz *et al.* (2020), protein content from autolysed spent yeast from brewery (41.08 g/100 g) was less than in its natural form (50.58 g/100 g).

The lipid contents were similar to those found by Vieira *et al.* (2019). A decrease in carbohydrate content with autolysis was observed, probably influenced by moisture and lipid content of AB. The values for the water activity are consistent for dehydrated foods, which should be less than 0.60, making it impossible to grow microorganisms, making them microbiologically stable (ZAMBRANO *et al.*, 2019).

Results for color analysis of natural biomass and autolysed biomass are presented in Table 3.

**Table 3.** Color analysis of natural biomass (NB) and autolysed biomass (AB).

	NB	AB
L*	53.46±0.40 <sup>a</sup>	49.17±0.29 <sup>b</sup>
a*	3.07±0.07 <sup>b</sup>	3.80±0.06 <sup>a</sup>
b*	14.96±0.17 <sup>a</sup>	14.63±0.03 <sup>b</sup>
ΔE		4.36
C*	15.27±0.18 <sup>a</sup>	15.11±0.03 <sup>a</sup>

NOTE: Values expressed as average (n=3) ± standard deviation. Different letters in the same line indicate significant differences ( $p \leq 0.05$ ) by Tukey test.

From the color analysis results made before and after the autolysis was observed that this process significantly changes the color of the biomass ( $p < 0.05$ ). The difference can be seen by the ΔE parameter, which resulted in a value greater than 2, it means that the difference between samples is visible to the human eye (FRANCIS; CLYDESDALE, 1975). Another important information was obtained by Chroma value. It is noted that, statistically, there was no difference between the two samples. Although the luminosity value decreases after the autolysis process, the biomass did not become darker.

Physical-chemical analyses were made in the cookies with spent brewer's yeast to investigate the final composition of the product. The results can be found in Table 4, with the centesimal composition and water activity for cookies formulations: SF, F1 and F2.

**Table 4.** Centesimal composition g/100 g (%) in dry basis and water activity of the cookies: SF (0% AB), F1 (10% AB) and F2 (20% AB).

	SF	F1	F2
Moisture	3.58±0.01 <sup>b</sup>	2.77±0.02 <sup>b</sup>	5.15±0.40 <sup>a</sup>
Ash	1.49±0.02 <sup>b</sup>	1.15±0.02 <sup>c</sup>	1.81±0.02 <sup>a</sup>
Proteins	7.88±0.62 <sup>a</sup>	8.97±0.93 <sup>a</sup>	9.63±1.24 <sup>a</sup>
Lipids	10.31±0.26 <sup>b</sup>	13.19±0.48 <sup>a</sup>	13.35±1.16 <sup>a</sup>
Carbohydrate	76.74±0.33 <sup>a</sup>	73.92±0.50 <sup>b</sup>	70.06±0.30 <sup>c</sup>
Aw	0.264±0.003 <sup>b</sup>	0.267±0.004 <sup>b</sup>	0.547±0.003 <sup>a</sup>

NOTE: Values expressed as average (n=3) ± standard deviation. Different letters in the same line indicate significant differences ( $p \leq 0.05$ ) by Tukey test.

The moisture content showed a significant difference between the samples (Table 4). F2 presented the highest value, probably due to the autolysed biomass added to it. However, the formulation F1 did not present the same behavior of the F2, and the moisture content was less than SF. The lower moisture content for F1 may have occurred due to the loss of moisture during baking due to the size of the cookies, which were manually shaped. The sample area exposed to the baking can influence its loss of mass. Literature addresses that cookies should have low amounts of water therefore, it is necessary to improve the cooking/dehydration process for F2 (JAN; PANESAR; SINGH, 2018). Also, for water activity, F2 presented the highest value, differing from the other samples. However, these values still agree with dehydrated foods (ZAMBRANO *et al.*, 2019).

All cookies formulations differed significantly in relation to the ash content, being F2 the highest value due to the ash content from autolysis process. Regarding lipids, with the addition of autolysed biomass, there was an increase in lipids. The autolysed biomass present 0.97% to lipid content (Table 2), this contribute to increased lipid content of the cookies. This behavior was also verified by Santucci *et al.* (2002) when they added yeast biomass to pasta.

The addition of 10% and 20% of AB did not significantly alter the protein content of the cookies. However, the addition of autolysed yeast biomass can improve the nutritional value of the food. In a study carried out by Viera *et al.* (2016), the extracts from inner contents of spent yeast biomass presented high content of vitamins B, minerals, and antioxidant activity being the use of yeast autolysed biomass as an ingredient in food applications encouraged.

Carbohydrate content also showed a significant difference between the formulations of cookies. The addition of autolysed biomass decreased the amount of carbohydrates. This behavior is related to the increase in the levels of lipids and moisture. The same situation also observed in the addition of autolysed yeast biomass in pasta (SANTUCCI *et al.*, 2002).

The use of autolysed biomass in the formulation of cookies significantly changed the samples colors. In Table 5 are presented the results of color analysis to SF, F1 and F2.

**Table 5.** Color analysis of cookies: SF (0% AB), F1 (10% AB) and F2 (20% AB).

	SF	F1	F2
L*	47.46±0.15 <sup>a</sup>	45.88±0.09 <sup>b</sup>	57.00±0.34 <sup>c</sup>
a*	16.45±0.17 <sup>a</sup>	14.80±0.81 <sup>a</sup>	11.49±0.03 <sup>b</sup>
b*	27.69±0.44 <sup>a</sup>	21.96±1.26 <sup>b</sup>	21.97±0.04 <sup>b</sup>
ΔE		6.00±1.41 <sup>a</sup>	12.00±0 <sup>b</sup>
C*	32.20±0.47 <sup>a</sup>	26.49±0.60 <sup>b</sup>	24.79±0.02 <sup>b</sup>

NOTE: Values expressed as average (n=3) ± standard deviation. Different letters in the same line indicate significant differences (p ≤ 0.05) by Tukey test.

The use of autolysed biomass in the formulation of cookies resulted in a significant lightening of the product. This behavior could be observed by the values of luminosity (L\*) and Chroma (C\*). Also, the Total Color Difference was twice times bigger for F2, presented a huge difference concerning the standard sample.

Results for all microbiological analyses of cookies are in accordance to the standards established by the legislation (BRASIL, 2001). All samples presented the following values, for Coliforms at 45 °C: <3.0 MPN/g (Legislation: 10 MPN/g), Staphylococcus: <1.0 × 10<sup>2</sup> CFU/g (Legislation: 5 × 10<sup>2</sup> CFU/g) and absence of Salmonella.

The cookies with different concentrations of biomass were submitted to sensory evaluation in order to verify the acceptance of the product in relation to the standard, without autolysed biomass (Table 6). The standard sample obtained the highest acceptability when compared to samples with added biomass.

**Table 6.** Sensorial analysis of the cookies: SF (0% AB), F1 (10% AB) an F2 (20% AB).

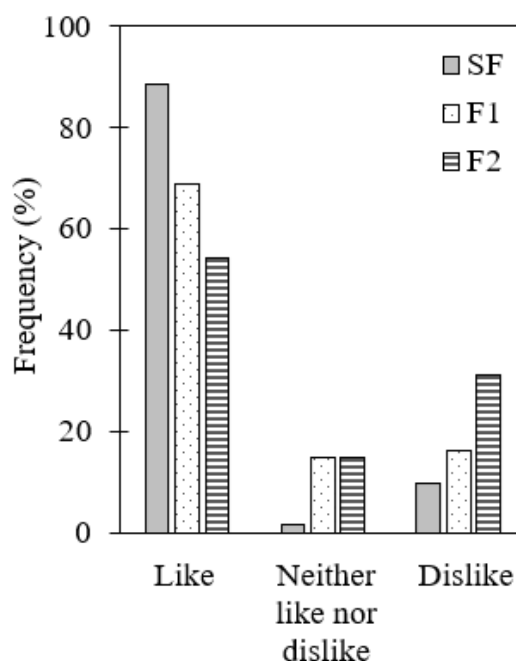
Formulation	Acceptability
SF	7.0±1.7 <sup>a</sup>
F1	6.0±1.6 <sup>b</sup>
F2	6.0±1.9 <sup>b</sup>

NOTE: Values expressed as average (n=3) ± standard deviation. Different letters in the same line indicate significant differences (p ≤ 0.05) by Tukey test.

To show with more details the answers from the acceptability test, Figure 1 was plotted. The frequency (%) were divided into 3 categories: like (6 - 9), neither like nor dislike (5) and dislike (1-4).

The cookie's acceptability values of all formulations were greater than the rejection (dislike), as can be seen in Figure 1. Therefore, there is a possibility to use the autolysed biomass from beer production as an ingredient in the formulation of cookies, creating a new destination for this residue. Nevertheless, new sensory studies are necessary to identify the most significant parameters for the acceptance of the cookies and, from there, adjust the product formulation.

Baptista *et al.* (2012) evaluated the partial substitution of wheat flour for the powder of *Moringa oleifera* leaf in cookies and obtained good marks (7.7 – 8.1) for the flavor and texture attributes, for which there was no significant difference between the three formulations with partial substitution of 3.64%, 7.27% and 9.09%. The possibility of replacing wheat flour with this vegetal leaf powder was verified in the percentages used without any unpleasant change in the flavor and texture of the cookies. In this study was verified minor values (6-7), and some judges commented on the sensory analysis sheets that cookies with the addition of autolysed biomass presented hard texture and extremely sweet taste than standard samples.



**Figure 1.** Frequency response for acceptability test.

### CONCLUSIONS

The physical-chemical characterization of spent yeast biomass from beer production in its natural and autolysed form revealed changes in some parameters, but there was no effective increase in the protein content. Therefore, the application of other cell disruption techniques would be necessary to increase the release of important inner components. Cookies with autolysed biomass have potential sensory acceptance. However, further studies are needed to adjust the formulation.



## Levedura de cerveja usada como ingrediente na elaboração de biscoitos

### RESUMO

Vários estudos foram conduzidos para encontrar maneiras de usar a levedura residual de cervejaria devido ao seu alto conteúdo nutricional (por exemplo, proteínas, vitaminas e minerais). Portanto, este estudo teve como objetivo caracterizar a levedura residual de cervejaria e adicioná-la aos biscoitos como ingrediente a fim de agregar valor a este resíduo da fermentação da cerveja. A levedura residual na sua forma natural e autolisada foi caracterizada por análises físico-químicas. Posteriormente, foram desenvolvidas formulações de biscoitos com biomassa autolisada (10% e 20%), os quais foram avaliados por análises físico-químicas e microbiológicas. Por fim, os biscoitos também foram submetidos à avaliação de aceitabilidade. A levedura de cerveja é uma fonte potencial de proteína, resultando em biscoitos com menor teor de carboidratos. Os biscoitos sem biomassa tiveram melhor aceitabilidade, portanto, é necessário aprimorar as formulações com a levedura residual. Porém, percebeu-se que a levedura de cerveja tem potencial para aplicação em alimentos e seu uso como ingrediente em formulações de alimentos pode ser uma forma de valorizar o resíduo da produção de cerveja.

**PALAVRAS-CHAVE:** levedura residual; cervejaria; biscoitos; análise sensorial.

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**Recebido:** 16 mai. 2020.

**Aprovado:** 09 set. 2020.

**DOI:** 10.3895/rebrapa.v11n1.12331

**Como citar:**

TESSARO, G. et al. Spent brewer's yeast as an ingredient on the elaboration of cookies. **Brazilian Journal of Food Research**, Campo Mourão, v. 11, n. 1, p. 32-44, jan./mar. 2020. Disponível em: <https://periodicos.utfpr.edu.br/rebrapa>

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