EXTRACTION AND QUANTIFICATION OF LIPIDS FROM BREWER'S SPENT GRAIN AND ITS POTENTIAL FOR LIPASE PRODUCTION

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ABSTRACT – Solid wastes from agro-industries may be used as culture medium for enzymes synthesis by filamentous fungi. The brewer's spent grain (BSG) is generated in abundance in the south of Brazil. Currently, most of this waste is used for animal feed. This work aims to extract and quantify the lipid presents in this residue. The lipids extraction was carried out by Soxhlet extraction method. Initially, the brewer's spent grain was maintained at 80°C for 30 minutes to remove the moisture. After, it was weighed a sample of 5 grams accurately. A flat-bottom flask, containing some glass beads, was dried at 80°C for 1 hour and it was weighed. The sample was introduced into the extractor unit in a sealed filter paper envelope. The solvent was added to flat-bottom flask (150 ml of ethanol). The scheme was assembled and maintained on a heating mantle for 4 hours. After, flat-bottom flask was maintained at 80°C for the complete solvent evaporation. Then, the flask was weighed again, to determinate the lipids mass presents in 5 grams of brewer's spent grain. We can conclude that this study provides the basis for further studies, since the percentage of lipids present in this residue (8.6%) justifies its use in experimental tests that aim lipase production.

1. INTRODUCTION

Lipases (glycerol ester hydrolase, E.C. 3.1.1.3) are from the hydrolases family and their main function is to hydrolyze triglycerides to produce diglycerides, monoglycerides, fatty acids and glycerol. The lipases are from animal origin (pancreatic, hepatic and gastric), vegetable or microbial origin (fungi and bacteria). Depending on its origin, the molecular mass and the enzymatic activity at different pHs and temperatures range (Atkinson and Mavituna, 1991; Pelizer *et al.*, 2007).

Currently, the lipases represent approximately 5% of the world market for enzymes, with significant growth trend due their many current and developing applications (Palma *et al.*, 2000; Saxena *et al.*, 2011).

Independent of their origin (plant, animal or microbial), lipases have numerous biotechnological applications in food, detergents, pharmaceutical and agrochemical industries (Sharma *et al.*, 2001).

The brewer's spent grain is an important by-product of brewing (White *et al.*, 2008). Mussatto and Roberto (2006) claim that the brewer's spent grain is approximately 85% of the waste generated in breweries and is commonly used for animal feed.

Mussatto *et al.* (2008) describe the results of the composition analysis of the brewer's spent grain from the procedure without addition of other cereals besides the malt. Santos *et al.* (2003) present different data for its composition, which can vary depending on the technology used in breweries, among other factors. Table 1 presents the experimental data obtained by the authors, with the percentage of dry weight. The missing values are related to components not quantified by the authors.

Table 1 - Composition of brewer's spent grain

Component	Mussatto *et al.* (2008) (%)	Santos *et al.* (2003) (%)
Pentosans	28,4	19
Lignin	27,8	16
Cellulose	16,8	9
Proteins	15,25	31
Ashes	4,6	4
Extractives*	5,8	----
Acetyl group	1,35	----
Lipids	----	9
Starch and β-glucan	----	12
Total	100	100

* consisting of waxes, fats, gums, starches, resins, tannin, essential oils and various other cytoplasmic components. Source: Adapted from Mussatto *et al.* (2008) and Santos *et al.* (2003).

There are reports in the literature about the use of agro-industrial wastes as fermentation medium for obtaining microbial lipase. Cordova *et al.* (1998) studied the use of sugar cane bagasse and olive cake as the medium for solid-state fermentation (SSF), conducted by the fungus *Rhizopus rhizopodiformis*. They obtained lipase activity of 79.6 U/g of dry residue.

Gombert *et al.* (1999) evaluated the lipase production by SSF using as a medium the babassu solid waste, supplemented with peptone and olive oil, and fermentation was conducted by *Penicillium restrictum*. The highest lipase activity (30.3 $U/g_{dry\ weight}$) was obtained after 24 hours of fermentation. Fernandes (2007) studied the production of lipase from *Burkholderia cepacia* LTEB1 by SSF using as substrate wheat husk, corn cake and sunflower seed meal, separately. The highest lipase activity (240 U per gram of dry solid) was obtained using sunflower seed meal.

In the work presented by Mahanta *et al.* (2008), the authors studied the production lipase by *Pseudomonas aeruginosa* in SSF using jatropha seed cake as substrate. The higher lipase production was in the medium increased with sodium nitrate (1084.2 $U/g_{dry\ substrate}$). Godoy *et al.* (2009) evaluated the lipase production by *Penicillium simplicissimum* in solid state fermentation of castor beans residue. After 96 hours of fermentation, the lipase activity resulted in 155 U/g of dry residue.

Rigo (2009) obtained lipase activity of 317 U/g using soybean meal (supplemented with 0.6% of urea plus soybean oil) as the culture medium for the SSF and the filamentous fungus *Penicillium* 58F. Sun and Xu (2008) studied the lipase production by SSF using the fungus *Rhizopus chinensis* CCTCC M201021. The fermentation medium was composed of wheat flour and wheat bran (supplemented with peptone (2%, v/v) and olive oil (2%, v/w)). The best result was 24.447 U/g of culture medium.

1.1. Objective

The main objective of the current study was extract and quantify the lipids present in the brewer's spent grain from breweries in Blumenau-SC, Brazil. This is an initial study, which will result in data to determining whether the brewer's spent grain has the potential to be used as a culture medium for further study in solid-state fermentation for obtaining microbial lipase.

2. METHODOLOGY

The lipid extraction, to quantify the same in the brewer's spent grain, was performed by Soxhlet extraction method (Castro and Priego-Capote, 2010). The BSG was obtained from different breweries, and homogenized for this experiment. Initially, the brewer's spent grain was ground and kept at 80°C for 30 minutes. Then, the residue remained in a desiccator to cool and an accurate sample of 5 grams was separated. A flat-bottom flask containing some glass beads were kept at 80°C for 1 hour, and subsequently its mass was determined.

The sample was introduced into the extractor in a stapled filter paper envelope to avoid direct contact of the sample with the solvent. As the solvent, it was used 150 ml of ethanol, which was added to flat-bottom flask. After, the scheme was set up and it was maintained on a heating mantle.

The extraction was maintained for 4 hours. The solvent was recovered after the conclusion of the process. After, the flat-bottom flask was maintained at 80°C for 8h for the complete solvent evaporation. Then, the flask was weighed again to determine the substrate mass extracted during the process. The Figure 1 illustrates the scheme used for the lipids extraction.

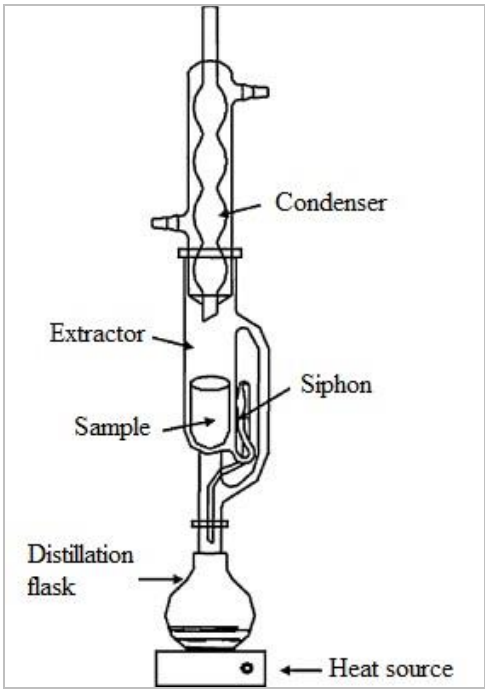

Figure 1 - Conventional scheme for Soxhlet extraction.
Source: Adapted from Castro and Priego-Capote (2010).

2.1. Calculation of Percentage of Lipids

The difference between the weight of the flask before and after extraction corresponds to the mass of lipids presents in 5 grams of brewer's spent grain (dry weight). With this, the percentage of the lipids in the sample can be calculated according to Equation 1.

$$\%\ Lipids = \frac{mass\ of\ lipids}{5} \times 100 \quad (1)$$

3. RESULTS AND DISCUSSION

The step of lipid extraction from the BSG was performed in duplicate for increased reliability of results. In both cases, the difference in the weight of the flask, before and after extraction, was 0.43 grams. Applying the result obtained in the Equation 1 we have:

$$\%Lipids = \frac{0{,}43g}{5g} \times 100 \quad (1)$$

$$\%Lipids = 8{,}6\%$$

The value obtained in this study approaches to the value presented by Santos *et al.* (2003), who reported that the brewer's spent grain valued by them contained 9% of lipids. Considering the fact that the BSG from this study is from different breweries, which do not necessarily use the same technology during the process, the result obtained is satisfactory for keeping close to the one presented in the literature. Also, considering that the addition of other ingredients during the process is unknown, which could make the BSG impure and change the amount of lipids present in the sample.

Ferraz *et al.* (2012) evaluated the lipase production by *Sporidiobolus pararoseus* using three different residues for SSF: soybean meal (8.5% lipids), sugar cane bagasse (0.63% lipids) and rice meal (16.43% lipids). Lipase activities of about 130.1 U/g, 164.2 U/g and 189.5 U/g were obtained using soybean meal, sugarcane bagasse and rice meal as substrates, respectively, without supplementation, at 30°C and 60% of moisture. Based on this, the brewer's spent grain of this study (considering the composition of 8.6% lipids) shows up as a residue in potential for future studies in obtaining microbial lipase.

4. CONCLUSIONS

From the literature review, we can observe the industrial importance of the lipase and the growing interest in researches for its producing. The use of residues as a culture medium for the production of several enzymes is of great interest for environmental and economic issues, and we can notice that the waste variety studied is increasing, regardless of the lipids percentage in their composition.

In this context, we conclude that the brewer's spent grain is viable for future studies to obtain microbial lipase, and it should be consider the study of the medium supplementation, as well as the microorganism to be used in solid state fermentation.

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