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Valorization of carob waste: Definition of a second-generation bioethanol production process

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HIGHLIGHTS

- Bioethanol production from carob solid waste of the food industry was studied.
- Sugar extraction efficiency was maximized at 60% on cut waste at room temperature.
- Liquid fermentation on these extracts achieved 45.0% yield and 78 g EtOH/kg waste.
- Solid-state fermentation maintained yield and achieved up to 155 g ethanol/(kg waste).
- Carob waste is an attractive feedstock for second-generation bioethanol production.

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ABSTRACT

The aim of this work was to develop a strategy for second-generation ethanol production from carob solid waste issued from Lebanese food industry. The pros and cons of submerged (SF) and solid-state fermentations (SSF) using *S. cerevisiae* on ethanol yield and productivity were compared, including the respective roles of upstream and downstream processes, such as the size reduction, or sugar and ethanol recovery processes. The design of experiments methodology was applied. Experimental results demonstrated that SSF applied to cut carob waste from carob syrup preparation was simpler to operate and more cost-effective, maintained yield and productivity (0.458 g ethanol/g consumed sugar and 4.3 g/(kg waste)/h) in comparison to SF (0.450 g ethanol/g consumed sugar and 5.7 g/(kg waste)/h), and was able to achieve ethanol production up to 155 g/(kg waste) at low water demand, while SF reached only 78 g/(kg waste) due to the limitations of the sugar extraction pretreatment.

1. Introduction

The carob tree, also referred to as locust bean tree, belongs to the legume family, is widely cultivated in Mediterranean countries for its indisputable ecological, industrial and ornamental importance (Race et al., 1999; Zengin et al., 2008). In Lebanon, carob trees are abundant and distributed along the coastal lower hills. For millennia, parts of the carob tree were used to treat diseases (Sidina et al., 2009), such as bark and leaves that are effective in folk medicine as an antidiarrheal treatment and against gastroenteritis in infants (Dhaouadi et al., 2014). In carob pods, the seeds are used for extracting galactomannan (Haddarah et al., 2013) and the pulp, rich in sugar, is used in the preparation of sweet juice, cacao and as a chocolate substitute. In recent years, carob pods have gained considerable attention because of their high carbohydrate and mineral content: Many high value-added products, such as lactic acid, mannitol, citric acid, and pullulans were produced from carob fermentation (Germec et al., 2015).

In parallel, carob pods have been used as a resource for bioethanol production. As a matter of fact, the consumption of energy in the world is constantly increasing, which encourages to seek renewable and sustainable energy resources and to use environment-friendly production processes for replacing progressively fossil fuels. Bioethanol fuel is a biodegradable product, less toxic than methanol; its combustion leads to a decrease in carbon dioxide emissions and is associated with a lower risk of ozone for-

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mation than gasoline and diesel (Thangavelu et al., 2016). Thus, it constitutes an alternative low-cost attractive energy vector able to replace fossil fuels. The most efficient microorganisms for bioethanol production are *Saccharomyces cerevisiae*, *Zymomonas mobilis* and *Pichia stipitis* (Brethauer and Wyman, 2010), either using the fermentation of biomass rich in fermentable sugars, or from lignocellulosic biomass and polysaccharides which require several pretreatment steps of hydrolysis to obtain fermentable sugars. Submerged fermentation (SF) and solid-state fermentation (SSF) can both be used for ethanol production. SSF is defined as a fermentation process occurring in the absence or near absence of free water. Even though SF is the most widely used, SSF offers some advantages: no extraction step, low water requirements (which is particularly interesting when water is scarce), lower mechanical energy input, and reduced investment cost.

In Lebanon, carob pulp is mainly used for the preparation of carob syrup or carob molasses denoted "dibs", which is consumed by Lebanese population as a sweetener (Haddarah et al., 2013). The traditional method used by Lebanese industries involves a double maceration of carob pods cut into pieces in water at room temperature with a mass ratio of liquid to solid L/S = 3, without agitation, for 3 h. Then, water is evaporated to obtain the final molasses. Currently, this solid waste is not subject to any type of recovery, and only a fraction is used for animal feed. With such a traditional process, a significant amount of sugars remains in the solid waste after the maceration step.

Consequently, the objective of this work was to set up a biofuel production process from the carbohydrates remaining in the carob solid waste from dibs production, by producing second generation bioethanol. To our knowledge, research works concerning the valorization of waste from carob molasses production had never been conducted yet. First, the composition of the carob waste was characterized and compared to that of carobs pulp used initially in the dibs production process. Then, solid-state fermentation (SSF) and submerged fermentation (SF) using *Saccharomyces cerevisiae* were optimized and compared using design of experiments methodology. In SF, sugar extraction usually constitutes a costly pretreatment. For the carob waste, this was optimized by investigating the respective effects of pH, temperature, particle size, liquid to solid mass ratio and contact time. For SSF, both the fermentation parameters (cell density, temperature, humidity, particle size and operation time) and ethanol recovery were analyzed. Finally, the conclusions defined a compromise between bioethanol productivity and yield, process simplicity and robustness, and environmental aspects.

2. Materials and methods

2.1. Analysis of the composition of carob pulp and carob waste

In Lebanon, nine carob varieties are available; a mixture of these varieties was used to analyze the composition of the carob pulp. Carobs and waste (in the form of 5 mm pieces) were both obtained from the Lebanese industry *Salloum Ekhwan*. Both were dried (70 °C, 24 h) and mechanically milled into fine particles using a Universal Machine UMC-5 (*Stephan Machinery GmbH*, Germany) equipped with knives (operating conditions: 500 g solid, 1500 rpm, 2.5 min). After sieving, the fraction of approximate size 0.5 mm was recovered for composition analysis.

Lipids were extracted using chloroform, mixing 0.5 g of ground sample with 4 mL chloroform, 2 mL methanol and 1 mL distilled water. The mixture was vortexed, and then, centrifuged at 2000*g* for 10 min. The lower phase was recovered and the solvent was evaporated in a vacuum concentrator at 40 °C before weighing (Bligh and Dyer, 1959). Total sugars were determined using the colorimetric Dubois method with glucose as the standard (Dubois et al., 1956). Insoluble fiber content was analyzed using the method adapted from Pádua et al. (2004) and Pierre et al. (2011); this consisted, first, in digesting 4 g carob powder in a 5% HCl solution (200 mL) during 30 min. under reflux. After subsequent filtration and washing, a second digestion step was carried out on the residue in a 5% NaOH solution (200 mL) during 30 min. under reflux. Then, the mixture was filtered, neutralized, and washed with ethanol and ethyl ether. Finally, the residue was dried at 100 °C for 2 h, and the residual mass was considered the insoluble fiber content, which should mainly consist of cellulose. Moisture content was estimated by the measure of the mass loss of 5 g of carob powder heated at 105 °C for 6 h., and the ash content using the AOAC 972.15 official method (AOAC, 2006). Total phenolic compounds were determined colorimetrically at 660 nm, and expressed as gallic acid equivalent (Singleton and Rossi, 1965). Total nitrogen was determined using the AOAC official method 945.46 (AOAC, 2007), which involved a Macro Kjeldahl unit for digestion and distillation.

2.2. Sugar extraction for submerged fermentation

2.2.1. Acid hydrolysis and extraction on dried waste

Extraction and hydrolysis with sulfuric acid was applied to the 0.5 mm size fraction of the dried and milled carob waste, obtained as described in Section 2.1. Experiments were carried out in a batch extraction device with total reflux under mechanical shaking during 1 h. The acid solution to carob waste mass ratio L/S was 5. Runs were conducted as a function of temperature (66, 83 and 100 °C) and acid concentration (1, 2 and 3% v/v), as summarized in Fig. 1. Design of experiments methodology was applied. A full factorial design with two factors and three levels was used to measure the effect of these parameters on the amount of extracted sugar. Each treatment was replicated three times, and total sugars (R_t) and reducing sugars (R_r) were chosen as the dependent variables. Total sugars were determined using the method of Dubois et al. (1956), and the reducing sugars were analyzed using the DNS method of Miller (1959).

2.2.2. Aqueous extraction on dried and fresh waste

Acid extraction requires further neutralization and is known to potentially produce fermentation inhibitors (Kuila et al., 2011). An alternative consists in reducing particle size for enhancing extraction at natural pH. However, the ability to reduce particle size depends on the moisture content in the waste. First, the fresh waste (approx. size 5 mm) could not be milled without forming a pasty medium. Thus, it could only be mechanically cut into pieces with an approximate size of 2.5 mm. Conversely, extraction could not be carried out at low L/S ratio with the waste dried at 70 °C and mechanically milled as in Section 2.1, as a swelling behavior could be observed during the aqueous extraction using the fine dried powder. To circumvent this issue, a minimum L/S ratio of 5 had to be used.

Consequently, extraction was performed, first, on milled dried waste (approx. size 0.5 mm), and then, as a reference for comparison purpose, on cut fresh waste from industry. Extraction was carried out in water at different conditions without pH adjustment, by changing the L/S ratio (5, 7.5 and 10), temperature (25, 58 and 90 °C) and contact time (30, 60 and 90 min). Each experiment was replicated three times and a 3^{3-1} fractional factorial design with replications was built. Each sample was filtered, then hydrolyzed in a 1 N HCl solution for 30 min at 90 °C under reflux, and neutralized by 1 N NaOH before analysis to determine total sugar content (R_t) as in Section 2.2.1. In parallel, a lower ratio L/S = 3 was only tested on the fresh waste (about 5 mm size), considering