Assessment of Tofu Whey's Potential for Use in Bioprocesses

Matheus. S. Erreira[1], Mariana F. Fabrício[2], Suse B. da Silva[3] Eliseu Rodrigues[2], Marco A.Z. Ayub[2] & Daniele M. Rossi[1*]

[1] *Department of Chemical Engineering, Federal University of Rio Grande do Sul, Brazil.*
[2] *Bioprocess and Biotechnology for Food Research Center (Biofood), Food Science and Technology Institute (ICTA), Federal University of Rio Grande do Sul, Av. Bento Gonçalves 9500, Porto Alegre, 91501-970, Brazil.*
[3] *Center for Exact and Technological Sciences, Area of Knowledge and Application of Food Technology and Processing. University of Vale do Rio dos Sinos, São Leopoldo, RS – Brazil*
** Corresponding author's email address: daniele.misturini@ufrgs.br*

ABSTRACT

Tofu whey, a byproduct of tofu production, has recently gained attention as an alternative to traditional whey and other by-products for cultivating lactic acid bacteria and yeasts, which utilize the sugars in tofu whey as an energy source. The objective of this study was to evaluate the potential of tofu whey as a culture medium for producing value-added bioproducts, specifically lactic acid and ethanol. To achieve this, potential microorganisms capable of utilizing tofu whey were screened. The microorganisms that showed promise in this first screening step were selected for batch cultures. Two microorganisms were selected: *Lactobacillus plantarum BL011* and *Kluyveromyces marxianus. L. plantarum BL011 and K. marxianus* obtained sucrose consumption of 37.71% and 100%, respectively. After 48 hours of culture, *L. plantarum BL011* were able to produce 7.06 g/L of lactic acid. Meanwhile, K. marxianus produced 19.05 g/L of ethanol in 24 hours. These findings suggest that these microorganisms have the potential to convert tofu whey residue into high value-added products.

Keywords: tofu, lactic acid bacteria, fermentation, ethanol, lactic acid

1 INTRODUCTION

Tofu is a millennia-old food consumed primarily by Asians, and to this day, its largest worldwide production is concentrated in Eastern countries. Tofu whey is a byproduct of the soy "cheese" manufacturing process and has recently been used as an alternative medium to milk whey and other growth media for the cultivation of lactic acid bacteria and yeasts. Tofu whey is a rich source of carbon, containing sugars such as sucrose, raffinose, stachyose and oligosaccharides, besides being considered a rich source of proteins and minerals.

Lactic acid bacteria (LAB) belong to a broad group of different bacteria found in nature, widely employed in food production, such as fermented milk, yogurts, cheeses and in the processing of meats and alcoholic beverages. Yeasts, on the other hand, have a long history of use with a wide range of applications in various areas, including food, beverages, pharmaceutical industries, as well as aquaculture, livestock, and biotechnology sectors.

The objective of this study was to evaluate the potential of tofu whey, a residue from tofu production, as an alternative carbon and nutrient source in bioprocesses. In the first stage of the study, potential microorganisms capable of using tofu whey without the addition of any other nutrient source were selected to verify the potential metabolites of interest.

2 MATERIAL & METHODS

In the first stage of the work, potential microorganisms capable of using tofu whey without the addition of any other nutrient source were selected to verify the potential metabolites of interest. *L.lactis, L.lactis 2* and *L. fermentum* was isolated from Kefir milk. *Lactobacillus plantarum BL011*, supplied by BIOTECLAB (ICTA-UFRGS) and *Kluyveromyces marxianus (formerly Kluyveromyces fragilis)* (BO399) was kindly supplied by Turval Company (Udine, Italy). The LAB (lactic acid bacteria) were previously growth in MRS (Man, Rogosa, and Sharpe) broth and the YPD (Yeast Peptone Dextrose) medium was used for the growth of the yeast *Kluyveromyces marxianus*. After the screening, *Lactobacillus plantarum BL011 and Kluyveromyces marxianus* were selected for fermentation. Fermentation assays were performed in 125 mL Erlenmeyer flasks on an orbital shaker with a useful volume of fermentative medium of 60 mL. The Erlenmeyer flasks were sealed with oxygen-permeable cotton plugs, simulating a microaerophilic condition with agitation at 150 rpm and a temperature of 30°C. The tofu whey, used directly as a carbon and nutrient source was autoclaved at 121°C for 15 minutes and the initial pH was adjusted to 6.7 with Sodium Hydroxide (NaOH). The culture was monitored by withdrawing 4 mL aliquot every 3 hours during the first 12 hours of cultivation and, thereafter, the same volume was withdrawn at different intervals until reaching the final cultivation time of 48 hours. The pH was measured and the samples were centrifuged for subsequent chromatography analysis. The same procedure described above was performed for the yeast *Kluyveromyces marxianus* with cultivation time of 24 hours. All assays were performed in duplicate.

The methodology used for the quantification of colony-forming units proceeded through the SP-SDS (Single Plate-Serial Dilution Spotting) method, adapted from Thomas et al. (2015). The concentration of fermentation products (lactic acid, acetic acid, and ethanol) and sugars was determined by High-Performance Liquid Chromatography (HPLC) using a Shimadzu brand equipment equipped with a refractive index detector (RID-10A). For the quantification of fermentation products, the column used was the Bio-Rad Aminex 87H, with a flow rate of 0.6 ml/min at 45°C, using a 5 mM sulfuric acid (H_2SO_4) solution as the mobile

phase. For quantification of sugar consumption, the conditions utilized were 85°C and a flow rate of 0.6 ml/min on the Bio-Rad Aminex 87C column with ultrapure water as the mobile phase.

3 RESULTS & DISCUSSION

In the initial fermentation assay, the objective was to evaluate which of the studied strains would exhibit production of the compounds of interest (lactic acid and ethanol) using only the sugars present in fresh tofu whey as a carbohydrate source. The cultivation time was 24 hours. The results are presented in Figure 1.

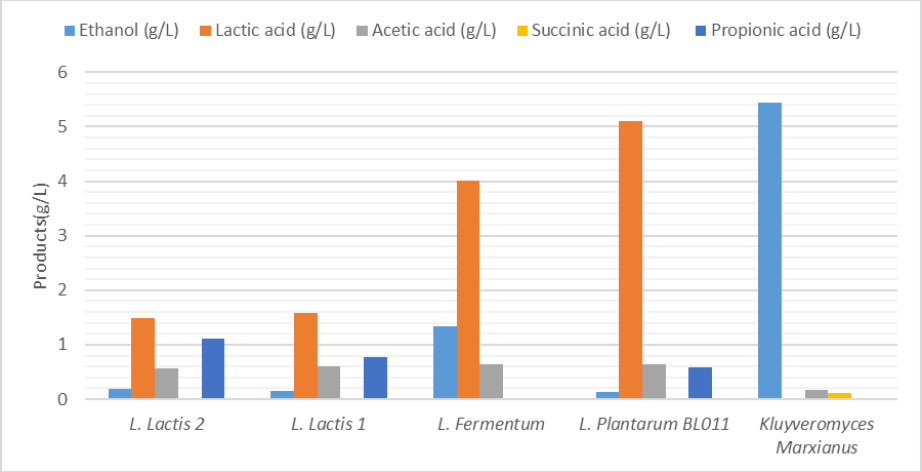

Figure 1 Microroganisms selection for lactic acid production and ethanol.

Analyzing the results from the fermentation assay of the five strains, it was observed that *L. fermentum* and *L. plantarum* BL011 exhibited the highest lactic acid production, with yields of 4.02 g/L and 5.10 g/L, respectively. They also produced other compounds in smaller amounts, such as ethanol and acetic acid. In contrast, the yeast *K. marxianus* was responsible for the highest ethanol production, yielding 5.45 g/L in the 24-hour, and also producing acetic acid and succinic acid in smaller concentrations. Based on the metabolite production, *L. plantarum BL011* and *K. marxianus* were selected for the next stage of the work.

In order to study the behavior of the yeast *K. marxianus* during its fermentation in tofu whey medium, a kinetics study was conducted over a period of 24 hours. Analysis of the results revealed that *K. marxianus* produced a significant amount of ethanol, along with smaller quantities of other bioproducts. Among the sugars present in tofu whey, the yeast was only able to consume sucrose, while the other sugars present, such as stachyose and raffinose, maintained their concentration until the end of the cultivation. The kinetics of the duplicate assay is presented in Figure 2. *K. marxianus*, obtained a yield of 0.44 g/g and productivity of 0.79 g/L.h.

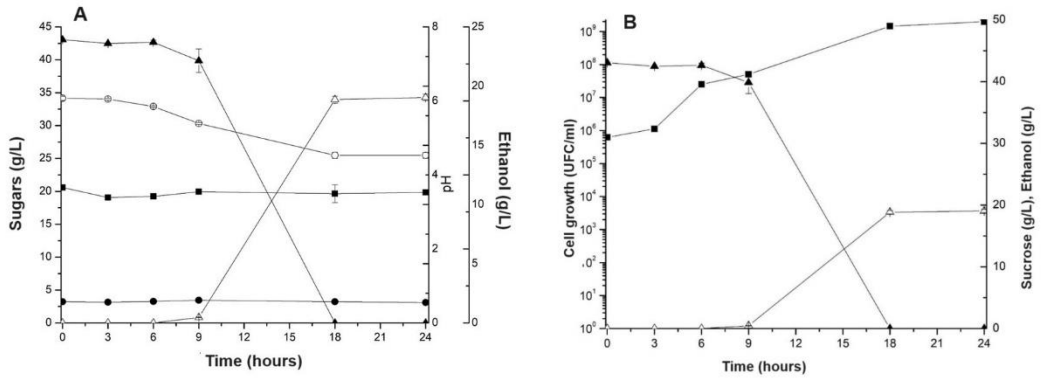

Figure 2 Kinetics of sugar consumption and ethanol production in tofu whey with *Kluyveromyces marxianus*. (A): (-■-) stachyose, (-●-) raffinose, (-▲-) sucrose; (-○-) pH, (-△-) Ethanol. (B): Cell growth of *Kluyveromyces marxianus* during fermentation assay in tofu whey medium. (-■-) CFU (Colony-Forming Units), (-▲-) sucrose, (-△-) Ethanol.

According to the results presented in Figure 3, we can observe that *Lactobacillus plantarum* BL011 showed a significant production of lactic acid, 7.06 g/L in 48 hours of fermentation. Also, a good cell growth can be observed during cultivation, more

pronounced in the first 12 hours. In the course of fermentation, a decrease in pH is also noticeable. This fact characterizes the antimicrobial activity of lactic acid bacteria (LAB). The antimicrobial effect of LAB is mainly due to the production of lactic acid and other organic acids, causing a decrease in the pH of the growth environment[1]. The low pH induces organic acids to become liposoluble and diffuse through the cell membrane into the cytoplasm[2].

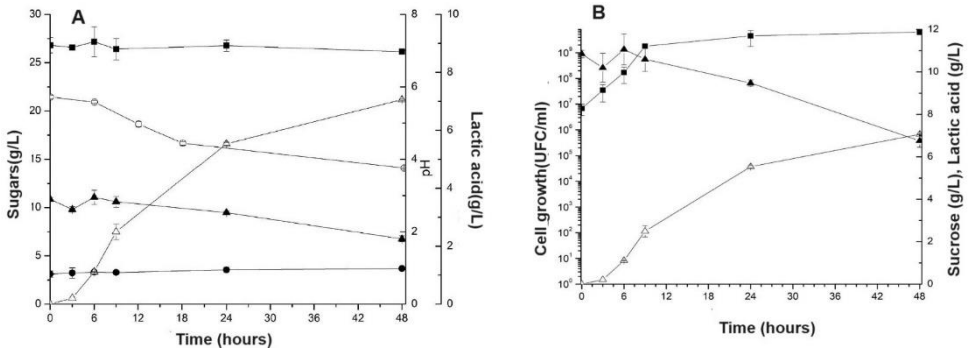

Figure 3 Kinetics of sugar consumption and lactic acid production in tofu whey with the bacterium *Lactobacillus plantarum BL011*. (-■-) Stachyose, (-●-) Rafinose, (-▲-) Sucrose; (-○-) pH, (-△-) Lactic Acid. (B) Cell growth of *Lactobacillus plantarum BL011* fermentation in tofu whey medium. (-■-) CFU (Colony-Forming Units), (-▲-) sucrose, (-△-) Lactic Acid

It was observed that, despite a significant production of lactic acid, the microorganism exhibited a slower consumption of sucrose, consuming only 32% of this sugar during the 48-hour assay and no consumption of the other sugars present, raffinose and stachyose. There was also a high yield despite the low sugar consumption, showing 1.99 g/g and productivity of 0.15 g/L.h. According to literature, growth parameters can undergo changes due to a variety of factors, including variations in the inoculum size that can cause alterations in the duration of the lag phase, a variable period where there is no significant increase in population, and in the maximum specific growth rate, depending on the method of cell quantification used[3,4,5]

4 CONCLUSION

L. plantarum BL011 proved capable of primarily producing lactic acid with good yield values and some other organic acids in smaller amounts from tofu whey medium, partially consuming sucrose and showing no changes in the concentrations of raffinose and stachyose. It also exhibited a good rate of cellular growth during its cultivation. Regarding the yeast *K. marxianus*, ethanol production during cultivation in tofu whey was considered satisfactory, demonstrating good yield and productivity values compared to other biomass sources used in the bioethanol industry, such as sugarcane. Sucrose was completely metabolized; however, the sugars raffinose and stachyose were not consumed. It also exhibited a high rate of cellular growth. Comparing the microorganisms mentioned in this study, the yeast *K. marxianus* emerges as the most promising for the production of biocompounds through biotechnological pathways. Not only did it consume all the sucrose present in tofu whey, but it also exhibited a good rate of cellular growth and furthermore showed significant ethanol production, standing out as an alternative for scaling up the production of clean and renewable fuel.

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