I COBBIND
I CONGRESSO BRASILEIRO DE BIOTECNOLOGIA INDUSTRIAL
XXIV SINAFERM ** XV SHEB ** XV ENZITEC FLORIANÓPOLIS ** 2024
Creating connections between biotechnology and industrial sustainability
August 25 to 28, 2024
Costão do Santinho Resort, Florianópolis, SC, Brazil
PPGEAL/UFLA

FERMENTATION OF ARROWROOT (*Maranta arundinacea*) AND PEANUTS (*Arachis hypogaea L.*) EXTRACTS WITH *Bifidobacterium longum*

Thiago Y. Neves[1], Giovanna F. Martins[1], Olga L. Mondragón-Bernal[2] & José G. L. F. Alves[2*]

[1] *Food Engineer/Department of Food Science/ Federal University of Lavras, Lavras, Brazil*
[2] *Department of Food Science, Federal University of Lavras, Lavras, Brazil.*
** Corresponding author's email address: jlembi@ufla.br*

ABSTRACT

Consumers are more aware that foods can contribute to the prevention of pathologies, promoting a healthier life. Arrowroot is valued for its nutritional properties and the absence of gluten, while peanuts, rich in protein, can be used in fermented drinks. This work aimed to evaluate the fermentative potential of arrowroot and peanut extracts with *Bifidobacterium longum* probiotic culture. A Central Composite Rotational Design was carried out with two medium formulation variables: hydrolyzed arrowroot extract (HAE) and peanut extract (PE). Fermentations were carried out in Erlenmeyer flasks with 50 mL of medium in triplicate with different formulations in an incubator without shaking at 37 ºC, until the medium pH reached 4.5. Cell counts were determined by plating on MRS agar, total titratable acidity, total reducing sugars (TRS) and pH at the beginning and end of fermentation. It was observed that TRS consumption and fermentation time were significantly influenced by HAE content and the quadratic term of PE content, respectively. Cell growth and production of organic acids were not significantly influenced by HAE and PE. It was found that greater cell growth (2.8 log CFU/mL) occurred in treatment 3 (30%v/v HAE and 45%v/v PE), reaching a final concentration of 8 log CFU/mL.

Keywords: *Maranta arundinacea*. *Arachis hypogaea L.* Probiotic. Lactic acid bacteria. Fermented beverage.

1 INTRODUCTION

Contemporary consumers recognize the importance of food not only to satisfy hunger, but also to promote health and prevent disease. In this context, functional foods emerge, which contain biologically active compounds capable of providing health benefits beyond basic nutritional functions. Among these foods, foods with probiotics stand out.

Probiotics, live microorganisms that confer health benefits when consumed in adequate quantities, have been the subject of increasing interest. However, to be considered probiotics, they must meet specific criteria, including resistance to digestive tract conditions.

Arrowroot (*Maranta arundinacea*) is a plant native to South and Central America. It has a tuberous rhizome, rich in starch (20%)[1] and is considered a non-conventional food plant (PANC). Starch is used to make biscuits and bread, and is highly desired due to its characteristics, digestibility and absence of gluten. Its fresh rhizome, in addition to starch, contains other nutrients such as proteins, phosphorus, sodium, potassium, magnesium, iron and calcium[2].

In a scenario where consumers are increasingly looking for foods of plant origin and with a low sugar content, unconventional food plants (PANC) have gained prominence as promising sources of research. Arrowroot and peanuts[3], for example, appear as interesting options for the development of milk substitute products and fermented drinks, meeting market demands for healthier and more sustainable alternatives.

2 MATERIAL & METHODS

The peanut extract was prepared following a modified methodology[4], where peeled peanut kernels were heated in a 0.5% sodium bicarbonate solution, drained, washed and disintegrated in a blender with water heated to 97°C. The aqueous extract was filtered, homogenized, frozen and stored at -15°C. For the enzymatic hydrolysis of arrowroot flour, the flour was dissolved in water (10% w/v), the pH was adjusted to 6.5, then the mixture was heated in a water bath until it reached 90 °C, remaining at that temperature. for 1 h to gel the starch. After this period, the enzyme α-amylase (Termamyl 2X ®) was added to the mixture (concentration of 7.5 mL/kg of arrowroot flour), and maintained at 90 ºC for another 2 hours for the hydrolysis of the linear starch chain. Then, the pH was adjusted between 4.0 and 4.5, and the temperature was reduced to 60 ºC.. Amyloglucosidase (AMG 300L ®) was added at the rate of 6.5 mL/kg of arrowroot flour and kept for another 5 hours before filtering, cooling and storing at -15°C.

The probiotic culture *Bifidobacterium longum* (BL04) was activated from its lyophilized form. This was done using Man, Rogosa, and Sharpe (MRS) broth. For activation, 1 mL of stock culture was added to 9 mL of MRS broth and incubated at 37°C for 48 h. Then, 10 mL of this medium was transferred to an Erlenmeyer flask containing 90 mL of MRS broth and incubated again for 24 hours to propagate the microorganism. To prepare the stock cultures, 1 mL of the growing inoculum was transferred to eppendorfs, centrifuged, the supernatant discarded, and the microorganism was maintained in freezing medium.

After obtaining the arrowroot and peanut flour extracts, both were mixed according to the proportions established in the Central Composite Rotational Design (CCRD), presented in Table 1. The pH was adjusted to 6.0 and pasteurization was carried out. of

the media added into Erlenmeyer flasks using autoclave (100 ºC/6 min). After cooling, the *Bifidobacterium longum* inoculum (BL04) was added and fermentations were carried out in a B.O.D oven without shaking at 37 ºC until reaching pH 4.5. The treatments were carried out in triplicate with samples collected at the beginning and end of fermentation for analysis.

Physicochemical analyzes were carried out in triplicate. The parameters analyzed were: pH, total titratable acidity and total reducing sugars. In addition, probiotic cultures were also counted by plating on MRS agar.

Table 1 – CCRD matrix for two independent variables in coded and real values.

.Treatment	Hydrolyzed Arrowroot Extract HAE (%v/v) - x1	anut Extract - PE (%v/v) - x2
1	-1 (30)	-1 (35)
2	1 (50)	-1 (35)
3	-1 (30)	1 (45)
4	1 (50)	1 (45)
5	-1.41 (25.9)	0 (40)
6	1.41 (54.1)	0 (40)
7	0 (40)	-1,41 (33)
8	0 (40)	1,41 (47)
9	0 (40)	0 (40)
10	0 (40)	0 (40)
11	0 (40)	0 (40)
12	0 (40)	0 (40)

The pH was monitored throughout the fermentation, measured initially and every 2 hours, using a calibrated bench pH meter. The titratable acidity was determined in the collected samples, diluted in distilled water and titrated with 0.01 mol/L NaOH solution, using phenolphthalein as an indicator. The determination of the concentration of total reducing sugars (TRS) was made using the 3,5-dinitrosalicylic acid/DNS method[5].

The total bacterial count was carried out by deep plating on MRS agar medium, in a B.O.D oven at 37°C for 48 hours in anaerobic conditions, using generators in hermetically sealed bottles[6]. The colony forming units count (CFU/mL) was performed by directly counting the colonies on the plates.

3 RESULTS & DISCUSSION

The DCCR results for fermentation time, consumption of total reducing sugars (TRS concentration at the beginning minus the end), acid production (total acidity at the end minus the beginning), cell growth (count at the end minus count at the beginning of fermentation) are presented in Table 2.

Table 2 – CCRD results for the analyzed variables.

Treatment	TRS Consumption (g/L)	Acid production (g/L)	Cell growth (log CFU/mL)	Fermentation time (h)
1	13.1	8.25	2.17	13
2	21.6	10.25	1.98	13
3	21	5.4	2.84	15
4	20.7	6.3	2.24	15
5	15.5	3.9	2.34	16
6	30.3	3.9	2.26	16
7	24	0.35	1.66	11
8	22.6	0.24	1.56	11
9	20	4.5	2.37	15
10	14.6	4.5	2.2	15
11	12.3	4.5	2.21	15
12	18.2	4.5	2.0	15

Results were analyzed using STATISTICA 8.0 software[7]. The consumption of total reducing sugars (TRS) was influenced by the content of arrowroot extract, so that the higher the arrowroot extract content in the fermentation medium, the greater the consumption of total sugars by bifidobacteria. In treatments 1 and 2, the highest productions of organic acids were observed, 8.25 g/L and 10.35 g/L, respectively. In treatments T2, T7 and T8, the lowest cell growth values were obtained, while T3 (30%v/v arrowroot extract and 45%v/v peanut extract) showed the highest cell growth values (2.84 log CFU/mL). It was observed that none of the variables (arrowroot extract and peanut extract) had statistically significant effects at 5% significance on the production

of organic acids and cell growth. In relation to fermentation time, the quadratic term for peanut extract showed a statistically significant effect, in the sense that at the extremes of the PE levels, the time tends to be shorter.

4 CONCLUSION

It was concluded that the consumption of total reducing sugars was influenced by the arrowroot extract content, increasing according to the concentration of this extract. However, none of the arrowroot or peanut extract contents significantly affected the production of organic acids or the cell growth of bifidobacteria. The fermentation medium with 30%v/v arrowroot extract and 45%v/v peanut extract showed the highest cell growth, around 2.8 CFU/mL. As for fermentation time, the peanut extract content significantly influenced it, with shorter times at the extremes of the contents. Therefore, it was concluded that bifidobacteria grow and ferment in media containing arrowroot and peanut extract, with the medium containing 30%v/v of arrowroot extract and 45%v/v of peanut extract being recommended for the production of a fermented beverage probiotic.

REFERENCES

1 JAYAKUMAR, A., SUGANTHI, A. 2017. Int.J.R.Pharm.Pharmac.Sci. 2 (3). 26-30.
2 AMANTE, P. R., SANTOS, E. C. Z., CORREIA, V. T. DA V., FANTE, C. A. 2020. J. Culin. Sci. Technol. *19* (6), 513–521.
3 UTAMI, T., GIYARTO, DJAAFAR, T.F., RAHAYU, E.S. 2014. Indones. Food and Nutr. Prog. 13 (1), 11-16.
4 BEUCHAT, L. R., NAIL, B. J. 1978. J. Food Sci. 43 (4), 1109-1112.
5 MILLER G.L 1959. Anal Chem 31:426–428.
6 MONDRAGÓN-BERNAL, O. L.; MAUGERI, F. F.; ALVES, J. G. L. F.; RODRIGUES, M. I. 2012. Symbiotic Soy Beverages: Principles and Sensory Attributes. In: Handbook of Plant-Based Fermented Food and Beverage Technology. HUI, Y. H.; EVRANUZ, E. Ö. (eds.) 2nd ed. Flórida: CRC Press.
7 STATISTICA ANALYSIS. 2010. Data Analysis Software System 10.0. Stat-Soft, Tulsa.

ACKNOWLEDGEMENTS

The authors would like to thank FAPEMIG for financing the project, CNPq for the Scientific Initiation scholarship and the Postgraduate Program in Food Engineering (PPGEAL)/UFLA.