Evaluation of gama-aminobutyric (GABA) production on plant based beverages using kefir cultures

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ABSTRACT

Plant extracts from peanuts and Brazil nuts were used to produce vegetable kefir drinks with bioactive potential. The results indicate that commercial Kefir culture was able produce kefir beverage and GABA via fermentation, in different substrates. The composition of plant extracts was essential to increase GABA productivity, as well as inulin, glutamic acid.

Keywords: Kefir; plant based; fermented beverages

1 INTRODUCTION

Kefir is a fermented drink, traditionally produced from the milk fermentation, provides a consortium of yeasts and bacteria (lactic acid bacteria and acetic acid bacteria) that live in symbiosis and can promote a set of biological actions, in addition to providing probiotics[1]. The fermentation of milk by lactic acid bacteria induces the production of a set of bioactive compounds, including the sometimes, gamma-aminobutyric acid (GABA). GABA is one of the main neurotransmitters of the central nervous system and has several biological activities reported, having antihypertensive action, antidiabetic effects, among others[2,3]. Microorganisms, including lactic acid bacteria and fungi, have the ability to synthesize GABA during the fermentation process of food matrices, depending on the substrate[4].

Kefir grains are capable of fermenting different non-dairy substrates such as fruit juices, and plant extracts[5,6]. In this scenario, the demand for products naturally enriched in GABA has increased and kefir, as it is drink rich in lactic acid bacteria, can be an interesting source of this bioactive compound. The objective of this study was to evaluate the effect of plant extract and addition of glutamic acid on GABA production on vegetable fermented beverages by kefir culture.

2 MATERIAL & METHODS

The water-soluble extracts of peanut and Brazil nut were produced according to the method described by from Lopes[7]. Both water-soluble plant extracts were pasteurized (90 °C/3 min), packed and stored in a freezer (-20 °C) for later use.

The drinks were prepared by inoculating 5% (w/v) kefir grains (eXact® Kefir 1 culture, Christian Hansen) in 50 g of water-soluble plant extracts. Fermentation took place at 25 ± 1 °C for 24 hours. 4 formulations were developed with 0.26% xanthan gum and 3% inulin, two of which were added 0.5% glutamic acid, based on the literature previous results[8,9].

The determination of the presence of GABA was carried out qualitatively, using the paper chromatography (PC) method[10], as previously described, with modifications. Solutions of glutamic acid 0.5% (w/v) and gamma-aminobutyric acid 0.05% (w/v) were used as analysis standards.

3 RESULTS & DISCUSSION

The addition of glutamic acid in the formulations was carried out aiming of enhance the production of GABA via fermentation by bacteria, since glutamic acid, as well as monosodium glutamate, is one of the main substrates to produce GABA[11,12]. Results are illustrated in Figure 1.

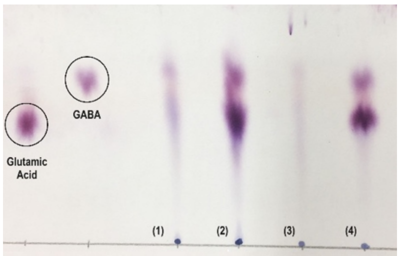

Figure 1 Chromatogram of vegetable kefir drinks fermented with commercial culture.

Glutamic acid: 0.5% glutamic acid solution; GABA: 0.05% gamma-aminobutyric acid solution; 1: WSP-CK formulation (WSP: water-soluble peanut extract, commercial kefir grains); 2: WSP-CKGA formulation (WSP, inulin, glutamic acid and commercial kefir grains); 3: WSBN-CK formulation (WSBN: water-soluble extract of Brazil nuts, commercial kefir grains); 4: WSBN-CKGA formulation (WSBN, inulin, glutamic acid and commercial kefir grains).

The chromatogram clearly showed the presence of GABA in the WSP-CKGA and WSBN-CKGA formulations, which commonly have the addition of glutamic acid in the formulation. Comparing WSP-CK and WSBN-CK (both without glutamic acid addition), the presence of GABA is more evident in the peanut-based formulation than in Brazil nut-based formulation. Apparently, peanut is better substrate for GABA production than Brazil nut, although the fresh Brazil nut has a higher glutamic acid content than raw peanuts (17.7% in Brazil nut against 5,39% in peanut)[14].

The identification of the presence of GABA in the WSP-CKGA and WSBN-CKGA formulations suggests that the microbial culture of commercial kefir has the capacity to produce GABA and that the addition of glutamic acid (the main substrate for GABA production) provided favorable conditions to produce this postbiotic during the fermentation of the beverages. The addition of 0.5% glutamic acid to vegetable kefir formulations resulted in higher GABA production than formulations fermented with the commercial culture without the addition of glutamic acid (Figure 1). As this is a qualitative determination, the concentration of glutamic acid was not calculated, however, studies suggest that GABA production may increase due to the presence of glutamic acid, which can be observed in the chromatogram above. Researchers developed GABA-enriched yogurt by fermenting a mixture of strains (Levilactobacillus brevis CGMCC1.5954, Streptococcus thermophilus ABT-T and Lactobacillus delbrueckii ssp. bulgaricus BNCC 336436) in a ratio of 3:1:1 with the addition of 0.1% of monosodium glutamate. The product contained 147.36 mg/100 mL of GABA, a content approximately 317% higher than that found in the control drink (produced only with the starter culture)[14]. The incorporation of monosodium glutamate also influenced the GABA content in yogurt, the authors varied the glutamate concentrations between 6 and 12 g/L and identified that the addition of 12 g/L resulted in a higher GABA content, in addition, factors such as Temperature and fermentation time also influenced the results found[15].

The microbial biosynthesis of GABA occurs through the decarboxylation of glutamate or its salts, through the enzyme glutamate decarboxylase (GAD)[12]. The substrate, fermentation conditions, in addition to the species of lactic acid bacteria used, directly influence the yield of GABA. It is clear that the commercial kefir microbiota used in this study (*Debaryomyces hansenii*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. lactis *biovar. diacetylactis*, *Leuconostoc* e *Streptococcus thermophilus*) proved to be effective in the production of GABA during the fermentation of plant extracts (water-soluble peanut extract and water-soluble Brazil nut extract) with the addition of glutamic acid.

4 CONCLUSION

This study demonstrated that it is possible to use plant extracts from peanuts and Brazil nuts to produce kefir drinks with bioactive potential. The results indicate that the incorporation of glutamic acid in the formulations enhanced the production of GABA via fermentation. Studies are needed to optimize the concentration of glutamic acid to increase the yield of GABA in beverages, as well as in vivo studies to prove the health effects, as well as the viability of GABA.

REFERENCES

1 COSTA, N. M. B., & ROSA, C. de O. B. Alimentos Funcionais: Componentes Bioativos e Efeitos Fisiológicos (2nd ed.). Editora Rubio.
2 NEJATI, F.; RIZZELLO, C. G.; CAGNO, R., SHEIKH-ZEINODDIN, M., DIVICCARO, A., MINERVINI, F., GOBBETTI, M. LWT. 51 (1). 183–189.
3 UNTEREINER, A., ABDO, S., BHATTACHARJEE, A., GOHIL, H., POURASGARI, F., IBEH, N., LAI, M., BATCHULUUN, B., WONG, A., KHUU, N. FASEB J. 33 (3) 3968-3984.
4 NIKMARAM, N., DAR, B. N., ROOHINEJAD, S., KOUBAA, M., BARBA, F. J., GREINER, S., JOHNSON, S. K. J. Sci. Food Agric. 97 (9) 2681-2689.
5 GOCER, E. M. C., KOPTAGEL, E. Food Chem. 402. 134252.
6 MENDES, R. M. L., ANDRADE, R. H. C., MARQUES, M. F. F., ANDRADE, E. R. Food Biosci. 39. 100809.
7 Lopes, G. A. Z. Caracterização química, física e sensorial de produtos à base de amendoim. Universidade Estadual Paulista (UNESP).
8 ALVES, V., SCAPINI, T., CAMARGO, A. F., BONATTO, C., STEFANSKI, F. S., JESUS, E. P. de., DINIZ, L. G. T., BERTAN, L. C., MALDONADO, R. R., TREICHEL, H. LWT. 145. 111364.
9 KANTACHOTE, D., RATANABUREE, A., HAYISAMA-AE, WE., SUKHOOM, A., NUNKAEW, T. J. Funct. Foods. 32. 401-408.
10 LI, H., QIU, T., CAO, Y., YANG, J., HUANG, Z. J. Chromatogr. A. 1216 (25) 5057-5060.
11 HASEGAWA, M., YAMANE, D., FUNATO, K., YOSHIDA, A., SAMBONGI, Y. J. Biosci. Bioeng. 125 (3) 316-319.
12 YOGESWARA, I. B. A., MANEERAT, S., HALTRICH, D. Microorganisms. 8 (2) 1923.
13 TAHERI, S.E. H., BAZARGAN, M., VOSOUGH, P. R., SADEGHIAN, A. J. Food Compos. Anal. 125. 105770.
14 FAN, X., YU, L., SHI, Z., LI, C., ZENG, X., WU, Z., PAN, D. J. Of Dairy Science. 106 (2) 852-867.
15 CHEN, L., ALCAZAR, J., YANG, T., LU, Z., LU, Y. J. Of Dairy Science. 101 (12) 10685-10693.

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