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FOOD TECHNOLOGY

Techno-functionalities and antioxidant capacity of chickpea flour fermented by *Lacticaseibacillus casei* or by co-culture (*Lactobacillus acidophilus, Bifidobacterium*, and *Streptococcus thermophilus*)

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ABSTRACT: Chickpea (*Cicer arietinum* L.; GB) is one of the most consumed pulses worldwide. Fermentation with lactic acid bacteria (LAB) has been a strategy to improve the nutritional and technological quality of chickpea flours, in addition to reducing the levels of antinutritional factors. Thus, this study evaluated the effect of fermentation with *Lacticaseibacillus casei* or co-culture on water activity (Aw), color, antioxidant capacity, and techno-functional properties of chickpea flour. Chickpeas were fermented at 28 °C for 96 h with *Lactobacillus Casei* or co-culture, resulting in the samples GB_{LC} and GB_{CC}, respectively, dried at 50 °C, and ground to obtain the flours. Fermentation reduced ($P < 0.05$) the Wa and color parameters (L^*, a^*, and b^*) of the fermented flours (GB_{CC} and GB_{LC}). In terms of techno-functional properties, GB_{CC} flours showed lower emulsifying capacity (EC, 80%) and emulsion stability (ES, 82.50%). GB_{LC} and GB_{CC} flours showed significant differences ($P < 0.05$) in swelling power only at 25 °C and a reduction in water solubility index (WSI) at all studied temperatures. LAB fermentation reduced the antioxidant capacity of GB_{LC} e GB_{CC} flours determined by the DPPH method, while in the FRAP method, there was an increase for GB_{CC} and a reduction for GB_{LC}. For the total phenolic content (TPC), there was an increase of 231% for GB_{CC} flour and 164% for GB_{LC} flour. Thus, it was concluded that the fermentation with *Lactobacillus Casei* and co-culture affected the Wa, color, EC, ES, WSI, the antioxidant capacity by the DPPH and FRAP assays, and the TPC of the fermented flours.
Key words: Legumes, plant based, lactic acid bacteria, bioprocessing.

Tecno-funcionalidades e capacidade antioxidante de farinha de grão-de-bico fermentada por *Lacticaseibacillus casei* ou por co-cultura (*Lactobacillus acidophilus, Bifidobacterium* e *Streptococcus thermophilus*)

RESUMO: O grão-de-bico (*Cicer arietinum* L.; GB) é um dos *pulses* mais consumidos no mundo. A fermentação com bactérias ácido-láticas (BAL) é uma estratégia eficaz para potencializar a qualidade nutricional e tecnológica de farinhas de GB, além de reduzir os teores de fatores antinutricionais. Desta forma, o objetivo do presente trabalho foi avaliar o efeito da fermentação com *Lactobacillus casei* ou por co-cultura na capacidade antioxidante e nas propriedades tecno-funcionais de farinha de GB. O GB foi fermentado a 28 °C por 96 h com *Lactobacillus Casei* ou a co-cultura, resultando nas amostras GB_{LC} e GB_{CC}, respectivamente, seco a 50 °C e moído para obtenção das farinhas. A fermentação reduziu a Aw e os parâmetros de cor (L^*, a^* e b^*) das farinhas fermentadas (GB_{CC} e GB_{LC}). Nas propriedades tecno-funcionais, a farinha GB_{CC} apresentou menor capacidade emulsificante (CE, 80%) e estabilidade da emulsão (EE, 82,50%). As farinhas GB_{LC} e GB_{CC} apresentaram diferenças significativas no poder de inchamento apenas a 25 °C e redução no índice de solubilidade em água (ISA) em todas as temperaturas estudadas. A fermentação com BAL reduziu a capacidade antioxidante das farinhas GB_{LC} e GB_{CC} determinada pelo método DPPH, já no método FRAP houve um aumento para GB_{CC} e uma redução para GB_{LC}. Quanto ao teor de fenólicos totais (TFT), observou-se um aumento de 231% da farinha de GB_{CC} e 164% da farinha de GB_{LC}. Assim, conclui-se que a fermentação com *Lactobacillus Casei* e co-cultura afetou a Aw, a cor, CE, EE , ISA, a capacidade antioxidante pelos ensaios de DPPH e FRAP e o TFT das farinhas fermentadas.
Palavras-chave: Leguminosas, *plant based*, bactérias ácido-láticas, bioprocessamento.

INTRODUCTION

In 2018, the Ministry of Agriculture, Livestock, and Supply of Brazil launched the National Plan for the Development of the Production Chain of Beans and Pulses, which has as its main objective the increase in the consumption and production of beans and diversified varieties of pulses (lentils, chickpeas, and peas) to serve the internal and external market (BRASIL, 2018). Chickpea (*Cicer arietinum* L.) is one of the most consumed pulses worldwide, in the form of cooked whole grain, flour, or as an ingredient in baked goods or pasta (FAO, 2019). It is a low-cost protein source that has high nutritional quality, containing

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amino acids, such as lysine, tyrosine, glutamic acid, and histidine, in addition to being an excellent source of complex carbohydrates and dietary fiber, with low levels of lipids and sodium (KAUR & PRASAD, 2021; KLONGKLAEW et al., 2022).

However, like most legumes, chickpea has consumption limitations due to the presence of antinutritional factors, such as trypsin inhibitors and oligosaccharides, like raffinose, stachyose, and verbascosis (AISA et al., 2019). In this sense, fermentation using lactic acid bacteria is an effective strategy in the bioprocessing of these legumes, enhancing their functional, nutritional, and technological qualities, in addition to contributing to the reduction of antinutritional factors (SARKAR et al., 2020). Additionally, fermentation increases the retention of total phenolic compounds and, consequently, the antioxidant capacity of chickpea flour. Moreover, it provides improved technological properties, such as water and oil absorption capacities and emulsifying potential (SENANAYAKE et al., 2020).

Despite the potential of this technique, many studies have focused on the effect of the fermentation type, conditions, and microorganisms that can be used aiming at the nutritional improvement of legumes (KAUR & PRASAD, 2021), and information on the effects of fermentation by *Lacticaseibacillus casei* or by co-culture (*Lactobacillus acidophilus*, *Bifidobacterium*, and *Streptococcus thermophilus*) on the antioxidant capacity and in the technological properties of chickpea flour are scarce. Thus, the present research studied the changes in the techno-functionalities and antioxidant capacity of chickpea flour fermented with *Lacticaseibacillus casei* or with co-culture.

MATERIALS AND METHODS

Materials

Chickpea and commercial co-culture (*Lactobacillus acidophillus* LA5® – 6 log CFU g^{-1}; *Bifidobacterium* BB12® – 6 log CFU g^{-1}; *Streptococcus termophilus* – 6 log CFU g^{-1}) (BioRich, Chr. Hansen, Valinhos, São Paulo, Brazil) were purchased in local stores from Seropédica, Rio de Janeiro, Brazil. The lyophilized culture of *Lacticaseibacillus casei* (Lyofast BGP93) was provided by SACCO Brazil (São Paulo, Brazil).

Preparation of chickpea flours fermented with Lacticaseibacillus casei or with co-culture

Chickpea (GB) samples were washed in running water and submerged in distilled water for 12 h, in the proportion of 500 g of GB per 2 liters of distilled water (Figure 1). Then, the water was discarded and replaced using a 1:1 ratio (GB:water), followed by sterilization in an autoclave at 121 °C for 15 minutes. After this step, GB was cooled to room temperature (25 °C) for 5 h. The GB was separated into 2 portions of 100 g. *Lacticaseibacillus Casei* (GB_{LC}) at a concentration of 0.2 g L^{-1} (9 log CFU g^{-1}) was added to the first portion, while commercial co-culture (GB_{CC}) at a concentration of 0.4 g L^{-1} was added to the second portion. Both additions were performed according to the manufacturer's specifications. The GB_{LC} and GB_{CC} samples were submitted to submerged fermentation in an incubator at 28 °C for 96 h, following the methodology proposed by XIAO et al. (2015), where the sterilized and non-fermented chickpea (GB_C) was used as control. Subsequently, fermented (GB_{LC} and GB_{CC}) and unfermented (GB_C) grains were pressed into sterile polyester fabric to remove excess water.

For the flour production (Figure 1), the fermented grains (GB_{LC} and GB_{CC}) and the control were subjected to drying in a dryer (Microfan E15CD) at 50 °C until constant weight (after 5 h of drying). The fermented and dried grains were ground in a knife mill for 40 seconds (IKA A11BS032, Staufen, Germany) and sieved using a 710 µm-sieve, obtaining 3 flours: GB_{LC} (GB fermented with *Lacticaseibacillus casei*), GB_{CC} (GB fermented with co-culture), and GB_C (control sample – not fermented). The flours obtained were stored in hermetically sealed glass jars and conditioned under refrigeration at 4 °C protected from light for approximately 4 hours, after which the analyses were performed.

Determinantion of water activity and instrumental color analysis of chickpea flours

Water activity (Aw) was determined using a digital dew point hygrometer (Aqualab CX2, Decagon Devices Inc., USA). The instrumental color analysis was performed using the MiniScan EZ 4500L colorimeter (HunterLab, Virginia, USA). The CIELab parameters L^*, a^*, and b^* and the total color difference (ΔE) magnitude values were determined for GB_{CC} and GB_{LC} in comparison with GB_C. Values of L^*, a^*, b^*, and ΔE were obtained according to the methodology of CHEKDID et al. (2021).

Techno-functional properties of chickpea flours

To determine the oil absorption index (OAI) of flours (GB_{LC}, GB_{CC}, and GB_C), the methodology of POIANI & MONTANUCI (2019) was used. The sample (2 g) was mixed with 13 mL of commercial corn oil, homogenized in a vortex at 100 rpm for 30 minutes at 25 °C, and centrifuged at 3,000 rpm for

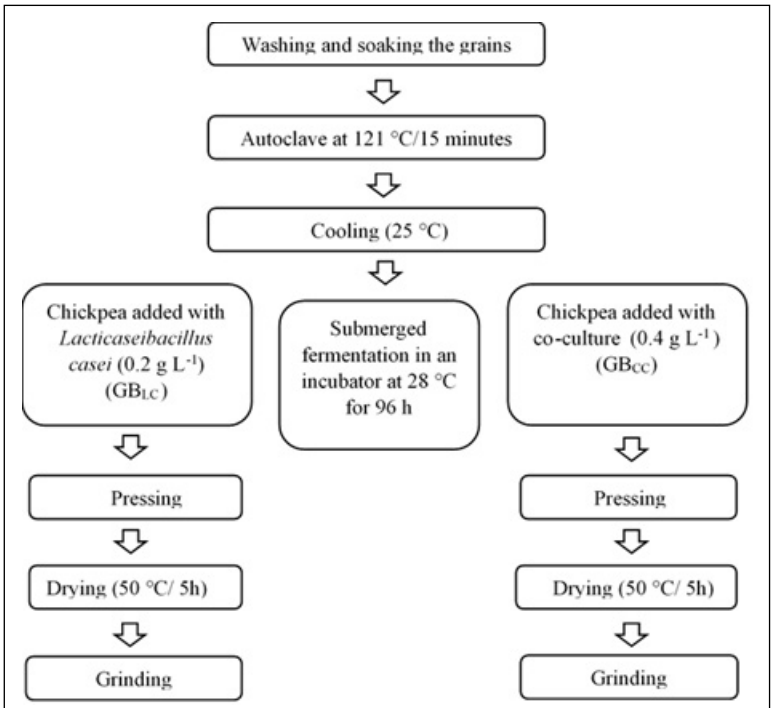

Figure 1 - Flowchart of procedures for chickpea fermentation and flour production.

20 minutes. The supernatants were discarded and the oily sediments were weighed.

The emulsifying capacity (EC) (%) and emulsion stability (ES) (%) of GB_{LC}, GB_{CC}, and GB_{C} flours were determined according to YASUMATSU et al. (1972). The sample (3.75 g) was mixed with 25 mL of distilled water and 25 mL of corn oil. Then, the mixture was emulsified using an agitator at 10,000 rpm for 10 minutes (Model 936 Drink Mixer, Scovill, USA). The emulsion obtained was distributed in centrifuge tubes (15 mL), which were centrifuged at 3,000 rpm for 10 minutes and heated to 80 °C for 30 minutes in a water bath with agitation. The ES (%) was calculated according to equation 1.

$$ES\ (\%) = \left(\frac{REL}{EL}\right) \times 100 \quad (1)$$

Where REL is the remaining emulsified layer (mL) and EL is the emulsified layer (mL).

After determining the ES, the tubes were again centrifuged at 3,000 rpm for 10 minutes to assess the EC (%), which was calculated according to equation 2.

$$EC\ (\%) = \left(\frac{EL}{TL}\right) \times 100 \quad (2)$$

Where EL is the emulsified layer (mL) and TL is the total layer (mL).

The swelling power (SP) was determined according to ROCHA et al. (2008) and KUSUMAYANTI et al. (2015). The water solubility index (WSI) was determined according to KUSUMAYANTI et al. (2015). The samples for SP and WSI determination were weighed (0.1 g) in centrifuge tubes and dispersed in 10 milliliters of distilled water, followed by homogenization and heating at different temperatures (25 °C, 50 °C, 60 °C, 70 °C, 80 °C, and 90 °C), in a water bath for 30 minutes. After cooling, the tubes were centrifuged at 3,000 rpm for 20 minutes, and the supernatant was collected and dried in an oven at 105 °C for 24 hours. The SP, in g/g starch, was calculated using equation 3, and the WSI (%) was determined using equation 4:

$$SP\ (g/g) = \left(\frac{SW}{DSW}\right) \quad (3)$$

$$WSI\ (\%) = \left(\frac{DSUW}{SW}\right) \times 100 \quad (4)$$

Where SW is the sediment weight, DSW is the dry starch weight, SW is the sample weight, and DSUW is the dry supernatant weight.

Antioxidant capacity and total level of phenolic compounds of flours

To determine the antioxidant capacity of flours, extracts were prepared using 2 g of sample and

20 mL of a 70% v/v methanolic solution. The mixture was homogenized and stirred for 12 h at 100 rpm. The extract was filtered and stored under refrigeration (4 °C). The DPPH radical scavenging assay was based on the methodology described by RUFINO et al. (2010). Trolox was used to construct a standard curve (from 5 to 70 µg/mL). The extract (150 µL) was added to 2.85 mL of DPPH solution and allowed to react for 1 h. Absorbances were measured at 517 nm in a spectrophotometer.

The antioxidant capacity was also determined by the Ferric Reducing Antioxidant Power (FRAP) assay according to THAIPONG et al. (2006), using Trolox as standard. The extract (90 µL) was added to 270 µL of distilled water, 2.7 mL of FRAP reagent, and 2.5 mL of a 20 mM aqueous solution of ferric chloride. The mixture was incubated at 37°C under agitation for 30 minutes. After cooling, the absorbances were measured at 595 nm in a spectrophotometer.

The contents of total phenolic compounds were determined by the method of RUFINO et al. (2010), with minor modifications. Gallic acid was used as a reference to create a standard curve. The extract (1 mL) was added to 1 mL of Folin-Ciocalteau aqueous solution (1:10), 1 mL of 70% methanol, and 1 mL of Na_2CO_3 aqueous solution. The mixture was left to rest for 2 h in the dark. Absorbances were measured at 725 nm with a spectrophotometer and the results were expressed in mg gallic acid equivalent per gram of sample (mg GAE/g).

STATISTICAL ANALYSIS

The analyses were performed in triplicate. Results were expressed as mean ± standard deviation. Data were analyzed using the STATISTICA 7® software. Analysis of variance (ANOVA) was performed and the means were compared using the Tukey's test, considering a significance level of 5% ($P < 0.05$).

RESULTS AND DISCUSSION

The GB_{CC} and GB_{LC} flours showed lower Aw ($P < 0.05$) when compared to the control (Table 1), indicating that the biochemical modifications that occurred during the fermentation process contributed to changes in the legume structure that facilitated the removal of water during drying. Similar behavior was observed in cassava flour fermented with *Lactiplantibacillus plantarum*, which had an Aw of 0.471, while the control (without fermentation) had 0.647 (WANG et al., 2019).

Aw is a fundamental parameter for food stability and quality control (CHISTÉ et al., 2006). Its reduction is important to inhibit the proliferation of undesirable microorganisms, maintain the chemical stability of food, and minimize non-enzymatic browning reactions, lipid oxidation reactions, and microorganism growth (ISA et al., 2021). According to CHISTÉ et al. (2006), Aw below 0.60 is the minimum limit capable of inhibiting the development of microorganisms. Thus, all chickpea flours of this study (Table 1) showed Aw values considered unfavorable for the development of bacteria and fungi.

The optical properties of fermented flours were evaluated due to the role of ingredient color in food formulations. When compared to the control (GB_C), fermentation with *Lacticaseibacillus casei* or co-culture significantly affected the color of chickpea flours. In GB_{CC} flour, the luminosity (L^*) increased by around 4% ($P < 0.05$), indicating a lighter color compared to the control (Table 1). However, a reduction of L^* was observed for GB_{LC} flour, suggesting that fermentation with *Lacticaseibacillus casei* slightly darkened the sample. The fermentation promoted a reduction of 16% for GB_{CC} flour and 30% for GB_{LC} flour of the chromatic coordinate a^* compared to the control. Otherwise, the reduction was less pronounced for the chromatic coordinate b^*, with approximately 12% for GB_{CC} flour and 1.25% for GB_{LC}.

Table 1 - Water activity (Aw), color parameters (L^*, a^*, and b^*) and total color difference (ΔE) of chickpea flour samples: unfermented (GB_C), fermented with co-culture (GB_{CC}), and fermented with *Lacticaseibacillus casei* (GB_{LC}).

Sample	Wa^*	L^*	a^*	b^*	ΔE^*
GB_C	$0.333^a \pm 0.002$	$64.50^a \pm 0.27$	$3.06^a \pm 0.05$	$27.15^a \pm 0.14$	-
GB_{CC}	$0.134^b \pm 0.001$	$67.15^b \pm 0.13$	$2.41^b \pm 0.03$	$23.82^b \pm 0.06$	$4.30^a \pm 0.03$
GB_{LC}	$0.207^c \pm 0.002$	$63.12^c \pm 0.13$	$2.55^c \pm 0.01$	$26.81^c \pm 0.07$	$1.50^b \pm 0.01$

Different letters in the same column indicate significant differences ($P < 0.05$).

The changes observed in the color parameters L^*, a^*, and b^* impacted the total color difference (ΔE), where the GB_{CC} flour samples were more affected than the control (Table 1). Despite the difference in ΔE presented by the fermented flours and the control, it can be considered that the color changes were not perceptible by the human eye, since according to WITZEL et al. (1973), this only occurs for ΔE values higher than 5.

To evaluate the techno-functionalities of flours, the OAI, EC, ES, SP, and WSI were determined (Table 2 and Figures 2 A and B). The OAI of flours was not affected by fermentation with *Lacticaseibacillus casei* or by co-culture. Conversely, CHANDRA-HIOE et al. (2016) reported that fermentation increased the OAI in the range of 37 to 42% for two chickpea cultivars fermented with co-culture (*Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophillus*) for 16 h at 30 °C, compared to the control. Generally, the OAI is associated with the interaction between the lipid fraction and hydrophobic amino acids. Thus, the increase in oil absorption capacity in fermented samples can be attributed to the higher availability of hydrophobic residues of protein molecules caused by the fermentation process (ELKHALIFA & BERNHARD, 2010).

Regarding the EC analysis, the GB_{CC} flour showed a lower capacity to form an emulsion, presenting a reduction of 11% in the EC compared to the control flour. Lower ES values were also determined for GB_{CC} and GB_{LC} flours in comparison to GB_C, with a significant difference between them. EC (%) is defined as the volume of oil that can be emulsified by the protein or peptides present in flours before phase inversion or emulsion collapse occurs (ARTEAGA et al., 2021), while ES (%) is an important technological parameter as it is linked to the prevention of oil droplet coalescence, flocculation, and cream formation (ENUJIUGHA et al., 2003).

Similar behavior was reported by ARTEAGA et al. (2021), who observed that the EC of pea protein decreased significantly after fermentation using six different strains of lactic acid bacteria (*Lactiplantibacillus plantarum*, *Lactobacillus perolens*, *Limosilactobacillus fermentum*, *Lacticaseibacillus casei*, *Cremoris de leuconostoc mesenteroides subsp*., and *Pediococcus pentosaceus*), and PEI et al. (2022), who reported a significant reduction in the emulsifying properties of flours obtained by peas fermented with *Lacticaseibacillus rhamnosus*.

Vegetable proteins have functional properties that make them suitable for the formulation of new ingredients and products, such as gluten-free or protein-enriched products. These properties depend on the structural chain of proteins and peptides, as well as the interaction with other molecules, like lipids, carbohydrates, and water. In addition, antinutritional factors can form insoluble complexes with proteins, reducing the availability of these nutrients, and consequently affecting some technological properties (MORA-UZETA et al., 2020).

The SP of flours was affected ($P < 0.05$) by the increase in temperature (Figure 2 A). The SP measures the hydration capacity of starch granules of flours during heating (RONKO et al., 2021). Significant changes in the SP ($P < 0.05$) were determined only when analyses were performed with heating at 25 °C and 60 °C for flours fermented with *Lacticaseibacillus casei* or with co-culture (GB_{LC} and GB_{CC}), compared to the control (Figure 2 A). Therefore, fermentation promoted an increase in the SP of GB_{CC} flour, while a reduction of this parameter was noticed in GB_{LC} flours at 60 °C (Figure 2 A).

The WSI (Figure 2 B) of chickpea flours was also affected ($P < 0.05$) by the increase in temperature. The fermented flours GB_{CC} and GB_{LC} showed a lower percentage of WSI compared to the non-fermented flours (GB_C) at all temperatures studied, indicating

Table 2 - Oil absorption index (OAI). emulsifying capacity (EC), and emulsion stability (ES) of chickpea flour samples: unfermented (GB_C), fermented with co-culture (GB_{CC}), and fermented with *Lacticaseibacillus casei* (GB_{LC}).

Sample	OAI (g/g)	EC (%)	ES (%)
GB_C	$4.37^a \pm 0.05$	$90.00^a \pm 0.01$	$90.00^a \pm 0.03$
GB_{CC}	$4.38^a \pm 0.06$	$80.00^b \pm 4.08$	$82.50^b \pm 2.50$
GB_{LC}	$4.43^a \pm 0.05$	$90.00^a \pm 0.05$	$88.33^c \pm 2.36$

Different letters in the same column indicate significant differences ($P < 0.05$).

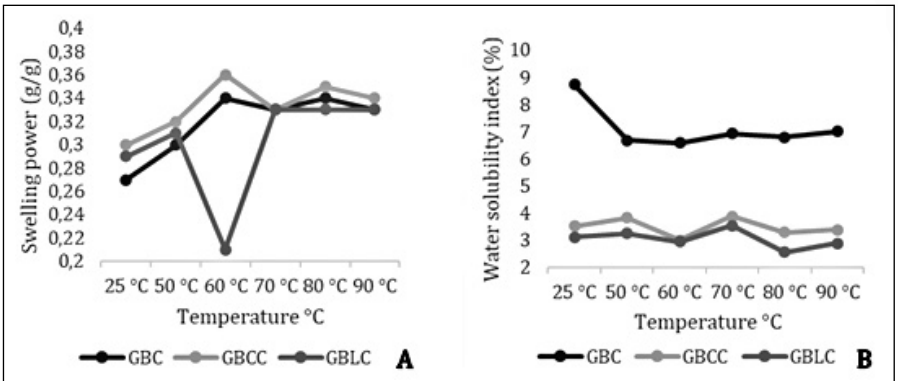

Figure 2 - Swelling power (SP) (A) and water solubility index (WSI) (B) of chickpea flour samples: unfermented (GB_C), fermented with co-culture (GB_{CC}), and fermented with *Lacticaseibacillus Casei* (GB_{LC}).

that fermentation may have led to a reduction in the number of hydrophilic groups available to interact with water molecules, contributing to a decrease in this property (REYES-BASTIDAS et al., 2010). This result is compatible with those reported for common bean flour (*Phaseolus vulgaris* L.) fermented in solid state (*Rhizopus oligosporus*) at 34 °C for 51 h, where a 44% increase in the SP and a 28% reduction in the WSI were observed in fermented flours compared to the control (REYES-BASTIDAS et al., 2010).

Fermentation significantly affected ($P < 0.05$) the antioxidant capacity of samples regarding both methods applied (DPPH and FRAP). For the DPPH assay, a significant reduction in the antioxidant capacity was determined for GB_{CC} and GB_{LC} compared to the control. As for the FRAP assays (Table 3), fermentation with *Lacticaseibacillus casei* increased the antioxidant capacity of GB_{LC} flour compared to the control. This same behavior was not observed for the flour samples fermented with co-culture (GB_{CC}), which showed lower ($P < 0.05$) antioxidant capacity compared to the control (GB_C). The differences in the antioxidant properties of flour samples after fermentation may be linked to qualitative and quantitative variations in the enzymatic activities with different strains of *Lactobacillus*, as well as in the fermentation time, since the fermentation process with the use of lactic acid bacteria can alter the enzymatic activity, which can promote an increase or reduction in the release of antioxidant compounds (LI & WANG, 2021).

For the content of total phenolic compounds, a significant increase was observed in GB_{CC} (231%) and GB_{LC} (164%) flour compared to the control (Table 3). This result may be attributed to the greater release of phenolic compounds from the plant cell wall due to the increased activity of protease enzymes that occurs during the fermentation process (GARRIDO-GALAND et al., 2021). It agrees with the behavior reported in a study conducted with 4 different varieties of fermented chickpeas, in which the phenolic content was significantly higher ($P < 0.05$) after 72 h of fermentation (KLONGKLAEW, et al., 2022).

Table 3 - Antioxidant capacity (DPPH, FRAP, and total phenolic compounds) of chickpea flour samples: unfermented (GB_C), fermented with co-culture (GB_{CC}), and fermented with *Lacticaseibacillus casei* (GB_{LC}).

Sample	DPPH (µmol TE/g)	FRAP (µmol TE/g)	Phenolic compounds (mg GAE/g)
GB_C	$126.68^{a} \pm 0.01$	$29.14^{a} \pm 0.01$	$4.97^{a} \pm 0.01$
GB_{CC}	$118.29^{ab} \pm 0.02$	$19.97^{b} \pm 0.01$	$16.45^{b} \pm 0.03$
GB_{LC}	$114.44^{b} \pm 0.03$	$35.97^{c} \pm 0.01$	$13.12^{b} \pm 0.01$

Different letters in the same column indicate significant differences ($P < 0.05$).

CONCLUSION

In general, the fermentation techniques studied (*Lacticaseibacillus casei* or with co-culture) proved to be feasible to promote improvements in the techno-functional properties and antioxidant capacity of chickpea flours. Moreover, both fermented flours (GB_{CC} and GB_{LC}) showed lower water activity compared to non-fermented flour. Fermentation with co-culture resulted in flours with lighter colors and higher content of total phenolic compounds; however, with lower emulsifying capacity and emulsion stability, compared to those fermented only with *Lacticaseibacillus casei*, highlighting the importance of selecting the microorganisms that will perform the fermentation. Indeed, studies aiming at optimizing the fermentation time and the selection of strains are important research areas for future legume-based fermented products.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors critically revised the manuscript and approved of the final version.

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