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Fermentation of the *Cucurbita ficifolia* Fruit Juice: Its Antioxidant Activity and Effects on the Glycemia

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Abstract: *Cucurbita ficifolia* is an edible plant whose fruits have hypoglycemic, anti-inflammatory, and antioxidant activities. Fermentation might improve these properties. This research aims to perform and characterize its fermentation in native and induced conditions with *Lactobacillus plantarum* (*Lp*) and evaluate its antioxidant activity and effect on glycemia. Fresh juice from mature fruits was characterized. One portion of this juice was spontaneously left to ferment (native fermentation), and the other was inoculated with *Lp* (controlled fermentation). Fermentation was monitored each 8 h by 56 h to measure microbial growth, pH, acidity, sugars, soluble protein, polyphenols and flavonoids, antioxidant activity, and effects on glycemia. In native fermentation, the growth of total microorganisms increased up to 32 h, decreasing at the end of the process. In *Lp* fermentation, total microorganisms increased until 16 h to stay constant at the end, with a predominance of *Lp*. The pH and the sugars decreased in the two fermentations, while polyphenol and flavonoid increased. In spontaneous fermentation, these changes were lesser. Both fermentations, like fresh juice, preserve functional properties (antioxidant, alpha-glucosidase inhibition, and hypoglycemia). The fermentation of this juice with *Lp* may develop functional beverages, which is significant due to its consumption as an edible fruit with medicinal properties.

Keywords: *Cucurbita ficifolia*; *Lactobacillus plantarum*; lactic fermentation; polyphenolic compounds; flavonoids; antioxidant activity

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1. Introduction

Cucurbita ficifolia (*C. ficifolia*), a plant known for its edible fruits, has been associated with antioxidant and anti-inflammatory activities and hypoglycemic effects [1,2]. Several fruit compounds were proposed as responsible for these biological activities, such as phenols, phytosterols, and D-chiro-inositol [3,4]. Therefore, the preparation of functional products from these fruits may be significant due to their consumption and proven medicinal properties.

The development of non-dairy fermented beverages from fruits with beneficial effects on health has increased, resulting in nutritious products and adjuvants in disease treatment [5,6]. Fruits of several plants have been used to produce fermented beverages, observing qualitative and quantitative modifications in the content of some of their components, especially polyphenols. Therefore, fermentation processes may impact many of the plants' previously reported biological activities [7,8].

Several chemical transformations occur during fermentation, either spontaneously or under controlled conditions. These transformations modify qualitative and quantitatively nutritional compounds and the secondary metabolites in many fruits, impacting in their

functional properties and organoleptic characteristics [9]. The controlled fermentation with lactic acid bacteria (LAB) has been a good process in developing functional beverages [10]. Primarily using *Lactobacillus plantarum* (*Lp*), a probiotic strain that hydrolyses phenolic compounds, modifying the composition and properties of the raw material [11].

C. ficifolia contains bioactive polyphenolic compounds with demonstrated antioxidant, anti-inflammatory, and anti-diabetic activities. It represents a potential platform for developing new functional products, with a high possibility of being used as adjuvants in treating diabetes mellitus (DM). Considering that it has not been yet explored acid fermentation of *C. ficifolia*, this research aims to characterize the fermentation of the *C. ficifolia* fruit juice in two conditions: spontaneously (native) and inoculating with *Lp* (controlled) and evaluate their antioxidant and hypoglycemic effects.

2. Materials and Methods

2.1. Physical Characterization of C. ficifolia Fruits and Juice Obtention

After harvesting in October 2019 at Ixtapaluca, Estado de Mexico, Mexico, the fruits were washed and weighed, measuring the longitudinal (LP) and equatorial (EP) perimeters and the sphericity coefficient ε (ε = EP/LP) [12]. The shell and seeds were removed, and the juice was obtained from the mesocarp with an electrical processor and filtered.

2.2. Fermentations of the C. ficifolia Juice: Native and Controlled

In native fermentation, the juice filtered was left spontaneously to ferment at 30 °C for 56 h. In the controlled fermentation, the strain of *Lp* was cultured in the broth Man Rogosa Sharp (MRS) (Difco®, Detroit, MI, USA) at 30 °C. After 24 h, 10 mL were transferred to sterile MRS broth and incubated at 30 °C until it reached an absorbance of 1.0 at 600 nm. Then, the juice was inoculated with *Lp* 5% (*v*/*v*) and incubated at 30 °C for 56 h. Samples of 30 mL of both fermentations were withdrawn every 8 h under aseptic conditions. All samples were filtered and frozen until further analysis.

2.3. Microbial Growth Determination

Since the fruit has microorganisms such as LAB and yeasts, the microbial growth was monitored each 8 h for 56 h. MRS agar was used for total LAB; a potato dextrose agar (PDA) was used (Becton Dickinson Bioxon™, Mexico City, Mexico) for total yeast and fungi, whereas for total mesophilic microorganisms, a plate count agar was used (PCA, Becton Dickinson Bioxon™, Mexico). In sterile NaCl (0.9% *w*/*v*) solution, the previously diluted samples were inoculated (5 µL) and incubated at 30 °C until the colonies' differentiation. Colonies' count was expressed as log CFU (colony forming units)/mL. Specific growth rate, µ (h^{-1}), was determined using the linear equation of the exponential growth (Equation (1)); in addition, Gompertz's predictive model was used (Equation (2)) [13]:

$$\text{Ln Xi} = (0.693\ t/t_d) + \text{Ln } X_0 \quad (1)$$

where Ln X_i = natural log of the cellular concentration at time "i"; t = time; td = time of duplication; Ln X_0 = natural log of the cellular concentration at time "cero".

$$\text{Log } N_{(t)} = A \times \exp(-\exp(B - M \times t) \quad (2)$$

where Log N = common log of bacterial populations (CUF/mL); t = time of incubation (h); A = common log of the initial bacterial populations (CUF/mL) (inoculum); B = growth rate; M = time with the faster growth rate.

2.4. Determination of pH, Total Titratable Acidity (TTA), and °Brix

The pH was measured (HANNA, Instruments®, Woonsocket, RI, USA), and TTA (%) determined (Equation (3)) [14]. Samples (5 mL) were titrated with NaOH 0.1 N up to reach

pH 7.0; the milliequivalents (mEq) of malic (0.067) and lactic (0.09) acids were used to determine TTA (%) in the fresh and fermented juices, respectively.

$$\% \text{ Acidity} = [(\text{Vol}_{\text{NaOH}} \text{ (mL)} \times \text{N}_{\text{NaOH}} \times \text{mEq predominant acid})/\text{mL juice}] \times 100 \quad (3)$$

where TTA = total titratable acidity; Vol_{NaOH} = NaOH (mL); N = normality acid mEq

Total soluble sugars expressed as °Brix were determined with a hand refractometer (PCE Instruments-0018, Southampton, UK) at room temperature (T = 24–28 °C).

2.5. Determination of Soluble Protein and Reducing Sugars

Reducing sugars were determined using the dinitro salicylic acid (DNS) method. A standard curve of bovine serum albumin (BSA, Sigma-Aldrich®, St. Louis, MO, USA) from a 1 mg/mL stock solution was prepared, measuring absorbance at 595 nm (UV–VIS Spectrophotometer, PerkinElmer®, Waltham, MA, USA) [15]. Results were expressed as mg/mL of soluble protein [16]. A standard curve of glucose (Sigma-Aldrich®, USA) from a 1 mg/mL stock solution was prepared, measuring absorbance at 540 nm. The results were expressed in mg/mL of glucose.

2.6. Determination of Total Polyphenols and Flavonoids

Polyphenols were determined using the Folin–Ciocalteu method [17]. Folin reagent (750 µL) (Sigma-Aldrich®, USA) (1:10) and 100 µL of the sample were mixed and left in the darkness for 5 min at room temperature (26 °C ± 2 °C). After, 750 µL of Na_2CO_3 (6%, J.K. Baker®, San Bernardino, CA, USA) was added and incubated in the dark for 90 min at room temperature (26 °C ± 2). The absorbance was measured at 765 nm. A standard curve of gallic acid (Sigma-Aldrich®, USA) from 0.350 mg/mL stock solution was prepared. The results were expressed as gallic acid (mg EqGA/mL).

In the case of total flavonoids, 300 µL of sample and 90 µL of $NaNO_2$ (5%, Merk®, Darmstadt, Germany) were mixed and incubated in the dark for 5 min at room temperature (26 °C ± 2). Then, 180 µL of $AlCl_3$ (10%, Meyer®, Mexico City, Mexico) were added and left in the dark for 5 min at room temperature (T = 24–28 °C); NaOH (600 µL, 1 M, J.T. Baker®) was added, and the absorbance immediately measured at 510 nm. A standard curve of quercetin (Sigma-Aldrich®, USA) from 0.200 mg/mL stock solution was prepared. Results were expressed as quercetin equivalents mg/mL (mg EqQ/mL) [18].

2.7. Determination of Antioxidant Activity by ABTS and DPPH Radical Scavenging Activities

Radical ABTS (2.2-azino-bis-3-ethylbenzotizolin-6-sulfonic) was generated from the 7 mM ABTS solution and 2.45 mM potassium persulfate left in the dark for 16 h at 4 °C. Then, the ABTS reagent was diluted with distilled water to give an absorbance value of 0.7 ± 0.01 at 734 nm; the sample (50 µL) was mixed with 1950 µL of ABTS and incubated in the dark for 10 min at room temperature (T = 24–28 °C), and absorbance was measured at 734 nm. The percentage (%) of inhibition was calculated with Equation (4) [19].

$$\% \text{ Inhibition} = [(\text{Abs } T_0 - \text{Abs } T_{10\text{ min}})/\text{Abs T0}] \times 100 \quad (4)$$

where T_0 = absorbance at time zero; $T_{10\text{ min}}$ = absorbance after 10 min reaction.

A solution of 0.1 mM of the radical DPPH (1,1-diphenyl-2-picryl hydrazine) was dissolved in methanol; the sample (50 µL) was mixed with 1950 µL of DPPH and left in the dark for 30 min at room temperature (26 ± 2 °C). The absorbance was measured at 517 nm. The percentage (%) of inhibition was calculated in Equation (5) [20].

$$\% \text{ Inhibition} = [(X_2 \times 100)/X_1] - 100 \quad (5)$$

where X_1 = initial value (t = 0, unfermented juice); X_2 = final value (fermented juice).

2.8. Alpha-Glucosidase Inhibitory In Vitro Assay

The juice of the *C. ficifolia* and samples at 48 h of fermentation (native and controlled) were subjected to an alpha glucosidase inhibitory in vitro assay. A p-nitrophenyl-α-D-glucopyranoside (pNPG) solution was prepared with phosphate buffer (0.1 M, pH 6.9). A total of 50 µL of α-glucosidase (1 U/mL) was preincubated with the juice of *C. ficifolia* and the native and controlled fermentations (50 µL of each) for 10 min. Then, 50 µL of both solutions were mixed and incubated at 37 °C for 5 min. The α-glucosidase activity was determined by measuring the absorbance at 405 nm (UV–VIS spectrometer, PerkinElmer®) and compared with pNPG solution. The percentage of inhibition was calculated with Equation (6).

$$\% \text{ Inibition} = [(\text{Control}_{\text{Absorbance}} - \text{Sample}_{\text{Absorbance}})/\text{Control}_{\text{Absorbance}}] \times 100 \quad (6)$$

2.9. Effects on the Glycemia of the Juice and Samples at the 48 h of Native and Controlled Fermentation

Fasted (12 h) normal male CD-1 mice of six-month-old provided by the Metropolitan Autonomous University Center, maintained with essential rodent diet, and cycles of 12 h light /darkness, were used. The juice of the *C. ficifolia* and samples at 48 h of fermentation (native and controlled) were also assayed in glycemic and glucose tolerance tests in normal mice. These studies were performed following the national and international standards of the Mexican Official Standard (NOM-062-ZOO-1991, revised 2001, Mexico). Metropolitan Autonomous University Ethic Committee approved this protocol on 31 January 2019 (DCBS.1857.2019).

In glycemic tests, fasted mice were grouped (n = 5): Group 1 received isotonic saline solution (ISS) as control (4 mL/kg); Group 2 glibenclamide (10 mg/kg); Group 4 juice of *C. ficifolia*; Groups 5 and 6 native fermented juice (25 and 50 mg/kg, respectively); Groups 7 and 8 controlled fermented juice (25 and 50 mg/kg, respectively). All the treatments were diluted in 4 mL/kg body weight of ISS and were administered per os. Glycemia was determined in tail vein blood samples by a puncture at the beginning (t = 0), 120, 240, and 360 min after administering the treatments. Glycemia was quantified by the dehydrogenase method (Accu-Chek, Performa, Roche, Mannheim, Germany).

In the glucose tolerance tests, fasted mice were grouped and received per os a 50% glucose solution (2 g/kg): Group 1 received isotonic saline solution (ISS) as control (4 mL/kg); Group 2 metformin (150 mg/kg); Group 3 juice of *C. ficifolia* (50 mg/kg); Groups 4 native fermented juice (50 mg/kg); Groups 5 controlled fermented juice (50 mg/kg). All the treatments were diluted in ISS (4 mL/kg) of and per os administered immediately after the administration of the glucose solution. Glycemia was determined by puncture of tail vein blood samples of the animals, which were obtained at the beginning (t = 0), 30, 60, 90, and 120 min after other administration of the treatments. Glycemia was quantified by the dehydrogenase method (Accu-Chek, Performa, Roche, Mannheim, Germany).

2.10. Statistical Analysis

Statistical analysis was performed using the NCSS software (NCSS® Inc., LLC, USA). A one-way analysis of variance (ANOVA) and Tukey's post hoc tests were used to evaluate differences ($p < 0.05$). Assays were performed triplicated, and results were expressed as mean ± S.D.

3. Results

3.1. Physical Characteristics of C. ficifolia Fruits

Figure 1 shows the sizes of the fruits and seeds of *C. ficifolia*. Other physical attributes of *C. ficifolia* fruits, including their weight, are shown in Table 1. Considering the sphericity coefficient ε, where 1.0 corresponds to a spherical shape, and lower values to an oval, the fruits used in this study had an oval shape and an average weight of almost 4.5 kg. The seeds were black with an average weight of 136.7 ± 35.1 g. The fiber content in the fruit was 12.2% (Table 1).

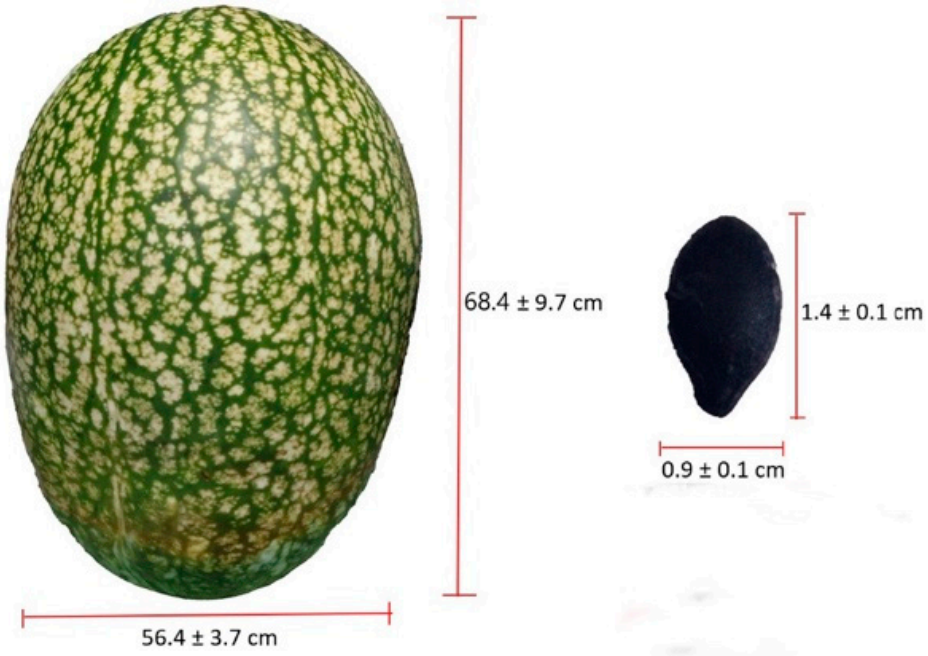

Figure 1. Sizes of the fruits and seeds of *C. ficifolia*.

Table 1. Physical characteristics of *C. ficifolia* fruits and seeds (Mean ± S.D., $n = 3$).

Characteristics	Fruits	Seeds
Longitudinal perimeter (LP, cm)	68.4 ± 9.7	1.4 ± 0.1
Equatorial perimeter (EP, cm)	56.4 ± 3.7	0.9 ± 0.1
Sphericity coefficient (ε)	0.8 ± 0.1	-
Weight (g)	4478.3 ± 527.7	136.7 ± 35.1
Juice (mL)	2025 ± 217	-
Yield juice (%)	45.2 ± 1.3	-
Fiber (g)	361.7 ± 114.5	-
Yield fiber (%)	12.2 ± 2.2	-
°Brix	4.1 ± 0.2	-

Sphericity coefficient ε (ε = EP/LP) and the weight of each fruit were measured. The juice was obtained and filtered to measure the volume, fiber, and °Brix.

3.2. Microbial Growth in Native and Controlled Fermentation of the C. ficifolia Juice

Figure 2 shows the microbial profile of the native fermentation (A) and controlled (B) of *C. ficifolia* juice for 56 h. Predominant native microbiota in the juice (t = 0): In LAB (MRS) was 12 ± 0.7 Log CFU/mL, followed by mesophiles (PCA) with 8 ± 0.01 Log CFU/mL, and fungi and yeast (PDA) 5 ± 1.4 Log CFU/mL. Figure 2A shows the microbial growth of the native fermentation. In this fermentation, the exponential growth of the LAB (MRS) was observed from 8 to 32 h (21 ± 2.0 Log CFU/mL) without reaching a stable phase, with a reduction in initial values at the end (56 h). Mesophiles exhibited an exponential growth from 0 to 8 h, with a maximal increase of 18 ± 2.0 CFU/mL, and gradually fell to the end (56 h). Fungi and yeast maximal microbial count was 25 ± 2.0 CFU/mL (32 h).

Figure 2B shows the microbial growth of induced lactic fermentation, reaching the maximal growth (27 ± 2.1 log CFU/mL) at 24 h; then, the LAB count decreased to 23 ± 1.0 log CFU/mL (56 h). In PDA, the highest growth occurred at 16 h (15.5 ± 2.1 Log CFU/mL), followed by a decrease at the end (12 ± 2.8 Log CFU/mL). The maximal microbial concentration observed in PCA was at 32 h (14.5 ± 4.1 Log CFU/mL), then reduced to 10 ± 0.1 log CFU/mL at the end (Figure 2B). Table 2 shows the kinetics parameters in the fermented juice of *C. ficifolia* with the two systems. Faster growth was observed in controlled fermentation in MRS medium, which accounts for LAB. While in native fermentation, yeast and fungi grew faster in the PDA medium. The results of the predictive Gompertz's model

showed that LAB microorganisms grew faster than yeast and fungi. The *Lp* growth shown by the model was the only one that matched well with the growth calculated experimentally (Table 3).

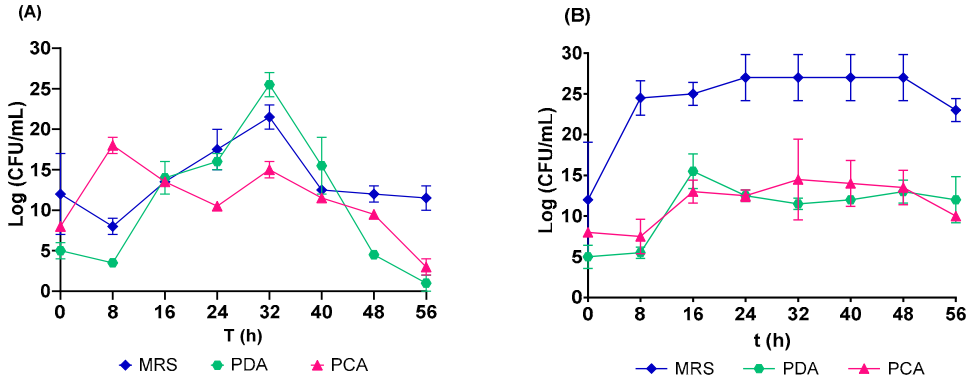

Figure 2. Microbial profile of the native fermentation (**A**) and controlled with *Lp* (**B**) of the *C. ficifolia* juice for 56 h (MRS = Man, Rogosa, and Sharpe, PDA = potato dextrose agar, PCA = standard count agar).

Table 2. Kinetic of growth of native and controlled fermentation (*L. plantarum*) of the *C. ficifolia* juice at 30 °C (Mean ± S.D., $n = 3$). Specific growth rate μ (h^{-1}) was determined using the linear equation of the exponential growth.

		Parameter Value			
		Controlled		Native	
Medium	**Microorganisms**	**μ**	**M**	**μ**	**M**
MRS	*L. plantarum*	0.46 ± 0.01	0.30 ± 0.30	0.26 ± 0.12	32.94 ± 0.23
PDA	Yeast and fungi	0.25 ± 0.17	1.77 ± 0.23	0.30 ± 0.02	1.24 ± 0.55
PCA	Mesophilic	0.33 ± 0.18	0.10 ± 0.03	0.21 ± 0.1	2.80 ± 1.26

μ: specific growth rate (h^{-1}) M: time in which the rate of growth is highest (h).

Table 3. Kinetic of growth with the Gompertz's predictive model (Equation (2)) [13] of native and controlled fermentation (*L. plantarum*) of the *C. ficifolia* juice (mean ± S.D., $n = 3$).

	Native			Controlled		
Parameter	***L. plantarum***	**Yeast-Fungi**	**Mesophilic**	***L. plantarum***	**Yeast-Fungi**	**Mesophilic**
A	14.19 ± 1.3	12.80 ± 1.5	11.57 ± 0.4	26.07 ± 2.4	12.69 ± 0.5	13.15 ± 0.6
B	29.08 ± 2.0	9.55 ± 1.3	−1.00 ± 0.1	0.47 ± 0.5	13.49 ± 1.9	−0.47 ± 0.04
M	32.94 ± 0.2	1.24 ± 0.6	2.80 ± 1.3	0.30 ± 0.3	1.77 ± 0.2	0.10 ± 0.03

A: logarithm of the initial population. B: growth rate. M: time in which the rate of growth was highest.

3.3. pH and TTA Profile

Figure 3 shows the temporal course of pH (A) and % TTA (B) of the native and controlled fermentation of the *C. ficifolia* juice for 56 h. In the controlled fermentation with *Lp*, a drop from pH 5.4 ± 0.2 was observed at 16 h to 4.1 ± 0.04; then, the pH value remained practically constant until 56 h. In native fermentation, the reduction in pH value was less in the controlled conditions, oscillating from 5.4 to 5.0 by the end. Concerning TTA in controlled conditions (Figure 3B), the initial value was 0.09 ± 0.02%, reaching a maximum of 0.91 ± 01% at 40 h and decreasing to 0.76 ± 0.07% at 56 h. One more time, in native fermentation, the values of TTA were lower than in controlled conditions, with a maximum acidity value reaching 0.4 ± 0.01%.

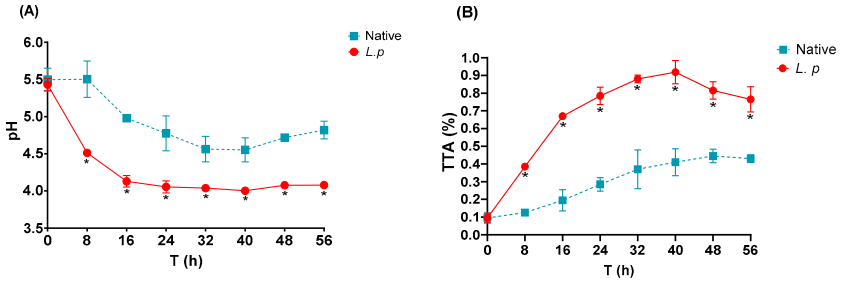

Figure 3. Temporal course of pH (**A**) and % TTA (**B**) of the native and controlled fermentation (*Lp*) of the *C. ficifolia* juice for 56 h. * Significant difference ($p < 0.05$) against native fermentation juice. T-paired test (Mean ± S.D., $n = 3$), Tukey's post hoc test.

3.4. Profile of Reducing Sugars and Soluble Protein

Figure 4 shows the temporal course of reducing sugars (A), protein (B), total polyphenols (C), and total flavonoids (D) of native and controlled fermentation of the *C. ficifolia* juice for 56 h. Initially, t = 0, reducing sugars were 2.4 ± 0.19. In the lactic fermentation, reducing sugars decreased 64% at 16 h and 77.5% at the fermentation ends, which also happened with the native fermentation. The protein content (Figure 4B) in the juice (t = 0) was 0.47 ± 0.05 mg/mL, reducing 79% until 40 h in the case of the controlled fermentation; at 56 h, it increased 12.7% from the initial value. In the native fermentation, the profile was similar, although the fluctuations were lesser than in controlled conditions.

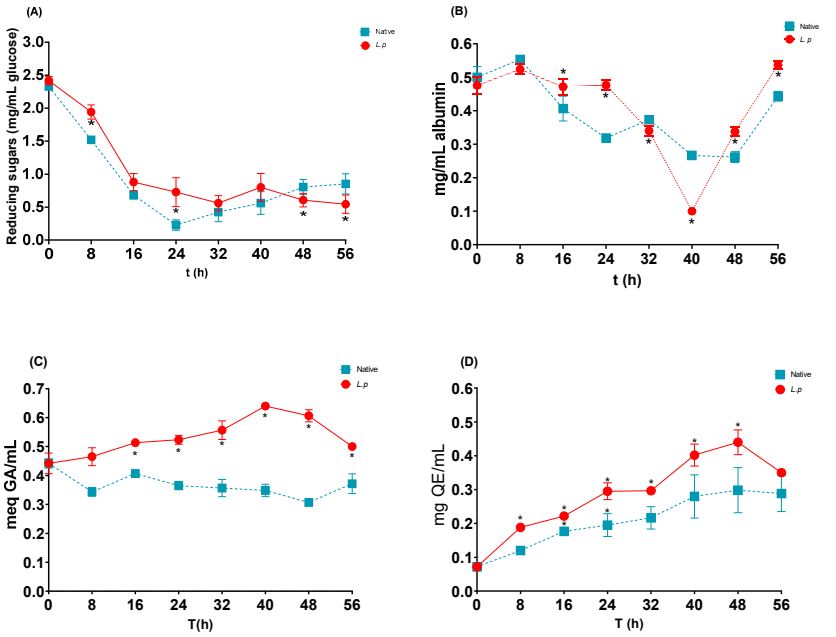

Figure 4. Temporal course of reducing sugars (**A**), protein (**B**), total polyphenols (**C**), and total flavonoids (**D**) of native and controlled fermentation (*Lp*) of the *C. ficifolia* juice for 56 h. * Significant difference ($p < 0.05$) against native fermentation juice. T-paired test (Mean ± S.D., $n = 3$), Tukey's post hoc test.

3.5. Polyphenols and Flavonoids

The content of polyphenols and flavonoids in the juice was 0.44 ± 0.01 mg EqGA/mL and 0.07 ± 0.003 mg EqQ/mL, respectively (Figure 4C,D). Polyphenols increased during the controlled fermentation, reaching 44.8% higher than the initial value. The quantity decreased to 0.50 ± 0.01 mg EqGA/mL, 13.1% over the initial value. At 48 h, flavonoids increased 514%, and finally, the increase was 388.3% over the initial value. In native fermentation, polyphenols were reduced compared with the controlled fermentation, staying

practically constant until 56 h. However, flavonoids were increased in native fermentation, although values were lesser than in controlled conditions.

3.6. In Vitro Antioxidant Activity of Native and Controlled Fermentations

Figure 5 shows the temporal course of the inhibition of DPPH (A) and ABTS (B) radicals during native and controlled fermentation of the *C. ficifolia* juice for 56 h. The juice exhibited percentages of the inhibition of DPPH (Figure 5A) and ABTS (Figure 5B) of 43.3 ± 1.4% and 86.5 ± 1.2%, respectively. With DPPH, inhibition did not change during the fermentation. ABTS inhibition decreased by 24% at 56 h, compared with the inhibition observed in the unfermented juice. In the case of native fermentation, the antioxidant activity did not change significantly in any of the assays.

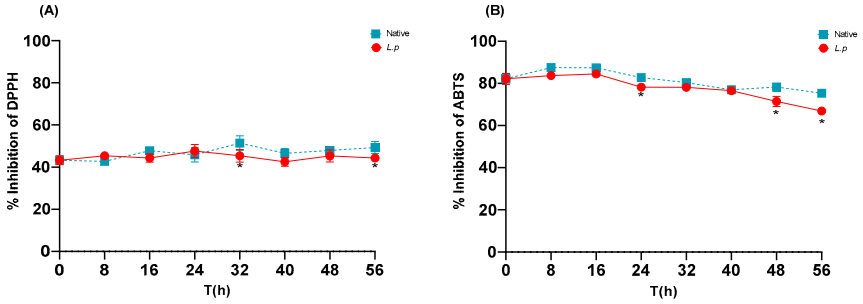

Figure 5. Temporal course of inhibition of DPPH (**A**) and ABTS (**B**) radicals during native and controlled fermentation (*Lp*) of the *C. ficifolia* juice for 56 h. * Significant difference ($p < 0.05$) concerning unfermented juice (t = 0). T-paired test (Mean ± S.D., $n = 3$). Tukey's post-hoc test.

3.7. Comparative Analysis at 48 h of the Native and Controlled Fermentations against the C. ficifolia Juice (t = 0)

In fermented juice with LAB, the exponential growth at 48 h reached a terminal stationary phase with 27 ± 2.1 log CFU/mL (Figure 2B) and a high content of flavonoids (0.44 ± 0.02 mg EqQ/mL) (Figure 4D). Therefore, values at 48 h were used to compare fermented juices (Table 4). At this time, the controlled fermentation decreased the pH, reducing sugars and proteins. In contrast, TTA, polyphenols, and flavonoids increased (800%, 38.6%, and 528%, respectively). DPPH did not change significantly, whereas inhibition of ABTS decreased by 17.4%. In the case of native fermentation, the reductions in pH and reducing sugars were lesser than in controlled fermentation. The rise in TTA and flavonoids also was less than in controlled fermentation. In native fermentation, reduced polyphenols were observed. Considering these values at 48 h, in this time were evaluated the effects of both fermented products on the in vitro alpha-glucosidase inhibition and the glycemia of fasted normal mice.

Table 4. Comparative between parameters at 48 h of native and lactic fermentation against fresh juice.

Assay	Fresh Juice (t = 0)	Native Ferm. (48 h)	% Variation	Lactic Ferm. (48-h)	% Variation
pH	5.4 ± 0.1	4.7 ± 0.018 #	−13	4.1 ± 0.04 *	−24.1
TTA (%)	0.09 ± 0.01	0.44 ± 0.03 #	+388.9	0.81 ± 0.07 *	+800
Reducing sugars (mg/mL)	2.4 ± 0.19	0.80 ± 0.28 #	−66.6	0.6 ± 0.41 *	−75
Protein (mg/mL)	0.47 ± 0.05	0.26 ± 0.04 #	−44.7	0.33 ± 0.02 *	−29.8
Polyphenols (mg EqGA/mL)	0.44 ± 0.01	0.30 ± 0.01 #	−31.8	0.61 ± 0.01 *	+38.6
Total flavonoids (mg EqQ/mL)	0.07 ± 0.003	0.29 ± 0.16 #	+314	0.44 ± 0.02 *	+528
Inhibition DPPH (%)	43.3 ± 1.4	47.91 ± 1.31 #	+10.6	45.29 ± 1.8	+4.6
Inhibition ABTS (%)	86.5 ± 1.2	78.25 ± 0.71 #	−9.5	71.43 ± 1.3 *	−17.4

#,* Significant difference against fresh juice ($p < 0.05$). ANOVA one-way test (mean ± S.D.; $n = 3$). Tukey's post hoc test. + Increase; − decrease.

3.8. Alpha-Glucosidase Inhibitory Effect and on the Glycemia

Table 5 shows the alpha-glucosidase inhibitory potential evaluated in vitro. There was no significant difference among all the treatments (juice of *C. ficifolia* and native fermentations). However, the controlled fermentation exhibited the highest inhibitory potential on the enzyme activity, significantly different from juice and native fermentation.

Table 5. In vitro α-glucosidase inhibitory effect (%) of the juice of *C. ficifolia* and samples at 48 h of native and controlled fermentation.

Treatment	Inhibition (%)
Juice	37.4 ± 1.0
Native fermentation	30.1 ± 2.9
Controlled fermentation	42.0 ± 1.0 *

* Significant difference against juice ($p < 0.05$). One-way ANOVA, mean ± S.D., $n = 3$. Tukey post hoc.

To the effect on the glycemia (Table 6), the results in fasted normal mice, at a dose of 25 mg/kg, none of the treatments showed an effect on the glycemia (Table 6). However, at a dose of 50 mg/kg, except for native fermentation, the juice and the controlled fermentation caused significant reductions in the glycemia compared with the ISS control ($p < 0.05$): at 360 min, the juice caused a considerable decrease in the glycemia; and at 240 and 360 min the controlled fermentation. The reductions caused by glibenclamide at any time were higher than the other treatments ($p < 0.05$). In the oral glucose tolerance test (Table 6), excepting native fermentation, all treatments caused significant reductions of the hyperglycemic peak at 30 min, compared with ISS control ($p < 0.05$). At 60 min, all treatments caused significant reductions in glycemia compared with ISS control ($p < 0.05$). The reductions caused by metformin (positive control) at any time were higher than the other treatments ($p < 0.05$).

Table 6. Effect on glycemia of the juice and samples at 48 h of native and controlled fermentations in: fasted normal mice and oral glucose tolerance test in mice.

Fasted normal mice					
		Glycemia (mg/dL)			
Treatment/Doses		**0 min**	**120 min**	**240 min**	**360 min**
ISS		101.9 ± 22.6	100.9 ± 12.2	101.6 ± 20.3	90.6 ± 20.9
Glibenclamide	10 mg/kg	102.6 ± 15.4	76.9 ± 12.4	56.6 ± 15.2 $^{+}$	62.3 ± 18.7 $^{+}$
Juice	25 mg/kg	95.5 ± 10.9	115.0 ± 9.9	92.5 ± 2.2	86.0 ± 15.6
	50 mg/kg	98.0 ± 21.0	106.7 ± 3.1	82.0 ± 9.8	77.0 ± 12.5 $^{+}$
Native	25 mg/kg	97.0 ± 5.3	126.0 ± 18.6	82.7 ± 15.0	80.7 ± 13.3
	50 mg/kg	90.0 ± 9.9	95.5 ± 23.2	79.0 ± 18.0	74.3 ± 9.4
Controlled	25 mg/kg	93.3 ± 2.1	116.0 ± 11.4	87.5 ± 19.1	83.0 ± 21.6
	50 mg/kg	95.7 ± 9.6	91.0 ± 12.2	76.0 ± 6.6 $^{+}$	72.5 ± 8.3 $^{+}$
Tolerance test in mice					
		Glycemia (mg/dL)			
Treatment	**0 min**	**30 min**	**60 min**	**90 min**	**120 min**
ISS	76.3 ± 11.7	254.7 ± 27.2	199.3 ± 42.8	140.17 ± 29.9	128.1 ± 24.6
Metformin 150 mg/kg	82.0 ± 13.6	128.4 ± 39.9 *	92.0 ± 19.5 *	90.2 ± 19.3 *	87.4 ± 20.9 *
Juice 50 mg/kg	83.6 ± 18.7	175.0 ± 25.5 *	104.5 ± 19.1 *	109.3 ± 24.0	115.5 ± 21.7
Native 50 mg/kg	73.7 ± 24.5	201.5 ± 37.5	134.0 ± 12.7 *	118.0 ± 10.4	112.3 ± 3.5
Controlled 50 mg/kg	79.0 ± 18.5	157.0 ± 45.3 *	108.0 ± 12.7 *	108.5 ± 33.2	106.0 ± 5.7

$^{+}$ Significant difference against initial glycemia (t = 0). * Significant difference against ISS ($p < 0.05$). One-way ANOVA, mean ± S.D., $n = 3$. Fisher post hoc.

4. Discussion

In the present study, mature fruits were used and characterized by their oval shape, mottled green color, hard shell, and black seeds, similar characteristics previously re-

ported [21]. The fiber content was high (12.2%) compared with other Cucurbitaceae fruits, whereas the *C. ficifolia* juice yield (45%) was similar [22,23]. The microbiological profile of the juice showed a LAB predominance, followed by the mesophiles, fungi, and yeast, which agrees with apple juice fermentation [24]. The pH was 5.4, whereas the TTA was low compared with other Cucurbitaceae [23]. The reducing sugar content was 2.4 mg/mL, whereas °Brix resulted in 4.1 ± 0.2. These data support the idea that the *C. ficifolia* juice is a potentially suitable matrix to perform a lactic fermentation with *Lp*.

Lp is the most widely used strain to produce fermented plant products in controlled conditions. It is usually found in plant products and is considered a probiotic that can survive in stomach conditions, inhibiting the growth of *Escherichia coli* and *Helicobacter pylori* [25]. *Lp* is one of the most used microorganisms in non-dairy fermentations with a high capacity to metabolize substrates, which induces changes in chemical composition, impacting the nutritional quality. The MRS selective medium for *Lp* growth was used in the present study, increasing similarly to the observed with other Cucurbitaceae juices [26,27]. The microbial growth in the non-fermented and fermented *C. ficifolia* juices corresponds well with other fermented juices, with a predominance of LAB [28].

In contrast, in the native fermentation, the predominant microorganisms were fungi, yeast, and LAB, followed by the mesophiles, all exhibiting an erratic growth that never reached a steady phase. The results of the predictive Gompertz's model, a mathematical model used to estimate the growth of different *Lactobacillus* strains on various substrates, describe the kinetics of growth of these microorganisms under controlled conditions to produce specific metabolites [13], showed that LAB grew faster than yeast and fungi. The *Lp* growth demonstrated by the model was the only one that matched well with the change calculated experimentally.

In the controlled fermented juice, the pH decreased, and the TTA increased, correlating well with glucose consumption from the medium by the microorganisms. Several reports indicate decreased pH during the lactic fermentation of fruit juices in the Cucurbitaceae family [29,30]. Lactic acid production and pH reduction during food fermentation can cause inhibition of Gram-positive and Gram-negative pathogen microorganisms [31,32]. Thus, reducing the growth of potential noxious microbiota, probably by increasing the lactic acid production during fermentation, contributing to carbohydrate hydrolysis. Since the values observed in these parameters for the native fermentation were clearly of a lesser scale, this might stimulate the growth of pathogen microorganisms. In further studies, the characterization of the specific strains of microorganisms in both fermented juices will be mandatory.

The reducing sugars are used as a primary source of carbon by *Lp*. They were diminished during the fermentation [33,34], as observed with both fermentations, which is relevant because beverages with low caloric content have a great demand as treatments for DM [35,36]. Although different types of fermented non-dairy beverages have been reported with hypoglycemic activity [36,37], the effect on glycemia of *C. ficifolia* fermented fruit has not yet been explored. The present investigation studied this effect in fasted normal mice and glucose oral tolerance tests in mice. In both models, the juice, and samples at 48 h of the fermented products exhibited significant reductions in the glycemia, as the controlled fermentation preserves the effect on the glycemia for the juice without fermentation and those previously reported for the juice in normal and diabetic mice [1,4,12].

The soluble protein in the *C. ficifolia* juice was decreased during the fermentation, necessary components for the growth of microorganisms, resulting in the modification of flavor due to transamination reactions. However, in the end, the protein content was like the initial values. Fermentation has been used to improve the protein nutrition quality in several products, facilitating the metabolic activity of the microorganisms, mobilizing the proteins, releasing proteolytic enzyme activities, and reducing the macromolecular complexes into simple amino acids [38–40]. These processes might explain the fluctuations in protein content in both fermentations of *C. ficifolia*.

C. ficifolia exhibited similar content of polyphenols and flavonoids to *C. pepo*, values lower than other Cucurbitaceae species [23]. During the controlled fermentation of *C. ficifolia* juice, the polyphenols were increased at 40 h. *Lp* contributes to enzymatic depolymerization of high molecular weight polyphenols to free polyphenols by carboxylases and tanases, improving the polyphenol content [41]. Contrarily, the polyphenols during all the native fermentation were observed reduced, reaching their lowest value at 48 h of the fermentation.

Polyphenols are associated with multiple beneficial effects [42]. *C. ficifolia* juice is rich in simple phenolic compounds, such as gallic acid, p-coumaric acid, and p-hydroxybenzoic acid, which significantly contribute to antioxidant activity [4]. Nevertheless, both fermentations did not modify DPPH compared to unfermented juice, remaining around 40% of inhibition, preserving the antioxidant activity. In contrast, a decrease in ABTS radical inhibition was observed at the end of both fermentations, probably due to the quantitative and qualitative changes in flavonoids [43]. In addition, the loss of antioxidant activity might be due to polyphenols' changes during fermentation, decreasing the original components [44,45]. Since antioxidant activity has not been reported in vitro in *C. ficifolia*, these data contribute to the knowledge of this fruit. Additionally, the fruit's fermentation probably does not alter the antioxidant activity reported in vivo [46]. However, this should be confirmed in further studies.

Polyphenols also have been attributed to the inhibitory activity of carbohydrate hydrolytic enzymes, reducing the absorption of simple sugars at the bowel level, which may be helpful in DM [47]. The present work evaluated the in vitro inhibitory effect potential on alpha-glucosidase. The different treatments exhibited a similar effect on inhibiting the enzyme, which might explain the antihyperglycemic effect observed in the glucose tolerance test. In addition, the product of the controlled fermentation also significantly reduced glycemia in fasted normal mice. These observed effects match previous studies in our laboratory with the juice, including diabetic experimental models [1,4,46]. In these studies, some compounds from the fruit were proposed as hypoglycemic agents, such as D-chiro inositol and chlorogenic acid [3,48], with potential utility in DM control. However, with the data until now, does not possible to know if these compounds remain in the fermented juices. Therefore, the chemical characterization of both fermented products should be considered in further studies.

Another aspect to consider is associated with the organoleptic properties of the *C. ficifolia* juice, which is lightly insipid. It is possible that with fermentation, this characteristic can be improved [49]. Finally, considering that the controlled fermentation was observed at 48 h, significant growth of *Lp*, with high flavonoid and polyphenol contents, and preserved the antioxidant activity, this period may be enough to stop the fermentation and generate a suitable product that improves the raw material's functional properties.

5. Conclusions

In conclusion, both fermentations modified the chemical composition of the unfermented juice of *C. ficifolia*. The data suggest that a controlled fermentation of *C. ficifolia* by *Lp* may improve its functional properties, particularly with glycemia; 48 h of fermentation might be sufficient to stop the process. These data can serve as a base for developing functional food with antioxidant properties, low sugar content, and effects on glycemia with possible utility in DM.

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