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Article

Effect of the Flash Vacuum Expansion (FVE) Process on the Response of *Limosilactobacillus fermentum* J24 to the Metabolism of Sugars and Organic Acids During the Development of a Papaya-Based Drink

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Abstract

The flash vacuum expansion (FVE) process is an unconventional technology that enables the generation of fruit purees by altering the state of the water stored in the vacuoles. The plant tissue is subjected to an increase in temperature (from 25 to 54 °C) while maintaining a constant pressure (101.3 kPa). The temperature and pressure are then rapidly reduced (25 °C and 5 kPa, respectively). This promotes the conversion of water from liquid to gas, increasing its volume, which causes cell rupture and efficiently releases cellular components, including compounds stored in the vacuole. Furthermore, fermentation with lactic acid bacteria (LAB) is a biotechnological strategy that allows the generation of beverages with specific characteristics derived from the metabolism of LAB. LAB are capable of consuming sugars as an energy source and producing organic acids as a means of defense against microbial competitors. This research analyzes the effect of the FVE process and the genetics of *Limosilactobacillus fermentum* J24 on sugars and organic acids in a papaya-based beverage. During the production of papaya puree, FVE affects the concentration of sugars and organic acids, leading the bacteria to a different metabolic response than when FVE is not used for papaya puree production. *Limosilactobacillus fermentum* J24 was found to activate genes that confer high potential for use in the fermentation of plant-based matrices, although it was isolated from cheese.

Keywords: flash vacuum expansion; metabolism; sugars; fermentation; papaya

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1. Introduction

The papaya (*Carica papaya* L.) is a tropical fruit valued for its flavor and nutritional benefits. It is high in fiber and simple sugars, which support the development of plant prebiotics and probiotics. Advances have been made in understanding its advantages and

potential uses; however, the fruit faces several challenges, such as post-harvest handling problems, pest and fungal infestations, and severe climate changes. These issues lead to considerable post-harvest papaya losses. An alternative method for fully utilizing the fruit is the flash expansion (FVE), which causes the disintegration of plant tissues through efficient cell rupture due to pressure and temperature differences caused by water changing from liquid to gas in the cell vacuoles. This enables the production of fruit purées. It significantly lowers microbial load, preserves the nutritional quality of the purées, and extends shelf life, all while consuming moderate energy and requiring low equipment investment [1–3]. FVE addresses post-harvest loss issues and the demand for efficient processing methods in producing countries, supporting the growth of functional foods with health benefits [4,5]. Although this process is used in winemaking to produce high-quality macerates, it has not yet been applied to industrial fruit processing [6]. Lactic acid bacteria (LAB) are recognized for their production of organic acids, vitamins, polyphenolic compounds, and enzymes, which enhance the consumer's diet [3]. Various LAB strains produce different metabolites. Recent research indicates that LAB strains found in fermented foods, such as *Lactiplantibacillus plantarum*, *Lactiplantibacillus pentosus*, *Lactococcus lactis*, *Lacticaseibacillus paracasei*, and *Limosilactobacillus fermentum*, can produce short-chain fatty acids and inhibit pathogenic microorganisms, such as *Salmonella* and *Escherichia coli* [7]. LAB ferment sugars, generating organic acids (e.g., lactic acid and acetic acid) and ethanol. These organic acids are important secondary carbon sources for several microbial genera that thrive during food fermentation [7]. Recent research evaluating organic acids and the microbiota present in a typical Chinese dish called Loatan suncai, which results from the fermentation of various vegetables, notes that organic acids (oxalic acid, malic acid, lactic acid, and acetic acid) are produced by bacteria during fermentation. The accumulation of organic acids can alter the pH of the environment and inhibit the growth of acid-sensitive bacteria, thereby changing the composition of the microbial population. Each fermentation parameter is related to organic acids and microbial population growth [8]. Heterofermentative bacteria produce lactic acid and other organic acids. Several LAB species are affected by their biotechnological attributes related to fermentation, including rapid bacterial activity and animal and human consumption safety.

Additionally, their diverse functional properties, such as the production of secondary metabolites and beneficial organic acids, make them excellent preservatives in fermentation, added to foods as flavorings, additives, and preservatives since they prevent the growth of pathogens during fermentation [9]. Several genes involved in organic acid biosynthesis, carbohydrate transport, and secondary metabolite biosynthesis have been characterized; in particular, genes encoding L-lactate dehydrogenase (EC 1.1.1.27) and acetate kinase (EC 2.7.2.1) are crucial in plant tissue fermentation for generating lactic acid, acetic acid, pyruvic acid, and more [9]. In addition, organic acids and secondary metabolites such as transformed phenolic compounds (ferulic, caffeic, coumaric acids), exopolysaccharides (EPS), vitamins, and bacteriocins are used as starting components in industrial applications, such as food and bio-based products. However, several LAB species have demonstrated the potential to replace synthetic-based technologies for organic acid production due to their ability to utilize inexpensive and renewable feedstocks [9]. Previous studies have examined the effects of *Limosilactobacillus fermentum* J24 on the fermentation of papaya puree obtained by FVE, including its impact on growth and physicochemical parameters, as well as its influence on phenolic compounds and antioxidant capacity [10]. However, no studies have shown the metabolic response of *Lmb. fermentum* J24 to free sugars and organic acids during the fermentation of papaya purée obtained by FVE. This study aimed to evaluate the effect of the FVE process on the metabolism of sugars and organic acids during the fermentation

of papaya purée with *Lmb. fermentum* J24, as well as the genes in the bacteria responsible for carbohydrate metabolism and the enzymes involved in metabolic activity.

2. Materials and Methods

2.1. Generation of Papaya Purees

The papaya (*Carica papaya* L.) variety "Maradol" used in the preparation of the purees was purchased at the consumption maturity stage from a local market in Veracruz, Mexico. The control puree (T0) was prepared by blending the pulp and peel of the papaya (without seeds) with an immersion blender (Braun®, 160 W, GE, Kronberg im Taunus, Germany). The fruits were processed using the Flash Vacuum Expansion (FVE) system at the Instituto Tecnologico de Veracruz. Longitudinal pieces of papaya, seedless and weighing between 0.5 and 1.2 kg, were placed in the heating chamber, which was kept at atmospheric pressure (101.3 kPa), where they were exposed to steam for 15 min (T1) and 20 min (T2) and reached temperatures in the heating chamber of 43 and 54 °C, respectively. Then, the plant material (T1 and T2) passed the expansion chamber (with a pressure of 5 kPa [10,11].

2.2. Papaya Beverages Formulation and Fermentation

The papaya beverages were prepared with 30% (*v*/*v*) papaya purée resuspended in sterile, purified water, following CODEX STAN 247-2005 [12]. Three treatments were prepared: a beverage with control puree (T0) and beverages with puree obtained by the FVE process (T1 and T2). After a 30 min heat treatment at 80 °C to reduce the microbial load. The strain was activated and cultured in MRS broth (DifcoTM, Franklin Lakes, NJ, USA), The biomass was recovered by centrifugation (4500× *g*, 15 min, 4 °C) in a centrifuge equipped with a conical tube rotor (Thermo Scientific™ SorvallTM St 16 R, DE, Waltham, MA, USA). The strain was washed twice with sterile phosphate-buffered saline (PBS, pH 7.2, 0.2 M), then resuspended in the same buffer to achieve a cell density of 1.57, corresponding to 9.0 Log CFU/mL. The cell density was measured at 600 nm using a SpectraMax M3 spectrophotometer (Molecular Devices, Sunnyvale, CA, Waltham, MA, USA). The beverages were inoculated with the previously washed strain at a concentration of 3% (*v*/*v*) and incubated at 37 °C for 24 h. For each treatment (T0, T1, and T2), two beverages were prepared, each with its respective duplicate. Four samples were taken per treatment.

According to previous publications by Castillo-Romero [10], the effective fermentation time is completed after 24 h of bacterial growth phases. In our study, we evaluated free sugars and organic acids content at the start (0 h) and end (24 h) of fermentation.

2.3. Identification and Quantification of Free Sugars and Organic Acids Using HPLC-DAD

The identification and quantification of free sugars and organic acids were performed according to Sigala-Robles [13] with slight modifications. Twenty mg of the sample (T0, T1, and T2) were weighed and dissolved in 1 mL of Milli-Q water. The mixture was stirred for 1 min until complete dissolution. Then, 30 µL of Carrez I and 30 µL of Carrez II were added and stirred for an additional 1 min. These solutions were used to precipitate the protein. The solution was centrifuged at 12,000× *g* for 10 min. The supernatant was filtered through a 0.22 µm nylon filter (Thermo Scientific) and injected into the HPLC system. Chromatographic analysis of sugars and organic acids was conducted with an isocratic flow rate of 5 mM H_2SO_4. The flow rate of this eluent was 0.3 mL/min, and the volume of sample injected was 20 µL. An Aminex HPX-87H ion exclusion column (300 mm × 7.8 mm; BioRad Laboratories, Hercules, CA, USA) was utilized for the analysis. The column was thermostatically maintained at 25 °C. The run time was 25 min and monitored at 196 and 210 nm for free sugars and organic acids, respectively. The peaks were identified by

comparing the retention times with those of the sugar and organic acid standards, and standard curves were prepared for quantification.

2.4. Functional Annotations and Gene Categorization

Genes associated with metabolic subsystems in *Limosilactobacillus fermentum* J24 were identified using the RAST (Rapid Annotations using Subsystems Technology) platform [14], based on the genomic sequences reported by Castillo-Romero [10]. The analysis focused on identifying genes related to carbohydrate metabolism, protein metabolism, and stress response.

2.5. Identification of Active Enzymes in Carbohydrates

Prediction of genes coding for carbohydrate-active enzymes (CAZymes) was performed using the dbCAN3 server (https://bcb.unl.edu/dbCAN2/, accessed on 12 October 2024), with the following parameters: HMMER dbCAN and HMMER dbCAN-sub (E-value < 1×10^{-15}, coverage > 0.35), and DIAMOND (E-value < 1×10^{-102}) [15]. Only CAZymes identified by at least two algorithms were considered for further analysis. The KEGG BlastKoala tool was used to infer the metabolic pathways associated with the predicted CAZyme.

2.6. Statistical Analysis

The Software Minitab® version 19.2020.2.0 was used for statistical analysis. The results of the chemical assays were expressed as mean ± SD of two independent analyses for each treatment and analysis. One-way analysis of variance (ANOVA) was used to verify the difference in free sugars and organic acids between sample groups, separately for the beginning and end. Statistical significance of the difference was tested using Tukey's test at $p \leq 0.05$.

3. Results and Discussion

3.1. Free Sugars

The net change in free sugar components was observed by evaluating the sample before the bacteria became active (0 h), before fermentation, and after fermentation (24 h), in three treatments (T0, T1, and T2).

Figures 1 and 2 show the glucose and fructose content, respectively, in grams per 100 g of dry weight. The data show that, at the beginning of fermentation, treatment T0 had the highest glucose content, followed by treatment T2, and then treatment T1, which had the lowest glucose content. However, after 24 h of fermentation, it was observed that the glucose in T0 had been depleted. The opposite was true for treatments T1 and T2; although these treatments did not consume the glucose, a decrease in its content was observed. At the beginning of fermentation, T0 presented the highest fructose content, followed by T2, and finally, T1. After 24 h of fermentation, total fructose consumption was observed in all three treatments.

Lactic acid bacteria (LAB) use the phosphotransferase system (PTS) to transport and phosphorylate sugars, such as fructose. This process involves using phosphoenolpyruvate (PEP) as a phosphate donor to transport the sugar into the cell. The PTS consists of several proteins that transfer this phosphate in a chain to the transported sugar [16]. The ability of LAB to metabolize carbohydrates is an essential indicator of gene functionality. Different carbohydrate transport pathways, including permeases, the PTS, and the corresponding genes involved in hexose utilization, have been found in LAB genomes [17].

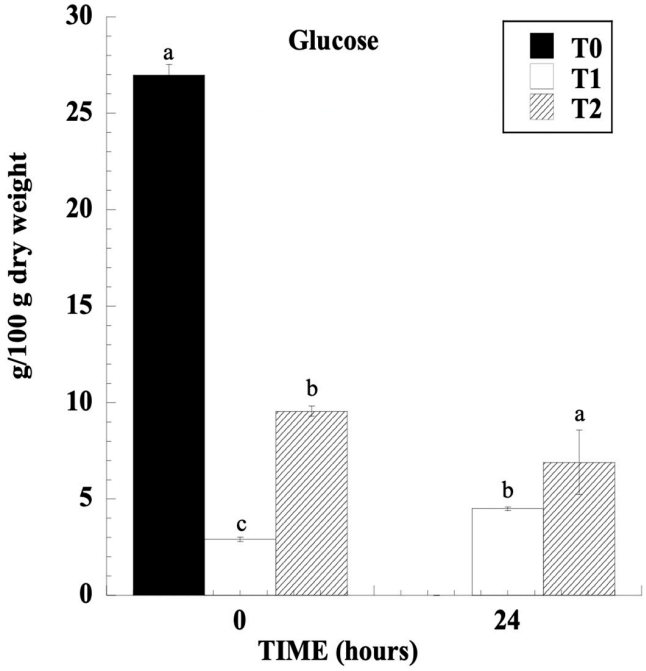

Figure 1. Glucose content in g/100 g dry weight in beverages made with papaya (*Carica papaya* L.) Maradol variety in control (T0), FVE 15 min (T1), and FVE 20 min (T2) treatments at 0 and 24 h of fermentation with *Limosilactobacillus fermentum* J24. Different letters indicate a significant difference at $p < 0.05$, according to Tukey's test for the same evaluation time (ANOVA, Tukey test, $p < 0.05$).

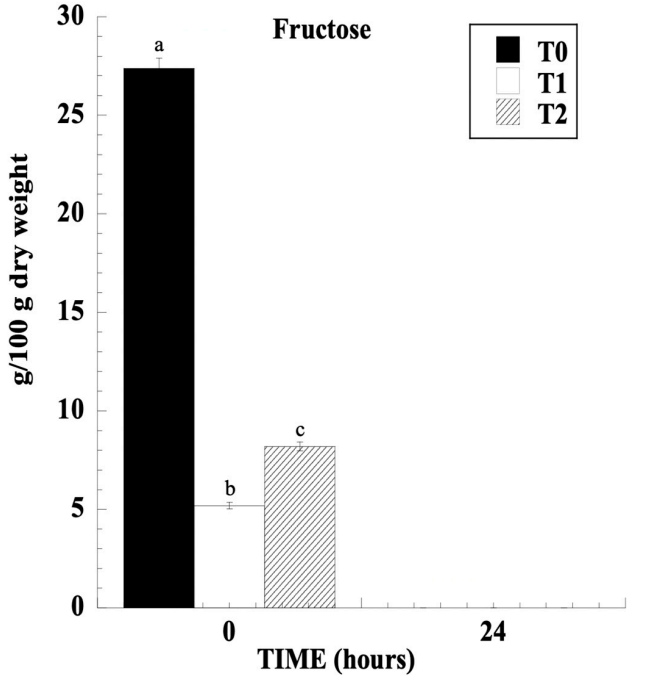

Figure 2. Fructose content in g/100 g dry weight in beverages made with papaya (*Carica papaya* L.) Maradol variety in control (T0), FVE 15 min (T1), and FVE 20 min (T2) treatments at 0 and 24 h of fermentation with *Limosilactobacillus fermentum* J24. Different letters indicate a significant difference at $p < 0.05$, according to Tukey's test for the same evaluation time (ANOVA, Tukey test, $p < 0.05$) (ANOVA, Tukey test, $p < 0.05$).

The PTS is the primary bacterial sugar translocation system. Through a phosphoenolpyruvate molecule, phosphorylation begins, which ends with the internalization of a specific sugar by a group of membrane transporter proteins [18]. Within the PTS, fructose is transported

into the bacterial cell by a membrane-associated diphosphoryl transfer protein (FruT). Fructose is simultaneously phosphorylated to fructose-1-phosphate through the transfer of a phosphoenolpyruvate group to fructose. The generated fructose-1-phosphate is then further phosphorylated to fructose-1,6-bisphosphate by ATP and FruK [19]. In a study by He [20], the researchers evaluated the genome of *Limosilactobacillus fermentum* KUB-D18 and found several sugar transporters, including the PTS. They identified 11 genes specifically related to fructose transport. *Limosilactobacillus fermentum* J24 may have a greater number of genes related to fructose uptake than glucose uptake. Although glucose is one of the main carbon sources for probiotic microorganisms, our study indicates that *Lmb. fermentum* 24 consumes fructose more than glucose. This suggests that *Lmb. fermentum* J24 has great potential for developing fructose-based functional foods and favoring fruit fermentation.

3.2. Organic Acids

The net change in organic acids components was observed by evaluating the sample before the bacteria became active (0 h), before fermentation, and after fermentation (24 h), in three treatments (T0, T1, and T2).

Ascorbic acid, also known as vitamin C, is a compound found in most fruits, particularly citrus fruits. While papaya contains ascorbic acid, it is not present in large quantities like in citrus fruits. Ascorbic acid is an important antioxidant found in various fruit juices, including tomato, pear, pineapple, carambola, and papaya [21]. Figure 3 shows the ascorbic acid content in beverages made with papaya purée. At the beginning of fermentation, T0 has the highest ascorbic acid content at 0.21 ± 0.004 g/100 g. T1 has the second highest content at 0.056 ± 0.0006 g/100 g, and T2 has the lowest content at 0.014 ± 0.022 g/100 g. There are significant differences between the treatments.

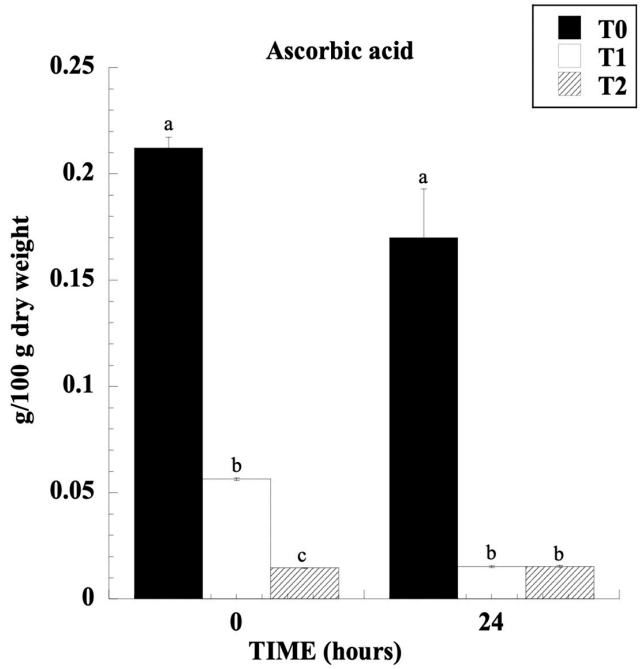

Figure 3. Ascorbic acid content in g/100 g dry weight in beverages made with papaya (*Carica papaya* L.) Maradol variety purees in control (T0), FVE 15 min (T1), and FVE 20 min (T2) treatments at 0 and 24 h after fermentation with *Limosilactobacillus fermentum* J24. Different letters indicate a significant difference at $p < 0.05$, according to Tukey's test for the same evaluation time (ANOVA, Tukey test, $p < 0.05$) (ANOVA, Tukey test, $p < 0.05$).

In the present study, at the beginning of fermentation, treatments T1 and T2 had lower ascorbic acid content than the control treatment (T0). Ascorbic acid is a quality control factor in

food because it degrades under various environmental conditions, such as exposure to light or temperatures above 40 °C [22]. In the purée obtained by FVE technology, the fruit (papaya) in treatments T1 and T2 reaches temperatures of 43 °C and 54 °C, respectively, during the heating stage [11]. This may explain the decrease in ascorbic acid content in treatments involving the FVE process. Conversely, the control puree is not subjected to thermal processes, which could benefit the stability or conservation of ascorbic acid. Therefore, in the control treatment (T0), the decrease in ascorbic acid content is minimal.

Figure 4 shows the citric acid content of beverages made with papaya purée. Before fermentation, T0 has the highest citric acid content at 2.033 ± 0.018 g/100 g dry weight. T1 has the second highest content at 1.21 ± 0.03 g/100 g dry weight, and T2 has the third highest content at 1.14 ± 0.02 g/100 g dry weight. T1 and T2 do not differ significantly from each other, but they differ significantly from T0. By the end of fermentation, T0 shows an absence of citric acid. T1 shows 1.29 ± 0.012 g/100 g dry weight. However, T2 shows an increase in citric acid, reaching 1.54 ± 0.02 g/100 g dry weight after 24 h of fermentation. In a study by Yu [23], it was mentioned that *Limosilactobacillus fermentum* strains can preferentially utilize citric acid from tangerines during fermentation of tangerine juice to support growth without consuming sugar. After six hours of fermentation, the sugar-to-acid ratio of the juice increased from 12:1 to 22:1. This resulted in higher hedonic sweetness, acidity, and overall acceptability scores for the fermented, pasteurized juice than for the unfermented, pasteurized juice. Therefore, light fermentation with *Lmb. fermentum* can improve the flavor of citrus juice by adjusting the sugar–acid ratio while maintaining good quality. Citric acid is used in the food industry because of its pleasant sour taste, high water solubility (62.07% at 25 °C), and slight hygroscopicity. The presence of citric acid in fruit-based beverages is desirable because it enhances the beverage's sensory quality. The presence of citric acid is associated with fruity flavors and a sensation of freshness, which positively impacts the beverage's pleasantness. Citric acid is primarily found in fruits such as lemons, tangerines, pineapples, plums, and peaches, among others [24,25].

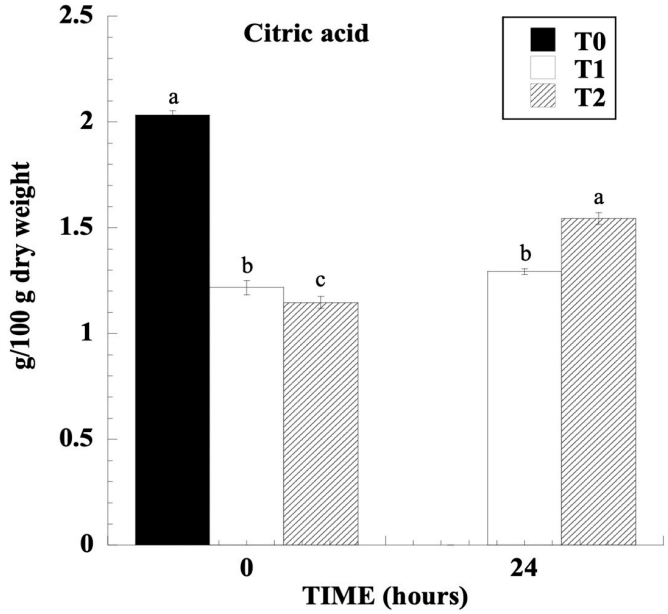

Figure 4. Citric acid content in g/100 g dry weight in beverages made with papaya (*Carica papaya* L.) Maradol variety purees in control (T0), FVE 15 min (T1), and FVE 20 min (T2) treatments at 0 and 24 h after fermentation with *Limosilactobacillus fermentum* J24. Different letters indicate a significant difference at $p < 0.05$, according to Tukey's test for the same evaluation time (ANOVA, Tukey test, $p < 0.05$) (ANOVA, Tukey test, $p < 0.05$).

Figure 5 shows the lactic acid content of beverages made with papaya purée. At hour 0, the lactic acid content was 0.15 ± 0.008 g/100 g dry weight for T0, 0.15 ± 0.004 g/100 g dry weight for T1, and 0.33 ± 0.005 g/100 g dry weight for T2, respectively. After 24 h of fermentation, T0 had the highest lactic acid content at 10.99 ± 0.11 g/100 g dry weight. T1 followed with 3.86 ± 0.07 g/100 g dry weight, and T2 followed with 2.85 ± 0.05 g/100 g dry weight. There were significant differences between the treatments. These results coincide with those previously reported by Castillo-Romero [10] in the quantification of titratable acidity. Treatment T0 presented the highest lactic acid content during papaya puree fermentation with *Limosilactobacillus fermentum* J24. For lactic acid bacteria, lactic acid production indirectly reflects microbial growth. The results in this paper are consistent with those reported for the same treatments, in which T0 exhibited the fastest growth rate and shortest generation time [10], which translates to higher lactic acid production than T1 and T2. In a study by Chen [3], papaya juice was fermented with *Lactobacillus acidophilus* GIM1.731 and *Lactiplantibacillus plantarum* GIM1.731. Lactic acid was the most abundant by the end of fermentation with both strains. This trend is similar to the results obtained in the present study, in which fermentation of papaya purée beverages with *Limosilactobacillus fermentum* J24 shows that lactic acid is the most abundant acid by the end of fermentation. Lactic acid, a distinctive product formed by LAB during fermentation, induces a local microenvironment that is unfavorable for pathogenic microorganisms [26]. According to a study by Wang [27], a lactic acid concentration of 0.5% (*v*/*v*) prevents the growth of pathogens such as *Salmonella*, *E. coli*, and *L. monocytogenes*.

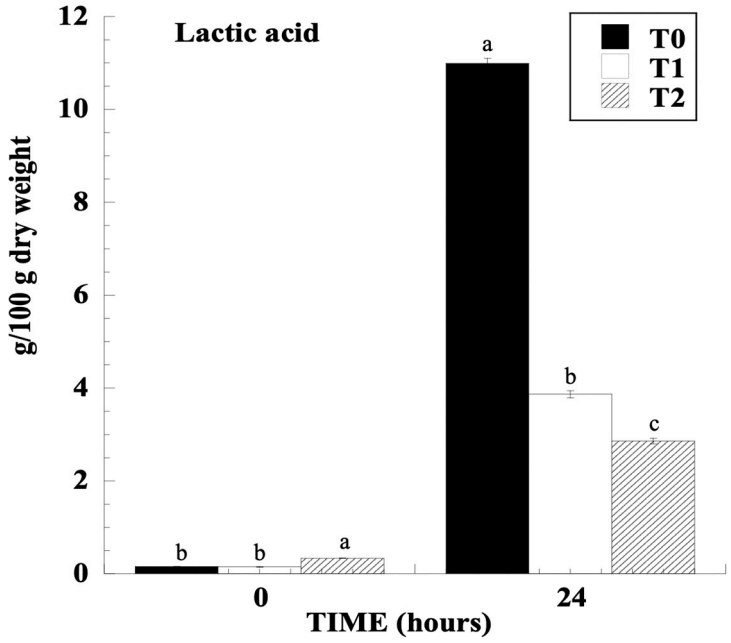

Figure 5. Lactic acid content in g/100 g dry weight in beverages made with papaya (*Carica papaya* L.) Maradol variety purees in control (T0), FVE 15 min (T1), and FVE 20 min (T2) treatments at 0 and 24 h after fermentation with *Limosilactobacillus fermentum* J24. Different letters indicate a significant difference at $p < 0.05$, according to Tukey's test for the same evaluation time (ANOVA, Tukey test, $p < 0.05$) (ANOVA, Tukey test, $p < 0.05$).

3.3. Genomic Data Associated with Limosilactobacillus fermentum J24

Functional genome analysis, which involves identifying and characterizing the genes of an organism, is essential for understanding its cellular and metabolic processes, as well as its beneficial characteristics for probiotic and industrial applications (Tables S1–S7). We further analyzed the metabolic genes identified in *Lmb. fermentum* J24, we classified the genes

into metabolic functional categories (Figure 6). The most abundant category was amino acid transport and metabolism (120 genes), followed by carbohydrate transport and metabolism (115 genes), protein transport and metabolism (100 genes), CAZymes (86 genes), lipid transport and metabolism (40 genes), and stress response (6 genes). Among the metabolic genes identified in *Lmb. fermentum* J24, one of the most abundant, was involved in carbohydrate transport and metabolism (115 genes), nine of which were associated with the pentose phosphate pathway. This pathway is associated with carbohydrate metabolism in heterofermentative bacteria, such as *Lmb. fermentum* J24.

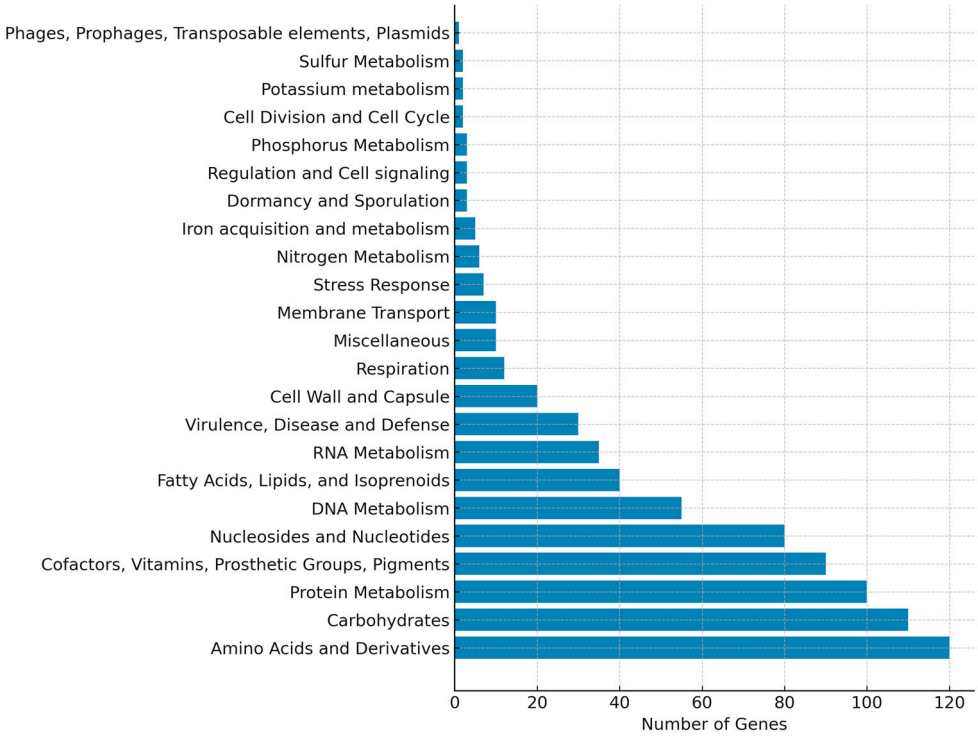

Figure 6. Distribution of annotated genes in the main functional categories predicted by the RAST server.

3.4. Sugar Metabolism

The presence of the pathways of central carbohydrate metabolism, including pyruvate oxidation and, pentose phosphate pathway, was observed. Although the strain carries all the genes necessary for the reconstruction of the intact pyruvate oxidation pathway (4 genes), the pentose phosphate pathway (9 genes). The other carbohydrate metabolic pathways, such as the uptake and utilization of both lactose and galactose, were identified, including the genes galactokinase, EC: 2.7.1.6, UDP-glucose 4-epimerase, EC: 5.1.3.2; aldose 1-epimerase, EC: 5.1.3.3; galactose-1-phosphate uridylyl transferase (EC: 3.2.1.26). The ability of a bacterium to metabolize lactose and galactose is an important criterion when carrying out starter culture selection, which makes the strain *Limosilactobacillus fermentum* J24 suitable for use as a starter culture [28].

3.5. Carbohydrate-Active Enzymes

Genes encoding carbohydrate-active enzymes (CAZymes) were identified in the genome of *Lmb. Fermentum* J24, which provides more information about carbohydrate utilization. CAZymes degrade, create, or modify glycosidic bonds and are essential in processes such as digestion, fermentation, biofuel production, and food biotechnology; they are classified into families according to their structure and function, and are organized in the CAZy database (www.cazy.org accessed on 12 October 2024) [29]. Among

the main classes of CAZymes in the genome of *Lmb. fermentum* J24 genome, 33 genes were detected, of which 7 belong to the family of glycosyl hydrolases (GH), responsible for breaking glycosidic bonds between sugars, for example, cellulases, xylanases, amylases, β-glucosidases; 8 belong to glycosyl transferases (GT), which form glycosidic bonds by transferring sugars from donors to acceptors and are important for the synthesis of polysaccharides and glycoconjugates; 2 are found in carbohydrate binding modules (CBM), which help enzymes to bind to insoluble substrates such as cellulose [30,31]. GH families identified in the analyzed strain are linked to potential substrates (dbCAN) and inferred metabolic pathways (BlastKoala). GH73 shows the highest frequency (4 genes), suggesting relevance in peptidoglycan degradation and a role in cell wall remodeling. GH13, GH14, GH15, GH2, GH32, GH36, and GH65 occur at lower frequency (1 gene each) and are mainly associated with the metabolism of sucrose, trehalose, starch, and galactans, which are polysaccharides composed mainly of galactose units, supporting the strain's ability to use common sugars from fermented foods or its environment (Figure 7).

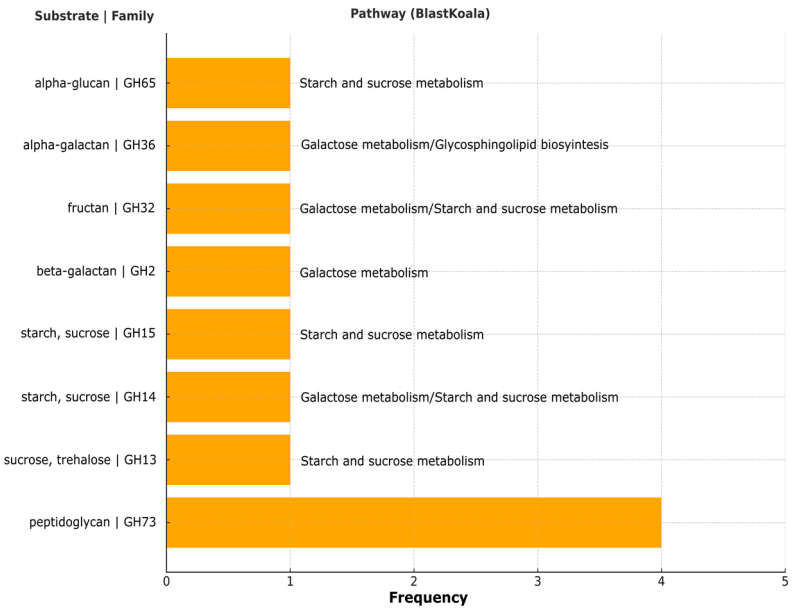

Figure 7. Frequency of glycoside hydrolase (GH) families, their predicted substrates (dbCAN), and associated metabolic pathways (BlastKoala). GH73 shows the highest count (4 genes), mainly linked to peptidoglycan hydrolysis. The other GH families occur once and are related to the metabolism of common carbohydrates such as sucrose, trehalose, starch, and galactans.

In a recent study by Wei [32], they identified CAZymes in the genome of *Limosilactobacillus fermentum* strain A51 by highlighting the presence of glycosyl hydrolases (GH) and carbohydrate esterases (CE), which play a crucial role in the degradation of polysaccharides and oligosaccharides present in fruit fermentation. Therefore, our results suggest that the presence of CAZymes in *Limosilactobacillus fermentum* J24 allows the bacteria to utilize polysaccharides and oligosaccharides as a carbon source in fruit fermentation.

3.6. Acetoin and Butanediol Production

Acetoin, an organic compound, plays a crucial role in several industries due to its chemical and sensory properties. It is associated with desirable sensory profiles in fermented products, such as cheeses and yogurts, and is used in the cosmetics industry as an aromatic ingredient [33]. Butanediol is a highly relevant compound in industrial applications, such as the production of plastics and as a biofuel and additive in plastics. It is also used as a wetting and stabilizing agent in food and cosmetic products due to its low

toxicity and biodegradability [34,35]. Four genes associated with the biosynthesis pathway of acetoin and butanediol production were identified in the genome of *Lmb. fermentum* J24. The first corresponds to catabolic acetatolactate synthase (EC 2.2.1.6), an enzyme that catalyzes the condensation of pyruvate to form acetolactate. The second gene encodes α-acetolactate decarboxylase (EC 4.1.1.5), responsible for the decarboxylation of acetolactate and the subsequent production of acetoin. Subsequently, acetoin can be reversibly reduced to 2,3-butanediol by the action of 2, 3-butanediol dehydrogenase S-formate, specific for acetoin (R) (EC 1.1.1.4). Finally, acetoin (diacetyl) reductase (EC 1.1.1.304) is involved in the irreversible reduction of diacetyl to acetoin. This information coincides with that reported by Hossain [17], where the same genes for the production of acetoin and butanediol were found in the genome of the strain *Limosilactobacillus fermentum* LAB-1.

3.7. Stress Response

Six genes associated with different stress responses were identified in the genome of Lmb. fermentum J24, including those involved in dimethyl arginine metabolism (NG, NG-dimethylarginine dimethylaminohydrolase 1 [EC 3.5.3.18]) and the periplasmic stress response (intramembrane protease RasP/YluC, which is involved in cell division through FtsL cleavage). The DedA protein is involved in maintaining membrane homeostasis and transporting toxic ions or compounds during selenite and selenate uptake. Although its function is not fully elucidated, recent studies in Ralstonia metallidurans suggest a link between the DedA protein and selenite resistance. This makes the DedA protein a promising candidate for bioremediation applications in selenium-contaminated environments [36,37]. Recent studies of lactic acid bacteria, such as *Lactobacillus casei* ATCC 393, have demonstrated their ability to absorb and transform inorganic selenium compounds, such as selenate and selenite, into less toxic and more bioavailable forms. These include selenium amino acids and selenium nanoparticles (SeNPs) [38]. Glutathione plays a crucial role in redox cycling and glutaredoxins (glutaredoxin-like protein NrdH, which is required for the reduction of class Ib ribonucleotide reductase). Glutathione also acts as a gamma-glutamyl and glutamate–cysteine ligase (EC 6.3.2.2). This enzyme, also known as γ-glutamylcysteine synthetase, plays a crucial role in glutathione biosynthesis. It catalyzes the first and limiting step in the synthesis of glutathione, an essential antioxidant that protects cells from oxidative stress. Several studies have shown that strains of Limosilactobacillus fermentum (CECT5716 and ME-3) can synthesize and transport glutathione, indicating a complete transport system and demonstrating the ability of these strains to utilize glutathione in protecting against oxidative stress [39,40]. The results suggest that *Limosilactobacillus fermentum* J24 may contribute to antioxidant protection in fermentative environments, as well as in probiotic applications.

4. Conclusions

Both the FVE process and fermentation with *Limosilactobacillus fermentum* J24 were found to influence the free sugar content at the beginning and end of the fermentation process in papaya-based beverages. Regarding organic acids, heat treatment primarily affected ascorbic acid levels, whereas fermentation significantly impacted lactic and citric acid concentrations. The FVE process and fermentation both contributed to lower glucose and fructose levels. This represents an advantage for developing functional beverages with low sugar content. The predominant presence of citric and lactic acids favors the microbiological stability of the final product. Conversely, the presence of CAZymes genes in *Limosilactobacillus fermentum* J24 enables the bacterium to utilize polysaccharides and oligosaccharides as carbon sources during fruit fermentation. Additionally, the stress re-

sponse genes in *Lmb. fermentum* J24 suggests that it can contribute to antioxidant protection in fermentative environments and probiotic applications.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/beverages11050150/s1, Table S1: Genes associated with amino acid metabolism L. fermentum J24; Table S2: Genes associated with protein metabolism L. fermentum J24; Table S3: Genes associated with carbohidrate metabolism L. fermentum J24; Table S4: Genes associated with lipid metabolism L. fermentum J24; Table S5: Genes associated with lipid metabolism L. fermentum J24; Table S6: Genes associated with stress response metabolism L. fermentum J24; Table S7: Genes of Cazymes.

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