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Sustainable Processes and Physico-Chemical Characterization of Artisanal Spontaneous Gluten Free Sourdough (Quinoa, Amaranth and Brown Rice) Compared to Wheat Sourdough

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Abstract: The industry predominantly depends on synthetic or artificial additives, occasionally permitting the inclusion of natural molecules sourced from plants or replicated from their original counterparts. The production of bakery products increasingly uses sourdough to improve the quality of bread or to obtain "clean label" products (free of artificial additives). The additive production sector contributes to this concern through the synthesis of potentially harmful compounds, the utilization of hazardous chemicals and solvents, the management of resulting by-products, and reliance on non-renewable resources for manufacturing. One percent of the world's population suffers from celiac disease. Celiac disease is treated by excluding gluten from the diet. Most gluten-free bakery products have low nutritional and sensory quality. Therefore, sourdough is being used to replace chemical yeast to improve the sensory and nutritional quality and increase the shelf life of gluten-free bakery products. Three gluten-free sourdoughs were prepared with different flours: brown rice, quinoa and amaranth, in order to compare them with traditional sourdough (wheat) and optimize the most suitable temperature for the conservation of sourdoughs. Physicochemical analysis (pH, titratable acidity and color), antioxidant activity (FRAP, ORAC and ABTS), total phenolic compound content (Folin–Ciocalteu), total aflatoxin content, lactic and acetic acid content and microbiological analysis (mold and yeast content and bacterial and fungal composition (microbiota composition)) were carried out during the elaboration process and at different storage temperatures. A higher microbiological quantity of molds and yeasts (7.97 log CFU/mL), non-*Saccharomyces* yeasts (7.78 log CFU/mL) and lactic acid bacteria (8.10 log CFU/mL) and fungal composition were observed in the amaranth sourdough. The wheat sourdough obtained a higher total content of phenolic compounds (33.03 mg GAE g^{-1}) and antioxidant capacity in ABTS and FRAP, but the quinoa sourdough had the highest ORAC content. In addition, it was observed that the adequate temperature for the conservation of the doughs is 25 °C, due to the predominance of *Lactobacillus* spp. and *Pediococcus* spp. bacteria in the sourdough. Therefore, pseudocereal sourdoughs (quinoa and amaranth) could be an alternative to incorporate into the preparation of gluten-free bread, since their microbial composition, physicochemical composition, antioxidant activity and total phenolic compounds would contribute to gluten-free bread and thus produce health benefits for people with celiac disease.

Keywords: sourdough; pseudocereals; microbiome; antioxidant; clean label

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1. Introduction

Sourdough is a mixture of flour and water spontaneously fermented by lactic bacteria and yeasts with acidifying and leavening capacity [1]. It is an ancient biotechnological process; this revival of natural fermentation has captured scientific interest for the positive

effects on breads, namely for improving their nutritional value, sensory quality and shelf life and for the absence of additives in the products with respect to conventional breads [2–5]. In fact, the production and consumption of sourdough bread have been positively affected by increasing demand for the use of sourdough as a replacement for baker's yeast in bakery products, encouraged by the interest of health-conscious consumers [6].

Sourdough fermentation could also be a tactic to improve several aspects of gluten-free bread, a product aimed at the celiac population or those with some gluten sensitivity. In this context, producing gluten-free breads with sensory characteristics that meet consumer expectations is a challenge for the bakery industry, since the absence of the viscoelastic gluten network hinders the entire bread-making process and negatively affects the sensory quality of the final product [7–9]. To solve this problem, the industry uses chemical yeasts, however, the demand for clean-label products is increasing, and the use of chemical yeasts is often criticized [10]. Therefore, sourdough fermentation has been considered a promising option to achieve gluten-free breads with a better texture, aroma and nutritional value, contributing to their greater acceptance [11].

The development of fermentation technologies for wheat-alternative flours such as pseudocereals (e.g., quinoa, amaranth) can be seen as an opportunity to meet the demand for more natural and healthier foods [2,12]. Pseudocereals such as quinoa and amaranth have triggered much interest in recent years because of their excellent nutritional profiles and health benefits [12]. Pseudocereals are noted for their high protein content with a balanced amino acid composition and also for being an important source of dietary fiber, vitamins and minerals [13–15]. In addition, quinoa and amaranth seeds are gluten-free pseudocereals and are considered healthy ingredients for developing gluten-free foods [3]. Additionally, pseudocereal crops are considered promising for the future due to their high genetic variability, which is advantageous for adapting to different environments, from tropical to temperate climatic conditions [16]. Therefore, this type of fermentation is a sustainable alternative, contributing to the development of innovative products with high nutritional value [17,18].

The main benefits linked to sourdough fermentation come from the microbiota composition of the sourdough. It is notable for containing mainly lactic acid bacteria (LAB), but also *Saccharomyces* and non-*Saccharomyces* yeasts [19]. The composition of the microbiota This microbiota depends primarily on the type of flour, the fermentation conditions and the processing environment. The metabolic process developed during sourdough processing results in a diverse and stable microbiota in mature sourdough, in which the species best adapted to the environmental conditions predominate [20,21]. Temperature and pH are the exogenous factors that most influence the diversity of the sourdough microbiota, being decisive in the selection of the most abundant species.

In this study, three gluten-free flours (quinoa, amaranth and brown rice) were used as substrates for sourdoughs, and three storage conditions (freezing, refrigeration and room temperature) were evaluated. The objective was to characterize the composition of gluten-free flour (quinoa, amaranth and brown rice) and gluten (wheat), such as the amount of polyphenols, in vitro antioxidant capacity, microbiology, microbiota characterization and physicochemical characteristics. In addition, the four sourdoughs were compared from the microbial point of view under three storage conditions.

2. Materials and Methods

2.1. Materials

The durum wheat flour and whole wheat flour were purchased in a local supermarket (Murcia, Spain). Amaranth flour was obtained from EcoAndesImportExport, Madrid, Spain. Quinoa flour was obtained from Legumbres Pedro, Cádiz, Spain. Brown rice flour was supplied from Biovitagral, Teglio, Italy.

2.2. Methods

2.2.1. Sourdough Preparation

The quinoa, amaranth, brown rice and wheat sourdoughs were made according to the method suggested by Katina et al. (2007) [22], with modifications. The four sourdoughs were made following the spontaneous fermentation method, which consists of mixing the flour with tap water in a 50:50 ratio, and the mixture was fermented at 25 °C for 24 h, refreshing it by renewing the flour and water for 4 days. For the preparation of wheat sourdough, everything is the same except that wheat and whole wheat flour are mixed in a 1:1 ratio.

2.2.2. Physico-Chemical Parameters

The pH was determined using a pH meter (Crison GLP22, Alella, Spain) after homogenizing the sourdough with distilled water at room temperature in a ratio of 1:10. Once the pH was measured, the total titratable acidity (TTA) was measured by titration with NaOH 0.01 N and expressed as ml NaOH/10 g. Color was measured using a Konica Minolta CR-410 colorimeter (Minolta Camera Co., Osaka, Japan), and the DP-400 data processor of the "AQ instrument" was used to measure slice color (CIE Lab* values). CIE L* values (lightness), CIE C* values (saturation), a* values (red–green), b* values (yellow–blue) and h (hue) were measured. Three replicates were averaged for each sample. These analyses were measured during sourdough processing (4 days).

2.2.3. Acetic Acid and Lactic Acid Content

The determination of L-lactic and acetic acid was carried out in mature sourdough using the specific acetic acid kit (acetate kinase) and a specific L-lactic acid kit (L-lactate dehydrogenase) (Byosistems S.A, Barcelona, Spain), respectively, following the manufacturer's guidelines. The results were expressed in g L^{-1}. Three replicates were averaged for each sample.

2.2.4. Microbiological Analysis for Molds, Yeast and Lactic Acid Bacteria

Non-*Saccharomyces* yeasts, lactic acid bacteria (LAB), total yeast and total molds were present in the four flour and sourdough samples on day 4. All the samples were analyzed in triplicate, and the counts were expressed as log colony forming units per milliliter (Log CFU/mL). Samples were prepared in a horizontal laminar flow cabinet (Telstar, BIO-II-A, Madrid, Spain) sterilized with UV irradiation. Under aseptic conditions, 10 g of sample was weighed into a stomacher bag with a filter, and 90 mL of sterile NaCl (0.9% w/v) serum was added and homogenized using the stomacher. Dilutions were obtained with this mixture. Total yeast and mold counts were performed on nutrient agar WL (Scharlab, Barcelona, Spain), non-*Saccharomyces* yeasts on lysine agar (Scharlab, Spain) and LAB on ManRogosa-Sharpe agar (MRS agar) (BIORAD, Madrid, Spain) with an adjusted pH of 5.5. WL plates were incubated at 28 ± 1 °C under anaerobic conditions for 3 days. Lysine plates were incubated at 25 ± 1 °C under aerobic conditions for 3 days. For MRS plates, they were incubated at 28 ± 1 °C for 3 days in an oxygen atmosphere reduced to less than 10% using a candle jar. The media used and NaCl serum were autoclaved at 121 °C for 20 min.

2.2.5. Sample Extraction

Extraction for the determination of total polyphenol content and antioxidant activity was performed using methanol (80% v/v). Eight ml of solvent were added to 2 g of sample (flour and sourdough) and left in the dark at 4 °C for 24 h. The samples were then centrifuged at 4500 rpm for 25 min at 4 °C. Finally, the supernatant was filtered through 0.45 mm filters and stored at −20 °C until analysis. The extraction was performed in triplicate.

2.2.6. Antioxidant Activity and Total Phenolic Content (TPC)

The total phenolic content (TPC) was determined according to the method suggested by Singleton and Rossi (1965) [23], using a Folin–Ciocalteau reagent, Na_2CO_3 (2%) and gallic acid as standard (20, 40, 60, 80, 100 mg/L). The absorbance of the extracts was measured at 750 nm. The analysis was performed in triplicate, and the TPCs were expressed as mg of gallic acid equivalents (GAE)/g. The radical cation scavenging activity (ABTS) was performed following the protocol described by Re et al. [24]. Then, ABTS radical cations were prepared by reacting 7 mM ABTS (2,2-azinobis(3-ethylbenzothiazolin)-6-sulfonic acid) with 2.45 mM potassium persulfate (1:1 v/v) pH = 7.4. This solution was adjusted to an absorbance of approximately 0.700 at 734 nm. In total, 1 mL of ABTS was added to 100 µL of sample. The absorbance of the mixture was measured at 734 nm in the spectrophotometer (Thermo Scientific Evolution 300 UV-Vis, Waltham, MA, USA) after reacting for 2 min at room temperature.

Ferric reducing power analysis was determined following the protocol of Benzie and Strain (1999) [25], with some modifications. Then, the FRAP reagent was prepared with 20 mL of 300 mM acetate buffer solution, pH = 3.6, 2 mL of 20 mM $FeCl_3$—6 H_2O and 2 mL of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in a 40 mM HCl solution. Then, 1 mL of FRAP reagent was mixed with 100 µL of sample or 500 mm of standard solution in plastic cuvettes. Finally, after 4 min of incubation at 37 °C in dark conditions, the absorbance was measured at 593 nm against a blank. The antioxidant activity of the sample was expressed as µM Trolox equivalents (TE) per g of sample.

The oxygen radical absorbance capacity (ORAC) method described by Prior et al. [26] was followed to measure the hydrophilic antioxidant capacity. All sample dilutions were prepared in triplicate. The results were obtained by means of GEN 5 software, in which the area under the curve was obtained and the data were extrapolated thanks to the Trolox standard curves. The antioxidant activity of the sample was expressed as Trolox equivalents (TE) µM per g of extract.

2.2.7. Content of Total Aflatoxins

Total aflatoxin concentration was analyzed using a specific Ridacreen total aflatoxin kit (R-Biopharm AG, Darmstadt, Germany) through an enzyme immunoassay for the quantitative determination of aflatoxin, following the respective manufacturer's instructions. Results were expressed in µg/kg. Three replicates were averaged for each sample.

2.2.8. Total Microbial Genomic DNA Extraction

The DNA sample was extracted from 200 mg of doughs at three times under three storage conditions: before fermentation (D0), after 4 days of fermentation (D4) and after 14 (D14) days of sourdough propagation when stored at 25 °C, stored at 4–5 °C (R) and stored at −20 °C (F). Extraction was performed with the specific FiberStool DNA extraction Kit (Universidad de San Sebastián, Santiago de Chile, Chile) according to the manufacturer's instructions. The extracted DNA was eluted in 50 µL of purified water and stored at −80 °C. The concentration of extracted DNA was determined using a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, DE, USA). A DNA concentration of ≥20 ng/µL, a total amount of ≥500 ng and a ratio of A260/280 of 1.8–2.0 were used for the subsequent polymerase chain reaction.

2.2.9. Illumina Sequencing and Bioinformatic Analysis

The sequencing of the V4 region of the 16S rRNA was performed through sequencing by synthesis with the Miseq illumina equipment using 50 ng of bacterial genomic DNA from each sample. This technique consists of the hybridization of a specific sequence of the V4 region of the 16S rRNA subunit through the formation of clusters, subsequently sequenced by sequencing by synthesis. This consists of the one by one detection of the fluorescent nucleotides that bind to the templated sequence. Once the sequencing is finished, it is compiled in an Excel file. The partitions used were 515F (5′-GTGCCAGCMGCCGCGGTAA-

3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), generating a product of 250 base pairs to subsequently perform the demultiplex of these sequences using MetaScope (v4.1). Subsequently, the sequences were cut in order to maintain 99% confidence using a quality score analysis [27]. Finally, existing chimeras were removed, and the sequences were grouped into operational taxonomic units, which allowed us to have an idea of evolutionary relatedness before assigning their taxonomy. The UNITE Database was used for the taxonomic assignment of the ITS.

2.2.10. Statistical Analysis

All statistical analysis was performed using IBM SPSS Statistics® 28 software (IBM Corporation, Armonk, NY, USA). Statistical analysis was performed using the one-way ANOVA test, and differences were considered statistically significant at $p < 0.05$. Values were represented as mean values ± standard deviations.

3. Results and Discussion

3.1. Physico-Chemical Parameters

Table 1 summarizes trends in selected processing indicators (pH, acidity (TTA) and color) during the maturation process. As expected, all indicators were affected by the maturation process of the sourdough over time. The pH of all the sourdoughs decreased during the 4 days of the elaboration process, and the greatest decrease was observed between day 1 and day 2, highlighting the brown rice sourdough with the greatest decrease, followed by amaranth sourdough, quinoa sourdough and wheat sourdough. From day 2 inclusive, the pH of all sourdoughs remained below 4.2, the optimum pH according to Regulation 308/2019 of 26 April, which approves the quality standard for bread [28]. On the last day of maturation, the lowest pH value was observed in the wheat sourdough (3.65 ± 0.17) and the highest value in the quinoa sourdough (4.11 ± 0.12). Lower values than those obtained by Carbó et al. [29], where they elaborated a gluten-free sourdough mixing quinoa, amaranth and buckwheat in 30 °C incubation and found values between 4.04 and 5.00. However, Harth et al. [30] in barley sourdough at an incubation temperature of 30 °C obtained a pH between 3.5–3.6, more similar to the value obtained for wheat sourdough (3.65 ± 0.17). Sterr et al. [31] in their study of amaranth sourdough elaboration in 30 °C incubation obtained lower pH values compared to the value obtained from amaranth sourdough (4.09 ± 0.08) in the present study. Therefore, it is deduced that the pH differences could be due to the protocol and recipe to be followed for the preparation of sourdoughs. Regarding TTA, an increase in acidity was observed in all sourdough samples as the maturation time passed. The samples showed an increasing trend in TTA as the pH decreased. Normally the pH is related to TTA, since the pH values represent the amount of strong acid metabolized by the microorganisms in the sourdough, while the TTA indicates the total acidity of the sourdough. However, there are contradictions, since the lowest pH was observed in the wheat dough and the highest acidity sourdough was observed in the amaranth dough, followed by the quinoa sourdough, the brown rice sourdough and wheat sourdough. Amaranth sourdough obtained the highest titratable acidity value (29.50 mL NaOH/10 g) on the last day of ripening, followed by quinoa sourdough (28.25 mL NaOH/10 g), brown rice sourdough (23.57 mL NaOH/10 g) and wheat sourdough (16.84 mL NaOH/10 g). However, in the study of Vogelmann et al. [32], where eleven cereal and pseudocereal sourdoughs were studied, higher values of titratable acidity were found, and the highest value was obtained in quinoa sourdough, followed by amaranth, and finally wheat, buckwheat and the remaining seven. The fact that the pH and TTA values in the present work coincide and differ, respectively, with those obtained by other authors could be explained by the buffering capacity of each pseudocereal and cereal. Some components present in flour, such as proteins, phytates or minerals, also have buffering capacity. It has been shown that TTA has a relationship with phytate concentration [33] and that high concentrations of minerals such as iron, sodium, potassium, magnesium and phosphorus have an action as a buffering agent [34]. Between days 2 and

3, the greatest increases in total titratable acidity greater than 10 mL of 0.1 M NaOH were found, the optimal acidity according to Regulation 308/2019, of April 26, which approves the quality standard for bread [28].

Table 1. Physical-chemical quality evolution of sourdough for four days of elaboration.

		Processing Days			
Parameters	**Sample**	**0**	**1**	**2**	**3**
pH	Quinoa	6.55 ± 0.46 ^{a,1}	5.91 ± 0.09 ^{a,1}	4.39 ± 0.08 ^{b,1}	4.11 ± 0.12 ^{b,1}
	Amaranth	6.72 ± 0.25 ^{a,1}	5.90 ± 0.35 ^{b,1}	4.27 ± 0.15 ^{c,1}	4.09 ± 0.08 ^{c,1}
	Brown rice	6.64 ± 0.25 ^{a,1}	5.70 ± 0.26 ^{b,1,2}	4.06 ± 0.20 ^{c,1}	3.97 ± 0.18 ^{c,1}
	Wheat	5.89 ± 0.14 ^{a,2}	5.24 ± 0.07 ^{b,2}	4.42 ±0.07 ^{c,1}	3.65 ± 0.17 ^{d,1}
TTA (ml NaOH/10 g)	Quinoa	7.33 ± 0.29 ^{a,1}	6.98 ± 0.75 ^{a,1}	17.47 ± 1.82 ^{b,2}	28.25 ± 2.96 ^{a,1}
	Amaranth	4.67 ± 1.26 ^{a,1}	10.83 ± 0.76 ^{a,3}	25.08 ± 1.48 ^{a,2}	29.50 ± 6.50 ^{c,a,2}
	Brown rice	3.93 ± 1.40 ^{a,1}	7.67 ± 2.25 ^{a,3}	10.73 ± 2.81 ^{b,3}	23.57 ± 4.01 ^{a,b,2}
	Wheat	2.70 ± 0.39 ^{b,1}	4.45 ± 0.31 ^{b,1}	11.41 ± 0.78 ^{b,2}	16.84 ± 1.31 ^{b,2}
L*	Quinoa	62.44 ± 1.35 ^{a,1}	65.05 ± 0.47 ^{a,1}	63.64 ± 1.43 ^{a,1}	72.71 ± 3.81 ^{b,2}
	Amaranth	80.09 ± 0.17 ^{b,2}	69.01 ± 0.61 ^{b,1,3}	72.63 ± 0.42 ^{b,1}	66.47 ± 1.06 ^{c,3}
	Brown rice	57.04 ± 0.50 ^{c,3}	62.27 ± 1.33 ^{a,1}	66.98 ± 0.76 ^{a,2}	65.39 ± 1.12 ^{c,1,2}
	Wheat	71.95 ± 0.35 ^{1}	73.71 ± 0.81 ^{c,1}	79.16 ± 0.72 ^{c,3}	81.59 ± 0.16 ^{a,3}
C*	Quinoa	17.43 ± 0.26 ^{a,1}	17.59 ± 0.17 ^{a,b,1}	14.51 ± 2.38 ^{a,1}	13.23 ± 0.91 ^{a,1}
	Amaranth	39.50 ± 0.49 ^{b,2}	29.48 ± 0.80 ^{b,1,2}	18.85 ± 0.79 ^{a,1}	18.36 ± 0.34 ^{a,1}
	Brown rice	13.09 ± 0.21 ^{a,1}	14.17 ± 0.42 ^{a,1}	15.12 ± 1.25 ^{a,1}	16.53 ± 0.24 ^{a,1}
	Wheat	11.67 ± 0.57 ^{a,1}	13.77 ± 0.22 ^{a,1}	13.77 ± 0.23 ^{a,1}	14.32 ± 0.08 ^{a,1}
h	Quinoa	84.42 ± 0.09 ^{a,1}	85.50 ± 0.14 ^{a,1,2}	87.30 ± 0.07 ^{b,2,3}	88.13 ± 0.72 ^{c,3}
	Amaranth	80.12 ± 0.06 ^{b,2}	82.85 ± 2.06 ^{b,1}	80.88 ± 0.09 ^{a,2}	79.09 ± 0.20 ^{a,2}
	Brown rice	79.85 ± 0.07 ^{b,2}	81.58 ± 0.28 ^{b,2}	87.76 ± 1.07 ^{b,1}	80.99 ± 0.13 $^{a\ b,3,2}$
	Wheat	80.97 ± 0.32 $^{b,1.2}$	82.77 ± 0.50 ^{b,1}	80.61 ± 0.09 ^{a,2}	82.20 ± 0.10 ^{b,1,2}
a*	Quinoa	1.17 ± 0.02 ^{a,1}	0.91 ± 0.04 ^{a,1,2}	0.61 ± 0.09 $^{b,2\ 3}$	0.43 ± 0.14 ^{c,3}
	Amaranth	1.18 ± 0.03 ^{a,1}	4.41 ± 0.38 ^{b,2}	2.99 ± 0.15 ^{c,3}	3.47 ± 0.08 ^{d,4}
	Brown rice	2.31 ± 0.03 ^{b,2}	2.07 ± 0.07 ^{c,2}	2.54 ± 0.17 ^{a,1}	2.59 ± 0.02 ^{a,1}
	Wheat	2.57 ± 0.04 ^{b,2}	2.43 ± 0.07 ^{c,2}	2.24 ± 0.02 ^{a,2,1}	1.95 ± 0.03 ^{b,1}
b*	Quinoa	12.04 ± 0.34 ^{a,1,2}	11.55 ± 0.17 ^{a,2}	11.45 ± 0.27 ^{a,2}	13.22 ± 0.92 ^{b,1}
	Amaranth	25.24 ± 0.59 ^{b,1}	29.40 ± 0.79 ^{b,2}	18.58 ± 0.82 ^{c,3}	18.02 ± 0.33 ^{c,3}
	Brown rice	12.88 ± 0.21 ^{a,1}	14.02 ± 0.42 ^{c,1}	16.25 ± 0.61 ^{b,2}	16.33 ± 0.25 ^{a,2}
	Wheat	10.90 ± 0.77 ^{c,1}	12.49 ± 0.66 ^{a,c,1}	13.59 ± 0.23 ^{d,2}	14.19 ± 0.08 ^{b,2}

TTA: total titratable acidity. $^{a–d}$: Different letters within the same column indicate significant differences between samples ($p < 0.05$). $^{1–4}$: Different numbers within the same row indicate significant differences between samples at different times of analysis ($p < 0.05$).

For color parameters, the brightness (L*) increased in quinoa, brown rice and wheat sourdoughs during the days of ripening, and the hue (h) increased during the days of maturation in brown rice and quinoa sourdough. However, in the amaranth sourdough, a decrease in the values of L* and h was observed comparing the initial and final days. In all samples, a significant difference was observed between day 0 and day 3. However, in parameters C and yellowish tones (b*) in the quinoa sourdough with respect to the

processing time, no modification was observed, but in the amaranth sourdough in both parameters, a decrease of both values was obtained during the time. Unlike in the brown rice sourdough, an increase in C and b* values was observed during the processing time. With respect to the wheat sourdough, the C value was not affected during processing, but the yellowish tone (b*) increased during the development time. Similarly, the reddish tone (a*) was also affected during the elaboration of the sourdoughs, where the quinoa and wheat sourdough decreased with respect to the maturation time of the sourdoughs, but the brown rice and amaranth sourdough did not follow a trend during the intermediate days, but an increase was observed comparing the initial and final days.

In the yellowish (b*) and reddish (a*) tones, the highest value was observed in the amaranth sourdough and the lowest value in the quinoa sourdough, as can be seen in Figure 1. This is due to the fact that pseudocereals contain an abundance of betalains, classified in two subcategories: orange-yellow betaxanthins and violet-red betacyanins [35]. Amaranth contains betacyanins such as amaranthine and isoamaranthine. Quinoa also contains betanins [36]. These are responsible for the shades of both sourdoughs.

Figure 1. Photographs of mature sourdoughs of wheat, amaranth, quinoa and brown rice.

3.2. Acetic Acid and Lactic Acid Content

The lactic acid and acetic acid content of the different sourdoughs are shown in Table 2. In general, more acetic rather than lactic acid content was observed in the sourdoughs. This may be due to the greater presence of heterofermentative bacteria in the sourdoughs, which, in addition to producing lactic acid, produce mostly acetic acid, ethanol and CO_2 [37]. The acetic acid in the sourdoughs in this study contains high values, with the highest value in the brown rice sourdough (4.45 g/L) and the lowest in the wheat sourdough (2.88 g/L). Therefore, according to Zhang et al. [38], the high content of acetic acid in the sourdough promotes its use in the bakery industry as a biopreservative since it is the most relevant antifungal metabolite of lactobacilli [39]. Furthermore, it has anti-ropiness [40] and may delay the rate of gastric emptying, thus prolonging the feeling of satiety [41]. Therefore, it is important that the sourdough contain a significant amount of acetic acid to produce bread. In fact, according to Spicher et al. [42], the lack of acetic acid in conventional sourdoughs is detrimental, since acetate improves the sensory quality of sourdough bread.

Table 2. Acid contents (g/L) in the mature sourdoughs.

Sample	Acetic Acid (g/L)	Lactic Acid (g/L)
Quinoa	3.92 ± 0.07^{a}	0.33 ± 0.01^{a}
Amaranth	3.90 ± 0.03^{a}	0.32 ± 0.01^{a}
Brown rice	4.45 ± 0.02^{b}	0.28 ± 0.01^{b}
Wheat	2.88 ± 0.05^{c}	0.42 ± 0.01^{c}

[a–c]: Different letters within the same column indicate significant differences between samples ($p < 0.05$).

Regarding the content of lactic acid in sourdoughs, which is lower than the content of acetic acid, the sourdough with the highest amount of lactic acid was observed to be wheat sourdough (0.42 g/L), and the lowest amount was observed to be brown rice sourdough (0.28 g/L). According to Liljeberg et al. [43], lactic acid reduces postprandial glucose and insulin responses in healthy individuals due to inhibition of their digestive amylolytic enzymes, and acetic acid is the other acid responsible for the microbiological extension of the shelf life of sourdough bread [44].

In relation to the lactic and acetic acid content, the differences observed in the different sourdough samples may be due to the amount of fermentable carbohydrates in the cereals and pseudocereals, since fermentable carbohydrates are the main factor in the production of lactic and acetic acid through carbohydrate metabolism [45].

3.3. Microbiological Analysis for Molds, Yeast and Lactic Acid Bacteria

The plate count results for total molds, total yeasts, non-*Saccharomyces* yeasts and lactic acid bacteria are shown in Table 3. In the TYMC (total yeast and mold count) counts in the flours, the lowest count was observed in the quinoa flour (<10 log CFU/mL) and the highest in the amaranth flour (4.58 log CFU/mL). On the contrary, the lowest LAB count was found in amaranth flour (>10 log CFU/mL) and the highest in brown rice flour (5.37 log CFU/mL). These results are superior to those obtained by Carbó et al. [29], where they developed gluten-free sourdough with a mixture of pseudocereal flours. Results superior to those obtained by Van Kerrebroeck et al. [46] were also obtained with wheat flour. Regarding the count, wheat flour (3.92 log CFU/mL) had the highest amount and whole wheat flour (2.69 log CFU/mL) the lowest.

In the TYC, populations ranging from 3.94–7.97 log CFU/mL (wheat and amaranth) were observed in the sourdough. Lower values than those obtained by Carbó et al. [29], at 88 h of fermentation, gluten-free sourdough developed with the mixture of several flours.

Regarding the NYC populations in the mother doughs, we observed populations between 6.41–7.78 log CFU/mL (wheat and amaranth) population counts at the upper lower limit of the TYC count. The values obtained were higher than those obtained by Carbó et al. [29] in the 256 h fermentation time (7.51 log CFU/g) in the development of gluten-free sourdough.

Table 3. Microbiological analysis of total yeast, total molds, non-Saccharomyces yeast and lactic acid bacteria (LAB) expressed as Log CFU/mL of sourdoughs and flours.

Type of Sample	Sample	TYMC	NSY	LAB
Sourdough	Quinoa	6.75 ± 0.08 ^{a}	7.39 ± 0.05 ^{a}	7.86 ± 0.10 ^{a,b}
	Amaranth	7.97 ± 0.08 ^{b}	7.78 ± 0.10 ^{b}	8.10 ± 0.06 ^{a}
	Brown rice	7.84 ± 0.04 ^{b}	7.67 ± 0.12 ^{a,b}	8.01 ± 0.02 ^{a,b}
	Wheat	3.94 ± 0.04 ^{c}	6.41 ± 0.03 ^{c}	7.40 ± 0.03 ^{b}
Flour	Quinoa	<10	3.25 ± 0.22 ^{d}	5.03 ± 0.08 ^{c}
	Amaranth	4.58 ± 0.06 ^{d}	3.11 ± 0.10 ^{d}	<10
	Brown rice	3.39 ± 0.35 ^{e}	3.66 ± 0.07 ^{e}	5.37 ± 0.08 ^{c}
	Wheat	3.98 ± 0.02 ^{c}	3.92 ± 0.05 ^{e}	3.79 ± 0.57 ^{d}
	Whole wheat	2.66 ± 0.03 ^{f}	2.69 ± 0.04 ^{f}	2.69 ± 2.71 ^{e}

$^{a–f}$: Different letters within the same column indicate significant differences between samples ($p < 0.05$). TYMC: total yeast and mold count; NSY: non-*Saccharomyces* yeast; LAB: lactic acid bacteria.

As for the results of LAB in general in all the sourdoughs, LAB predominated over the other microorganisms analyzed. The highest LAB content was observed in the amaranth sourdough (8.10 log CFU/mL) and the lowest in the wheat sourdough (7.40 log CFU/mL) after 72 h of fermentation. The results obtained are not similar to those obtained by other authors, for example, Rizzello et al. [47] obtained LAB populations between 9.3 and 9.7 log CFU/mL in a quinoa sourdough after 16 h of fermentation, while Rühmkorf et al. [48] obtained values around 8.48 and 9.85 log CFU/g in quinoa sourdoughs (inoculated with different lactic acid bacteria strains) after 24 h of fermentation. On the other hand, Sterr et al. [31] obtained final populations of lactic acid bacteria between 9.45 and 9.75 log CFU/g in amaranth sourdoughs after 10 days with daily refreshments, while in amaranth sourdough a final LAB population of 8.10 log CFU/mL was obtained, so the results are lower than those of the other authors. This may be due to the fact that the growth of microorganisms depends on endogenous factors (pH and temperature) and exogenous factors (flour and water) [21].

3.4. Antioxidant Activity and Total Phenolic Content (TPC)

The content of total polyphenols and antioxidant capacity are shown in Table 4. In general, both TPC and antioxidant capacity values were higher in the sourdoughs than in the flours. This is due to the fermentation process, as it increases the levels of extractable phenolic compounds [49]. In fact, many authors recognized that this process has a positive impact on TPC and the antioxidant activity of cereals and pseudocereals [50,51]. Whereas normally, the increase of extractable phenolic compounds is responsible for the improvement of antioxidant capacity [52].

In the total phenolic content (TPC), the highest total phenolic content was observed in quinoa flour (10.30 mg GAE g^{-1}), since it is one of the pseudocereals with the highest content of phenolic compounds, compounds that are mostly found in free form and range between 167.2 and 308.3 mg of gallic acid equivalents/100 g dry weight, with gallic and ferulic acids being the dominant compounds [53]. The flour with the lowest total phenolic compound content was found to be brown rice (2.75 mg GAE g^{-1}). However, in the sourdoughs, wheat sourdough (33.03 GAE g^{-1}) had the highest content of total phenolic compounds. This could be due to the pH when the sourdough is fermented, as can be seen in Table 1, the pH of wheat sourdough is 3.65, the lowest of all sourdoughs, and according to Lancetti et al. [54], the decrease in pH improves the extraction of polyphenols since it activates the endogenous enzymes of the flour (amylases, xylanases and proteases) that contribute to the modification of the composition of the grain and release the phenolics bound before extraction. In addition, esterase activities produced by lactic acid bacteria,

which hydrolyze complex phenolic compounds into the corresponding phenolic acids, have also been described [55]. Both events may explain the increased extraction of polyphenols after sourdough acidification and fermentation. The brown rice sourdough had the lowest content of total phenolic compounds (9.77 mg GAE g^{-1}) as did the brown rice flour, which had the lowest content of total phenolic compounds.

Table 4. Total phenolic content (TPC) (mg GAE g^{-1}) and antioxidant capacity (µmol TE g^{-1}) of sourdoughs and flours.

Type of Sample	Sample	TPC	Antioxidant Capacity		
			ABTS	ORAC	FRAP
Sourdough	Quinoa	23.45 ± 0.61 ^{a}	12.00 ± 0.31 ^{a}	269.48 ± 0.58 ^{c}	8.52 ± 0.31 ^{a}
	Amaranth	18.16 ± 0.34 ^{b}	11.60 ± 0.39 ^{a}	188.67 ± 0.55 ^{d}	7.35 ± 0.41 ^{b}
	Brown rice	9.77 ± 0.12 ^{c}	10.28 ± 0.40 ^{a,b}	125.00 ± 0.12 ^{b,f}	6.05 ± 0.32 ^{c,f}
	Wheat	33.03 ± 0.41 ^{d}	31.84 ± 2.45 ^{c}	142.93 ± 0.68 ^{a}	8.65 ± 0.36 ^{a}
Flour	Quinoa	10.30 ± 0.22 ^{c}	12.15 ± 1.18 ^{a}	161.33 ± 5.98 ^{a}	7.36 ± 0.43 ^{b}
	Amaranth	5.28 ± 0.97 ^{e}	8.57 ± 0.26 $^{b\,d}$	120.68 ± 15.42 ^{b,e}	4.40 ± 0.19 ^{d,e}
	Brown rice	2.75 ± 0.10 ^{f}	9.47 ± 0.15 ^{a,d}	142.91 ± 18.73 ^{a}	5.30 ± 0.36 ^{c,d}
	Wheat	3.78 ± 0.20 ^{g}	6.85 ± 0.11 ^{d}	100.58 ± 0.25 ^{e}	3.48 ± 0.34 ^{e}
	Whole wheat	5.33 ± 0.21 ^{e}	8.60 ± 0.32 ^{b,d}	104.94 ± 1.42 ^{e,f}	6.76 ± 0.59 ^{b,f}

TPC: Total phenolic compounds. $^{a–g}$: Different letters within the same column indicate significant differences between samples ($p < 0.05$). GAE: gallic acid; TE: trolox equivalent.

Regarding the antioxidant capacity in ABTS, FRAP and ORAC, the lowest level of antioxidant capacity in the flours was observed in wheat flour (6.85 µmol TE g^{-1}; 3.48 µmol TE g^{-1} and 100.58 µmol TE g^{-1}) and the highest antioxidant capacity was observed in quinoa flour (12.15 µmol TE g^{-1}; 7.36 µmol TE g^{-1} and 161.33 µmol TE g^{-1}) as well as in the TPC, since usually TPC and antioxidant capacity are correlated.

Regarding the sourdough, the lowest antioxidant capacity was observed in the brown rice sourdough in the ABTS, FRAP and ORAC (10.28 µmol TE g^{-1}; 6.05 µmol TE g^{-1} and 125.00 µmol TE g^{-1}) and the wheat sourdough with the highest antioxidant capacity in the ABTS and FRAP (31.84 µmol TE g^{-1} and 8.65 µmol TE g^{-1}) and in the ORAC the quinoa sourdough (269.48 µmol TE g^{-1}). This is because the decrease in pH, in addition to increasing the extractable phenolic compounds, also influences the increase in antioxidant capacity, but the antioxidant capacity can be influenced by several factors such as temperature, water content, fermentation time, aerobic conditions and the composition of the cereal or pseudocereal [56,57].

3.5. Content of Total Aflatoxins

The total aflatoxin contents of sourdoughs are shown in Table 5. No detectable total aflatoxin content was observed in quinoa, wheat, brown rice and wheat sourdough; however, total aflatoxin content was observed in amaranth sourdough (0.66 µg/kg), which is not very high. Aflatoxins are carcinogenic mycotoxins found naturally in cereals. Even though they exist in cereal products, the sourdoughs of the present study have an absence of total aflatoxins due to the antiaflatoigenic capacity of sourdough LAB [58]. In fact, Gerbaldo et al. [59] reported that in the presence of BAL, there is a relationship between fungal growth and aflatoxin production. Consequently, low mycelial biomass formation during fungal growth could directly reduce mycotoxin synthesis. Detoxifying strains of LAB can decrease aflatoxins by two degradation mechanisms: an enzyme-dependent reaction or a physical binding process [60].

Table 5. Total aflatoxin content (μg/kg) of mature sourdoughs.

Sample	Total Aflatoxins
Quinoa	<LoQ [b]
Amaranth	0.66 ± 0.23 [a]
Brown rice	<LoQ [b]
Wheat	<LoQ [b]

[a,b]: Different letters within the same row indicate significant differences between samples ($p < 0.05$).

Regarding total aflatoxin content, sourdoughs comply with Regulation (EC) No. 1881/2006 [61] on the minimum acceptable content fit for human consumption, since in cereal or derived foods, the permitted level is 4 μg/kg of total aflatoxins.

3.6. Microbial Composition

3.6.1. Fungi

The results of the fungal composition of the different sourdoughs in different forms of preservation are shown in Figure 2A,B. ITS identified 13 dominant fungal genera, including 1 yeast, 6 pathogenic fungi, 5 non-pathogenic fungi and 1 unidentified fungus, of which the most abundant genera in the samples are as follows: *Saccharomycopsis*, *Alternaria*, *Nigrospora* and *Holleya*. The results indicated that the dominant yeast genus identified by ITS, *Saccharomycopsis*, may be the dominant fermentation fungus in the samples, while the other dominant genera, as seen in Table 6: *Alternaria*, *Nigrospora* and *Holleya*, may be due to environmental contamination or plant-derived ingredients rather than the dominant fermentation fungi in the samples.

Regarding the fungal composition of the sourdoughs in the different storage conditions, a higher proportion of fungi and yeasts was observed in all the sourdoughs at room temperature (25 °C), followed by −20 °C, except for the brown rice sourdough, which had a higher proportion of fungi and yeasts at 4 °C. At room temperature (25 °C), it was observed that in the amaranth, wheat and quinoa sourdough, the predominant genus was *Saccharomycopsis*, specifically *Saccharomycopsis fibuligera*; however, in the brown rice sourdough, the Mucor genus, specifically the species *Mucor circinelloides*, stood out. *Saccharomycopsis fibuligera*, a dimorphic yeast species very common in fermented foods, such as traditional sourdough [62]. According to Jin et al. [63], sourdough fermented by *S.fibuligera* gives sourdough products aromatic esters and also protects the product from the spoilage fungus, *Aspergillus flavus*. The genus *Mucor* is a fungus that, as in our study, we found in brown rice sourdough; other authors, such as Sun et al. [64], found it in Niandoubao (millet food); and Charlotte et al. [65], in faba bean sourdough.

In the temperature conditions of 4 °C and −20 °C, the Alternaria genus predominated in the amaranth and wheat sourdough. However, in the amaranth sourdough, the *Holleya* genus, specifically the *Holleya sinecauda* species, and in the brown rice sourdough, the *Nigrospora* genus, specifically the *Nigrospora vesicularifera* species, were predominant.

Figure 3 shows the fungal community during the preparation of the sourdoughs. In the amaranth and wheat sourdough, it was observed that during all the days of elaboration, the fungus of the genus Alternaria predominated. However, in the quinoa sourdough, during the days of elaboration, the fungus of the genus *Holleya* abounded, specifically the spice *Holleya sinecauda;* and in the brown rice sourdough, the fungus of the genus *Epicoccum* prevailed, specifically the species *Epicoccum thailandicum*. *Alternaria* is a plant pathogen that is prone to contamination during sourdough processing [66].

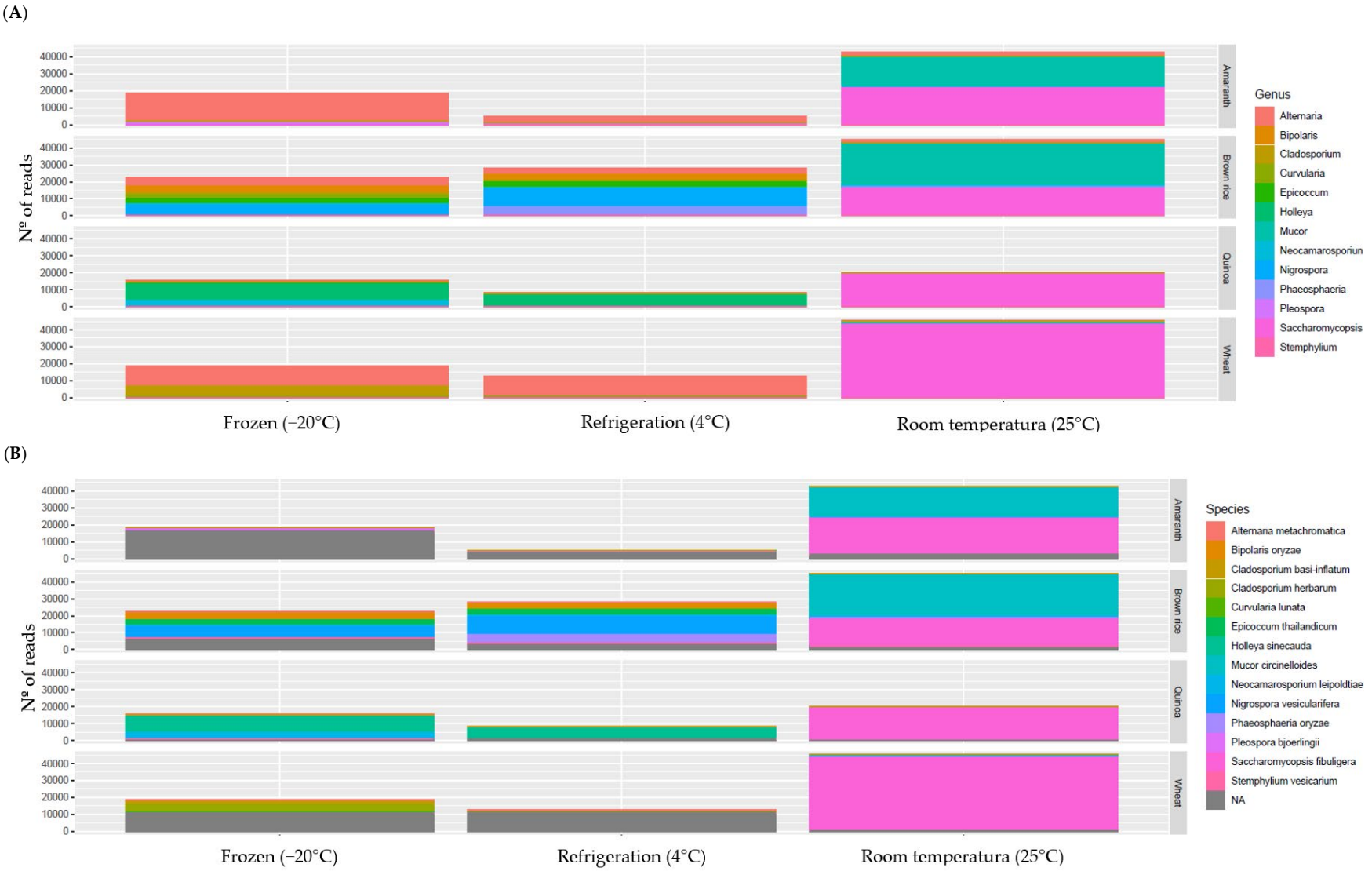

Figure 2. (**A**) Number of readings of fungi at the genus level of sourdough communities at different preservation temperatures. (**B**) Number of readings of fungi at the phylum level of sourdough communities at different preservation temperatures.

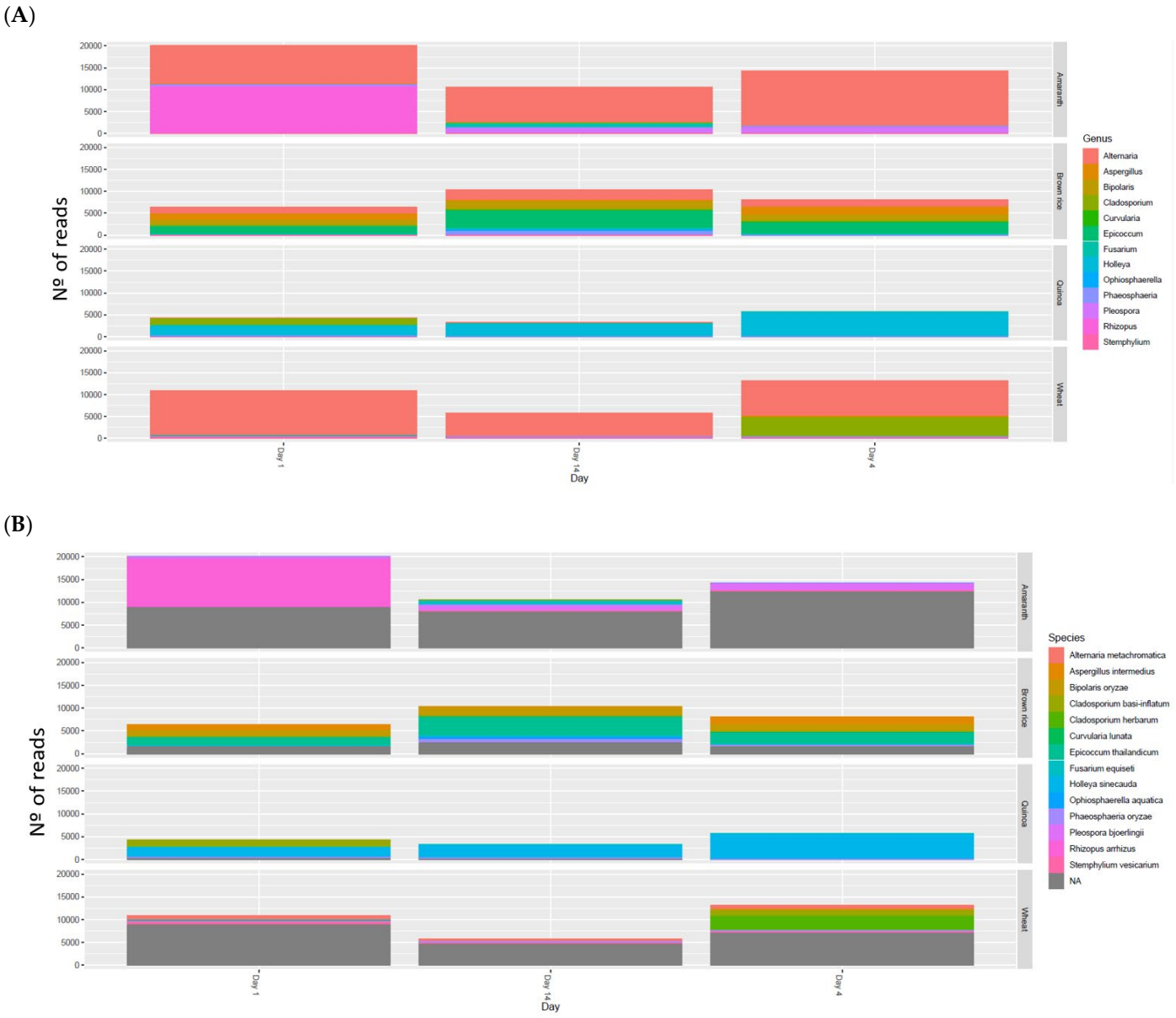

Figure 3. (**A**) Number of fungal readings at the genus level of sourdough communities during processing. (**B**) Number of fungal readings at the species level of sourdough communities during processing.

Table 6. Characterization of fungi and yeasts identified in samples analyzed during the processing of spontaneously fermented sourdoughs and during preservation.

Genus and Species	Type	Relationship with Food	Normally Found in	Pathogen	Metabolites and Biological Activity	References
***Alternaria* spp.** *A. destruens* *A. metachromatia* *A. rosae* *A. subcucurbitae*	Filamentous fungi	Cereals, oilseeds, tomatoes, cucumbers, cauliflowers, peppers, apples, melons, tangerines, oranges, lemons and sunflower seeds	Soil and plants	Yes (Opportunistic)	Alternariol (AOH), altenariol monomethyl ether (AME), altenuene (ALT), tenuazonic acid (TeA), tentoxin (TEN), altertoxins I, II and III, dehydrocurvularin, pyrenochaetic acid, alternarienonic acid and altechromoneA	[67,68]
***Aspergillus* spp.** *A. intermedius* *A. penicillioides* *A. ruber*	Filamentous fungi	Tea, coffee, rice and soybeans, meju (dried fermented soybeans), syrups, jams, jellies and salted meat products	Water and soil	Yes (Opportunistic)	Asperflavin, auroglaucin, dihydroauroglaucin, echinulins, epiheveadrides, flavoglaucin, isoechinulins, LL-S491β, neoechinulins, physcion, questin, tetrahydroauroglaucin, bisanthrons, catenarin, erythroglaucin, questin, questinol, tetracyclic	[69–71]
***Bipolaris* spp.** *B. oryzae* *B. yamadae*	Filamentous fungi	Corn, rice and oatmeal	Soil	Yes (Opportunistic)	Bipolahidroquinonas A-C, coclioquinonas I–N, isococlioquinonas F y G. Anticancer activity	[72]
***Bullera* spp.** *B. alba*	Fungi	n/a	n/a	No	n/a	
***Cladosporium* spp.** *C. basi-inflatum* *C. herbarum*	Filamentous fungi	May cause food spoilage	Soil and air	No	Alkaloids, azaphilones, benzofluorantheneones, benzopyrones, binaphtopyrones, butanolides, butenolides, cinnamic acid, citrinin, coumarins, isocoumarins, diketopiperazines, flavonoids, gibberellins, fusicoccane, diterpene, glycosides, lactones, macrolides, naphthalene, naphthalenones, naphtoquinones, anthraquinones, perylenquinones, pyrones, sterols, tetramic acids, tropolones and xanthones.	[73,74]

Table 6. *Cont.*

Genus and Species	Type	Relationship with Food	Normally Found in	Pathogen	Metabolites and Biological Activity	References
***Curvularia* spp.** *C. lunata*	Phytopathogenic fungus	Rice, sugarcane, rice, millet and maize (corn).	Plants and soil	Yes	Radicinin, radicinol and 3-epiradicinol (radicinol diastereomer).	[75,76]
***Di oszegia* spp.** *D. hungarica* *D. takashimae*	Yeast	n/a	Plants and insects	No	Antimicrobial activity	[77]
***Epicoccum* spp.** *E. thailandicum*	Fungi	Cheese	Air, soil and plant	No	Diketopiperazines, epicorazines, epicoccolides, epicocconigrones, epicocconones, epicolactone dimers, epipyrones, flavipins, triornicins epicoccamides, meroterpenoids and taxol. Antimicrobial activity and anticancer activity	[78]
***Eremothecium* spp.** *E. gossypii*	Filamentous fungi	n/a	Cotton	n/a	Riboflavin (vitamin B2) quinones, flavins and melanin	[79]
***Exserohilum* spp.** *E. gedarefense* *E. monoceras*	Fungi	n/a	Plant material like grasses, rotten wood and in the soil	n/a	n/a	[80]
***Filobasidium* spp.** *F. wieringae*	Fungi	n/a	n/a	n/a	n/a	
***Fusarium* spp.** *F. equiseti* *F. graminearum* *F. tricinctum*	Filamentous fungi	Cereals, fruits, nuts, spices, processed juices, grasses and vegetables	Soil, air and plants	Yes (Opportunistic)	Polyketides, alkaloids, terpenoids, peptides and steroids. antifungal activity	[81,82]
Hannaella spp. *H. oryzae*	Yeast	n/a	Soil and plants	No	n/a	[83]
Holleya spp. *H. sinecauda*	Fungi	Mustard seeds	n/a	Yes	n/a	[84]
***Microdochium* spp.** *M.Seminicola*	Fungi	Cereals	Plants	Yes	n/a	[85]

Table 6. *Cont.*

Genus and Species	Type	Relationship with Food	Normally Found in	Pathogen	Metabolites and Biological Activity	References
Mucor spp. *M. circinelloides*	Filamentous fungi	n/a	Soil	No	Alkaloid, pigment, benzoic acid, terpenoid, cinnamic acid, benzopyran, aspalathin and phloretin, arachidonic acid and ecosanoic acid	[86]
***Neocamarosporium* spp.** *N. leipoldtiae*	Fungi	n/a	n/a	n/a	n/a	
***Nigrospora* spp.** *N. hainanensis* *N. vesicularifera*	Fungi	Fruits and oils	Soil and sea	No	Polyketides, terpenoids, steroids, N-containing compounds and fatty acids.	[87]
***Ophiosphaerella* spp.** *O. aquatica*	Fungi	n/a	n/a	Yes	n/a	
***Papiliotrema* spp.** *P. rajasthanensis*	Yeast	n/a	Soil and plants	n/a	n/a	
***Parastagonospora* spp.** *P. nodorum*	Fungi	Wheat and cereals	n/a	Yes	n/a	[88]
***Penicillium* spp.** *P. citrinum*	Fungi	Tea	Soil and sea	No	Citrinin and tanzawaic acid. Antimicrobial and antioxidant acitivity	[89–91]
***Periconia* spp.** *P. echinochloae*	Fungi	Rice	Soil, detoriating or dead herbaceous stems, leaves, grasses, rushes and sedges	Yes	Diterpenes, sesquiterpenes, sesterterpenes and steroids	[92–94]
***Phaeosphaeria* spp.** *P. oryzae*	Fungi	n/a	n/a	Yes	n/a	
***Plenodomus* spp.** *P. fallaciosus*	Fungi	Grape	n/a	n/a	n/a	[95]
***Pleospora* spp.** *P. bjoerlingii*	Fungi	Garlic	Air	n/a	n/a	[96]
***Ramichloridium* spp.** *R. cucurbitae*	Fungi	n/a	n/a	No	n/a	

Table 6. *Cont.*

Genus and Species	Type	Relationship with Food	Normally Found in	Pathogen	Metabolites and Biological Activity	References
***Rhizopus* spp.** *R. arrhizus*	Fungi	Vegetables and fruits	Soil	Yes	Fumaric acid	[97]
***Saccharomycopsis* spp.** *S. fibuligera*	Yeast	Cereal-based fermented foods and beverages	Plants	No	Ethanol, carbon dioxide and diverse compounds including fusel alcohols and esters	[98–100]
***Saitozyma* spp.** *S. paraflava*	Yeast	n/a	n/a	n/a	n/a	
***Sporobolomyces* spp.** *S. roseus*	Yeast	Smoked dried sausages, nectarine fruits, fermented tea, Chinese miscanthus, grapefruit, citrus fruits and apple must	Environment, tree leaves and soil	No	β-carotene, torulene and torularhodin	[101]
***Stemphylium* spp.** *S. vesicarium*	Fungi	Cucumber, garlic, pear, parsley, asparagus, spinach and lettuce.	n/a	Yes	n/a	[102]
***Vishniacozyma* spp.** *V. tephrensis* *V. victoriae*	Yeast	Grape and kiwi	n/a	No	n/a	[103]

n/a: not identificated.

3.6.2. Bacterial Community Composition

The results of the analysis of the bacterial composition during the manufacturing process are shown in Figure 4A,B. Figure 4A shows the abundance of bacteria during the processing of the different sourdoughs. Eleven bacterial phyla were detected, but the species with the highest number of readings found in the different sourdoughs and during the different processing days were *Bacillus*, *Pantoea* and *Clostridium*. With respect to day 1, the brown rice dough stands out with the highest number of bacteria compared to the rest, with a predominance of bacteria of the *Bacillus* genus, except in the wheat dough, in which bacteria of the *Clostridium* genus abound. As for day 4, the amaranth sourdough stands out with the highest number of bacteria. In the quinoa and wheat sourdough, the *Clostridium* genus predominated, in the brown rice sourdough, the bacteria of the *Bacillus* genus abounded and in the amaranth sourdough, the bacteria of the *Pantoea* genus predominated.

However, on day 14, the *Bacillus* genus no longer predominates among the sourdoughs; in the quinoa, brown rice and wheat sourdoughs, the bacteria of the *Clostridium* genus stand out, and in the amaranth sourdough, the bacteria of the *Pantoea* genus. In the first phase, *Bacillus* prevails. This undesirable bacterium is usually present in raw materials during grain storage and processing [104,105] and in unripe sourdoughs [106,107], so flour contamination is not considered accidental but inevitable, but is quickly overcome by the fermentation process, which causes other bacteria to appear that regress *Bacillus* bacteria. In the second and third phases, *Clostridium* and *Pantoea* were presented. Similarly, the genus *Clostridium* was the most abundant in western sourdoughs, especially in sourdoughs from Tibet [108]. However, this genus has never been reported in sourdoughs anywhere in the world, although it can be found in cereals and flours [109]. Interestingly, the *Clostridium* genus produces butyric acid and acetic acid, and the acids are converted to butanol, acetone and ethanol [108]. These volatile compounds contribute a richer flavor and more aroma to sourdough wheat breads [110]. This suggests that these doughs are potentially good choices for making bread and other fermented products. Figure 4B shows the beta diversity, where it was observed that the wheat sourdough of days 4 and 14 obtained the diversity of bacterial composition the most similar to the rest of the sourdoughs in the different days of processing.

Figure 5A shows the bacterial composition depending on the preservation conditions of the different sourdoughs. In general, it was observed that depending on the storage condition of the sourdough, the bacterial composition was affected, as in the quinoa and wheat sourdough, the bacterial composition was higher at 4 °C, in the amaranth sourdough at 25 °C and in the brown rice sourdough at −20 °C. Regarding the storage conditions, both at 4 °C and −20 °C, the quinoa, brown rice and wheat sourdough were dominated by the *Clostridium* bacteria genus, and in the amaranth sourdough, the *Pantoea* genus was predominant. However, at 25 °C of storage in the quinoa, brown rice and wheat sourdough, the *Lactobacillus* genus abounded, and in the amaranth sourdough, the *Pediococcus* genus predominated. Therefore, taking into account that *Clostridium* and *Pantoea* bacteria are undesirable bacteria [105], it could be said that it would be more advisable to conserve the dough at 25 °C, although these bacteria, due to fermentation processes, regress as a consequence of the acidification of the sourdough by the intervention of LAB bacteria and can also disappear with temperatures during baking [111,112].

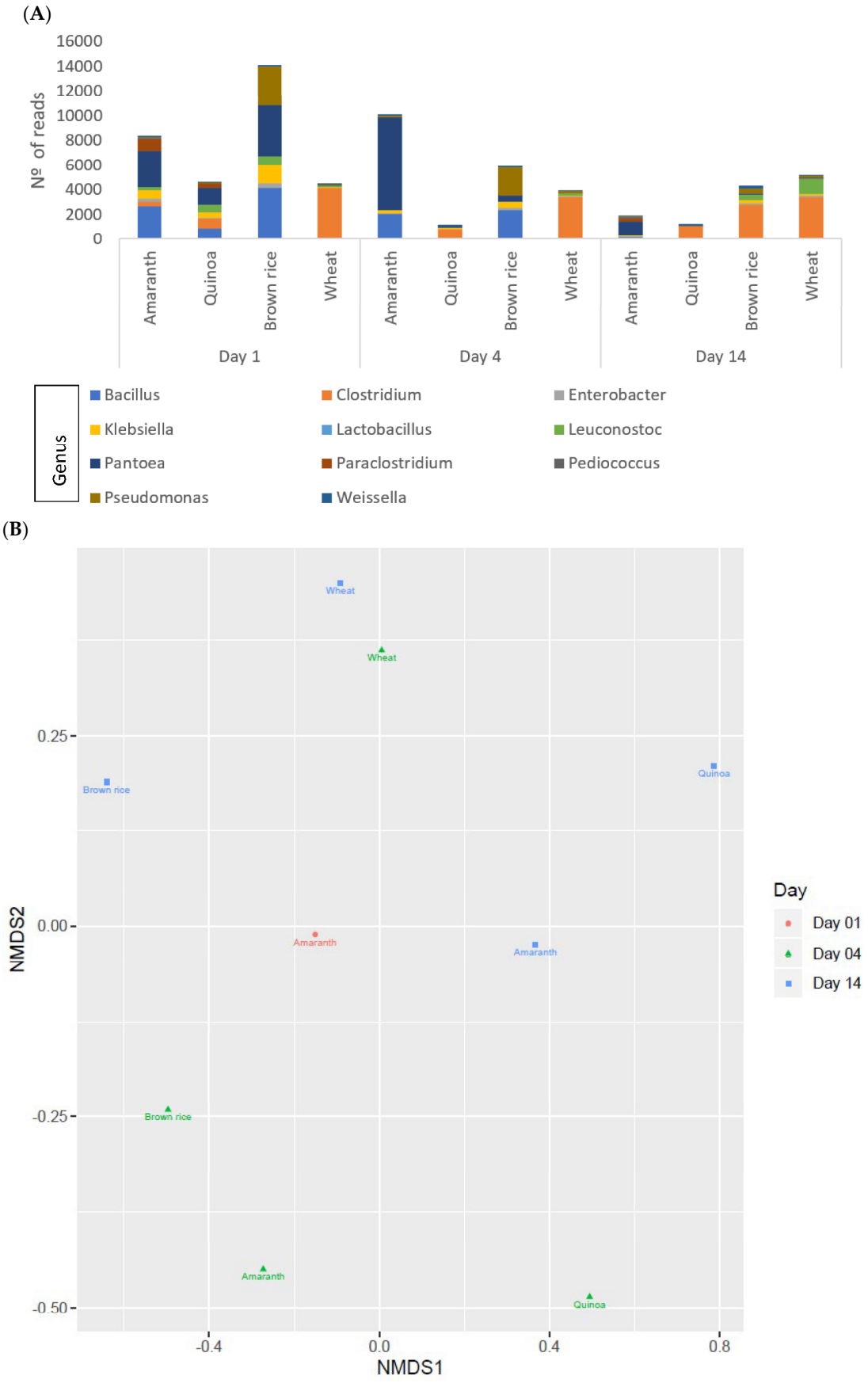

Figure 4. (**A**) Composition of bacterial species in the different sourdoughs during processing. (**B**) Characterization of beta diversity of the bacterial communities in the different sourdoughs during processing.

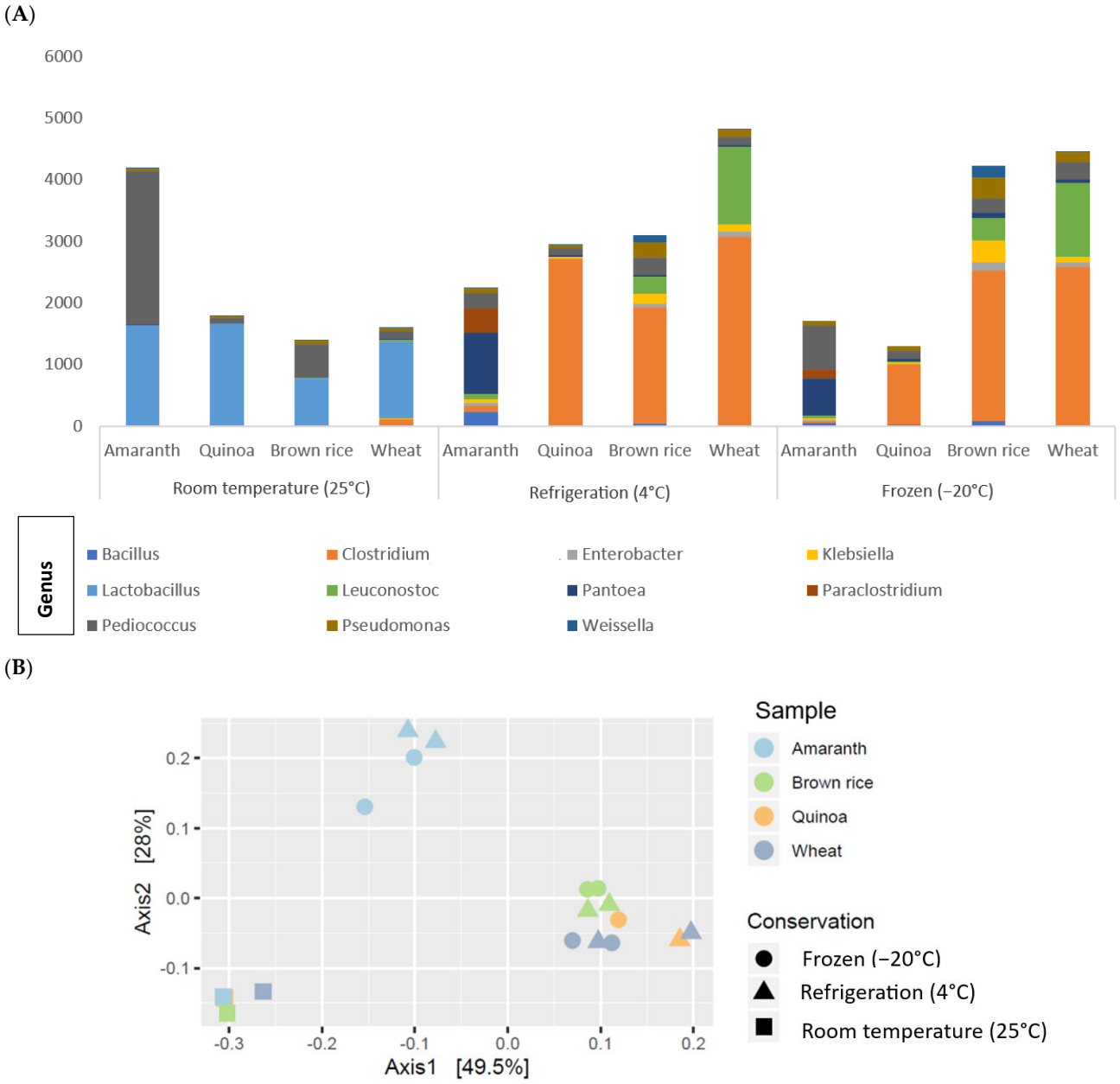

Figure 5. (**A**) Composition of bacterial species in the different sourdoughs in different storage conditions. (**B**) Characterization of the beta diversity of the bacterial communities of different sourdoughs under different preservation conditions.

Lactobacillus spp. were abundant in most sourdoughs at 25 °C due to their adaptability to acidic, dehydrated environments and nutrient-depleted situations during propagation, allowing their natural selection and ultimate dominance in the sourdough ecosystem [113]. *Lactobacillus* spp. widely detected in sourdough are producers of GABA (gamma-aminobutyric acid), a neurotransmitter with important physiological functions and beneficial effects in the treatment of anxiety and depression. Therefore, the activity of these bacteria can increase the levels and availability of GABA in the product, whose consumption can contribute to the reduction of symptoms related to mental disorders [21]. *Pediococcus* spp. provides intense proteolytic activity, phytic acid degradation and increased phenols and antioxidants to sourdough [114].

Lactobacillus, *Weissella*, *Enterococcus*, *Streptococcus*, *Leuconostoc*, *Lactococcus*, *Pediococcus*, *Bacillus*, *Paenibacillus* and *Staphylococcus* are the genera of Firmicutes that inhabit the flours [115]. Because of the ability of fermented cereal products to promote the growth of

beneficial bacteria such as *Lactobacillus* spp., they are considered novel sources of probiotics, prebiotics or both, as well as potential functional foods [116]. Furthermore, sourdough bacteria such as *Lactobacillus* and *Pediococcus* spp. are able to synthesize and excrete exopolysaccharides (EPS) from sucrose into the medium via extracellular glucansucrases or fructansucrases [117,118] and even provide antifungal activity [118]. Figure 5B shows the beta diversity of the different sourdoughs in different forms of preservation, where it was observed that in the amaranth sourdough, the bacterial composition is very similar at −20 °C and at 4 °C. Regarding the quinoa, brown rice and wheat sourdough, both sourdoughs had a very similar beta diversity both at 4 °C and at −20 °C. At 25 °C, a beta diversity of similar bacterial composition was observed in all the sourdoughs.

4. Conclusions

In conclusion, this study clarified the properties of gluten-free sourdoughs from pseudocereals and gluten-free cereals, selected a fermentation microbiota suitable for making gluten-free breads and also determined the most suitable temperature for the conservation of sourdoughs. Quinoa and amaranth sourdoughs are the most similar to traditional (wheat) sourdoughs: higher lactic acid, higher antimicrobial capacity, higher total phenolic content, higher antioxidant capacity and higher bacterial and fungal composition. Therefore, they are a good alternative to be used as a substitute for chemical yeast as an adjuvant in the production of sustainable gluten-free breads, since their long fermentation process provides beneficial health effects, mainly due to the bacterial composition dominated by bacteria of the genus *Lactobacillus*, bacteria that, according to the results obtained, are best adapted to 25 °C, so the best method of preservation of sourdoughs is at room temperature. Examining sourdough in the field of fermentation processes and product development is crucial to improving the sensory and nutritional quality of bread production. It also contributes to improving our understanding of the scientific principles behind this culinary tradition.

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