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Screening of Antioxidant Effect of Spontaneous and Bioinoculated with *Gluconobacter oxydans* Fermented Papaya: A Comparative Study

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Abstract: Fermented papaya is recognized as a nutraceutical with a diverse and rich composition. Fermentation of fruit with desirable microorganisms could be a strategy to improve the nutritional quality and profile of the fruit. Despite the popularity of fermented papaya, there is still a lack of knowledge on the effects of various fermentation parameters. The goal of this study was to screen the antioxidant and other properties of the products obtained through a variety of fermentation experiments, as well as the impact of adding *Gluconobacter oxydans* on their physicochemical properties. The strategies used to produce the fermented papaya extracts were spontaneous fermentation and bioinoculation with *G. oxydans*. Different fermentation tests were performed to measure pH, total soluble solids, reducing sugars, sodium pyruvate content, total phenolic content (TPC), and ferric reducing antioxidant power (FRAP). There was a decrease in TPC during spontaneous fermentations (five assays). However, it can be observed that in the fermentation assays with *G. oxydans*, there was an increase in TPC and antioxidant properties. The highest content of TPC was observed on the eighth day of P7 (260.18 ± 0.02 µg gallic acid equivalents mL^{-1}) which was fermented with the bacteria and supplemented with glucose. Therefore, phenolic compounds in fermented papaya were found to increase antioxidant capacity as a result of bioinoculation with *G. oxydans*.

Keywords: antioxidant activity; phenolic compounds; acetic acid bacteria; papaya; fermented papaya

Citation: Leitão, M.; Ferreira, B.; Guedes, B.; Moreira, D.; García, P.A.; Barreiros, L.; Correia, P. Screening of Antioxidant Effect of Spontaneous and Bioinoculated with *Gluconobacter oxydans* Fermented Papaya: A Comparative Study. *Fermentation* **2023**, *9*, 124. https://doi.org/10.3390/fermentation9020124

Academic Editors: Federica Tonolo and Maria Pia Rigobello

Received: 17 December 2022
Revised: 9 January 2023
Accepted: 23 January 2023
Published: 27 January 2023

1. Introduction

Fermentation is a central metabolic process in which an organism, mainly yeasts and some bacteria, obtains energy from carbohydrates, such as starch or sugar, converting it mainly into alcohols (alcoholic fermentation) or carboxylic acids (lactic or acetic fermentation), in oxygen-limited conditions. During this conversion, an intermediate product is formed, for instance pyruvate (or acetaldehyde) produced from glucose metabolism [1,2]. The most famous application of this process is in the field of food and nutrition, namely yogurt and beer.

Carica papaya, also known as papaya or pawpaw, belongs to the *Caricaceae* family that is divided in four genera. The genus *Carica* Linn is the most cultivated and includes the best-known species [3,4]. The papaya taxonomical classification includes: kingdom (*Plantae*); order (*Brassicales*); family (*Caricaceae*); genus (*Carica*); and species (*Carica papaya*) [4]. Fermented papaya is recognized as a nutraceutical with an exceptionally diverse composition.

Our research team gathered the different benefits of the existing fermented papaya in the current market in a review article that highlights the benefits of several brands of fermented papaya supplements in human health [5]. Various experiments to determine its biological activity have been executed. Several studies have shown immunomodulatory, antioxidant, and anticancer properties [6–11].

Spontaneous fermentation is carried out with indigenous microorganisms of the used fruit, but bioinoculation with desirable microorganisms could be a strategy to improve the nutritional quality and profile of the product, namely the polyphenol and antioxidant levels [12]. *G. oxydans* is a Gram-negative bacterium belonging to the family *Acetobacteraceae*, an acetic acid bacteria (AAB) group that converts glucose to glucuronic acid and fructose into acetic acid. *Gluconobacter* is an industrially important genus to produce L-sorbose from D-sorbitol, as well as D-gluconic acid, 5-keto- and 2-keto-gluconic acids from D-glucose. Over the past centuries, *G. oxydans* has been used in the industrial production of food-related products, pharmaceuticals, and cosmetics, not only for the previously referred to compounds but also because it promotes the production of a vitamin C precursor [13].

Despite the growing popularity of fermented papaya, there is still a lack of knowledge on the effects of various production parameters, such as fermentation time, the sugar content, and temperature. As a result, the goal of this study was to control some process parameters and to conduct a series of various spontaneous fermentation tests and fermentation assays with the bioinoculation of *Gluconobacter oxydans*, to obtain a product that could be used in the future as a beverage or even as an ingredient incorporated into pharmaceuticals or cosmetic products formulations. The antioxidant properties of the obtained fermented fruit extracts were also examined, namely the ferric ion reducing antioxidant power (FRAP), and some physicochemical parameters such as the total phenolic compounds (TPC) content, pH, °Brix, reducing sugars, and sodium pyruvate content. These parameters were evaluated using methodologies commonly applied in laboratories worldwide and previously described for the analysis of fermented products and beverages [14,15].

2. Materials and Methods

i. Chemicals and bacterial culture

Culture media were purchased from VWR Chemicals® (Leuven, Belgium) and Liofil Chem® (Roseto degli Abruzzi, Italy). Reagents were obtained from Sigma Aldrich® (St. Louis, MO, USA) VWR Chemicals® (Fontenay-sous-Bois, France), and Scharlau® (Barcelona, Spain). The *Gluconobacter oxydans* strain (DSMZ 2343) was obtained from DSMZ®-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany.

ii. Preparation of fermented papaya extracts

In this study, the assays were carried out under various experimental conditions to evaluate the impact of those conditions on the fermentation process and the properties of the obtained products, including the antioxidant effect. Two major assay conditions were evaluated: spontaneous fermentation (SF) and fermentation with *Gluconobacter oxydans* (FGO). Moreover, the influence of biostimulation with glucose, the use of fresh or thawed fruit after storage by freezing, and the use of fruit in different forms (puree and pieces) were also assessed (Table 1).

a. Spontaneous fermentation

Fresh papaya was purchased from local markets (Portugal) and was taken to the laboratory for experimental analysis. Fruit width and length were determined by using a digital caliper (Mitutoyo Absolute AOS®, Kanagawa, Japan). Papaya was washed with tap water, chopped into medium cubes, and the papaya parts were weighed. The remains of the papaya used were stored at −20 °C until further analysis. A portion of fruit (fresh or thawed) was placed in a 250 mL sterile Erlenmeyer, the volume was made up to 100 mL with ultrapure sterile water, and the pH was measured directly. The Erlenmeyer

was placed in an incubator at 30 °C and the fermentation was allowed to start without any stirring. The pH measurements were made every 24 h, always removing an aliquot for a Falcon® tube (frozen at −20 °C for later quantitative analysis), stirring slightly and allowing the fermentation process to continue, until the pH reached the minimum value. At the end of each test, the samples were submitted to clean-up processes, being centrifuged (4000 rpm, 15 min, 4 °C) and gravitationally filtered. Each fermentation process was performed in duplicate.

b. Fermentation with *Gluconobacter oxydans*

For the fermentation tests with *Gluconobacter oxydans* (P6 and P7), this acetic acid bacteria (AAB) was reactivated in medium (Glucose 100 $g \cdot L^{-1}$; yeast extract 10 $g \cdot L^{-1}$; $CaCO_3$ 20 $g \cdot L^{-1}$, and bacteriological agar 15 $g \cdot L^{-1}$) at 28 °C for 5–7 d. After achieving an active growth, some cellular mass (Abs = 0.664 at 610 nm) was placed in saline solution (0.85% (m/v) and added to the Erlenmeyer at the beginning of the fermentation cycle. The concentration of cultures was determined using the turbidity method on a UV–visible spectrophotometer. The remaining procedure was performed as described above.

Table 1. Conditions of the assays carried out in the different types of fermentation.

	Assay	Conditions
Spontaneous fermentation	P1	Fresh fruit in pieces
	P2	Fresh fruit in puree
	P3	Thawed fruit in pieces
	P4	Thawed fruit in puree
	P5	Thawed fruit in puree + 200 g of glucose/1000 g of papaya
Fermentation with *Gluconobacter oxydans*	P6	Fresh fruit in pieces
	P7	Fresh fruit in pieces + 200 g of glucose/1000 g of papaya

iii. Sample analysis

a. pH

The pH measurement was carried out in a Bante Instruments 900 Multiparameter Meter® (Shanghai, China).

b. Total Soluble Solid (TSS)

The TSS content of the fruit was determined using a pre-calibrated VWR® portable refractometer with automatic temperature compensation. A drop of homogenized fermented papaya was placed at the prism of the refractometer, the lid was closed, the TSS was read directly from the digital scale at 20 ± 1 °C, and the results were expressed in °Brix.

c. Reducing sugar content

Reducing sugar content was determined using the dinitrosalicylic acid (DNS) method [16], preparing the reagent by mixing 10 g of DNS and 300 g sodium potassium tartrate (Rochelle salt) into 800 mL of 0.5 N sodium hydroxide and then warming it slowly and adding distilled water until it reached 1 L. A sample (0.5 mL) was mixed with 0.5 mL of DNS reagent inside a test tube placed in boiling water for 5 min and then allowed to cool to room temperature. Absorbance (Abs) at 540 nm was measured using a UV–visible spectropho-

tometer. A calibration curve was prepared following the same procedure as above, by replacing the sample with glucose at a concentration (C) between 0.20–2.00 $g \cdot L^{-1}$. The calibration curve obtained was Abs = 0.5563C − 0.0271 (Correlation Coefficient-(R) R = 0.9965).

d. Sodium pyruvate content
Sodium pyruvate content was determined based on the method described by Metrani et al. [17] with some modifications. Stock solution (10 mM) was prepared by accurately transferring 110.04 mg of sodium pyruvate to a 100 mL volumetric flask and completing the volume with distilled water. The other standards were obtained by successive dilution from a stock solution with distilled water to obtain the different concentrations (4; 2; 1; 0.5; 0.25 and 0.15 mM). DNPH (125 mg) was dissolved in a 50 mL volumetric flask with a portion of 0.5 $mol \cdot L^{-1}$ H_2SO_4 solvent solution, placed in an ultrasound bath for 30 min at 60 °C in order to dissolve the DNPH, and then the total volume was completed to 50 mL with the same solvent solution.
For sodium pyruvate calibration/determination, 10 µL of fermented papaya extract/ various concentrations (C) of sodium pyruvate (4, 2, 1, 0.5 and 0.25 mM) were pipetted into 96-well plates in triplicate and 90 µL of DNPH was added. After incubation for 30 min at 25 °C, 50 µL of 5 mol4050-313 L^{-1} KOH was added. The absorbance (Abs) was immediately read at 490 nm and 37 °C. The calibration curve obtained was Abs = 0.2729C + 0.0982 (R = 0.9992).

e. Total Phenolic Content (TPC)
TPC was spectrophotometrically determined according to the Folin–Ciocalteu (FC) procedure [18] with minor modifications [19]. A volume of 250 µL of the sample was mixed with 2.5 mL of FC (diluted 1:10 with ultrapure water) followed by an addition of 2 mL of Na_2CO_3 (7.5 % w/v). The mixture was incubated for 15 min at 45 °C and then kept in the dark at room temperature for 30 min. The absorbance (Abs) of the resulting blue color was measured at 765 nm against a reagent blank using a UV-1600 PC Spectrophotometer-VWR®. Gallic acid was used as a reference standard to plot the calibration curve (linearity range 5−100 $\mu g \cdot mL^{-1}$). The calibration curve obtained was Abs = 0.0121C + 0.0247; R = 0.9996). The results are expressed as the mass of gallic acid equivalents (GAE) in $\mu g \cdot mL^{-1}$ of the fermented papaya sample.

f. Ferric Reducing Antioxidant Power Assay (FRAP)
The FRAP method, adopted by Benzie and Strain, was employed [20]: 1.2 µL of fresh FRAP reagent (300 mM acetate buffer pH 3.6: 10 mM of 2,4,6-Tris(2-pyridyl)-s-triazine dissolved in HCl 40 mM: 20 mM $FeCl_3 \cdot 6H_2O$ in a 10: 1: 1 ratio) was mixed with 40 µL of the sample and then incubated for 15 min at 37 °C. The absorbance (Abs) was read at 593 nm. A calibration curve for the standard dried ferrous sulfate ($FeSO_4$) was used to obtain a correlation between sample absorbance and standard concentration (linearity range 100–1800 µM). The calibration curve was of the form Abs = 0.0006C − 0.0206; R = 0.9983. The analyses were run in triplicate and the results were expressed as equivalent µmol of the $Fe^{2+} \cdot L^{-1}$ fermented papaya sample.

iv. Data Analysis
The experiment was randomly conducted, with three replicates per analysis except for the pH analysis, which was only carried out in duplicate.

3. Results

The analysis of the pH, TSS content (°Brix), reducing sugar and sodium pyruvate content of the studied samples' fermentation cycles are represented in Table 2. The analysis of these parameters revealed that pH, °Brix, and reducing sugars decreased throughout fermentation and the sodium pyruvate content increased slightly throughout the fermentation process. Regarding pH values, it was observed that it decreased during fermentation

and that the minimum possible value was achieved in six of the total seven assays because afterward the pH increased: in P1 the minimum pH value was obtained between the 4th and the 6th day; in P3 and P4 it occurred on the 7th day; in P5 it was achieved on the 8th day; and in the last two assays, the minimum pH was reached on the 9th day. Only in P2 was it not possible to verify the minimum value because pH values decreased in all the fermentation assays. Therefore, this assay should be repeated for a longer period to determine the exact moment the minimum pH value is achieved. The TSS content declines but does not oscillate, staying within the same order of magnitude in all the fermentation cycles. The analysis of reducing sugars shows that sugar content reduces more substantially from the 1st to 2nd days in P2, P3, and, more substantially, in P4. The lowest value of reducing sugars from all the analyses was also detected in P4 (0.87 ± 0.01 g·L^{-1}). During the fermentation process, the reducing sugar content in the P5 and P7 assays (tests with the addition of glucose) remained approximately constant. During the fermentation process, the sodium pyruvate content increased. On the 9th day of P7, sodium pyruvate content was the highest for all tested fermented papaya samples (0.87 ± 0.10 mM). The second-highest value (0.64 ± 0.04 mM) was also obtained on the 9th day of the P6 test (bacterial bioinoculation experiments).

Table 2. Changes in pH, total soluble solid content (TSS), reducing sugar and sodium pyruvate concentrations during papaya fermentation assays (P1 to P7).

	Parameter	Fermentation Time (d)										
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
P1	pH	5.978 ±0.020	4.787 ±0.002	4.456 ±0.001	4.233 ±0.002	4.053 ±0.001	-	-	4.148 ±0.001	-	-	-
	TSS (°Brix)	-	3.50	3.50	2.88	2.6	-	-	2.75	-	-	-
	Reducing sugars (g·L^{-1})	-	26.869 ±0.592	20.017 ±0.385	10.877 ±0.068	15.084 ±0.030	-	-	12.441 ±0.012	-	-	-
	Sodium pyruvate (mM)	-	0.047 ±0.039	-	-	0.085 ±0.050	-	-	0.115 ±0.041	-	-	-
P2	pH	5.585 ±0.003	4.857 ±0.006	4.018 ±0.001	3.831 ±0.002	3.700 ±0.000	-	-	3.486 ±0.002	-	-	-
	TSS (°Brix)	-	3.5	3.5	3.15	2.93	-	-	2.1	-	-	-
	Reducing sugars (g·L^{-1})	-	19.728 ±0.062	14.832 ±0.022	15.415 ±0.077	11.169 ±0.004	-	-	6.022 ±0.222	-	-	-
	Sodium pyruvate (mM)	-	0.065 ±0.043	-	-	0.163 ±0.044	-	-	0.134 ±0.027	-	-	-
P3	pH	5.431 ±0.001	5.171 ±0.004	4.546 ±0.001	4.201 ±0.002	3.971 ±0.003	-	-	3.520 ±0.002	3.524 ±0.002	3.590 ±0.002	-
	TSS (°Brix)	-	3.85	3.85	2.8	2.95	-	-	2.45	2.43	2.45	-
	Reducing sugars (g·L^{-1})	-	22.166 ±1.161	14.317 ±0.045	10.818 ±0.031	4.048 ±0.025	-	-	3.713 ±0.014	-	-	-
	Sodium pyruvate (mM)	-	0.071 ±0.113	-	-	0.257 ±0.070	-	-	0.571 ±0.061	-		-
P4	pH	5.497 ±0.003	5.227 ±0.002	4.856 ±0.002	4.372 ±0.002	4.229 ±0.001	-	-	4.188 ±0.002	4.215 ±0.007	4.268 ±0.002	-
	TSS (°Brix)	-	3.3	1.75	1.75	1.75	-	-	1.7	1.8	1.75	-
	Reducing sugar (g·L^{-1})	-	23.960 ±0.013	3.386 ±0.010	1.067 ±0.006	0.873 ±0.007	-	-	0.869 ±0.005	-	-	-
	Sodium pyruvate (mM)	-	0.226 ±0.068	-	-	0.115 ±0.151	-	-	0.301 ±0.044	-	-	-

Table 2. *Cont.*

	Parameter	Fermentation Time (d)										
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
P5	pH	5.240 ±0.002	4.685 ±0.000	4.552 ±0.002	-	-	4.218 ±0.001	4.166 ±0.004	4.150 ±0.005	4.124 ±0.002	4.134 ±0.004	-
	TSS (°Brix)	-	13.05	12.2	-	-	10.85	9.75	10.1	10.45	10.2	-
	Reducing sugars ($g \cdot L^{-1}$)	-	87.949 ±0.158	79.957 ±0.077	-	-	83.327 ±0.241	77.650 ±0.058	77.920 ±0.424	-	-	-
	Sodium pyruvate (mM)	-	n.d	-	-	-	0.12 ±0.014	-	0.12 ±0.023	-	-	-
P6	pH	5.855 ±0.009	5.379 ±0.002	4,995 ±0.001	4.968 ±.001	-	-	4.895 ±0.002	4.885 ±0.000	4.870 ±0.001	4.775 ±0.002	4.844 ±0.001
	TSS (°Brix)	-	3.35	2.35	2	-	-	2.25	2.2	2.3	2.2	-
	Reducing sugars ($g \cdot L^{-1}$)	-	20.657 ±0.177	-	38.342 ±0.125	-	-	3.771 ±0.114	-	4.013 ±0.031	-	-
	Sodium pyruvate (mM)	-	0.177 ±0.044	-	0.365 ±0.081	-	-	-	0.413 ±0.021	-	0.639 ±0.042	-
P7	pH	5.828 ±0.003	5.065 ±0.007	4.162 ±0.002	3.903 ±0.000	-	-	3.741 ±0.003	3.736 ±0.004	3.640 ±0.003	3.370 ±0.001	3.464 ±0.000
	TSS (°Brix)	-	10.25	10.25	10.25	-	-	11.3	11.25	11.25	11.3	-
	Reducing sugars ($g \cdot L^{-1}$)	-	90.218 ±0.113	-	69.935 ±0.965	-	-	73.186 ±0.169	-	61.537 ±0.277	-	-
	Sodium pyruvate (mM)	-	n.d	-	0.071 ±0.032	-	-	-	0.278 ±0.036	-	0.874 ±0.099	-

n.d—non-defined.

The analysis of TPC in fermented papaya samples during fermentation is represented in Table 3. This analysis revealed that the lowest TPC was observed on the 7th day of P4 (121. ± 0.12 µg GAE·mL^{-1}) and the highest content was observed on the 8th day of P7 (260.18 ± 0.02 µg GAE· mL^{-1}). The TPC decreased from the first to the last day of fermentation in all tests, except for the tests where bacteria were used (P6 and P7).

Table 3. Changes in total phenolic content (TPC) of the fermented papaya samples during fermentation assays (P1 to P7).

Fermentation Time (d)	TPC of the Fermented Papaya (µg GAE·mL^{-1} of Fermented Papaya Product)						
	P1	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**
1	203.29 ± 0.04	173.61 ± 0.66	214.31 ± 0.02	235.73 ± 0.02	138.59 ± 0.75	157.98 ± 0.04	119.89 ± 0.02
7	175.26 ± 3.53	166.10 ± 0.47	182.01 ± 0.02	121.20 ± 0.1	134.08 ± 0.54	-	-
8	-	-	-	-	-	221.13 ± 0.04	260.18 ± 0.02

GAE—gallic acid equivalents.

The analysis the antioxidant activity of fermented papaya samples during fermentation, labelled using the FRAP method, is represented in Table 4. This analysis shows that the antioxidant activity ranged between 0.79 ± 0.02 equivalent µmoles of $Fe^{2+} \cdot mL^{-1}$ (on the 7th day of P4) and 6.56 ± 0.18 equivalent µmoles of $Fe^{2+} \cdot mL^{-1}$ (in 9th day of P7). From the 1st to the 7th day, antioxidant activity slightly increased in P1, P2, and P3. However, in P4, there was a decrease from the 1st to the 7th day, but it was followed by a slight increase from the 7th to the 9th day. In the P5 and P6 assays, antioxidant activity increased from the 1st to 5th day and then decreased. Antioxidant activity became increasingly pronounced over time in the P7 assay.

Table 4. Changes in total antioxidant activity (FRAP method) of the fermented papaya samples during fermentation assays (P1 to P7).

Fermentation Time (d)	Total Antioxidant Activity of the Fermented Papaya (Equivalent µmoles of Fe^{2+}·mL^{-1} of Fermented Papaya Product)						
	P1	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**
1	2.16 ± 0.05	1.51 ± 0.05	1.64 ± 0.06	2.22 ± 0.13	1.52 ± 0.77	3.57 ± 0.39	2.43 ± 0.11
3	-	-	-	-	-	3.46 ± 0.23	5.38 ± 0.16
4	1.87 ± 0.07	1.70 ± 0.06	1.58 ± 0.15	0.93 ± 0.03	-	-	-
5	-	-	-	-	1.99 ± 0.07	-	-
7	2.19 ± 0.07	1.92 ± 0.06	1.76 ± 0.06	0.79 ± 0.02	1.73 ± 0.05	3.69 ± 0.14	6.39 ± 0.17
9	-	-	1.53 ± 0.17	0.82 ± 0.06	0.99 ± 0.05	2.22 ± 0.31	6.56 ± 0.18

4. Discussion

After 48 h of fermentation, a reduction in reducing sugars was observed from day 1 to day 2. The glucose consumption was 26%, 25%, 35%, 86%, and 9%, in P1, P2, P3, P4, and P5, respectively. In P6, after 72 h, there was an increase in reducing sugars (glucose consumption = 86%), probably due to the release of compounds from the fruit to the puree. After the same period, in P7, there was a glucose consumption of 22% (Table 2). In P5 and P7, glucose was added to the medium, so the initial glucose content is similar. Sugar consumption was not complete, leaving some residual reducing sugars in the medium at the end of fermentation. In assays with the glucose addition (P5 and P7), the fermentation cycle starts with a mean sugar concentration of 11.65 °Brix and it is possible to observe that the °Brix is proportional to the reducing sugars consumed. In the remaining tests, the fermentation cycle starts with a mean sugar content of 3.50 °Brix (Table 2). Chen and their colleagues (2018) also observed a decrease in reducing sugars of 36.79% and 34.55% after a 48 h fermentation in fermented papaya juices prepared with *Lactobacillus acidophilus* and *Lactobacillus plantarum*, respectively [21].

During these papaya fermentation assays, the conversion of substrates (sugars, organic acids) into metabolites causes a change in the medium equilibrium and, consequently, in the pH: for instance, in P1, the pH decreased from 5.97 to 4.15 on day 7; in P6, the pH decreased from 5.86 to 4.78 on day 9, while on day 10 the pH started to rise, indicating the end of the fermentation cycle; in P7, the decrease was more accentuated (from 5.83 to 3.74 on day 7), but, as observed in P6, on day 10, the pH started to rise (Table 2). The decreased pH value could be attributed to sugar consumption and acid production. Similar results were reported by Chen and their colleagues in fermented papaya juices with lactic acid bacteria [21]. Food acidification is also important because it preserves and ensures the safety of foods. The pH value could be used not only at the end of the process, to qualify the product, but also as an indicator throughout fermentation [22].

The values of sodium pyruvate increased during the fermentation process. The increase of sodium pyruvate was more accentuated in the assays where the bacteria *Gluconobacter oxydans* was added (Table 2), indicating that, possibly, the fermentation process was more effective because the bacteria produced more organic compounds. For example, in P6, the sodium pyruvate content increased from 0.37 ± 0.08 mM on day 3 to 0.64 ± 0.04 mM on day 9, and in P7, the pyruvate content increased from 0.07 ± 0.03 mM on day 3 to 0.87 ± 0.10 mM on day 9. This may be due to the possible fact that the presence of sugars that were added to the medium in P7 increased the production of pyruvate in its metabolic pathway [23].

In P1, P2, P3, P4, and P5, there was a decrease in TPC during fermentation. For example, in P1, TPC reduced from 203.29 ± 0.04 to 175.26 ± 3.53 µg·mL^{-1} on day 7, representing a decrease of 14%. This may occur due to the degradation of phenolic compounds or their hydrolysis oxidation during the fermentation time [24]. However, it can be observed that in P6 and P7, there was an increase in TPC: in P6, with the addition of *Gluconobacter oxidans* only, TPC increased by 40%, while fermentation with *Gluconobacter* and glucose (P7) led to

an increase in TPC of 54%. Indeed, in P6 and P7, it was possible to observe that whether with sugar added to the fermentation process (P7), or without sugar (P6), the TPC increased (Table 3). Therefore, this gain was possibly due to the addition of the bacteria. However, the increase was more pronounced with the addition of both the *Gluconobacter* strain and glucose (P7). Other authors also observed an increase in TPC content during fermentation [25]. For example, in a study related to the fermented beverage kombucha, recognized for its health benefits, an increase in TPC was also observed during fermentation, and the values observed on day 8 of P6 and P7 (221.13 $\pm$ 0.04 mg·L^{-1} and 260.18 $\pm$ 0.02 mg·L^{-1}, respectively) (Table 3) were similar to those recorded in kombucha obtained from red tea (270.5 $\pm$ 2.4 mg L^{-1} on day 7) [14]. Phenolic compounds are the key substances to the antioxidant properties found in fruits and, as often found in cell walls, fermentation can release them and enhance their conversion into more active forms. The rise in TPC can be linked to events that occur during papaya fermentation, such as the oxidation of polyphenolic compounds by certain enzymes, which results in the synthesis of flavonoids and other beneficial substances [26].

Fermented papaya has many reported benefits in human health, related to its antioxidant activity [6,27]. In spontaneous fermentation (P2), the antioxidant activity increased from 1.51 $\pm$ 0.05 to 1.92 $\pm$ 0.20 equivalents µmoles of Fe^{2+}·mL^{-1} of the fermented papaya product on day 7. It was possible to observe that with spontaneous fermentation, the antioxidant activity increased slightly, except for the P4 assay (Table 4). In this type of fermentation, no starter was added. Fermentation occurs due to the plant-autochthonous microorganisms. It is described that there is a bacterium present in papaya (*Leuconostoc mesenteroides*) that is related to the increase in the antioxidant activity of the fermented pulp [28]. However, the increase was more pronounced when bacteria was added (P6), and even more when the assay was also supplemented with glucose (P7) (Table 4). In fact, some studies reported the various benefits of fermented papaya related to its strong antioxidant properties [6,7]. Fermented papaya with the addition of bacteria showed a higher antioxidant effect than spontaneous fermentation. The bioinoculation of *G. oxydans* combined with glucose supplementation biotransformed papaya polyphenols into active phenol metabolites with strong antioxidant capacities. This increase in antioxidant activity may be related to the increase in TPC also observed in P6 and P7 (Table 3). In fact, it has been explained that phenolic compounds are responsible for antioxidant capacity [29]. Another possible reason for the increase in antioxidant activity during fermentation is the fact that *G. oxydans* promotes the production of a L-ascorbic acid (vitamin C) precursor and prefers environments rich in sugars [13].

5. Conclusions

In this study, phenolic compounds in papaya were found to increase antioxidant capacity as a result of fermentation with *G. oxydans*. It appears that fermented papaya has a high concentration of phenolic compounds, which is a promising source of natural antioxidants and may be used in the future as a therapeutic beverage, or even in pharmaceuticals and cosmetic preparations.

In order to perform a rigorous and comparative evaluation of the assays, it will be needed to specify the days on which the analyses are withdrawn in future work. Future research would also need to control additional fermentation conditions, such as: the type of plant material—rotting fruit, the seeds, or even the peel can be saved to prevent food waste; the temperature; and the use of different bacteria. Furthermore, it will be useful for future studies to identify and characterize the species of bacteria present in the fermented papaya product and in the starting medium used as substrate, to relate the bacterial community with the obtained compounds. It is also critical to identify and determine the phytonutrients produced as a result of fermentation that boost the increase in antioxidant activity provided by fermentation with bacteria, namely by the exact quantification of each phenolic compound.

Author Contributions: Conceptualization, M.L. and P.C.; methodology, M.L. and P.C.; writing—original draft preparation, M.L. and P.C., writing—review and editing M.L., B.F., B.G., D.M., L.B., P.A.G. and P.C.; supervision, P.A.G., P.C. and L.B.; funding acquisition, P.A.G., P.C. and L.B. All authors have read and agreed to the published version of the manuscript.

Funding: Financial support from PT national funds (FCT/MCTES, Fundação para a Ciência e a Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the projects UIDB/50006/2020 and UIDP/50006/2020 is acknowledged. L.B. Acknowledges funding from FCT through program DL 57/2016-Norma transitória. Financial support was granted to P.C. by the "Support Program for Training and Technical-Scientific Update of Teaching Staff of ESS-P.Porto" (Order ESS/PR-23/2019, Order ESS/PR-5/2020 and Order ESS/P-20/2021).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors gratefully acknowledge the Research Centre on Health and Environment, Department of Pharmacy, School of Allied Health Sciences, Polytechnic Institute of Porto, Porto, Portugal, for providing installations, and supervisors for helpful suggestions.

Conflicts of Interest: The authors declare no conflict of interest.

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