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Study of the Antioxidant and Chelating Properties of a Chickpea Aquafaba-Based Postbiotic Ingredient

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Abstract

According to the main principles of the circular economy, recovering bioactive compounds from food waste has significant potential to deliver financial, environmental, and health benefits by incorporating them into food products as additives, emulsifiers, antioxidants, and antimicrobial agents to enhance nutritional and functional value and/or extend product shelf life. This work investigated the potential of a food waste material, such as chickpea aquafaba from industrial processing, as a fermentation substrate using promising lactic acid bacteria, including *L. plantarum* AME-01, *L. paracasei* CBA L74, and *L. mesenteroides*, under non-controlled pH conditions, and evaluated the antioxidant/chelating capacity of the postbiotic obtained from the process. Fermented substrates were thermally inactivated to produce postbiotic preparations, which were characterized in terms of inactivated biomass and lactic acid concentration. Subsequently, liquid postbiotics were spray-dried using a lab-scale spray dryer, and the resulting powders were characterized in terms of antioxidant and chelating properties. While the postbiotic preparations using *L. paracasei* CBA L74 showed significantly lower activity (23.78 ± 7.25%) compared to non-fermented aquafaba (56.85 ± 2.79%), those using *L. plantarum* AME-01 (84.60 ± 5.57%) and *L. mesenteroides* (82.56 ± 1.11%) exhibited increased chelating activity. The antioxidant power of all fermented powders was significantly improved, confirming the potential of postbiotic ingredients as stabilizing additives in food formulations.

Keywords: food waste; chickpeas aquafaba; postbiotic; antioxidant properties; chelating activity; legumes; fermentation; spray drying

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1. Introduction

Food waste management is an important global challenge, given that the EU alone generates more than 58 million tons of food waste annually [1].

In the current global scenario, the sustainable utilization of agri-food waste and/or by-products to produce value-added products for several uses can provide considerable opportunities to earn additional income for industries [2]. Furthermore, the associated

environmental stress is enormous, as are the challenges of safe disposal and the resulting economic burden on the agri-food companies involved [3]. The use of leguminous matrices provides a valuable plant-based alternative to animal proteins [4]. In particular, chickpea (*Cicer arietinum* L.) represents the second most consumed pulse worldwide. It is an essential food for growing populations, especially in developing countries. In 2017, global chickpea production was 14.8 million tons [5]. Aquafaba (AQ) is a viscous waste liquid drained from canned pulses or traditionally produced by soaking and cooking dried chickpeas [6]. Its upcycling offers a sustainable strategy to mitigate the high disposal costs associated with legume processing wastewater, which is typically characterized by a heavy organic load and a consequent severe environmental impact [7]. Aquafaba has recently gained significant interest as a vegan, allergen-friendly, and cholesterol-free rheological additive in a wide range of food products, including egg-free mayonnaise, meringues, bakery goods, and ice creams [8,9]. Therefore, the use of aquafaba as an eco-friendly, plant-based additive in food formulation could promote sustainable agriculture; it would encourage the cultivation of legumes, which contribute little to greenhouse gas emissions due to their nitrogen-fixing capability, thereby reducing the need for nitrogen-based fertilizers [9,10]. Chickpea-derived aquafaba comprises approximately 92–95% water and 5–8% dry matter. This solid fraction contains saponins, carbohydrates (including soluble and insoluble fibers and sugars), and low-molecular-weight proteins ($\leq$24 kDa) at 0.95–1.5% w/v [9,11]. Additionally, it includes phenolic compounds leached from the seeds during the soaking and boiling processes [12]. Moreover, it contains unique γ-glutamyl peptides, including γ-Glu-Phe and γ-Glu-Tyr, that possess anti-inflammatory properties [13]. Additionally, aquafaba can be obtained from canned chickpeas, which comprise 16% oligosaccharides, 46–65% vitamins, and 16% dietary fiber [14]. Several studies indicate that regular consumption of protein from legumes provides health benefits, including reduced risks of hypertension, cardiovascular disease, and cancer [15,16]. It is known that fermentation can modify the nutritional and functional quality of several substrates, such as legumes, enriching them with bioactive components, such as vitamins, natural phenolics, bioactive peptides, and reducing anti-nutritional factors (ANFs), like oligosaccharides, proteinase inhibitors, trypsin inhibitors, saponins, and phytates [17–19]. Therefore, the enzymatic activity of microorganisms, such as amylases, xylanases, and glucosidases, can significantly enrich vegetable matrices with value-added components while effectively degrading ANFs. Furthermore, crucial techno-functional properties, including water- and oil-holding capacities (WAC and OHC), emulsifying activity (EA), and foaming capacity (FC), which are essential for food processing and formulation [17], can be improved through fermentation. Recently, an important category of fermented products that has been gaining interest is postbiotics, defined as "preparation of inanimate microorganisms and/or their components that confer a health benefit on the host" [20]. Postbiotics can be obtained through thermal inactivation of probiotics. They minimize the risks associated with their intake and represent a safer option for immunodeficient patients or infants [21].

They stimulate the growth of beneficial microbes [22–24], inhibit the growth of pathogens [25], modulate electrolyte and water absorption [26,27], and promote multiple beneficial health effects, including immunomodulatory, anti-inflammatory, antioxidant, and anticancer properties [28]. Bellomo et al. [29] demonstrated that sustainable milk-based postbiotic beverages can prevent P31-43-induced inflammation in Caco-2 cells and reduce constitutive inflammation in Celiac Disease (CeD) organoids.

Therefore, lactic acid fermentation could be considered one of the most promising routes for valorization to investigate. The selection of fermentation/inactivation conditions and the identification of the best starter cultures rely on the characteristics of the food

substrate, the functional compounds of interest, and the technological and organoleptic properties of the final fermented product.

For example, the valorization of industrial wastewaters from the blanching of dried navy beans through lactic acid fermentation was reported in the work of Colucci Cante et al. [30], where the improvement of the antioxidant properties (approximately 25.32–37.72%) and the foaming capacity of the lactic-fermented waters was demonstrated. Moreover, lactic acid fermentation of chickpea aquafaba and its potential as an enhanced texture-modifying ingredient with antioxidant properties have also been investigated in several other literature studies [31,32]. However, from an industrial perspective, the liquid nature of aquafaba poses challenges in handling, storage, transportation, and microbiological stability [33]. One solution is to dry the matrix to obtain a concentrated powder, thereby extending its shelf life [9]. Conventional spray drying is a valuable method for industrial applications, offering an effective compromise between powder quality and production efficiency.

The primary aim of the study was to investigate the potential of a food waste material, such as chickpea aquafaba, resulting from industrial processing, as a fermentation substrate using promising lactic acid bacteria such as *L. plantarum* AME-01, *L. paracasei* CBA L74, and *L. mesenteroides*, under non-controlled pH conditions, and to evaluate the antioxidant/chelating capacity of the postbiotic obtained from the process. Since food producers traditionally use synthetic antioxidants such as ethylenediaminetetraacetic acid (EDTA) to combat lipid oxidation in emulsions, postbiotic aquafaba could be proposed as a powerful natural emulsifier with significant metal-chelating and antioxidant properties, capable of meeting the increasing consumer demand for natural ingredients and clean-label products. Aquafaba's potential as a functional additive in novel formulations of plant-based mayonnaises was tested in several scientific studies [34,35], which demonstrated a reduced tendency toward lipid oxidation in aquafaba-based emulsions, attributed to their radical-scavenging properties and the resulting action against oxidative phenomena compared to conventional mayonnaises. In this perspective, this study involved a preliminary identification of three lactic acid strains with potential antioxidant/chelating properties from the literature, namely *Lactobacillus* paracasei CBA L74, *Lactobacillus plantarum* AME-01, and *Leuconostoc mesenteroides* spp. [30,36–39]. Subsequently, liquid aquafaba-based postbiotics using the selected bacteria were produced via fermentation and thermal inactivation, then spray-dried. Finally, the resulting postbiotic powders were characterized for antioxidant and chelating activities to select the best microorganism capable of effectively functionalizing the matrix and yielding an ingredient with optimal preservative characteristics.

2. Materials and Methods

2.1. Strains and Fermentation Substrate

Three different lactic acid bacteria (LABs), namely *Lactobacillus paracasei* CBA L74, *Lactobacillus plantarum* AME-01, and *Leuconostoc mesenteroides* spp., provided by University of Naples Federico II (UNINA)/Innovation & Technology Provider (I.T.P. s.r.l.), were used as starter cultures for aquafaba fermentations. These LABs (stored at −80 °C in glycerol) were revitalized in De Man, Rogosa, and Sharpe (MRS) broth (Sigma Aldrich, Milan, Italy) and incubated at 37 °C for 24 h, reaching a bacterial charge of 10^9 CFU/mL. The matrix used for the fermentations was Veggò chickpeas aquafaba, provided by Euro S.P.I.D. srl (Messina, Italy), and stored in sealed sterile bottles at 25 °C.

2.2. Experimental Campaign and Equipment

The study is structured in several experimental phases, as shown in Figure 1:

i. **Fermented chickpea aquafaba-based postbiotic production:** Each strain was used to ferment aquafaba under uncontrolled pH conditions, and the resulting biomass was thermally inactivated to produce postbiotics.
ii. **Dry postbiotic production:** Liquid aquafaba postbiotic was dried using a laboratory spray dryer to obtain a dry functional ingredient. Various operating conditions were tested to select those that maximized powder yield while maintaining low moisture content.
iii. **Functional characterization**: The dried postbiotic powders were characterized in terms of antioxidant and chelating activity to identify the best microorganism and the best fermentation conditions for the intended purpose.

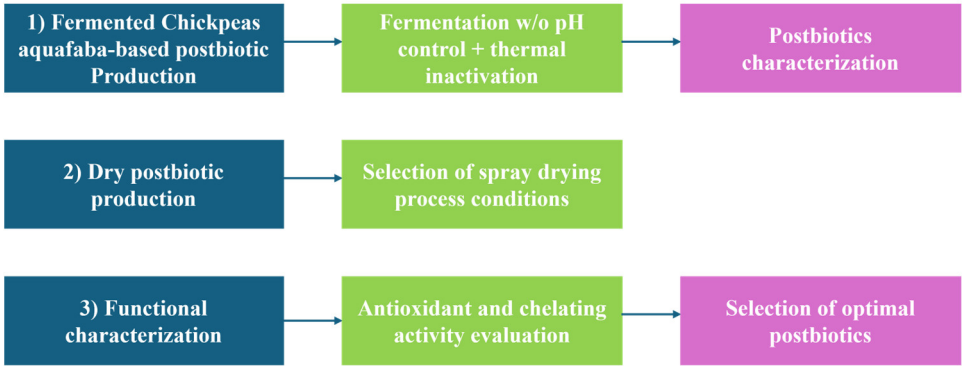

Figure 1. Experimental plan.

2.2.1. Production of Liquid Aquafaba-Based Postbiotics

Fermented Aquafaba (FAQ) was obtained by fermenting at 37 °C for 24 h using a 1% (*v*/*v*) inoculum (108 CFU/mL) in a stirred batch reactor previously described by Colucci Cante et al. [17], which was loaded with 1 L of matrix. Briefly, it consisted of a glass vessel (height of 20 cm and internal diameter of 10 cm), equipped with a jacket for the recirculation of heated water from a thermostatic bath, a 3 Rushton turbines impeller, and an inPro 3100 probe (Mettler Toledo, Milan, Italy), connected to an M300 transmitter for inline temperature/pH measurements. Subsequently, for each strain, a thermal inactivation step was performed inside the same bioreactor at 90 °C for 5 min to obtain a postbiotic. Liquid postbiotics were characterized for lactic acid content, pH reduction, and bacterial growth, and subsequently dried using a lab-scale spray-drying apparatus.

2.2.2. Production of Dried Aquafaba-Based Postbiotics

Fermented aquafaba postbiotics, with 4.5% of total solids, were used as feed in a spray drying system (Ollital Model "OLT-SD8000B"—Lab 2L Centrifugal Spray Dryer, Fujian, China). The product was heated at 30 °C and kept mixed using a lab hot plate magnetic stirrer before being fed into the system in a downward co-current configuration with air. It consists of a spray chamber connected to a cyclone separator and a downstream receiving tank, all made of transparent, heat-resistant high-borosilicate glass. An automatic jet cleaner prevents blockage of the 2.0 mm nozzle.

Preliminary tests were conducted using non-fermented AQ to evaluate operating variables; subsequently, new trials were performed on all fermented AQ matrices using the previously determined best parameters.

The drying tests were performed by varying certain drying process parameters [11,40,41] while keeping the atomizer pressure and feeding temperature constant at 3 bar and 30 °C, respectively (Table 1). Furthermore, different percentages (relative to the amount of solids of the matrix) of maltodextrins (18–20 DE, 100% pure powder, BulkTM, Colchester, UK),

used as a carrier agent, were tested to make up for possible high chamber fouling and stickiness phenomena due to the low total solids content in AQ [40].

Table 1. List of spray drying trials and related operating conditions adopted on raw AQ (tests 1–8) and FAQ postbiotics (tests 9–15). AQ: non-fermented aquafaba; FAQ: fermented aquafaba. Each trial was conducted in duplicate.

# Trial	Matrix	V_{feed} (mL)	AQ Solids (g)	Maltodextrins (%)	T_{air} (inlet) (°C)	Q_{air} (m^3/h)	Q_{feed} (mL/min)
1	AQ	100	4.50	5	160	280.5	4.5
2	AQ	100	4.50	5	170	280.5	4.5
3	AQ	100	4.50	5	170	330	4.5
4	AQ	100	4.50	0	170	330	4.5
5	AQ	100	4.50	5	170	313.5	4.5
6	AQ	100	4.50	10	170	313.5	4.5
7	AQ	110	4.95	5	170	313.5	4.5
8	AQ	100	4.50	5	170	313.5	5.5
9	FAQ postbiotic from *L. plantarum AME-01*	100	4.50	5	170	330	4.5
10	FAQ postbiotic from *L. plantarum AME-01*	493	22.18	5	170	330	4.5
11	FAQ postbiotic from *L. plantarum AME-01*	100	4.5	0	170	330	4.5
12	FAQ postbiotic from *Leuconostoc* spp.	500	22.50	5	170	214.5	4.5
13	FAQ postbiotic from *Leuconostoc* spp.	450	20.25	5	170	316.8	4.5
14	FAQ postbiotic from *L. paracasei CBA L74*	507	22.82	5	175	316.8	4.5
15	FAQ postbiotic from *L. paracasei CBA L74*	453	20.39	5	175	316.8	4.5

Drying performances were evaluated in terms of drying yield (DY) and residual moisture (RM) of the resulting powders, calculated as follows:

$$DY\ (\%) = \text{g of obtained postbiotic powder}/(\text{g of solids AQ} + \text{g maltodextrins}) \quad (1)$$

$$RM\ (\%) = 100 - [(W_f/W_i) * 100] \quad (2)$$

The RM was evaluated through a thermobalance. W_f is the final weight of the sample after the heating cycle, and W_i is the initial weight of the sample.

2.2.3. Functional Characterization of the Postbiotic Powders

The postbiotic powders derived from *L. paracasei* CBA L74, *Leuconostoc mesenteroides*, and *L. plantarum* AME-01 strains were then analyzed for antioxidant capacity and chelating activity, according to the analytical protocols described in the sections below.

2.3. Analytical Methods

2.3.1. Microbiological, pH, and Lactic Acid Concentration Evaluation

The microbial load of lactic acid bacteria was analyzed by serial dilution and the spread plate method on Petri plates filled with De Man, Rogosa, and Sharpe agar (Oxoid, Basingstoke, UK)) for lactobacilli counts and Yeast agar for yeast counts [42].

MacConkey agar (Oxoid, Basingstoke, UK)) and Gelatin Peptone agar (Biolife, Milan, Italy) were used to monitor the growth of microbial contaminants in the fermenting medium. All plates were incubated at 37 °C and 28 °C for lactobacilli and yeast, respectively, for 48 h before reading. The measured bacterial load was expressed as CFU/mL.

The pH of the fermented AQ samples was measured in-line throughout the process using an InPro 3100 probe (Mettler Toledo), connected to the fermentation apparatus and to an M300 transmitter (Mettler Toledo), which is also used for in-line temperature measurements.

Before High-Performance Liquid Chromatography (HPLC) analysis, postbiotic samples underwent a pre-treatment step to extract and accurately quantify lactic acid by removing interfering dietary fibers. Therefore, 1 g of the sample was mixed with 3 mL of 50 mM phosphate buffer (pH 7) and kept at 40 °C for 1 h with gentle mixing on a rotating wheel. Afterward, samples were centrifuged at 10,000× *g* for 10 min, and the supernatant was filtered using a 0.2 µm membrane filter and injected into a 1 mL vial for HPLC analysis.

Lactic acid content was measured by HPLC (Agilent Technologies 1100, Milan, Italy; adapted method from TN-1189 Phenomenex, Torrance, CA, USA) using a Synergy Hydro-RP 80 A column 250 mm × 4.6 mm, 5 µm particle size, Phenomenex), and 0.27% KH_2PO_4 aqueous solution with a pH value of 1.5 (adjusted with phosphoric acid), as mobile phase. The flow rate was set to 1 mL/min, the injection volume to 20 µL, and UV detection at 210 nm [31]. Information about the calibration curve is reported in Figure S1.

2.3.2. Antioxidant and Chelating Properties of AQ Postbiotics

The antioxidant and chelating power of aquafaba-based postbiotics was analyzed using spectrophotometric methods, namely the ABTS and Ferrozine assays.

Extracts Preparation

About 0.5 g of dry samples, obtained by spray-drying the liquid FAQ, was weighed and added to 7 mL of 70% (*w*/*v*) ethanol. The mixture was then subjected to 15 min of sonication using a SONOREX SUPER RK 100 H (Bandelin, Berlin, Germany) at an ultrasonic frequency of 35 kHz and a nominal power of 80 W. The sample was then centrifuged at 640× *g* for 15 min, and the supernatant was recovered. The sedimented pellet was redissolved in 7 mL of 70% (*w*/*v*) ethanol, and the same extraction procedure was repeated two more times [17]. Finally, all the supernatants were combined, and the resulting extracts were used for the subsequent evaluations.

Antioxidant Activity—ABTS Assay

The 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay was performed to measure the antioxidant capacity of FAQ postbiotic extracts by evaluating their scavenging ability toward the ABTS radical cation [43,44].

A stock solution of 7 mM ABTS (Sigma-Aldrich, Milan, Italy) was mixed with a 2.45 mM potassium persulfate; the resulting working solution (S1) was stored in the dark at room temperature for 12 h. The working ABTS reagent (S2) was diluted in pure ethanol to reach an absorbance of 0.7 at 734 nm.

Subsequently, 2.8 mL of S2 was added to 0.15 mL of each sample extract, and the corresponding absorbances (read at 734 nm) were read after 10 min and 1 h in the dark, using 100% ethanol as a blank. Antioxidant activity was evaluated as μmol of Trolox equivalent (TE) per 100 mL of aquafaba, using a calibration curve previously prepared with known solutions of Trolox (Sigma-Aldrich, Milan, Italy) (25–600 μM), used as a reference antioxidant compound.

The results are reported as a radical-scavenging activity percentage using the following equation (Equation (3)):

$$\text{ABTS radical scavenging activity (\%)} = (A_0 - A_s)/A_0 \times 100 \quad (3)$$

where A_s expresses the absorbance of the sample, and A_0 represents the absorbance of the blank (100% ethanol).

Chelating Activity—Ferrozine Assay

The chelating activity of the postbiotics was evaluated using the ferrozine assay. Ferrozine forms a purple complex with ferrous iron, and in the presence of chelating agents, this complex is disrupted, reducing the purple color. The extent of color reduction estimates the binding ability of the coexisting chelator. The capacity of the FAQ postbiotic extracts to chelate Fe^{2+} was determined using the method of Gu et al. [45].

Briefly, 1.85 mL of H_2O and 0.05 mL of $FeSO_4$ (2.0 mmol/L) were added to 1 mL of FAQ postbiotic extracts; the mixture was incubated at room temperature for 30 s, and then 0.1 mL of ferrozine (5 mmol/L) was added. The sample was allowed to stand at room temperature for 10 min, and the absorbance was measured at 562 nm. As a control, distilled water replaced the FAQ postbiotic extracts in the solution. The results were expressed as radical-scavenging activity percentage relative to the control solution, according to Equation (4), using a calibration curve prepared with known EDTA solutions (0.4 mg/L–1 g/L) as the reference antioxidant.

$$\text{Chelating activity (\%)} = (1 - As/A_0) \times 100 \quad (4)$$

where A_s represents the absorbance of the sample and A_0 expresses the absorbance of the control (mixture $FeSO_4$, H_2O, ferrozine).

2.4. Statistical Analysis

Each fermented sample was obtained from two independent productions.

Standard deviations, averages, and graphs were calculated using Microsoft Excel, and all the statistical evaluations were carried out with GraphPad Prism (Version 9.0.0). In particular, statistical significance was evaluated by one-way ANOVA followed by Tukey's multiple comparisons test, accepting as significant only results with $p < 0.05$.

3. Results and Discussion

3.1. Fermentation, Inactivation, and Drying Performances

Aquafaba was fermented using three lactic acid strains (*Lactobacillus paracasei* CBA L74, *Lactobacillus plantarum* AME-01, and *Leuconostoc mesenteroides* spp.) without controlling pH. The resulting probiotic and postbiotic products were then characterized for bacterial

growth, pH, and lactic acid concentration before and after the microbial inactivation phase, as shown in Table 2.

Table 2. Characterization of fermented aquafaba (FAQ) in terms of bacterial growth, pH, and lactic acid concentration before and after the biomass inactivation. Results are expressed as mean ± standard deviation according to the statistical analysis performed. Different lowercase letters in the same column indicate significant differences ($p < 0.05$) according to one-way ANOVA followed by Tukey's test.

	Probiotic FAQ			Postbiotic FAQ		
	Bacterial Growth [CFU/mL]	pH	Lactic Acid [g/L]	Bacterial Growth [CFU/mL]	pH	Lactic Acid [g/L]
Leuconostoc spp.	$5.00 \times 10^8 \pm 1.41 \times 10^8$ a	6.09 ± 0.04 b	3.19 ± 0.54 c	1.55 ± 0.49 e	6.01 ± 0.01 f	3.00 ± 0.57 g
L. plantarum AME-01	$5.50 \times 10^8 \pm 7.07 \times 10^7$ a	6.12 ± 0.03 b	3.40 ± 0.14 c	1.50 ± 0.71 e	6.06 ± 0.06 f	3.25 ± 0.07 g
L. paracasei CBA L74	$1.80 \times 10^8 \pm 1.84 \times 10^8$ a	5.51 ± 0.42 b	1.74 ± 0.01 d	1.70 ± 0.57 e	5.40 ± 0.42 f	1.50 ± 0.14 h

As seen from the results reported above, a final microbial load of approximately 8–9 Logs was observed after 24 h of fermentation, similar to Colucci Cante et al. [30].

Despite the high cellular viability achieved, the pH did not decrease significantly, indicating moderate lactic acid production. This behavior can be hypothesized as a result of the specific metabolic profiles of the selected LAB strains (*L. plantarum AME-01*, *L. paracasei CBA L74*, and *L. mesenteroides*) when inoculated into a protein-rich legume matrix. Facultative heterofermentative strains like *L. plantarum AME-01* and *L. paracasei CBA L74* are renowned for their metabolic flexibility and robust proteolytic systems, including cell-envelope proteinases [46]. In complex matrices lacking abundant simple sugars, these strains can slowly hydrolyze leguminous oligosaccharides (such as raffinose and stachyose) and heavily rely on amino acid catabolism to fuel massive biomass accumulation [47]. This intense proteolytic activity can support exponential cellular growth and release free amino acids, peptides, and ammonia.

These compounds significantly increase the substrate's buffering capacity, exerting a strong neutralizing effect that counteracts the pH drop, as observed in lactic acid fermentations of several legume-based suspensions [48].

Furthermore, *L. mesenteroides* is an obligate heterofermentative strain that produces ethanol, carbon dioxide, and exopolysaccharides (EPS), along with lactic acid, thereby resulting in a lower acidification rate per unit of sugar consumed [49].

However, approximately 2.7 g/L of lactic acid was produced, which aligns with typical legume fermentations [50–52], which intrinsically generate less lactic acid compared to milk, cereal, or fruit matrices [52–55]. Moreover, the thermal step was effective in inactivating the strains but did not significantly affect the characteristics of the FAQ, as can be seen from the lactic acid values [56]. Finally, it was possible to state that aquafaba served as a suitable fermenting substrate for all the tested microorganisms, ensuring high proliferation capacity and lactic metabolism [30], comparable to that commonly observed during lactic fermentation of other legume matrices [50–52]. No significant differences were observed among the strains regarding bacterial growth and pH. On the contrary, *L. plantarum AME-01* and *Leuconostoc* spp. showed a higher lactic acid production with respect to *L. paracasei CBA L74* ($p < 0.05$).

The drying yield and residual humidity of the final powders deriving from each spray-drying trial are reported in the following Table 3.

Table 3. Spray drying results obtained from experimental tests on raw AQ (tests 1–8) and FAQ postbiotics (tests 9–15). RM: residual moisture content in the final powders; DY: drying yield; AQ: non-fermented aquafaba; FAQ: fermented aquafaba. Results expressed as mean of the duplicate (n = 2) ± standard deviation.

#Trial	Matrix	T_{air} (out) (°C)	RM (%)	DY (%)	Selected Operating Parameters
1	AQ	71.80 ± 1.13	5.40 ± 0.14	52.91 ± 0.01	T_{air} (inlet) → 170 °C Q_{air} → 96–100 m^3/h Q_{feed} → 4.5 mL/min Maltodextrins → between 0–5%
2		73.55 ± 0.07	4.45 ± 0.07	55.02 ± 0.02	
3		77.50 ± 0.71	3.24 ± 0.05	70.23 ± 0.04	
4		78.50 ± 0.71	3.61 ± 0.01	74.37 ± 0.10	
5		76.50 ± 0.71	2.93 ± 0.04	68.74 ± 0.06	
6		77.50 ± 0.71	3.29 ± 0.02	67.74 ± 0.08	
7		77.50 ± 0.71	3.77 ± 0.03	65.31 ± 0.01	
8		74.50 ± 0.71	3.86 ± 0.01	64.53 ± 0.04	
9	FAQ postbiotic from *L.plantarum* AME-01	79.50 ± 0.71	3.64 ± 0.06	69.82 ± 0.03	
10		80.00 ± 1.41	3.82 ± 0.03	72.94 ± 0.06	
11		78.00 ± 1.41	4.45 ± 0.07	67.53 ± 0.04	
12	FAQ postbiotic from *Leuconostoc* spp.	69.00 ± 1.41	7.45 ± 0.07	45.60 ± 0.01	
13		79.50 ± 3.54	5.55 ± 0.06	56.21 ± 0.01	
14	FAQ postbiotic from *L.paracasei* CBA L74	81.50 ± 2.12	4.42 ± 0.02	67.17 ± 0.01	
15		81.50 ± 2.12	4.52 ± 0.03	63.39 ± 0.01	

A total of fifteen trials were conducted on both non-fermented and fermented aquafaba, using *L. plantarum* AME-01, *Leuconostoc* spp., and *L. paracasei* CBA L74. The objective was to select the most suitable conditions that maximized powder yield while maintaining a low moisture content.

Across all experiments, the final moisture content remained consistently low (between 3 and 5.6%), as reported by He et al. [11] and Begliyev et al. [41], indicating effective water removal. Yield variations were primarily due to product losses within the equipment, caused by matrix stickiness, chamber fouling, and incomplete separation in the cyclone.

The first trials showed a clear influence of inlet temperature and airflow on process performance. At 160 °C and a low airflow rate (280.5 m^3/h), the outlet temperature was around 71–73 °C, resulting in a moderate yield of 52.9% (trial 1). Increasing the inlet temperature to 170 °C [40,41] (trial 2) and the airflow rate to 330 m^3/h (trial 3) raised the outlet temperature to 76–80 °C and substantially improved final yield (up to 69–74%).

Lowering the airflow rate to 313.5 m^3/h, as in trials 5–8, reduced final yield compared with the previous trials. These findings confirmed that an outlet temperature of 78–82 °C is critical for efficient drying and minimal wall deposition. The feeding flow rate was maintained at approximately 4.5 mL/min, as suggested by Begliyev et al. [41], since increasing it, as in trial 8, did not increase the final yield or reduce the powder moisture content. Also in the studies by He et al. [11] and Silva-Padilha [40], a feeding flow rate of 5–6 mL/min was used to perform efficiently the spray drying of chickpea aquafaba.

Maltodextrins were added in different percentages (0, 5, 10%) [40] to assess their potential as a drying aid. Although higher amounts of carrier agent were expected to improve powder recovery, this trend was not observed. One of the highest yields (74.44%) was obtained under the optimized drying conditions (170 °C, drying air flow rate 8–330 m^3/h, feeding flow rate 4.5 mL/min) without adding maltodextrin.

This result suggests that the matrix can be effectively spray-dried on its own when the thermal profile is properly controlled [11,41]. While maltodextrin is commonly added to improve powder flowability and reduce stickiness, the present results showed no substantial increase in yield attributable to this addition. In fact, higher yields were obtained without adding maltodextrins. Similarly, Begliyev et al. [41] achieved a drying yield of about 46% without the addition of a carrier agent to the matrix. This suggests that, for aquafaba-based matrices, maltodextrin may be more relevant for particle morphology and powder stability rather than for improving mass recovery.

3.2. Antioxidant and Chelating Properties of Dry FAQ Postbiotics

Dry FAQ postbiotics were characterized for antioxidant and chelating properties and compared with non-fermented AQ using ABTS and ferrozine assays.

3.2.1. Antioxidant Activity

Results of the antioxidant activity are reported in Figure 2 in terms of µmol of Trolox equivalent (TE) per g of powder, after 10 min and 1 h of reaction.

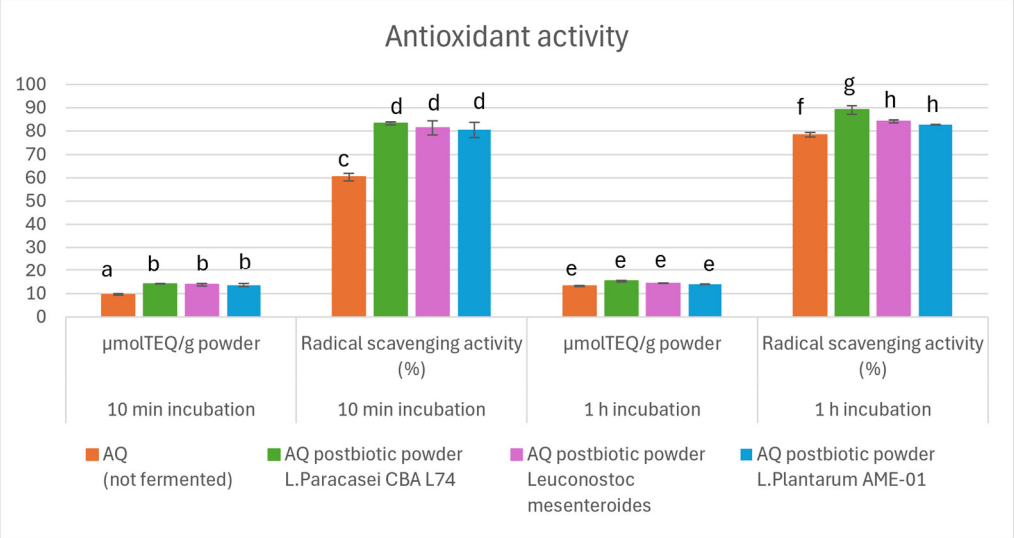

Figure 2. Antioxidant activity (ABTS) of FAQ postbiotics and not fermented aquafaba. Each result reported is a mean value of a duplicate analysis, and error bars indicate the corresponding standard deviations. Values marked with different lowercase letters are significantly different ($p < 0.05$), according to Tukey's test.

The ability of fermentation to enhance radical-scavenging activity has been reported in various studies. For instance, Lee et al. [57] and Juan and Chou [58] observed greater radical scavenging in fermented pigeon pea and soybean.

Similarly, Xiao et al. [59] reported 38% higher ABTS scavenging activity in chickpea fermented by *Cordyceps militaris* SN-18. In our work, after 10 min of incubation, all fermented samples (*L. paracasei* CBA L74 83.29 ± 0.64%, *Leuconostoc* spp. 81.43 ± 2.99%, and *L. plantarum* AME-01 80.57 ± 3.21%) exhibited significantly higher antioxidant activity than the unfermented control (60.29 ± 1.64%). According to several literature studies on the fermentation of plant-based matrices [60,61], this can be attributable to the production of bioactive molecules, such as enzymes, antioxidant peptides, or exopolysaccharides, during fermentation [31].

Furthermore, the observed antioxidant activity increased over time, likely due to prolonged exposure and reaction time between the samples and the ABTS solution.

After 1 h of incubation, the sample fermented with *L. paracasei* CBA L74 exhibited the highest antioxidant activity (89.14 ± 1.81%), followed by *Leuconostoc* spp. (84.29 ± 0.65%) and *L. plantarum* AME-01 (82.86 ± 0.05%). The time-dependent behavior can be related to the reaction kinetics of the different antioxidant compounds present in the matrix.

Unlike simple antioxidants, complex molecules such as high-molecular-weight polyphenols and fermentation-derived bioactive peptides are slow-reacting [62], requiring prolonged incubation to fully reduce the ABTS radical and accurately measure total antioxidant capacity.

3.2.2. Chelating Activity

Results of the chelating activity of aquafaba-based postbiotics and non-fermented AQ are reported in Figure 3:

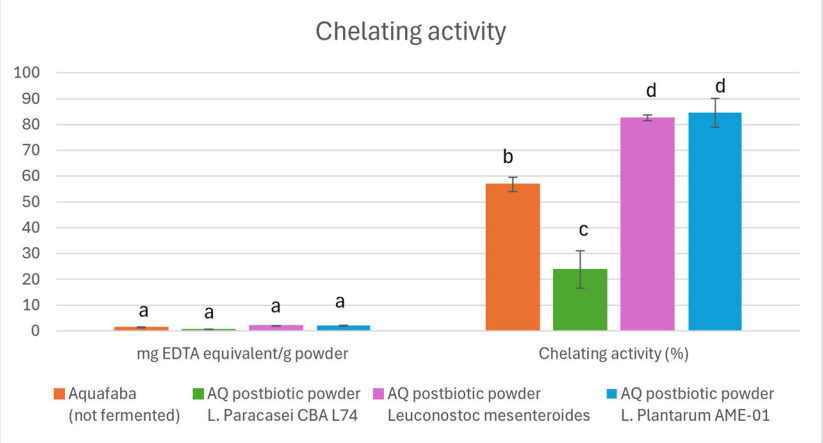

Figure 3. Chelating activity (mg EDTA equivalent/g powder and %) of FAQ postbiotics and not fermented powder AQ. Each result reported is a mean value of a duplicate analysis, and error bars indicate the corresponding standard deviations. Values marked with different lowercase letters are significantly different ($p < 0.05$), according to Tukey's test.

Based on the results, there is a significant difference in the chelating activity among the fermented samples. Postbiotic powder fermented with *L. paracasei* CBA L74 showed a significantly lower activity (23.78 ± 7.25%) compared to non-fermented AQ (56.85 ± 2.79%); whereas fermenting aquafaba with *L. plantarum* AME-01 or *Leuconostoc* spp. resulted in improved chelating activity compared to the non-fermented one, with values of 84.60 ± 5.57% and 82.56 ± 1.11%, respectively.

Similarly, Xiao et al. [63] and Li et al. [64] demonstrated the positive impact of fermentation on the chelating activity of legume matrices.

The improved chelating activity of fermented samples can be attributed to metabolites such as bioactive peptides, organic acids, or exopolysaccharides, which can act not only as radical scavengers but also as metal chelators [65,66]. Although the sample fermented with *L. paracasei* CBA L74 exhibited the highest antioxidant capacity, it showed lower metal-chelating activity than the other strains and the unfermented control. This divergent behavior highlights that radical scavenging and metal chelation rely on different molecular structures and strain-specific metabolisms [66,67].

Furthermore, it can be hypothesized that *L. paracasei* CBA L74 either produced insufficient amounts of chelating metabolites or converted strong chelators into non-chelating compounds, thereby reducing its chelating power [68].

4. Conclusions

This work focused on the valorization of chickpea aquafaba through lactic acid fermentation to obtain functional ingredients with potential health benefits.

In addition to the role of fermentation in enhancing the matrix's technological properties, as already demonstrated in recent literature studies, the primary aim of the present work was to confirm lactic acid fermentation as also an effective biotechnological strategy for upcycling agro-industrial by-products like aquafaba into value-added products with preserving effects.

Therefore, a key achievement of this study was the successful combination of fermentation with a carrier-free spray-drying process, showing that a low-value by-product such as chickpea cooking water can be transformed into a stable, dry postbiotic powder.

The postbiotic nature of the final semi-finished product, combined with its demonstrated antioxidant-preserving effect, represents the real novelty of this research.

In fact, thanks to its improved antioxidant and chelating properties, the resulting ingredient showed strong potential as a natural additive for food, cosmetic, and nutraceutical applications. Nevertheless, some limitations must be acknowledged.

The study was conducted at laboratory scale, and the functional properties of the postbiotic powders were assessed only through in vitro analyses. Future research should therefore focus on industrial scale-up, sensory evaluation in real food matrices, and in vivo studies to confirm the actual health benefits and application potential of this novel ingredient.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app16147078/s1, Figure S1: Calibration curve for lactic acid determination through HPLC analysis.

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