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# Controlled fermentation in improving the functional properties of *Brassica* with *Undaria*

**Romina B. Parada[1,2,*], Emilio R. Marguet[1], Carmen A. Campos[3,4], Marisol Vallejo[1]**

[1] National University of Patagonia San Juan Bosco, Chubut, Argentina
[2] National Scientific and Technical Research Council, Buenos Aires, Argentina
[3] University of Buenos Aires, Buenos Aires, Argentina
[4] Institute of Food Technology and Chemical Processes, CONICET – University of Buenos Aires, Buenos Aires, Argentina

* e-mail: parada.ro91@gmail.com

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**Abstract:**
Fermentation improves the nutritional and sensory properties of food. Despite the challenges of fermenting algae individually, incorporating it into vegetable matrices offers a great opportunity for the development of new products.
This study aimed to investigate changes in the antioxidant capacity and total phenolic content of Chinese, white, and red cabbages supplemented with *Undaria pinnatifida* throughout controlled fermentation. These values were then compared to those for the respective spontaneous process (mixed models), as well as to previously published data on cabbage fermentation without algae (simple models). Controlled fermentation was carried out in a two-step process using previously selected autochthonous starter cultures. Antioxidant activity was measured using the DPPH scavenging assay and the CUPRAC assay. The total phenolic content was determined using the Folin-Ciocalteu method.
The total phenolic content varied across the different fermentation processes depending on the vegetable matrix. The antioxidant capacity was significantly higher in the controlled process than in the spontaneous one in all mixed models. Red cabbage with algae exhibited higher total phenolics and antioxidant capacity than white and Chinese cabbages with algae. Furthermore, all the mixed models showed higher or comparable total phenolics and antioxidant capacity compared to the respective simple models under similar controlled fermentation and extraction conditions, except for Chinese cabbage with *Undaria* under the CUPRAC method.
Controlled fermentation of the studied cabbages improved their antioxidant capacity to a greater extent than spontaneous in all mixed models. In general, the mixed models showed higher nutritional properties than the simple models.

**Keywords:** *Lactiplantibacillus* sp., *Leuconostoc* sp., algae, total phenolics, antioxidant capacity, lactic fermentation

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## INTRODUCTION

*Undaria pinnatifida* (Harvey) Suringar (*Laminariales*, *Phaeophyta*) is native to the coast of Japan, China, and Korea [1]. This is a highly invasive species that has colonized different coastal areas worldwide [2]. In 1992, it was introduced to the coast of Golfo Nuevo (Puerto Madryn, Argentina) by shipping traffic [3], causing a significant impact on the ecosystem [4]. Currently, this alga is distributed continuously from Puerto Deseado, Province of Santa Cruz, to the west bank of the San Matías Gulf, Province of Río Negro [4]. As its invasion progresses and abundance increases, its eradication becomes a challenge. In this sense, *Undaria* becomes an alternative source for use in industries such as textile, food, cosmetics, and pharmaceuticals, among others.

*U. pinnatifida* is mainly consumed in Asia as wakame (dried seaweed), which is used as a supplement in soups and salads [5]. This seaweed is a natural source of nutritional compounds such as fatty acids, dietary fiber, protein, polysaccharides, minerals, vitamins, and

sterols. Furthermore, this alga also contains bioactive compounds, such as carotenoids and polyphenols, with high antioxidant activity [6]. The nutritive properties of *U. pinnatifida* exert numerous health benefits, making the alga a potential source of functional foods [7]. These powerful properties may contribute to formulating innovative natural alternatives that cater to the customers' growing demand for healthier products.

In response to this trend, seaweed fermentation has gained considerable interest due to its potential to promote the products' nutritional and sensory qualities [8]. Furthermore, this biotechnological approach can enhance the products' safety, health-promoting properties, and overall appeal [9]. Uchida *et al.* [10] used starter cultures for *U. pinnatifida* fermentation and, in all the treatments relying on native microflora, observed spoilage due to a slight decrease in the pH values. *U. pinnatifida* contains complex polysaccharides, such as fucoidans, laminarins, and alginates, which are challenging to hydrolyze by the metabolism of lactic acid bacteria (LAB) [11]. However, it could be used in a seaweed-sauerkraut style product with a broader spectrum of bioactive molecules [12], generating an alternative for vegans, vegetarians, lactose intolerants, and those requiring a cholesterol-free diet.

Sauerkraut is mainly consumed in Central and Eastern Europe. This traditional food is developed through the natural lactic fermentation of shredded white cabbage (*Brassica oleracea* var. *capitata* f. *alba*) preserved in salt, with sugars transformed to lactic acid by the action of LAB. However, this fermentation process has been adapted globally to include different types of cabbage, such as red cabbage (*B. oleracea* var. *capitata* f. *rubra*) and Chinese cabbage (*B. rapa* ssp. *pekinensis* (Lour.) Hanelt). These vegetables differ in their physicochemical properties due to variations in structure and composition. White and red cabbage have a moderate carbohydrate content between 6 and 7 g/100 g FW (fresh weight), primarily as simple sugars, whereas Chinese cabbage has a lower carbohydrate concentration, 3 g/100 g FW [13, 14]. Chinese cabbage stands out for calcium and iron. In addition, it exhibits diversity in the polyphenolic contents, with the outer leaves having the highest levels, and related antioxidant activities. In contrast, white and red cabbage are rich in Vitamin C, potassium, and glucosinolates, with red cabbage containing additional anthocyanins and polyphenols, enhancing its antioxidant properties [14]. Nevertheless, the profile and concentration of phytochemicals are influenced by such factors as cultivar, agricultural conditions, and environment [15], while fermentation can variably affect total phenolic compounds and antioxidant activity [13].

Sauerkraut is recognized for its numerous health benefits, which stem from the fermentation process used to produce it [9]. For example, the hydrolysis of polyphenols, naturally present in raw cabbage, enhances antioxidant activity, and organic acid production inhibits the development of pathogenic or spoilage bacteria [16]. Red cabbage, which is also used to produce sauerkraut, has an advantage over white cabbage due to its high concentration of anthocyanins [17]. These naturally occurring flavonoid pigment molecules have health-promoting properties such as antioxidant, anti-inflammatory, and anti-diabetic activities [14, 17]. Chinese cabbage, widely used to make kimchi, is also linked to health-promoting properties. Its effectiveness has been demonstrated in treating obesity and irritable bowel syndrome [18].

While many manufacturers incorporate seaweed in sauerkraut, it is used primarily as a seasoning to enhance flavor rather than as a fundamental ingredient. Unlike cabbage, seaweed does not contain simple carbohydrates and has a high buffering capacity, which affects the lactic fermentation process [11]. The LAB, which play a key role in this fermentation, are Generally Recognized as Safe (GRAS) and have a Qualified Presumption of Safety (QPS) status according to the U.S. Food and Drug Administration (FDA) and the European Food and Safety Authority (EFSA), respectively [19, 20]. The species belonging to *Lactiplantibacillus*, *Lactobacillus*, *Leuconostoc*, *Enterococcus*, *Pediococcus*, and *Weissella* genera are often involved in the spontaneous fermentation of vegetables [21]. Even though spontaneous fermentation is widely used to preserve raw vegetables, it can exhibit alterations during the process. These risks include inadequate inhibition of pathogenic and spoilage microorganisms, the development of undesirable sensory characteristics, and changes in the nutrient composition [22].

An alternative approach is to use selected starter cultures for controlled fermentation. Unlike other traditional products (cheese, sausages), starter cultures in vegetable matrixes have only been developed in recent years [23]. Previous studies widely recommend autochthonous cultures, which adapt quickly to the fermentation matrix, guarantee safety, and improve the fermented product's sensory and functional properties [21, 23].

In this study, we aimed to assess the development of fermentation, total phenolic content, and antioxidant capacity (DPPH and CUPRAC methods) of three varieties of *Brassica* vegetables (Chinese cabbage, white cabbage, red cabbage) supplemented with *U. pinnatifida* (mixed models). The fermentation process was controlled and included two steps. In addition, we compared these parameters with those for the respective spontaneous processes (mixed models) and previously published data for cabbage fermentation without algae (simple models).

## STUDY OBJECTS AND METHODS

**Cabbage and *Undaria* samples.** White cabbage (*Brassica oleracea* var. *capitata* f. *alba*), red cabbage (*B. oleracea* var. *capitata* f. *rubra*), and Chinese cabbage (*Brassica rapa* subsp. *pekinensis* (Lour.) Hanelt) were grown in autumn (March–June 2022) in the Valle Inferior del Río Chubut, Patagonia, Argentina (−43.31 latitude, −65.53 longitude). The cabbages were bought from a local farm and stored for less than three days at 4°C before fermentation. *U. pinnatifida* algae were

provided by the National Technological University, Puerto Madryn-Chubut, Argentina. The seaweeds were harvested in winter (July–August 2022) in the coastal areas of Golfo Nuevo at a depth of 3–15 m. After that, the samples were placed in sterile bags and stored at –20°C until used. Only the blade structure was used for the experiments.

**Bacterial strains.** *Leuconostoc mesenteroides* ssp. *jonggajibkimchii* RCTw1.1, *Ln. mesenteroides* ssp. *dextranicum* RBTw100, *Lactiplantibacillus plantarum* ssp. *argentoratensis* RBTw102*, L. plantarum* AKTw180, and *L. pentosus* AKTw332 were obtained from the Bacterial Biotechnology Laboratory (BBL, Trelew-Chubut, Argentina). The strains were previously isolated from the three spontaneously fermented cabbages and selected based on their technological properties [24]. They were propagated in MRS broth and incubated overnight at 30°C. After centrifugation (4,000×*g*, 10 min), the microbial cells were washed twice in a sterile saline solution (0.9% NaCl) before their inoculation.

**Fermentation trial.** A controlled fermentation trial was carried out with Chinese, white, and red cabbage supplemented with 20% (w/w) of shredded *U. pinnatifida* blades and 3% (w/w) of common salt. The cabbage heads were processed according to the methodology proposed by Parada *et al*. [24]. The algae blades were previously sanitized for 5 min (NaClO, 100 ppm) and washed. After removing the algae midrib, the blades were shredded for 2 min using a processor (Atma LM852, 400 Watt). Each salted cabbage jar (2 L) supplemented with the algae was heated at 100°C for 5 min and cooled at room temperature. The inoculation process was completed in two stages as described by Parada *et al.* [24]. At the same time, the spontaneous fermentation of Chinese, white, and red cabbage was carried out under the same conditions mentioned by Parada *et al*. [24]. The assays were performed in triplicate.

**Fermentation parameters.** The LAB count and pH were determined according to Parada *et al.* [24]. These parameters were monitored on days 0, 1, 3, 5, 10, 15, 20, 25, and 30 of the fermentation process. The results of the LAB count were expressed as CFU/g sample. All the trials were performed in triplicate.

**Antioxidant analysis.** ***Sample preparation.*** The samples collected on days 0, 1, 3, 5, 10, 15, 20, 25, and 30 were processed following the recommendation of Parada *et al.* [24]. They were dried at 37°C until constant weight and ground. Water extracts (1:10 w/v dilution) were obtained using an autoclave at 120°C for 15 min. After centrifuging the extracts (12,000×*g* for 15 min), the supernatants were stored at –20°C.

***Total phenolic content.*** The total phenolic content was determined by the Folin-Ciocalteu method with slight modifications [25]. For this, 50 µL of the extract was added to 100 µL of the Folin-Ciocalteu phenol reagent (Sigma Aldrich, St. Louis, USA). After 10 min incubation, 2 mL of $Na_2CO_3$ (1.0% w/v) was added to the mixture and allowed it to react for 90 min. The absorbance was measured at 750 nm using a spectrophotometer (Jenway, ColePalmer, St. Neots, UK). Gallic acid was used as a standard (50–800 µg/mL). The results were expressed as milligram gallic acid equivalents per 100 g dry weight (mg GAE/100 g DW).

***DPPH radical scavenging assay.*** The determination of DPPH (2.2-Diphenyl-1-picrylhydrazyl) radical scavenging capacities was carried out in agreement with the method outlined by Chen *et al.* [26] with some modifications. For this, 100 µL of the extract was mixed with a DPPH ethanolic solution (900 µL, 100 µM) (Sigma Aldrich, St. Louis, USA) and incubated at 25°C for 30 min in darkness. The absorbance was measured at 517 nm using a spectrophotometer, and ascorbic acid was used as a standard (10.5–176 µg/mL). The results were expressed as milligram ascorbic acid (Sigma Aldrich, St. Louis, USA) equivalents per 100 g dry weight (mg AAE/100 g DW).

***Cupric reducing antioxidant capacity.*** The antioxidant capacity of the water extracts was determined according to the cupric reducing antioxidant capacity (CUPRAC) method described by Gouda and Amin [27] with minor modifications. The reaction mixture was prepared with 3 mL of an acetate buffer (50 mM, pH 5.0) (Sigma Aldrich, St. Louis, USA), 2 mL of a neocuproine solution (5 mM) (Sigma Aldrich, St. Louis, USA), and 1 mL of 0.01 M $CuCl_2$ (Cicarelli, Cordoba, Argentina). Then, 100 µL of the extract and 900 µL of the reaction mixture were mixed and manually shaken. After 1 h of incubation in darkness, the absorbance was measured at 450 nm in a spectrophotometer. Ascorbic acid was used as a standard (10.5–176 µg/mL). The results were expressed as mg AAE/100 g DW.

**Statistical data analysis.** The numerical values represent mean values derived from triplicate samples and presented as mean ± standard deviation. The data were subjected to two- and one-way ANOVA, and significant differences between the means ($p < 0.05$) were determined by Tukey's test. InfoStat statistical software was used for all data analysis.

## RESULTS AND DISCUSSION

The fermentation processes were monitored through LAB count and pH measurement. The spontaneous and controlled fermentations exhibited a similar trend, namely an increase in LAB during the initial period and a decrease in pH caused by organic acid production during carbohydrate metabolism (Fig. 1).

The spontaneous processes exhibited pH of ≈ 6 and LAB counts of ≈ 4 log CFU/g at the initial time for the three cabbages. These results are consistent with prior research on fermented cabbage [23, 24]. The LAB population increased (≈ 7.8 log CFU/g), while the pH values dropped (≈ 4.6) after three days for all the cases. Later, the pH values continued to decrease in the three vegetables, exhibiting values of ≈ 4 after 30 days of the assay. At the same time, the LAB count dropped gradually to ≈ 6.6 log CFU/g after 15 and 20 days of fermentation in Chinese cabbage supplemented with *U. pinnatifida* and red cabbage supplemented with *U. pinnatifida*, respectively ($p \leq 0.05$). Meanwhile, the decrease of LAB was

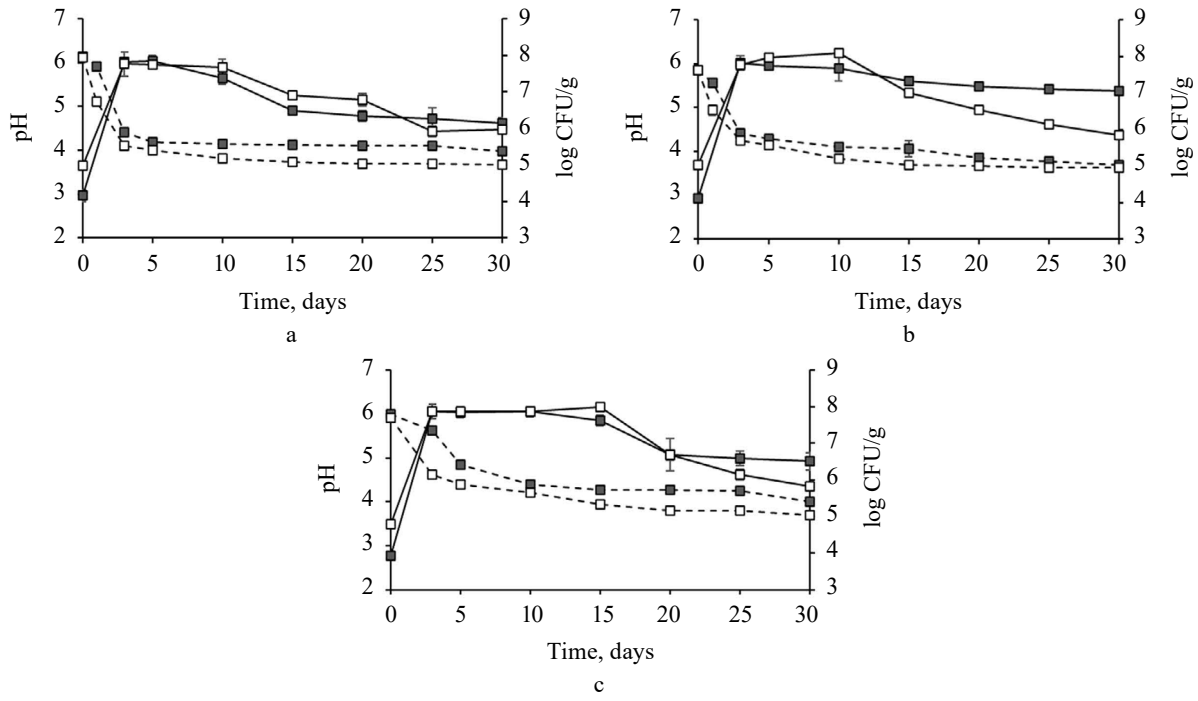


**Figure 1** Total lactic acid bacteria (–) and pH changes (– – –) determined in cabbage during controlled (□) and spontaneous (■) fermentation of: a – Chinese cabbage, b – white cabbage, and c – red cabbage, each supplemented with *U. pinnatifida*

slighter in white cabbage supplemented with *U. pinnatifida* than in the other mixed models, at ≈ 7.3 log CFU/g after 15 days of the process ($p \leq 0.05$). These values remained stable until the end of the trial for the three vegetables ($p > 0.05$).

The controlled fermentation showed pH of ≈ 6 and LAB counts of ≈ 5 log CFU/g at the initial fermentation period. The indigenous biota was previously reduced by the thermal treatment, and a starter culture with *Leuconostoc* species was added, as suggested by Parada *et al*. [24]. This process showed a significant drop in pH after one day for all the cabbages supplemented with *Undaria* ($p \leq 0.05$). Recent studies have observed a notable decrease in pH levels when *Ln. mesenteroides* strains were used as starter cultures in vegetable fermentations [24, 28]. Later, a second starter culture with selected *Lactiplantibacillus* strains was added after three days, when the pH values dropped to ≈ 4, and the LAB cell counts increased to ≈ 8 log CFU/g. Then, LAB populations decreased gradually in all the samples, exhibiting values of ≈ 5.8 log CFU/g at the end of the assay. Meanwhile, the pH reached < 4, which was lower than in the spontaneous fermentation of the Chinese and red cabbages supplemented with *U. pinnatifida* ($p \leq 0.05$), with the white cabbage sample showing no differences ($p > 0.05$).

Previous reports showed a maximum LAB count on the seventh day of fermentation followed by a slight decrease [29, 30]. We observed a similar trend in the three mixed models. According to Brochu [29], the algae significantly affected LAB development in sauerkraut due to the presence of complex polysaccharides that are difficult to metabolize. However, the fermentation conditions in our study did not significantly affect LAB growth, which showed similar values to those obtained in the previously published research on cabbage fermentations (simple model) [24]. Even though *Undaria* did not provide fermentable carbohydrates to the matrices [29], it did increase the content and diversity of vitamins and minerals [31, 32], contributing to the complex nutritional requirement of LAB.

The beneficial effects of cabbage on human health have been linked to phytochemicals, mainly phenolic compounds [13]. Furthermore, previous reports stressed the effect of polyphenols in *U. pinnatifida* on human health, highlighting their antioxidant activity [6, 7, 31]. In this study, we evaluated the evolution of the total phenolic content in the water extracts obtained from the Chinese, white, and red cabbages supplemented with *Undaria* during spontaneous and controlled fermentation processes (Fig. 2).

The red cabbage sample showed higher values of the total phenolic content (TPC) than the Chinese and white samples, regardless of the fermentation type ($p \leq 0.05$). Similar differences were reported for the fresh samples of Chinese cabbage (347.46 mg GAE/100 g DW) [33], white cabbage (980–1220 mg GAE/100 g DW) [34], and red cabbage (1851 mg GAE/100 g DW) [35], as well as for their fermented samples without seaweed (simple model) [24]. The *Brassica* vegetables contain a great diversity of phenolic compounds, with over 30 anthocyanin pigments previously identified only in red cabbage extract [17].

The evolution of the TPC in the supplemented Chinese cabbage was similar between the spontaneous and controlled processes (Fig. 2a). The TPC increased during the first three days of fermentation and then

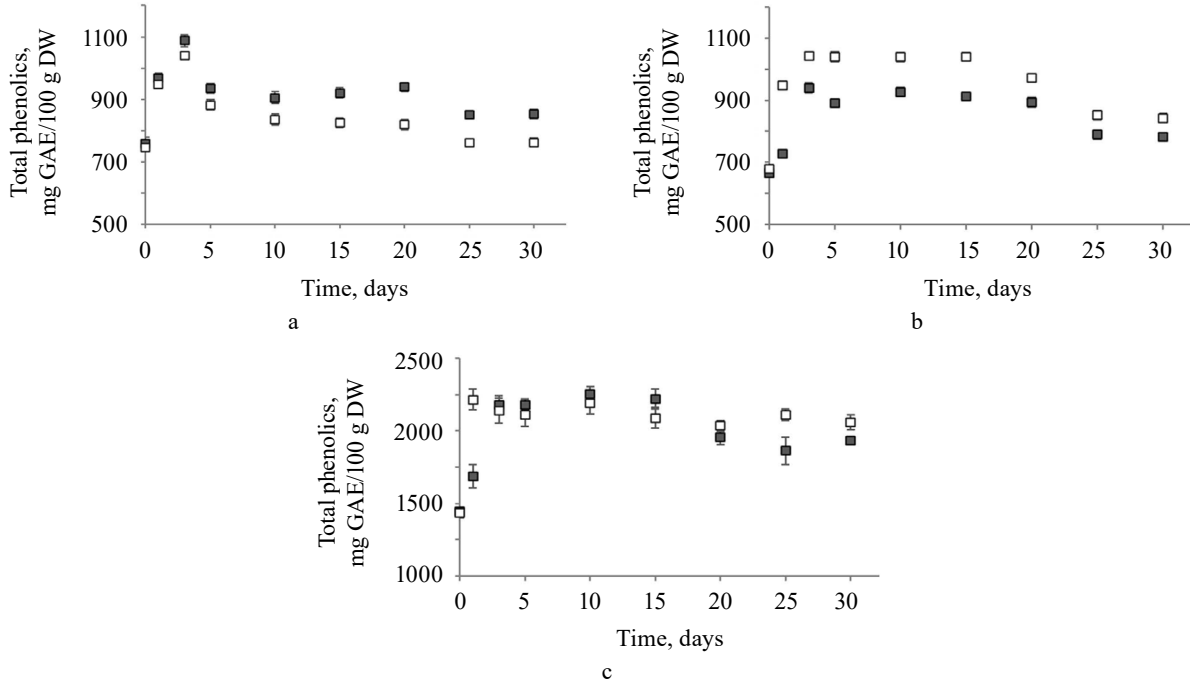


**Figure 2** Total phenolic content in water extracts of: a – Chinese cabbage, b – white cabbage, and c – red cabbage during spontaneous (■) and controlled (□) fermentation, each supplemented with *U. pinnatifida*

gradually decreased ($p \leq 0.05$). The spontaneous fermentation exhibited higher TPC values than the controlled process at 30 days. When comparing both models (mixed and simple) during controlled fermentation, we found higher TPC values in the Chinese cabbage with *U. pinnatifida* compared to the one without the algae during the initial period. After that, the difference was no longer significant ($p > 0.05$) (761.9 ± 14.2 and 726.5 ± 25.1 mg GAE/100 g DW for the Chinese cabbage with and without *U. pinnatifida*, respectively, on day 30) [24].

The controlled fermentation of the white cabbage with *U. pinnatifida* showed a significant increase in the TPC on day one of the fermentation. These values remained stable until day 20 and then decreased until the end of the assay. However, the values were significantly higher than the ones for the fresh vegetables. Parada *et al*. [24] reported a similar tendency in the simple model of while cabbage (without algae) fermented with the same starter strains. Meanwhile, the spontaneous fermentation of the supplemented white cabbage showed a significant increase in the TPC on day three of the process, followed by a gradual decrease after 20 days. The controlled fermentation of the WCU showed higher TPC values than the spontaneous process after the end of the assay ($p \leq 0.05$). These TPC values were not significantly different ($p > 0.05$) from the values previously reported in the simple model of white cabbage (without algae) during the controlled process (842.9 ± 15.0 and 847.7 ± 37.5 mg GAE/100 g DW for the white cabbage with and without *U. pinnatifida*, respectively, on day 30) [24].

Lastly, the red cabbage supplemented with *U. pinnatifida* had significantly higher TPC values on the first day of controlled fermentation, and these values remained stable until day 30. Meanwhile, the spontaneous process showed similar TPC values after three days, with a decrease on day 20. However, the TPC values achieved on day 30 did not show a significant difference between both fermentations ($p > 0.05$). Parada *et al*. [24] reported lower TPC values in the simple model of red cabbage compared to the model mixed with *U. pinnatifida*. These differences were exhibited from day five to the end of the controlled fermentation trial ($p \leq 0.05$) (2059.4 ± 62.7 and 1657.1 ± 118,2 mg GAE/100 g DW for the red cabbage with and without *U. pinnatifida*, respectively, on day 30). Notably, the TPC values at the end of fermentation were higher than the ones for the fresh vegetables.

The TPC showed a similar tendency during controlled fermentation of the mixed models, increasing in the first days and, in some cases, decreasing significantly during the intermediate and final stages of the process. Meanwhile, the LAB count was high and pH values were low. However, variations in the TPC values were observed between the vegetable species. Hunaefi *et al*. [36], who used an *L. plantarum* strain to ferment red cabbage, reported a similar tendency. Furthermore, the controlled fermentations of the same vegetables (without adding *Undaria*), with the inoculation of identical starter cultures in a two-step process, exhibited similar variations [24]. Lactobacilli have been observed to exhibit a higher tolerance towards phenolic compounds compared to other bacterial groups.

The strains of *L. plantarum* stand out mainly because of their mechanisms for mitigating the impact of these metabolites [37]. In this study, the strains used in the second inoculum exhibited tolerance to phenol, gallic acid, and tannic acid, as well as pectinase and tannase activity in the previous trials [24]. Thus, the selected strains could contribute to the metabolization of the phenolics in the vegetable matrix (*U. pinnatifida* and *Brassica*), as well as an increment in organic acids, consequently causing a decrease in TPC values and an increase in other biologically active compounds [33, 36–38].

*Undaria* contains a wide variety of bioactive compounds, such as phenolic compounds and carotenoids [31, 32], while cabbages can also display glucosinolates, anthocyanins, and tocopherols [39]. However, most antioxidant compounds in plants are phenols that act as reducing agents, metal chelators, and singlet oxygen inhibitors [40].

In this study, we used the DPPH radical scavenging method and the CUPRAC reduction assay to determine the antioxidant capacity. These electron transfer-based methods are commonly employed to assess phenolic compounds' antioxidant capacity in various food sources [41]. Due to its versatility and accuracy, the DPPH radical scavenging assay is one of the most frequently used methods for evaluating antioxidant activity. This method is based on the electron donation of antioxidants that induces the loss of color in the DPPH. The change in the optical density is proportional to the antioxidant activity. The CUPRAC method, a variant of the FRAP (ferric reducing antioxidant power) assay, measures the reduction of cupric ($Cu^{2+}$) to cuprous ($Cu^{+}$) ions by antioxidants [41, 42].

The antiradical activity of the cabbage samples against the DPPH radical can be observed in Figure 3. In the Chinese cabbage supplemented with *U. pinnatifida*, the controlled fermentation exhibited a higher reducing power than the spontaneous process ($p \leq 0.05$) (Fig. 3a). The antioxidant capacity of the supplemented Chinese cabbage sample increased significantly during the first ten days, remaining stable until the end of the controlled process. In contrast, the spontaneous fermentation of the CCU did not exhibit significant variations during all the assays ($p > 0.05$). According to Parada *et al.* [24], the antioxidant capacity of the simple model of fermented Chinese cabbage was comparable with that of the mixed model (with *U. pinnatifida*) in similar conditions ($p > 0.05$) (219.1 ± 6.7 and 199.6 ± 7.7 mg AAE/100 g DW for the Chinese cabbage with and without *U. pinnatifida*, respectively, on day 30).

In the white cabbage supplemented with *U. pinnatifida*, the antioxidant capacity increased significantly during both fermentation types ($p \leq 0.05$). However, the controlled process exhibited a higher reducing power than the spontaneous fermentation ($p \leq 0.05$) (Fig. 3b). In the controlled and spontaneous processes, the extract displayed a significant increase in the reduction power until day ten and day three, respectively ($p \leq 0.05$). Then, the values obtained in each process remained stable until day 30.

The antioxidant capacity of the mixed model of white cabbage was higher than that of the simple model, as previously reported by [24], during both controlled processes ($p \leq 0.05$) (319.3 ± 12.3 and 202.9 ± 7.2 mg AAE/100 g DW for the white cabbage with and without *U. pinnatifida*, respectively, on day 30).

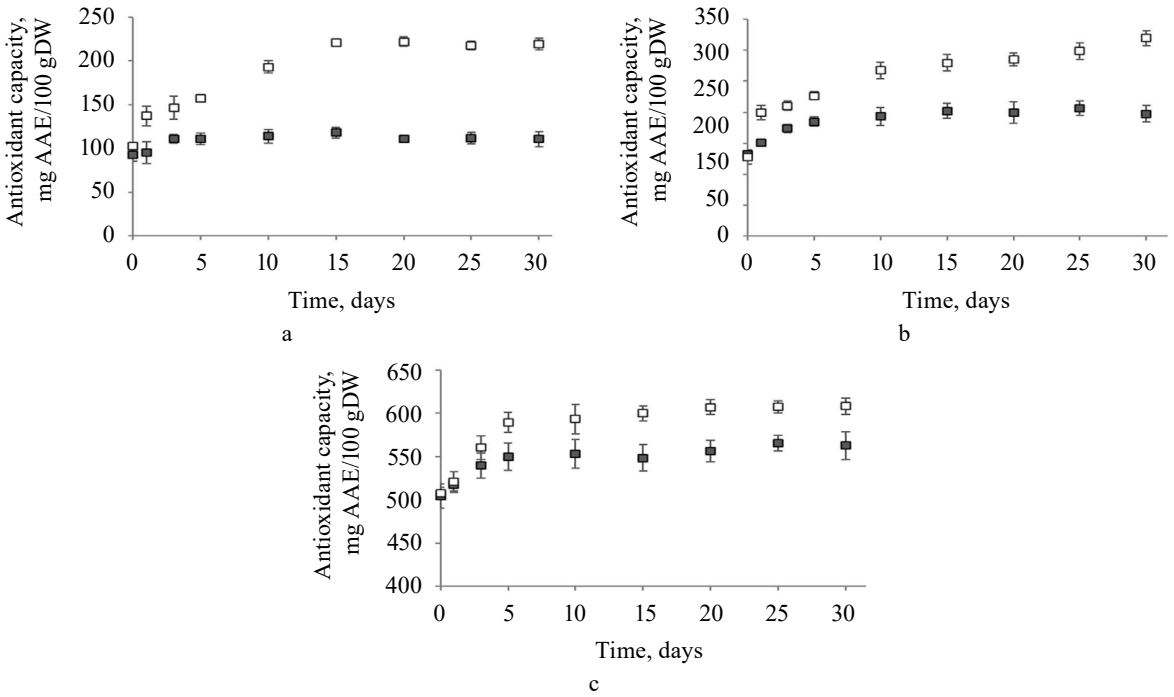


**Figure 3** Antioxidant capacity (DPPH method) in water extracts of: a – Chinese cabbage, b – white cabbage, and c – red cabbage supplemented with *U. pinnatifida* during spontaneous (■) and controlled (□) fermentation

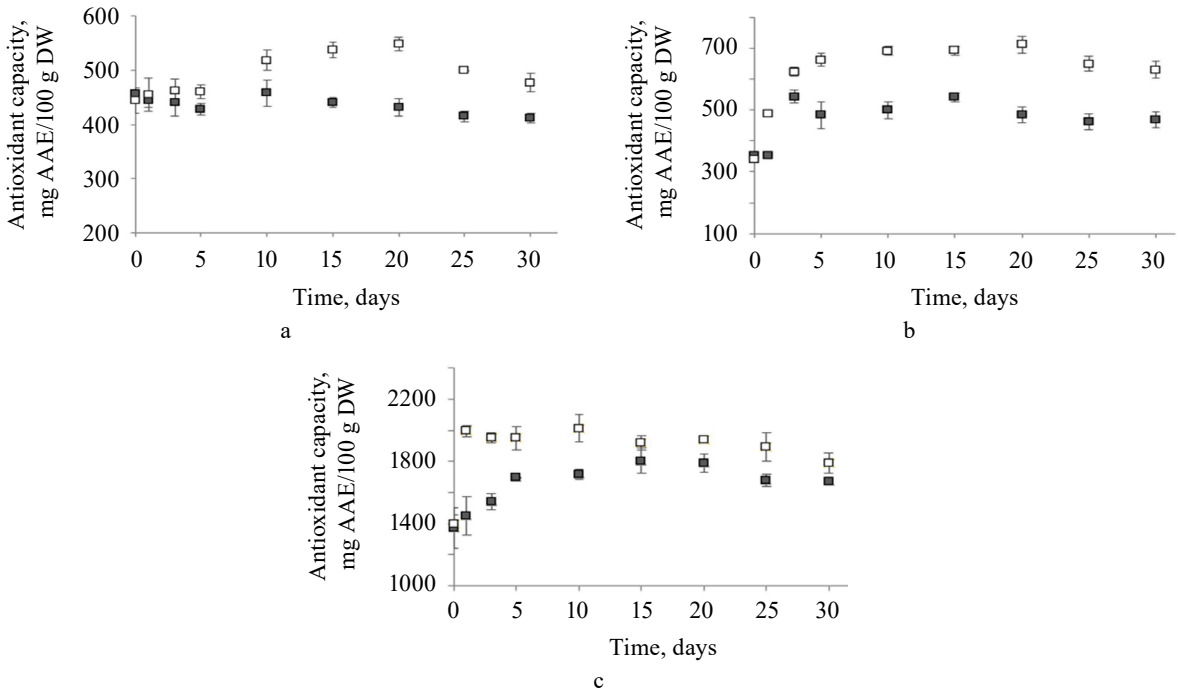


**Figure 4** Antioxidant capacity (CUPRAC method) in water extracts of: a – Chinese cabbage, b – white cabbage, and c – red cabbage supplemented with *U. pinnatifida* during spontaneous (■) and controlled (□) fermentation

Regarding the supplemented red cabbage, its water extracts exhibited a higher reducing power than the CCU and WCU, regardless of the fermentation type. Parada *et al.* [24] showed a similar tendency between the fermented simple models. In the controlled fermentation, the supplemented red cabbage sample showed a significant increase in the antioxidant capacity until day five, with no changes observed afterwards. However, the spontaneous process showed an increase during the last period of the assay (25 days) (Fig. 3c). After 20 days, the controlled fermentation of the RCU exhibited higher antioxidant capacity than the spontaneous process ($p \leq 0.05$). The antioxidant capacity of the mixed model of red cabbage during controlled fermentation was comparable with the values previously reported for the simple model under similar process conditions [24] ($p > 0.05$) (608.0 ± 9.3 and 567.5 ± 16.8 mg AAE/100 g DW for the red cabbage with and without *U. pinnatifida*, respectively, on day 30).

In all the cases, the DPPH radical scavenging capacity increased as natural and inoculated fermentation progressed. This tendency agrees with the results reported by other authors [29, 38]. Furthermore, in all the cases, controlled fermentation displayed higher values of antioxidant activity than spontaneous fermentation at the end of the process ($p \leq 0.05$). According to previous reports, the inoculation with *L. plantarum* has a significative effect on antioxidant activity [24, 36]. *Lactiplantarum* spp. strains as a starter culture can hydrolyze the ester bonds in hydrolysable tannins, releasing potent antioxidant compounds, such as gallic acid and pyrogallol, through the tannase activity [38]. In addition, the pectinase activity can modify the texture of cabbage during fermentation [33], allowing the release of phenolic compounds.

The antiradical activity of the samples determined by the CUPRAC method can be observed in Figure 4. The AAE values obtained through the CUPRAC assay were higher than those exhibited by DPPH in all the cases ($p \leq 0.05$). These dissimilarities were related to the capacity of each method. The CUPRAC method can detect hydrophilic and lipophilic antioxidants in the sample, whereas the DPPH method can determine only those molecules which are soluble in organic solvents [42].

The water extracts of the Chinese cabbage supplemented with *U. pinnatifida* exhibited different antioxidant capacity depending on the type of fermentation (Fig. 4a). In the controlled process, their antioxidant capacity increased significantly until day 20, followed by a decrease in the last days of the assay ($p \leq 0.05$). However, the spontaneous fermentation did not exhibit significant differences during the process ($p > 0.05$). The controlled fermentation displayed higher reduction power than the spontaneous process ($p \leq 0.05$). Parada *et al.* [24] reported higher antioxidant capacity in the Chinese cabbage simple model (without algae) than in the mixed model exposed to similar controlled fermentation conditions ($p \leq 0.05$) (477.6 ± 16.9 and 676.4 ± 37.2 mg AAE/100 g DW for the Chinese cabbage with and without *U. pinnatifida*, respectively, on day 30).

In the spontaneous and controlled fermentation, the white cabbage samples supplemented with *U. pinnatifida* showed increasing antioxidant capacity until day three

and day five, respectively ($p \leq 0.05$) (Fig. 4b). Then, the antioxidant capacity remained constant until day 30 ($p > 0.05$) for both types of fermentation. Notably, the controlled process displayed a higher antioxidant capacity than the spontaneous fermentation ($p \leq 0.05$). The mixed model of white cabbage exhibited a comparable evolution of the antioxidant activity to the one of the simple model, as previously reported [24], under similar controlled fermentation conditions ($p > 0.05$) (631.1 ± 28.5 and 694.5 ± 21.9 mg AAE/100 g DW for the white cabbage with and without *U. pinnatifida*, respectively, on day 30).

Lastly, the supplemented red cabbage extracts displayed similar tendencies during the controlled and spontaneous fermentation (Fig. 4c), with an increase in antioxidant capacity observed on days one and five, respectively ($p \leq 0.05$). Then, these values remained stable until day 30 in both processes ($p > 0.05$). Only during the initial stage did the controlled fermentation show higher values of the antioxidant capacity than the spontaneous process ($p \leq 0.05$). In the controlled fermentation, the mixed model of red cabbage showed similar values to those for the simple model, as previously reported [24], ($p > 0.05$) (1788.2 ± 63.2 and 1890.7 ± 77.4 mg AAE/100 g DW for the red cabbage with and without *U. pinnatifida*, respectively, on day 30).

The red cabbage supplemented with *U. pinnatifida* exhibited a higher antioxidant capacity than the supplemented Chinese and white cabbage samples, regardless of the fermentation type or method (DPPH/CUPRAC). This tendency was observed previously in the simple-model fermentations (without *Undaria*) [24]. Additionally, the antioxidant capacity of the fermented cabbages at the end of the assay was significantly higher than that for the fresh vegetables, except for the spontaneously fermented Chinese cabbage.

After analyzing the simple and mixed models under controlled fermentation conditions, we found that adding *U. pinnatifida* did not significantly increase the antioxidant capacity of any of the fermented vegetable matrices studied. However, *U. pinnatifida* supplementation in these types of matrices provides a greater diversity of available minerals and metabolic compounds (fucoidans, alginates, polyphenols, fucosterols, and carotenoids, among others) with nutritional, functional, and biological properties beneficial to the consumer's health [5, 6, 31]. Furthermore, controlled fermentation exhibited significantly higher antioxidant activity than spontaneous fermentation in all the mixed models, which was consistent with Parada's analysis [24] for the simple model.

## CONCLUSION

Current research aimed to improve the nutritional properties of foods and develop functional foods through supplementation with seaweed to increase their health benefits. This study was the first to use *U. pinnatifida* in the fermentation of cabbages in Argentina. Notably, supplementing the vegetable matrix with 20% of the algae did not affect the development of lactic fermentation. While the total phenolic content varied between the cabbages and fermentation types, the antioxidant capacity obtained by both methods (DPPH and CUPRAC) was significantly higher in the controlled process than in the spontaneous one in all the cases. All the mixed models (Chinese, white, and red cabbages supplemented with *Undaria*) exhibited higher or similar values of the total phenolic content and antioxidant capacity compared to the simple models (without the algae) under similar conditions of controlled fermentation and extraction (except for the supplemented Chinese cabbage when applying the CUPRAC method). The high total phenolic content and antioxidant activity exhibited by the supplemented red cabbage sample suggest its use as a functional food product and a new alternative for consuming *Undaria* algae, taking advantage of the scarcely exploited resources on our coasts.

## CONTRIBUTION

Romina B. Parada – conceptualization, methodology, software, validation, formal analysis, investigation, writing – reviewing and editing, and visualization. Emilio R. Marguet – conceptualization, methodology, formal analysis, investigation, and writing the original draft. Carmen A. Campos – conceptualization, software, formal analysis, writing-reviewing and editing. Marisol Vallejo – conceptualization, methodology, writing-reviewing and editing, and visualization. All the authors participated in developing the research concept and writing the original draft. All the authors approved the final version of the manuscript.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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**ORCID IDs**
Romina B. Parada https://orcid.org/0000-0002-1089-9625
Emilio R. Marguet https://orcid.org/0000-0002-9790-8511
Carmen A. Campos https://orcid.org/0000-0002-6665-8011
Marisol Vallejo https://orcid.org/0000-0002-4393-3615