Effect of cooking conditions and storage on phenolic contents of bottled Capia red pepper

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Abstract

Cooking (traditional and industrial-type roasting) and storage (bottling) can affect fresh Capia red peppers' (CRP) total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (AA). The present work aimed to determine the effect of cooking methods and storage on phenolic and flavonoid contents, and the change in antioxidant activity of samples. The changes in phenolic, flavonoid, and antioxidant activity of (1) fresh mature CRP pericarp of fruit, (2a) industrial fire roasted-bottled CRP pericarp at 700°C at 30 s, (2b) after 12-week storage at 4°C, (3a) traditional roasted-bottled CRP pericarp at 550°C at 10', and (3b) after 12-week storage at 4°C were investigated. Fresh CRPs' mean TPC and TFC were 118.57 mg GAE/g and 34.68 µg QE/g, respectively. Traditional bottled CRPs initial and post-storage mean TPC and TFC were 137.8 and 81.90 GAE/g, and 58.87 and 107.56 µg QE/g ($p < 0.05$), respectively. Industrial bottled CRPs' initial and post-storage mean TPC and TFC were 73.89 and 46.26 GAE/g, and 25.19 and 87.0µg QE/g ($p < 0.05$). AA decreased due to both traditional and industrial roasting methods after 12-week storage based on CUPRAC ($p < 0.05$). In traditional roasting method, 12-week storage negatively affected CRPs TPC. However, TFC positively affected more than industrial roasting method ($p < 0.05$). In traditional roasted method, 12-week storage negatively affected TPC. However, TFC positively affected more than industrial roasting.

Introduction

Biologically active components found in fruits and vegetables exert significant impact on health. Numerous studies have indicated that adopting a healthy dietary approach that emphasises the consumption of plant-based foods plays a crucial role in the prevention of various diseases, including cardiovascular diseases, cancers, diabetes, and age-related functional decline (Boeing *et al*., 2012; Petersen *et al*., 2017). Capia red pepper (*Capsicum annuum* L.), CRP, stands as a significant commercial crop with expanding cultivation on a global scale. It is characterised by its rich content of vitamins C and A, along with essential minerals. Consequently, a daily intake of 60 - 80 g of CRP fulfils the recommended daily requirements for vitamin C (100%) and vitamin A (25%) (Samtiya *et al*., 2021). Freshly consumed CRP exerts notable nutraceutical

potential owing to their abundance of antioxidants and a wealth of phytochemicals. Numerous studies have concentrated on the phenolic fraction of plants due to their antioxidant properties (Yu *et al*., 2021). Red pepper ranks second in phenolic content among 27 common vegetables, after spinach. It contains substantial quantities of capsaicinoids, luteolin, and quercetin (Song *et al*., 2010). Antioxidants are bioactive compounds that promote health by mitigating the accumulation of oxidants in the human body, which may result from oxidative stress. Elevated levels of oxidants can lead to various diseases, including mutagenic changes, cellular aging, cancers, and cardiovascular diseases. Consequently, the assessment of total antioxidant capacity in plant-based foods holds significant importance (Lobo *et al*., 2010).

Antioxidant assays can be categorised based on the type of antioxidant being assessed, the type of

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assay reagent utilised, the nature of the assay medium, or the mechanism of action. Among these categorisation methods, the most frequently employed is based on the type of antioxidant being assessed (Özyürek *et al.*, 2011). Several methods for quantifying antioxidant potential through free radical scavenging have been established over the past 15 years. Common tests such as ABTS/TEAC, DPPH, and the Folin-Ciocalteu total phenolic assay are widely employed for assessing the antioxidant capacity of various samples (Apak *et al.*, 2007).

Capia red pepper can be consumed in various forms: raw, cooked (roasted or boiled), or incorporated into tomato paste or sauce. Nevertheless, CRP is notably perishable, primarily due to moisture loss when stored in low relative humidity environments. Under optimal conditions, CRP can be stored for up to two weeks, provided the storage temperature falls within the range of 7.5 - 13°C, and the relative humidity level remains between 95 - 98%. It is important to note that temperatures exceeding 13°C accelerate the ripening process and promote microbial decay, whereas temperatures below 7.5°C can lead to chilling injury (Gil and Tudela, 2020). To mitigate the reduction in CRP shelf life caused by post-harvest water loss, processing is often used. Employing methods such as heat treatment or freezing, peppers can be preserved for extended durations, whether in the food industry or within a home environment (Azder *et al.*, 2020). The significance of the present work lies in its comprehensive examination of the phenolic, flavonoid, and antioxidant activity profiles of raw, roasted, and stored red peppers. We have systematically evaluated the impact of various processing techniques on these key parameters. Notably, our findings revealed a reduction in antioxidant activity (AA) following 12-week storage, both in traditional and industrial roasting methods, as assessed by the CUPRAC method.

Furthermore, our investigation suggested that traditional roasting had a negative influence on the total phenolic content (TPC) of CRP after 12-week storage, while the total flavonoid content (TFC) was more profoundly affected by this method compared to industrial roasting. Therefore, oven-roasting could have potential compared to industrial methods, primarily due to its preservation of TPC, TFC, and AA levels.

The present work was primarily designed to investigate the impact of different cooking methods, specifically traditional and industrial bottling techniques, as well as 12-week storage, on TPC, TFC, and AA in fresh CRP samples. The stability of phenolic compounds under different storage conditions plays a crucial role in supporting nutritional quality. Factors such as temperature, oxygen, and moisture can significantly affect the storage stability of CRP in terms of TPCA and AA. Hence, we evaluated the stability of TPC and AA in CRP under various storage conditions, including different cooking methods (traditional and industrial bottling) and storage durations (12 weeks). To achieve this goal, we formulated two key hypotheses. Firstly, we hypothesised that exposure to different cooking conditions would lead to significant changes in TPC of CRP. Secondly, we hypothesised that the duration of storage over a 12-week period would exert a negative influence on TPC of CRP. This would contribute to deeper understanding of the culinary and storage dynamics impacting this important agricultural commodity.

Materials and methods

We calculated antioxidant capacity of total phenol (mg GAE/g) as well as total flavonoid (µg QE/g) using three different well-known analysis methods (DPPH, CUPRAC, and ABTS). The examination of the impact of storage on phenolic and flavonoid contents, as well as antioxidant capacity, constituted a pivotal facet of our study. This investigation assumes considerable significance, as it offers valuable insights into the temporal dynamics of these vital compounds within bottled CRP. These observed alterations hold direct implications for the product's nutritional composition, and consequently, its potential health-related advantages. All analyses were conducted in triplicate.

Sample preparation

Generally, consumers tend to discard the placenta and seeds, opting to consume only the pericarp of CRP. Consequently, our samples encompassed the following categories: (1) fresh CRP pericarp, harvested at the maturity stage of full fruit size; (2a) industrial fire-roasted and bottled CRP pericarp at 700°C for 30 s; (2b) industrial-bottled CRP pericarp subjected to 12-week storage at 4°C; (3a) traditional fire-roasted and bottled CRP pericarp at 550°C for 10 min; and (3b) traditional-bottled CRP pericarp subjected to 12-week period at 4°C.

In the case of industrial CRPs, the roasting process was carried out using the 'Roasting with the Pepper Roasting Naasting Machine', whereas the traditional method involved roasting CRPs directly over an open flame on the stove. Irrespective of the cooking method employed, all cooked CRP samples were bottled in unused jars.

All CRP samples, both fresh and roasted, were sourced from the 2022 summer season harvest within the same infield as the local industry. To prepare the samples for analysis, we homogenised fresh CRP pericarp samples (isolated from seed and stem tissue) and processed CRP samples (2 g) using 80% methanol (20 mL). The homogenate was then filtered through filter paper in four layers to obtain supernatant. Any residue left was subsequently mixed with methanol for another round of extraction. The resulting filtrates were combined and subjected to centrifugation at 3000 g for 10 min at 4°C. The antioxidant capacity was determined using the clear supernatant.

Total phenolic content of Capia red pepper

The TPC of CRP samples was determined spectrophotometrically using the Folin-Ciocalteu method, employing gallic acid as the standard (Singleton *et al*., 1999). To prepare the crude sample, we liquefied 2 mg of the extract in 2 mL of solvent, resulting in a concentration of 1 mg/mL. Subsequently, we combined 100 µL of varying concentrations, depending on the extract's solubility (1 mg/mL), with Folin-Ciocalteu reagent (0.75 mL) in a tube. After 5 min incubation at 25°C, we introduced Na_2CO_3 solution (0.75 mL) and allowed it to rest in darkness for 90 min. Optical density was measured against a blank at 760 nm. The TPC was determined using a calibration curve based on gallic acid (0.01 to 500 µg/mL) with the equation $y = 0.54x$ and an R^2 of 0.9791. The results were expressed as gallic acid equivalents (GAE) in milligrams per gram of a hundred-gram fresh CRP sample. Figure 1 illustrates a typical calibration curve for phenols.
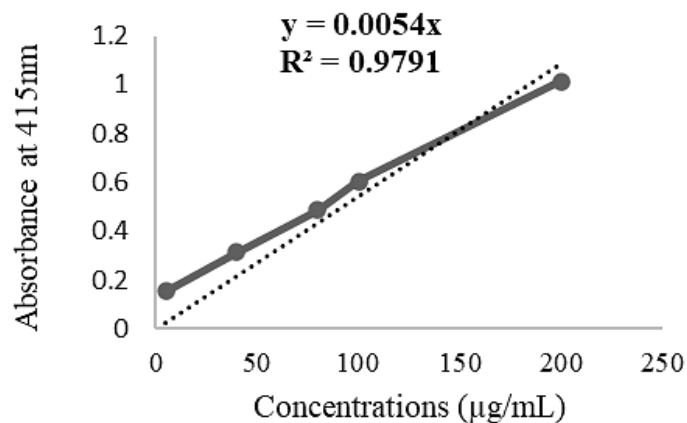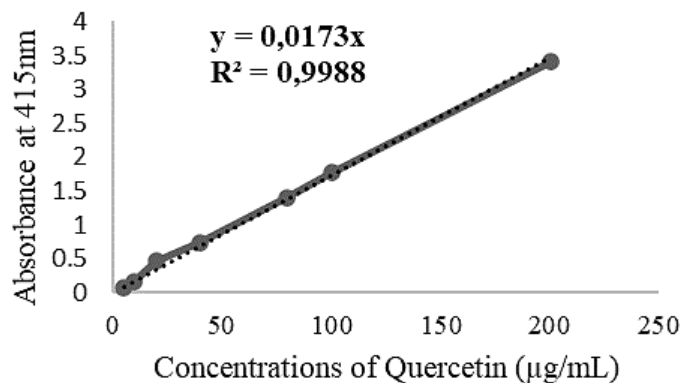

Figure 1. Calibration curve for total phenols.

Total flavonoid content of Capia red pepper

The TFC of CRP was determined using the Dowd method, and expressed the results in quercetin equivalents (QE) per gram of extract weight. This determination was made using a calibration curve with the equation $y = 0.137x$ and an R^2 of 0.9988, based on quercetin.

We initiated the process by preparing a stock quercetin solution. Then, we took 2 mL of CRP and mixed it with an equivalent amount of 2% aluminium chloride ($AlCl_3$), allowing it to stand for 10 min at room temperature. Following this, the absorbance of the CRP samples was measured at 415 nm, compared to a blank sample containing methanol and CRP

extract, but excluding $AlCl_3$ (Turan and Mammadov, 2018). While flavonoids exhibit stability in acidic solutions, they tend to become unstable and undergo discoloration in alkaline environments. Therefore, when dealing with plant-based foods, the utilisation of methanol is deemed suitable under neutral pH conditions (Yamazaki *et al*., 2007). Identical procedures were carried out for quercetin, serving as the standard. The TFC of the samples was subsequently determined and expressed as quercetin equivalents (mg QE/g) per 100 g of fresh CRP (Turan and Mammadov, 2018). Figure 2 illustrates the calibration curve for flavonoids.

Figure 2. Calibration curve for total flavonoids.

Determination antioxidant activity of Capia red pepper

DPPH• (2,2'-diphenyl-1-picrylhydrazyl radical) scavenging activity

To determine DPPH• activity, we introduced 0.25 mL of various extract concentrations into a solution containing 0.05 mM DPPH, freshly prepared in methanol. After incubating the mixture for 30 min in the dark at 25°C, we measured the absorbances at 517 nm. The results were reported in milligrams of ascorbic acid equivalents per gram of weight (mg AAE/g) (Brand-Williams *et al*., 1995). The percentage inhibition for each sample was calculated using Eq.1:

$$\text{\% Inhibition} = [(A_{DPPH} - A_{extract}) / A_{DPPH}] \times 100$$
(Eq. 1)

ABTS•+ scavenging activity

The ABTS scavenging activity was assessed using the TEAC (Trolox equivalent antioxidant capacity) method. The decolourisation of the ABTS•+ radical cation induced by the action of CRPs was monitored using a spectrophotometer (Model Cary 60 UV-Vis Spectrophotometer; Agilent, US) at 734 nm until the absorbance reached 0.7. The TEAC assay evaluates the capacity of the samples to quench ABTS•+ radical formation relative to Trolox (Yeh and Yen, 2003).

The reduction in absorbance for each CRP was continuously monitored, and each supernatant was subjected to assessment three times at varying sample volumes. Subsequently, the percentage inhibitions were plotted against the sample volume at specific time intervals of 1, 3, and 6 min. The slope of each line, corresponding to these time points, was calculated, and plotted against time to depict the percentage inhibition per microliter. Additionally, we computed the area under the curve. This value, along with the area under the curve derived from the Trolox standard curve, was employed to determine the antioxidant capacity values. These values were expressed as millimoles of Trolox per kilogram of dried weight of peppers.

CUPRAC (Cupric-reducing antioxidant capacity) method

In CUPRAC (cupric ion reducing antioxidant capacity) assay, antioxidants facilitate the reduction of cupric ions to cuprous ions. To measure the Cu(I)-neocuproine chelate formed as a result of the redox interaction between antioxidants and the CUPRAC reagent, the absorbance was recorded at the maximum wavelength of 450 nm. This wavelength was utilised for quantifying the levels of antioxidants (Özyürek *et al.*, 2011).

To initiate the process, copper(II) chloride, neocuproine alcohol solution, and an ammonium acetate buffer solution were combined, followed by the addition of extract at concentrations ranging from 100 to 1000 µg. Deionised water was subsequently added to reach the desired total volume of 4.1 mL, and the mixture was thoroughly blended. After 30 min incubation, absorbance was measured at 450 nm against a reagent blank. The antioxidant activity was expressed as Trolox equivalent per milligram of extract.

Statistical analysis

All experiments were conducted in triplicate, and the results were presented as mean values

accompanied by their respective standard deviations. Statistical analysis of non-parametric measurements was performed using the Mann-Whitney U test, Kruskal-Wallis test, and the Wilcoxon Rank Sum test. A *p*-value below 0.05 was considered statistically significant. For the statistical analyses, SPSS 25 IBM software was employed.

Results

The TPC of fresh, traditionally bottled, and industrially bottled CRP was determined to be 118.57 ± 18.2, 137.80 ± 31.2, and 73.89 ± 18 mg GAE/g, respectively. We observed no statistically significant differences ($p > 0.05$) in TPC between fresh CRP and traditionally bottled CRP, but we did find a significant difference between fresh CRP and industrially bottled CRP ($p = 0.001$).

After a twelve-week storage period at 4°C, the TPC of traditionally and industrially bottled CRPs were measured at 81.90 ± 19.82 and 46.26 ± 8.77 mg GAE/g, respectively. Notably, there was a significant statistical decrease in TPC in both traditionally and industrially bottled CRP due to the storage time ($p < 0.05$), as depicted in Figure 3.

The TFC was found to be 34.68 ± 4.41 mg GAE/g for fresh CRP, 58.87 ± 13.35 mg GAE/g for traditionally bottled CRP, and 25.19 ± 10.25 mg GAE/g for industrially bottled CRP. Statistically, TFC varied significantly based on the processing method and storage time ($p = 0.001$). Interestingly,

we observed a significant increase in flavonoid concentration in all stored samples ($p < 0.05$), as depicted in Figure 3.

The AA, assessed using the DPPH radical scavenging method, ranged from 73.09 to 81.14 mg AAE/g. The mean ± SD values for fresh, traditionally bottled, and industrially bottled CRP were 78.43 ± 11.81, 81.14 ± 8.42, and 73.09 ± 22.57 mg AAE/g, respectively. No statistically significant differences in these values were detected ($p > 0.05$). Furthermore, even after 12-week storage, AA of the samples remained unchanged ($p > 0.05$).

The ABTS radical cation scavenging activity across different CRP ranged from 27.84 to 76.23 µmol Trolox/g. Notably, there were no statistically significant differences in AA among fresh, traditionally bottled, and industrially bottled CRPs ($p > 0.05$). However, for traditionally bottled CRPs, a statistically significant change was observed between the initial and 12-week storage samples ($p < 0.05$), whereas no difference was noted for industrially bottled CRPs.

Using the CUPRAC method, AA was found to be 74.95 ± 0.89 mg AAE/g for fresh CRP, 77.88 ± 0.27 mg AAE/g for traditionally bottled CRP, and 63.92 ± 0.14 mg AAE/g for industrially bottled CRP. No statistically significant differences were observed ($p > 0.05$). However, after 12-week storage, a significant change in AA was noted for both traditionally bottled and industrially bottled CRPs ($p < 0.05$).
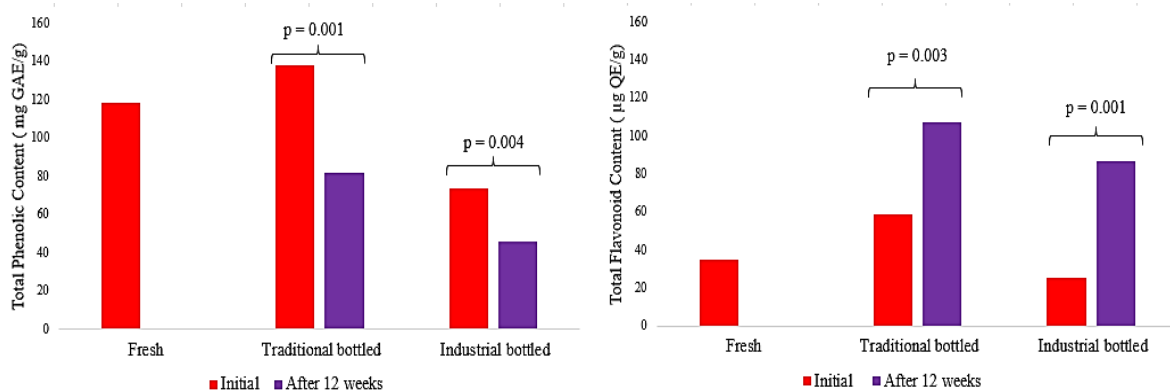
Figure 3. Total phenolic content (mg GAE/g) of fresh and bottled Capia red pepper. Mann-Whitney U test ($p < 0.05$); TPC (total phenolic content) expressed as mg gallic acid equivalent of 100 g of fresh weight (mg GAE/g); TFC (total flavonoid content) expressed as µg/mg quercetin equivalent (QE) of 100 g of fresh weight.

Discussion

We observed significant variations in TPC, TFC, and AA of CRP due to differences in traditional

and industrial-type roasting methods and subsequent storage in bottles. However, we found no significant difference between fresh CRP and traditionally bottled CRP for TPC ($p > 0.05$). Notably, we did

identify a statistically significant difference between fresh CRP and industrially bottled CRP ($p = 0.001$).

Comparing our findings with earlier studies, we noticed differences in the TPC of the pericarp of fresh peppers. For instance, Chen and Kang (2013) reported a TPC of 52.27 mg GAE/g dw in pepper pericarp, which differed from our results, while Sim and Sil (2008) found 47.52 mg/g as GAE. The measurement of TPC using the Folin-Ciocalteu reagent is subject to considerations regarding the method's specificity. This method relies on a redox reaction that could potentially occur with other reducing substances present in the analysed materials, including sugars, carotenoids, or amino acids. Such interactions might lead to an overestimation of the results obtained. Nonetheless, it is important to note that the Folin-Ciocalteu method is widely employed for determining TPC in food samples (Pereira *et al.*, 2018). Additionally, the observed reduction in TPC may be attributed to the leaching of antioxidant compounds from CRP during exposure to heat, washing, and/or peeling processes. Notably, TPC exhibited a slight decrease as they transitioned from the green to red stage, with plant age and maturity being the primary influencing factors.

Following 12-week storage at 4°C, both traditionally and industrially bottled CRPs experienced a significant statistical decrease in TPC due to the duration of storage ($p < 0.05$). This phenomenon suggested that polyphenols may lose their activity over time during storage. Our findings indicated that the decrease in TPC attributed to heat processing methods and storage may be associated with increased polyphenol oxidase activity in CRP during the heat treatment, particularly in the less preferred method and storage conditions. Flavonoid, a collection of organic compounds with varying phenolic structures are antioxidant-activated secondary metabolites whose effectiveness is based on the number and position of the free OH group (Panche *et al.*, 2016). These compounds have been acknowledged for their effectiveness in scavenging free radicals, their ability to chelate trace metals, their role as inhibitors of enzymes involved in free radical generation, and their capacity to enhance the body's own antioxidant defences (Ghasemzadeh and Ghasemzadeh, 2011). Pure methanol, ethanol, or a combination of both is often employed for the extraction of these compounds from plant materials (Naczk and Shahidi, 2004; Molnár-Perl and Füzfai, 2005). However, in certain instances, researchers have utilised ethyl acetate (Kuganesan *et al.*, 2017) or acetone (Schieber *et al.*, 2002) for this purpose.

The hydrolysis of flavonoids from vegetables and fruits is conducted using 1.2 M HCl at 90°C for a duration of 2 h (Pan *et al.*, 2018). Genetic diversity, as well as biological, environmental, and seasonal factors, including year-to-year variations, can significantly impact the flavonoid content of vegetables (Kumar and Roy, 2018). Hence, we exclusively utilised CRP sourced from the same cultivated fields as the industry. The levels of TFC in CRP varied based on the processing method and storage duration ($p = 0.001$). Distinct mechanisms for flavonoid degradation are influenced by factors such as temperature and light (Ioannou *et al.*, 2020).

Flavonoids are characterised by C-glycoside bonds, and typically found as dimers and oligomers in the majority of fruits and vegetables. During processing, such as heating, these C-glycoside bonds undergo hydrolysis, leading to the formation of monomers (Manach *et al.*, 2004). However, in the present work, the TFC of fresh and industrially bottled CRP did not exhibit significant differences ($p > 0.05$). Natural fermentation is characterised by the diverse and uncharacterised microflora it involves, resulting in unspecified metabolic processes occurring in products produced through this method (Antoniewicz *et al.*, 2021). We observed a significant increase in TFC for all stored samples ($p < 0.05$). Our findings indicated that TFC increased after storage at a specific temperature (4°C), and the processing methods employed influenced this increment. The most substantial increase was observed in CRP subjected to industrial bottling processes.

Numerous methods have been employed to evaluate the antioxidant properties of foods. Antioxidant activity, when present in low concentrations, pertains to the capacity to inhibit the auto-oxidation and free radical-mediated oxidation of any substrate (Munteanu and Apetrei, 2021). There is a consensus in the scientific community that phenolic compounds, especially flavonoids, are strongly linked to antioxidant capacity (Connor *et al.*, 2002). The present work examined AA of CRP in response to various heat treatments, encompassing both traditional and industrial processes. To evaluate AA, we employed three distinct methods: the free radical ABTS, DPPH, and CUPRAC scavenging assays. These tests are widely recognised for assessing AA.

The AA of CRP methanolic extracts was determined by assessing their ability to scavenge

DPPH• radical, as previously described by Brand-Williams *et al*. (1995). The DPPH assay is commonly favoured for its cost-effectiveness (Amarowicz and Pegg, 2019). In the present work, AA ranged from 73.09 to 81.14 mg AAE/g. We did not observe any statistically significant difference in AA among fresh, traditional-bottled, and industrial-bottled CRP samples ($p > 0.05$). Furthermore, the AA of the samples did not change significantly after 12-week storage ($p > 0.05$).

The ABTS assay was also employed to assess AA. Notably, the ABTS radical cation scavenging activity varied among several CRP samples, ranging from 27.84 to 76.23 µmol Trolox/g. Surprisingly, there was no significant difference in AA observed among the fresh, traditionally bottled, and industrially bottled CRPs ($p > 0.05$). However, we did find a notable difference between AA before and after 12-week storage for the traditionally bottled samples ($p < 0.05$), whereas the industrially bottled samples showed no such variation. This difference might be attributed to the alterations in pepper composition induced by the cooking process, as previously indicated (Hwang *et al*., 2012).

CUPRAC is a stable and user-friendly chromogenic reagent, making it a preferable choice compared to other chromogenic reagents such as

ABTS and DPPH (Özyürek *et al*., 2011). We did not find any significant difference in AA among fresh, traditional-bottled, and industrial-bottled CRPs ($p > 0.05$). However, after 12-week storage, there was a significant decrease in AA for both traditional-bottled and industrial-bottled CRPs ($p < 0.05$). While the processing method (industrial and traditional) did not affect AA of CRP ($p > 0.05$), storage time had a negative impact on AA of both traditional-bottled and industrial-bottled CRP ($p < 0.05$).

The AA of CRP varied between the applied methods, both initially and after 12-week storage (Table 1). Several factors could have contributed to these variations: (1) hydrophilic and lipophilic antioxidants can be detected by ABTS and CUPRAC methods, while the DPPH method primarily measures hydrophilic systems. (2) CUPRAC method is designed to function under neutral conditions which can influence the results. (3) Background colour in the food matrix may lead to absorbance changes, thus affecting discoloration assays (ABTS, DPPH) more significantly than colour formation reactions (CUPRAC) (Apak *et al*., 2017; Munteanu and Apetrei, 2021). (4) Difference in the potency or concentration of reducing agents, particularly phenols.

Table 1. Antioxidant capacity (DPPH, ABTS, and CUPRAC) of Capia red pepper.

Method	Fresh	Traditional bottling	Industrial bottling	*p*-value
DPPH				
Initial	78.43 ± 11.81	81.14 ± 8.42	73.09 ± 22.57	0.827*
After 12 weeks	-	78.95 ± 3.76	74.78 ± 1.66	0.200**
p-value	-	0.536***	0.998***	-
ABTS				
Initial	68.5 ± 36.82	76.23 ± 29.14	60.41 ± 38.62	0.195*
After 12 weeks	-	38.00 ± 16.56	27.84 ± 14.60	0.258**
p-value	-	0.007***	0.095***	-
CUPRAC				
Initial	74.95 ± 0.89[a]	77.88 ± 0.27[b]	63.92 ± 0.14[c]	0.001*
After 12 weeks	-	66.98 ± 13.42	60.39 ± 13.42	0.394**
p-value	-	0.001***	0.001***	-

*Kruskal Wallis test ($p < 0.01$); **Mann-Whitney U test ($p < 0.05$); ***Willcoxon Rank Sum Test ($p < 0.05$). Values are mean ± SD. Means followed by different lowercase superscripts in the same column are significantly different ($p < 0.05$) by Mann-Whitney U Test.

Conclusion

Understanding the influence of various cooking processes on phenolic compounds and their consequential impact on preserving antioxidant

activity in CRP holds substantial technological significance. This knowledge empowers decision-making in the selection of appropriate culinary techniques and preservation methods. When consumers are informed about the antioxidant

properties inherent in plant-based foods, they can make more informed choices regarding the healthiest food processing methods for storage purposes. Results of the present work indicated that in terms of preservation, the traditional bottling method may be preferable over industrial methods. This underscored the importance of informed decision-making in food processing and storage to optimise both nutritional value and overall health benefits.

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