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# Sustainable Recovery of Antioxidant Compounds from Rossa Di Tropea Onion Waste and Application as Ingredient for White Bread Production

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**Abstract:** From a sustainability and circular economy point of view, this study evaluated the possibility of recovering antioxidant compounds from Rossa di Tropea onion waste for application in the food industry as natural ingredients. In particular, the aim was to investigate the effect of adding natural antioxidants recovered from 'Rossa di Tropea' onion waste to maintain/improve the functional and qualitative characteristics of white bread. Total phenolic content, antioxidant activity, and sensorial aspects were studied on the different enriched samples during the storage period. The 'Rossa di Tropea' onion skins proved to be a good source of natural polyphenols, and their use in white bread production has resulted in a significant increase in bioactive compound content and antioxidant activity (ABTS and DPPH assays). Moreover, the enriched bread showed acceptable quality attributes in terms of odor, colour, and taste, despite the increase in firmness during the storage time compared to the control sample. The obtained results suggest the possibility of applying the antioxidants recovered by 'Rossa di Tropea' onion waste as ingredients in the formulation of bakery products to obtain new food with functional characteristics.

**Keywords:** antioxidant activity; bread; circular economy; phenols; recovery; 'Rossa di Tropea' onion; sustainability; waste



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## 1. Introduction

Nowadays, the concepts of circular economy and sustainability are of great interest to the scientific community in order to reduce the generation of waste through its recycle and reuse. This topic is widely studied by the scientific community, since the agro-food industries produce significant amounts of waste, which have been proven to be rich in bioactive compounds [1]. These bioactive compounds present in several food by-products can represent a natural and valid source of antioxidants for different applications, such as cosmetic, pharmaceutical, and food additives. Phytochemicals are considered the main bioactive compounds from fruits, vegetables, and grains with health advantages [2–4]. In this contest, onion is one of the world's widely cultivated crops, with a production of approximately 98 million tons per year; this produces a huge amount of by-product material, counting different portions such as external layers as well as the apical and basal parts [5]. In particular, the 'Rossa di Tropea' onion is a characteristic onion cultivar, planted in the Calabria region (Italy) [6], which in recent years has received a lot of attention on the part of the scientific community for its peculiar qualitative characteristics [7]. Moreover, this cultivar represents a valuable crop, granted with the protected designation of origin (PDO) and protected geographical indication (PGI) trademarks. Recently, some authors have

optimized a green extraction process on 'Rossa di Tropea' onion waste, in order to obtain a useful and active antioxidant extract to use for the preservation and functionalization of food [8]. In fact, the by-products obtained from the industrial processing of onions contain a large number of phenolic compounds, mainly quercetin and its derivatives, mostly represented by glucosides [9], with well-known anti-inflammatory, antioxidant, and anticancer properties [3,10–12]. Modern consumers tend to prefer healthier and more functional foods that can help prevent diseases; so, both industry and scientific researchers are interested in promoting food production technology to increase the quality and taste and the functional and bioavailable aspects of food, such as bakery products. Besides herbs and spices, which are used as ingredients in bakery good formulations to improve their nutraceutical potential [13], many studies have reported that the onion peel is a good source of bioactive compounds, such as quercetin and dietary fiber. The use of natural antioxidants in food formulations such as biscuits [14], breadsticks [15], and other hydrophilic and lipophilic food systems has already been examined in several studies. Among the different studies reported in the literature, the antioxidants extracted by onion and derivate were used for the formulation of wheat bread [3,16], showing an increase in antioxidant activity compared to the control. Moreover, this addition promoted the fortification of bread due to bio-accessible lipid oxidation inhibitors and compounds with reducing and chelating capacities [17].

The enrichment with bioactive compounds represents a valid solution in the food field; such compounds can be used not only as substitutes for synthetic preservatives but also for the formulation of foods with incremented antioxidant activity and therefore with potential healthy properties.

This study explores the possibility of using natural antioxidants recovered from 'Rossa di Tropea' onion solid waste as a functional ingredient in white bread production, evaluating their effects not only on environmental sustainability determined by the decrease of pollution but also on the replacement of synthetic antioxidants, generally used in the food industry.

## 2. Materials and Methods

### *2.1. Onion Solid Waste Extract Preparation*

'Rossa di Tropea' onion (*Allium cepa* L., *cv. Tropea*) was obtained a local market (Reggio Calabria, Italy) and immediately transported to the laboratory. The 'Rossa di Tropea' Onion solid waste (OSW) was represented by the onion peel, the external layer of the bulb, and the apical and basal trimmings. Before being used, the OSW was subjected to a dehydration process through a dryer ("Scirocco" model, Società Italiana Essiccatoi, Milan, Italy) until the sample reached a humidity of 17% at 50° C, then powdered with the help of a high-speed blender.

Fifty mL of water and 2.5 g of OSW (1:20 *w*/*v* ratio) were mixed and extracted for 30 min at 70 °C by an ultrasonic homogeniser (Sonoplus Series 2000.2, HD 2200.2, BANDELIN electronic GmbH & Co. KG, Berlin, Germany), following the method developed by us in a previous study [9]. The sample was then centrifuged (5000 rpm, 5 min, 4 °C, in a NF 1200R apparatus, Nüve, Ankara, Turkey) and filtered (0.45 µm filter paper). The resulting filtrate extract was made up to 50 mL of volume with water to obtain the 'Rossa di Tropea' onion solid waste extract (OSWE) for the bread enrichment.

### *2.2. Formulation of White Bread Enriched (WBE) with 'Rossa di Tropea' Onion Solid Waste Extract (OSWE)*

The white bread samples (WB, type toast bread) were prepared in the FoodTec Laboratory of the University of Reggio Calabria, according to the following recipe for the control bread (SB): 250 g wheat flour, 250 g Manitoba flour, 200 mL water, 50 g milk, 8 g yeast, 50 g sunflower oil, 5 g sugar, 12.5 g salt (Table 1). All the ingredients used in the formulation were purchased from a local supermarket. The enriched bread (WBE) was prepared using

the ingredients listed above, replacing 100 mL of water with 100 mL of OSWE obtained as previously described (Section 2.1).

**Table 1.** Sliced bread formulation.

| Ingredients | WB | WBE |
|---|---|---|
| Wheat flour (g) | 250 | 250 |
| Manitoba flour (g) | 250 | 250 |
| Sunflower oil (g) | 50 | 50 |
| White sugar (g) | 5 | 5 |
| Skimmed milk (g) | 50 | 50 |
| Yeast (g) | 8 | 8 |
| Salt (g) | 12.5 | 12.5 |
| Water (mL) | 200 | 100 |
| OSWE (mL) | 0 | 100 |

The ingredients were mixed using a spiral planetary mixer (Sigma Srl, Torbole Casaglia, Brescia, Italy) until a well-blended and elastic dough was obtained, which was then subjected to two leavening phases. The first occurred in leavening cells for 90 min, at 25 °C (constant temperature) and 75% of relative humidity. The two leavening phases were interspersed with the manual operations of dough breaking and shaping, which were necessary to give the product the correct final shape and a structure as homogeneous as possible, favoring a more appropriate distribution of the alveolations during the baking process.

At the end of the second leavening phase, which took place under the same conditions as the first one described above, the doughs were baked in an oven (Angelo Po Combistar FX, Carpi, Modena, Italy) for 35 min at 180 °C (humidity 40%), which represents a crucial step for the acquisition of the appropriate structural, sensory, and nutritional characteristics of the final products. The final products were allowed to cool down at room temperature, portioned into slices of about 2 cm thickness, and packed in polypropylene bags (Figure 1).

**Figure 1.** Preparation phases of sliced bread (WBE) enriched with OSWE.

### *2.3. Characterization of Physicochemical Properties of OSWE*

#### 2.3.1. Physicochemical Properties of WB

The pH of OSWE was measured with a pH-meter (Crison basic 20), according to the AOAC International Method [18]. Color parameters (CIE L*a*b* system) were determined using a tristimulus colorimeter (Minolta CR 300, Osaka, Japan), analyzing 15 mL of OSWE, placed in an optical glass.

#### 2.3.2. Antioxidant Characterization of White Bread Samples

The total polyphenol content (TPC) of the OSWE was analyzed applying the Folin-Ciocalteau method, following the method described by Imeneo et al. [14]. The results were expressed as mg of gallic acid equivalents $g^{-1}$ dry weight of 'Rossa di Tropea' onion solid waste (mg GAE $g^{-1}$ d.w.).

The total antioxidant activity by DPPH assay was analyzed following the reaction between the radical (DPPH, 2,2-diphenyl-1-picrylhydrazyl) and antioxidant compounds [9], appropriately modified. 50 µL of OSWE were mixed with 2950 µL of a $6 \times 10^{-5}$ M of DPPH (methanol solution), and after 30 min in the dark, the absorbance was measured at 515 nm.

The total antioxidant activity by ABTS+ (2,2′-azino-bis acid (3- ethylbenzothiazolin-6-sulfonic acid) assay was determined following Imeneo et al. method [9]. 25 µL of OSWE were mixed with 2975 µL of the ethanol solution of ABTS+, and the absorbance at 734 nm was measured after 6 min using a spectrophotometer.

For both total antioxidant assays (DPPH and ABTS), the quenching of initial absorbance was plotted against the Trolox concentration, and the results were expressed as µmol Trolox $g^{-1}$ dry weight of 'Rossa di Tropea' onion solid waste for OSWE (µmol TE $g^{-1}$ d.w.).

### *2.4. Characterization of Physicochemical and Microbiological Properties of WB*

#### 2.4.1. Physicochemical Properties of WB

The physicochemical characterization was carried out on WB and WBE, immediately after the baking and after 3 and 7 days of storage at room temperature. The moisture content percentage of the WB and WBE samples was determined using a thermal balance (Sartorius Moisture Analyzer MA37, Sartorius Italy S.r.l. Muggiò (MB), Italy), by the gravimetric method. The water activity ($a_w$) was detected using a hygrometer (Aqualab LITE Decagon, Nelson Court, Pullman, Washington). Color analysis was carried out as described in Section 2.3.1 both on crumbs and crusts of bread slices in ten different points.

#### 2.4.2. Microbiological Analysis

10 g of the sample were homogenized with 10 mL of ringer solution using a BagMixer 400 (Interscience, 30 Ch. Bois Arpents F. 78860 St. Nom, France), under sterile conditions. The total bacterial count (Plant Count Agar, Oxoid, at 25 ± 2 °C for 48 h), the lactic acid bacteria count (MRS Agar, Liofilchem, at 37 °C for 48 h in anaerobiosis), and the DRBC agar specific Petri plates for yeast and mould count (Dichloran Rose Bengal Chloramphenicol, 28 ± 2 °C for 4–5 days) were used for the analysis of the microbiological parameters. After the incubation period, the microbial load was determined and expressed in a $Log_{10}$ colony-forming unit (CFU) $g^{-1}$ of WB. All the microbial analyses were performed in duplicate for each monitoring time and for both sliced bread samples.

#### 2.4.3. Antioxidant Characterization of White Bread Samples (WB and WBE)

The antioxidant extraction was carried out following the method described by Zielinski et al., [19], with few modifications. Five g of ground bread (previously dried, 40 °C for 24 h, in a laboratory oven, FD115 Binder, Tuttlingen, Germany) were mixed with 50 mL of methanol: water (80:20, *v*:*v*) and homogenized for 2 h at 37 °C. Then the mixture was centrifuged (9000 rpm, 15 min, 10° C), and the supernatant was recovered. The resulting extract was filtered through a 0.45 µm NY (nylon) filter before the analyses. The determination of TPC in WB and WBE followed the method described by Ibrahim et al. [20], with appropriate modifications. 1 mL of WB or WBE was mixed with 5 mL of water and 1 mL of Folin-Ciocalteu reagent in a volumetric flask (25 mL). After 8 min, 10 mL of $Na_2CO_3$, 7.5% (saturated sodium carbonate solution) were added and made up to volume with water. The mixture was left to settle for two hours at room temperature in the dark, and afterward the absorbance was measured at 750 nm and compared with a gallic acid calibration curve. The results were expressed as mg of gallic acid equivalents $kg^{-1}$ dry weight of bread (mg GAE $kg^{-1}$ d.w.).

For the DPPH assay (DPPH), 150 µL of WB or WBE extract were mixed with 2850 µL of DPPH, and after 30 min, the absorbance was measured at 515 nm.

For the ABTS+ assay (ABTS), 100 µL of WB or WBE extract were mixed with 2900 µL of ABTS+, and the absorbance at 734 nm was measured after 6 min.

For the DPPH and ABTS, the results were expressed as µmol Trolox $kg^{-1}$ dry weight of bread (µmol TE $kg^{-1}$ d.w.).

#### 2.4.4. Evaluation of Sensorial Parameters

The sensorial parameters were carried out after 2 h of cooling from the end of production and after 7 days of storage to assess the overall acceptability of the two types of bread, evaluating their appearance, flavour, and texture and using a preference test to allow comparison between the WB and WBE. The bread samples were scored, on a 10-point hedonic scale (where score 0 and 10 were not considered), as compared to various qualitative factors related on both the crumb and the crust, such as: appearance attributes (crumb and crust color, alveolation distribution, crust–crumb area), aromatic aspects (fragrance, stale bread, cereal, yeast, onion), flavour (salty, onion, stale bread, aftertaste), and structural aspects (firmness, compactness, adhesiveness, crumb cohesiveness). The test was conducted by 18 judges (from 25 to 60 years old and of both genders), recruited among the staff of the FoodTec Unit, with previous experience in sensorial testing. The judges were trained before the sessions to identify the attributes to evaluate. The obtained data were processed through the median calculation.

### *2.5. Statistical Analysis*

All the analytical determinations executed in this study were performed in triples (n = 3), and the results were reported as mean value ± standard deviation. The statistical differences were determined by one-way ANOVA analysis through SPSS software (Version 15.0, SPSS Inc., Chicago, IL, USA), and the Tukey's post hoc test was used to determine significant differences ($p < 0.05$). Moreover, the correlation coefficients (r) among the extracted polyphenolic compounds and antioxidant assays were determined using the Pearson's correlation test.

## 3. Results and Discussions

### *3.1. Characterisation of 'Rossa di Tropea' Onion Solid Waste Extract*

For the formulation of enriched white bread (WBE) we used an antioxidant extract obtained from Rossa di Tropea onion solid waste (OSWE). The choice to use this antioxidant extract was made after having carried out a previous study on the evaluation of different extraction methods to recover a high amount of antioxidant compound material from OSWE [9]. In particular, the selected extract was obtained through ultrasound-assisted extraction and using water an as extraction solvent; as such, this OSWE represented the best solution in terms of antioxidant properties.

The results of the physicochemical characterization of OSWE were reported in Table 2. The extract showed an amount of 6.27 ± 0.13 mg GAE $g^{-1}$ d.w of total polyphenols and 5.62 ± 0.15 mg QE $g^{-1}$ d.w. of total flavonoids. The total antioxidant activity has been better highlighted by the ABTS assay, with a value of 19.03 ± 2.78 µmol TE $g^{-1}$ d.w. These results agree with the ranges reported in the literature [21–23], although the same food matrix could exhibit different values of bioactive compound content and antioxidant activity depending on the type of raw material used and the extraction procedure applied. For example, Munir et al. [21] in their study obtain an amount of total flavonoid content that ranged between 4.37 and 16.62 mg quercetin equivalent $g^{-1}$ d.w.

**Table 2.** Physicochemical characterization of OSWE.

| | |
|---|---|
| **pH** | 4.20 ± 0.02 |
| Colour: | L*: 33.23 ± 0.12 |
| | a*: 16.1 ± 0.23 |
| | b*: 0.14 ± 0.02 |
| TPC (mg [1] GAE $g^{-1}$ d.w.) | 6.2297 ± 0.13 |
| TFC (mg [2] QE $g^{-1}$ d.w.) | 5.62 ± 0.15 |
| DPPH (µmol TE $g^{-1}$ d.w.) | 1.34 ± 0.38 |
| ABTS (µmol TE $g^{-1}$ d.w.) | 19.03 ± 2.78 |

[1] GAE: Gallic acid equivalent; [2] QE: quercetin equivalent.

### *3.2. Physicochemical Analysis of White Bread Samples (WB and WBE)*

During the monitoring time, none of the analysed bread samples showed significant variations ($p > 0.05$) regarding water activity (Table 3), with WB characterised by slightly higher values. Contrary to $a_w$, a significant variation ($p < 0.05$) was highlighted for the moisture content (MC). The MC decreased similarly over time in both samples, probably caused by the staling process, which is attributed to the humidity balance between crust and crumb. Indeed, during storage, the crust was inclined to trapping moisture from the crumb, resulting in a dehydration of the crumb and a rapid staling in the case of bread with a thick crust, as in this study [24].

**Table 3.** $a_w$ and moisture content values of WB and WBE.

| | $a_w$ | | | MC (%) | | |
|---|---|---|---|---|---|---|
| **Time (Days)** | **WB** | **$WB_E$** | **Sign.** | **WB** | **$WB_E$** | **Sign.** |
| 0 | 0.91 ± 0.00 | 0.90 ± 0.00 | * | 31.20 ± 0.45 $^{ab}$ | 32.20 ± 0.82 $^{ab}$ | ns |
| 3 | 0.92 ± 0.01 | 0.91 ± 0.01 | ns | 32.29 ± 0.38 $^{a}$ | 32.57 ± 0.17 $^{a}$ | ns |
| 7 | 0.92 ± 0.00 | 0.90 ± 0.01 | * | 30.50 ± 0.99 $^{b}$ | 31.29 ± 0.63 $^{b}$ | ns |
| Sign | ns | ns | | * | * | |

Data are presented as means ± SD ($n = 3$). Means within a column with different letters are significantly different by Tukey's post hoc test. Abbreviation: ns, not significant, * Significance at $p < 0.05$.

Significant differences were found between the two samples with regard to colour (Table 4) on both the bread crumb and crust, which highlighted the influence of the OSWE on the final product. In fact, the addition of the extract caused a significant decrease in the crumb L* value, giving a darker colour to the enriched sample compared to the control and a higher contribution of red component at the day of production. Similar results were also found by Bedrníček et al. [25] after adding waste from a variety of red onion. Unlike what was described for the crumb, a different situation was found on the crust of the two bread samples. In this case, L* values did not show any significant differences for most of the time, as also noted by Altamirano-Fortoul et al. [26], whereas a clear reduction of a* value was detected after 7 days only in the WBE. Regarding the crust of the two different samples (WB and WBE), no statistical differences were detected for either of the colour parameters evaluating (L*, a* and b*) at time 0 (corresponding to production day). In general, there were no changes in colorimetric parameters L* and a* ($p > 0.05$) during monitoring time (0, 3, 7 days) and between the samples (WB and WBE). Only the b* parameter showed lower values in WBE. In fact, the data showed that the WBE was characterized by a darker crumb due to the obvious influence of the extract and a lighter crust.

**Table 4.** Colour parameters of sliced bread samples (WB and $WB_E$).

| CRUMB | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | L* | | | a* | | | b* | | |
| Time (Days) | WB | WBE | Sign. | WB | WBE | Sign. | WB | WBE | Sign. |
| 0 | 75.66 ± 2.01 | 68.82 ± 2.22 | ** | 0.60 ± 0.30 | 2.06 ± 0.33 $^{ab}$ | ** | 18.73 ± 1.42 | 20.67 ± 0.72 | ** |
| 3 | 76.11 ± 2.59 | 69.84 ± 1.11 | ** | 0.56 ± 0.20 | 1.91 ± 0.22 $^{b}$ | ** | 18.57 ± 1.44 | 20.87 ± 0.85 | ** |
| 7 | 74.96 ± 1.00 | 68.48 ± 1.99 | ** | 0.53 ± 0.11 | 2.29 ± 0.26 $^{a}$ | ** | 18.20 ± 0,67 | 21.42 ± 0.54 | ** |
| Sign. | ns | ns | | ns | * | | ns | ns | |
| CRUST | | | | | | | | | |
| | L* | | | a* | | | b* | | |
| Time (days) | WB | WBE | Sign. | WB | WBE | Sign. | WB | WBE | Sign. |
| 0 | 56.63 ± 10.20 | 55.06 ± 11.48 | ns | 15.45 ± 3.37 | 15.98 ± 2.98 | ns | 30.73 ± 2.92 | 27.41 ± 3.77 | ns |
| 3 | 63.17 ± 8.77 | 57.71 ± 9.90 | ns | 13.21 ± 2.96 | 15.32 ± 2.27 | ns | 32.27 ± 3.24 | 28.66 ± 3.53 | * |
| 7 | 61.81 ± 9.74 | 61.48 ± 9.73 | ns | 13.49 ± 2.73 | 15.30 ± 1.97 | ns | 31.44 ± 3.85 | 26.14 ± 4.69 | * |
| Sign. | ns | ns | | ns | ns | | ns | ns | |

Data are presented as means ± SD. Abbreviation: ns, not significant, * Significance at $p < 0.05$, ** Significance at $p < 0.01$.

### 3.3. Microbiological Analysis

A significant increase in total bacterial count (TBC) values was observed in both bread samples over time (Table 5), with similar values at the last day of storage (2.23–2.46 $Log_{10}$ UFC $g^{-1}$). The lactic acid bacteria count showed a similar increasing trend during storage, with significant differences between the samples in all the monitoring times: The enriched bread showed a lower lactic acid bacteria (LB) count compared to the control. Contrarily, mould and yeast were not detected during the monitoring days, but to the 7th day of storage there was a gradual increase particularly in the sample WB. The appearance of mould in the product defined the end of shelf life.

**Table 5.** Total bacterial count (TBC), mould (M) and yeast (Y) growth, lactic acid bacteria count (LB) on WB samples.

| | **TBC ($Log_{10}$ UFC $g^{-1}$)** | | | **M and Y ($Log_{10}$ UFC $g^{-1}$)** | | | **LB ($Log_{10}$ UFC $g^{-1}$)** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Time (Days)** | **WB** | **WBE** | **Sign** | **WB** | **WBE** | **Sign** | **WB** | **WBE** | **Sign** |
| 0 | 0.00 ± 0.00 $^{b}$ | 0.00 ± 0.00 $^{c}$ | ns | 0.00 ± 0.00 $^{b}$ | 0.00 ± 0.00 $^{b}$ | ns | 1.46 ± 0.04 $^{c}$ | 0.47 ± 0.05 $^{c}$ | ** |
| 3 | 1.46 ± 0.03 $^{a}$ | 1.22 ± 0.05 $^{b}$ | ** | 0.00 ± 0.00 $^{b}$ | 0.00 ± 0.00 $^{b}$ | ns | 1.95 ± 0.01 $^{b}$ | 0.95 ± 0.08 $^{b}$ | ** |
| 7 | 2.46 ± 0.70 $^{a}$ | 2.23 ± 0.53 $^{a}$ | ns | 0.46 ± 0.03 $^{a}$ | 0.19 ± 0.03 $^{a}$ | ** | 2.04 ± 0.02 $^{a}$ | 1.80 ± 0.02 $^{a}$ | ** |
| Sign. | ** | ** | | | ** | | ** | ** | |

Data are presented as means ± SD ($n = 3$). Means within a column with different letters are significantly different by Tukey's post hoc test. Abbreviation: ns, not significant, ** Significance at $p < 0.01$.

So, comparing the two different samples showed that enrichment with natural antioxidant compounds (OSWE) led to a good effect on the microbiological control during the shelf-life of the food samples.

### 3.4. Total Phenolic Content (TPC) and Antioxidant Activity of Bread Samples

The TPC and total antioxidant activity measured in the bread samples were reported in Table 6.

The TPC value was significantly higher in WBE in the production day, related to the presence of the onion solid waste extract, with a value of 435.20 ± 18.68 mg GAE $kg^{-1}$ d.w., which decreased to 363.95 ± 24.59 mg GAE $kg^{-1}$ d.w. at the 7th day of storage. Han and Koh [27] demonstrated that the phenolics compounds responsible for the antioxidant capacity of enriched breads are closely linked to the matrix components at the forming and

kneading steps of the dough. They noted a decline in phenolic acid content of about 20–30% in the breads compared to initial product. The reduction in TPC observed in our study could be due to the aptitude of phenolics to precipitate proteins through various mechanisms, such as hydrophobic and ionic interactions or hydrogen and covalent bindings. Thus, the bioavailability of phenolic substances and, consequently, the expression of the antioxidant activity might be significantly reduced respect to the initial content in the OSWE, as also observed by Swieca et al. [28]. In model food systems, relations between phenolic compounds (mainly flavonoids) and protein were reported also by Sivam et al. [29], Siebert et al., [30] and Arts et al. [31]. Several studies related to the effect of these interactions on antioxidant capacity highlighted that a part of that activity is hidden, depending on both the proteins and the phenolics used, emphasising a key role of protein–phenolic interactions [27,30–34]. Moreover, Sivam et al. [29] suggested that a low phenolic extraction from enriched breads could be influenced by the stability of these compounds during bread formulation and their extractability from the bread matrix system, depending on the polyphenol–protein or polyphenol–polysaccharide complexes via hydrogen bonding and/or hydrophobic interactions. In addition, it is known that antioxidant compounds could be damaged or degraded as a consequence of the thermal process during baking, due to the fact that most bioactive compounds become unstable when exposed to heat; this could be an explanation for the lower-than-expected antioxidant activity determination [28,33,35,36]. Besides that, the antioxidant capacity of the bread samples could be influenced also by the activity of oxidative enzymes present in the ingredients used in bread formulation or oxidized by environmental oxygen. The addition of water will begin enzyme activities, and a considerable inclusion of oxygen will take place during kneading. [37]. In this study, in spite of thermal treatment needed for bread preparation, OSWE addition increased the antioxidant capacity of the final product (enriched bread). The WBE showed significantly higher values of antioxidant activity on the day of production by ABTS and DPPH assays, even if no linear trend was found between the level of TPC and expression of the antioxidant activity. Similar values of antioxidant activity between the two samples were noted on the last day of monitoring. The WBE showed greater stability in terms of antioxidant activity expressed by the ABTS assay, showing no significant change in values over time compared with the WB. In accordance with the above, different studies showed a significant improvement in the antioxidant activity of enriched bread [37–41]. The increase in DPPH values at the 7th day could be explained by the presence of Maillard reaction products as antioxidants. This aspect is crucial because phenolics are quite heat-unstable and the baking process may have damaged them [33,42]. Indeed, thermally processed foods may be characterized by various levels of Maillard reaction products that have been reported to have antioxidant activity through scavenging oxygen peroxyl, hydroxyl, and DPPH radicals, copper, and $Fe^{2+}$ chelators [39].

**Table 6.** TPC values and antioxidant activity of bread samples.

| | TPC (mg GAE $kg^{-1}$ d.w.) | | | ABTS (μM TE $kg^{-1}$ d.w.) | | | DPPH (μM TE $kg^{-1}$ d.w.) | | |
|---|---|---|---|---|---|---|---|---|---|
| **Days** | **WB** | **WBE** | **Sign.** | **WB** | **WBE** | **Sign.** | **WB** | **WBE** | **Sign.** |
| 0 | $333.9 \pm 12.7^{b}$ | $435.2 \pm 18.7^{a}$ | ** | $1227.7 \pm 187.2^{b}$ | $2298.3 \pm 182.2$ | ** | $942.8 \pm 44.7^{a}$ | $1043.8 \pm 16.4^{a}$ | * |
| 3 | $379.4 \pm 2.8^{a}$ | $404.5 \pm 7.3^{ab}$ | ** | $1829.7 \pm 184.8^{a}$ | $2054.7 \pm 175.8$ | ns | $367.7 \pm 28.2^{c}$ | $484.9 \pm 7.4^{c}$ | ** |
| 7 | $277.8 \pm 10.7^{c}$ | $363.9 \pm 24.6^{b}$ | ** | $1664.8 \pm 52.8^{a}$ | $1960.6 \pm 363.8$ | ns | $656.6 \pm 1.5^{b}$ | $671.6 \pm 54.6^{b}$ | ns |
| Sign. | ** | ** | | ** | ns | | ** | ** | |

Data are presented as means ± SD ($n$ = 3). Means within a column with different letters are significantly different by Tukey's post hoc test. Abbreviation: ns, not significant, * Significance at $p < 0.05$, ** Significance at $p < 0.01$.

Considering the results obtained in this study, it is clear that enrichment with OSWE determines an improvement in the antioxidant properties in white bread. Thus, the added extract can be considered important for different aims, as a natural preservative and from the point of view of functionally for the consumer.

### *3.5. Sensorial Parameters*

The bread samples were also analysed for the determination of the sensory attributes on the day of production and on the 7th day of storage, as showed in Figures 2 and 3.

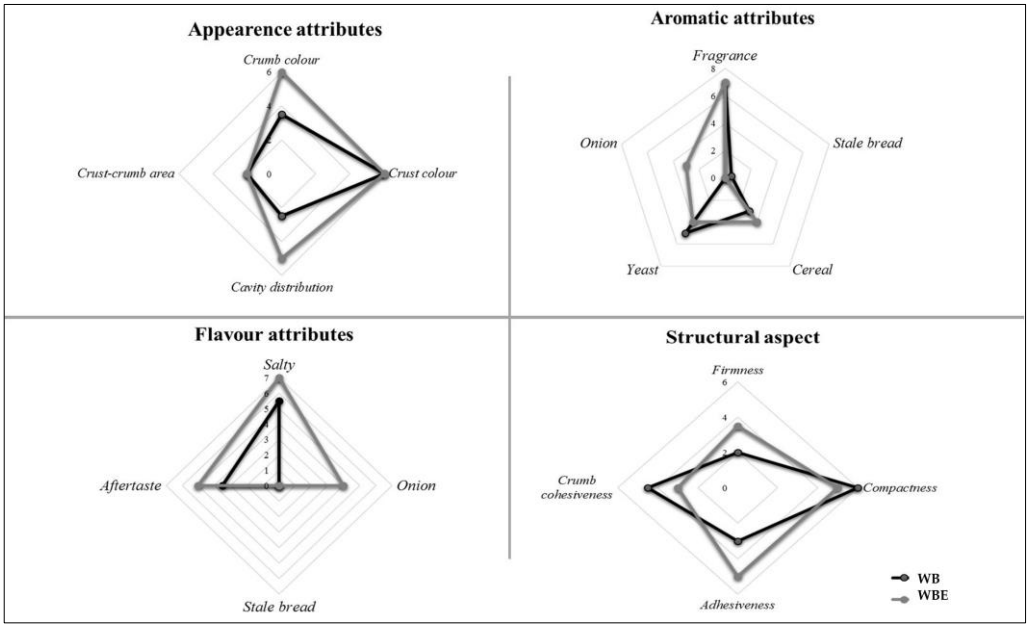


**Figure 2.** Median value of white bread sensorial evaluation at time 0.

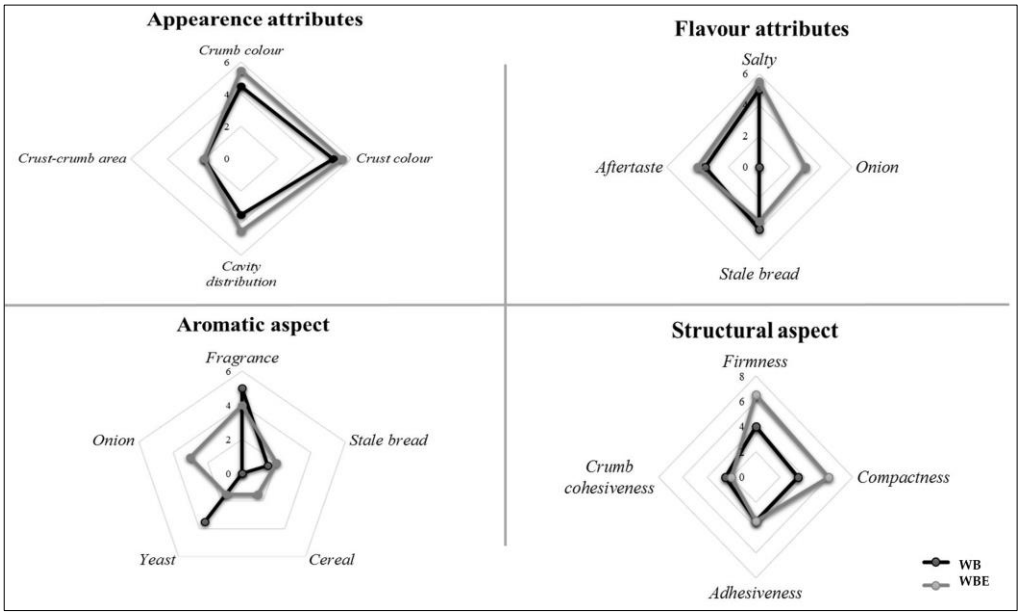


**Figure 3.** Median value of white bread sensorial evaluation at time 7.

In particular, looking at the appearance attributes, the WB crumb colour showed an increase in terms of superficial brown colour over time, in contrast to the enriched bread, whose crumb colour remained fairly stable over time. Contrarily, the crust of both samples showed a slight discolouration, confirming what was described previously in the colorimetric analysis for the WBE (Table 4). Also in terms of alveolation distribution, the WBE showed greater stability than the WB. Low crust–crumb area values, which is considered one of the major bread defects, were recorded for both bread samples. Regarding the aromatic aspect, the enriched bread preserved the onion aroma imparted by the addition

of the 'Rossa di Tropea' onion solid waste extract, as well as a greater cereal aroma than the control sample, which was found to have a greater yeast sensor. Low water activity values of WBE and high processing temperatures could accelerate the Maillard reaction, favouring the production of some typical flavours and aromas of bread, such as 2-acetyl-1-pyrroline, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 2,3-butanedione, methional, (E)-2-nonenal and methylpropanal [41,43]. As expected, both samples at the end of the monitoring were characterised by a lesser perception of fragrance and a greater hint of stale bread. This type of phenomenon is very common in bread, due to the staling process which occurs over time, due to the progressive loss of moisture and starch retrogradation. As reported by Sullivan et al. [44], the different starch fractions retrograde at different times and percentages: Amylose crystallises first, taking from a few minutes to hours, whereas the variations of amylopectin happen at a slower rate, in some cases days. Regarding the flavour attributes, the WBE showed a good level of onion taste perception with a slight decrease at the end of the monitoring period. According to what we described for aromatic attributes, both samples showed an increase in stale bread perception. Moreover, significant changes were found in the structure of the two bread samples, which showed a clear decrease in terms of compactness, adhesiveness, and crumb cohesiveness.

## 4. Conclusions

This study showed that the recovery of antioxidant compounds from 'Rossa di Tropea' onion waste can represent a valid and sustainable method to obtain natural antioxidant to use as an alternative to the synthetic preservatives generally used in the food industry. The applied antioxidant extract (OSWE) has been shown to be a good source of bioactive compounds with antioxidant and antimicrobial activity. The results obtained in this study have confirmed the possibility of using them as functional ingredients to produce new food products, such as bread, without compromising sensory quality. Indeed, the WBE was characterized by a higher level of TPC as well as in vitro antioxidant activity, showing positive results in terms of sensory acceptability. These results confirmed the possibility of producing functional foods by incorporating useful compounds obtained from food industry by-products, reducing their environmental impact and at the same time meeting the growing consumer demand for healthy food. It should be considered that the possible bioactivity of this kind of enriched products could be influenced by many factors, and there are no clear procedures for formulating functional products with a definite nutritional and nutraceutical quality. Thus, to obtain healthy and functional baked goods, further studies in this field are necessary, as is the optimization of processing conditions to limit the loss of useful compounds.

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