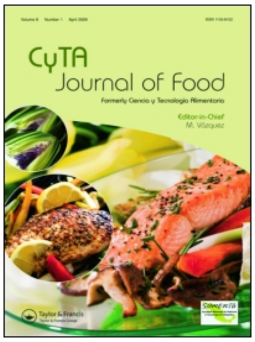

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Opuntia cladodes as functional ingredient in durum wheat bread: rheological, sensory, and chemical characterization

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ABSTRACT

Cladodes are considered by-products of *Opuntia ficus-indica* cultivation. Their addition as source of antioxidants to durum wheat breads could have effects on preventing cardiovascular diseases, cancers, and inflammation. The inclusion of 0-5-10-15% cladodes, harvested in three different locations, on quality and antioxidant properties of fortified durum wheat breads has been evaluated. The enrichment with 10% of cladodes resulted in an increase in the content of total phenolics (14.8 vs 2.7 mg GAE/100 g of control bread), a decrease of IC_{50} (3.28 vs 49.7 mg/ml of control bread), good rheological characteristics of loaves and largely positive evaluation by panel test. Fortification with 15% of cladodes caused an increase in the resistance to the mixture with a reduction of dough extensibility. In addition, the evaluation of different populations of *Opuntia* gave information about the valorization of the crop and could be a strategy to increase bioactive compounds in durum wheat breads.

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Durum wheat bread; cladodes; prickly pear; sensory characteristics; antioxidants

PALABRAS CLAVE
Pan de trigo duro; cladodios; nopal; tuna; características sensoriales; antioxidantes

Cladodios de *Opuntia* como ingrediente funcional en el pan de trigo duro: características reológicas, sensoriales y químicas

Los cladodios se consideran subproductos del cultivo de *Opuntia ficus-indica*. Su adición a los panes de trigo duro como fuente de antioxidantes podría tener efectos en la prevención de enfermedades cardiovasculares, cánceres e inflamaciones. Este estudio evaluó la inclusión al 0-5-10-15% de cladodios cosechados en tres lugares diferentes en la calidad y las propiedades antioxidantes de los panes de trigo duro fortificados. Se pudo constatar que el enriquecimiento con 10% de cladodios produjo aumento del contenido de fenólicos totales (14.8 frente a 2.7 mg GAE/100 g de pan de control), disminución del IC_{50} (3.28 frente a 49.7 mg/ml de pan de control), buenas características reológicas de los panes y una evaluación ampliamente positiva en una prueba de panel. El enriquecimiento con 15% de cladodios provocó un aumento en la resistencia de la mezcla con reducción de la extensibilidad de la masa. Además, la evaluación de diferentes poblaciones de *Opuntia* arrojó información sobre la valorización del cultivo, por lo que su inclusión en los panes de trigo duro podría servir como estrategia para aumentar los compuestos bioactivos en estos.

1. Introduction

Opuntia ficus-indica, also known as the prickly pear or nopal, is part of the *Cactaceae* family. The species origins are from Central America, from desert areas but its natural habitat is also the Mediterranean basin. This species has an erect bearing and it can even reach 5 m in height. The branches are globular and are commonly called paddles or cladodes, covered with a wax that prevents water perspiration, with a size that reaches 40 cm in length, while the leaves are a few millimeters in size and are placed on the paddles; the plant produces edible fruits. The fruits of *Opuntia ficus-indica* are of various colors, depending on the species and the state of ripeness, covered with thorns and many seeds inside. The fruit derives from beautiful yellow, white, or orange flowers, which bloom in a scaled way, starting in spring and going on all summer (Azucena Nazareno, 2014; Las Casas et al., 2018). The main Italian production is in Sicily (over 90%). In this regard, "Ficodindia di San Cono" is a product with a protected designation of origin (PDO), with big fruits, with a weight ranging from 150 to 270 g (European Comm, 2013).

The prickly pear fruit is used for nutritional, cosmetic, and ethno-pharmacological purposes in the form of tea, jam, juice. Even the small seeds, squeezed, give a wonderful and precious anti-aging oil, rich in polyunsaturated fatty acids (over 80%) (Altunkaya et al., 2013; Sawaya et al., 2007).

Recently, many literature data have highlighted the possibility of using the aerial parts of prickly pears as sources of phytochemicals with biological activities and high added value for the food and nutraceutical industry (Andreu et al., 2018; Azucena Nazareno, 2014; Barba et al., 2017; Bensadón et al., 2010; El-Mostafa et al., 2014).

Tender cladodes of *Opuntia* are used as a food and in traditional Mesoamerican medicine to treat a number of conditions such as asthma, cancer, diabetes, gastric mucosa diseases, heart conditions, hypercholesterolemia, hypertension, obesity, and rheumatic pain (Ventura-Aguilar et al., 2017). The cladodes contain high quantities of fiber, including mucilage, pectin, lignin, cellulose, and hemicellulose, substances that are able to bring well-being to the

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metabolism of lipids and sugars (Ayadi et al., 2009; Bayar et al., 2016). Particularly important is the presence of β-polysaccharides (glucose units connected (1 → 4) -β bonds connected with (1 → 3) -β-bonds), characterized by an irregular structure that leads to a water-soluble structure (Lovegrove et al., 2017). This type of water-soluble fiber is capable of absorbing large quantities of water and leads to the formation of viscous and gelatinous colloids which improve the absorption of many organic molecules.

Phenolic profile in cladode extracts was performed and flavonoids (anthocyanins, flavones, and flavonols) were definitely the most abundant of polyphenols detected, followed by phenolic acids, lignans, alkylphenols, and stilbenes derivatives, showing high radical scavenging activity. (Msaddak et al., 2017; Rocchetti et al., 2018).

Extracts of *O. ficus-indica* cladodes have also antimalarial in vitro activity (Bargougui et al., 2013) and reduce gene expression related to endothelial cell inflammation (Armijos & A. G. & G., A, 2013). The positive effects of the *Opuntia* cladodes, fruits, and flowers on consumer physiology have been related to both type and content of secondary metabolites in their tissue (Armijos & A. G. & G., A, 2013; Azucena Nazareno, 2014).

As a powder, sold in capsules, cladodes are used to regulate weight and blood sugar or to increase the general fiber intake. After hydration, the resulting gel exerts a cooling effect, will ease the skin, and thus contribute to accelerated wound healing (Stintzing & Carle, 2005). Different products have been developed with cactus pear peel and cladodes, such as yogurts, snacks, pasta, and drinks (Aiello et al., 2018; Attanzio et al., 2019; Bensadón et al., 2010; Gurrieri et al., 2000; Manzur-Valdespino et al., 2020; Msaddak et al., 2015; Palmeri et al., 2020; Sáenz-Hernández et al., 2002; Sawaya et al., 2007).

Today great attention is paid to isolation of bioactive compounds from natural sources for their antioxidant properties. In particular, the interest is directed to the study and use of products obtained from the transformation processes of vegetable raw materials. Also, the development of new functional bread and fortified flour products can have a demanding task capable of influencing metabolism and other health-related conditions.

Worldwide wheat flour bread is regularly consumed in significant quantities and is one of the main components of the human diet. From a nutritional point of view, it has a high content in complex sugars and very low lipid content; for this reason, the nutraceutical value of this product is low. Bread, thanks to its low cost and its formulation, is a good carrier for functional molecules. Fortified bread offers the possibility of introducing substances with beneficial properties for health through the diet. The potential use of bread for the delivery of antioxidant molecules by adding natural raw materials to enhance the functional properties is a quite common practice (Altunkaya et al., 2013; Ayadi et al., 2009; Liguori et al., 2020; Melilli et al., 2020; Msaddak et al., 2017; Mukkundur Vasudevaiah et al., 2017). Durum wheat is widespread in the Mediterranean area and it is traditionally used, for bread making from re-milled semolina (Arena et al., 2020). Due to the high content of bioactive compounds, prickly pear cladodes could be conveniently used as a nutraceutical and functional ingredient in some food preparations, such as bakery products. In the view of the full potential of *Opuntia*'s bioeconomy (Ciriminna et al., 2019) and due to its economic importance in Sicily, the introduction of added value crop residues to obtain new functional foods could be one way for disposal recovery and valorization.

The use of natural raw materials in bread could strongly influence the organoleptic characteristic of this product, affecting rheological characteristics and consumer acceptability. Therefore, the aim of this study was to evaluate the effect of the addition of dry *O. ficus-indica* cladodes, collected in different areas of Sicily, on the rheological and sensory and antioxidant aspects of durum wheat bread.

2. Materials and methods

2.1. Raw material

The stems of *Opuntia ficus-indica* (cladodes) were collected in three different sites of eastern Sicily: Caltagirone (Cal; 37° 11′07″ N-14°13′19″W; 608 m a.s.l.), Syracuse (Syr; 36°58′33″ N-15°12′18″W; 17 m a.s.l.) and Sortino (Sor; 37°9′24″ N 15° 1′39″; 438 m a.s.l.) during Autumn 2017 Spine of cladodes were removed, after they were washed and dried in a thermoventilated oven at 60°C; the dried material was milled in fine powdered and kept in a hermetic bottle until use. The final moisture content was less than 5%. Durum wheat semolina was purchased from a local artisan mill (Caltagirone, Catania, Sicily). The chemical characteristics, expressed in g 100 g^{-1} were as follows: Fats (1.4); Carbohydrates (65.2); Proteins (11.8); Fiber (10.0).

2.2. Bread making test

Each form of bread was obtained adding to 400 g of commercial durum wheat semolina (xg semolina + xg dried *Opuntia* cladodes) 8 g of sucrose, 8 g of salt, 24 g dehydrated mother yeast, and x ml of distilled water, calculated according to water absorption index (WA) by Brabender farinograph analysis. The process was performed according to a previous study (Melilli et al., 2020). Briefly, the obtained mixture was divided into two 200 g shapes and placed in rectangular aluminum pans. For each population, three levels of fortification (5%, 10%, and 15% w/w) on total weight of the durum wheat semolina were studied. Bread without *Opuntia* cladodes was used as control (CTRL). The leavening conditions were 30°C for 1 h, 75% r.h. Samples were baked in an experimental oven (Wind Pierre, Ing. Polin e C. S.P.A., Verona, Italy), at 180°C, for 18 min. Bread samples (Cal5, Cal10, Cal15, Syr5, Syr10, Syr15, Sor5, Sor10, and Sor15) are shown in Figure 1. Samples were subjected to the instrumental measurement for volume (Volumometer 2 L, Geass S. R.L., Torino, Italy), expressed in cm^3 and height of the loaf of breads (Vernier Caliper, cm).

2.3. Rheological characteristics

The dough mixing properties of the CTRL and different mixes were examined with the Brabender farinograph (Brabender, Duisburg, West Germany) according to the constant flour weight procedure (AACC n° 54–21). Three hundred grams of durum wheat semolina was mixed at optimum water absorption and the farinograph curve was calibrated on the 500 BU line. According to the standard procedure, the following farinograph indices were

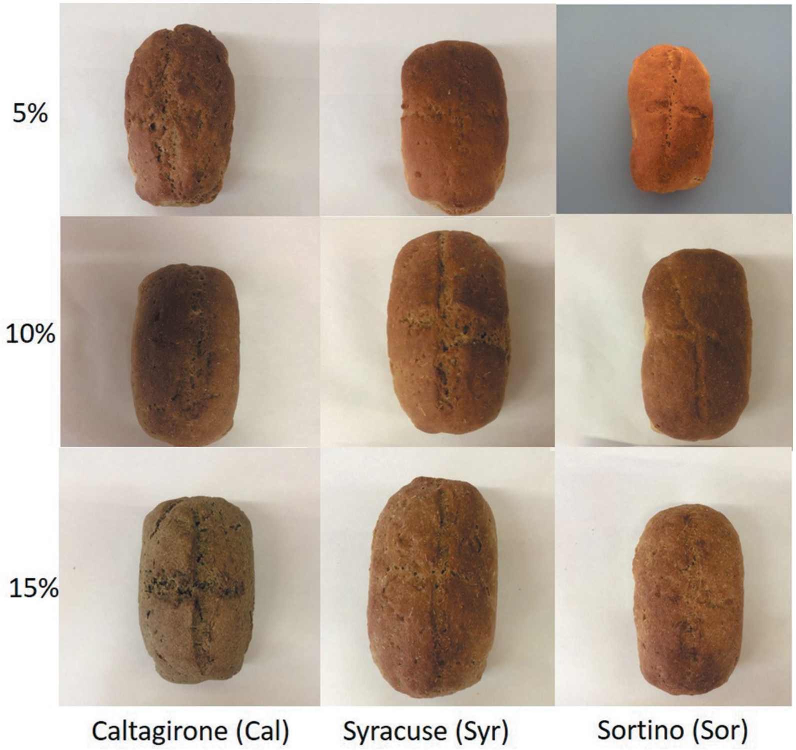

Figure 1. Cladodes collected in three different sites of Sicily.

Figura 1. Cladodios recogidos en tres sitios diferentes de Sicilia.

determined: (1) water absorption of blend (WA), (2) development time of dough (DT), (3) stability of dough (S) and (4) the degree of softening of dough (DS) (Table 2). The alveographic test was used to analyze the effect of additions on the dough rheological behavior performed by Chopin alveograph (Chopin, Villeneuve La Garenne, France) according to the standard alveographic method (UNI n° 10453) (American Association of Cereal Chemists (AACC) International 2000). Each sample was analyzed in triplicate and deformation energy W (strength) and P/L (tenacity/extensibility ratio) were calculated.

2.4. Sensory analysis of bread

In order to evaluate the sensory attributes, each bread sample was submitted to a panel of 10-trained tasters (five men and five women, aged between 27 and 60 years). The panel group is an on-going panel with prior training. The samples were served in dishes labeled randomly with three-digit random numbers for all panelists. Breads were sliced (1 cm thick) and were offered in distinct dishes at the same time. Water was provided for rinsing purposes. Panel group evaluated on crust thickness, elasticity, hardness, friability, apparent softness (force required for compressing the bread slice on a flat surface with a finger, to obtain a deformation about 50% of crust); on crumb elasticity, friability, cohesiveness, humidity, optical evaluation of the average size and homogeneity of the alveoli, cohesiveness to the crust. We asked panelist to give a bread overall judgment as overall taste and odor. A 9-point scale was used: 1 low sensation, 9 high sensation while for final overall judgment 1 corresponds to extremely unpleasant, 9 to extremely pleasant. The threshold of acceptability was set at 5.

2.5. Bread color evaluation

On dried *Opuntia* powders and on bread samples, color data were collected with the use of a Chroma Meter (Minolta CR – 400, Milan, Italy), as previously described by Melilli et al. (Melilli et al., 2016). The colorimeter was calibrated using the manufacturer's standard white plate (L* = 96.55; a* = −0.35; b* = −0.16), where the L* value represents light-dark spectrum with a range from 0 (black) to 100 (white), the a* value represents the green-red spectrum with a range from −60 (green) +60 (red). The b* value represents the blue-yellow spectrum with a range from −60 (blue) +60 (yellow) (Di Stefano et al., 2019).

2.6. Total phenols content

Total phenol content in bread samples (TPC) was determined using Folin-Ciocalteu method as reported by Singleton, et al. (Singleton et al., 1999) with some modifications (Gentile et al., 2019), on *Opuntia* cladodes and on bread samples. One gram of the sample was extracted with 10 ml of a solution MeOH:H_2O (80:20), sonicated for 40 minutes, and filtered. The extract was stored at – 20°C overnight. For the determination of TPC 625 μl of Folin–Ciocalteu reagent (Merck KGaA, Darmstadt, Germany), diluted 5 times and 1.2 ml of Na_2CO_3 (7% w/v) solution was added to 125 μl of sample extract. Mixtures were vortexed for 2 min and incubated in the dark for 1 h. Absorbance at 760 nm was measured using a spectrophotometer (Biospectrometer® basic, Eppendorf AG). The TPC was expressed as mg gallic acid equivalent per g of samples (mgGAE/100 g). the determinations were performed in triplicates.

2.7. In vitro antioxidant activity

Cladodes, CTRL bread, and fortified bread samples were submitted to analysis, using the DPPH• (2,2-diphenyl-1-picryhydrazyl) radical scavenging activity (Brand-Williams et al., 1995). Briefly, 1 g of the sample was extracted with 4 ml of Methanol. The solutions were centrifuged. The ability of a compound to donate a hydrogen atom was assessed on the basis of the scavenging activity of the stable DPPH• radical. Three ml 0.06 mM DPPH• was added to 100 µl of extracts. A control was prepared by adding the same quantity of DPPH• to methanol. The contents of the tubes were mixed and allowed to stand for 20 min at 37°C and absorbance was measured at 515 nm by a spectrophotometer (Eppendorf). The results were expressed as IC50 (mg/ml), the concentration required to cause 50% DPPH• inhibition. For every sample, the protocol was repeated three times.

2.8. Data analysis

Data were submitted to Bartlett's test for the homogeneity of variance and then analyzed using analysis of variance (ANOVA). Means were statistically separated on the basis of Student-Newmann-Kewls test, when the 'F' test of ANOVA for treatment was significant at least at the 0.05 probability.

3. Results and discussion

3.1. Opuntia cladodes characterization

The results of color indices are reported in Table 1. Cladodes of Cal had the highest L* index, 55.3 vs 47.1 (Syr) and vs 46.0 (Sor). Cladodes collected at Syracuse resulted greener than those of the other two populations. The TPC was measured in the dry powders in order to determine the final amount of total phenols in produced breads. Cladodes from Sortino and Syracuse showed the same TPC content while, it decreased in cladodes from Caltagirone. DPPH• scavenging activity did not result significantly different among three *Opuntia* cladodes flours samples.

3.2. Rheological characteristics

Both the origin of the cladodes and levels of fortification influence the properties of the doughs both on alveographic and on farinographic parameters (Table 2).

Gluten is composed of glutenin and gliadin giving the dough toughness and dough extensibility. The alveographic index *P* value indicates the dough tenacity and L value, its extensibility. The P/L configuration ratio indicates the balance between the two factors and greatly affects the bread-making quality. A value of P/L close to 1 is favorable to the baking process (Palumbo et al., 2002).

Table 2 shows that a partial substitution (from 5% to 15%) of durum wheat semolina with dried cladodes has induced significant modifications on alveographic parameters (W and P/L). The data agree with Ayadi et al. (Ayadi et al., 2009). The comparison concerning the average of populations shows that the W value has decreased by about 22% compared to the CTRL in Cal, by about 50% in Syr, and by about 34% in Sor. Regarding the different average of cladodes concentrations, the W value sample containing 5% of dried cladodes has decreased by 34% in comparison to the CTRL, while about 35% of decrease was observed for 10% substitution. By increasing the replacement percentage up to 15%, the W value decreased by about 32%. Cladodes powder enrichment induces important modifications on the dough rheological parameters in terms of deformation energy (W) decrease (Msaddak et al., 2017). The different

Table 1. *Opuntia* cladode characteristics. LSD was calculated at $p < 0.05$, according to Duncan test, using one-way analyses.

Tabla 1. Características de los cladodios de *Opuntia*. La LDE se calculó a $p < 0.05$, según la prueba de Duncan, usando análisis de una sola vía.

Cladodes	L*	a*	b*	TPC (mg GAE/100 g)	IC50 mg/ml
Cal	75.4	−6.3	20.3	6.4	10.9
Syr	79.9	−4.5	16.3	7.6	10.8
Sor	84.4	−6.0	19.2	7.6	10.8
Means	79.9	−5.6	18.6	7.19	10.9

Cal: Caltagirone cladodes; Syr: Syracuse cladodes; Sor: Sortino cladodes.

Cal: cladodios de Caltagirone; Syr: cladodios de Siracusa; Sor: cladodios de Sortino.

Table 2. Alveographic and farinographic indices of doughs enriched with *Opuntia* cladodes. LSD was calculated at $p < 0.05$, according to Duncan test, using one-way (all samples) or two ways (mean values) analyses.

Tabla 2. Índices alveográficos y farinográficos de masas enriquecidas con cladodios de *Opuntia*. La LDE se calculó a $p < 0.05$, según la prueba de Duncan, utilizando análisis de una vía (todas las muestras) o de dos vías (valores medios).

	Alveograph analysis		Farinograph analysis		
Sample	W (10^{-4} J)	P/L	Water Absorption %	Development Time (min)	Dough Stability (min)
CTRL	135.0	3.6	62.9	2.0	4.2
Cal5	95.0	5.2	62.1	1.7	3.7
Cal10	102.0	6.8	62.2	1.8	5.8
Cal15	119.0	6.4	62.9	2.8	7.0
Syr5	80.0	4.7	62.4	1.8	3.8
Syr10	80.0	3.5	62.5	1.8	3.8
Syr15	70.0	4.3	62.9	2.7	4.5
Sor5	93.0	4.0	61.4	1.7	4.2
Sor10	88.0	4.9	61.6	1.8	4.4
Sor15	88.0	4.8	63.4	2.5	6.8
LSD	*5.4*	*0.9*	*1.4*	*1.3*	*1.1*
			Average of populations		
Cal	105.3	6.1	62.4	2.1	5.5
Syr	76.7	4.2	62.6	2.1	4.0
Sor	89.7	4.6	62.1	2.0	5.1
LSD	*9.7*	*0.4*	*ns*	*ns*	*0.9*
			Average of concentrations		
5%	89.3	4.6	62.0	1.7	3.9
10%	90.0	5.0	62.1	1.8	4.7
15%	92.3	5.2	63.1	2.7	6.1
LSD	*1.9*	*0.4*	*0.5*	*0.9*	*0.9*

CTRL: bread without fortification; Cal5: bread fortified with 5% of Caltagirone cladodes; Cal10: bread fortified with 10% of Caltagirone cladodes; Cal15: bread fortified with 15% of Caltagirone cladodes; Syr5: bread fortified with 5% of Syracuse cladodes; Syr10: bread fortified with 10% of Syracuse cladodes; Syr15: bread fortified with 15% of Syracuse cladodes; Sor5: bread fortified with 5% of Sortino cladodes; Sor10: bread fortified with 10% of Sortino cladodes and Sor15: bread fortified with 15% of Sortino cladodes. Cal: bread fortified with Caltagirone cladodes; Syr: bread fortified with Syracuse cladodes; Sor: bread fortified with Sortino cladodes.

CTRL: pan sin fortificar; Cal5: pan fortificado con 5% de cladodios de Caltagirone; Cal10: pan fortificado con 10% de cladodios de Caltagirone; Cal15: pan fortificado con 15% de cladodios de Caltagirone; Syr5: pan fortificado con 5% de cladodios de Siracusa; Syr10: pan fortificado con 10% de cladodios de Siracusa; Syr15: pan fortificado con 15% de cladodios de Siracusa; Sor5: pan fortificado con 5% de cladodios de Sortino; Sor10: pan fortificado con 10% de cladodios de Sortino y Sor15: pan fortificado con 15% de cladodios de Sortino. Cal: pan fortificado con cladodios de Caltagirone; Syr: pan fortificado con cladodios de Siracusa; Sor: pan fortificado con cladodios de Sortino.

Table 3. L*, a*, b* in the breads enriched with Opuntia cladodes. LSD was calculated at $p < 0.05$, according to Duncan test, using one-way (all samples) or two ways (mean values) analyses.

Tabla 3. L*, a*, b* en los panes enriquecidos con cladodios de *Opuntia*. La LDE se calculó en $p < 0.05$, según la prueba de Duncan, utilizando análisis de una vía (todas las muestras) o de dos vías (valores medios).

Sample	Crust L*	Crust a*	Crust b*	Crumb L*	Crumb a*	Crumb b*	Height cm	Volume cm^3
CTRL	42.72	16.47	25.49	69.32	−1.73	22.35	7.07	337.5
Cal5	43.46	12.96	22.83	59.39	−0.66	23.79	5.8	327.5
Cal10	44.55	10.06	23.14	55.25	−0.62	24.51	5.6	298.33
Cal15	44.1	9.37	21.81	45.45	−1.32	22.6	5.55	247.5
Syr5	44.18	15.42	24.7	64.64	−0.99	23.04	6.13	405.0
Syr10	46.25	12.94	26.22	63.54	−1.43	24.27	5.92	348.33
Syr15	45.89	10.6	22.93	61.08	−1.58	24.32	5.33	198.33
Sor5	44.33	14.24	24.38	66.48	−0.11	24.03	5.95	345.83
Sor10	42.82	14.51	22.61	64.45	−1.93	25.76	5.78	291.17
Sor15	43.26	11.44	22.46	62.77	−2,0	26.87	5.3	275.83
LSD	1.17	2.41	1.74	7.95	0.65	1.5	0.61	68.9
				Average of populations				
Cal	44.04	10.8	22.59	53.36	−0.87	23.63	5.65	291.11
Syr	45.44	12.99	24.61	63.08	−1.33	23.88	5.79	317.22
Sor	43.47	13.4	23.15	64.56	−1.35	25.55	5.68	304.28
LSD	*1.25*	*2.75*	*1.95*	*8.02*	*0.56*	1.45	0.84	69.4
				Average of concentrations				
5%	43.99	14.21	23.97	63.5	−0.59	23.62	5.96	359.44
10%	44.54	12.5	23.99	61.08	−1.33	24.85	5.77	312.61
15%	44.42	10.47	22.4	56.43	−1.64	24.6	5.39	240.56
LSD	*1.21*	*2.02*	*1.45*	*7.41*	*0.54*	1.54	0.62	65.4

CTRL: bread without fortification; Cal5: bread fortified with 5% of Caltagirone cladodes; Cal10: bread fortified with 10% of Caltagirone cladodes; Cal15: bread fortified with 15% of Caltagirone cladodes; Syr5: bread fortified with 5% of Syracuse cladodes; Syr10: bread fortified with 10% of Syracuse cladodes; Syr15: bread fortified with 15% of Syracuse cladodes; Sor5: bread fortified with 5% of Sortino cladodes; Sor10: bread fortified with 10% of Sortino cladodes and Sor15: bread fortified with 15% of Sortino cladodes. Cal: bread fortified with Caltagirone cladodes; Syr: bread fortified with Syracuse cladodes; Sor: bread fortified with Sortino cladodes.

CTRL: pan sin fortificar; Cal5: pan fortificado con 5% de cladodios de Caltagirone; Cal10: pan fortificado con 10% de cladodios de Caltagirone; Cal15: pan fortificado con 15% de cladodios de Caltagirone; Syr5: pan fortificado con 5% de cladodios de Siracusa; Syr10: pan fortificado con 10% de cladodios de Siracusa; Syr15: pan fortificado con 15% de cladodios de Siracusa; Sor5: pan fortificado con 5% de cladodios de Sortino; Sor10: pan fortificado con 10% de cladodios de Sortino y Sor15: pan fortificado con 15% de cladodios de Sortino. Cal: pan fortificado con cladodios de Caltagirone; Syr: pan fortificado con cladodios de Siracusa; Sor: pan fortificado con cladodios de Sortino.

origin of the cladodes has influenced the P/L values, as showed in Table 2. All three populations have led to an increase of P/L value, but the higher increase has been recorded in Cal sample. Regarding the average of concentrations, the partial replacement from 5% to 15% of wheat semolina by the dried cladodes led to a proportional increase in P/L value.

In Table 2 are reported water absorption, development time, and dough stability. The different origin of *Opuntia* cladodes influences mainly dough stability. Water absorption and development time parameters have not shown significant differences. Also, concerning the average of concentrations, the level of fortification of 15% led to a higher increase of all farinographic parameters, in particular for stability.

3.3. Color, form and organoleptic characteristics of the fortified breads

The first parameter of quality evaluated by consumers is the color of the food. The objective values of CIELAB, on crust and crumb, the height, and the volume of the bread samples are reported in Table 3. The color indices were affected by type of cladodes and levels of fortification. "Syr" gives darker crust, while "Cal" gives darker crumb. Independently of the type of cladodes used, all samples have L* values lower than CTRL both for crust and crumb, and in general, increasing from 0 (CTRL) to 15% of fortification samples gave the lowest L* values. The a* value represents the green-red spectrum. Negative values go towards green. For crust, the values ranged from 10.5 (15%) to 14.2 (5%), while for crumb the values ranged from −1.6 (15%) −0.6 (5%). The blue-yellow spectrum (b*) resulted in about 24.0 both for crust and crumb at the different levels of fortification. The CTRL shows values of L* 42.7, a* 16.5, and b* 25.5, for crust, while L* 69.3, a* −1.7 and b* 22.3 are recorded on the crumb. The visual appearance of the loaf of CTRL is characterized by a high 7.1 cm with a volume of 337.5 cm^3. The height of the loaf of bread results improved adding 5% of cladodes flours (359.4 cm^3, mean of the type of *Opuntia*). Msaddak et al. (Msaddak et al., 2017) also report the 5% supplementation resulted in an increase of the bread yield and bread-specific volume. The best results for the visual appearance of the loaf are recorded adding "Syr" and "Sor" cladodes.

The addition of plant or their extract to flours to obtain bread is expected to influence its structure, altering the organoleptic characteristics. For this reason, the sensory properties of bread samples investigated in this work are addressed (Figures 2 and 3).

Figure 2 shows the sensory characteristics recorded in all bread samples separately for crust (a) and crumb (b). For crust, the fortification higher than 10% negatively affects elasticity and hardness. Until 10% of fortification, friability and apparent softness show the same scores of CTRL. Concerning crumb, the addition of *Opuntia* cladodes led to a higher sensation of softness and humidity. If we look at the judgment of aver quality score, reported in Figure 3, all bread samples show scores higher than 5

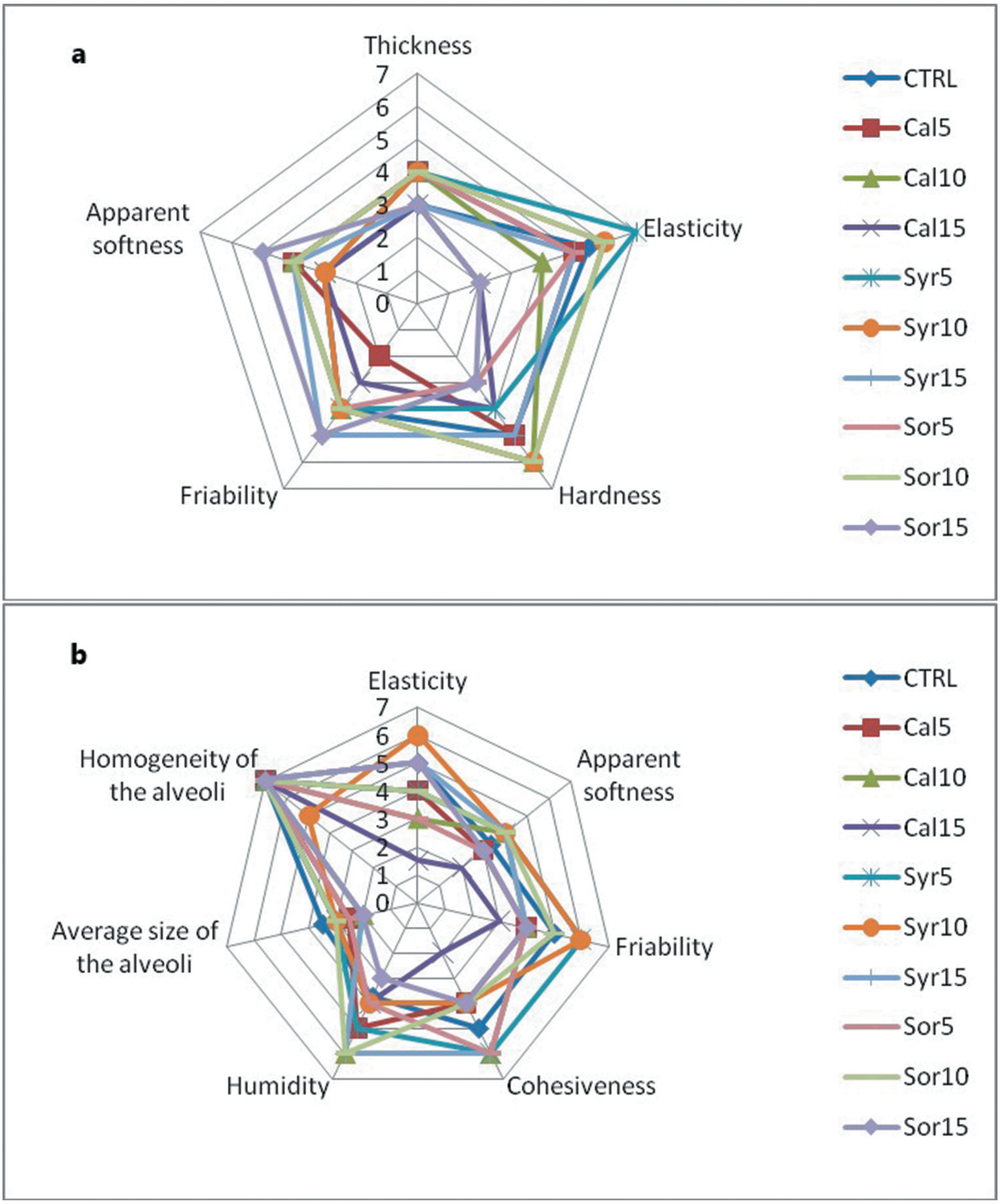

Figure 2. Sensory attributes of crust (a) and crumb (b) of the fortified breads. 1 good feeling and 9 bad feeling. CTRL: bread without fortification; Cal5: bread fortified with 5% of Caltagirone cladodes; Cal10: bread fortified with 10% of Caltagirone cladodes; Cal15: bread fortified with 15% of Caltagirone cladodes; Syr5: bread fortified with 5% of Syracuse cladodes; Syr10: bread fortified with 10% of Syracuse cladodes; Syr15: bread fortified with 15% of Syracuse cladodes; Sor5: bread fortified with 5% of Sortino cladodes; Sor10: bread fortified with 10% of Sortino cladodes and Sor15: bread fortified with 15% of Sortino cladodes.

Figura 2. Atributos sensoriales de la corteza (a) y de la miga (b) de los panes fortificados. 1 buena sensación y 9 mala sensación. CTRL: pan sin fortificar; Cal5: pan fortificado con 5% de cladodios de Caltagirone; Cal10: pan fortificado con 10% de cladodios de Caltagirone; Cal15: pan fortificado con 15% de cladodios de Caltagirone; Syr5: pan fortificado con 5% de cladodios de Siracusa; Syr10: pan fortificado con 10% de cladodios de Siracusa; Syr15: pan fortificado con 15% de cladodios de Siracusa; Sor5: pan fortificado con 5% de cladodios de Sortino; Sor10: pan fortificado con 10% de cladodios de Sortino y Sor15: pan fortificado con 15% de cladodios de Sortino.

(threshold of acceptability), except Sor15. For all types of *Opuntia* cladodes, it is possible to obtain good results until 10% of fortification. Score decay for all samples is noticed at 15% of substitution.

3.4. Chemical characterization and antioxidant activity

TPC content, reported in Figure 4, increased with the levels of *Opuntia* cladode fortification. CTRL has a TPC content of 2.7 mg GAE/100 g. On average of the three

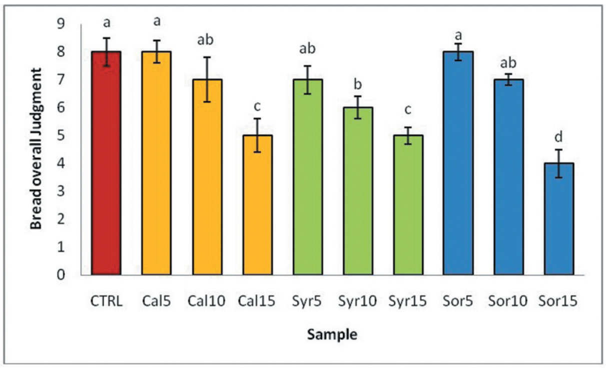

Figure 3. Overall judgment of bread samples (Means ± SD). The threshold of acceptability for bread overall judgment is 5. 1 corresponds to extremely unpleasant, 9 to extremely pleasant. CTRL: bread without fortification; Cal5: bread fortified with 5% of Caltagirone cladodes; Cal10: bread fortified with 10% of Caltagirone cladodes; Cal15: bread fortified with 15% of Caltagirone cladodes; Syr5: bread fortified with 5% of Syracuse cladodes; Syr10: bread fortified with 10% of Syracuse cladodes; Syr15: bread fortified with 15% of Syracuse cladodes; Sor5: bread fortified with 5% of Sortino cladodes; Sor10: bread fortified with 10% of Sortino cladodes and Sor15: bread fortified with 15% of Sortino cladodes.

Figura 3. Valoración global de las muestras de pan (medias ± DE). El umbral de aceptabilidad para la valoración global del pan es 5. 1 corresponde a extremadamente desagradable, 9 a extremadamente agradable. CTRL: pan sin fortificar; Cal5: pan fortificado con 5% de cladodios de Caltagirone; Cal10: pan fortificado con 10% de cladodios de Caltagirone; Cal15: pan fortificado con 15% de cladodios de Caltagirone; Syr5: pan fortificado con 5% de cladodios de Siracusa; Syr10: pan fortificado con 10% de cladodios de Siracusa; Syr15: pan fortificado con 15% de cladodios de Siracusa; Sor5: pan fortificado con 5% de cladodios de Sortino; Sor10: pan fortificado con 10% de cladodios de Sortino y Sor15: pan fortificado con 15% de cladodios de Sortino.

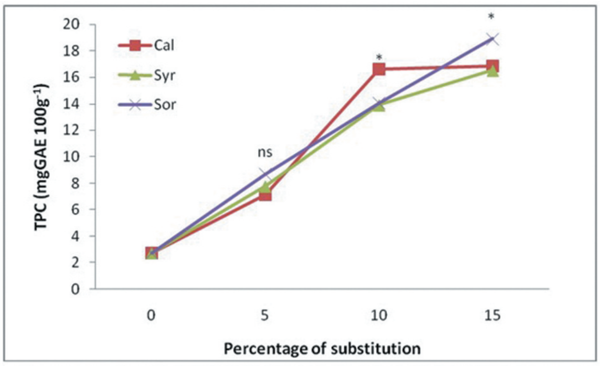

Figure 4. TPC (mgGAE/100 g) of fortified breads, obtained with durum wheat semolina and *Opuntia* cladodes at three different concentrations. (ns) indicates not statistically different. (*) significant at 0.05 probability level, among the three types of *Opuntia* cladodes. Cal: bread fortified with Caltagirone cladodes; Syr: bread fortified with Syracuse cladodes; Sor: bread fortified with Sortino cladodes.

Figura 4. TPC (mgGAE/100 g) de panes enriquecidos, obtenidos con sémola de trigo duro y cladodios de *Opuntia* en tres concentraciones diferentes. (ns) indica que no son estadísticamente diferentes. (*) significativo a un nivel de probabilidad de 0.05, entre los tres tipos de cladodios de *Opuntia*. Cal: pan fortificado con cladodios de Caltagirone; Syr: pan fortificado con cladodios de Siracusa; Sor: pan fortificado con cladodios de Sortino.

types of *Opuntia* cladodes, the TPC content is increased by 3 (5% fortification), 5.5 (10% fortification), and 6.8 times compared to CTRL. At 5% and 10% levels of fortification, samples fortified with Syr and Sor cladodes do not give statistical differences. Samples fortified with Cal cladodes give an increase in TPC content higher at 10% of fortification. The sample Sor 15 records the major increase in TPC (Figure 4).

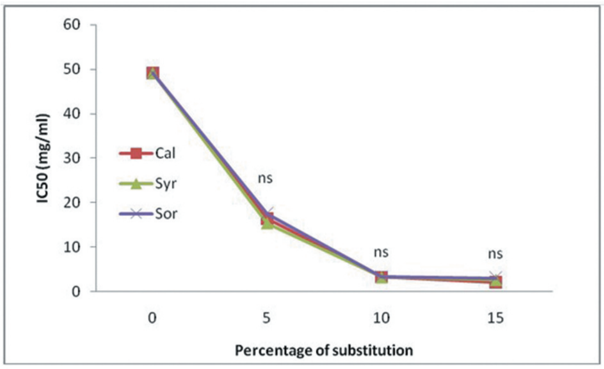

Figure 5. IC_{50} (mg/ml) antioxidant activity of fortified breads, obtained with durum wheat semolina and *Opuntia* cladodes at three different concentrations. (ns) indicates not statistically different, among the three types of *Opuntia* cladodes. Cal: bread fortified with Caltagirone cladodes; Syr: bread fortified with Syracuse cladodes; Sor: bread fortified with Sortino cladodes.

Figura 5. Actividad antioxidante IC_{50} (mg/ml) de los panes enriquecidos, obtenidos con sémola de trigo duro y cladodios de *Opuntia* en tres concentraciones diferentes. (ns) indica que no es estadísticamente diferente, entre los tres tipos de cladodios de *Opuntia*. Cal: pan fortificado con cladodios de Caltagirone; Syr: pan fortificado con cladodios de Siracusa; Sor: pan fortificado con cladodios de Sortino.

Results of TPC result well correlated with the values of antioxidant activity, expressed as IC50 and graphed in Figure 5. An increase of antioxidant activity is one of the main aims of food supplementation and DPPH• assay highlighted the capacity of *Opuntia* cladodes to increase the antioxidant potential of enriched samples vs CTRL. The IC50 of CTRL bread was 49.1 mg/ml. A valuable decrease in IC50 was noticeable at the three levels of substitutions. The mean values were 16.4 (5% fortification), 3.3 (10% fortification) and 2.5 mg/ml (15% fortification). The three types of prickly pear added do not influence the IC50 values, which did not result different also in dry cladodes used for bread fortification (Table 1). Also, bread enriched with roasted prickly pear seed flour results in a fourfold increase of antioxidant activity and TPC content compared to wheat flour (Ali et al., 2020). The addition of fresh mucilage (in substitution to water) showed a biological role of the cactus mucilage, because their antioxidant activity was higher than that of control wheat bread (Liguori et al., 2020).

4. Conclusions

The increasing interest of consumers to foods rich in bioactive compounds must find a good compromise between the levels of plant/herbs or spice fortification and the sensory properties of products. In this study, the fortification of durum wheat flour by up to 10% of *Opuntia* cladodes resulted in a good compromise to obtain good rheological characteristics of loaves and breads with an increase in TPC and decreased IC50. The obtained results represent the key step for developing this product, which could potentially provide antioxidant properties and health benefits to the consumers. Of course, further studies are necessary to assess the effect on human health benefits and the bread shelf – life. Considering the lands involved in the cultivation of prickly pear in Sicily and cladode production, due to the common agricultural practice, the addition of 10% of dried cladodes could be a strategy to increase bioactive compounds with potential health benefits in durum wheat bread and reduce/valorize crop disposals.

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