Quality Characteristics of Wholemeal Flour and Bread from Durum Wheat (*Triticum turgidum* L subsp. *durum* Desf.) after Field Treatment with Plant Water Extracts

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Abstract: The use of selected plant water extracts to control pests and weeds is gaining growing attention in organic and sustainable agriculture, but the effects that such extracts may exert on the quality aspects of durum wheat are still unexplored. In 2014, 5 plant water extracts *(Artemisia arborescens*, *Euphorbia characias, Rhus coriaria, Thymus vulgaris, Lantana camara*) were prepared and distributed on durum wheat cv Valbelice to evaluate their potential herbicidal effects. After crop harvesting, the major physicochemical and technological parameters of wholemeal flours obtained from each treatment were measured and compared with those from chemical weeding and untreated controls. A baking test was also performed to evaluate the breadmaking quality. In wholemeal flours obtained after the treatment with plant extracts protein and dry gluten content were higher than in control and chemical weeding. Wholemeal flours obtained after chemical weeding reached the highest Mixograph parameters, and that from durum wheat treated with *R. coriaria* extract demonstrated a very high α-amylase activity.

We concluded that the treatments with plant water extracts may influence many quality traits of durum wheat. This occurrence must be taken into account in overall decisions concerning the use of plant extracts in pest and weed management practice.

Keywords: bread, cereal quality, Maillard reaction, phytochemicals, wholemeal flour

Practical Application: The results of this work may be useful for a proper use of natural products for weed management, in the frame of sustainable and organic cropping techniques.

Introduction

Growing knowledge on the environmental and health risks linked to the widespread use of synthetically derived products for pests and weed management has caused a general change in the behavior of many farmers, who are increasingly utilizing sustainable synthetic products or making environmentally friendly technical choices (Vyvyan 2002; Özçatalbaş 2014). In fact, in many countries (for example, in the European Union, where this topic is ruled by the EU Reg. 834/2007), the only allowed products for organic production are natural inorganic or plant-derived materials (Isman 2006). As a general rule, these products have a shorter shelf-life in comparison with the analogous synthetic products, as they show poor chemical stability to air, light, moisture, and high temperatures (Kühne 2008; Flamini 2012). Such a trait is typical of natural products. Furthermore, although there is no certainty that a "natural" product is also a "safe" product, they are not expected to pose a hazard to non-target organisms (including humans) or to the environment in most cases (Duke and others 2010). Hence, the active compounds that are contained inside some plants seem to ensure compliance with the safety and low-persistence requirements that are so strongly advocated for. Currently, intense experimental activity is performed all over the world to identify plant products that meet strict safety requirements and have high efficacy (Isman 2006; Isman and Grieneisen 2014). Of course, plant extracts have a complex chemical nature, as they are composed of many different compounds whose presence in the final item may vary greatly according to the extraction method, the conditions of the starting plant material and so on (Verpoorte 1998). Obviously, to ascertain which specific compound is actually responsible for a given biological activity has great practical interest, and this approach has led to interesting results in a number of cases (Rates 2001; Copping and Duke 2007; Li and Vederas 2009; Duke and others 2010). However, in many cases, the search for a unique active product has led to the frustrating result that the effects shown for the whole plant extract were significantly different from its individual components. The occurrence of synergistic phenomena was advocated to justify this outcome. As such, a number of natural extracts are produced and used "as they are," by considering them as unique products mostly identified with their botanical source, rather than as the complex mixture that they actually are (Verpoorte 1998; Rates 2001; Copping and Duke 2007; Li and Vederas 2009; Appendino and Pollastro 2010). Of course, further improvement in the technical procedures for plant extraction, purification and analysis will allow some remediation for this problem.

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Durum wheat (*Triticum turgidum* L subsp. *durum* Desf.) is used all over the word mainly to produce pasta (Spina and others 2015). This crop bears a major interest in all Mediterranean countries, where it is cultivated for making high-quality products including, other than pasta, also couscous, bulghur and bread (Rharrabti and others 2003; Giannone and others 2016). In many cultivation areas of Italy, including Southern Italy, Sicily and Sardinia, the use of durum wheat in breadmaking is an old practice as well. In these areas, durum wheat is traditionally used to obtain a specialty flat bread, endowed with a characteristic taste and smell, a yellowish color, a fine and uniform crumb structure, and a quite prolonged shelf-life (Boyacioğlu and D'Appolonia 1994; Liu and others 1996; Nachit 1998; Pasqualone 2012). Such a specialty bread is greatly appreciated by consumers, and, also because of the preeminent role of cereals intake in Mediterranean diet, an increasing share of Italian durum wheat production is addressed to breadmaking (Dexter and Marchylo 2001; Palumbo and others 2002). Actually, in Italy more than 200 traditional wheat breads have been classified (INSOR 2000); only in Sicily, more than 50 traditional durum wheat breads have been listed in the "Atlante del pane di Sicilia" ("Atlas of Sicilian Bread"; CRGPB 2001). Being obtained by means of different production technologies, their features vary according to local habits (Palumbo and others 2002; Spina and others 2003). As far, about 50% of durum wheat harvested in Sicily is used in breadmaking (Giannone and others 2010 and 2015; Spina and others 2015).

It is generally assumed that many quality properties of wheat flour also depend on environmental factors. Nevertheless, most studies on this topic concentrate on a few environmental issues, namely soil fertility, rainfall, and temperature during the growing season and at harvest (Rharrabti and others 2003; Edwards 2010). Scarce literature is available about the effects of weed management on wheat seed quality, and to our knowledge, no work has been dedicated, as far, to the study of natural extracts on durum wheat seed and flour quality.

With the aim of searching for new products to be used in organic and environmentally friendly cropping techniques, we evaluated the suitability of using several water extracts obtained from 5 different donor species on arable crops, with a special interest in evaluating their potential herbicidal effects.

This work provides the results of an experimental study carried out in 2014 aimed to evaluate the effects exerted by these extracts on the major quality traits, for bread-making purposes, of durum wheat cv Valbelice.

Materials and Methods

Preparation and use of plant water extracts

The extracts that were used for the trials were obtained from 5 donor plants (Table 1) that were selected based on their biological activity and the availability of plant biomass. Plant material included both leaves and inflorescences picked from wild *(A. arborescens, E. characias, R. coriaria)* or cultivated plants (*T. vulgaris, L. camara*) growing near Ciminna (Palermo, Sicily) and Sparacia (Cammarata, Agrigento, Sicily). The extraction procedure was the same that had been successfully used in previous works (Militello and Carrubba 2016). Accordingly, all plant material was first air dried at room temperature for at least 5 d, with the exception of *Thymus*, which was already dried. All extracts were obtained by grinding tissues with distilled water at a ratio of 1:10 (w/v), followed by constant stirring with a speed rotation of 70 rounds/min for at least 10 h. At the end of the extraction, the mass was filtered with filter paper (Whatman n. 4), and the obtained extracts were refrigerated at 4 °C until use.

The experimental plots were arranged in the field according to a randomized block design with 2 repetitions. Each treatment, including the 5 water extracts and the control with only water, was tested on experimental plots sized 1×1 m located on 2 experimental areas (repetitions) 1 m apart. To avoid drift phenomena between the plots, each plot was separated from the other by a strip area (0.5×1 m) which was excluded by all measurements. Two treatments were performed for each plot during crop post-emergence by applying 4 L of each previously prepared extract. The first treatment was applied after 25 DAS (days after sowing), when the durum wheat plants were at the stage of 2 to 3 leaves unfolded (Zadoks' scale: Z13; Zadoks and others 1974), whereas the second was applied after 88 DAS, as the crop was reaching the stem elongation stage (Zadoks' scale: Z31).

Field management

The field trial was carried out in the experimental farm "Sparacia" (Cammarata, AG, Sicily; 37°38'N to 13°46'E; 415 m a.s.l.) of the Department of Agricultural and Forest Sciences of the University of Palermo. The trial environment is typical of the inner hilly Sicily (meso-thermo-Mediterranean climate), with a long and dry summer period and a colder winter, with very limited snow days and irregular rainfall. The experiment was performed on a plot cultivated with durum wheat according to the cropping techniques ordinarily applied in the cereal areas of the site. The chosen durum wheat variety was Valbelice (0111 x BC5). This cultivar had been obtained in 1992 by the Dept. of Agricultural and Forest Sciences of the Univ. of Palermo. It is a early, highly yielding and tall genotype, which is able to compete with weeds and is strongly suitable to organic farming and low-input cropping systems (Boggini and others 2001; SINAB 2016). Cv Valbelice has already been compared to other durum wheat cultivars, and many aspects related to its yield performance, and to the chemical, rheological and technological traits of its semolina, have been studied (Spina and others 2001a, 2001b; Palumbo and others 2002).

The preceding crop was Berseem clover *(Trifolium alexandrinum* L.). The soil was prepared by means of a summer work (25-30 cm deep) and 2 following shallow works. Sowing was made mechanically on December 19, 2013 by spreading at a soil depth of approximately 5 cm, on rows 30 cm apart, an amount of seed as to obtain a seeding density of 350 viable seeds per m^2 (approximately 200 kg/ha). At sowing time, 1.5 t/ha of diammonium phosphate (18/46) was distributed for fertilization. Next, 1.1 t/ha of urea (46) was spread when the crop had reached the phase of full tillering (Zadoks' scale: Z 22). Chemical weeding was performed in the remaining part of the field by supplying a mixture of mesosulfuron – methyl 3% + iodosulfuron-methyl-sodium 0.6% + Mefenpirdiethyl 9% (ATLANTIS®, Bayer AG, Leverkusen, Germany), a postemergence weeding against all graminaceous weeds and some important dicots.

To avoid any effect due to the additional presence of water in the tested extracts, an additional set of control plots was arranged where 4 L/m^2 of water was spread contemporary to the distribution of the extracts in the other plots.

Each plot was hand-harvested separately on June 22, 2014, with the exclusion of the above mentioned border areas.

Table 1–Basic information about the donor plants used for the preparation of water extracts.

Species/Family	Origin	Used plant part	Reported activity	Major components	References
Artemisia arborescens (Vaill.) L. (*Asteraceae*)	Mediterranean	Flowering tops	Bactericidal Fungicidal Insecticidal Herbicidal/germination inhibitor	Essential oil Phenolic compounds Lignans and flavonoids	Militello and Carrubba 2016
Euphorbia characias L. (*Euphorbiaceae*)	Mediterranean	Leaves	Fungicidal Illegal fishing	Latex Polyphenols (flavonoids) Proteins Lectins	Barbieri and others 1983; Savo and others 2013
Lantana camara L. (*Verbenaceae*)	Southern America	Leaves	Fungicidal Repellent Insecticidal Nematicidal Herbicidal (aquatic weeds and various plant species)	Essential oil Phenolic compounds Aromatic alkaloids	Ghisalberti 2000; Kong and others 2006
Rhus coriaria L. (*Anacardiaceae*)	Mediterranean	Leaves	Fungicidal	Phenolic compounds	Rayne and Mazza 2007
Thymus vulgaris L. (*Lamiaceae*)	Mediterranean	Flowering tops	Bactericidal Fungicidal Insecticidal	Essential oil Phenolic compounds	Rota and others 2008

Biochemical characterization of kernels

In order to check the varietal behavior and the level of genetic purity of the genotype used for the experiment, the electrophoresis of storage proteins composition of kernels was carried out. The analysis was performed on 10 kernels which had been sorted out from the untreated control. SDS-PAGE electrophoretic patterns for High Molecular Weight Glutenin Subunits (HMW-GS) were determined according to the method described by Payne and others (1980), and those for Low Molecular Weight Glutenin Subunits (LMW-GS) were determined according to Payne and others (1984).

Grain milling and wholemeal flour quality

Samples of seeds obtained from both treated plots and controls were analyzed for the main quality features of seeds and flours. Protein content (% dry matter) was determined by means of Infratec 1241 Grain Analyzer (Foss Tecator, Höganas, Sweden) by near infrared transmittance using a calibration (range 8.3 to 15.3) based on the Kjeldahl nitrogen method ISO 20483 (2013). Calibration was validated in accordance with ISO 12099 method (2010) using different sets of test samples of durum wheat grain with a linear correlation coefficient of $r = 0.99$.

The grain obtained from each treatment was milled to obtain wholemeal flour by an experimental mill, Cyclotec type 120 (Falling Number, Huddinge, Sweden), with a sieve of 0.5 mm.

Gluten quantity (dry gluten content) and quality (gluten index) were measured using a Glutomatic 2200 apparatus, a Centrifuge 2015 and a Glutork 2020 (Perten Instruments AB, Huddinge, Sweden) according to the ICC Standard No. 158 (1995). Centrifugation was carried out to force the wet gluten through a specially constructed sieve under standardized conditions. The percentage of wet gluten remaining on the sieve after centrifugation is defined as the Gluten Index, whereas the wet gluten that passes through the grid is termed "B fraction," and when highly represented, it indicates a poor technological gluten quality.

The α-amylase activity was determined using the Falling Number 1500 apparatus (Perten Instruments AB, Huddinge, Sweden), following the ISO 3093 method (2009). The sedimentation test in sodium dodecyl sulfate (SDS) [Sigma-Aldrich, Milan, Italy] is a preliminary test that is useful to estimate gluten quality. This test was performed according to the method of Dick and Quick (1983).

Colorimetric measurements on wholemeal flours were performed following the method described by Sgrulletta and others (1999), and they were expressed using the standard CIE 1976 $L^*a^*b^*$ system (Robertson 1977). Accordingly, the measured indexes were: L^* (lightness in the range between black = 0 and white = 100), a^* (the difference between red and green tones), and b^* (direct measurement of the yellow color). Those indexes were determined by means of a CR 200 Minolta Colorimeter Chroma (Minolta, Osaka, Japan), using illuminant D_{65}.

Mixograph analyses allow the analysis of small quantities of flour for measurement of dough gluten strength. Flour water absorption evaluated by a mixograph often serves as a measure of bake absorption in bread baking tests. A mixograph curve was obtained using the National Mfg. Co. (Lincoln, Nebr., U.S.A.) standard, according to AACC method 54–40.02 (1999).

Baking test

The breadmaking test was performed on the flours obtained from each treatment according to the AACC 10-10 procedure (1979), as modified for durum wheat by Boggini and Pogna (1989), to obtain 2 loaves using 100 g of flour each. Hence, a total of 16 loaves were obtained, onto which the following traits were individually measured: volume, height and weight, moisture, loaf firmness, crumb porosity, internal structure, crumb and crust color. The volume was determined according to the rapeseed displacement in a loaf volume meter; the loaf height was measured by using a digital caliper (Digi-Max™, Scienceware®, N.J., U.S.A.). The crumb porosity was estimated through the Mohs scale and the internal structure was visually estimated. The loaf firmness was measured using a texture analyzer (Zwick Z 0.5 Roëll, Ulm, Germany) equipped with an aluminum 8-mm diameter cylindrical probe. The resulting peak force was measured in Newton (N). The CIE $L^*a^*b^*$ color parameters were measured for the crumbs in the transversely cut bread and on the crust surface, averaging

Table 2–Storage proteins composition of the durum wheat variety used for the trial.

	Glutenin composition			Gliadin composition
	HMW-GS		LMW-GS	
Variety	***Glu-A1***	***Glu-B1***	***Glu-B3***	***Gli-B1***
Valbelice	*Null*	20	Type-2	γ-45

10 distinct points in each case, using a Chroma Meter (CR-200, Minolta) with illuminant D_{65}. The moisture content was determined in triplicate by gravimetric analysis. The bread samples were ground in a home grinder DJ2002, © Moulinex, France, and then portions of the ground bread samples were placed in an oven at 105 °C until constant weight.

Statistical treatment of the data

All data were submitted to statistical analysis according to the planned experimental layout using the SAS 9.0 software package (proc. GLM; SAS Institute Inc., Cary, N.C., U.S.A., 2002). A preliminary ANOVA was carried out for all tested variables, and when the *F*-test indicated statistical significance at the $P \leq 0.05$ level, Tukey's HSD test was used to evaluate the differences between treatments. To detect any significant differences among the major experimental groups (all natural extracts; chemical weeding; and the 2 controls), an orthogonal contrast (OC) test was performed (Steel and Torrie 1980). The OC technique allows number of independent groups comparisons equal to the number of degrees of freedom inside the examined factor to be performed (in our case, 8 treatments). Because 7 is the maximum number of allowed comparisons, 3 were chosen as the most meaningful, namely, plant extracts compared with controls, plant extracts compared with chemical and chemical compared with controls.

Results and Discussion

Storage protein composition of kernels

All analyzed kernels showed a uniform protein composition, that was typical of cv Valbelice (Table 2), in that confirming the genetic purity of the used variety. The tested samples showed "*null*" type at HMW-GS at *Glu-A1* locus, a composition that is usual in the Italian durum wheat genotypes (Boggini and Pogna 1989). As for the HMW-GS at *Glu-B1* locus, the samples showed the subunit "20." This feature, although recognised in cv Valbelice, is seldom represented in the other Italian durum wheat genotypes, and it is often associated with a weak gluten and a low bread volume (Boggini and Pogna 1989). As for the LMW-GS at *Glu-B3* locus, the tested kernels showed the LMW-2, that is associated with γ-gliadin "45" (Payne and others 1984; Pogna and others 1990).

Durum wheat wholemeal flour quality parameters

As shown in Table 3, the highest protein content (>14%) was reached after the treatment with extracts of *R. coriaria*, whereas the lowest (11.30%) was found in the chemically weeded crop. Noticeably, this value was statistically lower than that of the controls group (12.04%) and the average of all 5 treatments with plant extracts (12.97%).

Total protein content and dry gluten content are the principal factors used to characterize durum wheat. In our study, the ratio dry gluten/protein content was 68.67, meaning that dry gluten

Table 3–Effect of treatment with plant extracts on some qualitative traits of grain and wholemeal flour in durum wheat (cv Valbelice), in comparison with 2 untreated controls and 1 chemical weeding, and calculated *F* values for the treatments and for the orthogonal contrasts.

	Protein content (%)	Dry gluten (%)	Gluten Index	Falling Number (s)	SDS (mm)	Mixing time (s)	Peak dough height (M.U.)	Overall score (1–8)*
Treatments with plant extracts (p.e.)								
A. arborescens	13.58 ± 1.93 b	9.45 ± 1.85 ab	10.93 ± 0.80 a	356.8 ± 7.8 cd	27.8 ± 3.2 e	117.0 ± 4.2	9.5 ± 0.4	3.0
E. characias	12.20 ± 1.04 d	8.30 ± 0.93 cd	11.23 ± 0.46 a	377.3 ± 8.2 ab	29.3 ± 5.5 d	129.0 ± 46.7	9.2 ± 0.2	3.5
T. vulgaris	11.85 ± 0.59 e	7.85 ± 0.58 d	7.91 ± 1.00 bc	370.5 ± 5.7 bc	31.5 ± 0.6 c	117.0 ± 4.2	7.8 ± 0.7	2.5
L. camara	12.98 ± 0.25 c	9.05 ± 0.29 b	9.49 ± 1.64 ab	389.0 ± 5.0 a	32.0 ± 1.2 c	132.0 ± 8.5	9.3 ± 0.9	4.0
R. coriaria	14.23 ± 1.01 a	9.93 ± 0.78 a	11.18 ± 1.94 a	65.5 ± 3.0 e	33.5 ± 2.4 ab	141.0 ± 12.7	9.9 ± 0.2	4.5
All p.e. treatments	***12.97***	***8.92***	***10.15***	***311.8***	***30.8***	***127.2***	***9.1***	***3.5***
Controls								
Water (control)	11.98 ± 0.49 de	8.28 ± 0.50 cd	7.68 ± 1.07 bc	361.3 ± 5.4 cd	31.5 ± 2.4 c	138.0 ± 8.5	7.5 ± 0.7	3.0
Untreated (C–)	12.10 ± 0.18 de	8.35 ± 0.17 c	6.44 ± 1.00 c	388.0 ± 7.0 a	32.5 ± 0.6 bc	114.0 ± 33.9	8.1 ± 0.6	2.5
All controls	***12.04***	***8.32***	***7.06***	***374.7***	***32.0***	***126.0***	***7.8***	***2.8***
Chemical (C+)	**11.30 ± 0.37 f**	**7.35 ± 0.42 e**	**8.75 ± 0.50 b**	**355.5 ± 32.4 d**	**34.0 ± 0.8 a**	**177.0 ± 21.2**	**9.6 ± 0.4**	**5.5**
ANOVA (treatments)	$F_{(7,16)}$: 285.12***	$F_{(7,16)}$: 65.68***	$F_{(7,16)}$: 21.72***	$F_{(7,16)}$: 1297.52***	$F_{(7,16)}$: 46.67***	$F_{(7,7)}$: 1.99 n.s.	$F_{(7,7)}$: 1.74 n.s.	
Orthogonal contrasts (O.C.)	$F_{(1,16)}$	$F_{(1,16)}$	$F_{(1,16)}$	$F_{(1,16)}$	$F_{(1,16)}$	$F_{(1,7)}$	$F_{(1,7)}$	
P.e. treatments compared with controls	355.00***	43.03***	90.27***	617.39***	21.94***	<1 n.s.	5.51 n.s.	
P.e. treatments compared with chemical	708.60***	193.60***	10.83**	174.25***	91.02***	9.87*	<1 n.s.	
Chemical compared with controls	119.64***	63.42***	12.58**	26.70***	28.44***	8.28*	4.51 n.s.	

*Range of possible values: 1 to 8; data not statistically analyzed.

Data are expressed as means ± standard deviation. Inside treatments, means followed by the same letter (including partials) are not significantly different at P ≤ 0.05 (HSD Tukey's test). Prior to analysis, percent data have been transformed into √(%).

represented about 70% of total protein content. Hence, the 2 parameters showed rather the same trend. The statistically highest and lowest values of dry gluten content were obtained after treatment with *R. coriaria* extracts and in chemically weeded plots, respectively. Gluten protein fraction, that corresponds to the prolamin storage proteins, has a number of about 100 individual protein components (Shewry 2009), that have been estimated to account for about 80% of total grain proteins in European bread wheats (Seilmeier and others 1991).

As evidenced by the OC analysis, the group of plant extract treatments averaged a dry gluten percentage (8.92%) that was higher than those of controls and chemical treatment.

Determination of the Gluten Index confirmed the presence of a rather weak gluten in flours of cv Valbelice, as already assessed by other works (Spina and others 2001a, 2001b; Palumbo and others 2002; Rizzo and others 2011). After centrifugation, almost all wet gluten passed through the grid (B fraction), indicating very low technological gluten characteristics. The flours obtained from samples treated with water extracts exhibited values of Gluten Index ranging from 7.91 to 11.23. As the OC analysis was able to show, the group of p.e. treatments (on average, 10.15) had an overall better performance than control samples (7.06) and chemical weeding (8.75).

The Falling Number (FN) in almost all of the wholemeal flour samples showed a weak α-amylase activity, and all treatments gave values higher than 350 s. A surprising exception was *R. coriaria*, which showed a very low value (65.5 s) corresponding to a very high enzymatic activity. This last metric is unusual in Sicilian durum wheat, which mostly shows values from 550 to 750 s due to the frequent drought conditions that occur during grain ripening (Rizzo and others 2011). According to the literature, an FN $>$ 300 indicates low α-amylase activity, whereas values of approximately 200 to 250 are indicative of normal enzyme activity (Caglar and others 2011; Bulut and others 2013). Although variations in FN values were found as a consequence of some aspects of crop management such as fertilizer type, weeding management (Bulut and others 2013) or seeding rates (Caglar and others 2011), no difference was found in relation to the application of different herbicides (Manthey and others 2004). A reduction in the FN is common in ripe wheat grain in which preharvest sprouting occurs (Humphreys and Noll 2002), but according to recent studies (Mares and Mrva 2014), a significant production of late maturity α-amylase (LMA) is frequently detected independent of sprouting in commercially grown wheat. Hence, the production of LMA in seeds is a genetic defect that is triggered by particular environmental conditions (for example, thermal shocks) that may occur during the grain filling period (Mares and Mrva 2014).

A reduction in α-amylase activity due to *R. coriaria* fruit extracts was reported in a study about the activity of this extract against insect pests of stored foods, where the hypothesis was that plant defense compounds act on insect gut enzymes by inhibiting insect α-amylases (Mehrabadi and others 2011).

The SDS sedimentation test showed a trend that was inversely associated with that of protein content, consistent with the findings of other authors (Rharrabti and others 2003). All treatments with water extracts and the controls showed the weakest gluten, with SDS values ranging from 27.8 to 33.5 mm for *A. arborescens* and *R. coriaria*, respectively. The chemical treatment produced the highest statistically relevant value (34.0 mm).

ANOVA applied to the results of the mixograph analyses did not show any significant difference among the treatments for mixing time and peak dough height. The OC analysis otherwise highlighted that in flours from chemically weeded crop, a higher mixing time (177 s) compared to the other treatments occurred. This value was associated to a good peak dough height value, 9.6 M.U. (Mixograph Units), and consequently, the highest score was given (5.5). *A. arborescens* and *T. vulgaris* and the flours obtained from the untreated plots recorded the lowest mixing time ($<$120 s), which correlated with low classification values (3 and 2.5). For mixing time only, the OC analysis confirmed this difference between chemical treatments and the other 2 group of samples (p.e. treatments and controls). The peak dough height did not exhibited any significant difference at OC analysis, and ranged from 7.8 M.U. for controls and 9.1 and 9.6 M.U. for p.e. and chemical treatment, respectively. Conclusively, the overall score for the wholemeal flours under observation were rather low, ranging from 2.5 (treatment with *T. vulgaris*) to 5.5 (chemical weeding).

ANOVA and OC analysis indicated that the colorimetric parameters of wholemeal flour (Table 4) showed significant differences both among individual treatments and between treatment groups. The highest values of lightness (L*) were recorded for *R. coriaria* treatment (89.34 L*) and for the untreated control (86.10 L*), while the lowest were found in *T. vulgaris*, *L. camara* (values $<$ 80 L*), water and *A. arborescens* treatments (values slightly $>$ 80 L*). On average, the lowest group value was reached by the chemical treatment (81.81 L*), whereas the highest was reached by the control group (83.39 L*). The a* index (brown hue) showed rather high values for all samples obtained after treatment with plant water extracts, with the exception of *T. vulgaris*. In fact, the a* value obtained on this treatment (0.46 a*) was not significantly different from that obtained after chemical weeding (0.41 a*). Control group showed a brown value intermediate to the previous 2 groups (0.66 a*). The b* values (yellow hue) showed the highest outcomes ($>$16 b*) in *A. arborescens* and *L. camara*, while the lowest value was obtained in *T. vulgaris* (14.65 b*). The OC analysis confirmed the statistical homogeneity ($F < 1$) between the treatments with water extracts and that with chemicals. The b* value averaged from the control group (15.28 b*) was statistically lower than those of the other treatment groups.

Breadmaking quality

Experimental baking is performed to assess breadmaking potential of a dough, and the bread volume may be reasonably considered the first and most important trait to evaluate the breadmaking quality of a flour. As reported in table 5, the chemically weeded and the group of untreated samples reported, on average, the highest loaf volume values (322.50 and 303.75 cm^3, respectively). In contrast, the group of p.e. treatments averaged the lowest result (299.75 cm^3). The smallest bread (270.00 cm^3) was obtained from flours treated with *R. coriaria*, due to a very high α-amylase activity, that allowed a quick conversion of starch in fermentable sugars, which were then immediately available for yeasts. The bread height, a parameter that is strictly correlated with the loaf volume, in all cases showed a trend similar to the preceding one. Hence, the highest value was detected in the chemical control (51.50 mm), whose bread was statistically greater than in the remaining 2 groups, that is the p.e. treatments (on average, 45.20 mm), and all controls (on average, 44.50 mm). A comparatively higher bread (48.50 mm) was also obtained from the untreated control (C-), although this value could not differentiate from those obtained in the majority of bread samples obtained from the p.e. treatments. No statistically relevant difference was measured among treatments in

Table 4–Effect of treatment with plant extracts on color parameters of wholemeal flour in durum wheat (cv Valbelice), in comparison with 2 untreated controls and 1 chemical weeding, and calculated *F* values for the treatments and for the orthogonal contrasts.

	L*	a*	b*
Treatments with plant extracts (p.e.)			
A. arborescens	80.89 ± 0.81 e	1.13 ± 0.13 a	16.16 ± 0.52 a
E. characias	82.08 ± 0.15 c	0.74 ± 0.31 c	15.65 ± 1.43 c
T. vulgaris	79.55 ± 4.20 h	0.46 ± 0.26 e	14.65 ± 0.42 f
L. camara	79.89 ± 1.54 g	1.03 ± 0.40 b	16.26 ± 0.23 a
R. coriaria	89.34 ± 9.05 a	0.76 ± 0.72 c	15.80 ± 0.07 b
All p.e. treatments	***82.35***	***0.82***	***15.70***
Controls			
Water (control)	80.67 ± 0.38 f	0.65 ± 0.39 d	15.07 ± 0.21 e
Untreated (C-)	86.10 ± 0.59 b	0.67 ± 0.05 d	15.48 ± 0.51 d
All controls	***83.39***	***0.66***	***15.28***
Chemical (C+)	***81.81*** ± 2.19 d	***0.41*** ± 0.16 e	***15.71*** ± 0.14 bc
ANOVA (treatments)	$F_{(7,16)}$: **11972.0*** **	$F_{(7,16)}$: **404.98*** **	$F_{(7,16)}$: **527.96*** **
Orthogonal contrasts (O.C.)	$F_{(1,16)}$	$F_{(1,16)}$	$F_{(1,16)}$
P.e. treatments *vs* controls	1545.71***	234.95***	488.61***
P.e. treatments *vs* chemical	250.74***	936.78***	<1 n.s.
Chemical *vs* controls	1682.69***	285.76***	230.67***

Data are expressed as means ± standard deviation. Inside treatments, means followed by the same letter (including partials) are not significantly different at P≤ 0.05 (HSD Tukey's test). Prior to analysis, percent data have been transformed into √(%).

Table 5–Effect of treatment with plant extracts, in comparison with 2 untreated controls and 1 chemical weeding, on physical properties of bread obtained at baking test, and calculated *F* values for the treatments and for the orthogonal contrasts.

	Volume (cm^3)	Height (mm)	Weight (g)	Moisture (%)	Loaf firmness (N)	Crumb porosity (1:8)[a]*	Internal structure (1: 2)[b]*
Treatments with plant extracts (p.e.)							
A. arborescens	293.75 ± 5.30 a–c	43.00 ± 1.41 b–d	145.18 ± 0.06	33.1 ± 0.2	9.77 ± 0.75 a–c	8	1
E. characias	307.50 ± 3.54 ab	47.50 ± 0.71 a–c	145.71 ± 0.74	33.0 ± 0.8	6.97 ± 1.10 cd	6	1
T. vulgaris	312.50 ± 3.54 ab	47.00 ± 2.83 a–c	147.13 ± 0.35	33.9 ± 0.1	5.91 ± 0.73 d	7	2
L. camara	315.00 ± 14.14 ab	46.50 ± 2.12 a–d	143.37 ± 4.93	33.8 ± 0.1	8.19 ± 0.17 b–d	6	1
R. coriaria	270.00 ± 7.07 c	42.00 ± 2.83 cd	144.32 ± 0.39	33.9 ± 1.1	12.75 ± 1.86 a	8	1
All p.e. treatments	***299.75***	***45.20***	***145.14***	***33.6***	***8.71***	***7***	***1.2***
Controls							
Water (control)	285.00 ± 14.14 bc	40.50 ± 0.71 d	142.39 ± 0.34	34.2 ± 1.2	10.88 ± 1.04 ab	7	2
Untreated (C-)	322.50 ± 3.54 a	48.50 ± 0.71 ab	141.74 ± 1.48	33.6 ± 0.5	10.01 ± 0.60 a–c	6	1
All controls	***303.75***	***44.50***	***142.07***	***33.9***	***10.45***	***6.5***	***1.5***
Chemical (C+)	***322.50*** ± 3.54 a	***51.50*** ± 0.71 a	***144.15*** ± 0.52	***32.5*** ± 0.2	***9.12*** ± 0.30 b–d	***5***	***1***
ANOVA (treatments)	$F_{(7,7)}$: **9.87** **	$F_{(7,7)}$: **11.21** **	$F_{(7,7)}$: **1.86** n.s.	$F_{(7,7)}$: **1.25** n.s.	$F_{(7,7)}$: **14.87** **		
Orthogonal contrasts (O.C.)		$F_{(1,7)}$	$F_{(1,7)}$	$F_{(1,7)}$	$F_{(1,7)}$	$F_{(1,7)}$	
P.e. treatments *vs* controls	<1 n.s.	<1 n.s.	8.05*	<1 n.s.	13.41 **		
P.e. treatments *vs* chemical	11.86*	27.34**	<1 n.s.	3.61 n.s.	<1 n.s.		
Chemical *vs* controls	6.45*	27.00**	1.72 n.s.	4.98 n.s.	3.70 n.s.		

[a]Range of possible values: 1 (most porous) to 8 (least porous)
[b]Possible values: 1 (regular); 2 (irregular).
*data not statistically analyzed.
Data are expressed as means ± standard deviation. Inside treatments, means followed by the same letter (including partials) are not significantly different at P≤ 0.05 (HSD Tukey's test). Prior to analysis, percent data have been transformed into √(%).

loaf weight, whose mean value across all samples averaged 144.25 g with very small variations between the lowest (141.74 g; C-) and the highest (147.13 g; *T. vulgaris*) values. The OC analysis, however, showed a significantly heavier loaf for the group of treatments with plant extracts, in comparison to the group of controls, highlighting different bread yields.

The bread moisture did not show significant differences, and all treatments ranged between 32.5% and 34.2% of C+ and water control, respectively.

The evaluation of loaf firmness highlighted, on average, a medium resistance in bread samples obtained from wheat treated with plant extracts and chemical herbicide (mean values of 8.71 and 9.12 N, respectively). An exception was the bread from the *R. coriaria* sample, whose resistance was found to reach a noticeable high value (nearly 13 N), as a consequence of its low volume and height. The firmness increased in the bread obtained from the controls (mean value of 10.45 N), particularly in the bread derived from the water control.

With regards to crumb characteristics, the porosity of the chemically treated group demonstrated the best crumb development (score = 5), the untreated control and treatment with *E. characias* and *L. camara* reported a good porosity (score = 6), while the *R. coriaria* and *A. arborescens* showed a very reduced crumb development (score = 8).

Almost all treatments showed a regular development of the internal structure (score = 1), with the exception of the water control and the treatment with *T. vulgaris,* which expressed an irregular crumb structure (score = 2).

Table 6–Effect of treatment with plant extracts, in comparison with 2 untreated controls and 1 chemical weeding, on color parameters of bread obtained at baking test, and calculated *F* values for the treatments and for the orthogonal contrasts.

	Crumb			Crust		
	L*	**a***	**b***	**L***	**a***	**b***
Treatments with plant extracts (p.e.)						
A. arborescens	58.23 ± 0.50 a–c	4.11 ± 0.21 ab	24.81 ± 0.16	36.18 ± 2.33 b	9.89 ± 0.70 b	14.92 ± 2.54
E. characias	57.05 ± 0.03 bc	4.11 ± 0.06 ab	23.56 ± 0.03	37.10 ± 1.29 b	9.74 ± 0.06 b	15.14 ± 0.66
T. vulgaris	59.92 ± 1.00 ab	3.73 ± 0.27 bc	23.62 ± 0.35	43.23 ± 0.05 a	10.08 ± 0.50 b	18.20 ± 0.40
L. camara	58.59 ± 0.49 ab	4.07 ± 0.04 ab	23.72 ± 0.08	35.10 ± 1.63 b	9.69 ± 0.06 b	14.45 ± 1.14
R. coriaria	58.33 ± 1.10 a–c	4.54 ± 0.18 a	24.29 ± 0.26	35.96 ± 1.49 b	10.21 ± 0.09 b	14.50 ± 0.34
All p.e. treatments	***58.42***	***4.11***	***24.00***	***37.51***	***9.92***	***15.44***
Controls						
Water (control)	54.68 ± 0.73 c	4.18 ± 0.04 ab	23.73 ± 0.17	36.94 ± 0.89 b	9.46 ± 0.66 b	15.37 ± 0.33
Untreated (C-)	61.20 ± 1.19 a	3.55 ± 0.13 bc	24.31 ± 0.01	37.22 ± 1.39 b	12.39 ± 0.30 a	17.36 ± 0.38
All controls	***57.94***	***3.86***	***24.02***	***37.08***	***10.92***	***16.36***
Chemical (C+)	***60.73*** ± 1.09 a	***3.21*** ± 0.21 c	***24.62*** ± 1.06	***37.42*** ± 0.02 ab	***11.51*** ± 0.67 ab	***18.25*** ± 0.40
ANOVA (treatments)	$F_{(7,7)}$: **11.14****	$F_{(7,7)}$: **11.27****	$F_{(7,7)}$: **2.90** n.s.	$F_{(7,7)}$: **5.86** *	$F_{(7,7)}$: **8.49** **	$F_{(7,7)}$: **3.78** n.s.
Orthogonal contrasts (O.C.)	$F_{(1,7)}$	$F_{(1,7)}$	$F_{(1,7)}$	$F_{(1,7)}$	$F_{(1,7)}$	$F_{(1,7)}$
P.e. treatments *vs* controls	<1 n.s.	5.65*	<1 n.s.	<1 n.s.	11.60 *	2.00 n.s.
P.e. treatments *vs* chemical	11.15*	43.84***	3.95 n.s.	<1 n.s.	16.87 **	10.82 *
Chemical *vs* controls	13.05**	18.47**	2.97 n.s.	<1 n.s.	<1 n.s.	3.90 n.s.

Data are expressed as means ± standard deviation.
Inside treatments, means followed by the same letter (including partials) are not significantly different at P≤ 0.05 (HSD Tukey's test). Prior to analysis, percent data have been transformed into √(%).

Concerning the color of the crumb and crust (Table 6), all parameters except for the yellow index (b*) showed at ANOVA the occurrence of statistically significant differences among the treatments. As is typical in wholemeal bread, the crumb brightness index (L*) reached rather low values. There was a tendency for the bread obtained using flour from chemically weeded (C+) and untreated (C-) plots to have a brighter crumb, while the flours obtained from the water control produced bread with a lower crumb brightness (54.68 L*). The crumb brown index (a*), which in wholemeal bread is more important than in semolina bread, showed the highest value (4.54 a*) after treatment with *R. coriaria* extract, which therefore produced bread with the darkest crumb. The lighter crumb (3.21 a*) was otherwise obtained after chemical weed control. The crumb yellow index (b*) in wholemeal bread bears a lower importance than in semolina bread; no significant difference was found among the different treatments for this variable, which ranged between 23.56 b* for *E. characias* extract and 24.81 b* for *A. arborescens* extract. Compared to the colorimetric values measured on the wholemeal flour, the crumb showed a decreased brightness and increased values of both red and yellow indexes. This is, firstly, due to the determination process, that requires a wholemeal flour passage through a sieve (200 μ mesh) to remove the bran fraction, and, secondly, to the addition of other ingredients during the dough formation (yeast, ascorbic acid, NaCl and sugar solutions, saturated fat).

Regarding crust color, *T. vulgaris* treatment showed the highest brightness value (43.23 L*), followed by chemical treatment (37.42 L*). Low red crust index (a*) was recorded for bread from the water control (9.46 a*), while the bread from the untreated plots showed the most red crust (12.39 a*). The crust yellow index (b*), whose importance is anyway lower than the other crust colorimetric parameters, did not show any significant difference among treatments. The occurrence of a dark color both in crumb and in crust, which is rather normal in wholemeal bread, is generated by the Maillard reaction, which involves the interaction between proteins and fermentable sugars obtained after starch cleaving. In our samples, high levels of Maillard indicators were due to the rather high amount of NaCl used for the baking test (2%). On one hand, salt induced higher starch degradation (Spina and others 2015) with an increased availability of sugars to the Maillard reaction, and on the other hand, it limited yeast growth, thereby reducing sugar consumption (Moreau and others 2011).

Conclusions

In our experiment, cv Valbelice showed a good protein and dry gluten content, but confirmed to deal with a very weak gluten, and most wholemeal flour parameters matched the expected values for this variety as well. However, the physical and chemical traits and the technological properties of this durum wheat genotype were differently affected by the tested treatments.

Chemical weeding had a slightly negative effect on protein and dry gluten content. However, this treatment achieved the best mixographic score and the highest loaf volume value. The supply of *R. coriaria* extract did significantly increase both the protein and dry gluten content of wholemeal flour and the SDS sedimentation value. Furthermore, the supply of this extract caused a very high enzymatic activity of durum wheat wholemeal flour. Probably because of this, *R. coriaria* extract yielded the lowest bread volume and a reduced crumb development, along with a high production of brown-colored compounds, that was found both in the crumb and in the crust of bread. As an overall result, a flat, hard and dark bread was obtained from that treatment, with an obviously lower quality. So far, no data are available in the literature on the eventual effects of *R. coriaria* extracts on the growth and physiology of durum wheat. However, the attractive hypothesis that *R. coriaria* extracts could act as triggering agents for late maturity α-amylase production must be verified with further experiments.

Being restricted to a 1-y trial only, the outcomes of this work need to be further deepened. It definitely appears the necessity to get additional information about many issues, including the mechanism of action of *R. coriaria* extracts in activating α-amylase activity, along with the possible influence of environmental factors. Notwithstanding, a meaningful overall finding is that the use of plant water extracts is not without consequences on durum wheat quality. In view of the need for diversity in weed management strategies (Jabran and others 2015), the use of plant extracts could surely play a major role. However, the occurrence of many unwelcome variations in the quality features of the final product must be taken into consideration when quality is of particular importance.

Author Contributions

A. Carrubba designed the study, planned field experiments and data collection, performed statistical analysis, interpreted and discussed field results. A. Comparato contributed in planning and design of field experiments, prepared and managed all plant extracts, surveyed the experimental plots and collected agronomical data. A. Labruzzo contributed in discussion, statistical analysis and interpretation of field data about plant extracts. S. Muccilli performed laboratory flour and baking tests, statistical analyses, interpretation and discussion on flour and bread quality data. V. Giannone performed kernels electrophoresis, laboratory flour (FN), interpretation and discussion on seed and flour quality data. A. Spina designed and planned the analytical part of the work, surveyed laboratory analyses, interpreted and discussed the results of quality tests.

Conflicts of Interest

The authors declare no conflict of interest in this research.

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