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Old and new common wheat (*Triticum aestivum* L.) varieties in organic zero-input: connecting agronomic, microorganism, phytochemical and bread sensory characteristics

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Abstract

On aim to connect phytochemical traits as related with bread sensory features, Italian Old varieties and one Modern variety of common wheat (*Triticum aestivum* L.) were compared in a framework taut to develop Micro Organism Consortium (MOC) under organic agriculture managements. Almost all the agronomic traits distinguished the Modern from the Old Italian varieties: reduced plant height, root weight, yield and lodging but increased harvest, tillering, and weeds attacks. Polyphenols in the modern variety Blasco were significantly lower than in the old varieties, Andriolo and Gentil Rosso but addition of MOC factor raised the content. The MOC factor dramatically modified the phytochemical traits, which in turn concurred to give the sensorial characteristics of the Old Wheat varieties Bread (OWB) to the Modern Wheat varieties Bread (MWB). A multivariate data elaboration highlighted some relationships between the classes of secondary components and the OWB scores, judged as favourable (polyphenols, bound flavonoids) or adverse (flavonoids, tannins, total anthocyans). Italian wheat varieties enhanced excellent bakery properties, based on the secondary compounds variation and strengthened by MOC factor modifications.

Key words: Old wheat varieties, micro-organism consortium, phytochemicals, bread sensory.

Introduction

Common wheat (*Triticum aestivum* L.) is amongst the leading crops for human nutrition in most temperate regions worldwide, and as such, is facing the challenge of being produced more sustainably, with reduced levels of external inputs. However, climatic change events are a major threat for wheat production and genetic diversification of the crop stand is part of an overall strategy to improve wheat performance based on the potential of diversity to buffer for biotic and abiotic stresses.

The large part of the common wheat is addressed to the production of bakery products, in particular bread. In Europe the median consumption of bread is 125 g/d compared to 27 g/d of pasta [1]. Although over the years the consumption of bread has fallen, this food is still among the most popular, consumed and loved. This food basically consists of complex carbohydrates, fibre and proteins. In fact, epidemiological studies have associated the consumption of whole grain and whole-grain products with reduced incidence of chronic diseases such as cardiovascular disease, diabetes and cancer [2]. It is widely accepted that phenolic acids including ferulic, vanillic, and p-coumaric acids are the major antioxidants in wheat and significantly contribute to the overall antioxidant properties of wheat grain. Dietary phenolics include phenolic acids, phenolic polymers (commonly known as tannins) and flavonoids. Phenolic acids are aromatic secondary plant metabolites, derivative from hydroxylated benzoic and cinnamic acids.

Microorganism (MO) fertilizers are an emerging input in sustainable agriculture. Mycorrhizal fungi in soil enter into symbiosis with plant roots by establishing a symbiosis called arbuscular mycorrhiza (AM). The associations crops-fungi are widely distributed in the plant kingdom [3]. Moreover, AM fungi and several bacteria species can improve the soil structure and water retention properties by forming and stabilizing soil aggregates through deposition of organic compounds such as glycoproteins or exopolysaccharides [4]. The function now recognized for this symbiosis is that the plant improves its mineral nutrition, recording a positive effect on its growth, and giving in return sugars to the fungus. Moreover, the plant appears to be more resistant to biotic and abiotic stresses, increases the tolerance to lack of water or presence of pollutants and leads to a reduction of sensitivity to common pathogens [3]. It is evident that any agronomic mean suitable to promote the accumulation of secondary metabolites with antioxidant capacity in wheat could be beneficial in nutritional value of bread and grain products in general. The symbiosis of a mycorrizhal factor with food plants resulted in a modification of the putative secondary constituents leading to an antioxidant fortification and increased nutritional and functional value in several plants and fruits [5]. This technology, as recently testified in low input corn by Sabia *et al.* [6], does not raise any issue by environmentalists because they are based on natural strains, and are addressed to a sustainable modern and economic agriculture.

As Hildermann *et al.* [7] suggested, in order to incorporate environmentally friendly methods into the farming system used for the production of whole grain bread, in recent times, there is a renewed attention in old varieties of wheat and in particular to their yield productivity and to baking quality under organic management. In Italy there is a growing interest in assessing the agronomic characteristics of old, modern and mixture of varieties and landrace of bread wheat (*Triticum aestivum* spp.) and their adaptability to organic farming in hilly and mountainous areas also for their potential functional properties [8, 9].

A preliminary sensorial study of Torri *et al.* [10] from this experiment have shown that some old varieties have a satisfactory attitude to bread and the sensory properties of bread were dramatically modified by the Micro Organism Consortium (MOC) factor associated to the plant.

Numerous purposes were at the basis of this research: 1) to compare agronomic and some phytochemical traits related with secondary components, between old varieties and one modern variety of common wheat (*Triticum aestivum*); 2) to evaluate the effect of the MOC factor on the modern variety; 3) to ascertain the versus and the force of the covariation of the phytochemical traits into the set of the bread sensory variables in the framework of Old Wheat varieties Bread (OWB) compared to the Modern Wheat varieties Bread (MWB).

Materials and Methods

The experimental site (44°56'N, 7°40'E, altitude 253 m a.s.l.) was located in a park. The organic field was previously a perennial grass meadow, that has just been broken after several decades. Experimental silty soil had optimum pH value (7.45) and very high content of organic matter (3.13%) and total nitrogen (2.1 ‰), also soil was rich in nutrients (CEC 9.90 meq 100 g^{-1}) with an excellent C/N ratio (8.76). The available phosphorus (15.75 mg/kg) and exchangeable potassium (0.15 meq 100 g^{-1}) were a bit low without posing any risk of leaching. The total lime (1.08 g/kg) is very poor. The conductivity is low (1.28 mS cm^{-1}).

The wheat varieties were I- Inallettabile (old), G- Gentil Rosso (old), A- Andriolo (old), S- Sieve (old), B- Blasco (modern) and Bm- Blasco with MOC (modern). The sowing date was the 21st of November. The plots of modern variety Blasco (CO.NA.SE, Conselice, Ravenna, Italy) were sown with 230 kg/ha of seed at a depth of 2-3 cm, while for old varieties, only a few hundred grams available and 450 seeds m^{-2} sown by hand in plots of 100 m^2 and then buried. The Bm with MOC is a modern Blasco variety inoculated with Micosat F © (CCS-Aosta s.r.l., Italy: *www.micosat.it*) a bio-fertilizer composed of three AM species (*Glomus caledonium* GM24, *Glomus intraradices* GG31, *Glomus coronatum* GU53, in form of spores, hyphae and root fragments), and three PGPR species (*Pseudomonas fluorescens* PA28, *Pseudomonas borealis* PA29, *Bacillus subtilis* BA41) providing a total concentration of 10^6 g^{-1} distributed into the seeding machine as powder at a dose of 400 g per 100 kg of seed. No fertilization and pest and weed control were done with external input. Noticeable was a presence of excessive weeds in the Micosat-treated plots (+228% *vs.* Blasco untreated). The harvest took place July 7 at full ripening of the grain (moisture content about 13%).

The following data were collected on the crops: plant height (cm), lodging (%), tillering index (number of stems m^{-2}, number of plants m^{-2}), harvest index (grain yield total plant weight $^{-1}$), capacity to control weeds (g DM ha^{-1} of weeds), sensitivity to virus (score 0-1-2), root weight (g m^{-2}), grain yield (t ha^{-1}). The C and N content in the dried samples was measured using the Flash Elemental Analyser 1112 NC (Thermo Fisher Scientific; Waltham, Massachusetts, USA) according to the manufacturers instructions. In order to evaluate the functional content, the extraction of soluble (free) and insoluble (bound) phenolic compounds was performed according to the method of Dinelli *et al.* [11, 12]. Polyphenol content in both the free and bound fractions was measured using the spectrophotometric Folin-Ciocalteu method (Lambda 25 Spectrophotometer, Perkin-Elmer Corporation, USA) with gallic acid as the reference standard. Similarly, as Adom *et al.* [13] reported, the flavonoid content was determined using a colorimetric method with catechin as the reference standard. The free and bound polyphenol and flavonoid contents, respectively, were summed to provide the total content. The total anthocyanin content was extracted and measured [14]. Total carotenoid content was estimated from the yellow pigment content, extracted and measured [15].

The experimental design for agronomic and phytochemical variables, featured three blocks with three randomized repetitions. Data were submitted to ANOVA analysis of the six varieties (I, G, A, S, B, Bm), by using a mixed model with random block, and providing a multiple Duncan mean comparison [16]. The MOC effect was ascertained according the Bm *vs.* B contrast, while the Epoch factor considered the Old varieties *vs*. the Blasco wheat plots. In the bread sensory framework, the Bread Type factor compared the Old Wheat varieties Bread (OWB) to the Modern Wheat varieties Bread (MWB). The phytochemical data of the flours were connected indirectly to the sensory properties of the bread using a chemometric partial least square (PLS) regression [17]. The relevance of the phytochemical *vs.* the sensory results was assessed by considering the sign and the amount of the standardized coefficients in the respective equations. A distance matrix between the six varieties was calculated by a PLS method [17] and a series of multiple regression analyses highlighted the significant discriminant variables [16].

Results

Almost all the traits (Table 1) except the sensitivity to virus, distinguished the Modern from the Old wheat varieties: reduced plant height, lodging, root weight and yield but increased harvest index, tillering indexes and weeds biomass (+151%).

The most discriminant trait of the MOC factor in Bm compared with B, was the excessive weed proliferation in the Micosat F-treated plots (+219%) that may be responsible of a reduced yield (-10%, not significant) together a slight more lodging. Among the groups several distinctions enriched the variability, the most implicated variables being the plant height, the lodging and the tillering index. As yield features Inallettabile (3.86 t ha^{-1}) and Sieve (3.42) outperformed the Gentil Rosso (2.85) and Andriolo varieties (2.53) together the modern Blasco. The Blasco, over the shortness, was distinguished from the Gentil Rosso because of its harvest index and from the Sieve because of its unlodging capacity (0% vs. 82%). In the framework of the old varieties the Sieve is distinguished from Gentil Rosso because its shortness, and from the Inallettabile because its lower unlodging performance, and from Andriolo because of a reduced tillering index.

The total polyphenol content (Table 2) varied significantly among the old varieties. This variation was attributable to differences in the free as well as the larger bound polyphenol contents in the respective varieties. Polyphenol content in the modern variety Blasco was significantly lower than the old varieties, Andriolo and Gentil Rosso. Interestingly, the addition

Table 1. Agronomic features of the wheat varieties. Linear model results, LSMeans of the varieties and estimates of the contrast for MOC and Epoch factors.

						Old varieties [A]				Modern[A]/MOC factor		Epoch factor	
Variables	Unit	R^2	CV (%)	SE	Mean	I	G	S	A	B	Bm	Old	Modern
Plant height	cm	0.84	12	13	106	121b	134a	117b	128ab	63c	70c	125.4a	67.0b
Lodging	%	0.66	64	23.0	35.7	23.8c	61.1b	82.2a	44.2b	0.0c	2.2bc	52.9a	1.1b
Sensitivity	Score[B]	0.14	100	0.56	0.56	0.4ab	1.0a	0.3b	0.4ab	0.5ab	0.5ab	0.41	0.55
Tillering Index	Score[C]	0.34	39	2.03	5.22	3.6b	5.6a	3.2b	5.6a	6.0a	7.1a	4.53b	6.58a
Harvest Index	HI[D]	0.63	10	0.04	0.39	0.32d	0.33d	0.37c	0.41b	0.45a	0.44a	0.36b	0.45a
Root Weight	$g*plant^{-1}$	0.17	34	10.7	31.85	36.9a	37.1a	30.2ab	33.6ab	28.2b	24.7b	34.5a	26.5b
Weeds	$g\ DM*m^{-2}$	0.26	149	66.7	44.7	17.0b	10.4b	17.8b	73.5ab	35.6b	113a	29.7b	74.7a
Yield	$t*ha^{-1}$	0.25	28	0.86	3.03	3.86a	2.85bc	3.42a	2.53c	2.90bc	2.61c	3.2a	2.7b

MOC = Micro Organism Consortium. (a > b > c; $p<0.05$). [A] Wheat varieties: I=Inallettabile; G =Gentil Rosso; S =Sieve; A =Andriolo; B =Blasco; Bm=Blasco with MOC factor; R^2 =r-square of the linear model; CV = coefficient of variation %; SE =standard error; [B] Sensitivity: 0 = no attack, 2=high attack. [B] Sensitivity score: 0=no attack, 2=high attack. [C] Tillering index (number of stems m^{-2} number of plants m^{-2}). [D] Harvest index (grain yield total plant $weight^{-1}$).

of MOC factor to Blasco raised the polyphenol content to a level comparable to that of Andriolo and Gentil Rosso (4.01). The total flavonoid content comprised approximately 30% of the total polyphenol content in the wheat varieties investigated. By difference, the remaining 70% of the total polyphenol content is likely comprised of phenolic acids. Interestingly, the addition of MOC factor to Blasco had the effect of raising the total flavonoid content to a level higher than that observed for the old varieties. Of the flavonoid classes present, MOC factor was not shown to improve the anthocyanin component and it was also shown to reduce yellow pigment.

The standardised partial least square coefficients of the sensory scores of the bread (Table 3, right side) fitted to OFB (pointed 2) and MB (pointed 1) are ranked in decreasing order; the adjacent column reports the significant difference between the two types of bread, as relative percentage. Six sensory variables featured significantly the OFB in positive sense: elasticity and crumbliness (texture), pore quantity (appearance), sweet (taste), yeasty and grain (odour). On the opposite side five traits characterised in negative sense the MB type: crust darkness (appearance), sour (taste), toasted and nutty (odour), hardness (texture). The whole PLS relationship for the sensory evaluation of the bread attained an R-square level of 0.66.

The left side of Table 3 collimates the OFB/MB factor to the grain phytochemical analyses: a high coefficient tends to OFB type, while a negative coefficient tends to MB bread type. The total polyphenols (free and bound) and the bound flavonoids featured the higher ranking, but without a difference significant between OFB and MB; as opposite, the modern bread type was related with total hydrolysable tannins, free flavonoids, total anthocyans and carbon content. All these last constituents had determined significant modifications between the two types of bread. The whole PLS relationships for phytochemical attained an R-square level of 0.59.

Discussion

It is noteworthy that modern breeding programs for genetic improvement have been primarily focused on yield and on the improvement of disease and pest resistance rather than nutritional and functional characteristics. Modern varieties are characterized by genetic uniformity and adaptation to conventional agriculture typically using high-energy inputs in terms of fertilizers, herbicides, insecticides and fungicides.

In a comparable zero-fertilization experience, as Dinelli *et al.* [18] analysed, the yield of the modern varieties (1.5 t ha^{-1}) was inferior to the old varieties (1.99 t ha^{-1}), a result which was also observed in this trial (2.7 *vs*. 3.2 respectively). In a field trial experiment comparing 30 wheat genotype in Piedmont, Blasco variety featured between 8.06 and 6.01 t ha^{-1} in conventional farming with late winter 140 kg N ha^{-1} [19].

Thus, the lower yield of Blasco in our plots (2.90 and 2.61 t ha^{-1} in Bm and B, respectively), could be accounted by the applied zero fertilization and to a great intrinsic need of mineral N. This is confirmed by Migliorini *et al.* [8], that in 2011-2013 field trials in similar pedo-climatic environments with zero-fertilization but in different organic farms, the old varieties, landrace and their non-modern mixture yielded 2.05 t ha^{-1} and the modern 3.05 t ha^{-1} as mean of Bolero, Blasco, Arabia, Bologna while Blasco 2.14 t ha^{-1}.

In a variety of ecosystems, interactions between soil microbiota and weedy plants can strongly affect population and community dynamics of these plants. Many weeds are strongly mycorrhizal. The relationship between AMF diversity and host species diversity/productivity has been examined in natural tropical ecosystems, as indicated in the studies of Husband *et al.*[20]. However, weed-soil microbe interactions are not well characterized in field-crop agroecosystems. In our trial weeds may have benefitted by the symbiosis with MOC.

As regards to phytochemicals, in old wheat varieties Dinelli *et al.* [18] showed a high level of polyphenols (on avg. +21%) and

Table 2. Phytochemical profiles of the wheat grain varieties [A]. Linear Model Results and LSMeans.

						Old varieties [A]				MOC factor		Epoch factor	
	Unit	R^2	CV	SED	Mean	I	G	S	A	B	Bm	Old	Modern
N	%	0.41	10	0.24	2.49	2.36bc	2.47bc	2.29c	2.73a	2.59ab	2.49b	2.47	2.54
C	%	0.57	2	0.70	42.4	41.6c	42.3b	42.2bc	42.5b	43.5a	42.2b	42.21b	42.90a
C/N	-	0.37	10	1.69	17.2	17.7ab	17.3b	18.7a	15.6c	16.8bc	17.0bc	17.3	16.9
Tot. Polyphenol	mg/g dry weight	0.81	7	0.26	3.76	3.74c	4.24a	3.02d	4.00b	3.55c	4.01ab	3.75	3.78
Free polyphenol	mg/g dry weight	0.69	11	0.14	1.28	1.36ab	1.44a	1.03c	1.36ab	1.18b	1.25b	1.30a	1.22b
Bound polyphenol	mg/g dry weight	0.74	9	0.23	2.49	2.38b	2.79a	1.98c	2.64ab	2.36b	2.75a	2.45	2.56
Total flavonoids	mg/g dry weight	0.73	7	0.07	1.13	1.05c	1.16b	1.03c	1.07c	1.15b	1.30a	1.08b	1.23a
Free flavonoids	mg/g dry weight	0.57	13	0.05	0.42	0.38c	0.41bc	0.39bc	0.37c	0.49a	0.43b	0.39bc	0.47a
Bound flavonoids	mg/g dry weight	0.73	9	0.06	0.72	0.66c	0.74b	0.64c	0.69bc	0.66c	0.86a	0.69bc	0.77a
Tot. Anthocians	mg/g dry weight	0.85	9	0.002	0.024	0.018d	0.027a	0.019d	0.025b	0.028a	0.022c	0.023b	0.026a
Yellow pigment	µg/g dry mass	0.69	11	0.001	0.011	0.010b	0.010b	0.009c	0.013a	0.011b	0.009c	0.0109a	0.0102b

[A] Wheat varieties: I=Inallettabile; G=Gentil Rosso; S=Sieve; A=Andriolo; B=Blasco; Bm =Blasco MOC fertilized with Micosat F factor; R^2 = r-square of the linear model; CV=coefficient of variation %; SED = standard error.

Table 3. Relationships between the phytochemical composition of the grain and the sensory characteristic of the bread by a PLS fitting of the Old Wheat varieties Bread (OWB), with the Blasco not MOC contrasted to all the other as Modern Wheat Bread (MWB). Values of the standardised PLS coefficients (st_PLS) and significant deviations of the OWB to MWB.

Grain Phytochemical	st_PLS	[A]Delta % OWB/MWB	Bread sensory	st_PLS	[A]Delta % OWB/MWB
			Elasticity	0.234	110%
Total_Polyphenols	0.157	7% [a]	Pore_quantity	0.162	36%
Free_Polyphenols	0.152	9% [a]	Sweet	0.145	28%
Bound_Polyphenols	0.123	6% [a]	Yeasty	0.112	33%
Bound_Flavonoids	0.105	9% [a]	Grain	0.109	31%
			Crumbliness	0.099	22%
			Moisture	0.050	
			Odor_intensity	0.028	
			Pore_dimension	0.027	
			Flavor intensity	0.017	
C_N	-0.005		Crumb_darkness	0.013	
Yellow_Pigment	-0.016		Adhesiveness	-0.011	
N	-0.031		Salty	-0.034	
Total_Flavonoids	-0.062		Crust_darkness	-0.037	-16%
Total_Hydrolisable_Tannins	-0.201	-23%	Sour	-0.086	-28%
Free_Flavonoids	-0.265	-18%	Toasted	-0.134	-30%
Total_Anthocians	-0.286	-22%	Nutty	-0.236	-50%
C	-0.301	-3%	Hardness	-0.303	-54%
Old Wheat varieties Bread (OWB) < > Modern Wheat varieties Bread (MWB)					
R^2	0.59		R^2	0.66	

[A]Delta % (LSMean TBT / LSMean ABT), reported when $P<0.05$ or [a] when $P<0.15$.

flavonoids, together with higher amounts of resistant starch (+200%), gluten and lipids (+30%). Also Leoncini *et al.* [21] observed an increase in total polyphenols (+21%) and flavonoids (+69%) in the same old varieties studied in this paper but with a different modern control compared with modern genotype. In Migliorini *et al.* [8] only old varieties were assessed for phytochemicals and significant differences were observed between years and varieties, confirming the dependency of biosynthesis and accumulation of phenolic compounds on both variety and environmental conditions (abiotic and biotic stress), showing that Gentil Rosso had a much higher amount of total, free and bound polyphenols. Otherwise, these trend is not a constant as verified in the present work. Shewry and Hey [22] reported that total phenolic compounds may achieve values of 2.71-3.16 mg/g dry weight for bread wheat cultivated in Poland, values in range with the present data (3.02-4.24 mg/g dry weight). Heimler *et al.* [23] have shown that atmospheric conditions play a big role in free polyphenols quantity and in the - uncorrelated - antiradical activity and high temperatures can cause a drop in polyphenol content. The presence of weeds may force the crops to react with secondary metabolites expression [24]. Thus, the results observed in the present study could be indirectly accounted for the weed increase promoted by the MOC factor, but the MO itself have to be considered as the primary factor for the antioxidant compound enhancement, as proved in several species by Raiola *et al.* [5]. Other results [11, 12] showed that mean values of total phenolic compounds and total flavonoid content in old wheat varieties were similar as in modern genotypes; however, the HPLCESI-TOF-MS analysis highlighted remarkable differences between modern and old cultivars.

The interpretation of the mass spectra allowed the identification of 70 phenolic compounds, including coumarins, phenolic acids, anthocyanins, flavones,isoflavones, proanthocyanidins, stilbenes and lignans. The free extracts of old wheat varieties showed the presence of a mean number of phenolic compounds more than double than in modern genotypes; a similar trend was observed also for the bound phenolic fraction. Moreover, the phytochemical profiles showed the presence of unique phenolic compounds in both free and bound fractions of some of the investigated wheat genotypes. In the present work a high degree of phytochemical variability exhibited: in reference to the lower side of the distance matrix (Table 4) the list of most discriminant variables, enumerated 30 cases where only C did not appear, when most represented are the total anthocyans (11 counts), N content (4 counts) and yellow pigment bound polyphenols and bound flavonoids (3 counts). In Dinelli *et al.* [9] lignin, a class of phytochemicals with proved health benefit effects, discriminated the new and the old varieties of wheat: in the old ones a double concentration with three exclusive lignin compounds (arctigenin, hinokinin, and syringaresinol) and higher number of glycosidic forms were observed. The study of the phytochemical contents and profiles of different wheat varieties could be a promising way to study the effects of MO in wheat, especially mycorrhiza.

Regarding to bread sensory aspects [10] (Table 2), it was enhanced that the Old Wheat varieties and Bm Bread differentiated from the Modern wheat variety bread because of significant increases in flavour intensity and grain odour, pore quantity appearance, elasticity, crumbliness texture and salty taste. Conversely, a decrease was ascertained in adhesiveness texture, in nutty and toasted odour and in sweet taste. In this paper, the total polyphenols, free polyphenols, bound polyphenols and bound

Table 4. Matrix of the between-group distances according to the chemical analyses of eleven constituents and polyphenols analyses (R^2 values; above the diagonal) with indication of the chief constituents[A] accounting of each contrast (below the diagonal).

Groups[B]	B	Bm	G	I	S	A	Avg.
B	0	0.82	0.78	0.89	0.86	0.76	0.83
Bm	7,9	0	0.84	0.80	0.91	0.78	
G	2,6	7,4	0	0.89	0.84	0.65	
I	7,3	3,6,7	7	0	0.90	0.77	
S	7,3	1,7	2,6,8	1,8,	0	0.94	
A	7	9,5	9,7	7,9	8,7	0	

1-VR =r-square in crossvalidation mode.[A] 1-Total polyphenols; 2-Free polyphenols; 3-Bound polyphenols; 4-Total flavonoids; 5-Free Flavonoids; 6-Bound Flavonoids; 7-Total Anthocians; 8-Yellow pigment; 9-N; 10-C; [B] Wheat varieties: I=Inallettabile; G=Gentil Rosso; S=Sieve; A=Andriolo; B=Blasco; Bm =Blasco Myc with Micosat F Mycorrhizal and microbial factor.

flavonoids were linked with the most positive features of a OWB and, by contraposition, the free flavonoids, tannins, total anthocyans and carbon contents appeared to be linked with the negative traits. Chlopicka *et al.* [25] reported that the addition of 15% and 30% of pseudocereals (buckwheat, amaranth and quinoa) high in phenolic content to the wheat flour was effective in enhancing antioxidant activity, and sensory properties of bread added by buckwheat showed an increase of acceptable quality attributes such as taste, colour or odour.

As to mycorrhizal, several studies of Beauregard *et al.* [26], based on 368 records in field and greenhouse, have been conducted to confirm AM symbiosis positive influence on major edible plants resulting an average yield increase of 26%. In early stage trial gas exchange parameters of the maize leaves were positively affected by inoculation of AM, leading to mass increment [4]. In the present trial, the MOC effect did not result in a significant yield increase but the high weed biomass in Bm plots may be responsible of the no quantitative effect. Otherwise a series of strong qualitative effects involved the secondary compounds. The Micosat F is a mycorrhizal-microbial consortium already affirmed in agriculture practices and recently discovered as an *in vivo* acidifier of the maize stem [27]. Some studies focused on the so-called Mycorrhiza Helper Bacteria (MHB) or Plant Growth Promoting Rhizobacteria (PGPR) distinguishing between the helper bacteria, which assist mycorrhiza formation, and those that interact positively with the functioning of the symbiosis. Berta *et al.* [22] by first highlighted the separated modifications promoted by the Pseudomonad bacteria and/or by the AM fungi on the maize grain quality. The bacterial activity sustained a higher level in degradability of the starch; on the contrary the AM strongly raised the insoluble zeins (prolamine) content, by 30%, and both, bacteria and AM increased the Fe and Zn levels by some 18-40%. This point concerning the complexity of the protein metabolism may be the basis to understand the properties in OWB and MWB. In tomato plants inoculated with AM, β-carotene, lycopene and luteine in fruits were increased by 11, 10 and 7 %, respectively [29]. The nutraceutical value of tomatoes, e.g. the phytochemical content of the fruits, has been increased with the establishment of beneficial MOC symbioses [30], out of the production of mutagenic compounds, since tomato extracts induced no *in vitro* genotoxic effects. Also Raiola *et al.* [5] observed in many important Mediterranean vegetables several improvement of functional compounds, but especially in durum wheat as regard to total carotenoids (+42%) and phenols (+230%).

Conclusions

The work has confirmed the morpho-biological separation of some old Italian wheat varieties from a modern one, while enhancing very good yield properties and especially distinctive features that lead to a distinguished Old Wheat variety Bread. The raising in both polyphenol and flavonoid content in wheat grain was also achieved by an adjuvant based on mycorrhizal and microorganism treatment of the modern Blasco variety to levels comparable to those of Gentil Rosso, the variety containing the highest contents. On the contrary, a significant decrease in total anthocyanins was noted after MOC treatment and in the old varieties. As to the final sensory quality of the OW bread, the total flavonoids seem to enhance the flavour intensity, the total polyphenols the pore quantity, the free polyphenols the grain odour, the yellow pigment the crumbliness texture and salty taste, while a high total anthocyans content was favourable to the OWB, because repressor of the negative nutty and toasted odour and of the sweet taste.

It was concluded that organic farming of old wheat varieties, or modern with MOC adjuvant, could promote healthy local and innovative products.

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Abbreviations

MOC: Micro Organism Consortium;
MWB: Modern Wheat varieties Bread;
OWB: Old Wheat varieties Bread.