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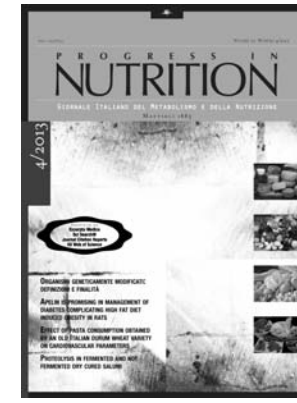

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Effect of pasta consumption obtained by an old Italian durum wheat variety on cardiovascular parameters: an intervention study

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TITOLO
Effetti del consumo di pasta ottenuta da una varietà antica di grano duro su parametri di rischio delle malattie cardiovascolari: uno studio di intervento

KEY WORDS
Whole-grain, pasta, haemorheological parameters, cardiovascular diseases

PAROLE CHIAVE
Pasta; frumento, malattie cardiovascolari, emoreologia

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Summary
Aim this study was to evaluate the influence of short-term dietary intake of pasta obtained by an old Italian durum wheat variety on parameters related to the atherosclerotic process. Twenty subjects were followed for 10 weeks a diet containing 70 g/die of test pasta (Test period) and for the same period a control pasta (Placebo period). The test period determined a significant improvement of total cholesterol (-10.3%; p=0.04), whereas no significant changes during the placebo period have been observed. With regard to haemorheological parameters, the test period significantly ameliorated whole blood viscosity at high shear rate (pre: 4.5 vs. post: 4.3 mPA*s; p=0.04) as well as erythrocytes' deformability (pre: 9.8 vs. post: 10.3; p=0.03). In conclusions, dietary intake of pasta obtained by an old variety of wheat seems to impose favourable biochemical changes, with regard to lower circulating levels of markers of atherosclerosis, such as lipid parameters, and haemorheological variables.

Riassunto
Scopo dello studio è stato di valutare l'influenza di un consumo a breve termine di pasta ottenuta da una varietà antica di grano duro Italiano su alcuni parametri relativi al processo aterosclerotico. Venti soggetti sono stati seguiti per 10 settimane con una dieta contenente 70 g / die di pasta ottenuta dalla varietà antica di grano (periodo di prova) e per lo stesso periodo una pasta di controllo (periodo placebo). Il periodo di prova ha determinato un significativo miglioramento del colesterolo totale (-10,3%, p = 0,04), mentre non sono stati osservati cambiamenti significativi nel periodo placebo. Relativamente ai parametri emoreologici, il periodo di prova ha migliorato significativamente la viscosità del sangue intero ad alto shear rate (pre: 4,5 vs post: 4,3 mPa * s, p = 0,04) e la deformabilità eritrocitaria (pre: 9.8 vs post: 10.3, p = 0,03). In conclusione, l'assunzione alimentare di pasta ottenuta da una vecchia varietà di frumento sembra imporre cambiamenti favorevoli a livello di alcuni parametri di rischo delle malattie cardiovascolari, come i parametri lipidici e le variabili emoreologiche.

Introduction

Diet can significantly influence health status of the population (1). In particular, Mediterranean diet has been extensively reported to be associated with a reduced risk of major chronic degenerative diseases (2). Actually, one of the most important dietary components of the traditional Mediterranean diet is certainly pasta, derived from durum wheat (*Triticum turgidum* L. subsp. *durum*), a tetraploid wheat with an annual global yield of 36 million tons. From the results presented in 2010 at the 13th Annual World Pasta Day it was shown that Italy (with a pasta production of 3.2 million tons) is responsible for approximately 26% of the pasta produced worldwide, and 75% within the EU.

In the last decades, the commercialization of durum wheat and its end-products have been defined predominantly on the basis of the quality and quantity of the proteins of the grain, which determine the technological characteristics (pasta-making qualities) of the semolina. Intensive genetic improvement programs on wheat, conducted so far, have been directed towards improving both yield and technological characteristics (pasta-making performance), resulting in a narrowing of the genetic base (3,4).

On the other hand, aspects related to secondary metabolite content, responsible for taste and functionality of the pasta, have been completely neglected. Previous findings from our research group have shown that although mean values of total polyphenol content in both old durum and soft wheat varieties did not differ significantly from those in modern varieties, the old varieties had more unique compounds as well as isomers of compounds not found in modern varieties (5-7). Hence, old varieties may provide an interesting genetic resource for utilization in breeding programs in order to maintain the wide genetic variability. Moreover, reduced contents of essential minerals, including iron and zinc, have also been reported in modern high yielding cultivars of wheat compared to older varieties (8).

The durum wheat variety *Senatore Cappelli* is representative old variety with a significantly higher number of both free and bound polyphenol compounds and isomers, including unique compounds in comparison to modern varieties (6). Recently we have demonstrated that the consumption of bread derived from wholegrain flour of an old soft wheat variety over a short period resulted in a significant improvement of certain parameters linked to the manifestation of cardiovascular diseases (9). However, no data on effects of consumption of pasta obtained by old durum wheat on biomarkers related to disease are available. The purpose of the present study was to investigate the effect of the consumption of pasta derived from wholegrain semolina using *Senatore Cappelli* on various blood parameters related to atherosclerosis.

Material and methods

Evaluation of old and modern durum wheat varieties

The wheat grain samples investigated included: 2 old (*Senatore Cappelli, Urria*), 7 modern (*Anco Marzio, Claudio, Iride, Levante, Orobel, Solex, Svevo*) varieties of durum wheat (*T. turgidum* ssp. *durum*) and an accession of *T. turgidum* ssp. *turanicum*. All varieties were cultivated under low-input conditions on a farm near Florence, Italy. The field trials included small plots of 1.5 m^2 with five rows within each plot and with two replicates per variety. Productive data were collected. On representative grain samples, characteristics including carbon and total protein content, gluten and mineral elements were analysed. The C and N content of wheat samples were conducted on three replicates of whole grain wheat flour (3-4

mg) obtained from grinding caryopses in a stone mill, and measured using the Elemental Analyser (CHN-Thermo Fisher Scientific) according to the Dumas combustion method. Total protein was obtained by multiplying the total nitrogen content by 5.62. Dry gluten composition was measured on 100 g of flour after separation of the starch fraction using a saline solution, and then dried in an oven and expressed as the % dry weight. Whole meal flour samples were digested with high purity HNO_3 (3 ml) and ultra-pure water milliQ (7 ml) using a closed-vessel microwave digestion protocol (Mars, CEM Corporation, Matthews, NC), and diluted to 25 ml with ultra-pure water milliQ. The concentrations of mineral elements were measured using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES IRIS Intrepid II XSP Radial) Thermo Fisher Scientific (Waltham, Massachusetts, USA). Mineral element concentration was expressed on a dry weight basis.
The analyses of protein, carbon and mineral element content in both modern and old wheat varieties are reported in Table 1 and Table 2.

The *Senatore Cappelli* variety, an old durum wheat Italian variety grown in organic cultivation, was used to produce the test pasta. The *Senatore Cappelli* variety was obtained in the early 1900s by the breeder Strampelli, through the selection of Jennah Khetifa. This variety was widespread in central and southern Italy in the first half of the last century, and widely used by Strampelli in genetic improvement programs for the attainment of varieties with improved lodging tolerance and suitability for pasta making. The pasta was produced from semi-integral semolina (0.9-1.2% ash) in an artisan pasta factory - *Pastificio Artigiano FABBRI s.a.s. (Strada in Chianti, Firenze, Italy)*,

Study population

Twenty clinically healthy volunteers (9 men, 11 women) were recruited from the staff of the University of Florence and from their family/friends. Inclusion criteria for subject participation in the study were: being of an age ranging between 20–70 years, being in good general health, being neither

Tabella 1 - Mean and standard error (±SE) of 1000 Kernel Weight (1000 K.W.), Total Protein (T. Prot.), Total Carbon (T. C) and Gluten content

Variety	1000 K. W. g	T. Prot. % s.s.	T. C % s.s.	Gluten % s.s.
AncoMarzio	45.10±1.89	15.06±0.22	44.75±0	7.41±0.77
Claudio	34.75±0.75	14.99±0.52	45.26±0.12	7.18±1.02
Iride	35.30±0.70	15.26±0.41	45.28±0.32	8.98±0.71
Levante	38.80±0.20	15.63±1.24	45.61±0.28	10.46±0.46
Orobel	42.45±1.04	14.30±1.55	45.37±0.26	8.12±1.53
S.Cappelli	**43.45±2.04**	**17.92±2.41**	**22.64±22.6**	**11.91±0.65**
Solex	39.20±0.19	15.05±1.26	45.47±0.54	6.78±2.80
Svevo	33.60±0.40	16.27±1.68	45.68±0.29	9.04±0.07
T.turanicum	53.40±1.09	18.43±2.49	22.54±22.5	11.70±1.64
Urria	43.30±1.70	16.26±2.16	45.55±0.24	8.98±0.70

Tabella 2 - Mean and standard error (±SE) of micro elements. The concentration is expressed as mg/kg of s.s.

Variety	Ca	Cu	F	K	Mg	Mn	Mo	Na	P	Se	V	Zn
AncoMarzio	395.95±67.3	5.16±0.4	28.44±4.2	5327±270	1034±15	15.98±2.7	0.38±0.2	15.79±0.9	4160±245	0.28±0.0	2.13±0.2	34.2±1.0
Claudio	460.80±64.3	5.77±1.5	31.65±4.0	5400±149	1062±39	25.88±6.3	0.47±0.2	16.59±4.6	3950±84	0.57±0.1	2.14±0.1	48.0±14.0
Iride	507.25±14.5	5.29±1.0	28.59±4.6	6266±38	1045±19	20.07±2.8	0.35±0.2	19.27±2.3	4355±27	0.38±0.1	2.03±0.3	44.0±10.0
Levante	371.40±5.6	4.94±1.6	26.62±5.6	6180±403	1053±29	20.11±5.8	0.57±0.1	13.33±5.0	4067±292	0.03±0.0	2.21±0.4	44.1±9.8
Orobel	511.00±34.7	3.96±1.3	21.44±3.5	5886±467	923±43	21.77±3.3	0.74±0.3	22.29±4.8	3725±263	0.25±0.1	1.90±0.3	38.8±8.0
S.Cappelli	**418.35±31.4**	**3.66±1.0**	**29.57±6.9**	**5381±161**	**1077±2.5**	**24.80±6.9**	**0.22±0.0**	**18.65±0.7**	**4269±103**	**0.44±0.0**	**2.07±0.2**	**43.4±4.8**
Solex	441.15±8.8	4.80±2.0	26.00±4.9	5319±340	982±41	19.34±5.9	0.51±0.1	10.49±1.8	4049±188	0.69±0.4	2.10±0.2	44.3±8.6
Svevo	527.25±30.3	4.64±1.2	28.56±4.7	5602±186	1008±11	27.68±8.8	0.27±0.1	15.71±4.0	3947±65	0.03±0.0	1.99±0.3	37.7±3.3
T.turanicum	411.00±0	4.48±0	33.95±0	5244±0	1187±0	33.29±0	0.58±0	16.79±0	4540±0	0.48±0	1.82±0	67.6±0
Urria	500.15±10.5	5.39±1.9	28.53±5.3	5273±272	947±29.	23.13±7.7	0.32±0.1	21.53±5.7	4000±251	0.16±0.03	1.85±0.3	44.9±7.4

pregnant nor lactating, and having neither a gluten allergy nor gastrointestinal disorders (e.g. chronic constipation, diarrhoea, inflammatory bowel disease, irritable bowel syndrome, or other chronic gastrointestinal complaints) and gall bladder problems. Volunteers were instructed not to alter their usual dietary or fluid intakes. All participants were selected on the basis of liking grain products, and being prepared to adhere to the dietary regime as prescribed by the study period. Subjects were required to be symptom-free of vascular and inflammatory diseases. This was ascertained prior to the experiment through a detailed interview, aimed at addressing both personal and familial history. Smoking status was determined at the time of the physical examination. Body Mass Index (BMI) was calculated as weight (kg) / height $(m)^2$.

Study design

The study was a randomized, single-blinded, crossover trial designed to investigate the effects of consuming pasta obtained by an old and a modern wheat variety.
The study was composed of two 10-week periods, interrupted by a 10-week washout period. Participants were individually instructed to include 70 g/day *Senatore Cappelli* pasta over a 10-week period (*Test period*). In the Mediterranean diet, 70 gram of pasta a day is representative of an average consumption and replacement of the normal pasta intake with *Senatore Cappelli* did not represent an extra energy intake. After the intervention period a washout period of 10 weeks was followed (*Washout period*). Thereafter, commercially available pasta obtained from a modern wheat variety of the same quantity as the test pasta was given to all the subjects for an additional 10-week period (*Control period*). Blood samples were obtained prior to and after both the Intervention and Control periods, respectively. Written informed consent was obtained from each participant before the initial screening visit and before randomization. The institutional review board at the University of Florence approved the study protocol.

Blood measurements

After an overnight fasting venous blood samples were collected from the antecubital vein into evacuated plastic tubes (Vacutainer), anticoagulated with 0.129 M sodium citrate (ratio 9:1). Venous blood was also collected in tubes without anticoagulant. Citrated and serum samples were centrifuged at 2000 g

for 10 minutes at 4 °C, and supernatants were stored in aliquots at –80 °C until analysis. Lipid variables were assessed by a nephelometric assay (ADVIA 2400; Siemens, Deerfield, IL, US).
Haemorheological variables, including whole blood viscosity (WBV), plasma viscosity (PLV) and erythrocyte deformability index (DI), were determined. WBV and PLV were measured using the Rotational Viscosimeter LS 30 (Contraves, Zurich, Switzerland). The filtration of erythrocytes was measured by a microcomputer-assisted filtrometer, model MF4 (Myrenne GmbH, Roetgen, Germany). DI was determined by a curve indicating erythrocyte filtration, by passing them through polycarbonate filters with 5-µm micropores (Nucleopore, Pleasanton, CA, USA) throughout a 10-minute recording, in order to determine rheological properties of erythrocytes. The initial flow rate from the microcomputer-generated curves was used for assessing erythrocyte DI. WBV was analysed at shear rates of 0.512 sec^{-1} and 94.5 sec^{-1}.

Statistical analysis

Statistical analysis was performed by using the SPSS (Statistical Package for Social Sciences Inc., Chicago, IL, USA) software for Windows (Version 19.0). Results were expressed either as mean ± standard error (SE) or as median and range, as appropriate. The analyses were simplified by calculating the absolute change for each variable tested on each subject (mean value at baseline subtracted from the mean value after intervention per subject) with independent *t* sample tests. No carry-over effect was observed. Therefore, all data were treated as paired samples. Data were analysed by using paired *t* tests for significant differences between changes observed during the test and control intervention periods, respectively. In order to compare the effect of the pasta test compared to both the baseline and the control pasta, data was subjected to analysis using a general linear model for repeated measurements were performed to compare the effect of the two different treatments. A model with adjustments for age, gender, BMI change was performed. Data for the general linear model were reported as geometric mean and range. A p-value < 0.05 was considered to indicate statistical significance.

Results

Test in vivo

Baseline and demographic characteristics of the subjects enrolled in the study are shown in Table 3. The median age of the studied population was 35 years (range: 29-53). Five subjects were current smokers. No significant differences for age, body mass index, smoking habit, and family history of cardiovascular diseases were observed between males and females. In order to evaluate the possible effects of dietary interventions on the investigated parameters, we performed a general linear model for repeated measurements, after adjustment for age, gender, and BMI change. Table 4 shows adjusted mean values of lipid parameters before and after dietary interventions with test and control pasta.
During the intervention period with the test pasta, there was a decrease of 10.3% of the total cholesterol, (p=0.04) (Table 4). Although not significant, a trend of

Tabella 3 - Demographic and baseline characteristics.

	Males (n=9)	Females (n=11)	p
Age, years*	36 (29-53)	35 (29-47)	0.3
BMI, kg/m^2	25.7 ± 2.3	24.8 ± 2.0	0.8
Smoking habit, n (%)	2 (22.2)	3 (27.3)	0.3
Positive family history of CVD, n (%)	2 (22.2)	2 (18.2)	0.4

** Median and (range)*

Tabella 4 - Effect of dietary interventions with test and control pasta on lipid profile.

Variable	Test Pre	Test Post	Change (post-pre)	p	Control Pre	Control Post	Change (post-pre)	p
Total , cholesterol mg/dL	214.8 (197.1; 229.6)	205 (177.2; 220.7)	-9.8 (-19.9; -8.9)	0.04	209.3 (184.8; 221.8)	205.1 (175.3; 222.9)	-4.2 (-9.5; 1.1)	0.1 0.1
LDL-Cholesterol, mg/dL	129.4 (112.2; 139.1)	124.9 (106.4; 139.6)	-4.5 (-5.8; 0.5)	0.8	124.6 (105.8; 154.7)	137.5 (103.2; 164.5)	2.9 (-2.6; 9.8)	0.6
Triglycerides, mg/dL	96.2 (73; 144.1)	105.2 (70.7; 144.5)	0.95 (-2.3; 0.4)	0.8	112.2 (98.7; 146.9)	131.9 (86.7; 180.6)	19.7 (-12; 33.7)	0.7
HDL-Cholesterol, mg/dL	66.3 (48.2; 73.8)	59.1 (42.3; 76.5)	-1.6 (-5.9; 2.7)	0.09	55.8 (49.6; 58.4)	52.5 (44.5; 59.2)	-3.3 (-5.1; 0.8)	0.2

Data are reported as geometric mean and (range). General linear model adjusted for age, gender, and BMI change

reduction for LDL-cholesterol was reported. Conversely, no significant changes in these parameters during the control period with the control pasta, were observed.
Variables related to the rheological characteristics of blood were also investigated according the two different dietary interventions. After adjustment for age, gender, and BMI change, respectively, WBV at high shear rates were significantly improved at the end of the intervention period (4.5 vs. 4.3 mPA*s; p=0.04 for pre and post-intervention, respectively), whereas no significant changes during the control period were observed. In addition, changes in DI during the test period were significantly improved (9.77 % vs. 10.3 %; p=0.03 for pre and post-test, respectively) with respect to those observed during the control period (Table 5). Fur-

Tabella 5 - Effect of dietary interventions with test and control pasta on haemorheological profile

Variable	Test Pre	Test Post	Change	p	Control Pre	Control Post	Change	p
			(post-pre)				(post-pre)	
WBV 94.500 sec-1, mPA*s	4.5 (4.3; 4.8)	4.3 (4.2; 4.4)	-0.2 (-0.1; -0.4)	0.04	4.6 (4.3; 4.9)	4.4 (4.2; 5.2)	0.1 (-0.1; 0.3)	0.4
WBV 0.512 sec-1, mPA*s	26.9 (25.7; 28.6)	25.4 (23.0; 29.1)	-1.5 (-2.7; 0.5)	0.5	21.8 (17.9; 25.1)	23.1 (21.2; 25.3)	1.3 (-0.2; 3.3)	0.3
EF, %	(8.2; 10.4) 9.8	(8.6; 11.2) 10.3	(0.4; 0.8) (0.4; 0.8)	0.03	(9.3; 11.7) (9.3; 11.7)	(8.5; 11.8) (8.5; 11.8)	(-0.7; 0.1) (-0.7; 0.1)	0.7 0.7
PLV, mPA*s	1.32 (1.29; 1.35)	1.32 (1.28; 1.37)	0 (-0.01; 0.02)	0.1	1.33 (1.30; 1.38)	1.38 (1.33; 1.45)	0.05 (0.03; 0.07)	0.02

Data are reported as geometric mean and (range). General linear model adjusted for age, gender, and BMI change

thermore, a significant worsening of the parameters related to PLV was reported in the control period (1.33 vs. 1.38 mPA*s; p=0.02 for pre and post-control, respectively), whereas in the intervention period no significant differences were obtained.

Discussion

The present is the first paper showing that pasta produced from a stone-ground flour obtained from an old grain variety (*Senatore Cappelli*) is able to result in moderate positive effects on lipid, and haemorheological profiles, which likely determine a reduction of the risk of atherosclerotic disease. Conversely, the consumption of commercially available pasta, for the same duration, was not able to produce significant effects on the atherosclerotic risk profile of the same participants.

Previous studies reported that consumption of whole-wheat products is inversely related to the mortality from and incidence of diabetes and ischemic heart disease (10, 11, 13). Conversely, the intake of refined products, (consisting mainly of the starchy endosperm), has not been shown to be associated with a reduced diabetes and ischemic heart disease risk.

The results obtained from the present experiment support the beneficial effects of consuming whole-wheat cereals on human health. Stone-grinding the entire seed produces semolina rich in fiber, resistant starch, vitamins (in particular B-group' vitamins), trace mineral elements and phytochemicals (phytoestrogens, antioxidants and phenols) that produce a synergistic effects on reducing various diseases, including cardiovascular disease. These beneficial effects are most likely attributable to a combination of effects rather than the effect of any single component. Replacing commercial pasta (refined semolina), which did not produce the same effects on the same participants, with whole-wheat pasta significantly reduced risk. Studies conducted by the US Nurses' Health Study showed that women who consumed a cereal fibre-rich diet had a 30% less chance of developing coronary heart disease (10,12). Interestingly, this effect was not observed in a vegetable-rich fiber diet, so indicating that of the various sources of fiber (cereals, fruit and vegetables), only those derived from the cereals were associated with a reduced risk of cardiovascular disease (12).

In addition to the benefits obtained from consuming a whole-wheat product, the benefits from consuming an old wheat variety cannot be excluded. As mentioned previously, ancient wheat varieties were reported to contain a greater variability in biologically active compounds with antioxidant activity than modern varieties (5,6). In particular, *Senatore Cappelli* is representative of an old variety in which there is a large diversity in polyphenolic compounds (6).

To the best of our knowledge, this is the first report of a possible influence of dietary intervention with whole-wheat pasta on a haemorheological profile. Epidemiological studies showed that increased blood viscosity is associated with several cardiovascular risk factors as well as with both prevalent and incident cardiovascular diseases. Mechanisms by which elevations in rheological factors may promote cardiovascular events are different and include increases in blood pressure, shear stress, ischemia, and blood vessel wall interactions. A relevant feature of the rheological flow is the erythrocyte morphology since the deformability of circulating cells greatly influences the rheological properties of the blood, so playing a key role in maintaining and regulating the microcirculation. Under pathologic conditions, the erythrocyte deformability is altered, thus affecting the rheological environment at the level of the microcirculation. We previously reported influence of diet on haemorheological parameters (9). In the present study a significant

improvement of all the haemorheological parameters after the dietary intervention with the test pasta was observed in comparison to the control pasta, thereby improving the deformability of red cells and rheological characteristics of the blood. The mechanisms by which *Senatore Cappelli* pasta may have contributed to the health benefits observed in the participants remains to be fully elucidated. As mentioned previously, whole grains are a rich source of fiber, minerals (magnesium, potassium, phosphorus, selenium, manganese, zinc, and iron), vitamins (B group, and vitamin E), and related antioxidants. These compounds may all produce distinctive biological functions, acting in a synergistic manner, which are important in reducing cardiovascular risk.

Although the present study provided positive results supporting the beneficial effects of consuming whole-wheat pasta produced from an ancient variety, it is important to note that the study has several limitations. The sample size of the study (20 participants) and the design of the study constitute a principle limitation. Larger studies involving more participants, in a blinded and randomized design, conducted over a longer time period need to be performed before it will be possible to draw any firm conclusion on the effects of such food products on human health. The present results merely represent a promising basis for a more comprehensive evaluation of this aspect of clinical nutrition. An additional limitation is the lack of knowledge relating to the dietary habits and physical activity habits of the study population. The possibility that changes in dietary and/or lifestyle habits may have significantly affected the parameters investigated cannot be excluded, even though participants were instructed by physicians and by an expert dietician to maintain their usual lifestyle habits during the course of the study. Although the test does not have a high statistical power (owing to the fact that the overall number of participants was a small number), the change in cholesterol was significant. These findings are relevant since an amelioration of the cholesterol levels (decrease of 10.3% in total cholesterol) determine a significant reduction of overall risk of incidence and/or mortality from cardiovascular disease.

Conclusions

In conclusion, this pilot intervention study reported a supposed beneficial effect of an old wheat variety of grain on some biomarkers of cardiovascular disease. The positive effects obtained from the consumption of *Senatore Cappelli* pasta are invariably attributed to both the use of whole-grain semolina and the synergistic effects of various phytochemical substances. Further investigations are necessary to verify these hypotheses. Nonetheless, old wheat varieties represent a genetic heritage that should not be neglected in genetic programs for the improvement, not only for the productive and technological characteristics, but also for the beneficial effects on health.

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