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Effects of Nitrogen plus Sulfur Fertilization and Seeding Density on Yield, Rheological Parameters, and Asparagine Content in Old Varieties of Common Wheat (*Triticum aestivum* L.)

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Abstract: Numerous epidemiological studies have highlighted the positive effects on health of wholegrain bakery products made from 'old' common wheat (*Triticum aestivum* L.) varieties. However, 'old' common wheat varieties display poor rheological properties, and there is limited information on its free asparagine (ASN) content, the main precursor to acrylamide during the baking process. This paper evaluates the effects of two seeding density levels (SD: 200 and 350 seed m^{-2}), three nitrogen levels (NL: 35, 80 and 135 kg N ha^{-1}), and two sulfur levels (SL: 0 and 6.4 kg S ha^{-1}) towards improving the grain yield (GY), rheological characteristics, and ASN content of 14 'old' common wheat varieties. SL and SD treatments significantly increased GY without decreasing the protein content (PC), while NL significantly increased the PC without affecting GY. The dough strength (W) increased significantly with increasing SL and NL but was significantly reduced with increasing SD. ASN significantly increased by 111% as NL increased from 35 to 135 kg ha^{-1}, while ASN significantly decreased by 85.1% with the SL treatment. The findings show that 135 kg N ha^{-1} combined with 6.4 kg S ha^{-1} can improve the technical performance of 'old' wheat wholegrain flours while maintaining the ASN as low as possible.

Keywords: old common wheat varieties; agronomic treatments; sulfur fertilization; free asparagine; rheological properties

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1. Introduction

Common wheat (*Triticum aestivum* L.) is one of the most important cereals worldwide for both human and livestock consumption, contributing towards enhancing the global economy [1,2]. Common wheat production amounted to 761 Mt in 2020 [3] and provides protein for the nutrition of both humans and livestock, estimated at around 60 Mt y^{-1}, as reported in Shewry (2009) [4]. After the Green Revolution, common wheat production increased, attributable to intensive fertilizer use and the breeding of cultivars, respectively, characterized by increased tolerance to diseases and pests, higher nutrient use efficiency, as well as a higher protein production per hectare, with a gluten composition suitable for industrial processing [5–9]. Conventionally, common wheat cultivars registered before the late 1960s are referred to as 'old', while those registered coinciding with the period of the Green Revolution are referred to as 'modern' [7].

In the past decades, 'old' common wheats varieties have been reintroduced, and many local micro-economies have been developed around 'old' cultivars [7,10]. In fact, the increase in pollution and food security problems has led us to reconsider common wheat production in terms of not only productivity but also of environmental and human health impacts [11]. Interest in low impact and sustainable agricultural practices, combined with functional (health-promoting) products, has permitted the rediscovery of 'old' common wheat varieties, considered to be more suited to unfavorable environmental factors and with improved functional value in comparison to the 'modern' varieties [12]. Numerous epidemiological studies have highlighted the positive effects on health and disease prevention of bread and other bakery products made from 'old' varieties [13,14]. In particular, the production of wholegrain bakery products is recommended as most bioactive compounds, associated with health benefits, are concentrated in the bran and aleurone layers, respectively [15,16]. However, although the aleurone layer also contains good quality free amino acids and proteins, it also stores free ASN, which is the predominant precursor of acrylamide formation in wholegrain bakery products [16,17]. As acrylamide is classified as a neurotoxin and "probably carcinogenic to humans" by the International Agency for Research on Cancer [18], free ASN concentration in grain should be monitored and maintained as low as possible. Corol et al. (2016) [19] found the free ASN contents in 150 genotypes of common wheat, ranging from 0.32 to 1.56 mg g^{-1} of dry matter (corresponding to 2.4–11.8 micromoles g^{-1} of dry matter) in wholemeal wheat flours. The 'old' cultivars are characterized by poor efficiency in converting assimilated nitrogen (N) to grain protein; this may contribute to an increased accumulation of ASN [20]. Furthermore, the grain ASN content may increase in relation to stress conditions such as waterlogging, drought, and plant diseases, as well as either nutrient excesses or deficiencies [21]. Of all the essential nutrients applied in the field, N is the most important for vegetative crop growth, productivity, and grain quality, thereby affecting plant development [22]. Sulfur (S) is an essential element for wheat nutrition, and S deficiency significantly affects the production and quality of wheat [23]. Interestingly, it was observed that ASN formation was correlated positively with N availability [24] but was increased in the presence of S deficiencies [20]. In this context, Wilson et al. (2020) detected free ASN concentrations ranging from 21.0 to 41.4 micromoles g^{-1} in S-deficient conditions. Aside from the effects on ASN, S affects not only N utilization and grain quality [25] but also plays an important role in baking quality [7]. Thus, optimized S and N fertilization practices can be implemented to reduce the ASN concentration in wholegrain common wheat and, consequently, act towards reducing the health concern of acrylamide in the baked products [26].

Despite the increased interest in old varieties for functional benefits and low input agricultural practices, these varieties are also usually characterized by a low dough strength (W) and an unbalanced ratio between dough tenacity and dough extensibility (P/L) compared to modern varieties. These rheological parameters render old varieties more difficult to bake [7]. In order to improve the rheological properties of both old common and durum varieties, research on fertilizer supplements is currently being investigated [7,27].

While ASN content in common wheat grain has been studied extensively on a global scale [20,21,28,29], only limited information on ASN concentrations in 'old' cultivars is available [30]. Given the increasing importance of 'old' cultivars and the success of crop management strategies in reducing ASN content in 'modern' cultivars, to the best of our knowledge, there is no work specifically focused on reducing the ASN concentration in the grain of 'old' cultivars. To address this aspect, the present study is aimed at investigating grain yield, dough rheology, and ASN concentration of 14 "old" Italian *Triticum aestivum* L. varieties in response to varying seeding density (SD) as well as N and S fertilization rates. The objective is to simultaneously evaluate the capacity of these agronomical practices in improving the technical performance of the dough whilst maintaining the lowest levels of ASN.

2. Materials and Methods

2.1. Field Experiment

The experimental field trials were conducted at the demo-farm "Tenuta di Cesa" in Marciano della Chiana, Tuscany (Lat. 43.3095; Lon. 11.8264; 246 m asl) from September 2017 to July 2019 under rainfed conditions on an alkaline clay-loam soil (Table 1).

Table 1. Soil properties.

Soil Parameters	Value
Sand (%)	37
Clay (%)	34
Silt (%)	27
pH	8.13
Organic matter (%)	0.88
Total N (%)	0.03
Olsen available P (mg kg^{-1})	0.42
Available S (mg kg^{-1})	3.3

The soil was characterized by a low organic matter content and low nutrient availability. In particular, the soil was both phosphorous- and sulfur-deficient, with less than 10 mg kg^{-1} available P [31] and S [32], respectively. Fourteen old Italian varieties of common wheat (*Triticum aestivum* L.) were investigated. The varieties were: Acciaio (AC), Andriolo (AN), Autonomia A (AU_A), Autonomia B (AU_B), Bianco Nostrale (BI), Frassineto 405 (FR), Gentil Bianco (GB), Gentil Rosso (GR), Gentil Rosso Aristato (GR_A), Gentil Rosso Mutico (GR_M), Inallettabile (IN), Mentana (ME), Sieve (SI), and Verna (VE) (Table 2).

Table 2. Release year and origin for the wheat cultivars used in this study. Data were obtained from the website of the seed bank in the Tuscany Region [33].

Variety	Year of Release	Origin
AC	1950	Selection of "Mara", in turn, selection of "Frassineto 405"
AN	1933	Selection of the local landrace "Andriolo"
AU_A	1938	"Frassineto 405" × "Mentana"
AU_B	1930	"Frassineto 405" × "Mentana"
BI	1927	Selection of the local landrace "Bianco Nostrale"
FR	1932	Pureline selection of "Gentil Rosso"
GB	1900	Local landrace dating back to the late 19th century
GR	1900	Local landrace dating back to the late 19th century
GR_A	1900	Selection of the local landrace "Gentil Rosso"
GR_M	1900	Selection of the local landrace "Gentil Rosso"
IN	1920	Selection of "Hatif Inversable"
ME	1913	("Wilhelmina" × "Rieti 21") × "Akakomugi"
SI	1960	"Est Mottin 72" × "Bellevue II"
VE	1953	"Est Mottin 72" × "Mont Calme"

Five of the old varieties in the trial were derived from the older varieties that were used as parental material. These included AU_A and AU_B, which were derived from crossing ME × FR, and FR, GR_A and GR_M, derived from the selection of the GR landrace. The genealogy and release dates of the varieties were obtained from the website of the seed bank in the Tuscany Region [33].

The 14 wheat genotypes (Gen) were evaluated during two growing seasons (Y) with 12 agronomic treatments comprising two seeding densities (SD) (200 and 350 kg seed m^{-2}, namely, SD200 and SD350, respectively), three nitrogen fertilization rates (NL) (35, 80 and 135 kg N ha^{-1}, namely, NL35, NL80, NL135, respectively), and two sulfur fertilization rates (SL) (0 and 6.4 kg S ha^{-1}, namely, SL0 and SL6.4, respectively) (Figure 1). The experiment was established as a strip-plot design with three replicate blocks per year. Gen was arranged in vertical strips as the main plot, SD was assigned to the vertical sub-plots, SL was applied horizontally in sub-sub-plots, and, lastly, NL was assigned to horizontal sub-sub-subplots, respectively. Each sub-sub-subplot was 14.4 m^2 (width of 1.44 m and length of 10 m).

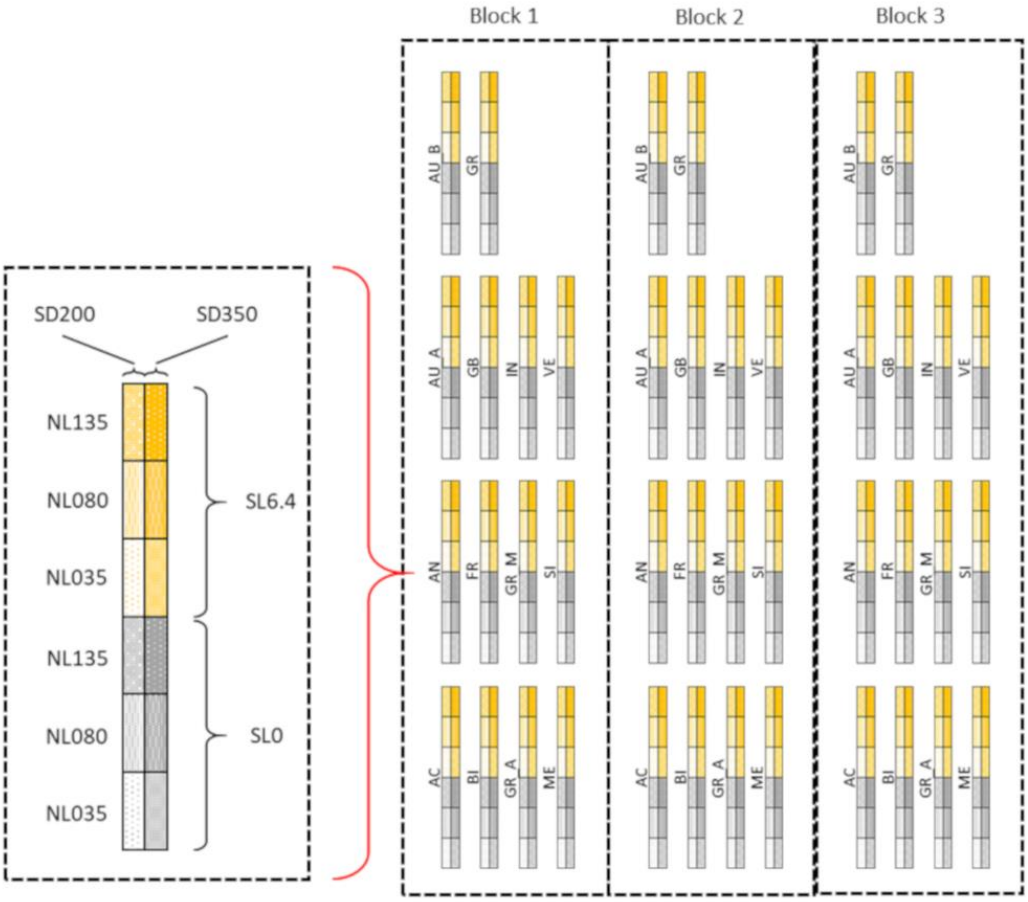

Figure 1. Experimental design and plot layout of trials (not in scale); On the left, the plot layout for each variety: SD200 and SD350 represent seeding density of 200 and 350 kg seed m^{-2}, respectively; NL35, NL80 and NL135 represent the three nitrogen fertilization rates of 35, 80 and 135 kg N ha^{-1}, respectively; SL0 and SL6.4 represent the two sulfur fertilization rates of 0 and 6.4 kg S ha^{-1}, respectively. On the right is the disposition of plots within the blocks for different varieties.

Soil tillage was carried out to a depth of 0.4 m with a moldboard plow in both September 2017 and 2018, followed by a tandem disk harrow (0.1 m depth) to break the clods. Before seeding, 174 kg ha^{-1} of triple superphosphate (P_2O_5: 46%) was broadcasted and immediately incorporated into the soil by means of a tandem disk harrow (0.05 m depth). The seeding was performed on 20 November and 15 November in the first and second year, respectively. Nitrogen application was implemented over three distinct periods. Initially, 20% nitrogen was broadcasted at seeding as ammonium nitrate (N: 26%). Thereafter, 40% was spread at tillering as ammonium nitrate (N: 26%), with a final 40% at the beginning of the stem elongation as urea (N: 46%). As suggested in Guerrini et al. (2020), in S6.4, a total of 6.4 kg S ha^{-1} was distributed at booting by spraying a solution containing 20 g L^{-1} of wettable sulfur powder (80% a.i.; Thiovit Jet 80WG®, Syngenta, Basel, Switzerland). At tillering, a broadleaf herbicide treatment was performed by distributing Manta Gold (Syngenta, Basel, Switzerland) at a dose of 2.5 L ha^{-1} (60 g L^{-1} fluroxipir acid, 23.3 Clopyralid, and 266.7 g L^{-1} MCPA acid). The monocot weeds were removed from each plot by performing manual weeding at tillering and at stem elongation. In both growing seasons, no crop damage by weeds, insects, or diseases was observed. Common wheat was harvested at commercial maturity (grain moisture <13%) on 12 July 2018 and

5 July 2019. For each sub-sub-subplot, the grain biomass was calculated to determine the grain yield per hectare (GY, t ha^{-1}).

2.2. Meteorological Conditions

The climatic conditions were typically Mediterranean, with average daily temperatures around 13 °C and approximately 750 mm of rain per year, mostly concentrated in autumn and spring, as well as the dry summer period (Figure 2).

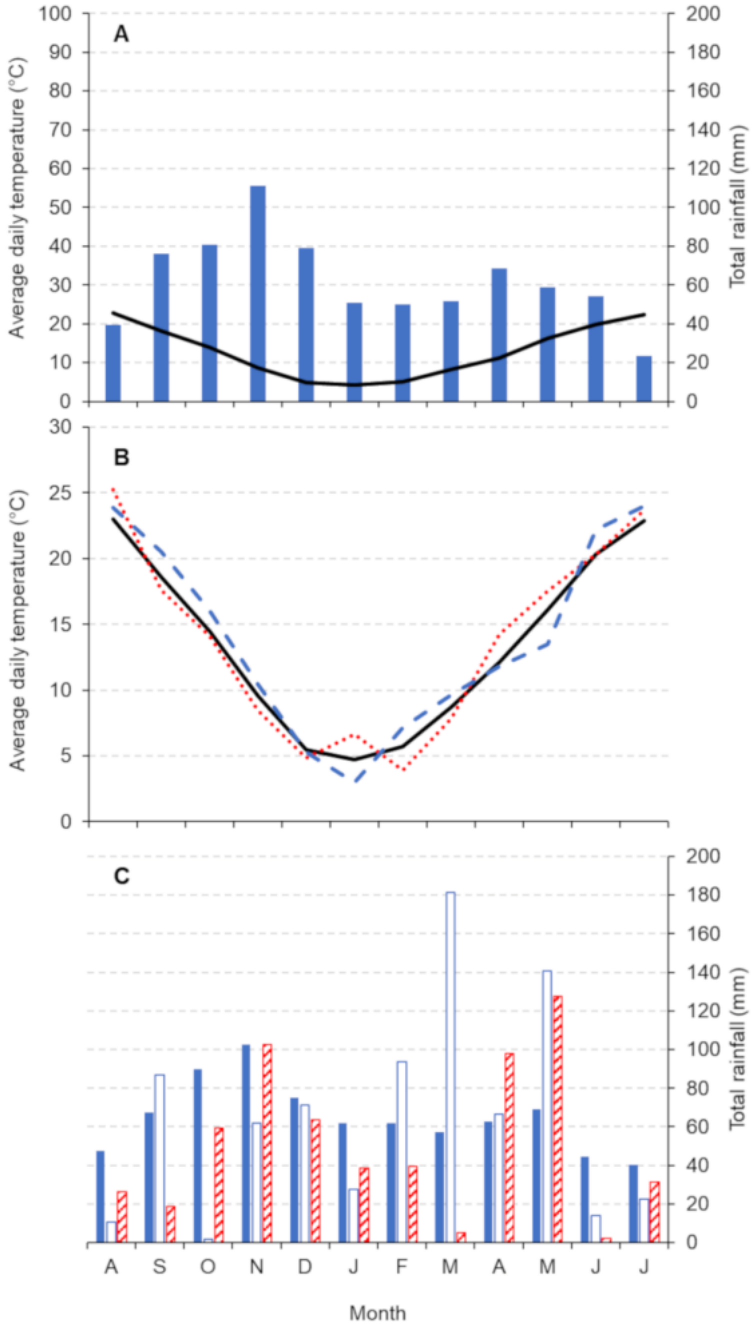

Figure 2. (**A**) Walter-Lieth climate diagram of the study site (data 2001–2020), with monthly daily average temperature (°C, black continuous line) and monthly average rainfall amount (mm, histograms). (**B**) Comparison of the monthly daily average temperature (mm) measured during 2001–2020 (continuous line), 1st growing season (dashed line) and 2nd growing season (dotted line); (**C**) comparison of the monthly rainfall amount (mm) measured during 2001–2020 (color-filled histograms), 1st growing season (hollow histograms) and 2nd growing season (diagonal-filled histograms).

The average temperature pattern during both growing seasons was consistent with the long-term temperature pattern. However, the average temperature values across the first and second growing seasons (13.7 and 13.9 °C, respectively) were higher than the long-term average (13.0 °C). In both years, rainfall distribution data fluctuated significantly with respect to the long-term rainfall pattern.

During the first growing season, excess rainfall was recorded from February to May, corresponding to the tillering to flowering phenological stage of common wheat. Then, a shortage of rainfall was experienced in June, the month coinciding with grain filling. The average temperature values at flowering and grain filling in the spring season of 2018 were slightly warmer than the long-term averages by about 1.2 and 0.5 °C, respectively.

During the second growing season, excess rainfall was recorded in April and May (from the booting to flowering phenological stage of common wheat), while a rainfall shortage was experienced in March, coinciding with stem elongation, as well as June. During the summer months of 2019, the daily average temperature at flowering was lower than the long-term average by 2.7 °C, while the average temperature at grain filling exceeded the long-term average by 2.4 °C. Therefore, between May and June 2019, there was a temperature increase of 8.7 °C, which could have resulted in stress for the plants during both the initiation and grain-filling phases.

2.3. Analysis of Kernels and Dough

The 1000 kernel weight (TKW, g 1000^{-1} seeds) and hectoliter weight (HW, kg hL^{-1}) were determined according to ISO 7971-1 (2009) and ISO 520 (2010) [34,35]. For each treatment, wholemeal flour samples were obtained by milling kernel samples in a grinder with a 0.5 mm screen (Cytolec 1093 lab mill, FOSS Tecator, Hoganas, Sweden), as reported in Guerrini et al. (2020) [7] and Žilić et al. (2011) [36]. The wholemeal flour samples (5 mg) were analyzed with a CHNS analyzer (CHN-S Flash E1112, Thermo-Finnigan LLC, San Jose, CA, USA) to determine total nitrogen percentage and then converted to total protein percentage (PC, %) by multiplying by 5.7, according to ICC Standard 167 (2000) [37]. The protein yield per hectare (PY, kg ha^{-1}) was calculated as the product of GY by PC. The ASN concentration in wholegrain flour (ASN, micromoles g^{-1}) was determined using an enzymatic method (K-ASNAM L-Asparagine/L-Glutamine/Ammonia kit; Megazyme, IL, USA) followed by spectrophotometric quantification (340 nm) using a Lambda 20 spectrophotometer (PerkinElmer Waltham, MA, USA), as reported by Lecart et al. (2018) [38].

Dough rheology was performed according to ISO 27971 (2015) [39]. Briefly, wholegrain flour (250 g) was mixed in the Chopin alveograph chamber with a NaCl solution (2.5% *w*/*w*) for 8 min without adding yeast. The resulting dough was extruded and allowed to rest for 20 min before performing the alveograph parameters: the ratio between dough tenacity and dough extensibility (P/L) and the dough strength (W; 10^{-4} J). TKW, HW, and PC were determined for each sub-sub-subplot, while ASN, W and P/L were determined for each treatment on a bulk from the three replicates.

2.4. Statistical Analysis

Data were analyzed using a mixed model analysis of variance. Both years' trial data were analyzed together. Data analysis was carried out in R studio (software version 1.1.456). A 4-way ANOVA was applied to determine the main effect of the four agronomical factors with their interactions. Significance was determined as: * = 0.05, ** = 0.01, *** = 0.001, n.s. = not significant. Differences between averages were compared for significance by means of the Tukey honest significant difference (Tukey HSD) test ($p < 0.05$).

3. Results

3.1. Agronomic Traits and Kernel Analyses

The Y was the dominant factor for GY, followed by SL, Gen and SD, while the NL did not significantly affect GY (Table 3).

Additionally, GY was significantly affected by the interaction Y × SD, whilst no interactions between Y × SL and Y × NL, respectively, were found to be statistically significant. Statistically significant differences were detected in the interaction genotype–environment. The highest average GY was measured in AU_A, followed by AU_B and SI, while the lowest average GY values were measured in AC, followed by FR and GB, respectively (Table 4). SD significantly affected average GY, which increased by 5.4% from SD200 to SD350 (Table 4). Results of the present study indicated that the SL6.4 treatment increased GY by 8.2% compared to SL0.

Table 3. Results of the ANOVA for grain yield (GY), hectoliter weight (HW), thousand kernel weight (TKW), protein concentration (PC) and protein yield (PY). The table columns report the Fisher F (F) and the significance levels: * = 0.05, ** = 0.01, *** = 0.001, ns = not significant.

Variability Sources	**DF**	**GY ($t\ ha^{-1}$)**		**HW ($kg\ hL^{-1}$)**		**TKW (g)**		**PC (%)**		**PY ($kg\ ha^{-1}$)**	
		F	**Sig**	**F**	**sig**	**F**	**sig**	**F**	**sig**	**F**	**sig**
Year	1	80.00	***	2.49	ns	40.60	***	170.00	***	32.40	***
NL	2	0.40	ns	0.53	ns	7.91	***	38.20	***	4.00	***
SL	1	34.20	***	0.3	ns	3.00	ns	2.39	ns	39.40	***
SD	1	15.50	***	0.17	ns	0.00	ns	15.00	***	23.10	***
Gen	13	32.40	***	6.54	***	23.90	***	10.70	***	32.80	***
SL × SD	1	0.23	ns	0.01	ns	0.02	ns	0.00	ns	0.30	ns
NL × SD	2	0.52	ns	0.02	ns	0.25	ns	0.37	ns	0.61	ns
NL × SL	2	2.76	ns	0.16	ns	0.80	ns	0.17	ns	3.02	*
Y × SD	1	9.48	*	0.47	ns	0.11	ns	5.30	*	13.40	**
Y × SL	1	0.24	ns	0.23	ns	5.79	*	1.07	ns	0.39	ns
Y × NL	2	1.97	ns	0.34	ns	0.48	ns	2.36	ns	2.29	ns
Residuals	980										

In the present study, Gen was the sole factor affecting HW (Table 4). Furthermore, Gen was the dominant factor for TKW, followed by Y, NL, the second-order interaction Y × SD and SL, respectively (Table 3). Among the 14 varieties, the highest HW was measured in AU_A, followed by AU_B, while the lowest HW was measured in GR_M, followed by VE and IN, respectively (Table 4).

The highest TKW was measured in GR, followed by GR_A and GR_M, while the lowest TKW was measured in AN and VE (Table 4). The TKW values were found to be significantly decreased by 9.6%, with the increase from NL35 to NL135.

According to the ANOVA, the PC was significantly dominated by Y, followed by NL, SD, and Gen, while SL did not have a significant effect (Table 3). On the contrary, SL was the dominant factor for PY, followed by Gen, Y, SD and NL (Table 3). As regards the second-order interaction, only Y × SD affected both PC and PY, while NL × SL significantly affected only PY (Table 3). Results indicated that sulfur application (SL6.4) increased PY by 8.7% with respect to SL0 (Table 4). The highest SD treatment significantly increased the PC and PY values with respect to the control by 1.4% and 6.6%, respectively. Furthermore, the PC and PY significantly increased by 3.8% and 4.5%, respectively, from NL35 to NL135.

Table 4. Grain quality parameter mean values (standard error in brackets) of 14 old common wheat varieties as a function of genotype (Gen), nitrogen (NL) and sulfur fertilization (SL), and seeding density (SD). First-order interactions are provided for SD, NL, SL and Y. Lowercase letters represent the Tukey HSD post hoc test results. The table columns report the significance levels: *** = 0.001, ns = not significant.

Variability Sources	**GY ($t\ ha^{-1}$)**		**HW ($kg\ hL^{-1}$)**		**TKW (g)**		**PC (%)**		**PY ($kg\ ha^{-1}$)**	
	Average	**sig**	**Average**	**sig**	**Average**	**sig**	**Average**	**sig**	**Average**	**sig**
Gen		***		***		***		***		***
AC	3.36 (0.15)	e	80.1 (0.86)	ab	43.57 (0.42)	e	15.17 (0.11)	a	502.67 (21.67)	gh
AN	4.14 (0.09)	bc	79.33 (0.85)	ab	38.72 (0.34)	f	14.74 (0.1)	abcd	612.39 (15.88)	def
AU_A	5.41 (0.14)	a	81.89 (0.77)	a	46.7 (0.52)	abcd	14.64 (0.12)	bcd	788.35 (20.06)	a
AU_B	5.03 (0.12)	a	81.75 (0.91)	a	44.52 (0.54)	de	14.7 (0.11)	bcd	736.59 (16.73)	ab
BI	4.2 (0.14)	bc	79.07 (0.82)	ab	47.06 (0.49)	abcd	14.97 (0.07)	ab	629.77 (21.6)	de
FR	3.44 (0.13)	de	72.75 (1.12)	c	47.73 (0.79)	ab	14.18 (0.15)	e	483 (17.65)	h
GB	3.78 (0.05)	cde	79.05 (0.72)	ab	47.26 (0.72)	abc	14.92 (0.08)	abc	563.99 (8.35)	efg
GR	4.39 (0.06)	b	78.13 (0.7)	ab	48.76 (0.53)	a	14.53 (0.07)	bcde	638.1 (10.51)	cd
GR_A	3.87 (0.07)	cd	78.7 (0.59)	ab	48.56 (0.51)	a	14.31 (0.11)	de	555.01 (11.78)	fg
GR_M	3.85 (0.07)	cde	77.05 (0.9)	b	48.11 (0.44)	a	14.63 (0.09)	bcd	564.74 (11.53)	efg
IN	3.93 (0.08)	bcd	77.57 (0.99)	b	47.03 (0.81)	abcd	14.13 (0.11)	e	554.14 (11.78)	fgh
ME	4.24 (0.15)	bc	79.87 (0.79)	ab	45.15 (0.5)	bcde	14.48 (0.13)	cde	607.21 (19.84)	def
SI	4.92 (0.11)	a	78.58 (0.94)	ab	44.72 (0.55)	cde	14.31 (0.09)	de	702.15 (15.3)	bc
VE	3.84 (0.09)	cde	77.33 (1.1)	b	43.69 (0.54)	e	14.84 (0.11)	abc	567.13 (12.7)	defg
SD		***		ns		ns		***		***
SD200	4.06 (0.05)	b	78.75 (0.35)		45.83 (0.23)		14.51 (0.05)	b	588.12 (6.65)	b
SD350	4.28 (0.05)	a	78.56 (0.33)		45.82 (0.25)		14.71 (0.04)	a	626.92 (7.32)	a
NL		ns		ns		***		***		***
NL35	4.14 (0.06)		78.97 (0.39)		46.56 (0.3)	a	14.34 (0.05)	c	591.88 (8.04)	b
NL80	4.2 (0.06)		78.61 (0.45)		45.8 (0.31)	ab	14.61 (0.05)	b	611.89 (8.8)	ab
NL135	4.17 (0.06)		78.38 (0.41)		45.12 (0.27)	b	14.88 (0.05)	a	618.78 (8.94)	a
SL		***		ns		ns		ns		***
SL0	4.01 (0.05)	b	78.53 (0.31)		45.57 (0.25)		14.57 (0.04)		582.18 (6.7)	b
SL6.4	4.34 (0.05)	a	78.78 (0.37)		46.08 (0.24)		14.65 (0.04)		632.85 (7.19)	a

Table 4. *Cont.*

Variability Sources	GY (t ha^{-1})		HW (kg hL^{-1})		TKW (g)		PC (%)		PY (kg ha^{-1})	
	Average	**sig**	**Average**	**sig**	**Average**	**sig**	**Average**	**sig**	**Average**	**sig**
Y		***		ns		***		***		***
2018	3.92 (0.04)	b	79.02 (0.34)		46.77 (0.26)	a	14.94 (0.04)	a	584.54 (6.48)	b
2019	4.42 (0.05)	a	78.29 (0.34)		44.89 (0.22)	b	14.28 (0.04)	b	630.5 (7.53)	a

3.2. Alveograph Parameters and Free Asparagine Content in Whole Flour

As regards the main factor, NL was the dominant factor for W, followed by SL, SD, Gen and finally Y in decreasing order, respectively (Table 5). Additionally, W was strongly affected by the second-order interaction NL × SL, while no interactions between Y and the agronomic treatments were detected.

Table 5. Results of the ANOVA for dough strength (W), the ratio between dough tenacity and dough extensibility (P/L), and ASN concentration in whole flour. The table columns report the Fisher F (F) and the significance levels: * = 0.05, ** = 0.01, *** = 0.001, ns = not significant.

Variability Sources	DF	W (10^{-4} J)		P/L		Asparagine (Micromoles g^{-1})	
		F	**sig**	**F**	**sig**	**F**	**sig**
Year	1	11.9	**	0.298	ns	215.0	***
NL	2	446.0	***	47.9	***	300.0	***
SL	1	77.4	***	5.66	*	3966.0	***
SD	1	67.0	***	35.5	***	0.0	ns
Gen	13	28.2	***	62.3	***	15.9	***
SL × SD	1	0.3	ns	11.6	***	0.0	ns
NL × SD	2	3.4	*	0.69	ns	0.0	ns
NL × SL	2	52.9	***	38.3	***	177.0	***
Y × SD	1	0.2	ns	0.11	ns	0.0	ns
Y × SL	1	0.3	ns	0.719	ns	53.7	***
Y × NL	2	0.5	ns	0.169	ns	6.1	***
Residuals	308						

The highest W was measured in SI, followed by GB and FR, while the lowest values were measured in AN, followed in increasing order by BI, VE, GR_M, and ME, respectively (Table 6). The W decreased by about 19.3% as SD increased from SD200 to SD350 (Table 6). In contrast, the W value increased by 84.4% and 15.9% with the NL treatment (from N35 to N135) and the SL treatment, respectively. The S fertilization did not affect the W at N35, while W increased when S was applied at the NL80 and NL135 treatments, respectively (Figure 3).

Thus, at S0, W increased from 25% at NL80 to 55.5% at NL135, while at S6.4, the W increased from 37.4% at NL80 to 112.7% at NL135 compared to the lowest N fertilization level.

Table 6. Averages (standard error in brackets) of dough strength (W), the ratio between dough tenacity and dough extensibility (P/L), and asparagine concentration in whole flour as a function of genotype (Gen), nitrogen (NL) and sulfur fertilization (SL), seeding density (SD), and first-order interaction. Lowercase letters represent the Tukey HSD post hoc test results. The table columns report the significance levels: ** = 0.01, *** = 0.001, ns = not significant.

Variability Sources	**W (10^{-4} J)**		**P/L**		**Asparagine (Micromoles g^{-1})**	
	Average	**sig**	**Average**	**sig**	**Average**	**sig**
Gen		***		***		***
AC	62.74 (3.72)	cd	0.61 (0.03)	efg	19.74 (3.5)	bcd
AN	52.41 (3.42)	e	0.84 (0.06)	cd	19.71 (3.45)	bcd
AU_A	78.94 (5.08)	b	0.6 (0.03)	efg	16.99 (3.06)	cde
AU_B	78.42 (5.77)	b	0.71 (0.02)	de	17.92 (2.97)	cd
BI	55.22 (3.41)	de	0.55 (0.02)	fg	16.52 (3.03)	de
FR	80.7 (6.83)	ab	0.88 (0.06)	bc	17.23 (3.22)	cde
GB	81.35 (6.25)	ab	0.71 (0.04)	de	23.69 (4.39)	ab
GR	67.96 (2.56)	c	0.68 (0.03)	ef	22.94 (3.83)	ab
GR_A	63.5 (2.72)	cd	0.69 (0.04)	def	25.12 (4.72)	a
GR_M	62.16 (3.29)	cde	0.7 (0.03)	def	24.52 (4.52)	a
IN	63.33 (3.68)	cd	0.52 (0.03)	g	21.01 (3.76)	abc
ME	61.97 (2.83)	cde	0.71 (0.05)	de	17.92 (3.11)	cd
SI	89.67 (7.16)	a	1.54 (0.07)	a	13.64 (2.43)	e
VE	58.18 (3.69)	cde	1.03 (0.05)	b	17.1 (3.06)	cde
SD		***		***		ns
SD200	73.17 (1.13)	a	0.72 (0.02)	b	19.59 (0.77)	
SD350	63.67 (1.01)	b	0.82 (0.01)	a	19.58 (0.78)	
NL		***		***		***
NL35	49.27 (0.48)	c	0.82 (0.02)	a	11.66 (0.56)	c
NL80	65.16 (0.66)	b	0.84 (0.02)	a	22.48 (1.01)	b
NL135	90.83 (1.44)	a	0.65 (0.02)	b	24.6 (1.04)	a
SL		***		**		***
SL0	63.37 (0.82)	b	0.79 (0.01)	a	34.08 (0.59)	b
SL6.4	73.46 (1.27)	a	0.75 (0.02)	b	5.08 (0.12)	a
Y		***		ns		***
2018	70.56 (0.79)	a	0.77 (0.01)		16.23 (0.67)	b
2019	66.28 (0.77)	b	0.77 (0.01)		22.93 (0.79)	a

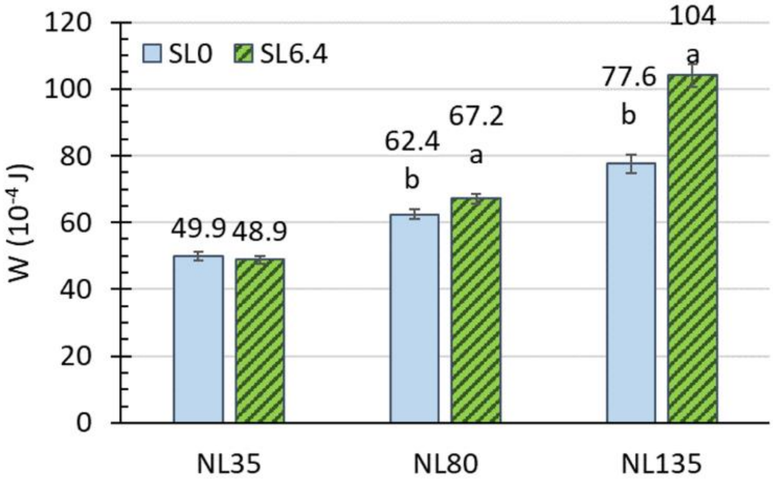

Figure 3. Effect of nitrogen fertilization level (NL) and sulfur fertilization level (SL) on dough strength (W). Lowercase letters represent the Tukey HSD post hoc test results.

In the present trial, P/L was significantly affected by genotype. Of the 14 varieties, 10 had optimal P/L ranges, while BI and IN showed lower values, with SI and VE showing higher values, respectively (Table 6). Regardless of the variety, P/L was not affected by Y, highlighting the strong genotype effect on this characteristic. Conversely, agronomical practices affected P/L. The increase in SD significantly increased the P/L (Table 6). As the main effect, SL significantly decreased P/L. However, the SL interactions with SD and NL need to be considered. SL decreased the P/L only at the lower SD, while no significant effect was found at the higher SD (Figure 4). P/L was also decreased at the higher NL, while no significant difference was found between NL35 and NL80. Instead, there was a significant decrease in P/L at NL135 in combination with the SL treatment (Figure 4). Moreover, the P/L value was shown to be below the 0.6 threshold with SL and NL135 treatments.

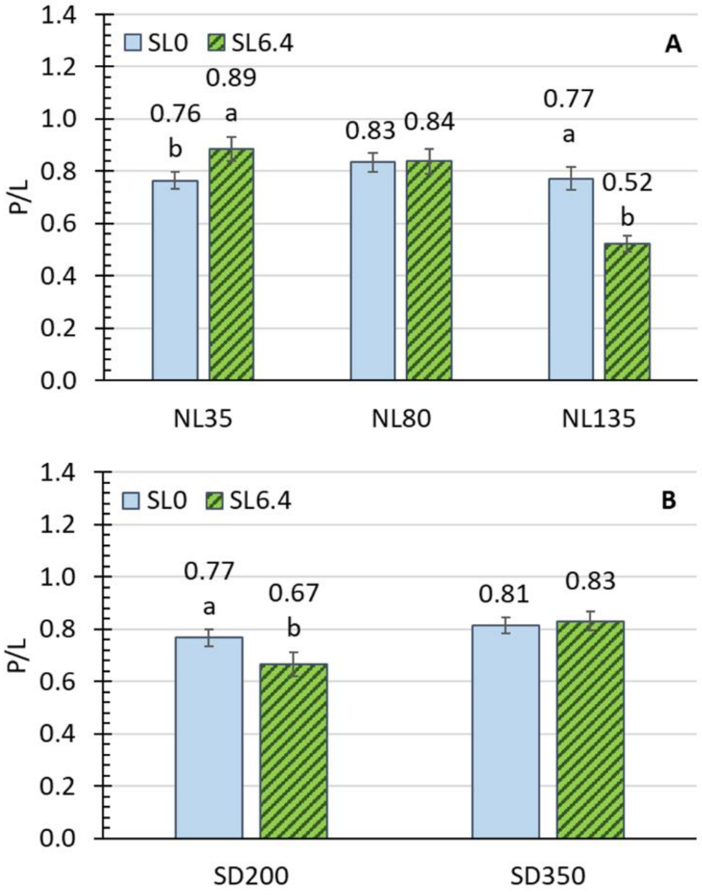

Figure 4. (**A**) Effect of nitrogen fertilization level (NL) and sulfur fertilization level (SL) on the ratio between dough tenacity and dough extensibility (P/L). (**B**) Effect of seeding density level (SD) and sulfur fertilization level (SL) on the ratio between dough tenacity and dough extensibility (P/L). Lowercase letters represent the Tukey HSD post hoc test results.

The SL treatment was by far the most important factor influencing the concentration of free ASN in grains, followed by NL, Y, and Gen in decreasing order, respectively (Table 5). SD was the only agronomic treatment not exerting a significant effect on free ASN concentration. The free ASN concentration in grain was affected by the second-order interaction NL × SL, followed by Y × SL and Y × NL (Table 5). Free ASN concentration in grain was significantly higher in 2019 than in 2018. When combining both years, the ASN content significantly increased from 92.8% at NL80 to 111% at NL135 compared to N35. Instead, the ASN content was shown to decrease by 85.1% with the SL treatment. In the present study, S fertilization was more effective in reducing the ASN concentration in 2018 than in 2019 (Figure 5). S treatment decreased the ASN concentration by 7.5 and 4.8 times in 2018 and 2019, respectively. At the same time, during the two growing seasons, N fertilization had a contrasting effect to that of S. In particular, the N fertilization increased the ASN concentration by 197% and 72% in 2018 and 2019, respectively. A more effective reduction in grain ASN concentration was observed at NL80 than at the remaining N fertilization levels (Figure 5). Particularly, the decrease in ASN content measured at SL0 and SL6.4, respectively, was not significantly different between NL35 and NL135 (6.09 and 6.01 times, respectively), while the decrease in ASN was significantly different at NL80 (8.2 times). The highest free asparagine concentration was measured in GR_A, followed by GR_M, GB, and GR, while the lowest values were measured in SI, followed in increasing order by BI, AU_A, VE, and FR, respectfully (Table 6).

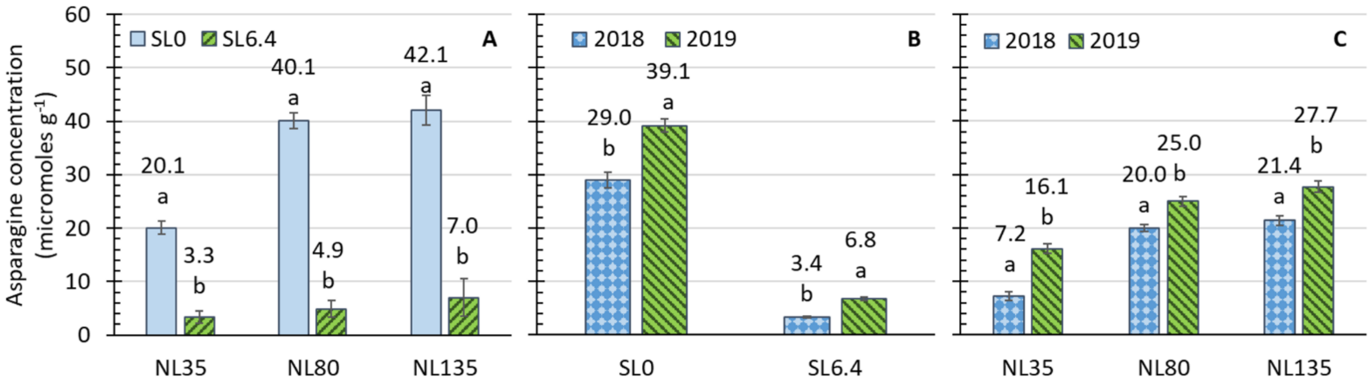

Figure 5. Interactions between years and agronomic treatments on asparagine content in grain; (**A**) Effect of nitrogen fertilization level (NL) and sulfur fertilization level (SL); (**B**) effect of year (Y) and sulfur fertilization level (SL); (**C**) effect of the year (Y) and nitrogen fertilization (NL). Lowercase letters represent the Tukey HSD post hoc test results.

Interestingly, when the average ASN levels determined in the present study were plotted against the date when the varieties were released, there was a significant decline ($R^2 = 0.69$, $p < 0.01$) in ASN content across the release year of the considered varieties (Figure 6).

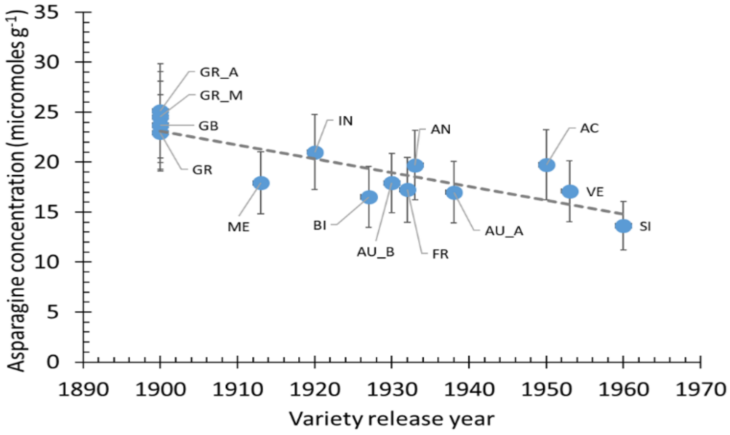

Figure 6. Scatter plots of free asparagine concentration in grain as a function of the variety year of release.

4. Discussion

In general, the results suggest that the N concentration in the soil of the study site was not a limiting factor for the growth and production of these 'old' common wheat genotypes. Gooding et al. (2002) [40] and Zhang et al. (2016) [41] found a significant interaction between N fertilization and seeding density in determining the kernel yield, whilst no interactions between SD × NL, respectively, were found to be statistically significant in this study. Our study corroborated previous results [42], indicating that genotypes having high tillering potential may benefit from SD up to 400 seeds m^{-2}. In contrast, Zhang et al. (2016) [41] found that SD increased from 120 to 180 plants m^{-2}, significantly increasing GY, with no further increases observed as SD increased from 180 to 240 plants m^{-2}. The present study suggests that the sulfur treatment can significantly increase GY. However, variable effects in response to sulfur treatment have been reported in previous literature. For example, Wilson et al. (2020) [20] found that foliar application of 20 kg S ha^{-1} increased GY by up to 55% compared to the control. Instead, Guerrini et al. (2020) [7] reported that sulfur treatment did not significantly affect GY of 'old' Italian common wheat landraces. In general, the present results corroborated those of Kilmer and Nearpass (1960) [32], indicating that crops respond to sulfur fertilization in sulfur-deficient soils. Salvagiotti and Miralles (2008) [43] showed that S fertilization increased grain yield in wheat by increasing nitrogen use efficiency (NUE). Further, Salvagiotti et al. (2009) [25] suggested that sulfur fertilization can increase the NUE in sulfur-deficient soils. In the 'old' varieties used in this study, the genotypic factor predominated on the HW and TKW, corroborating previous results for Italian landraces [7]. In contrast, in modern varieties, HW values were shown to increase, with increasing N up to 150 kg ha^{-1} [44]. Our study indicated a strong effect of N and S fertilization on PC and PY production. These results were consistent with previous findings in 'modern' common wheat varieties [45–49]. Likewise, Guerrini et al. (2020) [7] reported that S and N fertilization substantially affected the PC in 'old' varieties. Yu et al. (2021) [50] observed a reduced efficiency of sole N fertilization in increasing both protein and grain yield in sulfur-deficient soils. Further, Yu et al. (2021) [50] suggested that sulfur application can result in protein and grain yield increases by regulating glutamine synthetase 1 and improving nitrogen-use efficiency.

Our results suggest that nitrogen fertilization may be used as a tool to modify the dough deformation energy (i.e., alveograph W) in these 'old' varieties and highlight a positive synergy between N and S. The W values were consistent with those measured in previous studies [7,8]. As 'old' common wheat flours are usually characterized by a low W, any increase in this value can be regarded with interest as it improves the flour's bread-making characteristics [7,51]. Therefore, the observed increases in W with the NL and SL treatments, respectively, are of particular interest for 'old' common wheat varieties. The effect of S and N fertilization on W was consistent with those measured previously [7,52]. Considering all the varieties, the agronomic treatments were unsuccessful in increasing the W values above 90×10^{-4} J, which, according to the common classification, distinguishes biscuit flours from flours suitable for bread-making. However, the 90×10^{-4} J threshold was exceeded by five varieties at NL135 (132.3, 118.4, 117.8, 110.7, and 108.6 $\times 10^{-4}$ J in SI, GB, FR, AU_B, and AU_A, respectively), thus attaining the status of weak flours, attributable to this level of nitrogen fertilization. A P/L range of 0.6–0.8 is usually considered the optimal ratio between dough tenacity and extensibility (i.e., P/L) in bread-making flours [53]. P/L ratios exceeding 0.8 are known to be lacking in old varieties for bread-making as unrefined flours [51]. SI and VE have been extensively studied in the literature and are popular among bakers using flour from 'old' varieties, already known for high tenacity and low extensibility doughs [51]. In the literature, there has been speculation on the advantages of a blending strategy between the "poor" P/L wheats, such as BI and IN, and the most commonly used higher P/L wheats (SI and VE) in order to improve the bread-making performances, thereby promoting the valorization of local germplasm characteristics [7,51]. The dough parameters highlight the importance of agronomical practices in modulating the technological performance of dough in old, weak varieties. Old varieties are widely reported as having weaker dough, with unbalanced tenacity–extensibility ratios, rendering baking difficult. Hence, the effect of agronomical practices on dough strength necessitates investigation, with careful selection of SD, NL, and SL to optimize rheological parameters for the baking industry. The ASN concentration determined in 2019 was higher than in 2018. This was attributable to the stress incurred by the higher temperatures combined with lower precipitation over the entire growing season and, in particular, during the grain-filling stage. Similar interactions between ASN content and environmental stress conditions were also reported previously [20]. Results indicated that the N fertilization increased the ASN content, while sulfur fertilization was able to reduce the ASN content by up to 85.1%. This result was consistent with that observed by Wilson et al. (2020) [20], showing an increase in ASN content in response to increasing N. Moreover, present results were similarly consistent with various studies reporting higher ASN contents in wheat grains cultivated in sulfur-deficient soils [4,54]. In contrast, in soil with satisfactory S availability, S fertilization does not impact on the ASN content in grain [29,55]. Previously, it was noted that in three 'old' common wheat varieties (namely, AN, SI and VE), the albumin, globulin, and gliadin fractions were decreased significantly, whilst the glutenin fraction was significantly increased in response to S fertilization [7]. Thus, it could be possible that these 'old' common wheat varieties were highly responsive to S deficiency and that changes in the protein composition resulted in a significant increase in ASN content. The ASN content was consistent with that measured previously for wheat [20,30]. Poudel et al. (2021) [30] suggested that despite the absence of a legal limit for ASN concentrations in grain, this should be as low as possible. This is the first time that a negative correlation between the ASN content and the release year has been shown for old Italian common wheat varieties. Furthermore, significant correlations between free ASN and grain protein content were reported previously and shown to be higher in the old varieties [56]. Corol et al. (2016) [19] reported a weak correlation between ASN concentration and the release year. However, those authors also found that free ASN content was positively correlated to plant height [19], which, interestingly, is generally higher in the old varieties. In contrast, more recent work, analyzing the free ASN content in grain of 19 cultivars released between 1870 and 2013 across two growing seasons in the USA, showed that the free ASN concentration in grain was significantly increased in the second growing season across the release years, whilst no trend across release year was detected during the first growing season [30]. Given the scarcity of information, the requisite for further investigating this aspect in future research programs is evidenced. Consequently, further studies involving a larger number of genotypes over a longer breeding period should be conducted to provide additional insights into the effect of previous breeding programs on the compositional properties of 'old' common wheat varieties. Nonetheless, the preliminary results suggest that breeding programs may have inadvertently selected against free ASN content. Overall, selection by breeding programs has improved nutrient-use efficiency, increased resistance to lodging by reducing the plant height, as well as resistance to stress conditions such as water stagnation, drought, and plant diseases, which are notorious for affecting the ability of wheat to convert assimilated nitrogen (N) into free amino acids and then proteins [20].

5. Conclusions

This paper was aimed at evaluating whether the grain yield and protein, rheological characteristics, as well as the ASN content in kernels of 'old' common wheat varieties grown on S-deficient soils could be improved with agronomical treatments, more specifically S fertilization, N fertilization, and SD. The experiment was conducted on 14 'old' common wheat varieties released between 1900 to 1960 in Italy. A higher seeding density was shown to increase the grain yield and protein concentration. S fertilization was found to increase the grain yield without decreasing grain protein concentration, while N fertilization was found to effectively increase the grain protein concentration and the protein yield by hectare. Regarding the dough rheological parameters, SD was shown to negatively affect the dough strength in all the varieties. Instead, dough strength was significantly increased in relation to increasing S and N fertilization. Free ASN concentration in 'old' common wheat varieties was found to be comparable to other studies investigating 'old' and 'modern' genotypes with low nitrogen-use efficiency under S-deficient conditions. Interestingly, free ASN concentration was negatively correlated with the year of release in the considered varieties. This may suggest that past breeding programs may have contributed to reducing the ASN content; however, more studies on old varieties need to be conducted to further investigate this aspect. N fertilization was found to significantly increase the ASN content, whereas

S application decreased the ASN content by 85.1%. In the present study, S fertilization successfully improved the grain yield and the technical parameters of the 'old' common wheat varieties while reducing the ASN concentration, thereby promoting food safety. Hence, these present results can be considered of particular interest for 'old' common wheat varieties characterized by poor technical performance when these varieties are grown on S-deficient soils. However, additional trials, including additional years within differing pedo-climatic conditions, are required in order to further evaluate the interaction between cultivars and the agronomical treatments.

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Valorization of an Old Variety of *Triticum aestivum*: A Study of Its Suitability for Breadmaking Focusing on Sensory and Nutritional Quality

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Abstract: "Avanzi 3-Grano 23" (G23) is an old variety of *Triticum aestivum* from the mountain areas of Lunigiana (north Tuscany, Italy), where traditional farming communities have contributed to its success and on-farm conservation. G23 flour, traditionally used for typical food products, is characterized by particular nutritional and sensory traits but has technological properties which limit its suitability for breadmaking. The aim of this work was to evaluate how to promote the use of G23 through the optimization of bread formulation by leveraging both flour blending and the leavening system. During the preliminary test, three different mixes of G23 flour and a strong flour (C) were tested in terms of their leavening power as a function of leavening agent (baker's yeast or sourdough). The selected M2 flour, composed of G23:C (1:1 *w*/*w*), was used for further breadmaking trials and 100% C flour was utilized as a control. The sourdough bread obtained with the M2 flour (SB-M2) showed an improved sensory profile compared with the related control (SB-C). Furthermore, SB-M2 exhibited the best aromatic (high content in aldehydes, pyrazines and carboxylic acids) and phytochemical profile (total polyphenols and flavonoids content and antioxidant activity). In contrast, the use of baker's yeast, although optimal from the point of view of breadmaking, did not result in the same levels of aromatic complexity because it tends to standardize the product without valorizing the sensory and nutritional qualities of the flour. In conclusion, in the experimental conditions adopted, this old wheat variety appears to be suitable for the production of sourdough bakery products.

Keywords: wheat flour; old variety; sensory quality; VOCs; bread color; nutritional quality; sourdough bread; baker's yeast bread; dough volume increase

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1. Introduction

One of the major cereal crops worldwide is common wheat (*Triticum aestivum*) [1]. Rich in calories, minerals, vitamins, dietary fiber, beneficial bioactive compounds and essential amino acids, wheat and its products are a staple in human nutrition [1–3]. However, to meet the industry demands in flour technological quality, modern wheat varieties with higher starch and protein content have been created, and this has led to a consequent decrease in other nutritional components [4,5]. Nevertheless, especially in recent years, consumers are focused on the sensory and nutritional quality aspects of wheat-based products [2,6–8]. In this context, old wheat varieties and local landraces have gained increasing attention, and many studies have suggested that they could offer a healthier and a better nutritional profile than modern varieties in terms of protein, lipids, soluble dietary fiber, minerals and different phytochemicals [1,3,9–12]. However, old wheats are known to have low technological quality, despite their high grain protein percentage, due to their weak gluten index [13,14].

"Avanzi 3-Grano 23" (G23) is an old variety of *Triticum aestivum* cultivated since the early decades of the twentieth century in the mountain areas of Lunigiana (north Tuscany, Italy) [15]. The traditional agricultural communities of this marginal area have contributed for decades to the protection and evolution of this variety of wheat through on-farm conservation to ensure its continuous evolution and diversification to meet the complex agro-environmental conditions and to provide a reliable livelihood and a sustainable food source to local communities [16]. The combined effects of natural and farmer selection have led to a genotype characterized by tall plants, long coleoptiles, early vigor, competition with weeds, cold tolerance, and quality traits suited for local food preferences [15,17].

G23 flour is characterized by particular nutritional and sensory features; nevertheless, it has technological properties which limit its suitability for baking. Indeed, this flour is traditionally used to make local products such as "testaroli" and "panigacci" historically produced in the Lunigiana area [18].

This old variety is now listed as an endangered species as its agricultural production has mostly been interrupted due to the massive focus on the cultivation of modern high-yielding wheat varieties. For these reasons, it is important to create a local food system to promote this variety of wheat through establishing a short supply chain, direct sales, exchange and purchase of specialty agricultural and food products in local markets, which will certainly contribute to a general process of economic revitalization of the territory [19].

A possible approach to increasing G23 economic valorization and thus spreading its use could be the optimization of its flour technological properties by blending with other strong flours and using a suitable leavening agent in order to obtain a bread with nutritional and sensory characteristics particular to this old variety.

Several studies [1,20–22] have shown how processes such as sourdough fermentation can boost the phenolic compound availability and antioxidant activity of raw material. In addition, the sourdough induces a high acidity in the final product, prolonging its shelf-life and increasing its nutritional and sensory profile [2,6,22,23].

On the other hand, baker's yeast is widely used, especially by industrial bakeries, due to its technological properties [24]. Baker's yeast has the advantage of simplifying the production process, getting the highest yields possible and reducing costs [25].

For these reasons, the aim of this work was to evaluate how to enhance this old wheat variety through the optimization of bread formulation, by leveraging both flour blending and a leavening system.

During the preliminary tests, doughs obtained from different mixes between G23 flour, and a strong flour (C) leavened by brewer's yeast or sourdough were evaluated considering their volume increase and their fermentation metabolites. At a later stage during further breadmaking trials, the blend of flour selected on the basis of leavening performance was compared with the control (100% strong flour) as a function of the leavening system (brewer's yeast vs. sourdough). In particular, our attention was focused on the bread's technological properties, bread sensory profile and compositional traits.

2. Materials and Methods

2.1. Raw Materials

Control (C) is a wheat flour consisting of a mix of four varieties (Verna, Bologna, Bolero and Pandas) of common wheat (*Triticum aestivum*) supplied by the Department of Agriculture, Food, Environment, and Forestry of the University of Florence.

G23 is a wheat flour of an old variety ("Avanzi 3-Grano 23") of common wheat (*Triticum aestivum*) and was provided by a small local farmer in Lunigiana (North Tuscany, Italy).

The wheat grains were ground with a commercial mill (Industry-Combi, Waldner Biotech, Lienz, Austria) at the Department of Agriculture, Food, and Environment (DAFE) of the University of Pisa. Table 1 shows the chemical composition and technological features of the flours obtained.

Table 1. Chemical and technological parameters of flours (C and G23). Results are expressed as mean ± SD (*n* = 4).

Parameters	Units	C	G23
Chemical			
Humidity	% *w*/*w*	10.93 ± 0.30	12.60 ± 0.20
Ashes	% *w*/*w*	1.35 ± 0.09	2.05 ± 0.18
Proteins	% *w*/*w*	12.42 ± 0.32	11.94 ± 0.82
Total fats	% *w*/*w*	2.53 ± 0.63	1.74 ± 0.52
Total dietary fiber	% *w*/*w*	5.72 ± 0.22	3.56 ± 0.32
Maltose	% *w*/*w*	6.28 ± 0.26	3.78 ± 0.36
Glucose	% *w*/*w*	0.43 ± 0.02	0.23 ± 0.05
Fructose	% *w*/*w*	0.14 ± 0.01	0.10 ± 0.03
Sucrose	% *w*/*w*	0.96 ± 0.05	0.46 ± 0.09
Wet gluten	% *w*/*w*	38.82 ± 2.02	29.02 ± 2.23
Dry gluten	% *w*/*w*	12.32 ± 1.62	9.82 ± 1.22
Gluten index	% *w*/*w*	72.22 ± 10.01	41.04 ± 15.12
Total Starch	% *w*/*w*	83.72 ± 0.52	72.54 ± 0.89
Falling number	seconds	333 ± 16	351 ± 18
Total polyphenol	mg GAE/kg dm	800 ± 17	415 ± 12
Total flavonoids	mg CE/kg dm	75.8 ± 0.9	47.4 ± 0.8
ABTS	µmol TE/g dm	1.17 ± 0.07	0.63 ± 0.02
DPPH	µmol TE/g dm	0.70 ± 0.05	0.45 ± 0.03
FRAP	µmol TE/g dm	1.57 ± 0.09	0.44 ± 0.02
Technological			
W	10^{-4} joules	263 ± 17	57 ± 14
P	mm	148 ± 14	27 ± 7
L	mm	50 ± 10	88 ± 28
P/L		2.96 ± 0.72	0.36 ± 0.12
G		18.4 ± 1.6	20.8 ± 3.1

The sourdough used was maintained over one year at the DAFE of the University of Pisa by a daily refreshment procedure as reported by Taglieri et al., 2020 [26], while the baker's yeast was a commercially available compressed yeast (Zeus Iba s.r.l., Firenze, Italy).

2.2. Chemical and Technological Parameters of Flours

The chemical and technological parameters of flours (humidity [27]; ashes [28]; proteins [29]; total fats [30]; falling number [31]; wet gluten and gluten index [32]; dry gluten [33]; total dietary fiber [34]; sugars (maltose; glucose, fructose, sucrose) [35]; total starch [36]; and Chopin alveogram (W, P, L, P/L, G) [37]) were determined through the methods accepted by the International Organization for Standardization (ISO), as previously reported by Bianchi et al., 2022 [6].

2.3. Biga Preparation

The leavening and acidifying performances of sourdough were periodically monitored in order to maintain constant and replicable conditions. The bread-making procedure was performed using the "biga" pre-ferment method using sourdough (S) or baker's yeast (Y).

Sourdough biga (S-biga) was prepared by mixing a strong wheat flour type 0 (56% *w*/*w*), sterile water (33% *w*/*w*), and sourdough (11% *w*/*w*). The mixture was then left to ferment for 18 h at 20 °C. Baker's yeast biga (Y-biga) was obtained by mixing a strong wheat flour type 0 (68% *w*/*w*), sterile water (31% *w*/*w*), and 1% (*w*/*w*) of baker's yeast and then fermenting for 21 h at 18 °C. The recipes of S-biga and Y-biga were defined in previous studies [26,38]. The chemical compositions of the two types of biga (S-biga and Y-biga) are reported in Table 2.

Table 2. Characterization of the two biga (S-biga and Y-biga) used during the research. Results are expressed as mean ± SD (*n* = 4).

Parameters	Units	S-Biga	Y-Biga
Dry matter	(% dm)	55.20 ± 0.12	59.80 ± 0.16
pH		4.06 ± 0.02	5.43 ± 0.03
TTA	(meq lactic acid/kg dm)	0.117 ± 0.002	0.038 ± 0.003
Acetic acid	(mmol/kg dm)	16.28 ± 0.26	2.56 ± 0.28
Lactic acid	(mmol/kg dm)	91.52 ± 0.62	4.22 ± 0.44
Ethanol	(mmol/kg dm)	56.24 ± 0.24	141.14 ± 0.34

2.4. Preliminary Leavening Tests

Preliminary leavening tests were conducted using two different types of leavening agent (sourdough (S) and baker's yeast (Y)) to test different mixes of G23 and C flours (M1 = 1:3 *w*/*w*, M2 = 1:1 *w*/*w*, M3 = 3:1 *w*/*w*) and identify the best blend on the basis of dough volume increase and fermentation metabolites (acetic acid, lactic acid, ethanol). The different doughs (Y-M1, Y-M2, Y-M3, S-M1, S-M2, and S-M3) were prepared following the protocol (formulation, times and temperatures) reported in Section 2.5.

To evaluate the volume increase, as reported in Balestra et al., 2015 [39], 20 g of dough was placed inside a 100 mL graduated cylinder. The dough was left in a prover for 4 h at 32 ± 1 °C. The dough volume increase (DVI) was expressed as a percentage according to the following equation:

$$\mathrm{DVI} = \frac{(v_1 - v_0)}{v_0} \times 100 \quad (1)$$

where:

v_0 = starting volume of the dough.

v_1 = volume after the leavening time.

2.5. Bread Preparation

Two formulations of sourdough bread (SB-M2, SB-C) and two formulation of baker's yeast bread (YB-M2, YB-C) were produced with water (32%), biga leavening agent (16%), and flour (52%).

The first leavening was allowed to occur for 90 min at 26 ± 1 °C, and then the dough was broken and shaped into 500 g loaves which were left for 2.5 h at 35 ± 1 °C (second leavening). Finally, the loaves were baked at 220 °C for 45 min. The bread was then cooled at room temperature (23 ± 1 °C) and sliced (20 mm) for the analysis.

2.6. Physico-Chemical Characterization of Dough, Biga and Bread Samples

The moisture content of samples (dough, biga or bread) was determined on an approximately 5 g sample dried at 105 °C until constant weight. The pH, total titratable acidity (TTA) and the fermentative metabolites (acetic acid, lactic acid, ethanol) were measured as previously reported by Bianchi et al., 2022 [6].

In addition, the flour, biga and bread samples were characterized from a phytochemical point of view. In particular, for total polyphenols, total flavonoids and anti-radical activity evaluation, 80% methanol solution was used to perform a solid/liquid extraction (ratio 1/20 *w*/*v*) from 0.5 g of fresh sample (flour, biga or bread), and the mixture was then sonicated for 30 min. All the extracts were subsequently centrifuged (15 min, 3500 rpm), filtered on a syringe filter (0.45 µm) and stored at 4 °C for immediate analysis.

The Folin–Ciocalteu colorimetric method was applied for the total polyphenols spectrophotometric analysis (wavelength = 765 nm), according to Macaluso et al., 2020 [40], with the results expressed as milligrams of gallic acid equivalents (GAE) per kilogram dry matter (dm) of sample.

The total flavonoids analyses were performed according to the procedure reported by Tavarini et al., 2020 [41], with the results expressed as milligrams of catechin equivalents

(CE) per kilogram of sample (dm) and the measures compared with a standard curve of catechin.

The anti-radical activity of the extracts was determined by the DPPH [42], ABTS [43] and FRAP [44] free radical methods. The results were expressed as µmol Trolox equivalents (TE) per gram dm of sample, according to different standard curves of Trolox: in the range 0–200 µmol L^{-1} for the DPPH assay, a range of 0.2–1.5 mM for ABTS and 0–2.0 mM for the FRAP assay.

In order to better evaluate the technological properties of the baked bread, the crumb was analyzed to assess water activity, softness and color, as reported below.

The water activity (a_w) of the crumb of bread was assessed by a HygroPalm HP23-AW-A device (Rotronic AG, Bassersdorf, Switzerland).

The softness of the crumb of bread was measured as compressibility by a PNR-12 penetrometer (Anton Paar, Rivoli (TO), Italy) using the method described by Taglieri et al., 2021 [38]. Each sample was compressed in five spots by a weight of 90 g for 10 s. The softness was measured in mm of penetration (0.1 mm = 1 penetration unit).

The crumb color of bread was measured according to the CIE L*a*b* color System by means of a tristimulus colorimeter (Eoptis, Mod. CLM-196 Benchtop, Trento, Italy). The Chroma value C* and hue value h* were also calculated as previously reported [45]. The color differences among samples (ΔE^*_{ab}) were calculated as previously reported [38] and expressed in CIELAB units.

The whiteness (WI) and yellowness (YI) indices of the samples were calculated, as reported by Alam et al., 2022 [46], according to the following equations:

$$\mathrm{WI} = 100 - \sqrt{(100 - \mathrm{L}^*)^2 + (\mathrm{a}^*)^2 + (\mathrm{b}^*)^2} \tag{2}$$

$$\mathrm{YI} = 142.86 \times \frac{\mathrm{b}^*}{\mathrm{L}^*} \tag{3}$$

2.7. Volatile Organic Compounds (VOCs) of Bread

The bread VOC profile (sliced bread samples) was assessed according to the protocol previously described in Sanmartin et al., 2018 [47], sampling the volatile analytes using a 50/30 µm coating thickness SPME (Supelco, St. Louis, MO, USA) and using a gas chromatography-electron impact mass spectrometer (GC-EIMS) (Agilent Technologies Inc., Santa Clara, CA, USA) for their determination.

2.8. Sensory Profile of Bread

The bread sensory profile was evaluated by a panel of 8 long-term members of the "Committee of Experts" of DAFE of the University of Pisa, according to the protocol previously described [38], including quantitative (color intensity, presence of lacerations, crumb structure, olfactory intensity, elasticity, resistance to chewing, juiciness, cohesiveness, sapidity, acidity, bitter, aftertaste) and hedonic (visual attractiveness, olfactory pleasantness, tasting pleasantness, global pleasantness) indices. The overall hedonic index of bread was calculated according to Bianchi et al., 2022 [6]. The research obtained the approval of the bioethical committee of the University of Pisa (protocol n. 0088081/2020).

2.9. Statistical Analysis

All the evaluations were performed in quadruplicate, and data are reported as mean values ± standard deviation (SD). A one-way ANOVA (CoStat, Cohort 6.0) on the physico-chemical data was performed, followed by the Tukey's HSD test at $p \leq 0.05$ significance.

Statistical analysis of volatile organic compounds and hierarchical cluster analysis (HCA) applying the Ward method and using two-way clustering were performed using the JMP Pro 17.0 software package (SAS Institute, Cary, NC, USA). The 3D principal component analysis (PCA) was performed by selecting the three principal components (PCs) obtained by the linear regressions operated on mean-centered, unscaled data.

Sensory analysis data were processed by Big Sensory Soft 2.0 (ver. 2018), carrying out a two-way ANOVA, with samples and panelist as main factors [48], followed by the Friedman test to identify significant descriptors to discriminate samples.

3. Results and Discussion

3.1. Preliminary Leavening Test

As a first step, we performed a leavening test comparing different percentages of G23 flour and leavening systems to assess their breadmaking suitability through the evaluation of the dough volume increase (DVI) and the fermentative metabolites (Table 3).

Table 3. Physico-chemical composition of six types of dough evaluated.

Parameters	Units	*p*-Value [1]	Y-M1	Y-M2	Y-M3	S-M1	S-M2	S-M3
Dry matter	(% dm)	*	58.22 ^{a}	57.23 ^{b}	57.83 ^{ab}	53.44 ^{d}	54.12 ^{c}	53.98 ^{cd}
pH		***	5.20 ^{b}	5.16 ^{b}	5.36 ^{a}	3.90 ^{e}	3.81 ^{d}	4.25 ^{c}
TTA	(meq lactic acid/kg dm)	***	0.040 ^{d}	0.042 ^{d}	0.035 ^{e}	0.121 ^{b}	0.129 ^{a}	0.108 ^{c}
Acetic acid	(mmol/kg dm)	**	2.56 ^{c}	2.62 ^{c}	2.59 ^{c}	19.83 ^{a}	20.15 ^{a}	12.24 ^{b}
Lactic acid	(mmol/kg dm)	***	4.71 ^{d}	4.62 ^{d}	4.42 ^{d}	108.52 ^{b}	117.54 ^{a}	73.5 ^{c}
Ethanol	(mmol/kg dm)	**	189.62 ^{a}	190.24 ^{a}	152.18 ^{b}	59.77 ^{c}	60.26 ^{c}	35.12 ^{d}
DVI	%	***	360 ^{a}	350 ^{a}	180 ^{d}	220 ^{c}	250 ^{b}	70 ^{e}

[1] Significance level: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. In the same row, different letters indicate significant differences among samples.

During the fermentation time, all the doughs underwent structural changes depending on the fermentation methods used (Y and S). The modifications are related mainly to an increase in resistance to the extension and a decrease in extensibility, with a noticeable change in volume and pH. As shown in Table 3, the two systems differ in terms of DVI, in favor of baker's yeast, which exhibits a greater leavening power, as reported also in the literature [7,26,39].

The mix M3 (G23:C = 3:1 *w*/*w*) exhibited statistically lower values in terms of DVI, as expected considering the low production of leavening metabolites (acetic acid, lactic acid and ethanol) both for baker's yeast (Y) and sourdough (S). The other two flour mixes behaved in a similar way when leavened with baker's yeast (Y-M1 and Y-M2). Conversely, M2 seems to be more favorable for sourdough fermentation than M1 since a higher content of lactic acid was observed in S-M2, with a consequent lower pH, higher TTA, and a significantly higher DVI (Table 3).

According to the results obtained, the ratio 1:1 between G23 flour and C flour allows the use of the greatest amount of G23 with both the leavening systems, resulting in a dough with acceptable results in terms of metabolite content and dough volume.

3.2. Physico-Chemical Characterization of Bread

On the basis of the results obtained during the leavening test (see Section 3.1), the M2 flour was thus selected for further breadmaking trials and 100% C flour was utilized as a control. The technological parameters of M2 flour are reported in Table S1. The aim of the breadmaking trial was, therefore, to assess the quality of sourdough bread (SB-M2) and baker's yeast bread (YB-M2) in comparison with their respective controls (SB-C and YB-C) from compositional, technological and sensory points of view.

As shown in Table 4, the breads obtained with M2 flour (YB-M2 and SB-M2) were characterized by the greatest softness, especially when the sourdough was used.

Table 4. Physico-chemical composition of bread.

Parameters	Units	*p*-Value ^{1}	YB-M2	YB-C	SB-M2	SB-C
Dry matter	(% dm)	***	56.53 ^{a}	55.42 ^{b}	53.82 ^{d}	54.54 ^{c}
Softness	mm	*	2.05 ^{ab}	1.81 ^{b}	2.15 ^{a}	1.73 ^{b}
a_w		ns	0.936	0.938	0.940	0.939
pH		**	5.99 ^{a}	6.03 ^{a}	4.28 ^{d}	4.41 ^{c}
TTA	(meq lactic acid/kg dm)	**	0.027 ^{c}	0.026 ^{c}	0.083 ^{a}	0.072 ^{b}
Acetic acid	(mmol/kg dm)	**	1.75 ^{c}	1.62 ^{c}	21.28 ^{a}	19.72 ^{b}
Lactic acid	(mmol/kg dm)	***	3.96 ^{c}	4.27 ^{c}	96.59 ^{a}	87.4 ^{b}
Ethanol	(mmol/kg dm)	**	17.41 ^{b}	18.31 ^{a}	12.06 ^{c}	11.94 ^{c}
Total polyphenol	mg GAE/kg dm	***	545 ^{d}	618 ^{c}	702 ^{b}	1054 ^{a}
Total flavonoids	mg CE/kg dm	***	72.9 ^{d}	84.5 ^{c}	109.8 ^{b}	113.8 ^{a}
ABTS	µmol TE/g dm	***	0.62 ^{d}	0.73 ^{c}	0.89 ^{b}	1.15 ^{a}
DDPH	µmol TE/g dm	***	0.36 ^{d}	0.42 ^{c}	0.55 ^{b}	0.72 ^{a}
FRAP	µmol TE/g dm	***	0.84 ^{d}	0.98 ^{c}	1.32 ^{b}	1.72 ^{a}

[1] Significance level *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns: not significant ($p > 0.05$). In the same row, different letters indicate significant differences among samples.

As expected, the use of sourdough resulted in different TTA and pH levels, due to the production of acetic and lactic acid compared with baker's yeast [49]. Instead, the M2 seems to be a better substrate for the sourdough since SB-M2 is significantly more acid than SB-C (Table 4). This difference was obviously not observed for the baker's yeast breads, which did not show any critical issues regardless the flour used.

Regarding the phytochemical features, it is interesting to note that the control breads were generally richer in polyphenols with a consequent higher antioxidant capacity, also derived from its high initial content of C flour (Table 1). However, it is interesting to note that the M2 flour combined with sourdough (SB-M2) underwent a greater increase in phytochemical value from its initial content (Table S1). This increase was not observed for the baker's yeast breads, which showed lower values than the starting ones. According to [1,3,20], sourdough fermentation promotes the release of bound phenolics, even from flours that showed a lower initial free phenolics content. Clearly, the low pH reached in the sourdough significantly increased the bioavailability of polyphenols in the breads obtained, also enhancing the G23 nutritional and phytochemical potential (Table 4).

3.3. Color Characterization of Bread

Figure 1 includes pictures of the four samples obtained using the two flours (M2 and C) with the two leavening agents (Y and S).

As can be observed in Figure 1, the chromatic characteristics of the samples showed significant differences which seem to be especially related to the flour used, as shown by the comparison of the components a* ($\geq$0 redness; $\leq$0 greenness) and b* ($\geq$0 yellowness; $\leq$0 blueness) for both the leavening systems (Table 5).

In general, the lightness (L*) seems to be influenced by the leavening agent and the flour used, as shown by the significant differences in the whiteness index (WI).

The a* index in bakery products is generally related to the Maillard reaction [50], but in our samples, this index is negligible, since in crumb the temperature never exceeds 100 °C and thus the color features of dough are partially retained [51].

The yellow color of crumb depends on both the carotenoid content of the flour and on the baking process that promotes the yellow hue [51].

The yellowness index (YI) of the evaluated flour seems to be more represented in the control samples (YB-C and SB-C), regardless the leavening system.

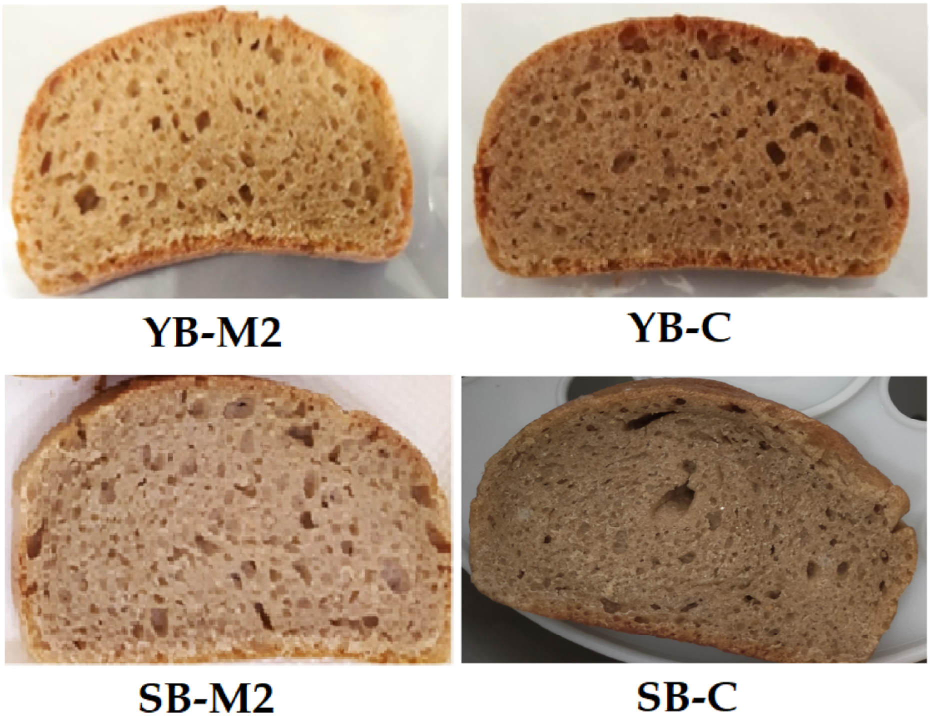


Figure 1. Pictures of the different bread sliced.

Table 5. Chromatic characteristics of bread samples.

Parameters	*p*-Value [1]	YB-M2	YB-C	SB-M2	SB-C
L*	***	53.69^{c}	51.05^{d}	57.32^{a}	54.85^{b}
a*	**	3.56^{b}	5.93^{a}	3.25^{b}	5.73^{a}
b*	***	19.28^{b}	23.06^{a}	17.36^{c}	22.76^{a}
C*	***	19.60^{b}	23.81^{a}	17.66^{c}	23.47^{a}
h*	**	79.53^{a}	75.57^{b}	79.39^{a}	75.86^{b}
WI	***	50.09^{b}	45.57^{d}	53.81^{a}	49.11^{c}
YI	***	51.30^{c}	64.53^{a}	43.27^{d}	59.28^{b}

[1] Significance level *** $p < 0.001$; ** $p < 0.01$. In the same row, different letters indicate significant differences among samples.

Moreover, the C samples, having a higher saturation (C*) and a lower hue value (h*), exhibit a color turning to a warm yellow, regardless the leavening system used [13].

Conversely, when M2 flour is used, a significant difference associated with the leavening system is observed, probably due to a higher sensitivity to the pH reduction linked to the use of sourdough [38].

In order to quantify the color differences among the samples, the ΔE^*_{ab} values were calculated and are reported in Table 6. All the samples showed a perceptible difference [52], with the greatest color distance (8.89) observed between SB-M2 and YB-C.

Table 6. Color differences (ΔE^*_{ab}) among sliced bread samples. The difference is expressed in CIELAB units. ΔE^*_{ab} values up to 2.7 represent chromatic changes perceptible to the human eye.

ΔE^*_{ab}	YB-C	YB-M2	SB-C	SB-M2
YB-C		5.18	3.82	8.89
YB-M2			4.26	4.12
SB-C				6.44
SB-M2				

A noticeable difference (Table 6) was found even with the same leavening system (SB-M2/SB-C and YB-C/YB-M2), confirming the strong effect of the flour in color determination.

3.4. Volatile Organic Profile of Bread Samples

From a purely aromatic point of view, bread is a complex product characterized by a multitude of volatile substances that influence the final aromatic profile. To date, in fact, many studies have focused on evaluating and quantifying the volatile substances of the final aroma of bread, coming to describe over 540 substances produced depending on the type of formulation, type of yeast and cooking method [53,54].

Quantitatively, the analysis of the spontaneous emission of the VOCs allowed the identification of about 62 different compounds (Table S2), where the main groups were alcohols, acids, aldehydes, ketones, esters, pyrazines and pyrrolines, but there were also furans, hydrocarbons and lactones.

The analysis of the main components (PC1 + PC2 = total variance of 88.8%) highlights the different volatile substances that allowed differentiation of the loaves into three different groups (Figure 2).

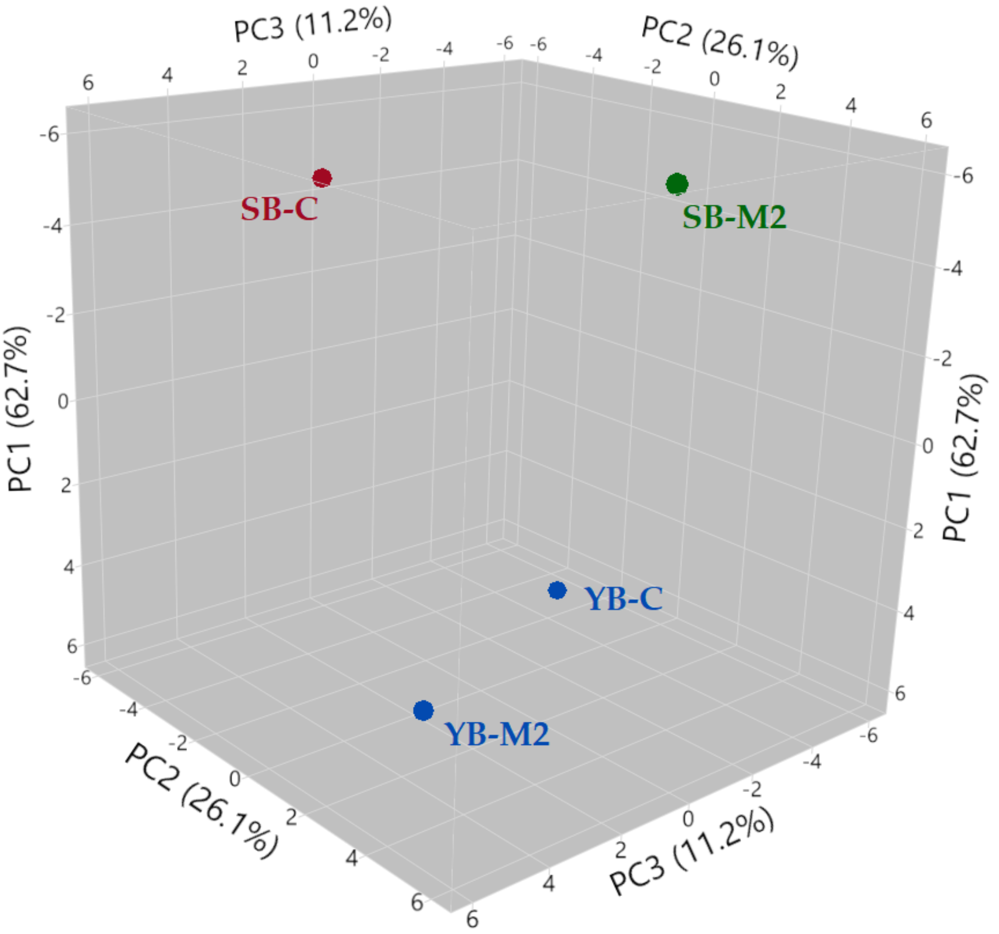
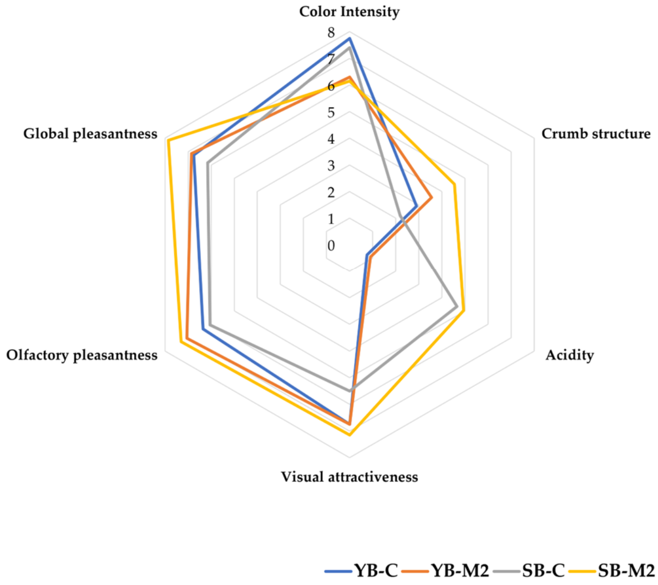


Figure 2. 3D version of the principal component analysis (PCA) of volatile organic compounds of the breads.

The gas chromatographic analysis shows that the type of leavening agent used led to the production of diverse VOCs. Breads made with baker's yeast (YB-C and YB-M2) showed a higher production of alcohols and aldehydes with slight differences related to the type of flour: YB-M2 was characterized by a good production of alcohols, such as isobutyl alcohol, 2-methyl-1-butanol, and phenylethyl alcohol, and aldehydes, such as 3-ethyl-2-methyl-1,3-hexadiene, whereas YB-C showed a greater production of aldehydes and alcohols (hexanal and 2-furanomethanol). On the other hand, sourdough breads (SB-C and SB-M2) had more complex VOC profiles with a greater variety of compounds, including pyrazines, pyrimidines, and carboxylic acids. In particular the flour used strongly influenced the volatile expression, allowing clear grouping of the two sourdough breads into separate clusters. (Figure S1). The different flours used have, therefore, led to a different production of volatile substances: (i) SB-M2 was characterized by compounds belonging mainly to aldehydes (i.e., (Z)-2-heptanal, benzaldehyde), pyrazines (i.e., 2,6-dimethylpyrazine and methoxypyrazine), alcohols (i.e., isopentyl alcohol), monoterpenes (i.e., p-cymene); and (ii) SB-C showed mainly alcohols (i.e., 1-pentanol), pyrimidines (i.e., 4-methylpyrimidine) and aldehydes (i.e., 3-methylbutanal).

3.5. Sensory Evaluation of Bread

Figure 3 reports the organoleptic profiles of the breads considering the sensory parameters that showed statistically significant differences.



Significant parameters	T
Color Intensity	12.07
Crumb structure	12.40
Acidity	15.62
Visual attractiveness	10.19
Olfactory pleasantness	12.86
Global pleasantness	11.7

Figure 3. Median of significant qualitative parameters. Friedman's ANOVA analysis ($T > \chi^2$; $\chi^2 = 9.49$).

The sensory profiles are fully in agreement with all the chemical-physical results obtained for all the samples, including color and VOCs, thus confirming that the differences in chemical composition among samples can be clearly perceived by consumers.

In general, the main parameter that enabled differentiation of the breads from a sensory point of view seems to be the formulation. Considering the quantitative parameters, the type of flour clearly influenced the color intensity, but also the crumb structure, while the leavening system strongly affected the acidity. As expected, sourdough breads had a greater perceived acidity, with the highest value reported for the SB-M2 sample, closely followed by SB-C. Regarding the hedonic descriptors, the bread produced with M2 flour was evaluated more positively than that produced with C flour, regardless the leavening agent utilized.

As shown in Figure 4, all the samples received a positive hedonic evaluation (HI > 6). In particular, the highest hedonic index was attributed to the SB-M2 sample, followed by both the breads leavened by baker's yeast. The baker's yeast seems to lead to a standardization of the product. For this reason, as also confirmed by the VOCs analysis, the bread showed the same level of aromatic complexity and sensory pleasure.

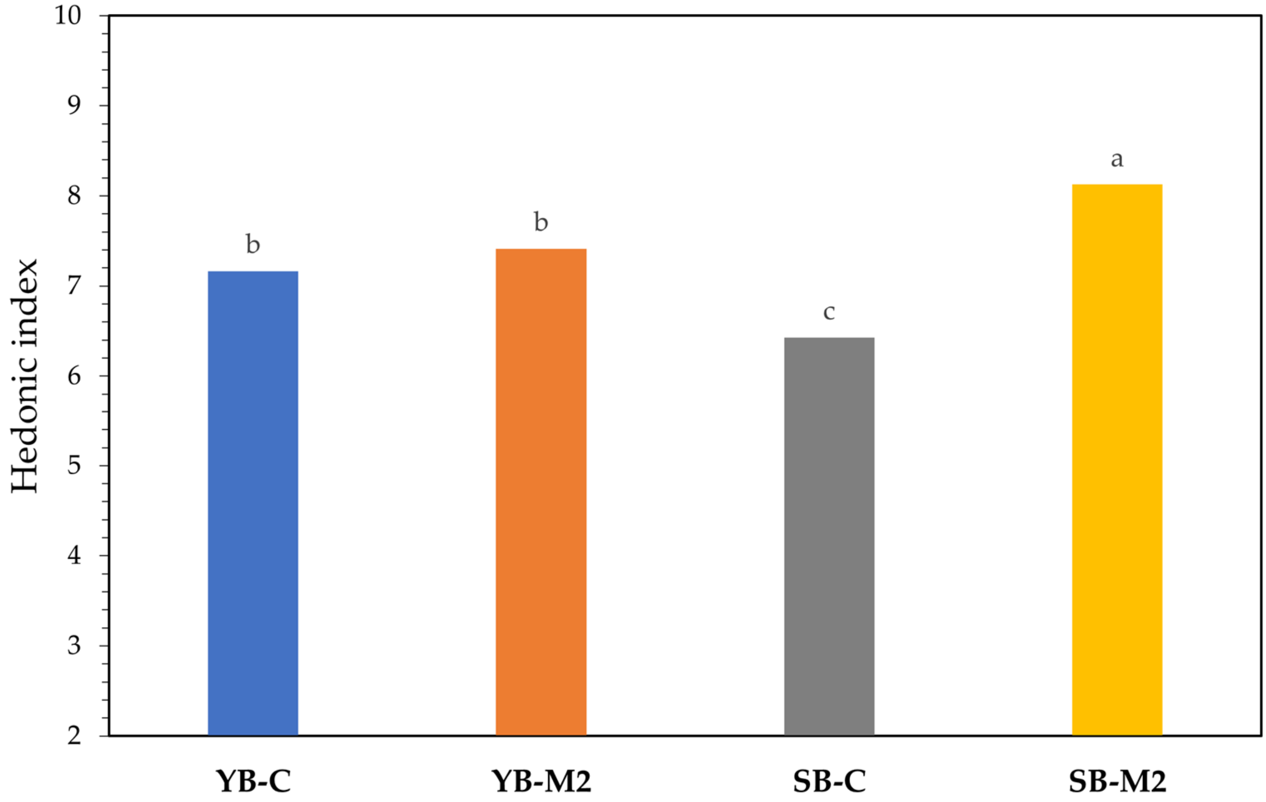


Figure 4. Hedonic index (HI) of different types of bread. Different letters indicate significant differences among samples ($p < 0.05$).

4. Conclusions

The results of this study suggest that the incorporation of flour of an old wheat variety such as "Avanzi 3-Grano 23" along with sourdough fermentation represents an important tool in the development of functional bakery products with improved antioxidant capacity and phenolics bio-accessibility. Moreover, the use of sourdough conferred to the bread a lower pH, due to the higher acidity, which could prolong its shelf-life.

The sourdough bread with the M2 mix in particular, achieved the best results, also from a sensory point of view, reaching a high level of acceptability, as expressed by the hedonic index (HI > 8).

Even if the combination of baker's yeast and M2 flour was optimal in terms of leavening, it did not achieve the same levels of aromatic complexity and sensory pleasure. According to our results, the baker's yeast tends to standardize the product, without valorizing the sensory and nutritional potential of the flour

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/foods12061351/s1, Table S1: chemical and technological parameters of M2 flour. Results are expressed as mean ± SD ($n = 4$); Table S2: complete headspace compositions of baked bread as a function of flour and leavening agent; Figure S1: hierarchical cluster analysis (HCA) based on VOCs of baked bread as a function of flour and leavening agent.

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Comparison between organic and conventional farming systems using Life Cycle Assessment (LCA): A case study with an ancient wheat variety

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Acidification

ABSTRACT

Assessing the environmental impact of agriculture is a key factor towards reducing human impacts on the food production chain. Because of the growing consumer interest in healthy foods cultivated with low-impact approach, a study assessing the impacts of ancient wheat variety in organic and conventional farming appears to be of great importance. Thus, this study was aimed at assessing and comparing the environmental impacts and resources consumptions of organic and conventional farming practices on an ancient soft wheat variety (var. Verna) in Tuscany, Italy. The fact that Verna wheat falls within the PDO bread specification, one of the few at the European level, increases the importance of this work. A cradle-to-grave life cycle assessment (LCA) from raw material extraction, through to industrial processing, field utilization, grain harvesting and finally transportation to storage centres was carried out. This study analysed data sampled over a five-year period (2014/2015 to 2018/2019) derived from five organic and conventional farms, respectively. The impact categories considered included: global warming, freshwater ecotoxicity, seawater ecotoxicity, terrestrial ecotoxicity, human toxicity, acidification, eutrophication, photo-oxidant formation, non-renewable energy resource consumption, renewable energy resource consumption, water consumption and land use. In almost all the impact categories, organic farming was shown to have lower environmental impacts, while conventional farming had a lower impact on land use. Results relating to acidification, photo-oxidant formation, ozone layer depletion and non-renewable energy resource consumption were considered similar for the two cultivation systems. Normalization of the results showed that seawater ecotoxicity had the greatest impact among all impact categories ($> 99\%$) for both cultivation systems. Moreover, major environmental problems in conventional farming and organic farming were the use of synthetic N fertilizers and low yields, respectively. Results showed that 192×10^6 hectares of organic farming would be needed to maintain current wheat production in the EU, compared to just 99×10^6 hectares cultivated with the conventional farming. Accordingly, yield increase in organic farming, and reduction of nutrient losses/emissions from conventional farming, are the two most promising strategies towards maintaining a high agricultural production with concomitant reductions in the related environmental impact.

1. Introduction

Wheat is the second most cultivated crop worldwide and is the staple food crop for a significant part of the global population (Anon, 2014). Modern wheat varieties are required to meet specific technological quality criteria for the processing industry. Meeting these criteria have been the subject of intense genetic breeding efforts. However, conventional breeding has caused losses in genetic variability with a reduction in crop adaptability to different ecosystems and pedoclimatic variations (Newton et al., 2010; Döring et al, 2015). In the last years, the use of ancient wheat varieties is increasing, attributable to a higher adaptability to climate variability, lower input requirements and improved nutraceutical properties (Lammerts van Bueren et al., 2011; Migliorini et al., 2016; Boukid et al., 2020; Fatholahi et al., 2020). The latter is a crucial aspect of interest for consumers, where there is an ever-increasing shifting preference towards local and healthy foods, as well as a specific interest in sustainability (Chiriacò et al., 2017). In order to meet both the above requirements, farmers are prompted to adopt organic farming systems based on ancient crop varieties.

In general, organic farming is growing significantly on a global level

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with increases in surface area coverage of approximately 24 million of hectares from 2006 to 71 million of hectares in 2018 (Tu et al., 2006; Willer et al., 2020). Consumer preferences for organic food is mainly due to the absence of chemical inputs (synthetic fertilizers and pesticides). Despite a higher use of machinery compared to conventional agriculture, organic farming is more environmentally sustainable in terms of pollution, biodiversity pressure, soil erosion and energy use, with positive impacts on soil and water quality (Brandão et al., 2010; Tuomisto et al., 2012). Nonetheless, the real impacts of organic farming on global warming and climate change mitigation have been widely debated and are currently under discussion (Gomiero et al., 2011; Tuomisto et al., 2012; Chiriacò et al., 2017; Giampieri et al., 2022).

On the one hand, organic farming is based on the maintenance of natural soil fertility with a reduced adoption of external inputs. However, on the other hand, the reduced yields per hectare and, consequentially, the higher amount of land required to satisfy food demand, represent a setback in the system. Recently, an intensive review of the literature was performed throughout the scientific community in order to assess the real environmental impacts of organic and conventional farming systems (Tuomisto et al., 2012; Meier et al., 2015; Chiriacò et al., 2017). Principal findings reported wide variability, mostly attributable to relevant differences in management practices, as well as differences in the methodological approaches between the two farming systems that render comparisons difficult (Chiriacò et al., 2017). In addition, the zoning of different agricultural aptitude groups may produce different results even when adopting similar agricultural practices. Recent findings affirmed that organic farming is not a sustainable strategy to optimize land use efficiency (Giampieri et al., 2022) confirming the observations of Tuomisto et al. (2012) reporting that approximately 84% more land is needed for organic farming compared to conventional farming (Tuomisto et al., 2012). This is principally due to the lower yields (crops and animals) that account for approximately 20 to 34% less than conventional farming (De Ponti et al., 2012; Seufert et al., 2012). Generally, lower yields in organic farming are determined by the lack of nutrients, presence of weeds, pests and diseases (Korsaeth et al., 2008).

Moreover, some authors reported that organic farming guarantees a higher soil organic matter (SOM) due to the continuous input of compost, manure and crop residues (Leite et al., 2010; Santos et al., 2012). From the analysis of cumulative greenhouse gas (GHG) emissions, olives, beef and some crops were shown to produce less emissions in organic farming (Casey and Holden, 2006; Tuomisto et al., 2012). In contrast, higher GHGs were produced in certain sectors of organic farming, such as milk production, due to lower yields and higher CH_4 and N_2O emissions (Thomassen et al., 2008), and cereal and pig productions, due to higher N_2O emissions. However, Tuomisto et al. (2012) observed that N_2O and NH_3 emissions are hugely variable based on the calculation approach. In particular, it was reported that organic farming produced roughly 31% and 18% lower emissions of N_2O and NH_3, respectively, per unit of area than conventional farming. However, from calculations based on unit of product, Tuomisto et al. (2012) also reported that organic farming produced 8% and 11% higher emissions of N_2O and NH_3, respectively. Lower yields also significantly affect the water footprint. A higher water consumption by approximately 15% was reported in dairy organic farming compared to conventional farming (Palhares et al., 2015). Different impacts between organic and conventional farming on winter wheat production in Belgium were also observed (Van Stappen et al., 2015). A better performance of organic farming in terms of aquatic ecotoxicity, land occupation, water deficiency potential and photo-oxidant formation have been reported (Van Stappen et al., 2015).

Conventional farming has been shown to have a higher impact on terrestrial ecotoxicity, acidification and eutrophication potential. However, this is only true when considering 1 ha as the functional unit. The worst performances in terms of acidification, eutrophication and land occupation were shown in organic farming when the analysis was performed using 1 kg (fresh matter) of wheat grain (Van Stappen et al., 2015). From a meta-analysis on the environmental impacts of organic and conventional farming systems in Europe, lower N leaching losses (approximately 31%) were shown in organic farming compared to conventional farming per unit of area, as a result of the lower N rate normally adopted in organic farming (Tuomisto et al., 2012). In contrast, the same authors affirmed that N leaching losses were roughly 49% higher in organic farming if calculated as a unit of product. According to Aronsson et al. (2007), this is due to the limited N availability in the soil for plant uptake. The use of a non-renewable energy resources in organic farming, as with grasslands, was calculated to be approximately 50% less than conventional farming due to the lower adoption of external inputs (Haas et al., 2001). Similarly, organic farming was reported to represent an effective strategy to reduce the consumption of non-renewable energy resources (with a net reduction of 60%) than conventional farming on barley, when expressed as functional unit of 1 ha (Tricase et al., 2018). In this sense, the energy requirement for the production of 1 kg of urea accounts for 35.1 MJ (Zegada-Lizarazu et al., 2010). However, from the analysis of terrestrial ecotoxicity, conventional farming was shown to produce only 33% of the impacts produced by organic farming (Tricase et al., 2018). As with other impact categories, this was mainly due to taking into account the higher yields obtained from conventional farming over that of organic farming.

Based on the inconsistencies reported in the literature, there is an important requisite for a comprehensive analysis, considering the most important impact categories. The available literature focuses primarily on only a few impact categories. Articles investigating all the available categories are relatively few, and rarely dedicated to the comparison between organic and conventional farming. The relevance of this study is emphasized by the lack of available studies dealing on the environmental impacts of ancient wheat varieties cultivation, both from organic and conventional farming systems.

The present study was aimed at providing a complete evaluation of the environmental performance of an ancient wheat variety, in organic (ORG) and conventional (CON) farming systems. The variety Verna was chosen as it is one of those varieties included into the PDO for Tuscany bread production and, therefore, it is quickly growing at a regional level and holds the potential to spread at a wider scale. The evaluation was carried out with LCA, that is a widely adopted technique in agriculture for the assessment of the environmental impacts of food production processes (Fallahpour et al., 2012; Van Stappen et al., 2015; Krzyzaniak et al., 2018). LCA examines different impact categories, including global warming, acidification, eutrophication, thereby permitting a complete investigation of different production processes and food products. In this sense, by using LCA, it is possible to evaluate the global impact and the critical phases of a specific food during the entire production process and to compare different production processes using a standard functional unit (FU) either as a unit of product or a unit of cultivated area (Brentrup et al., 2004a; Meier et al., 2015).

The impact of the present study is primarily linked to the following aspects: the relevant amount of considered impact categories, the amplitude of the studied area, the time length of the experiment and the limited literature currently available on the topic.

2. Materials and methods

2.1. Goal and scope definition

This study was aimed at performing an environmental sustainability assessment for the comparison of ORG and CON farming on the production chain of an ancient soft wheat variety (var. Verna). From the analysis of the processes, it was possible to identify the critical phases of the processes in order to propose improvement actions to increase the level of sustainability of Verna wheat agricultural systems. Despite all farms were mainly focused on cereals production, all of them produced additional products as legumes, cattle and forages. However, this study was limited on the assessment of Verna wheat cultivation process.

2.2. Functional unit and system boundaries

The functional unit (FU) indicates the reference factor of the study allowing the comparison of different production systems. Since agriculture is a multifunctional sector, different FU may be adopted according to the aim of the study (Nemecek et al., 2011).

Considering that we compared two production systems in homogenous pedoclimatic area (central Tuscany region) on the same wheat variety, Verna, the functional unit was the mass of product expressed as 1 kg of grain.

Verna is an ancient soft wheat variety, typically of Tuscany, and it was selected due to the following aspects: (i) an effective weed control capacity due to the high plant height; (ii) a sensitive tillering potential that allows it to produce a broad rooting system with a higher nutrient and water use efficiency, compared to modern varieties; (iii) late ripening; (iv) better stress resistance than modern varieties (Lammerts van Beuren at al., 2011) and, (v) high level of nutraceutical properties (Dinelli et al., 2008).

The analysis was performed using data acquired from a five-year period, spanning the growing season from 2014/2015–2018/2019. In order to obtain a representative overview of the wheat-systems in the Tuscany region, five organic and five conventional farms, homogenously distributed over the region to include the provinces of Arezzo, Florence, Grosseto and Siena, were included (Fig. 1). The soil and agro-climatic environment can be considered similar in relation to the productivity of Verna, whereas the random distribution of the farms guaranteed data representativity. The climatic conditions of the study were typically Mediterranean, characterized by rainy and cold winters, and dry and hot summers with precipitation concentrated in both autumn and spring (average annual precipitation of 700 mm). Wheat yields are linked to the higher inter-annual meteo-climatic variability (Dalla Marta et al., 2011a, 2012, 2011b). The maximum vegetative growth period of winter wheat occurs between stem elongation and anthesis, coinciding with the period between February to mid-May. Verna has a later ripening of 7–10 days, compared to modern varieties, that typically occurs between mid-end July. Early heat waves represent a serious issue causing the interruption of the starch accumulation phase with yield losses. However, given the low sowing densities and deeper rooting systems, plants are better able to overcome short-term periods with anomalous high temperatures.

Furthermore, in this study the productive chain with wheat grains as outputs was considered. The straw was not taken into consideration. Straw is used as a source of organic matter for the soil, both directly during harvesting operations and indirectly as manure in livestock farms. The system boundaries encompassed all wheat cultivation activities, from seeds to yields (wheat grains) and transport to the storage centre, including: (i) seed production; (ii) production and consumption of fuels; (iii) production and use of fertilizers; (iv) production and distribution of plant protection products for treatments; (v) transport of cultivation inputs; (vi) water consumption for the dilution of

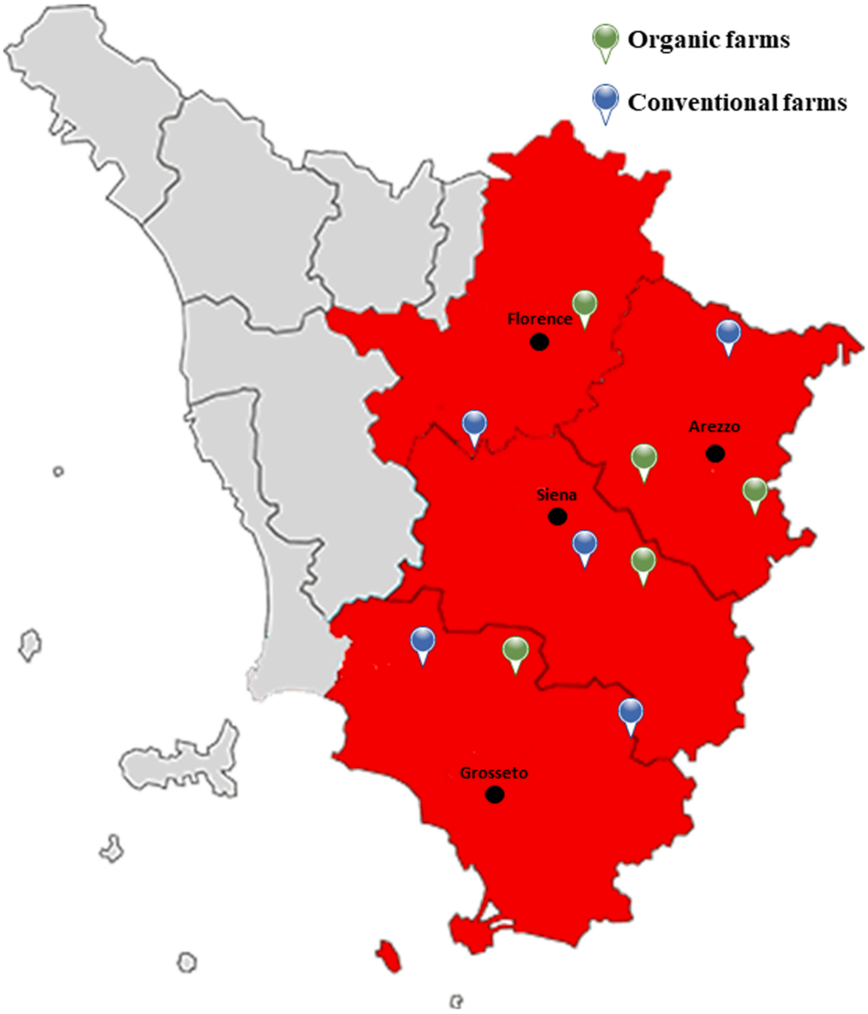

Fig. 1. Representation of the study area in Tuscany region (Italy) composed of the Arezzo, Florence, Grosseto and Siena provinces, respectively.

components used for phytosanitary product preparations; (vii) entire life scenarios for cultivation input packaging; (vii) transport of yields from the farm to the storage centre. As Verna is a rainfed crop, water consumption was only necessary for the dilution of phytosanitary products. Farm infrastructures (both agricultural and related factories), production of machinery and tractors for agricultural operations, human labour, and maintenance phases were not considered in the present study. Further, impacts of pesticide use were not considered because an appropriate model is currently unavailable (Naudin et al., 2014).

2.3. Impact assessment

In the present study, the SimaPro v8.5 software was used for the impact assessment of ORG and CON production systems of Verna wheat. Through CML vs 3.06 (2016) methodology, the following impact categories were evaluated: global warming, freshwater ecotoxicity, seawater ecotoxicity, terrestrial ecotoxicity, human toxicity, acidification, eutrophication, photo-oxidant formation and ozone layer depletion. Moreover, resource consumption indicators including, non-renewable energy resource consumption and renewable energy resource consumption were calculated using Cumulative Energy Demand (CED) vs. 1.11 (2018). In order to implement the wheat production process impact assessment for Verna (Table 1), water consumption and land use were calculated starting with the reporting of water volumes and surfaces used in the life cycle. Previous listed impact categories were chosen since they are the most representative to analyse the sustainability and the environmental impacts of farming systems.

2.4. Life cycle inventory

Data collection for the Life Cycle Inventory (LCI) was carried out by means of specific check-lists developed ad hoc for wheat cultivation. “Survey scheme” summarized the data collected. Inventories refer to the two farming systems, each with its own phases, consumption and yields, respectively (Fig. 2). Primary data from the inventories, were analysed with Ecoinvent v.3.4 database processes using geographical and technological analogies.

The inventory analysis included all the exploited resources and environmental emissions for wheat cultivation, starting from seed production for sowing to the harvest of yields and subsequent transportation to the storage centre.

The observed data from the different farms showed differences in the used inputs and in the agricultural management strategies for both for ORG and CON. In particular, the most relevant differences were observed between organic farms. Each farm followed the same method throughout the years. In order to define a reference cultivation model for the two systems, all the inputs used by the five farms were considered by attributing a respective relative weight. For instance, ploughing was carried out by all organic farms and 100% of the “weight” of impacts was assigned. However, three out of the five organic farms performed disc harrowing, to which 60% of impacts was assigned.

Inputs and outputs, relative to the cultivation activity, were divided into 6 different factors in order to investigate the specific contribution of each one:

- Mechanical Practices (MP). This factor included the use of machinery for tillage, input supplying, as well as harvest and yield transportation (grain and straw) from the farm to the storage centre. Tractor type and power, as well as time and fuel consumption were considered for each mechanical operation (“Survey scheme”). A high degree of homogeneity regarding mechanical practices was observed between the two farming systems, with the exception of fertilization strategies that showed a relevant variability in ORG.

- Fertilizer Manufacturing (FM). This factor considered the impacts generated from the production of fertilizers. In both farming systems, nitrogen-based and phosphorus-based fertilizers were adopted. In ORG, we observed a high variability in agricultural practices and adopted inputs. In particular, for each of the five farms, five different types of fertilizers were used before sowing (Ravel 27, Opengreen; Biosiapor 3.12, Unimer; Endurance N7, Unimer; Endurance N8, Unimer; Siapton, Siapa) (“Survey scheme”). In contrast, in CON, a nitrogen-phosphate fertilizer was adopted for the fertilization treatment before sowing (diammonium phosphate - Siapor, Unimer). Thereafter, a nitrogen-based fertilizer (ammonium nitrate - Sulfan, Yara) was adopted for two top dressing fertilization treatments, respectively (“Survey scheme”).

- Use of Fertilizers (UF). Direct impacts from the use of fertilizers in the field were considered in this category. Both N and P were the elements adopted by both farming systems. However, the dispersion of the fertilizers was carried out differently. Due to their organic source, N-based fertilizers adopted in ORG can be assumed to be more stable than those adopted in CON (“Survey scheme”). In fact, N fertilizers adopted in CON have a synthetic source with a faster degradation rate and higher risk of environmental losses.

- Manufacturing of Herbicides and Fungicides and Seed tanning (HFS). This factor included the production of seeds for sowing, seed tanning, as well as the manufacturing of herbicides and fungicides (for CON only). Seed tanning was performed using copper-based products for ORG, and a mixture of prothioconazole + fluoxastrobin + tebuconazole for CON (“Survey scheme”). Furthermore, in CON, one

Table 1
Impact categories.

	Impact categories	Abbreviations	Reference factor	Units	Reference method
Environmental Impact Categories	Global Warming	GLW	Carbon Dioxide	kg CO_2 eq	CML vs 3.06 (2016)
	Freshwater Ecotoxicity	FET	1,4-Dichlorobenzene	kg 1,4-DB eq	“
	Seawater Ecotoxicity	SET	1,4-Dichlorobenzene	kg 1,4-DB eq	“
	Terrestrial Ecotoxicity	TET	1,4-Dichlorobenzene	kg 1,4-DB eq	“
	Human Toxicity	HUT	1,4-Dichlorobenzene	kg 1,4-DB eq	“
	Acidification	ACD	Sulphur Dioxide	kg SO_2 eq	“
	Eutrophication	EUT	Phosphates	Kg PO_4^- eq	“
	Photo-Oxidant Formation	POF	Ethylene	kg C_2H_4 eq	“
	Ozone Layer Depletion	OLD	Chlorofluorocarbon-11	kg CFC-11 eq	“
Resource Consumption Indicator	Non-Renewable Energy Resources Consumption	NRC	Mega Joule	MJ	Cumulative Energy Demand (CED) vs. 1.11 (2018)
	Renewable Energy Resources Consumption	RRC	Mega Joule	MJ	“
	Water Consumption	WAC	Litres	L	Substances Inventory
	Land Use	LAU	Square meters	m^2	“

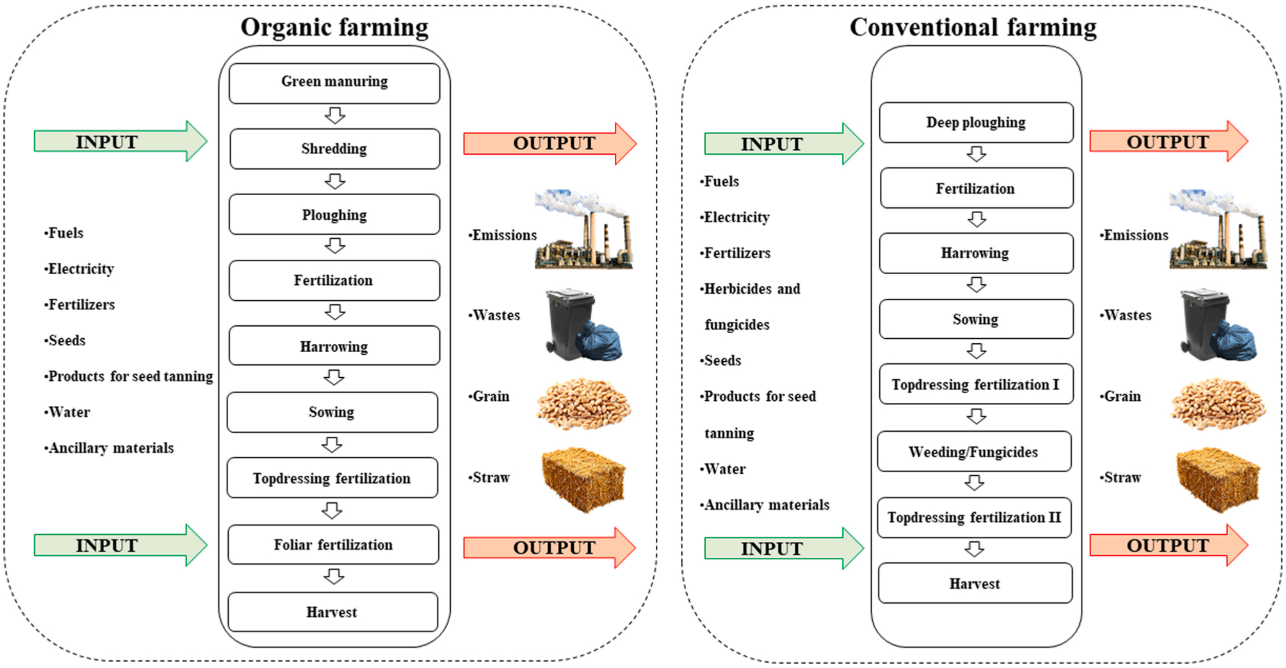

Fig. 2. System boundaries for Verna wheat cultivation in ORG and CON farming system of Verna wheat.

fungicide (BUMPER P: prochloraz + propiconazole) and different herbicides (MAROX: thifensulfuron methyl + tribenuron methyl; AXIAL PRONTO: pinoxaden + cloquintocet mexyl; MANTA GOLD: fluroxipir + clopiralid + mcpa; ATLANTIS: methyl iodosulfuron + diethyl mefenpir + mesosulfuron methyl) treatments were adopted during the growing season ("Survey scheme").

- Ancillary Materials (AM). In this category, we included the impacts for polypropylene thread production, used for tying straw bales. Furthermore, impacts for transport were considered.

- Waste Materials (WM). This category included all the materials used for the packaging of products. In particular, the analysis was conducted based on both the weight and the type of packaging materials, standardized on FU. In addition, disposal methods, as well as the distances between farms and disposal centres were considered. For both farming systems, an average distance of 50 km between the farms and the disposal centre was considered using the Ecoinvent "Transport, freight, lorry 3.5–7.5 metric ton, EURO4 {RER}.

- Direct Land Occupation (DLO). For the LAU impact category, the effective cultivated surface was computed.

Within each impact factor, the substances responsible for the impacts were identified.

2.5. *Life cycle impact assessment (LCIA)*

The LCIA (carried out using the SimaPro v8.5 software) highlighted the impacts of each impact category starting from the data collected in the LCI. The combination of inventory data, with specific equivalent factors, permitted the attainment of characterization factors for each impact category. The impacts were reported by specific indicators for each category.

Results from the LCIA were standardized based on their respective reference scales on the EU25 level (CML vs 3.06, 2016). Therefore, dimensionless values that permitted a comparison between the different impact categories were obtained (Table 3). The normalized values allowed for an objective characterization of the phenomenon extent with respect to the amplitude of its reference scale. During normalization, the indicator values per functional unit for impact category were related to specific normalization factors (Brentrup et al., 2004a; Fallahpour et al., 2012; Krzyzaniak et al., 2018).

3. Results

From the analysis of results on yields a significant difference ($p < 0.001$) was observed between the two farming systems. In particular, average yields throughout five years were 1524 (± 521) and 2815 (± 294) kg of wheat grain for ORG and CON, respectively.

3.1. *Global warming*

Emissions of greenhouse gases (GHGs) are the driving factor causing the increase in the Earth's surface temperature, known as "Global Warming". The main GHGs from agriculture are principally represented by carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). GHG emissions are standardized on CO_2 global warming effects and computed as kg CO_2 equivalents (eq) on a 100-year time scale, using IPCC guidelines (IPCC, 2013). In ORG, MP represented the main factor affecting GLW, accounting for 76% of 0.359 kg CO_2 eq FU^{-1} (Fig. 3). HFS produced a significant impact, due to the products for seed tanning and the environmental costs involved in seed production for sowing. The FM and UF provided a small contribution due to the limited adoption of synthetic fertilizers. Nevertheless, in CON the main contributors to GLW were FM (39% of the total), MP (29% of the total) and UF (25% of the total) (Fig. 3). Compared to ORG, CON showed higher impacts in terms of GLW, accounting for 0.518 kg CO_2 eq FU^{-1} with a net contribution of more than 162% (Table 2).

3.2. *Freshwater ecotoxicity*

FET involves the impact of toxic substances produced by the various processes on freshwater organisms. As with the other ecotoxicity categories (see Sections 4.3 and 4.4), FET was reported as kg 1,4 dichlorobenzene equivalents (1,4-DB eq). FM showed a relevant impact on ORG, accounting for 35% of the total. The second was represented by HFS, with an impact of 33% of the total. MP characterized 20% of the total impacts in ORG. Progressively, in descending order, the main impacts were shown to be linked to nickel (26%), beryllium (21%), copper (13%) and cobalt (12%). FM accounted for 88% of the total impacts in CON, with HFS amounting to 7% (Fig. 3). Likewise in ORG, the main impacts were related to heavy metals, such as nickel (29%), beryllium

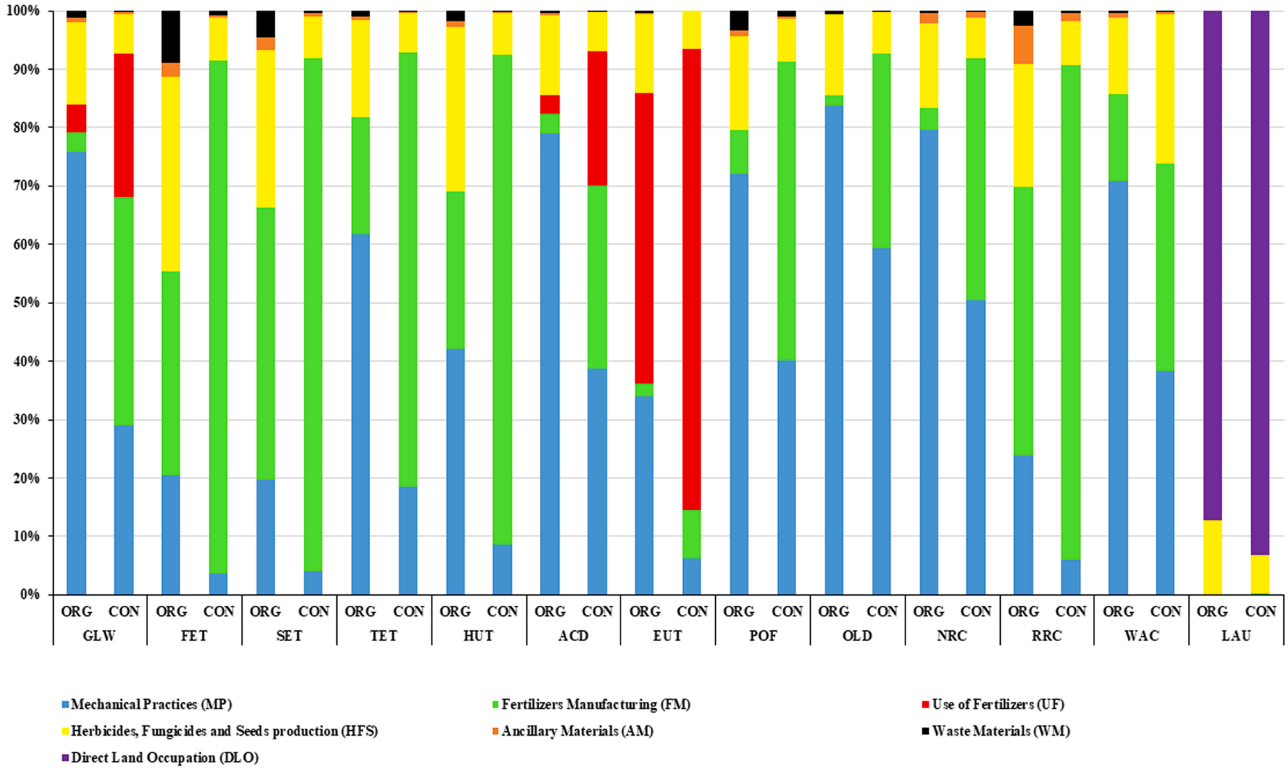

Fig. 3. Relative contribution (percentage) of cultivation factors to impact categories in Verna wheat production between organic (ORG) and conventional (CON) farming systems.

Table 2
Environmental impacts of Verna wheat production processes between organic (ORG) and conventional (CON) farming systems from each of considered factors expressed as per one kg of wheat grain.

			MP	FM	UF	HFS	AM	WM	DLO	TOTAL
GLW	kg CO_2 eq	ORG	2.70E-01	1.00E-02	2.00E-02	5.00E-02	3.08 E-03	4.20 E-03	–	3.60E-01
		CON	1.70E-01	2.30E-01	1.40E-01	4.00E-02	1.69 E-03	1.31 E-03	–	5.80E-01
FET	kg 1,4-DB eq	ORG	3.03 E-03	5.18 E-03	0.00E+ 00	4.96 E-03	3.51 E-04	1.31 E-03	–	1.00E-02
		CON	1.88 E-03	4.00E-02	0.00E+ 00	3.68 E-03	1.91 E-04	4.07 E-04	–	5.00E-02
SET	kg 1,4-DB eq	ORG	1.10E+ 01	2.62E+ 01	0.00E+ 00	1.51E+ 01	1.30E+ 00	2.50E+ 00	–	5.61E+ 01
		CON	6.85E+ 00	1.50E+ 02	0.00E+ 00	1.23E+ 01	7.10E-01	7.80E-01	–	1.70E+ 02
TET	kg 1,4-DB eq	ORG	1.72 E-04	5.56 E-05	0.00E+ 00	4.66 E-05	1.59 E-06	2.78 E-06	–	2.79 E-04
		CON	1.07 E-04	4.32 E-04	0.00E+ 00	4.00 E-05	8.57 E-07	8.71 E-07	–	5.80 E-04
HUT	kg 1,4-DB eq	ORG	1.00E-02	9.83 E-03	6.26 E-06	1.00E-02	3.51 E-04	6.69 E-04	–	4.00E-02
		CON	9.47 E-03	9.00E-02	5.61 E-05	7.86 E-03	1.89 E-04	2.09 E-04	–	1.10E-01
ACD	kg SO_2 eq	ORG	2.44 E-03	9.98 E-05	1.00 E-04	4.24 E-04	1.05 E-05	1.13 E-05	–	3.08 E-03
		CON	1.51 E-03	1.22 E-03	8.98 E-04	2.61 E-04	5.72 E-06	3.51 E-06	–	3.90 E-03
EUT	kg PO_4^- eq	ORG	5.51 E-04	3.52 E-05	8.03 E-04	2.20 E-04	2.08 E-06	6.05 E-06	–	1.62 E-03
		CON	3.42 E-04	4.47 E-04	4.26 E-03	3.55 E-04	1.13 E-06	1.88 E-06	–	5.41 E-03
POF	kg C_2H_4 eq	ORG	4.74 E-05	4.98 E-06	0.00E+ 00	1.05 E-05	6.62 E-07	2.22 E-06	–	6.58 E-05
		CON	2.94 E-05	3.75 E-05	0.00E+ 00	5.27 E-06	3.61 E-07	6.88 E-07	–	7.32 E-05
OLD	kg CFC-11 eq	ORG	5.18 E-08	1.04 E-09	0.00E+ 00	8.55 E-09	5.11 E-11	3.33 E-10	–	6.18 E-08
		CON	3.22 E-08	1.80 E-08	0.00E+ 00	3.87 E-09	2.61 E-11	1.04 E-10	–	5.41 E-08
NRC	MJ	ORG	4.24E+ 00	2.06 E-01	0.00E+ 00	7.70E-01	9.00E-02	2.00E-02	–	5.34E+ 00
		CON	2.64E+ 00	2.17E+ 00	0.00E+ 00	3.60E-01	5.00E-02	5.56 E-03	–	5.23E+ 00
RRC	MJ	ORG	8.36 E-03	2.00E-02	0.00E+ 00	7.34 E-03	2.30 E-03	8.94 E-04	–	3.00E-02
		CON	5.19 E-03	7.00E-02	0.00E+ 00	6.45 E-03	1.27 E-03	2.78 E-04	–	9.00E-02
WAC	litres	ORG	3.73E-01	7.80E-02	0.00E+ 00	6.90E-02	4.00E-03	2.00E-03	–	5.26E-01
		CON	2.32 E-01	2.16E-01	0.00E+ 00	1.55E-01	2.00E-03	1.00E-03	–	6.06E-01
LAU	m^2	ORG	3.30 E-04	1.09 E-03	0.00E+ 00	9.60E-01	1.45 E-04	6.89 E-05	6.56E+ 00	7.53E+ 00
		CON	2.05 E-04	1.00E-02	0.00E+ 00	2.50E-01	7.93 E-05	2.17 E-05	3.62E+ 00	3.89E+ 00

Legend: GLW = Global Warming; FET = Freshwater Ecotoxicity; SET = Seawater Ecotoxicity; TET = Terrestrial Ecotoxicity; HUT = Human Toxicity; ACD = Acidification; EUT = Eutrophication; POF = Photo-oxidant Formation; OLD = Ozone Layer Depletion; NRC = Non-renewable energy Resources Consumption; RRC = Renewable energy Resources Consumption; WAC = Water Consumption; LAU = Land Use. MP = Mechanical Practices; FM = Fertilizers Manufacturing; UF = Use of Fertilizers; HFS = Herbicides and Fungicides manufacturing and Seeds use; AM = Ancillary Materials; WM = Waste Materials; DLO = Direct Land Occupation (only for LAU impact category). DLO refers only to LAU and therefore does not show any correspondence with the other impact categories.

(21%), copper (14%) and cobalt (12%), respectively. The impacts of ORG were approximately 30% (0.015 kg 1,4-DBeq FU^{-1}) of those of CON (0.050 kg 1,4-DBeq FU^{-1}) (Table 2).

3.3. Seawater ecotoxicity

SET refers to the effect of toxic substances, emitted in the environment, on seawater organisms. Similar to FET, SET is highly dependent on FM and HFS. In ORG, we observed an impact of 47% and 27% for FM and HFS, respectively. Emissions of hydrogen fluoride (41%) and beryllium release in the aquifer (33%), due to FM and HFS, were the principle impact factors. To the contrary, in CON the main impacts were primarily from FM, and secondarily from HFS, accounting for 88% and 7% of the total impacts, respectively (Fig. 3). Likewise in ORG, hydrogen fluoride and beryllium represented the main impact factors contributing to 38% and 37% from FM, respectively. Total impacts from CON (170.304 kg 1,4-DBeq FU^{-1}) were approximately three times higher than ORG (56.069 kg 1,4-DBeq FU^{-1}). Furthermore, SET had $10^3/10^4$ higher impacts compared to FET in both farming systems (Table 2).

3.4. Terrestrial ecotoxicity

TET considers the effects of toxic substances on land organisms. TET is mainly derived from MP, FM and HFS in both farming systems. In particular, MP contributed to 62%, FM to 20% and HFS to 17% of the total impacts in ORG, respectively. In CON, FM contributed to 74% and MP to 18% of the total impacts, respectively (Fig. 3). Heavy metals (zinc and mercury) had higher impacts in ORG accounting for approximately to 80% of the total. In CON, mercury (35%), cypermethrin (25%), zinc (16%) and nickel (10%) were the main impacts. In total, CON produced 208% (5.80E^{-4} kg 1,4-DBeq FU^{-1}) higher impacts than ORG (2.79E^{-4} kg 1,4-DBeq FU^{-1}) (Table 2).

3.5. Human toxicity

HUT refers to the effects of toxic substances (released from wheat cultivation into the environment) on human health. Similarly to the ecotoxicity categories, HUT was also expressed as kg 1,4-DBeq. In ORG, the main impacts were related to MP, HFS and FM that accounted for 42%, 28% and 27% of the total, respectively. In particular, nitrogen oxides (15%), selenium (12%) and chromium VI (10%) were shown to incur the main impacts. Instead, in CON the main impacts were related to FM that accounted for 84% of the total (Fig. 3). Chromium VI (26%), selenium (17%) and nickel (16%) represented the main source of impact for CON. Cumulative impacts of ORG (0.036 kg 1,4-DBeq FU^{-1}) were approximately one third of those of CON (0.110 kg 1,4-DBeq FU^{-1}) (Table 2).

3.6. Acidification

This impact category is caused by the release of protons into aquatic and terrestrial ecosystems, mainly through rain, with effects on the development of life. The acidification potential is assessed as SO_2 or H^+. In this study kg SO_2 eq was adopted. Principally, ACD is determined as the release of sulphur dioxide, nitrogen oxides and ammonia. MP embodied the main impact factor from ORG, accounting for 79% of the total impacts. This is mainly attributable to fuel usage that represented a significant source of nitrogen oxide (76%) and sulphur dioxide (SO_2) (20%) emissions. Nevertheless, HFS embodied a relevant impact factor with 14% of the total. In CON, acidification was affected by several factors. In particular, MP showed the higher impact (39%) following fuel use. However, FM and UF represented relevant factors accounting for 31% and 23% of the total impacts (Fig. 3), respectively. In total, ORG and CON contributed to 0.003 kg SO_2 eq FU^{-1} and 0.004 kg SO_2 eq FU^{-1}, respectively, with a higher impact of 27% from CON (Table 2).

3.7. Eutrophication

ETP is the undesired proliferation of biomass in ecosystems following a nutrient enrichment process. In this study, ETP was mainly linked to the following factors: release of nitrates and phosphates (via leaching and erosion) release of NH_3 following (over) fertilization of fields, and NOx production from the use of tractors. Normally, ETP is referred as kg of PO_4^- eq.

In ORG, the highest contribution was from UF, with 50% of the total impacts. Nevertheless, MP and HFS showed a relevant impact with 34% and 14% of the total, respectively. Nitrogen oxides represented 37% of the total impacts followed by nitrate (30%) and phosphate (28%). In CON, the main impacts were from UF with 79% of the total. This aspect represented one of the main issues related to the environmental pressures of agriculture. FM (8%), HFS (6%) and MP (6%) all showed a small influence on ETP in CON (Fig. 3). Nitrates characterized the main impact source (74% of the total), with a lower contribution from both phosphates (8%) and nitrogen oxides (8%). Overall, ORG presented an impact of 0.002 kg of PO_4^- eq FU^{-1} corresponding to 30% of the impact of CON (0.005 kg of PO_4^- eq FU^{-1}) (Table 2).

3.8. Photo-oxidant formation

POF is an indicator related principally to the formation of tropospheric ozone, caused by the reactions of organic components in the presence of light and heat. Generally, it is formed during hot periods (eg. summer). This impact category is largely affected by air pollutants such as nitrogen oxides (NO_x), SO_2 and non-methane volatile organic compounds (NMVOC). These compounds are mainly produced by extraction and distribution of fossil fuels, vehicle exhausts and combustion processes (Derwent et al., 2007; Preiss, 2015). Photochemical Ozone Creation Potential (POCP) represents the contribution of a substance on photochemical ozone production and is expressed as kg of C_2H_4 eq. In ORG, MP and HFS embodied the main impact factors with 72% and 16% of the total, respectively. The high impact of MP was due to carbon monoxide (43%) and SO_2 (38%) emissions. In CON, FM showed the highest impact with 51% of the total, attributable to emissions following fertilizer synthesis. However, MP produced relevant impacts with 40% of the total (Fig. 3). The predominant impact sources were represented by SO_2 (57%) and carbon monoxide (25%) emissions. Higher yields of CON reduced the impacts from MP, but fertilization balanced the total impacts of the two farming systems. Total impacts were 6.58 E^{-5} kg C_2H_4 eq FU^{-1} and 7.32E^{-5} kg C_2H_4 eq FU^{-1} from ORG and CON respectively. For this impact category, ORG produced 10% lower impacts than CON (Table 2).

3.9. Ozone layer depletion

Stratospheric ozone depletion causes the increase of ultraviolet ray incidence, harmful to living organisms. For a specific evaluation of this phenomenon, the Ozone Depletion Potential (ODP) index was proposed. This impact category is referred to the amount of ozone depleting substances, and includes chlorofluorocarbons (CFCs) and Hydrofluorocarbons (HFCs), expressed as kg of CFC-11 eq.

In ORG, MP embodied 84% of the total impacts, and was followed by HFS with a net contribution of 14%. Methane, bromotrifluoro- and Halon 1301 were found to be responsible for 98% of the total impacts. Even in CON, MP represented the main impact factor, contributing to 59% of the total. However, FM also played an important role with 33% of the total impacts (Fig. 3). Methane, bromotrifluoro-, Halon 1301 and methane, bromochlorodifluoro-, Halon 1211 were responsible for 83% and 13% of the total impacts, respectively. In total, CON showed an impact of 5.41E^{-8} kg of CFC-11 eq FU^{-1} while ORG produced 6.18E^{-8} kg of CFC-11 eq FU^{-1}, with a higher impact of 14% (Table 2).

3.10. Non-renewable energy resources consumption

NRC was assessed as the budget of primary non-renewable energy resources, used for the production processes of wheat. In the present study non-renewable energy resource consumption was reported in mega joules (MJs).

Following the lower yields, a MP, constituting 80% of the total impact was found in ORG. In addition, 14% of the total impact was represented by HFS. Crude oil represented 93% of the impacts. MP (50%) and FM (42%) mainly caused NRC in CON (Fig. 3). This was mainly due to fuel consumption, attributable to both mechanization and processes for the production of chemical fertilizers. Crude oil and natural gas consumption represented 69% and 26% of total impacts, respectively. Nevertheless, higher total impacts were produced by ORG (5.34 MJ FU^{-1}) than CON (5.23 MJ FU^{-1}) with a net increase of 2% (Table 2).

3.11. Renewable energy resources consumption

This impact category assesses the consumption of primary renewable energy resources used within the production processes, not calculated thus far. Similar to NRC, this indicator is reported as MJs.

In ORG, the highest impact were derived from FM, with 46% of the total. MP and HFS represented 24% and 21% of the total impacts, respectively. The highest use of renewable resources was water (50%). Biomass (35%), as well as wind, solar and geothermic factors (15%) produced a lower impact. The total consumption of RRC in ORG accounted for 0.035 MJ FU^{-1}. In CON, FM represented the predominant part of the impacts (85%), whereas HFS and MP accounted for 7% and 6% (Fig. 3), respectively. Energy from water and biomass resources produced similar impacts (45% and 42%, respectively). Wind, solar, geothermic factors then represented the residual part of the impacts (13%). RRC in CON accounted for 0.087 MJ FU^{-1}. Thus, ORG consumed 40% more of renewable energy resources than CON (Table 2).

3.12. Water consumption

This impact category refers only to the water used for the production processes relating to cultivation inputs. In addition, WAC for herbicide and fungicide dilutions was considered. However, given that wheat is a rainfed crop in Italy, there was no consumption of water for irrigation. The adopted methodology did not consider the water required for the dilution of pollutants to the legal values, as indicated in the water footprint methodology (e.g. Available WAter Remaining, vs 1.02 2016 - AWARE).

MP that accounted for 71% of 0.526 l H_2O FU^{-1} represented the predominant part of WAC in ORG. Instead, FM and HFS characterized 15% and 13%, respectively. In CON, MP represented 38% of the total (0.606 l H_2O FU^{-1}). In this case, FM and HFS were shown to have a higher impact representing 36% and 26%, respectively (Fig. 3). The impact gap between the two farming systems was 115.21% higher in CON (Table 2). The relevant impact of MP in ORG was related to the lower yields compared to CON. However, in CON the lower impact of MP was replaced by the higher impacts from both FM and HFS than ORG.

3.13. Land Use

This impact category is calculated for land occupation, for both the cultivation phase and the production of adopted external inputs, and is reported as square meters per year (m^2 y).

In both farming systems, approximately the 90% of land occupation was linked to DLO (Fig. 3). The residual part referred to the external input production and logistic services on farms. CON required 52% of LAU than ORG (3.89 and 7.53 m^2 y, respectively) (Table 2).

3.14. Normalization

As previously mentioned, to standardize the impacts of the analysed system on the reference scale of the Europe 25 level a normalization step was adopted. This provided a dimensionless data allowing an objective comparison between different impact categories. To do that, during the normalization phase characterized data were related to dedicated normalization factors (Krzyzaniak et al., 2018).

The analysis showed that CON generated a higher impact compared to ORG. From all considered environmental impact categories, in both farming systems, SET had the greatest impact followed by EUT, ACD and GLW, respectively. FET, TET, HUT and POF generated lower impacts. Nevertheless, wheat production showed a negligible impact in both farming systems for OLD.

4. Discussion

Of the goals of the EU “Farm to Fork Strategy” (COM 381/2020) (Anon, 2020), a 50% reduction in nutrient losses with no deterioration to soil fertility, and a 20% reduction in the use of synthetic fertilizers by 2030 are central to rendering make food systems fair, healthy and environmentally-friendly.

Ancient cereals cultivation is growing rapidly in the recent years due to their high variable genetic heritage and, thus greater resilience, higher biotic and abiotic stress tolerance and growing interest of consumers due to their nutraceutical value. These characteristics make Verna, and the ancient cereals in general, interesting crops. Nevertheless, Verna cannot represent the whole wheat cropping systems due to the lower yields compared to those of modern varieties. A comparison between them may risk to overestimate the environmental impacts of ancient wheat varieties.

The comparison between a LCA analysis on organic and conventional Verna wheat cultivation is an effective tool to assess the environmental performances of different farming systems in the light of the “Farm to Fork Strategy”.

4.1. Global warming

From the present study regarding GLW, the most relevant impact factor was MP, attributable to fuel consumption from agricultural machinery (Fig. 3) (Fallahpour et al., 2012). Of the MP impacts, ploughing was shown to produce the greatest impact, amounting to approximately 29% and 32% of the total fuel consumption for ORG and CON, respectively (“Survey scheme”). The present results are corroborated by recent publications, investigating the environmental impacts of agriculture, in which a relevant effect of mechanization to on-farm emissions was reported (Lovarelli et al., 2017). However, the contribution of fuel consumption to the environmental impacts of agricultural activity was shown to be liable to strong fluctuations based on different factors such as pedo-climatic conditions, production systems (organic or conventional farming), tillage systems (conventional tillage, minimum tillage, no tillage etc.) and yields (Lovarelli et al., 2017; Carranza-Gallego et al., 2018). This latter aspect was shown to represent a critical feature. The reason was that despite the comparable fuel consumption use between ORG and CON (“Survey scheme”), the greater impact generated by ORG was related to lower yields (Fig. 3) (Meisterling et al., 2009; Chiriacò et al., 2017; Carranza-Gallego et al., 2018; Tricase et al., 2018). In this sense, we observed an impact of 0.27 and 0.17 kg CO_2eq FU^{-1} for ORG and CON, respectively (Table 2). However, when considering a hectare as a functional unit, the net contribution of GLW from fuel consumption appeared similar between the two farming systems (415.62 and 476.67 kg CO_2 eq ha^{-1}, for ORG and CON, respectively). The present findings are in line with Ali et al. (2017) when compared to CON (MP=29% of total impacts), but significantly higher when compared to ORG (MP=76% of total impacts) (Fig. 3). This highlighted that in order to ensure comparable yields with CON farming, the ORG system, requiring a greater surface area of land, is at risk in causing significant impacts from the use of machinery. Our results, were shown to be higher than previous observations, that reported an average impact of 67.4 kg C ha^{-1} using conventional tillage on wheat in USA (West and Marland, 2002). The relevant impacts of FM and UF are predominantly related to the energy consumption for the production of N-based compounds (e.g. nitrates, ammonium, urea) and to N_2O emissions in fields by fertilization treatments. Recent studies reported that from 1% to 5% of the N distributed during fertilization is lost as N_2O, contributing greatly to global warming (Meisterling et al., 2009; Venterea et al., 2012; Verdi et al., 2019a). Thus, the impacts related to fertilization represents a serious issue for conventional farming. Previous research supported the present findings, showing a significantly higher contribution of fertilization (fertilizers production and use) to GLW in CON (0.37 kg CO_2eq FU^{-1}) than ORG (0.03 kg CO_2eq FU^{-1}) (Table 2). Fallahpour et al. (2012) reported similar findings, with a global warming mitigation potential from organic fertilizer use of approximately 80% compared to chemical fertilizers. Therefore, the potential of precision agriculture, or better precision fertilization, to manage soil nutrient variability in space and time appears to be very high by directly affecting the main sources of impact on GLW. However, the technological barriers to adopting this management strategy and the high costs are limiting its diffusion, especially in less developed agricultural systems. Slow-release N fertilizers and nitrification/urease inhibitors, may contribute to long-term sustainability of agriculture production. Furthermore, the increase in soil fertility through the adoption of functional cover crops and intercropping, as well as conservative tillage represent additional essential elements to reduce GLW.

Nowadays, soil organic carbon levels are low and are also considered unchanged. There were no significant changes between the two systems. Therefore, in the balance, it was not possible to consider the greater potential for organic C sequestration in ORG soils. This was attributable to the adoption of non-conservative tillage practices and to the hot, dry summers, which expose soil organic matter to rapid oxidation. In addition, as affirmed by the present study ("Survey scheme" 1), green manure practices had been abandoned, despite ORG protocol suggestions. If from one hand, MP is the main source of impact for GLW, from the other the adoption of specific management strategies aiming to improve soil organic carbon sequestration should counterbalance this trend improving the environmental performances of agriculture while maintaining soil fertility.

4.2. Freshwater ecotoxicity

The lower impact on FET in ORG was mainly due to the reduced use of pesticides, fungicides, and fertilizers compared to CON. According to Prechsl et al. (2017), heavy metal emissions into the environment from fertilizers represent the predominant impact. However, copper used for seed tanning, as well as the metals linked to its extraction, all generate a relevant impact on freshwater ecotoxicity in organic farming (Sydow et al., 2020). The direct use of copper was not shown to generate impacts, although recent studies reported the movement of this element from the soil to the aquifer with relevant impacts on the environment (Rocha et. al., 2011). Krzyzaniak et al. (2018) showed lower impacts (kg 1,4-DB) on a mallow cultivation in Poland. Similar to our observations, the main impacts were related to the fertilizer sector with a lesser impact attributed to chemical weeding. In an irrigated-rainfed wheat experiment, Taki et al. (2018) observed a relevant impact on FET from chemicals use (averagely 0.05 kg 1,4-DB per kg of wheat grain). The intense adoption of chemicals (pesticides, fungicides and fertilizers) was primarily responsible for the higher impact on FET from wheat cultivated in CON (Table 2). Similarly to GLW, an improved N and P-based fertilizers, through site-specific fertilization strategies, in order to reduce the release of heavy metals from FM, may represent an effective strategy to reduce FET impacts in CON.

Table 3
Normalization values based on the Europe 25 scale (CML vs 3.06, 2016) for the different environmental impact categories.

Impact category	Units	Normalization Factors	Organic farming	Conventional farming
GLW	kg CO_2 eq	1.99E-13	71.5E-15	115.7E-15
FET	kg 1,4-DB eq	1.93E-12	28.6E-15	96.7E-15
SET	kg 1,4-DB eq	8.57E-15	480.5E-15	1459.5E-15
TET	kg 1,4-DB eq	2.06E-11	5.7E-15	11.9E-15
HUT	kg 1,4-DB eq	1.29E-13	4.7E-15	14.2E-15
ACD	kg SO_2 eq	3.55E-11	109.4E-15	138.6E-15
EUT	kg PO_4^{-} eq	7.58E-11	122.6E-15	410.2E-15
POF	kg C_2H_4 eq	1.18E-10	7.8E-15	8.6E-15
OLD	kg CFC-11 eq	1.12E-08	0.7E-15	0.6E-15

4.3. Seawater ecotoxicity

Corroborating recent literature, SET in the present study represented the major impact category in wheat cultivation accounting for 99.77% and 99.73% of the total impacts for ORG and CON, respectively (Table 3). An average impact of 320 kg 1,4-DB per kg wheat grains on 75 farms in Iran was reported recently (Ghasemi-Mobtaker et al., 2020). Those authors reported that N and P-based fertilizers represented the sector with the greatest impact. In over 210 farms in Iran, an average impact on seawater ecotoxicity of 239.5 kg 1,4-DB per kg wheat grain was reported (Taki et al., 2018). The most relevant contribution was due to P-based fertilizers, accounting for over 70% of the total impacts. Monti et al. (2009), comparing four perennial energy crops and wheat-maize rotations in Italy, found a 10–30 times higher impact on seawater ecotoxicity compared to other impact categories. Regardless of the system considered, seawater ecotoxicity represented the most affected impact category regardless of the farming system. In the present study, the relevant impact of ORG on SET was related to copper used for seed tanning (Table 2). As with other heavy metals, copper has a strong impact on marine ecosystem (Zhang et al., 2017). Similar to FET, the adoption of those fertilization strategies allowing the improvement of efficiency use (precision farming, slow release fertilizers etc.) ensures the reduction of SET impact of wheat cultivation in CON. Even for SET, including cover crops/intercropping would contribute on limiting weeds and thus herbicides amounts that, in CON, are relevant source of impact.

4.4. Terrestrial ecotoxicity

Terrestrial ecotoxicity is predominantly related to fertilizer production, fuel combustion and pesticide use (including herbicides and fungicides) (Charles et al., 2006; Alaphilippe et al., 2013; Krzyzaniak et al., 2018; Ghasemi-Mobtaker et al., 2020). Fertilization treatments were reported to have a relevant impact on terrestrial ecotoxicity when comparing British and Swiss wheat production systems (Charles et al., 2006), By comparing the different fertilization strategies, those authors observed that by lowering fertilization, only terrestrial ecotoxicity was shown to decrease. Similarly, in the present study CON showed a relevant impact of FM due to the elevated adoption of fertilizers. The present results were consistent with those presented in the literature, in which total impacts from FM amounted to 20% and 74% for ORG and CON, respectively. (Krzyzaniak et al., 2018; Ghasemi-Mobtaker et al., 2020). There was a relevant impact of MP in ORG (Fig. 3), mainly due to mercury (Chen et al., 2016) and zinc emissions produced by fuel combustion combined with the lower yields. In the present study, a significant impact from HFS on terrestrial ecotoxicity in ORG was noted (Fig. 3), consistent with previous findings showing that mineral fungicides contaminate soil following copper release (Alaphilippe et al., 2013). Regarding ORG, tillage efficiency improvements represented a key factor in reducing impacts of TET through the decrease of fuel consumption. Additionally, the use of alternative seed tanning products may represent an additional strategy to reduce TET impacts. In CON, similar to FET and SET, environmental performance improvements were strongly linked to nutrient use efficiency.

4.5. Human toxicity

The resultant production of Chromium VI, NOx, Nickel (air) and Selenium (water) from FM and HFS, indicated that industrial processes impacted on HUT from both farming systems. Recent literature is consistent with our results, suggesting that the reduction of chemicals (fertilizers, herbicides, pesticides and products for seed tanning) may represent an effective strategy to reduce the HUT impact magnitude of wheat cultivation (Taki et al., 2018; Ghasemi-Mobtaker et al., 2020). MP was shown to represent an additional source of toxic compounds, especially from ORG, thereby highlighting the environmental impact of fuel combustion (Fig. 3). Reducing mechanization (e.g. adopting conservative-tillage practices) was reported to reduce toxic substances in both air and water, and to contribute to the maintenance of SOM levels and the intrinsic fertility of the soil (Ding et al., 2002). Rationalizing the use of mechanization and chemicals appear to be two performing strategies for creating sustainable supply chains that produce healthy food with low environmental impacts and reduced impacts on human health.

4.6. Acidification

Acidification is primarily attributable to the combustion of fossil fuels at power stations and industrial plants, vehicle exhausts, and agriculture (van Zelm et al., 2015). The results of the present study corroborated recent literature where acidification impacts from wheat cultivation were shown to range between 1.95 and 7 g SO_2 kg^{-1} wheat grain (Achten and Van Acker, 2016; Holka et al., 2016; Fallahpour et al., 2012; Ghasemi-Mobtaker et al., 2018). More than 60% of acidification from wheat cultivation was shown to be attributable to on-farm emissions from diesel and fertilizers into the air, water and soil, and from heavy metals of fertilizers into the soil, respectively (Ghasemi-Mobtaker et al., 2018). Environmental performance in ORG was improved, as fuel combustion from MP formed approximately 80% of the total impacts (Fig. 3). CON showed more than 90% of ACD due to the combustion of fuels and fertilizers (production and use).

Similar to previous reports, we observed that sulphur oxides (SOx) and NOx production from exhaust, fertilizers production processes and use constituted the main components of ACD (Holka et al., 2017; Taki et al., 2018). Generally, organic fertilizers have a weak tendency of soil acidification. In contrast, chemical fertilizers used in conventional systems have different properties based on their composition. In some fertilizers such as YARA SULFAN the acidity caused by ammonium nitrate is additive due to the high sulphur (S) content (15%). In similar cultivation conditions, Fallahpour et al. (2012) observed that roughly 48% of the "total acidification gases" were due to NH_4, whereas 15% and 36% were from N_2O and $SO_{2,}$ respectively. The use of fertilizers in CON did not seem to offset the greater use of diesel per kg of product in ORG. For this reason, the impacts between the two farming systems were shown to be similar. According to Houshyar and Grundmann (2017) reducing the ACD implicated the adoption of minimum tillage strategies under conditions not negatively affecting yield. Straw return into the soil would be an effective strategy to mitigate soil acidification and maintain soil fertility (Hao et al., 2020; Shan et al., 2021). However, N dynamics (intrinsic soil content and fertilization) must be taken into account to ensure N availability at the soil level for microbial biodiversity conservation and to maintain high yields from the upcoming crops.

4.7. Eutrophication

EUT is directly connected to nutrient dynamics and the intense use of N and P- based fertilizers (Brentrup et al., 2004b; Fallahpour et al., 2012; Huang et al., 2017). Generally, in order to offset the nutrient losses related to different factors (site-specific pedo-climatic conditions, fertilizers dispersion methods etc), fertilizers rate exceeds the needs of plants. However, fertilizer use efficiency is highly dependent on the farming system, where anthropic actions drive plant-environment interactions (Fabbri et al., 2020). We observed a positive correlation between N and P rate and EUT, resulting in a higher impact in CON. Intense tillage has a relevant effect on N and P losses, both by increasing N and P organic mineralization and by promoting erosion losses (Pulighe et al., 2020). Thus, minimum tillage may represent an effective strategy to mitigate losses. The opportunity to mitigate organic N and P losses and to protect the soil from erosion renders the adoption of cover crops an additional effective strategy to reduce EUT (Houshyar and Grundmann, 2017; Prechsl et al., 2017; Taki et al., 2018). In ORG, fertilizers have a lower impact. This is due both to the lower quantity used and to the form of N, which was organic. However, as was observed previously, the use of manure and sewage in ORG is a critical factor in increasing the impacts on EUT due to higher NH_3 emissions (Van Stappen et al., 2015; Prechsl et al., 2017). The adoption of slow release fertilizers may favour N use efficiency by hampering leaching losses that represent the principle impact factor. In this study we observed a certain abandonment of green manuring in ORG (only one in five farms adopted this strategy). Development of cover cropping coupled to green manuring may reduce fertilizers needs and thus EUT impacts in organic farming while maintaining high yields.

4.8. Photo-oxidant formation

The use of fossil energy, fuel combustion and fertilizer manufacturing all constitute the primary factors affecting POF (Taki et al., 2018; Ghasemi-Mobtaker et al., 2020). From the analysis of our results, we observed that fuel consumption and input manufacturing (fertilizers and pesticides) affected POF the most (Fig. 3). ORG has a great impact from fuel consumption following the high impact of MP when compared to the low usage of synthetic inputs (Derwent et al., 2007). According to Queiros et al. (2015), CON is characterized by a relevant impact from FM and MP due to environmental emissions from both industrial plants and exhaust. Recent findings proposed the use of activated charcoal enriched filters in catalytic converters to abate exhaust impacts from diesel engines (Naveenkumar et al., 2020). Conversely, the use of urea to reduce NOx emissions produced an insignificant effect since the latter show a negligible contribution on POF impacts. Lessening $SO_{2,}$ linked to diesel exhaust, is challenging since available technologies are only used in large-scale facilities (Osaka et al., 2015). SO_2 emissions from the production of P-based fertilizers represent an additional source of impact affecting POF (Salam, 2013). This was emphasized by the global sulphur consumption that accounted for more than 50% with P-based fertilizer manufacturing (Ceccotti et al., 1998). Moreover, climatic conditions strongly affect photo-oxidant formation (Yang et al., 2020). The present study was based on the EU 25 scale (CML vs 3.06, 2016) for average European conditions. Nevertheless, it should be noted that the study area, with a typical Mediterranean climate, has unfavourable irradiation and temperature conditions for the impact of this category. Thus, the adoption of the CML methodology may underestimate POF impacts than the reality.

Recent findings reported different soil NOx emission levels based on N-compounds into the soil (Yang et al., 2020). NO_2^- and NO_3^- compounds shows higher NOx emission potentials than NH_4^+ under intense solar radiation condition. Thus, the combined effect of different forms of N

(NO_3^-) derived from fertilizers and solar radiation both represented a key factor on the POF process. In this sense, the adoption of different N fertilizers (e.g. NH_4^+) would have a direct effect on POF impacts mitigation.

4.9. Ozone layer depletion

The contribution of agriculture to stratospheric ozone depletion is mainly related to fuel combustion, fertilizers and pesticide production (Queiros et al., 2015). Following the regulation proposed in the Montreal Protocol, the atmospheric concentration of halocarbon compounds was significantly reduced and is under control nowadays. Nevertheless, novel anthropogenic emission sources represent a critical issue hampering the stratospheric ozone layer recovery (Revell et al., 2012). The WMO Scientific Assessment Panel (WMO, 2011) recently recognized a negative effect of N_2O on ozone layer recovery. Recent literature reported that approximately 90% of anthropogenic N_2O reaches the stratosphere, thus contributing to the catalytic destruction of ozone. Nevertheless, Fleming et al. (2011) affirmed that anthropogenic CO_2 and CH_4 emissions hampered negative N_2O effects, thereby encouraging stratospheric ozone recovery. However, the combustion processes were still shown to represent a serious issue in both farming systems due to the emissions of ozone layer depleting substances such as halocarbons. Despite the reduced application of fertilizers and pesticides in ORG ("Survey scheme"), the lower yields contributed to a higher impact compared to CON. However, from comparisons to other impact categories, analysed in the present study, OLD was negligible (Monti et al., 2009) (Table 3).

4.10. Non-renewable energy resource consumption

NRC impacts of wheat cultivation are mainly related to fuel consumption (80% and 50% for ORG and CON respectively) and, in CON, fertilizer production (42%). Optimization of mechanization (conservative agriculture or minimum tillage) and fertilizers use (precision farming) have direct effect on NRC environmental impacts but also on the economic aspect at the farm level due to reduced resources use. Tillage had a significant impact, not only in fuel consumption, but also due to effect on yields. In fact, yields were strongly affected by the climatic conditions of the area. In the same location, inter-annual variability is one of the main causes of differing yields from year to year and, consequently, of a differing energy source consumption for FU (Failla et al., 2020). The climatic conditions (temperature and humidity) of different areas have a direct impact on yields, energy consumption and indirect impacts on cultivation practices, such as irrigation, that have to be adopted in order to overcome environmental limits. In this sense, for climatic areas similar to central Italy, Mondani et al. (2017) reported an energy consumption of 8.45–9.05 MJ kg^{-1} of grain. Such consumption increases when passing into arid and semi-arid regions where irrigation is used. Likewise, because of the variability in climatic conditions and agricultural management strategies, a wide variability of NRC impacts is available in the literature (Achten and Van Acker, 2016; Ghasemi-Mobtaker et al., 2020). This is emphasized by effect of soil types and the spatial variability of soils. In similar climatic conditions a clay soil would require higher energy costs to perform the same tillage, e.g. ploughing, than those needed in a loam or sandy soil. Given the continuous growth in food demand, agricultural efforts have been focused on maximizing yields with the increased adoption in fertilizer usage, particularly in conventional farming systems. The production of synthetic fertilizers has caused a direct effect on fossil energy consumption, increasing NRC impacts of conventional farming (Achten and Van Acker, 2016).

4.11. Renewable energy resource consumption

Renewable energy sources are mainly related to biofuels and electricity (Nguyen et al., 2013). The present results, 0.03 and 0.09 MJ kg wheat $grain^{-1}$ for ORG and CON respectively, corroborated those in the literature, reporting an average RRC from 0.03 to 0.15 MJ kg wheat $grain^{-1}$ (Achten and Van Acker, 2016; Mondani et al., 2017; Ghasemi-Mobtaker et al., 2020). In wheat production, straw represents one of the main by-products and its usage to produce electricity is common practice (Nguyen et al., 2013). However, alternative uses of this by-product should be considered, along with the respective repercussions on various impact categories within the LCA. From an agronomical point of view, straw has a significant importance in maintaining the physical, chemical and biological fertility of the soils. Removal would require compensation through the extra input of nutrients, which in turn would necessitate the increased use of fertilizers, with additional impacts in terms of emissions attributable to the production and use of these products. Nguyen et al. (2013), calculated that the removal of straw would necessitate an extra input of fertilizers by 1.5 kg N, 0.77 kg P and 12.8 kg K, respectively. Those authors also reported that the incorporation of 1 t of straw produced a carbon sequestration rate of approximately 80 kg C, thereby demonstrating the relevant contribution towards mitigating climate change. Energy crops were proposed as alternative strategy to reduce the adoption of non-renewable resources. However, energy crops introduce a new LCA linked to the cultivation activity, which in turn lead to additional burdens on different impact categories, and which also require soil and water consumption creating an unsustainable energy balance. Biogas, represents an effective option for the energetic valorisation of straw and maintenance of soil fertility as the by-product of the process, digestate, is a valuable organic fertilizer to maintain soil fertility. Several studies reported that the appropriate management of digestate produced comparable yields to those generated from the use of mineral fertilizers, but with limited environmental impacts (Alburquerque et al., 2012; Verdi et al., 2018, 2019a, 2019b).

4.12. Water consumption

Fertilizer manufacturing is a process that requires a significant amount of water (Table.2). The N-fertilizer synthesis process requires an energy vector representing water in the form of steam (Madanhire et al., 2015). Moreover, water is also the basis for cooling systems on an industrial scale. Given that water is used for washing processes that dramatically decreases water quality, consumption increases. To date, water is an irreplaceable resource for fertilizer production, as is evident for many other industrial processes. In this sense, water consumption represents a critical environmental issue for CON, given the application of synthetic fertilizers (2.16E-01 litres FU^{-1}). Moreover, agriculture exerts a high impact on water consumption for fuel production. In the present study, results on WAC from MP are a relevant amount of total WAC in both farming system (Table.2). Due to the lower yields, ORG shows a relevant impact on WAC from fuel consumptions in MP. In CON, WAC impacts are related also to FM and HFS due to the higher adoption of chemicals than ORG. Furthermore, oil extraction and the refining of fuel require significant water usage (Carter, 2015). In the year 2010, on a global scale, approximately 66 billion of cubic meters of water were consumed from the energy sector amounting to 15% of global water withdrawal (IEA, 2012). Mielke et al. (2010) reported that water consumption for oil extraction exceeded seven times the volume of oil produced. Fracking processes (U.S. EPA, 2016) represent a relevant amount of water usage. Carter (2015) reported an average consumption of 1,000 to 10,000 litres of water to produce one tonne of fuel from an oil source. Biofuels may represent an alternative to the use of fossil resources. Nevertheless, it was shown that in order to produce one tonne of biofuel, from irrigated crops, more than 10,000 litres of water were consumed via both direct and indirect uses (irrigation, raw materials extraction, cultivation input production etc.) (Dalla Marta et al., 2015). In this way, production potentials related to pedo-climatic conditions and the adoption of cultivation inputs play a key role on water demand for biofuel production (Dalla Marta et al., 2011a, 2014, 2011b). In addition, energy crops for biofuel production were reported to be in conflict with food crops, and the use of marginal fields is not sustainable from an energetic point of view (Dalla Marta et al., 2010).

4.13. Land use

In the period 2015–2018, European areas cultivated with wheat accounted for approximately 62 million hectares with average yields of 4141 kg ha^{-1} grain (FAO, 2020). Consistent with the observations of the present study, recent research reported an average land occupation of 4.5 m^2 kg wheat grain^{-1} (Holka et al., 2016; Ridoutt and Garcia, 2020). Yields of Verna wheat are lower than those of modern varieties grown in Tuscany, where average yields are similar to those generally produced in Europe. According to our observations, to ensure the current European total yields of wheat, about 91 and 168 million of ha, for CON and ORG, respectively, would be needed, in terms of DLO. Therefore, a LAU of roughly 99 and 192 million ha for CON and ORG, respectively, would be needed. Tuomisto et al. (2012) stated that organic farming ensures higher levels of biodiversity (from 30% to 50%) than those observed in conventional systems. However, same authors reported a higher land use of about 84% than that for the conventional system with relative impacts on ecosystems. This is consistent to our observations, reporting 94% more LAU in ORG than CON. Lower yields in ORG would imply a strong increase in DLO for agricultural use with a higher impact on forestry ecosystems and biodiversity.

5. Conclusions

The relevance of the present study is linked to the lack of available literature dealing on the environmental impacts of ancient wheat varieties cultivation. From the results of this study it is confirmed that despite the potential of ancient cereal cultivation, there are critical points for both organic and conventional farming. In general, regardless of the production system, the lower yields of Verna wheat are the main critical issue compared to selected modern varieties cultivation. This is exacerbated by comparing the two production systems where the lower yields observed in the organic system (- 46%) result in greater impacts. For almost all the impact categories, conventional farming showed the worse performances due to the production and consumption of non-renewable resources. On the other hands, the EU goal of reaching 25% of agricultural land under organic farming by 2030, must account with the necessity to offset the low yield by increasing cultivation surface areas.

The development and adoption of innovative strategies that increase the resource use efficiency play a key role in improving environmental performance of conventional agriculture (precision farming, nitrification/urease inhibitors, slow release fertilizers etc.). However, the effort toward the creation of sustainable farming systems needs different actions that must run in parallel to support agricultural production.

Climate changes will worsen crop yields and, consequentially, cultivation input use efficiency. Drought effects on the latest phenological phases of wheat growing season play a key role on productivity and crop failure. The optimized management of water resources has also the potential to increase the water-use efficiency of all system inputs, through the adoption of specific storage and irrigation strategies, especially in those areas where precipitation fluctuates.

Energy was also identified to be a key factor towards improving the performance of both cultivation systems (especially for tillage and chemicals manufacturing). Raw material extraction and energy consumption, linked to the industrial sector, could potentially adopt natural energy resources (solar, wind and hydroelectric).

In order to reduce the energy consumptions, the reduction of N-synthetic fertilizers through site-specific fertilization is crucial. This is emphasized by the current geopolitical situation in Europe due to the Russia-Ukraine conflict. The war stressed Europe's dependence to external source of energy and raw materials with a relevant increase of prices. This reveal the fragility of the food supply chain that is still strongly linked to external synthetic input. Thus, capability of Europe to produce food is a central aspect in a view of human population growth. Therefore, it is essential that the programming of EU policy address future investments towards technological and organizational renewal in line with the identified solutions.

CRediT authorship contribution statement

Verdi Leonardo: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Dalla Marta Anna**: Data curation, Project administration, Validation, Writing – review & editing. **Falconi Francesca**: Formal analysis, Investigation, Software, Validation. **Orlandini Simone**: Project administration, Supervision, Validation, Writing – review & editing. **Mancini Marco**: Conceptualization, Investigation, Methodology, Roles/Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eja.2022.126638.

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