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INNOVATIVE PROCESSING PLANTS:

Technological and Nutritional quality of unrefined durum wheat air-classified fractions

Scientific-disciplinary sector

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Summary

Abstract

Durum wheat is of primary importance to human nutrition. Its main milling product, semolina, is used as a raw material for manufacturing several types of food of the Mediterranean diet. Durum wheat grain contains minerals and bioactive compounds in the external layers that end up as waste products (bran fraction). Various studies have highlighted the benefits of the consumption of foods obtained from whole flour or poorly refined milling products, which are richer in fibre, minerals, and antioxidant compounds than the refined milling fractions, for human health. However, the inclusion of bran particles in whole wheat flour or unrefined semolina could increase the risk of the presence of inorganic (e.g., heavy metals) and organic contaminants (e.g., mycotoxins) along the production chain.

This study aimed to develop less-refined milling fractions suitable for manufacturing high-quality end-products with enhanced health-promoting effects, mainly pasta, using latest micronisation and air-classification processes.

Results revealed that the obtained air-classified fractions offered diverse choices to make a good compromise between the yield percentage, particle-size composition, and health benefits associated with a higher content of bran components, such as fibre and antioxidants, while ensuring the containment of organic and inorganic contaminants.

The following chapters explain the studies carried out, starting from the micronisation and air-classification processes applied to the pilot plants up to the assessment of both beneficial compounds and contaminants in the obtained unrefined products.

Keywords: air-classification plant; micronization plant; durum wheat; inorganic contaminants; organic contaminants; antioxidant; polyphenolic acids

Introduction

In recent years, the agro-food research sector has developed new technologies to make the best use of the characteristics of the raw material. Advanced technologies have been developed to reduce the particle sizes of both liquids and solids in order to improve the physicochemical and functional characteristics of food products [**1**]. The development of these new technologies has made it possible to obtain products of excellent quality without compromising food safety. In fact, recent studies have shown that the particle size of the product obtained from grinding and the type of grinding can improve the quality of the product and, in some cases, even maintain its sensory properties [**2-4**].

Particularly, it has been shown that a reduction in particle size increases the surface-volume ratio, thereby increasing the accessibility of the dietary components of the biomaterial. Furthermore, the micron technology helps in improving the functional properties and bio-accessibility of bioactive compounds associated with dietary fibre [**5**].

It has also been shown that ultrafine grinding has a positive impact on the antioxidant activity of wheat bran fractions. It has been demonstrated that the antioxidant capacity increases from 30 to 45 mmol (ratio of the antioxidant capacity/kg equivalent of Trolox) as the surface area of the wheat bran increases from 0.09 to 0.26–0.30 m^2/g. Furthermore, it has been reported that a reduction in the particle size leads to a slight increase in water absorption (farinograph) and a decrease in viscosity, even in the final peak, which is associated with an increase in starch damage. [**6-8**].

During the economic boom, there was an increase in the consumption of animal proteins and industrial products, which led to an increase in per capita caloric intake and the increased risks of developing metabolic and cardiovascular diseases such as diabetes, obesity, stroke, heart attack, and ischaemia.

In recent years, owing to the unhealthy eating habits, the models recommended for maintaining a good health re-propose the consumption of foods based on less-refined grains, preferably whole grains, which are the basis of the healthy diet. In fact, numerous studies have shown that a diet rich in cereals, legumes, vegetables, and fruit protects against the incidence of various diseases widely spread in developed countries, particularly various forms of cancer and cardiovascular diseases (pathologies of the coronary arteries, hypertension, and heart attack), cataract, diseases of the respiratory system (asthma and bronchitis), and diseases of the digestive system (diverticulosis and constipation) [**9,10**].

Different strategies have been developed to improve and/or increase the contents of bioactive compounds in the first processing products. For example, debranning, which through a pre-milling

treatment partially removes the external teguments and the aleurone layer of the kernels, allows for the selective recovery of bioactive compounds such as fibre and phenolic compounds [**11**].

However, the only use of debranning does not allow to modulate the characteristics of milling products. Instead, these latter can be managed adopting different operating conditions both in micronisation and air-classification processes which, however, don't exclude the preliminary debranning treatment followed by the micronisation step.

Furthermore, considering the composition of durum wheat grain, which contains several interesting bioactive compounds mostly found within the coating structure of the kernel, with the use of fine grinding technologies (microniser) and subsequent air-classification, it is possible to enhance the quality of the final product while limiting the concentrations of organic and inorganic contaminants, such as heavy metals, metalloids, and mycotoxins [**12-14**].

Chapter 1

Content of minerals and deoxynivalenol in the air-classified fractions of durum wheat

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Abstract

Background and objectives: Durum wheat (*Triticum durum* Desf.), such as other cereals, contains several interesting bioactive compounds mostly found within the coating structure of the kernel; also, these structures included in the bran fractions, contain the highest concentration of both minerals and organic contaminants (mycotoxins). The purpose of this project was to employ the micronization and air-classification technology to obtain fewer refined milling fractions with an adequate good quality in regard to safety and nutritional aspects to manufacture end products (e.g., pasta).

Findings: The results have identified the milling fraction as the best compromise among satisfactory technological traits, high decrease of cadmium (−83%) and lead (−59%) in comparison to the whole grain, high content of iron (+37%), and the maintenance of useful elements (calcium, potassium, and magnesium). A significant decrease of the deoxynivalenol (−16%) content, as compared with the micronized sample, and a not significant increase (+19%), if compared with semolina, have occurred.

Conclusions: The air-classification process has been used as an appropriate tool to reach a good compromise between minerals and deoxynivalenol content in milling fractions.

Significance and novelty: The same process has proved to be a proper technology to produce high-quality mixtures suitable to obtain both healthier end products and better raw material exploitation.

Keywords: air-classification, deoxynivalenol, durum wheat, micronization, minerals

Introduction

Epidemiological studies have linked the consumption of the whole grain and derivatives to a reduced incidence of cardiovascular diseases, cancer, and diabetes [**1**; **2**]; all these health consequences have mainly been associated both with the antioxidant activity of phytochemicals, also contained within cereals, and the dietary fiber intake [**3**; **4**]. Therefore, the consumption of rich- in fiber food is commonly recommended as an important strategy a healthy diet is based upon [**5**; **6**; **7**].

The maintenance of several bioactive compounds within the coating structures of the kernel of wheat is highly interesting with regard to the biological and nutritional properties: Indeed, several compounds (e.g., β- glucans, lignans, folates, fructans, phytosterols, polyphenols, and arabinoxylans) that ease health benefits (e.g., prebiotic, antioxidant, hypoglycemic, hypocholesterolemic) are mostly present in the whole grain [**8**; **9**]; nevertheless, the outer structures of the kernel end up in the milling waste products (i.e., bran, middlings). The identification of milling fractions suitable to be employed for the production of functional food has been enriched with beneficial components that can be achieved by using appropriate technologies during the initial phase in the milling process or through a focused mixture of the selected fractions. However, the beneficial bioactive compounds can be associated with the presence of minerals, some of whom are toxic elements, and of organic compounds such as mycotoxins. In more detail, the final content of several minerals within the milling products depends on both the type of the element and its distribution within the structures of the kernel. Concerning metals, cadmium (Cd) is uniformly distributed within the endosperm, unlike copper (Cu), zinc (Zn), and iron (Fe)— all of these can be easily found in the aleurone layer— whereas lead (Pb), manganese (Mn), and nickel (Ni) are mainly located in the outer teguments [**10**; **11**; **12**]. As far as the mycotoxin contamination is concerned, deoxynivalenol (DON) is the most widespread Fusarium- toxin in small grain cereals and constitutes the most relevant source of exposure to this type of mycotoxin for humans and animals. Deoxynivalenol (DON) belongs to the type B group of trichothecenes, which have been mainly discovered in cereal crops, whose toxic effects are of great concern for human health [**13**; **14**; **15**]. This form of mycotoxin is produced by toxic varieties (e.g., *F. graminearum* and *F. culmorum*), which colonize the kernel from its outer layer progressively establishing the main source of grain contamination by DON and its modified forms [**16**; **17**]. The estimated mean chronic dietary exposure for humans was over the TDI (1 µg/kgb.w.) concerning infants, toddlers and other children, and also at high exposure in adolescents and adults, and it represents a potential problem for people's health [**18]**. Maximum tolerable limits havebeen set by European Commission for Cd, Pb, and DON in food products [**19**; **20**].

As a result of the traditional milling process, all the outer layer structures of the kernel form the so-called “by-products fractions” (e.g., bran, fine bran, fine middlings), where both inorganic and organic compounds tend to concentrate [**21**; **22**]; on the contrary, although the decrease in semolina has resulted higher for nickel, it has been gradually less substantial for arsenic, cadmium, and lead

[**12**]. Therefore, in the case of the manufacturing of less refined end products a grain processing technology is needed in such a way to manage negative and positive aspects efficiently [**23**; **11**]. On the other hand, the use of the whole milled wheat as a consequence of the manufacturing process can have a negative impact on products in terms of technological and sensorial properties due to the high content of bran [**3**]: An innovative approach toward the employment of proper technological solutions and new milled mixtures with improved functional and healthy properties would result as making high-quality products in terms of taste, and they would be more attractive to end users.

To resort to modern technologies, such as debranning, micronization, and air-classification during the initial phase of the production process could reveal itself as an effective method to properly contain undesirable compounds within the milling products [**24**; **25**]. For example, by removing a small part of the outer layers (3% – 5%) through the debranning process, a 10% – 20% decrease of Cd and Zn was observed in cereal products that reached 80% for Pb [**26**]; the fate of several detrimental components was assessed in durum wheat and a 11% and 24% decrease concerning several traces of toxic elements was achieved when debranning treatment was applied, respectively, for 30s and 60s [**27**; **11**]. Furthermore, the employment of micronization and air- classification technologies during the early phase of durum wheat's manufacturing process might constitute an appropriate way to obtain final products enriched in bioactive compounds. [**4**]. The micronization consists of a milling process that transforms the initial kernels into a mixture of fine particles; the air-classification is an effective method to separate the particles of the micronized mixture making fine and coarse fractions. By many decades, this kind of wheat processing has been widely used in the wheat milling industry, both to better exploit raw material and promote innovative end products. Theapplication of by-products in the food industry results as an added value toward both industry and consumer: the industry benefits from economic incomes and the consumer from the excellent nutritional value of these materials with potential health claims [**28**; **24**].

Materials and Methods

Samples

Two grain samples (Triticum durum Desf. “Anco Marzio” and “Svevo”) have been collected in different growing areas of Italy (Central regions, Sardinia and Sicily); according to representativeness criteria, one 8.0 kg grain sample of Anco Marzio has been divided in the following way: 3.0 kg have been submitted to the traditional roller milling plant (TMP) in a single proof as specified inSection 2.2 [**29**], while the remaining part has been further divided into three sub-samples (I, II, and III) and then reduced to a fine wholemeal employing a micronizer; each of these three micronized sub-samples has been then just made subjected to one of the three setting conditions of the air-

classification treatment (220, 250, and 280), according to the rate of the airflow inlet within the air-classification plant (ACP). The main milling products derived from TMP and the three fractions from ACP all have collected and analysed both for minerals and mycotoxin content (Figure 1). In a similar way, four grain samples (n = 4) belonging to cv. Svevo have been submitted to the micronization treatment followed by the air-classification process: It was confined to the setting conditions that were more effective to the aim of this project.

Traditional roller milling plant process (TMP)

The 17% of the moisture content of the 3.0 kg sample (cv. Anco Marzio) was conditioned by adding water letting it stand 24 hr. Such a specific treatment was helpful to simplify both the undressing process of the kernel and the softening of the endosperm; therefore, it was required to improve the quality of the milling treatment through the use of the traditional roller milling plant (Bühler, model MLU 202, Uzwil, Switzerland). The main milling products have collected afterward: semolina, semolina waste, the latter is obtained by sieving semolina in order to remove the bran residues due to the use of the experimental system (NAMAD Impianti, Rome, Italy) equipped with some varieties of sieve (38GG, 40GG and 44GG), fractions of bran (both coarse and refined bran), and fine middlings-the content of ash has been analysed by the use of the UNI- ISO 2,171 method (two repetitions minimum).

Air- classification plant process (ACP)

The previously mentioned three sub- samples (I, II, and III) of cv. Anco Marzio— whose weight was 1.0, 3.0, and 1.0 kg, respectively— have been subjected to a micronization procedure by using a milling pilot plant in the laboratory (mod. "Pulverisette," Fritsch, Kitzingen, Germany), helpful both for small and medium amounts of grain; the micronizer (the size of the sieve was set at 0.7 mm), which belonged to the mill cutting procedures, has been equipped with a rotor (ø 110 mm) and an engine power (2,800 revolutions per minute) capable of achieving the operating conditions (18,000 revolutions/minute): The heating has been prevented due to pre-set operative appropriate conditions. Indeed, with the same size setting conditions (0.7 mm), this system has been able to ensure results comparable to technological methods that are opportune for high volumes of raw material (data not available). The micronization phase did not require a preventive conditioning of grains: Therefore,

the aforesaid sub- samples have been submitted to an integrated air- classifying system (turbo-separator unit, SX- LAB model, Separ Micro System, Flero, BS, Italy), extremely adequate to size a specific setting limit up (Ø ≤ 1.5 mm). The functioning criteria of the air- classifying plant were based on the consequences among opposite forces, air- traction and centrifugal forces, by the use of an airflow variation according to the setting of the inlet valve (220, 250, and 280). The pneumatic transport of the whole milled wheat particles has caused a flow of product along circular orbits wherein a series of ascending air currents have been dosed by different airflow opening conditions' settings, which has allowed a progressive increase of the amount of the air and its connection with the entire milled wheat from 220 to 280 in the setting rate of airflow of the valve.

Due to the combined action of different forces, the most lightweight particles have failed to precipitate and consequently have been rated as a "F" (fine), subdivided into F1 (heavier fine particles), and F2 (lighter fine particles) sub- fractions; the remaining heavier particles have been rated as "G" (heavier gross particles). At the end of the air- classification treatment, the G, F1, and F2 fractions have been collected for each sample; also, particular sizes of all fractions have been measured through certified test sieves (Giuliani Tecnologie S.r.l., Turin, Italy), and the ash content has been assessed by following the aforesaid approach. By comparison, four durum wheat grain samples (cv. Svevo)—from 1.1kg to 2.0 kg each— have been processed with the identical procedure, but all of them have only performed at one selected airflow opening condition. Indeed, the setting rate of the incoming airflow within the air- classification system was the same as for the previously chosen fractions, since it was the most appropriate to achieve the aim of this project.

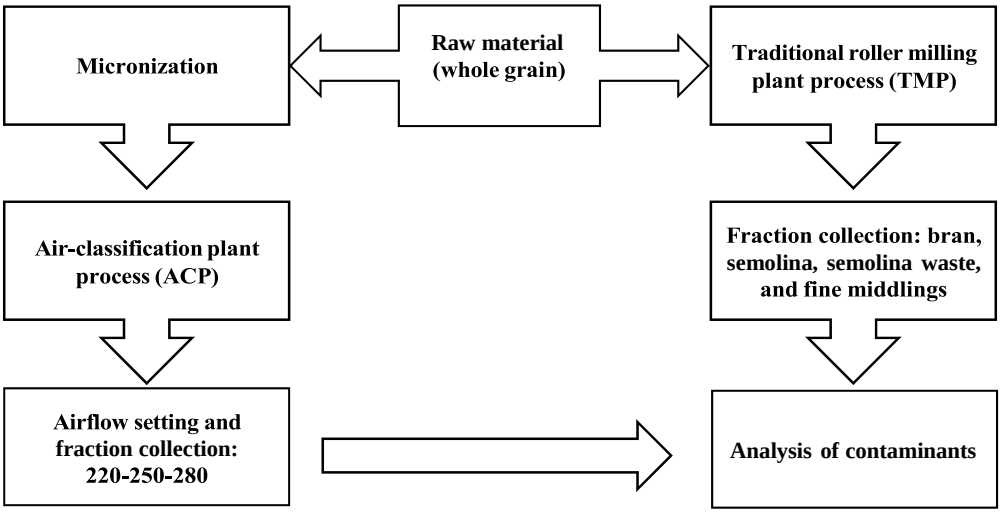

Figure 1. Flow chart of traditional roller milling, micronization, and air- classification processes used to obtain the fractions of interest; 220–250–280: setting points of the airflow incoming.

Analysis of minerals and deoxynivalenol

Each grain milled samples (Retsch pm 100, GmbH, Germany, accessorized with a "comfort" stainless steel grinding jar with 250ml; a 10mm ø stainless steel grinding ball, set at 200 rpm for 5 min), micronized samples (the whole entire milled wheat), and other first-processing products (which belong to traditional and air-classification plants). The analysis of minerals has been carried out to quantify the concentration level of calcium (Ca), phosphorus (P), potassium (K), sodium (Na), magnesium (Mg), iron (Fe), copper (Cu), cadmium (Cd), lead (Pb), and nickel (Ni): All the grain samples have been processed to estimate the concentration of each element in the source material.

Therefore, every sample has been subjected to an initial phase of acid digestion by the use of an economical high-pressure microwave oven in the laboratory (Mars plus CEM, Italy) employed with a 1,800 W energy output; approximately, 200 mg per every dry sample has been directly im-planted in a microwave- closed tubes (100 ml PFA HP- 500 Plus), two milliliters of 30% (w/w) H_2O_2 (Merck, Darmstadt, Germany), 0.5 ml of 37% HCl (Merck), and 7.5 ml of HNO_3 (Merck) and 69% of the entire solution have been added to each vessel. Also, a predigestion has been carried out with 7.5 ml of HNO_3 at 67% v/v for one hour. Afterward, a 0.5 ml HCl amount has been added and left to act for an additional hour; in conclusion, 2 ml of H_2O_2 has also added and left to digest for 30 min, with an entire final volume of 10ml. At the end of the predigestion process, the acid solution obtained has been mineralized within the microwave, the heating program has been performed through a single step: Temperature has regularly increased from 25℃ to 180℃ in 37 min, pre-serving it at 180℃ for 15 min afterward.

At the end of the digestion procedure and the subsequent cooling process, the three samples have been transferred into a Teflon® beaker, and their entire volume had turned into 25ml with high-purity water (18 MΩ/cm) from a Milli-Q water purification system (Millipore, Bedford, USA); the digest solution have consequently filtered (DISMIC 25HP PTFE syringe filter, pore size = 0.45 mm, Toyo Roshi Kaisha, Ltd.) and stored within a screw cap plastic tube (Nalgene, New York, USA). Furthermore, each experiment has been carried out three times more. The super pure grade reagents, employed for the microwave- assisted digestions, were as follows: hydrochloric acid (36% HCl), nitric acid (69% HNO_3), and hydrogen peroxide (30% H_2O_2); highly pure water (18 MΩ/cm) has been used for the standards' dilution and to prepare samples throughout the chemical procedure.

Trace elements' quantifications have performed by using an Inductively Coupled Plasma Optical Emission 184 Spectrometer (ICP- OES) with an axially configuration (8,000 DV, Perkin Elmer) equipped with an ultrasonic nebulizer; the Limit of Detection (LOD) for each analysed element (µg/kg) has resulted: 8.0 (Ca), 2.0 (P), 20.0 (K), 2.0 (Na), 2.0 (Mg), 0.2 (Fe), 0.1 (Cu), 0.3 (Cd), 3.0 (Pb), and 0.9 (Ni).

To assess the trace elements' concentration level, the calibration standards have been prepared and equally treated to samples before dilution (multi-element standard solution, CaPurAn, CPAchem, Stara Zagora, Bulgaria). For detection, it has been necessary to employ the frequency with the lowest interferences regarding every single element: Mg 279.0 nm, Cd 228.8 nm, Fe 259.9 nm, Cu 324.7 nm, Ca 317.9, P 213.6, Pb 220.3, Na 588.9, Ni 231.6 nm, K 766.4, through a specific accuracy analysed with the use of ERM- BC21 (the European reference material).

Every main milling product derived from the traditional plant, and the fractions obtained from the air-classification system have been further sampled according to representative criteria and then assayed for the DON content [**29**]. According to the aforesaid criteria, it is assumed that that specificcontent (consequently divided into the three sub-samples) was the same for the grain sample employed for the roller milling process (TMP); the analysis has been carried out by using the enzyme-linked immuno- sorbent assay (ELISA) (Ridascreen® DON method, R- Biopharm AG, Darmstadt) with a limit of detection (LOD) of 18.5 μg/kg. The recovery range stated in the method was 85%- 110%. Deionized water has been acquired from Water Purification System Zener Power I (Human Corporation, Seoul, Korea). The Basic Robotic Immunoassay Operator (BRIO, SEAC, Radim Group, Florence, Italy) has been successfully employed and the absorbance data have been acquired by Sirio-S Microplate Reader (SEAC, Radim Group, Florence, Italy). The ELISA methodological process for the DON assay content in durum wheat samples had already been submitted to a validation phase through the comparison with the chromatographic (HPLC) analysis [**30**].

The RIDA® Soft Win software (R- Biopharm A Darmstadt, Germany) has been employed to quantify the DON in those samples.

Statistical analysis

The employed samples for the cv. Anco Marzio's experimental project have involved a TMP trial as control (n = 1) and both micronization (n = 3) and ACP trials: These latter have been carried out with a 220, 250, or 280 setting rate for the airflow. All the collected fractions (n = 3) have been analyzed according to the content of the contaminants. The air-classification performance of the F 250 fraction has been further tested by comparing yield and ash contents with samples of cv. Svevo (n = 5); the variance analysis has been made applying ANOVA (post hoc: Tukey test) and using the STATISTICA software for the results deriving from elemental analysis and PAST 2.12 for data referring to the DON content [**31**]: In the latter circumstance, the LOD half detection limits (9.25 μg/kg) have been employed for the results below [**32**].

Results and Discussion

Technological results (TMP and ACP processes)

The cv. Anco Marzio sample was submitted to the traditional roller milling plant (TMP) for a preliminary evaluation from both milling and technological point of view, through the measurement of yield and ash contents within the main milling fractions. The equivalent sample was further intended as a reference since compared to the results reached from the ACP process. Overall, the results referred to the TMP employment have substantially confirmed the expected performance: More specifically, the entire recovery of the sum of all fractions achieved due to the whole milling products amounted to 96.87% of the initial grain sample with a total loss of matter, which was equal to 3.13%. Furthermore, the yields of the fractions obtained were: semolina (at 37.95%), coarse bran (at 17.65%), refined bran (at 14.45%), fine middlings (at 13.51%), and purified offal (at 13.31%); the results obtained have substantially been those expected by taking into account the variability according to quality and features of the raw material [**33**]. In particular, the semolina fraction was compliant with the current national regulations mainly regarding the ash content (<0.90% db), with an average value of 0.83% ± 0.005 (*SD*) on dry basis [**34**]. Due to the employment of the micronizing process, high yield values have been measured for all the three trials that have been carried out (average value ± *SD*: 97.48% ± 0.81—Table 1). Moreover, good yields have been achieved for F1 fractions at all the opening airflow setting conditions (220, 250, and 280), which were extremely appropriate for the F1 250 (93.70%) and F1 280 (95.18%) fractions, whereas the F1 220 fraction has been obtained at the 60% value (63.98%) above: Furthermore, the G 220 fractions have shown a maximum value of 33.06%; the entire recovery yield of each opening airflow condition has reached high values between 97.13% and 98.44%. In addition, the F2 fractions have not been further considered to achieve the purpose of this project, essentially due to the scarce amount collected (range: <0.01%–0.68%) in each assessed condition. Therefore, they have been considered inappropriate to carry out the expected analytical assessments. The percentage of the most important particle size according to a technological viewpoint (between 180 µm and 425 µm) with a range from 10.68% to 14.64% (G) and from 41.73% to 61.46% (F1) within the assayed opening conditions of the flow. Since the presence of bran particles within the air-classified fractions, the range size between180 µm and 425 µm included not less than a minimum part of the refined bran fractions (<180 µm, 180 µm, and 250 µm), a rather efficient detail for a better quality of pasta added with bran fractions [**35**]; furthermore, the positive effects of medium coarse and coarse particle sizes of semolina for theend product (pasta) quality had been already analyzed by the experts' **36**; also, the attention has been focused on both F1 and G fractions that have been assessed for the ash content, which is a properly appropriate trait of fewer refined products [according to **37** and **38**]. The percentage of the ash content regarding the dry basis has revealed the following average values (±*SD*): 2.04 ± 0.003 (F1 220), 1.84 ± 0.021 (F1 250), 1.80 ± 0.002 (F1 280), 1.28 ± 0.002 (G 220), 1.19 ± 0.011 (G 250), and 1.23 ± 0.009 (G 280). According to these parameters, the statistical analysis has shown a considerable

difference ($p < 0.05$) between F1 250/280 and G 220 fractions: The first mentioned have resulted of great interest due to its slightly high ash content (i.e., fewer refined product) than semolina added to the milling yield showed, whereas G 220 referred to a good performance pointed out in the air-classification procedure; however, the two F1 250 and 280 fractions have substantially resulted equivalent for analogous parameters regarding ash and milling yield. Nevertheless, that slight difference in the ash content has been considered as a trend to- ward a higher fiber content, as well as a valuable key feature for a fewer refined product; in addition, the F1 250 fraction has been deemed appropriate for a more specific analysis. Regarding the milling yield and ash content, additional data have been acquired comparing the F1 250 fraction that referring to cv. Anco Marzio ($n = 1$) and the F1 250 fractions referring to the air-classified samples belonging to cv. Svevo ($n = 4$); regarding the whole samples ($n = 5$), for F1 250 the average values obtained for the percentage of the yield and ash content were 88.41% ± 3.13 (*SD*) and 1.99 ± 0.14 (*SD*), respectively: Therefore, it has been possible by saving a good performance reached for the two parameters even in the case of different cultivars employed.

Table 1. Weights (kg), yields (%), and particle size range percentages of the main milling products through the micronizer and air- classification plant (ACP) of durum wheat sub-samples (cv. Anco Marzio)

Micronization process						
	Trials (n = 3)					
Sub-samples	**I**		**II**		**III**	
Starting weight (kg)	10.000		30.000		10.000	
Micronized weight (kg)	0.9832		29.226		0.9670	
Yield (%)	98.32		97.42		96.70	
Main yield (%) ± *SD* 97.48 ± 0.81						
Air-classification process						
Airflow opening degree	**220 (I)**		**250 (II)**		**280 (III)**	
Fractions	**Weight (kg)**	**Yield (%)**	**Weight (kg)**	**Yield (%)**	**Weight (kg)**	**Yield (%)**
G	0.2926	33.06	0.1000	3.42	0.0276	2.85
F1	0.5662	63.98	27.384	93.70	0.9204	95.18
F2	0.0060	0.68	0.0002	0.01	0.0040	0.41
Total	0.8648	97.72	28.386	97.13	0.9520	98.44
Airflow opening degree	**220 (I)**		**250 (II)**		**280 (III)**	
Fractions	**Particle size range (425 µm > Ø > 180 µm) (%)**					
G	11.44		14.64		10.68	
F1	61.46		41.73		45.10	
F2[a]	–		–		–	

Note: G, heavy gross fraction; F1, intermediate fraction; F2, fine fraction.

[a]Insufficient quantity.

Analysis of minerals (ICP-OES)

Minerals are generally classified as macroelements (i.e., Ca, K, Mg, Na, P) and microelements (i.e., Fe, Cu, Co, Zn, Mn, Mo, Cr, Se) based on the recommended daily intake (respectively around 100 mg/die). If inhaled, by skin contact or ingestion, some trace elements might be potentially noxious to human health [**39**].

The TMP process has reduced Ca values both in semolina (−52%) and in the semolina waste (−39%); in fine middlings fractions, the Ca content has been characterized by a 10% reduction, whereas in bran fractions it has remained unchanged. Beneath the ACP process, a significant difference in Ca content has been detected in F1 and G fractions, obtained by operating at 220, if compared to the other two 250 and 280 conditions, which have been more substantial for F1 220 analogous to more than 36% and for the G 220 fraction equal to more than 9%.

The results regarding the TMP employment highlight that P has been maintained in the bran fractions (8.8 mg/kg), whereas its content significantly has decreased in semolina with a reduction up to 95%: analogously, the P trend to an improved content in bran-rich fractions has been highlighted through the ACP employment. Furthermore, the F1 220 and 250 fractions, which are those with the highest content of the aforesaid element, despite the fact that a significant reduction of respectively 63% and 70% if compared to the whole grain. In addition, the P presence in F1 and G fractions set at 280 has not endured a significant difference, probably due to a higher homogeneous distribution within those two fractions.

The bran section derived from the TMP process has high- lighted a significant K decrease when compared to the whole grain sample; a higher K reduction has been registered by the fraction related to the semolina (−65%): It might have been possible due to the conditioning phase adding of water. On the contrary, the K content has substantially remained identical with fewer differences in each ACP fraction.

The TMP milling treatment has caused an increase of the Na content within the fine middlings fraction: a 5% higher than the Na amount of the entire wheat, whereas it has been fundamental to notice a highly relevant decrease in semolina (−91%). However, each result regarding the air-classified products has highlighted a decrease in the Na within all the fractions obtained with different airflow opening conditions, except for the G 280 fraction that does not significantly differ from the Na content within the whole analyzed grain.

When the TMP treatment was completed, the amount of Mg increased by +44% in the bran fractions whereas the same element decreased by −75% in semolina, considering the Mg content in the initial entire grain. Regarding the ACP technology, a substantial maintenance of Mg has been detected in the F1 fraction, whereas the same element has significantly decreased in all G fractions, with the minimum amount in G 250 (350.8 mg/kg) equal to 43% of loss in relation to the whole grain.

The TMP employment caused a higher Fe presence both in the bran fractions and in the fine middlings (+25% and +31% each) differently from the Fe value in the whole grain sample, whereas the same element has shown a 58% decrease in semolina fraction. Furthermore, the ACP process' outcome has shown an unchanged Fe content in F1 and G fractions, except for the 250 condition that showed a + 37% increase with the F1 fraction and a −47% decrease in the G fraction. Thus, that trend toward the maintenance or increase of macroelements and iron in every single fraction containing bran particle material (e.g., pericarp and aleurone) has confirmed the interest in rich-in-fiber food [**40**].

Copper (Cu) has shown a comparable decreasing trend in all parts both regarding the TMP and the ACP procedures: A huge amount of Cu has been lost (−93%) in the G 250 section.

In addition, Cd has not been detected in the analyzed parts, except for the fine middlings: Here, the Cd concentration has not changed. The ACP process has led to a total reduction of the Cd element in the G fractions, and its localization in the F1 parts; a decreasing trend of content has occurred along with the increase of the airflow opening rate (−67% in F1 220 and – 83% in F1 250), with a minimum value in F1 280 equal to 91% of abatement: This factor might be considered as a technological effect on the processed matrix.

As confirmed by the Pb localization in the tegument layers of the kernel, the same element has been detected only in bran (0.7 mg/kg) and fine middlings fractions (1.0 mg/kg): that coincides with the previous outcomes [**26**]. Moreover, the ACP procedure has registered a high Pb concentration decrease in each fraction, except for the F1 220 condition which has identified a + 60% increase and a significant −59% reduction in F1 250.

Analogously to the Pb trend, Ni content has not been undetected in semolina, whereas a more than 75% decrease has been reached both in the bran fractions and in the fine middlings; in the ACP processing, the Ni concentration was higher both in the F1 220 and F1 250 fractions with an in-crease of 33% and 16%, respectively.

The outcomes have shown a non-negligible effect within the procedure, starting from the whole grain sample to the micronized sample regarding P, Na, Cd, and Cu: The latter element has resulted highly reduced along the aforesaid passage, and these differences which might be caused by the employment of pilot plants within the wheat process indicate that additional studies are required.

Overall, the distribution of contaminants within the milling fractions includes several factors, among which the opening contamination rate is highly important [**27**]. In this study, the outcome has highlighted a different trend regarding the content of minerals in fractions between air- classifier (ACP) and traditional milling (TMP) processes (Table 2). In the first case, the evaluation of the distribution trend in F and G fractions should consider the presence of bran particles within all fractions, although in different amounts and types. Conversely, in the second case (TMP) the marked

difference of distribution between the bran-free semolina and the remaining bran-containing fractions was evident: In particular, the majority of the analyzed macroelements (Ca, K, Na, and Mg) except of P and Fe have resulted more concentrated in all bran-containing fractions, such as bran, middlings, and semolina waste. This detail might be caused by the high content of minerals in the outer layers structures, above the aleurone that constitutes the phosphorus reserve within the phytates form, and all of them end up in the bran particles [**40**; **11**; **41**]. However, a loss of P has occurred in the TMP rich-in-bran parts, and even in the micronized fraction during the ACP process. Nevertheless, although this event might not be completely explained, several factors such as process effects, sampling, and sample treatment be- fore milling might have been involved.

A possible explanation to the whole analyzed heavy metals decrease except for Cd might be found in a marked effect during that procedure due to the peculiarly constructive features of the experimental system employed: the latter factor which is at the basis of the not negligible process's effects, and complicated to be completely explained has already been highlighted in literature, though it requires further investigations [**12**].

Table 2. Mean content values (n = 3) of the minerals analyzed in the main fractions of durum wheat samples.

	Traditional roller milling plant (TMP), (mg/kg) (d.m.) referred to each type of fraction					Air-classification plant (ACP), (mg/kg) (d.m.) referred to each type of fraction						
Minerals	**Whole grain**	**Bran fraction**	**Fine middlings**	**Semolina**	**Semolina waste**	**Micronized sample**	**F1 220**	**G 220**	**F1 250**	**G 250**	**F1 280**	**G 280**
Ca	296.6 ^{bc}	309.4 ^{bc}	266.1 ^{c}	141.7 ^{de}	181.2 ^{d}	135.1 ^{bc}	404.7 ^{a}	325.7 ^{ab}	271.3 ^{cd}	150.3 ^{e}	203.3 ^{d}	232.5 ^{d}
P	28.7 ^{a}	8.8 ^{bc}	5.1 ^{de}	1.3 ^{f}	3.4 ^{def}	11.1 ^{b}	10.5 ^{b}	6.2 ^{cd}	8.5 ^{bc}	2.2 ^{ef}	4.6 ^{de}	3.7 ^{def}
K	1999.0 ^{a}	1,218.6 ^{de}	1951.2 ^{a}	701.9 ^{f}	2038.3 ^{a}	1731.9 ^{ab}	1,377.1 ^{d}	1557.1 ^{bc}	1632.3 ^{b}	1,114.4 ^{e}	2033.0 ^{a}	1508.3 ^{c}
Na	716.1 ^{a}	507.2 ^{c}	753.6 ^{a}	67.3 ^{d}	109.1 ^{d}	508.6 ^{bc}	511.0 ^{bc}	479.3 ^{c}	483.3 ^{c}	517.8 ^{bc}	485.1 ^{c}	638.2 ^{a}
Mg	618.2 ^{bc}	890.7 ^{a}	437.3 ^{def}	156.5 ^{g}	400.2 ^{ef}	626.4 ^{bc}	725.4 ^{b}	465.1 ^{def}	554.9 ^{cd}	350.8 ^{f}	548.7 ^{cd}	429.6 ^{def}
Fe	31.3 ^{b}	39.2 ^{a}	41.1 ^{a}	13.2 ^{d}	18.0 ^{c}	25.5 ^{bc}	23.9 ^{bc}	24.3 ^{bc}	43.0 ^{a}	16.7 ^{c}	26.0 ^{bc}	27.5 ^{bc}
Cu	33.5 ^{a}	5.8 ^{bcd}	4.8 ^{bcd}	1.5 ^{f}	2.9 ^{de}	7.3 ^{bc}	7.9 ^{b}	4.3 ^{cd}	6.8 ^{bc}	2.4 ^{e}	3.2 ^{de}	2.9 ^{de}
Cd	0.6 ^{a}	<L.R.	0.5 ^{ab}	<L.R.	<L.R.	0.5 ^{b}	0.2 ^{c}	<L.R.	0.1 ^{d}	<L.R.	0.1 ^{d}	<L.R.

Note: Different letters = values not significantly different ($p < 0.05$).

Abbreviations: G, heavy gross fraction; F1, intermediate fraction; d.m. = dry matter; LOD, limit of detection.

Analysis of deoxynivalenol (DON)

As far as the DON mycotoxin presence within the traditionalmilling products is concerned, further detailed informationis available [**21**]; therefore, this project has concentrated on the mainly important milling fractions, such as semolina, sum of coarse bran and refined bran compared with the air-classified fractions (Figure 2); in particular, a significant reduced presence (−29.3%) of DON in semolina (558 µg/kg) compared to the whole micronized sample(789 µg/kg) has been pointed out, whereas a considerable higher average concentration (1,064 µg/kg) within the entirebran fraction has been detected (+34.9%): that represents a further confirmation of the already acquired information regarding the distribution of DON within the wheat kernel structures and the role the

external teguments have since they are a first defensive line against a pathogenic fungi's attack [**42**; **43**]. Furthermore, it might clarify the outcomes regarding the micronizing and air- classifying processes. Within the F1 parts obtained by operating in each of the three different airflow opening conditions (220, 250, and 280), the residual bran content was superior than the G fractions, as confirmed by the measurement of the ash content within each type of fraction as already explained. As expected, the range of the average DON concentration (666–899 µg/kg) has had an increasing trend if compared with the G fractions (515–689 µg/kg), which have shown a decrease if compared to the micronized sample (789 µg/kg). Thus, it has been possible to analyze the smaller but substantial differences of mycotoxin accumulation in F1 250 (666 µg/ kg), equal to −16% in relation to the micronized sample, and in G 250 (515 µg/kg) in relation to the corresponding opening airflow conditions (220 and 280) left: The F1 250 fraction has proved that it is the most appropriate for the purposes of this project focused to fewer refined end products (e.g., pasta) in relation to the F1 220 and the 280; in addition, the G250 has provided good outcomes for an eventual manufacturing employment due to the lowest accumulation of DON; however, it has shown a reduced content of bran residues, greater content of semolina and fewer transformation yield in relation to the corresponding F1 fraction. After considered this, both the −16% reduction of the average DON content in the F1 250 fraction and the irrelevant +19% increase in relation to semolina necessitate to be intended as outcomes referred to an initial sample with a DON content that does not exceed the mandatory limits (durum wheat: 1,750 µg/kg). Therefore, the application of the air-classification technology might be a satisfactory method for innovative products during the first durum wheat processing phase to obtain a safer milling fraction than the entire milled wheat in terms of DON content. However, this argument necessitates further analysis.

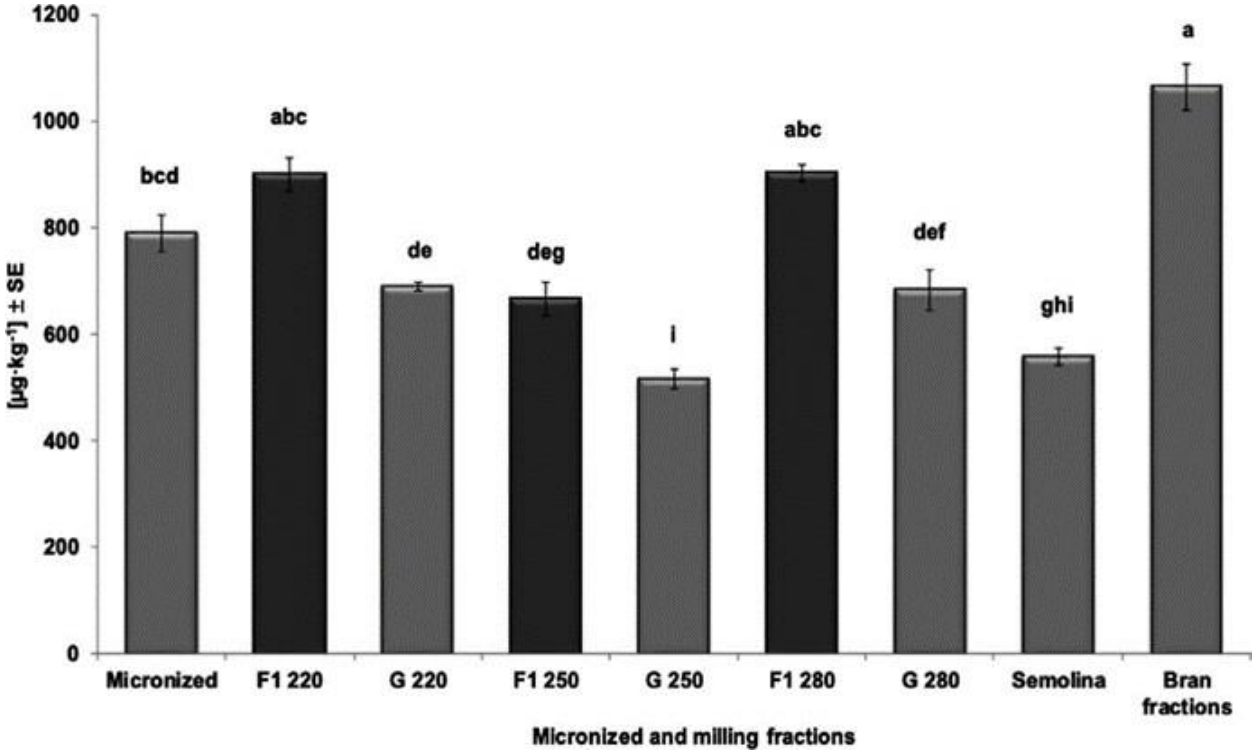

Figure 2. Mean values of repeated analysis (n = 3) of DON content ± SE (corrected for recovery) of the main fractions (cv. Anco Marzio) from traditional milling plant (semolina and bran fractions), air-classification plant (F and G fractions at several setting conditions), and micronized samples.

Conclusions

The minimally processed wheat food improvement in consumption a healthy parameter for humankind, needs innovative mixtures with high-quality properties; it is a challenge that requires a proper technology for grain fractionation, in such a way to efficiently separate negative and positive features. The outcomes reached with this project have allowed to identify an air-classified fraction (the F1 250), which is the most proper choice for an excellent transformation yield, a good content of bran residues and the maintenance of several macroelements (Ca, K, Mg) and Fe. In addition, a significant decrease of toxic trace elements (i.e., Cd and Pb) has occurred; in the same fraction, a remarkably reduced content of deoxynivalenol has been detected in relation to the micronized sample, which has resulted almost similar to semolina.

In conclusion, these outcomes have provided a valid response to the increasing demands regarding healthier not highly refined food: A better raw material exploitation might prove to be an added value for both food industry and end users through the employment of milling by-products.

References

1. Aune, D., Keum, N. N., Giovannucci, E., Fadnes, L. T., Boffetta, P., Greenwood, D. C., Tonstad, S., Vatten, L. J., Riboli, E., & Norat, T. (2016). Whole grain consumption and risk of cardiovascular dis- ease, cancer, and all cause and cause specific mortality: Systematic review and dose-response meta-analysis of prospective studies. BMJ, 353, i2716. https://doi.org/10.1136/bmj.i2716.
2. Călinoiu, L. F., & Vodnar, D. C. (2018). Whole grains and phenolic acids: a review on bioactivity, functionality, health benefits and bioavailability. Nutrients, 10(11), 1615. https://doi.org/10.3390/ nu10111615
3. Onipe, O. O., Jideani, A. I. O., & Beswa, D. (2015). Composition and functionality of wheat bran and its application in some cereal food products. International Journal of Food Science and Technology, 50, 2509–2518. https://doi.org/10.1111/ijfs.12935
4. Ficco, D. B. M., Borrelli, G. M., Giovanniello, V., Platani, C., & De Vita, P. (2018). Production of anthocyanin-enriched flours of durum and soft pigmented wheats by air-classification, as a potential ingredient for functional bread. Journal of Cereal Science, 79, 118–126. https://doi.org/10.1016/j.jcs.2017.09.007
5. Ötles, S., & Ozgoz, S. (2014). Health effects of dietary fiber. Acta Scientiarum Polonorum Technologia Alimentaria, 13(2), 191–202.
6. Probst, Y. C., Guan, V. X., & Kent, K. (2017). Dietary phytochemical in- take from foods and health outcomes: A systematic review protocol and preliminary scoping. British Medical Journal Open, 2017(7), e013337. https://doi.org/10.1136/bmjopen-2016-013337
7. Stephen, A. M., Champ, M. M. J., Cloran, S. J., Fleith, M., van Lieshout, L., Mejborn, H., & Burley, V. J. (2017). Dietary fibre in Europe: Current state of knowledge on definitions, sources, recommendations, intakes and relationships to health. Nutrition Research Reviews, 30, 149–190. https://doi.org/10.1017/S095442241700004X
8. Giordano, D., Reyneri, A., & Blandino, M. (2016). Folate distribution in barley (Hordeum vulgare L.), common wheat (Triticum aestivum L.) and durum wheat (Triticum turgidum durum Desf.) pearled fractions. Journal of the Science of Food and Agriculture, 96, 1709–1715.
9. Fu, B. X., Chiremba, C., Pozniak, C. J., Wang, K., & Nam, S. (2017). Total phenolic and yellow pigment contents and antioxidant activities of durum wheat milling fractions. Antioxidants, 6, 78. https:// doi.org/10.3390/antiox6040078
10. Conti, M. E., Cubadda, F., & Carcea, M. (2000). Trace metals in soft and durum wheat from Italy. Food Additives and Contaminants, 17(1), 45–53. https://doi.org/10.1080/026520300283577

11. Ficco, D. B. M., Borrelli, G. M., Miedico, O., Giovanniello, V., Tarallo, M., Pompa, C., De Vita, P., & Chiaravalle, A. E. (2020). Effects of grain debranning on bioactive compounds, antioxidant capacity and essential and toxic trace elements in purple durum wheats. LWT-Food Science and Technology, 118, https://doi.org/10.1016/j. lwt.2019.108734

12. Cubadda, F., Raggi, A., Zanasi, F., & Carcea, M. (2003). From durum wheat to pasta: effect of technological processing on the levels of arsenic, cadmium, lead and nickel-a pilot study. Food Additives and Contaminants, 20(4), 353–360. https://doi.org/10.1080/0265203031000121996

13. Gauthier, T., Waché, Y., Laffitte, J., Taranu, I., Saeedikouzehkonani, N., Mori, Y., & Oswald, I. P. (2013). Deoxynivalenol impairs the immune functions of neutrophils. Molecular Nutrition & Food Research, 57, 1026–1036. https://doi.org/10.1002/mnfr.201200755

14. Maresca, M. (2013). From the gut to the brain: Journey and pathophysiological effects of the food-associated trichothecene mycotoxin deoxynivalenol. Toxins (Basel), 5, 784–820. https://doi.org/10.3390/ toxins5040784

15. Pinton, P., & Oswald, I. (2014). Effect of deoxynivalenol and other type B trichothecenes on the intestine: a review. Toxins (Basel), 6, 1615– 1643. https://doi.org/10.3390/toxins6051615

16. Khaneghah, A. M., Martins, L. M., von Hertwig, A. M., Bertoldo, R., & Sant'Ana, A. S. (2018). Deoxynivalenol and its masked forms: Characteristics, incidence, control and fate during wheat and wheat based products processing – A review Trends. Food Science & Technology, 71, 13–24. https://doi.org/10.1016/j.tifs.2017.10.012

17. Freire, L., & Sant'Ana, A. S. (2018). Modified mycotoxins: An updated review on their formation, detection, occurrence, and toxic effects. Food and Chemical Toxicology, 111, 189–205.

18. EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA); Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre. European Food Safety Authority (2017) Scientific opinion on the risks to human and animal health related to the presence of deoxynivalenol and its acetylated and modified forms in food and feed. EFSA Journal, 15(9), 1–345. https://doi. org/10.2903/j.efsa.2017.4718

19. European Commission (2007). Commission regulation of 28 September 2007 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards Fusarium toxins in maize and maize products, 1126/2007/EC. Official Journal of the European Union L 255/14. http://data.europa.eu/eli/ reg/2007/1126/oj

20. European Commission (2006) Commission regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union, L, 364, 5–24. http://data.europa.eu/eli/reg/2006/1881/oj

21. Brera, C., Peduto, A., Debegnach, F., Pannunzi, E., Prantera, E., Gregori, E., De Giacomo, M., & De Santis, B. (2013). Study of the influence of the milling process on the distribution of deoxynivalenol content from the caryopsis to cooked pasta. Food Control, 32, 309–312. https://doi.org/10.1016/j.foodcont.2012.12.005.

22. Schaarschmidt, S., & Fauhl-Hassek, C. (2018). The fate of mycotoxins during the processing of wheat for human consumption. Comprehensive Reviews in Food Science and Food Safety, 17, 556–593.

23. Fares, C., Platani, C., Baiano, A., & Menga, V. (2010). Effect of pro- cessing and cooking on phenolic acid profile and antioxidant capacity of durum wheat pasta enriched with debranning fractions of wheat. Food Chemistry, 119, 1023–1029.

24. Wang, J., Xie, A., & Zhang, C. (2013). Feature of air classification product in wheat milling: Physicochemical, rheological properties of filter flour. Journal of Cereal Science, 57, 537–542. https://doi. org/10.1016/j.jcs.2013.03.001

25. Brera, C., Gregori, E., De Santis, B., Pantanali, A., & Monti, M. (2019). Ricerca e sviluppo di una farina innovativa destinata ai bambini di seconda e terza infanzia. In: ISTISAN Congressi, Riassunti: Istituto Superiore di Sanità, 2019 19/C4. IV Congresso Nazionale. Micotossine e Tossine Vegetali nella filiera agro-alimentare. Roma, 10-12 giugno 2019 (p.61). ISSN 0393-5620.

26. Brüggemann, J., & Kumpulainen, J. Z. (1995). The status of trace elements in staple foods. Lebensm Unters Forch, 201, 7–11. https://doi. org/10.1007/BF01193190

27. Cheli, F., Campagnoli, A., Ventura, V., Brera, C., Berdini, C., Palmaccio, E., & Dell'Orto, V. (2010). Effects of industrial processing on the distributions of deoxynivalenol, cadmium and lead in durum wheat milling fractions. Food Science and Technology, 43, 1050–1057. https://doi.org/10.1016/j.lwt.2010.01.024

28. Stringfellow, A. C., Brekke, O. I., Pfeifer, V. F., Burbridge, L. H., & Griffin, E. I. (1977). Fractionation of defatted wheat-and corn-germ flours by air classification. Cereal Chemistry, 54(3), 415–428.

29. European Commission (2006). Commission Regulation (EC) No. 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Official Journal of the European Union. L 70/12. http:// data.europa.eu/eli/reg/2006/401/oj

30. Brera, C., Debegnach, F., De Santis, B., Pannunzi, E., Berdini, C., Prantera, E., & Miraglia, M. (2009). Validazione di metodi immunoenzimatici per la determinazione delle micotossine in campioni di cereali. In Polistampa (Ed.), I Georgofili Quaderni 2008, IV (pp. 3–45). Edizioni Polistampa.

31. Hammer, O., Harper, D. A. T., & Ryan, P. D. (2001). PAST: Paleontological Statistic software package for education and data analysis. Paleontologia Electronica, 4(1), 1–9.

32. Anon (2003). Scoop Task 3.2.10. Collection of occurrence data of Fusarium toxins in food and assessment of dietary intake by the population of EU member states. http://ec.europa.eu/food/fs/scoop/ task3210.pdf/

33. Troccoli, A., Borelli, G. M., De Vita, P., Fares, C., & Di Fonzo, N. (2000). Durum wheat quality: A multidisciplinary concept. Journal of Cereal Science, 32(2), 99–113. https://doi.org/10.1006/ jcrs.2000.0322

34. Presidential Decree n. 187, dated 9 February 2001 (2001). Regulation for the revision of laws concerning the production and sale of milling products and pasta, pursuant to Article 50 of Law n 146, dated 22 February 1994. Official Journal 117, 6–12. https://www.gazzettaufficiale.it/eli/gu/2001/05/22/117/sg/pdf

35. Alzuwaid, N. T., Fellows, C. M., Laddomada, B., & Sissons, M. (2020). Impact of wheat bran particle size on the technological and phytochemical properties of durum wheat pasta. Journal of Cereal Science, 95, 103033. https://doi.org/10.1016/j.jcs.2020.103033

36. Sacchetti, G., Cocco, G., Cocco, D., Neri, L., & Mastrocola, D. (2011). Effect of semolina particle size on the cooking kinetics and quality of spaghetti. Procedia Food Science, 1, 1740–1745.

37. Flagella, Z. (2006). Qualità nutrizionale e tecnologica del frumento duro. Italian Journal of Agronomy 1, 203–239. https://doi.org/10.4081/ ija. 2006.s1.203

38. Padalino, L., Mastromatteo, M., Lecce, L., Spinelli, S., Conte, A., & Del Nobile, M. A. (2015). Effect of raw material on cooking quality and nutritional composition of durum wheat spaghetti. International Journal of Food Sciences and Nutrition, 66(3), 266–274. https://doi.org/10.3109/09637486.2014.1000838

39. Fernández-Caliani, J. C., Giráldez, M. I., & Barba-Brioso, C. (2019). Oral bioaccessibility and human health risk assessment of trace elements in agricultural soils impacted by acid mine drainage. Chemosphere, 237, 124441.

40. Brouns, F., Hemery, Y., Price, R., & Anson, N. M. (2012). Wheat aleurone: Separation, composition, health aspects, and potential food use. Critical Reviews in Food Science and Nutrition, 52, 553–568.

41. Madsen, C. K., & Brinch-Pedersen, H. (2020). Globoids and Phytase: The mineral storage and release system in seeds. International Journal of Molecular. Science, 21, 7519. https://doi.org/10.3390/ ijms21207519

42. Rios, G., Zakhia-Rozis, N., Chaurand, M., Richard-Forget, F., Samson, M. F., Abecassis, J., & Lullien-Pellerin, V. (2009). Impact of durum wheat milling on the deoxynivalenol distribution

in the outcoming fractions. Food Additives and Contaminants, 26(4), 487–495. https://doi.org/10.1080/02652030802382717

43. Visconti, A., & Pascale, M. (2010). An Overview on Fusarium Mycotoxins in the Durum Wheat Pasta Production Chain. Cereal Chemistry, 87(1), 21–27.

Chapter 2

Bran-Enriched Milled Durum Wheat Fractions Obtained Using Innovative Micronization and Air-Classification Pilot Plants

Adapted to: Cammerata, A. et al. (**2021**); Foods 2021, 10, 1796. https://doi.org/10.3390/foods10081796

Abstract

Dietary guidelines recommend the consumption of unprocessed, or minimally processed, wheat foods because they are richer in health-promoting components (i.e., minerals, vitamins, lignans, phytoestrogens, and phenolic compounds) compared to traditionally refined products. The design and implementation of technological solutions applied to the milling process are becoming a key requirement to obtain less refined mill products characterized by healthier nutritional profiles. This study presents the development of an upgraded micronization plant and of a modified air-classification plant to produce several novel types of durum wheat milling fractions, each enriched in bran particles of different sizes (from 425 µm > Ø to Ø < 180 µm) and percentage ratios. A preliminary quality assessment of the milling fractions was carried out by measuring yield percentages and ash content, the latter being related to detect the presence of bran particles. A wide array of milling fractions with different original particle size compositions was provided through the study of the process. Results indicate the ability of the novel pilot plants to produce several types of less refined milling fractions of potential interest for manufacturing durum wheat end-products beneficial for human health.

Keywords: durum wheat; milling fractions; air-classification plant; micronization plant

Introduction

Durum wheat (Triticum turgidum L. ssp. durum) is the most important cereal staple food in Mediterranean climates and regions. Traditionally, it is the primary raw material used in the production of pasta, couscous, bulgur, and different types of leavened and unleavened breads [**1**]. Overall, these products provide a significant portion of calories and proteins to human diets; also, they are an important source of bioactive compounds that may contribute to a healthy diet [**2**]. Among others, the most common bioactive compounds of wheat include dietary fiber, vitamins, micronutrients, and phytochemicals, which are mainly located in the outer layers of the kernel, typically in the bran, aleurone, and germ tissues [**3**]. A high number of in vitro, in vivo, and epidemiological studies have shown the significant health-related benefits associated with the consumption of bran-rich or whole-wheat foods [**4**], together with a decreased risk of non-communicable diseases, such as type 2 diabetes mellitus, cardiovascular disorders, and colorectal cancer [**5**]. Despite the known positive effects, the consumption of whole wheat food products is still limited in many countries. Such a hindrance may depend on consumer knowledge and behaviors [**6**], but also on the negative impact of wheat bran on the sensorial quality of end products [**7**]. Several studies have evaluated the effect of bran particle size, bran pre-treatment, and cooking method on the sensorial quality of final products [**8**]. The interfering effect of bran on both protein hydration and dilution of gluten network in the dough is due to the occurrence of hydroxyl groups in the bran structure reacting with water through hydrogen bonds, resulting in an increase in water absorption. In fact, arabinoxylans reduce the amount of water available for the gluten network, thus affecting dough stability and development time [**9**]. New technological solutions in wheat milling processing have many advantages as compared to traditional milling, which, by removing germ and bran, causethe loss of several beneficial compounds that are located in the bran fraction. Industrial pre-treatments, such as debranning, micronization, and air-classification processes, can reduce thenegative effects of wheat bran [**10–13**]. Debranning is a dry separation technology based on consecutive abrasions of cereal kernels; the progressive bran removal through the detachment of theouter, intermediate, and inner layers of pericarp leads to different byproduct classes, which can be removed by using pressurized air flowing through the screens and outlets of the debranner [**7**].

The reduction in particle size obtained through micronization can be followed by an air-classifier treatment in order to obtain two or more fractions that are collected separately. Micronization consists of a milling process able to reduce the starting matrix such as cereal grains in a fine particle product through the use of different technologies (e.g., hammer mill, knife mill). The range of the particle size of the milled product depends on the sieve diameter employed [**11**]. The general functioning criteria of the air-classification system is based on the pneumatic transportation of the milled particlesinside the plant, which operates in depression, thus promoting a pneumatic flow along circular orbits.

Inside the orbits, a series of ascending airflows was controlled by setting the airflow inlet valve to different opening conditions. Due to the combined action of different forces, the heavier particles fall in the first "G" housing (heavier gross particles), whereas the lighter particles fail to be collected in the first step but are gathered in the second "F" housing (fine particles). Bran fractions derived from both debranning and micronization were used to enrich semolina, obtaining pasta products with enhanced health properties and minimal impacts on sensory quality [**14**]. The above technologies are also useful to better manage the natural or chemical contaminants (e.g., mycotoxins, heavy metals, and pesticides) that are typically concentrated in the outermost layers of the wheat kernel [**15–17**].

The aim of this study was to further improve both the micronization and air-classification of plants by introducing ad hoc modifications into the process in order to obtain a larger choice of less-refined milling fractions. More in detail, the micronizer pilot plant improvement was carried out through the addition of a grinding chamber, equipped with a hammer crusher impeller and a decanting collector, whereas the air-classifier pilot plant was added with both a programmable logic controller (PLC) for the pneumatic flow management and a chamber specially designed with a decreasing section. These latest improvements of pilot plants already supplied in our laboratories allowed us to produce innovative mixtures of milling products. Particular focus was put on studying the process to develop diverse milling fractions, each characterized by a peculiar content and composition in bran particles that could be used to make durum wheat end-products characterized by a higher bran content and healthier nutritional content.

Materials and Methods

Durum Wheat Cultivars

Three Italian durum wheat cultivars, namely Saragolla, Maestà, and Iride, were grown under conventional farming in the experimental field of CREA, Foggia (Italy) in the 2018– 2019 growing season. Single grain samples were evaluated for test weight, moisture, protein content, gluten content, and yellow color through near-infrared analysis in transmission mode (NIT) by using Infratec™ mod 1241 (FOSS, Hillerød, Denmark) and the results were expressed as mean value of replicates (n = 10). Figure 1 shows the flow chart of the experimental plan.

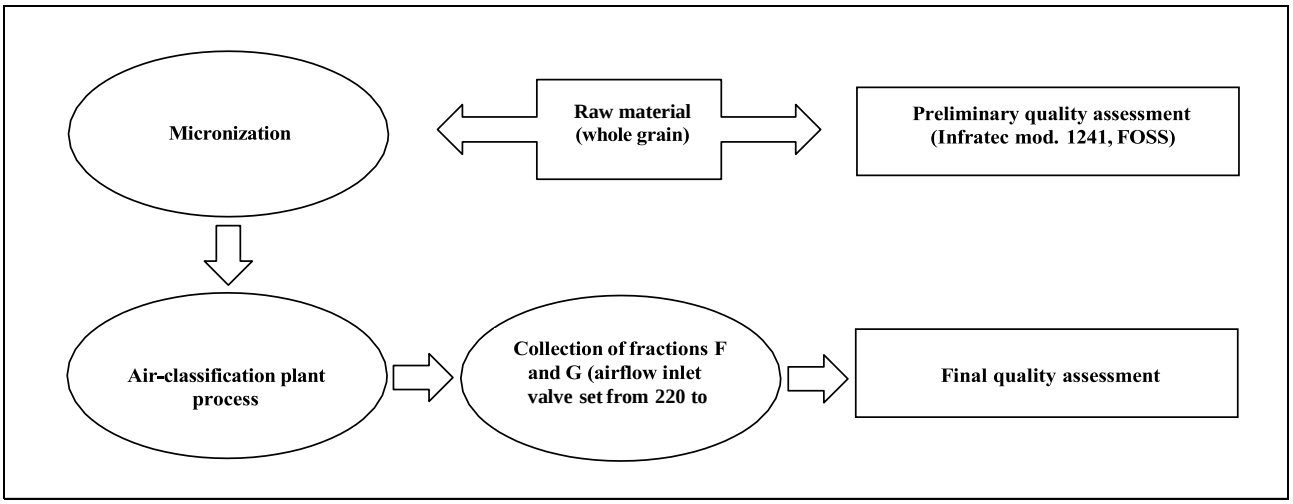

Figure 1. Flow chart of the experimental plan.

Micronization of Grain Samples and Air Classification of Milling Fractions

Durum wheat grain samples (11 kg for each cultivar) were micronized using a micronizer pilot plan (mod. 32300, KMXi-300-7,5; Separ Microsystem S.a.s, Brescia, Italy). The micronization step did not require preventive conditioning of the grains. An improvement in the micronizing pilot plant was achieved by adding a hammer crusher impeller with a reduced cross-section inside the grinding chamber compared to the normal type. This latter was equipped with a sieving grid (Ø = 0.7 mm) suitable for obtaining a more homogeneous product. In addition, the grinding chamber was connected to a decanting chamber suitable for collecting the ground product. Moreover, with the aim to better detach the milled product from the plant walls, an electric vibrator was placed on the top side of the same chamber (Figure 2).

Figure 2. Micronizer pilot plant improved through the addition of a grinding chamber including a hammer crusher impeller (A) and a decanting collector (B).

Afterwards, 10.5 kg of micronized sample was submitted to an air-classifier pilot plant (turbo-separator unit, model SX-LAB; Separ Microsystem S.a.s, Brescia, Italy) suitable for particle sizes up to a set limit (Ø ≤ 1.5 mm).

The plant was improved through the insertion of a programmable logic control (PLC) able to manage both the frequency and the action time of the airflow inside the separation chamber. Additionally, the chamber was further improved by the modification of the internal orbits that had been made according to a progressive decrease in the internal section. The latter modification allowed us to enhance the separation between the fine fractions and the coarse ones (Figure 3).

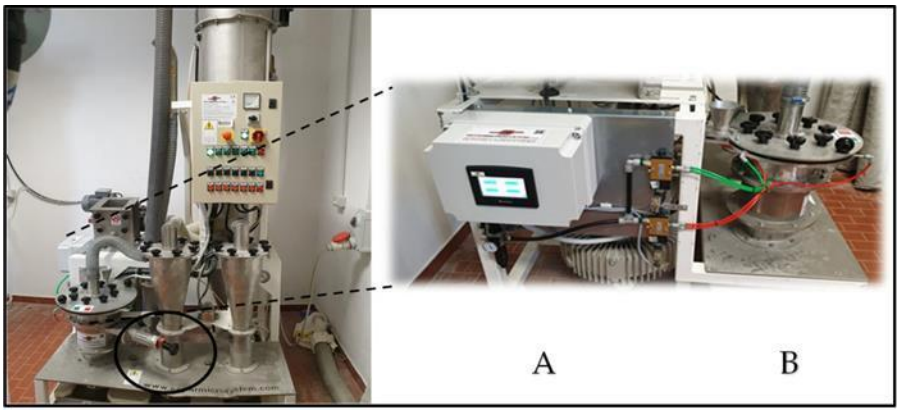

Figure 3. Air-classifier pilot plant augmented through the addition of a programmable logic controller (PLC) for the pneumatic flow management (A) and a chamber designed with a decreasing section (B). The circle indicates the setting airflow inlet valve. The F and G housings are placed below the steel plan located under theinlet valve.

Micronized aliquots of 1.5 kg were air-classified for one cycle at a time by setting the airflow inlet valve to 220, 230, 240, 250, 260, 270, or 280. At the end of each cycle the fractions of type G and F were collected and submitted for analysis.

Quality Analysis

Mean yield percentages of samples were evaluated based on the weight percentage of each sample in relation to the starting weight of the same sample. Ash content was also determined by following the official method [**18**]. The particle sizes of all fractions were measured by using certified test sieves (Giuliani Tecnologie S.r.l., Turin, Italy).

Statistical Analysis

The analysis of variance was performed on transformed data applying ANOVA (post hoc: Tukey test and Bonferroni correction) using the statistical software PAST 2.12 [**19**]. The data transformation (log or root square) was needed to achieve a normal distribution of the same data suitable for statistical analysis. The Bonferroni correction was used to counteract the incorrect rejection of a null hypothesis.

Results

The qualitative characterization of Saragolla, Maestà, and Iride grains are summarized in Table 1. Test weights among the three cultivars ranged from 81.0 to 84.4 kg/hL, namely in the first-class group according to the official standards [**20**], revealing the absence of significant quantities of shrivelled kernels. The moisture content of the grains varied from 10.2% to 10.9%, below the maximum limit of 14.0%.

Table 1. Durum wheat cultivars, origin (region of Italy), and proximate quality parameters measured by NIT. Mean values of replicated analyses (n = 10); d.m. = dry matter.

Cultivar	Origin	Test Weight (kg/hl)	Moisture %	Protein Content % d.m.	Gluten Content % d.m.	Yellow Color
Saragolla	Puglia	81.6	10.9	12.9	8.9	13.9
Maestà	Puglia	81.0	10.2	15.6	11.0	15.4
Iride	Puglia	84.4	10.7	12.6	9.1	14.2

Except for yellow color, which was low for each of the three cultivars, protein and gluten percentages were satisfactory in all samples varying from 12.6 to 15.6, and from 8.9 to 11.0, respectively, suggesting good potential nutritional and technological quality of the cultivars.

Milling yield percentages were determined to test the efficiency of the milling process. The mean recovery of micronized samples was equal to 98.8%, showing a negligible loss of starting sample.

As expected, the air-classified fractions showed marked differences between the F and G fractions (Figure 4). In detail, the F fraction showed yield values with an ascending trend from 23% (registered for setting 220) to 93% (for setting 280). Conversely, a descending trend was observed for the G fractions, varying from 77% (setting 220) to 6% (setting 280). Yield recovery of the F fractions clearly exceeded 60% only for setting conditions ranging from 250 to 280. In the G fractions, this value exceeded only at 220 and 230 settings.

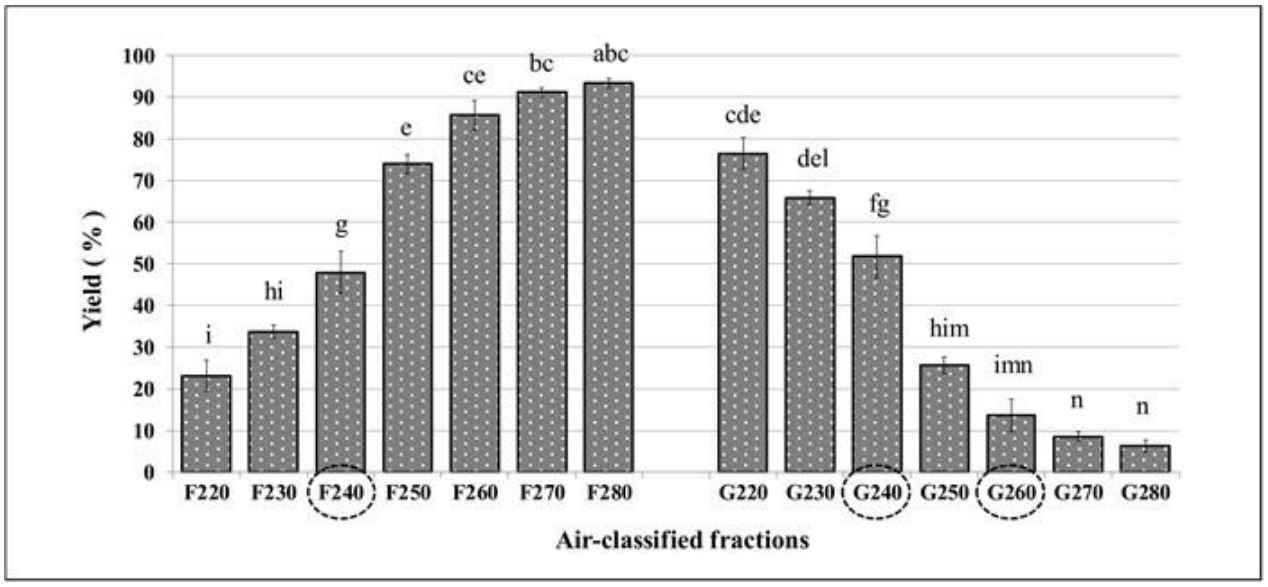

Figure 4. Mean yield percentage (%) of the air-classified fractions of samples belonging to the cultivars Saragolla, Maestà, and Iride. Different letters indicate statistically significant difference ($p < 0.05$, $n = 3$). Circles highlight the fractions with significant differences ($p < 0.05$) among cultivars ($n = 2$).

We did not observe significant differences ($p > 0.05$) among the three cultivars at any of the operating airflow conditions, with the exception of F240, G240, and G260 (Figure 4). Results obtained for each cultivar are presented in Table S1.

Further investigations are in progress to test the reliability of the process at those conditions.

The F and G type fractions obtained through the micronization and air-classification of whole durum grains contained a different percentage distribution of bran particles, due to the applied opening rate of the inlet valve. Ash content of F fractions was higher compared to that of G fractions, due to the higher content of bran particles in F fractions, while the G were richer in semolina (Figure 5). Considering the ash percentages across the F fractions, a descending trend was observed, with F220 (3.16%) and F230 (2.74%) showing the highest levels. Conversely, no significant differences ($p > 0.05$) were pointed out across the G fractions, observing a range from 1.67% (G220) to 1.42% (G280). Ash percentage of the air-classified fractions for each cultivar showed a trend that was similar to that of cultivar mean values with no significant differences ($p > 0.05$) from G220 to G280 (Table S2). Results concerning the particle size distributions (Ø > 425 µm, 425 µm > Ø > 180 µm, Ø < 180 µm) within the air-classified fractions are shown in Figure 6. As expected, heavier particles (Ø > 425 µm) were found mainly in the G fractions, because these contained more semolina than bran residues as compared to the F fractions. The percentage of the heavier particles in G fractions ranged from 11.4% (G220) to 53.4% (G280). Conversely, heavy particles were scarcely present in the F millings, varying from 4.3% (F250) to 19.9% (F220). Intermediate (425 µm > Ø > 180 µm) and fine (Ø < 180 µm) particles were predominant in all F fractions.

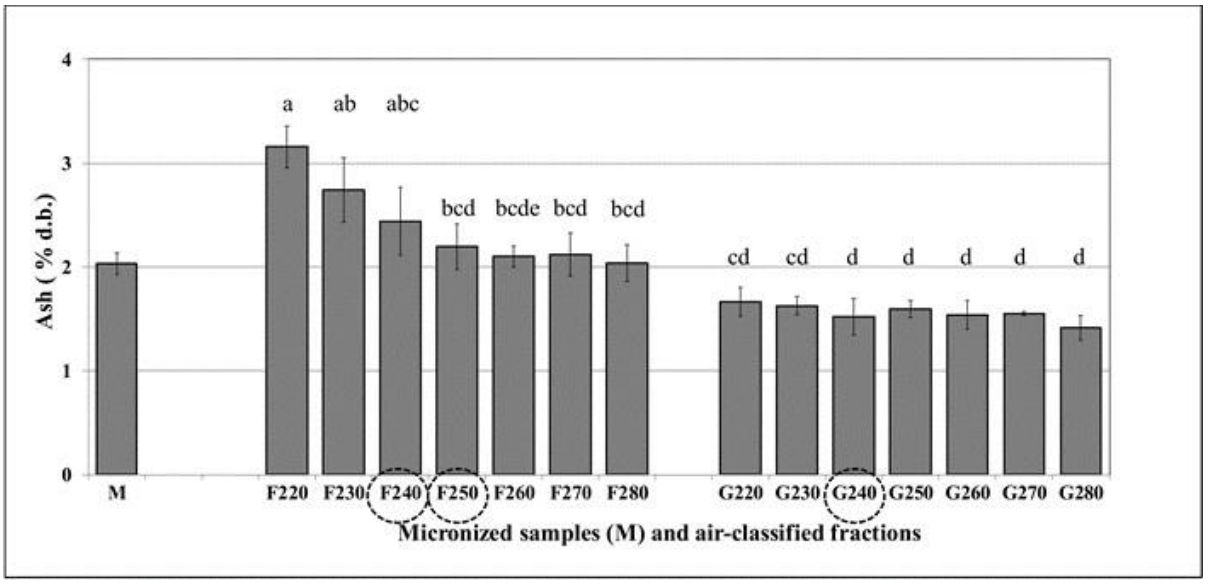

Figure 5. Mean ash percentages (% dry basis) of three durum cultivars: micronized wholemeals (M); air-classified fractions (F and G types). Different letters indicate statistically significant difference ($p < 0.05$); circles highlight the fractions with significant differences ($p < 0.05$) among cultivars ($n = 2$).

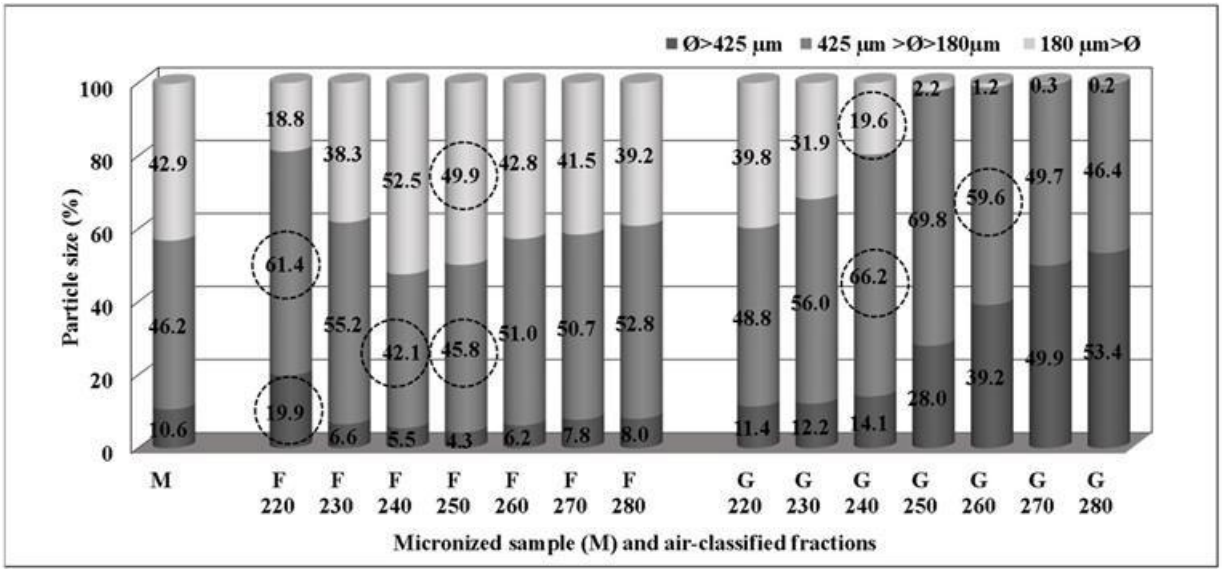

Figure 6. Mean percentages of particle size in micronized samples (M) and air-classified fractions (F and G types) of the mean of three cultivars. Circles highlight the fractions with significant differences ($p < 0.05$) among cultivars ($n = 2$).

Together, the intermediate and fine particles were prevalent in all G fractions, with the exception of G280 where their amount was 46.6% of the total, which could be explained by the less open inlet valve and the consequent different dynamics of the incoming airflow. Notably, a low percentage of finer particles was detected in F220 and F230 (18.8% and 38.3%, respectively). Detailed data on each cultivar are presented in Table S3, showing a marked trend towards no significant differences ($p > 0.05$) of percentage values from G250 to G280 fractions of all particle sizes assayed. The statistical analysis highlighted the influence of the two sources of variation (cultivar and setting valve point) related to the afore mentioned three particle sizes assayed (heavier, intermediate, and finer). The results showed that each source of variation had a significant ($p < 0.05$) influence on heavier size particles (Ø > 425 µm) in the fractions F, whereas their interaction was significant ($p < 0.05$) in the case of finer size particles both in F and in G. In the case of the fractions G, only the setting valve point factor was significant ($p < 0.05$) for heavier size particles (Ø > 425 µm).

Discussion

The measure of yield percentages allowed us to evaluate the air-classified fractions from the point of view of milling quality, this latter intended as an important aspect for the choice of more suitable fractions also for large scale production.

The ash content is an important quality parameter influenced both by genetic and environmental factors and associated with bran content [**21**]. In this study, the assay of the ash percentage proved useful to better characterize each air-classified fraction in any case containing both bran and semolina particles in different ratios.

The evaluation of particle size distribution within each fraction type (F and G) revealed the key role played by the involved physical factors (i.e., weight, air-pressure inside the circuits, turbulence, etc.). The resultant force obtained by the air-classification process, intended as a whole system, determined the distribution of the particles in F or G collectors. In any case, all air-classified millings were less refined compared to traditional semolina and were characterized by a specific percentage of bran residues. In studying the processing plants performance, the use of more than one cultivar with different grain characteristics was considered to test if they could introduce possible “critical” issues influencing the reliability of the system. Although the results need to be confirmed on a higher number of cultivars grown in different years and environments, the preliminary data pointed out that the F220, F250, and G240 fractions showed significant differences ($p < 0.05$) among the cultivars. Nevertheless, the use of the innovative plants allowed the production of several different types of air-classified fractions, allowing us to choose the more suitable for making less refined and more attractive end-products with innovative properties from among them due to the different combinations of heavier,

intermediate and finer bran particles. Previous studies already highlighted that the addition of durum wheat bran with a particle size range of 150–500 µm, at levels of 10%, 20%, and 30%, had a negative impact on pasta sensory and technological properties [**22**]. However, through the addition of 30% of the same size range of bran particles, a comparable texture to that of commercial whole milled wheat pasta was achieved. Conversely, the addition of 10% had similar sensory texture scores to regular durum wheat pasta not supplemented with bran. The use of milling fractions enriched with more fine bran particles is expected to be more suitable for the release and bioavailability of bioactive compounds, due to both the major exposition of bran particle surfaces and aleurone cells to disruption, resulting in a better release of the intra cellular contents [**23**]. Therefore, the different air-classified fractions here described offer a suitable tool to produce innovative food-products characterized by new technological properties and improved nutritional value that could make these products more attractive to consumers. The inclusion of bran particles in wheat millings leads to technological disadvantages and worsens end-products quality compared to refined semolina or flour-based processes and products, causing, among other effects, a decrease in bread loaf volume, textural changes, and color changes [**24**]. These effects are more severe, especially at 20% incorporated bran, reducing pasta quality; yet, a reduced impact occurs at the same percentage of incorporation using finer bran [**21**]. In general, bran supplementation also has a positive effect due to the increase in polyphenols and phytosterols in end-products. These parameters are not influenced by bran particle size above 10% incorporation, except for phenolic acids, which increase at a higher rate of finer bran particles [**21**]. Therefore, the range size between 180 µm and 425 µm, which is included in the fractions here assayed, has a key role due to the presence of a part of the fine bran fractions (<180 µm, 180 µm, and 250 µm) suitable for better nutritional quality of pasta due to the presence of health-promoting compounds (from the outer kernel layers) compared to traditional pasta produced with refined semolina. The influence of the bran particle size on the water absorption of cooked pasta has been deeply studied. Finer bran particles produce a lower and significant degree of water absorption compared to particles with a larger diameter, regardless of the derivation of the particles (e.g., bran or middlings) [**25**]. On the other hand, the positive effects of medium coarse and high coarse particle sizes of semolina on the end product (pasta) quality has already been described [**26**]. Therefore, the particle size composition of less-refined fractions constitutes a very important aspect to be considered, especially in relation to technological aspects and the intended use of the end-products.

With regard to the detailed results concerning the single cultivars, it should be under-lined that the choice to use them was aimed only at increasing the variability of samples tested. In any case the results are intended as a study on the cultivar behavior in the process, which would require a different experimental design (greater number of cultivars grown under different and controlled environmental conditions over multiple seasons). Indeed, our study was aimed at assessing the application of new

technological solutions to current micronization and air-classification plants in order to improve the quality of the overall milling performances.

Conclusions

The improvement of the micronization and air-classified plants yielded a wide range of less-refined milling fractions that could be suitable to make durum wheat end-products characterized by enhanced health-promoting qualities. The technological modifications of the pilot plants already in use in our laboratories regarding both the micronizer (through the addition of a grinding chamber including a hammer crusher impeller and a decanting collector) and the air-classifier (through the inclusion of PLC and a suitable chamber) allowed us to deepen some quality aspects linked to the new mixtures that were developed. Indeed, milling fractions (F and G) have been characterized by new particle size content and composition as compared to those obtained before the update of milling plants. A preliminary assessment of the quality of the milling fractions was based on yield per- centages, ash content, and particle size composition. The results revealed that the obtained air-classified fractions offer diverse choices to make a good compromise between high yield percentage, particle size composition, and the health benefits associated with a higher content of bran (fiber, antioxidants, minerals, etc.). Further investigations on technological and qualitative features of the air classified fractions are ongoing and will be the subject of upcoming papers.

References

1. Hammami, R.; Sissons, M. Durum wheat Products: Couscous. In *Wheat Quality for Improving Processing and Human Health*; Igregas, G., Ikeda, T., Guzman, C., Eds.; Springer: Cham, Switzerland, **2020**; pp. 345–366.

2. Laddomada, B.; Durante, M.; Minervini, F.; Garbetta, A.; Cardinali, A.; D'Antuono, I.; Caretto, S.; Blanco, A.; Mita, G. Phytochem- ical characterization and anti-inflammatory activity of extracts from the whole-meal flour of Italian durum wheat cultivars. *Int. J. Mol. Sci.* **2015**, *16*, 3512–3527. [CrossRef]

3. Shewry, P.R.; Hassall, K.L.; Grausgruber, H.; Andersson, A.A.M.; Lampi, A.M.; Piironen, V.; Rakszegi, M.; Ward, J.L.; Lovegrove, A. Do modern types of wheat have lower quality for human health? *Nutr. Bull.* **2020**, *45*, 362–373. [CrossRef] [PubMed]

4. Calabriso, N.; Massaro, M.; Scoditti, E.; Pasqualone, A.; Laddomada, B.; Carluccio, M.A. Phenolic extracts from whole wheat biofortified bread dampen overwhelming 1 inflammatory response in human endothelial cells and monocytes: Major role of VCAM-1 and CXCL-10. *Eur. J. Nutr.* **2020**, *59*, 2603–2615. [CrossRef]

5. Budreviciute, A.; Damiati, S.; Sabir, D.K.; Onder, K.; Schuller-Goetzburg, P.; Plakys, G.; Katileviciute, A.; Khoja, S.; Kodzius, R. Management and Prevention Strategies for Non-communicable Diseases (NCDs) and Their Risk Factors. *Front. Public Health* **2020**, *8*, 788. [CrossRef] [PubMed]

6. Foster, S.; Beck, E.; Hughes, J.; Grafenauer, S. Whole Grains and Consumer Understanding: Investigating Consumers' Identifica- tion, Knowledge and Attitudes to Whole Grains. *Nutrients* **2020**, *12*, 2170. [CrossRef] [PubMed]

7. Pasqualone, A.; Laddomada, B.; Centomani, I.; Paradiso, V.M.; Minervini, D.; Caponio, F.; Summo, C. Bread making aptitude of mixtures of re-milled semolina and selected durum wheat milling by-products. *LWT Food Sci. Technol.* **2017**, *78*, 151–159. [CrossRef]

8. Onipe, O.O.; Jideani, A.I.O.; Beswa, D. Composition and functionality of wheat bran and its application in some cereal food products. *Int. J. Food Sci. Technol.* **2015**, *50*, 2509–2518. [CrossRef]

9. Coda, R.; Kärki, I.; Nordlund, E.; Heiniö, R.-L.; Poutanen, K.; Katina, K. Influence of particle size on bioprocess induced changes on technological functionality of wheat bran. *Food Microbiol.* **2014**, *37*, 69–77. [CrossRef]

10. Fares, C.; Platani, C.; Baiano, A.; Menga, V. Effects of processing and cooking on phenolic acid profile and antioxidant capacity of durum wheat pasta enriched with debranning fractions of wheat. *Food Chem.* **2010**, *119*, 1023–1029. [CrossRef]

11. Cammerata, A.; Marabottini RAllevato, E.; Aureli, G. Content of minerals and deoxynivalenol in the air-classified fractions of durum wheat. *Cereal Chem.* **2021**. [CrossRef]

12. Taddei, F.; Galassi, E.; Nocente, F.; Gazza, L. Innovative milling processes to Improve the technological and nutritional quality of parboiled brown rice pasta from contrasting amylose content cultivars. *Foods* **2021**, *10*, 1316. [CrossRef] [PubMed]

13. Martín-García, B.; Verardo, V.; de Cerio, E.D.; del Carmen Razola-Díaz, M.; Messia, M.C.; Marconi, E.; Gómez-Caravaca, A.M. Air classification as a useful technology to obtain phenolics-enriched buckwheat flour fractions. *Food Sci. Technol.* **2021**, *150*, 111893. [CrossRef]

14. Ciccoritti, R.; Taddei, F.; Nicoletti, I.; Gazza, L.; Corradini, D.; D'Egidio, M.G.; Martini, D. Use of bran fractions and debranned kernels for the development of pasta with high nutritional and healthy potential. *Food Chem.* **2017**, *225*, 77–86. [CrossRef] [PubMed]

15. Ficco, D.B.M.; Borrelli, G.M.; Miedico, O.; Giovanniello, V.; Tarallo, M.; Pompa, C.; De Vita, P.; Chiaravalle, A.E. Effects of grain debranning on bioactive compounds, antioxidant capacity and essential and toxinc trace elements in purple durum wheats. *LWT Food Sci. Technol.* **2020**, *118*, 108734. [CrossRef]

16. Sovrani, V.; Blandino, M.; Scarpino, V.; Redyneri, A.; Coïsson, J.D.; Travaglia, F.; Locatelli, M.; Bordiga, M.; Montella, R.; Arlorio, M. Bioactive compound content, antioxidant activity, deoxynivalenol and heavy metal contamination of pearled wheat fractions. *Food Chem.* **2012**, *135*, 39–46. [CrossRef]

17. Laddomada, B.; Del Coco, L.; Durante, M.; Presicce, D.S.; Siciliano, P.A.; Fanizzi, F.P.; Logrieco, A.F. Volatile metabolite profiling of durum wheat kernels contaminated by Fusarium poae. *Metabolites* **2014**, *4*, 932–945. [CrossRef]

18. EN ISO 2171:2010. *Cereals, Pulses and By-Products—Determination of Ash Yield by Incineration (ISO 2171:2007)*; ISO: Brussels, Belgium, 2007.

19. Hammer, O.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological Statistic software package for education and data analysis. *Paleontol. Electron.* **2001**, *4*, 1–9. Available online: http://palaeo-electronica.org/2001_1/past/issue1_01.htm. (accessed on 22 June 2001).

20. UNI 10709:1998. *Durum Wheat Grains. Qualitative Requirements, Classification and Test Methods*; UNI: Rome, Italy, 1998.

21. Alzuwaid, N.T.; Fellows, C.M.; Laddomada, B.; Sissons, M. Impact of wheat bran particle size on the technological and phytochemical properties of durum wheat pasta. *J. Cereal Sci.* **2020**, *95*, 103033. [CrossRef]

22. Aravind, N.; Sissons, M.; Egan, N.; Fellows, C. Effect of insoluble dietary fibre addition on technological, sensory, and structural properties of durum wheat spaghetti. *Food Chem.* **2012**, *130*, 299–309. [CrossRef]

23. Hemery, Y.M.; Anson, N.M.; Havenaar, R.; Haenen, G.R.M.M.; Noort, M.W.J.; Rouau, X. Dry fractionation of wheat bran increases the bioaccessibility of phenolics acids. *Food Res. Int.* **2010**, *43*, 1429–1438. [CrossRef]

24. Hemdane, S.; Jacobs, P.J.; Dornez, E.; Verspreet, J.; Delcour, J.A.; Courtin, C.M. Wheat (*Triticum aestivum* L.) Bran in Bread Making: A Critical Review. *Compr. Rev. Food Sci. food Saf.* **2016**, *15*, 28–42. [CrossRef] [PubMed]

25. Sandberg, E. The Effect of Durum Wheat Bran Particle Size on the Quality of Bran Enriched Pasta; Swedish University of Agricultural Sciences, Department of Food Science: Uppsala, Sweden, 2015; Series no: 405. Available online: http://stud.epsilon.slu.se (accessed on 8 July 2015).

26. Sacchetti, G.; Cocco, G.; Cocco, D.; Neri, L.; Mastrocola, D. Effect of semolina particle size on the cooking kinetics and quality of spaghetti. Procedia Food Sci. 2011, 1, 1740–1745. [CrossRef]

Chapter 3

Qualitative Characterization of Unrefined Durum Wheat Air-Classified Fractions

Adapted to: Cammerata, A. et al. (**2021**); Foods 2021, 10, 2817. https://doi.org/10.3390/foods10112817

Abstract

Durum wheat milling is a key process step to improve the quality and safety of final products. The aim of this study was to characterize three bran-enriched milling fractions (i.e., F250, G230 and G250), obtained from three durum wheat grain samples, by using an innovative micronization and air-classification technology. Milling fractions were characterized for main standard quality parameters and for alveographic properties, starch composition and content, phenolic acids, antioxidant activity and ATIs. Results showed that yield recovery, ash content and particle size distributions were influenced either by the operating conditions (230 or 250) or by the grain samples. While total starch content was lower in the micronized sample and air-classified fractions, the P/L ratio increased in air-classified fractions as compared to semolina. Six main individual phenolic acids were identified through HPLC-DAD analysis (i.e., ferulic acid, vanillic acid, p-coumaric acid, sinapic acid, syringic and p-hydroxybenzoic acids). Compared to semolina, higher contents of all individual phenolic components were found in all bran-enriched fractions. The highest rise of TPAs occurred in the F250 fraction, which was maintained in the derived pasta. Moreover, bran-enriched fractions showed significant reductions of ATIs content versus semolina. Overall, our data suggest the potential health benefits of F250, G230 and G250 and support their use to make durum-based foods.

Keywords: durum wheat; air-classified fractions; alveographic properties; antioxidants; starch; ATI

Introduction

Durum wheat (Triticum turgidum L. ssp. durum) is an important crop, especially in the Mediterranean basin, as it is used for the production of daily foods, such as pasta, couscous, bulgur, and unleavened and leavened bread. The main component of the kernel is starch, followed by storage proteins, lipids and other minor compounds. Durum wheat grain also contains several bioactive compounds of health interest, such as insoluble fiber, phenolic acids, and alkylresorcinols, which are mostly concentrated within the coating structure of the kernel [**1**]. Phenolic acids are among the most abundant and studied components promoting human health. As dietary antioxidants, they act as free-radical scavengers [**2,3**], and reduce the inflammatory response in endothelial cells and monocytes [**4**]. Besides their antioxidant and antiradical activity, phenolic acids participate in plant cell walls as structural components and are involved in plant adaptation to abiotic and biotic stresses [**5,6**]. Among other wheat constituents that recently have become a major target of research in wheat there are the Amylase/Trypsin Inhibitors (ATIs) [**7**]. ATIs contribute about 3% of total kernel proteins and, similarly to phenolic acids, they are involved in plant defense mechanisms against pests andpathogens [**7**]. However, differently from them, ATI proteins are concentrated mainly in the endosperm and are associated with negative effects on human health [**7**]. ATIs, in fact, are putative factors triggering Non-Celiac Wheat Sensitivity (NCWS), one of the most common adverse reactionsto wheat, and are responsible for baker's asthma, the most common occupational Ig-E mediated allergy in Europe [**7**]. So far, the use of raw materials rich in health-promoting compounds and poorin toxic components constitutes a key point for a proper nutritional approach [**8–10**]. Diets rich in

whole cereals, legumes, vegetables and fruit guarantee greater protection against the onset of age-related metabolic diseases that are mainly spread in developed countries [**11,12**]. Compared to refined products, whole grain-based foods, besides having higher contents of dietary fiber and bioactive compounds, are characterized by reduced amounts of starch, which is associated with postprandial responses, the risk of type 2 diabetes and the incidence of other non-communicable diseases, such as cardiovascular disorders and colon cancer [**13–15**].

Several attempts were made to improve the poor quality of refined wheat milling products. Among these, the technology based on micronization and subsequent air-classification of milling particles was among the most promising [**1,16**]. The air-classification technology is a suitable tool to this purpose giving rise to sub-fractions with diverse particle size and composition that can be selected to enhance the beneficial health effects associated with bran particles, while limiting the content of toxic contaminants [**17–21**]. Through the use of this latter technology, several types of unrefined milling products can be directly obtained from the air-classified plants and evaluated on the basis of

qualitative tests without the need for adding selected fractions enriched with specific outer layers such as aleurone [**22,23**].

Air-classification is based on pneumatic transportation of micronized samples inside the plant, where a series of ascending airflows are controlled by setting the airflow inlet valve at different opening conditions [**17**]. At the end of each cycle, G fractions (heavier gross particles) and F fractions (fine particles), are collected. The use of micronization and subsequent air-classification treatment has expanded the possibility of selecting milling fractions with technological and nutritional characteristics that are more suitable to make healthier and safer unrefined wheat-based products, also taking into account good testing results and consumer acceptability [**16**]. These latter aspects are of great interest because durum end-products (e.g., pasta, bread) must meet good technological requirements and consumers' taste. Moreover, constant upgrading of micronization and air-classification technologies significantly improved their efficiency over the last decade. Among the main innovative solutions, a Programmable Logic Control (PLC) used to manage the airflow inside the separation chamber and a decreasing section chamber were adopted [**17**]. The latter modifications allowed to better split heavier gross fractions (G) from fine fractions (F). Three air-classified fractions of major interest resulted, namely F250, G230 and G250, showing a good compromise between technological properties and bran enrichment [**17**]. More in detail, these three fractions proved to be the most interesting on the basis of the following criteria:

- F250 (unrefined product with higher bran content): excellent yield (60–70%), good particle size composition, higher ash percentage than micronized samples, and mycotoxin presence;

- G250 (unrefined product with more semolina particles and less fine middlings): insufficient yield (20–30%) but excellent particle size composition, good ash content (higher than semolina), and strong reduction of the mycotoxin content;

- G230 (unrefined product containing both more semolina and more fine middlings): excellent yield (>60%), balanced particle size composition, good ash content (higher than semolina), and strong reduction of the mycotoxin content.

In the present work, the air-classified fractions F250, G230 and G250 were thoroughly characterized in terms of main quality parameters, alveographic properties, starch composition, phenolic acids profile and antioxidant activity, along with the measure of ATIs amount.

Materials and Methods

Plant Materials

Three grain sample were considered in the present study, each belonging to: (i) cv. Saragolla, grown in the Lazio region (hereafter named: Saragolla_LA); (ii) cv. Antalis, grown in the Basilicata region (hereafter named: Antalis_BA); and (iii) cv. Antalis, grown in the Marche region (hereafter named: Antalis_MA). The plants were grown in experimental fields under conventional farming during the 2018-2019 growing season.

Grain Characterization

Grain samples were evaluated for test weight, moisture and protein content, gluten percentage and yellow color through near-infrared analysis in transmission mode (NIT) by using Infratec™ mod 1241 (FOSS, Hillerød, Denmark). Figure 1 shows the flow chart of the experimental plan.

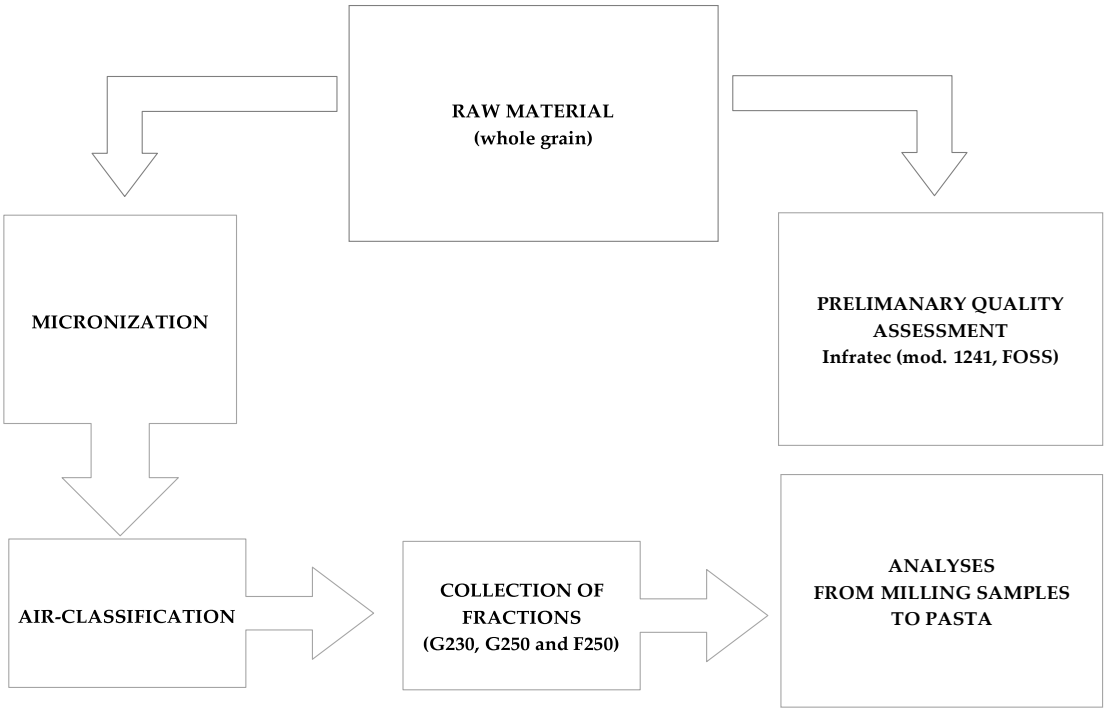

Figure 1. Flow chart of the experimental plan.

Whole Grain Micronization and Air Classification

Durum wheat grain samples (11 kg for each grain sample) were micronized using a micronizer pilot plan (mod. 32300, KMXi-300-7,5; Separ Microsystem S.a.s, Brescia, Italia). The micronization step did not require a preventive conditioning of the grains. The micronizing pilot plant was equipped with a hammer crusher impeller with a reduced cross-section inside the grinding chamber. Compared to the traditional type, a sieving grid (Ø = 0.7 mm) suitable to obtain a more homogeneous product was added to the plan.

Afterwards, the micronized sample was submitted to an air-classifier pilot plant (model SX-LAB; Separ Microsystem S.a.s, Brescia, Italia) suitable for a particle size up to a setting limit (Ø 1.5 mm). More in detail, micronized aliquots of 2.0 kg were air-classified for each cycle (in total 3–5 cycles) at a time by setting the airflow inlet valve at 230 and 250. At the end of each cycle the fractions of type G (heavier gross particles) and F (fine particles) were collected but only G230, G250 and F250 were submitted to analysis. All steps of the milling process were carried out both using the plants already updated and following the detailed procedure recently described [**17**].

Traditional Roller Milling Process

Grain samples were conditioned by adding water until a moisture value of 17% was achieved and left to rest for 24 h. Such a specific treatment was helpful to favor both the undressing process of kernels and softening the endosperm, thus being suitable to submit grain samples to the traditional roller milling plant (Bühler, model MLU 202, Uzwil, Switzerland). The main milling products collected were semolina refined through a sieving treatment (sieve types: 38GG, 40GG and 44GG) by the use of a suitable pilot plant sieving system (NAMAD Impianti, Rome, Italy), bran fractions (coarse and refined types) and fine middlings.

Yield, Ash and particle Size

Mean yield percentages of the milling fractions were calculated as the weight percentage over the weight of the starting sample. Ash content was determined by following the official method EN ISO 2171:2010 [**24**]. Particle size analysis of each fraction was carried out using certified test sieves (Giuliani Tecnologie S.r.l., Turin, Italy). Concerning this last point, an electronic sieve (Giuliani Tecnologie S.r.l., Turin, Italy, mod. IG/3) consisting of six stacked sieves of different screens (500-425-355-250-180-125 µm) was used. The milling fraction retained in each sieve was then weighed.

The yield parameter was used as a key indicator of the milling quality along with ash and grain size parameters; these last two are expressly mentioned in the Italian legislation [**25**].

Alveographic Parameters

The alveographic parameters were evaluated on the basis of the viscoelastic behavior of the dough. The analyses were performed using the Chopin alveograph (model NG) AACC Method 54-30.02 and UNI 10453 (Cedex, France) [**26**].

Total Starch, Damage Starch and Amylose

Starch content and damaged starch were determined using the Total Starch Assay Kit (K-TSTA, Megazyme, Irishtown, Ireland) and the Starch Damage Assay Kit (K-SDAM, Megazyme, Irishtown, Ireland), respectively. Total starch contents were determined using the protocol specific for "samples containing also resistant starch". For each fraction, the values represented the mean of four technical replicates.

Amylose content was determined from 15 mg of sample using a colorimetric assay based on the iodine–amylose reaction [**27**]. The standard curve was created using a mixture of potato amylose (Fluka, Neu-Ulm, Germany) and wheat amylopectin (Sigma Aldrich, St. Louis, MO, USA). Each value represented the mean of four technical replicates.

Phenolic Acids Analysis

Phenolic acid analysis was performed on the raw materials (micronized samples; F250, G250 and G230 air classification fractions; and semolina) and on uncooked and cooked pasta made using the F250, G250 and G230 air classification fractions and semolina as a control. Total phenolic acids (TPA), comprising the soluble and insoluble fractions, were extracted from samples and analyzed by HPLC according to the procedure detailed in Laddomada et al. [**28**]. In brief, samples underwent delipidation using hexane, hydrolyzation with 2M NaOH, and acidification with HCl 12Mup to pH 2 prior to ethyl acetate extraction. Ethyl acetate extracts were dried under nitrogen flux, dissolved in 80:20 methanol/water and analyzed using an Agilent 1100 Series HPLC-DAD system (Agilent Technologies, Santa Clara, CA, USA). Individual phenolic acids were identified by comparing their retention times and UV–Vis spectra to those of authentic phenolic standards and quantified via their ratio to the internal standard (3, 5-dichloro-4-hydroxybenzoic acid) added to every sample and using calibration curves for this standard. All analyses were performed on duplicate extracts.

Pasta making Process

Semolina (control) and the F250, G250 and G230 air-classified fractions (1 kg) were mixed with 280 mL water in a premixing chamber for 15 min; afterwards the dough was transferred to a pilot plan extruder (NAMAD impianti, Rome, Italy) equipped with a 1.6 mm Teflon-coated spaghetti die. Fresh spaghetti were dried using a pilot plan drier (AFREM, Lyon, France), at 50 C for 18 h. Dried pasta samples were stored in sealed plastic bags at room temperature.

Antioxidant Activity Assayes

Trolox equivalent antioxidant capacity (TEAC) was measured for all extracts using the ABTS decolorization assay according to Durante et al. [**29**] with modifications. ABTS+ stock solution was prepared by incubating overnight in the dark 7 mM ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) and 2.45 mM potassium persulfate in water. Trolox standard solutions in the interval of 0–300 µM were prepared by diluting in 80:20 methanol/water the ethanolic 30 mM stock solution. Samples were diluted in 80:20 methanol/water by a factor varying between 10 and 80 according to their TPA content and mixed with diluted $ABTS^{+}$ (A734 = 0.7) solution in PBS (Phosphate Buffer Solution) (50 µL samples in duplicate or Trolox standard in 950 µL $ABTS^{+}$). After 5 min of incubation at 25° C, the absorbance at 734 nm was measured by means of a spectrometer (Shimadzu UV-1800). TEAC values were calculated from the Trolox standard curve and values in µeq/mL were converted into µeq/g dry matter considering the initial amount of samples used for the TPA extraction.

Extraction of Albumin and Globulin Fractions (A/G)

The A/G fraction was extracted according to Lupi et al. [**30**]. Briefly, 1 g of wheat semolina or air-classified fractions were suspended in 27 mL of 0.05 M phosphate buffer/0.1 M NaCl (pH = 7.8) and incubated for 2 h at 4° C. After centrifugation at 8000x g for 15 min at 15° C, the supernatant was recovered, and the proteins (A/G fraction) were precipitated with four volumes of cold (-20 °C) acetone. After 1 h, the supernatant was discarded and the protein pellet was dissolved in 50 mM carbonate buffer (pH = 9.6).

Indirect Enzyme- Linked Immunosorbent Assay (ELISA) with Anti-ATI Antibodies

The wells of a microtiter plate (ELISA plate 82.1582.100, Sarstedt, Nümbrecht, Germany) were coated with 5 µg/mL of antigen prepared as above in 50 mM carbonate buffer (pH = 9.6) overnight at 4° C. The plate was washed three times with PBS-0.05% Tween 20, and then the wells were blocked with PBS-BSA 4% for 1 h at 37° C. After three washes with PBS-0.05% Tween 20, the platewas incubated for 1 h at 37° C with serial dilutions (from 1:2 to 1:20,000) in PBS-BSA 2% of anti- ATI polyclonal antibodies (developed by BIA-INRA, Nantes, France). Following three washes withPBS-0.05% Tween 20, the secondary antibody (anti-rabbit IgG conjugated with horseradish peroxidase) diluted 1:3000 in PBS-BSA 2% was added to each well and the plate was incubated for 1 h at 37 C. After three additional washings with PBS-0.05% Tween 20, the plate was incubated at room temperature with the colorimetric substrate, composed of o-phenylenediamine (OPD) in 0.05 M citrate buffer (pH = 5.5) plus hydrogen peroxide. After 30 min, the reaction was stopped with H2SO4 4N and the plate was read at 492 nm (Multiskan GO, Thermo Scientific). Three technical replicates were used and the data were subjected to one-way ANOVA followed by Tukey's post hoctest. When one outlier was present, this was excluded from analysis.

Statistical Analysis

With regards to all data the analysis of variance was performed applying ANOVA (post-hoc: Tukey test) by using the statistical software PAST 2.12 [**31**].

Results

Grain Characterization

The main qualitative parameters of grain samples used are summarized in Table 1. Test weight values ranged from 81.0 to 84.4 kg/hl, revealing the absence of significant quantities of shrivelled kernels, thus falling in the first-class group according to the official standards UNI 10709:1998 [**32**]. The moisture content of the grains varied from 10.2% to 10.3%, therefore falling below the maximum limit of 14.0%. Except for a low yellow color, protein and gluten percentages were satisfactory, varying from 12.7% to 15.0 % (protein content), and from 8.7% to 10.5% (gluten).

Table 1. *Infratec*™ analysis: test weight, moisture, protein content, gluten and yellow color of durum wheat grain samples Saragolla_LA, Antalis_BA, Antalis_MA. Results indicate mean values of replicated analyses (n = 10); d.m. = dry matter.

Durum Wheat Grain Sample	Test Weight (kg/hl)	Moisture (%)	Protein Content (% d.m.)	Gluten (% d.m.)	Yellow Color Index
Saragolla_LA	83.4	10.2	12.7	8.7	14.1
Antalis_BA	80.2	10.2	13.9	9.8	15.0
Antalis_MA	81.7	10.3	15.0	10.5	13.6

Milling Yield and Particle Size Parameters

The average yield recovery of micronized samples exceeded 99%, thus showing a negligible loss of the starting grain samples. Yield percentages of air-classified fractions were high for the F250 and G230 milling fractions (Figure 2). Some significant differences were observed within each F and G fraction as depending on grain samples. In fact, within G milling products, Saragolla_LA yields varied from 42% (G250 setting) to 70% (G230 setting), whereas Antalis_BA yield recovery was lower and comprised between 25% (G250) and 61% (G230), and that of Antalis_MA ranged from 33% (G250) to 66% (G230). Among F fractions, yields varied from a minimum of 56% (Saragolla_LA) to a maximum of 74% (Antalis_BA) (Figure 2).

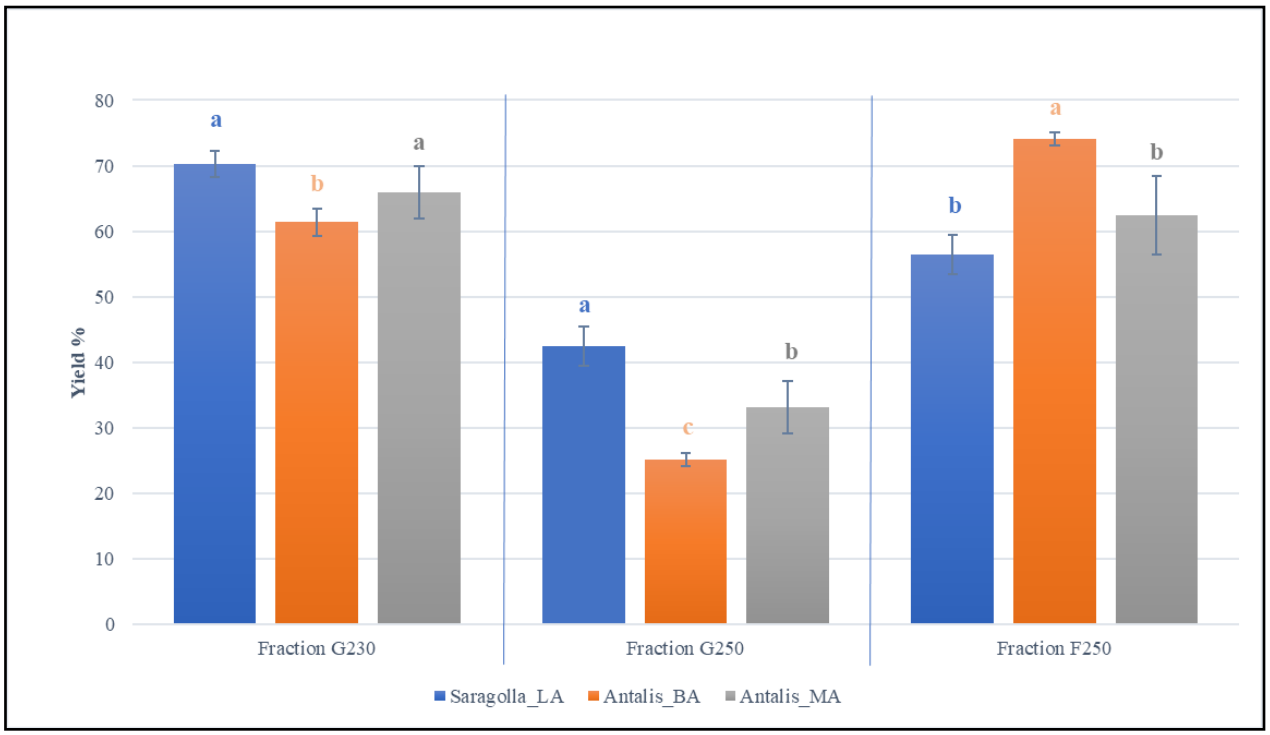

Figure 2. Mean yield (%) of F250, G250 and G230 air-classified fractions obtained from Saragolla_LA, Antalis_BA and Antalis_MA samples. Different letters indicate statistically significant differences ($p < 0.05$, $n = 3$) within each fraction type. F250: Saragolla_LA = 3.4 kg, Antalis_BA = 4.5 kg, Antalis_MA = 3.9 kg; G250: Saragolla_LA = 2.5 kg, Antalis_BA = 1.5 kg, Antalis_MA = 1.9 kg; G230: Saragolla_LA = 3.5 kg, Antalis_BA = 3.0 kg, Antalis_MA = 3.3 kg.

About particle size (Ø), in semolina samples the range was between 425 and 180 µm, whereas in air-classified fractions varied depending on the type of fraction (G or F) (Figure 3).

F fractions had a higher concentration of fine particles (Ø < 180 µm), which was be- tween 49% (Antalis_MA) and 68% (Saragolla_LA). By contrast, G fractions contained more coarse particles (Ø > 425 µm) with contents ranging from 12% to 28% (Antalis_BA). The content of intermediate particles and fine particles of all air-classified fractions (except for G250) was significantly different ($p < 0.05$) from that found in semolina samples (Figure 3).

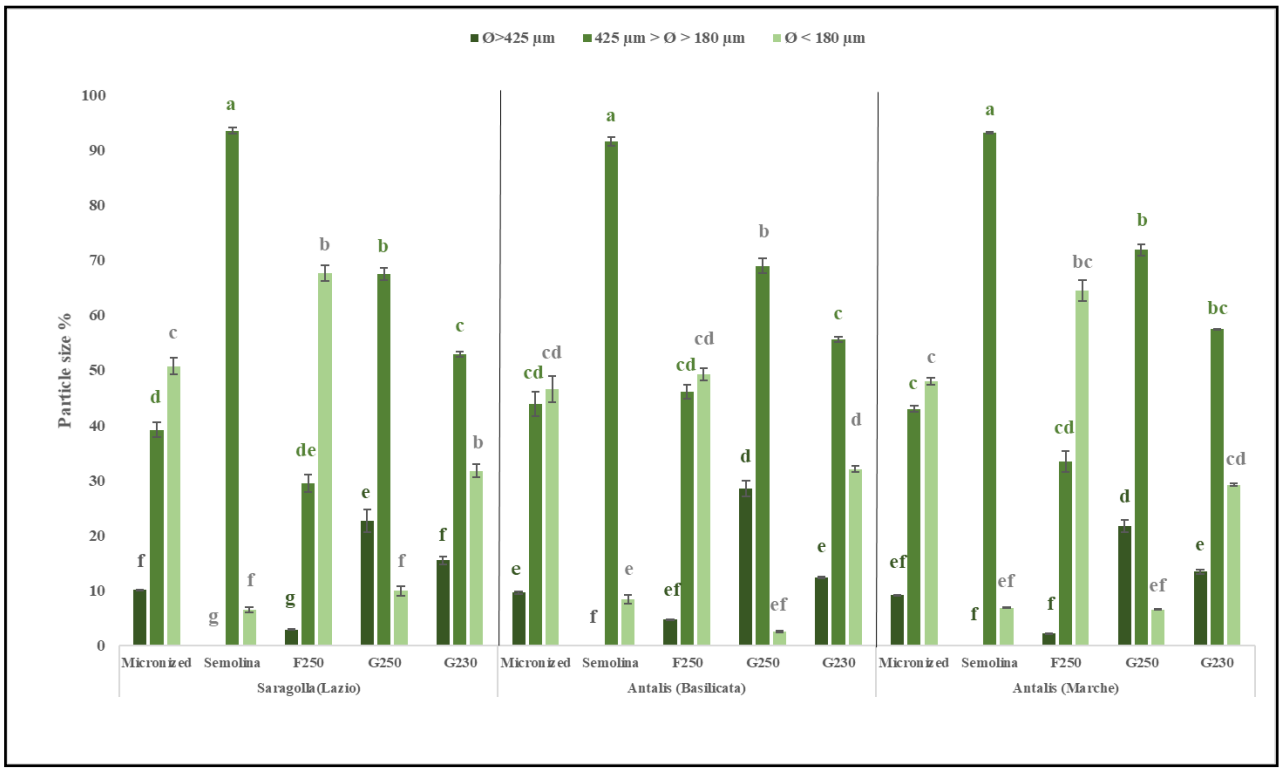

Figure 3. Mean particle size fractions (%) of micronized samples, semolina and air-classified fractions (F250, G250 and G230) obtained from Saragolla_LA, Antalis_BA and Antalis_MA. Different letters indicate statistically significant difference ($p < 0.05$, $n = 2$) within each cultivar sample.

Ash Content

In general, ash content of micronized samples and bran-enriched fractions was significantly ($p < 0.05$) higher compared to that of semolina (Figure 4). Among F fractions, ash content ranged from 2.46 (Antalis_BA) to 2.10 (Antalis_MA), which was significantly higher ($p < 0.05$) than in G, varying from 1.89 (Antalis BA) to 1.59 (Antalis_MA).

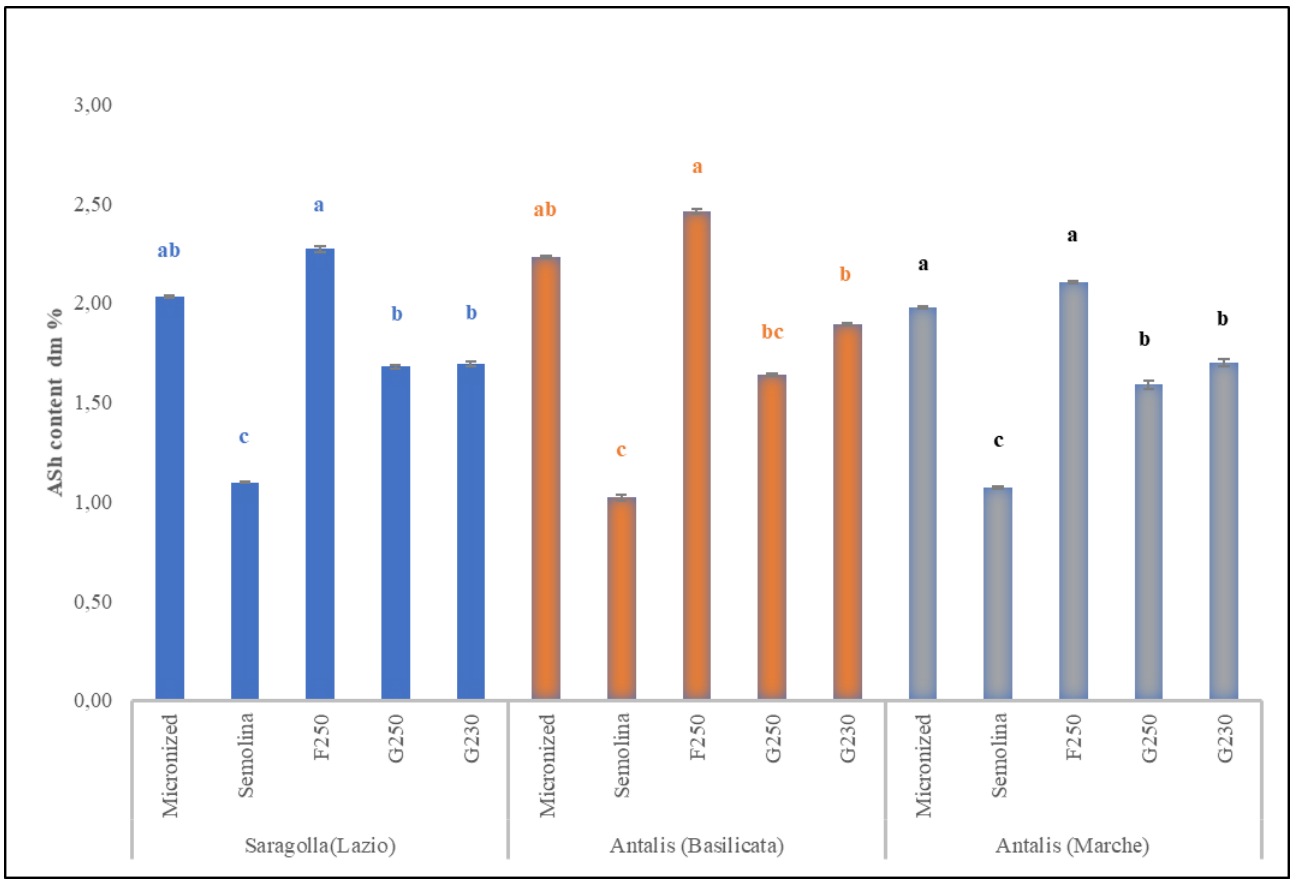

Figure 4. Mean values of ash content (d.m.%) of five milling products: micronized sample, semolina, F250, G250 and G230 air-classified fractions obtained from Saragolla_LA, Antalis_BA and Antalis_MA. Different letters indicate statistically significant differences ($p < 0.05$, $n = 3$) within each cultivar sample.

Alveographic Properties of Air-Classified Fractions

Alveographic parameters of air-classified fractions, in comparison with the corresponding semolina samples, showed an increased P/L ratio (Table 2). While semolina samples showed P/L ratios ranging from a minimum of 1.49 ± 0.15 (Antalis_BA) to a maximum of 3.96 ± 0.38 (Saragolla_LA), higher values were observed in air-classified fractions, varying from 4.69 ± 0.21 (Antalis_BA G250) to 11.32 0.08 (Antalis_MA F250). The W parameters showed more homogeneous values between air-classified fractions and semolina and a narrower range of variation (i.e., from 133 ± 20 $J \cdot 10^{-4}$ (Antalis_BA F250) to 286 ± 48 $J \cdot 10^{-4}$ (Antalis_MA semolina).

Table 2. Alveographic parameters of semolina and F250, G250 and G230 air-classified fractions obtained from three durum grain samples (Saragolla_LA, Antalis_BA, Antalis_MA). Different letters indicate significant differences ($p < 0.05$) within each milling products; n = 5.

Durum Wheat Grain Samples	Milling Products	W(J·10−4)	P/L
Saragolla_LA	Semolina	241 ± 18 [a]	3.96 ± 0.38 [a]
Antalis_BA	Semolina	162 ± 7 [b]	1.49 ± 0.15 [b]
Antalis_MA	Semolina	286 ± 48 [a]	3.85 ± 0.93 [a]
Saragolla_LA	F250	129 ± 13 [a]	10.80 ± 0.37 [a]
Antalis_BA	F250	133 ± 20 [a]	9.32 ± 0.66 [b]
Antalis_MA	F250	156 ± 13 [a]	11.32 ± 0.08 [a]
Saragolla_LA	G250	144 ± 10 [b]	8.35 ± 0.60 [a]
Antalis_BA	G250	137 ± 5 [b]	4.69 ± 0.21 [c]
Antalis_MA	G250	189 ± 4 [a]	7.50 ± 0.31 [b]
Saragolla_LA	G230	144 ± 28 [b]	8.93 ± 0.93 [a]
Antalis_BA	G230	151 ± 6 [b]	6.82 ± 0.58 [b]
Antalis_MA	G230	217 ± 19 [a]	7.59 ± 0.95 [ab]

Starch Composition of Air-Classified Fractions

Starch, amylose and damaged starch contents were determined in the air-classified fractions (F250, G230 and G250) along with semolina and micronized samples (Table 3). Total starch content was significantly lower in micronized samples and in all air-classified samples as compared to semolina ($p < 0.05$). Saragolla_LA showed the highest amount of total starch (between 61.19% and 74.87%), followed by Antalis_BA (55.09–67.68%) and Antalis_MA (53.01–64.83%). G fractions had superior amounts of total starch than F fractions (Table 3). Interestingly, damaged starch values were not significantly different ($p > 0.05$) between F50 fraction and semolina, whereas significant differences ($p < 0.05$) resulted between each G fraction and the corresponding semolina sample.

Table 3. Total starch, damaged starch and amylose (g/100g) of five milling products: micronized sample, air-classified fractions (F250, G250, G230) and semolina obtained from three durum grain samples (Saragolla_LA, Antalis_BA, Antalis_MA). Different letters indicate statistically significant differences ($p < 0.05$, $n = 3$) within each milling product ($n = 4$).

Durum Wheat Grain Samples	Milling Products	Total Starch	Damage Starch	Amylose
	Micronized	61.82 ± 0.94 ^{cd}	4.88 ± 0.21 ^{d}	15.23 ± 2.99 ^{hi}
	Semolina	74.87 ± 1.45 ^{a}	5.96 ± 0.56 ^{abc}	26.70 ± 3.41 ^{ab}
Saragolla_LA	F250	61.19 ± 1.23 ^{d}	6.41 ± 0.68 ^{ab}	14.02 ± 1.17 ^{i}
	G250	67.49 ± 1.20 ^{b}	2.62 ± 0.27 ^{e}	21.56 ± 2.29 ^{defg}
	G230	65.18 ± 1.44 ^{b}	2.93 ± 0.11 ^{e}	17.41 ± 1.02 ^{ghi}
	Micronized	56.46 ± 1.11 ^{fg}	5.32 ± 0.39 ^{cd}	21.67 ± 3.82 ^{def}
	Semolina	67.68 ± 1.61 ^{b}	5.80 ± 0.48 ^{bcd}	28.84 ± 2.18 ^{a}
Antalis_BA	F250	55.09 ± 1.07 ^{gh}	5.91 ± 0.22 ^{abc}	18.39 ± 2.05 ^{fgh}
	G250	60.26 ± 1.78 ^{de}	2.76 ± 0.13 ^{e}	23.80 ± 0.76 ^{bcde}
	G230	59.26 ± 1.58 ^{def}	3.51 ± 0.10 ^{e}	25.54 ± 4.11 ^{abcd}
	Micronized	54.70 ± 1.36 ^{gh}	5.38 ± 0.54 ^{cd}	20.30 ± 1.58 ^{efg}
	Semolina	64.83 ± 0.93 ^{bc}	6.36 ± 0.29 ^{ab}	26.09 ± 1.42 ^{abc}
Antalis_MA	F250	55.14 ± 1.25 ^{gh}	6.83 ± 0.43 ^{a}	18.82 ± 1.31 ^{fgh}
	G250	53.01 ± 1.01 ^{h}	3.02 ± 0.27 ^{e}	23.75 ± 3.05 ^{bcde}
	G230	57.13 ± 0.67 ^{efg}	3.29 ± 0.08 ^{e}	22.46 ± 2.43 ^{cdef}

The amylose content was directly determined on semolina, micronized samples and air-classified fractions. Results showed a significant reduction of amylose content in all air-classified fractions and micronized samples due to the reduced amount of starch as compared to semolina (Table 3). However, this reduction was not significant ($p > 0.05$) in the case of evaluation of the amylose/total starch ratio (data not shown).

Phenolic Acids Analysis

Phenolic acids content and composition of micronized samples, F250, G250 and G230 air classified fractions, and semolina from three grain samples are shown in Table 4. Overall, each milling product exhibited a typical phenolic acid profile, differing significantly ($p < 0.05$) for almost all individual components. Independently of the grain sample and milling type, ferulic acid was the most abundant phenolic acid being comprised between 63.52 µg/g dry matter (Antalis_BA, semolina) and 569.60 µg/g dry matter (Antalis_BA, F250). Second in abundancy was sinapic acid, ranging from 8.28 µg/g dry matter (Saragolla_LA, semolina) to 68.26 µg/g dry matter (Antalis_MA, F250).

Table 4. Phenolic acid profiles (µg/g dry matter) and antioxidant activity (µeq Trolox/g dry matter) of micronized samples, air-classified fractions (F250, G250, G230) and semolina obtained from three durum grain samples (Saragolla_LA, Antalis_BA, Antalis_MA). Different letters within columns indicate significant differences ($p < 0.05$).

Durum Wheat Grain Samples	Milling Product	*p*-Hydroxy Benzoic Acid	Syringic Acid	Vanillic Acid	*p*-Coumaric Acid	Ferulic Acid	Sinapic Acid	TEAC
Saragolla_LA	Micronized	4.37±0.50^{ab}	5.98±0.21^{abc}	8.51±0.48^{bc}	7.96±0.52^{cd}	502.19±8.05^{b}	51.04±1.64^{abc}	8.10±1.54^{cde}
	Semolina	1.12±0.01^{cd}	1.43±0.04^{fg}	1.50±0.42^{f}	0.14±0.01^{f}	80.32±2.79^{e}	8.28±0.66^{g}	2.67±0.18^{gh}
	F250	6.22±0.36^{a}	8.12±0.4^{a}	11.83±0.35^{a}	11.60±0.73^{ab}	498.04±5.08^{b}	61.72±3.83^{ab}	15.22±0.27^{a}
	G250	2.96±0.47^{bcd}	3.23±0.57^{defg}	5.07±0.83^{de}	3.52±0.62^{ef}	383.78±7.31^{c}	32.38±4.01^{bcde}	7.05±0.58^{cdef}
	G230	3.07±0.44^{bcd}	4.12±0.51^{cde}	5.59±0.65^{cd}	4.33±0.47^{e}	408.18±10.25^{c}	30.50±3.49^{cdef}	8.58±0.38^{bcd}
Antalis_BA	Micronized	3.36±0.75^{bc}	5.12±0.19^{bcd}	5.92±0.26^{cd}	8.59±0.11^{bc}	409.65±4.24^{c}	41.47±4.12^{bcde}	9.45±0.05^{bc}
	Semolina	0.76±0.15^{d}	0.76±0.18^{g}	0.73±0.09^{f}	0.09±0.00^{f}	63.52±3.32^{e}	9.81±0.47^{f}	2.47±0.06^{gh}
	F250	4.72±0.56^{ab}	8.15±0.20^{a}	7.53±0.62^{bc}	13.02±0.59^{a}	569.60±5.41^{a}	44.21±4.77^{bcd}	12.26±0.17^{ab}
	G250	2.07±0.08^{bcd}	1.60±0.75^{fg}	2.12±0.70^{f}	2.17±1.00^{ef}	240.90±6.59^{d}	25.36±1.38^{def}	5.03±0.15^{efgh}
	G230	1.53±0.12^{cd}	1.64±0.23^{efg}	2.22±0.28^{f}	2.81±0.74^{ef}	203.66±5.87^{d}	20.59±2.11^{ef}	5.48±0.17^{defg}
Antalis_MA	Micronized	3.12±0.01^{bc}	4.69±0.33^{cd}	6.76±0.15^{bcd}	8.49±0.30^{bc}	394.44±4.44^{c}	54.71±5.03^{ab}	9.07±0.39^{bc}
	Semolina	0.83±0.01^{cd}	1.01±0.01^{fg}	1.19±0.11^{f}	0.16±0.00^{f}	67.51±3.48^{e}	10.99±0.05^{ef}	2.07±0.10^{h}
	F250	4.04±0.11^{ab}	7.73±0.58^{ab}	9.09±0.49^{ab}	12.40±0.40^{a}	512.48±4.37^{b}	68.26±3.61^{a}	11.90±0.67^{ab}
	G250	1.91±0.01^{bcd}	4.49±0.53^{cd}	3.42±0.03^{def}	4.57±0.21^{de}	241.48±5.66^{d}	29.74±2.59^{cdef}	4.26±0.02^{fgh}
	G230	1.88±0.01^{cd}	3.49±0.04^{cdef}	3.18±0.27^{def}	4.28±0.35^{e}	214.11±4.38^{d}	25.47±1.62^{def}	4.32±0.17^{fgh}

Four minor compounds were also detected, namely sinapic, *p*-coumaric, vanillic, syringic and *p*-hydroxybenzoic acids (Table 4).

Semolina had the lowest concentration of all phenolic acids, whereas the micronized samples and the F250 fraction had the highest (Table 4).

The antioxidant activity associated with phenolic acids was lower in semolina samples (ranging from 2.07 to 2.67 µeq Trolox/g dry matter) compared to that of all air-classified fractions; the highest value was in Saragolla_LA, for the F250 fraction (15.22 µeq Trolox/g dry matter) (Table 4). Considering the average values of TPAs evaluated on the overall data across cultivars and environments, significant differences ($p < 0.05$) were found

among the different milling products (Figure 5). The observed variation ranged from a minimum of 82.48 µg/g dry matter (semolina) to a maximum of 619.57 µg/g dry matter (F250). Compared to semolina, the F250 fraction had the highest TPAs content (7.5-fold vs. semolina), whereas the G250 and G230 fractions showed a four-fold higher content. The pasta-making and cooking processes

caused a slight decrease of TPAs content compared to that of raw materials (Figure 5). Nevertheless, uncooked pasta made with air-classified fractions had significant enrichments of TPAs compared to traditional pasta made with semolina; such an increase varied from seven-fold (F250) to four-fold (G250 and G230) (Figure 5). A complete and detailed view of the phenolic acids profiles and antioxidant activity of uncooked and cooked pasta is presented in Supplementary Tables S1 and S2.

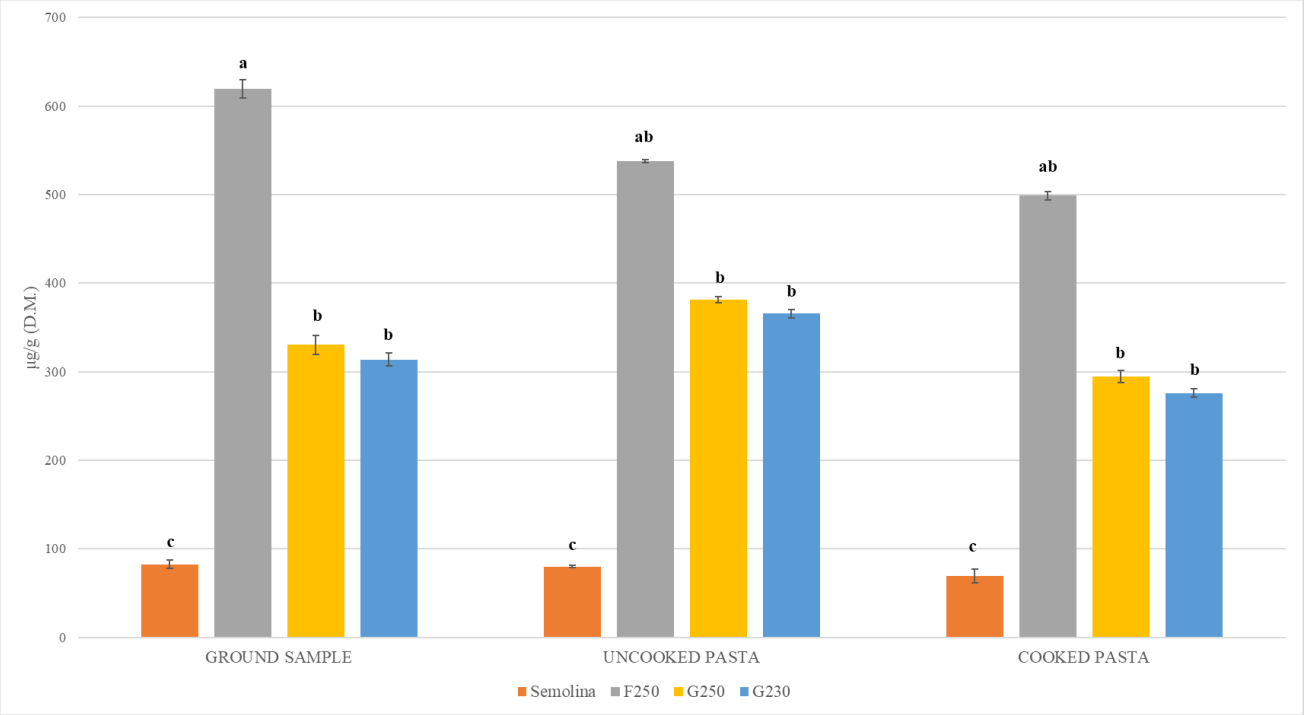

Figure 5. Average contents (n = 3) of total phenolic acids (TPAs), expressed as µg/g dry matter (d.m.) of four milling types: semolina, F250, G250 and G230 air classified fractions, obtained from the overall durum grain samples used in this study, and derived uncooked and cooked spaghetti. Different letters show significant differences ($p < 0.05$).

ATIs Content

An ELISA test was performed for detecting differences in the amount of ATI on the albumin and globulin fractions (A/G) of the different milling types (micronized grains, F250, G250, and G230 air-classified fractions, and semolina). Data relative to the four antibody dilutions used are reported in Supplementary Table S3. Since the dilution 1:200 corresponded to the most linear region, it was used to build the histograms reported in Figure 6. Overall, results showed that semolina had higher ATIs contents compared to the other milling products, excepting for the Antalis_BA G230 air-classified fraction that had slightly higher, though not significant ($p < 0.05$), amounts (Figure 6). Among the five milling types obtained from the Saragolla_LA grain sample, the G230 fraction showed the lowest

amount of ATI, whereas both Antalis_BA and Antalis_MA displayed the lowest content of ATI in the F250 air classified fraction (Figure 6).

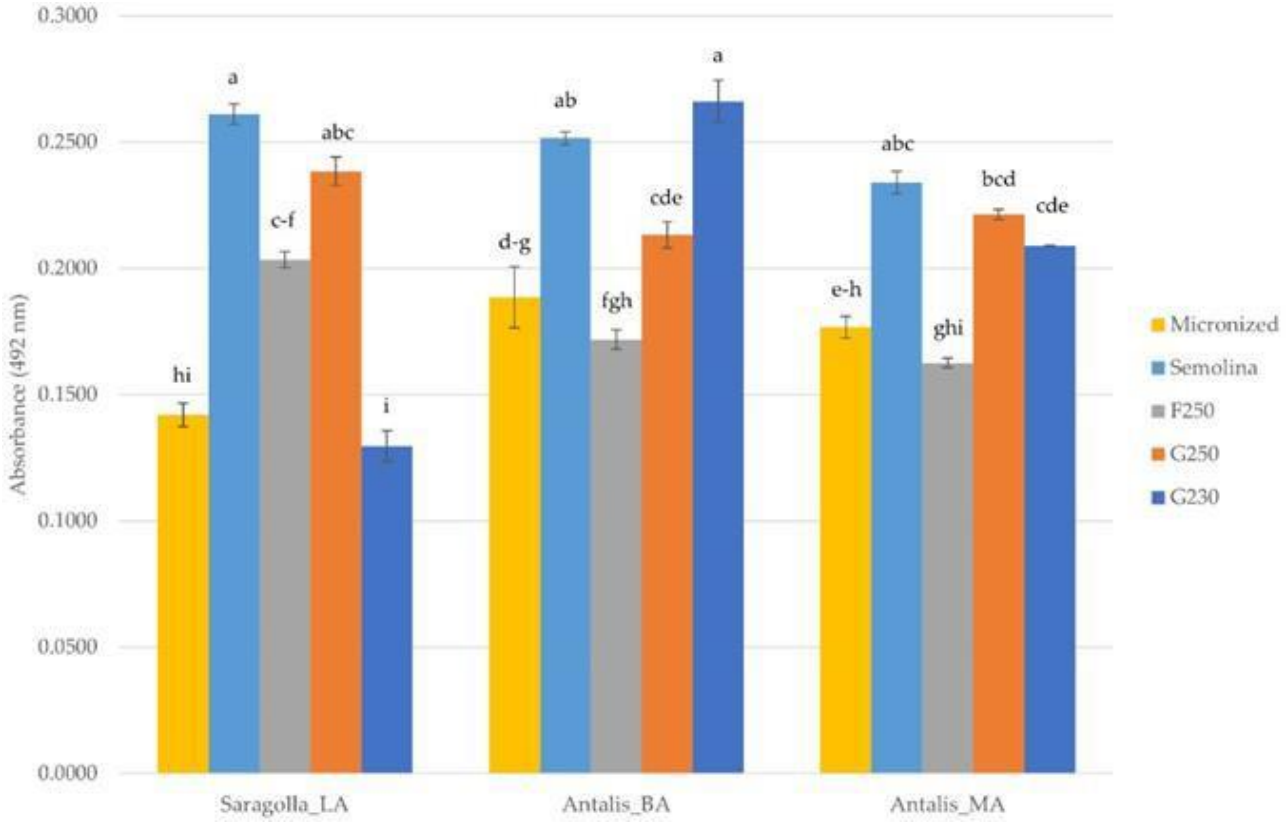

Figure 6. Indirect ELISA performed with anti-ATI polyclonal antibodies dilution 1:200 of micronized samples, semolina and air-classified fractions (F250, G250, G230) obtained from the durum grain samples used in this study. Different letters indicate significant differences ($p < 0.05$).

Discussion

One of the key challenges to enhance the health value of durum wheat foods is the use of raw materials rich in health-promoting compounds and poor in toxic components. Bran is the compartment accounting for only 15% of the wheat grain, but it is an important source of components associated with health benefits, such as phenolic acids. To maintain these healthy bran components, and reduce the negative impacts associated with some endosperm proteins, such as ATIs, diverse bran-rich streams can be used [**16,17**].

In this paper, we evaluated several qualitative features of F250, G230 and G250 air-classified fractions as compared to semolina and micronized samples. The use of this updated technology directly provided several unrefined milling products, each having different characteristics, and

without adding fractions rich in bioactive compounds [**19**]. This fact could lead to time and money savings, especially in a large-scale application.

First, milling yield percentages were assessed as they are among the major milling quality features [**33**]. The total recovery of milling products (F + G fractions) along the micronizing and air classification processes reached values around 99%, thus ensuring a minimal loss of raw material. The present results were comparable to those observed in previous evaluations [**17**]; slight differences detected here could depend on diverse grain size and texture [**34**]. The operating conditions that we used gave rise to G230 and F250 fractions with yield values that were near or above 60%, with some differences depending on grain samples ($p < 0.05$) [**17**].

The distribution of coarse, intermediate and fine particles within each G and F fraction resulted in agreement with a preliminary study [**17**]. The variability observed among the overall samples depended on grain samples and the setting valve point, supporting previous findings [**17**]. The typical particle size distribution characterizing each fraction type suggested that each could be more suitable to make specific unrefined end-products (pasta, bread, biscuits, etc.). The presence of bran particles in different rates within each air-classified fraction could lead to durum end-products (such as pasta) of higher quality than those obtained by adding bran aliquots to semolina [**35**]. The occurrence of coarse semolina particles in milling products could be important to enhance the gluten index and yellow color of end products [**36**]. Results regarding ash characterization suggested a higher content of minerals in the F fractions than in G fractions [**37**]. Overall, ash values were higher in air-classified fractions and micronized samples than in semolina. On the whole, results concerning yield, ash content and particle sizes' composition proved to be in agreement with previous studies [**17**]. The alveographic behavior of the unrefined samples revealed a great reduction in the alveographic parameters, especially with regard to a P/L ratio increase. This latter observation confirms previous results on the quality effects of the addition of bran aliquots to semolina [**38**]. Total starch content was lower in micronized flour and in all air-classified fractions compared to semolina. G fractions had superior amounts of total starch compared to two out of three F250 samples. These data were expected, since F fractions contain a higher amount of bran and a reduced content of endosperm. Results of amylose content showed a significant reduction in all air-classified fractions and micronized samples compared to semolina due to the reduced amount of starch in these fractions. In this regard, when considering the amylose/total starch, no significant differences were detected. The reduction of starch, observed in the air-classified fraction compared to semolina, is potentially interesting for the realization of low glycemic foods, useful for the prevention of some diet-related diseases (type II diabetes, obesity and cardiovascular disorders) [**13–15**].

Based on phenolic acids analysis of raw materials and derived uncooked and cooked pasta, six main individual phenolic acids were identified, namely ferulic acid, vanillic acid, *p*-coumaric acid, sinapic acid, and syringic and *p*-hydroxybenzoic acids. These findings were in line with previous works carried out along the durum chain from seed to pasta [**22,39**]. Literature studies established that phenolic acids have important biological effects, independently if they are easily absorbed by the small intestine as free forms [**40**] or reach the colon intact as bound forms [**41**]. Upon these evidences, we estimated the overall content of free and bound forms for each individual phenolic acid [**22,39**]. Results showed that semolina had the lowest concentration of all individual phenolic components, whereas micronized samples and the F250 fraction had the highest (Table 4). That was expected for the diverse endosperm concentration in semolina and milling in which bran occurred, though at a different rate [**38**]. Considering the total sum of individual phenolic acids, the F250 fraction had the highest increase over semolina (650%) (Figure 5), suggesting that it would be the best to use for enriching the content of phenolic acids of pasta. In fact, the F250-derived pasta had the highest content of TPAs with an increase by up to 616% over that made with semolina (Figure 5). A slight, though not significant TPAs reduction (between 11% and 19%) resulted in cooked pasta compared to raw materials (Figure 5). These findings were expected and in line with other studies [**39,42**]. The antioxidant activity of phenolic extracts from raw materials and derived pasta were in good agreement with predictions considering the ferulic acid content with a TEAC of 3.0, indicating that the main source of antioxidant power of tested samples are the extracted phenolic acids.

Regarding ATIs, the highest amounts were found in semolina, which was expected since these components are mostly present in the starchy endosperm as reviewed [**7**]. So far, it can be concluded that our data show that micronization and air-classification treatment decrease the amount of ATIs, giving a potential added value to derived pasta products. The importance of ATIs in triggering adverse reactions to wheat is increasing, since they seem to be not only involved in allergies and sensitivity, but also in apparently different pathologies, such as Alzheimer's disease, at least in murine models [**43**]. Thus, increasing attention is paid to the different procedures that can be used to achieve the goal of decreasing ATIs amounts. ATIs can be reduced in wheat flours through food processing, but the results are ambiguous (reviewed in [**7**]). Moreover, most of these processing procedures include fermentation, which is not typically used for pasta production. A genetic approach could theoretically be the method of choice; for example, transgenic and genome edited plants were produced in previous papers [**44,45**]. However, due to legislation restrictions, these genotypes cannot be used for commercial purposes. In addition, the production of new plant lines/varieties takes a long time. The air-fractionation procedure, described in this paper, has the advantage that it can be applied to different genotypes without the issues related to the above reported strategies, giving the possibility to produce pasta with high technological quality.

Conclusions

In the present work, F250, G230 and G250 air-classified fractions were characterized for standard quality parameters, starch, phenolic acids and ATIs content. The percent- age distribution of coarse, intermediate and fine particles within each G and F fraction depended both on grain samples and setting valve points. While the rheological behavior revealed a reduction in the alveographic parameters, all fractions had significant improvements in other qualitative properties. In fact, total starch content diminished in micronized samples and in all air-classified fractions compared to semolina, suggesting their use for the production of low glycemic foods. All air-fractionated millings, especially F250, also showed strong improvements in phenolic acids content and antioxidant activity versus semolina and traditional pasta. Finally, micronization and air-classification treatments decreased the amount of ATIs. Overall, our data suggest the potential use of the F250, G230 and G250 air-classified fractions to make more nutritious, healthier and safer foods.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/foods10112817/s1, Table S1: Phenolic acid profiles (µg/g dry matter) and antioxidant activity (µeq Trolox/g dry matter) of un-cooked pasta made with air-classified fractions (F250, G250, G230) and semolina obtained from three durum grain samples (Saragolla_LA, Antalis_BA, Antalis_MA), Table S2: Phenolic acid profiles (µg/g dry matter) and antioxidant activity (µeq Trolox/g dry matter) of cooked pasta made with air-classified fractions (F250, G250, G230) and semolina obtained from three durum grain samples (Saragolla_LA, Antalis_BA, Antalis_MA), Table S3: Anti-ATI polyclonal antibodies on micronized wholemeal, F250, G250, G230 air-classified fractions, and semolina from three durum grain samples.

Table S1. Phenolic acid profiles (μg/g dry matter) and antioxidant activity (μeq Trolox/g dry matter) of un-cooked pasta made with air-classified fractions (F250, G250, G230) and semolina obtained from three durum grain samples (Saragolla_LA, Antalis_BA, Antalis_MA). Same letters within columns indicate not significant difference ($p < 0.05$).

Grain sample	Milling product	*p*-Hydroxy benzoic acid	Syringic acid	Vanillic acid	*p*-Coumaric acid	Ferulic acid	Sinapic acid	TEAC
Saragolla_LA	F250	5.89±0.03^{a}	9.84±0.02^{a}	12.94±0.08^{a}	10.53±0.14^{a}	540.75±1.41^{a}	33.17±0.76^{a}	10.80±0.14^{a}
	G250	3.64±0.03^{b}	5.64±0.04^{b}	7.17±0.06^{bc}	4.87±0.08^{cd}	443.80±1.92^{c}	24.03±1.88^{b}	7.87±0.06^{b}
	G230	3.53±0.03^{b}	5.41±0.17^{b}	7.27±0.06^{bc}	4.92±0.06^{cd}	430.65±5.00^{c}	26.05±0.59^{b}	5.35±0.17^{c}
	Semolina	1.24±0.01^{cd}	1.88±0.01^{e}	1.22±0.01^{d}	0.07±0.01^{f}	70.20±2.23^{h}	3.32±0.03^{c}	1.14±0.03^{e}
Antalis_BA	F250	2.41±0.54^{bcd}	1.51±0.05^{e}	9.94±0.04^{ab}	8.38±0.14^{b}	506.15±4.86^{b}	23.36±0.52^{b}	8.52±0.12^{b}
	G250	2.57±0.01^{bc}	2.57±0.35^{de}	4.59±0.45^{cd}	5.48±0.02^{cd}	310.47±5.02^{ef}	28.15±1.27^{ab}	5.23±0.00^{c}
	G230	1.24±0.33^{cd}	1.51±0.06^{e}	2.39±0.12^{d}	2.39±0.56e	287.06±4.48^{f}	11.55±0.59^{c}	5.15±0.07^{c}
	Semolina	1.09±0.04^{d}	1.56±0.22^{e}	1.34±0.08^{d}	0.74±0.05^{ef}	69.25±0.05^{h}	6.63±0.35^{c}	1.11±0.01^{e}
Antalis_MA	F250	3.53±0.02^{b}	4.18±0.00^{bcd}	6.69±1.69^{bc}	6.73±0.11^{bc}	397.31±2.62^{d}	30.30±0.78^{ab}	6.24±0.26^{c}
	G250	3.36±0.21^{b}	4.92±0.64^{bc}	6.05±0.15^{c}	7.23±0.62^{bc}	260.09±4.64^{g}	18.49±0.33^{b}	3.80±0.43^{d}
	G230	2.73±0.12^{b}	3.57±0.12^{cd}	4.29±0.35^{cd}	4.73±0.16^{d}	273.84±3.36^{fg}	23.21±1.20^{b}	5.35±0.17^{c}
	Semolina	1.04±0.23^{d}	1.63±0.24^{e}	1.18±0.23^{d}	0.69±0.32^{ef}	71.77±1.40^{h}	6.33±0.33^{c}	1.11±0.01^{e}

Table S2. Phenolic acid profiles (μg/g dry matter) and antioxidant activity (μeq Trolox/g dry matter) of cooked pasta made with air-classified fractions (F250, G250, G230) and semolina obtained from three durum grain samples (Saragolla_LA, Antalis_BA, Antalis_MA). Same letters within columns indicate not significant difference ($p < 0.05$).

Grain sample	Milling product	*p*-Hydroxy benzoic acid	Syringic acid	Vanillic acid	*p*-Coumaric acid	Ferulic acid	Sinapic acid	TEAC
Saragolla_LA	F250	4.35±0.23 ^{a}	7.72±0.24^{a}	8.58±0.61^{a}	9.05±0.^{47a}	504.66±8.03^{a}	31.28±0.89^{a}	8.33±0.02^{a}
	G250	2.14±0.02^{bc}	3.38±0.04^{bc}	4.12±0.01^{bc}	3.13±0.01^{bcd}	340.66±7.48^{d}	24.55±0.07^{abc}	5.71±0.14^{b}
	G230	1.06±0.13^{cd}	1.23±0.04^{c}	1.68±0.21bc	1.39±0.66^{cde}	282.30±3.99^{e}	12.75±2.76^{ef}	5.17±0.05^{bc}
	Semolina	0.43±0.05^{d}	0.76±0.23^{c}	0.75±0.16^{c}	0.24±0.07^{e}	45.53±7.85^{h}	6.55±1.59^{f}	0.90±0.09^{f}
Antalis_BA	F250	1.73±0.03^{bc}	2.58±0.30^{bc}	4.08±0.93^{bc}	5.02±0.67^{b}	460.15±2.38^{b}	19.00±0.08^{bcde}	7.62±0.12^{a}
	G250	1.56±0.16^{bcd}	1.47±0.16^{bc}	2.36±0.45^{bc}	2.76±0.15^{bcde}	164.07±6.21^{g}	16.15±1.76^{de}	2.88±0.03^{e}
	G230	1.79±0.12^{bc}	2.16±0.16^{bc}	2.77±0.18^{bc}	3.92±0.37^{bc}	212.07±6.41^{f}	19.76±0.11^{bcde}	4.74±0.12^{cd}
	Semolina	0.80±0.13^{d}	0.95±0.10^{c}	0.83±0.12^{c}	0.43±0.28^{de}	57.26±8.06^{h}	6.95±1.01^{f}	0.90±0.00^{f}
Antalis_MA	F250	2.64±0.44^{b}	4.06±1.32^{b}	4.77±1.49^{ab}	8.33±0.54^{a}	402.29±5.75^{c}	16.71±0.43^{cde}	1.11±0.03^{f}
	G250	2.66±0.02^{b}	2.93±0.01^{bc}	3.44±0.04^{bc}	4.46±0.45^{b}	277.95±4.38^{e}	26.08±0.17^{ab}	5.19±0.15^{bc}
	G230	2.09±0.13^{bc}	2.36±0.08^{bc}	2.70±0.15^{bc}	3.47±0.06^{bcd}	252.14±5.37^{e}	21.76±1.28^{bcd}	4.74±0.01^{cd}
	Semolina	1.25±0.01^{cd}	1.56±0.04^{bc}	1.05±0.03^{bc}	0.83±0.03^{de}	73.25±3.69^{h}	9.57±0.12^{ef}	4.41±0.01^{d}

Table S3. Anti-ATI polyclonal antibodies on micronized wholemeal, F250, G250, G230 air-classified fractions, and semolina from three durum grain samples. Different letters indicate significant differences ($p<0.05$) within columns.

Durum wheat grain sample	Milling type	Dilution			
		1:20	1:200	1:2000	1:20000
Saragolla_LA	Micronized	0.25 ± 0.03^{efg}	0.14 ± 0.01^{hi}	0.08 ± 0.01^{g}	0.06 ± 0.00^{a}
	Semolina	0.36 ± 0.02^{ab}	0.26 ± 0.01^{a}	0.17 ± 0.01^{a}	0.08 ± 0.00^{a}
	F250	0.31 ± 0.01^{bcd}	$0.20\pm0.01^{c-f}$	0.11 ± 0.01^{def}	0.06 ± 0.00^{a}
	G250	0.33 ± 0.01^{bc}	0.24 ± 0.01^{abc}	0.14 ± 0.01^{bc}	0.08 ± 0.00^{a}
	G230	0.19 ± 0.00^{hi}	0.13 ± 0.01^{i}	0.14 ± 0.01^{bc}	0.09 ± 0.02^{a}
Antalis_BA	Micronized	0.30 ± 0.01^{cde}	$0.19\pm0.02^{d-g}$	0.10 ± 0.01^{ef}	0.06 ± 0.00^{a}
	Semolina	0.38 ± 0.00^{a}	0.25 ± 0.00^{ab}	0.15 ± 0.00^{b}	0.08 ± 0.01^{a}
	F250	$0.27\pm0.01^{d-g}$	0.17 ± 0.01^{fgh}	0.11 ± 0.01^{ef}	0.09 ± 0.02^{a}
	G250	0.33 ± 0.02^{bc}	0.21 ± 0.01^{cde}	0.12 ± 0.01^{cde}	0.08 ± 0.00^{a}
	G230	0.33 ± 0.00^{abc}	0.27 ± 0.01^{a}	0.15 ± 0.00^{ab}	0.09 ± 0.04^{a}
Antalis_MA	Micronized	0.22 ± 0.00^{gh}	$0.18\pm0.01^{e-h}$	0.11 ± 0.01^{ef}	0.06 ± 0.00^{a}
	Semolina	0.31 ± 0.00^{cd}	0.23 ± 0.01^{abc}	0.14 ± 0.00^{b}	0.08 ± 0.00^{a}
	F250	0.24 ± 0.01^{fg}	0.16 ± 0.00^{ghi}	0.10 ± 0.00^{fg}	0.06 ± 0.00^{a}
	G250	0.27 ± 0.01^{def}	0.22 ± 0.00^{bcd}	0.13 ± 0.00^{bcd}	0.07 ± 0.00^{a}
	G230	0.15 ± 0.01^{i}	0.21 ± 0.00^{cde}	0.13 ± 0.01^{bcd}	0.08 ± 0.01^{a}

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References

1. Hemery, Y.; Rouau, X.; Lullien-Pellerin, V.; Barron, J.C.; Abecassis, J. Dry processes to develop wheat fractions and products with enhanced nutritional quality. *J. Cereal Sci.* **2007**, *46*, 327–347. [CrossRef]

2. Sevgi, K.; Tepe, B.; Sarikurkcu, C. Antioxidant and DNA damage protection potentials of selected phenolic acids. *Food Chem. Toxicol.* **2015**, *77*, 12–21. [CrossRef] [PubMed]

3. Pandey, K.B.; Rizvi, S.I. Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Med. Cell. Longev.* **2009**, *2*, 270–278. [CrossRef] [PubMed]

4. Calabriso, N.; Massaro, M.; Scoditti, E.; Pasqualone, A.; Laddomada, B.; Carluccio, M.A. Phenolic extracts from whole wheat biofortified bread dampen overwhelming 1 inflammatory response in human endothelial cells and monocytes: Major role of VCAM-1 and CXCL-10. *Eur. J. Nutr.* **2020**, *59*, 2603–2615. [CrossRef] [PubMed]

5. Laddomada, B.; Blanco, A.; Mita, G.; D'Amico LSingh, R.P.; Ammar, K.; Crossa, J.; Guzmán, C. Drought and heat stress impacts on phenolic acids accumulation in durum wheat cultivars. *Foods* **2021**, *10*, 2142. [CrossRef] [PubMed]

6. Nigro, D.; Grausgruberb, H.; Guzmàn, C.; Laddomada, B. Phenolic compounds in wheat kernels: Genetic and genomic studies of biosynthesis and regulation. In *Wheat Quality for Improving Processing and Health*; Igrejas, G., Ikeda, T., Guzmàn, C., Eds.; Springer Sciences + Business Media: Dordrecht, The Netherlands, 2020; pp. 223–251, ISBN 978-3-030-34162-6.

7. Geisslitz, S.; Shewry, P.; Brouns, F.; America, A.H.P.; Caio, G.P.I.; Daly, M.; D'Amico, S.; De Giorgio, R.; Gilissen, L.; Grausgruber, H.; et al. Wheat ATIs: Characteristics and Role in Human Disease. *Front. Nutr.* **2021**, *8*, 667370. [CrossRef]

8. Otles, S.; Ozgoz, S. Health effects of dietary fiber. *Acta Sci. Pol. Technol. Aliment.* **2014**, *13*, 191–202. [CrossRef] [PubMed]

9. Probst, Y.C.; Guan, V.X.; Kent, K. Dietary phytochemical intake from foods and health outcomes: A systematic review protocol and preliminary scoping. *Br. Med. J.* **2017**, *7*, e013337. [CrossRef] [PubMed]

10. Stephen, A.M.; Champ, M.M.J.; Cloran, S.J.; Fleith, M.; van Lieshout, L.; Mejborn, H.; Burley, V.J. Dietary fibre in Europe: Current state of knowledge on definitions, sources, recommendations, intakes and relationships to health. *Nutr. Res. Rev.* **2017**, *30*, 149–190. [CrossRef]

11. Jacobs, D.; Anderson, F.L.; Blomhoff, R. Wholegrain consumption is associated with a reduced risk of non cardiovascu- lar, non cancer death attributed to inflammatory disease in the Iowa Women's Health Study. *Am. J. Clin. Nutr.* **2007**, *85*, 1606–1614. [CrossRef]

12. Kushi, L.H.; Meyer, K.A.; Jacobs, D.R. Cereals, legumes, and chronic disease risk reduction: Evidence from epidemiologic studies. *Am. J. Clin. Nutr.* **1999**, *70*, 451s–458s. [CrossRef]

13. Aune, D.; Keum, N.N.; Giovannucci, E.; Fadnes, L.T.; Boffetta, P.; Greenwood, D.C.; Tonstad, S.; Vatten, L.J.; Riboli, E.; Norat, T. Whole grain consumption and risk of cardiovascular disease, cancer, and all cause and cause specific mortality: Systematic review and dose-response meta-analysis of prospective studies. *Br. Med. J.* **2016**, *353*, i2716. [CrossRef]

14. Călinoiu, L.F.; Vodnar, D.C. Whole grains and phenolic acids: A review on bioactivity, functionality, health benefits and bioavailability. *Nutrients* **2018**, *10*, 1615. [CrossRef]

15. Giacco, R.; Costabile, G.; Della Pepa, G.; Anniballi, G.; Griffo, E.; Mangione, A.; Cipriano, P.; Viscovo, D.; Clemente, G.; Landberg,R.; et al. A whole-grain cereal-based diet lowers postprandial plasma insulin and triglyceride levels in individuals with metabolic syndrome. *Nutr. Metab. Cardiovasc. Dis.* **2014**, *24*, 837–844. [CrossRef] [PubMed]

16. Hemery, Y.; Chaurand, M.; Holopainen, U.; Lampi, A.-M.; Lehtinen, P.; Piironen, V.;Sadoudi, A.; Rouau, X. Potential of dry fractionation of wheat bran for the development of food ingredients, part I: Influence of ultra-fine grinding. *J. Cereal Sci.* **2011**, *53*,1–8. [CrossRef]

17. Cammerata, A.; Sestili, F.; Laddomada, B.; Aureli, G. Bran-Enriched Milled Durum Wheat Fractions Obtained Using InnovativeMicronization and Air-Classification Pilot Plants. *Foods* **2021**, *10*, 1796. [CrossRef] [PubMed]

18. Cammerata, A.; Marabottini, R.; Allevato, E.; Aureli, G.; Stazi, S.R. Content of minerals and deoxynivalenol in the air-classifiedfractions of durum wheat. *Cereal Chem. J.* **2021**, *98*, 1101–1111. [CrossRef]

19. Ficco, D.B.M.; Borrelli, G.M.; Giovanniello, V.; Platani, C.; De Vita, P. Production of anthocyanin-enriched flours of durum and soft pigmented wheats by air-classification, as a potential ingredient for functional bread. *J. Cereal Sci.* **2018**, *79*, 118–126. [CrossRef]

20. Gomez-Caravaca, A.M.; Verardo, V.; Candigliota, T.; Marconi, E.; Segura-Carretero, A.; Fernandez-Gutierrez, A.; Caboni, M.F. Use of air classification technology as green process to produce functional barley flours naturally enriched of alkylresorcinols, β-glucans and phenolic compounds. *Food Res. Int.* **2015**, *73*, 88–96. [CrossRef]

21. Laudadio, V.; Bastoni, E.; Introna, M.; Tufarelli, V. Production of low-fiber sunflower (*Helianthus annuus* L.) meal by micronization and air classification processes. *CyTA-J. Food* **2013**, *11*, 398–403. [CrossRef]

22. Chen, Z.; Li, W.; Ren, W.; Li, Y.; Chen, Z. Triboelectric separation of aleurone cell-cluster from wheat bran fragments in nonuniformelectric field. *Food Res. Int.* **2014**, *62*, 111–120. [CrossRef]

23. Chen, Z.; Zha, B.; Wang, L.; Wang, R.; Chen, Z.; Tian, Y. Dissociation of aleurone cell cluster from wheat bran by centrifugal impact milling. *Food Res. Int.* **2013**, *54*, 63–71. [CrossRef]

24. EN ISO 2171:2010. *Cereals, Pulses and by-Products-Determination of Ash Yield by Incineration (ISO 2171:2007)*. European Directive, **2010**.

25. Decreto del Presidente della Repubblica 9 febbraio 2001, n. 187. In *Regolamento per la Revisione Della Normativa Sulla Produzione e Commercializzazione di Sfarinati e Paste Alimentari, a Norma Dell'articolo 50 Della Legge 22 feb-Braio 1994, n. 146*; Italian Legislation:Italy, **2001**.

26. AACC Method 54-30.02 and UNI 10453. In *Alveograph Method for Soft and Hard Wheat Flour*; AACC Method 54-30.02: International Method, First approval October 3, 1984, Reapproval 3 November 1999; UNI 10453; Italian National Unification Bod: Italy, **1995**.

27. Chrastil, J. Improved colorimetric determination of amylose in starches or flours. *Carbohydr. Res.* **1987**, *159*, 154–158. [CrossRef]

28. Laddomada, B.; Durante, M.; Mangini, G.; D'Amico, L.; Lenucci, M.S.; Simeone, R.; Piarulli, L.; Mita, G.; Blanco, A. Genetic variation for phenolic acids concentration and composition in a tetraploid wheat (*Triticum turgidum* L.) collection. *Genet. Resour.Crop. Evol.* **2017**, *64*, 587–597. [CrossRef]

29. Durante, M.; Milano, F.; Caroli, M.; Giotta, L.; Piro, G.; Mita, G.; Frigione, M.; Lenucci, M.S. Tomato Oil Encapsulation by α-, β-,and γ-Cyclodextrins: A Comparative Study on the Formation of Supramolecular Structures, Antioxidant Activity, and Carotenoid Stability. *Foods* **2020**, *9*, 1553. [CrossRef] [PubMed]

30. Lupi, R.; Denery-Papini, S.; Rogniaux, H.; Lafiandra, D.; Rizzi, C.; De Carli, M.; Moneret-Vautrin, D.A.; Masci, S.; Larré, C. Howmuch does transgenesis affect wheat allergenicity?: Assessment in two GM lines over-expressing endogenous genes. *J. Proteom.* **2013**, *80*, 281–291. [CrossRef] [PubMed]

31. Hammer, O.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological Statistic Software Package for Education and Data Analysis. *Paleontol. Electron.* **2001**, *4*, 1–9. Available online: http://palaeo-electronica.org/2001_1/past/issue1_01.htm (accessed on 14 November 2021).

32. UNI 10709:1998. *Durum Wheat Grains. Qualitative Requirements, Classification and Test Methods*; Italian National Unification Body:Italy, **1998**.

33. Sabanci, K.; Aydin, N.; Sayaslan, A.; Sonmez, M.E.; Aslan, M.F.; Demir, L.; Sermet, C. Wheat Flour Milling Yield Estimation Basedon Wheat Kernel Physical Properties Using Artificial Neural Networks. *Int. J. Intell. Syst. Appl. Eng.* **2020**, *8*, 78–83. [CrossRef]

34. Marshall, D.R.; Mares, D.J.; Moss, H.I.; Ellison, F.W. Effects of grain shape and size on milling yields in wheat. II. Experimental studies. *Aust. J. Agric. Res.* **1986**, *37*, 331–342. [CrossRef]

35. Alzuwaid, N.T.; Fellows, C.M.; Laddomada, B.; Sissons, M. Impact of wheat bran particle size on the technological and phytochemical properties of durum wheat pasta. *J. Cereal Sci.* **2020**, *95*, 103033. [CrossRef]

36. Sacchetti, G.; Cocco, G.; Cocco, D.; Neri, L.; Mastrocola, D. Effect of semolina particle size on the cooking kinetics and quality of spaghetti. *Procedia Food Sci.* **2011**, *1*, 1740–1745. [CrossRef]

37. Padalino, L.; Mastromatteo, M.; Lecce, L.; Spinelli, S.; Conte, A.; Del Nobile, M.A. Effect of raw material on cooking quality andnutritional composition of durum wheat spaghetti. *Int. J. Food Sci. Nutr.* **2015**, *66*, 266–274. [CrossRef] [PubMed]

38. Pasqualone, A.; Laddomada, B.; Centomani, I.; Paradiso, V.M.; Minervini, D.; Caponio, F.; Summo, C. Bread making ap- titude of mixtures of re-milled semolina and selected durum wheat milling by-products. *LWT-Food Sci. Technol.* **2017**, *78*, 151–159. [CrossRef]

39. Martini, D.; Ciccoritti, R.; Nicoletti, I.; Nocente, F.; Corradini, D.; D'Egidio, M.G.; Taddei, F. From seed to cooked pasta: Influence of traditional and non-conventional transformation processes on total antioxidant capacity and phenolic acid content. *Int. J. FoodSci. Nutr.* **2017**, *69*, 24–32. [CrossRef] [PubMed]

40. Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jiménez, L. Polyphenols: Food sources and bioavailability. *Am. J. Clin. Nutr.***2004**, *79*, 727–747. [CrossRef]

41. Cardona, F.; Andres-Lacueva, C.; Tulipani, S.; Tinahones, F.J.; Queipo-Ortuno, I.M. Benefits of polyphenols on gut microbiota and implications in human health. *J. Nutr. Biochem.* **2013**, *24*, 1415–1422. [CrossRef]

42. Fares, C.; Platani, C.; Baiano, A.; Menga, V. Effect of processing and cooking on phenolic acid profile and antioxidant capacity of durum wheat pasta enriched with debranning fractions of wheat. *Food Chem.* **2010**, *119*, 1023–1029. [CrossRef]

43. Dos Santos Guilherme, M.; Zevallos, V.F.; Pesi, A.; Stoye, N.M.; Nguyen, V.T.T.; Radyushkin, K.; Schwiertz, A.; Schmitt, U.; Schuppan, D.; Endres, K. Dietary Wheat Amylase Trypsin Inhibitors Impact Alzheimer's Disease Pathology in 5xFAD Model Mice. *Int. J. Mol. Sci.* **2020**, *21*, 6288. [CrossRef]

44. Kalunke, R.M.; Tundo, S.; Sestili, F.; Camerlengo, F.; Lafiandra, D.; Lupi, R.; Larrè, C.; Denery-Papini, S.; Islam, S.; Ma, W.; et al. Reduction of allergenic potential in bread wheat RNAi transgenic lines silenced for CM3, CM16 and 0.28 ATI genes. *Int. J. Mol.Sci.* **2020**, *21*, 5817. [CrossRef]

45. Camerlengo, F.; Frittelli, A.; Sparks, C.; Doherty, A.; Martignago, D.; Larré, C.; Lupi, R.; Sestili, F.; Masci, S. CRISPR-Cas9 multiplex editing of the α-amylase/trypsin inhibitor genes to reduce allergen proteins in durum wheat. *Front Sustain. Food Syst.* **2020**,*4*, 104. [CrossRef]

Chapter 4

Use of Air-Classification Technology to Manage Mycotoxin and Arsenic Contaminations in Durum Wheat-Derived Products

Adapted to: Cammerata, A. et al. (**2022**); Foods 2022, 11, 304. https://doi.org/10.3390/foods11030304

Abstract

Mycotoxins are the most common natural contaminants and include different types of organic compounds, such deoxynivalenol (DON) and T-2 and HT-2 toxins. The major inorganic toxic elements include those commonly known as heavy metals such as cadmium, nickel and lead and other minerals such as arsenic. In this study, micronisation and air-classification technologies were applied to durum wheat (*Triticum turgidum* ssp. *durum* L.*)* samples to mitigate inorganic (arsenic) and organic contaminants in unrefined milling fractions and final products (pasta). The results showed the suitability of milling plants for providing less refined milling products lowering the amount of mycotoxins (DON and the sum of T-2 and HT-2 toxins) and inorganic toxic elements (As, Cd, Ni, and Pb). The results showed an As content (in end products) similar to that obtained using semolina as raw material. In samples showing high organic contamination, the content rate detected in the more bran-enriched fractions ranged from 74% to 150% (DON) and from 119% to 151% (sum of T2 and HT-2 toxins), as compared to the micronised samples. Therefore, this technology may be useful for manufacturing unrefined products with reduced levels of organic and inorganic contaminants, minimising health risk to consumers.

Keywords: durum wheat; air-classification; inorganic contaminants; organic contaminants; arsenic; mycotoxins

Introduction

Currently, cereal grains are the primary source of carbohydrates in human nutrition worldwide. The Food and Agriculture Organization (FAO) forecasts that world grain utilisation in 2021/22 will reach a record level of 2809 million tons. The FAO's forecast for total wheat utilisation has been 777 million tons, which is 2.4% (18.5 million tons) higher than in 2020/21 [**1**]. Durum wheat is a crop of primary importance, as semolina (obtained by milling durum wheat grain) is the basic raw material for the realisation of several end-products (pasta, couscous, bulgur, local breads) of large consumption and is mainly used as a traditional food for Mediterranean populations while is highly appreciated worldwide.

Cereals such as wheat provide most of the energy in diets, and their consumption in the form of unrefined end products provides health advantages because they are rich in bioactive compounds. Among these, antioxidant molecules are of great interest because they are involved in lowering the risk of cardiovascular diseases, cancers, gastrointestinal disorders, and type 2 diabetes [**2-6**].

In general, wholemeal products are realised by mixing suitable amounts of bran, germ, and endosperm fractions as similar as possible to the natural grains [**7**]. In any case, the consumption of bran-enriched products could increase the risk to consumers' exposure to food-borne organic and inorganic contaminants, the main ones being mycotoxins, toxic elements such as arsenic, and heavy metals. All these compounds could remain from the raw material to the end products, in addition to the so-called processing compounds, such as acrylamide [**8**]. The environment plays a key role as the main source of contamination in grain, such as wheat, starting from the cultivation field up to storage. Even though minerals are ubiquitous in the earth's crust, anthropogenic activities are increasingly result in the contamination of water and soil with toxic metals and metalloids [**9-11**]. Among toxic minerals, arsenic (As), cadmium (Cd), and lead (Pb) are ranked as the most hazardous substances owing to their toxicity, prevalence, and potential for human exposure [**12**]. Among the metals of great concern, cadmium (Cd) is uniformly distributed within the endosperm, whereas lead (Pb) and nickel (Ni) are mainly located in the outer teguments [**13-15**]. European legislation has established maximum tolerable levels of several metals and metalloids in foodstuffs. However, the maximum levels of Pb, Cd, and tin (Sn) are currently established and monitored in cereals, whereas the maximum arsenic content has been established only for rice (0.200 µg/g) [**16,17**].

Mycotoxins, produced by several fungi growing on cereal grains, are of great concern as they add organic contaminants in a wide range of food commodities [**17,18**]. Deoxynivalenol (DON) and T-2/HT-2 toxins belong to type B and A, respectively, group of chemically related compounds named trichothecenes. They are the most widespread *Fusarium toxins* in small grain cereals, including durum

wheat (*Triticum turgidum* ssp. *durum* L.*)*, which constitutes the main source of exposure of humans and animals to these types of mycotoxins [**19,20**]. These mycotoxins are produced by fungi which colonise the kernel starting from its outer layers, where they mostly accumulate. Since the coating structures end up in the bran milling fraction, the bran-enriched foods represent an increased risk of exposure of humans to mycotoxins [**21-25**].

To date, the maximum tolerable limits for DON in food products have been set by the European Commission [**17**]. However, there are no legal limits for T-2 and HT-2 toxins in food and feed. Nevertheless, the European Commission recommendation on the content of T-2 and HT-2 in cereals and cereal indicates 100 μg/kg for the sum of T-2 and HT-2 as the maximum level in cereal grains [**26**].

The use of new technologies, such as debranning, micronisation, and air-classification at the starting phase of wheat processing is effective in reducing the presence of undesirable compounds within the milling products [**27-29**]. In fact, micronisation combined with air-classification provides suitable solutions to obtain less refined products with regard to safety and nutritional aspects, ensuring optimal technological quality. Recently, a study of the process carried out using updated pilot plants allowed the selection of several unrefined milling fractions obtained from micronised and air-classified durum wheat samples. Therefore, three types of fractions were selected based on the results obtained in previous assays [**30,31**]. These fractions proved to be of interest regarding yield percentage, particle size composition, and health benefits associated with a higher content of bran (fibre, antioxidants, minerals, etc.).

The goal of this study was to assess the reliability of recently updated milling plants (microniser and air-classifier) to minimise the presence of DON, T-2, HT-2 toxins and toxic elements (mainly arsenic) along the production chain.

Materials and Methods

Durum wheat samples

A process study was carried out on three durum wheat grain samples (group I) belonging to cultivars Saragolla, Maestà, and Iride. All of them grown in conventional farming fields in Apulia (Southern Italy) during the season 2018-2019 (Figure 1). The use of these latter three samples allowed to identify the more suitable air-classifying conditions for the aim of this study. More in detail, the group I samples were selected from those collected within the "AsFRUM" research project. The criteria adopted in the choice concerned both the type and the rate of contamination. Moreover, the use of three different cultivars only served to achieve greater variability in the grain characteristics without taking into account the cultivar as a factor. On the basis of the results obtained in the process study,

three additional durum wheat grain samples (group II) were used only under the selected air-classifying conditions previously identified in the process study. These group II samples were collected within the aforesaid research project during the same cropping year (2018-2019) in conventional growing fields located in three regions of the Centre-South of Italy. More specifically, one durum wheat sample (Saragolla) was grown in the Lazio region (Central-Italy), and the other two samples belonging to the cv Antalis were grown in the Marche region (Central-Italy) and in the region Basilicata (Southern-Italy) (Figure 1). Also in this latter case (group II) the selection criteria adopted for grain samples have been concerned both the type and the rate of contamination. Three analytical replicates have been carried out for all the materials tested as below detailed.

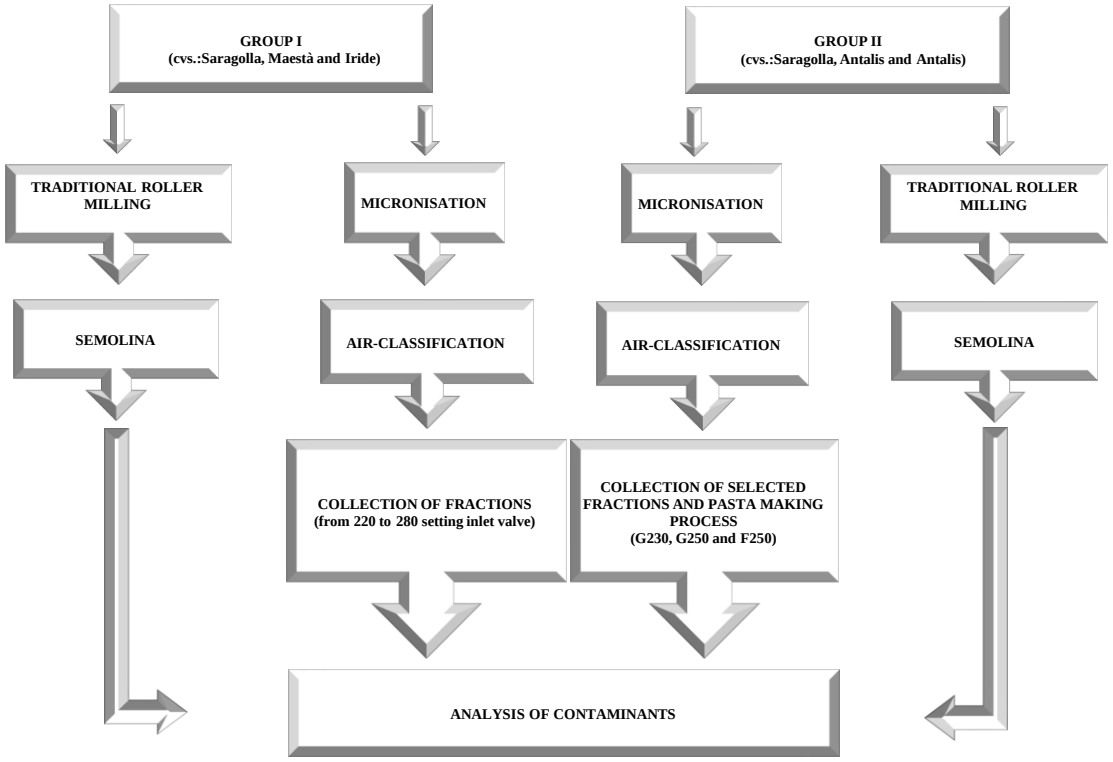

Figure 1. Flow chart of the experimental plan.

Micronisation and air-classification processes

The milling plants (micronizer and air-classifier) used in this study were already supplied to our laboratories and recently updated [**30**].

Briefly, innovative advance in the micronizer pilot plant (mod. 32300, KMXi-300-7,5; Separ Microsystem S.a.s, Brescia, Italy) were the introduction of the hammer crusher impeller, which reduced the internal cross-section and a sieving grid (Ø = 0.7 mm), which produced a more

homogeneous milled product than the traditional type. The air-classifier pilot plant (model SX-LAB; Separ Microsystem S.a.s, Brescia, Italy) designed for a sample particle size up to a setting limit (Ø ≤ 1.5 mm) was improved through the insertion of a programmable logic control (PLC) capable of managing the airflow inside the separation chamber. Moreover, the orbits located in the inner part of the chamber were improved by designing a progressively decreasing inside diameter. Each micronised sample (groups I and II) was split into 1.5 kg aliquots. The micronisation step did not require preventive conditioning of the grains. The samples included in group I were subjected to the air-classification process by setting the airflow inlet valve from 220 to 280 opening conditions for each cycle at a time. At the end of each cycle, the fractions of type G (heavier gross particles) and F (fine particles) were collected. The samples of group II were only air-classified at 230 and 250 settings based on previous evaluation (see Figure 1). Therefore, the three air-classified fractions (F250, G250, and G230) were collected and used in the subsequent steps.

Traditional roller milling process

The traditional roller milling process was carried out as a reference process. Grain samples were conditioned by adding tap water until a moisture value of 17% was achieved, and the samples were left to rest for 24 h. This treatment facilitated both the undressing process of kernels and softening of the endosperm, making it suitable for submitting grain samples to the traditional roller milling plant (Bühler, model MLU 202, Uzwil, Switzerland). The main milling products were collected: semolina refined through a sieving treatment (sieve types: 38GG, 40GG, and 44GG) using a suitable pilot plant sieving system (NAMAD Impianti, Rome, Italy), bran fractions (coarse and refined types), and fine middlings.

Pasta making process

A pilot press plant (NAMAD Impianti, Rome, Italy) was used and the “spaghetti” format (Ø = 1.6 mm) was adopted in the pasta making process. The pasta samples were submitted to a drying cycle for 18 h at low temperature (50 °C) using a suitable dryer plan (AFREM, Lyon, France).

Mycotoxin analysis

Sampling was carried out based on representative criteria and all milled samples (from micronisation, air-classification and traditional roller milling) were stored at 2-8°C until analysis [**32**]. DON content was assessed using the Ridascreen® DON kit (Ridascreen® DON method, R-Biopharm AG, Darmstadt, Germany) following the manufacturers. More in detail, extraction at ratio of 1 part sample

with 5 parts of deionised water was performed and then sample shaked vigorously for 3 minutes. The filtered extract through Whatman n. 1 filter was collected as a sample. The limit of detection (LOD) of the enzyme-linked immunosorbent assay (ELISA) is 18.5 µg/kg. The recovery range declared in this method was 85-110% in the sample. Deionised water was obtained from the Water Purification System Zeener Power I (Human Corporation, Seoul, Korea). The basic robotic immunoassay operator (BRIO, SEAC, Radim Group, Florence, Italy) was employed, and the absorbance data were acquired using a Sirio-S Microplate Reader (SEAC, Radim Group, Florence, Italy). The ELISA method for the DON assay content in durum wheat samples had already been submitted to a validation step by comparison with chromatographic (HPLC) analysis [**33**]. The sum of T-2 and HT-2 toxins was measured by ELISA analysis (Veratox® T2/HT2 toxin, Neogen, Lansing, MI, USA) according to the manufacturer's instructions. Extraction at ratio of 1 part sample with 5 parts 70% methanol was carried out and then sample shaked vigorously for 3 minutes. The extract was filtered through Whatman n. 1 filter and the filtrate was collected as a sample. The limit of quantification (LOQ) was 25 µg/kg. The ELISA method for measuring the sum of T-2 and HT-2 toxin content in durum wheat samples was assessed by comparison with Ultra Performance Liquid Chromatography (UPLC) analysis [**34**]. Absorbance values were read using the Sirio-S Microplate Reader (SEAC, Radim Group, Florence, Italy) and the RIDA® Soft Win software (R-Biopharm AG, Darmstadt, Germany) was used for quantification of mycotoxins in samples.

Toxic elements

Micronised samples and all air-classified G and F fractions were processed to determine the concentrations of As, Cd, Pb, and Ni. Each sample underwent a first step of acid digestion using a laboratory high-pressure microwave oven (Mars plus CEM, Cologno al Serio (BG) Italy) with an energy power of 1.200 W. Approximately 200 mg of the dry sample were placed in contact with 7.5 mL of 67% v/v HNO_3 for one hour, then 0.5 mL of HCl were added and left to digest for one hour; finally, 2 mL of H_2O_2 were added and left to digest for 30 min. At the end of the predigestion process, the obtained acid solution was mineralised inside a microwave, with a heating program in which the temperature of 180 °C was reached in 37 min and incubation at 180 °C continued for 15 min. At the end of the digestion procedure, the samples were appropriately diluted with high purity water (18 MΩ/cm) obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA), and then filtered (DISMIC 25HP PTFE syringe filter, pore size = 0. 45 mm, Toyo Roshi Kaisha, Ltd., Tokyo Japan) and stored in a plastic screw-cap tube (Nalgene, New York, USA). Each experiment was performed in triplicate.

Elemental quantification was performed using an inductively coupled plasma optical emission spectrometer (ICP-OES) with an axial configuration (8,000 DV, Perkin Elmer Inc. Waltham, MA, USA) equipped with an ultrasonic nebuliser (Teledyne Cetac Technologies, Omaha, USA). To assess the concentration level of trace elements, calibration standards were prepared and treated in the same way as the samples before dilution (multi-element standard solution, CaPurAn, CPAchem, Stara Zagora, Bulgaria). The frequencies used for the determinations were: Cd 228.8 nm, Fe 259.9 nm, Cu 324.7 nm, Pb 220.3, Ni 231.6 nm, As 197.69 nm.

The European reference material ERM-BC21 was used as the standard material to assess the accuracy of the measurements.

The super-pure grade reagents used for the microwave-assisted digestion were as follows: hydrochloric acid (36% HCl), nitric acid (69% HNO_3), and hydrogen peroxide (30% H_2O_2); highly pure water (18 MΩ/cm) was used to dilute the standards and prepare the samples throughout the chemical procedure.

Statistical analysis

The results concerning the mycotoxin content were submitted to analysis of variance (ANOVA and Tukey test) using the statistical software PAST v. 2.12, Oslo, Norway [**35**].

Results

3.1 Determination of mycotoxin concentration

All air-classified G and F fractions (220-280) collected from group I samples were analysed to show the distribution of DON and T-2 and HT-2 toxins (sum of the two types) within the same samples. The results clearly highlighted the influence of the process conditions on the DON content in the milling fractions collected (Figure 2).

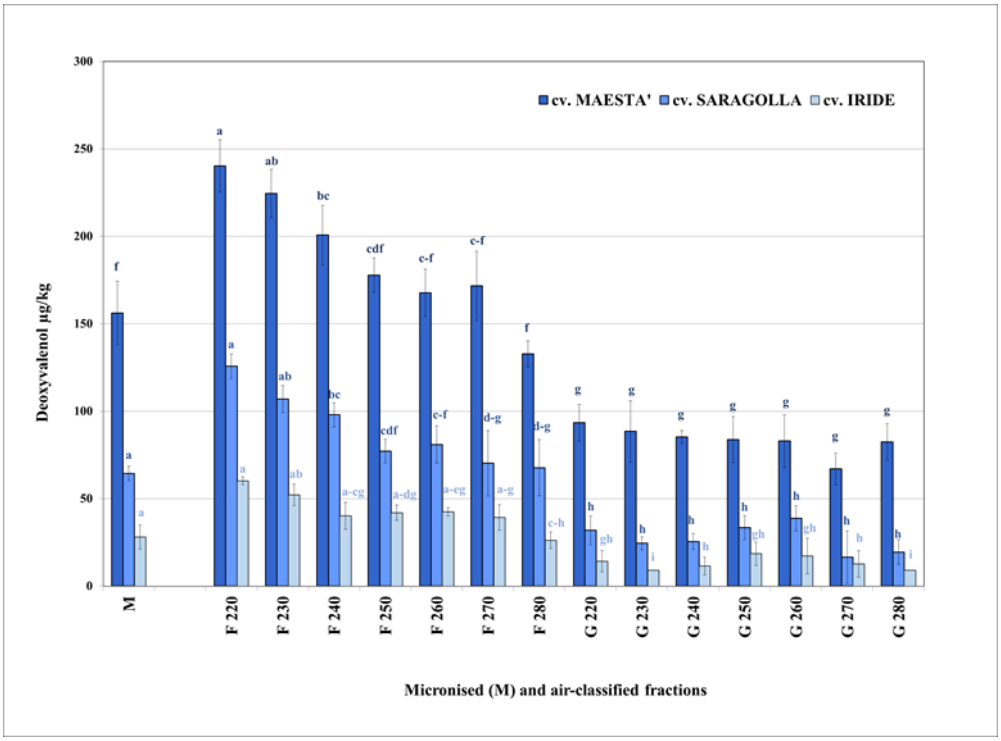

Figure 2. Average content of DON in the micronised (M) and air-classified fractions (F and G) of the durum wheat samples belonging to the group 1; different letters within each cultivar indicate significant differences ($p < 0.05$), n = 3.

The average levels of DON in micronised samples ranged between 28 µg/kg (cv Iride) and 156 µg/kg (cv Maestà). The highest mycotoxin content was detected in the F fractions, with a clear decreasing trend from fraction F220 to fraction F280 in all samples tested. From F220 to F240, which had the highest degree of contamination, the differences were statistically significant ($p < 0.05$) as compared with the corresponding micronised samples. A marked reduction in mycotoxin content was also found in all G fractions, with no significant differences ($p > 0.05$) between the most contaminated cultivar samples (Saragolla and Maestà). Concerning to the latter, all G fractions were significantly different ($p < 0.05$) from the F fractions.

The aforementioned general trend was even more marked in the case of the residual content, expressed as a percentage, compared to the micronised samples, all of which were set equal to 100%. In fact, the content rate varied from a minimum of 155% (cv Maestà) to a maximum of 215% (cv Iride) in the F220 fractions, whereas it de-creased in the F280 fractions to values even lower than those of the micronised samples (cv Maestà = 86% and cv Iride = 94% both in F280). Among the G fractions, the maximum rate achieved was 66% (G250) as compared to the micronised sample (cv Iride).

A trend similar to DON was also found for the sum of T-2 and HT-2 toxin content (Figure 3). In fact, the F fractions F220, F230, and F240, showed significantly higher levels of contamination ($p < 0.05$) with a clear further reduction in the remaining F fractions as compared to the average values of the corresponding micronised samples, ranging from 58 µg/kg (cv Iride) to 256 µg/kg (cv Saragolla). With regard to the G fractions, a marked reduction in mycotoxin content was detected in all G fractions without significant differences ($p > 0.05$) in all samples between fractions G240 and G280, but in any case, were significantly different ($p < 0.05$) from the F fractions. Moreover, similarly to DON, a marked effect of the air-classification process was highlighted in the case of the percentage evaluation as compared to the micronised samples set equal to 100% (data not shown). In fact, within all the F220 fractions, the percentage rates varied from a minimum of 164% (cv Maestà) to a maximum of 225% (cv Iride), with a tendency to decrease as the opening of the air-flow inlet valve increased until reaching levels close to the micronised samples (Maestà = 105% in F270). A further decreasing trend was observed in fractions G, which did not exceed the percentage value of 46% for G230 (cv Maestà), except for G220 in all samples (maximum value = 67% for cv Iride).

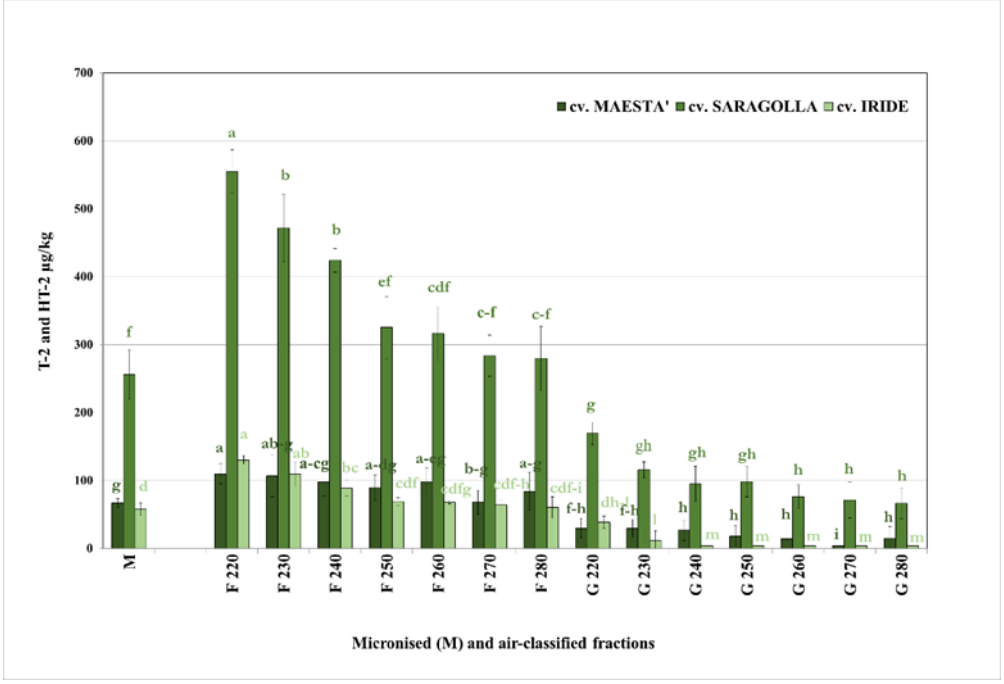

Figure 3. Average content of the sum of T-2 and HT-2 toxins in the micronised (M) and air-classified fractions (F and G) of the durum wheat samples belonging to group 1; different letters within each cultivar indicate significant differences ($p < 0.05$), n = 3.

The results concerning the DON contents only in the selected fractions (group II) showed a trend similar to that of group I (Figure 4). In fact, in the case of the more contaminated sample (cv Antalis,

region Marche) showing the highest DON content (1350 µg/kg) in the micronised sample, there was no significant difference ($p > 0.05$) between micronised and F250 samples, and significant differences ($p < 0.05$) between micronised and G fractions were confirmed. The pasta making process effect tended to limit the concentration of DON in the F fractions as compared to the micronised sample, and only the F250 fraction (1043 µg/kg) was significantly different ($p < 0.05$) from semolina in Antalis (Marche).

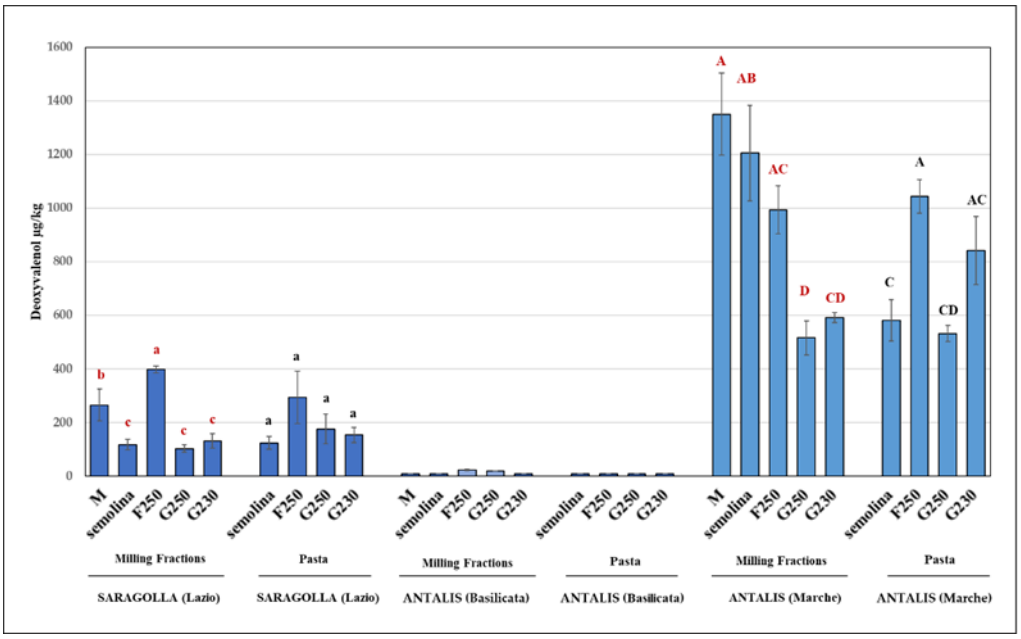

Figure 4. Group II: average content of DON in the micronised (M) and air-classified fractions (F and G) of the durum wheat samples tested as positive; same different letter within each group indicate significant difference ($p < 0.05$), different coloured letters are referred to different sample types, n = 3.

As regards the contamination by toxins T-2 and HT-2, which is defined as the sum of them, in the case of the most contaminated sample (cv Antalis, Basilicata) there was a trend for highest contamination rate in the bran-enriched F250 fraction (97 µg/kg) but not significantly different ($p > 0.05$) compared to the micronised sample (64 µg/kg) (Figure 5). Conversely, a marked decrease from 34 µg/kg in the G230 fraction to undetectable levels less than the limit of quantification (< LOQ) in the G250 fraction was observed. Moreover, in the case of micronised samples that did not show detectable levels of the sum of T-2 and HT-2 toxins (cv. Saragolla, region Lazio and cv Antalis, region Marche), the "latent" presence of toxins, just below the LOQ (25 µg/kg) led to its detection only in the F250 fraction (maximum value: 37 µg/kg). The absence of contamination was confirmedin all G250 fractions analysed, whereas a marked effect of the pasta making process on the most contaminated fractions (F250) was observed.

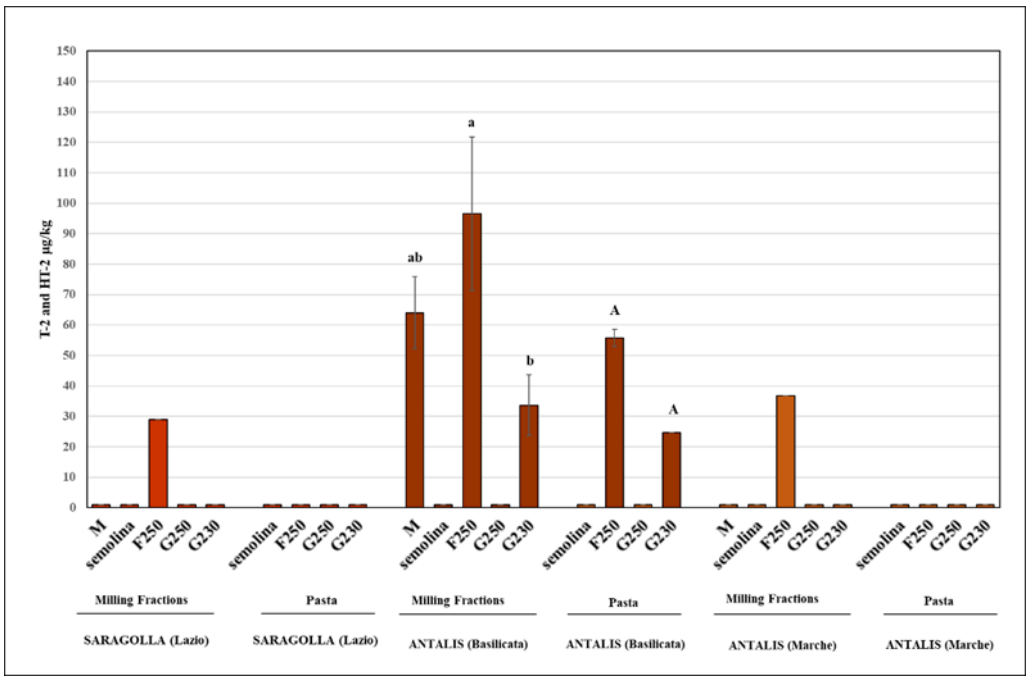

Figure 5. Group II: average values of the sum of the T-2 and HT-2 toxins in the micronised (M) and in the air-classified fractions (F and G) of the durum wheat samples tested as positive; different letter within each group indicate significant difference ($p < 0.05$), n = 3; no letter for single values (F250).

3.2 Determination of Toxic Elements

All air-classified G and F fractions (220-280) collected from the Group I samples were analysed to determine the distribution of As, Cd, Ni, and Pb (Tables 1 and 2).

The results clearly showed the influence of the process conditions on the As content of the milling fractions. The highest concentration of As was detected in fractions F. Among these, significantly lower values were highlighted in all samples between fractions F220 and F240 as compared to the corresponding micronised sample. The lowest content was detected in F220 (cv Maestà, 0.143 µg/g; cv Saragolla, 0.073 µg/g; cv Iride 0,110 µg/g), whereas a clear trend toward higher amounts was observed in the remaining F fractions, with the highest value detected in F280 (cv Iride 0.638 µg/g) and F270 (cv Saragolla, 0.590 µg/g; cv Maestà 0.511 µg/g). As a whole, the G fractions showed lower levels of As than both the F fractions and the micronised samples, with a clear tendency to decrease from G220 to G280. The highest values were detected in G220 (cv Iride and cv Saragolla, 0.420 and 0.455 µg/g, respectively) and G240 (cv Maestà, 0.325 µg/g). Conversely, F280 showed the lowest levels of As (cv Maestà, 0.025 µg/g; cv Saragolla, 0.034 µg/g; cv Iride, 0.050 µg/g).

Cd content was reduced significantly ($p < 0.05$) in all F fractions tested, with the exception of F270 (cv Maestà, 0.074 µg/g), F260 (cv Saragolla, 0.013 µg/g), and F280 (cv Iride, 0.027 µg/g), all of

which were higher than those of the micronised samples by 67, 9, and 37 times, respectively. Similar to As content behaviour, G fractions showed a greater reduction in contamination than F fractions. In fact, except for G220 (cv Saragolla) and G250 (cv Iride), the Cd content was lower than that of the micronised sample or even undetectable.

As in the case of As and Cd, a more marked reduction in the Ni content of the G fractions than that in the F fractions was shown. In particular, F220 and F230 (cv Maestà and cv Saragolla, respectively) showed a trend toward lower values of Ni content than the corresponding micronised samples, even significantly ($p < 0.05$) only for the cv Saragolla samples. This latter trend was not confirmed in the case of the cv Iride samples because all F fractions showed values higher than the micronised ones, with relevance for F260 (0.031 µg/g). In addition, this latter fraction type tended towards higher levels of Cd in all the cultivar samples tested. The G fractions had a lower rate of contamination than the corresponding micronised samples especially with regard to G280, which reached a maximum value of 0.005 µg/g (cv Saragolla). Regarding the cv Iride G fractions, only G250 (0.035 µg/g) and G260 (0.004 µg/g) had detectable Ni content, which was undetectable for the micronised and the remaining G samples.

Higher Pb content in micronised samples belonging to cv Maestà (0.157 µg/g) and cv Saragolla (0.152 µg/g) than cv Iride (0.022 µg/g) was detected. An appreciable trend to a reducing rate due to the technological process in milling fractions was confirmed in all cultivar samples, especially for G fractions from G250 to G280. Although not always significant, a general tendency toward values lower than those of the micronised samples was observed from F230 to F250 in all cultivar samples. However, in the case of cv Iride, only the F230 value (0.016 µg/g) was significantly lower than that of the micronised sample. Table 3 shows the results for As, Cd, Ni, and Pb for group II. The As contamination was significantly decreased in all fractions as compared to the corresponding micronising samples. More specifically, the F250 and G250 fractions maintained the lowest rate of contamination (maximum percentage residue: 52% in F250, cv. Saragolla), which is even lower than that of semolina.

Regarding Cd contamination, the presence of the element was unchanged compared to micronised samples. Undetectable Ni content was observed in all micronised and milled samples, and the same results were obtained for all fraction types tested.

Regarding Pb contamination, the fractions F250 and G250 had undetectable levels of this element (content<LOD) in micronised samples. Moreover, the fraction F250 and G250 showed a significantly ($p < 0.05$) lower rate of contamination than the micronised sample (0.138 µg/g) belonging to cv Antalis (Basilicata).

Through the pasta making process, a slight decrease in the content of As in semolina was achieved, which proved to be not significantly ($p > 0.05$) different from the air-classified fractions (Table 4).

Table 1. Group I: average values ± standard error (SE) of arsenic (As), cadmium (Cd), nickel (Ni) and lead (Pb) contents in the micronised samples and F type air-classified fractions; d.m.: dry matter; LOD: limit of detection, different letters indicate significant difference ($p < 0.05$) n=3.

		cv Maestà (region Apulia)			
		As (µg/g d.m.) ± SE	Cd (µg/g d.m.) ± SE	Ni (µg/g d.m.) ± SE	Pb (µg/g d.m.) ± SE
Micronised sample		0.478±0.037 ^{a}	0.002±0.0004 ^{a}	0.043±0.001 ^{b}	0.157±0.019 ^{a}
Air-classified fractions	F 220	0.143±0.004 ^{d}	0.001±0.0002 ^{a}	0.031±0.001 ^{b}	0.059±0.020 ^{bc}
	F 230	0.190±0.008 ^{cd}	< LOD	0.027±0.003 ^{b}	0.030±0.005 ^{c}
	F 240	0.274±0.012 ^{bc}	0.001±0.0002 ^{a}	0.034±0.002 ^{b}	0.039±0.004 ^{c}
	F 250	0.359±0.022 ^{b}	< LOD	< LOD	0.005±0.001 ^{c}
	F 260	0.522±0.016 ^{a}	< LOD	0.355±0.012 ^{a}	0.038±0.005 ^{bc}
	F 270	0.511±0.018 ^{a}	0.074±0.025 ^{a}	0.058±0.012 ^{b}	0.103±0.004 ^{ab}
	F 280	0.513±0.018 ^{a}	0.001±0.0003 ^{a}	0.041±0.013 ^{b}	0.025±0.006 ^{c}
		cv Saragolla (region Apulia)			
Micronised sample		0.518±0.069 ^{a}	0.008±0.0013 ^{abc}	0.143±0.004 ^{b}	0.152±0.007 ^{ab}
Air-classified fractions	F 220	0.073±0.012 ^{c}	0.001±0.002 ^{c}	0.014±0.001 ^{e}	0.074±0.002 ^{c}
	F 230	0.207±0.009 ^{bc}	0.005±0.0004 ^{bc}	0.039±0.004 ^{d}	0.077±0.004 ^{c}
	F 240	0.286±0.014 ^{b}	0.011±0.003 ^{a}	0.051±0.002 ^{cd}	0.081±0.015 ^{bc}
	F 250	0.486±0.023 ^{a}	0.005±0.001 ^{bc}	0.055±0.002 ^{c}	0.086±0.011 ^{bc}
	F 260	0.555±0.029 ^{a}	0.013±0.003 ^{ab}	0.057±0.001 ^{c}	0.163±0.031 ^{a}
	F 270	0.590±0.038 ^{a}	0.007±0.002 ^{abc}	0.126±0.001 ^{b}	0.118±0.011 ^{abc}
	F 280	0.297±0.061 ^{b}	< LOD	0.171±0.003 ^{a}	0.101±0.026 ^{abc}
		cv Iride (region Apulia)			
Micronised sample		0.582±0.013 ^{a}	< LOD	< LOD	0.022±0.005 ^{b}
Air-classified fractions	F 220	0.110±0.008 ^{f}	0.001±0.0002 ^{b}	0.014±0.001 ^{d}	0.074±0.002 ^{d}
	F 230	0.246±0.008 ^{e}	< LOD	0.007±0.002 ^{d}	0.016±0.001 ^{d}
	F 240	0.342±0.013 ^{d}	< L.R.	0.009±0.001 ^{bcd}	0.038±0.002 ^{cd}
	F 250	0.469±0.022 ^{c}	< LOD	0.009±0.001 ^{cd}	0.038±0.002 ^{bc}
	F 260	0.502±0.033 ^{bc}	0.005±0.0007 ^{b}	0.031±0.003 ^{a}	0.055±0.009 ^{ab}
	F 270	0.618±0.021 ^{a}	< LOD	0.019±0.003 ^{bc}	0.044±0.005 ^{ab}
	F 280	0.638±0.021 ^{a}	0.028±0.001 ^{a}	0.021±0.001 ^{ab}	0.063±0.006 ^{a}

Table 2. Group I: average values ± standard error (SE) of arsenic (As), cadmium (Cd), nickel (Ni) and lead (Pb) contents in the micronised samples and G type air-classified fractions; d.m.: dry matter; LOD: limit of detection, different letters indicate significant difference ($p < 0.05$) n=3.

		cv Maestà (region Apulia)			
		As (µg/g d.m.) ± SE	Cd (µg/g d.m.) ± SE	Ni (µg/g d.m.) ± SE	Pb (µg/g d.m.) ± SE
Micronised sample		0.478±0.037 ^{a}	0.002±0.000 ^{a}	0.043±0.001 ^{b}	0.157±0.019 ^{a}
	G 220	0.325±0.034 ^{b}	0.001±0.000 ^{ab}	0.102±0.024 ^{a}	0.109±0.034 ^{ab}
	G 230	0.313± 0.009 ^{b}	0.002±0.000 ^{ab}	0.019±0.001 ^{c}	0.049±0.0059 ^{bc}
	G 240	0.372±0.003 ^{b}	< LOD	0.019±0.002 ^{c}	0.026±0.003 ^{c}
Air-classified fractions	G 250	0.145±0.002 ^{c}	0.001±0.000 ^{b}	0.001±0.000 ^{d}	0.006±0.002 ^{c}
	G 260	0.055±0.000 ^{d}	< LOD	0.003±0.001 ^{d}	0.003±0.000 ^{c}
	G 270	0.037±0.001 ^{d}	0.001±0.000 ^{ab}	0.002±0.001 ^{d}	0.007±0.001 ^{c}
	G 280	0.025±0.001 ^{d}	0.0001±0.000 ^{b}	0.003±0.000 ^{d}	0.007±0.001 ^{c}
		cv Saragolla (region Apulia)			
Micronised sample		0.518±0.069 ^{a}	0.008±0.0013 ^{b}	0.143±0.004 ^{a}	0.152±0.007 ^{a}
	G 220	0.420±0.044 ^{ab}	0.021±0.0056 ^{a}	0.014±0.001 ^{bc}	0.213±0.044 ^{a}
	G 230	0.363±0.001 ^{bc}	< LOD	0.058±0.0017 ^{b}	0.061±0.001 ^{b}
	G 240	0.243±0.005 ^{cd}	0.001±0.000 ^{c}	0.008±0.001 ^{c}	0.036±0.005 ^{b}
Air-classified fractions	G 250	0.119±0.002 ^{de}	< LOD	0.007±0.001 ^{c}	0.015±0.002 ^{b}
	G 260	0.100±0.002 ^{de}	0.0007±0.0001 ^{c}	0.057±0.001 ^{bc}	0.020±0.002 ^{b}
	G 270	0.045±0.001 ^{e}	0.0005±0.0001 ^{c}	0.009±0.003 ^{c}	0.019±0.001 ^{b}
	G 280	0.034±0.001 ^{e}	0.0002±0.000 ^{c}	0.005±0.001 ^{c}	0.010±0.001 ^{b}
		cv Iride (region Apulia)			
Micronised sample		0.582±0.013 ^{a}	< LOD	< LOD	0.022±0.005 ^{b}
	G 220	0.455±0.038 ^{b}	< LOD	< LOD	0.044±0.003 ^{a}
	G 230	0.412± 0.002 ^{b}	< LOD	< LOD	0.023±0.0012 ^{b}
	G 240	0.322±0.002 ^{c}	< LOD	< LOD	0.022±0.002 ^{b}
air-classified fractions	G 250	0.160±0.001 ^{d}	0.008±0.000 ^{a}	0.035±0.010 ^{a}	0.015±0.001 ^{bc}
	G 260	0.088±0.002 ^{de}	< LOD	0.004±0.001 ^{b}	0.015±0.002 ^{bc}
	G 270	0.065±0.001 ^{e}	< LOD	< LOD	0.015±0.001 ^{bc}
	G 280	0.050±0.001 ^{e}	0.0003±0.000 ^{b}	< LOD	0.008±0.001 ^{c}

Table 3. Group II: average values ± standard error (SE) of arsenic (As), cadmium (Cd), nickel (Ni) and lead (Pb) contents in the micronised, semolina and selected air-classified fractions (F250, G250 and G230); d.m.: dry matter; LOD: limit of detection, different letters indicate significant difference ($p < 0.05$) n=3.

		As (µg/g d.m.) ± SE	Cd (µg/g d.m.) ± SE	Ni (µg/g d.m.) ± SE	Pb (µg/g d.m.) ± SE
cv Saragolla (region Lazio)	Micronised sample	0.100±0.002 [a]	0.01±0.002 [b]	< LOD	< LOD
	Semolina	0.053±0.002 [c]	0.002±0.000 [b]	< LOD	0.013±0.001 [a]
	F250	0.052±0.004 [c]	< LOD	< LOD	< LOD
	G250	0.025±0.001 [d]	0.01±0.000 [a]	< LOD	< LOD
	G230	0.063± 0.005 [b]	0.002±0.001 [b]	< LOD	0.008±0.003 [a]
cv Antalis (region Basilicata)	Micronised sample	0.111±0.05 [a]	0.01±0.001 [a]	< LOD	0.138±0.006 [a]
	Semolina	0.066±0.005 [b]	0.001±0.000 [a]	< LOD	0.144±0.026 [a]
	F250	0.039±0.001 [c]	0.001±0.000 [a]	< LOD	0.042±0.009 [b]
	G250	0.015±0.001 [d]	0.001±0.000 [a]	< LOD	0.016±0.002 [c]
	G230	0.062± 0.007 [b]	0.002±0.000 [a]	< LOD	0.038±0.004 [b]
cv Antalis (region Marche)	Micronised sample	0.164±0.016 [a]	< LOD	< LOD	< LOD
	Semolina	0.089±0.007 [b]	< LOD	< LOD	0.042±0.002
	F250	0.041±0.001 [d]	< LOD	< LOD	< LOD
	G250	0.034±0.002 [e]	< LOD	< LOD	< LOD
	G230	0.054±0.004 [c]	< LOD	< LOD	< LOD

Table 4. Pasta: average values ± standard error (SE) of arsenic (As) content in semolina and selected air-classified fractions (F250, G250 and G230); d.m.: dry matter, different letters indicate significant difference ($p < 0.05$) n=3.

	As (µg/g d.m.) ± SE		
	cv Saragolla (region Lazio)	cv Antalis (region Basilicata)	cv Antalis (region Marche)
Semolina	0.050±0.012 ^{ab}	0.063±0.009 ^{ab}	0.048±0.011 ^{a}
F250	0.026±0.004 ^{b}	0.084±0.019 ^{a}	0.037±0.004 ^{a}
G250	0.023±0.004 ^{b}	0.031±0.006 ^{ab}	0.044±0.010 ^{a}
G230	0.053± 0.003 ^{a}	0.029± 0.013 ^{b}	0.039±0.013 ^{a}

Discussion

This study aimed to address the issue of organic and inorganic contamination in durum wheat-derived food products, which has already been preliminarily examined [**29**]. In the present assay, updated micronising and air-classification plants were used as reliable tools to improve the quality characteristics of products. Moreover, the choice of the three fractions assayed in the second series samples (group II) has also taken into account the previous assessment of the main quality characteristics (i.e.: milling yield, particle size composition and ash content). These latter characteristics showed by all air-classified fractions (from 220 to 280 setting) were already assayed as process study [**30**].

As a whole the three air-classified fractions assayed herein were the best compromise between the presence of bran particles and the several contaminants in the products.

Moreover, the use of several cultivars showing different grain characteristics was deemed suitable only to introduce more variability within the durum wheat samples tested.

The results obtained showed a clear effect of the process on mycotoxin contamination of both milling and final products while retaining a non-negligible rate of bran particles in the air-classified mixes. Both types of mycotoxins were concentrated in the F250 fraction, with a clear trend toward a strong reduction in the G fractions. However, different behaviours of DON and T-2/HT-2 toxins were observed (Figures 4 and 5). In fact, only in the case of a slight contamination of DON (cv Saragolla, region Lazio), the semolina sample was significantly different ($p < 0.05$) from both micronised and F250 fractions, whereas in the case of high contamination (cv Antalis, region Marche), no significant difference ($p > 0.05$) was detected for these same fractions. These results confirmed the high capacity

of DON-producing fungi to penetrate the inner parts of the kernel, thus colonising the endosperm structure with negative effects on semolina, as previously shown [**36**]. Moreover, the pasta making process did not produce significant differences ($p > 0.05$) among the semolina and G fractions, probably due to the high content of semolina particles in the G fractions [**30**].

In the case of a higher rate of contamination of the sum of T-2 and HT-2 toxins (cv. Antalis, region Basilicata), a strong mycotoxin accumulation in F250 (151%) and a decreased amount in G230 (53%) were noted as compared to the micronised sample. These latter air-classified fractions (F250 and G230) maintained mycotoxin contamination, albeit to a lesser extent (88% and 39%, respectively) in the end product (pasta). Conversely, the presence of toxins was not detected in semolina. The latter was consistent with previous studies showing the greater difficulty of the toxin-producing fungi (i.e. *F. langsethiae*) in colonising the inner endosperm of the kernel and thus strongly limiting semolina contamination [**21-37**]. Interestingly, the toxin content (sum of T-2 and HT-2) tended to concentrate in the fraction F250 and the higher bran content was clearly highlighted in the case of a measurement around the limit of quantification of the ELISA test (25 µg/g). This latter limit must be defined as the "zone" with the greatest uncertainty of quantification. However, this was not observed in the corresponding pasta samples analysed.

As a whole, the results concerning both types of mycotoxins outlined the importance of the starting levels of grain contamination for determining the rate of contamination both in the air-classified fractions and final product (pasta). For this reason, the air-classification technology intended as a management tool for mycotoxin contamination in products must be used only in the case of grain samples with absent or slight contamination and properly below the mandatory limits [**16-26**]. In fact, only in this latter manner, the residual contamination rate in milling and final products can be contained below the established safe levels.

The data from the detection of inorganic contaminants showed an important effect of the technological process on the content of toxic elements in the different fractions, which is in agreement with previous assays [**14-15, 38**]. Particularly, these latter assays highlighted the effect of processing under controlled pilot plant conditions on the levels of As, Cd, Pb and Ni, and mainly the marked trend toward reduction of As content in the milling fractions. This latter aspect has been confirmed in our study both for pasta manufactured with semolina and with the use of each of the three selected fractions (F250, G250 and G230).

The residual content of elements assayed in the fractions decreased as compared to the micronised samples, except for several fractions where an opposite trend was observed. Moreover, considering the lower starting contamination rates in micronised samples concerning Cd, Ni, and Pb, as compared

to As, only the latter element was analysed in the end product (pasta) as the inorganic contaminant of major interest for the purposes of this work.

From the results obtained, we cannot select a fraction where the average content of all toxic elements analysed was in any case significantly lower than that of the micronised sample. However, as shown by the first series tested (Group I), a significant trend of the G fractions toward a reduction in inorganic element contents, as compared to the F fractions, was clearly observed. This could be due to the higher content of bran particles in F fractions than in G fractions, which conversely containedhigher amounts of semolina residues. Furthermore, the latter effect was evident as the progressive airflow opening increased. In addition, based on the contamination rates observed, the three air- classified fractions (F250, G250, and G230), previously selected based on qualitative evaluation, in any case were confirmed as suitable products for the pasta making process [**30,31**].

The results concerning the second series of samples (group II) proved to be of particular interest because it seemed to confirm the process effect behaviour shown in the first series (group I), mainly regarding As contamination. In fact, in all samples, the lowest As content was found in the G250 fraction, with decreasing rates ranging from 75% (cv Saragolla, region Lazio) to 86% (cv Antalis, region Basilicata). The presence of Cd and Pb was reduced and thus lower levels were present in the chosen fractions than in the semolina samples.

The assessment of As content in the end product was of great interest due to its role as an important source of consumers' direct exposure to food-borne contaminants [**11**]. The clear trend toward lower rates of As contamination showed by the three selected fractions (F250, G250, and G230) provided greater safety in manufacturing unrefined end products. In fact, the same three air-classified fractions showed not significant difference as compared to semolina even in the case of higher content of bran residue (F250). The lower content of As content in the pasta manufacturing was in agreement with previous assay as a process effect [**15**].

Conclusions

This work addressed the management of several organic (mycotoxins) and inorganic (mainly arsenic) contaminants in the manufacturing of unrefined durum wheat products. The importance of reducing the risk of consumers' exposure to toxic compounds derives from the role of pasta as an appreciated and widely consumed food worldwide. The results obtained confirmed the reliability of the upgraded technological process to provide air-classified milling fractions characterised by a strong containment of several contaminants, and thus the process is suitable for manufacturing high-quality, unrefined end products. Therefore, from a technological point of view, suitable management protocols and desirable results have been achieved for preparing the main product of durum wheat (pasta), with a focus on ensuring consumers' safety.

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References

1. FAO. World Food Situation. Available online: http://www.fao.org/worldfoodsituation/csdb/en/ (accessed on 13 August **2018**).
2. Barrett, E.M.; Foster, S.I.; Beck, E.J. Whole grain and high-fibre grain foods: How do knowledge, perceptions and attitudes affect food choice? *Appetite* **2020**, Vol. 149, 104630.
3. Reynolds, A.; Mann, J.; Cummings, J.; Winter, N.; Mete, E.; Te Morenga, L. Carbohydrate quality and human health: A series of systematic reviews and meta-analyses. *Lancet* **2019**, *393*, 434–445.
4. He, M.; Van Dam, R.M.; Rimm, E.; Hu, F.B.; Qi, L. Response to letter regarding article, whole-grain, cereal fiber, bran, and germ intake and the risks of all-cause and cardiovascular disease-specific mortality among women with type 2 diabetes mellitus. *Circulation* March 8, **2011,** Vol 123, Issue 9.
5. Ferlay, J.; Soerjomataram, I.; Dikshit, R.; Eser, S.; Mathers, C.; Rebelo, M.; Parkin D.M.; Forman D.; Bray, F. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* **2015**, *136*, E359–E386.
6. Torre, L.A.; Bray, F.; Siegel, R.L.; Ferlay, J.; Lortet-Tieulent, J.; Jemal, A. Global cancer statistics, 2012. *CA: A Cancer J Clin* **2015**, *65*, 87–108.
7. Barrett, E.M.; Batterham, M.J.; Ray, S.; Beck, E.J. Whole grain, bran and cereal fibre consumption and CVD: A systematic review. *Br J Nutr* **2019**, *121*, 914–937.
8. F Thielecke; Nugent A.P. Contaminants in grain—A major risk for whole grain safety? *Nutrients* **2018**, *10*, 1213.
9. Bowell, R.J.; Alpers, C.N.; Jamieson, H.E.; Nordstrom, D.K.; Majzlan, J. The environmental geochemistry of arsenic—An overview. *Rev Mineral Geochem* 2014, *79*, 1–16.
10. Zhu, Y.G.; Yoshinaga, M.; Zhao, F.-J.; Rosen, B.P. Earth abides arsenic biotransformations. *Annu Rev Earth Planet Sci* **2014**, *42*, 443–467.
11. Zhao, F.J.; Ma, Y.; Zhu, Y.G.; Tang, Z.; McGrath, S.P. Soil contamination in China: current status and mitigation strategies. *Environ Sci Technol* **2015**, *49*, 750–759.
12. Clemens, S.; Ma, J.F. Toxic heavy metal and metalloid accumulation in crop plants and foods. *Annu Rev Plant Biol* **2016**, *67*, 489–512.
13. Conti, M.E.; Cubadda, F.; and Carcea, M. Trace metals in soft and durum wheat from Italy. *Food Addit Contam* **2000**, *17(1),* 45–53.
14. Ficco, D.B.M.; Borrelli, G.M.; Miedico, O.; Giovanniello, V.; Tarallo, M.; Pompa, C.; De Vita, P.; Chiaravalle, A.E. Effects of grain debranning on bioactive compounds, antioxidant capacity and essential and toxinc trace elements in purple durum wheats. LWT-Food Sci Technol **2020**, *118*, 108734.

15. Cubadda, F.; Raggi, A.; Zanasi, F.; Carcea, M. From durum wheat to pasta: effect of technological processing on the levels of arsenic, cadmium, lead and nickel-a pilot study. *Food Addit Contam* **2003**, *20(4)*, 353–360.

16. European Commission. Commission regulation (EC) No 1881/2006 of 19 December **2006** setting maximum levels for certain contaminants in foodstuffs. *Official J Eur Union*, L *364*, 5–24.

17. Commission Regulation (EU) 2015/1006 of 25 June **2015** amending Regulation (EC) No 1881/2006 as regards maximum levels of inorganic arsenic in foodstuffs.

18. Bennett, J.W.; Klich, M. Mycotoxins. *Clin Microbiol Rev* **2003**, *16*, 497–516.

19. European Food Safety Authority. Scientific opinion on the risks for animal and public health related to the presence of T-2 and HT-2 toxin in food and feed. *EFSA J* **2011**, *9(12)*, 2481.

20. European Food Safety Authority. Scientific opinion on the risks to human and animal health related to the presence of deoxynivalenol and its acetylated and modified forms in food and feed. *EFSA J* **2017**, *15(9)*, 4718.

21. Pascale M.; Haidukowski M.; Lattanzio V.M.T.; Silvestri, M.; Ranieri, R.; Visconti A. Distribution of T-2 and HT-2 toxins in milling fractions of durum wheat. *J Food Prot* **2011**, *74*, 1700–1707.

22. Tibola C.S.; Guarient E.M.; Guerra Dias A.R.; Nicolau M.; Barboza Devos R.J.; Dalbosco Teixeira, D. Effect of debranning process on deoxynivalenol content in whole-wheat flours. *Cereal Chem* **2019**, *96*, 717–724.

23. Khaneghah, A.M.; Martins, L.M.; von Hertwig, A.M.; Bertoldo, R.; Sant'Ana, A.S. Deoxynivalenol and its masked forms: Characteristics, incidence, control and fate during wheat and wheat based products processing - A review. *Trends Food Sci Tech* **2018**, *71*, 13–24.

24. Freire, L.; Sant'Ana, A. S. Modified mycotoxins: An updated review on their formation, detection, occurrence, and toxic effects. *Food Chem Toxicol* **2018**, *111*, 189–205.

25. Aureli, G.; D'Egidio, M.G. Efficacy of debranning on lowering of deoxynivalenol (DON) level in manufacturing processes of durum wheat. *Tecnica Molitoria* **2007,** *58*, 729–733.

26. Commission Recommendation (**2013**/165/EU) on the presence of T-2 and HT-2 toxin in cereals and cereal products. *Official J European Union*, L91/12.

27. Brera, C.; Gregori, E.; De Santis, B.; Pantanali, A.; Monti, M. Ricerca e sviluppo di una farina innovativa destinata ai bambini di seconda e terza infanzia. In: ISTISAN Congressi, Riassunti: Istituto Superiore di Sanità, **2019** 19/C4. IV Congresso Nazionale. Micotossine e Tossine Vegetali nella filiera agro-alimentare. Roma, 10-12 giugno 2019 (p.61). ISSN 0393-5620.

28. Wang, J.; Xie, A.; Zhang, C. Feature of air classification product in wheat milling: Physicochemical, rheological properties of filter flour. *J Cereal Sci* **2013**, *57*, 537–542.

29. Cammerata, A.; Marabottini, R.; Allevato, E.; Aureli, G.; Stazi, S. R. Content of minerals and deoxynivalenol in the air-classified fractions of durum wheat. *Cereal Chem* **2021,** *00*, 1–11.

30. Cammerata, A.; Sestili, F.; Laddomada, B.; Aureli, G. Bran-enriched milled durum wheat fractions obtained using innovative micronization and air-classification pilot plants. *Foods* **2021**, *10*, 1796.

31. Cammerata, A.; Laddomada, B.; Milano, F.; Camerlengo, F.; Bonarrigo, M.; Masci, S.; Sestili, F. Qualitative characterization of unrefined durum wheat air-classified fractions. *Foods* **2021**, *10*, 2817.

32. European Commission (**2006**). Commission Regulation (EC) No. 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. In: Official Journal of the European Union, L 70/12.

33. Brera, C.; Debegnach, F.; De Santis, B.; Pannunzi, E.; Berdini, C.; Prantera, E.; Miraglia, M. Validazione di metodi immunoenzimatici per la determinazione delle micotossine in campioni di cereali. In: Polistampa (Ed.), I Georgofili Quaderni 2008, **2009**, IV (pp. 3–45). Edizioni Polistampa.

34. Pascale, M.; Aureli, G.; Haidukowski, M.; Melloni, S.; D'Egidio, M.G.; Visconti, A. Comparison between ELISA and UPLC methods for the determination of T-2 and HT-2 toxins in wheat. International Mycotoxin Conference, 19-23 May, **2014**, Beijing, P.R. China, Book of Abstracts, 258.

35. Hammer, O.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological Statistic software package for education and data analysis. *Paleontol Electron*, **2001**, *4(1)*, 1–9.

36. Ríos, G.; Zakhia-Rozis, N.; Chaurand, M.; Richard-Forget, F.; Samson, M.F.; Abecassis, J.; Lullien-Pellerin, V. Impact of durum wheat milling on deoxynivalenol distribution in the outcoming fractions. *Food Addit Contam* **2009**, *26*, Issue 4

37. Aureli, G.; Melloni, S.;. D'Egidio, M.G.; Quaranta, F.; Haidukowski, M.; Pascale, M.; Lattanzio, V.M.T. 2015. Effect of debranning on T-2 and HT-2 toxin content in durum wheat kernels and milling fractions. Proc. 10th AISTEC Conference "Grain for feeding the world" 1-3 **2015**, 156–161.

38. Cheli, F.; Campagnoli, A.; Ventura, V.; Brera, C.; Berdini, C.; Palmaccio, E.; Dell'Orto, V. Effects of industrial processing on the distributions of deoxynivalenol, cadmium and lead in durum wheat milling fractions. *Food Sci Technol* **2010,** *43*, 1050–1057.

Discussions

Dietary guidelines recommend the consumption of unrefined or minimally refined wheat-based foods for achieving better effects of their health-promoting components, i.e., minerals, vitamins, lignans, phytoestrogens, and phenolic compounds, over traditionally refined end-products. The improvement of technological solutions applied to the wheat milling process is becoming a key requirement to obtain less-refined milling products with healthier nutritional profiles. Among wheat species, the durum wheat (*Triticum turgidum* L. ssp. *durum*) is the most important cereal staple food in the Mediterranean area and is used as the primary raw material for the production of different types of wheat-based foods such as pasta, couscous, bulgur, and bread [**15**].

Overall, these products comprise a major portion of human diets and are an important source of bioactive compounds that may contribute to a healthy diet [**16**]. The most common bioactive compounds in wheat are dietary fibre, vitamins, micronutrients, and phytochemicals, which are mainly present in the outer layers of the kernel, typically in the bran, aleurone, and germ tissues [**17**]. Nevertheless, the components of the outer layers of the kernel end up as the milling waste products (i.e., bran and middlings). Durum wheat grain contains several bioactive compounds of health interest, such as insoluble fibre, phenolic acids, and alkylresorcinols, which are mostly concentrated within the coating structure of the kernel [**18**]. Phenolic acids are among the most abundant and studied components that promote human health. As dietary antioxidants, they act as free-radical scavengers [**19,20**] and reduce the inflammatory response in endothelial cells and monocytes [**21**]. In addition to performing antioxidant and antiradical activities, phenolic acids participate in plant cell wall formation as structural components and are involved in plant adaptation to abiotic and biotic stresses [**22,23**]. Among other wheat constituents that have recently become a major interest of research in wheat, there are Amylase/Trypsin-Inhibitors (ATIs) [**24**]. An important challenge in enhancing the health value of durum wheat foods is the use of raw materials rich in health-promoting compounds and poor in toxic components. Bran accounts for only 15% of the wheat grain, but it is an important source of components associated with health benefits, such as phenolic acids. To retain these healthy components of bran and reduce the negative impacts associated with some endosperm proteins, such as ATIs, diverse bran-rich streams can be used [**25,26**]. The preparation of milling fractions suitable for the production of functional food and enriched with beneficial components can be achieved by using appropriate technologies for the milling process or through using an optimum mixture of the selected fractions.

In general, wholemeal products are realised by mixing suitable amounts of bran, germ, and endosperm fractions so that they are as similar as possible to the natural grains [**27**]. However, the consumption of bran-enriched products could increase the risk of consumer exposure to food-borne organic and inorganic contaminants, particularly mycotoxins and toxic elements such as arsenic and heavy metals. All these compounds can persist from the raw material to the end-products, in addition to so-called processing compounds, such as acrylamide [**28**]. Among toxic minerals, arsenic (As), cadmium (Cd), and lead (Pb) are considered the most hazardous substances owing to their toxicity, prevalence, and potential for human exposure [**29**]. Among the metals, Cd is uniformly distributed within the endosperm, whereas lead (Pb) and nickel (Ni) are mainly present in the outer teguments [**14,30,31**]. The European legislation has established maximum tolerable levels of several metals and metalloids in foodstuffs. However, while the maximum levels of Pb, Cd, and tin (Sn) are currently established and monitored in all cereals, the maximum arsenic content has only been established for rice (0.200 µg/g) [**32,33**].

Despite their known beneficial effects, the consumption of whole wheat food products is still limited in many countries. Such a hindrance depends not only on consumer knowledge and behaviours but also on the negative impact of wheat bran on the sensorial quality of end-products [**34,35**]. Several studies have evaluated the effects of bran particle size, bran pre-treatment, and cooking method on the sensorial quality of the final products [**36**]. The interfering effect of bran on both protein hydration and dilution of gluten network in the dough is due to the occurrence of hydroxyl groups in bran structure, which react with water through hydrogen bonds, resulting in an increase in water absorption. In fact, arabinoxylans reduce the amount of water available for the gluten network, thus affecting dough stability and development time [**8**].

New technological advances in wheat milling processing have many advantages compared to the traditional milling process, which, by removing germ and bran, results in the loss of several beneficial compounds that are present in the bran fraction. Industrial pre-treatments, such as debranning, micronisation, and air classification, can reduce the negative effects of wheat bran processing [**13,37,38,39**]. Debranning is a dry separation technology based on consecutive abrasions of cereal kernels; progressive bran removal through detachment of the outer, intermediate, and inner layers of the pericarp results in the formation of different by-product classes, which can be separated by using pressurised air flowing through the screens and outlets of the debranner [**35**].

After the reduction of particle size through micronisation, the air classification treatment was applied to obtain more fractions. Micronisation comprises a milling process that can convert the starting matrix, such as cereal grains, to a fine particle product through the use of different technologies (e.g., hammer mill and knife mill). The particle size range of the milled product depends on the sieve diameter employed [**13**]. The general functioning criteria of the air classification system are based on the pneumatic transportation of the milled particles inside the plant, which operates in a depression, thus promoting a pneumatic flow along circular orbits. Inside the orbits, a series of ascending airflows is controlled by setting the airflow inlet valve to different opening conditions. Owing to the combined action of different forces, the heavier particles fall in the first "G" housing (heavier gross particles), whereas the lighter particles fail to be collected in the first step, but are gathered in the second "F" housing (fine particles). Bran fractions derived from both debranning and micronisation are used to enrich semolina and obtain pasta products with enhanced health properties and minimal impacts on sensory quality [**40**]. The above-mentioned technologies are also useful for better management of natural or chemical contaminants (e.g., mycotoxins and heavy metals) that are typically concentrated in the outermost layers of the wheat kernel [**41–43**].

The aim of this study was to further improve both the micronisation and air classification of plants by introducing ad hoc modifications into the process to obtain diverse less-refined milling fractions.More specifically, microniser pilot plant improvement was carried out through the addition of a grinding chamber, equipped with a hammer crusher impeller and a decanting collector, whereas the air-classifier pilot plant was equipped with both a programmable logic controller (PLC) for pneumaticflow management and a chamber specially designed with a decreasing section. These latest improvements in pilot plants, already implemented in our laboratories, allowed us to produceinnovative mixtures of milling products. Particular focus was placed on studying the process of developing diverse milling fractions, each characterised by a peculiar content and composition in branparticles that could be used to make durum wheat end-products with high bran content and nutritionalvalue and low presence of both mycotoxins, such as deoxynivalenol (DON) and T-2/ HT-2 toxins, and toxic elements (mainly arsenic) along the production chain.

The measure of yield percentages allowed us to evaluate the air-classified fractions from the perspective of milling quality, which is an important aspect for the selection of more suitable fractions for large-scale production.

Ash content is an important quality parameter that is influenced by both genetic and environmental factors and is associated with bran content [**44**]. In this study, the assay of the ash percentage proved useful to better characterise each air-classified fraction in cases where both bran and semolina particles were present in different ratios. The evaluation of particle size distribution within each fraction type (F and G) revealed the key role played by the involved physical factors (i.e., weight, air pressure inside the circuits, turbulence, etc.). The resultant force achieved using the air-classification process, intended as a whole system, determines the distribution of particles in the F or G collectors. Here, all air-classified millings were less refined compared to the traditional semolina and were characterised by a specific percentage of bran residues. Although the results need to be confirmed in a higher number of cultivars grown in different years and environments, the preliminary data indicated that the F220, F250, and G240 fractions showed significant differences ($p < 0.05$) among the cultivars. Nevertheless, the use of innovative plants allowed the production of several different types of air-classified fractions, allowing us to select the most suitable fraction for making less refinedand more attractive end-products with innovative properties because of the different combinations ofheavier, intermediate, and finer bran particles. Previous studies have highlighted that the addition ofdurum wheat bran with a particle size of 150–500 µm, at levels of 10%, 20%, and 30%, has a negativeimpact on pasta sensory and technological properties [**45**]. However, through the addition of 30% bran particles of the same size range, a texture comparable to that of commercial whole milled wheatpasta was achieved. Conversely, the addition of 10% bran particles resulted in sensory texture scoressimilar to those of regular durum wheat pasta not supplemented with bran. The use of milling fractionsenriched with fine bran particles is expected to be more suitable for enhancing the release and bioavailability of bioactive compounds, owing to both the major exposure of bran particle surfaces and aleurone cells to disruption, resulting in a better release of the intracellular contents [**46**]. Overall,the different air-classified fractions described here offer a suitable tool to produce innovative food products with new technological properties and improved nutritional value that could make these products more attractive to consumers. The inclusion of bran particles in wheat millings results in technological disadvantages and worsens the quality of end-products compared to that of refined semolina or flour-based products, causing a reduction in bread loaf volume, textural changes, and colour changes [**47**]. These effects are more severe, especially with 20% incorporated bran, and reduce the quality of pasta; however, a reduced impact occurs at the same percentage of incorporationusing finer bran.

In general, bran supplementation also has a positive effect owing to the increased contents of polyphenols and phytosterols in end-products. These parameters are not influenced by bran particle size above 10% incorporation, except for phenolic acids, whose content increases at a higher concentration of finer bran particles [**44**]. Therefore, the range size between 180 and 425 µm, which is included in the fractions assayed here, plays a key role because of the presence of a part of the fine bran fractions suitable for better nutritional quality of pasta due to the presence of health-promoting compounds (from the outer kernel layers) compared to traditional pasta produced with refined semolina. The influence of bran particle size on the water absorption of cooked pasta has been extensively studied. Finer bran particles result in a low and significant degree of water absorption compared to particles with a larger diameter, regardless of the derivation of the particles (e.g., bran and middlings) [**48**]. The positive effects of medium coarse and high coarse particle sizes of semolina on the quality of end-products (pasta) have been described previously [**49**]. The particle-size composition of less-refined fractions is a crucial aspect to be considered, especially in relation to technological aspects and the intended use of the end-products.

With regard to the detailed results concerning the single cultivars, it should be noted that the choice to use them was aimed only at increasing the variability of samples tested. Indeed, our study aimed to assess the application of new technological solutions to current micronisation and air-classification plants to improve the quality of the overall milling performance.

Three air-classified fractions of major interest, namely F250, G230, and G250, showed a good compromise between technological properties and bran enrichment [**26**].

These three fractions proved to be the most interesting based on the following criteria:

- F250 (unrefined product with higher bran content): excellent yield (60%-70%), good particle-size composition, higher ash percentage than micronised samples, and containment of mycotoxin presence.

- G250 (unrefined product with more semolina particles and less fine middlings), insufficient yield (20%-30%), excellent particle-size composition, good ash content (higher than semolina), and high reduction in mycotoxin content.

- G230 (unrefined product containing more semolina and finer middlings): excellent yield (>60%), balanced particle-size composition, good ash content (higher than semolina), and high reduction in mycotoxin content.

The air-classified fractions F250, G230, and G250 were characterised in terms of main quality parameters, alveographic properties, starch composition, phenolic acid profile, antioxidant activity, and the amount of ATIs.

First, milling yield percentages were assessed, as they are among the major milling quality parameters [**50**]. The total recovery of milling products (F+G fractions) along the micronising and air classification processes reached the value of approximately 99%, thus ensuring a minimal loss of raw material. The present results were comparable to those observed in previous studies [**26**]; the slight differences detected may be due to diverse grain sizes and textures [**51**]. The operating conditions that we used resulted in G230 and F250 fractions, with yield values ≥60%, with some differences depending on the grain samples ($p< 0.05$) [**26**].

The distribution of coarse, intermediate, and fine particles within each G and F fraction was in agreement with that in the preliminary study. In fact, the variability observed among the overall samples depended on the grain samples and the set valve point, consistent with the results of a previous study [**26**]. The typical particle size distribution characterising each fraction type suggested that each of them could be more suitable for making specific unrefined end-products (pasta, bread, biscuits, etc.). The presence of bran particles at different rates within each air-classified fraction results in durum end-products of higher quality than those obtained by adding bran aliquots to semolina [**33**]. The occurrence of coarse semolina particles in milling products could be important for enhancing the gluten index and yellow colour of end-products. The results of ash content characterisation suggested the presence of a higher content of minerals in the F fractions than in the G fractions [**52**]. Overall, ash content values were higher in the air-classified fractions and micronised samples than in semolina. Overall, the results regarding yield, ash content, and particle-size composition were in agreement with those of previous studies [**26**]. The rheological behaviour of the unrefined samples revealed a significant reduction in the alveographic parameters, especially with regard to the increase in the P/L ratio. This latter observation confirms the results of previous assays regarding the effects of the addition of bran aliquots to semolina [**53**]. The total starch content was lower in micronised flour and in all the air-classified fractions than in semolina. The G fractions had higher amounts of total starch than two of the three F250 samples. These results were expected because the F fractions contained higher amounts of bran and reduced contents of endosperm. Amylose content was significantly reduced in all the air-classified fractions and micronised samples compared to that in semolina because of the reduced amount of starch in these fractions. No significant differences were observed in the amylose/total starch ratio. The reduction in starch content, observed in the air-classified fraction compared to that in semolina, is potentially interestingfor the realisation of low glycaemic foods and useful for the prevention of some diet-related diseases(type II diabetes, obesity, and cardiovascular disorders) [**54-56**].

Based on phenolic acid analysis of raw materials and the derived uncooked and cooked pasta, six main individual phenolic acids, namely ferulic acid, vanillic acid, *p*-coumaric acid, sinapic acid, syringic acid, and *p*-hydroxybenzoic acid, were identified. These findings are in line with those of previous studies [**57,58**]. Numerous studies have established that phenolic acids have important biological effects, regardless of whether they are easily absorbed by the small intestine as free forms or reach the colon intact in bound forms [**59**]. Based on this evidence, we estimated the overall content of free and bound forms for each phenolic acid [**57,58**]. The results showed that semolina had the lowest contents of all individual phenolic components, whereas micronised samples and the F250 fraction had the highest contents. This was expected because of the diverse endosperm concentrations in semolina and the milled samples, in which bran occurred at a different rate [**53**]. Considering the total sum of individual phenolic acids, the F250 fraction had the highest increase over semolina (650%), suggesting that it would be the best choice for enriching the content of phenolic acids in pasta. In fact, the F250-derived pasta had the highest content of total phenolic acids (TPA), with an increase of up to 616% compared to that in the pasta made with semolina. A significant reduction in TPA content (between 11% and 19%) was observed in cooked pasta compared to that in raw materials. These findings are consistent with those of other studies [**25,58**].

The highest amounts of ATIs were observed in semolina, which was expected because these components are mostly present in the starchy endosperm [**24**]. Together, our data indicate that micronisation and air-classification treatments result in reduced amounts of ATIs, providing a potential added value to the derived pasta products in terms of food safety.

Furthermore, this study aimed to address the issue of organic and inorganic contamination in durum wheat-derived food products, which have already been preliminarily examined [**13**]. In the present study, updated micronising and air classification plants were used as reliable tools to improve the quality characteristics of the products. Moreover, the choice of the three fractions assayed in the second series of samples (group II) was also taken into account for assessment of the primary quality characteristics (i.e., milling yield, particle size composition, and ash content). These characteristics shown by all air-classified fractions (from the 220 to 280 settings) were previously assayed in a process study [**26**].

Overall, the three air-classified fractions assayed in this study were the best compromise between the presence of bran particles and several contaminants in the end-products.

The results obtained show a clear effect of the process on mycotoxin contamination of both milling products and end-products while retaining a non-negligible amount of bran particles in the air-classified mixes. Both types of mycotoxins were concentrated in the F250 fraction, with a clear trend toward a strong reduction in the G fractions. However, different behaviours of DON and T-2/HT-2 toxins were observed. In fact, the semolina sample showed a slight DON contamination (cultivar 'Saragolla', region Lazio), whereas in the case of high contamination (cultivar 'Antalis', Marche region), no significant difference ($p > 0.05$) was detected for these fractions. These results confirm the high capacity of DON-producing fungi to penetrate the inner parts of the kernel and colonise the endosperm structure, with negative effects on semolina, in the case of heavy infection rates [**60**]. Moreover, the pasta-making process did not show significant differences ($p > 0.05$) between the semolina and G fractions, probably because of the high content of semolina particles in the G fractions [**26**].

In the case of a higher rate of contamination, the sum of T-2 and HT-2 toxins ('Antalis', Basilicata region), a strong mycotoxin accumulation in F250 (151%) and a decreased amount in G230 (53%) were compared to those of the micronised sample. Mycotoxin contamination persisted in the air-classified fractions F250 and G230, albeit to a lesser extent (88% and 39%, respectively) in the end-product (pasta). Conversely, the presence of toxins was not detected in semolina. This result is consistent with the results of previous studies showing the greater difficulty of managing toxin-producing fungi (i.e., *F. langsethiae*) colonising the inner endosperm of the kernel, thus strongly limiting semolina contamination [**61–64**]. Interestingly, the toxin content (sum of T-2 and HT-2) tended to concentrate in the F250 fraction, and a higher bran content was clearly demonstrated by the measurement that was close to the limit of quantification of the ELISA test (25 µg/g). This limit must be defined as the 'zone' with the greatest uncertainty of quantification. However, this was not observed in the corresponding pasta samples. Overall, the results for both types of mycotoxins underline the importance of the initial levels of grain contamination, for determining the rate of contamination in both the air-classified fractions and the final product (pasta). For this reason, the air-classification technology, when intended as a management tool for mycotoxin contamination in products, must be used only for grain samples without or a slight contamination below the mandatory limits [**32**]. In fact, this is the only way to maintain the residual contamination rate during milling and in the final products below the established safe levels.

The data from the detection of inorganic contaminants showed an important effect of the technological process on the contents of toxic elements in the different fractions, which is in agreement with the results of previous studies [**12,14,31**]. In particular, the latter assays highlighted the effect of processing under controlled pilot plant conditions on the levels of As, Cd, Pb, and Ni, mainly by showing a marked trend toward a reduction in As content in the milled fractions. This latter aspect was confirmed in our study for pasta manufactured with semolina and with the use of each of the three selected fractions (F250, G250, and G230).

The residual content of elements assayed in the fractions decreased as compared to that in the micronised samples, except for several fractions, in which the opposite trend was observed. Moreover, considering the low initial contamination of micronised samples with Cd, Ni, and Pb as compared to As, only the latter element was analysed in the end-product (pasta) as the inorganic contaminant of major interest for the purpose of this study.

From the results obtained, we could not select a fraction wherein the average content of all the toxic elements analysed was consistently lower than that in the micronised sample. However, as shown by the first series tested (group I), a reduction in inorganic element content was clearly observed in the G fractions compared to that in the F fractions. This could be due to the higher content of bran particles in F fractions than in G fractions, which conversely contained higher amounts of endospermresidues. Furthermore, the latter effect was evident as the progressive airflow opening increased. In addition, based on the contamination rates observed, the three air-classified fractions (F250, G250, and G230), previously selected based on qualitative evaluation, were confirmed as suitable material for the pasta-making process [**26,65**].

The results for the second series of samples (group II) were of particular interest because they seemed to confirm the process effect shown in the first series (group I), mainly regarding As contamination. In fact, in all samples, the lowest As content was found in the G250 fraction, with decreasing rates ranging from 75% (‘Saragolla’, Lazio region) to 86% (‘Antalis’ Basilicata region). The presence of Cd and Pb was reduced, and thus, lower levels were present in the selected fractions than those in the semolina samples.

The assessment of As content in the end-products is of great interest because of its role as an important source of direct exposure of consumers to food-borne contaminants [**66**]. The clear trend toward lower rates of As contamination shown by the three selected fractions (F250, G250, and G230) indicates enhanced safety of the manufactured unrefined end-products. In fact, the same three air-classified fractions showed no significant difference as compared to semolina, even in the fraction with a higher content of bran residue (F250). The lower As content as a process effect in pasta manufacturing is in agreement with the results of a previous study [**14**].

The results obtained in the present study confirmed the reliability of the upgraded technological process to provide air-classified milling fractions with health benefits associated with a higher content of bran (fibre, antioxidants, minerals, etc.) and a clear containment of several contaminants. For these reasons, the same process is suitable for manufacturing high-quality, unrefined end-products. From a technological point of view, suitable management protocols and desirable results have been achieved for producing the main durum wheat-based food (pasta), with a focus on ensuring consumer safety. Furthermore, the present study highlighted the advantages to use the aforesaid technologies in the first processing step of several durum wheat grains of different genotypes. However, the real effects of the genotype factor on the processing results will need to be further investigated.

Conclusions

We demonstrated the micronisation and air-classification pilot plants as suitable milling technologies to obtain both healthier end-products and ensure better exploitation of raw material. Indeed, owing to the use of these technologies, it was possible to select unrefined milling fractions as a good compromise between the maintenance of bioactive compounds and the limited content of organic and inorganic contaminants.

In addition, the updated processes of pilot plants used in this study improved production yields, thereby favouring better management of bran fractions. Therefore, the goal of obtaining unrefined products with high contents of bioactive compounds without neglecting consumer safety was achieved. In fact, several types of milling fractions showed a good containment of mycotoxins (DON and T2/H-T2), metals, and metalloids. With regard to these contaminants, the control of arsenic (As) content is of great importance, as highlighted in Chapter 4.

Moreover, as described in Chapter 3, other parameters, such as starch content, which in unrefined fractions was found to be lower than that in semolina, were also assessed. Similarly, the presence of damaged starch was lower than that in semolina. The assessment of the main technological properties showed lower values of the alveographic parameters than those in semolina without compromising on the quality of the main end-product (pasta) of durum wheat.

References

1- Chen T., Zhanga M., Bhandaric B. and Yangd Z. (2018). Micronization and nanosizing of particles for an enhanced quality of food: A review. Critical Reviews in Food Science and Nutrition, vol. 58, no. 6, 993–1001.

2- Meghwal M. and Goswami, T. K. (2014). Comparative study on ambient and cryogenic grinding of fenugreek and black pepper seeds using rotor, ball, hammer and Pin mill. Powder Technol. 267:245–255.

3- Liu H., Zeng, F. Wang, Q., Ou S., Tan L. and Gu F. (2013). The effect of cryogenic grinding and hammer milling on the flavour quality of ground pepper (Piper nigrum L.). Food Chem. 141(4):3402–3408.

4- Chen Y., Zhang B. C., Sun Y. H., Zhang J. G., Sun H. J. and Wei Z. J. (2015). Physicochemical properties and adsorption of cholesterol by okra (Abelmoschus esculentus) powder. Food Funct. 6(12):3728–3736.

5- Du B., Meenu M., and Xu B. (2019). Insights into Improvement of Physiochemical and Biological Properties of Dietary Fibers from Different Sources via Micron Technology. Food Review International. https://doi.org/10.1080/87559129.2019.1649690.

6- Rosa N. N., Dufour C., Micard V., Gaiani C., Barron C. (2013). Ultra-fine Grinding Increases the Antioxidant Capacity of Wheat Bran. J. Cereal Sci. 2013, 57(1), 84–90. DOI: 10.1016/j. jcs.2012.10.002.

7- Niu M., Hou G. G., Wang L., Chen Z. (2014). Effects of Superfine Grinding on the Quality Characteristics of Whole-wheat Flour and Its Raw Noodle Product. J. Cereal Sci. 2014, 60 (2), 382–388. DOI: 10.1016/j.jcs.2014.05.007.

8- Coda, R.; Kärki, I.; Nordlund, E.; Heiniö, R.-L.; Poutanen, K.; Katina, K. Influence of particle size on bioprocess induced changes on technological functionality of wheat bran. Food Microbiol. 2014, 37, 69–77. [CrossRef]

9- Jacobs D., Anderson F.L., Blomhoff R. (2007). Wholegrain consumption is associated with a reduced risk of non cardiovascular, non cancer death attributed to inflammatory disease in the Iowa Women's Health Study. American Journal Clinical Nutrition, 85:1606-1614.

10- Kushi L.H., Meyer K.A., Jacobs D.R., (1999). Cereals, legumes, and chronic disease risk reduction: evidence from epidemiologic studies. The American Journal of Clinical Nutrition, 70(3):451S-458.

11- Zanoletti M., Parizad P. A., Lavelli V., Cecchini C., Menesatti P., Marti A., Paganini A. (2017). Debranning of purple wheat: recovery of anthocyanin-rich fractions and their use in pasta production. LWT - Food Science and Technology, Volume 75, January 2017, Pages 663-669.

12- Cheli, F., Campagnoli, A., Ventura, V., Brera, C., Berdini, C., Palmaccio, E., & Dell'Orto, V. (2010). Effects of industrial processing on the distributions of deoxynivalenol, cadmium and lead in durum wheat milling fractions. *Food Science and Technology*, *43*, 1050‑1057. https://doi.org/10.1016/j.lwt.2010.01.024.

13- Cammerata, A., Marabottini, R., Allevato, E., Aureli, G., & Stazi, S. R. Content of minerals and deoxynivalenol in the air-classified fractions of durum wheat. Cereal Chemistry. 2021; 00:1–11. https://doi.org/10.1002/cche.10458.

14- Cubadda, F., Raggi, A., Zanasi, F., & Carcea, M. (2003). From durum wheat to pasta: effect of technological processing on the levels of arsenic, cadmium, lead and nickel-a pilot study. *Food Additives and Contaminants*, *20*(4), 353‑360. https://doi.org/10.1080/0265203031 00012 1996.

15- Hammami, R.; Sissons, M. (2020). Durum wheat Products: Couscous. In Wheat Quality for Improving Processing and Human Health; Igregas, G., Ikeda, T., Guzman, C., Eds.; Cham, Switzerland, pp. 345–366.

16- Laddomada, B.; Durante, M.; Minervini, F.; Garbetta, A.; Cardinali, A.; D'Antuono, I.; Caretto, S.; Blanco, A.; Mita, G. (2015). Phytochemical characterization and anti-inflammatory activity of extracts from the whole-meal flour of Italian durum wheat cultivars. Int. J. Mol. Sci., 16, 3512–3527.

17- Shewry, P.R.; Hassall, K.L.; Grausgruber, H.; Andersson, A.A.M.; Lampi, A.M.; Piironen, V.; Rakszegi, M.; Ward, J.L.; Lovegrove, A. (2020). Do modern types of wheat have lower quality for human health? Nutr. Bull., 45, 362–373.

18- Hemery Y, Rouau X, Lullien-Pellerin V, Barron, JC, Abecassis J. (2007). Dry processes to develop wheat fractions and products with enhanced nutritional quality. J Cereal Sci, 46, 327–347.

19- Sevgi K, Tepe B, Sarikurkcu C. (2015). Antioxidant and DNA damage protection potentials of selected phenolic acids. Food Chem Toxicol, 77: 12–21.

20- Pandey KB, Rizvi SI. (2009). Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev., 2(5), 270–278.

21- Calabriso N, Massaro M, Scoditti E, Pasqualone A, Laddomada B, Carluccio MA (2020). Phenolic extracts from whole wheat biofortified bread dampen overwhelming 1 inflammatory response in human endothelial cells and monocytes: major role of VCAM-1 and CXCL-10. Eur J Nutr, 59(6), 2603–2615.

22- Laddomada B, Blanco A, Mita G, D'Amico LSingh RP, Ammar K, Crossa J, Guzmán C. (2021). Drought and heat stress impacts on phenolic acids accumulation in durum wheat cultivars. Foods, 10, 2142.

23- Nigro D, Grausgruberb H, Guzmàn C, Laddomada B. (2020). Phenolic compounds in wheat kernels: genetic and genomic studies of biosynthesis and regulation. In: Wheat quality for improving processing and health. Eds: Igrejas G, Ikeda T, Guzmàn C. Springer Sciences+Business Media Dordrecht, Netherlands, pp 223–251, ISBN 978-3-030-34162-6.

24- Geisslitz S, Shewry P, Brouns F, America AHP, Caio GPI et al. (2021). Wheat ATIs: Characteristics and Role in Human Disease. Front Nutr, 8, 667370.

25- Hemery Y, Chaurand M, Holopainen U, Lampi AM, Lehtinen P, et al. (2011). Potential of dry fractionation of wheat bran for the development of food ingredients, part I: Influence of ultra-fine grinding. Journal of Cereal Science, 53, 1-8.

26- Cammerata A, Sestili F, Laddomada B, Aureli G. (2021). Bran-Enriched Milled Durum Wheat Fractions Obtained Using Innovative Micronization and Air-Classification Pilot Plants. Foods, 10, 1796.

27- Barrett, E.M.; Batterham, M.J.; Ray, S.; Beck, E.J. (2019). Whole Grain, Bran and Cereal Fibre Consumption and CVD: A Systematic Review. Br. J. Nutr., 121, 914–937.

28- Thielecke, F.; Nugent, A.P. (2018). Contaminants in Grain—A Major Risk for Whole Grain Safety? Nutrients, 10, 1213.

29- Clemens, S.; Ma, J.F. (2016). Toxic Heavy Metal and Metalloid Accumulation in Crop Plants and Foods. Annu. Rev. Plant Biol., 67, 489–512.

30- Conti, M.E.; Cubadda, F.; and Carcea, M. (2000). Trace Metals in Soft and Durum Wheat from ITALY. Food Addit. Contam., 17, 45–53.

31- Ficco, D.B.M.; Borrelli, G.M.; Miedico, O.; Giovanniello, V.; Tarallo, M.; Pompa, C.; De Vita, P.; Chiaravalle, A.E. (2020). Effects of Grain Debranning on Bioactive Compounds, Antioxidant Capacity and Essential and Toxinc Trace Elements in Purple Du-rum Wheats. LWT-Food Sci. Technol., 118, 108734.

32- European Commission. Commission regulation (EC) No 1881/2006 of 19 December 2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs. Official J. Eur. Union 2006, L364, 5–24.

33- No.1881/2006; Commission Regulation (EU) 2015/1006 of 25 June 2015 Amending Regulation (EC) No 1881/2006 as Regards Maximum Levels of Inorganic Arsenic in Foodstuffs.

34- Foster, S.; Beck, E.; Hughes, J.; Grafenauer, S. (2020). Whole Grains and Consumer Understanding: Investigating Consumers' Identification, Knowledge and Attitudes to Whole Grains. Nutrients, 12, 2170.

35- Pasqualone, A.; Laddomada, B.; Centomani, I.; Paradiso, V.M.; Minervini, D.; Caponio, F.; Summo, C. (2017). Bread making aptitude of mixtures of re-milled semolina and selected durum wheat milling by-products. LWT Food Sci. Technol., 78, 151–159.

36- Onipe, O.O.; Jideani, A.I.O.; Beswa, D. (2015). Composition and functionality of wheat bran and its application in some cereal food products. Int. J. Food Sci. Technol., 50, 2509–2518.

37- Fares, C.; Platani, C.; Baiano, A.; Menga, V. (2010). Effects of processing and cooking on phenolic acid profile and antioxidant capacity of durum wheat pasta enriched with debranning fractions of wheat. Food Chem., 119, 1023–1029.

38- Taddei, F.; Galassi, E.; Nocente, F.; Gazza, L. (2021). Innovative milling processes to Improve the technological and nutritional quality of parboiled brown rice pasta from contrasting amylose content cultivars. Foods, 10, 1316, doi:10.3390/foods10061316.

39- Martín-García, B.; Verardo, V.; de Cerio, E.D.; del Carmen Razola-Díaz, M.; Messia, M.C.; Marconi, E.; Gómez-Caravaca, A.M. (2021). Air classification as a useful technology to obtain phenolics-enriched buckwheat flour fractions. Food Sci. Technol., 150, 111893, doi: 10.1016/j.lwt.2021.111893.

40- Ciccoritti, R.; Taddei, F.; Nicoletti, I.; Gazza, L.; Corradini, D.; D'Egidio, M.G.; Martini, D. (2017). Use of bran fractions and debranned kernels for the development of pasta with high nutritional and healthy potential. Food Chem., 225, 77–86.

41- Ficco, D.B.M.; Borrelli, G.M.; Miedico, O.; Giovanniello, V.; Tarallo, M.; Pompa, C.; De Vita, P.; Chiaravalle, A.E. (2020). Effects of grain debranning on bioactive compounds, antioxidant capacity and essential and toxic trace elements in purple durum wheats. LWT Food Sci. Technol., 118, 108734.

42- Sovrani, V.; Blandino, M.; Scarpino, V.; Redyneri, A.; Coïsson, J.D.; Travaglia, F.; Locatelli, M.; Bordiga, M.; Montella, R.; Arlorio, M. (2012). Bioactive compound content, antioxidant activity, deoxynivalenol and heavy metal contamination of pearled wheat fractions. Food Chem., 135, 39–46.

43- Laddomada, B.; Del Coco, L.; Durante, M.; Presicce, D.S.; Siciliano, P.A.; Fanizzi, F.P.; Logrieco, A.F. (2014). Volatile metabolite profiling of durum wheat kernels contaminated by Fusarium poae. Metabolites, 4, 932–945.

44- Alzuwaid, N.T.; Fellows, C.M.; Laddomada, B.; Sissons, M. (2020). Impact of wheat bran particle size on the technological and phytochemical properties of durum wheat pasta. J. Cereal Sci., 95, 103033.

45- Aravind, N.; Sissons, M.; Egan, N.; Fellows, C. (2012). Effect of insoluble dietary fibre addition on technological, sensory, and structural properties of durum wheat spaghetti. Food Chem., 130, 299–309.

46- Hemery, Y.M.; Anson, N.M.; Havenaar, R.; Haenen, G.R.M.M.; Noort, M.W.J.; Rouau, X. (2010). Dry fractionation of wheat bran increases the bioaccessibility of phenolics acids. Food Res. Int., 43, 1429–1438.

47- Hemdane, S.; Jacobs, P.J.; Dornez, E.; Verspreet, J.; Delcour, J.A.; Courtin, C.M. (2016). Wheat (Triticum aestivum L.) Bran in Bread Making: A Critical Review. Compr. Rev. Food Sci. food Saf., 15, 28–42.

48- Sandberg, E. (2015). The Effect of Durum Wheat Bran Particle Size on the Quality of Bran Enriched Pasta; Swedish University of Agricultural Sciences, Department of Food Science: City, Country, Series no: 405. Online publication: http://stud.epsilon.slu.se. (accessed on Day, Month, Year).

49- Sacchetti, G.; Cocco, G.; Cocco, D.; Neri, L.; Mastrocola, D. (2011). Effect of semolina particle size on the cooking kinetics and quality of spaghetti. Procedia Food Sci., 1, 1740–1745.

50- Sabanci K, Aydin N, Sayaslan A, Sonmez ME, Aslan MF, Demir L, Sermet C. (2020). Wheat Flour Milling Yield Estimation Based on Wheat Kernel Physical Properties Using Artificial Neural Networks. Int J Intell. Syst. Appl Eng, 8, 78–83.

51- Marshall DR, Mares DJ, Moss HI, Ellison FW. (1986). Effects of grain shape and size on milling yields in wheat. II. Experimental studies. Aust. J Agric Res, 37, 331–342.

52- Padalino L, Mastromatteo M, Lecce L, Spinelli S, Conte A, Del Nobile MA. (2015). Effect of raw material on cooking quality and nutritional composition of durum wheat spaghetti. Int. J. of Food Sciences and Nutrition, 66 (3), 266-274.

53- Sacchetti, G., Cocco, G., Cocco, D., Neri, L., & Mastrocola, D. (2011). Effect of semolina particle size on the cooking kinetics and quality of spaghetti. Procedia Food Science, 1, 1740–1745.

54- Aune D, Keum NN, Giovannucci E, Fadnes LT, Boffetta P, Greenwood DC, Tonstad S, Vatten LJ, Riboli E, Norat T. (2016). Whole grain consumption and risk of cardiovascular disease, cancer, and all cause and cause specific mortality: Systematic review and dose-response meta-analysis of prospective studies. Br Med J, 353, i2716. https://doi.org/10.1136/bmj.i2716.

55- Călinoiu LF, Vodnar DC. (2018). Whole grains and phenolic acids: a review on bioactivity, functionality, health benefits and bioavailability. Nutrients, 10(11), 1615. https://doi.org/10.3390/ nu101 11615.

56- Giacco R, Costabile G, Della Pepa G, Anniballi G, Griffo E, Mangione A, Cipriano P, Viscovo D, Clemente G, Landberg R, Pacini G, Rivellese AA, Riccardi G. (2014). A whole-grain cereal-based diet lowers postprandial plasma insulin and triglyceride levels in individuals with metabolic syndrome. Nutr Metab Cardiovasc Dis, 24(8):837-44.

57- Martini D, Ciccoritti R, Nicoletti I, Nocente F, Corradini D, D'Egidio MG, Taddei F. (2018). From seed to cooked pasta: influence of traditional and non-conventional transformation processes on total antioxidant capacity and phenolic acid content. Int J Food Sci Nutr, 69, 4–32.

58- Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. (2004). Polyphenols: food sources and bioavailability. Am J Clin Nutrion, 79, 727–747.

59- Cardona F, Andres-Lacueva C, Tulipani S, Tinahones FJ, Queipo-Ortuno IM. (2013). Benefits of polyphenols on gut microbiota and implications in human health. J Nutr Biochem, 24, 1415–1422.

60- Ríos, G.; Zakhia-Rozis, N.; Chaurand, M.; Richard-Forget, F.; Samson, M.F.; Abecassis, J.; Lullien-Pellerin, V. (2009). Impact of Du-rum Wheat Milling on Deoxynivalenol Distribution in the Outcoming Fractions. Food Addit. Contam., 26.

61- Pascale, M.; Haidukowski, M.; Lattanzio, V.M.T.; Silvestri, M.; Ranieri, R.; Visconti, A. (2011). Distribution of T-2 and HT-2 Toxins in Milling Fractions of Durum Wheat. J. Food Prot., 74, 1700–1707.

62- Freire, L.; Sant'Ana, A.S. (2018). Modified Mycotoxins: An Updated Review on Their Formation, Detection, Occurrence, and Toxic Effects. Food Chem. Toxicol., 111, 189–205.

63- Pascale, M.; Aureli, G.; Haidukowski, M.; Melloni, S.; D'Egidio, M.G.; Visconti, A. (2014). Comparison between ELISA and UPLC Methods for the Determination of T-2 and HT-2 Toxins in Wheat. In Proceedings of the International Mycotoxin Conference, Beijing, China, 19–23 May 2014; Book of Abstracts, p. 258.

64- Aureli, G., Melloni, S., D'Egidio, M.G.; Quaranta, F.; Haidukowski, M.; Pascale, M.; Lattanzio, V.M.T. (2015). Effect of Debranning on T-2 and HT-2 Toxin Content in Durum Wheat Kernels and Milling Fractions. In Proceedings of the 10th AISTEC Conference "Grain for Feeding the World", 1–3 2015; pp. 156–161.

65- Cammerata, A.; Laddomada, B.; Milano, F.; Camerlengo, F.; Bonarrigo, M.; Masci, S.; Sestili, F. (2021). Qualitative Characterization of Unrefined Durum Wheat Air-Classified Fractions. Foods, 10, 2817.

66- Zhao, F.J.; Ma, Y.; Zhu, Y.G.; Tang, Z.; McGrath, S.P. (2015). Soil Contamination in China: Current Status and Mitigation Strategies. Environ. Sci. Technol., 49, 750–759.

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"*L'innovazione è frutto della tecnologia e la tecnologia è frutto della ricerca applicata, la quale a sua volta deriva dalla ricerca di base: la ricerca, cioè, che non si pone problemi di applicazioni pratiche, ma si occupa solo di scoprire le leggi che regolano l'universo, il nostro pianeta, il nostro corpo, la ricerca che soddisfa la curiosità e insegue la conoscenza per la conoscenza.*"

Cit.: Margherita Hack, Libera scienza in libero Stato, 2010