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Evaluation of biogenic amines, phenolic and antioxidant compounds in "Senatore Cappelli" durum wheat products

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Abstract---"*Senatore Cappelli*" durum wheat products have attracted much interest in recent years because of their nutritional and quality characteristics, as they are a source of different bioactive compounds (i.e., polyphenols, serotonin, etc.). This study examines the content of biogenic amines (BAs), ß-phenylethylamine, putrescine, cadaverine, histamine, tyramine, serotonin and spermine, total polyphenols content (TPC) and antioxidant capacity (AC) of different products of the "*Senatore Cappelli*" durum wheat chain (SCDW): seeds, chaff, flour, and pasta. BAs were detected and quantified by HPLC-RF. While UV-Vis spectrophotometer was applied for the determination of TPC and AC. All BAs investigated were found in the samples analyzed at different concentrations. Particularly, the presence of serotonin (21.71 ± 0.15 - 42.66 ±0 .03 mg/kg), an BA with positive effects on human health, was found in all SCDW products. In addition, a higher concentration in SCDW pasta of histamine (36.37 ± 0.01 mg/kg) and cadaverine (11.43 ± 0.36 mg/kg), which are the main BAs involved in

allergic processes, compared to the other SCDW products was found. TPC was significantly higher in bran samples (780.35 ± 1.4 mg GAE/kg); while pasta had a lower content (343.1 2± 3.35 mg GAE/kg) than flour and seeds. This information could be important for the wheat industry to obtain products with higher nutritional and functional characteristics. The chosen compounds were found to be a suitable marker for assessing the quality of SCDW products.

Keywords---antioxidant compounds, biogenic amines, durum wheat, food quality, food safety.

Introduction

Senatore Cappelli (*Triticum turgidum* ssp. *durum*) (SC) is an autumnal durum wheat cultivar obtained by geneticist Nazareno Strampelli, by selecting individual dwarf genes from Italian, North African, and Syrian – Palestinian landraces [1]. Selection procedures have been carried out to obtain intensive crop management – resistant genotypes, performing wide adaptability, and good agronomic performances also in border areas. In this regard, Senatore Cappelli is generally considered an *ancient grain*, because of its characteristic of never undergoing modern plant breeding programs of intensive farming. This could be of strategic importance in the selection of low environmental impact agricultural cultivars with high yield and better technological quality (i.e., gluten quality) [2, 3]. Moreover, breeding programs have always focused on improving yield and technological properties, without considering the nutritional and nutraceutical importance of wheat consumption in the human diet [2].

Cereals and cereal products are placed at the base of the food pyramid, accounting for more than 55% of total consumption in the Mediterranean Diet [4]. The ancient grain SC is generally used in mixture as raw material to produce cereal-based products, such as pasta, flours, etc., of which Italy represents the first world producer, with the highest pro capita consumption ratios [5]. For this reason, a quality assessment on its nutritional value is highly recommended. Durum wheat products (*T. turgidum* ssp. *durum*) are a rich source of bioactive compounds, presenting an excellent amount of dietary fiber, proteins, and antioxidants [2], which can exert beneficial effects on human health (i.e., chronic diseases prevention, cholesterol-lowering properties, anti-inflammatory, antioxidant properties, etc.) [6]. However, the production process to which cereal products are subjected is determined by the quality of the final product. In this regard, food markers (i.e., phenolic compounds, biogenic amines, etc.) are allowed to provide corrective action in the event of noncompliance with product safety or quality standards. Biogenic amines are ubiquitous bioactive compounds, which originate from microbial decarboxylation of amino acids. They are widely used as food safety markers as their presence in foods could be either associated with physiological and health-promoting functions (i.e., nucleic acid regulation, membrane stabilization, etc.) or negative inflammatory reactions, such as “Histamine poisoning” [7]. In grains, these markers can be formed from endogenous enzymes or microorganisms contained in the raw material or added during processing [8], allowing food production to be monitored at all stages of

processing and storage.

Moreover, grain processing leads to the production of by-products (such as chaff), which are often not used, but could be applied in alternative productions and other supply chains (Fig. 1). To address this gap, over the last decade the EU has adopted a shift towards a circular economic model by 2030 for the food and beverage industry and aims to avoid processing waste going to landfills as much as possible [9]. In Europe, wheat chaff could provide an annual biomass potential of about 54.8 Megatons (Mt). However, advances in harvesting technology play a key role in transforming unexploited by-products into valuable raw materials [10]. Some studies have examined the potentialities of the valorization of durum wheat by-product production [9–11]. Most of them only provided a microbiological assessment of mycotoxin contamination of commercial-scale cereal grain cleaning operations [11, 12]. Since the low availability of literature research focusing on the nutritional and quality features of SC durum wheat products, this study aimed to provide a quality and safety assessment of the Senatore Cappelli durum wheat chain (SCDW) (seeds, flour, pasta, and chaff), through the evaluation of biogenic amines, phenolic and antioxidant compounds.

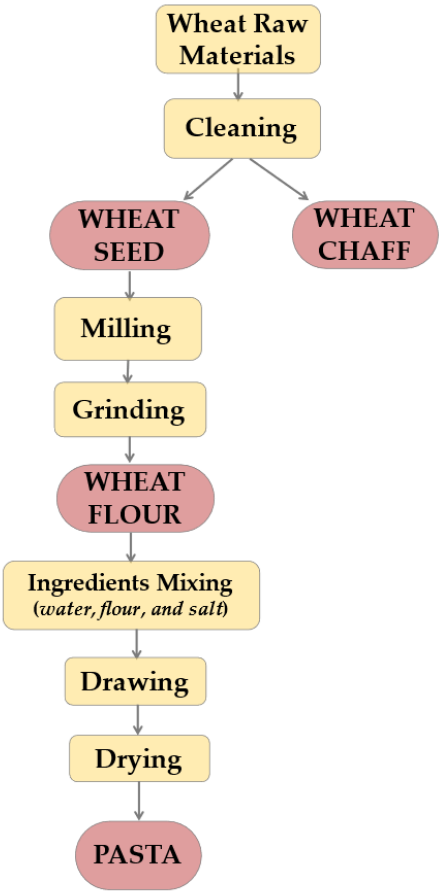

Figure 1. The production process of durum wheat products

Eight Bas was evaluated in SCDW samples by high-performance liquid chromatography with fluorescence detection (HPLC-FD) with pre-column derivatization. The BAs studied were putrescine (PUT), cadaverine (CAD), spermine (SPM), and spermidine (SPD), for polyamines, whereas β-phenylethylamine (β-PEA), SER, TYR, and HIS were studied for monoamines. Thereafter, the evaluation of antioxidant compounds by means of Folin-Ciocâlteu, ABTS and DPPH assays was carried out by UV-Vis spectrophotometric analysis.

Materials and Methods

Chemicals

β-PEA, PUT, CAD, HIS, TYR, SER, SPM, SPD were supplied by Supelco (Bellefonte, PA, USA) as well as the derivatizing agent, dansyl chloride. Ethanol (C_2H_5OH), methanol CH_3OH), water (HPLC grade), acetonitrile (HPLC grade), Folin–Ciocâlteu reagent (H3[P(W3O10)4]/H3[P(Mo3O10)4]), ABTS (2,2-azino-bis [3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt), DPPH (2,2-diphenyl-1-picrylhydrazyl), potassium persulfate, sodium bicarbonate (NaHCO3), gallic acid (C7H6O5), perchloric acid (HClO4), sodium hydroxide (NaOH), sodium carbonate (Na2CO3), ammonium hydroxide (NH4OH), were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Instruments

Bandelin Sonorex RK100H water and ultrasonic thermostatic bath, IKA T18 digital Ultra–Turrax (IKA-group, Saufen, Germany), and Whatman 0.45 μm 100 (PTFE) syringe filters (Sigma Aldrich, Milan, Italy), UV-Vis spectrophotometer (Jenway, Stone, UK), NEYA 10R refrigerate centrifuge (Exacta Optech, Modena, Italy). Chromatographic analysis was performed using an ATVP LC-10 HPV binary pump with an RF-10° XL fluorimetric (FD) detector (Shimadzu, Kyoto, Japan) operating to $\lambda_{emission}$=320 nm, and $\lambda_{excitation}$= 523 nm. A Supelcosil LC-18 column (250 mm × 4.6 mm, 5 μm) with a Supelguard LC-18 (Supelco, Bellefonte, PA, USA) pre-column were used for the determination of BAs.

Sampling

Different Senatore Cappelli durum wheat products were analyzed: seeds, flour, pasta and chaff. The samples were purchased from a cereal farm located in the Campania region, in Italy. After acquisition, all samples were homogenized by grinding and sifting with a 0.7 ÷ 2 mm diameters holes – sieve. The obtained particle size fractions were collected and stored at refrigerated temperature, T= -18 °C until the day of analysis.

Determination of Biogenic Amines

BAs detection was performed according to a previously optimized method with some modifications [13]. Approximately 1 g of durum wheat sample was extracted with 12 mL of 0.6 M $HClO_4$, homogenized with an Ultra-Turrax T-18 tissue homogenizer at 3000x g for 3 min, and centrifuged at 2700×g for 10 min, at T= 25 °C. The supernatant was collected in a flask. The residue was added with 12 mL

of 0.6 M $HClO_4$, mixed, and centrifuged again for 10 min. Then, the second extract was added to the first one and filtered through a syringe filter with a 0.45 µm membrane. The final volume was adjusted to 25 mL with 0.6 M $HClO_4$. An aliquot part of 1 mL of the final extract was then derivatized by adding 200 µL of 2 M NaOH, 300 µL of saturated $NaHCO_3$ solution, and 2 mL of dansyl chloride solution (10 mg/mL in acetone). After stirring, the samples were left in the dark for 60 min at 45 °C. To stop the dansyl-chloride reaction about 100 µL of 25% NH_4OH was added. The final volume was adjusted to 5 mL by adding acetonitrile. The dansylated extract was filtered using 0.45-µm filter (Whatman® Puradisc filters, Sigma Aldrich, Milan, Italy), injected into the HPLC system, and analyzed with a previous standardized method [13].

For the chromatographic detection of BAs content in SCDW samples, a volume aliquot of 20 µL (loop 20 µL) was injected. Analyses were performed by using a Supelcosil LC-18 column (250 mm × 4.6 mm, 5 µm), Supelco, Bellefonte, PA, USA) coupled with an FD detector ($\lambda_{emission}$=320 nm, and $\lambda_{excitation}$=523 nm). The analyses were carried out maintaining a fixed temperature of 30 °C. The solvents used for the chromatographic separation were: (A) purified water and (B) acetonitrile. The elution program started with 3 min of isocratic elution (50% A; 50% B) reaching 100% B after 18 min to finish with a further 3 min of isocratic elution. Finally, it took 5 min to restore the initial isocratic conditions (50% A 50% B). The flow was kept constant at 1.0 mL/min, for a total analysis time of 30 min. The results were obtained through a calibration curve for each BA, ranging from 0.1 and 25 mg/l.

Total Phenolic Content (TPC) and Antioxidant Activity (ABTS and DPPH) evaluation

Sample extraction for TPC and antioxidant activity were carried out according to a previously published method with some modifications [14]. Briefly, 1g of each homogenized durum wheat sample (seeds, flour, pasta, and chaff), was extracted with 4 ml of refrigerated–ethanol (T= 4 °C) in an aqueous solution (EtOH:H_2O 80:20, *v:v*), homogenized in an ultrasonic bath for 5 min at room temperature, and centrifuged at 2800×*g* for 10 min, at T= 4 °C. The supernatant was collected in an amber vial. The residue was added with 4 ml of refrigerated EtOH:H_2O (80:20, *v:v*), homogenized and centrifuged again for 10 min. The second extract was collected with the first one, and adjusted to a final volume of 10 ml with EtOH:H_2O (80:20, *v:v*). The final extracts were then filtered by 0.22 µm membrane syringe filter, and stored at T= -20 °C, until the day of analysis.

The Folin-Ciocâlteu method was used for the determination of TPC [15], some modifications were made for SCDW samples as follows: 1 ml of hydroalcoholic extract was added to 0.25 mL of Folin–Ciocâlteu reagent and 0.5 mL of Na_2CO_3 water solution (7.5%, *w/v*) in a 10-mL volumetric flask. Purified water was added to arrive at the final volume. Spectrophotometric analysis was performed at λ= 750 nm after 30 min of incubation in dark at room temperature. TPC was expressed as milligrams of gallic acid equivalent (GAE) per kg. The results were obtained through a calibration curve ranging from 10 to 100 mg/l (R^2 = 0.9997). Antioxidant activities were determined by means of DPPH and ABTS assays, according to the methods of Preti et al. [15]. Free radical scavenging activity of

SCDW hydroalcoholic extracts was evaluated by measuring the decrease in absorbance at 515 nm (DPPH), and 734 nm (ABTS). The absorbance was measured in 1-cm path length cuvettes against ethanol in aqueous solution (80:20, *v:v*), through a UV-Vis spectrophotometer. Results were expressed as inhibition percentage (I %) and were calculated based on Equation 1:

$$I\% = \frac{Ao - Af}{Ao} \; x \; 100 \qquad (1)$$

where A_0 is the radical cation's initial absorbance, and A_f is the absorbance after the addition of hydroalcoholic SCDW sample extracts.

Results and Discussion

Biogenic Amines Content in Senatore Cappelli Durum Wheat products

Numerous studies showed that BAs concentration in cereal-based products (i.e., flour, pasta, etc.) are largely dependent on varietal features, such as cultivar, pedoclimatic conditions, cultivation, and post-harvest treatment, including wheat milling and processing [6, 8, 16]. In this study, Bas content were determined in SCDW products by means of HPLC-FD. The quantification of BAs in SCDW samples was summarized in Table 1. Among wheat products, the highest total BAs contents were detected in flour (172.72 mg/kg), and pasta (143.08 mg/kg). Among SCDW products, HIS has always been detected with the highest amounts in pasta (36.37 ± 0.01 mg/kg) and flour (17.45 ± 0.02 mg/kg), nevertheless within the limit established by European Regulation 2073/2005 [17]. These processed durum wheat-based products also presented the highest concentrations of SPD (70.71 ± 0.01mg/kg in SCDW flour, and 33.32 ± 0.02 mg/kg in SCDW pasta), and SPM (21.30 ± 0.01 mg/kg in flour, and 10.01 ± 0.01 mg/kg in pasta), respectively. SPD and SPM are natural polyamines, which synthesis is endogenous in all animals and plants, including cereals [18]. Different authors reported that the occurrence of these polyamines is significantly affected by the transformation processes of cereal, such as milling [8, 16]. This could be a positive feature since a high polyamines (SPD and SPM) intake could be linked with a decreased risk of allergic reaction [19].

The lowest amount of TYR, PUT, and CAD was detected in SCDW seeds (0.01 ± 0.01 mg/kg, 0.73 ± 0.01 mg/kg, and 2.25 ± 0.01 mg/kg, respectively), thus enhancing good preservation status [7]. After all, an interesting remark should be done about SER content, which represented the 36% of total BAs detected in all SCDW samples, with the highest amount in pasta (42.66 ± 0.03 mg/kg), and flour (42.17 ± 0.15 mg/kg), followed by chaff (31.84 ± 0.12 mg/kg). Durum wheat seeds presented the lowest content (21.71 ± 0.03 mg/kg). These results agreed with previously published results [8], founding a similar trend in SER content only in durum wheat pasta (40 – 90 mg/kg) and semolina (0 – 130 mg/kg). However, the excellent SER amounts in Senatore Cappelli durum wheat products, may induce some health-increasing effects, such as stress and mood modulation, muscle contraction and blood pressure regulation [8, 20].

Total Phenolic Content and Antioxidant evaluation in Senatore Cappelli Durum Wheat products

Phenolic compounds are considered as important givers to antioxidant activity in durum wheat, due to the presence of hydroxyl groups that react and stabilize free radicals [16]. In this regard, a remarkable highlight has been given to the health-promoting effects of introducing these bioactive components into the daily diet, leading to renewed interest in selecting varieties, including ancient cultivars, for their nutritional potential. Table 2 summarized antioxidant evaluation in SCDW products, by means of Folin-Ciocâlteu reaction, ABTS and DPPH assays. TPC was evaluated by means of Folin-Ciocâlteu assay. This assay establishes the total amount of decreasing substances by measuring the change in color due to the reduction of metal oxides operated by phenolic antioxidants, and other reducing substances, such as nitrogen compounds and proteins [21]. This approach would also provide indication of antioxidant capacity for free or bound substances present in the extracts. Among all samples, durum wheat chaff showed the highest amounts of TPC with 780.35 ± 2.7 mg GAE/kg, followed by SCDW flour and seeds (418.17 ± 1.6 mg GAE/kg, and 415.35 ± 3.2 mg GAE/kg, respectively); while SCDW pasta presented the lowest TPC values (343.61 ± 1.4), showing a 20% reduction in total phenolic content, compared to flour and seeds. De Pula et al., [22]. also showed similar TPC trends in barley flour and pasta, with 808.01 ± 26.45 mg/kg, and 735.9 ± 21,59 mg/kg, thus highlighting a reduction of 9% in TPC content in pasta compared to flour. Compared to cereals, the lower polyphenol content in pasta may be attributable to the pasta production process, which promotes the bond interference between the phenolic compounds and the food matrix components, thus facilitating the extraction of phenolic.

The antioxidant activity was also evaluated by means of free radical scavenging, ABTS and DPPH assays [23]. The trend of TPC resulted in agreement with ABTS radical scavenging activity, proving the highest radical inhibition % (I%) in Senatore Cappelli Durum Wheat chaff (96.57, I%), and in SCDW seeds (66.89, I%). The lowest result was achieved by pasta (12.14, I%). A similar trend was reported by Fares et al., [24], who comparing semolina and durum wheat pasta observed a decrease in phenolic and antioxidant contents. This could be attributable to the oxidative degradation of antioxidants induced by heat treatment, and drying conditions during pasta processing [22]. Nevertheless, the values obtained with DPPH assay were significantly lower than those of ABTS assay, even if the trends of I% among SCDW products were similar: chaff (75.76, I%) > seeds (52.18, I%) > flour (13.46, I%) > pasta (8.17, I%). This could be related to the different scavenging activity of these two *in vitro* antis–radical assays. In fact, ABTS is mainly oxidized by peroxyl radicals and is soluble in both aqueous and organic solvents, thus reacting both with hydrophilic and lipophilic compounds. While DPPH reagent is a stable nitrogen radical that bears no resemblance to the peroxyl radicals involved in lipid peroxidation. Therefore, its reactivity is limited to the lipophilic fraction [25]. The highest presence of antioxidant compounds in chaff could be of interest for the wheat industry, thus valorizing a product destined to become 'waste', giving it the role of a 'new resource' to be reused in other production chains, such as agri-food, cosmetics, pharmaceutical, agro-industrial, environmental sectors, etc. [10].

Table 1
Biogenic amines contents (mg/kg) ± standard deviation values in *Senatore Cappelli* durum wheat products

	SCDW Seeds	SCDW Flour	SCDW Chaff	SCDW Pasta
ß-PEA	*n.d.*	*n.d.*	*n.d.*	*n.d.*
SER	21.71±0.03	42.17±0.15	31.84±0.12	42.66±0.03
TYR	0.01±0.01	*n.d.*	0.56±0.01	3.63±0.16
PUT	0.73±0.01	15.84±0.01	0.75±0.01	5.67±0.16
CAD	2.25±0.01	5.25±0.02	2.33±0.01	11.43±0.36
HIS	3.18±0.02	17.45±0.02	6.61±0.01	36.37±0.01
SPD	0.32±0.01	70.71±0.01	0.08±0.01	33.32±0.02
SPM	0.79±0.01	21.30±0.01	0.67±0.01	10.01±0.01
Total BAs	28.98	172.72	42.83	143.08

β-PEA: β-phenylethylamine; SER: serotonin; TYR: tyramine; PUT: putrescine; CAD: cadaverine; HIS: histamine; SPD: spermidine; SPM: spermine; Total BAs: Total amount of biogenic amines; n.d.: not detectable.

Table 2
The results of evaluation of antioxidant compounds in Senatore Cappelli Durum Wheat (SCDW) products. Values are expressed as mg GAE/kg for TPC, and Inhibition % for ABTS and DPPH assays ± standard deviation

	SCDW Seeds	SCDW Flour	SCDW Chaff	SCDW Pasta
TPC (mg GAE/kg)	415.35 ± 3.2	418.17 ± 1.6	780.35 ± 2.7	343.61 ± 1.4
ABTS (I%)	66.89 ± 0.76	27.44 ± 0.31	96.57 ± 0.12	12.14 ± 0.54
DPPH (I%)	52.18 ± 1.6	13.46 ± 0.11	75.76 ± 0.71	8.17 ± 0.16

TPC: Total Phenolic Content; GAE: Gallic Acid Equivalent; ABTS: diammonium salt; DPPH: 2,2-diphenyl-1-picrylhydrazyl.

Conclusions

The study aimed at assessing the quality of Senatore Cappelli Durum Wheat products (seeds, flour, chaff, and pasta), through the quantitative determination of biogenic amines content and antioxidant properties. The results showed a great variability of BAs content among all samples analyzed. Among SCDW products, the highest total BAs content was detected in processed cereal-based products, flour (172.72 mg/kg), and pasta (143.08 mg/kg, because of their transformation processes. These processed durum wheat-based products also presented the highest concentrations of SPD (33.32–70.71 mg/kg), and SPM (10.01–21.30 mg/kg in flour), thus being associated with a decreased risk of food allergy. Meanwhile, an interesting remark should be made about SER content (21.71 – 42.66 mg/kg), which represented 56% of the total BAs detected in all samples.

Furthermore, the highest TPC and antioxidant compounds in chaff, which represented 51% of the total phenolic content, with 780.35±2.7 mg GAE/kg for TPC and 96.57, ABTS I%, respectively, could be of interest for the wheat industry both for nutritional and technological aspect. Senatore Cappelli Durum Wheat products relatively rich in these beneficial BA could be of interest to wheat producers these bioactive compounds in functional foods are designed to produce certain health benefits (anti-inflammatory, anti-allergenic, mood regulator, etc.). Likewise, enhancing and re-using by-products could be an opportunity for wheat industries to reduce 'waste', giving it the role of a 'new resource' to be reused in other production chains, such as agri-food, cosmetics, pharmaceutical, agro-industrial, environmental sectors, etc.

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