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Proceeding Paper

Comparative Evaluation of Antioxidant Activities of Flours from Durum Wheat Varieties †

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Abstract: Antioxidants are known to play a crucial role in maintaining cellular health by neutralizing harmful free radicals. Among various dietary sources of antioxidants, wheat-based products, particularly flours, have gained significant attention due to their potential health benefits. Durum wheat, a widely cultivated species, serves as a primary ingredient in numerous food products. However, limited research has been conducted to assess the antioxidant activity of flours obtained from durum wheat varieties. In this study, we aimed to comparatively evaluate the antioxidant and antiplatelet potential of flours from 22 selected durum wheat varieties cultivated in Greece. We focused on three major parameters for antioxidant activity measurement: total phenolic content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and ferric-reducing antioxidant power (FRAP). The total phenolic content was determined using the Folin–Ciocalteu method, while DPPH and FRAP assays were employed to assess the ability of flours to scavenge free radicals and reduce ferric ions, respectively. The antiplatelet activity was evaluated using a platelet-activating factor inhibition assay (PAF) in platelet-rich plasma. Analysis of the data revealed notable differences in the total phenolics and antioxidant and antiplatelet activities among the tested samples. The total phenolic content ranged from 624.0 ± 3.5 to 950.0 ± 5.3 μg of gallic acid equivalent/g flour with the variety Zeta E having the highest content. The antioxidant activities based on the DPPH and FRAP assays ranged from 0.56 ± 0.02 to 2.26 ± 0.08 and 1.93 ± 0.02 to 3.65 ± 0.03 μmol of Trolox equivalent/g flour, respectively, with the varieties Marco Aurelio and Zeta E exerting the highest antioxidant activities in the DPPH and FRAP tests, respectively. In addition, the IC50 values for the antiplatelet activity ranged from 0.72 ± 0.21 to 3.06 ± 0.17 in mg of flour, with the variety of Zoi exhibiting the highest antiplatelet activity. Overall, this comparative evaluation highlights the differences in the antioxidant and antiplatelet activities among flours obtained from 22 different durum wheat varieties cultivated in Greece. The results from this study aid in the selection of wheat varieties with superior antioxidant and antiplatelet capacities for use in bakery food formulation and dietary recommendations.

Keywords: durum wheat flour; in vitro antioxidant activity; total phenolics; DPPH; FRAP; antiplatelet activity; platelet-activating factor

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1. Introduction

There is a growing focus on the connection between modern diets and lifestyles and various health issues, such as cardiovascular disease and malignancies in the digestive system. In recent years, there has been a notable rise in the consumption of nutritious food due to heightened consumer awareness and health-related considerations.

At the same time, it is projected that the global production of wheat will surpass 770 million tons in the year 2024. Wheat constitutes a significant proportion of Greece's

agricultural output. According to a reliable source, it was projected that Greek wheat output would exceed the threshold of one million tons in the year 2024 [1]. One of the primary agricultural crops cultivated in Greece is durum wheat (*Triticum turgidum* L. var. durum). The use of durum wheat flour in the food industry provides both nutritional and economic advantages to Greece.

Bakery products from durum wheat are considered staple foods due to their significant contribution to energy and nutrition, mostly attributed to their substantial carbohydrate and protein composition. Furthermore, wheat has essential nutrients and phytochemical compounds that are crucial for maintaining optimal health. Several phytochemicals included in wheat grains have been well acknowledged for their substantial biological impacts [2–4]. Wheat has the potential to possess secondary metabolites, such as polyphenols. Chemical compounds such as phenolic acids, coumarins, flavonoids, stilbenes and lignans are included under the aforementioned group [3,4]. Previous studies have shown evidence of the antioxidant, anti-inflammatory, antimutagenic and anticarcinogenic characteristics associated with polyphenols [4,5].

It has been shown that oxidative stress, inflammation and thrombosis are implicated in the development of several health conditions, including cancer, atherosclerosis, rheumatoid arthritis and neurological disorders [6,7]. The capacity of antioxidants to eliminate free radicals and mitigate oxidative harm is well recognized [6]. Therefore, it is recommended to increase the consumption of foodstuffs that are high in antioxidants.

The aim of the study was to comparatively evaluate the antioxidant and antiplatelet potential of flours from 22 selected durum wheat varieties (*Triticum turgidum* L. var. durum) cultivated in Greece so that it is possible to select flours of those varieties that have the greatest possible antioxidant and antiplatelet activities.

2. Materials and Methods

2.1. Materials

There were 22 flour samples from different durum wheat (*Triticum turgidum* L. var. durum) varieties kindly offered by the Institute of Applied Biosciences at the Centre for Research and Technology Hellas (INAB | CERTH, Thessaloniki, Greece).

2.2. Extract Preparation

The flour samples were extracted with aquas methanol 80% (*v*/*v*) according to Armelin et al. [8] with some modifications. Briefly, the samples were agitated in an orbital shaker (GFL 3017, GFL; Burgwedel, Germany) at 200 rpm for 2 h at room temperature and then centrifuged at 1200× *g* force for 10 min (Ortoalresa, Digicen 21R, Madrid, Spain). The supernatant was stored at −40 °C until being further analyzed for total phenolics, antioxidant activities and antiplatelet activity.

2.3. Total Phenolics

The total phenolics were measured in triplicate by using the Folin–Ciocalteu method as previously described [9] using a spectrophotometer LAMBDA 25 (Perkin Elmer, Norwalk, CT, USA). The results were expressed as equivalent concentrations of gallic acid (mg GAE per g flour).

2.4. In Vitro Antioxidant Activities

The antioxidant activities were evaluated using the DPPH and FRAP assays as previously described [9]. Each sample was examined in triplicate. Trolox solutions were prepared in the appropriate concentrations for quantitation purposes and the results expressed as Trolox equivalents in μmol per g of flour.

2.5. In Vitro Antiplatelet Activity

The in vitro antiplatelet activity was evaluated on an aggregometer (CHRONO-LOG Corporation, 500CA, Havertown, PA, USA) according to the ability of the samples to inhibit

the lipid mediator of platelet-activating factor (PAF) toward human platelet-rich plasma (PRP) as described previously [10]. The in vitro antiplatelet activity was expressed as mg^{-1} required for 50% inhibition of PAF activity for human PRP.

2.6. Statistics

Statistical analysis was performed using the SPSS software (IBM© SPSS® ver 28.0, IBM UK Ltd., Portsmouth, UK). The data were analyzed for normality using the Shapiro–Wilk test. Homogeneity of variance was checked using Levene's test. Data between the flours from different cultivars were compared statistically using one-way analysis of variance with a post hoc Bonferroni test for $p < 0.05$.

3. Results and Discussion

The data for the total phenolics, antioxidant activities and antiplatelet activity were all normally distributed, as assessed using the Shapiro–Wilk test ($p > 0.05$). They passed Levene's test for homogeneity of variance ($p > 0.05$), and one-way ANOVA with post hoc Bonferroni test was applied to find any statistical differences in total phenolics, antioxidant activities and antiplatelet activity between the flour samples.

3.1. Total Phenolics

The total phenolic content for the flour samples ranged from as low as 0.620 ± 0.003 mg gallic acid/g (Thraki) to as high as 0.950 ± 0.005 mg gallic acid/g (Zeta E). This indicates a significant variation in the phenolic content among the different samples. Svevo, with a total phenolic content of 0.89 mg gallic acid/g, has one of the highest phenolic contents among the samples. Lemnos, Canavaro and Vendeta all have a phenolic content value of 0.73 mg gallic acid/g, showing that these three samples have a similar phenolic content. The results are presented in Figure 1. This variation of phenolic compounds among the different samples indicate possible differences in their antioxidant potential.

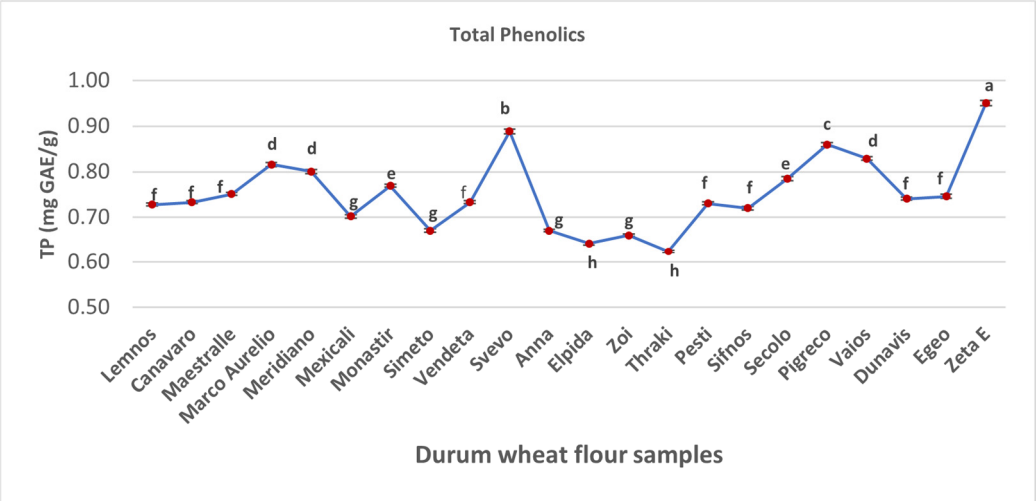

Figure 1. Total phenolics in 22 durum wheat (*Triticum turgidum* L. var. durum) flour samples. Different letters denote statistical difference based on one-way ANOVA with a post hoc Bonferroni test for $p < 0.05$.

3.2. In Vitro Antioxidant Activities

The DPPH values also vary widely among the samples, with values ranging from 0.56 ± 0.02 to 2.26 ± 0.08. Higher Trolox equivalent (TE) values (µmol per g of flour) based on DPPH assay indicate better scavenging of free radicals. Samples like "Marco Aurelio", "Simeto", "Svevo", "Secolo", "Dunavis" and "Zeta E" have notably higher values, suggesting stronger antioxidant activity in these samples (Figure 2). Similar to DPPH, TE (µmol per g of flour) based on FRAP assay vary across the samples, ranging from

2.12 ± 0.03 to 3.65 ± 0.02. Again, "Marco Aurelio" exhibits the highest TE value, followed by samples like "Mexicali", "Zoi", "Pesti", "Sifnos", "Secolo" and "Zeta E", indicating stronger antioxidant potential for those flours (Figure 2).

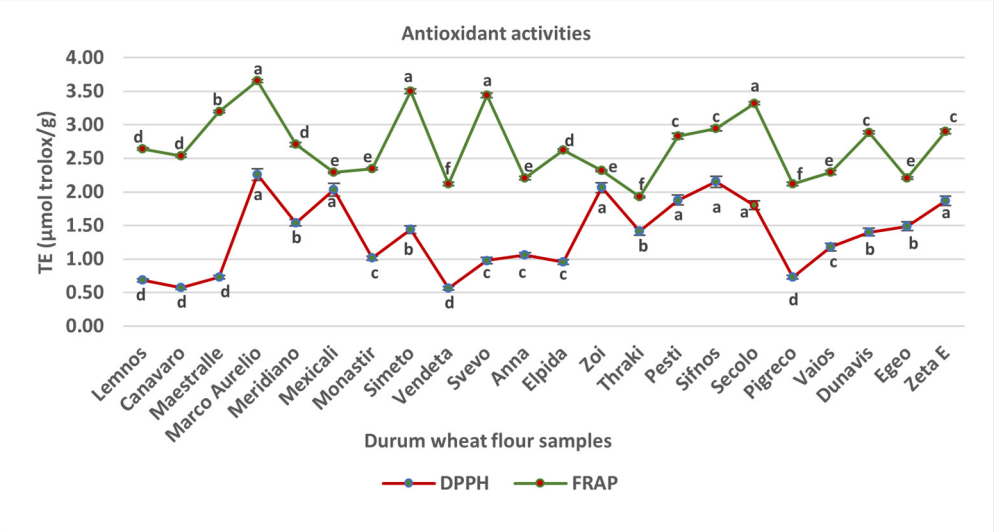

Figure 2. Antioxidant activities in 22 durum wheat (*Triticum turgidum* L. var. durum) flour samples based on DPPH and FRAP assays. The results are expressed as mean ± SD of triplicate measurements and were expressed as Trolox equivalents (TE) in µmol Trolox per one gram of flour sample. Different letters denote statistical differences based on one-way ANOVA with a post hoc Bonferroni test for $p < 0.05$.

3.3. In Vitro Antiplatelet Activity

Higher values (in mg^{-1}) suggest stronger antiplatelet activity. The sample with the highest antiplatelet activity is "Zoi" with a value of 1.38, followed closely by "Pigreco" and "Anna" with equal values of 1.31 (Figure 3). On the other hand, the sample with the lowest antiplatelet activity is "Vendeta" with a value of 0.32.

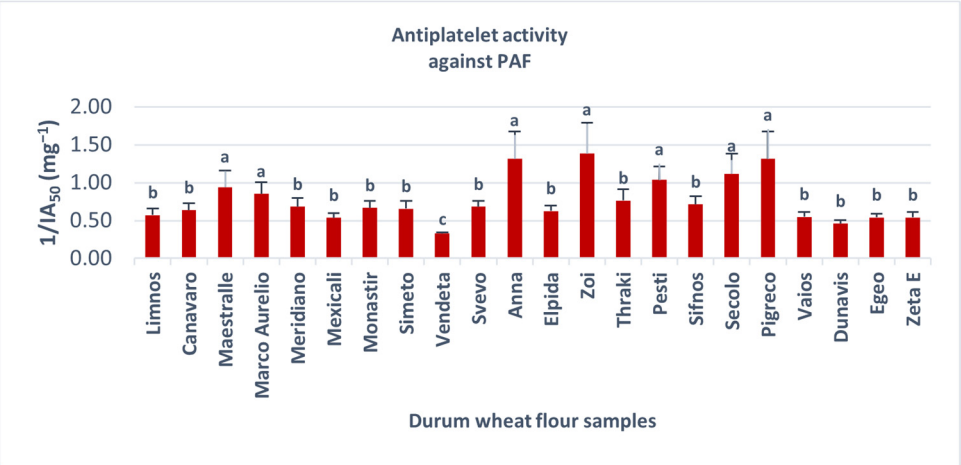

Figure 3. Antiplatelet activity in 22 durum wheat (*Triticum turgidum* L. var. durum) flour samples based on in vitro inhibition of platelet-activating factor (PAF). The results are expressed as mean ± SD of triplicate measurements and were expressed as the reverse of the amount of flour (mg^{-1}) required for 50% inhibition of PAF activity ($1/IA_{50}$) in human platelet-rich plasma (hPRP). Different letters denote statistical differences based on one-way ANOVA with a post hoc Bonferroni test for $p < 0.05$.

4. Conclusions

The data provide valuable information about the antioxidant and antiplatelet potential of flour samples from different durum wheat (*Triticum turgidum* L. var. durum) cultivars. The community can use these data to make informed decisions about which samples may offer greater health benefits in terms of antioxidant and antiplatelet properties, which are implicated in the development of various chronic diseases [6,7].

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