## Unlocking the Antioxidant potential of Kamut Wheat: Insights from Triple Detector Analysis of Phenolic Compounds

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This study examined the antioxidant properties of bound and free phenolic extracts from Kamut wheat (*Triticum* *turgidum* ssp. *turanicum*). Bound extract exhibited superior antioxidant activity, phenolic content, and flavonoid content compared to free extract. Using a triple detector system, the study identified and quantified antioxidant compounds. Phenylalanine, tyrosine, tryptophan, and apigenin 6-C-arabinoside-8-C-glucoside contributed to the antioxidant capacity of the free extracts. The bound extract, after alkaline hydrolysis, contained hydroxycinnamic acids, ferulic acid, and derivatives, demonstrating stronger antioxidant activity. These findings highlight Kamut wheat's potential as a valuable source of dietary antioxidants for the development of functional foods with health benefits.

### Svelare il potenziale antiossidante del frumento Kamut: Studio dall'analisi a triplo rilevatore dei composti fenolici

Questo studio ha esaminato le proprietà antiossidanti degli estratti fenolici legati e liberi del grano Kamut (Triticum turgidum ssp. turanicum). L'estratto legato ha mostrato un'attività antiossidante, un contenuto fenolico e di flavonoidi maggiore rispetto all'estratto libero. Utilizzando un sistema a triplo rivelatore, lo studio ha identificato e quantificato i composti antiossidanti, come fenilalanina, tirosina, triptofano e apigenina 6-C-arabinoside-8-C-glucoside. L'estratto legato, dopo idrolisi alcalina, conteneva acidi idrossicinnamici, acido ferulico e derivati, dimostrando una maggiore attività antiossidante. Questi risultati evidenziano il potenziale del grano Kamut come fonte preziosa di antiossidanti alimentari per lo sviluppo di cibi funzionali con benefici per la salute.

### Introduction

In accordance with the PhD thesis project previously described (Razem 2022), this poster reports the main results of the first two activities concerning: (A1) Extraction of antioxidant compounds from Kamut wheat, (A2) The study of the antioxidant activity and characterization of bound and free antioxidant compounds present in Kamut wheat with a triple detector.

### Materials and Methods

Kamut wheat samples were obtained from Molino Merano, Italy, and processed by drying, grinding, and sieving. Free phenolic compounds were extracted from defatted flour fractions using methanol (80%), followed by centrifugation and concentration. Bound phenolic compounds were extracted from the remaining free phenolic pellets through alkaline hydrolysis. The total phenolic and flavonoid contents of the extracts were determined using the Folin-Ciocalteu reagent and total Flavonoid assays. The oxygen radical absorbance capacity and DPPH radical scavenging activity were evaluated as measures of antioxidant activity. A triple detector analysis approach was used, which included an HPLC system coupled with a diode array detector (DAD), a CoulArray detector (CAD), and a mass spectrometer (MS) detector. The use of this approach allowed the characterization of bound and free phenolic extracts of Kamut wheat and identified the most potent antioxidant compounds present in each.

### Results and Discussion

#### 3.1 Antioxidant assays

The primary antioxidants that may contribute to cereals' antioxidant activity are phytochemicals, amino acids, tocopherols, and tocotrienols (Okarter *et al.*, 2010). In the antioxidant assays conducted, the bound phenolic extracts of Kamut wheat showed significantly higher total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity compared to the free phenolic extracts. The TPC of the bound extracts was six times higher than that of the free extracts, with values of 1024 ± 55 µmol GAE/100g DW and 169.5 ± 13 µmol GAE/100g DW, respectively. The TPC values of the refined Kamut wheat flour were also higher than those reported in previous studies (Dinelli *et al*., 2009). Similarly, the bound extracts exhibited more than six times the TFC compared to the free extracts, with values of 148.8 ± 12 µmol QE/100g DW and 23.8 ± 2.9 µmol QE/100g DW, respectively. Moreover, the bound phenolic extracts demonstrated higher DPPH radical scavenging activity and ORAC values, indicating greater antioxidant capacity. These findings suggest that the bound phenolic extracts of Kamut wheat have superior antioxidant potential, attributed to their higher phenolic content.

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| **Extract** | **TPC** | **TFC** | **DPPH** | **ORAC** |
| **µmol GAE / 100 g DW** | **µmol QE / 100 g DW** | **µmol TE / 100 g DW** | **µmol TE / 100 g DW** |
| **Free Phenols** | 169.5±13B | 23.8±2.9B | 46.7±1.8B | 767.5±88B |
| **Bound Phenols** | 1024±55A | 148.8±12A | 268.4±4.2A | 3736±274A |

**Table 1:** *Total phenol content, total flavonoid content, DPPH, and ORAC of free and bound phenolic extracts of Kamut wheat.*

TPC: Total phenolic content; DPPH: DPPH antioxidant assay; TFC: Total flavonoid content; ORAC: Oxygen radical absorbance capacity; GAE: Gallic acid equivalent: TE; Trolox equivalent; QE: Quercetin equivalent; DW: Dry weight. In a column mean ± SD (n=3) that do not share a letter in the superscript are significantly different (*p* < 0.05).

#### Characterization of antioxidant compounds using a triple detector system

The HPLC-DAD-CAD-MS2 analysis was used to characterize the phenolic compounds in the free and bound extracts of Kamut wheat. The DAD displayed all compounds from the extracts of free and bound phenols from Kamut wheat, while the CAD selectively displays peaks with redox activity (Razem *et al*., 2022). The main antioxidant peaks observed in the HPLC-CAD, were subsequently identified based on retention times, and fragmentation patterns using HPLC- MS2. In the free extracts, four peaks with redox activity were found, tentatively identified as amino acids (tyrosine, phenylalanine, and tryptophan) and a flavonoid (apigenin 6-C-arabinoside 8-C-glucoside). Among these compounds, tyrosine exhibited the strongest antioxidant activity according to the hydrodynamic voltammogram (HDV) analysis. In the bound extracts (Figure 1), 15 antioxidant compounds were identified, with five of them being identified using reference standards, and having ferulic acid and it’s derivatives the most distinctive. The antioxidant compounds identified included phenolic aldehydes, phenolic acids, hydroxycinnamic acids, and a flavonoid. The HDV analysis showed that the bound extracts contained more electroactive compounds with lower half-wave potentials, indicating a greater capability for electron transfer compared to the free extracts.



**Figure 1:** HPLC-DAD chromatogram of bound extracts of Kamut wheat measured at 280 nm (top chromatogram), and the accumulated sum of current obtained from the 16 CAD channels (bottom mirrorred chromatogram).(1: Protocatechuic aldehyde, 2: vanillic acid, 3: syringic acid, 4: p-coumaric acid, 5: vanillin, 6: ferulic acid, 7: 8-8'-diferulic acid, 8: 8-5'-diferulic acid, 9: quercetin, 10: 8-O-4′-DFA benzo form, 11: 8-O-4′-DFA linear form, 12: 3-4-Dimethoxycinnamic acid, 13: Tri ferulic acid, 14: gamma-tocotrienol, 15: Heliannuol D).

1. **References**

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