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ORIGINAL ARTICLE

Sprouting bioprocess as a sustainable tool for enhancing durum wheat (*Triticum durum*) nutrients and bioactive compounds

Sarra Jribi [1]*, Otilia Tamara Antal [2], Helga Molnàr [2], Nora Adànyi [2], Zoltan Fustos [2], Zoltan Naàr [2] Oussema Kheriji [3], Hajer Amara [4], Hajer Debbabi [1]

[1] National Institute of Agronomy of Tunisia (INAT), Research Unit UR17AGR01 "Valorization of the Tunisian natural and agro-food heritage through innovation", University of Carthage, 43 Avenue Charles Nicolle, 1082 Tunis, Tunisia.
[2] Food Science Research Institute, National Agricultural Research and Innovation Centre, Herman Otto utca 15, H-1022 Budapest, Hungary.
[3] National Institute of Cereal crops (INGC), 8170 Bou Salem, Tunisia.
[4] National Institute of Agronomy of Tunisia (INAT), Genetic and Plant Breeding Laboratory, University of Carthage, 43 Avenue Charles Nicolle, 1082 Tunis, Tunisia.

Abstract

Background: Consumers are more aware of the role of healthy diet in preventing food-related diseases (Cancer, cardiovascular diseases, diabetes, etc.). Consequently, they are looking for products with beneficial nutritional attributes that encourage the food industry to develop functional foods. **Aims:** In this study, we aimed at using a natural bioprocess to improve durum wheat "*Triticum durum*" nutritional properties for its further use as a functional ingredient. **Materials and Methods:** Six durum wheat cultivars were tested: four high yielding and two landrace ones. Seeds were germinated for 48 hours at 22°C. Nutritional properties were evaluated through proximate composition and bioactive compounds (carotenoids, total phenol, vitamin C and tocopherols) levels. **Results:** Biochemical characterization of sprouted seeds showed significant modifications with a decrease in ash, starch contents and an increase in reducing sugars, and in proteins. Improvements in bioactive compounds were also observed in sprouted seeds. Vitamin C, tocopherols, total phenols, carotenoid pigments as well as antioxidant activity significantly increased after sprouting. Interestingly, durum wheat landrace cultivars showed the best performances. **Conclusions:** Results provided by our study proved that sprouting is an interesting natural tool to use in the food industry for the development of cereal products with added nutritional value.

Keywords: Durum wheat, bioactive compounds, nutritional properties, sprouting, vitamins.

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1 Introduction

According to the World Health Organization (WHO) global health estimate in 2015, cardiovascular diseases (31.3%), malignant neoplasms (15.5%) and diabetes (2.8%) caused almost half of the deaths all over the world. These rates increased considerably if compared to those of 2000. Lifestyle, new eating habits and lack of regular physical activity are among the reasons explaining this fact. Consequently, consumers are more and more looking for a healthy lifestyle. Functional foods could satisfy these requirements. This trend started in the 1980s mainly in Japan with FOSHU (Foods for Specified Health Uses) [1] and since the market of functional foods spread all over the world [2] and took its consideration in the food industry. Functional foods could be defined as "Foods with physiological benefits that can reduce the risk of chronic diseases" [3]. Thus, they could be obtained by (i) adding substances (already present on the original products), (ii) substituting a component by healthier analog or (iii) removing a component known for its undesirable effects[4]. Functional foods' health effect requires their consumption among a varied diet, regularly and at acceptable levels [1].

Cereals are an important part of the human diet due to their composition offering carbohydrates, proteins, fibers,

* **Corresponding author:** Dr. Sarra JRIBI, University of Carthage, 43 Avenue Charles Nicolle, 1082 Tunis, Tunisia. Tel. +216 21 927 237 Email: sarra.jribi@gmail.com

vitamins, etc. Wheat is the first cereal cultivated all over the world with a surface of more than 218 million hectares in 2017 [5] and the second in terms of consumption. Tunisians are among the highest consumers of wheat worldwide (148.7 kg/person/year). Considering specifically durum wheat consumption, they are in the second position (12.4 kg/person/year) just after Italians [6].

Consequently, improving wheat seeds nutritional properties has a high interest. Breeding over the past century aimed to improve yielding and adaptability to climate change conditions. Previous studies compared Landrace (old) and high yielding (modern) durum wheat genotypes. Daaloul-Bouacha *et al.* [7] showed a better quantitative quality in landrace varieties while others suggested that breeding did not affect health-promoting components such as gluten and dietary fibers [8,9].

Sprouting being an old food engineering tool, is utilized mainly in Eastern countries such as China and Japan [10]. "Sprouts" are obtained from the germination of seeds and their development in water, collected before the development of leaves. The final product still contains the seed [11]. Sprouting bioprocess is known for its effect on improving the nutritional properties of different kinds of seeds such as pulses and cereal grains [12]. Numerous studies investigated the effect of sprouting on wheat (*Triticum aestivum*) nutritional properties and bioactive compounds [13-17]. Therefore, it would be interesting to develop a cereal functional ingredient from durum wheat, naturally fortified with bioactive molecules, by the use of sprouting bioprocess.

To the best of our knowledge, there is a scarcity of studies evaluating the effect of sprouting on the quality of durum wheat *(Triticum durum*). The following research aimed to evaluate and compare the effect of this bioprocess on different landrace and high yielding durum wheat cultivars for their further use in the food industry as a functional ingredient.

2 Material and Methods

2.1 Materials

Six Tunisian cultivars of durum wheat (*Triticum durum*) were selected for this study including four high yielding varieties: *Karim*, *Razzek*, *Khiar*, and *Maali*, and Two landraces: *Chili* and *Chili*.

Samples (Harvested in 2015) were kindly provided by the National Institute of Cereal crops (INGC Bou Salem, Tunisia) and the National Gene Bank of Tunisia (BNG, Tunisia). Samples were stored at 4°C on closed bags until use.

2.2 Materials

2.1.1 In vitro sprouting

Seeds (50g) were initially sterilized with 1% (V/V) hypochlorite sodium solution for 30 minutes, then rinsed three times with distilled water-soaked again in distilled water and finally spread into plates with three layers of "Blotting paper". Samples were watered after 24 hours. Sprouting was conducted at a temperature of 22.5 ± 0.5°C for 48 hours.

After sprouting samples were immediately subjected to lyophilization (Christ freeze dryer alpha 1-4 LCS, Germany) then milled (Retsch Grindomix GM 200, Germany) and stored at -18°C until analysis.

2.1.2 Proximate composition

Ash content was determined according to AACC methods [18] (AACC 08-01.01), crude fat (AACC 30-10.01) and protein content (AACC 46-30.01), a value of 5.7 was used as a factor of conversion to estimate protein content. Reducing sugar measurement was carried out through the Fehling method, and starch measurement, through a hydrolytic method and titration with $Na_2S_2O_3$.

2.1.3 Enzyme activity determination

Amylolytic and α-amylase activities were measured as suggested by Kalita *et al.* [19]. One unit of enzyme activity (U) was defined as the amount of micro-moles of maltose produced per minute under the assay conditions and calculated using formula (1):

$$\text{Activity (U/mL)} \left(\frac{(\text{mg/ ml in terms of maltose})*1000}{\text{Molecular weight of maltose}*\text{Time (min}}=\right)*2 \quad ..(1)$$

2.1.4 Vitamins and Bioactive molecules

Tocopherols and Vitamin C contents were assessed by the HPLC procedure as described by Molnar *et al.* [20]: Tocopherols were separated on Nucleosil-100 (5 mm, 250 4.6 mm) column with isocratic elution (99.6:0.4 n-hexane-ethanol) and detected using a Shimadzu RF-535 Fluorescence HPLC Detector (excitation wavelength: 295 nm and emission wavelength: 320 nm).

Total carotenoid pigments were determined by extraction with butanol as described by Pasqualone *et al.* [21].

Total phenol content was assessed using the Folin-Ciocalteu method [22]. Gallic acid was used as standard (0-1 mg/ml; r^2=0.987).

2.1.5 Antioxidant activity: DPPH-radical scavenging activity (DPPH RSA

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity (DPPH RSA) was measured using the method proposed by Aprodu & Banu [22] with a slight modification during the extraction procedure for antioxidant activity measurement. The extraction was performed with 80% (v/v) aqueous methanol solution, for 2 h at 37°C. Samples were afterward centrifuged at 6,000 RPM for 30min and the supernatant was used for the determination of antioxidant capacity.

Antioxidant activity was calculated according to the formula (2):

$$\%DPPAH\ RSA = (1 - A_{Sample/t=30}/A_{Control/t=0}) * 100 \ldots. (2)$$

2.1.6 Statistical analysis

Statistical analysis was carried out using the Minitab software (Minitab 17, USA). All experiments were carried out in triplicate and the average values were reported together with standard deviations. Analysis of variance (ANOVA) was performed using the Fisher test. Significance was defined at $p<0.05$.

3 Results and Discussion

3.1 Effect of sprouting on proximate composition and enzyme activities

In this study, besides the effect of germination, an interest was accorded to the genetic background (landrace and high yielding cultivars). As shown in Table 1 sprouting led to modifications in wheat seeds composition.

Ash content was significantly different from one cultivar to another and ranged between 1.65 and 2.06 % before sprouting. Sprouting contributed to a decrease in ash content. This reduction was only significant for *Razzek*, *Maali* (high yielding varieties) and *Chili* (durum wheat landrace). A previous study of Singh *et al.* [23] reported a decrease in ash content for sprouted sorghum while other results showed that three days germination could not affect the ash content of wheat [24]. This difference could be explained by a change in some sprouting parameters (mainly soaking time and temperature). An increase in ash content of high-amylose wheat after 48 hours of sprouting was observed [25]. Thus, ash content evolution after sprouting could be probably related to the genetic background of the seeds used [26].

The amounts of proteins were significantly different among varieties. Cultivars could be divided into two main groups: The first one with high protein content (over 17%), represented by the landrace genotypes *Chili* and *Chili* and the second with lower protein content. Tunisian landrace durum wheat is recognized for its better quantitative quality parameters compared to the high yielding ones [7]. Our data clearly showed that sprouting increased protein content. This increase was probably due to proteolytic enzyme activity. In fact, protein hydrolysis depends on sprouting conditions (temperature, duration)[27]. The significant increase observed in our study was in agreement with previous results [24,25]. The effect of different sprouting conditions on alpha amylase activity, functional properties of wheat flour and on shelf-life of bread supplemented with sprouted wheat was evaluated[28]. Results showed that after two days of sprouting, with SDS-Page, a hydrolysis in protein was observed. The author reported that an increase in sprouting time leads to an increase low molecular weight protein and a decrease in high molecular weight ones [28]. Similarly, another study dealing with effects of enzyme activities during steeping and sprouting on the solubility and composition of proteins, their bioactivity and relationship with the bread making quality of wheat flour, showed a decrease in peptides with high molecular weight and an increase in low molecular weight in sprouted wheat flour [29]. In this study, five days sprouting led to changes in proteins proportions and solubility rather than their amounts. According to the same authors, the release of low molecular weight proteins could present a high nutritional interest due to the bioactive role of these peptides (anti-bacterial, anti-carcinogenic, anti-thrombocytic or stimulating biological activity) [29].

Lipid content was significantly different from one cultivar to another. The highest average was noticed for *Khiar* (2.28%±0.41) and the lowest for the landrace *Chili* (1.34%±0.11) before sprouting. In fact, cultivars showed different behaviors after sprouting. The two varieties of *Karim* and *Chili* showed a significant decrease ($p<0.05$) in lipid content while a significant increase ($p<0.05$) was observed with landrace genotype *Chili*. However, sprouting did not affect the lipid content for the high yielding varieties *Khiar*, *Razzek*, and *Maali*. It has been reported that germination for three days did not affect wheat lipid content [24]. Nevertheless, some qualitative results could suggest that sprouting would modify the fatty acid composition of soft wheat (*Triticum aestivum*) [16]. The fatty acid composition after sprouting evolved differently, according to the tested varieties highlighting the role of genetic properties [16].

Table 1: Effect of sprouting on wheat seeds composition (n=3)

Cultivar		Ash (%) (g.100 g^{-1} dm)	Lipid (%) (g.100 g^{-1} dm)	Protein (%) (g.100 g^{-1} dm)	Starch (%) (g.100 g^{-1} dm)	Reducing sugar (mg.g^{-1} dm)
Khiar	Raw	1.65 ± 0.07^{FG}	2.28 ± 0.41^{A}	14.04 ± 0.04^{GH}	45.46 ± 0.81^{E}	35.04 ± 1.55^{C}
	Sprouted	1.55 ± 0.04^{G}	2.20 ± 0.05^{A}	14.22 ± 0.20^{FG}	35.37 ± 0.91^{F}	57.09 ± 1.36^{B}
Razzek	Raw	1.96 ± 0.07^{BC}	1.76 ± 0.03^{BC}	13.88 ± 0.11^{HI}	49.34 ± 0.46^{D}	15.71 ± 0.13^{F}
	Sprouted	1.79 ± 0.03^{DE}	1.74 ± 0.13^{BC}	14.55 ± 0.20^{E}	31.36 ± 0.72^{G}	60.41 ± 0.73^{A}
Maali	Raw	2.00 ± 0.05^{AB}	1.43 ± 0.07^{DEF}	14.29 ± 0.16^{F}	48.60 ± 0.36^{D}	26.13 ± 1.18^{E}
	Sprouted	1.81 ± 0.04^{DE}	1.61 ± 0.04^{BCD}	14.39 ± 0.09^{EF}	36.03 ± 0.70^{F}	56.85 ± 0.84^{B}
Karim	Raw	1.79 ± 0.05^{DE}	1.58 ± 0.11^{CDE}	13.70 ± 0.05^{I}	64.11 ± 0.63^{A}	32.52 ± 1.29^{D}
	Sprouted	1.72 ± 0.11^{EF}	1.32 ± 0.07^{F}	14.28 ± 0.08^{F}	28.98 ± 0.68^{H}	55.47 ± 1.02^{B}
Chili	Raw	2.06 ± 0.06^{A}	1.59 ± 0.09^{CD}	17.91 ± 0.11^{B}	54.62 ± 0.95^{B}	33.96 ± 1.38^{CD}
	Sprouted	1.87 ± 0.06^{CD}	1.33 ± 0.03^{F}	18.60 ± 0.03^{A}	31.11 ± 0.46^{G}	60.01 ± 1.84^{A}
Chili	Raw	1.85 ± 0.07^{D}	1.34 ± 0.11^{EF}	17.04 ± 0.05^{D}	52.93 ± 0.26^{C}	27.57 ± 0.32^{E}
	Sprouted	1.84 ± 0.04^{D}	1.84 ± 0.09^{B}	17.35 ± 0.03^{C}	29.48 ± 0.37^{H}	61.42 ± 2.22^{A}

Means in the same column that do not share the same letters are significantly different, according to Fisher's test, ($p<0.05$).

Consequently, the evolution of endogenous lipids after sprouting may be associated to the selectivity of lipases [30]. Rose & Pike [31] measured lipase activity in wheat and wheat bran and observed that the lipolytic activity was linked to the pool of free fatty acids in the stored wheat tested. They also highlighted that optimal conditions for lipase should be specified to the variety tested.

Table 2: Effect of sprouting on enzyme activity (n=3)

Cultivar		Amylolytic activity	α-amylase activity
Khiar	Raw	4.88 ± 0.12^{F}	1.79 ± 0.15^{I}
	Sprouted	6.48 ± 0.36^{C}	7.13 ± 0.30^{E}
Razzek	Raw	5.33 ± 0.13^{E}	2.08 ± 0.05^{H}
	Sprouted	6.53 ± 0.23^{C}	11.86 ± 0.09^{A}
Maali	Raw	4.54 ± 0.22^{G}	2.38 ± 0.07^{G}
	Sprouted	7.54 ± 0.14^{A}	9.57 ± 0.24^{C}
Karim	Raw	4.56 ± 0.04^{G}	2.28 ± 0.08^{GH}
	Sprouted	5.85 ± 0.07^{D}	8.40 ± 0.27^{D}
Chili	Raw	4.82 ± 0.22^{FG}	2.14 ± 0.04^{GH}
	Sprouted	7.36 ± 0.19^{A}	10.24 ± 0.10^{B}
Chili	Raw	4.83 ± 0.03^{FG}	3.30 ± 0.10^{F}
	Sprouted	6.98 ± 0.19^{B}	8.52 ± 0.27^{D}

Means in the same column that do not share the same letters are significantly different, according to Fisher's test ($p<0.05$).

Starch is an energy storage molecule in plants. Its levels ranged between 45.46 and 64.11% for raw seeds and 28.98 to 36.03% for sprouted ones. This significant decrease in starch content was followed by a significant increase ($p<0.05$) in reducing sugars (Table 1). According to cultivars, this increase ranged between 2 and 4 folds was related to the amylolytic enzyme's action [32]. In fact, sprouting is a physiological event where seeds move from a dormant state to an active one. Therefore, this event requires energy for the new embryo. Degradation of macromolecules under enzymatic activity is a way to provide the embryo with necessary nutrients [15]. In our study, results of amylolytic enzymes, as summarized in Table 2, are confirming the results of starch and reducing sugar measurements. Although we observed differences among cultivars in enzymatic activity in raw seeds, the trend was the same after germination with a significant increase ($p<0.05$). In terms of enzymatic activity, our results are in line with previous dealing with malted rice [19].

The first step of sprouting consists of water absorption and tissue rehydration or "Imbibition". This procedure stimulates gibellirin hormone synthesis, which then stimulates hydrolytic enzymes activities [33]. Enzymatic activity depends not only on genotypes and environmental area but also on sprouting conditions (Temperature, duration) [19].

3.2 Vitamins and bioactive molecules

Results of vitamins C and E measurement are summarized in Table 3. Vitamin C is a water-soluble vitamin that is naturally present in some foods. Humans are unable to synthesize vitamin C endogenously. Therefore, it is an essential dietary component. Wheat is not commonly known as a source for vitamin C. In our study, this vitamin has been detected only in the cultivar *Khiar* at a low level (Table 3). Interestingly, sprouting increased significantly ($p<0.05$) vitamin C content of durum wheat seeds. Averages ranged between 15.10 µg/g dm (durum wheat landrace *Chili*) and 43.9 µg/g dm (for the high yielding variety *Khiar*). Previous works reported that vitamin C content increases gradually with sprouting time. Optimal conditions to obtain the maximal content of vitamin C differ among authors from 4 up to 7 days [10,34]. Biosynthesis of vitamin C includes several enzymatic reactions to transform D-glucose to L-ascorbic acid [35]. Consequently, carbohydrates level (glucose, sucrose) plays a key role in this process. Sprouting increased significantly reducing sugars amounts (Table 1) which probably contributed to increasing vitamin C levels as a strong positive correlation was observed in our study (Pearson correlation r=0.81, p=0.00).

Table 3: Effect of sprouting on vitamin C and tocopherols contents (n=3)

Cultivar		Vitamin C (µg/g dm)	α-tocopherol (µg.g^{-1} dm)	ß-tocopherol (µg.g^{-1} dm)	α-tocotrienol (µg.g^{-1} dm)	ß-tocotrienol (µg.g^{-1} dm)
Khiar	Raw	5.35±0.04^{F}	5.62±0.28^{DE}	2.78±0.09^{CD}	3.32±0.34^{CDE}	17.94 ±0.61^{BC}
	Sprouted	43.9±1.50^{A}	6.33±0.07^{AB}	3.24±0.09^{A}	3.72±0.14^{AB}	16.59±0.28^{D}
Razzek	Raw	ND	5.56±0.19^{DEF}	2.71±0.05^{DE}	3.17±0.15^{DE}	17.26±0.48^{CD}
	Sprouted	19.48±0.50^{C}	6.59±0.20^{A}	3.29±0.15^{A}	3.94±0.17^{A}	16.87±0.55^{D}
Maali	Raw	ND	5.51±0.13^{EF}	2.90±0.07^{BC}	3.10±0.04^{DE}	17.36±0.45^{BCD}
	Sprouted	16..42±0.52^{D}	6.05±0.21^{BC}	2.98±0.04^{B}	3.73±0.13^{AB}	16.60±0.46^{D}
Karim	Raw	ND	5.88±0.20^{CD}	2.87±0.10^{BCD}	3.59±0.16^{BC}	19.17±0.69^{A}
	Sprouted	23.92±0.55^{B}	6.13±0.24^{BC}	3.00±0.15^{B}	3.78±0.21^{AB}	15.46±0.67^{E}
Chili	Raw	ND	5.23±0.13^{F}	2.55±0.10^{EF}	2.53±0.13^{F}	12.94±0.36^{F}
	Sprouted	15.10±0.27^{E}	6.05±0.14^{BC}	2.92±0.10^{BC}	3.04±0.03^{E}	11.87±0.30^{G}
Chili	Raw	ND	6.09±0.22^{BC}	2.49±0.10^{F}	3.39±0.20^{CD}	18.21±0.58^{B}
	Sprouted	24.62±1.17^{B}	6.20±0.28^{BC}	2.52±0.07^{F}	3.61±0.20^{BC}	15.67±0.64^{E}

Means in the same column that do not share the same letters are significantly different, according to Fisher's test (p<0.05).
ND: Not Detected.

Vitamin E is a liposoluble vitamin including a group of eight organic compounds (four tocopherols and four tocotrienols) [36]. Results of vitamin E measurement (Table 3) showed that amounts of α-tocopherol were significantly different from one cultivar to another before and after germination. Sprouting led to an increase in α-tocopherol amount, ranging between 12.63% and 18.53%. A same trend was also observed for ß-tocopherol. An increase of 50% in α-tocopherol after 9 days of soft wheat sprouting was previously reported [16] while another study showed an increase in vitamin E of 25% after 4 days of germination [37]. Moreover, Yang *et al.* [34] reported that the maximum of vitamin E was obtained after sprouting for eight days. Thus, the increase in tocopherol would probably depend on germination conditions used as well as the genetic background of the tested seeds. The biosynthesis pathway of vitamin E has not been reported previously [38].

In our study, 48 hours of germination also increased α-tocotrienol levels. In fact, sprouting increased significantly (p<0.05) α-tocopherol, ß-tocopherol, and α-tocotrienol, whereas a significant decrease in ß-tocotrienol was observed after sprouting. Luckily, α-tocopherol, ß-tocopherol, and α-tocotrienol display higher biological activity than ß-tocotrienol [36].

Total phenol content ranged between 15.9 and 29.44 mg GAE.g^{-1} dm for raw seeds (Table 4). Averages were significantly different among cultivars. Durum wheat landrace showed the highest amounts. Dordevic *et al.* [39] reported an average of 16.2 mg GAE.g^{-1} dm for total phenol content for wheat seeds. Bioactive molecules amount, such as polyphenols are related geographic zones where plants were grown, genotypes and the procedure used for extraction and quantification [26]. Thus, results may differ from one study to another. In our study, all cultivars followed the same trend after sprouting: a significant increase (p<0.05) was observed in total phenol content. The highest averages were observed for landrace genotypes *Chili* and *Chili*. The differences observed between landrace and high yielding durum wheat varieties could be related to the genetic differences as well as the agronomic practice and geographical origin. Glucose is the precursor for the phenolic compounds' synthesis [38]. As sprouting is marked by the degradation of macromolecules like starch, amounts of simple sugars may increase. Hence, such increase in total phenol content could be expected. According to our results, the increase in total phenol content was positively correlated with reducing sugars content (Pearson correlation r=0.80; p=0.00). Moreover, during germination, there is an increase in phenylalanine ammonia-lyase (PAL), a key enzyme for the accumulation of phenolic compounds, with a significant positive correlation between PAL activity and total phenols content [14].

Carotenoids are organic bioactive molecules produced by plants. They may play a role of provitamin A and contribute to the antioxidant properties of foods. The high yielding variety *Khiar* and the landrace *Chili* and *Chili* showed the highest total carotenoid pigments (19.55; 18.53; 17.72 mg.kg^{-1} dm respectively). Sprouting increased significantly (p<0.05) carotenoid pigments content for all tested cultivars. These contents are higher than those reported for remilled semolina (5.5 mg.kg^{-1} dm) [21]. This difference might be related to different genetic backgrounds and environmental conditions of wheat seeds. In the same study, some by-products like debranning fractions also showed higher values (9.7 mg.kg^{-1} dm), suggesting that whole grain flour would be also characterized by higher amounts. Our study showed that durum wheat sprouting increases vitamins C & E as well as carotenoid pigments contents. These changes may improve the antioxidant properties of sprouted seeds.

Table 4: Effect of sprouting on bioactive molecules and antioxidant properties

Cultivar		Total phenol content (mg GAE.g^{-1} dm)	Carotenoid (mg β-carotene.kg^{-1} dm)	DPPH RSA (%)
Khiar	Raw	16.56±0.34^{GH}	19.55±0.41^{E}	29.19±0.79^{G}
	Sprouted	28.72±0.89^{D}	24.37±0.31^{A}	37.80±0.62^{D}
Razzek	Raw	15.9±0.38^{H}	16.88±0.32^{H}	32.66±0.60^{EF}
	Sprouted	26.40±0.51^{E}	21.89±0.28^{C}	43.93±0.67^{B}
Maali	Raw	17.04±0.27^{G}	16.85±0.36^{H}	31.77±0.89^{F}
	Sprouted	39.47±0.68^{B}	22.62±0.59^{B}	41.95±0.69^{C}
Karim	Raw	15.85±0.97^{H}	15.31±0.29^{I}	18.86±0.35^{H}
	Sprouted	35.14±0.16^{C}	20.28±0.32^{D}	33.46±0.41^{E}
Chili	Raw	19.93±0.50^{F}	18.53±0.24^{F}	32.51±0.82^{EF}
	Sprouted	43.59±0.34^{A}	21.99±0.43^{C}	42.78±0.40^{C}
Chili	Raw	29.44±0.08^{D}	17.72±0.32^{G}	36.84±0.31^{D}
	Sprouted	42.97±0.48^{A}	20.73±0.25^{D}	53.19±0.63^{A}

Means in the same column that do not share the same letters are significantly different, according to Fisher's test ($p<0.05$).
GAE: Gallic Acid Equivalent, dm: dry matter bases, DPPH RSA: (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity.

For the DPPH RSA, the lowest value for raw seeds was obtained for *Karim* genotype with 18.86% while the highest value was observed for *Chili* (36.87%). An average of 12.7% was previously reported for remilled semolina[21]. In our study, we used whole grain flour thus our results could be higher: some metabolites and bioactive molecules are concentrated in the outer parts and germ of seeds [40]. Sprouting also induced a significant increase ($p<0.05$) in antioxidant properties for all cultivars tested. The highest averages were for landrace cultivars probably because of their highest total phenols content. In fact, our results showed a positive correlation between DPPH Radical Scavenging Activity and total phenol content (Pearson correlation coefficient r=0.78; p=0.00). The improvement in antioxidant properties could be also explained by the enhancement of bioactive compounds as they were positively correlated with DPPH RSA according to our results (Carotenoid: r=0.66, p=0.00; Vitamin C: r=0.54, α-tocopherol: r= 0.49, p=0.00).

On the other hand, it is fundamental to remind that plants possess some antioxidant enzymes such as peroxidase (POD), catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD). Germination may activate these enzymes [41]. For example, a significant increase in catalase, peroxidase, and ascorbate peroxidase activities in sprouted soft wheat was observed [14].

In conclusion, our data proved a nutritional improvement of durum wheat quality after sprouting. This bioprocess could be suggested as an efficient, natural and low-cost method for supplying vitamins and bioactive molecules.

4 Conclusion

Sprouting of Tunisian landrace and high yielding durum wheat (*Triticum durum*) cultivars induced degradation of some macromolecules such as starch and proteins suggesting an improvement in digestibility. The increase in vitamins, total phenols, and carotenoid pigments led to an improvement in antioxidant properties. Interestingly, among Tunisian cultivars, durum wheat landrace genotypes showed better performance than the high yielding. Altogether, our study highlighted the contribution of sprouting to improving the nutritional properties of durum wheat seeds. Thus, it could be suggested as a green tool for the development of functional ingredients and cereal products with added nutritional value for the food industry.

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