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Original article

Effect of Sprouting Temperature on Durum Wheat (*Triticum Durum*) Sprouts Nutritional Properties and Bioactive Compounds [1]

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Abstract

Sprouting is an old food engineering tool to improve edible seeds nutritional value. It can improve carbohydrate digestibility and enhance levels of bioactive compounds. These changes are strongly related to sprouting conditions: temperature, light, duration. The aim of this research was to evaluate the effect of sprouting temperature on sprouts bioactive molecules and proximate composition of Chili Tunisian durum wheat (*Triticum durum*) seeds. Hence, two temperatures were tested: 18°C and 25°C. Analysis of ashes, proteins, lipids, reducing sugars, carotenoids, vitamin C, vitamin E, and total phenols contents and DPPH radical scavenging activity were done. Our results indicated that temperature influenced significantly proximate composition of sprouts, as well as bioactive compounds. Vitamin C and tocopherols levels were higher at 25°C, than in raw seeds. In contrast, a temperature of 18°C led to highest amounts of carotenoids and total phenols. In addition, antioxidant properties of durum wheat seeds were improved by sprouting only at 18°C. In conclusion, evolution of nutritional properties and bioactive compounds in sprouts were strongly dependent on sprouting temperature used.

Keywords: Durum wheat, Sprouting, Temperature, Nutritional properties, Bioactive compounds.

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INTRODUCTION

Cereals are an important part of human diet. In fact, they have a varied composition offering consumers macro and micro-nutrients (carbohydrate, protein, fiber, minerals, vitamins). Wheat is the first cereal cultivated all over the world with a surface of more than 220 million hectares in 2014 (FAO STAT, 2017). It is also the second in terms of consumption.

Sprouting is an old process used mainly in eastern countries (Plaza et al., 2003). Several cereals and legumes could be used (wheat, rice, sorghum, soybean, mung bean, chickpea). Interestingly this bioprocess leads to enhancing nutritional properties of raw seeds (Gan et al., 2017). Germination is a physiological event where seeds move from a dormant state to an active one after the imbibition phase. Tissues are hydrated with water and enzymes are activated (Hopkins, 2003). To provide embryo with required nutrients, catabolism of existing molecules takes place and synthesis of new metabolites too (Mak et al., 2009). Consequently, levels of simple nutrients and bioactive compounds increas (Jribi et al., 2018). Sprouts could be consumed raw or processed (Hubner and Arendt, 2013).

Despite the great number of research assessing the improvements of cereals and legumes nutritional properties after sprouting, the details of protocols used are quite different among authors. Basically the three main steps used are: disinfection, soaking and sprouting. However, parameters used (disinfection agent, concentration, soaking duration, water temperature, sprouting duration, sprouting temperature) are different (Gan et al., 2017). These differences might be related to the nature of seeds used or to the specific purposes of sprouting process: For example, Yang et al. (2001) have previously showed that the maximum of vitamin E in wheat sprouts could be obtained after sprouting for 8 days while 7 days were enough to obtain the highest levels of vitamin C. Koehler et al. (2007) reported that protein degradation during wheat germination depends on sprouting conditions used. Sing et al. (2001) proved that sprouting conditions (soaking duration, sprouting duration and sprouting temperature) have significant effect on functional and dynamic rheological properties of wheat.

To the best of our knowledge there is a scarcity of works evaluating durum wheat (*Triticum durum*) sprouting interests. The aim of this research was particularly to assess the effect of sprouting temperature in enhancing durum wheat sprouts nutritional properties and bioactive compounds.

Material and methods

Plant materials

Tunisian landrace durum wheat (*Triticum durum*) “Chili” was used in this study. This variety was introduced from France and pure line was registered in 1953 (Ammar et al., 2011). Samples (harvested in 2015) were kindly provided by the National Gene Bank of Tunisia (BNG, Tunisia).

In vitro sprouting

Durum wheat seeds (50g) were first sterilized with 1% (V/V) hypochlorite sodium solution during 30 minutes (Wei et al., 2012). Then rinsed three times with distilled water and spread in plates with three layers of "Blotting paper". Samples were watered after 24 hours. Sprouting experiments was conducted at a temperature of 18±0.5°C and 25±0.5°C during 48 hours (Hung et al., 2015).

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

Proximate composition

Ash content was determined by incinerating the sample in a muffle furnace at 550 °C (Nobertherm GmbH, Germany) cooling it in a desiccator and weighing. Crude fat was determined by acid hydrolysis of the sample with HCl followed by extraction of hydrolyzed lipid materials with mixed ethers. Ethers were evaporated, and lipid residue was heated to constant weight at 100°C. Residue was expressed as percentage (%) of crude fat (AACC International Method 30-10.01). Protein content was determined using Dumas combustion method (Elementar Rapid N cube, Switzerland) (AACC 46-30.01). A value of 5.7 was used as factor of conversion to estimate protein content (Ciccoritti et al., 2017). The carbohydrate content (%) was calculated by subtracting the contents of crude ash, fat and protein from 100% of dry matter. Reducing sugar measurements were carried out through the Nelson-Somogyi reducing sugar method (McCleary and McGeough, 2015).

The energy value was determined by computation and expressed in calories. It was calculated from protein, fat and carbohydrate contents using the Atwater's conversion factors:

1kcal/100g= [(4 x Carbohydrate)+(4 x Protein)+(9 x Fat)].

Bioactive compounds and vitamins determination

Tocopherols and Vitamin C contents were assessed by HPLC following the procedure described by Molnar et al. (2018)

Total phenol content

Total phenol content was assessed using Folin-Ciocalteu method as suggested by Aprodu and Banu (2012). Gallic acid was used as standard (0-1mg/ml; r^2=0.987).

Carotenoid pigments

Total carotenoid pigments were determined according to the procedure described by Pasqualone et al. (2017).

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 and Banu (2012) with slight modification during extraction procedure for antioxidant activity measurement: the extraction was made with 80% (v/v) aqueous methanol solution, for 2h at 37°C. Samples were afterwards centrifuged at 6000 rpm for 30 min. The supernatant was used for the determination of antioxidant capacity.

Antioxidant activity was calculated according to the following formula:

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

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 are reported together with standard deviations. Analysis of variance (ANOVA) was used to test the statistical differences among results followed by a Tukey multiple comparison test. Significance was defined at $p<0.05$.

Results

Effect of temperature on proximate composition of sprouts

Proximate composition of raw seeds and sprouts is presented in Table 1. Sprouting bioprocess induced significant changes. In fact, it led to an increase in ash content. This increase seems related to sprouting temperature used as it was significant ($p<0.05$) at 25°C only. Similarly, if compared to raw seeds durum wheat sprouts had higher protein content. However no significant ($p<0.05$) differences were observed between sprouts germinated at 18 or 25°C. In contrast, crude fat content decreased after sprouting. Significant decrease ($p<0.05$) was observed at 18°C in our study if considering raw seeds as reference. As seen on Table 1, regardless sprouting temperature used no significant quantitative changes were observed in carbohydrates. However, amounts of reducing sugars were significantly different ($p<0.05$). In fact, levels in sprouts ranged from 29.17 to 49.94 mg/g dm versus 27.92 for raw seeds. Temperature affected reducing sugars levels significantly. Increasing sprouting temperature increased also durum wheat reducing sugars levels. Taking together, our results clearly indicate that sprouting temperature could affect levels of ashes, total proteins and lipids in sprouts and consequently sprouts energy value.

Table 1. Effect of sprouting temperature on nutritional properties

Sample	Ashes (%)	Proteins (%)	Crude fat (%)	Carbohydrates (%)	Reducing sugars (mg/g dm)	Energy (Kcal/100g)
Raw	1.87±0.05b	17.91±0.10b	1.59±0.08a	78.31±0.15a	27.92±0.27c	407.95±0.43a
Sprouted at 18°C	1.97±0.01ab	18.32±0.23a	1.40±0.05b	78.30±0.20a	29.17±0.12b	406.99±0.24b
Sprouted at 25°C	2.07±0.07a	18.24±0.11ab	1.47±0.07ab	78.22±0.18a	49.94±0.28a	407.35±0.38ab

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

Effect of sprouting temperature on vitamins

Results of vitamin C and tocopherols measurement are summarized in Table 2. Vitamin C was not detected in raw seeds while sprouting bioprocess enhanced its levels. Moreover, an increase in sprouting temperature from 18 to 25°C increased vitamin C content from 44.11 to 47.64 (µg/g dm).

As shown on Table 2, sprouting increased significantly ($p<0.05$) α-tocopherol, β-tocopherol, α-tocotrienol while a decrease in β-tocotrienol was observed. Temperature had significant impact only on α-tocopherol and β-tocotrienol evolution.

Table 2. Effect of sprouting temperature on vitamins

Sample	Vitamin C (µg/g dm)	α-tocopherol (µg/g dm)	β-tocopherol (µg/g dm)	α-tocotrienol (µg/g dm)	β-tocotrienol (µg/g dm)
Raw seeds	0.00±0.00c	5.23±0.13c	2.55±0.10b	2.53±0.12b	12.94±0.35a
Sprouted seeds at 18°C	44.11±1.52b	6.14±0.15b	2.88±0.06a	2.84±0.07a	11.89±0.60b
Sprouted seeds at 25°C	47.64±1.18a	6.40±0.10a	2.90±0.11a	2.92±0.07a	10.76±0.27c

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

Effect of sprouting temperature on bioactive compounds and antioxidant activity

Total phenol and carotenoids content increased significantly ($p<0.05$) after sprouting (Table 3). An increase of 52.77% in total phenolic compounds was observed when grains were germinated at 18°C whereas a germination at 25°C led to an increase of 43.34%. The same trend was also observed with carotenoids since the increase reached 49.65% at 18°C versus 16.30% only at 25°C.

The evolution of bioactive molecules and vitamins led to an improvement of the antioxidant properties of sprouts compared to raw seeds. An increase in temperature decreased DPPH radical scavenging activity.

Table 3. Effect of sprouting temperature on bioactive compounds and antioxydant activity

Sample	Total phenols (mg GAE/g dm)	Carotenoids (mg β-carotene/kg dm)	DPPH RSA (%)
Raw seeds	13.05±0.14c	18.53±0.23c	31.11±0.98b
Sprouted seeds at 18°C	19.93±0.49a	27.73 ± 0.32a	34.18±0.28a
Sprouted seeds at 25°C	18.71±0.24b	21.55± 0.37b	32.51±0.82b

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

GAE: Gallic Acid Equivalent, dm: dry matter bases; DPPH RSA: (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity

Discussion

Sprouting bioprocess as well as temperature used affected durum wheat sprouts proximate composition and bioactive compounds levels. A significant increase in ash content ($p<0.05$) after sprouting was only observed at 25°C, in accordance with previous studies (Ozturk et al., 2012; Hung et al., 2015). Total protein content was significantly ($p<0.05$) enhanced by sprouting at 18°C, confirming studies of Sighkornart et al. (2014) and those of Hung et al. (2015) while no significant increase was observed at 25°C if compared to raw seeds. The decrease in fat content observed in our study is in agreement with previous results of Sighkornart et al. (2014). However, only low temperature (18°C) had significant effect. Taking together, our results clearly indicate that sprouting temperature could affect levels of ashes, total proteins and lipids in sprouts. During germination, catabolism of macromolecules such as proteins and lipids takes place under enzymes activity (mainly proteolytic and lipolytic enzymes) (Hopkins, 2003; Koehler et al., 2007). Consequently, the effect of temperature on sprouts nutrients might be linked to these enzymes optimal temperatures. For example, our results clearly showed that temperature affected reducing sugars levels significantly. The increase in reducing sugars amounts is probably linked to starch degradation under enzymatic activities, mainly amylases (Eskin and Shahidi, 2013). Singh and Kayastha (2014) showed that optimal temperature for α-amylase activity is around 68°C. Consequently, it would be expected in our study that grains sprouted at 25°C could show higher amounts of reducing sugars.

Low temperature (18°C) decreased energy content of sprouts if compared to raw seeds. Energy content depends on the composition. The decrease seen at low temperature might be linked to the significant decrease in fat content at 18°C. Even though, no significant changes on energy content was observed after sprouting at 25°C this temperature could be suggested to improve durum wheat nutritional properties: In fact, weather sprouted at 18 or 25°C no significant difference are observed in protein, crude fat and carbohydrate contents. However, reducing sugar amounts amounts increased by 78.87% at 25°C. Sprouts obtained at 25°C are consequently easier to digest as they contain more proteins and less starch (Dziki et al., 2015).

Vitamin C is a water-soluble vitamin that is naturally present in some foods. Wheat is not known as a source for vitamin C. In our study also, this vitamin has not been detected in raw seeds. Pérez-Balibrea et al. (2011) observed also a positive effect of temperature on broccoli sprouts. Added to, Plaza et al. (2003) reported that Vitamin C content increased gradually with sprouting time. Biosynthesis of vitamin C includes several enzymatic reactions to transform D-glucose to L-ascorbic acid (Pérez-Balibrea et al., 2011). Consequently, carbohydrate level (glucose, sucrose) plays a key role in this process. Our results showed clearly that higher temperature led to higher amounts of reducing sugar. Probably, for this reason increasing temperature increased vitamin C content.

Vitamin E is a liposoluble vitamin present by eight molecules of tocopherols and tocotrienol. In our study we assessed α- and β-tocopherols as well as tocotrienol. Authors studied previously the role of sprouting duration on Vitamin E enhancement and proved that tocopherols content increase gradually with sprouting duration (Yang et al., 2001; Ozturk et al., 2012). Richards et al. (2008) observed that an increase in monthly temperature averages induced an increase in α-tocopherol content of canola seeds. Almonor et al. (1998) reported also a positive effect of temperature on soybean tocopherols content.

Total phenol and carotenoids content increased significantly ($p<0.05$) after sprouting (Table 3). Results of Paucar-Menacho et al. (2010) showed that temperature and time modified sprouted soybean the concentrations of bioactive compounds. Previous study of Schonhof et al. (2007) also showed that low temperatures led to an increase in broccoli lutein content (greenhouse production). Schreiner et al. (2000) reported also the same with lutein and β carotene amounts in broccoli.

The evolution of bioactive molecules and vitamins led to an improvement of the antioxidant properties of sprouts compared to raw seeds. An increase in temperature decreased DPPH radical scavenging activity. Bioactive compounds might have different antioxidant properties. Our results showed that carotenoids had the highest contribution on Radical Scavenging Activity (Pearson correlation coefficient 0.63) in comparison to the other molecules assessed. As highest carotenoids levels were obtained at 18°C it would be expected to measure the highest Radical Scavenging Activity at this temperature.

Conclusion

Sprouting is an environmental-friendly, effective and inexpensive tool to improve durum wheat nutritional properties. Temperature used for sprouting influenced significantly Chili landrace durum wheat (*Triticum durum*) composition as well as bioactive compounds levels within the range studied: A temperature of 25°C was suitable for improving digestibility, vitamin C and tocopherols synthesis however high amounts of carotenoids and total phenol content required lower temperature of 18°C. Thus, the choice of sprouting temperature is an important parameter to take into account according to the main purposes of sprouting (improving antioxidant properties, improving digestibility).

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