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Spécialité : Agroalimentaire

Développement technologique d'un ingrédient fonctionnel, le blé germé, et formulation de produit céréalier à valeur nutritionnelle ajoutée

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Development of cereal products with added nutritional value through the use of sprouted wheat

“***Success means doing the best we can with what we have. Success is the doing, not the getting; in the trying, not the triumph. Success is a personal standard, reaching for the highest that is in us, becoming all that we can be***”

Zig Ziglar

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Declaration of Authorship

I, Sarra JRIBI, declare that this dissertation titled, "**Development of cereal products with added nutritional value through the use of sprouted wheat"** and the work presented in it are my own. I confirm that:

- This work was done wholly or mainly while in candidature for a research degree at this University.
- Where I have consulted the published work of others, this is always clearly attributed.
- Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work.
- I have acknowledged all main sources of help.
- Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself.

Sarra JRIBI

ملخص

يحتل القمح الصلب مكانة هامة في النظام الغذائي التونسي نظرا لإستعماله في عدة وصفات (معجنات, كسكسي, خبز, بسيسة...) لذلك يعتبر تحسين القيمة الغذائية للقمح الصلب طريقة تمكن من الإستجابة إلى الحاجيات الغذائية للمستهلك و لكن في اطار ندرة الموارد الطبيعية فإن الأخذ بعين الإعتبار بمبادئ التنمية المستدامة أثناء الانتاج والتحويل أصبح حاجة ملحة .

تندرج هذه الدراسة ضمن إطار تحسين القيمة الغذائية للقمح من خلال استعمال الإنتاش. تأثير الإنتاش لمدة 48 ساعة تمت دراسته على المستوى الغذائي, الميكروبيولوجي والوظيفي. بينت النتائج تحسنا ملحوظا للقيمة الغذائية خاصة من خلال ارتفاع معدلات "Molécule bioactives" و" Indexe prébiotique. ولكن رغم تحسن القيمة الغذائية فإن توفر كميات هامة من الماء أثناء الإنتاش يعتبرملائم لانتشار و تكاثر البكتيريا. في هذا الإطار قيمنا دور استعمال الزنك في تطهير حبوب القمح المنتشة. تم تحديد الظروف التجريبية باستعمال طريقة " Plan d'expériences" وقد مكنت هذه الطريقة من تحسين كل من الجودة الغذائية و الميكروبيولوجية . في مرحلة ثانية وقع استعمال التجفيف للحفاظ على القمح المنتش من خلال تجربة ثلاث طرق : "Micro-ondes sous vide" "étuvage à 50°C" "lyophilisarion". و قد بينت النتائج أن تطور الخاصيات الوظيفية, الحرارية والغذائية مرتبط بطريقة التجفيف المعتمدة.

اضافة إلى تأثير التجفيف على هذه الخصائص فإن مدة الإنتاش لها تأثيرأيضا على هذه الخصائص لذلك قمنا بمتابعة تطور الخصائص الجيلاتينية , الحرارية و الوظيفية من 12إلى 72 ساعة. و قد أظهرت النتائج انخفاضا في الخصائص الجيلاتينية و القدرة على إستيعاب الماء اعتبارا من 12 ساعة في حين أن القدرة على امتصاص الزيت و المعلمات الحرارية قد ارتفعت.

في آخر قسم من هذه الدراسة قمنا باستعمال دقيق القمح المنتش في إعداد البسكويت. في هذا السياق استعملنا دقيق القمح الكامل, دقيق القمح الكامل المنتش (لمدة 24 و 48 ساعة) , الدقيق العادي و المزيج بينهما (50:50). بالنسبة للعجين فإن استعمال دقيق القمح الكامل قد ساهم في الترفيع من الخاصيات اللزجة المرنة أما بالنسبة للبسكويت فقد أظهرت النتائج ازديادا في الصلابة و تقلصا في الحجم مع استعمال دقيق القمح الكامل رغم ذلك فقد لاحظنا تحسنا في قبول البسكويت من قبل المستهلك. في الختام فقد بينت نتائج هذا البحث فاعلية الإنتاش في التحسين من الخاصيات الغذائية للقمح مما يشجع على استعماله كون ذا قيمة مضافة.

الكلمات المفاتيح: القمح الصلب ; الإنتاش ; الجودة الصحية; الخصائص الغذائية و الوظيفية; بسكويت

Résumé

Le blé dur occupe une place importante dans le régime alimentaire tunisien puisqu'il est présent dans plusieurs produits de consommation courante (pates, couscous, pain, bsissa...). L'amélioration de la valeur nutritionnelle du blé dur contribue donc à mieux satisfaire les besoins du consommateur tunisien. Cependant, vu la rareté des ressources naturelles, la durabilité lors de la production et transformation s'impose. Cette étude s'inscrit dans le cadre d'une amélioration de la qualité nutritionelle du blé dur par le biais d'un bioprocédé, la germination. L'impact de la germination pendant 48 h a été étudié à differents niveaux : nutritionnel, microbiologique et fonctionnel. Les résultats ont montré que la germination a amélioré significativement les propriétés nutritionnelles, suite à l'augmentation des teneurs en molécules bioactives et de l'index prebiotique. Cependant, l'augmentation de la teneur en eau lors de la germination a favorisé une prolifération bactérienne. Dans ce contexte, l'utilisation du zinc pour décontaminer les graines de blé germé a été testée. Les conditions expérimentales ont été optimisées grâce à un plan d'expériences. L'utilisation de cette approche a permis une amélioration de la qualité hygiénique et nutritionnelle du blé germé. Le séchage est la deuxième approche étudiée pour conserver le blé germé. Trois différentes méthodes ont été testées : l'étuvage à 50°C, la lyophilisation et le séchage par micro-onde sous vide. L'évolution des propriétés thermiques, fonctionnelles et nutritionnelles a révélé la dépendance de ces paramètres à la méthode de séchage utilisée. D'autre part, la germination a affecté aussi les propriétés gélatineuses, thermiques et fonctionnelles de la farine de blé germé. L'étude des propriétés de 12 à 72 h de germination a montré une diminution des propriétés gélatineuse et d'hydratation à partir de 12 h de germination. En revanche, la capacité d'absorption d'huile ainsi que les paramètres thermiques ont augmenté. Finalement, des essais d'incorporation de la farine complète de blé germé dans des biscuits ont été menés. Dans ces essais, différentes farines ont été testées : complète, complète de blé germé pendant 24 h et 48 h, ainsi que des mélanges de farine raffinée et farine complète de blé germé (50 :50). Concernant la pate, les paramètres visco-élastique G' et G'' ont augmenté suite à l'utilisation de farines germées. Quant au biscuit, l'incorporation des farines germées a conduit à une augmentation de la dureté et une diminution de la taille des biscuits. L'analyse sensorielle a revelé une amélioration de l'acceptabilité grace à l'utilisation de farine complète de blé germé. En conclusion, notre travail a conduit au developpement d'un ingrédient fonctionnel, le blé germé pouvant etre incorporé dans des aliments fonctionnels à valeur ajoutée.

Mots clé : Blé dur, germination, qualité hygiénique, nutrition, propriétés fonctionnelles, farine, biscuit.

Abstract

Durum wheat represents a millstone of Tunisan diet as it is used in many products (pasta, couscous, bread, bsissa…). Improving durum wheat nutritional leads to a better satisfaction of Tunisian consumers' needs. Because of natural resources scarcity, sustainability becomes a primordial concept to take into account in production and transformation. In this context, the main objective of this research was the use of a sustainable approach to enhance durum wheat nutritional attributes. The effects of sprouting bioprocess (for 48 h) on durum wheat (*Triticum durum*) were studied at different levels: nutritionally, microbiologically and functionally. Results showed that sprouting for 48 h significantly improved durum wheat nutrients, bioactive compounds levels and prebiotic index. However, the increase in water content led to an increase on bacterial growth. Thus, the use of zinc soaking was suggested for sprouts decontamination. Experimental conditions were optimized through a factorial design. The use of this approach was effective to limit bacterial growth and also ameliorate nutritional approach. The second approach used for sprouts conservation was drying through different methods: lyophilisation, microwave vacuum drying and oven drying (at 50°C). Evolution of thermal, functional and nutritional were dependent on drying method use. Sprouting time also played a key role on pasting, thermal and functional properties. Following these parameters from 12 to 72 h showed a significant decrease on hydration and pasting properties from 12 h while oil absorption capacity and determined parameters from DSC analysis (onset temperature T_0 and peak temperature T_p) increased. Finally, trials to incorporate sprouted whole wheat flour in cookies elaboration were conducted: The use of whole wheat flour sprouted for 24 and 48 h, and sprouted whole wheat flour-refined flour blends (50:50) in cookies elaboration was investigated. Regarding the dough, an increase in visco-elastic moduli (G' and G") was recorded when whole wheat flour and sprouted whole wheat were used instead refined flour. For cookies, both whole wheat flour and sprouted whole wheat decreased spread factor and increased hardness compared to control. Sensory analysis results highlighted an improvement on overall acceptability was improved when both whole wheat flour and sprouted whole wheat were used.

Key words: Durum wheat, sprouting, hygienic quality, nutritional and functional properties, cookie.

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Chapter 1: General Introduction

1. Introduction

Food security is among century's issues. Several actions should be taken to meet the second goal of sustainable development: zero hunger while respecting natural resources and their scarcity. Functional foods developement is among options allowing an improvement of foods nutritional quality. Consumer would be willing to use functional foods as they are looking for the benefits of quality rather than excessive quantities (Szakály et al., 2019). The global functional foods market size was estimated at USD 161.49 billion in 2018 and it is expected to reach USD 275.77 billion by 2025 (Grand View Research, Inc (online), 2019).

Cereals and cereal products are staple foods in most human diets offering an important part of nutrients and energy due their composition (Laskowski et al., 2019). Improving cereals quality could be achieved through different strategies: agronomic practice, crop breeding and genetic engineering (Wang *et al.,* 2015; Wei *et al.,* 2012; Saha *et al.,* 2017). In all cases, trials are time consuming and costy. Accordingly, it would be vital to explore other options allowing sustainable production of better quality cereals. These cereals could be suggested as portential functional ingredient.

2. Sustainable production

World population grew continuously to reach 7.7 billion in 2019 (Figure 1; 1,860-times the size of what it was 12 millennia ago when world population was around 4 million) and it is expected to reach 10 billion people by 2050 (Vandermeer et al., 2018). On 2017, around 821 million people were undernourished (United Nation (online), 2019). Particularly, on developing countries 12.9% of the population is undernourished (United Nation (online), 2019). Accordingly there is a huge effort to make to overcome this situation and reach the 2030 agenda goals, particularly goal 2: zero hunger. Meanwhile, planetary boundaries for sustainable food supply has been already exceed (Campbell et al., 2017). Consumption of animal derived products (digestive system emission) and intensive farming production (farm equipment fuel, fertilizer manufacture, greenhouse gas emission…) are illustrations of planetary boundaries exceed (Dawson et al., 2019). Unfortunately, such facts contribute on global warming and climate change.

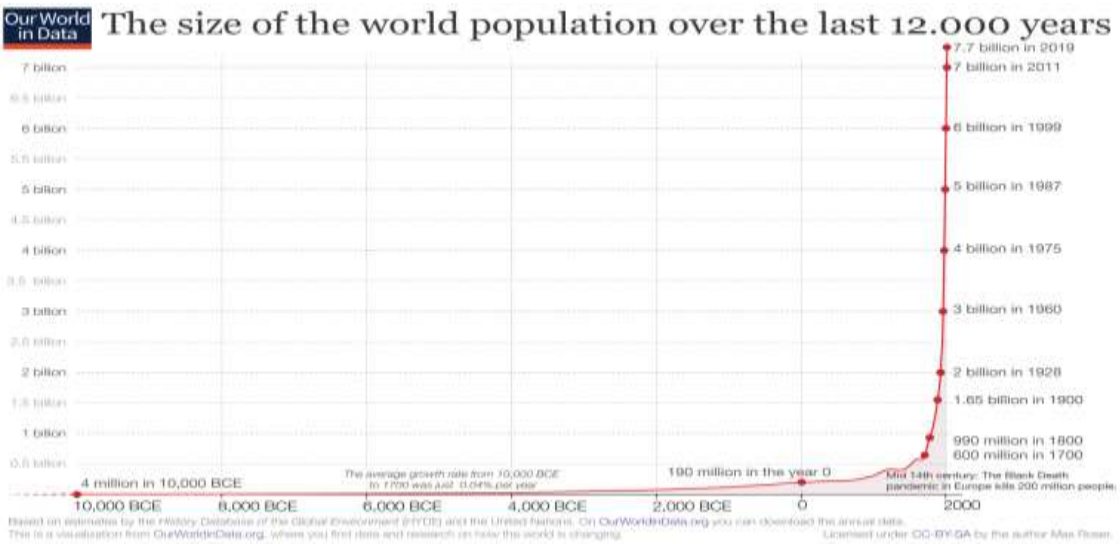


Figure 1: World population evolution during years (Our World In Data, 2019 online)

Thus, food production is more problematic and challenged. On one hand, the environmental factor is crucial to take into consideration on a global sustainable approach. On the other hand, the increase in diet related diseases rate as obesity, type 2 diabetes, cardiovascular disease, cancer...and their contribution in mortality all over the world raised consumers' awareness. They become more aware about the link between the diet they adapt and their health. Accordingly, food products need to satisfy consumers' expectancy: staple foods have to fulfill basic requirements (in terms of calories and macro-nutrients) and also micronutrients and bioactive compounds.

To face environmental challenges and food security a rising interest is accorded to 'sustainable intensification' (SI) of farming practices (Dwson et al., 2019). In others words, the use of process achieving "higher [and/or more stable] agricultural [food] yields whilst simultaneously reducing [or reversing] the negative impact of farming [food production] on the environment" (Dawson et al., 2019). Somehow, it is necessary to produce with respect to the goal 12 of 2030 sustainable development goals: Responsible consumption and production. Accordingly, there is an urgent need to use approaches reducing ecological footprint to achieve economic growth.

3. Cereals

Cereals play a key role on human nutrition. In fact, they represent a food staple with high nutritional interest (Zuchowski et al., 2011). Moreover, consumption of whole grains has been

associated with a decrease on prevalence of obesity, type 2 diabetes, cardiovascular diseases and some cancers (Bjork et al., 2012; Liu, 2007; Okarter & Liu, 2010; Zhu et al., 2012).

Wheat is the first cultivated cereal allover the world, harvested area exceeded 218 million hectars on 2017 (Faostat, 2019). It is the second on terms of consumption. Wheat comes from a type of grass (*Triticump*) that is grown in countless varities world wide. Bread wheat or common wheat is the primary specie. Several other closely related species include durum, spelt, emmer, einkorn…

Durum wheat (*Triticum turgidum L. ssp durum*) is worldwide cultivated on about 30-35 million hectar particularly in the Mediterranean basin (Guzmàn et al., 2016). In Tunisa cultivated surface with durum wheat reached 515 000 hectar on 2015-2016 (INGC (online), 2020). For the same period production was estimated at 812 000 tones which represents only 53% of Tunisian market needs (INGC (online), 2020). The remaining needs are imported. Wheat kernel is composed from three main fractions (Figure 2)

- Bran
- Germ
- Endosperm (Onipe et al., 2015)

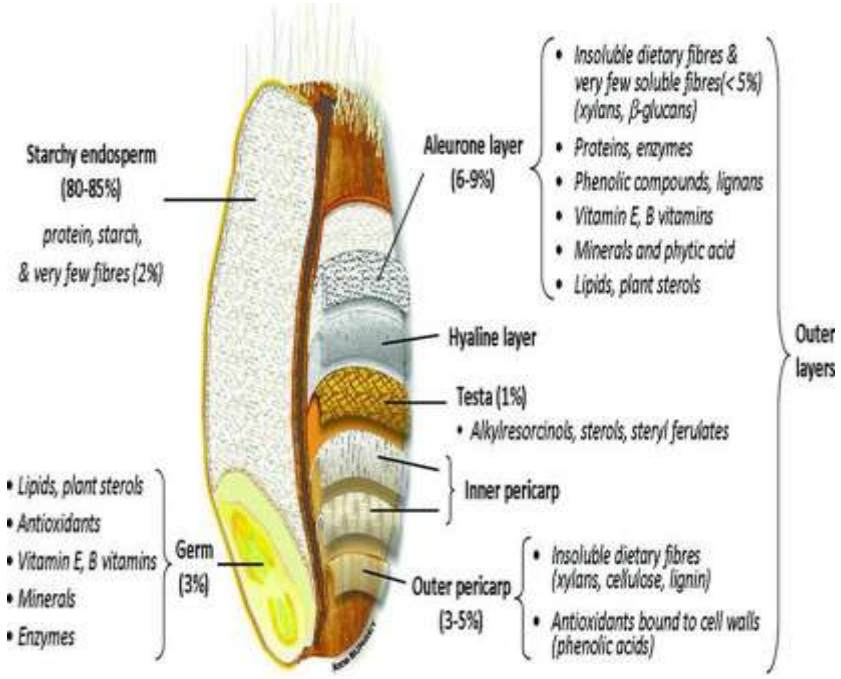


Figure 2: Wheat grain structure (Onipe et al., 2015)

In terms of composition, wheat grain contains carbohydrates (65-75%: starch, fibers), proteins (7-12%), lipid (2-6%), water (12-14%), minerals, vitamins and bioactive compounds (polyphenol, carotenoids…) (Hemery et al., 2007).

Considering their solubility, wheat proteins are divided into four groups:

- Albumin (water soluble)
- Globulin (soluble on salty solutions)
- Gliadin (alcohol soluble)
- Glutenin (soluble on acid solution) (Feuillet, 2000).

Albumin and globulin are described as soluble or metabolic proteins while gliadin and glutenin, main components of gluten, are storage molecules offering amino acids during germination (Feuillet, 2000). Consumption of wheat as only protein source is not enough to satisfy body needs of essential amino acids like leucin (Jiang et al., 2008). Meanwhile, composition of amino acids on wheat grain relies on many factors: genetic background, geographic position and agronomic practices (Zuckowski et al., 2011).

Fibers represent 13% of wheat grain (Fardet, 2010). They are mainly concentrated on outer parts, germ and bran (Hung et al., 2010).

Polyphenols are secondary metabolites playing role on plant growth and protection against parasites and predators (Liu, 2007). They are structured by hydroxyl groups linking phenols (Okarter et al., 2010). The nutritional interest of polyphenols is linked to their anti-oxydant acticity. What grain is rich on phenolic acids, basically, ferruli acid (Okarter et al., 2010) representing 90% of total polyphenols (Zuckowski et al., 2011). Among other phenolic acids we can find: caffeic acid, p-coumaric, gentisic, p-hydroxybenzoic, salycilic, sinapic, syringic and vanillic acid (Liu, 2007; Hung et al., 2010; Zuckowski et al., 2011).

Wheat grain is a good source of nutrients and bioactive compounds. These nutrients are concentrated on the outer parts of the grain. Phenolic acids for example are more concentrated on the bran than the starchy endosperm (Liu, 2007). For this reason consumption of whole grain would be more recommended than refined flour as shown by result of “Health Grain” (2005-2010) (Anderson et al., 2013). “US Nurses study” also linked consumption of whole grains to a decrease on cardio-vascular diseases prevalence for women (Okarter and Liu, 2010).

Despite its nutritional interest, some components on wheat grain could be jugged as "anti-nutritional". This might be the case of phytate available on the aleurone layer (Hemery et al., 2007).

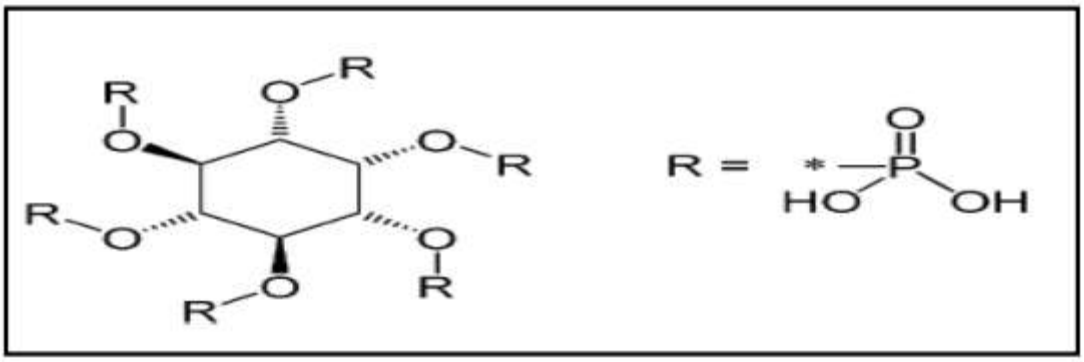


Figure 3: Phytic acid structure (Kumar et al., 2009)

About 90% of phosphate is accumulated as phytate form on mature grain (Bohn et al., 2008). Unfortunately, the structure of phytic acid (Figure 3) allows chelation of cations like calcium, magnesium, zinc, potassium… and thus obtaining insoluble salts. Consequently, absorption and digestion of these elements become difficult (Kumar et al., 2009). Phytates react negatively also with carbohydrate, lipid and proteins. Some of these interactions are presented on Table 1.

Table 1: Interactions of phytate with nutrients (Kumar et al., 2009)

Component	Action
Mineral ions	Decrease on minerals availability after their chelating and liberation of unsoluble salts.
Proteins	Formation of nonspecific phytate-protein complex, not hydrolysed by proteolytic enzymes.
Carbohydrates	Formation of phytate carbohydrate complexes making carbohydrate less degradable. Inhibition of amylase activity by complexing with Ca^{++} ion and decrease of carbohydrate degradation.
Lipids	Formation of 'lipophytin' complexes, may lead to metallic soaps in gut lumen, resulting in lower lipid availability.

3.1Durum wheat (*Triticum durum*)

Durum wheat (*Triticum turgidum subsp. Durum*) is a tetraploid species of wheat (Feillet, 2000). It is the first cultivated species in Tunisia in termes of all cereals harvested area (55% in 2018) (ONAGRI, 2020). In 2018, the national production reached 962 thousand tons (versus 113 for soft wheat) wich represents 81.2% of local needs and the rest is imported (however for soft wheat importation levels exceeds 49%) (ONAGRI, 2020).

Due its caracteritics durum wheat is mainly used for pasta elaboration and some traditional tunisian products (couscous, pain artisanal, nouasser, makroudh, bsissa...). Protein, carotenoids, gluten quality and stregth play a key role in durum wheat transformation (Babay et al., 2014; Daaloul-Bouacha, 2014). These parameters might be different among cultivars. In Tunisia, despite high quality attributes of landrace cultivars, mainly high yielding cultivars like Karim, Razzek, Maali, Khiar ... are used due their resistance and yield (Babay et al., 2014; Daaloul-bouacha, 2014). It is important in this context to keep in mind that quality attributes are not only related to genetic background but also environmental conditions and agronomic practices (Flagella et al., 2010; Lerner et al., 2006; Li et al., 2013; Rharrabti et al., 2003). Accordingly, no specific conclusions could be done just from genetic background: each cultivar might behave differently according conditions used.

4. Sprouting

Sprouting, germination or malting are ambiguous terms used revealing slight differences according to the use of end products (Old wheat grain council, 2007). Malting is a specific form of sprouting used in brewing and distilling industries. Meanwhile, sprouting and germination might be used as synonyms. To the best of our knowledge, there is no globally definition and regulation of sprouting. According to The European Sprouted Seeds Association (ESSA), “Sprouts means the product obtained from the germination of seeds and their development in water or another medium, harvested before the development of true leaves and which is intended to be eaten whole, including the seed” The European Union (EU) regulation defines sprouted grains as grains in which the embryo has undergone clearly visible changes (EU 1272/2009), thereby focusing on the agricultural aspect of sprouting as a quality characteristic in intervention trade (EU 742/2010). “Sprouted grains” are defined by the American Association of Cereal Chemists (AACC) with the endorsement of the United States Department of Agriculture (USDA) as follows: “malted or sprouted grains containing all of the original bran, germ, and endosperm

shall be considered whole grains as long as sprout growth does not exceed kernel length and nutrient values have not diminished. These grains should be labelled as malted or sprouted whole grain. In this context other terms could be revealed like shoots and cress: “Shoots are sprouted seeds obtained from the germination and the development of seeds to produce a green shoot with very young leaves and/or cotyledons. The shoots and the leaves are harvested at the end of the production process and the final product does not include the seed integuments and the roots”; “Cresses are sprouted seeds obtained from the germination and development of true seeds in soil or in hydroponic substrate, to produce a green shoot with very young leaves and/or cotyledons. Cress is sold as the entire plants in its substrate or soil.” (ESSA, 2016). Despite these various definitions, there is no specific indication on the approach to adapt for obtaining sprouts. Germination procedure is basically carried through three main steps: sterilization, soaking and sprouting (Gan et al., 2017). Huge differences might be observed on each among authors. Sterilization is the first step performed in order to inhibit microbial growth. Sodium hypochlorite (NaClO) and ethanol (pure or 70%) has been reported on scientific literature as sanitizer for seeds (Gan et al., 2017). The second step consists on soaking seeds on water for hydrating them before allowing seeds to sprouts. Conditions adapted (soaking duration, water temperature, sprouting temperature and duration, relative humidity, light) are determinant on seeds growth.

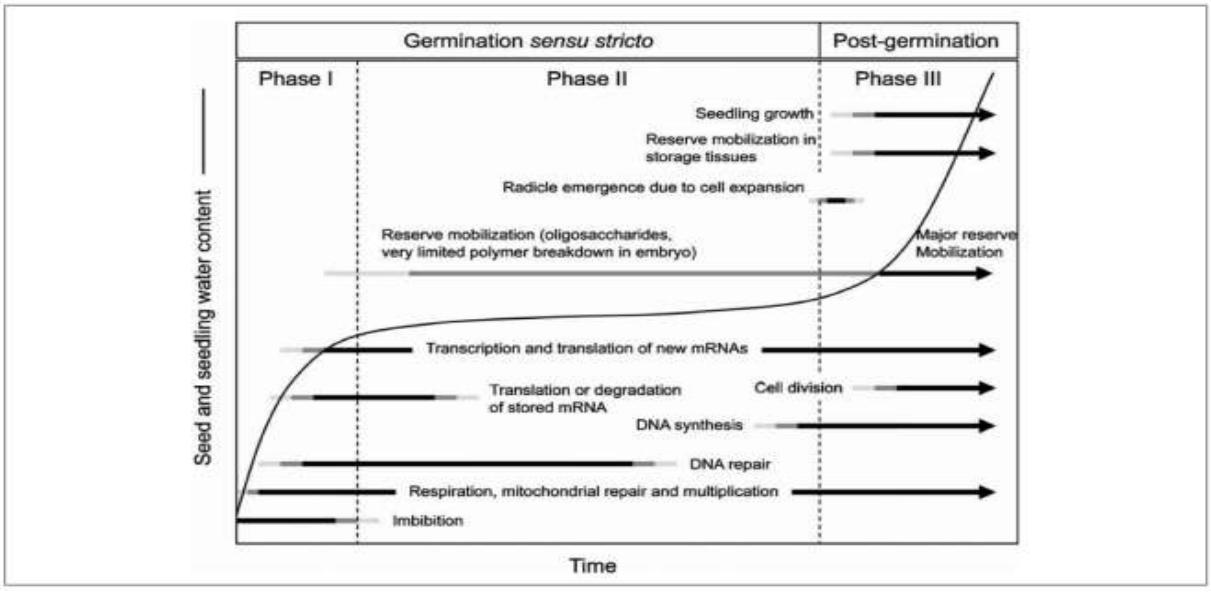


Figure 4: Physiological changes during germination and water uptake (Lemmens et al., 2018)

From a plant physiological point of view, germination is the evolution releasing seeds from dormancy (Figure 4). It starts by water uptake and is completed with the appearance of the radicle (Nonogaki, Bassel, & Bewley, 2010).

4.1 Impact of sprouting on nutritional properties

Sprouting is a natural process known by improving pulses and cereals nutritional properties through an increase on nutrients and bioactive compounds levels (Donkor et al., 2012) (Table 2). Evolution of nutrients depends on sprouting condition used (Yang et al., 2001) and sample characteristics (genetic background, geographic origin, agronomic practices) (Lee et al., 2016).

During sprouting several changes take place: degradation of some molecules, delocalization of nutrients and synthesis of new metabolites (Lorenz and D'Appolonia, 1980). Consequently, digestibility of some macro-molecules (proteins, starch) could be improved. The impact of sprouting on wheat nutrients are summarized on Table 2.

Previous results of Zilic et al. (2016) showed that sprouting led to significant quantitative and qualitative changes on whole wheat flour proteins: In fact, there was an increase on the content of high molecular weight polypeptides and an increase in low molecular weight polypeptides. Moreover, the authors reported beneficial effects of steeping pre-treatment, previous to sprouting, on the antigenicity of the glutenin fraction (Zilic et al., 2016).

The lipid reserves on wheat are stored mostly in oil droplets in the scutellum and aleurone (Morrison, 1994). The principal fatty acids on wheat lipid fraction are palmitic (16: 0), stearic (18: 0), oleic (18: 1), linoleic (18: 2) and linolenic (18: 3), and the composition of the major lipid groups is highly unsaturated. Although, sprouting did not affect wheat total lipid content (Hung et al., 2011) changes on fatty acids composition could be observed after sprouting as highlighted by Marton et al. (2010): In fact, results of this study showed that after sprouting wheat for three days there was a decrease on stearic and oleic acid proportions while there was an increase on palmitic, linoleic, α linolenic ones (Marton et al., 2010). Such changes improve wheat nutritional properties.

Ash content is related to the mineral composition. Minerals are mainly concentrated in bran layer and the portion of endosperm adjacent to bran on wheat kernel (Sezer et al., 2017). Semolina or flour ash content is an important parameter to know as it affects cereal products formulation (color, fermentation, dough strength). Ash content depends on wheat (genotype,

growing conditions, fertilization, weather conditions, disease) and milling (Sezer et al., 2017). Probably, for these reasons evolution of ash content after sprouting is different on previous studies.

Table 2: Effect of sprouting on wheat nutrients and bioactive compounds

Nutrient	Evolution	References
Protein	No effect	Hung et al., 2011
	Increase	Mak et al., 2009; Hung et al., 2015 ; Sighkornart et al., 2014
Free amino acids	Increase	Hung et al., 2011
Albumin/Globulin	Increase	Koheler et al., 2007
Gluten	Decrease	Koheler et al.,2007
Lipid	No effect	Hung et al., 2011 ; Sighkornart et al., 2014
Ash	Increase	Hung et al., 2011 ; Hung et al., 2015 ; Ozturk et al., 2012
	Decrease	Aryama & Khan, 1990 ; Sighkornart et al.,2014
Fiber	Increase	Hung et al., 2011 ; Koheler et al., 2007
β Glucan	Decrease	Sighkornart et al., 2014
Arabinoxylan	No effect	Sighkornart et al., 2014
GABA	Increase	Hung et al., 2015
Starch	Decrease	Hung et al., 2011
Reducing sugars	Increase	Sighkornart et al., 2014
Total phenol content	Increase	Alvarez-Jubete et al., 2010 ;Hung et al., 2010 ; Hung et al., 2011 ; Panfil et al., 2014 ; Zilic et al., 2014
Anti-oxydant activity	Increase	Alvarez-Jubete et al., 2010 ; Hung et al., 2010 ; Hung et al., 2011 ; Zilic et al., 2014
Vitamin E	Increase	Yang et al., 2001
	Decrease	Plaza et al., 2003
α-tocophérol	Increase	Ozturk et al., 2012. Yang et al., 2001 Zilic et al., 2014
	Decrease	Plaza et al., 2003
β + δ Tocopherol	Increase	Zilic et al., 2014
β-caroténe	Increase	Yang et al., 2001
Vitamin A	Increase	Plaza et al., 2003
Vitamin B1	Increase	Plaza et al., 2003
	Decrease	Zilic et al., 2014
Vitamin B2	Increase	Plaza et al., 2003 ; Zilic et al., 2014
Vitamin B6	Increase	Plaza et al., 2003
	Decrease	Zilic et al., 2014
Vitamin C	Increase	Plaza et al. 2003 ; Yang et al. 2001
Folate	Increase	Hefni and Witthoft 2012; Hefni and Witthoft 2011 ; Koheler et al.2007

Wheat carbohydrates include starch and fibers. Sprouting induces starch degradation under α amylase action (Singh et al., 2001). This bioprocess leads also to an increase on fiber content (Hung et al., 2015), particularly arabinoxylan fraction (Sighkornart et al., 2014). The role of fibers on the prevention from some diet related diseases such as cardiovascular diseases or

type 2 diabete is well known (Fardet, 2010). Arabinoxylan present a nutritional interest as it contributes on decreasing cholesterol and improving mineral adsorption (Donkor et al., 2012).

Micronutrients (minerals and vitamins) play an important role on human nutrition for a normal developement. For most low-income rural and urban population groups plant origin foods are the main sources of micronutrients (Ortiz-Monasterio et al., 2007). Interestingly, sprouting increases vitamins (A, B9, C, E) and bioactive compounds (total phenols, carotenoids) levels. Consequently, wheat antoxidant properties are improved.

Regarding health benifits of sprouted wheat consumption, to the best of our knowledge, there are no preclinical and clinical studies investigating the effect of sprouted wheat consumption on human health. Most papers describe biochemichal changes induced by sprouting and extrapolate potential health benifits of specific nutrients based on a cause-effect links described previously in literature. Previous epidemiological studies found correlation between whole grain consumption and reduced risk of developing cardiovascular diseases, type 2 diabete and some cancers (Lemmens et al., 2018). Moreover, sprouts are known as easier to digest thanks to enzyme activity leading to macromolecules degradation (Pagand et al., 2017). Thus, cereals sprouts contain oligosaccharides as well as fibers, minerals which may stimulate probiotic bacteria growth (Hubner and Arendt, 2013). Besides positive effect on probiotics, sprouted edible seeds have been reported with a number of bioactivities such as antiinflamatory, antibacterial, antidiabetic and anticancer (Gan et al., 2017). These bioactivities can be associated with the accumulation of different bioactive compounds (polyphenol, vitamins, and peptids). Consequently, sprouts could be consumed as a part of our diet to prevent some chronic diseases.

4.2 Sprouts, a minimally processed food?

Minimally processed food is a generic term. In fact, this denomination includes: fresh-cut, ready-to-serve, ready-to-eat, ready-to-cook, cook-chill, cook-freeze and part baked products (Siddiqui & Rahman, 2015). These minimally processed foods could be plant based (vegetables, fruits…) or animal based (fermented milk, curd…). During the last decades, there was a growing demand for these products (Bansal et al., 2015). In fact, consumers are looking for “fresh-like” products with no are less synthetic additives (Siddiqi & Rahman, 2015). Consequently, minimally processed foods should meet the consumers’ expectancy in terms of freshness, nutritional and sensory attributes. Meanwhile, these products must be safe. Generally, refrigeration is the main method used for minimally food preservation. As it is

hard to maintain the temperature sufficiently low throughout the chain of production and processing to consumption further methods are required to control the growth of spoilage and pathogenic microorganisms (Gorris, 1998). The used methods should be adapted to fresh like products. Table 5 presents some examples of techniques used to extend minimally processed foods shelf life. Besides the use of suitable methods it is important to select well raw materials (hygienic quality) and to respect good manufacturing practices.

Table 3: Examples of methods used for shelf-life extension of minimally processed products (Bansal et al., 2010; Ohlsson, 2003)

	Method	**Target**
Chemical agent	Acidulants (Citric acid)	Enzymatic browning
	Antioxidant (Ascorbic acid)	Enzymatic browning
	Antimicrobials (Hypochlorite)	Microbial contamination
	Eseential oils	Enzymatic browning, microbial growth
Non thermal processing methods	High-pressure treatment	Microbial contamination
	Gamma irradiation	Microbial contamination
	High-electric-field pulses	Microbial contamination
	Thermosonication	Microbial contamination
New thermal processing methods	Ohmic heating	Optimized heating regime reduces levels of microorganisms while minimizing thermally induced quality losses
	High-frequency heating	
	Microwave heating	
New packaging technologies	Modified-atmosphere packaging and active packaging	Antimicrobial effect
	Edible films	Protection against oxygen ingress, moisture loss, and flavor loss

Sprouts could be considered a minimal processed food: ready-to-eat. Consumption of sprouted seeds might be recommended to the nutritional interest of these products. Unfortunately, several outbreaks related to raw sprouts consumption. For example, from 1996 to Auguste 2018, there were 50 reported outbreaks of foodborne illness in the United States

associated with sprouts (FDA (online), 2019). *Salmonella* and *Escherichia coli* O157:H7 have been consistently linked with sprout-associated outbreaks (EC, 2002). In fact, sprouts production relies on four main steps:

- Seed receipt and storage
- Seed treatment and rinsing: Seeds are treated to reduce pathogens.
- Pre-germination soak: Seeds are pre-soaked in water to initiate sprouting.
- Germination and growth: Seeds are transferred to growing containers such as bins, trays or rotary drums for germination.

Water availability during the different steps may lead to bacterial proliferation. For this reason, it is important to select acceptable raw seeds and to respect good manufacturing practices.

5. Conclusion

Cereal grains constitute a major source of dietary nutrients for all people, all over the world. However, the nutritional quality of cereal grains and sensory properties of their products are considered as inferior because of their lower protein content and availability, deficiency of certain essential amino acids, lower starch availability, and the existence of certain anti nutritional compounds, as well as the coarse nature of the grains. Sprouted cereal, legume, and other seeds are used for human consumption. Controlled sprouting of grains leads to increase activities of hydrolytic enzymes, to improve the contents of certain essential amino acids, total sugars, and B group vitamins, and to decrease dry matter, starch, contents as well as to reduce anti nutritional factors. The digestibility of storage proteins and starch are enhanced by their partial hydrolysis during sprouting. Therefore nutritional improvement of cereals can be achieved by sprouting and utilization of sprouted cereals in traditional and processed foods.

6. Aims of the thesis

In recent years, whole grains consumption is recommended in the overall dietary guidelines of many countries around the world. Although they are characterized by many benefits on human health, whole grains contain several anti-nutritional factors which lessen their nutritional quality leading to a poor use in human diet.

Sprouting is a low-cost technology which starts with seed water uptake and ends at the protusion of radicle from the seed. Literature has reported to be associated with improvements in the nutritive value of seeds, and the decrease in anti nutritional compounds. Sprouted grains are widely accepted as a functional food because of their nutritious and health benefits. In fact, consumers are showing an increasing positive attitude toward sprouted products and expect them to be "natural", "better taste", "more nutritious", and "healthier" (Lemmens et al., 2018). Therefore, using sprouted grains in food formulations is becoming increasingly popular in the marketplace and represents an emerging trend in health foods.

The overall objective of this thesis is the contribution in meeting current dietary goals through the improvement of wheat nutritional properties, particularly of durum wheat (*Triticum durum*). The choice of durum wheat was based on its availability in market: Tunisian production of durum wheat exceeds soft wheat. For exemple in 2018-2019 durum reached around 1.2 millon of tones while soft wheat production was did not exceed 185 300 (Kapitalis (online), 2019). An other encaraging factor was the widespread use of durum weat in different Tunisian products: pastas (couscous,nwasser, hlelem…), bsissa, bread, makroudh…

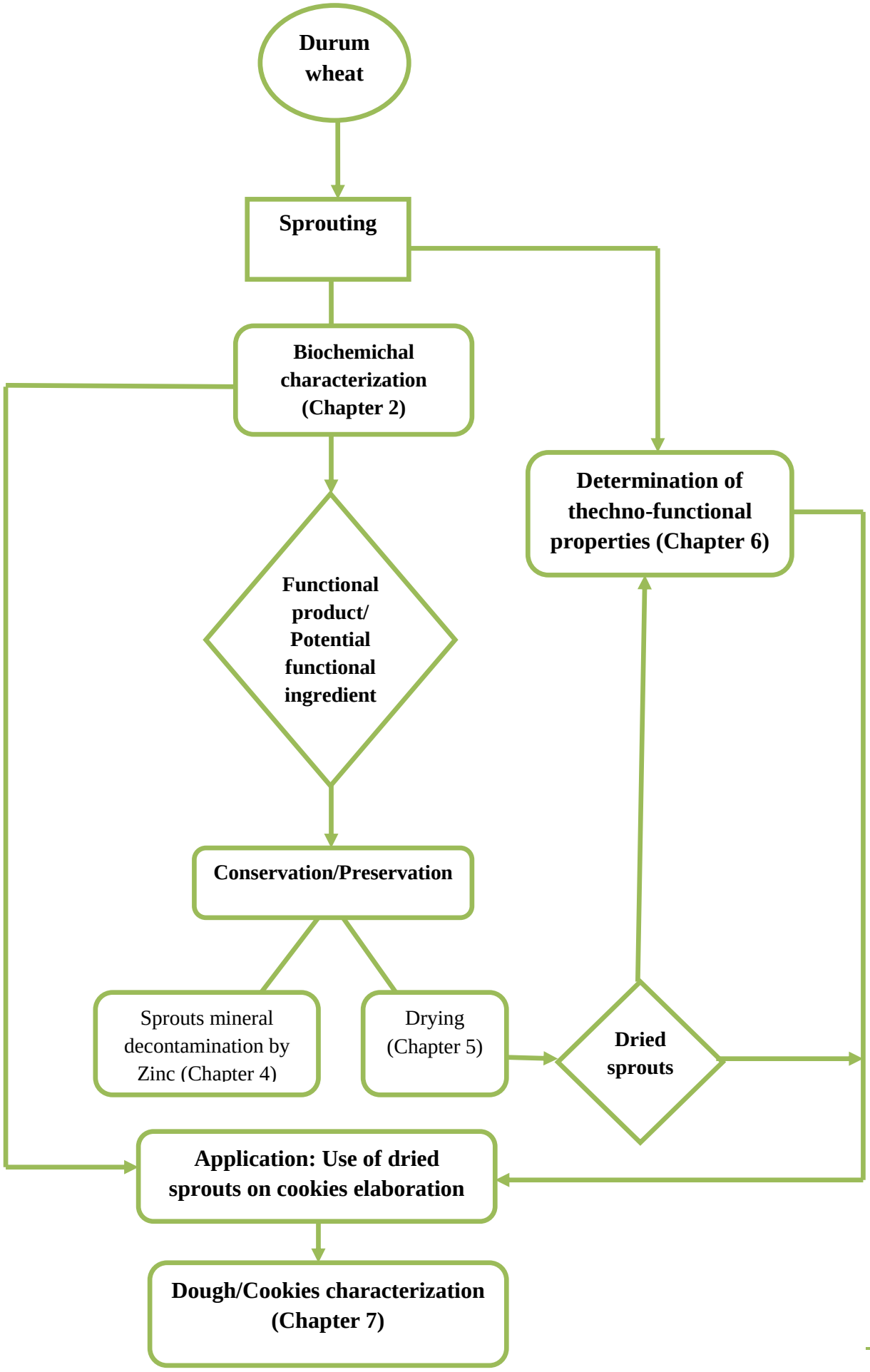
The studies conducted on this thesis are described on chapter 2-7 while chapter 8 is reserved to discuss the main findings, conclusions and implications of the studies described in the preceding chapters. In fact, this research relies on three main parts:

- The first part was reserved to investigating the impact of sprouting bioprocess on durum wheat seeds. An interest was accorded also to the genetic background of the tested samples: 6 cultivars were evaluated: two landrace ones ("Chili" and "Hadhba") and four high yielding cultivar ("Karim", "Khiar", "Maali" & "Razzek"). Accordingly, in this part seeds were sprouted and nutritional attributes were assessed through proximate composition (Ash, lipid, protein, starch, reducing sugars), bioactive compounds (carotenoids, total phenol content, tocopherols and vitamin C) and antioxidant activity (Chapter 2). Added to nutritional properties, the impact of sprouting on prebiotic index was investigated through an in vitro digestion experiment (Chapter 3). The significant increase on bacterial growth after sprouting led to second part.
- The second part focused on sprouts preservation/ conservation. Sprouting improved nutritional attributes. Thus sprouts might be suggested as a functional food. Meanwhile, the increase on bacterial growth makes crude sprouts consumption risky.

In this context two approaches were tested: Firstly, for crude sprouts preservation, mineral decontamination through Zinc use was suggested (Chapter 4). Factorial design was used for experimental condition (Zinc solution concentration, soaking duration and sprouting time) optimization. Microbiological and nutritional properties were assessed to evaluate the approach efficiency. Secondly, drying was used for sprouts conservation and their further use as a functional ingredient on cereal products elaboration (Chapter 5). Different drying methods were tested: lyophilisation, oven drying and micro-wave vacuum drying. The impact of drying method on physic-chemical, thermal and functional properties was studied.

- The third part explored the potential use of dried whole wheat flour (24 and 48 h) on cookies elaboration (Chapter 7). On a first step, evolution of sprouted whole wheat flour functional, thermal and pasting properties with sprouting time (from 12 to 72 h) were assessed (Chapter 6). Then, sprouted whole wheat flour (24 and 48 h) and blends of refined/ sprouted whole wheat flour (50:50) were used for cookies making. Flour functional properties were determined as well as dough rheological parameters. Cookies physical properties and consumers' acceptance were evaluated.

Materials used and methods are specified in each chapter separately.


Durum wheat
Sprouting
Biochemichal characterization (Chapter 2)
Determination of thechno-functional properties (Chapter 6)
Functional product/ Potential functional ingredient
Conservation/Preservation
Sprouts mineral decontamination by Zinc (Chapter 4)
Drying (Chapter 5)
Dried sprouts
Application: Use of dried sprouts on cookies elaboration
Dough/Cookies characterization (Chapter 7)

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Chapter 2: Sprouting bioprocess as a sustainable tool for enhancing durum wheat (Triticum durum) nutrients and bioactive compounds

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Original Article

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

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

Abstract

Consumers are more aware of the role of healthy diet on preventing food related diseases (Cancer, cardiovascular diseases, diabetes…). In this study we aimed at using a natural bioprocess to improve durum wheat "*Triticum durum*" nutritional properties for its further use as functional ingredient. Six Tunisian cultivars were tested: four high yielding and two landrace ones. Seeds were germinated for 48 hours at 22°C. 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 showed the best performances. Results provided by our study proved that sprouting is an interesting natural tool to use in food industry for the development of cereal products with added nutritional value.

Key words: Durum wheat, nutritional properties, sprouting, vitamins, bioactive compounds

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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) (Delgado-Andrade, 2017) and since the market of functional foods spread all over the world (Martirosyan and Singh, 2015) and took its consideration in food industry. Functional foods could be defined as "Foods with physiological benefits that can reduce the risk of chronic diseases" (Austria *et al.*, 2016). Thus, they could be obtained by: (i) adding substances (already present on the original products), (ii) substituting a component by healthier analogue or (iii) removing a component known for its undesirable effects (Poulsen, 1999). Functional foods' health effect requires their consumption among a varied diet, regularly and at acceptable levels (Delgado-Andrade, 2017).

Cereals are an important part of human diet due their composition offering carbohydrates, proteins, fibers, vitamins… Wheat is the first cereal cultivated all over the world with a surface of more than 220 million hectares in 2014 (FAO 2017) 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 (Ammar *et al.*, 2011).

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. (2014) have shown a better quantitative quality in landrace varieties while others have suggested that breeding did not affect health promoting components such as gluten and dietary fibers (De Santis *et al.*, 2017; De Santis *et al.*, 2018)

Sprouting is an old food engineering tool, used mainly in Eastern countries (China, Japan…) (Plaza *et al.*, 2003). "Sprouts" are obtained from the germination of true seeds and their development in water, collected before the development of leaves. The final product still contains the seed (EFSA, 2011).

Sprouting Bioprocess is known by its effect on improving nutritional properties of different kind of seeds such as pulses and cereal grains (Donkor *et al.,* 2012). Numerous studies investigated the effect of sprouting on wheat (*Triticum aestivum*) nutritional properties and bioactive compounds (Alvarez-Jubet *et al.,* 2009; Chen *et al.,* 2017; Mak *et al.,* 2009; Ozturk *et al.,* 2012; Zilic *et al.,* 2014). 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 aim of the following research was to evaluate and compare the effect of this bioprocess on different Tunisian Landrace and high yielding durum wheat cultivars for their further use in food industry as a functional ingredient.

2. Materials and methods

2.1Materials

Six Tunisian cultivars of durum wheat (*Triticum durum*) were selected for this study:

- Four high yielding varieties:

-Karim: The most grown cultivar in Tunisia, made at the International Maize and Wheat Improvement Center (CIMMYT) introduced in 1973 and registered in 1982.

-Razzek: Obtained from a cross made in Tunisia in 1976, registered in 1987.

-Khiar: Introduced in 1987 from a cross made at CYMMYT and registered in 1992.

-Maali: Obtained from a cross made in Tunisia in 1992, registered in 2008.

- Two landraces:

-Chili: Introduced from France, pure line registered in 1953.

-Hadhba: Landrace first introduced from Algeria in 1924 (Ammar *et al.,* 2011).

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.

2.2Methods

2.2.1In vitro sprouting

Seeds (50g) were firstly sterilized with 1% (V/V) hypochlorite sodium solution during 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 during 48 hours (Hung et al. 2015).

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

2.2.2Proximate composition

Ash content was determined according AACC method (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 Fehling method, and starch measurement, through an hydrolytic method and titration with $Na_2S_2O_3$.

2.2.3Enzyme activity determination

Amylolytic and α-amylase activities were measured as suggested by Kalita et al. (2017). 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 the following formula:

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

2.2.4Vitamins and Bioactive molecules

Tocopherols and Vitamin C contents were assessed by the HPLC procedure as described by Molnar et al. (2017): 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. (2017).

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

2.2.5Antioxidant 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 the extraction procedure for antioxidant activity measurement: The extraction was made with 80% (v/v) aqueous methanol solution, for 2 h at 37 °C. Samples were afterwards centrifuged at 6,000 RPM for 30min. The supernatant was used for the determination of antioxidant capacity.

Antioxidant activity was calculated according to the following formula:

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

2.3 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

3.1Effect 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 4 sprouting led to modifications in the wheat seeds composition.

Ash content was significantly different from one cultivar to another. It ranged between 1.65 and 2.06 % before sprouting. Sprouting contributed to a decrease in ash content. The decrease was only significant for Razzek, Maali (high yielding varieties) and Chili (durum wheat landrace). A previous study of Singh et al. (2017) reported a decrease in ash content for sprouted sorghum while other results showed that three days germination could not affect ash content of wheat (Singkhornart *et al.,* 2014). 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 (Hung *et al.,* 2015). Thus, ash content evolution after sprouting could be probably related to the genetic background of the seeds used (Lee *et al.,* 2016).

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 Hadhba and the second with lower protein content. Tunisian landrace durum wheat are recognized for their better quantitative quality parameters compared to the high yielding ones (Daaloul-Bouacha *et al.,* 2014). Our data clearly showed that sprouting increased protein content. This increase was probably due to proteolytic enzyme activity. In fact, protein degradation depends on sprouting conditions (temperature, duration) (Koehler *et al.,* 2007). The significant increase observed in our study was in agreement with previous results (Hung, Maeda & Morita 2015; Singkhornart *et al.,* 2014). 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

(Shafqat, 2013). Results showed that after two days of sprouting with SDS-Page an hydrolysis in protein was observed. The author reported that an increase in sprouting time leads to an increase low molecular weight proteins and a decrease in high molecular weight ones (Shafqat, 2013). 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 (Zilic *et al.*, 2016). In this study, five days sprouting led to changes in proteins proportions and solubility rather than their amounts (Zilic *et al.*, 2016). According to the authors, the release of low molecular weight proteins could present a high nutritional interest thanks to the bioactive role of these peptides (anti-bacterial, anti-carcinogenic, anti-thrombocytic or stimulating biological activity…) (Zilic *et al.*, 2016).

Table 4: Effect of sprouting on durum wheat seeds composition

Cultivar		Ash (%)	Lipid(%)	Protein (%)	Starch (%)	Reducing sugar (mg/g 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}
Hadhba	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 same column that do not share same letters are significantly different, according to Fisher's test.($p<0.05$).

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 Hadhba (1.34%±0.11) before sprouting. In fact, cultivars showed different behaviors after sprouting. The two varieties 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 Hadhba. 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 lead to a change in wheat lipid content (Singkhornart *et al.,* 2014). Nevertheless, some qualitative results could suggest that sprouting would change fatty acid composition of soft wheat (*Triticum aestivum*) (Ozturk *et al.,* 2012). The fatty acid composition after sprouting evolved differently, according to the tested varieties, highlighting the role of genetic properties (Ozturk *et al.,* 2012). Consequently, the evolution of endogenous lipids after sprouting may be linked to selectivity of lipases (Gertis *et al.,* 2015). Rose and Pike (2006) 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 condition for lipase should be specified to the variety tested.

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 4). This increase ranged between 2 and 4 folds according to cultivars and it was related to amylolytic enzyme's action (Fardet, 2010). 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 (Mak *et al.,* 2009). In our study results of amylolytic enzymes (Table 5) 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 (Kalita *et al.,* 2017).

The first step of sprouting consists in water absorption and tissue rehydratation or "Imbibition". Imbibition stimulates gibellirin hormone synthesis, which then stimulates hydrolytic enzymes activities (Nelson *et al.,* 2013). Enzymatic activity depends not only on genotypes and environmental area, but also on sprouting conditions (Temperature, duration) (Kalita *et al.,* 2017).

Table 5: Effect of sprouting on enzyme activity

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}
Hadhba	Raw	4.83±0.03 ^{FG}	3.30±0.10 ^{F}
	Sprouted	6.98±0.19 ^{B}	8.52±0.27 ^{D}

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

3.2Vitamins and bioactive molecules

Results of vitamins C and E measurement are summarized in Table 6. Vitamin C is a water-soluble vitamin that is naturally present in some foods. Humans are unable to synthesize vitamin C endogenously. So, 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 6). Interestingly, sprouting increased significantly ($p<0.05$) vitamin C content of durum wheat seeds. Averages ranged between 15.10 $\mu g.g^{-1}$ dm (durum wheat landrace Chili) and 43.9 $\mu g.g^{-1}$ 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 differs among authors from 4 up to 7 days (Plaza *et al.,* 2003; Yang *et al.,* 2001). Biosynthesis of vitamin C includes several enzymatic reactions to transform D-glucose to L-ascorbic acid (Pérez-Balibrea *et al.,* 2011). Consequently, carbohydrates level (glucose, sucrose) plays a key role in this process. Sprouting increased significantly reducing sugars amounts (Table 4) which probably contributed in increasing vitamin C levels as a strong positive correlation was observed in our study (Pearson correlation r=0.81, p=0.00).

Table 6: Effect of sprouting on vitamin C and tocopherols contents

Cultivar		Vitamin C (µg.g^{-1} 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}
Hadhba	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 same column that do not share 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) (Méne-Saffrané and Pellaud, 2017). Results of vitamin E measurement (Table 6) 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 (Ozturk *et al.,* 2012) while another study showed an increase in vitamin E of 25% after 4 days of germination (Finney 1985). Moreover, Yang et al. (2001) showed that the maximum of vitamin E was obtained after sprouting for 8 days. Thus the increase in tocopherol would probably depend on germination conditions used as well as genetic background of the tested seeds. The biosynthesis pathway of vitamin E has not been reported previously (Gan *et al.*, 2017).

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 have higher biological activity than ß-tocotrienol (Méne-Saffrané and Pellaud, 2017).

Total phenol content ranged between 15.9 and 29.44 mg GAE.g^{-1} dm for raw seeds (Table 7). Averages were significantly different among cultivars. Durum wheat landrace showed the highest amounts. Dordevic et al. (2010) reported an average of 16.2mg GAE.g^{-1} dm for total phenol content for wheat seeds. Bioactive molecules amounts, such as polyphenols are related geographic zones where plants were grown, genotypes and the procedure used for extraction and quantification (Lee *et al.*, 2016). 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 Hadhba 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 (Gan *et al.*, 2017). As sprouting is marked by degradation of macromolecules like starch, amounts of simple sugars may increase. Thus 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 (Chen *et al.*, 2017).

Carotenoids are organic bioactive molecules produced by plants. They may play a role of provitamin A and contribute in the antioxidant properties of foods. The high yielding variety Khiar and the landrace Chili and Hadhba showed the highest total carotenoid pigments (19.55; 18.53; 17.72mg.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) (Pasqualone *et al.*, 2017). This difference might be related to different genetic background 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 antioxidant properties of sprouted seeds.

Table 7: 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}
Hadhba	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 same column that do not share 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 Hadhba (36.87%). An average of 12.7% was previously reported for remilled semolina (Pasqualone *et al.*, 2017). 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 (Hung *et al.*, 2011). 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).

In the other hand, it is important to remind also that plants have some antioxidant enzymes such as peroxidase (POD), catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD). Germination may activate these enzymes (Jin *et al.*, 2017). For example, a significant increase in catalase, peroxidase and ascorbate peroxidase activities in sprouted soft wheat were observed (Chen *et al.*, 2017).

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 a 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 of antioxidant properties. Interestingly, among Tunisian cultivars durum wheat landrace genotypes showed better performance than the high yielding. Altogether, our study clearly showed that sprouting contributed in improving nutritional properties of durum wheat seeds. Thus, it could be suggested as a green tool for the development of functional ingredient and cereal products with added nutritional value for food industry.

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Chapter 3: Influence of sprouting bioprocess on durum wheat (Triticum durum) prebiotic properties

Influence of sprouting bioprocess on durum wheat (*Triticum durum*) prebiotic properties

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Influence of sprouting bioprocess on durum wheat (*Triticum durum*) prebiotic properties*

Abstract

Sprouting has been widely used as a green engineering tool improving cereals and pulses nutritional properties. Thus, sprouts could be suggested as a functional food. In this study, we aimed to evaluate the role of sprouting bioprocess in enhancing durum wheat (*Triticum durum*) prebiotic properties, through the use of an *in vitro* digestion model. The methodology consisted in sprouting two different cultivars of durum wheat "Karim" (a modern cultivar) and "Chili" (a landrace old one) for 48 hours and then digest them to calculate the prebiotic index. Results showed that the tested cultivars had a positive prebiotic index either before or after sprouting. Interestingly, this bioprocess increased prebiotic index (+62.7% for "Karim" and +14.4% for "Chili"). However, the intensity of evolution for this parameter was dependent on the genetic background. In conclusion, our study showed that sprouting is a sustainable tool for enhancing prebiotic properties, and therefore gut health.

Key words: Durum wheat, sprouting, in vitro digestion, prebiotic index.

Options Méditerranéennes : Série A. Séminaires Méditerranéens, 124, 84-94.*

1. Introduction

Nowadays, consumers' awareness about the link between their health status and the diet they adapt rose considerably. Since the human gut microbiota has been shown to play a major role in the health of the host (Markowiak and Śliżewska 2017), the manipulation of the composition of the intestinal flora is currently attracting interest for a potentially more healing community. Dietary fibres, and prebiotics are all dietary components that can play a critical role in maintaining a healthy gut microflora. Prebiotics are non-digestible food ingredients that beneficially stimulates growth or/and activity of one or a limited number of beneficial bacteria in the colon (Grootaert *et al.,* 2007). In fact, any foodstuff that reaches the colon, such as non-digestible carbohydrates, can be a prebiotic candidate. Various types of fibres and prebiotics could influence specifically Lactobacillus and Bifidobacterium populations. Lactobacillus spp. and Bifidobacterium spp. are common markers for gut health since they could down-regulate gut inflammation, alleviate irritable bowel syndrome symptoms, stimulate immune functions, help in mineral absorption and produce little, if any, gas or known carcinogenic substances (Krumbeck *et al.,* 2016; Markowiak and Śliżewska, 2017). Thus, improving products with functional food ingredients such as fibres and prebiotics can satisfy consumer demands for foods with benefits beyond basic nutrition.

Sprouting is an old green tool used to improve cereals and pulses nutritional properties (Donkor *et al.,* 2012). This bioprocess is marked by a degradation of some storage molecules (proteins, starch...) under enzymatic activities (Mak *et al.*, 2009) and synthesis of bioactive molecules (Carotenoids, polyphenols, vitamins...) (Jribi *et al.*, 2019 a; Plaza *et al.,* 2003). Consequently, digestibility could be improved. Wheat and its by-products (such as bran) are recognized by their promoting prebiotic effect on probiotic microorganisms (Al-Sheraji *et al.,* 2013; Terpou *et al.,* 2018). The role of sprouting in improving nutritional properties has been highlighted previously (Gan *et al.,* 2017). However, to the best of our knowledge, durum wheat sprouts behavior during digestion and the impact of genetic background have not been reported. Experimental in vitro digestion models are widely used for the study of structural changes, digestibility and release of food compounds in gastrointestinal-like conditions. In fact, clinical trials are quite expensive and time consuming, and may raise ethical concerns (Minekus *et al.*, 2014; Ting *et al.,* 2015). Added to nutritional characterization of functional foodstuffs, an understanding of food components behavior during digestion is needed to prove the suggested physiological effects. Therefore, *in vitro* digestion model could be suggested as a useful alternative to overcome these problems. Dupont (2016) specified in his review, the

composition of the simulated gastrointestinal media. Most of these media contain digestive enzymes (pancreatin, pepsin, trypsin, chymotrypsin, peptidase, α-amylase and lipase), bile salts and mucin. The experimental conditions in these models are a digestion temperature of 37 °C and an incubation time of 2 hours.

Thus, the aim of this study was to evaluate the effect of sprouting bioprocess in durum wheat (*Triticum durum*) prebiotic properties, through the use of an *in vitro* digestion model. As some studies reported differences between old and modern cultivars due the impact of breeding (Morris and Sands, 2006), an interest was also accorded also to the genetic background of samples.

2. Materials and Methods

2.1 Plant material

Two Tunisian cultivars of durum wheat (*Triticum durum*) were selected for this study: a high yielding one, Karim, (the most grown cultivar in Tunisia), and a landrace Chili (an old cultivar). Samples (harvested in 2015) were kindly provided by the National Institute of Cereal crops (INGC) (Bou salem, Tunisia) and the Bank of Genes (Tunis, Tunisia).

2.2Sprouting procedure

Sprouting was conducted exactly as described in previous study of Jribi et al (2019)[b]. After sprouting, samples were immediately subjected to lyophilisation (Christ freeze dryer alpha 1-4 LCS, Germany) then milled (Retsch Grindomix GM 200, Germany) and stored at -18°C until analysis.

2.3In vitro digestion

Samples were digested according to the model developed by the Food Science Research Institute (Budapest, Hungary) (Figure 5). The model was mainly based on Versantvoort et al. (2005) protocol (without glucose in the gastric juice). It also contained elements from the COST Infogest model (Minekus *et al.*, 2014), like snap-freezing samples in liquid nitrogen.

The colon phase was modelled by inoculating the digested samples with a bacterial mixture made of *Bifidobacterium longum* subsp. *infantis* NCAIM B.01821, *Lactobacillus casei* 2756, *Escherichia coli* ATCC 8739, *Clostridium perfringens* ATCC 13124 at 10^6 CFU (colony-forming unit)$\cdot$ ml^{-1} concentration for each. This step was followed by an anaerobic incubation for 24 h. Plate counting was performed on selective media: *Bifidobacterium* - BSM agar (Fluka Analytical 88517, SIGMA-ALDRICH CHEMIE Gmbh, Riedstr. 2-D89555 Steinheim,

Germany. Product of Switzerland), *Lactobacillus* – Rogosa agar (Rogosa Agar, Fluka Analytical 83920, SIGMA-ALDRICH CHEMIE Gmbh, Riedstr. 2-D89555 Steinheim, Germany. Product of Switzerland) , *E. coli* – Harlequin™ E.coli/Coliform agar (Harlequin™ E.coli/Coliform Medium, LABM HAL008, Lab M Limited, Heywood, United Kingdom) , *Clostridium* – TSC agar (Tryptose Sulfite Cycloserine Agar (TSC Agar), Scharlab 01-278, Barcelona, Spain).

In vitro digestion experiments were conducted in duplicate.

2.4Prebiotic index

The growth rate is based on the rate between the number measured at the end and at the beginning of the experiment. Almost every research group (exception: Vulevic et al. 2004) compares the growth rate of a given bacteria with the growth rate of total bacteria.

In the equations presented below the following abbreviations will be used: FA= the number of favourable bacterium unit; AD= the number of adverse bacterium unit; TOT=the number of total bacteria in the system; t (h) = the final moment of the measurement; 0 h=the beginning of the measurement.

The following equations can be found in the scientific literature. Some equations are based on colony forming unit (CFU), others on its natural or 10 based logarithm:

$$\mathbf{PI= (FA_{t(h)}-FA_{0h})/TOT_{t(h)}- (AD_{t(h)}-AD_{0h})/ TOT_{t(h)}}$$ **(Equation 1)** (Manderson *et al.*, 2005; Olano-Martin *et al.*, 2002).

(The abbreviations represent the number of the given bacterial group at the given time point in logarithm base 10 of CFU).

$$\mathbf{PI = (FA_{t(h)}-FA_{0h})/t-(AD_{t(h)}-AD_{0h})/t}$$ **(Equation 2)** (Vulevic *et al.*, 2004).

where t means the incubation time , the abbreviations represent the number of the given bacterial group at the given time point in natural logarithm of CFU.

$$\mathbf{PI=(FA_{t(h)}/FA_{0h})/(TOT_{t(h)}/TOTt_{0h}) - (AD_{t(h)}/AD_{0h})/(TOT_{t(h)}/TOT_{0h})}$$ (Equation 3)

(Barczyńska *et al. 2015*; Depeint *et al. 2008*; Mandalari *et al.*, 2008; Śliżewska, 2013).

The abbreviations represent the number of the given bacterial group at the given time point in CFU.

The method applied in this research is the combination of the 1st and the 3rd method. We count with the logarithm base 10 of the CFU's, but the equation is the same as in the 3rd method:

$$\mathbf{PI = (FA_{t(h)}/FA_{0h})/(TOT_{t(h)}/TOTt_{0h}) - (AD_{t(h)}/AD_{0h})/(TOT_{t(h)}/TOT_{0h})} \text{ (Equation 4)}$$

In other words:

$$\mathbf{PI = Bif + Lac - Eco – Clos} \text{ (Equation 5)}$$

Where **Bif = (log Bif Tx / log Bif T0) / (log Tot Tx / log Tot T0)** (Equation 6).

Equation (6) was applied for all terms of Equation (5) and **Bif -** number of *Bifidobacterium* CFUs, **Lac -** number of *Lactobacillus* CFUs, **Eco** - number of *Escherichia* CFUs, **Clos -** number of *Clostridium* CFUs; **Tx** – at sample time; **T0** – at inoculation time.

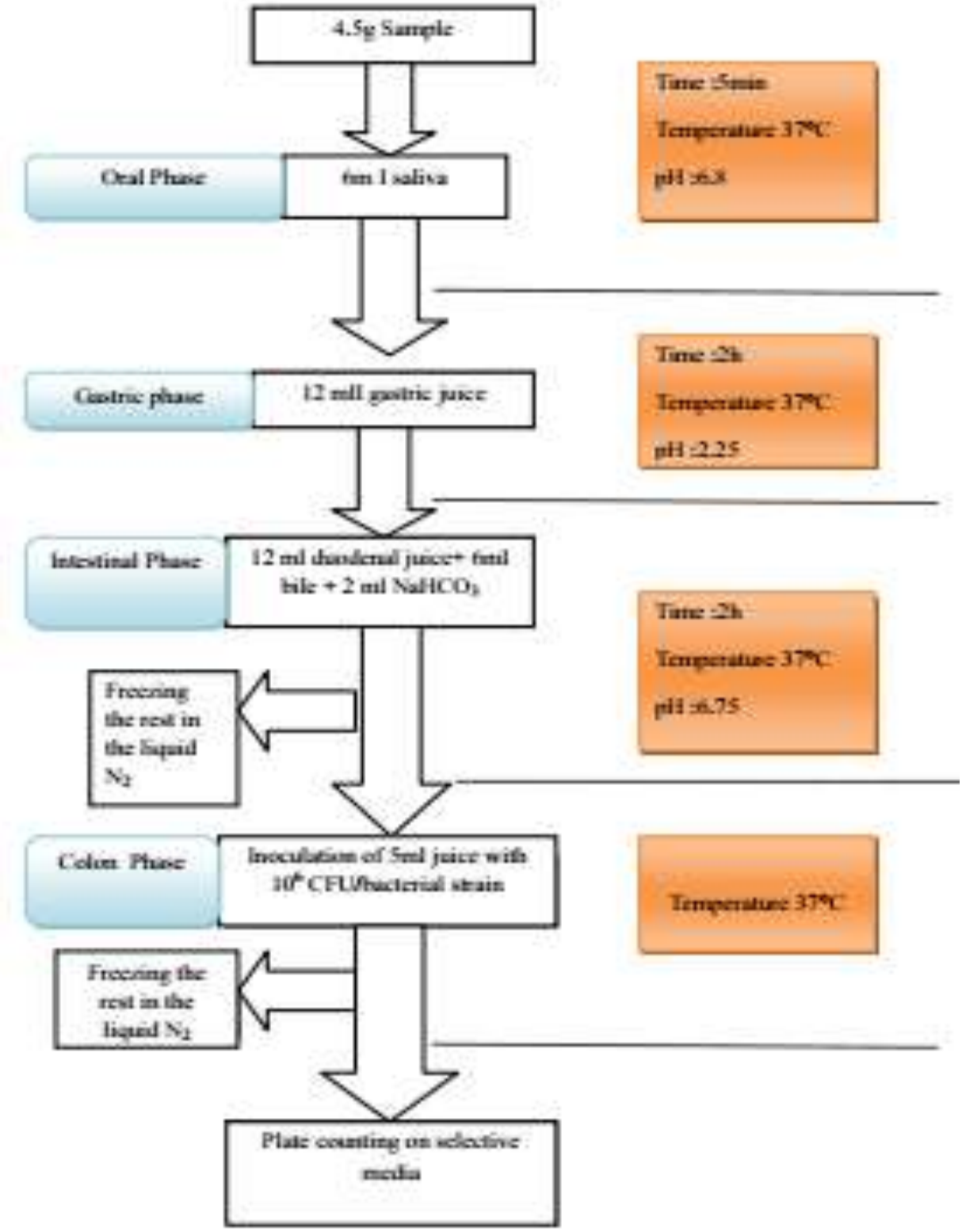

Figure 5: Shematic presentation of the *in vitro* digestion model

3. Results and discussion

As shown in Figure 6, all tested samples had a positive PI. Referring to Equation (5) these results indicate a preferential growth of *Bifidobacterium* and *Lactobacillus*, known as main health promoting bacterial groups. Such nutritional properties might be related to the presence of prebiotic carbohydrates in wheat. Wheat prebiotic properties could be associated to the presence of fibers (Al-Sheraji *et al.*, 2013). Fibers represent 13% of the wheat grain (Fardet, 2010). Particularly, in this study whole meal flour was used. Vulevic *et al.* (2004) evaluated specific growth rates and PI with different substrates. They showed that the highest *Bifidobacterium* growth rates were obtained with trans-galacto-oligosaccharides and fructo-oligosaccharides with trans-galacto-oligosaccharides (50:50) for *Bifidobacterium.* Soya oligosaccharides and isomalto-oligosaccharides led to a maximal growth for *Lactobacillus.* The use of simple sugars like sucrose led to a negative PI. Moreover, the preferential growth of *Bifidobacterium* and *Lactobacillus* could also be attributed to the presence of resistant starch. Zeng et al. (2018) investigated the prebiotic activities of fractionated lotus seed resistant starches. They reported that resistant starch promoted the growth of *Bifidobacterium adolescentis* and *Lactobacillus acidophilus*.

Interestingly, PI of raw Chili (old) wheat was higher than Karim one (modern) ($p<0.05$). This difference could be related to genetic differences between old and modern genotypes. In fact, previous results of Ficco *et al.* (2018), comparing different modern and old durum wheat cultivars, showed that modern ones have negligible amounts of resistant starch if compared to old ones.

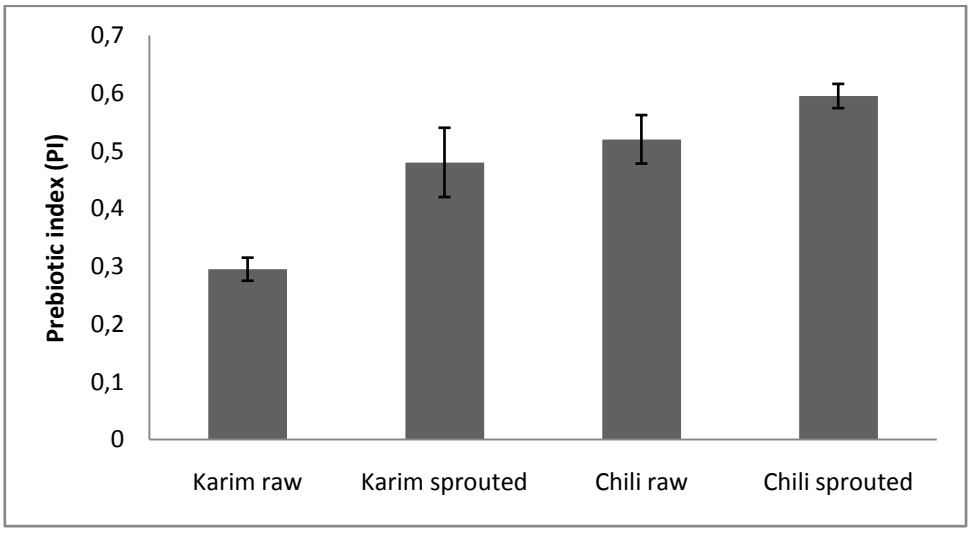

Figure 6: Evolution of prebiotic index (PI) after sprouting of two wheat cultivars

The effect of sprouting on PI depended on the genetic background of the sample used. The increase of PI was significant ($p<0.05$) only for the high yielding cultivar Karim (+62.7%). The difference observed among the two cultivars could be explained by the different proportions of nutrients of seeds. In fact, geographical location, agronomic practices and genetic background had an effect on seeds composition (Lee *et al.*, 2016). Regarding samples used in this study, the promoting effect of sprouting on PI might be related to the evolution of nutritional properties during this bioprocess (increase in bioactive compounds, peptides…). Particularly, sprouting leads to an increase in fibers (Hung *et al.*, 2011; Koheler *et al.*, 2007) which may have a positive effect on *Bifidobacterium* and *Lactobacillus* growth. Results of this research suggest that the evolution of nutrients during sprouting depends on the genotype tested.

4. Conclusion

Our *in vitro* results have shown that prebiotic effects in the human colon could be induced not only by whole mill flour obtained from raw durum wheat seeds (*Triticum durum*), but also from sprouted seeds. Interestingly, sprouting could significantly enhance this positive effect in high yielding cultivar. This finding highlights the interest to use sprouts as functional ingredient. In conclusion, sprouted wheat flour could be suggested as a potential source of prebiotics as they can similarly satisfy consumers' demands for natural products and functional foods in relation with human gut health.

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Chapter 4: Zinc fortification as a tool for improving sprouts hygienic and nutritional quality: A factorial design approach

Research Article

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Zinc fortification as a tool for improving sprout hygienic and nutritional quality: a factorial design approach

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Zinc fortification as a tool for improving sprouts hygienic and nutritional quality: A factorial design approach*

Abstract

Sprouting is known by improving cereals and pulses nutritional properties. However, several outbreaks were reported after raw sprouts consumption. This research aimed to improve wheat sprouts hygienic properties through the use of zinc diacetate. Sprouting conditions (Sprouting temperature, soaking time, zinc diacetate solution concentration) were optimized to decrease total plate count, coliforms and molds & yeasts using a factorial design approach and desirability function. Based on the responses, the effects of variables were calculated and the interactions between them were determined. Optimal conditions were defined as follows: sprouting temperature 18 °C, soaking time 0.66 h and zinc diacetate concentration 400 $mg.l^{-1}$). These conditions led to eliminating coliforms and decreasing total flora count by 2 log. Interestingly, zinc sprouting increased sprouts zinc content and improved their nutritional properties. Results provided showed that the use of zinc solution is a useful tool to improve sprouts hygienic and nutritional properties.

Key words: Sprouting; Durum wheat; Zinc; hygienic properties; factorial design.

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1. Introduction

Food market has been signed by a considerable evolution during the last decades to satisfy consumers' requirements as they become looking for more "natural" products that are minimally processed with no/less synthetic additives (Bansal *et al.*, 2015; Fellows, 2017). In order to meet these conveniences, manufacturers are trying to balance food products safety (chemically and microbiologically) with their quality (nutritional and sensory) (Bansal *et al.*, 2015). Consequently, several techniques could be used for minimally processed foods: non-thermal treatments, low temperature storage, new packaging and treatment with natural antimicrobials (Bansal *et al.*, 2015; Fellows, 2017).

Sprouting is an old practice that improves cereals nutritional properties (Donkor *et al.*, 2012). In fact, sprouting increases the amounts of simple nutrients after macromolecules degradation (Gan *et al.*, 2017). This bioprocess enhances also vitamins and bioactive compounds levels (Jribi *et al.*, 2018; Plaza, De Ancos and Cano, 2003). Added to, sprouting decreases some anti-nutritional factors like phytates (Wei *et al.*, 2013). Thus, it contributes in increasing minerals bioaccessibility.

Although sprouted seeds are ready-to-eat products with a high nutritional value, they represent a microbial food safety concern. Potentially, certain pathogenic bacteria may contaminate the raw materials and grow during germination. Between 1988 and 2008 there have been over 40 reported outbreaks worldwide attributed to consumption of contaminated seed sprouts (FSANZ, 2010). *Salmonella* and *E. coli* O157:H7 have consistently been linked with sprout-associated outbreaks (EC, 2002).

Tornuk et al. (2010) improved microbial safety of wheat sprouts through using chemical sanitizers (H_2O_2 and NaOCl). Luksiene et al. (2007) used aminolevulinic acid for fungal decontamination of wheat sprouts.

The use of zinc derivatives in pharmaceutical, agrochemical, perfumes and petroleum industry has been reported (Wu *et al.*, 2014). Zinc derivatives showed a strong antimicrobial activity at low concentrations (Pasquet et al., 2014; Savi *et al.*, 2015). Ibrahim et al. (2017) reported an inhibition of fungi cells growth due the presence of zinc. Zinc derivatives (Zinc: acetate, chloride, citrate, carbonate, sulfate) are considered safe and authorized for food fortification (Savi *et al.*, 2015).

From a metabolic point of view, zinc reduces oxidative stress (Ackland and Michalczyck, 2016). It is also involved in more than 300 enzymatic reaction as a cofactor (Pasquet et al., 2014; Tomat, De Los Angeles Costa and Arranz, 2011), regulates structural functions, gene

expression, cell division and organ development, neuronal transmission, immune response and cognitive functions (Ackland and Michalczyck, 2016; Tomat *et al.*, 2011). Recommended amounts range between 8 and 11 mg per day for men and women, respectively (Savi *et al.*, 2015). Zinc deficiency represents a risk factor for morbidity and mortality (Tomat *et al.*, 2011). Unfortunately, almost 30 % of world's population suffers from this deficiency (Gharibzahedi and Jafari, 2017). This rate is more calamitous in developing countries where zinc deficiency is the 5th risk factor of illness and death (Wei *et al.*, 2012). This fact could be explained by the high use of cereals as staple foods in developing countries (Wei *et al.*, 2012). As a result, phytate are consumed at high levels and zinc availability decreases (Kumar *et al.*, 2009).

Food fortification could be therefore an effective option to solve micronutrients malnutrition (Oghbaei and Prakash, 2017). Salt ionidation and fluor fortification of toothpaste are successful trials with this strategy (Poletti *et al.*, 2004). Cereals biofortification with zinc could be achieved through different strategies: agronomic practice, crop breeding and genetic engineering (Wang *et al.*, 2015; Wei *et al.*, 2012; Saha *et al.*, 2017). However, such trials are expensive and time consuming.

To the best of our knowledge, improvement of row sprouts hygienic properties through the use of minerals has not been reported previously. The aim of this study was firstly to investigate the effect of zinc solution soaking on durum wheat (*Triticum durum*) sprouts hygienic quality through the use of a factorial design. Secondly sprouting conditions (Temperature, Zinc solution concentration and soaking time) were optimized through desirability function. Thirdly, an interest was accorded to the role of this procedure in improving sprouts nutritional properties in terms of zinc biofortification and nutrients enhancement.

2. Materials and methods

2.1Plant material

The cultivar "Karim" of durum wheat (*Triticum durum*) was used in this study. Samples were kindly provided by The National Institute of Cereal crops (INGC Bou Salem, Tunisia)

2.2Sprouting

Sprouting was performed exactly as described by previous study of Jribi et al. (2019): Briefly, seeds (50 g) were firstly sterilized with 1% (V/V) hypochlorite sodium solution during 30 min, then rinsed with distilled water, soaked again in distilled water for control or in zinc

solutions. After removing distilled water or Zinc solution, seeds were spread into plates with three layers of "Blotting paper". Samples were watered after 24 h. Sprouting was conducted for 48 h in darkness.

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

2.3Germinated grains

To estimate germination percentage, germinated grains (GG) of durum wheat were calculated from the following equation with four replicates:

GG = (germinated grains /total grains) × 100. (Wei *et al.*, 2012) (Equation 1)

2.4Factorial design methodology

A factorial design approach was used in our study. Responses selected were: The percentage of germinated grains (GG), Total Mesophilic Aerobic, Molds and yeasts and Coliforms counts. The control factors were: zinc concentration, soaking time on zinc solution and sprouting temperature (8 experiments were conducted, 2^3 factorial design). Factors levels (Table 8) were selected according to previous studies (Lazo-Velez *et al.*, 2016; Prom-u-thai *et al.*, 2009; Wei *et al.*, 2012) . The quadratic model applied to predict the response variables is given by Equation 2.

$$Y_j = b_0 + b_1x_1 + b_2 x_2 + b_3 x_3 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{32} x_3 x_2 + b_{11} x_1^2 + b_{22} x_2^2 + b_{33} x_3^2 + e$$

(Equation 2)

Where: j responses; i variables; Y dependent variables; b_o constant term; b_i, b_{ii} coefficients; x_i linear effect; x_{ii} quadratic effects; and x_ix_i interactions.

Desirability function was used to optimize responses with a target of maximizing germinated grains and minimizing bacterial growth (Total Mesophilic Aerobic bacteria, Molds and Yeasts and Coliforms).

2.5Microbiological methods

Ninety milliliters of peptone-water (peptone 1 $g.l^{-1}$, NaCl 9 $g.l^{-1}$) were added to 10 g of fresh sprouts, homogenized in Stomacher 400 Circulator (England), then tenfold dilution series were performed in test tubes. Total Mesophilic Aerobic plate counts were determined on Plate Count Agar (PCA) (LAB M, UK) incubated at 37°C for 48 h. Molds and Yeasts were enumerated on Peptone Yeast Extract Agar (Sigma-Aldrich, Switzerland) incubated for 5 days at 25 °C. Coliforms were counted on Harlequin Medium (Lab M, UK) after incubation at

37 °C for 48 h. The presence of *Salmonella spp*. was determined by the corresponding EU - Hungarian standard procedure (EN ISO 6579:2002).

Table 8: Coded levels for independant variables used in experimentation.

Variables	Units	Coded level	
		-1	1
Zinc diacetate solution concentration	$mg.l^{-1}$	25	400
Soaking time	h	0.66	10
Temperature	°C	18	25

2.6Proximate composition

Ash content was determined according AACC method (AACC 08-01.01), crude fat (AACC 30-10.01) and protein content (AACC 46-30.01). Reducing sugar measurement was carried out through the Nelson-Somogyi Method (McCleary and McGeough, 2015).

2.7Bioactive compounds

Vitamin C content was assessed following the HPLC procedure as described by Molnar et al. (2017) Total carotenoids pigments were determined as described by Pasqualone et al. (2017) Total phenol content was assessed using the Folin-Ciocalteu method (Aprodu and Banu, 2012). Gallic acid was used as standard (0-1 $mg.ml^{-1}$; r^2=0.987).

2.8Antioxidant 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 the extraction procedure for antioxidant activity measurement: The extraction was made with 80% (v/v) aqueous methanol solution, for 2 h at 37 °C. Samples were afterwards centrifuged at 4000x g for 30 min. The supernatant was used for the determination of antioxidant capacity.

Antioxidant activity was calculated according to the equation 4:

$$\%DPPAH\ RSA = (1 - A_{Sample/t=30}/A_{Control/t=0}) * 100 \quad \text{(Equation 4)}$$

2.9Determination of Zinc content

Zinc content was assessed by Inductively Coupled Plasma-Atomic Emission (CPA-AE) (Horiba Jobin Yvon, France).

2.10Statistical 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) were performed using the Fisher multiple comparison test. Significance was defined at $p < 0.05$. The same software was used for factorial design analysis.

3. Results and discussion

3.1Fitting responses to factorial model

Factorial design is an approach allowing a better organization of experimental trials. Thanks to its use, it is possible to understand the effect of two or more independent variables and their interaction upon a single dependent variable (Goupy, 2001).

The analysis of variance (ANOVA) analysis for the different variables (Y) was done to determine the significance of the model. The analytical expression correlating the studied factors with the different responses are summarized in Table 9.

Table 9 indicates that different quadratic model equations had an $R^2 > 85\%$ (All R^2 adj were higher than 80%). Accordingly, the suggested models fitted experimental and predicted values as in all case R^2 were higher than 80% (Karazhiyan, Razavi and Phillips, 2011). On a second time, the statistical significance of each coefficient was checked with p-values to obtain the reduced models as followed:

Germinated grains (%)=87.71+2.04T+1.29-2.46[c]+0.46Tt+1.46Tt[c] (Equation 5)

Total mesophilic aerobic flora (log_{10} CFU.g^{-1})=7.32+0.25T+1.05t-0.23Tt+0.07T[c]-0.06Tt[c] (Equation 6)

Molds and Yeasts (log_{10} CFU.g^{-1})= 3.33+0.07T-0.19t-0.06[c]+0.07Tt+0.05T[c] (Equation 7)

Coliforms (log_{10} CFU.g^{-1})=1.36-0.06T-0.84t-0.74[c]-0.46Tt+0.67T[c]+0.22t[c]-0.15T[c] (Equation 8)

Where: T: Temperature; t: Soaking time and [c]: Zinc solution concentration

Table 9: Quadratic model equations and statistical parameters

Output variable	Original equation	S	R^2	R^2 adj
Germinated grains(%)	=87.71+2.04T+1.29t-2.46[c]+0.46Tt+0.04T[c]-0.21t[c]+1.46Tt[c]	0.96	95.86	94.09
Total mesophilic aerobic flora (log10 CFU.g^{-1})	=7.32+0.25T+1.05t+0.01[c]-0.23Tt+0.07T[c]+0.01t[c]-0.06Tt[c]	0.05	99.87	99.82
Molds and Yeasts (log10 CFU.g^{-1})	=3.33+0.07T-0.19t-0.06[c]+0.07Tt+0.05T[c]-0.02t[c]-0.03Tt[c]	0.10	89.26	84.55
Coliforms (log10 CFU.g^{-1})	=1.36-0.06T-0.84t-0.74[c]-0.46Tt+0.67T[c]+0.22t[c]-0.15T[c]	0.10	99.63	99.47

T: Temperature; t: Soaking time; [c]: Zinc solution concentration, CFU : Colony Forming Colony.

3.2Effect of sprouting conditions on durum wheat germination ability

Statistical models are useful for prediction of variables. Graphical tools could be helpful for an easier understanding of the effects. Pareto chart may be used to determine the magnitude and the importance of the effects. On the Pareto chart, bars that cross the reference line are statistically significant. However, Pareto chart can not determine which effects increase or decrease the response. The use of the normal probability plot of the standardized effects allows an examination of the magnitude and direction of the effects on one plot. As shown on Figure 7, almost all factors and their interactions have a significant effect on grains germination ability except the interaction Temperature*Zinc concentration and Soaking duration*Zinc concentration. It is clearly observed, the concentration used has the highest

effect. The normal plot indicates that an increase in Zinc concentration decreases grains germination ability. Contrarily, temperature and interaction of the three factors have a positive significant effect on this property.

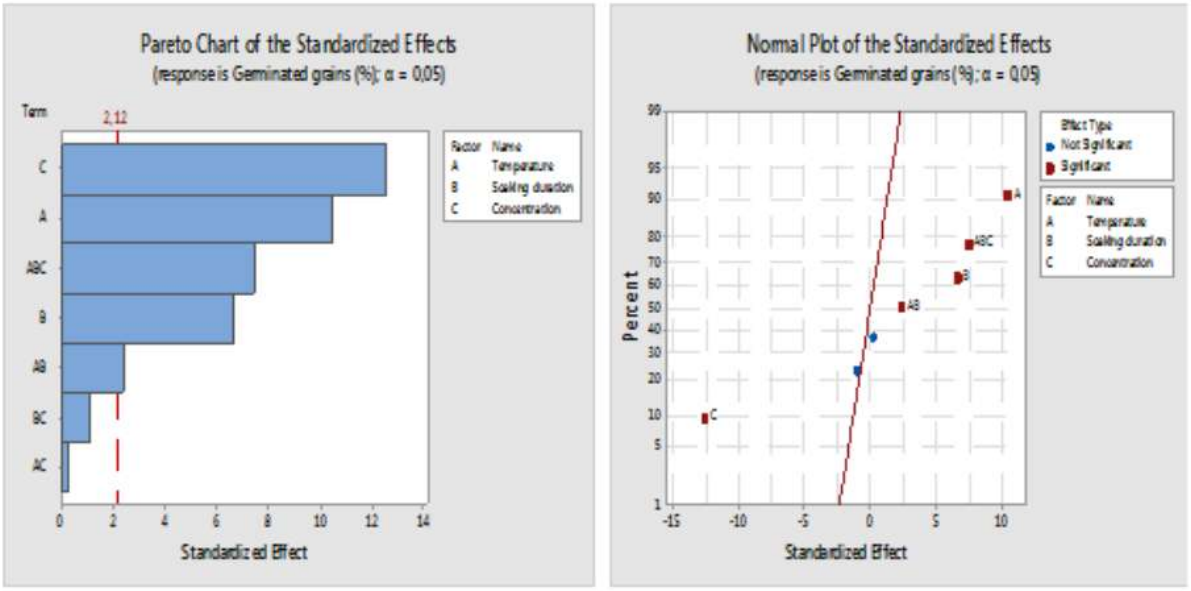

Figure 7: Effect of germination conditions on germinated grains (%)

Germination is commonly performed at a range temperature between (20-30 °C) (Gan *et al., 2017*). Our results are in agreement with this as an increase of sprouting temperature (from 18 to 25 °C) promoted grains germination. Despite the negative effect of zinc concentration, the use of 400 mg.l^{-1} did not induce toxicity since the amount of germinated grains was more than 83%. Wei et al. (2012) reported that the use of zinc sulfate solutions up to 150 mg.l^{-1} did not show a significant inhibition of brown rice seeds germination. In the same study authors reported that a concentration up to 250 mg.l^{-1} could be suitable for fortification without toxic effects on sprouts growth. According to our results, it would be possible to use a concentration up to 400 mg.l^{-1} of the diacetate zinc without inhibiting wheat sprouts growth.

3.3Effect of germination factors on microorganisms proliferation

Figure 8 groups the Pareto charts and Normal plot for counts of Total Mesophilic Aerobic flora, Molds and Yeasts and Coliforms. Their analysis indicates that the factors do not have the same effect on microorganisms proliferation. Soaking time and temperature promoted Total Mesophilic Aerobic bacteria growth whereas no significant effect of zinc concentration was observed. Interestingly, interaction between temperature and soaking time has a negative effect on their growth.

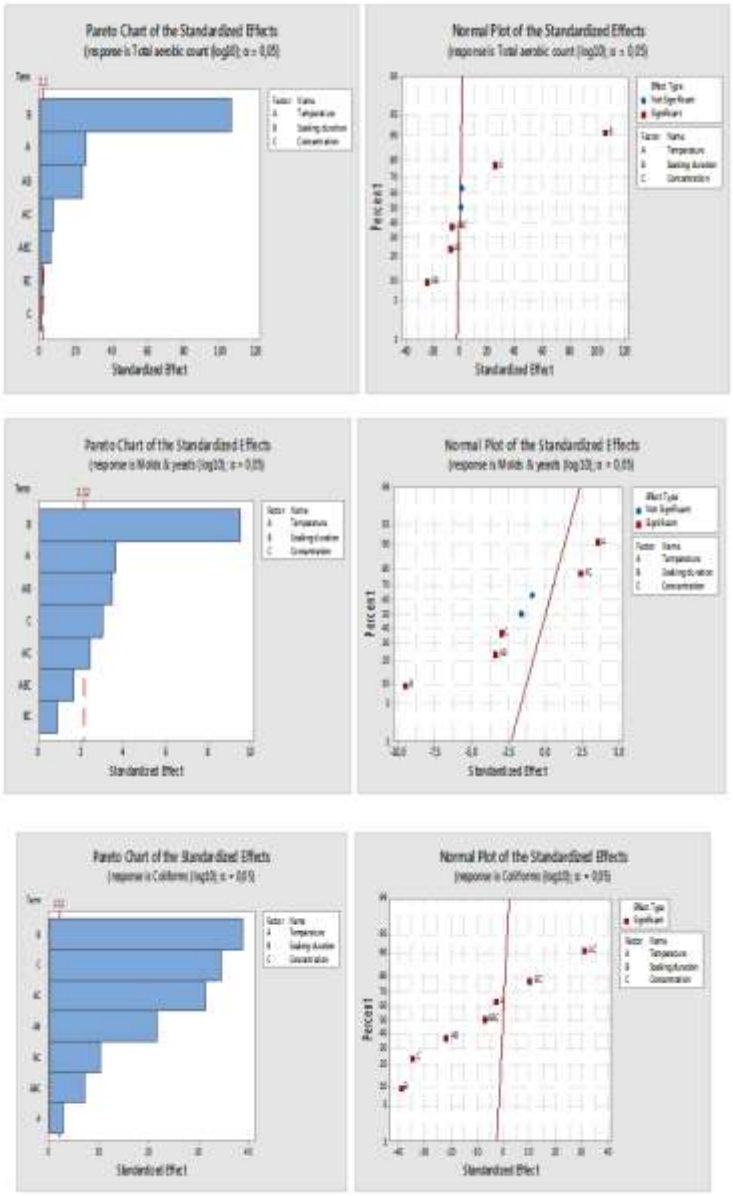

Figure 8: Effect of germination factors on Total Aerobic baacteria, Molds and Yeasts and Colifoms growth.

Coliforms growth was not significantly affected by temperature. In fact, in our experiments temperature ranged between 18 and 25 °C which is far from the optimal temperature for their growth, known to be around 37 °C (Boniece and Mallmann, 1950). Thus, even an increase of temperature in the studied range would not promote Coliforms proliferation. However, soaking time and zinc concentration had a significant negative effect. In fact, an increase in these factors completely inhibited Coliforms growth.

Increasing temperature stimulated Molds and Yeasts growth while increasing soaking time and zinc concentration significantly induced an inhibitory effect. Previous results of Tornuk et

al. (2011) also showed that increasing temperature (from 18 to 22 °C) accelerated also proliferation of microorganisms.

3.5 Responses optimization

In this study 4 variables were considered. Optimizing each one separately is challenging as the optimal conditions for one variable could be the minimal for another (Buratti *et al.*, 2018). Desirability function is an alternative to solve this problem. The approach is based on mathematical transformation converting a multiple response problem to a single response problem (Lazo-Velez et al., 2016).

Optimal conditions were defined as follow: Temperature 18 °C, Soaking time 0.66 h and Zinc concentration 400 mg.l^{-1}. These conditions gave a global desirability of 55.16 %. Responses values according to these conditions are detailed in Table 10. Replicates confirmed adequacy of the model.

Table 10: Desirability values (δ) in optimal conditions.

Response	δ %	Value
Germinated grains (%)	16.67	84
Total mesophilic aerobic flora (log10 CFU.g^{-1})	96.52	5.79
Molds and yeasts (log10 CFU.g^{-1})	57.54	3.26
Coliforms (log10 CFU.g^{-1})	100	0
Total desirability	55.16	

CFU: Colony Forming Colony.

3.6Effectiveness of Zinc soaking treatment on microorganisms' growth of durum wheat sprouts

The effectiveness of zinc in controlling pathogenic microorganisms on sprouted wheat grains was investigated in previously optimized conditions. Results are summarized in Table 11. Sprouting increased microorganisms growth as reported previously (Peles *et al.*, 2012). *E.coli* and *Salmonella spp.* were not detected in raw and sprouted seeds. Interestingly, the use of zinc decreased microorganisms proliferation after sprouting, when compared to water treatment (control). This result in agreement with results of previous studies (Atmaca, Gul,

and Cicek, 1998; Chandra *et al.,* 2012; Fluhr *et al.,* 1999; Zelenak, Gyoryova, and Mlynarcik, 2002).

This inhibitory effect of Zinc solution was more pronounced for Total Mesophilic Aerobic flora and Coliforms, which were completely eliminated, than Molds and Yeasts. In fact, previous studies reported that the presence of zinc ions (nanoparticles) may cause wall disintegration and lysis of bacterial and fungi cell membranes (Ibrahim *et al.,* 2017; Kairyte, Kadys, and Luksiene, 2013). In addition, these antimicrobial effects could be explained by a reduction of microbial metabolic activity: Zinc ions can bind in a non-specific way to polar side-chains of microbial enzymes, inducing either a reduction in substrate availability as a result of altered charge, or a decrease in enzyme activity as a result of configurational changes (Klotz, 1954).

Table 11: Recovery of total mesophilic aerobic bacteria, coliforms and molds and yeasts in raw durum wheat seeds and sprouted seeds after soaking either with water (control) or with zinc solution

	Total mesophilic aerobic flora (log10 CFU.g^{-1})	Molds and yeasts (log10 CFU.g^{-1})	Coliforms (log10 CFU.g^{-1})	*E. coli* (log10 CFU.g^{-1})	*Salmonella spp* (log10 CFU.g^{-1})
Raw seeds	4.14±0.37^{c}	2.47±0.23^{a}	2.44±0.15^{b}	ND	ND
Water soaked sprouted seeds	7.48±0.08^{a}	2.24±0.02^{ab}	3.88±0.02^{a}	ND	ND
Zinc soaked sprouted seeds	5.47±0.05^{b}	2.13±0.11^{b}	0.00±0.00^{c}	ND	ND

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

3.7Effect of zinc disinfection treatment and sprouting on nutritional properties

As shown in Table 12, durum wheat sprouting led to some modifications on nutritional properties mainly through an increase on protein and reducing sugar contents, as reported

previously (Hung, Maeda and Morita, 2015; Singkhornart, Edou-ondo and Ryu, 2014). Ash and lipid content were not affected by sprouting. These results are in line with those of Singkhornart et al. (2014). Nevertheless, Zinc content decreased after sprouting. Plaza et al. (2003) observed an increase in Zinc content after sprouting soft wheat (*Triticum aestivum*) for 4 days at 28 °C. The authors reported that mineral content of sprouts was highly reliant on sprouting conditions adapted. Contrarily, results of Platel et al. (2010), showed a decrease in zinc content after 48 h of germination as observed in our study.

Soaking seeds in zinc solution instead of distilled water modified proximate composition of sprouts. In fact, a significant increase in ash content was detected which might be related to the metallic properties of zinc. Lipid and protein contents were higher in sprouted seeds soaked in zinc solution than control. Evolution of these molecules during sprouting is mainly due enzymatic mechanisms aiming to provide embryo with nutrients (Mak *et al.,* 2009). The increase in lipid and protein contents might be related to an effect of zinc on lipases and proteases: In fact, zinc plays a key role in structural and catalytic functions of numerous enzymes (Ackland and Michalczyck, 2016). In addition, it is involved in several enzymatic reactions (Pasquet, 2014). Still, levels of reducing sugars were lower in Zinc treated sprouted seeds than control ones. The difference can probably be related to a non specific binding of Zinc ion on reducing sugars as zinc binding properties are modulated by surrounding conditions (Krezel and Maret, 2016). Impressively, soaking in zinc solution significantly increased spouts Zinc content (+247.23 %). Such results are expected and are in agreement with those of Pongrac et al. (2016). In this study the use of water with high zinc content increased wheat sprouts zinc content (+232.9 %). Moreover, the increase in Zinc content might be related to the evolution of phytates after sprouting: In fact, previous study of Lemmens et al. (2018) showed an increase in phytase activity exceeding 3 folds in wheat germinated for 48 h. Consequently a decrease in phytate content is recorded and more Zinc ions, previously chelated by phytate, are released. Luckily, sprouting bioprocess also increases zinc bioaccessibility in wheat as demonstrated (Lemmens *et al.,* 2018; Platel *et al.,* 2010).

Table 12: Effect of zinc disinfection treatment on wheat sprouts nutritional properties

Sample	Ash ($g.kg^{-1}$ dm)	Lipids ($g.kg^{-1}$ dm)	Proteins ($g.kg^{-1}$ dm)	Reducing sugars ($g.kg^{-1}$ dm)	Zinc content ($mg.kg^{-1}$)
Raw seeds	17.86 ± 0.51^{b}	16.41 ± 0.15^{b}	137.00 ± 0.54^{c}	26.69 ± 0.49^{b}	25.09 ± 0.08^{b}
Water soaked sprouted seeds	17.43 ± 0.40^{b}	16.48 ± 0.36^{b}	140.83 ± 0.30^{b}	29.12 ± 0.13^{a}	22.63 ± 0.06^{c}
Zinc soaked sprouted seeds	19.03 ± 0.20^{a}	18.70 ± 0.58^{a}	147.67 ± 0.20^{a}	27.16 ± 0.11^{b}	87.12 ± 0.18^{a}

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

3.8Effect of Zinc disinfection treatment and sprouting on bioactive compounds and antioxidant properties

Evolution of bioactive compounds after wheat seed sprouting and zinc soaking treatment are presented in Table 13. Sprouting improved durum wheat seeds antioxidant properties, thanks to the elevation of tested bioactive compounds levels as reported previously (Plaza et al., 2003; Yang, Basu and Ooraikul, 2001). Soaking seeds in Zinc solution enhanced significantly vitamin C and total phenol contents, whereas no significant effect was observed on carotenoids and DPPH Radical Scavenging Activity.

Although antioxidant properties of sprouted wheat seeds are, commonly, positively correlated with total phenol contents, our results did not show a significant correlation between total phenol content and DPPH RSA as well as vitamin C and DPPH RSA. To overcome the toxic effect of oxygen species, plants may use an enzymatic defense system through the use of Superoxide dismutase (SOD) (to scavenge superoxide radicals), Catalase (to transform H_2O_2 into H_2O) or the ascorbate–glutathione (ASC–GSH) (Ma et al., 2017). Durum wheat has three isoforms of SOD: Mn-,Fe- and Cu/Zn-containing SOD (Huseynov et al., 2014). Results of Bharti et al. (2014) showed that an increase in zinc levels was beneficial for wheat SOD activity. However, these results are from a field experiment using soil and foliar fertilizers. Availability of zinc is lower if compared to soaking seeds on zinc solution. Accordingly, it is probable that the concentration used on our study led to a partial inhibition on SOD.

Moreover, the contribution of zinc, as cofacteur, in maximizing catalytic activity was probably related to SOD structure and availability of free metal binding sites (Stephenie et al., 2020): at saturation, all SOD units are binding zinc, increasing its concentration would no more accelerate reactions.

Table 13: Effect of sprouting conditions on bioactive compounds

Sample	Vitamin C ($\mu g.g^{-1}$ dm)	Carotenoids (mg β-carotene.kg^{-1} dm)	Total phenol content (mg GAE.g^{-1} dm)	DPPH RSA (%)
Raw seeds	0.00 ± 0.00^{c}	15.17 ± 0.07^{b}	14.66 ± 0.11^{b}	19.19 ± 0.79^{b}
Water soaked sprouted seeds	45.60 ± 0.28^{b}	17.29 ± 0.28^{a}	15.18 ± 0.24^{b}	22.85 ± 0.27^{a}
Zinc soaked sprouted seeds	77.19 ± 0.56^{a}	15.05 ± 0.17^{b}	17.40 ± 0.33^{a}	19.96 ± 0.62^{b}

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

4. Conclusion

In this study, sprouting condition using a zinc solution were optimized through a factorial design to improve durum wheat (*Triticum durum*) sprouts hygienic properties without affecting grains viability. Our results showed that the use of zinc was significantly effective in inhibiting Total Mesophilic Aerobic flora, Coliforms and Fungal flora, and has improved nutritional properties of sprouted seeds. Sprouting wheat seeds using zinc solution could be thus suggested as an effective, quick and low-cost strategy for disinfection and mineral biofortification of wheat.

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Chapter 5: Dried sprouts as a functional ingredient: Impact of drying method on bioactive compounds, functional and thermal properties

Dried sprouts as a functional ingredient: Impact of drying method on bioactive compounds, functional and thermal properties*

Abstract

This research investigated the impact of different drying method (Oven, lyophilisation and micro-wave vacuum drying (MVD)) on bioactive compounds, physico-chemical (Water content, aw, color), functional (Bulk density, Water Absorption Capacity (WAC), Oil Absorption Capacity (OAC), Swelling power (SP), Least Gelation Concentration (LGC)) and thermal properties through DSC. Results showed that sprouting and drying significantly affected evolution of all properties. In all case, water content and aw decreased to an acceptable level. Oven and MVD dried samples were darker than freeze dried ones. Compared to raw sample, bulk density and swelling power decreased while OAC increased. WAC decreased after lypohilisation (-14.3%) while MVD significantly increased it (+90.5%). DSC measurement showed two endotherms with peak temperatures between 39.7 and 46.6 °C for the first one and 101.9 to 108.5 °C for the second. Regarding nutritional properties lyophilisation preserved them the best. However, MVD induced less losses than oven drying.

Key words: Sprouts, Drying, Functional properties, DSC, Bioactive compounds.

Submitted*

1. Introduction

Cereals are an important part of human diet as they are found in varied range of products: bread, pasta, cookies… Sprouting is a green engineering tool contributing in improving cereals and pulses nutritional properties (Donkor *et al.,* 2012). Thus, sprouts could be suggested as potential functional ingredient for the development of food products with added nutritional value. Unfortunately, sprouting increases seeds water content. This may reduce their shelf life and make their use difficult in many food products, especially cereal ones. To overcome this limit, drying could be suggested as a solution. In fact, drying is one of the oldest ways used for food preservation (Maskan, 2000) as it reduces water activity and consequently extends shelf-life (Zhang *et al.,* 2006). Meanwhile, quality degradation could occur (Maskan, 2000). In this context, studies have compared several drying technologies impact on foods functional properties, as reviewed by Denhnad et al. (2016). Convective drying is an ancient and the common method used (Dorofejeva et al., 2011) thanks to its simplicity and low cost. However, it might cause quality alteration (Giri and Prasad, 2007). Lyophilisation process is known by preserving plant material composition (Georgé et al., 2011). Consequently, freeze dried products have high nutritional and sensory properties (Wojdyło et al., 2016). Though, the use of this method might be restricted due to its long duration and cost. To reduce the long drying time, the use of microwave could be suggested (Bondaruk et al., 2007). Despite the improvement of drying parameters (diffusion coefficient, dehydration rate…) with microwave, products characteristics could be modified: color, loss of nutrients, texture…(Therdthai and Zhou, 2009). During the last decade, researchers investigated Microwave-Vacuum Drying (MVD) as a potential alternative to optimize microwave drying (Giri and Prasad, 2007). In fact, it combines the advantage of both technologies. In one hand, drying time is shortened. In the other hand, the process takes place at low temperature (Therdthai and Zhou, 2009). Consequently, the use of MVD may contribute in improving dried products quality. Functional properties play a key role in food conception. These properties are affected by several parameters as storage, ingredients composition (proteins, carbohydrate, lipid amounts) (Denhnad et al., 2016) and also processing, among other drying. In this context, kilning or drying of germinated seeds is an important step in the sprouting process. In fact, it allows the product stability, handling and milling. Meanwhile, some biochemical changes may occur according to the parameters used in drying (Woffenden et al., 2002). The work of Shingare and Thorat (2014) focused on the effect of

fluidized bed drying of sprouted wheat with different operating conditions on color, physical properties, proximate composition and effective diffusivity. However, to the best of our knowledge, no previous work compared the effect of different drying methods on sprouted wheat seeds properties. Therefore, this research investigated the impact of drying technology on sprouts nutritional, functional and thermal properties in order to use dried sprouted wheat seeds as a functional ingredient in cereal products.

2. Materials and methods

2.1Materials

A Tunisian cultivar of durum wheat (*Triticum durum*) "Karim" was used in this study. Samples (Harvested in 2015) were kindly provided by the National Institute of Cereal crops (INGC) (Bou salem, Tunisia).

2.2 Sprouting

Seeds were sprouted as described in a previous study (Jribi et al., 2019). Briefly seeds were disinfected with hypochlorite sodium solution, soaked in distilled water and finally spread in plates with "Blotting paper" Samples were watered after 24 hours with distilled water. Sprouting was conducted at a temperature of 22 ± 1 °C during 48 hours.

2.3Drying methods of sprouted seeds

Sprouted seeds were freezed at -80 °C then lyophilisation was carried out in a freeze dryer (Christ freeze dryer alpha 1-4 LCS, Germany) at a reduced pressure. Microwave vacuum drying (MVD) was performed with a custom-designed MVD dryer. The apparatus contains a cylindrical stainless steel vacuum chamber with a conical dome for better vapor removal. The samples were located in a rotary polytetrafluoroethylene (PTFE) tray. Microwaves were generated by two 850 W rated output magnetrons, operating at 2450 MHz. The vacuum was kept constant at 1 kPa by a rotary vane vacuum pump, connected to a shell and tube heat exchanger for vapor condensation. The cooling water for the heat exchanger was provided by a compressor and was kept circulated by a pump (Ferenczi et al., 2017). Intermitent drying was applied: Samples were dried with 60 s microwave pulses followed by 60 s break. For convective hot air drying, a laboratory scale hot-air dryer (L-MIM 320, Hungary) was used: Temperature was set at 50 °C and airflow was 0.9 $m.s^{-1}$. For oven drying and MVD experiments were stopped when samples reached at least 15% water content. All drying experiments were performed in triplicate. After drying, all samples

were milled (Retsch Grindomix GM 200, Germany). Milled raw seed and dried sprouted wheat powders were hermetically packed and stored at 4 °C until analysis. Physico-chemical, functional and thermal properties, as well as bioactive compounds and antioxidant activity were investigated on both raw and sprouted wheat seeds.

2.4Physico-chemical properties

Moisture content of all samples was analyzed using the AOAC oven method. Water activity was measured by Novasina LabMaster aw (Switzerland) device at 25 °C. Color parameters were measured with a Konica Minolta Croma Meter CR 400 (Japan). The device uses CIELAB color space, where L* stands for lightness (0 for black, 100 for white) –a* is green, +a* is red, –b* is blue and +b* is yellow tint.

2.5Functional properties

Bulk density was assessed as described by Singh et al. (2017). Water absorption capacity (WAC) and Oil absorption capacity (OAC) were determined according the procedure described by Kaushal et al. (2012). Swelling power and least gelation concentration (LGC) were evaluated as suggested by Singh et al. (2017).

2.6Bioactive compounds and antioxidant activity

Total phenol content was assessed using Folin-Ciocalteu method as suggested by Aprodu and Banu (2012). Gallic acid was used as standard (0-1 mg/ml; r_2=0.987). Total carotenoid pigments were determined according to the procedure described previously (Pasqualone et al., 2017). 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 2 h 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:

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

2.7Differential scanning calorimetry (DSC) measurements

Thermal properties were determined using a DSC 131 (SETARAM, France). Flour samples (from raw and sprouted seeds) (20 mg) were weighed into aluminum pans. Samples were hermetically sealed and allowed to stand for 2h at room temperature before testing. An empty pan was used as a reference. DSC analyses were carried through 3 cycles of heating-cooling (Ciesla et al., 2007): Samples were kept at 30 °C for 5 min then heated from 30 to 110 °C at

5 °C.min^{-1} (heating cycle 1) (Martinez et al., 2014). Samples were then cooled from 150 to 30 °C at 20 °C.min^{-1}. Two successive heating/cooling runs were conducted in similar conditions. The Universal Analysis 2000 software was used to analyze the main endotherm of the DSC traces for onset temperature (T_0), peak temperature (Tp) and enthalpy change (ΔH). As no significant differences were observed between the second and the third heating cycle, only results of the first and second heating cycle will be presented.

2.8Statistical 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 Physico-chemical properties

Compared to raw seeds, sprouted ones have a high water content (56.74 %) as well as water activity (0.989) (Table 14). This significant increase ($p<0.05$) reaching 5 folds for water content, may reduce the shelf life of sprouted seeds as the conditions are optimal for microbial growth. For dried samples, the averages of moisture content were significantly ($p<0.05$) different according to the technology used. The lowest averages were obtained with lyophilisation (6.89 %). The differences could be explained by the nature of transfers occurring during drying, mainly, heat and mass transfer. Water activity (a_w) level decreased significantly after drying following the same trend as moisture content: The highest averages was observed with convective drying (0.524) while lowest corresponded to freeze drying (0.322). In all cases, final levels are enough to stop bacterial growth.
Color parameters after drying are presented on Table 14. As seen, drying method is determinant on color evolution. MVD contributed on samples darkening mainly through a decrease in lightness (L*) and an increase in redness (a*). Contrarily, freeze drying led to an increase in lightness (L*) and a decrease in redness (a*) and yellow index (b*). Regarding oven drying, there was only a decrease in redness and yellow index. Korkida et al. (2001) investigated the effect of different drying methods on fruits and vegetables color. The authors reported a significant effect of the method used on color evolution. They also highlighted the role of oven and microwave drying on browning.

Table 14: Effect of drying method on physical properties of sprouted wheat flour (n=3)

Treatement	Water content (%)	Water activity (a_w)	Lightness index (L*)	Redness index (a^*)	Yellow index (b*)
Raw	11.34±0.11^{b}	0.558±0.00^{b}	76.09±0.04^{b}	1.84±0.04^{b}	19.23±0.08^{a}
Sprouted	56.74±0.14^{a}	0.989±0.00^{a}	--	--	--
Oven drying	11.17±0.05^{bc}	0.524±0.00^{bc}	76.13±0.05^{b}	1.42±0.01^{c}	18.12±0.04^{b}
Lyophilisation	6.89±0.15^{d}	0.322±0.00^{d}	80.70±0.04^{a}	0.69±0.04^{d}	16.13±0.04^{c}
Micro-waves vacuum drying	10.97±0.10^{c}	0.490±0.00^{c}	73.03±0.14^{c}	2.32±0.04^{a}	19.22±0.18^{a}

Means in the same colon that do not share same letters are significantly different, according to Fisher's test.

3.2Functional properties

Bulk density, as an indicator of flour particles heaviness (Singh et al., 2017), is an important factor to decide whether a flour is more suitable for use in food preparations (flours with high bulk density) or for complementary products (low bulk density)(Kaushal et al., 2012). As seen on Table 15, bulk density of dried samples ranged between 0.65 and 0.72 g/ml. This parameter decreased significantly ($p<0.05$) if compared to raw seed. Previous results of Singh et al. (2017) dealing with flours obtained from germinated sorghum seeds showed that this bioprocess decreased bulk density. In our study, it could be suggested that the significant decrease seen was not only due to germination but also due to the drying method. According to these results, whole mill flour from sprouted wheat could be used for both food preparations and complementary products (Kaushal et al., 2012). Comparatively to raw whole mill flour, drying method was determinant in Water Absorbance Capacity (WAC) evolution (Table 2). In fact, MVD increased this property; lyophilisation decreased it while no significant changes were seen after oven drying. According to Dehnad et al. (2016), oven drying at low temperature (40-50 °C) increases water retention capacity. These results are not necessarily contradictory with ours because, in this study, the effect of drying is combined with the effect of the germination process. In fact, functional properties of dried products depend on drying parameters used (mainly temperature and time). In this case, these properties depend also on germination and conditions used (temperature, duration, soaking time) which are known to negatively affect WAC (Singh et al., 2017; Singh et al., 2001). Probably, for this reason, WAC does not show differences between raw seeds and oven dried

samples. However, the effect of germination on WAC was more seen on freeze dried samples (Table 15). In fact, lyophilisation is a dehydration process that preserves products properties and quality. Thus, the effect of the germination process (decrease of WAC) will be more highlighted. Regarding, the increase of WAC seen on MVD dried samples, this evolution might be related to the impact of microwave on decreasing particle size and protein alteration as demonstrated (Walde et al., 2002). In fact, water holding capacity increases as particle size decreases (Protonotariou et al., 2016). Results of Berton et al. (2002) showed a significant negative correlation between particle size (d90) and water hydration capacity. It could be suggested that the decrease in particle size increases specific surface area per unit weight. Consequently, there is more available surface to interact (Protonotariou et al., 2016).

Table 15: Impact of drying method on sprouted wheat flour functional properties (n=3)

Treatement	Bulk density ($g.ml^{-1}$)	Water absorption capacity (WAC) ($g.g^{-1}$ dm)	Oil absorption capacity (OAC) ($g.g^{-1}$ dm)	Swelling power (SP) ($g.g^{-1}$ dm)
Raw	0.75 ± 0.01^{a}	1.05 ± 0.01^{b}	1.15 ± 0.02^{c}	6.67 ± 0.11^{a}
Oven drying	0.72 ± 0.00^{b}	1.04 ± 0.01^{b}	1.18 ± 0.01^{c}	4.44 ± 0.08^{c}
Lyophilisation	0.71 ± 0.00^{c}	0.9 ± 0.01^{c}	1.37 ± 0.01^{b}	4.15 ± 0.03^{d}
Microwaves vacuum drying	0.65 ± 0.00^{d}	2.00 ± 0.05^{a}	1.60 ± 0.02^{a}	5.62 ± 0.08^{b}

Means in the same colon that do not share same letters are significantly different, according to Fisher's test. dm: dry matter basis

As seen on Table 15, raw seeds flour and oven dried ones showed the lowest averages of Oil Absorption Capacity (OAC). When lyophilisation and MVD methods were used, a significant increase was observed on OAC. Previous study, dealing with sprouted cereals, showed that sprouting increases OAC (Singh et al., 2017; Elkhalifa and Bernhardt, 2010). This increase could be explained by protein degradation taking place during sprouting under proteolytic enzyme activity (Alvarez-Jubete et al., 2009). Moreover, the observed increase in OAC could be also associated to the role of gluten proteins. In fact, previous results showed that freeze and micro-wave drying induce morphological and structural modifications on wheat proteins, particularly on gluten (Gianfrani et al., 2017; Liao et al., 2013). However, temperature less than 60-75 °C do not affect these proteins (Which is the case for oven drying in our study) (Singh and MacRitchie, 2004). This is in agreement with our results as the

highest OAC were recorded after freeze and micro-wave vacuum drying. The increase in OAC would be an advantage in foods formulation as flavour retention and palatability might be improved (Hussain and Uddin, 2012). Recorded averages of Swelling Power (SP) (Table 2) ranged between 3.95 and 5.91 g/g dm. Raw seeds flour had the highest values while a significant decrease was observed with sprouted dried samples. Considering dried samples, oven dying decreased the most the SP contrarily to micro-wave vacuum drying (Table 15). The significant decrease in SP is probably due the starch degradation under amylasic enzyme activity (observed also in our study, data not shown) and also drying time. In fact, oven drying is low a process if compared to MVD, availability of free water at the first stage of drying contributes to more degradation of starch molecules under amylasic enzyme. Added to, previous results of Singh and Kayastha (2014) dealing with purified α amylase from germinated wheat showed that optimum temperature for this enzyme is 68 °C. The temperature used with oven drying (50 °C) is the closest one to the optimum. The decrease of SP after sprouting has been previously reported (Singh et al., 2017). In addition, Chinma et al. (2015) reported that the size of particles, genetic background and types of processing methods or unit operations may influence the flour swelling capacity. The least gelation concentration (LGC) corresponds to the lowest concentration at which gel remained in the inverted tubes. It reflects the minimum amount required of starch or blends of starch to form a gel: An increase in gelation concentration means an increase in needed mounts of starch to form a gel (Eke-Ejiofor , 2015). It is also an indicator for the ability of proteins to provide a structure able to hold water and thus form a gel (Appiah et al., 2011). LGC could be considered as a reflection of sample denaturated molecules (Appiah et al., 2011). Dried samples showed different results according to the drying method used (Table 16). The use of lyophilisation and oven led to an increase in LGC (from 8 % for raw seeds to 12 % and 15 %, respectively) while no difference between raw seeds and MVD sprouted seeds flour was observed. Gelation properties are related to flours composition, mainly protein and starch. In fact, competition for water between starch gelatinization and protein gelation is the main phenomenon influencing flour's gelation properties (Kaushal et al., 2012). An increase in LGC is followed by a decrease in swelling power (Kaushal et al., 2012). As swelling power reflects the ability of starch granules to hold water, an increase in this indicator means than low starch concentration will be needed to form a gel. The same trend was observed in our study. Germination process induces an increase in gelation properties as some macro-molecules like starch and proteins are degraded under enzymatic action (Singh et al., 2016). This effect was observed in freeze and oven dried samples at different extent (Probaly due to evolution of

starch and proteins during the drying process). However, the MVD samples showed the same LGC as raw one. Considering swelling power (SP) (Table 15), it is seen that the lowest decrease in this parameter is for MVD. It might be proposed that this method has a lower impact on starch compared to other method used. Meanwhile, microwave affects gluten protein structure (Liao et al., 2013). Thus, it may suggest that a combined role between proteins and starch erased the effect of sprouting on LGC.

Table 16: Effect of drying on least gelation concentration (LGC) (n=3)

Treatment	2%	4%	6%	8%	10%	12%	15%	20%	25%	30%
Raw	-	-	-	+	+	+	+	+	+	+
Oven drying	-	-	-	-	-	±	+	+	+	+
Lyophilisation	-	-	-	-	±	+	+	+	+	+
Microwave vacuum drying	-	-	-	+	+	+	+	+	+	+

-: Not gelled; ±: Slightly gelled ; +: Gelled completely

3.3Bioactive compounds and anti-oxidant activity

Final products quality is among concerns during drying. As shown on Table 17, freeze dried samples had the highest carotenoids content. This increase, comparatively to raw seeds, reflects the role of sprouting in enhancing carotenoids levels as reported previously (Plaza et al., 2003). The use of MVD induced losses leading carotenoids amounts to similar level as raw seeds. However, oven drying drastically reduced its levels. Regarding total phenol content, freeze dried samples had also the highest averages. However, MVD and oven drying affected these compounds as recorded levels are lower than raw seeds ones. The decrease is more important with oven drying. The increase in total phenol content in freeze dried samples could be attributed to the effect of sprouting as reported (Alvarez-Jubete et al., 2009). The difference seen among oven and MVD could be explained by the role of drying time on phenols and carotenoids degradation. Multari et al. (2018) investigated the effect of different drying temperatures (40, 50, 60 and 70 °C) on the quinoa seeds carotenoids and phenol contents. The authors reported that highest recovery of total phenolics compounds was at 70 °C while highest recovery of cumulative carotenoids was at 60 °C. DPPH Radical Scavenging Activity increased also after sprouting, as demonstrated by freeze dried samples (Table 17) which suggests the role of this bioprocess in improving antioxidant properties. Meanwhile, oven and MVD significantly affected DPPH RSA. According to our results, DPPH RSA

evolution is related to the evolution of carotenoids and total phenols ones (Pearson correlation coefficient 0.904 and 0.916 respectively).

Table 17: Evolution of sprouted wheat flour bioactive compounds and antioxidant activity after dryin (n=3)

Treatment	Carotenoid (mg β carotene. Kg^{-1} dm)	Total phenol content (mg GAE. g^{-1} dm)	DPPH RSA (%)
Raw	15.31 ± 0.28^{b}	15.84 ± 0.94^{b}	18.64 ± 0.34^{c}
Oven drying	6.16 ± 0.37^{c}	6.54 ± 0.03^{d}	13.47 ± 0.37^{d}
Lyophilisation	20.28 ± 0.32^{a}	35.14 ± 0.16^{a}	33.46 ± 0.41^{a}
Microwave vacuum drying	15.52 ± 0.30^{b}	11.62 ± 0.03^{c}	23.65 ± 0.37^{b}

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

3.4Thermal properties

The effect of sprouting and drying methods on flour's thermal properties is shown on Table 18. In the range of tested temperature (30-110 °C), two events; two endotherms, were detected on the first and second scan. Previous studies dealing with starch and wheat flour at low water content detected also two endotherms (Liu et al., 2006; Wang et al., 2014). Noda et al. (2004) attributed the first endotherm to starch gelatinization and the second to amylose-lipid complex dissociation. Gelatinization peak temperature (T_{P1}) was significantly different among tested samples. It ranged between 39.7-45.1 °C for the first scan and between 46.6-41.8 °C for the second one. Commonly, gelatinization of wheat starch takes place at a range of 54-73 °C (Liu et al., 2006). Measurement conditions and water content may affect endotherms (Liu et al., 2006). An increase in water ratio increases peak temperature (Wang et al., 2014) which was not the case in our experiment as no water was added. Regarding the second heating cycle, raw seeds flour and sprouted MVD dried seeds flours showed the highest averages, for the first endotherm, followed by freeze dried and oven dried samples. These findings are in agreement with our previous ones dealing with LGC where lowest concentrations were those of raw and MVD samples followed by freeze dried and oven. An increase in LGC reflects that more starch is required to form a structured medium. The similar results obtained with raw and sprouted MVD flours may reflect the effect of microwave on starch molecules. Despite the effect of sprouting on starch degradation, microwave may affect molecule structure. In fact, previous study of Xie et al. (2013) showed that even a short time treatment with

microwave modified potato starch crystalline structure which may modify thermal properties. Similarly, Ndife et al. (1998) observed an increase on the degree of gelatinization of starch after a short time microwave treatment. The difference seen among freeze and oven dried samples could be explained by drying history: Sprouted seeds were freezed at -80 °C before lyophilisation. Thus, enzymatic reactions were stopped. However, oven drying is a slow process (at least about 4 h to reach required water content). Consequently, as long as there is available water, starch degradation could be continued during the first steps. Peaks of the second endothem ranged between 101.9-105.3 °C during the first heating cycle and 104.8-108.5 °C during the second. The trend observed was the same: No significant difference between samples except MVD ones who showed the lowest values. Admitting that this peak corresponds to amylose-lipid complex dissociation, our results seem in agreement with those of Noda et al. (2004). We can suggest that two days germination did not affect the amylose-lipid complex. However drying method, mainly MVD, may affect the structure of this complex and thus peak temperature. A previous study dealing with gamma irradiated wheat starch samples showed difference on peak temperature of amylose-lipid complex transition between control and irradiated samples (Ciesla and Eliasson, 2003). During the first heating cycle, MVD induced a significant decrease in enthalpy if compared to raw seeds. However, no differences were seen among all samples during the second heat scan. The impact of MVD on enthalpy during the first heating cycle could be attributed to the structural changes (mainly on starch and protein) induced by this process. Then, since all the samples experienced the same heating-cooling steps no differences were seen.

Table 18: Effect of drying on sprouted wheat flour thermal properties

Heating cycle	Treatment	T_{01}	T_{p1}	ΔH_1	T_{02}	T_{p2}	ΔH_2
1	Raw	32.5^{e}	40.2^{e}	3.1^{a}	95.3^{c}	105.0^{b}	5.4^{bc}
	Oven drying	34.4^{cd}	41.5^{d}	3.2^{a}	95.7^{c}	105.1^{b}	4.6^{de}
	Lyophilisation	33.3^{de}	39.7^{e}	2.8^{ab}	96.0^{c}	105.3^{b}	4.8^{cd}
	Microwave vacuum drying	37.1^{b}	45.1^{b}	2.0^{b}	93.5^{d}	101.9^{c}	2.9^{f}
2	Raw	40.5^{a}	46.6^{a}	1.9^{b}	99.3^{a}	108.0^{a}	6.2^{ab}
	Oven drying	35.5^{c}	41.8^{d}	1.9^{b}	98.1^{b}	108.5^{a}	4.4^{de}
	Lyophilisation	37.1^{b}	43.8^{c}	2.8^{ab}	99.5^{a}	108.0^{a}	6.4^{a}
	Microwave vacuum drying	40.5^{a}	46.6^{a}	1.9^{b}	97.2^{b}	104.8^{b}	3.9^{e}

Means in the same colon that do not share same letters are significantly different, according to Fisher's test.

4.Conclusion

In this study, sprouted wheat seeds (*Triticum durum*) were dried according three technologies: Freeze drying, convective oven drying and Microwave vacuum drying (MVD). Our results clearly showed that evolution of bioactive compounds, physical, functional and thermal properties was strongly related to germination process and the technology used. Lyophilisation led to the highest nutritional quality. Interestingly, MVD induced less losses in bioactive compounds than oven drying (-23.5% for carotenoids, -67% for TPC -29.32% for DPPH RSA). Considering drying time and energy consumption, to reach desired moisture content, 24 hours were required for lyophilisation, 4 hours for oven and only 25 minutes for MVD. Thus, MVD could be suggested as the suitable technology to use for drying sprouts.

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Chapter 6: Evolution of functional, thermal and pasting properties of sprouted whole durum wheat flour with sprouting time

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Original article

Evolution of functional, thermal and pasting properties of sprouted whole durum wheat flour with sprouting time

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Evolution of functional, thermal and pasting properties of sprouted whole durum wheat flour with sprouting time*

Abstract

This research investigated the evolution of functional, pasting and thermal properties of durum wheat (*Triticum durum*) with sprouting time. Particle size, flour and flour gel hydration properties (Water Holding Capacity (WHC), Swelling Volume (SV), Water Absorption Index (WAI), Water Solubility Index (WSI), Swelling Power (SP)), Oil Absorption Capacity (OAC), Pasting and thermal properties were evaluated on different sprouting time: 12, 24, 36, 48 and 72 h. Results showed that more than 12 h decreased significantly particle size, WHC(-14.8%), SV (-19%), SP (-14%) and WAI (-36.5%) while WSI (+383%) and OAC (+7.3%) increased. Pasting properties drastically decreased with sprouting time. DSC results showed a significant increase in onset temperature (T_0) (from 55.2 to 58.2 °C), peak temperature (T_p) (from 62.4 to 63.8 °C) while conclusion temperature T_c decreased (from 76 to 72.6 °C). Despite these changes, sprouted whole wheat flour could be suggested as an improver of some cereal products' functionality.

Key words: Durum wheat, whole flour, sprouting, hydration properties, pasting properties.

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1. Introduction

Bread, pasta, cookies, cakes or noodles are common products in our diet. Wheat is the main ingredient contributing to all these products nutritional properties. In fact, wheat is the main source of carbohydrates, proteins, fibers, minerals and vitamins in human diet (Singh *et al*., 2015).

Consumers' perception of nutrition has markedly changed as they become more demanding for products with health promoting effect. Sprouting could be suggested as an alternative to satisfy this need. This bioprocess is recognized as an effective and inexpensive tool to enhance nutrients and bioactive compounds (Liu *et al*., 2017). Many previous studies (Donkor *et al*., 2012; Koehler *et al*., 2007; Plaza, Ancos, & Cano, 2003) and reviews (Gan *et al*., 2017; Hubner & Arendt, 2013; Lemmens *et al*., 2018; Singh *et al*., 2015) reported the improvement of cereals nutritional value through the use of sprouting bioprocess.

Sprouting is a physiological event that starts by water absorption by kernels which activate some enzymes to break down storage molecules (starch, protein) (Fu, Hatcher & Schlichting, 2014) in order to provide embryo with nutrients. Consequently, analysis of sprouted seeds shows a higher amount of reducing sugar, free amino acids and low molecular weight molecules (Singh *et al*., 2015). Some bioactive compounds like polyphenols could be newly formed during germination (Chen *et al*., 2017).

In cereal industry, carbohydrates (particularly starch) and proteins (mainly gluten) have a high interest not only for their nutritional value but also for their contribution to products' functional and technological properties. Thus, it would be expected that sprouting affects cereal products functional properties. The use of sprouted wheat flour had been suggested in cereal products, as bread and tortilla, with an improvement of their quality attributes (Afifiy *et al*., 2016; Liu *et al*., 2017; Marti *et al*., 2017; Marti *et al.*, 2018). The knowledge of functional properties is a key factor in cereal products' formulation. Ding *et al*. (2018) reported a decrease of whole wheat flour pasting properties after sprouting. Moreover, this bioprocess leads to a decrease in water absorption capacity (Fu, Hatcher & Schlichting, 2014).

Previous research investigated the effect of germination on some rheological and functional properties (Afify *et al*., 2016; Baranzelli *et al*., 2018; Hussain & Uddin, 2012). However, all these studies used soft wheat (*Triticum aestivum).* Durum wheat *(Triticum durum)* is the second most cultivated species of wheat after common wheat. It is used in several cereal products. To the best of our knowledge, no previous study evaluated the evolution of

hydration, pasting and thermal properties of durum wheat (*Triticum durum*) with sprouting time between the range of 12-72 h. Thus, the aim of this research is to investigate evolution of functional, pasting and thermal properties of sprouted whole durum wheat flour (*Triticum durum*) with sprouting time for its potential use as functional ingredient.

2. Material and methods

2.1Material

Two durum wheat (*Triticum durum*) cultivars were used in this study according to their different genetic background: Karim, a high yielding cultivar (14% protein (AACC 46-30.01), 64% starch (hydrolytic method and titration with $Na_2S_2O_3$), 9% moisture content (AACC 44-15.02), 1.8% ash (AACC 08-01.01)) and Chili, a landrace one, (17.9% protein, 54.6% starch, 7.3% moisture content, 2.6% ash). The National Institute of Cereal crops (INGC Bou Salem, Tunisia) and the National Gene Bank of Tunisia (BNG, Tunisia) kindly provided the samples.

2.2Methods

2.2.1Sprouting

Samples were firstly sterilized with 1% (V/V) hypochlorite sodium solution during 30 min, and then rinsed three times with distilled water. After this step, seeds were soaked (kernels: water ratio of 1:5) again in distilled water for 40 min and finally spread into plates with three layers of "Blotting paper". The seeds were germinated for different times: 12, 24, 36, 48 and 72 h in darkness at 25 °C (30% RH). For each germination time, at least 3 single batches were prepared. The same was applied for each cultivar. During germination seeds were static. Finally, sprouts were oven dried at 50°C for 8 h and milled (Ultra-Centrifugal Mill, Retsch, ZM 200, Germany with screen size of 500 µm). Milled samples were stored at 4 °C until further analysis.

2.2.2Microstructure and particle size distribution

Environmental scanning electron microscopy (ESEM) of raw and germinated whole wheat flour were taken on a Quanta 200 F microscope (Hillsboro, Oregon, USA). The sample was placed on a specimen holder with the help of double-sided scotch tape. ESEM was achieved under high vacuum at an acceleration voltage of 1.3 kV and using a back scattered electron detector (BSED) at a magnification of 500 x, and the particularities were shown at a magnification of 200 x and 1000 x.

Particle size distribution of raw and dried sprouted seeds was determined through a laser diffraction technique with a Malvern Mastersizer 3000 (Malvern Instruments Ltd., Malvern, Worcestershire, UK).

2.2.3Hydration properties

Water holding capacity (WHC) and swelling volume (SV)

Water holding capacity (WHC) and swelling volume (SV) were determined as follows: 5 g of sample were weighed in a graduated cylinder. Then, 100 ml of distilled water were added and the samples were kept at room temperature for 24 h. The water holding capacity corresponds to the amount of water retained by the sample without being subjected to any stress (AACC method 88-04, 2012). The swelling volume (SV) was calculated by dividing the total volume of swollen sample by the original dry weight of the sample.

Water binding capacity (WBC)

Water binding capacity, defined as the amount of water retained by the sample under centrifugation, was determined according to the AACC method 56-30.01 (AACC, 2012).

Flour gel hydration properties

Water absorbance index (WAI), Water solubility index (WSI) and Swelling power (SP) were assessed as described by Cornejo & Rosell (2015): Samples (50 mg ± 0.1) were dispersed in 1 ml of distilled water in Eppendorf tubes then cooked at 90°C for 15 min. The cooked paste was cooled with ice and then centrifuged at 3000x g at 4°C for 10 min. The supernatant was decanted into an evaporating dish and the weight of dry solids was recovered by evaporating the supernatant at 110 °C. Residues and dried supernatants were weighed and WAI, WSI and SP were calculated as follows:

$$\text{WAI (g/g)} = \frac{\text{Wheight of sediment}}{\text{Wheight of sample}} \quad (1)$$

$$\text{WSI (g/100g)} = \frac{\text{Weight of dried supernatant}}{\text{Wheight of sample}} \quad (2)$$

$$\text{SP (g/g)} = \frac{\text{Wheight of sediment}}{(\text{Sample weight} - \text{Weight of dried supernatant})} \quad (3)$$

2.2.4Oil absorption capacity (OAC)

Oil absorption capacity (OAC) was determined according to the protocol suggested by Lin, Humbert, & Sosulski (1974). Briefly, flour samples (100 mg ± 0.2) were mixed with 1 ml of sunflower oil. The content was stirred for 1 min to disperse the sample. The samples were vortexed for 30 min then centrifuged at 3000x g and 4 °C for 10 min. The supernatant was

removed with a pipette and tubes were inverted for 25 min and residues were weighed. The oil absorption capacity was expressed as grams of oil bound per gram of sample on dry basis. OAC was calculated according to equation (4):

$$OAC = \frac{\text{Residue weight}}{\text{Sample weight (g db)}} \quad (4)$$

2.2.5 Pasting properties

Pasting properties of sprouted seeds flour were analyzed in duplicate using the AACC method61-02.01 (AACC, 2012) with a Rapid ViscoAnalyser (RVA-4) (Perten Instruments, Hägersten, Sweden) controlled by Thermocline software (Perten Instruments, Hägersten, Sweden) for Windows. Sample (3.5 g) was added to 25 ml of water. The mixture was heated at 50 °C for 1 min then heated up to 95 °C at a rate of 12 °C/min. The temperature of 95 °C was held for 2.5 min then the suspension was cooled up to 50 °C using the same rate. The rotational speed of the paddle was 960 rpm at the first 10 s then it was maintained at 160 rpm during the trial.

2.2.6Thermal properties

Thermal properties of whole wheat sprouted flour were determined according to the procedure described by Roman *et al*. (2017) with slight modification. Briefly, flour (6 mg) was loaded into the aluminum pan and distilled water (18 µL) was added using a micro syringe. Samples were hermetically sealed and allowed to equilibrate for 30 min at 30 °C before heating in the DSC oven. Samples were kept at 30 °C for 2 min, heated from 30 to 100 °C at 10 °C/min. Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy (ΔH) (expressed as J/g of sample in dry basis) of starch gelatinization were determined. A differential scanning calorimeter Q-20 (TA instruments, Crawley, UK) equipped with a refrigerated cooling system (RCS 40) was used. An empty pan was used as reference and dry nitrogen at a flow rate of 50 ml/min was used as the purge gas.

2.3Statistical analysis

Statistical analysis was carried out using the Minitab software (Minitab 17, USA). The average values of experiments 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

3.1Microstructure and particle size distribution

Particle size distribution is presented in Figure 9. As shown, germination affected particle size distribution with an increase in mass fraction of fine particles (<0.2 mm) and a decrease in the mass fraction of coarse particles (>1.0 mm) without differences with sprouting time. The same trend of evolution was observed with both varieties. These results are in line with previous ones dealing with sprouted soft wheat (*Triticum aestivum*) for 3 and 4 days (Dziki *et al*., 2015; Dziki & Laskowski, 2010). Evolution of particle size distribution could be linked to role of sprouting in decreasing grains hardness (Mis & Grundas, 2002). This decrease was due both physical and biochemical changes occurring during germination. In one hand, to provide embryo with nutrients macromolecules are degraded under enzymatic activity (Mak *et al*., 2009). In the other hand, the first step in sprouting consists on soaking seeds in water. This rapid moistening induces a mechanical change "cracking" leading to grains' softening (Mis & Grundas, 2002). Overall, it is known, the bran gives rise coarser particles than endosperm during milling (Dziki & Laskowski, 2010). In our case, only significant differences were observed, in the coarsest fractions, between the raw wheat flour and the sprouted ones, independently of the sprouting time. This fact could state that the differences between samples are caused mainly by the drying process after sprouting. The bran becomes more brittle when dried and the percentage of coarse particles produced in milling is lower thanks to the decrease of hardness after drying (Mis & Grundas, 2004). For his part, a higher percentage of fine particles (<0.2 mm) was observed when the sprouting time was increased.

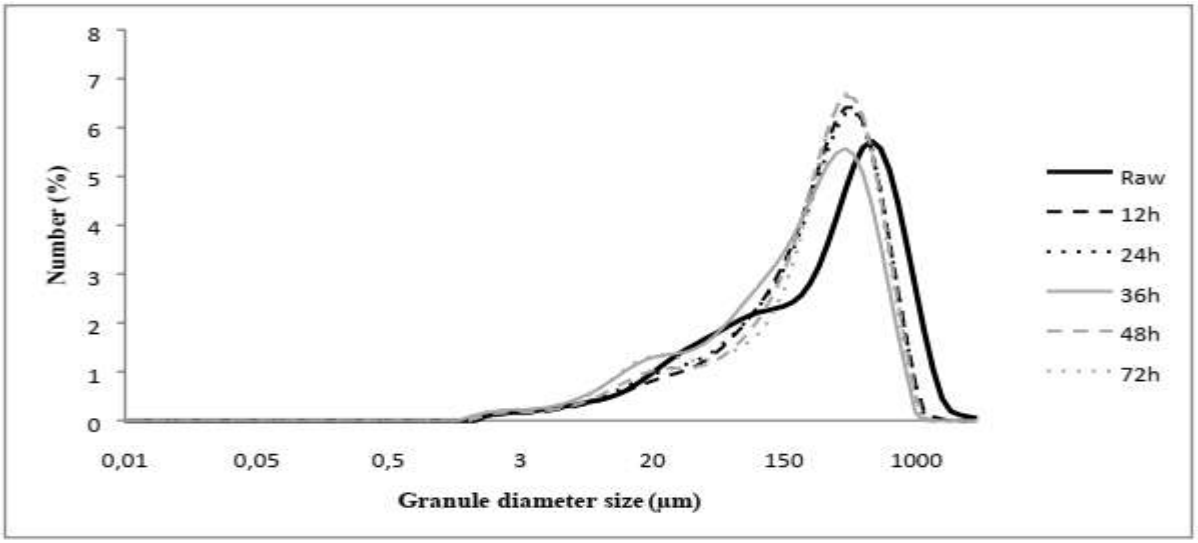

Figure 9: Particle size distribution for raw and germinated samples for the cultivar "Karim"

3.2Functional properties: Flour and flour gel hydration properties

Evolution of wheat flour and flour gel hydration properties after sprouting are summarized in Table 19. As it can be seen, for both cultivars sprouting decreased significantly ($p<0.05$) water holding capacity (WHC) and swelling volume (SV) while no difference on water binding capacity (WBC) was observed. These results agree with those obtained with brown rice flours (Cornejo & Rosell, 2015) and sorghum flours (Singh, Sharma, & Singh, 2017). Germination bioprocess induces catabolism of storage molecules to provide the embryo with nutrients. Particularly, starch is degraded under amylasic enzymes activity (Mak *et al*., 2009). Thus, starch structural modifications affect hydration properties (Alcazar-Alay & Almeida Meireles, 2015).

Regarding flour gel hydration properties, results showed that germination process reduced significantly swelling power from 36 h. The decrease in swelling power has been previously reported with germinated brown rice (Cornejo & Rosell, 2015) and germinated sorghum (Singh *et al*., 2017). Such decrease was related to starch degradation. As germination time increases starch content may decrease while low molecular weight starch molecules and oligosaccharides amounts increase. These sugars have not the swelling power of starch granules (Singh *et al*., 2017). Consequently, a decrease in this parameter is detected on sprouted wheat flour.

Regardless the cultivar tested, WAI significantly decreased from 24 to 36 h of germination. The same duration was required to detect a significant increase in WSI. These findings are in line with those of Cornejo & Rosell (2015) for germinated brown rice. However, results of this study showed that short germination time (12 h) induced significant changes in these parameters which was not the case in our study. The divergence is probably related to genetic differences between durum wheat and brown rice and sprouting procedure adopted (soaking time 12 h versus 40 min in our study, sprouting temperature 28 °C while we used 25 °C). Results of Singh *et al*. (2001) also clearly showed that WSI increased with the increase in soaking and sprouting duration while the WAI decreased with them. The authors explained these results by the role of amylasic enzymes as reported by Cornejo & Rosell (2015).

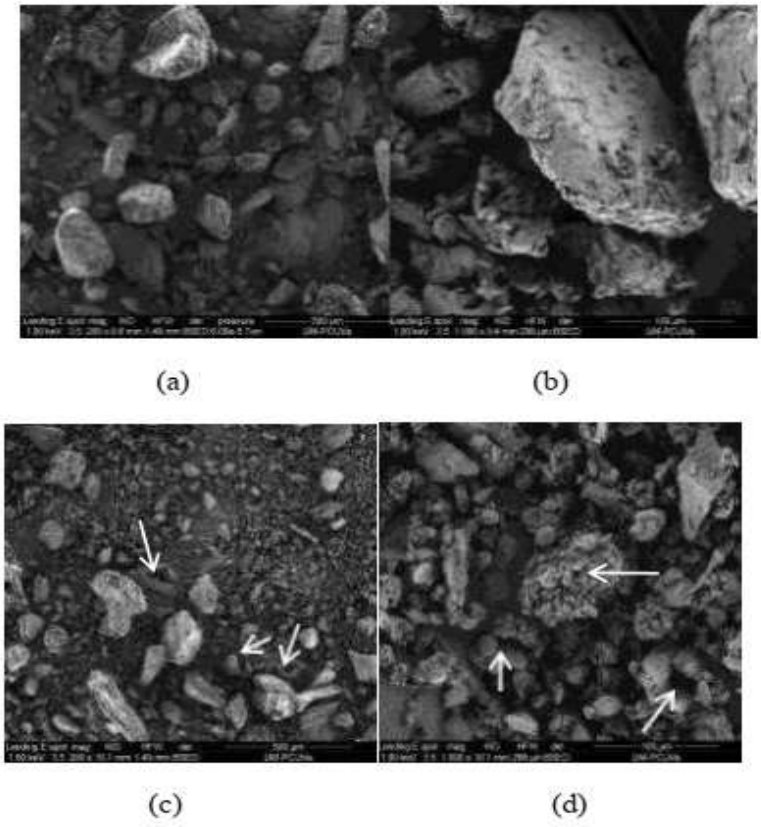

Figure 10: Scanning electron micrographs of native and germinated whole wheat flour cultivar "Chili": (a) Raw sample(x200), (b): Raw sample (x1000); (c): Germinated sample for 72 h (x200); (d) Germinated sample for 72 h (x1000)

Table 19: Evolution of sprouted whole wheat flour functional properties with sprouting time

Cultivar	Germination time (h)	WBC(g/g dm)	WHC (g/g dm)	SV (cm^3/g)	SP(g/g dm)	WAI (g/g dm)	WSI (g/100g dm)	OAC (g/g dm)
Karim	Raw	2.22±0.07^{a}	3.56±0.22^{abcde}	3.95±0.07^{b}	8.05±0.3^{a}	7.66±0.09^{a}	7.93±0.27^{ef}	1.98±0.02^{d}
	12	2.16±0.08^{a}	3.66±0.09^{abcd}	3.97±0.03^{b}	8.09±0.14^{a}	7.47±0.26^{a}	5.66±1.02^{f}	1.99±0.08^{cd}
	24	2.17±0.06^{a}	3.19±0.40^{ef}	3.45±0.22^{cd}	7.96±0.24^{a}	7.45±0.20^{a}	6.87±0.80^{ef}	2.04±0.12^{bcd}
	36	2.16±0.07^{a}	3.31±0.30^{cdef}	3.34±0.24^{cde}	6.52±0.20^{c}	5.31±0.37^{c}	21.20±0.52^{cd}	2.08±0.02^{bcd}
	48	2.16±0.02^{a}	3.15±0.38^{ef}	3.20±0.02^{de}	6.61±0.46^{bc}	5.16±0.26^{cd}	22.35±2.01^{c}	2.09±0.01^{abcd}
	72	2.19±0.00^{a}	3.76±0.09^{ab}	3.47±0.26^{cd}	6.22±0.48^{c}	4.69±0.35^{d}	26.35±1.17^{b}	2.11±0.02^{abc}
Chili	Raw	2.20±0.01^{a}	3.93±0.44^{a}	4.24±0.21^{a}	7.78±0.21^{a}	7.77±0.19^{a}	6.83±0.53^{ef}	2.05±0.04^{bcd}
	12	2.17±0.04^{a}	3.68±0.24^{abc}	4.05±0.01^{ab}	8.23±0.24^{a}	7.29±0.22^{a}	6.01±0.71^{f}	2.09±0.07^{abcd}
	24	2.19±0.01^{a}	3.10±0.12^{f}	3.24±0.01^{cde}	8.03±0.48^{a}	7.38±0.46^{a}	8.73±0.57^{e}	2.06±0.01^{bcd}
	36	2.18±0.07^{a}	2.92±0.20^{f}	3.15±0.11^{e}	7.11±0.35^{b}	5.82±0.40^{b}	19.41±2.30^{d}	2.07±0.09^{bcd}
	48	2.17±0.02^{a}	3.24±0.11^{def}	3.14±0.11^{e}	7.08±0.38^{b}	5.39±0.24^{bc}	20.90±2.87^{cd}	2.12±0.1^{ab}
	72	2.18±0.03^{a}	3.35±0.07^{bcdef}	3.44±0.20^{cd}	6.69±0.08^{bc}	4.93±0.28^{cd}	32.95±0.23^{a}	2.20±0.08^{a}

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

Oil absorption capacity (OAC) increased gradually with sprouting time for both tested cultivars. Similar findings were previously reported with germinated brown rice (Cornejo & Rosell, 2015) and germinated sorghum (Singh *et al*., 2017). The increase in oil absorption capacity could be related to the role of proteolytic enzymes on protein quality changes during

sprouting (Koehler *et al*., 2007) as high lipophylic surface could increase OAC (Singh *et al.*, 2015). The use of sprouted whole wheat flour would be recommended for products with high fat content. Moreover, it may contribute in improving products flavour retaining and mouthfeel (Kaushal, Kumar & Sharma, 2012).

3.3Pasting properties

Pasting properties evolution with germination time is presented in Figure 11. A significant increase in peak viscosity was observed after 12 h of germination. Then, it drastically decreased for both cultivars from 36 h. An increase in germination time decreased viscosity. Although peak values are different between tested cultivars, the same evolution was observed after sprouting. These findings, concerning the decrease in peak viscosity, are in agreement with those of Baranzelli *et al*. (2018) and Grassi *et al*. (2018). The decrease in peak viscosity was probably related to the degradation of starch to low molecular weight particles under amylasic enzyme activity during germination process (Ding *et al*., 2018). This increase in α-amylase activity during germination and decrease in viscosity has been previously studied (Ichinose *et al*., 2001). Moreover, according to Zhang & Hamaker (2005), evolution of proteins during germination also could affect pasting profiles. The modification of proteins network structure by enzymatic hydrolysis might increase the weakness of swollen starch granules inducing a decrease in viscosity (Zilic *et al*., 2016). Regarding the increase in peak viscosity after 12 h, Grassi *et al.* (2018) observed an increase in peak viscosity of whole wheat flour after 24 h if compared to longer sprouting time. This evolution might be explained by the role of sprouting on decreasing kernels hardness (Mis & Grundas, 2002) and kernels hardness increases starch damage (Li, Dhital & Hasjim, 2014; Roman *et al*., 2017). Accordingly, it might be possible that 12 h of germination did induce significant starch degradation. However, sprouted kernels are softer than raw ones which increased starch damage in raw seeds. Thus, a higher peak viscosity was observed with sprouted samples than raw as starch damage decreases peak viscosity (Sharif Hossen *et al*., 2011). Increasing sprouting time would not be recommended if the flour would be used for elaboration of products, where viscosity plays a key role in final product quality, like cakes or bread.

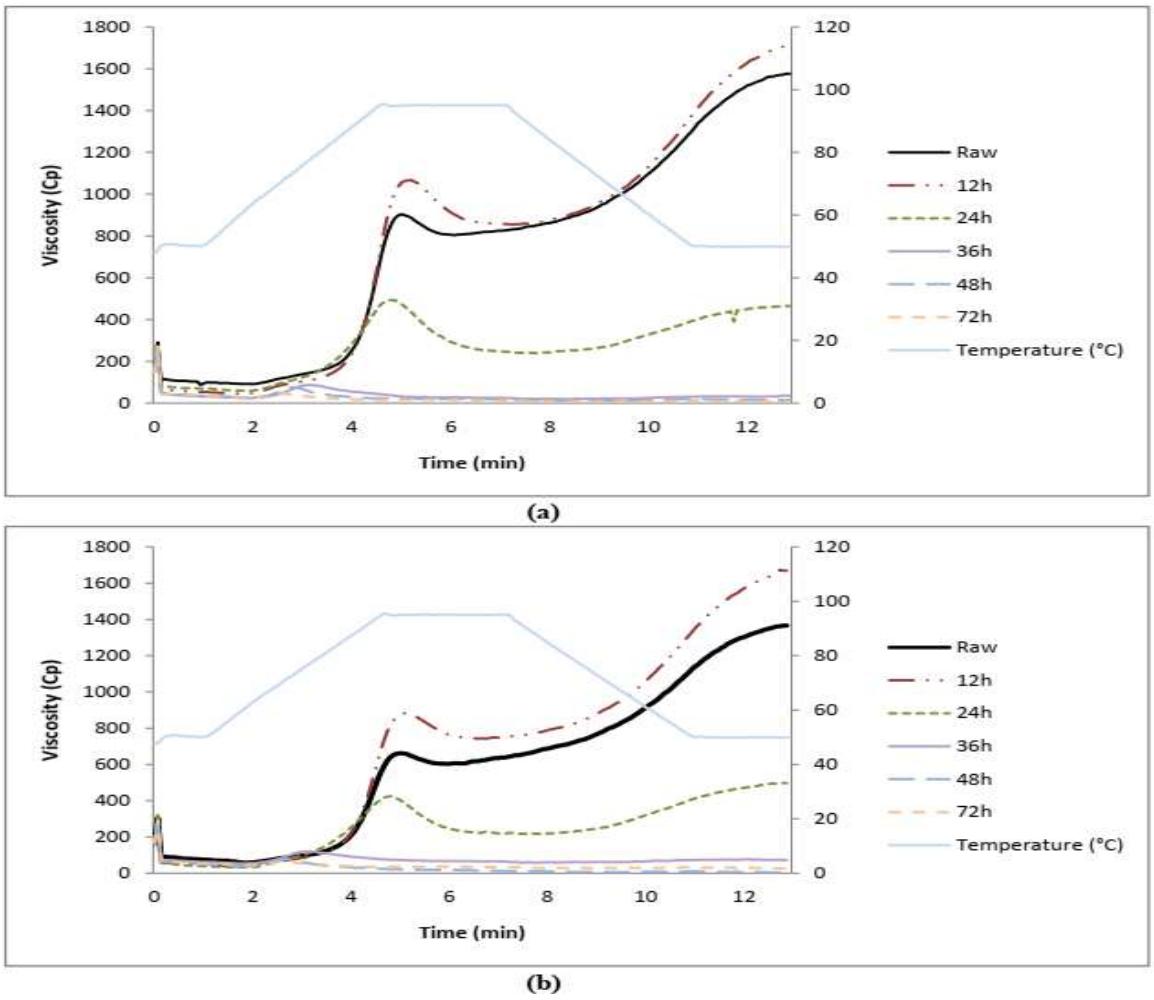

Figure 11: Evolution of pasting profile of whole wheat flour with germination time: (a) cultivar "Chili"; (b) cultivar "Karim"

3.4Thermal properties

The effect of sprouting time on whole wheat flour thermal properties is shown on Table 20. In the range of tested temperature (30-110 °C), an endotherm peak was observed for all tested samples between 62.36 and 65.08 °C. It could consequently be attributed to starch gelatinization as wheat starch gelatinization takes place between 54 and 73 °C (Liu *et al*., 2006). Significant differences are observed between the two tested cultivars on thermal properties, probably due the genetic background of each (Bao *et al*., 2009) and differences in composition. Despite these differences, both cultivars showed the same evolution of thermal properties after sprouting. An increase in sprouting times increased T_0 and T_p while it decreased T_c. The enthalpy increased after 12 h then decreased gradually with sprouting time. A similar decrease in enthalpy was previously observed in germinated brown rice starch (Li *et al*., 2017). In other words, after sprouting endotherms are shorter and peaks occur at higher temperature. The gelatinization endotherm is an indicator of partial dissolution of starch polymers (Wang & Copeland, 2013). An increase in gelatinization temperature might be

linked to a modification of starch granule structure (Martinez, Rosell & Gomez, 2014). Previous results of Li *et al*. (2017), showed evolution on morphological properties after grain germination, mainly changes on surface and increase in pores. Furthermore, sprouting process is known by starch degradation and therefore, by the generation of oligosaccharides and simple sugars. Results of Johnson, Davis & Gordon (1990) showed that an increase in simple sugar concentration of a starch water mixture raised gelatinization temperature, what would explain our results. The decrease in native starch granules for longer sprouting time could explain the decrease in enthalpy. Li *et al*. (2017) observed a decrease in gelatinization enthalpy of brown rice after germination, mainly changes on surface and increase in pores (Figure 10). They attributed this decrease to structural changes of starch during germination, particularly the disruption of some of the hydrogen bonds linking adjacent double helices.

Table 20: Evolution of thermal properties with germination time

Cultivar	Germination time (h)	T_0 (°C)	T_p (°C)	T_c (°C)	ΔH (J/g dm)
Karim	Raw	55.53±0.42^{g}	62.36±0.24^{g}	76.04±0.07^{b}	3.12±0.09^{g}
	12	55.87±0.18^{g}	62.50±0.14^{fg}	76.96±0.56^{a}	5.09±0.08^{a}
	24	56.71±0.29^{f}	62.75±0.14^{fg}	76.43±0.31^{ab}	4.16±0.07^{cd}
	36	57.43±0.07^{e}	63.43±0.00^{cde}	74.08±0.27^{c}	4.45±0.16^{b}
	48	57.56±0.22^{e}	63.33±0.42^{de}	73.67±0.26^{c}	4.34±0.28^{bc}
	72	58.18±0.07^{cd}	63.82±0.17^{bcd}	72.64±0.52^{d}	3.45±0.03^{f}
Chili	Raw	57.29±0.19^{e}	63.46±0.43^{cd}	77.09±0.15^{a}	3.99±0.09^{d}
	12	57.35±0.07^{e}	62.93±0.06^{ef}	77.25±0.09^{a}	4.51±0.04^{b}
	24	57.79±0.05^{de}	63.38±0.24^{cde}	75.99±0.75^{b}	4.17±0.09^{cd}
	36	58.37±0.34^{bc}	63.85±0.14^{bc}	74.09±0,34^{c}	3.97±0.01^{d}
	48	58.80±0.14^{b}	63.99±0.17^{b}	73.87±0.19^{c}	3.71±0.06^{e}
	72	59.78±0.36^{a}	65.08±0.21^{a}	73.95±0.50^{c}	2.55±0.06^{h}

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

4. Conclusion

Germination time plays an important role in evolution of durum wheat (*Triticum durum*) functional, thermal and pasting properties. The changes observed in the durum wheat after sprouting as starch gelatinization, batter viscosity after gelatinization or water holding capacity are especially important at 24 h and, above all, from 36 h of sprouting. Therefore, for elaborations where the starch gelatinizes, such as sauces or cakes, it would be better to use flours with lower sprouting times. For other elaborations, in which the starch gelatinization does not occur, as cookies, the use of flour with higher sprouting time could be more

interesting. Meanwhile, it is something that should be studied. In the case of products like breads or pasta, where the gluten network is crucial, it is important to keep in mind the effect of sprouting on wheat properties.

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Chapter 7: Effect of sprouting time on dough and cookies properties

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ORIGINAL PAPER

Effect of sprouting time on dough and cookies properties

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Effect of sprouting time on dough and cookies properties*

Abstract

The role of sprouting in improving cereals nutritional properties is known. However its use in baked products is limited due the functional modifications occurring. In this study, the use of whole wheat flour sprouted for 24 and 48 h (SWWF), and SWWF-refined flour blends (50:50) in cookies elaboration was investigated. Sprouting decreased water holding capacity (WHC) and swelling volume (SV) and increased oil absorption capacity (OAC) gradually with sprouting time. For the dough, an increase in visco-elastic moduli (G' and G") was recorded when whole wheat flour (WWF) and SWWF were used instead refined flour. However, no significant differences were observed between SWWF, weather for 24 or 48 h, and raw WWF. Regarding cookies, both WWF and SWWF decreased spread factor and increased hardness compared to control. Cookies color parameters were also affected with a decrease in lightness (L*) and yellowness (b) and an increase in redness (a). Cookie color changes were more pronounced with 100% 48 h sample. Despite these changes, consumers overall acceptability was improved when both WWF and SWWF were used.

Key words: Whole-wheat flour, sprouting, flour properties, rheology, cookies characteristic.

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1. Introduction

The widespread of several diseases, such as obesity, type 2 diabetes, cardiovascular diseases and cancers, raised consumers' awareness about the importance of their diet on their health. Consequently, the demand for health-oriented food products is steadily increasing in food market (Baumgartner *et al.*, 2018) and the use of whole grain could be suggested in this context due to its nutritional composition. Whole grains have a high level of nutrients like fibers, minerals, vitamins and phytochemicals (Liu, 2007). For this reason, consumption of whole grain cereals may reduce some diet related diseases as proved by several epidemiological studies (Fardet, 2010).

Sprouting bioprocess is known by improving cereals nutritional properties by enhancing nutrients and bioactive content amounts (Gan *et al.*, 2017). Bioactive compounds like carotenoids, phenols and vitamins (A, B, C & E) are newly formed during sprouting (Lemmens *et al.*, 2018). Furthermore, sprouting decreases phytate content (Bohn et al., 2008) which improves minerals bioaccessibility (Lemmens *et al.*, 2018). On the other hand, the evolution of storage molecules during this bioprocess improves flour digestibility thanks to starch and protein degradation (Dziki *et al.*, 2015).

Cookies are widely consumed all over the world due ready to eat and easy storage nature, long shelf life, availability of several varieties and low cost (Jan, Panesar, & Singh, 2018; Pareyt and Delcour, 2008). Thus, they might be suggested as an effective vehicle of nutritional fortification (Rao, Kulkarni, & Kavitha, 2018).

Despite the nutritional interest of sprouted wheat flour, its use in cereal products is challenging as its functional properties are affected after starch and protein degradation. Previous studies suggested the use of sprouted wheat flour in different bakery products such as tortillas (Liu *et al.*, 2017), breads (Marti *et al.*, 2018; Sweica, Dziki & Gawlik-Dziki, 2017) and cakes (Yaqoob *et al.*, 2018). However, no previous study investigated the use of sprouted whole wheat flour (SWWF) in cookies. Thus, the aim of this study is to evaluate the influence of sprouted whole wheat flour use in cookies formulation. Durum wheat (*Triticum durum*) was selected as grain to sproute since it is more cultivated in some North African areas, like Tunisia, compared to bread wheat (*Triticum aestivum*) (Chedli *et al.*, 2018). In this way, durum wheat was sprouted for 24 and 48 h and the obtained SWWF together with their blends at 50% with refined flour were used in cookies elaboration. Flour hydration properties and oil absorption capacity were measured. Dough properties were assessed through visco-elastic

moduli. Cookies were characterized by their physical properties (spread factor, color, texture) and consumer acceptability.

2. Material and methods

2.1Materials

Wheat (*Triticum durum*) (13.91 g/100 g protein) was supplied by Harinera Villamayor (Huesca, Spain). The rest of ingredients were: refined flour (11.82 g/100 g moisture, 10.40 g/100 g protein) (Haricaman, Toledo, Spain), white sugar (AB Azucarera Iberica, Valladolid, Spain), sodium bicarbonate (Manuel Riesgo S.A., Madrid, Spain) and margarine (Argenta Crema, Puratos, Barcelona, Spain).

2.2Methods

2.2.1 Preparation of flours: sprouting and milling

Durum wheat seeds were sprouted exactly as described previously by Jribi *et al.* (2019a). Briefly, samples were disinfected with 1% (V/V) hypochlorite sodium solution during 30 min. Then, the grains were rinsed three times with distilled water to remove completely solution residues. After this step, seeds were soaked again in distilled water for 40 min and finally spread into plates with three layers of "Blotting paper". The seeds were germinated in darkness for 24 or 48 hours at 25 °C at 30% R.H. Finally, sprouts were oven dried at 50 °C for 8 h and milled with a screen size of 500 µm (Ultra-Centrifugal Mill, Retsch, ZM 200, Germany). Milled samples were stored at 4°C until further analysis.

2.2.2 Flour characteristics

Water holding capacity (WHC) and swelling volume (SV)

Water holding capacity (WHC) and swelling volume (SV) were determined in duplicate as follows: 5 g of sample were weighed in a graduated cylinder. Then, 100 ml of distilled water were added and the samples were kept at room temperature for 24 h. After that, the water was removed and the weight and volume of swollen sample were recorded. In this way, the water holding capacity corresponds to the amount of water retained by the sample without being subjected to any stress (AACC method 88-04, 2012). The swelling volume (SV) was calculated by dividing the total volume of swollen sample by the original dry weight of the sample.

Oil absorption capacity (OAC)

Oil absorption capacity (OAC) was determined in triplicate according to the protocol suggested by Lin, Humbert, & Sosulski (1974). Briefly, flour samples (100 ± 0.2 mg) were

mixed with 1 ml of sunflower oil. The content was stirred for 1 min to disperse the sample. The samples were vortexed for 30min then centrifuged at 3000x g and 4 °C for 10 min. The supernatant was removed with a pipette and tubes were inverted for 25 min. Residues were weighed. The oil absorption capacity was expressed as grams of oil bound per gram of sample on dry basis. OAC was calculated according to equation (1):

$$\text{OAC} = \frac{\text{Residue weight}}{\text{Sample weight (g db)}} \quad (1)$$

2.2.3Cookie making

Six different flours were used for cookies formulation: 100% refined flour (Control), 100% whole wheat flour (WWF), 100% sprouted whole wheat flour (SWWF) for 24 h (100% 24 h), 100% SWWF for 48 h (100% 48 h), and 50:50 mixtures of refined flour with SWWF for 24 h (50% 24 h) and 48h (50% 48 h). The following ingredients proportions were used (g/100g dough basis): 42.8 flour, 19.2 margarine, 30.8 sugar, 0.9 sodium bicarbonate and 6.2 of water. Cookies were elaborated as described by Bravo-Nuñez *et al.* (2018). Briefly, on a first step the moisture of the flour blends was adjusted to 15 g/100 g. Then, the ingredients were mixed accordingly: The margarine was heated in the microwave (1000 watts for 1 min). The melted margarine together with the sugar were mixed in a Kitchen Aid 5KPM50 mixer (Kitchen Aid, Benton Harbor, MI, USA) using a flat beater at speed 4 for 180 s, with intermediate scraping every 60 s. The water was then added and mixed at speed 4 for 120 s, with a scraping steep at the end. Finally, the flour and the sodium bicarbonate were included and mixed at speed 2 for 120 s, with scraping every 30 s. The obtained dough was covered with plastic film and allowed to rest for 30 min at 25 °C. After the resting period, the dough was laminated with a Salva L-500-J sheeter (Salva, Lezo, Spain) using a gap of 6.00 mm and cut with a circular cookie cutter (internal diameter: 40 mm). The resulting dough pieces were baked in an electric modular oven for 14 min at 185 °C. Finally, cookies were cooled down for 60 min at room temperature before packing them in plastic bags and storing them at 24 °C. All cookies were made in duplicate.

2.2.4Dough rheology

For the rheological behavior of doughs a controlled strain rheometer (Thermo Fisher Scientific, Schwerte, Germany) equipped with a parallel- plate geometry (60 mm diameter titanium serrated plate-PP60 Ti) and a water bath (Thermo Fisher Scientific) at 25 °C were used following the methodology suggested by Mancebo *et al.* (2016).Circular dough pieces (3 mm height and 60 mm width) were placed in the rheometer and compressed with a gap of 3 mm. Samples were rested for 300 s before measuring. A strain sweep test in the range of 0.1–

100 Pa at a constant frequency (1 Hz) was conducted to identify the strain value included in the linear viscoelastic region. This strain value was used to perform a frequency sweep test in a frequency range from 10 to 0.1 Hz. Elastic modulus (G'[Pa]), viscous modulus (G″[Pa]) and loss factor (tan δ) were obtained. Doughs were analyzed in duplicate.

2.2.5Physical properties of cookies

From each elaboration, the dimensions (diameter and height) of six cookies were measured with a caliper. Cookie diameter was measured twice, perpendicularly, in order to calculate an average diameter. Spread factor of cookies was determined by dividing the average diameter by the thickness.

Cookie color was measured using a Minolta CN-508i spectrophotometer (Minolta, Co. LTD, Tokyo, Japan) with the D65 standard illuminant and the 2° standard observer. Measurements were carried out at the cookie centre of the upper surface (crust) for six cookies from each elaboration. Results were expressed in the CIE L*a*b* color space.

Cookies texture was determined by a 'three-point bending' test, using a TA-XT2 texture analyser (Stable Microsystems, Surrey, UK) and a sounding line HDP/ 3PB with a test speed of 2.0 mm/s. The maximum force (N) to break the cookies (hardness) was measured. From each elaboration, eight cookies were used for textural analysis. All physical properties were determined seven days after baking.

2.2.6Sensory analysis

Hedonic sensory evaluation of the cookies was conducted with 79 volunteers who were habitual cookie consumers. For sensory evaluation, samples were presented as whole pieces on white plastic dishes coded with four-digit random numbers and served in random order. A nine-point hedonic scale was used to evaluate cookies acceptability on the basis of their appearance, odor, texture, taste and overall appreciation. The scale of values ranged from "like extremely" to "dislike extremely", corresponding to the highest and lowest scores of "9" and "1" respectively.

2.3Statistical analysis

One way Analysis of variance (ANOVA) was performed using the Fisher test for determination of differences between means. Significance was defined at $p < 0.05$. Statistical analysis was carried out using the Minitab software (Minitab 17, Pennsylvania, USA).

3. Results and discussion

3.1 Flour properties

Flour properties are summarized on Table 21. WHC and SV, WWF had the highest value. An increase in sprouting time decreased these two parameters as shown by 100% 24 h and 100% 48 h samples. Cornejo and Rosell (2015) reported also a decreasing trend in hydration properties (WHC and SV) for germinated brown rice. The differences in hydration properties between refined flour and sprouted and/or whole wheat flour might be attributed to the ability of the bran in binding water (Li *et al.,* 2016). Starch also plays a key role in hydration properties. In fact, starch holds water through hydrogen bonding between amylose and amylopectin branches and inter amylopectin helices (Roozendaal, Abu-hardan, & Frazier, 2012). Accordingly, the decrease in WHC and SV in sprouted samples is expected as sprouting is known by starch degradation under amylasic enzymes (Hung *et al.*, 2011).

Regarding oil absorption capacity (OAC), WWF had higher values than refined flour since fibre of wheat bran has a high capacity to hold oil (Elleuch *et al.*, 2011). For SWWF, OAC increased gradually with sprouting time. The role of sprouting in increasing OAC has been reported previously with germinated sorghum (Singh, Sharma & Singh, 2017) and germinated brown rice (Cornejo & Rosell, 2015). This trend after sprouting might be related to the protein degradation under proteolytic enzymes which may increase lipophylic surfaces (Singh *et al.*, 2015).

Table 21: Flour functional properties

Flour	WHC (g/g db)	SV	OAC (g/g db)
Control	1.50 ± 0.06^{e}	2.25 ± 0.13^{de}	1.82 ± 0.05^{d}
WWF	3.73 ± 0.31^{a}	3.88 ± 0.23^{a}	1.97 ± 0.02^{b}
50% 24 h	2.21 ± 0.08^{d}	2.68 ± 0.22^{c}	1.93 ± 0.02^{bc}
100% 24 h	3.24 ± 0.09^{b}	3.59 ± 0.01^{b}	2.03 ± 0.03^{b}
50% 48 h	2.15 ± 0.22^{d}	2.14 ± 0.12^{e}	1.87 ± 0.04^{cd}
100% 48 h	2.71 ± 0.19^{c}	2.42 ± 0.00^{cd}	2.20 ± 0.03^{a}

Means in same column that do not share same letters are significantly different, according to Fisher's test.($p<0.05$). Control: refined flour; WWF: whole wheat flour; 50% 24 h: blend containing 50% refined flour and 50% sprouted whole wheat flour for 24 h; 100% 24 h: 100% sprouted whole wheat flour for 24 h; 50% 48 h:blend containing 50% refined flour and 50% sprouted whole wheat flour for 48 h. 100% 48 h:100% sprouted whole wheat flour for 48 h; WHC: water holding capacity; SV: swelling volume; OAC: oil absorption capacity, db: dry basis

3.2Dough rheological properties

As seen on Table 22, for all samples, G' is higher than G'', which confirms the elastic behavior of the dough. Refined flour had lower values of G', G'' and G^{*} than WWF and SWWF. In this way, the presence wheat bran increased the rheological dough values which agree with the higher water absorption capacity of WWF samples. Contrarily, refined flour

had the highest value of Tan δ due to the greater rise of G' compared to G''. Regarding the effect of sprouting time, this factor did not induce significant ($p<0.05$) changes in G' and G'' and mixtures of refined and SWWF (weather for 24 or 48 h) had intermediate values. These results are not in line with those of Singh *et al.* (2001) where significant changes in viscolelastic moduli were observed after sprouting bread wheat up to 24 h. However, in this study the dough was only composed by wheat flour and water and, therefore, there was a great gluten network development being the responsible of the dough rheology (Singh & Singh, 2013). In this way, the proteolytic activity manifested during sprouting (Gan *et al.*, 2017) can hydrolyze the gluten network (Koehler *et al.*, 2007) and modifies dough rheology. In our case, the high fat and sugar quantities hinder the gluten development and the rheological behavior is influenced not only by the flour but also by the rest of ingredients. The use of WWF induced a significant decrease in Tan δ if compared to control. Meanwhile, sprouting did not affect Tan δ. The difference between refined and WWF might be related to the composition of each as higher fiber content are detected in WWF. In fact, results of Li *et al.* (2018) showed that an increase in aleurone-rich fraction (ALF) decreased dough Tan δ values. Such findings reflect an improvement in dough stability thanks to bran fibers (Li *et al.*, 2016).

Table 22: Rheological characteristic of the dough

Sample	G'(Pa)* 10^5	G''(Pa)*10^5	G*(Pa)* 10^5	Tan δ
Control	0.66 ± 0.13^{d}	0.24 ± 0.05^{c}	0.71 ± 0.14^{d}	0.36 ± 0.01^{a}
WWF	1.97 ± 0.16^{a}	0.56 ± 0.01^{a}	2.05 ± 0.16^{a}	0.29 ± 0.03^{d}
50% 24h	1.32 ± 0.27^{bc}	0.42 ± 0.07^{b}	1.39 ± 0.28^{bc}	0.32 ± 0.01^{bc}
100% 24h	1.88 ± 0.35^{a}	0.55 ± 0.09^{a}	1.96 ± 0.36^{a}	0.30 ± 0.02^{cd}
50% 48 h	1.13 ± 0.28^{c}	0.37 ± 0.08^{b}	1.18 ± 0.29^{c}	0.33 ± 0.01^{b}
100% 48 h	1.63 ± 0.15^{ab}	0.46 ± 0.03^{ab}	1.70 ± 0.14^{ab}	0.30 ± 0.02^{cd}

Means in same column that do not share same letters are significantly different, according to Fisher's test.($p<0.05$). Control: refined flour; WWF: whole wheat flour; 50% 24 h: blend containing 50% refined flour and 50% sprouted whole wheat flour for 24 h; 100% 24 h: 100% sprouted whole wheat flour for 24 h; 50% 48 h:blend containing 50% refined flour and 50% sprouted whole wheat flour for 48 h. 100% 48 h:100% sprouted whole wheat flour for 48 h.

3.3Cookies properties

3.3.1Physical properties

Compared to control, the use of WWF decreased significantly spread factor. In fact, this decrease was due the increase in thickness and decrease in cookie diameter (Table 3). Sprouting did not affect this parameter as no significant differences were seen between WWF, 100% 24 h and 100% 48 h samples. The effect of WWF on decreasing cookie spread factor has been reported previously (Demir, 2014), just like the incorporation of wheat bran (Sudha, Vetrimani, & Leelavathi, 2007). Similarly, Jan *et al.* (2016) observed that the use of germinated chenopodium flour did not affect spread factor significantly. Evolution of spread factor highlights previous results of hydration and rheological properties. Compared to control, the use of WWF and SWWF increased hydration capacity (Table 21) and dough viscosity (as G'' increased) (Table 22). Such increase in hydration capacity and viscosity might decrease expansion during baking (Chauhan, Saxena, & Singh, 2016; Mancebo *et al.*, 2016) which decreases cookies diameter (Table 23) and consequently spread factor.

Table 23: Cookies physical characteristic

Flour used	Thickness (mm)	Width (mm)	Spread factor	Hardness (N)	L*	a	b
Control	7.57 ± 0.4^{d}	61.73±1.0^{a}	8.15±0.4^{a}	37.18±8.0^{d}	55.8±7.8^{a}	4.07±0.6^{e}	15.55±4.9^{b}
WWF	10.57 ±0.3^{a}	46.25±0.8^{d}	4.37±0.1^{d}	64.50±8.6^{b}	59.3±3.1^{a}	5.25±0.18^{c}	19.14±2.0^{a}
50% 24 h	8.99 ± 0.4^{c}	51.41±0.5^{c}	5.72±0.3^{c}	43.79±7.1^{c}	46.7±4.9^{c}	4.34±0.38^{e}	12.66±3.6^{c}
100% 24 h	10.27±0.5^{ab}	45.01±0.4^{e}	4.38±0.2^{d}	76.56±10.0^{a}	50.6±2.1^{b}	4.79±0.37^{d}	15.61±1.3^{b}
50% 48 h	8.98 ± 0.6^{c}	53.94±0.8^{b}	6.01±0.4^{b}	42.94±5.5^{c}	47.9±3.8^{bc}	5.89±0.54^{b}	10.92±3.0^{c}
100% 48 h	10.15 ±0.4^{b}	46.58±1.0^{d}	4.59±0.2^{d}	60.50±8.2^{b}	46.7±2.0^{c}	7.24±0.49^{a}	11.04±1.9^{c}

Means in same column that do not share same letters are significantly different, according to Fisher's test.($p<0.05$). Control: refined flour; WWF: whole wheat flour; 50% 24 h: blend containing 50% refined flour and 50% sprouted whole wheat flour for 24 h; 100% 24 h: 100% sprouted whole wheat flour for 24 h; 50% 48 h:blend containing 50% refined flour and 50% sprouted whole wheat flour for 48 h. 100% 48 h:100% sprouted whole wheat flour for 48 h.

Cookies hardness is among parameters influencing consumers' acceptability. The use of WWF increased cookies hardness by 73.4% if compared to control. Similarly, using sprouted flours increased hardness while the use of blends with refined flour led to intermediate values. These results could be related to the lower spread factor of the cookies and, therefore, a more compact structure, as it has been confirmed in prior studies (Mancebo *et al.*, 2015). Previous studies also showed that the use of germinated flours (brown rice and chenopodium) did not modify cookies hardness comparatively to their raw seeds flour (Chung, Cho, & Lim, 2014; Jan et al., 2016). While results of Sudha *et al.* (2007) highlighted the role of wheat bran incorporation on increasing cookies hardness and also decreasing spread factor as shown by our results. Accordingly, the increase in hardness might be attributed to the difference in flour composition between refined and WWF. Particularly, WWF (and SWWF) might have a

higher fiber content than control. The contribution of insoluble fibers on increasing cookies hardness has been previously reported (Mancebo *et al.,* 2018; Sudha *et al.,* 2007). This increase might be attributed to the contribution of fibers in increasing water absorption capacity increasing cookie hardness (Ajila, Leelavathi, & Prasada Rao, 2008).

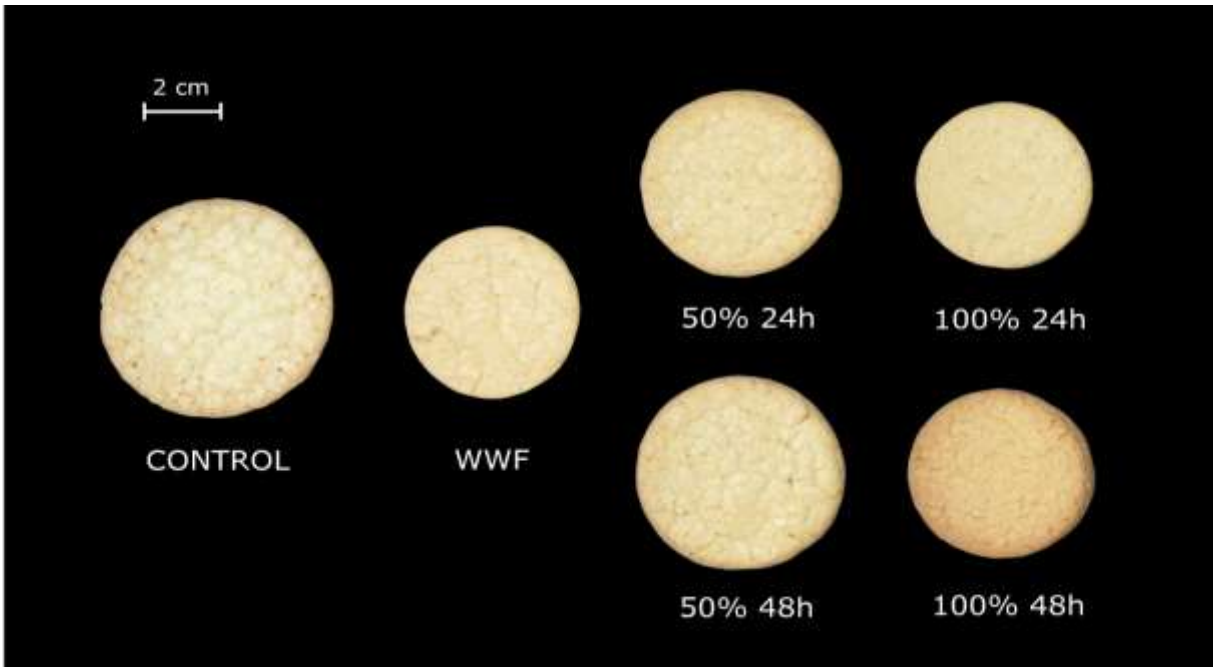

Figure 12: Image of cookies made with different flours: Control: refined flour; WWF: whole wheat flour; 50% 24 h: blend containing 50% refined flour and 50% sprouted whole wheat flour for 24 h; 100% 24 h: 100% sprouted whole wheat flour for 24 h; 50% 48 h: blend containing 50% refined flour and 50% sprouted whole wheat flour for 48 h. 100% 48 h: 100% sprouted whole wheat flour for 48 h**.**

The use of WWF did not affect cookies lightness (L*). However, the use of SWWF decreased it gradually with sprouting time. Regarding redness (a*), both WWF and SWWF flour increased its averages significantly. The highest values were recorded when 100% 48 h SWWF was used. Such decrease was previously reported with germinated amaranth and germinated brown rice flours (Chauhan *et al.,* 2016; Chung *et al.,* 2014). Yellowness (b*) increased when WWF is used while its value decreased when sprouted whole wheat flour is used. Yaqoob *et al.,* (2018) observed the same trend in unsprouted/sprouted barley-wheat flour blend. Evolution of cookies color is related to baking and Maillard reaction. During sprouting starch is degraded under enzymatic activity (Mak *et al.,* 2009) which may lead to a pronounced Maillard reaction.

3.3.2Sensory analysis

As shown on Table 24, the use of WWF and SWWF increased the appearance values, although there were not significant different between them. Therefore, the improvement of appearance is probably due to the use of the whole grain and its impact on cookies size and/or color, since cookies made with WWF and SWWF were smaller (spread factor, Table 23) and darker. Regarding odor, this parameter was affected by the flour used. In general, the use of sprouted whole wheat flour improved cookies odor and taste versus control. This could be attributed to role of Maillard reaction in enhancing aromatic compounds, mainly because more reducing sugars are available on SWWF after starch degradation (Jribi *et al.,* 2019b). Coming to texture, cookies made with WWF got the highest mark while no significant differences were seen among all the other samples. Altogether, substitution of refined flour by WWF or SWWF improved overall acceptability.

Table 24: Effect of raw and sprouted whole wheat flour use on the organoleptic acceptability

Elaboration	Appearance	Odor	Taste	Texture	Overall acceptability
Control	5.69±1.79^{a}	4.97±1.68^{a}	5.11±2.16^{a}	5.44±1.65^{a}	5.4±1.76^{a}
WWF	6.55±1.54^{b}	5.39±1.39^{ab}	5.68±1.71^{ab}	6.33±1.49^{b}	6.03±1.37^{b}
50%24h	6.45±1.25^{b}	5.53±1.44^{bc}	5.93±1.73^{b}	5.79±1.60^{a}	6.11±1.37^{b}
100%24h	6.45±1.22^{b}	5.56±1.56^{bc}	5.96±1.70^{b}	5.73±1.52^{a}	6.17±1.41^{b}
50%48h	6.61±1.45^{b}	5.44±1.49^{abc}	5.57±1.83^{ab}	5.92±1.62^{ab}	5.8±1.54^{ab}
100%48h	6.73±1.51^{b}	5.91±1.51^{c}	5.96±1.64^{b}	5.63±1.78^{a}	6.11±1.37^{b}

Means in same column that do not share same letters are significantly different, according to Fisher's test.($p<0.05$). Control: refined flour; WWF: whole wheat flour; 50% 24 h: blend containing 50% refined flour and 50% sprouted whole wheat flour for 24 h; 100% 24 h: 100% sprouted whole wheat flour for 24 h; 50% 48 h: blend containing 50% refined flour and 50% sprouted whole wheat flour for 48 h; 100% 48 h:100% sprouted whole wheat flour for 48 h.

4. Conclusion

Sprouting is recognized as an effective tool to improve cereals nutritional properties. Moreover, the sprouted flours are whole grain flours, which have also nutritional benefits. The sprouting process decreased the WHC of whole wheat flours, but it did not modify the dough rheology. This led to cookies with the same spread factor and similar hardness, although the spread factor is lower than control cookies and their hardness is higher.

However, the sprouting changes (grain components hydrolysis) influence the Maillard reactions and color cookies. The use of WWF improved the cookie acceptability, but the sprouted flour cookies had not significant differences respect to WWF cookies. Therefore, sprouted flours could be an interesting alternative to WWF due to their nutritional benefits.

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Chapter 8 : General conclusion

General conclusion

Consumers become more caring about their diet as it influences their health statue. To satisfy health-conscious consumers' food market may focus on improving products nutritional quality. Consumption of "natural" or "minimally processed foods" is one of available options. Another option is the use of whole grains instead of refined flour to enhance cereal products nutritional attributes. Sprouting is a green choice combining both previous cited ones. The objective of this thesis was to improve durum wheat "*Triticum durum*" nutritional properties through the use of sprouting bioprocess and its potential use. Accordingly, on a first step, the impact of 48 h of sprouting on durum wheat was investigated on landrace and high yielding Tunisian cultivars. Results showed firstly significant differences in terms of proximate composition and bioactive active levels among cultivars. Particularly, landrace cultivars showed highest averages. Despite the differences between raw samples sprouting for 48 h led to significant improvement of nutritional attributes according to the same trend. Mainly, sprouting contributed in an increase in protein (from +0.7 to + 4.83% depending on cultivars) and reducing sugars contents (after starch degradation under amylasic activity) (from +62.93 to +284.5%). Similarly, antioxidant molecules were improved after sprouting, particularly carotenoids (from +17 to +34.24%), total phenol content (from +45.96 to 131.63%), vitamin C (was not detected on raw samples only on sprouted ones) and α tocopherol (from +1.8 to +18.52%). Similarly, determination of raw and sprouted wheat seeds prebiotic index by *in vitro* digestion model has showed a positive effect of sprouting bioprocess. In this context, further analysis would be recommended to validate these results: Evolution of prebiotic index is probably related to proximate composition and its impact on remaining bacterial growth. Particularly, specifications of different phenolic compounds, peptides and oligosaccharids would be helpful to understand growth of tested bacteria. Analysis of nutrients on digested samples might also be recommended. Considering obtained results on the first part, sprouts are a functional food. Consumption of sprouted wheat could therefore improve consumers'health by preventing NTD.

Despite the significant improvements on sprouts nutritional attributes, the increase in water content during sprouting increases microorganisms growth. Therefore, consumption of raw sprouts could be risky. To overcome this problem, on the second part of this thesis we focused on improving sprouts hygienic properties through the use of two different approaches. On a first step, we evaluated the use zinc during sprouting. In this part, experimental conditions were optimized by a factorial design. Then microbiological and nutritional quality were

evaluated. Interestingly, results showed that this approach improved sprouts hygienic properties as well as nutritional ones: bacterial growth significantly decreased while zinc and bioactive compounds levels increased. Accordingly, the use of this approach might be suggested as a tool for cereals mineral fortification. In perspective, it would be interesting to compare effectiveness of different minerals on sprouts decontamination and their impact on sprouts shelf life.

Apart from consumed raw, sprouts could be incorporated as a functional ingredient in cereal products formulation. To do so and to avoid sprouts spoilage they could also be dried. The knowledge of the ingredients properties is crucial on food formulation. In this context, on a second step of sprouts preservation, we evaluated the impact of different drying method used (lyophilisation, microwave vacuum drying and oven drying) on bioactive compounds (caroteneoids, polyphenols, antioxidant activity), functional (water absorption capacity, oil absorption capacity, swelling power, least gelation concentration) and thermal properties (On set temperature, peak temperature). Our results showed an evolution on tested parameters according to the drying method used. In terms of nutritional properties lyophilisation would be recommended as losses were observed with other tested methods. However, results obtained with microwave vacuum drying could be highlighted, particularly, in terms of time and energy consumption. Other drying methods (or combined drying methods) could be tested as well. Conclusions would not be determinant in such study as optimal drying method and conditions should be defined by combining different parameters (duration, energy consumption, cost, induced losses…).

After studying the role of sprouting in enhancing durum wheat nutritional attributes, sprouts preservation and conservation (through drying and zinc decontamination) we intended to use sprouted whole wheat flour on cookies elaboration. We firstly, studied the evolution of functional (flour hydration properties, swelling volume, oil absorption capacity), thermal and pasting properties with sprouting time (for 12, 24, 36, 48 and 72 h). Results provided by this research, showed significant changes from 24 h and above 36 h. In fact, during sprouting bioprocess there is a degradation of some macromolecules and synthesis of new ones (as shown on chapter 2). Meanwhile, the role of macromolecules like starch and proteins is not only nutritional but also functional. Despite observed changes, it is hard to define optimal sprouting time. The use of long sprouting time could be suggested for products in which starch gelatinization does not occur as cookies. Short sprouting time would be rather recommended in case of cakes. These findings need to be more studied in case of partial and

total incorporation of sprouted wheat flour. In case of our application, we used whole wheat flour sprouted for 24 and 48 h. Our findings showed that the use of sprouted whole wheat flour did not induce changes on dough rheological properties if compared to raw whole wheat flour. Cookies physical properties (width, thickness, hardness) were modified according to the use of whole wheat flour rather than the impact of sprouting. Moreover, the use of whole wheat flour and sprouted whole wheat flour improved the cookie acceptability. These promoting results are encouraging for the use of sprouted wheat flour due its nutritional interest on cereal products elaboration. In perspective, other application could be tested to evaluate the possibility of sprouted wheat flour use and /or partial substitution on other cereal products.

Taken together, our research showed that sprouting is sustainable tool to improve durum wheat nutritional properties. The use of zinc may contribute in sprouts decontamination and also improving sprouts zinc content and antioxidant properties. Dried sprouts, particularly when used as whole wheat flour, is good alternative to ameliorate cereal products quality. For this option, it is important to consider sprouting time used as it may significantly affect functional properties and consequently end-product properties.

In perspective, further researches should be conducted. It would be useful to understand the impact of different sprouting conditions (such as light, relative humidity level, soaking time, sprouting time) on evolution of nutrients and bioactive compounds. Such findings might allow an optimization of these conditions. In terms of sprouts preservation, as many techniques are used to extend minimally processed foods shelf life and considering the sensibility of raw sprouts, some non thermal processing methods (High-pressure treatment, High-electric-field pulses, Thermosonication...) and new packaging technologies might be tested and compared. It would be important also to assess acceptability of raw sprouts consumption. In fact, consumption of such products is not known on Tunisian diet. Consequently, the nutritional improvement would not guarantee their acceptance by consumers. Hedonic sensory analysis should be carried out. Finally, sprouting can be also studied on other cereals and legumes, to improve nutritional and sensory quality of cereal products.

Appendix

List of publications

Articles:

- **Jribi S, Chabbouh M., Sassi K., Sfeyhi D., Marzougui S., Slim Amara H., & Debbabi H. (2018).** Sprouting, a bioprocess supporting food Industry? Journal of New Sciences, 57, 3725-3737.
- **Jribi S, Molnàr H., Adanyi N., Marzougui S., Naàr Z., & Debbabi H. (2019).** Effect of Sprouting Temperature on Durum Wheat (Triticum Durum) Sprouts Nutritional Properties and Bioactive Compounds. International Journal of Innovative Approaches in Agricultural Research, 3(1) 87-95.
- **Jribi S., Sahagùn M., Debbabi H., & Gomez M. (2019).** Evolution of functional, thermal and pasting properties of sprouted whole durum wheat flour with sprouting time. International Journal of Food Science and Technology, 54, 2718-2724**.**
- **Jribi S., Molnàr H., Antal O., Adanyi N., Kheriji O., Naàr Z., & Debbabi H. (2019).** Zinc fortification as a tool for improving sprouts hygienic and nutritional quality: A factorial design approach. Journal of the Science of Food and Agriculture, 99, 5187-5194.
- **Jribi S., Sahagùn M., Belorio, M., Debbabi H., & Gomez M.(2020).** Effect of sprouting time on dough and cookies properties. Journal of Food Measurement and Characterization, 14, 1595-1600.
- **Jribi S., Antal O.T., Molnàr H., Adanyi N., Fustos Z., Naàr Z., Amara H., & Debbabi H. (2020).** Sprouting bioprocess as a sustainable tool for enhancing durum wheat (*Triticum durum*) nutrients and bioactive compounds. The North African Journal of Food and Nutrition Research, 4, 252-259.

Talks and posters

- **Jribi S., Sassi K., Sfayhi D., & Debbabi H. (2018).** Sprouting, an Eco-Friendly Technology for Improving Nutritional Quality of Tunisian Wheat Cultivar "Khiar". In: Kallel A., Ksibi M., Ben Dhia H., Khélifi N. (eds) Recent Advances in Environmental Science from the Euro-Mediterranean and Surrounding Regions. EMCEI 2017.

Advances in Science, Technology & Innovation (IEREK Interdisciplinary Series for Sustainable Development). Springer, Cham. DOI: 10.1007/978-3-319-70548-4_41.

- **Jribi S., Molnàr H., Adanyi N., Marzougui S., Naàr Z., & Debbabi H. (2018).** Sprouting an effective tool to enhance wheat bioactive compounds: Role of sprouting temperature. International Agricultural, Biological and Life science conference**,** Edirne, Turkey, September 2-5th.
- **Jribi S., Gliguem H., Nagy A., Zsolt N.G., Szalóki-Dorkó L., Naàr Z., Bata-Vidàcs I., Marzougui S., Cserhalmi Z., & Debbabi H. (2018).** Evolution of "Chili" Tunisian landrace durum wheat sprouts properties after drying. 21st International Drying Symposium, Valencia, Spain, September 11-14th.
- **Jribi S., Antal O.T., Fustos, Z., Papai G., Naàr Z., Marzougui S., & Debbabi H. (2018).** Sprouting: A sustainable tool for enhancing prebiotic properties of durum wheat seeds (*Triticum durum*). 2nd Mediterranean forum for PhD students and young researchers: Research and innovation as tools for sustainable Agriculture, Food and Nutrition security, CIHEAM Bari, Italy, September 18-20th.
- **Jribi S., Molnar H., Adanyi N., Naàr Z., Kheriji O., & Debbabi H. (2019).** Durum wheat (*Triticum durum*) sprouts a natural food additive?. International symposium on toxicology, food and environmental health, Mahdia, Tunisia April 26-27th.