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

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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.

Keywords. Durum wheat - Sprouting - In vitro digestion - Prebiotic index.

Influence du bioprocédé de la germination sur les propriétés prébiotiques du blé dur (Triticum durum)

Résumé. *La germination est un bioprocédé utilisé pour l'amélioration des propriétés nutritionnelles es céréales et légumineuses. De ce fait, les graines germées peuvent être proposées comme aliment fonctionnel. Cette étude a pour objectif l'évaluation du rôle de la germination dans l'amélioration des propriétés prébiotiques du blé dur (Triticum durum) via un modèle de digestion in vitro. La méthodologie a consisté à germer deux variétés de blé dur: «Karim» (une variété améliorée) et «Chili» (une variété autochtone) pendant 48h ensuite calculer l'index prébiotique. Les résultats ont montré que les deux variétés testées avaient un index prébiotique positif que ce soit avant ou après germination. Les bioprocédés de la germination a contribué à une augmentation des valeurs de l'index prébiotique (+62,7% pour «Karim» et +14,4% pour «Chili»). Cependant, l'intensité de cette évolution dépendait de la variété testée. En conclusion, cette étude a montré que la germination est moyen durable contribuant à l'amélioration des propriétés prébiotiques et ainsi du confort intestinal.*

Mots-clés. *Blé dur – Germination - Digestion in vitro - Index prébiotique.*

I - 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*., 2019a; 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 has 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, an interest was accorded also to the genetic background of samples.

II - Materials and Methods

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. Sprouting procedure

Sprouting was conducted exactly as described in previous study of Jribi and co-workers (2019b). 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.

3. In vitro digestion

Samples were digested according to the model developed by the Food Science Research Institute (Budapest, Hungary). The model was mainly based on Versantvoort and co-workers (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.

4. Prebiotic 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 base 10 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 base 10 logarithm 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:

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

(**Equation 4**)

In other words:

PI = Bif + Lac - Eco – Clos
(**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.

III - Results and discussion

As shown in Figure 1, 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.

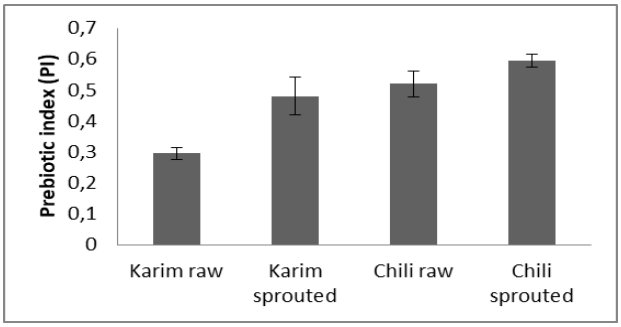

Figure 1. **Evolution of prebiotic index (PI) after sprouting of two wheat cultivars.**

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.

IV - Conclusions

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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