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Original article

Incorporation of Sprouted Wheat Seeds as Functional Ingredient in a Mediterranean Breakfast Cereal Based Product, Bsissa: A Mixture Design Approach

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Abstract

Evolution of life style markedly modified people's eating habits. Fortunately, consumers are more aware by the importance of a healthy diet and are more demanding for functional foods. The Mediterranean Diet is a dietary pattern recognized by its positive health effect. However, this diet is disappearing in Mediterranean societies. The aim of this study was to promote a local Tunisian breakfast cereal product by improving its nutritional properties. The methodology consisted on using dried sprouted durum wheat seeds (Triticum durum) as a functional ingredient. The proportion to use without affecting Bsissa functional properties was defined by optimization of a mixture design where the used components were: wheat, dried sprouted wheat and chickpea. The selected responses were water and oil absorption capacity.

Based on a mathematical analysis, germinated dried wheat decreased water absorption capacity and had no effect on oil absorption capacity. The optimal formula was composed of: 17.2% wheat, 41.4% germinated dried wheat and 41.4% chickpea. With such composition an increase of +32% in total phenol content and +228.5% in reducing sugar were obtained. However no significant changes on protein content were seen. Interestingly, the use of dried sprouted wheat (not roasted) did not affect the consumers' acceptance of Bsissa.

In conclusion, the use of dried sprouts successfully improved nutritional properties of Bsissa. The use of mixture design approach allowed attempting a balance between functional and nutritional properties.

Keywords: Bsissa, Dried sprouted wheat, Formulation, Mixture design, Nutritional properties.

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INTRODUCTION

The Mediterranean Diet (MedDiet) is a dietary pattern where fruits, vegetables, cereals and olive oil are the main elements (Benhammou et al., 2016). MedDiet has been recognized by UNESCO as "Intangible cultural heritage of humanity" (Rizza et al., 2016; Farineti et al., 2017). Health benefits of this diet are probably among the main reasons for this recognition. In fact, several reviews and studies reported that adherence to this healthy-eating lifestyle is inversely associated to the risk of obesity, diabetes, metabolic syndrome, cancer, cardiovascular and cognitive diseases (Benhammou et al., 2016; Veronese et al., 2017; Salas-Salvado et al., 2018). Unfortunately, the Western diet becomes more widespread in Mediterranean societies to the detriment of MedDiet (Rizza et al., 2016). Innovation and promotion of local Mediterranean healthy foods could be suggested as an effective tool to overcome this problem.

Germination is a bioprocess that might increase cereal protein digestibility (Alvarez-Jubete et al., 2009) and nutritional value. In particular, an increase in bioactive compounds carotenoids (Plaza et al. 2003), vitamin C (Yang et al., 2001), vitamin E (Ozturk et al., 2012) and total phenol contents (Donkor et al., 2012) were reported in sprouted cereals. Such evolution of bioactive compounds was associated to an improvement of antioxidant properties of sprouted seeds (Hung et al., 2011). Moreover, sprouting leads to a decrease in anti-nutritional components such as phytates (Kumar et al., 2009).

Durum wheat (*Triticum durum*) is an important cereal crop whose grains are predominantly used in Mediterranean diet: pasta, bread… Whole grain wheat has been found to be a good source of nutritionally valuable substances, such as antioxidants, minerals, vitamins, and dietary fibers (Liu, 2007). Its contribution in human diets has led to a renewed interest in whole mill durum-based products. Particularly, in Tunisia, consumption of wheat based products such as: couscous, traditional bread, frik, zummita, assida, bazin, and bsissa, is a part of gastronomic cultural heritage (Jedidi et al., 2016; Gharbi Yahiaoui et al., 2018). Bsissa is a Mediterranean breakfast product: It is an aromatized powder obtained from the mixture of roasted cereals, pulses, aromatized herbs and spices (Gharbi Yahiaoui et al., 2018). Depending on geographical location and living standards, many recipes of Bsissa could be found (Gharbi Yahiaoui et al., 2018). This product could be consumed in different forms: liquid or as paste, with water, olive oil or milk.

Sprouting is an eco-friendly technology known by enhancing pulses and cereals nutritional properties (Mbithi Mwikya et al., 2001; Jribi et al., 2018). During this bioprocess storage molecules like starch and proteins are degraded under enzymatic activity (Delcour and Hoseney, 2000).

Mixture of ingredients is a common fact in food formulation. In such cases, what matters the most is the proportions of ingredients rather than their amounts (Cornell, 1990). Mixture design is an effective tool to deal with these cases. The method is based on statistical and mathematical approach to define

combinations of ingredients achieving specific targets (Quality parameters, product shelf life…) (Dou et al., 1993). In mixture design, the components studied are interconnected as the sum of their proportion must be equal to one.

In this study, we aimed to investigate the potential use of dried sprouts as functional ingredient in a traditional Tunisian breakfast cereals: "Bsissa", while preserving the original product functional properties. A mixture design methodology was used for formulation.

Material and Methods

Plant material

Durum wheat seeds (*Triticum durum*) from the genotype "Khiar" were used in this study. Seeds harvested in 2014 (Bizerte, Tunisia) were provided by The National Agricultural Research Institute of Tunisia (INRAT).

Sprouting

Durum wheat seeds were sterilized in a 1% sodium hypochlorite for 30 minutes then germinated for 48 hours (Hung et al. 2011). After sprouting, seeds were solar dried until reaching at least 15% moisture content.

Formulation

Bsissa preparation

Bsissa preparation was based on four main steps: cleaning ingredients, roasting them, grinding and sieving the obtained powder, finally storing. Roasting enhances flavor and consequently could improve sensory properties. Unfortunately, some undesirable changes on nutritional properties may occur during roasting mainly the Maillard reaction and production of some toxic components like hydroxymethylfurfural, glucosylisomaltol and furfural (Rufián-Henares et al., 2009). For this reason, sprouted wheat seeds used in the mixture design were dried only and not roasted like other ingredients.

Mixture design

Wheat, dried sprouted wheat and chickpea were used to develop a ready-to-eat cereal breakfast. A screening mixture design was chosen for the experiment because all the components have the same range, which between 0–100%, and there were no constraints on the design space. All proportions of all substrates in each mixture were sum to 100% for a mixture load of 100 g (Table 1).

Responses analyses

The searched responses in this study were two functional properties of "Bsissa": water capacity absorption and oil absorption capacity.

Water and oil absorption capacities of the "Bsissa" were determined by the modified method of Jitngarmkusol et al. (2008). Briefly, for the water absorption capacity (WAC) test, 2g of each flour sample were weighed into a pre-weighed centrifuge tube and 5 ml of distilled water were added. For the oil absorption capacity (OAC) test, 5 ml of olive oil were added, instead of distilled water. The dispersions were stirred and allowed to stand for 30 min before being centrifuged at 3000 rpm for 30 min. The supernatant was decanted, and sample was weighed again. The water and oil absorption capacities were expressed as grams of water or oil bound per gram of sample on a dry basis.

Quality analysis

Total phenol content was assessed as described by Aprodu and Banu (2012).The reducing sugar content was determined using a colorimetric method (3,5 dinitrosalicilic acid, DNS) (Singkhornart et al., 2014). Protein content was evaluated by means of the standard Kjeldhal method (%N*5.7) (Ciccoritti et al. 2017).

Sensory analysis

For sensory analysis two products were tested: Bsissa prepared with sprouted wheat according to the optimized formula, Bsissa with the same previous formula and where sprouted wheat was substituted by common wheat (Control). These products were subjected by 61 panelists to an hedonic sensory evaluation for color, odor, texture, taste, after-taste and overall acceptability. Ratings were carried out using a 5 point hedonic scale ranging from 5 (like extremely) to 1 (dislike extremely).

Statistical analysis

All values are presented as mean ±Standard deviation of 3 samples. All determinations were carried out in triplicates. The statistical analyses and the mixture design were performed using (Minitab 17, USA). Numerical optimization was carried out to determine the exact optimum value of independent variables. One-way ANOVA was used to determine whether proportion of the used ingredients affected quality parameters of the Bsissa significantly ($P < 0.05$).

Results

The aim of this study was to incorporate dried sprouted wheat to enhance Bsissa nutritional properties without affecting functional ones. Evolution of water absorption capacity and oil absorption capacity according to the different formulations tested through the mixture design are presented in table1.

Table 1. Mixture design and responses values

Formulation	X1: Wheat	X2: Sprouted dried wheat	X3: Chickpea	Water absorption capacity (g/g)*	Oil absorption capacity (g/g)*
1	0	0	1	1.13 ± 0.07^{a}	1.14 ± 0.02^{a}
2	0	1	0	0.73 ± 0.18^{bc}	0.92 ± 0.04^{bc}
3	1	0	0	1.78 ± 0.14^{e}	1.13 ± 0.08^{b}
4	0	0.5	0.5	0.66 ± 0.07^{c}	1.06 ± 0.02^{bc}
5	0.5	0	0.5	1.45 ± 0.05^{d}	1.01 ± 0.02^{bc}
6	0.5	0.5	0	0.85 ± 0.05^{b}	1.09 ± 0.02^{bc}
7	0.67	0.16	0.16	1.23 ± 0.10^{a}	0.98 ± 0.02^{bc}
8	0.16	0.67	0.16	0.78 ± 0.07^{bc}	1.00 ± 0.06^{bc}
9	0.16	0.16	0.67	1.28 ± 0.02^{ad}	0.89 ± 0.03^{c}
10	0.33	0.33	0.33	1.16 ± 0.05^{a}	1.04 ± 0.04^{bc}

^{abcd}The values designated by the different letters in the column of the table are significantly different ($\alpha = 0.05$)

* g/g: grams of water or oil bound per gram of sample on a dry basis

Water absorption capacity

Water absorption capacity (WAC) is an important parameter to take into account in Bsissa preparation as several recipes mix the powder with water only. A significant modification in this parameter may influence consumers' acceptability of the product. As it could be seen on table1, the use of sprouted wheat seeds induced a decrease in water absorption capacity.

The regression for mixtures shows that the full cubic model describes significantly ($p<0.05$) the evolution of water absorption capacity according to wheat, sprouted wheat and chickpea proportions (R^2=94.46%). The quantitative relation between wheat, sprouted wheat and chickpea proportions and water absorbance capacity is represented by equation 1:

$$WAC= 1.78X_1+0.73X_2+1.13X_3-1.64X_1X_2-0.04X_1X_3-1.07X_2X_3+6.69X_1X_2X_3 \text{ (Equation 1)}$$

Where X_1: Wheat proportion; X_2: Dried sprouted wheat proportion; X_3: Chickpea proportion

Based on equation 1 a graphical presentation of WAC evolution with mixture component proportions (wheat, dried sprouted wheat and chickpea) could be presented due the mixture contour plot (Figure 1).

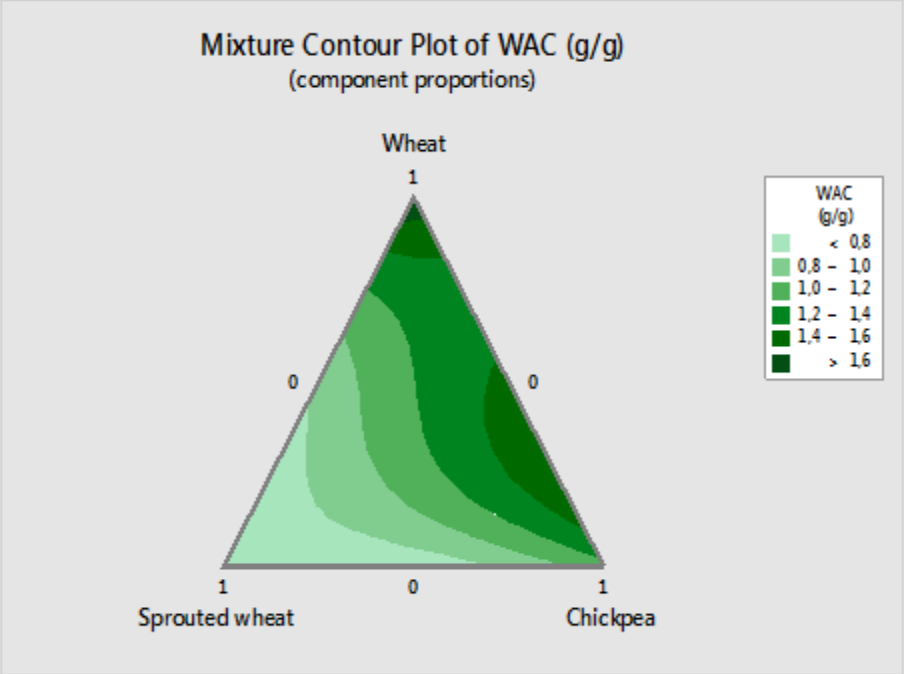

Figure 1. Contour plot for water absorbance capacity response

Oil absorption capacity

Oil absorption capacity (OAC) ranged between 0.92 and 1.14g/g depending on the mixture. Considering pure mixtures (Table 1) there was a significant difference ($p<0.05$) between the three tested components. The highest averages were obtained when chickpea was used by its own. However, no significant difference on oil absorption capacity was observed when only wheat or dried sprouted wheat is used.

The regression for mixtures also shows the significance of the full cubic model as with water absorbance capacity with a lower R^2 ($R^2 = 65.05\%$). Full equation describing oil absorbance evolution is presented on Equation 2:

$$OAC= 1.12X_1+0.91X_2+1.14X_3-0.24X_1X_2-0.54X_1X_3+0.10X_2X_3-1.84X_1X_2X_3 \quad \text{(Equation 2)}$$

Where X_1: Wheat proportion; X_2: Dried sprouted wheat proportion; X_3: Chickpea proportion

Considering the significance of each term on the model, a reduced equation might be suggested in the equation 3:

$$OAC= 1.12X_1+0.91X_2+1.14X_3-0.54X_1X_3 \quad \text{(Equation 3)}$$

As seen on Equation 3, there was a negative interaction between wheat and chickpea factors.

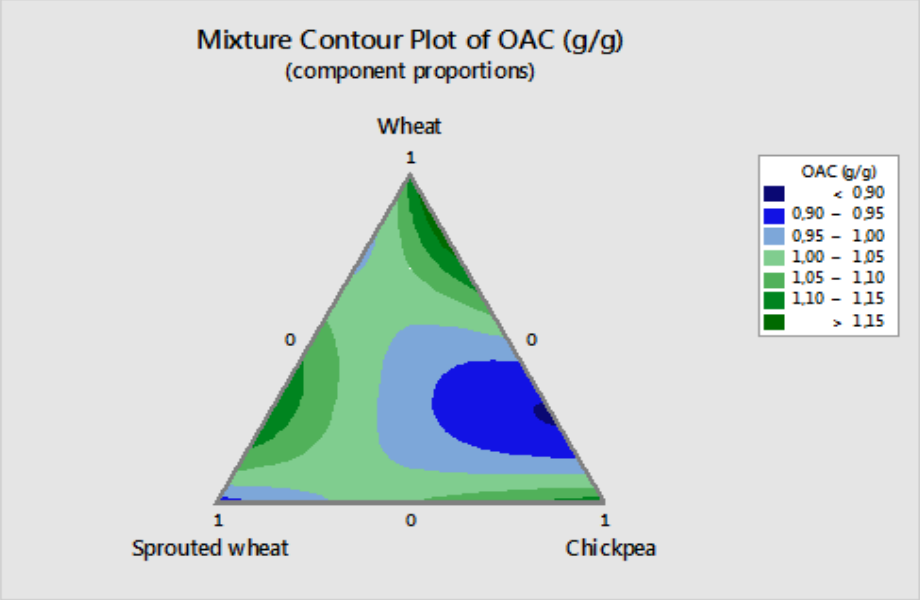

Figure 2. Contour plot for oil absorption (OAC) capacity response

Optimization of the mixture design base on functional properties

Improving nutritional properties of Bsissa through the use of dried sprouted wheat is not the only aim of this research. Maintaining functional properties similar to available products on the market was also taken into account. To do so, firstly, functional properties of two Bsissa formulas available on the market were evaluated. As oil absorption capacity was not significantly affected by the use of dried sprouted wheat, only water absorption capacity was considered for optimization. (With obtained results, Minitab software was used to define an interval of desired water absorption values as shown on Figure 3).

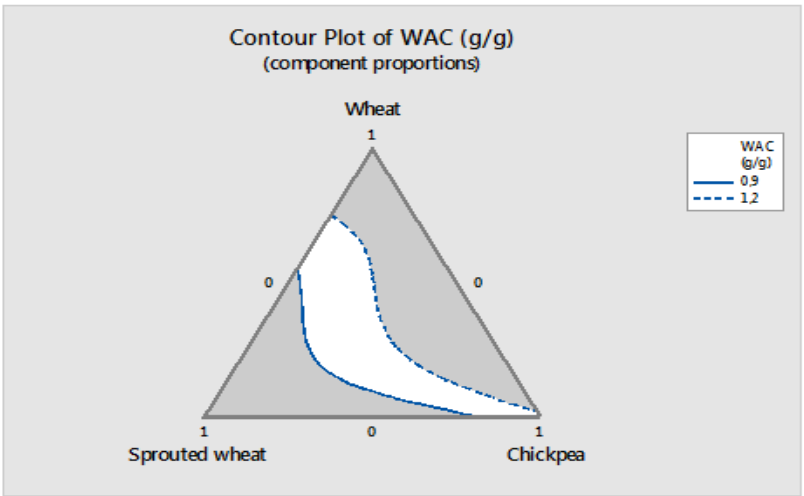

Figure 3. Optimization of Bsissa mixture composition

On the other hand, specific targets of functional properties were determined based on average of available products, in order to define optimal proportions. The use of Minitab software allowed to define the proportions of optimal Bsissa formula. Maximum global desirability was 0.81. This desirability was reached with 17.2% of wheat, 41.4% sprouted dried wheat and 41.4% of chickpea. These proportions, according to the mathematical model suggested, would lead to a water absorption capacity of 1.03g/g and an oil absorption capacity of 0.91g/g. Results of replicates using the optimums, confirmed that the model was appropriate.

Effect of incorporating dried sprouted wheat on quality of Bsissa

To investigate the effect of using dried sprouts on Bsissa physicochemical properties, the optimal recipe was compared to a formula where dried sprouts were substituted by common unsprouted wheat. As shown on Table 2, incorporating dried sprouted wheat on Bsissa induced several significant changes on product quality.

Table 2. Impact of incorporation of dried sprouts on Bsissa physicochemical properties (n=3)

Parameter	**Bsissa with common wheat**	**Bsissa with dried sprouted wheat**
Density	0.67 ± 0.01^{a}	0.63 ± 0.01^{b}
Water absorption capacity (g/g)	1.13 ± 0.03^{a}	0.95 ± 0.02^{b}
Oil absorption capacity (g/g)	0.81 ± 0.05	0.85 ± 0.02
Proteins (%)	13.35 ± 0.16	13.33 ± 0.04
Reducing sugars (mg/g dm)	7 ± 0.01^{b}	23 ± 0.01^{a}
Total phenols (mg GAE/g dm)	16.96 ± 0.22^{b}	22.34 ± 0.27^{a}

dm: dry matter, GAE: Gallic acid equivalent, The values designated by the different letters in the same raw of the table are significantly different ($\alpha = 0.05$)

Physicochemical properties of Bsissa were mightily depending on the properties of ingredients used. The use of dried sprouts induced a significant ($p<0.05$) decrease in density. As seen through the mixture design, using dried sprouts did not affect oil absorption capacity. However, it led to a significant decrease on water absorption capacity. No changes were observed in Bsissa's protein content. In fact, no quantitative changes on protein content were observed after 48 hours of durum wheat sprouting (Jribi et al. 2018). Incorporating dried sprouts induced an increase of about 3 folds in reducing sugars, leading to an increase of the energetic value of the obtained product. Such increase in reducing sugars was probably due the composition of dried sprouts. Previous results from our lab showed an increase in reducing sugars of dried sprouts, when compared to unsprouted ones (Jribi et al., 2018). Our results

showed also an increase of about +32% in total phenols in dried sprouted wheat based Bsissa formula, when compared to control formula, as similarly reported for dried durum wheat sprouts (Jribi et al., 2018).

Effect of incorporation of dried sprouted wheat into Bsissa on consumers' acceptance

The main purpose of food formulation is satisfying consumers' requirements. Such approach should attempt a balance between nutritional, functional and sensory properties. Hedonic evaluation could be suggested as an indicator of consumers' acceptability of products. Results of consumers' appreciation are presented in table 3.

Table 3: Consumers' appreciation marks

	Odor	**Color**	**Texture**	**Taste**	**After taste**	**Overall appreciation**
Control	3.18/5	3.16/5	3.28/5	3.30/5	2.84/5	3.12/5
Developed formula with sprouted wheat	3.38/5	3.42/5	3.24/5	3.34/5	2.94/5	3.38/5

Statistical analysis showed that incorporating sprouted wheat into Bsissa formula did not significantly impair appreciation of sensory descriptors (ie. color, odor, texture, taste and after taste). Taste appreciation of Bsissa with sprouted wheat exceeded the average (3.34). Interestingly the absence of difference on texture in both formulas confirmed the effectiveness of the mixture design approach used since functional properties of the product were not affected. Moreover, incorporating sprouted wheat did not impair consumers' acceptance.

Discussion

As seen, the use of dried sprouted wheat led to a significant decrease on water absorption capacity. This decrease could be probably related to the evolution of seeds properties during sprouting. In fact, during this bioprocess some macromolecules such as starch and proteins are degraded under enzymatic mechanisms (Hopkins, 2003). Previous studies of Singh et al. (2001) and Singh et al. (2017) reported the negative effect of sprouting and also the sprouting conditions adapted on water absorption capacity.

Regarding oil absorption capacity, the evolution could be explained by the ability of the powder proteins to bind oil. Accordingly it might be expected that chickpea have a higher contribution on this properties in Bsissa's mixture. It has been shown previously that sprouting increases cereals oil absorbance capacity (Elkhalifa and Bernhardt, 2010; Singh et al., 2017). The role of sprouting in increasing oil absorbance capacity could be related to the effect of this bioprocess on protein content: Sprouting increases protein content (Koehler et al., 2007). Such evolution was not significantly observed

in our results. The previous studies evaluated this property on pure cereals while in our study mixture were used. Interaction between ingredients could be then seen. Moreover, the evolution of proteins during sprouting depends on conditions used, mainly on temperature used (Koehler et al., 2007).

Using dried sprouts induced a significant ($p<0.05$) decrease in density. Sprouting has been previously associated to the decrease of density of flour (Singh et al., 2017). Such changes could be linked to macromolecules degradation such as starch and proteins (Hopkins, 2003; Koehler et al., 2007).

The decrease on water absorption capacity indicates an easier preparation of Bsissa for consumers. Evolution of lifestyle makes consumers more looking for products simple to use (Free and Forge, 2000) which may explain the considerable evolution of ready-to-use products in food market.

Despite the lack of quantitative changes on protein content, Bsissa still a good source of proteins. In fact, the use of a combination of wheat and chickpea improves protein quality value, thanks to the complementarities in amino-acids between both ingredients (Quillien and Gueguen, 1997). In fact, wheat proteins are deficient on some essential amino acids such as lysine, in the contrary of chickpea proteins. In addition, sprouting increases amino acids concentration (Singkhornart et al., 2014), particularly lysine and tryptophan (Lorenz and D'Appolonia, 1980). This significant increase in reducing sugars through incorporation of dried sprouted wheat could be beneficial for children's immature digestive system (Rohleder and Nater, 2009). Similarly, an increase on total phenol content was seen. Phenolic compounds are inversely associated to the risk of cardiovascular diseases, some cancers and chronic diseases caused by oxidative stress and degenerative illnesses (Derbel and Ghedira, 2005; Quideau et al., 2011; Pallauf and Rimbach, 2012;Yamagata et al., 2014).

In conclusion, Bsissa nutritional properties were significantly improved through the use of dried sprouted wheat without altering sensory quality.

Conclusion

The present work aimed to incorporate dried sprouted seeds as functional ingredient in Bsissa formula. The use of mixture design approach allowed defining the proportion of dried sprouts to use without affecting functional properties. The optimized formula showed a significant improvement in Bsissa nutritional properties, as well as a satisfying consumers' acceptance. Such innovation may satisfy consumers' needs. On the same time, it is a way to preserve a traditional product and avoid its disappearance. Further studies are needed to assess the effect of dried sprouted wheat seeds incorporation in other cereal products.

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