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TRADITIONAL FERMENTED WHEAT: NUTRITIONAL QUALITY AND SENSORY EVALUATION OF BREAD PRODUCED FROM COMPOSITE FERMENTED WHEAT FLOUR

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ABSTRACT

In this study, nutritional quality of traditional fermented wheat was determined, followed by assessment of organoleptic acceptance of the bread produced from composite traditional fermented wheat flour. The results showed that samples of traditional fermented wheat are more or less rich in proteins (4.59%), crude fibers (1%), fat (11.29%), polyphenols (10.48 mg AGE/g) and flavonoids (7.99 mg QE/g). They also show interesting antioxidant activity. Furthermore, the bread made with a mixture of 10% traditional fermented wheat flour was highly appreciated and had the best sensory qualities.

1.Introduction

Cereals are considered one of the most important sources of dietary protein, carbohydrates, vitamins, minerals and fiber for people all around the world (Kohajdovà and Karovicovà, 2007), however, Chavan *et al.* (1989); reported that the nutritional quality of cereal grains and the sensory properties of their products are inferior. In this regards, fermentation of cereals comes to extend shelf-life, improve palatability, digestibility, nutritive value (Shekib, 1994; Holzapfel, 2002) texture, taste and aroma (Deshpande, 2000; Kohajdovà and Karovicovà, 2007).

Several authors have demonstrated that the spontaneous fermentation of cereals can involve lactic acid bacteria, yeasts and molds (Kohajdovà and Karovicovà, 2007; Viéra-Dalodé *et al.,* 2007). Lactic acid fermentation processes are the oldest and most important economical forms of production and preservation of food for human consumption (Blandino *et al.,* 2003). This fermentation exerts chemical changes in food accelerated by enzymes of lactic acid bacteria. It is estimated that the largest spectrum of lactic acid fermented foods exists in Africa (Holzapfel, 2002). Moreover, spontaneous fermentation processes at the household or small industry level are often used to prepare fermented foods and beverages using maize, sorghum and millet as the main cereals in Africa (Blandino *et al.,* 2003). These spontaneous fermentation processes also take place in parts of Eastern Algeria to produce fermented wheat in order to prepare traditional foods.

To store cereals, several methods are employed by Algerian farmers but the traditional techniques are still used and give special products. Such techniques are based on the use of underground holes or silos built near the farm at generally high places. These stuffs are called ‘Matmours’. This method gives to durum wheat brown color and very strong acid odor, following the natural fermentation due to native micro-organisms, giving rise to the traditional fermented wheat locally named 'Mzeyet' and in other parts of Algeria it is called 'Elhammoum', which is used as the main ingredient in the preparation of a valued traditional Algerian dish (Couscous).

The aim of this paper is to examine the current knowledge on a traditional Algerian fermented food (fermented wheat, Mzeyet or Elhammoum), to provide more information regarding its biochemical and nutritional quality, as well as to evaluate its potential to be used as a supplement in bread technology.

2. Materials and methods

2.1. Sample collection

The different samples of traditional fermented wheat (TFW) were collected from two regions situated in Eastern Algeria namely Jijel and Mila. The eight samples were obtained after being subjected to traditional spontaneous fermentation in Matmours during approximately 10 months in rural areas.

2.2. Biochemical analysis

The traditional fermented wheat samples were subjected to biochemical evaluation; the pH and the greasy acidity were measured according to Multon (1982) and AFNOR (NF V 033-712 1982) procedure respectively. The protein contents (Kjeldhal method) and the starch content were determined according to Lecoq (1965), crude fiber content was measured employing AOAC procedure (1995) and fat content according to Serna-Saldivar (2012) procedure. Carbohydrates were determined by difference as described by Srivastava *et al.* (2002):

$$\text{Carbohydrate (\%)} = 100 - [\text{protein (\%)} + \text{fat (\%)} + \text{ash (\%)} + \text{moisture (\%)}]. \quad (1)$$

2.3. Antioxidant activity of methanolic wheat extract

Lyophilized traditional fermented wheat was homogenized with 70 % methanol during 48 h and the mixture was filtered using a filter paper. The filtrate was concentrated at the temperature of 45 °C (Bruneton, 1999).

2.3.1. Total polyphenolic and flavonoid contents

The total phenolic content (TPC) was investigated using the Folin-Ciocalteau assay (Othman *et al.*, 2007). Briefly: 0.2 ml of sample was mixed with 1.5 ml of Folin-Ciocalteau reagent. After 5 min, 1.5 ml of 7 % Na_2CO_3 solution was added and the mixture was incubated for 90 min, then the absorbance was measured at 750 nm. The total phenolic content was expressed as mg gallic acid equivalents per g of dry weight (mg GAE / g). To determine the total flavonoid content (TFC), aluminium chloride complex forming assay was used according to Djeridane *et al.* (2006). In this test, 1.5 ml of sample was added to 1.5 ml of aluminum chloride solution (2 %). The mixture was allowed to stand in darkness for 30 min. The absorbance of this reaction mixture was recorded at 430 nm and the results are expressed as mg quercetin equivalents per g of dry weight (mg QE/g).

2.3.2. DPPH (1,1-diphenyl-2-picrylhydrazyl) assay

Antioxidant activity of the Traditional fermented wheat extracts (TFWE) was measured as scavenging free radical potential in ethanolic solution of DPPH, as described by Brand-Williams *et al.* (1995). 100 µl of TFWE were added to 3 ml of 0.025 g / l DPPH ethanolic solution freshly prepared. After incubation for 30 min at room temperature and in darkness, the absorbance was recorded at 517 nm and the antiradical activity was calculated as percentage of DPPH discoloration compared to the control using following formula:

$$\text{Inhibition percentage} = [(A-B) / A] \times 100$$

Where (A) is the absorbance of pure DPPH in oxidized form and (B) is the absorbance of the sample.

2.4. Bread preparation and analysis
2.4.1. Flour blends and backing process

Three flour blends were prepared by mixing wheat flour with traditional fermented wheat flour (TFWF) in the proportions 90 / 10, 80 / 20 and 70 / 30 (w / w), while 100 % unfermented wheat flour (UFWF) was used as control. The four flour samples were stored at room temperature for bread production.

Bread was backed from the flour samples using the usual dough method in a commercial bakery, located in Jijel, Algeria. The formulations of the breads with TFWF were developed by modification of the traditional formulation, using different levels of TFWF addition (0 %, 10 %, 20 % and 30 %) in substitution for part of the UFWF (Table 1).

To obtain bread, the ingredients (UFWF, TFWF, fresh yeast, salt, flour improver and water) were mixed manually in laboratory then kneaded. After kneading, the dough was divided into 250 g dough pieces and these were balled and allowed to rise at room temperature for 20 min. The dough pieces were then shaped before being placed for 40 min in a fermentation chamber (humidity - temperature: 75 % - 35 °C). The baking takes place at 210 °C for 40 min in a plate oven equipped with a steam injection system. Once baked, breads were allowed to cool down for 30 min, and kept at room temperature for further assessment (Abdourahamane *et al.,* 1999).

Table 1. Bread making ingredients

Ingredients	Formulation			
	UFWF	10 % TFWF	20 % TFWF	30 % TFWF
UFWF	250 g	225 g	200 g	175 g
TFWF	00 g	25 g	50 g	75 g
Yeast	12.5 g	12.5 g	12.5 g	12.5 g
Salt	4.25 g	4.25 g	4.25 g	4.25 g
Flour improver	0.1 g	0.1 g	0.1 g	0.1 g
water	-	-	-	-

UFWF: unfermented wheat flour, TFWF: traditional fermented wheat flour, -: variable amounts

2.4.2. Gas retention and sensory evaluation of bread

The properties of gas retention during fermentation were evaluated by manually measuring the dough height during 60 min.

The bread samples were subjected to sensory evaluation about 1 h after baking by a jury composed of 6 tasters that were very familiar with bread. The sensory characteristics of the breads were evaluated according to the following criteria: loaf color, crust, crumb texture, aroma, taste and overall acceptability of the bread sample. The panelists rated their acceptability of the product on a 01-09 point hedonic scale (Ijah *et al.*, 2014).

2.5. Statistical analysis

All data were performed using SPSS software version 22.0 for windows. The data obtained from the analyses are expressed as the mean ± standard (SD). Statistical differences were analyzed by one way analysis of variance (ANOVA) at $p < 0.05$. Correlation analysis between some parameters was performed using Pearson correlation at $p < 0.05$.

3. Results and discussions
3.1. Biochemical analysis of traditional fermented wheat

The eight samples under study showed significant differences in pH values ($p < 0.001$***), these values varied from 3.94 to 6.89 with an average of 5.25. The most acidic sample was WM4 (3.94 ± 0.09), while, the least acidic one was WM5 (6.89 ± 0.17) (Table 2). Similar results were reported in an earlier study conducted by Gourchala *et al.* (2014) (5.63 ± 0.014), in which they compared naturally fermented wheat samples to unfermented ones. However, these values were higher than those found by Doukani *et al.* (2013), where a value of 4.45 was recorded as being the most acidic. The lowest pH value (3.94) makes the WM4 sample very acidic. This can be explained by a long fermentation time in Matmour, which leads to a large bacterial activity responsible for the accumulation of organic, lactic and other acids, subsequently causing a decrease in pH (Kohajdovà and Karovicovà, 2007).

Table 2. Biochemical characteristics of traditional fermented wheat

Means ± SD	WM1	WM2	WJ1	WM3	WM4	WJ2	WM5	WM6
pH (***)	5.61 ± 0.30	5.75 ± 0.02	4.93 ± 0.09	4.57 ± 0.02	3.94 ± 0.09	4.04 ± 0.03	6.89 ± 0.17	6.33 ± 0.26
Greasy acidity (***)	10.86 ± 3.74	10.43 ± 3.21	8.39 ± 3.24	9.18 ± 0.62	7.44 ± 0.00	12.19 ± 1.00	2.19 ± 0.27	6.46 ± 0.69
Total sugars (%) (***)	80.52 ± 0.57	49.08 ± 0.29	79.13 ± 0.02	55.10 ± 0.72	79.81 ± 0.73	65.86 ± 0.67	87.70 ± 0.65	81.83 ± 0.65
Starch (%)	38.60 ± 0.07	13.20 ± 0.60	31.20 ± 0.07	10.00 ± 0.04	11.50 ± 0.12	13.70 ± 0.07	39.00 ± 1.00	56.00 ± 8.00
Crude fiber (%)	1.00 ± 0.00	2.01 ± 0.00	0.29 ± 0.00	0.26 ± 0.02	0.51 ± 0.02	0.29 ± 0.03	1.37 ± 0.20	2.30 ± 0.30
Total proteins (%)	0.17 ± 0.00	5.46 ± 0.00	5.84 ± 0.00	18.81 ± 0.00	1.96 ± 0.00	1.75 ± 0.00	1.42 ± 0.00	1.31 ± 0.00
Total fat content (%)	ND	ND	5.30 ± 0.00	ND	6.13 ± 0.00	ND	0.75 ± 0.00	1.30 ± 0.00
Total phenolic content (mg GAE/g) (***)	10.36 ±0.11	9.37 ±0.09	9.72 ± 0.10	9.81 ±0.14	10.61 ±0.08	10.79 ±0.16	11.21 ±0.12	11.97 ±0.03
Total flavonoid content (mg QE/g) (***)	8.36 ±1.13	8.25 ±0.28	9.11 ±0.41	9.22 ±0.17	9.24 ±0.07	8.51 ±0.42	3.77 ±0.00	7.51 ±0.00

WM1, WM2, WM3, WM4, WM5, WM6, WJ1, WJ2: traditional fermented wheat samples. ND: not determined. Values are mean ± SD, differences were evaluated by one-way analysis of variance (ANOVA) ($p < 0.05$).

In the same table, greasy acidity values range from 2.19 ± 0.27 to 12.19 ± 1 ($p < 0.001$***). The lowest value was found in sample WM5 and the highest one in sample WJ2. According to Doukani *et al.* (2013), the wheat fermented in a Matmour has a greasy acidity of about 1.51 %. The increase of greasy acidity level in the eight samples may be explained by inadequate grain storage conditions, which lead to hydrolysis of triglycerides by endogenous and exogenous lipases, and thereafter, the accumulation of free fatty acids (Feillet, 2000). Indeed, we noted a significant correlation between greasy acidity values, and the recorded pH values (r = -0,611**, p = 0.002**).

Sugar contents in the eight samples varied significantly ($p < 0.001$***). They ranged from 49.08 ± 0.29 % to 87.70 ± 0.65 %. We noticed that sample WM5 had the highest carbohydrate content (87.70 ± 0.65 %) followed by WM6, WM1, WM4 and WJ1. However, low values were registered in samples WM2, WM3 and WJ2 (49.08 ± 0.29 %, 55.10 ± 0.72 % and 65.86 ± 67 % respectively). The low content of sugar in TFW samples is due probably to excessive fermentation in underground silos. The same results were found in previous studies (Deshpande, 2000; Doukani *et al.*, 2013; Gourchala *et al.*, 2014). Wheat seeds contain amylolytic enzymes which release maltodextrins, maltose and glucose. These endogenous enzymatic activities play an important role in starch degradation, which is considered a source of fermentable sugars (Ganzle, 2014). The decrease in carbohydrate content may be due to their use as fermentable substrate by wheat microflora during fermentation. For this reason, fermented wheat is suitable for diabetics.

Starch represents a major part of carbohydrate in the mature wheat kernel (Matz, 1991; Bushuk and Rasper, 2012). As shown in Table 2; starch contents of TFW samples ranged from 10 ± 0.04 % to 56 ± 8 % (26.68 % in average). These values are low compared to that found by Gourchala *et al.* (2014) who recorded a value of 48.17 %. According to Matz (1991), starch content in unfermented wheat varied from 60 to 68 %. During fermentation, the starch is the mostly degraded substrate (Bekhouche *et al.,* 2014), and this is due to high amylase activity (Ganzle, 2014), which explains the low starch content in TFW samples.

The protein content is an important criterion for assessing the quality of wheat. As shown in Table 2, values of total protein contents varied from 0.17 % to 18.81 %. WM1 protein content (0.17 %) seemed to be very far from the limit suggested by Matz (1991) and Feillet (2000), who required a protein content of 7 to 18 % and 10 to 15 % respectively. The low protein content in the seven samples can be linked to protein degradation during fermentative process, which favors the action of certain endogenous cereal proteases (Kamal Eldin, 2012). On the other hand, the production of free amino acids resulting from hydrolysis of cereal proteins during fermentation (Thiele *et al.,* 2004) improves cereal nutritional quality (Blandino *et al.,* 2003) by increasing the content of the essential amino acids such as lysine, methionine and tryptophan (Adams, 1990). Otherwise, the high level of protein content in WM3 sample (18,81 %) can be explained by bacterial synthesis of new protein products during spontaneous natural fermentation (Kohajdovà and Karovicovà, 2007).

The results revealed that total fat contents values varied between 0.75 % and 6.13 % with an average of 3.37 %. The values appeared to be higher than those found by Doukani *et al.* (2013) (1.08 %). In fact, Matz (1991) and Feillet (2000) suggested a fat content of 1.5 to 2 % and 2 to 3 % respectively. WM5 and WM6 fat contents (0.75 % and 1.3 %) are lower than the values pointed to above; this can be explained as hydrolysis of triglycerides and liberation of fat acids during storage.

The data given in Table 2 indicate that TFW samples have a crude fiber content ranging from 0.26 and 2.3 % with an average of 0.71 %, which is low compared to the limits given by Matz (1991) and Feillet (2000), which are respectively 1.5 to 2 % and 2 to 4 %. However, these results are in agreement with those obtained by Doukani *et al.* (2013), who noted a decrease in crude fiber content in fermented wheat grains contrary to the control sample. The decrease can be explained as cellulose hydrolysis due to yeast activity during grain fermentation (Jesperson, 2003).

3.2. Antioxidant activity of wheat extract

Phenolic compounds play a role in free radical scavenging capacities. They are one of the most effective antioxidative constituents that contribute to the antioxidant activity (Govindarajan *et al.*, 2007). According to Table 2, the TPC of the samples varied significantly ($p < 0.001$***), the highest TPC was obtained in wheat extract of sample WM6 (11.97 ± 0.03 mg EAG / g). These results are not in perfect agreement with those found by Gourchala *et al.* (2014). In fact, they quantified total polyphenols in Elhammoum 'durum wheat fermented product', and they noted a significant increase in the total polyphenols content in the fermented wheat (23,75 mg EAG / g) compared to the unfermented wheat sample (18,32 mg EAG / g). The same results were found by Zhang *et al.* (2012). On the other hand, our present results seem to be in agreement with those reported by El hag *et al.* (2002), who found a decrease in total polyphenols during fermentation but after shelling.

The same table shows total flavonoid contents in the eight samples of TFW ($p < 0.001$***). The sample coded WM1 has the higher value, but compared to previous studies, these values are very low. Zhang *et al.* (2012) and Sandhu *et al.* (2016); proved that fermentation using fungus species promotes the increase of total polyphenols and flavonoids contents, Dordevic *et al.* (2010), found that fermentation using lactic acid bacteria and yeast can enhance polyphenol and flavonoid contents, which is not confirmed by our results.

Figure 1 shows the DPPH antioxidant activity of TFWE samples. The decrease in the absorbance of DPPH radicals at 517 nm induced by antioxidants determines its reduction capacity. During radical scavenging assay, DPPH radical without extracts of TFW was stable over the time. However, in the presence of several concentrations of TFWE, DPPH radical is reduced to non-radical DPPH-H. This reduction depends on the used concentrations. It was found that the DPPH scavenging effect of TFWE increased with the increase of their concentration.

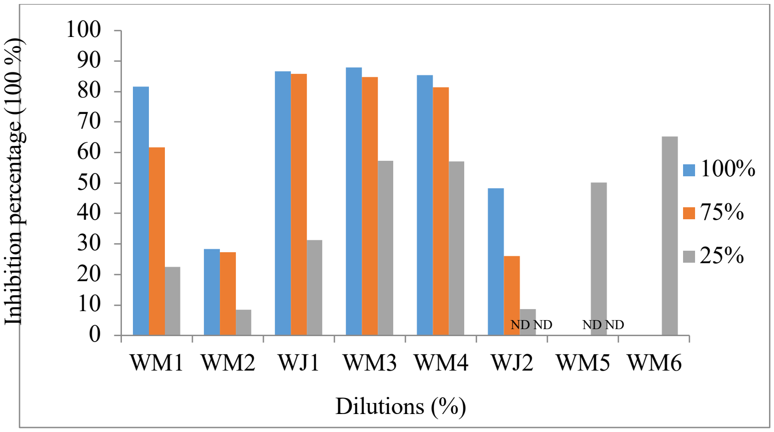

Figure 1. DPPH scavenging activity of different concentrations of TFW extracts
ND : not determined

According to Figure 1, the highest DPPH antioxidant capacity was attributed to WM3 fermented wheat sample (87.97 %) followed by WJ1 (86.55 %). At 75 % dilution, WJ1 also recorded the highest activity (85.77 %). The lowest activity was abserved in WM2 sample at all dilutions. A positive correlation was also noted between flavonoid contents and antioxidant activity (r = 0.856*, p = 0.03*). DPPH antioxidant activity is effective in traditional fermented wheat, this can be explained by enhancement of antioxidants like polyphenols and flavonoids by fermentation. According to Zhang *et al.* (2012); DPPH antioxidant activity was more effective in wheat fermented using *Cordyceps militaris* than in unfermented wheat.

3.3. Bread analysis

The breads obtained are presented in Figure 2.

Table 3 shows measurement of dough height at 0, 20, 40 and 60 min. The dough height increases as time fermentation increases. At 20 min, the sample with (90:10 %) has the highest value while the bread obtained from (70:30 %) has the lowest value. The same results are noted at 40 and 60 min. It seems that the bread from (90:10 %) and (80:20 %) has better gas retention than that from unfermented wheat (100%) and from (70:30 %). The decrease in gas retention as fermented wheat flour proportions increase is probably due to a decrease in the elastic property of the flours, which depends on the gluten content. Such proteins could undergo an enzymatic degradation during the fermentation of the grains in the Matmour. The results obtained in our study are in agreement with those found by Okafor *et al.* (2012) for wheat mushroom powder bread, and Oloyede *et al.* (2013) for fermented unripe plantain flour, in regards to measurement of loaf volume of bread samples. Comparing the results for TFW dough gas retention to those of UFW dough, heighst values in TFW could be explained by the fact that the TFW flour is rich in simple products (such as fermentable sugars) used during fermentative process of *Saccharomyces cerevisiae*.

According to Figure 3 which represents the mean sensory scores of experimental and control bread samples, after the bread from unfermented wheat, the bread from (90:10 %) was the most appreciated with a score of 7.03 ± 0.21, while the bread obtained from the (70:30 %) mixture was the least appreciated with a score of 4.83 ± 0.00. It is possible that the most brownish color of the crumb (Figure 2), which is dependent on percentage incorporation of TFWF, is the cause of the decrease in the acceptance of the products by consumers, who prefer bread with a lighter color (Gomes *et al.*, 2016), this result is confirmed by negative correlation between percentage incorporation of TFWF and sensory score of bread samples (r = - 0.991**, p < 0.001***). Oloyede *et al.* (2013); also reported low overall acceptability for bread produced from 30 % fermented unripe plaintain flour substitution. Although the bread from (90:10 %) had a score close to that of the bread from 100 % unfermented wheat, it is less acceptable than the latter. This result is similar to that found by Ameh *et al.* (2013), who noticed that the 100 % wheat bread was organoleptically more acceptable than the wheat bread supplemented with rice bran. So, breads of good sensory qualities could be produced from up to 10 % fermented wheat flour substitution in unfermented wheat flour. It is the same conclusion obtained by Olaoye *et al.* (2006) when they used soy flour substitution in wheat flour.

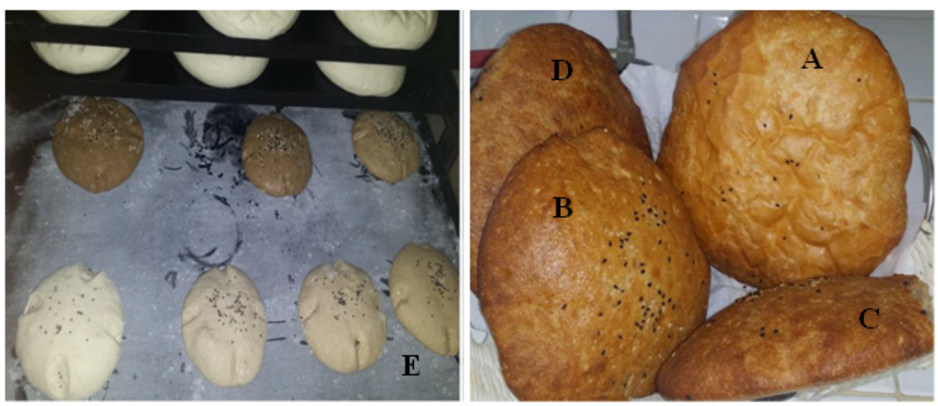

Figure 2. Aspect of produced bread
A: UFW (100 %), B: TFW (10 %), C: TFW (20 %), D: TFW (30 %) and E: aspect of bread during fermentation

Table 3. Physical properties of bread samples (dough height) (cm)

Fermentation time (min)	UFW (100%) (Cm) NS	UFW / TFW (90:10%) (Cm) NS	UFW / TFW (80:20%) (Cm) *	UFW / TFW (70:30%) (Cm) **
00	2 ± 0.00	2 ± 0.00	2 ± 0.00	2 ± 0.00
20	2.3 ± 0.00	3.15 ± 0.21	3.05 ±0.63	2 ± 0.00
40	2.6 ± 0.00	3.85 ± 1.06	3.5 ± 0.28	2.9 ± 0.14
60	3.2 ± 0.00	4.25 ± 1.06	3.95 ± 0.21	3.1 ± 0.14

UFW: unfermented wheat, TFW: traditional fermented wheat. Values are mean ± SD, differences were evaluated by one-way analysis of variance (ANOVA) ($p < 0.05$)

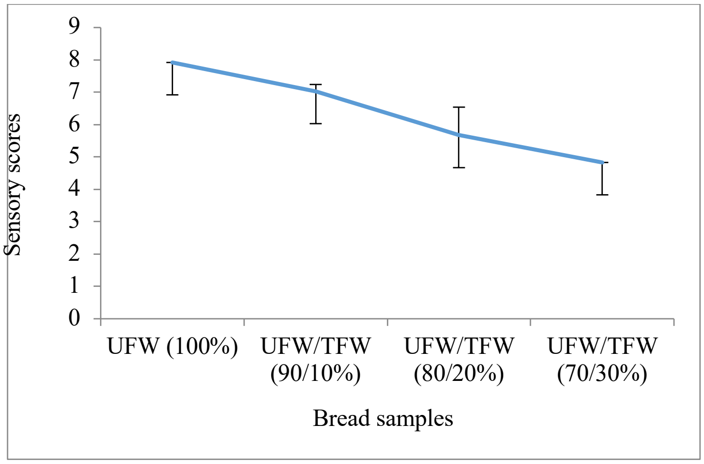

Figure 3. Sensory scores of bread samples
UFW: unfermented wheat, TFW: traditional fermented wheat

4. Conclusions

The fermentation of wheat in underground silos gives the grains special organoleptic characteristics and allows to obtain the Algerian traditional fermented product (Mzeyet or Elhammoum), which is much appreciated by Algerians and especially diabetics because it has a moderate carbohydrate content. Samples of traditional fermented wheat represent a satisfactory quality from a biochemical and nutritional point of view. The use of the flour obtained after milling the samples as supplementation in bread technology represents a very interesting task and makes it possible to obtain breads of good quality and organoleptic acceptability through the use of a proportion of incorporation at a ratio of 90:10 (unfermented wheat : traditional fermented wheat).

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