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THE GLIADIN ANALYSIS BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF TRADITIONAL FERMENTED "*HAMOUM*" WHEAT IN WEST ALGERIA

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Abstract: *Our ancestors had always used food fermentation without having a scientific explanation. Their natural phenomena with therapeutic potential became interesting only in the early twentieth century. A comparative study between the electrophoretic profiles of wheat proteins of fermented wheat type Hamoum (FWH) and regular unfermented wheat (NUW) was performed to identify the effect of fermentation realized by the traditional technique using underground storage (locally named Matmora) for 12 months. SDS-PAGE electrophoresis was performed after the sequential extraction of different proteins (albumins/globulins, gliadins and glutenins). The results showed a total degradation of the regular wheat proteins after fermentation. Fermented wheat type Hamoum (FWH) could be a good alternative for people with disorders related to gluten ingestion as compared to regular unfermented wheat (NUW).*

Key words: *FHW, fermentation; albumin; globulin; gliadins; SDS-PAGE; RP-HPLC.*

1. Introduction

Wheat is considered worldwide to be the most consumed foodstuff after rice. It provides about 15% of human energy needs, hence the importance of its storage to ensure its annual consumption [6, 25].

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In Algerian rural areas, wheat is stored in underground granaries locally called "Matmora". It is highly appreciated for its organoleptic and especially medicinal properties due essentially to the action of fermentation by the endogenous lactic bacterial flora and to the underground conditions [13, 18, 19]. During the storage period, the wheat ferments in the outer layer, which is in close contact with the soil. Soil flora and fauna contribute to the emergence of lactic acid bacteria during the wheat fermentation process [2]. Regular consumption of fermented food may have beneficial effects on body weight regulation and certain metabolic functions through several mechanisms [12]. Fermented wheat has previously been used as an alternative therapy for several intestinal complications, inflammatory and metabolic pathophysiological problems [15, 23]. The lactic microbial flora obtained during FWH fermentation (fermented Hamoum-type wheat) could benefit the digestive system in certain pathophysiological situations. Recent works have shown that FWHcontributes to the mucosal barrier restructuring during intestinal bacterial translocation [1], and also a restructuring of intestinal morphometry induced by specific protein malnutrition for an improvement of the volatile fatty acids' synthesis, which is an energy source for colonic cells and the regulation of the physiology of the gastrointestinal tract in malnourished rats [29].

This work aims to evaluate the electrophoretic and chromatographic profile of albumin/globulin and gliadin issued from FWH compared to regular unfermented wheat (NUW) by the SDS-PAGE electrophoresis and reverse-phase high-performance liquid chromatography (RP-HPLC) techniques. Also, by studying different protein fractions of fermented and non-fermented wheat. These fractions are involved in pathophysiological cases such as celiac disease (gluten intolerance), wheat protein allergy and non-celiac gluten sensitization [3, 17]. Therefore, finding a solution to these problems is always a wise choice regarding the nutritional and therapeutic value of natural food products like FWH.

2. Materials and Methods

2.1. Plant Material

Two types of common wheat (*Tritichum aestivium*) were sampled under aseptic conditions in the west of Algeria in June 2021 after one year's storage: fermented Hamoum type wheat (FHW) and unfermented wheat (NUW). After a year's storage in a natural granary away from vegetation and water run-off, the fermented wheat was taken from the peripheral zone of the granary (Matmora) in contact with the soil. The NUW was taken from the central spot.

2.2. Macroscopic Study

For the macroscopic study, an organoleptic test (generally, taste and smell) concerning visual macroscopic parameters such as the size and the color was carried out using a binocular magnifying glass.

2.3. Sequential Extraction of Albumin/Globulin, Gliadin and Glutenin Fractions

A protein extraction from FHW and NUW samples was performed from wheat

grain flour obtained after milling and collected in sterile tubes. The technique was based on the difference in solubility of the three main classes of wheat proteins.

Albumin and globulin were extracted in a NaCl phosphate buffer according to the protocol described by Kheroua et al. [13], while gliadins and glutenins precipitated. The latter were extracted according to the protocol proposed by Singh et atl. [25] with an alcoholic solubilization for gliadins and solubilization in the presence of acetic acid and a reducing agent, i.e. Dithiothreitol (DTT), for glutenin subunits [25].

2.4. The Sequential Extraction of Gliadin Fractions

1.0 g of flour was put for solubilisation in a phosphate/Nacl buffer solution at pH 7.8, under agitation at 4°C for 2 h, then centrifuged at 8000 rpm for 15 min; the supernatant was dialysed, frozen and freeze-dried.

The pellet was then suspended in a 70% ethanol solution to extract the gliadins and centrifuged at 12,000 rpm for 20 min at room temperature. The supernatant was dialyzed, frozen and lyophilized.

2.5. SDS-PAGE Electrophoresis

Sample preparation for electrophoresis involves adding a sample buffer (bromphenol blue) to the lyophilisates. A volume of 20 µL is added to each well. Proteins are separated into gels containing 10% acrylamide for gliadins and 15% acrylamide for albumin/globulins. After migration, the gels are stained with a staining solution of Coomassie Blue R250 and then bleached [21, 27].

2.5. Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC)

A reverse-phase chromatography separation was carried out to verify the presence of gliadins from NUW and FHW. The analysis used a C18 analytical column (250 mm x 4.5 mm internal diameter, 5 µm particle size).

The samples were mixed in eluent A. The flow rate was 1 mL/min, and the sample volume injected into the column was 20 µL. A linear elution gradient was applied using two mobile eluents: eluent A containing 0.1% trifluoroacetic acid (TFA) (v/v) in ultrapure water and eluent B containing 0.1% trifluoroacetic acid (TFA) (v/v) and acetonitrile. The gradient applied was 20-60% eluent B for 70 min. The temperature during separation was 60°C. Optical density was recorded at 214 nm.

3. Results and Discussion

3.1 Macroscopic Analysis

Macroscopically, FHW grains are oval, more or less elongated, 6 to 8 mm long and 2 to 3 mm wide. They have a strong, slightly acidic odour. These observations are consistent with the work of Feillet [8]. Morphological parameters depend on grain development conditions. The FHW sample was metadined/ metallic color (slightly brown) with a non-vitreous appearance.

Compared with NUW, the dark brown or even blackish discoloration may be caused by the microbiome of FHW, as storage conditions may lead to a darkening of the wheat germ [11].

Recent work on the endogenous flora of FHW has shown that it contains lactic acid

bacterial flora such as *Lactobacillus plantarum* and yeasts such as *Schizosaccharomyces pombe* and *Saccharomyces pasturianus*which which are beneficial for digestive health [2], as well as *Lactococcus lactis* LLGKC18 having an important proteolytic power because it is capable of cleaving a certain number of gliadin epitopes responsible for wheat allergy by its proteolytic action [7].

3.2. Biochemical Analysis

The different electrophoretic profiles corresponding to the different fractions of wheat proteins (albumins/globulins and gliadins) are revealed by the SDS-PAGE electrophoretic technique. The FHW results show the presence of polypeptide fragments of albumins/globulins and gliadins that are very distinct and different from those of the NUW.

Electrophoresis shows that NUW contains different albumin/globulin fractions, unlike FHW. The latter has undergone total degradation, explained by its fermentation of endogenous bacterial and fungal flora [5]. According to the study by Thiele et al. [26], albumin is 83.4% degraded [22]. This difference in degradation can be explained by the duration of fermentation, which may involve one or more fermentation stages ranging from a few hours to several months, depending on the nature of the wheat and the bacterial species [22]. However, the balance of the total endogenous bacterial flora presented in wheat grains can be affected by numerous factors, influencing the development of certain bacteria, yeasts and moulds responsible for the spontaneous fermentation of the grains [28].

The microorganisms' hydrolysis of proteins into polypeptides and amino acids occurs very slowly under storage conditions [18]. Most microbial proteases are specific. They act on both proteins and oligopeptides and are generally exocellular enzymes [9] (Figure 1).

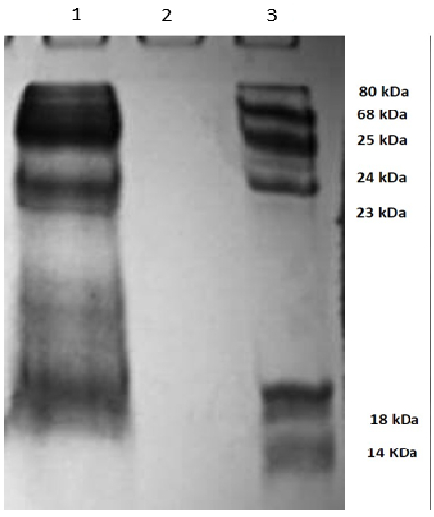

Fig. 1. *Electrophoresis on polyacrylamide gel (15%) in the presence of SDS of albumin/globulin with β-mercaptoethanol:* 1- NUW with β-mercaptoethanol; 2- FHW with β-mercaptoethanol; 3- Protein kit with β-mercaptoethanol

Electrophoresis on a 10% SDS-PAGE gel also showed an almost total degradation of gliadins, which are soluble proteins in dilute ethanol solutions and have a molecular weight of 30-80 kDa. Gourchala et al. [10] showed that the degradation of gliadins was 57.3%, partly explaining the depolymerisation of gluten [9] (Figure 2).

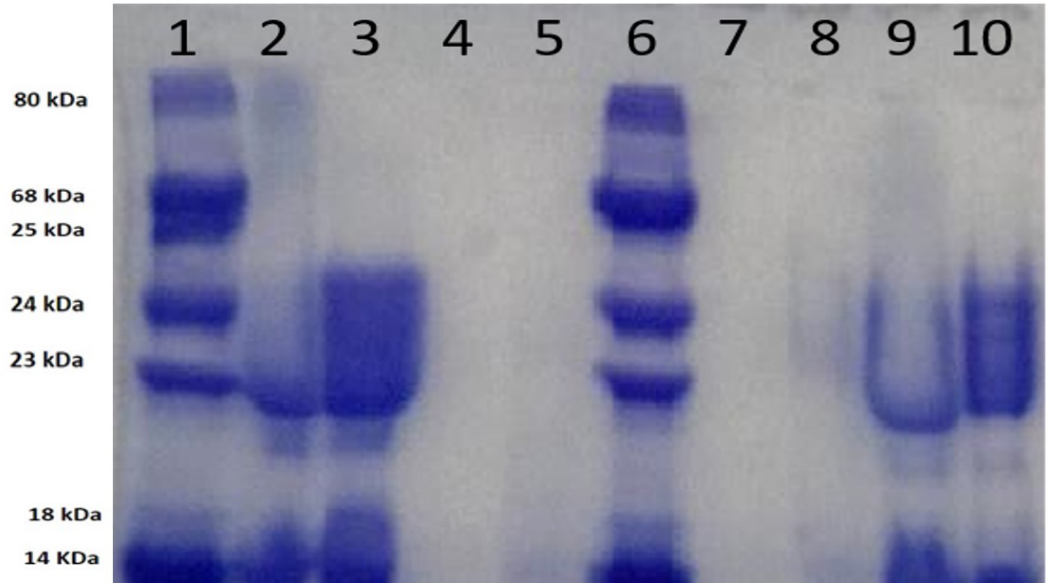

Fig. 2. *Polyacrylamide gel electrophoresis (10%) in the presence of SDS of gliadins in the presence and absence of β-mercaptoethanol: 1. Protein kit without β-mercaptoethanol (80 – 14 kDa); 2. NUW without β-mercaptoethanol; 3. NUW with β-mercaptoethanol; 4. FHW without β-mercaptoethanol; 5. FHW with β-mercaptoethanol; 6. Protein kit with β-mercaptoethanol (Lactoferrin 80 kDa, SAB 68 kDa, Ovalbumin 45 kDa, Caseins 19-25 kDa, β -Lactoglobulin 18 kDa, α-Lactalbumin 14 kDa); 7. NUW without β-mercaptoethanol; 8. NUW with β-mercaptoethanol; 9. FHW without β-mercaptoethanol; 10. FHW with β-mercaptoethanol*

Gourchala et al. [10] showed that the degradation of glutenins was 20%, which partly explains the depolymerisation of gluten [9]. This was contrary to our obtained results, which showed 100% gluten degradation, which reflects the good biochemical and, in this case, the nutritional quality of the FHW. This could be explained by the nature of the wheat, the storage period, the seed, the climate and the soil (biotope). Its richness in mineral elements and the diversity of the endogenous lactic bacterial flora and yeasts are beneficial to the health of the digestive system [22].

Previous studies from the literature [4, 16] showed that fermentation, due to the action of bacteria and fungi, is responsible for changes in the biochemical composition and nutritional value of wheat during storage [4]. FHW has an acid pH, is rich in water, ash, reduced sugars and total polyphenols, and is lower in

lipids, total sugars, fiber and protein than unfermented NUW wheat.

3.3. Chromatographic Profiles by RP-HPLC

Reverse-phase high-performance liquid chromatography provides excellent separations of gliadin polypeptides based on surface hydrophobicity differences.

This technique separates proteins based on hydrophobic forces between amino acids on the surface of polypeptides and apolar chromatographic supports under specific elution conditions. The chromatographic profiles of native gliadins and those of FHW and NUW are shown in Figures 3 and 4.

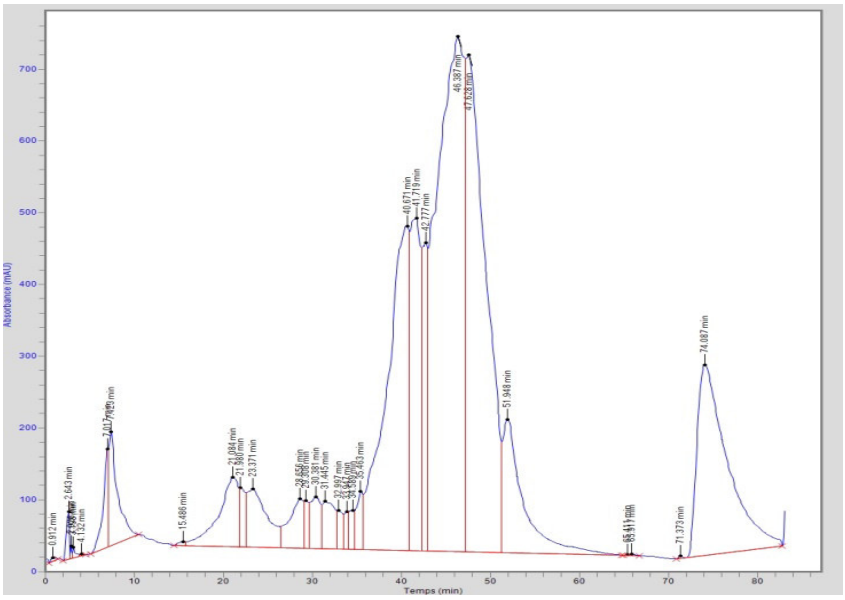

Fig. 3. *Chromatographic profiles of native gliadins by RP-HPLC*

We observe from these figures that the chromatographic profile of native gliadins is represented by several peaks, which can be divided into two major groups according to their retention time and interaction with the stationary phase support. The ω-gliadins are the polypeptides matched first on the chromatogram at a retention time ranging between 20 and 26 min, which is explained by their low hydrophobicity.

The mixture of α, β and γ-gliadins emerges after the ω-gliadins between a retention time of 28 and 54 min. The peak area is more significant than that of the ω-gliadins, which leads us to say that the interaction between the protein and the support is higher (a high degree of hydrophobicity).

The chromatographic profile of FHW compared with NUW shows an almost total degradation of these fractions and the disappearance of the gliadin peaks (20-54 min), which is explained by the degradation of gliadin by the endogenous bacterial flora rich in lactic acid bacteria present in FHW [2, 19, 20]. According to El Mecherfi et al. [7], **the RP-HPLC analysis of gliadins after fermentation** by *Lactococcus lactis* LLGKC18 revealed that

this strain hydrolyzed more than 90% of the gliadins. This is confirmed by the electrophoretic profile by both SDS PAGE and RP-HPLC. Cross-tabulating the results obtained by electrophoresis and the profiles obtained by HPLC; we conclude that gliadins are almost absent in FHW as compared to NUW [3].

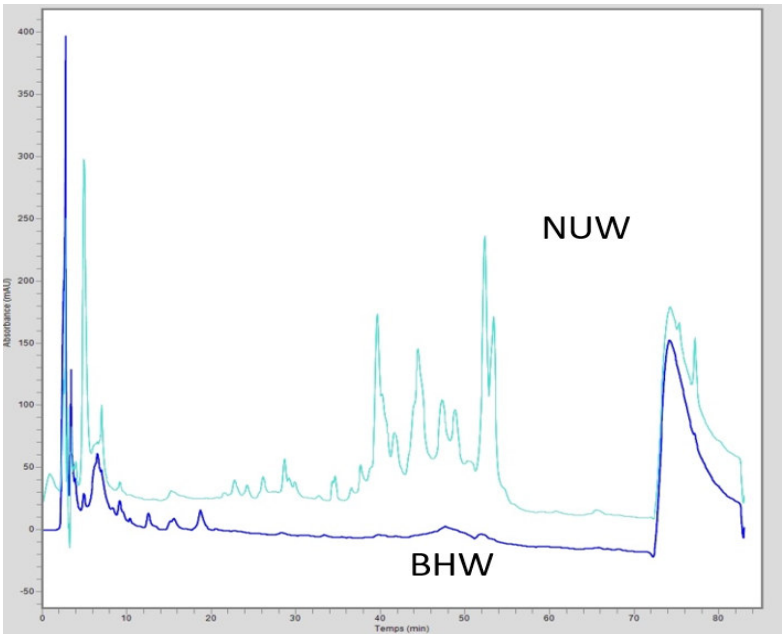

Fig. 4. *Chromatographic profiles of FHW and NUW by RP-HPLC*

4. Conclusion

This study aimed to compare the biochemical composition and nutritional value of two soft wheat samples before and after fermentation under traditional Matmora underground storage conditions for 12 months in western Algeria. Very little work has been done on FHW throughout the literature survey, which gave an idea on how to carry out this issue to determine the influence of fermentation by endogenous flora (lactic acid bacteria, yeasts and fungi) on the biochemical modification of soft wheat proteins.

The results obtained by SDS-PAGE gel electrophoresis for the different protein fractions after sequential extraction showed total degradation. This difference between FHW and NUW is due to fermentation, which partially or totally degrades wheat proteins, sugars, etc.

By cross-referencing the results obtained by electrophoresis and the profiles obtained by HPLC, we conclude that gliadins are virtually absent in FHW as compared with NUW.

FHW can be considered a dietary and medicinal product for people with diabetes, gluten allergy and intolerance.

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