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Biochemical and Microbiological Changes Associated with Fermentation of Durum Wheat for *Lemzeïet* Processing, a Traditional Algerian Fermented Food

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Abstract: In Algeria, "*Lemzeïet*" is prepared by the natural fermentation of wheat. This study aimed to follow the evolution of microbiological and biochemical properties of *Lemzeïet* with and without vinegar addition for 3, 6, 9 and 12 months. Lactic acid bacteria (LAB) were identified and the microbial count, as well as pH, acidity, protein, fat, ash and starch contents were determined. Results showed that *Lemzeïet* samples represented a safe product after the gradual absence of fungi. It also contained a significant load of LAB that were cocci or rods, white or yellow, grouped in chain, pair and tetrad. LAB isolates were mannitol positive, grew between 10 and 45 °C, showed resistance at 63.5 °C and the majority were homo-fermentative. Results showed a significant decrease in pH during fermentation regardless of the vinegar addition. Protein content increased up to 14.90% and 15.50% at the end of fermentation. The fat and starch contents decreased after 12 months of fermentation, regardless of the vinegar addition. Ash content remained high (1.41% and 1.45%) after six months of fermentation with and without vinegar, respectively. The microbiological and the biochemical characteristics of *Lemzeïet* make it a very interesting raw material in the manufacturing of healthy foods.

Keywords: durum wheat; fermentation; *Lemzeïet*; acid lactic bacteria; biochemical properties

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1. Introduction

Fermentation is one of the oldest biotechnological processes used in food production [1]. Fermented foods contain numerous lactic acid bacteria (LAB) that play a substantial role in the development of organoleptic characteristics [2] and offer other benefits such as the ability to kill pathogens as well as modulate the immune system [3].

In Algeria, in some rural areas, a variety of traditional fermented foods is produced at the household level or in the small enterprise using spontaneous fermentation [4]. These foods include: dairy products, such as beverages (e.g., *Raib* and *Lben*), cheeses (e.g., *Jben*, *Klila*, *Takammarit*, *Bouhezza*, *M'chouna* and *Medghessa* in the Northeast of Algeria and *Igounenes* and *Aghoughlou* in the North Center of Algeria) and fat dairy products (e.g., *Zebda* or butter, *Smen* or *Dhan* in Southeast of Algeria) [4,5], fermented vegetables of many ripe products available only at given periods of the year, such as lemons, onions, green peppers, carrots, figs, grapes and olives in many regions of Western Algeria, fermented sausages made from minced meat called *Merguez* [6] and fermented cereals like sourdough bread and confectionery (e.g., *Zlabia*) [7].

Throughout history, durum wheat (*Triticum durum*) was stored in underground silos (*Matmor*) for 4 to 9 years, leading to a new fermented product. Fermentation in *Matmor* is a

technique applied by farmers. In recent times, the *Matmor* approach has almost disappeared due to the difficulty that the traditional method presents during *Matmor* emptying, as well as the settlement of rural populations in urban areas. Currently, the fermentation of wheat is carried out outside of the *Matmor*, according to a new rapid uncontrolled process which generally occurs in plastic containers of different shapes and sizes, by small producers to respond to the consumers' demands [8,9].

Fermented durum wheat (FDW), "*Lemzeïet,*" is used to manufacture the traditional Algerian couscous, also called *Lemzeïet* or "*El-Machroub*" or "*El-Hammoum,*" translatable as "black ash" [8–11]. It is appreciated for its organoleptic characteristics and health benefits.

Information about the properties of durum wheat fermented in plastic containers is limited. Therefore, this study aimed to identify LAB, evaluate the product safety regarding this uncontrolled fermentation and determine microbial and biochemical changes during the fermentation process of durum wheat with and without the addition of vinegar for up to one year of storage.

2. Materials and Methods

2.1. Raw Material

Durum wheat (*Triticum durum* Desf.) was obtained from the June 2016 harvest of the local variety, "*Mohamed Ben Bachir*" grown in Bouhatem City (province of Mila, North-East of Algeria). Durum wheat was manually dry-cleaned to remove foreign matter and inedible parts and then stored in jute bags at room temperature. The unfermented durum wheat (UFDW) was considered a control (fermentation time: 0 months).

2.2. Preparation of Fermented Durum Wheat

Durum wheat fermentation was conducted following the protocol described by Merabti et al. [8]. On a laboratory scale, two series of fermentation with or without the initial addition of vinegar were reproduced in eight 10 L plastic canisters. For both fermentation batches, 4 L of spring water and 6 kg of durum wheat were introduced into the canisters. For fermentation with the vinegar addition, water was mixed with 150 mL of commercial vinegar solution (5%). The canisters were sealed using sanitary silicone to create anaerobiosis and were then kept at ambient room temperature in a dark place. After the required fermentation time (3, 6, 9 and 12 months), canisters were opened, the content (FDW) was stirred, washed, spread on a clean tissue in thin layers and dried outdoors in a sunny place with a regular, manual turning up of the moisture content in the final product below 11% (Figure 1). Their visual appearance was similar to that of UFDW except for the color, due to browning during fermentation. About 500 g of each sample was collected in sterile glass containers before being analyzed. Fermentation was repeated in triplicate for each series.

2.3. Preparation of Stock Solution and Decimal Dilutions

Ten grams of each ground sample was homogenized with 90 mL of sterile peptone water for 2 min at maximum speed in a Stomacher 400 (Seward Lab-blender, Worthing, UK). Decimal dilutions were prepared by incorporating 1 mL of the obtained stock solution into 9 mL of the diluents [12,13]. Microbiological analyses were carried out in triplicate.

2.4. Enumeration of Total Flora, Yeasts, Molds and Lactic Acid Bacteria

Enumeration of total flora (TF) was performed on PCA (Plate Count Agar) after incubation at 30 °C for 72 h [12]. Yeasts on OGA (Oxytetracycline Glucose Agar) and molds on PDA (Potato Dextrose Agar) were incubated at 25 °C for three days and seven days, respectively. All mediums were from Biokar Diagnostics (Beauvais, France). Fungi isolation required an antibacterial Gentamicin (0.004 g/L) [14].

MRS agar (pH 5.4) (Biokar Diagnostics, Beauvais, France) was used to enumerate rod LAB. In doing so, it was incubated at 35 °C for 72 h under anaerobic conditions (Anaerocult®

A Merck, Darmstadt, Germany), while M17 agar (Biokar Diagnostics, Beauvais, France) was employed to enumerate cocci LAB at 30 °C for 72 h [15].

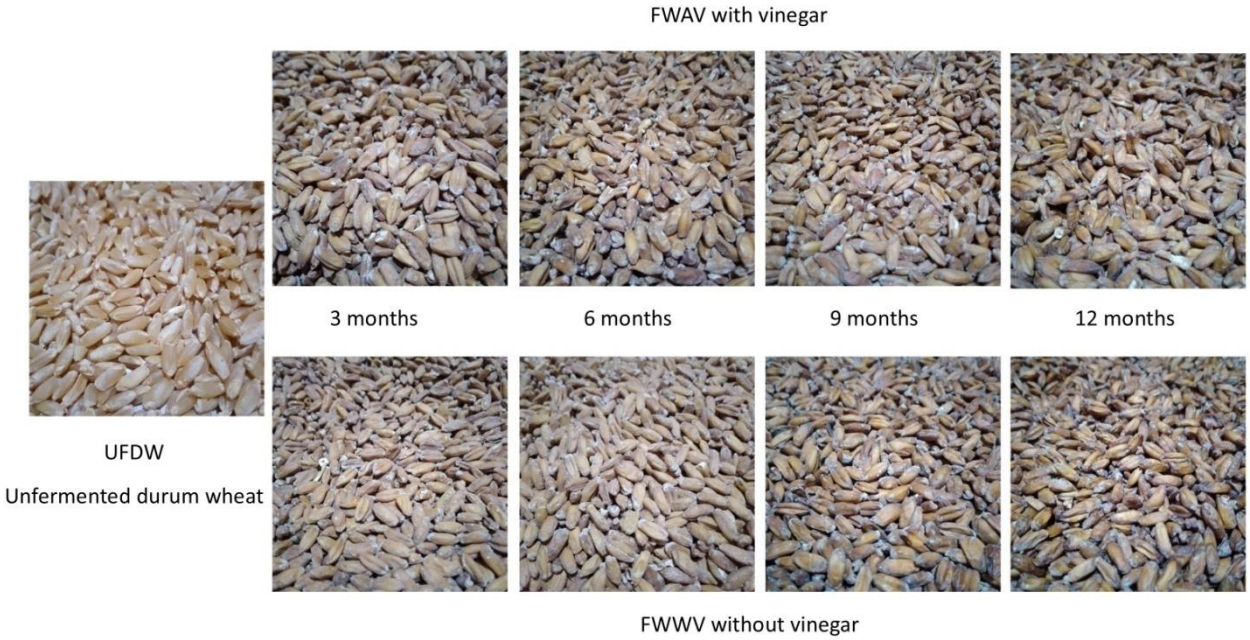

Figure 1. Unfermented durum wheat (UFDW), fermented durum wheat with vinegar (FWAV) and fermented wheat without vinegar (FWWV) after 3, 6, 9 and 12 months of fermentation.

2.5. Isolation and Identification of LAB

LAB were isolated in M17 and MRS agars by picking only colonies of differing morphologies, and then purified by raising three subcultures in the same media at 30 °C. The purity was checked by microscopic observations (Motic BA-210, Chapelle-Sur-Erdren, France), Gram test and catalase formation by adding hydrogen peroxide (H_2O_2) 3% (v/v) (Sigma-Aldrich, St. Louis, MO, USA) onto the cultured colonies [2]. Non-motile, Gram-positive and catalase-negative strains were selected [16]. Pure isolates were stored at −20 °C in isolation broth (MRS or M17) with 20% (v/v) glycerol.

Phenotypic characterization was carried out for the initial identification of isolates. The cell morphology type was determined using a binocular loupe to describe the macroscopic characters of colonies (cell shape, cell color and surface), and a microscope linked to a camera was used to acquire pictures (×1000 magnification) for microscopic observation and grouping mode [17]. A Mannitol-Mobility test was performed using phenol red mannitol agar (Sigma-Aldrich, St. Louis, MO, USA) based on mannitol fermentation [12]. Assessment of LAB growth ability was realized at different temperatures (10 °C, 15 °C and 45 °C) in broth media (MRS or M17) for 24 h [15].

The survival of LAB was evaluated after the inoculation of the strains at 63.5 °C for 30 min, then after cooling at ambient temperature, they were incubated at 30 °C for 72 h. Trouble tubes indicated bacterial growth and resistant strains [18]. The fermentation profile (homo-fermentative (HoF) or hetero-fermentative (HeF)) of the isolates was studied based on their ability, or lack thereof, to produce CO_2 from glucose. The test was performed using inverted Durham tubes on MRS and M17 broth medium [2,12].

2.6. Biochemical Analysis

Biochemical analyses were determined in triplicate. Approved methods of AACC [19] were applied to determine pH (AACC 02-52-01) and total titratable acidity (AACC 02-31-01). ISO Standard methods [20–23] were used to determine moisture content (ISO 712, 2009), protein content by a Kjeldahl method with a nitrogen-to-protein conversion factor of 5.7 (ISO 1871, 2009), total fat content (ISO 7302, 1982) and ash content (ISO 2171, 2010). The

Polarimetric method was utilized to determine starch content. This method is based on the partial acid hydrolysis of starch during 15 min boiling in a calcium chloride solution (0.15 M), followed by a measurement of the optical rotation of the resulting solution by a polarimeter (MCP 150, Anton Paar GmbH, Les Ulis, France) [24].

2.7. Statistical Analysis

Statistical analysis of the data was performed using SPSS software (IBM SPSS Statistics 23). Data were expressed as means ± standard deviation. One-way analysis of variance (ANOVA), followed by Tukey's *post-hoc* test, was used to determine significant differences ($p < 0.05$) between mean values.

3. Results and Discussion

3.1. Microbiological Characteristics

3.1.1. Microbial Flora

The microbial count of the UFDW sample was represented by 71% (1.54×10^2 CFU/g), 25% (0.54×10^2 CFU/g) and 4% (11 CFU/g) of TF, yeasts and LAB, respectively (Table 1). Moreover, we noticed the absence of molds in the starting material (UFDW) confirming the good mycological quality and safe storage conditions according to the Codex Alimentarius Standards [25].

Table 1. Evolution of microbial flora (CFU/g) of durum wheat during fermentation without vinegar and with vinegar.

Time (Months)	Total Flora ($\times 10^2$)	Molds ($\times 10^2$)	Yeasts ($\times 10^2$)	Lactic Acid Bacteria	
				Bacilli ($\times 10^4$)	*Cocci* ($\times 10^4$)
0	1.54 ± 0.05 ^{a}	0.00 ± 0.00 ^{a}	0.54 ± 0.05 ^{bcd}	0.0007 ± 1.15 ^{a}	0.0004 ± 0.57 ^{a}
Without vinegar (FWWV)					
3	4.80 ± 0.20 ^{a}	0.09 ± 0.01 ^{b}	0.64 ± 0.02 ^{bcd}	Uncountable	112.50 ± 0.03 ^{a}
6	9.66 ± 0.05 ^{b}	0.10 ± 0.01 ^{b}	0.74 ± 0.02 ^{cd}	12.3 ± 0.41 ^{ab}	2.93 ± 0.04 ^{a}
9	14.00 ± 0.20 ^{c}	0.16 ± 0.01 ^{bc}	0.82 ± 0.03 ^{d}	333 ± 0. 57 ^{d}	174 ± 0.05 ^{a}
12	1.65 ± 0. 32 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	26.2 ± 0. 12 ^{bc}	4.41 ± 0.01 ^{a}
With vinegar (FWAV)					
3	3.30 ± 0.10 ^{a}	0.14 ± 0.02 ^{cd}	0.37 ± 0.05 ^{b}	0.34 ± 0.20 ^{a}	44,002.55 ± 0.31 ^{d}
6	14.66 ± 3.05 ^{c}	0.00 ± 0.00 ^{a}	3.06 ± 0.30 ^{f}	753 ± 0.11 ^{e}	12,400 ± 0.05 ^{c}
9	9.00 ± 0.30 ^{b}	0.16 ± 0.02 ^{d}	14.00 ± 0.20 ^{e}	Uncountable	1700 ± 0.05 ^{b}
12	3.08 ± 1.09 ^{a}	0.07 ± 0.02 ^{b}	0.41 ± 0.03 ^{bc}	36 ± 0.16 ^{c}	9.21 ± 0.02 ^{a}

Data are expressed as mean values ± standard deviation; $^{a–f}$ values in columns with the same letter were not significantly different ($p < 0.05$); CFU/g: colony forming units per gram; Uncountable: >300 colony per plate.

TF count increased ($p < 0.05$) during fermentation time from 1.54 CFU/g to 14.00 CFU/g after 9 months of fermentation without vinegar and to 14.66 CFU/g after 6 months of fermentation with vinegar. A subsequent decrease ($p < 0.05$) was observed reaching 1.65 CFU/g and 3.08 CFU/g for fermentation without vinegar and with vinegar, respectively. This flora has no relation to fermentation but represents a hygiene indicator of the product and provides information on its age [26]. Although the mold load of the UFDW sample was below its detection threshold, it was detected in almost all FDW samples and could be associated with contamination during drying or storage periods. The species of molds belong to *Aspergillus*, *Mucor* and *Rhizopus*, as reported by Merabti [26].

The UFDW sample contained a minor yeast load (0.54×10^2 CFU/g). An increase ($p < 0.05$) was noted for FDW samples with vinegar after six and nine months of fermentation (3.06×10^2 and 14×10^2 CFU/g, respectively). At the end of the fermentation period, the yeast load decreased significantly to reach 0.41×10^2 CFU/g. The species of yeasts belong to *Candida*, *Pichia* and *Hansenula* [26].

The fungi count in FDW samples did not exceed 10^3 CFU/g. This value is in line with the standard microbiological limits (10^2–10^3 CFU/g) [27]. During fermentation, the fungi gradually disappeared due to the production of natural antifungal compounds (organic acids, CO_2, hydrogen peroxide, diacetyl, ethanol and bacteriocins) that made the product safer for consumption [28–33].

LAB charge increased significantly and rapidly despite their minor load in the UFDW sample (7 CFU/g and 4 CFU/g for *bacilli LAB* and *cocci LAB*, respectively). It became the major microbial group in FDW samples. The species of LAB belong to *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* (arriving naturally from the soil) [8,34]. LAB are the principal players in the natural transformation of agricultural, primary products into safe, delicious and shelf-stable foods for human consumption [35], whose *Lactobacilli* and *Lactococci* are GRAS (generally regarded as safe). In anaerobic conditions, most LAB species are compelled to live on fermentation metabolism with lower energy yields. However, natural stress, like the acidity generated by LAB, could have also resulted in a dramatic decrease in their growth, as we noted during the fermentation period [8,36–39]. The LAB count in FDW samples is lower than that reported in previous studies [40–42] on other fermented foods because LAB counts were usually monitored for hours, days or a maximum of 1 month. Although our results were after a long fermentation period (1 year), they were satisfactory. Rezac et al. [43] reported that fermented cereals contained LAB and TF, which ranged between 10^5 and 10^9 CFU/g.

3.1.2. Morphological and Physiological Characteristics of the Isolates of LAB

A total of 13 LAB isolates were selected from the two fermentation types of durum wheat (without vinegar and with vinegar) (Figure 2, Table 2).

The retained strains are cocci (7 strains from 13) or short rod shape (6 strains from 13). Strains showed a white (8 strains from 13) or yellow (5 strains from 13) color with a smooth and shiny surface. Cell grouping modes are in short and long-chain (i), in pair, tetrad, short-chain (ii) and pair and chain (iii).

Table 2. Morphological and physiological characteristics of LAB isolates from FW samples after various fermentation time without or with the addition of vinegar.

LAB Isolates Code	Time (Months)	Vinegar Presence	Media	Color and Shape of Colony	Cell Arrangement	Growth at (°C)			Surviving at 63.5 °C	Mannitol Fermentation	Glucose Fermentation
						10	15	45			
1	3	+	M17	W/Co/SS	A	+	+	+	+	+	HoF
2	3	−	MRS	W/SRB/SS	B	+	+	+	+	+	HoF
3	6	+	MRS	Y/SRB/SS	C	+	+	+	+	+	HeF
4	6	−	M17	Y/Co/SS	C	−	+	+	+	+	HeF
5	9	+	MRS	W/SRB/SS	A	+	+	+	+	+	HoF
6	9	+	M17	Y/Co/SS	B	+	+	+	+	+	HoF
7	9	−	MRS	Y/SRB/SS	C	+	+	+	+	+	HoF
8	12	+	M17	W/Co/SS	A	+	+	+	+	+	HeF
9	12	+	MRS	Y/SRB/SS	B	+	+	+	+	+	HoF
10	12	+	MRS	Y/SRB/SS	B	+	+	+	+	+	HeF
11	12	−	M17	W/Co/SS	A	+	+	+	+	+	HoF
12	12	−	M17	W/Co/SS	C	+	+	+	+	+	HoF
13	12	+	M17	Y/Co/SS	C	−	+	+	+	+	HoF

MRS (deMan, Rogosa and Sharpe Medium) and M17: bacterial growth medium for LAB; W: white; Y: yellow; Co: cocci; SRB: short rod bacilli; SS: smooth and shiny; A: short and long chain; B: pairs and chain; C: pair, tetrad and short chain; HoF: homo-fermentative; HeF: hetero-fermentative; +: positive result; −: negative result.

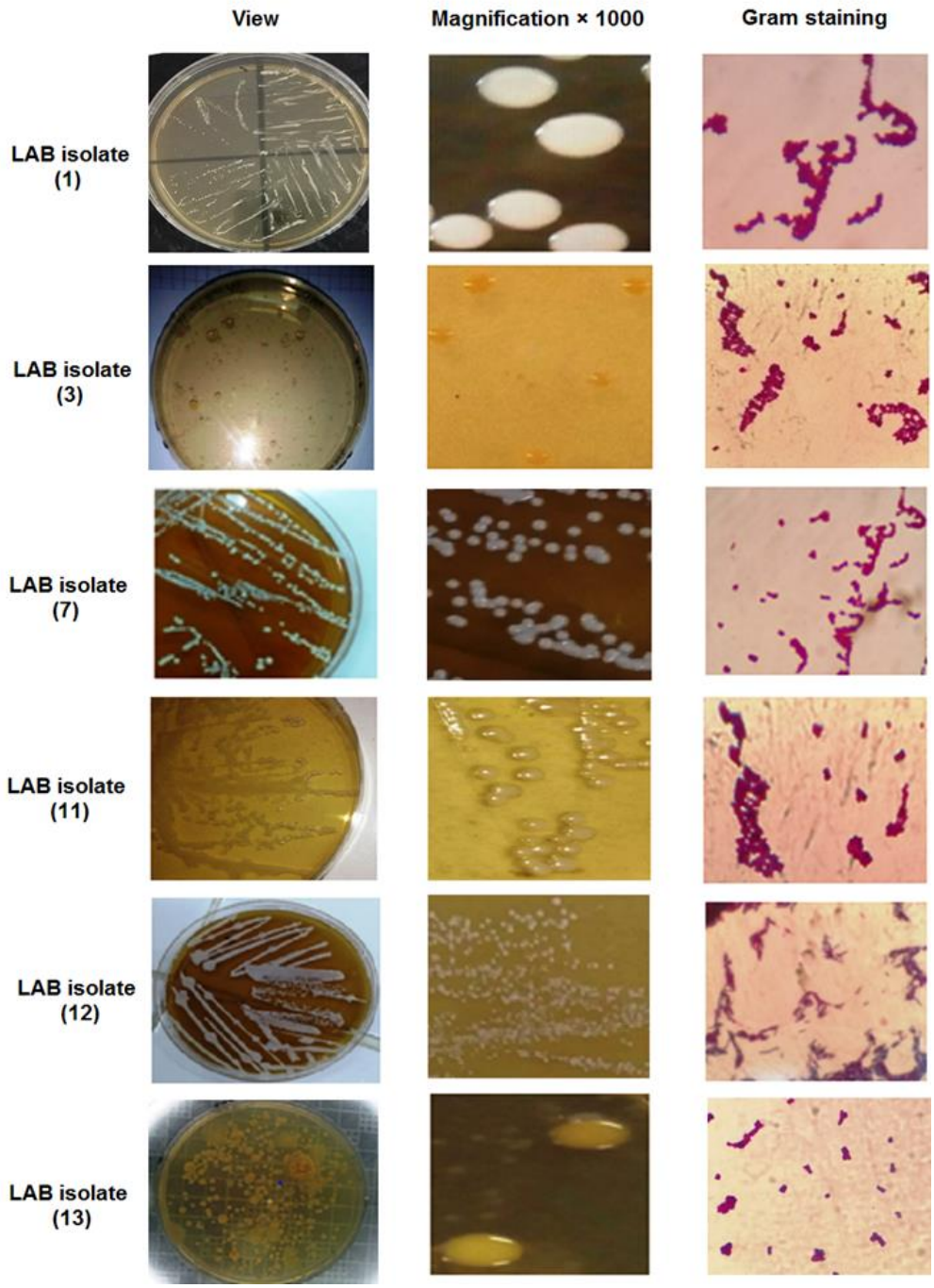

Figure 2. Characteristics of the colonies and microscopic view (×1000) of the LAB isolate after Gram staining.

All isolates were mannitol-positive, and almost all strains (11 strains from 13) grew between 10 and 45 °C and showed resistance at 63.5 °C. Regarding optimum growth temperatures, most lactobacilli multiply in a temperature range between 15 and 42 °C; certain strains of so-called "thermophilic lactobacilli" remain viable at 55 °C [44,45]. Growth ability at a high temperature is a desirable trait that could increase bacteria growth and lactic acid, acetic acid, flavor and aroma production. It also affects the shelf life and taste of the product due to the continuous production of lactic acid, which could reduce the chances of contamination by other microorganisms [46].

The majority of isolates (9 strains from 13) were HoF and only four isolates were HeF. This result can be contributed to the creation of a confined atmosphere by the accumulation of CO_2 that could have a considerable impact on their viability and metabolic activity [28].

It could also affect fungal growth in the presence of other factors such as acidity and osmotic pressure, revealing its harmfulness to molds and yeasts as antifungal activity [29,30,46]. Commonly, HoF and HeF LAB species are associated together, and most groups of HoF include *Lactococcus*, *Streptococcus* and *Pediococcus*, as well as some *Lactobacillus*; HeF bacteria are *Leuconostoc*, *Betabacterium* and *Lactobacillus* [45].

3.2. Biochemical Properties

3.2.1. PH and Total Titratable Acidity

pH values decreased significantly during fermentation regardless of the addition of vinegar. pH passed from 6.48 for UFDW to 5.47 and 5.50 after 12 months, respectively, of fermentation without and with vinegar (Table 3). Our results are higher than those reported in the study of Merabti [26] for FDW samples during 3–24 months in plastic containers (4.02–4.95). When comparing our results with those studying the FDW samples inside the *Matmor*, it seems that pH values are higher than those found by Doukani et al. (2013) [47] for three months (4.45) and by Mokhtari et al. [11] for nine years (5.23). However, our results are lower than those reported by Gourchala et al. [48] for five years (5.63).

Table 3. Evolution of biochemical properties of durum wheat after various fermentation times without or with the addition of vinegar.

Time (Months)	pH	TTA (%)	Proteins (%)	Fat (%)	Starch (%)	Ash (%)
0	6.48 ± 0.24^{d}	0.01 ± 0.01^{a}	13.20 ± 0.10^{b}	4.66 ± 0.06^{e}	70.69 ± 0.20^{d}	0.91 ± 0.01^{bc}
Without vinegar (FWWV)						
3	5.76 ± 0.01^{ab}	0.16 ± 0.15^{ab}	12.90 ± 0.10^{a}	1.00 ± 0.01^{a}	68.90 ± 0.95^{cd}	0.96 ± 0.04^{cd}
6	5.77 ± 0.05^{ab}	0.24 ± 0.15^{ab}	13.82 ± 0.04^{c}	1.94 ± 0.04^{d}	70.48 ± 0.89^{d}	1.45 ± 0.01^{g}
9	5.80 ± 0.10^{bc}	0.24 ± 0.10^{ab}	15.82 ± 0.03^{f}	1.54 ± 0.12^{c}	57.84 ± 1.85^{b}	1.00 ± 0.06^{d}
12	5.47 ± 0.06^{a}	0.44 ± 0.27^{c}	15.50 ± 0.10^{e}	1.50 ± 0.20^{bc}	67.30 ± 0.80^{cd}	0.76 ± 0.02^{a}
With vinegar (FWAV)						
3	5.87 ± 0.01^{c}	0.25 ± 0.15^{ab}	12.80 ± 0.10^{a}	1.00 ± 0.17^{a}	65.00 ± 3.00^{c}	0.93 ± 0.01^{bc}
6	5.89 ± 0.01^{c}	0.16 ± 0.15^{ab}	17.46 ± 0.06^{g}	1.86 ± 0.26^{d}	50.08 ± 0.88^{a}	1.41 ± 0.01^{f}
9	5.80 ± 0.17^{bc}	0.15 ± 0.01^{ab}	15.40 ± 0.10^{e}	1.28 ± 0.02^{b}	68.96 ± 0.94^{cd}	1.15 ± 0.06^{e}
12	5.50 ± 0.10^{a}	0.44 ± 0.28^{c}	14.90 ± 0.10^{d}	1.50 ± 0.10^{bc}	55.10 ± 0.86^{b}	0.90 ± 0.01^{b}

Data are expressed as mean values ± standard deviation; $^{a–g}$ values in a column with the same letter were not significantly different ($p < 0.05$). TTA: total titratable acidity.

For TTA, it increased from 0.01% for UFDW to 0.44% after 12 months of fermentation, regardless of the addition of vinegar. Our results are similar to those reported in previous studies ranging from 0.01% to 0.39% [11,47,48]. The accumulation of organic acids leads to a reduction in pH values, inhibiting pathogenic microorganisms and food conservation. Moreover, LAB strains have effects of this chemical acidification via the fermentation of carbohydrates into organic acids, which contribute to the pleasantly sour taste of many fermented foods [8,40–42,47–49].

3.2.2. Protein Content

Protein content was marked by two steps regardless of the vinegar addition: a decrease phase ($p < 0.05$) from 13.20% to 12.90% and 12.80% for three months of fermentation without vinegar and with vinegar, respectively, a subsequent increase in protein content from the third month to the end of fermentation, reaching 14.90% and 15.50% for FDW samples with and without vinegar, respectively. Similar tendencies have been reported in previous studies, although with different duration and methods of fermentation: from 17.17 to 10.29% [47], from 15.04 to 12.76% [11], from 10.01 to 9.33% [26] as a decrease phase and from 15.33 to 16.08% [48] as an increasing phase.

The decrease in protein levels during the first months of fermentation is likely linked to their hydrolysis by the catabolic activity of yeasts and LAB (proteolytic), not only as a nitrogen source but also for energy metabolism [50]. Low pH promotes the endogenous cereal proteases to solubilize gluten proteins [48].

The increase in protein content is likely due to the accumulation of amino acids that act as aroma precursors and other nitrogen products from metabolism of LAB. The lysis of cells by a high microbial load can cause an increase in nitrogen molecules. Indeed, the Kjeldahl method evaluated total nitrogen after mineralization and made it possible to quantify non-protein nitrogen [40]. The different proteolytic activities improve the digestibility of protein and nutritional value by the availability of lysine, methionine and tryptophan from cereals [11,47,49,51–54].

3.2.3. Fat Content

Fat content decreased significantly from 4.66% for UFDW sample to 1.50% after 12 months of fermentation, regardless of vinegar addition. Doukani et al. [47] reported similar results, while Mokhtari et al. [11] reported an increase in fat content after fermentation. Lipids of the grains prove to be particularly sensitive to microorganism-induced degradation: *Pseudomonas* and *Flavobacterium*, some molds (*Rhizopus*, *Aspergillus*, *Geotrichum*, *Penicillium*) and yeasts (*Pichia*) synthesize extracellular lipases that can metabolize the lipids present in the environment [47,55,56].

Lipolysis is important in the texture and flavor development of fermented products. The degradation is appreciable because it releases fatty acids, aldehydes and esters that contribute to developing the sour taste and, therefore, the acceptability of the product by the consumer [50,57].

3.2.4. Starch Content

A significant decrease in starch content was observed for vinegar fermentation; it passed from 70.69% for UFDW sample to 50.08% after six months of fermentation (Table 3). The TSC reduction was also mentioned by Gourchala et al. [48]. Many studies confirmed the starch degradation by amylolytic LAB (*Lactobacilli and Lactococci*), yeasts, or endogenous enzymatic activities of the wheat grain. It seems to play an influential role in the microbiota of fermented cereals by making the necessary substrates available for the growth of non-amylolytic LAB [12,36,37,54,58–62].

The modification of starch that would be linked to a significant degradation of amylose and amylopectin content, which is the dominant fraction and more assimilable, results in a low glycemic load after *Lemzeïet* consumption, which leads to the production of improved products for the health, hence the interest of diabetics for this traditional product [48].

3.2.5. Ash Content

After 6 and 9 months of fermentation, regardless of vinegar addition, FDW samples had significantly more ash content (1.41–1.45%) than the UFDW sample (0.91%). After the 9th month of fermentation, the ash content decreased significantly, reaching 0.90% and 0.76% for fermentation with and without vinegar, respectively. Doukani et al. [47] and Gourchala et al. [48] reported an increase in ash content for FDW samples, respectively 0.6 to 0.85% and 1.71 to 1.72%. This increase could be related to the liberation of polyvalent cations such as iron, zinc, calcium and magnesium following the reduction of phytates [41,51,63]. In contrast, other studies recorded lower ash content in FDW samples [11]. Our results are in concordance with the Codex Alimentarius Standard [25] for durum wheat ($\leq$2.1%).

4. Conclusions

The present study showed that fermentation induced a significant change in the microbial load, where LAB increased significantly and became the principal fermentation factor.

Compared to the UFDW sample, results highlighted the safety of the FDW *Lemzeïet* by the low fungi load. Thirteen LAB strains were isolated from FDW samples, and they

belonged to 2 *bacilli* and *cocci* LAB. Morphologically, they were cocci or rods, white or yellow, commonly forming in a chain, pair and tetrad. Most isolates were HoF, mannitol-positive, mesophilic and thermophilic and could grow at different temperatures. Fermentation also affected the biochemical characteristics by the accumulation of organic acids, leading to a significant decrease in pH and improving the nutritional properties.

FDW *Lemzeïet* would be a very interesting raw material for new functional fermented wheat-based products such as couscous, pasta, etc. Therefore, LAB that has potentially imparted health benefits could be included in food processing procedures to increase product appeal and safety. Moreover, the identification of selected LAB isolated from fermented wheat *Lemzeïet* (diversity, functional properties, growth, dynamic, and survival) will contribute to a better control of the fermentation process. These LAB could be used as starter cultures for the rationally-controlled food fermentation processes of the industrial production of *Lemzeïet* by selecting safe, accurate and efficient starters using starter cultures composed of several strains, each having a specific functionality when used in association within wheat matrix, and confirming the properties of the final fermented product.

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