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Promotion of Antioxidant and Antibacterial Activities of Fermented Oat Products

Asem Mahmoud Abdelshafy [a*], Eid, A. El-Naggar [a] and Mohamed, N. Kenawi [b]

[a] Department of Food Science and Technology, Faculty of Agriculture, Al-Azhar University - Assiut Branch, Assiut 71524, Egypt.
[b] Department of Food Science, Faculty of Agriculture, Minia University, Minia 61519, Egypt.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Fermentation of oats by probiotics provides higher nutritional value and can be considered as a significant source of bioactive compounds for the human body. Moringa leaves powder (MLP) at the levels of 0.25 and 0.50% were used as an additional prebiotic source to supply oat fermentation by *Lactobacillus plantaram* ATCC 14917 and *Lactobacillus delbrueckii ssp. Bulgaricus* EMCC 11102. The results indicated that oat products supplemented with MLP (0.50%) and fermented by *L. delbrueckii ssp. Bulgaricus* EMCC 11102 showed the highest values of free phenolic content and antioxidant activity (30.87 mg Gallic acid (GAL) /100 g and 7.64%, respectively), followed by oat products supplemented with MLP at level 0.50% and fermented by *L. plantaram* (28.38 mg GAL /100 g and 5.31%, respectively). Also, oat products fermented by probiotics showed different antibacterial activity by well-diffusion agar method against selected pathogenic bacteria. It is thus concluded that supplementation of fermented oat products with MLP will improve the nutritional value and health benefits of fermented oat products.

Keywords: Oat fermentation; antioxidants; phenolic compounds; prebiotics; probiotics.

**Corresponding author: Email: asemmahmoud.2149@azhar.edu.eg*

1. INTRODUCTION

Oats is the sixth most significant cereal in the world and consumed as a piece of daily diet in many countries. Among approximately seventy species of oats, the common oat (*Avena sativa* L.) is one of the most consumed species around the world [1]. It contains good amounts of dietary fibers (55% soluble fiber and 45% insoluble fiber), proteins (high level of lysine), unsaturated fatty acids (linolenic, linoleic and oleic acids), vitamins (A, E, D and B12), minerals (Ca, P and Fe) and bioactive compounds [2-4]. Because of its significant content of bioactive compounds such as phenolics and β-glucan, oats consumption has been related to several health benefits, such as antioxidant, anti-inflammatory, anticancer, reducing blood cholesterol and blood glucose levels [5,6].

Probiotics are live microorganisms which when added in suitable amounts give health benefits on the host such as alleviation of gastrointestinal infections, stimulation of immune system, serum cholesterol lowering, prevention and treatment of allergies, antimutagenic effects and stabilization of the gut mucosal barrier [7,8]. Lactobacilli strains are one of the most generally used probiotics in functional foods [9]. Prebiotics are nondigestible ingredients of food that selectively stimulate the growth of beneficial microorganisms (probiotics) in the gastrointestinal tract of the host [10]. Synbiotics are food combination of probiotics and prebiotics that may be more effective than the individual components in the host colon [11].

Probiotics are mainly used in dairy products and show the biggest share of the probiotic food market [12]. However, some disadvantages of dairy products containing probiotics have been recorded for many consumers worldwide such as lactose intolerance; allergy to β-caseine in cow's milk; high content of cholesterol and saturated fatty acids in dairy products as well as high costs of milk [13]. Probiotic cereal foods may be produced as a good alternative to avoid drawbacks of the dairy products [12]. It has been shown that oats can promote the growth of probiotics due to its content of prebiotics such as β-glucan [14].

Moringa oleifera leaves powder (MLP) produce numerous health benefits and have good amounts of crude protein, crude fiber, extract ether, carbohydrates, energy, minerals, vitamins, β-carotene and polysaccharides [15,16]. Also, MLP displays a prebiotic effect which may be due to its significant content of prebiotic compounds such as oligosaccharides [17,18]. This study is carried out to evaluate the effect of supplementing oat fermentation with MLP as a source of prebiotic so as to produce healthier fermented oat products.

2. MATERIALS AND METHODS

2.1 Materials

Oat seeds were purchased from Agricultural Research Center, Giza, Egypt. Moringa *(Moringa oleifera)* leaves were obtained from a local farm located in Albalyana city, Sohag, Egypt, and sugar was purchased from a local market in Assiut city, Egypt. *Lactobacillus plantarum* ATCC 14917 and *L. delbrueckii ssp. Bulgaricus* EMCC 11102 were purchased from Microbiological Resources center (Cairo MIRCEN) Ain Shams University, Cairo, Egypt. Strains of *Escherichia coli O157:H7, Klebsiella pneumonia, Bacillus sp. Proteus vulgaris, Staphylococcus aureus,* and *Pseudomonas sp.* were obtained from Department of food science and nutrition, Faculty of agriculture, Sohag University, Sohag, Egypt.

2.2 Methods

2.2.1 Preparation of oat fermentation

2.2.1.1 Preparation of raw materials and oat blends

Grain oat seeds were washed and dried at 60°C for 8 h and milled to get whole oat flour (WOF). The Moringa leaves were dried and milled to produce MLP, which were stored in a cool dry place until commencement of the experimental work. Blends of WOF, MLP and sugar were prepared to produce the final formulas as shown in Table 1. Subsequently, the blends were gelatinized in a water bath, and then sterilized in an autoclave at 121°C for 15 min.

2.2.1.2 Fermentation of WOF and MLP blends

The Lactobacillus bacteria strains were activated by inoculating broth culture into 9 mL sterile deMan, Rogosa and Sharpe (MRS) broth and incubation at 37°C for 24 h. The cells were separated from the broth by centrifuging, and re-suspended in sterile saline solution (9 ml) with final concentration 10^8 CFU/mL [19].

Table 1. Formulas of fermented oat products

	Oat %	Sugar %	MLP %	Water %
Control	10	2	---	88
FOP	10	2	---	88
FOP1	10	2	0.25	87.75
FOP2	10	2	0.50	87.5
FOD	10	2	---	88
FOD1	10	2	0.25	87.75
FOD2	10	2	0.50	87.5

Control, Non-fermented oat; FOP, Fermented oat by L. plantaram ATCC 14917; FOP1, Fermented oat with 0.25% MLP by L. plantaram ATCC 14917; FOP2, Fermented oat with 0.5% MLP by L. plantaram ATCC 14917; FOD, Fermented oat by L. delbrueckii ssp. Bulgaricus EMCC 11102; FOD1, Fermented oat with 0.25% MLP by L. delbrueckii ssp. Bulgaricus EMCC 11102; FOD2, Fermented oat with 0.5% MLP by L. delbrueckii ssp. Bulgaricus EMCC 11102

The resulting probiotic suspensions were added to the prepared WOF and MLP blends at a concentration of 1%. All treatments were incubated at 37°C for 24 h for *L. plantarum* fermentation and only 8 h for *L. delbrueckii ssp. Bulgaricus* fermentation because it decreases the pH faster than *L. plantarum*. The fermented oat products were stored after fermentation at 4 ±1°C for 21 days so as to determine the chemical properties on day 0, 7, 14 and 21.

2.2.2 Determination of free phenolics and antioxidant activity

2.2.2.1 Extract preparation

Free phenolic compounds were extracted from samples using the method of Acosta-Estrada et al., [20]. Briefly, 1 g of fermented oat samples were mixed with 10 mL of chilled ethanol/water (80:20 v/v) and shaken at 250 rpm for 10 min at 25°C, then centrifuged at 3000 g for 10 min. The supernatant was collected and the extraction repeated. Supernatants were pooled and evaporated at 50°C and 20 mbar. The resulting extracts were stored at -20°C until required for use.

2.2.2.2 Determination of free phenolic content

Free phenolic contents in the extracts were then determined using the Folin-Ciocalteu method as described in Jaramillo-Flores et al. [21]. Aliquot of 100 µL of ethanolic extract was mixed with 900 µL of Folin-Ciocalteu reagent (diluted 1:10 with distilled water) and was allowed to stand for 5 minute at room temperature; 0.75 mL of sodium bicarbonate solution (7%) was added to the mixture and vortexed for 30 second, and allowed to stand at room temperature (25–30°C) for 90 min. The absorbance was measured at 725 nm using a spectrophotometer (6505 UV/Vis, Jenway LTD., Felsted, Dunmow, UK). A calibration curve of gallic acid (ranging from 0 to 1 mg/mL) was prepared and tested under similar conditions. All values were expressed as mean (mg of Gallic acid equivalents/100 g wet weight fermented oats).

2.2.2.3 Determination of free radical scavenging activity

Free radical scavenging activity was determined using the 1,1-diphenyl-2 picrylhydrazyl (DPPH) assay according to Elfahri et al. [22] with slight modification. Briefly, 900 µL of the DPPH reagent (0.1 mM DPPH dissolved in 95% methanol) was added to 100 µL of fermented product extracts in glass test tubes. The samples were shaken vigorously and incubated for 30 min in the dark at room temperature. The absorbance of the incubated samples was measured at 517 nm. The percentage of radical scavenging activity was expressed as scavenging (%) using the equation below:

$$\% \text{ Scavenging} = \frac{Abs\ blank - Abs\ sample}{Abs\ blank} \times 100$$

2.2.3 Determination of the antibacterial activity of fermented oat products

The antibacterial activity of fermented oat products were estimated using the well-diffusion method as described by Zhong et al. [23] with slight modification. Briefly, 200 µL of activated culture (containing 10^8- 10^9 CFU/ mL) of selected pathogens were spread inoculated on nutrient agar plates separately. Wells (6 mm diameter) were bored into the agar using sterile cork-borer. Next, 100 µL of the different fermented oat products were carefully added into the wells. The

plates were then incubated at 35°C for 24 h. Assessment of the antibacterial activity was based on measurement of the diameter of inhibition zone formed around the wells. Determination of antibacterial activity was performed in triplicates.

2.2.4 Sensory evaluation

Based on the ability of describing and sensitivity to sensory attributes, 10 members from Department of food science and technology, Faculty of Agriculture, Al-Azhar University were screened and asked to evaluate the sensory properties of fermented oat products, and gave scores for color, texture, taste, odor and overall acceptability, using a hedonic number scale from 1-10 points (from dislike to like) according to Sudha et al. [24].

2.2.5 Statistical Analysis

Basic statistics and analysis of variance (ANOVA) were performed on data obtained in the research using IBM SPSS software version 22**.** Duncan test was used to determine the differences among calculated means at the significance level of 0.05%.

3. RESULTS AND DISCUSSION

3.1 Changes in Free Phenolic Content of Fermented Oat Products

Generally, fermentation of oats by probiotics increased the free phenolic content of fermented oat products in all treatments compared to non-fermented oat product (Table 2). Also, the results showed that the adding of MLP at the two levels (0.25 and 0.50%) significantly enhanced the free phenolic content of fermented oat products more than fermented oat products without MLP, and the samples containing the higher level of MLP (0.50%) displayed the highest values of free phenolic content (30.87 and 28.38 for FOD2 and FOP2, respectively). During the first and second weeks, the free phenolic content of fermented oat continuously increased through the storage period may be because of the activity of probiotics. Similar results were reported by Chen et al., and Bei et al. [25,26], they stated that fermentation of oats by probiotics promoted the phenolic content of oats. Călinoiu et al., and Hole et al. [27,28], reported that the increase of antioxidants such as phenolic compounds after fermentation may be due to its increased release or synthesis by some possible enzymes, such as β-glucosidases, esterase, cellulose, β-glucosidases and glycoside hydrolase that is produced by some probiotic strains. The production of these enzymes during fermentation could release esterified and insoluble-bound phenolcs in a time-dependent manner.

3.2 Changes in Free Radical Scavenging Activity of Fermented Oat Products

Changes in free radicals in the fermented oat during storage are presented in Table 3. Significant differences were recorded in the free antioxidant activity of fermented oat supplemented and non-supplemented with MLP. Oat products containing MLP at the level of 0.50% and fermented by *L. delbrueckii ssp. Bulgaricus* showed the highest antioxidant activity (7.64 %) followed by oat product containing 0.50 % MLP fermented by L. plantaram, (5.31 %). The increase in free phenolic content appears to have resulted in improving the antioxidant activity of the fermented products. Correlation between phenolic content and antioxidant activity has been reported in previous studies [26,27].

Table 2. Changes in free phenolic content (mg GAL /100 g ww) of fermented oat during storage

Treatments	Storage periods (days)			
	0	**7**	**14**	**21**
Control	12.42^{Ea}	13.15^{Da}	14.20^{Ea}	14.03^{Ea}
FOP	14.93^{Db}	16.64^{CDab}	18.97^{Da}	18.15^{Cab}
FOP1	21.03^{Cb}	25.82^{Ba}	29.53^{Ca}	28.08^{Ba}
FOP2	28.38^{ABb}	32.08^{Aab}	33.45^{Ba}	33.82^{Aa}
FOD	15.51^{Da}	17.48^{Ca}	17.71^{Da}	16.80^{CEa}
FOD1	26.11^{Bb}	28.42^{Bab}	31.45^{BCa}	29.00^{Bab}
FOD2	30.87^{Ab}	34.90^{Aa}	37.33^{Aa}	35.42^{Aa}

Means within a column with different superscript capital letters are significantly different (P > 0.05); means within a row with different superscript small letters are significantly different (P > 0.05)

Table 3. Changes in free radical scavenging activity of fermented oat during storage

Treatments	Storage periods (days)			
	0	**7**	**14**	**21**
Control	2.24^{Ea}	2.49^{Da}	1.92^{Da}	1.68^{Ea}
FOP	2.68^{DEb}	4.44^{Ca}	3.20^{Cb}	2.71^{Db}
FOP1	3.41^{CDc}	6.73^{Ba}	4.95^{Bb}	3.81^{Cc}
FOP2	5.31^{Bd}	10.70^{Aa}	8.31^{Ab}	7.01^{Ac}
FOD	3.12^{DEb}	5.02^{Ca}	4.21^{Ba}	4.25^{Ca}
FOD1	4.24^{Cb}	6.93^{Ba}	4.52^{Bb}	3.90^{Cb}
FOD2	7.64^{Ab}	10.77^{Aa}	7.63^{Ab}	6.10^{Bc}

Table 4. Diameters of inhibition zones (mm) of fermented oat products against some pathogenic bacteria

Treatments	***Escherichia coli O157 :H7***	***Klebsiella pneumoniae***	***Bacillus sp.***	***Proteus vulgaris***	***Staphylococcus aureus***	***Pseudomonas sp.***
Control	18^{D}	0	0	0	0	14^{E}
FOP	21^{B}	0	20^{A}	0	0	25^{B}
FOP1	22^{A}	0	17^{C}	0	0	26^{A}
FOP2	21^{B}	0	18^{B}	0	0	24^{C}
FOD	20^{C}	0	10^{E}	0	0	15^{D}
FOD1	20^{C}	0	13^{D}	0	0	15^{D}
FOD2	22^{A}	0	13^{D}	0	0	13^{F}

3.3 Antibacterial Activity of Fermented Oat Products

Fermented oat products showed varied antibacterial activity against selected pathogenic bacteria (Table 4). It was observed that the fermentation by probiotics significantly enhanced the antibacterial activity of the oat products against the investigated pathogenic bacteria, and oat products fermented by *L. plantaram* exhibited more antibacterial activity than oat products fermented by *L. delbrueckii ssp. Bulgaricus*. All fermented oat products presented higher antibacterial activity (20-22 mm) against *Escherichia coli O157:H7* than the control sample (18 mm), and the most effective treatment was FOD2 (23 mm) followed by FOP1 (22 mm). No antibacterial activity was observed for non-fermented oat against *Bacillus sp*, while all fermented oat products displayed various antibacterial activities ranging between 13 to 20 mm against the *Bacillus sp*. *Pseudomonas sp* was the most sensitive bacteria for oat products fermented by *L. plantaram* with higher inhibition zones 26, 25 and 24 mm for FOP1, FOP and FOP2, respectively. However, *Klebsiella pneumonia, Proteus vulgaris and Staphylococcus aureus* were resistant to non-fermented and all fermented oat products.

It has been reported that the antimicrobial activity of fermented oat products by lactic acid bacteria could be due to decreased pH and higher content of organic acids specially lactic acid which might aid the antimicrobial activity of phenolic compounds present in fermented products [23,29]. In many cases, MLP slightly improved the antibacterial effect of fermented oat products with significant differences ($p > 0.05$) between its two concentrations (Table 4).

Table 5. Organoleptic evaluation of fermented oat products

Treatments	Color	Taste	Odor	Texture	Overall acceptability
Control	9.40^{A}	6.03^{A}	5.56^{A}	8.52^{A}	5.58^{B}
FOP	9.23^{A}	6.24^{A}	6.02^{A}	9.13^{A}	7.30^{A}
FOPM1	7.58^{B}	6.10^{A}	6.15^{A}	9.17^{A}	6.61^{AB}
FOPM2	6.90^{B}	6.07^{A}	6.19^{A}	9.23^{A}	6.25^{AB}
FOD	9.11^{A}	6.11^{A}	6.00^{A}	9.11^{A}	7.12^{A}
FODM1	8.01^{AB}	6.15^{A}	5.90^{A}	9.30^{A}	6.22^{AB}
FODM2	6.68^{B}	5.91^{A}	5.88^{A}	9.18^{A}	6.15^{AB}

3.4 Organoleptic Evaluation of Fermented Oat Products

Data presented in Table 5 shows the results of the organoleptic evaluation carried out to determine the acceptability of oat fermented products compared with non-fermented oat products (control sample).

From the results in the table, it could be stated that oat fermented products mixed with MLP, including FOP1, FOP2, FOD1 and FOD2 showed low color scores compared with control sample, and FOD2 as well as FOP2 which containing high MLP level (0.50%), had the lowest scores of color (6.68 and 6.90 respectively). No significant differences were observed in the taste, odor and texture scores between the fermented products and control sample. Also, oat products fermented by *L. plantaram* and *L. delbrueckii ssp. Bulgaricus* without MLP displayed the highest overall acceptability scores (7.30 and 7.12, respectively).

4. CONCLUSION

Fermented oat foods by probiotics were produced as a significant source of bioactive compounds and probiotics for the human body. Supplementation with MLP resulted in improving the nutritional value and healthy benefits of fermented oat products. More studies are needed in the future for testing more kinds of probiotics with different concentration of MLP in oat fermentation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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