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Enhancing the Antibacterial Activity of Quinoa Fermented by Probiotics: *In vitro* and *In vivo* Study

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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 quinoa by probiotics provides higher nutritional value and can be considered as a significant source of bioactive compounds and alive probiotics 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 quinoa fermentation by *Lactobacillus plantaram* ATCC 14917 and *Lactobacillus delbrueckii subsp. Bulgaricus* EMCC 11102 and produce healthier quinoa products. The results indicated that quinoa products fermented by probiotics showed different antibacterial activity against selected pathogenic bacteria, and supplementation of fermented quinoa products with MLP at bath levels improved its antibacterial activity in most cases. Also, the coliform group counts of rat feces were reduced after feeding for 30 d with fermented quinoa products supplemented with MLP (0.50%). Furthermore, Fermented quinoa products with MLP exhibited acceptable sensory properties compared with fermented quinoa products without MLP. Supplementation of fermented quinoa products with MLP resulted in improving its antibacterial activity with acceptable sensory properties.

Keywords: Quinoa fermentation; probiotics; prebiotics; antibacterial activity; experimental rats.

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1. INTRODUCTION

Quinoa (*Chenopodium quinoa*) is a pseudo-cereal plant domesticated in the Indian region 5000 years ago [1]. It is known for its high nutritional value and high contents of essential amino acids, minerals and dietary fibre. Due to its significant content of phenolic compounds, quinoa showed antioxidant activity which associated with many health benefits [2, 3].

Probiotic bacteria are mainly used in dairy products and show the biggest share of the probiotic food market [4]. 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 [5]. Probiotic cereal foods may be produced as a good alternative to avoid drawbacks of fermented dairy products [4]. Several studies have evaluated the fermentation of quinoa by probiotics for production of fermented quinoa products with higher healthy benefits [6-8].

Moringa oleifera leaves powder (MLP) produce numerous healthy benefits and have good amounts of crude protein, crude fiber, extract ether, carbohydrates, energy, minerals, vitamins, β-carotene and polysaccharides [9, 10]. Also, MLP displayed a prebiotic effect may be due to its significant content of prebiotic compounds such as oligosaccharides [11, 12].

This study aimed to evaluate the effect of supplementation with MLP as a source of prebiotics to enhance the antibacterial activity of fermented quinoa products by *in vitro* and *in vivo study*.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Raw materials and reagents

Quinoa 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. Microbiological media used in this study were obtained from El-Gomhouria Trading Chemicals and Drugs Company, Assiut city, Egypt.

2.1.2 Probiotics and pathogenic bacteria strains

Lactobacillus plantarum ATCC 14917 and *L. delbrueckii subsp. Bulgaricus* EMCC 11102 were purchased from Microbiological Resources center (Cairo MIRCEN) Ain Shams University, Cairo, Egypt. The strains of *Escherichia coli* O157:H7, *Klebsiella pneumonia*, *Bacillus* sp. *Proteus vulgaris*, *Staphylococcus aureus*, *Psudomonas* 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 quinoa fermentation

2.2.1.1 Preparation of raw materials and quinoa blends

Grain quinoa seeds were washed and soaking for 24 h in water which was discarded and changed every 3 h. The soaked quinoa seeds was dried at 60 °C for 8 h and milled to get the whole quinoa flour (WQF). The moringa leaves were dried and milled to produce MLP, which stored in a cool dry place until the experimental work. The blends of WQF, MLP and sugar were prepared and tap water was added to produce the final formulas as follow in Table 1.

In the next step, the mixtures were gelatinized using water bath and autoclaved at 121 °C for 15 min.

2.2.1.2 Inoculant strains and quinoa fermentation conditions

The Lactobacillus bacteria strains were activated by inoculating in sterile MRS broth (9 ml) 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 [13].

The 24 h activated culture of probiotic bacteria (10^8 CFU/mL) were added to the previous quinoa mixtures by a concentration of 1%. A control sample without inoculation of bacteria was prepared. All treatments were incubated at 37 °C for 24 h for *L. plantarum* fermentation and 8 h for *L. delbrueckii subsp. Bulgaricus* fermentation. The fermented quinoa products were stored after fermentation at 4 ±1 °C for 21 days. Chemical and microbiological properties of fermented quinoa products were estimated at 0, 7, 14 and 21 days.

2.2.2 Determination of the antibacterial activity of fermented quinoa products

The pathogenic bacteria were selected as representative to Gram-positive and Gram-negative bacteria. The antibacterial activity of fermented quinoa products were estimated using the well-diffusion method as described by Zhong et al., [14] with slight modification. Briefly, 200 µL of activated culture (containing 10^8- 10^9 CFU/ mL) of selected pathogens added into petri plates containing 20 ml of nutrient agar medium. Wells (6 mm diameter) were cut into the agar by the corkborer tool that had been sterilized previously. Next, 100 µL of different fermented quinoa products were carefully added into the wells. The plates containing various samples were incubated at 35 °C for 24 h. The assessment of the antibacterial activity was based on measurement of the diameter of inhibition zone formed around the wells. All experiments were performed in triplicates.

2.2.3 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 quinoa 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., [15].

2.2.4 Biological experiment

2.2.4.1 Adaptation and distributing of experimental animals

The animals (Forty adult male white Wistar albino rats) were housed as groups in wire cages under the normal laboratory conditions and fed on basal diets for 10 days as adaptation period. The rats were distributed to 4 groups containing control group and fed during experimental period (30 days).

2.2.4.2 Experimental design

The rats were randomly divided into 4 groups (1, 2, 3 and 4), each group was contained 10 rats as described in Table 2.

2.2.4.3 Microbiological analysis of rat feces

2.2.4.3.1 Determination of total bacterial count of rat feces

Total bacterial count of rat feces was determined using the plate counts technique on a nutrient agar medium according to procedures by [16] Difco. The plates were incubated at 37°C for 48 h.

Table 1. Formulas of fermented quinoa products

	Quinoa %	Sugar %	MLP %	Water %
Control	10	2	---	88
FQP	10	2	---	88
FQP1	10	2	0.25	87.75
FQP2	10	2	0.50	87.5
FQD	10	2	---	88
FQD1	10	2	0.25	87.75
FQD2	10	2	0.50	87.5

Control, Non-fermented quinoa; FQP, Fermented quinoa by L. plantaram ATCC 14917; FQP1, Fermented quinoa with 0.25% MLP by L. plantaram ATCC 14917; FQP2, Fermented quinoa with 0.5% MLP by L. plantaram ATCC 14917; FQD, Fermented quinoa by L. delbrueckii subsp. Bulgaricus EMCC 11102; FQD1, Fermented quinoa with 0.25% MLP by L. delbrueckii subsp. Bulgaricus EMCC 11102; FQD2, Fermented quinoa with 0.5% MLP by L. delbrueckii subsp. Bulgaricus EMCC 11102

Table 2. Experimental groups and feeding diets

Groups	The experimental diets
Group 1 (control)	Fed on basal diet
Group 2	Fed on basal diet containing of 30% of non-fermented quinoa
Group 3	Fed on basal diet containing of 30% of FQP2
Group 4	Fed on basal diet containing of 30% of FQD2

FQP2, Fermented quinoa with 0.5% MLP by L. plantaram ATCC 14917; FQD2, Fermented quinoa with 0.5% MLP by L. delbrueckii subsp. Bulgaricus EMCC 11102

2.2.4.3.2 Determination of Lactobacillus counts of rat feces

Lactobacillus bacteria counts of fermented quinoa and rat feces were determined using serial dilution technique and de Man-Rogosa-Sharpe (MRS) agar medium and the plates were anaerobically incubated at 37 °C for 48 h [17].

2.2.4.3.3 Determination of coliform group of rat feces

Violet red bile agar (VRBA) medium was used for determination of coliform group in rat feces and the plates were incubated at 37 °C for 24-48 h [18].

2.3 Statistical Analysis

Basic statistics and analysis of variance (ANOVA) were performed to data analysis and test the significance within replications and between treatments by using IBM SPSS software version 22**.** Duncan test was used to determine the differences among the means at the significance level of 0.05%.

3. RESULTS

3.1 Antibacterial Activity of Fermented Quinoa Products

Fermented quinoa products showed varied antibacterial activity against selected pathogenic bacteria (Table 3). It has been observed that the fermentation by probiotic bacteria significantly enhanced the antibacterial activity of quinoa products against some pathogenic bacteria. All fermented quinoa products presented higher antibacterial activity (23-29 mm) against *Escherichia coli O157:H7* than control sample (22 mm), and the most effective treatment was FQP1 (29 mm) followed by FQD1 (27 mm). Only fermented quinoa products containing MLP (0.25 or 0.50 %) showed antibacterial activity against *Klebsiella pneumoni* and *Proteus vulgaris*. The inhibition zones of fermented quinoa products against *Bacillus sp*. ranged between (17-18 mm) with insignificant differences compared with non-fermented quinoa (16 mm). There is no antibacterial activity was observed for FQP against *Staphylococcus aureus*, while all fermented quinoa products displayed various antibacterial activities ranged between 8 to 10 mm inhibition zones against the same bacteria.

FQP, FQP1, FQP2, FQD, FQD1 and FQD2 presented various antibacterial activities against *Psudomonas sp* and showed 21, 21, 20, 17, 16 and 20 mm inhibition zones compared with 15 mm for control sample (non-fermented quinoa).

3.2 Organoleptic Evaluation of Fermented Quinoa Products

Data presented in Table (4) showed the organoleptic evaluation which carried out to evaluate color, taste, odor, texture and overall acceptability of quinoa fermented products compared with non-fermented quinoa products (control sample). From the results in this table, it could be stated that quinoa fermented products mixed with MLP, including FQP1, FQP2, FQD1 and FQD2 showed low color scores compared with control sample, and FQD2 as well as FQP2 which containing high MLP level (0.50%), had the lowest scores of color (6.88 and 6.75 respectively). No significant differences were observed in the taste, odor, texture and overall acceptability scores between the fermented products and control sample.

3.3 Microbiological Counts of Rat Feces (Log CFU/g) before and After Feeding with Fermented Quinoa Products

Total bacterial count, total Lactobacillus bacteria count and coliform group count of tested groups before and after feeding were illustrated in Figs. 1, 2 and 3. TBC recorded 8.47, 8.6, 9.10 and 8.2 Log CFU/g rat feces before feeding, while it recorded 9.19, 9.16, 10.22 and 10.05 Log CFU/g rat feces after feeding with fermented quinoa products for groups 1, 2, 3 and 4, respectively (Fig. 1). Groups 3 and 4 showed higher increases in the total Lactobacillus bacteria count (1.21 and 1.31 Log CFU/g rat feces, respectively) than groups 1 and 2 (0.38 and 0.43 Log CFU/g rat feces, respectively) (Fig. 2). On the other hand, coliform group count was increased in groups 1 (from 4.01 to 4.63 Log CFU/g rat feces) and 2 (from 3.90 to 4.32 Log CFU/g rat feces), while it decreased in the groups 3 (from 4.19 to 3.35 Log CFU/g rat feces) and 4 (from 4.21 to 3.20 Log CFU/g rat feces) (Fig. 3).

4. DISCUSSION

MLP was used at the levels of 0.25 and 0.50 % to supply the fermented quinoa by selected probiotics and study its effect on the antibacterial properties of fermented products by *in vitro* and *in vivo* investigation.

Table 3. Diameters of inhibition zones (mm) of fermented quinoa products against some pathogenic bacteria

Treatments	*Escherichia coli O157 :H7*	*Klebsiella pneumoniae*	*Bacillus sp.*	*Proteus vulgaris*	*Staphylococcus aureus*	*Psudomonas sp.*
Control	22^{C}	0	16^{A}	0	0	15^{C}
FQP	23^{C}	0	18^{A}	0	0	21^{A}
FQP1	29^{A}	9^{A}	17^{A}	10^{A}	9^{A}	21^{A}
FQP2	23^{C}	10^{A}	17^{A}	11^{A}	10^{A}	20^{AB}
FQD	23^{C}	0	17^{A}	0	8^{A}	17^{BC}
FQD1	27^{AB}	9^{A}	18^{A}	10^{A}	10^{A}	16^{C}
FQD2	25^{BC}	10^{A}	17^{A}	9^{A}	8^{A}	20^{AB}

Control, Non-fermented quinoa; FQP, Fermented quinoa by L. plantaram ATCC 14917; FQP1, Fermented quinoa with 0.25% MLP by L. plantaram ATCC 14917; FQP2, Fermented quinoa with 0.5% MLP by L. plantaram ATCC 14917; FQD, Fermented quinoa by L. delbrueckii subsp. Bulgaricus EMCC 11102; FQD1, Fermented quinoa with 0.25% MLP by L. delbrueckii subsp. Bulgaricus EMCC 11102; FQD2, Fermented quinoa with 0.5% MLP by L. delbrueckii subsp. Bulgaricus EMCC 11102; 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)

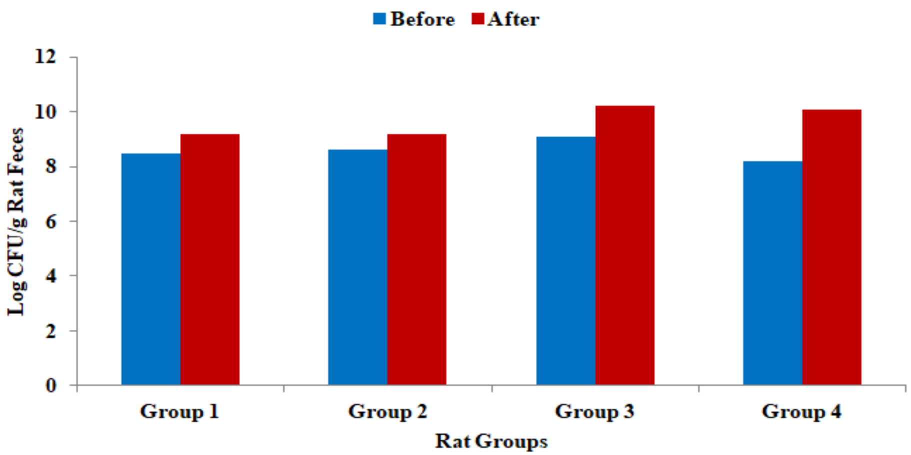

Fig. 1. Total bacterial count of rat feces before and after feeding with fermented quinoa products for 30 d

Group (1), Control group; Group (2), Fed with non-fermented quinoa; Group (3), Fed with FQP2; Group (4), Fed with FQD2

Table 4. Organoleptic evaluation of fermented quinoa products

Treatments	Color	Taste	Odor	Texture	Overall acceptability
Control	9.21^{A}	5.66^{A}	5.71^{A}	8.82^{A}	5.31^{A}
FQP	9.03^{A}	6.00^{A}	6.13^{A}	9.30^{A}	6.80^{A}
FQP1	7.38^{AB}	6.03^{A}	6.01^{A}	9.26^{A}	6.51^{A}
FQP2	6.75^{B}	6.09^{A}	5.97^{A}	9.37^{A}	6.66^{A}
FQD	9.02^{A}	5.90^{A}	6.20^{A}	9.11^{A}	6.93^{A}
FQD1	7.66^{AB}	5.88^{A}	6.00^{A}	9.09^{A}	6.02^{A}
FQD2	6.88^{B}	5.95^{A}	5.90^{A}	9.20^{A}	5.88^{A}

Control, Non-fermented quinoa; FQP, Fermented quinoa by L. plantaram ATCC 14917; FQP1, Fermented quinoa with 0.25% MLP by L. plantaram ATCC 14917; FQP2, Fermented quinoa with 0.5% MLP by L. plantaram ATCC 14917; FQD, Fermented quinoa by L. delbrueckii subsp. Bulgaricus EMCC 11102; FQD1, Fermented quinoa with 0.25% MLP by L. delbrueckii subsp. Bulgaricus EMCC 11102; FQD2, Fermented quinoa with 0.5% MLP by L. delbrueckii subsp. Bulgaricus EMCC 11102; 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)

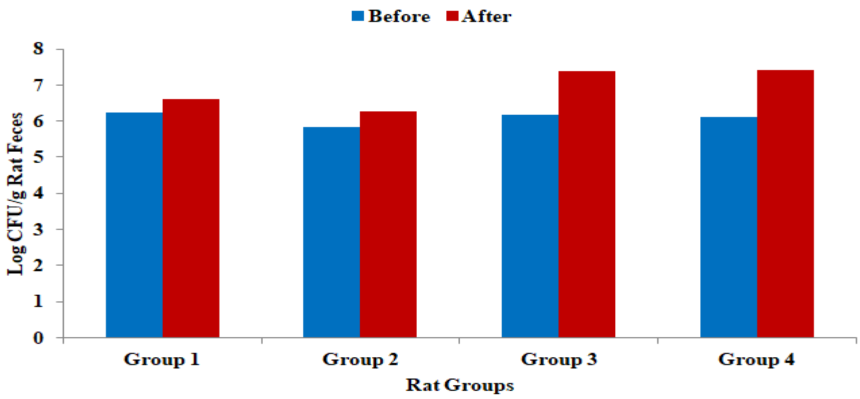

Fig. 2. Total Lactobacilli count of rat feces before and after feeding with fermented quinoa products for 30 d

Group (1), Control group; Group (2), Fed with non-fermented quinoa; Group (3), Fed with FQP2; Group (4), Fed with FQD2

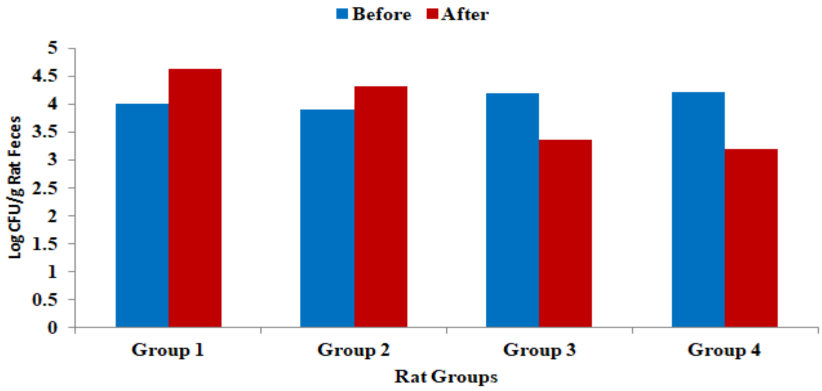

Fig. 3. Coliform group count of rat feces before and after feeding with fermented quinoa products for 30 d

Group (1), Control group; Group (2), Fed with non-fermented quinoa; Group (3), Fed with FQP2; Group (4), Fed with FQD2

In most cases, MLP improved the antibacterial effect of fermented quinoa products as shown in Table 3. It has been reported that the antimicrobial activity of fermented quinoa products by lactic acid bacteria due to the decreased pH and higher content of organic acids specially lactic acid which might aid the antimicrobial activity of phenolic compounds present in fermented products [14, 19]. Except color property, data indicated to there is no significant differences were recorded in other sensory properties, including taste, odor, texture and overall acceptability scores between the fermented products with MLP and fermented products without MLP. Also, the adding of MLP at the level of 0.25% to fermented quinoa showed no significant differences on the color compared to fermented quinoa without MLP or control sample.

After rat feeding with fermented quinoa product for 30 days, the increase of total bacterial count in the rate feces of groups 1 and 2 was lower than the increase of total bacterial count in the rate feces of group 3 and 4. Similar results were observed in the total Lactobacilli count which displayed higher increase in rat feces of group 3 and 4 than rat feces of group 1 and 2 may be due to the presence of probiotics in the rat diets.

Also, the decrease in the coliform group counts in the groups 3 and 4 may be due to the presence of probiotic bacteria in the diets which showed antimicrobial activity against pathogenic bacteria by many previous studies [14, 20, 21]. The antimicrobial activity of lactic acid bacteria is due to production of many antimicrobials, including organic acids (Mostly lactate, acetate, phenyllactate, formate and propionate), CO_2, ethyl alcohol, H_2O_2, diacetyl, fungicins, bacteriocins and fatty acids, which extent food shelf-life [22, 23]. These results were in the same line with those obtained from [24], they reported that the adding of MLP to fermented cereals may enhance their health benefits.

5. CONCLUSION

The results showed that quinoa products fermented by selected probiotics displayed different antibacterial activity against selected pathogenic bacteria, and supplementation of fermented quinoa products with MLP at bath levels improved its antibacterial activity in most cases. Coliform group counts of rat feces were decreased after feeding with fermented quinoa products supplemented with MLP (0.50%). Supplementation of fermented quinoa products with MLP resulted in improving its antibacterial activity with acceptable sensory properties. More studies are needed in the future to test more kinds of probiotics with different concentration of MLP in quinoa fermentation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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