Emirates Journal of Food and Agriculture. 2024. 36(11): 1-8
doi: 10.9755/ejfa.2024.v36.i11.3209
http://www.ejfa.me/

RESEARCH ARTICLE

Effects of *Lactobacillus fermentum* fermentation of Quinoa milk on its phytochemistry and health benefits.

Bababode Adesegun Kehinde[1]*, Poorva Sharma[2], Shafiya Rafiq[2], Ishrat Majid[2], Adetokunbo Adekoya[3]

[1]Department of Biosystems and Agricultural Engineering, University of Kentucky, Lexington, 40546, Kentucky, USA, [2]Department of Food Technology and Nutrition, Lovely Professional University, Phagwara, 144001, Punjab, India, [3]Department of Food Technology, Islamic University of Science and Technology, 192122, Awantipora, India, [4]New Hampshire Department of Health and Human Services, Concord, 03301, New Hampshire, USA

ABSTRACT

Quinoa, a pseudocereal containing essential amino acids has been appraised as a functional food, however, with relatively scarce scientific studies focused on the isolation of bioactives from it. In this study, *Lactobacillus fermentum* was optimally selected for the fermentation of quinoa milk to induce proteolysis of its concomitant proteins for the synthesis of peptides and improve its overall phytochemical profile. The protein hydrolysates were further fractionated by ultrafiltration and their antidiabetic potentials examined and the antioxidative potential of the quinoa milk was also monitored in the course of the fermentation. Results obtained showed that after 9 days of controlled fermentation at 37 °C, there was observable antidiabetic potentials of obtained quinoa protein hydrolysates, with the 5 kDa fraction showing an α-amylase inhibition of 87.11 %, and the <5 kDa fraction with 73.97 %. There was remarkable increase in the antioxidant profile as tested by the DPPH scavenging potential from 22.29 % to 88.2 %; a trend associated with the increase in polyphenolic contents as confirmed by FTIR analysis. GC-MS showed the fermentative removal of antinutritional factors binding to different bioactive components. This study has shown the possibility of a cost-effective methodology for the isolation of bioactive peptides from a pseudocereal and the multiple enhancement of the biofunctional profile of the grain.

Keywords: Quinoa; *Lactobacillus fermentum*; Proteolysis; Hydrolysates; Antidiabetic

INTRODUCTION

The demand for vegetable "milk" preparations as dairy substitutes has gained considerable attention and increase in demand in recent years. Being plant extracts, they do not meet with the technical definition of milks as lacteal secretions from animals, (Marco et al., 2017). When a food material is the substrate, the minor and major constituents are converted, particularly by the enzymes secreted by the probiotic. A review of historical timeline places food fermentation and drying as the two most-ancient techniques of food processing, primarily adopted for preservation purposes, but as scientific civilization ensued, other benefits of fermentation such as improvement in organoleptic properties, enhancement of nutritional constituents, and bio-removal of toxicants and antinutritional factors were discovered (Lavefve et al., 2018, Abdalla et al., 2022, Ayyash et al., 2022). Besides the syntheses of antimicrobial metabolites, the metabolomic production of organic acids, biogenic amines, vitamins and bioactive peptides have been scientifically established (Ozogul and Hamed, 2018). Bioactive peptides (BPs) have been described as protein fractions possessing desirable influences on the health status of living beings (Korhonen and Pihlanto, 2006). The desirable and beneficial impacts of bioactive peptides have been classified based on their biofunctionalities as antithrombotic, antioxidative, antipathogenic, immunomodulatory, antidiabetic, and antihypertensive amongst several others (Kehinde and Sharma, 2018, Ayyash et al., 2021).

Bioactive peptides can also be viewed as amino acid fractions of proteins and bonded with covalent linkages known as peptide or amide bonds. A few peptides are in natural existence in their free states; however, others are protein-encrypted and have to be enzymatically released or chemically synthesized. BP's play significant roles in the body through their influences on cardinal systems such as;

***Corresponding author:**
Bababode Adesegun Kehinde, Department of Biosystems and Agricultural Engineering, University of Kentucky, Lexington, 40546, Kentucky, USA. **E-mail:** mrkenny05@gmail.com

Received: 21 April 2023; **Accepted:** 12 November 2023

nervous, immune, cardiovascular, endocrine and digestive systems. Peptides are appraised as the modern metabolites with the remarkable importance of regulating organic systems; they have been proven effective in preventing both the microbial spoilage and oxidation in foods and also for the management of metabolic syndrome diseases. The continuously-increasing focus on peptides has motivated scientific researchers and food industrialists for their adoption in the formulation of novel and functional food products. They are versatile, and in addition to their proven potentials in food preservation, they have been integrated into food packages to exert their antimicrobial effects and extend shelf lives of such food products (Appendini and Hotchkiss, 2002).

Quinoa (*Chenopodium quinoa* Willd) is an ancient pseudocereal of the dicotyledoneae family of having a significant nutritional and dietetic value based on the biological value of its concomitant protein. It is appraised as one of the optimal sources of vegetable proteins, having a protein profile somewhat relative to milk and comparably higher proteins in staple cereals such as maize, wheat and rice, along with the advantage of being gluten-free (Nisar et al., 2017). Pineli et al. (2017) prepared quinoa milk but added enzymes such as amuloglucosidase and α-amylase for starch hydrolysis. Lorusso et al. (2018) prepared a yogurt-like beverage from quinoa flour using *L. plantarum* and *L rhamnosus* but did not examine the fatty acid profile of fermented preparations. The use of probiotics as substitutes for enzymes for food processing has been reviewed to possess advantages such as cheaper costs, less requirement for technical expertise, and availability (Kehinde et al., 2022). In addition, there is need for investigation on the changes in functional groups and fatty acid profiles of quinoa caused by probiotic fermentation. Accordingly, this study is designed to examine the effect of probiotic fermentation on the phytochemistry and fatty acid profile of quinoa milk and to obtain protein hydrolysate fractions of potential antidiabetic biofunctionality.

MATERIALS AND METHOD

Reagents and equipment

All reagents used for this research were analytical grade and procured from Hi media, SRL, Sigma and CDH and are as follows: o-phthaldialdehyde OPA), β-mercapto-ethanol (Sigma-aldrich), Methanol (SRL), Sodium tetraborate (Hi-Media), Potassium chloride, Starch, Sodium dodecyl sulfate (SDS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trichloroacetic acid (TCA), Dinitrosalicyclic acid (DNS), and Sodium hydroxide. Fourier transform infrared spectrometer (SHIMADZU Corporation, Japan) equipped with a cuvette rack. Ultrafiltration membranes of 5 kDa, (Sartorius, United Kingdom). GC/MS 5900 series (Nucon, India).

Growth medium and probiotics

The growth medium adopted for the growth of studied probiotic strains was MRS-Cysteine medium (M369) and it was prepared as prescribed by the manufacturer followed by autoclave sterilization for 15 min at 15 psi before usage. The probiotic strains in their lyophilized states used for this study were obtained from Imtech Chandigarh, India. Quinoa was obtained from a local market in Jalandhar, India.

Optimized preparation of quinoa milk

Quinoa grains were broken by shear impact in a laboratory-grade mortar and pestle and then defatted, dried at room temperature for complete evaporation of the solvent and further milled to powder using a high-speed blender. The powder was used for milk preparation by dissolving in distilled water in concentrations of 5 and 10 % respectively in schott bottles with pasteurization at 80 °C for 30 mins (Fig. 1). The prepared milk samples were comparatively evaluated for their fermentation suitability on the basis of pH, proteolysis and antioxidant activities

Probiotic strains and proteolytic studies

Three probiotic Lactic acid bacteria strains were examined namely, *Lactobacillus plantarum* (MTCC 1325), *Lactobacillus fermentum* (MTCC 903), and *Lactobacillus acidophilus* (MTCC 10307) were acquired in their lyophilized states from Microbial Type Culture Collection (MTCC), Chandigarh, India. They were revived by individual aseptic inoculation in MRS Broth media, followed by incubation at 37 °C for 48 h. The formation of a turbid suspension in the broths confirmed the revival of the strains. Subsequent to incubation, the broths were aseptically transferred into sterilized centrifuge tubes and centrifuged at 15,000 rpm for 10 minutes. The pellets were collected, washed in saline solutions and aseptically inoculated individually into 10 % quinoa milk preparation to examine their proteolytic potentials in the substrate. After 48 h of fermentation, the three samples were examined for proteolytic activity using the OPA test and the MTCC 903 preparation was found to have undergone the highest proteolysis and the strain was selected for further studies.

Quinoa milk fermentation

Lactobacillus fermentum (MTCC 903), the LAB strain with the highest proteolytic activity was used as seed culture and purified by serial inoculation as described by Sharma et al. (2021). The washed and incubated cell pellets were aseptically inoculated in equal volumes (50 ml) of 5 and 10 % quinoa milk preparations and incubated at 37°C for the fermentation process. In the course of fermentation,

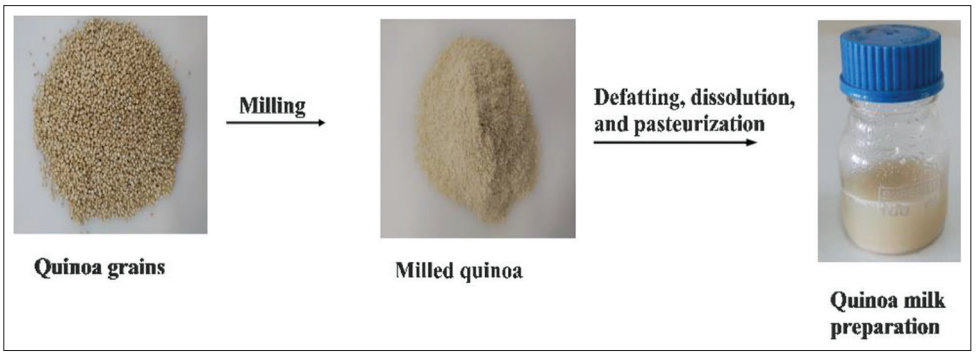

Fig 1. Preparation procedure of Quinoa mil prior to inoculation.

at every 24 h time interval, the pH, proteolysis, and antioxidant activities of the milks were evaluated from the aqueous and trichloroacetic acid (TCA) extracts. pH was measured directly using an electronic pH meter.

Proteolysis examination

This was conducted as conducted by Ayyash et al. 2018, with few modifications, however. TCA solution was prepared by dissolving 0.75 mol of TCA crystals in 1 L of water. For each proteolytic analysis, the milk was withdrawn aseptically, mixed with TCA solution at 1:3 and centrifuged for 15 mins at 10,000 rpm. The supernatant was obtained, syringe filtered and used for the OPA (o-phthalaldehyde) test. This test functions on the biochemical principle that α – amino groups released during proteolysis reacts with o-phthalaldehyde and 2-mercaptoethanol to synthesizean adduct of strong absorption at 340nm, using distilled water as blank. This absorption principle is analogous for all α – amino groups.

Antioxidant examination

The progress in antioxidative influence of the fermentation operation was evaluated with the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging procedure as described by Ayyash et al., 2018 with minor alterations. Concisely, 1 ml of DPPH (0.1 mM solubilized in 95 % methanol) was incorporated into 250 µl of the quinoa aqueous extract (QAE) in test tubes with a subsequent vigorous shaking and dark incubation for 30 mins at room temperature. The methanol solvent was adopted as blank and the absorbance was spectrophotometrically determined at 517 nm. At the attainment of unchanging proteolytic activity in combination with a pH of less than 3.5, the fermentation was halted.

FTIR spectroscopy

The effect of fermentation on the phytochemical profile of the quinoa milk was examined with the Fourier transform infrared spectroscopy. Fermented and unfermented quinoa milk (10 %) were centrifuged and their supernatant analyzed at an absorption spectra range of about 415 to 4000/cm, at a resolution of 4/cm and for 12 scans.

Fractionation of peptides

After optimal fermentation, the TCA peptide precipitate of the 10 % quinoa milk preparation was collected, and syringe filtered. The crude peptide mixture was fractionated using ultrafiltration membranes into 5 kDa and ≥ 5kDa fractions. This was done by cold microcentrifugation at 15,000 rpm for 30 mins. Further tests were conducted on the functionalities of the fractions.

Antidiabetic functionality of peptide fractions

This was examined on the basis of their α-amylase inhibition. An α-amylase biochemical assay was prepared as suggested by Kim, Wang, and Rhee (2004), however, with slight changes. 200 µl of α-amylase was mixed separately with 200 µl of each peptide fraction and incubated for 5 mins at 37 °C. 500 µl of 1% starch in a saline phosphate buffer was added as a substrate to initiate the reaction for 5 mins at 37 °C. The reaction was thereafter terminated with 400 µl of DNS reagent (12% sodium potassium tartrate in 0.4 M NaOH and 1% of 3,5- dinitrosalicylic acid). The reaction mixture was heated for 15 min at 100°C and further diluted with deionized water (2 ml) in an ice bath. The α-amylase inhibitory activity was determined using the equation.

GC-MS analysis of fatty acids

This was conducted as described by Ghosh et al. (2015) with few modifications. Briefly, 5 ml of homogenized quinoa preparations were dried under vacuum to dryness and their methanolic extracts obtained. Fatty acids from chromatographs were identified through comparison of their spectral peaks to those from installed libraries.

Statistical analysis

Test of significance of examined variables and their correlations were conducted using the Pearson Product Correlation in Sigmaplot 14.0 (SPSS, Chicago). All studies were carried out in triplicates

RESULTS AND DISCUSSION

Preliminary proteolytic evaluation

The initial screening of the proteolytic activities on quinoa milk substrates showed that *Lactobacillus fermentum* (MTCC 903) with absorbance readings of 0.236±0.014 and 0.427±0.012 for 5 and 10 % Quinoa preparations was superlative after 48 h of fermentation as shown in Fig. 2. In several studies relating to the comparative proteolytic potentials of food proteins by probiotics, especially several dairy substrates, *L. plantarum* seemingly shows the highest proteolytic strength. However, this trend is not fixed for all dairy and non-dairy substrates. The results obtained in this study are analogous to those reported by Akabanda et al., 2014 in a study regarding the comparative proteolysis of 4 probiotic strains namely, *Lactobacillus plantarum* 8-2, *Leuconostoc mesenteroides* 14-11, *Lactobacillus helveticus* 22-7 and *Lactobacillus fermentum* 22-16 on a traditional dairy product indigenous to the West-African people. Their o-phthaldialdehyde study showed that *L. plantarum* had the lowest proteolytic potential of 10 % while *L. fermentum* showed the second-highest proteolytic strength of 40 % with *Leu. mesenteroides* displaying the highest (50 %). On another note, the concomitant proteinases in *L. fermentum* have been experimentally confirmed as formidable for the proteolysis of food-borne protein. Ramesh et al., 2012 examined the proteolytic activity of proteinases in the cell-free extracts of skim milk samples fermented individually by several probiotics including *L. plantarum* and *L. fermentum*. Their investigation showed that the *L. plantarum* had a lower cell-free proteolytic potential relative to *L. fermentum*. Furthermore, the proteinase proteolytic activity of *L. fermentum* has been attributed to its biofilm formation in its antimicrobial action against pathogens. Carmo et al., 2016 conducted a research on the antimicrobial potential of 7 probiotic strains namely; *L. rhamnosus* (ATCC 9595), *L. plantarum* (ATCC 8014), *L. paracasei* (ATCC 335), *L. fermentum* (ATCC 23271), *L. delbrueckii* ssp. *delbrueckii* (ATCC 9645), *L. brevis* (ATCC 367), and *L. acidophilus* (ATCC 4356) for the inhibition of *Candida albicans* (ATCC 18804), through the formation of biofilms and found only the *L. fermentum* strain possessing the potential.

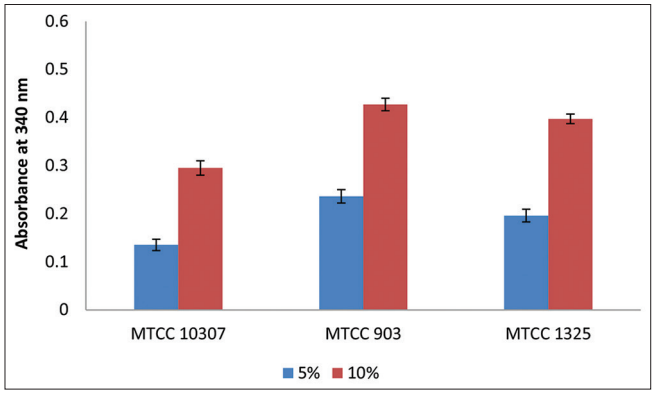

Fig 2. Comparison of proteolytic activities of different probiotics on Quinoa milk.

Proteolysis and pH

Results obtained for the proteolytic breakdown of quinoa proteins by *L. fermentum* are shown in Fig. 3 with the initial and final absorbance being 0.302 and 0.794 respectively. Proteolysis is the irreversible degradation of proteins into amino acids or peptides of relatively lesser molecular weights, typically by proteases (Rodgers and Overall, 2013).

The OPA method was extensively used for the evaluation of proteolysis in this study due to its relative advantages of not requiring prolonged cooling and heating that could affect the quinoa proteins and also due to the low absorbances of the reagent blanks. It has proven to be efficacious and optimally sensitive for experimental studies involving amino acids, peptide chains and proteins. The progressive increase in proteolysis as indicated by the increasing absorbance obtained is attributable to the action of proteases released by *L. fermentum* on the quinoa proteins during fermentation. Proteases that have been experimentally linked with this probiotic include phophopyruvate hydratase, ornithine carbamoyltransferase, fumarate hydratase, arginine deiminase and O-sialoglycoprotein endopeptidase (Wickström et al., 2013).

The absorbance readings remained fairly constant after 7 days of fermentation showing that optimal proteolysis has been obtained. This trend of increasing proteolysis with fermentation time was obtained by Hati et al., 2017 in a research involving the use of different probiotics for the fermentation of soymilk supplemented with varying concentrations of whey protein concentrate. For probiotics employed in the study namely, *L. rhamnosus* MTCC 5945 (NS4), *L. helveticus* MTCC 5463 and *Streptococcus thermophilus* MTCC 5462, there was a progressive rise in the optical density results obtained in the course of the fermentation.

The pH decrease signifies the fermentative conversion of quinoa carbohydrates to organic acids, unlike the control preparation. Correlation studies showed a significant

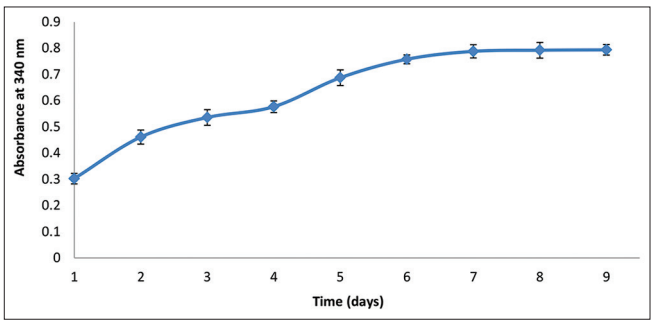

Fig 3. Proteolytic examination of *L. fermentum* on Quinoa milk over time.

positive correlation (>0.7) between proteolysis and time, but a negative correlation between pH and time, however both parameters being statistically significant at $p < 0.05$. A strong negative correlation of -0.9 between proteolysis and pH was found at a statistically significant $p < 0.05$.

Similar pH readings were obtained according to Ayyash et al., 2018 for the solid-state fermentation of quinoa with the probiotics *L. plantarum* K779 and *L. reuteri* K777. Initial and final pH readings of 6.7±0.05 and 3.2±0.02 were obtained for 0 and 72 h fermentation time respectively.

Antioxidant activity

The antioxidant profile of the prepared quinoa milk was monitored on a daily basis throughout the fermentation period as displayed in Fig. 4. The in vitro biochemical procedure involving the scavenged reduction and decrease in stability of DPPH is prominently used for evaluating the antioxidative potentials of food materials. The fundamental mechanism involves the donation of an electron to the DPPH radical complex, thus reducing it. The percentage DPPH radical scavenging potential of fermented quinoa milk in this study was found to increase from 11.29±2.02 to 88.76±1.92 % from day-1 to day-9 of fermentation respectively. The increase in antioxidant activity of the quinoa preparation could be related to the rising concentration of polyphenols and other antioxidant compounds metabolically synthesized by the probiotic in the course of fermentation and this trend is similar for typical fermentation operations irrespective of the strains adopted. Starzyńska-Janiszewska et al., 2016 studied the effect of Rhizopus oligosporus ATCC 64063 fermentation on different varieties of cooked quinoa seeds with regards to its phytonutrient and antioxidative profile. For most preparations, there was increase in the total phenolic content of the substrates used with increasing fermentation time. There was an increase from 7.65±0.04 to 8.46±0.11 mg/g d.m and 8.71±0.02 to 9.12±0.05 mg/g d.m for black and white quinoa seeds from 30 h to 40 h fermentation respectively.

Consequently, their antioxidant functionality increased from 1.67±0.05 to 2.11 ±0.03 DPPH mg trolox/mg d.m and 1.42±0.04 to 1.61±0.01 DPPH mg trolox/mg d.m for both preparations. Similar readings were obtained by Xu, Guo and Zhang, 2019 for the solid-state fermentation of quinoa using three distinct strains of filamentous fungi namely, Fomitiporia yanbeiensis G1, Agaricus bisporus AS2796 and Helvella lacunosa X1 and reported an increasing total phenolic content for each strain.

α-amylase inhibition

The α-amylase inhibitory potentials of the <5 and 5 kDa hydrolysate fraction samples are shown in Fig. 5. The proteolytic degradation of the proteins had a positive effect on their α-amylase inhibitory potential; however, the ultrafiltration did not. The <5 kDa fraction displayed less inhibition for α-amylase relative to the 5 kDa fraction. Similar results were obtained by Nongonierma et al., 2015 for the enzymatic digestion of quinoa proteins with a papain and another similar enzyme and a subsequent analysis of the DPP-IV inhibitory activity of the hydrolysates. They reported that the hydrolysates from the enzymatic treatments had a more potent inhibitory activity against the digestive enzyme in comparison with the protein isolate. Both enzymatic treatments showed half maximal inhibitory concentration (IC50) values of 0.88±0.05 and 0.98±0.04 mg/mL respectively with the control (undigested protein isolate) having > 2.00 mg/mL. The inhibition of digestive enzymes such as α-amylase, α-glucosidase and dipeptidyl peptidase-IV (DPP-IV) has been the fundamental mechanism for the development of food-based antidiabetic products (Kehinde and Sharma, 2018). α-amylase is an endoenzyme, functional in the digestive and hydrolytic catalysis of ingested polysaccharides (Wang, Li and Lu, 2018). The retardation of its activity in the body would be consequently beneficial for the reduction of blood sugar and accordingly for the management of diabetes (Chonlatid, Opeyemi, and Chitchamai 2018).

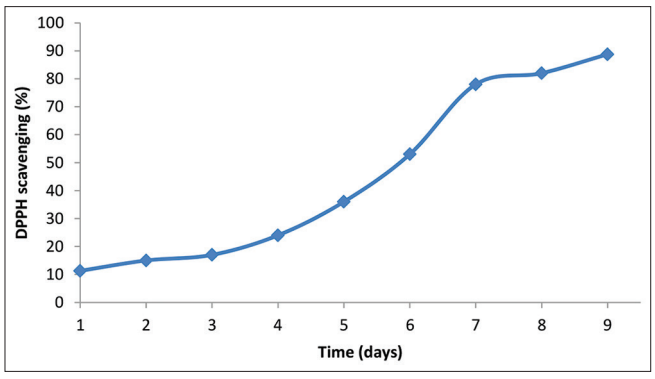

Fig 4. Antioxidant potential of fermented quinoa milk over time.

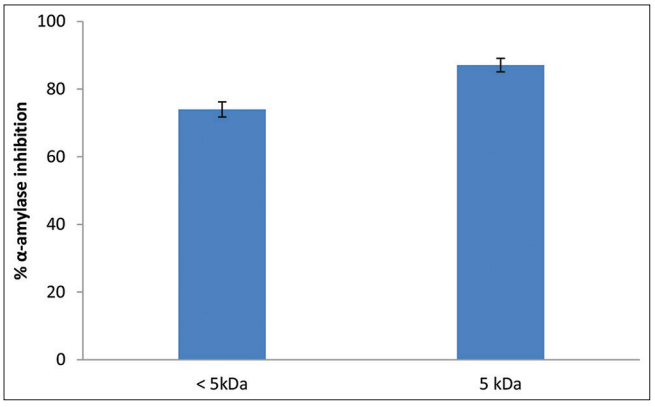

Fig 5. Antidiabetic potential of quinoa hydrolysate fractions.

FTIR phytochemical analyses

The mid-infrared spectrum images are shown in Figs. 6 (a and b) for control and optimally fermented samples. Changes in the intensities and areas of generated peaks proffer information on the quantitative and qualitative changes resulting from fermentation metabolism. The control sample peak of 418.57/cm with an intensity of 12.722 changed to 416.64/cm and 3.153 after fermentation. Spectral peaks generated in this region correlate to the C-N-C deformation; nitrogen-containing compounds such as choline which is remarkably resent in quinoa (Sheela et al., 2010). Accordingly, the changes signify the consumption of nitrogen-containing compounds during fermentation by the probiotic bacteria. The control sample also had a spectral peak at 1645.33/cm with a corresponding 1635.69/cm peak for the fermented samples. Though their intensities were similar (72.261 and 72.421 respectively), their areas were significantly different with the control having 5.239 and the fermented sample with 7.991 respectively. The peaks in this region represent the amines and the increase in area correspond to increased amide groups from peptidic bonds, thus further confirming the proteolysis of quinoa proteins (Wang et al., 2016). A significant shift in peak position for the O-H group region representing polyphenols and other alcohols was detected from 3292.6/cm to 3315.74/cm. This change is possibly due to the reduction in mass and bond length of alcoholic compounds due to the fermentative metabolism by the probiotic since higher peaks are scientifically correlated with lower bond lengths and masses. Consequently, there is less requirement for energy for stronger vibrations and an eventual increase in peak position (Papageorgiou et al., 2010).

GC-MS fatty acids

Figs. 7a and 7b show the chromatographs of the unfermented and fermented quinoa milk preparations respectively. The figures indicate the fewer peaks of the fermented sample indicate nutrient metabolism and consumption of anti-nutritional factors by the fermenting probiotics along with the synthesis of alkanes and other organic compounds present in essential oils. More

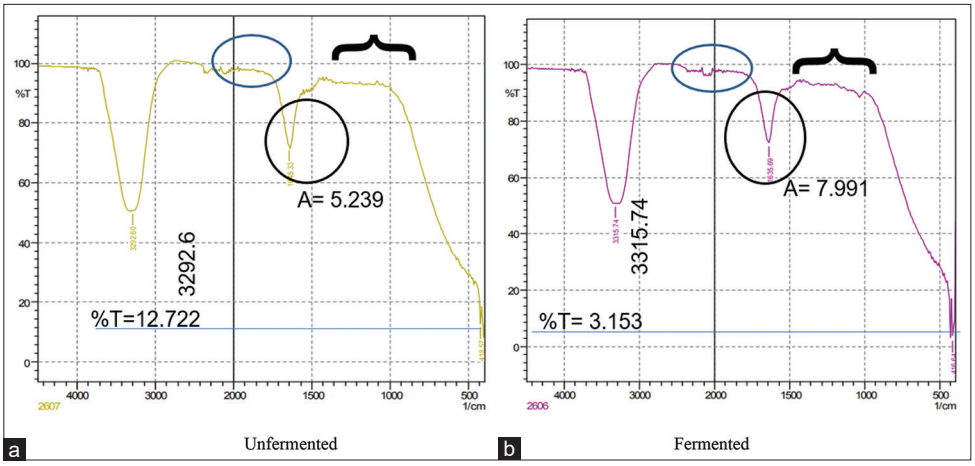

Fig 6. (a and b) FTIR examination of fermented quinoa milk.

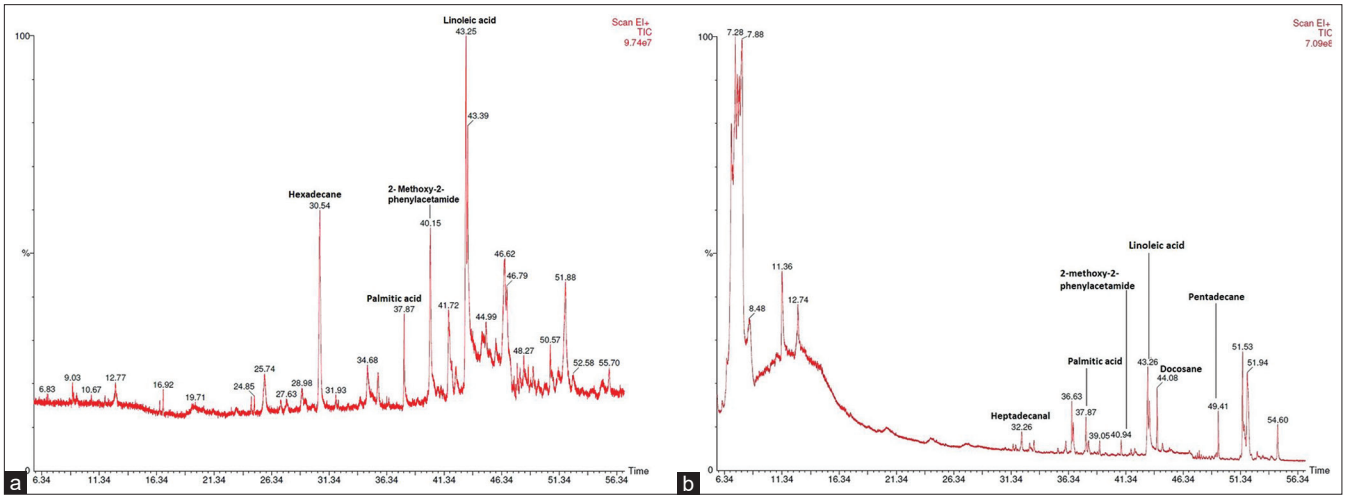

Fig 7. (a) Gas chromatograph of unfermented quinoa milk. (b) Gas chromatograph of fermented quinoa milk.

importantly, the bioactive fatty acids became biologically available for their biochemical activities in the body at the cellular level. Saturated and unsaturated fatty acids such as linoleic and palmitic acids affect the taste and mouthfeel of the product. Quinoa is known to contain a good proportion of fatty acids especially linolenic acid which is functional for reducing the risks of metabolic disease syndromes related to inflammation, cancer and cardiovascular disorders amongst several others (Alvarez-Juvete, Arendt and Gallagher, 2010). Medina-Meza et al. (2016) analyzed the GC-MS profile of saponic antinutritional factors in quinoa and identified the presence of phytolaccagenic acid, serjanic acid, hederagenin and Oleanolic acids as the prominent saponins.

CONCLUSIONS

The isolation of bioactive components by probiotic fermentation is methodology for the food-based syntheses of products functional for human health. The comparative advantages of cost-effectiveness and natural style are possible rationales behind this. Furthermore, the minimal risks and likelihood of severe aftereffects on health associated with the ingestion or administration of food-originated therapeutic materials are formidable reasons behind their optimized selection for the management of health-related issues. In this research study, bioactive peptides were productively isolated from quinoa milk by optimized fermentation with the probiotic – *Lactobacillus fermentum*. The hydrolysates were fractionated on the basis of their molecular weight and were further biofunctionally characterized as potentially antioxidative and antidiabetic. In addition, extensive analyses showed that the fermentation process had positive effects on the overall phytonutrient profile of the fermented milk. The adopted probiotic efficaciously metabolized the food constituents and removed antinutritional factors for the release of multiple bioactive components. Despite being a timelessly employed biotechnological process, fermentation remains a superlative and efficient tool for food and health purposes. However, only a few segments of its versatile scope are utilized on the industrial scale, globally. An increased focus on the fermentation route for the isolation of bioactive peptides with the proper integration of industrialization would doubtlessly improve the pharmaceutical and health supply of peptide products around the globe. Consequently, the food-peptide approach for the management of health disorders could possibly the much-desired panacea for in this regard.

Author's contributions

Bababode Adesegun Kehinde: Conceptualization, experimentation, writing, review, and organization. Poorva Sharma: Supervision. Shafiya Rafiq: Experimentation. Ishrat Majid: Experimentation. Adetokunbo Adekoya: Statistical analysis.

REFERENCES

Abdalla, A., B. Abu-Jdayil, S. AlMadhani, F. Hamed, A. Kamal-Eldin, T. Huppertz and M. Ayyash. 2022. Low-fat akawi cheese made from bovine-camel milk blends: Rheological properties and microstructural characteristics. J. Dairy Sci. 105: 4843-4856.

Akabanda, F., J. Owusu-Kwarteng, K. Tano-Debrah, C. Parkouda and L. Jespersen. 2014. The use of lactic acid bacteria starter culture in the production of nunu, a spontaneously fermented milk product in Ghana. Int. J. Food Sci. 2014: 1-11.

Alvarez-Jubete, L., E. Arendt and E. Gallagher. 2010. Nutritive value of pseudocereals and their increasing use as functional gluten-free ingredients. Trends Food Sci. Technol. 21: 106-113.

Appendini, P. and J. H. Hotchkiss. 2002. Review of antimicrobial food packaging. Innov. Food Sci. Emerg. Technol. 3: 113-126.

Ayyash, M., A. Abdalla, A. Alhammadi, C. S. Ranadheera, M. A. Baig, B. Al-Ramadi, G. Chen, A. Kamal-Eldin and T. Huppertz. 2021. Probiotic survival, biological functionality and untargeted metabolomics of the bioaccessible compounds in fermented camel and bovine milk after *in vitro* digestion. Food Chem. 363: 130243.

Ayyash, M., A. Abdalla, B. Abu-Jdayil, T. Huppertz, R. Bhaskaracharya, S. Al-Mardeai, A. Mairpady, A. Ranasinghe and A. Al-Nabulsi. 2022. Rheological properties of fermented milk from heated and high pressure-treated camel milk and bovine milk. LWT. 156: 113029.

Ayyash, M., A. S. Al-Dhaheri, S. Al Mahadin, J. Kizhakkayil and A. Abushelaibi. 2017. *In vitro* investigation of anticancer, antihypertensive, antidiabetic, and antioxidant activities of camel milk fermented with camel milk probiotic: A comparative study with fermented bovine milk. J. Dairy Sci. 101: 900-911.

Ghosh, G., P. Panda, M. Rath, A. Pal, T. Sharma and D. Das. 2015. GC-MS analysis of bioactive compounds in the methanol extract of *Clerodendrum viscosum* leaves. Pharmacognsy Res. 7: 110-113.

Hati, S., N. Patel, K. Patel and J. B. Prajapati. 2017. Impact of whey protein concentrate on proteolytic lactic cultures for the production of isoflavones during fermentation of soy milk. J. Food Process. Preserv. 41: e13287.

Kehinde, B. A., I. Majid and S. Hussain. 2022. Isolation of bioactive peptides and multiple nutraceuticals of antidiabetic and antioxidant functionalities through sprouting: Recent advances. J. Food Biochem. 46: e14317.

Kim, Y., M. H. Wang and H. I. Rhee. 2004. A novel alpha-glucosidase inhibitor from pine bark. Carbohydr. Res. 339: 715-717.

Korhonen, H. and A. Pihlanto. 2006. Bioactive peptides: Production and functionality. Int. Dairy J. 16: 945-960.

Lavefve, L., D. Marasini and F. Carbonero. 2019. Microbial ecology of fermented vegetables and non-alcoholic drinks and current knowledge on their impact on human health. Adv. Food Nutr. Res. 87: 147-185.

Lorusso, A., R. Coda, M. Montemurro and C. C. Rizzello. 2018. Use of selected lactic acid bacteria and quinoa flour for manufacturing novel yogurt-like beverages. Foods. 7: 51.

Marco, M. L., D. Heeney, S. Binda, C. J. Cifelli, P. D. Cotter, B. Foligné, M. Gänzle, R. Kort, G. Pasin, A. Pihlanto, E. J. Smid and R. Hutkins. 2017. Health benefits of fermented foods: Microbiota and beyond. Curr. Opin. Biotechnol. 44: 94-102.

Medina-Meza, I. G., N. A. Aluwi, S. R. Saunders and G. M. Ganjyal.

2016. GC-MS profiling of triterpenoid saponins from 28 quinoa varieties (*Chenopodium quinoa* Willd.) grown in Washington State. J. Agric. Food Chem. 64: 8583-8591.

Nisar, M., D. R. More and S. I. Hashmi. 2017. Physico-chemical and nutritional properties of quinoa seed: A review. J. Pharmacogn. Phytochem. 6: 2067-2069.

Nongonierma, A. B., S. Le Maux, C. Dubrulle, C. Barre and R. J. FitzGerald. 2015. Quinoa (*Chenopodium quinoa* Willd.) protein hydrolysates with *in vitro* dipeptidyl peptidase IV (DPP-IV) inhibitory and antioxidant properties. J. Cereal Sci. 65: 112-118.

Özogul, F. and I. Hamed. 2018. The importance of lactic acid bacteria for the prevention of bacterial growth and their biogenic amines formation: A review. Crit. Rev. Food Sci. Nutr. 58: 1660-1670.

Papageorgiou, S. K., E. P. Kouvelos, E. P. Favvas, A. A. Sapalidis, G. E. Romanos and F. K. Katsaros. 2010. Metal-carboxylate interactions in metal-alginate complexes studied with FTIR spectroscopy. Carbohydr. Res. 345: 469-473.

Pineli, L. L. O., R. B. A. Botelho, R. P. Zandonadi, J. L. Solorzano, G. T. De Oliveira, C. E. G. Reis and D. S. Teixeira. 2015. Low glycemic index and increased protein content in a novel quinoa milk. LWT Food Sci. Technol. 63: 1261-1267.

Rogers, L. D. and C. M. Overall. 2013. Proteolytic post-translational modification of proteins: Proteomic tools and methodology. Mol. Cell Proteomics. 12: 3532-3542.

Sharma, P., B. A. Kehinde, N. Chhikara and A. Panghal. 2021. Development of whey and turmeric based functional synbiotic product. Environ. Sustain. 4: 861-872.

Starzyńska-Janiszewska, A., R. Duliński, B. Stodolak, B. Mickowska and A. Wikiera. 2016. Prolonged tempe-type fermentation in order to improve bioactive potential and nutritional parameters of quinoa seeds. J. Cereal Sci. 7: 116-121.

Wang, J., Y. Li and F. Lu. 2018. Molecular cloning and biochemical characterization of an α- amylase family from *Aspergillus niger*. Electron. J. Biotechnol. 32: 52-62.

Wang, J., Y. Yue, T. Liu, B. Zhang, Z. Wang and C. Zhang. 2016. Change in glutenin macropolymer secondary structure in wheat sourdough fermentation by FTIR. Interdiscip. Sci. Comput. Life Sci. 9: 247-253.

Wickström, C., L. Chávez de Paz, J. R. Davies and G. Svensäter. 2013. Surface-associated MUC5B mucins promote protease activity in *Lactobacillus fermentum* biofilms. BMC Oral Health. 13: 43.

Xu, L., N. S. Guo and S. W. Zhang. 2019. Effects of solid-state fermentation on the nutritional components and antioxidant properties from Quinoa. Emir. J. Food Agric. 31: 39-45.