Inhibition of acrylamide in gluten-free quinoa biscuits by supplementation with microbial dextran

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**ABSTRACT**

The microbial dextran (MD) as a natural bacterial hydrocolloid was used for acrylamide (AA) inhibition in gluten-free quinoa biscuits. The European council approved the use of MD as a safe functional additive in bakery products. AA was spontaneously produced during the baking of 100% quinoa biscuit dough which was quantified at 2142 ± 3 μg kg\textsuperscript{-1} dry biscuit. This amount of AA in quinoa biscuits was about two times higher than the reported highest content of AA in bakery products (1044 μg kg\textsuperscript{-1}) in some previous studies and six times higher than the recommended benchmark level of AA by European commission regulation in biscuits (350 μg kg\textsuperscript{-1}). The effect of MD on the AA inhibition was first studied at four concentration levels (1, 3, 5, and 7%, v/v) in quinoa dough. The Leuconostoc mesenteroides NCIM-2198 synthesized MD was investigated by Fourier Transform Infrared Spectroscopy (FT-IR), 13C Nuclear Magnetic Resonance (13C NMR) and 1H NMR depicting the formation of α-(1-6) glycosidic linear chain without branches. Moreover, MD solution was a high viscous solution at pH 6.8. Interestingly, 5% (v/v) MD solution supplemented in the quinoa dough was successfully applied to inhibition rate 89.1% of AA formation (down to 233 ± 6 μg kg\textsuperscript{-1} dry biscuit) without deteriorating the quality of quinoa biscuits. This is the first AA mitigation approach in quinoa biscuits with an achievable result of about half the recommended EU benchmark level of AA. This AA mitigation could be due to the ability of MD to retain water molecules during baking process. Furthermore, the presence of hydroxyl groups in MD polymer could cross-link AA via hydrogen bonding. Therefore, the MD fortified quinoa biscuits could serve as low-acrylamide and health-promoting food products.

**INTRODUCTION**

Recently, it has been found out the impact of microbial hydrocolloids as safe food ingredients due to easy production, better stability characteristics and cost-effectiveness.\textsuperscript{[1]} Microbial hydrocolloids are a type of hydrocolloids that are obtained by fermenting polysaccharides using generally lactic acid bacteria (LAB). LAB produces two types of exopolysaccharides (EPS): heteropolysaccharides and homopolysaccharides. Dextran production by LAB is an example of homopolysaccharide.\textsuperscript{[2]} Microbial dextran (MD) usually produced by various strains of LAB such as Leuconostoc, Lactobacillus and Streptococcus, differs in their glucosidic linkage, mass, degree of branching and chain length.\textsuperscript{[3]} Leuconostoc species bacteria are one of the main group of EPS-producing mesophilic LAB.\textsuperscript{[2]} To date, MD synthesized by L. mesenteroides NRRL B-640 comprised of D-glucose residues with consecutive α-(1→6) linkages in a linear chain.\textsuperscript{[4]} Whereas, MD synthesized by L. mesenteroides NRRL B-512 F and

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NRRL B-523 included 95% α-(1→6) and 5% α-(1→3).[4] *L. mesenteroides* NRRL B-742 produced MD with 87% α-(1→6)-linear linkages and 13% α-(1→4)-branched linkage. The strain *L. mesenteroides* NRRL B-1299 produced MD with 63% α-(1→6), 27% of α-(1→2), and 8% of α-(1→3).[2]

The European council received an application for approval of MD as a safe functional ingredient in bakery products.[5] MD has interesting characterization due to the flexibility of its structure via free rotation of glycosidic bonds and its solubility in water.[6] It was found that MD produced from *L. mesenteroides* with high molecular weight, high viscosity and few/no branched linkages improved markedly the quality of wheat dough.[6,7] Hence, it is expected that MD produced from *L. mesenteroides* has to hold potential applications in baking industry.

Recently, gluten-free bakery products have markedly increased in sales with expectation to reach more than 7 billion dollars in 2023.[8] Recently, gluten-free bakery products are considered the main eating sources with individuals of gluten allergy and celiac disease.[9,10] Of many different bakery products in market, biscuits have commercially great attention because of their ease composition, high nutritional content, long shelf stability and relatively low cost.[11] Quinoa pseudocereal seed was successfully used to prepare 100% gluten-free biscuits without adversely affecting its baking performance.[12] Quinoa is also characterized with high content of amino acids which are the main blocking units of protein formation (16–18%).[13] Amino acids are also considered as a source of the formation of unhealthy compounds.[14] During the processing of biscuit dough at a high temperature,[15] Maillard’s reaction should be formed between amino acids (e.g. asparagine) and reducing sugars (e.g. glucose or fructose) producing a harmful compound acrylamide.

Acrylamide (AA) is a chemical hazard in food chain and is classified by the International Agency of Research on Cancer (IARC) as a potential carcinogenic (class 2A) compound.[10,15] AA usually forms from the naturally occurring its precursors asparagine and sugars in baked foods when prepared at temperatures typically higher than 120°C and low moisture.[15] EU commission regulation, 2017/2158 establishes a regulation that the benchmark level of AA in biscuits must be less than 350 µg kg⁻¹.[16, article 1 and annex IV]. Moreover, some reported results indicated that the highest content of AA in bakery products could be 1044 µg kg⁻¹. On the same hand, authors in some previous studies on quinoa baked products observed that the color lightness decreased with increasing of quinoa level, but, most of them failed to explain the reasons and others stated that this could be attributed to the formation of non-enzymatic browning compounds (majorly AA) which accelerated by the higher content of amino acids in quinoa flour.[10–12] Therefore, it is interesting to investigate AA in quinoa bakery products (commonly biscuits) and it is still a challenge to ensure that AA level is as low as reasonably achievable.

Recently, food hydrocolloids have attracted the food researchers attributed to their effective action for the mitigation of AA formation.[16–18] Hydrocolloids usually formed protective layers on the surface of food products due to thermally induced gelation above 60°C leading to inhibit the escape of unbound water from sample. Moreover, hydrocolloids could interfere with the Maillard’s reaction and subsequently could mitigate AA content.[10,15,16] Therefore, it is expected that the addition of MD in quinoa biscuits could effectively mitigate AA formation. However, literature contained no information about this important issue in quinoa biscuits. Therefore, the ultimate objective of the current work is to measure AA content in quinoa biscuits (QBs) and to first investigate the effect of MD synthesized by *L. mesenteroides* NCIM-2198 on the decreasing of AA formation in QBs. This is the first time establishment of a simple mitigation approach of AA formation in quinoa biscuits.

**Materials and Methods**

*L. mesenteroides* NCIM-2198 was obtained by NCIM (National Center for Industrial Microbiology, Pune, India). Quinoa seeds were obtained from The Egyptian Ministry of Agriculture (they were grown at 2019) and were saved at 4°C. Quinoa flour (QF) was prepared by washing seeds many times with cold water for removing of saponin[19] and then milling them by Blendtec Kitchen Mill Model 91 at medium setting (Blendtec Inc., Wichita, KS, USA). Wheat flour (72% extraction) was purchased from the North Cairo Flour Mills Company. Acrylamide (AA, ≥ 99.8%) and standard dextran were purchased from
Sigma (Steinheim, Germany). Sucrose (Nile Sugar Company), palm oil, salt, sodium bicarbonate, and ammonium bicarbonate were brought from a local supermarket. Other chemicals were analytical grades. The used deionized water was prepared by Millipore water system (Millipore, Billerica, MA).

**Production, isolation, and purification of microbial dextran (MD)**

The method was performed as described earlier. The microbial strain (L. mesenteroides NCIMB 2198) was sub-cultured in MRS broth (HiMedia specialist Ltd., India) and incubated for 24 h at 26°C. After that streak plating was done for future preservation purpose. For cultivation media, 5 mL of sterilized MRS broth was inoculated for 24 h subculture and incubated for 24 h at 26°C. After 24 h the inoculums were transferred in 45 mL culture medium and incubated again as described above. Then, the inoculums were transferred in 450 mL sucrose broth (its composition was described in) and incubated at 26°C for 24 h. After that, the culture medium was centrifuged at 5000 rpm for approximately 5 min to remove the cells. The supernatant was decanted followed by the addition of chilled ethanol with constant stirring till the precipitation of MD. The supernatant was again decanted. After 10 min, chilled ethanol was added again and MD was precipitated in very fine form followed by drying over calcium chloride at 30°C. For the purification of MD, 8 g of isolated MD was dissolved in 250 mL water and re-precipitated with cold ethanol. This cycle of dissolving, precipitation and washing was repeated three times and finally the purified MD powder was dried at 30°C and was preserved in small capped bottle for further use.

**Characterization of microbial dextran (MD)**

**FT-IR analysis of MD**: The functional groups of MD were obtained by Fourier transform infrared coupled with attenuated total reflectance spectroscopy (ATR-FTIR) in the range of wave number from 4000 to 400 cm⁻¹ using a SHIMADZU model spectrophotometer.

**NMR analysis of MD**: NMR spectroscopy was used to investigate the structural analysis of MD (Bruker DMX-400 spectrometer). Purified MD (5 mg) was dissolved in 0.5 mL D₂O solvent. This solution was used for ¹H NMR analysis which operated at 400 MHz and 40°C. However, ¹³C NMR analysis, heteronuclear single-quantum coherence was performed at 125 MHz with the same temperature.

**Quantification of MD using HPLC**: Microbial dextran (MD) was analyzed and quantified in its solution using the reversed phase-HPLC method. Agilent Series 1100 HPLC system was used for chromatographic quantification of dextran with UV detection at 275 nm. Ascentis® C18 column (4.6 mm x 250 mm, 5 μm) was used as a separation column. Mobile phase was composed of a mixture of acetonitrile and ultrapure water (10:90 (v/v)). Isocratic elution was performed at a flow rate of 2.4 mL min⁻¹ and 25°C column temperature. The injection volume was 20 μL. The mobile phase and MD solution were filtered through a 0.45 mm filter (Type HVLP, Millipore) and ultrasonicated for 10 min before using. The MD was identified in the chromatograms by comparing the retention time of its peak in the sample solution to the corresponding peak of standard MD solution.

**Viscosity and pH measurements of MD solution**

The viscosity measurement of MD solution was investigated using a rotational viscometer (Haake Viscometer, Germany). The average of three readings were recorded. pH meter (Lovibond, UK) equipped with the electrode type 330 containing gel electrolyte was used for pH measurements. It was regularly calibrated with universal phosphate buffer solutions (pHs 4 and 7) at room temperature. The average of three reading was recorded.
Table 1. The studied biscuit control samples and biscuit samples supplemented with variant concentrations of MD.

| Samples | Concentration (%) | Usage levels (mL/ 17.6 mL water) |
|---------|------------------|---------------------------------|
| CW      | 0.0              | 0.000                           |
| CQ      | 0.0              | 0.000                           |
| D1      | 1.0              | 0.176                           |
| D2      | 3.0              | 0.528                           |
| D3      | 5.0              | 0.880                           |
| D4      | 7.0              | 1.232                           |

**Preparation of biscuits**

The 100% quinoa biscuit dough (control quinoa, CQ) was prepared by the following steps. At the first step, 35.0 g sucrose was mixed with 1.0 g NaCl, 0.8 g of NaHCO₃ and 0.4 g NH₄HCO₃; the obtained mixture was creamed with 20.0 g palm oil for 3 min at 60 rpm using a dough mixer Artisan Kitchen Aid 5KSM150 (MI, USA). Then, 17.6 mL water was added and thoroughly hand mixed followed by the addition of 80.0 g quinoa flour with stirring for 4 min at 150 rpm so as to obtain homogenous dough. As an effective comparison with the most common wheat biscuits, the 100% wheat dough (control wheat, CW) was prepared by the same procedure with using refined wheat flour instead of quinoa flour.

For the preparation of MD modified quinoa dough, water (17.6 mL) in control quinoa (CQ) was replaced with different MD solutions. These solutions were prepared by dissolving the appropriate weight of MD in water giving the concentration levels of 1, 3, 5 and 7% (w/v) as described in Table 1. Then, the dough of each sample was rolled in 3 mm thickness by Atlas Brand rolling machine. After that, the 50 mm diameter cutter was used to cut the sheeted dough into round shape. The obtained dough was baked in an oven (Memmert UNE 400, Germany) at 180°C for 10 min. After 5 min, biscuits were rotated from front to back. At the end of process, biscuits were left to cool at the room temperature. The obtained biscuits were packed in low-density polyethylene bags for further uses.

**Proximate analysis**

Proximate analysis of MD, quinoa flour, wheat flour, and treated biscuits were carried out in triplicate based on AOAC method\(^{[21]}\) including moisture, crude fat, crude fiber, crude protein, and ash content. Kjeldahl method was used for crude protein analysis with factor of 6.25 for conversion of nitrogen to crude protein. The carbohydrate content of the biscuits was calculated by subtracting 100 from the sum of moisture, ash, fat, fiber and protein. The total energy value was calculated by the following equation:

Total energy value (kcal/100 g) = (protein % × 4 kcal/g) + (fat % × 9 kcal/g) + (carbohydrate % × 4 kcal/g) ... .................................................. (1)

**Amino acids analysis**

Analysis of amino acid was carried out by following the published work\(^{[22]}\). The quinoa flour and wheat flour were treated with 6 N hydrochloric acid followed by derivatization of free amino acids with phenylisothiocyanate. High performance liquid chromatography (HPLC VARIAN, Waters 2690, California, USA) was used for the separation and determination of derived phenylthiocarbamyl amino acids. A reversed-phase Nova-Pak C 18 (3.9 × 150 mm, 5 µm) was used as a separation column. The mobile phase consists of two eluents labeled A and B. A bottle comprises 940 mL of 0.14 M sodium acetate, pH 6.4, containing 0.05% triethylamine, mixed with 60 mL acetonitrile. B bottle is 60% acetonitrile and 40% water by volume. A gradient elution was employed to detect amino acids at a flow rate of 1.0 mL min\(^{-1}\) and a wavelength of 254 nm at 35°C.
**Water loss (WL) evaluation**

Water contents (WC) of all biscuits under the study were measured before and after baking process on the dried basis. Samples were placed on metal plates and were put in an oven for 65 min at 180°C. The following equation was used for the calculation of water loss percent (% WL):

\[
% \text{WL} = \frac{(\text{WC before baking} - \text{WC after baking})}{\text{WC before baking}} \times 100 \quad \text{(2)}
\]

The following equation was used for the calculation of inhibition rate:

\[
% \text{Inhibition rate in WL} = \frac{(\text{WL}_{\text{CQ}} - \text{WL}_4)}{\text{WL}_{\text{CQ}}} \times 100 \quad \text{(3)}
\]

WL\text{CQ} and WL\text{4} are the water loss of the control quinoa and the water loss of biscuits treated with MD, respectively. All measurements were repeated three times.

**Acrylamide quantification by LC/MS/MS**

AA was extracted from the studied biscuits and quantified by following the validated LC/MS/MS method as described elsewhere. An Agilent 1100 HPLC system equipped with MS detector and atmospheric pressure chemical ionization (APCI) mode (Waldbronn, Germany) was utilized to perform the LC/MS/MS experiments. The Sunfire™ C18 column (250 x 4.6 mm, 5 μm, Waters Corporation, Ireland) was used. Isocratic mobile phase of 0.01 mmol L\textsuperscript{−1} acetic acid and 0.2% formic acid was also used at flow rate of 0.9 mL min\textsuperscript{−1}. The selected ion monitoring mode was used for data acquisition. AA ions were detected at m/z 55 and 72. Working solutions of AA were daily prepared from stock solution (1 mg mL\textsuperscript{−1} water) within the linearity range between 1.0 and 200.0 ng mL\textsuperscript{−1}. All measurements were repeated three times. The following equation was used for the calculation of inhibition rate:

\[
% \text{Inhibition rate in AA} = \frac{(\text{AA}_{\text{CQ}} - \text{AA}_4)}{\text{AA}_{\text{CQ}}} \times 100 \quad \text{(4)}
\]

AA\text{CQ} and AA\text{4} are AA amount of the control quinoa and AA amount of biscuits treated with MD, respectively.

**Instrumental Texture Profile Analysis (TPA)**

Texture analyzer (TA1, LLOYD Instruments, AMETEK S.A.S., France) was used for TPA investigation as described elsewhere. In these experiments, a cylinder length of 125 mm and a disc diameter of 45 mm were attached to a 50 N load cell. Two-cycle compression test was executed to 50% compression of the original height. A cross head speed of 2 mm s\textsuperscript{−1} with a 30 s delay between compressions was applied. TPA test replicated the effect of two bites on a biscuit sample. Lloyd Instruments software appeared the TPA graph including the force, distance, area and time as well as allowing the calculation of hardness, cohesiveness and springiness.

**Color measurement**

Hunterlab model Precise Color Reader TCR 200 (BAMR Ltd., Claremont, South Africa) with 8-mm diameter circle and the specular component included (SCI) mode was utilized for color measurement. The colorimeter was standardized against a white calibration plate (lightness (L\textsuperscript{*}) = 97.79, redness (a\textsuperscript{*}) = −0.11, yellowness (b\textsuperscript{*}) = 2.69) using D65 standard daylight illuminant and 10° standard observer position. Three replicates were performed for each biscuit sample per treatment.
**Statistical analysis**

All measurements were carried out in triplicates at each experiment, and all data were shown as the means ± standard error (mean ± SE). SPSS package program (SPSS version) was used for statistical analysis of measurements. Linear mixed model was used to analyze the effect of MD concentration as a fixed effect along with the interaction between them and replication as a random effect. Duncan’s multiple comparison test was employed to compare between mean values considering \( P < .05 \) as a significant difference among mean values.

**RESULTS AND DISCUSSION**

**Characterization of microbial dextran (MD)**

In this work, MD was produced by a bacterium *L. mesenteroides* NCIM-2198. An amorphous white powder of MD with ash content (7.15%) and moisture (9.8%) was obtained. The FT-IR of MD produced by the bacterium *L. mesenteroides* (Figure 1) showed a broad and intense absorption band in the region of 3292.49 cm\(^{-1}\), which is vibrational stretch of (OH) group. This observation indicated the existence of a polyhydroxyl compound. The band of 2949.16 cm\(^{-1}\) and 1635.64 cm\(^{-1}\) are due to stretch vibrations of C-H bonds. The absorption bands that characterized MD (1→6) EPSs were established at 1155.36 cm\(^{-1}\), being related to the vibrational frequency of the glycoside C-O-C links in alpha confirmation. The absorption peak at 1016.49 cm\(^{-1}\) showed the flexibility of dextran chain.

The proton spectrum (\(^1\)H NMR) of the MD (Figure 2) shows the corresponding resonance of hydrogen to the glycosyl units. Carbonyl proton of D-glucopyranose (H-4) was resonating as triplet (t) at \( \delta \) 3.247 ppm, with coupling constant \((J)\) of 12 Hz; H-2 and H-3 are resonating at \( \delta \) 3.451 ppm as a broad peak, H-5 was resonating as doublet at \( \delta \) 3.641 ppm \((J = 6.2 \text{ Hz})\) and H-6 was resonating as doublet at \( \delta \) 3.755 ppm \((J = 6.6 \text{ Hz})\). Based on the coupling constants \((12, 6.2 \text{ and } 6.6 \text{ Hz})\), it was concluded that the alpha confirmation from D-glucopyranose. The peak resonating at \( \delta \) 4.979 ppm confirmed that the substance was not ramified.

In the \(^{13}\)C NMR spectrum of MD (Figure 3), C-1 was resonating at \( \delta \) 98.13 ppm, C-2 at \( \delta \) 71.79 ppm, C-3 at \( \delta \) 73.29 ppm, C-4 at 70.30 ppm, C-5 at \( \delta \) 70.07 ppm and C-6 at \( \delta \) 65.93 ppm. The peaks are resonating in the \( \delta \) 65 to 73 ppm region shows free position at C-2, C-3 and C-4.

*Figure 1.* FT-IR spectrum of MD produced from *L. mesenteroides* NCIM-2198. Structure formula of MD insided figure.
Moreover, additional peaks were not observed at 77–85 ppm, confirmed the absence of branched linkages and further confirmed the MD synthesized by the L. mesenteroides is a linear dextran with α-(1-6) glycosidic bonds. This property of MD, which reflects high water solubility and Newtonian fluids, is expected to be important for the ease supplementation in quinoa dough.

The MD content was also investigated by HPLC. The linearity range of MD was achieved between 0.1 and 100.0 μg mL⁻¹. Limit of detection (LOD) and limit of quantification (LOQ) of MD were 0.03 μg mL⁻¹ and 0.1 μg mL⁻¹, respectively. The retention time of standard dextran was observed at 2.435 min. Five different standard dextran concentrations were injected into column and after that with the given results calibration curve was formed. Then, the synthesized MD solution was quantified from the calibration curve giving the MD concentration of 1.1 μg mL⁻¹. In another published study, size exclusion chromatography was used for the quantification of dextran 20 from sucrose giving a short time similar to the current work.

The viscosity and pH of MD solution were also measured. The pH of MD solution was 6.8, and the viscosity of MD solution was 12.69 cp at 1% (w/v). Viscosity increased with increasing MD concentrations from 210.23 cp at 3% (w/v) up to 530.23 cp at 7% (w/v). It was observed that viscosity values largely increased from 1% (w/v) to 3% (w/v), while, from 5% (w/v) to 7% (w/v) MD, the viscosity increased to a lesser extent. High viscosity of MD solution has suggested that it has long chain of α-(1→6) linkage in the main dextran chain. In the current work, it was noted that the MD synthesized by L. mesenteroides NCIM-2198 strain is more viscous than the earlier findings (approximately 200 cp). Dextran with a few branched linkages or with a long main chain is required for the application in dough. These results are similar to those obtained in the previous study.
Chemical characterization of flours and treated biscuits

The chemical analysis of quinoa and wheat flours was investigated. The values of crude protein, crude fiber, crude fat, moisture, ash and carbohydrate were 17.81 ± 0.52, 8.65 ± 0.35, 3.75 ± 0.60, 11.74 ± 0.23, 2.34 ± 0.25 and 55.61 ± 0.40 g/100 g, respectively in quinoa flour. Moreover, the values of crude protein, crude fiber, crude fat, moisture, ash and carbohydrate were 7.95 ± 0.62, 0.48 ± 0.75, 0.84 ± 0.53, 9.25 ± 0.31, 0.55 ± 0.16 and 90.97 ± 0.35 g/100 g, respectively in wheat flour. It was observed that QF had higher fiber (18 times), fat (4.7 times), protein (2.2 times) and moisture (1.2 times) than those in WF which are in agreement with those reported in literature.\textsuperscript{[10–12]} Moreover, the analysis of amino acids in QF and WF showed that arginine (8.52 ± 0.41 g/100 g) and lysine (5.93 ± 0.26 g/100 g) in QF are higher than those in WF (arginine 4.21 ± 0.23 g/100 g and lysine 3.65 ± 0.53 g/100 g). However, glutamine (32.95 ± 0.64 g/100 g) is the highest amino acid in WF which is about 2.2 times higher than that in QF (14.80 ± 0.39 g/100 g). Furthermore, proline (10.37 ± 0.07 g/100 g) and tryptophan (1.82 ± 0.43 g/100 g) in WF are higher than those in QF (proline 4.09 ± 0.33 g/100 g and tryptophan 0.17 ± 0.88 g/100 g). It was interesting to observe that the highest amount of asparagine (20.53 ± 0.26 g/100 g) appeared in QF which is about 15 times higher than that in WF (1.38 ± 0.43 g/100 g). It is well known that asparagine is a limiting factor for AA formation via Maillard’s reaction.

Furthermore, the values of moisture, crude protein, crude fiber, crude fat, ash, carbohydrate and energy values were investigated in CQ and CW control samples as shown in Table 2. Moisture, crude protein, crude fiber, crude fat and ash are significantly (P < .05) higher in CQ samples than those in CW samples. However, the values of carbohydrate and energy in CQ are significantly (P < .05) lower than those in CW. After supplementation with different concentrations of MD from 1% to 5% (v/v),
the values of moisture, fiber and ash markedly \((P < .05)\) increased against control samples. Further increasing of MD concentration to 7% (v/v) did not appear any significant change \((P > .05)\). A previous study revealed that the addition of hydrocolloid produced slightly moister biscuits. The highest moisture contents were appeared in the D3 and D4 samples containing 5% and 7% (v/v) MD, respectively. The highest amounts of MD in treated samples could be able to capture more water molecules. As aforementioned, NMR investigation (Figures 2 and 3) confirmed the absence of branched linkages in MD synthesized by the \(L.\ mesenteroides\) and it is a linear dextran with \(\alpha-1(6)\) glycosidic bonds. Furthermore, the use of MD increased largely the viscosity values of dough. All these characteristics could contribute for the effective capturing of water molecules by MD at neutral pH.

**Water loss (WL) and inhibition of acrylamide (AA)**

Table 3 indicates the changes of water loss (WL) in all treated and control biscuits. It was found that the supplementation of dough with MD (D1→D4 samples) appeared significant changes \((P < .05)\) in % WL versus control samples. Moreover, D3 biscuit samples showed the highest \((P < .05)\) inhibition rate \((45.5\%)\) in WL. This result should be due to the formation of a polymeric network of MD, which retains large amount of water. These findings are in good agreement with moisture measurements as depicted in Table 2. Moreover, our previous studies indicated that food hydrocolloids could be able to form rigid networks on the exterior surface of food sample which could entrap water during baking.

The AA content was also investigated by LC/MS/MS technique in all control and treated samples. AA ions were detected at \(m/z\) 55 and 72. The linearity range of AA was achieved between 1.0 and 200.0 ng mL\(^{-1}\). Limit of detection (LOD) and limit of quantification (LOQ) of AA were 0.3 ng mL\(^{-1}\) and 0.7 ng mL\(^{-1}\), respectively. It was found that there is no AA in the dough before baking of biscuits which also affirmed by the previous studies. Results in Table 3 indicate that AA content in CQ control samples after baking process at 180°C was 2142 ± 3 μg kg\(^{-1}\) which was about two times higher than that in CW control samples. This result is in agreement with the presence of highest amount of asparagine in quinoa flour against wheat flour. This amount of AA is considered to be toxic based on the approval limit in bakery products by European commission regulation. On the other side, the fortification of biscuits with MD caused a significant inhibition \((P < .05)\) in AA formation (Table 3).

**Table 2.** Proximate chemical composition and caloric value (mean value ± SE, \(n = 3\)) of the studied samples.

| Sample | Moisture (g/100 g) | Crude protein (g/100 g) | Crude fat (g/100 g) | Ash (g/100 g) | Crude fiber (g/100 g) | Carbohydrates (g/100 g) | Caloric value (kcal/100 g) |
|--------|-------------------|------------------------|---------------------|--------------|----------------------|-------------------------|--------------------------|
| CW     | 3.88 ± 0.05\(^a\) | 5.02 ± 0.31\(^b\)    | 1.77 ± 0.05\(^c\)  | 0.62 ± 0.10\(^c\) | 0.45 ± 0.22\(^c\)  | 88.26 ± 0.25\(^c\)    | 389.05 ± 0.21\(^c\)     |
| CQ     | 7.23 ± 0.08\(^d\) | 14.66 ± 0.28\(^e\)  | 6.91 ± 0.23\(^f\)  | 2.17 ± 0.43\(^f\) | 8.71 ± 0.21\(^f\)  | 60.32 ± 0.51\(^f\)    | 362.11 ± 0.32\(^f\)     |
| D1     | 8.29 ± 0.32\(^c\) | 14.99 ± 0.33\(^d\)  | 6.12 ± 0.31\(^e\)  | 3.01 ± 0.42\(^e\) | 10.22 ± 0.51\(^e\) | 57.37 ± 0.25\(^e\)    | 344.52 ± 0.23\(^e\)     |
| D2     | 10.05 ± 0.12\(^c\) | 15.13 ± 0.55\(^d\)  | 6.10 ± 0.54\(^e\)  | 3.65 ± 0.12\(^e\) | 18.48 ± 0.45\(^e\) | 46.61 ± 0.46\(^e\)    | 301.78 ± 0.21\(^e\)     |
| D3     | 12.01 ± 0.13\(^c\) | 15.31 ± 0.11\(^d\)  | 6.15 ± 0.64\(^e\)  | 4.45 ± 0.14\(^e\) | 22.81 ± 0.22\(^e\) | 39.26 ± 0.68\(^e\)    | 273.67 ± 0.23\(^e\)     |
| D4     | 12.01 ± 0.14\(^c\) | 15.22 ± 0.18\(^d\)  | 6.33 ± 0.39\(^e\)  | 4.49 ± 0.16\(^e\) | 22.89 ± 0.41\(^e\) | 39.06 ± 0.12\(^e\)    | 274.09 ± 0.18\(^e\)     |

Values which don’t share the same letter in each column are significantly different \((P < 0.05)\).

**Table 3.** Water loss (WL) and acrylamide (AA) determination (mean value ± SE, \(n = 3\)) in the studied samples.

| Sample | WL % (m/m) | Inhibition rate in WL (%) | Acrylamide (μg/kg)±SD | Inhibition rate in AA (%) |
|--------|------------|--------------------------|-----------------------|--------------------------|
| CW     | 43.2 ± 0.6\(^d\) | --           | 922 ± 2\(^a\)     | --                      |
| CQ     | 27.5 ± 0.7\(^b\) | --           | 2142 ± 3\(^b\)   | --                      |
| D1     | 22.5 ± 0.1\(^c\) | 18.2         | 1453 ± 7 \(^c\)  | 32.2                    |
| D2     | 19.1 ± 0.8\(^d\) | 30.5         | 925 ± 9\(^d\)    | 56.9                    |
| D3     | 15.0 ± 0.5\(^e\) | 45.5         | 233 ± 6\(^e\)    | 89.1                    |
| D4     | 16.2 ± 0.7\(^h\) | 41.1         | 340 ± 6\(^f\)    | 88.8                    |

Different letters within a column indicate significantly different values \((P < 0.05)\).

Inhibition rates were calculated by the equations as described in experimental section.
The addition of 1% MD (D1 sample) decreased markedly ($P < .05$) the AA content to $1453 \pm 7 \, \mu g \, kg^{-1}$ dry biscuit (32.2% inhibition rate). The use of 3% MD (D2 sample) decreased significantly the amount of AA to $925 \pm 9 \, \mu g/kg$ dry biscuit (about 56.9% inhibition rate), which is lower than the EU-acceptable limit. Further significant ($P < .05$) reduction of AA amount was achieved by the addition of 5% MD (sample D3) to $233 \pm 6 \, \mu g/kg$ dry biscuit giving the highest inhibition rate of AA (89.1%). This behavior could be attributed to the presence of functional (OH) groups in MD polymer, as confirmed by FT-IR analysis (Figure 1), which is crucial for chemically cross-linked hydrogel system. In other words, the presence of hydroxyl groups in MD could motivate the formation of stable hydrogen bonding with carbonyl and/or amino group in AA at pH value of 6.8 and subsequently reduced the amount of free AA in formulated biscuits. This explanation was confirmed in non-bacterial hydrocolloid used as AA inhibitor.$^{10,15}$ Moreover, NMR investigation (Figures 2 and 3) confirmed the formation of linear dextran with α-(1-6) glycosidic bonds in MD synthesized by the *L. mesenteroides*. Furthermore, the use of MD increased largely the viscosity values of dough. All these characteristics of MD could contribute for the effective capturing of water molecules and subsequently could reduce the AA formation.

**Instrumental Texture Profile Analysis (TPA)**

Table 4 investigated the TPA of proposed biscuits. As observed, the quinoa control biscuits showed higher values of hardness, cohesiveness and springiness in comparison with wheat control biscuits. The fiber and protein high contents detected in quinoa flour compared to wheat flour could have influenced the TPA of quinoa biscuits.$^{14}$ However, the addition of MD from 1% to 7% (D1→D4 samples) in biscuit dough lowered significantly ($P < .05$) the values of hardness, cohesiveness and springiness (Table 4). The lowest hardness value was achieved at 5% (D3 sample) and 7% MD (D4 sample), which was not significantly different from 100% wheat biscuits (CW sample). This reduction in hardness could be attributed to the dilution effect. It is well-documented that water-binding properties of polysaccharides could appear a dilution effect on food system giving a softer texture.$^{30}$ These observations are correlated to the findings in D3 and D4 samples for WL values (Table 2) and moisture content (Table 1).

**Color analyses**

The values of whiteness ($L^*$), redness ($a^*$) and yellowness ($b^*$) in control and treated biscuits were shown in Table 4. It was observed that there is a color difference between CW and CQ control samples. The CQ samples had higher yellow ($P < .05$) values than CW samples because quinoa flour is yellowish in color. The $L^*$ value of CQ was lower than CW due to the possibility of Maillard’s products formation.$^{33}$ However, the addition of MD in quinoa biscuits (D1→D4 samples) rose significantly ($P < .05$) the $L^*$ values in a comparison with CQ sample. The highest $L^*$ values were achieved in the D3 and D4 samples, which were not significantly different from 100% wheat control biscuits (CW).

### Table 4. Texture profile and color analyses of biscuits (mean value ± SE, n = 3).

| Sample | Hardness (N) | Cohesiveness | Springiness (%) | $L^*$ | $a^*$ | $b^*$ |
|--------|-------------|--------------|-----------------|------|------|------|
| CW     | 36.18 ± 0.76 f | 0.61 ± 1.21 c | 61.61 ± 1.32 c  | 50.20 ± 1.05 a | 11.72 ± 0.82 a | 12.98 ± 0.52 a |
| CQ     | 53.18 ± 0.07 g | 0.72 ± 1.32 b | 72.53 ± 1.24 f  | 34.85 ± 1.05 d | 11.17 ± 0.26 d | 15.97 ± 0.53 d |
| D1     | 50.61 ± 0.33 c | 0.68 ± 1.76 f | 70.66 ± 1.27 f  | 40.84 ± 1.40 a | 11.17 ± 0.60 d | 15.97 ± 0.51 d |
| D2     | 46.43 ± 0.11 i | 0.64 ± 1.33 a | 64.46 ± 1.41 e  | 46.67 ± 0.03 j | 11.18 ± 0.60 d | 15.97 ± 0.78 d |
| D3     | 36.09 ± 0.71 f | 0.57 ± 1.31 g | 60.31 ± 1.44 b  | 51.72 ± 1.26 c | 11.16 ± 0.02 d | 15.97 ± 0.53 d |
| D4     | 36.20 ± 0.34 f | 0.58 ± 1.13 g | 60.90 ± 1.01 b  | 52.18 ± 0.70 e | 11.15 ± 0.13 d | 15.97 ± 0.73 d |

Averages followed by distinct letters, on the column, differed significantly one from another ($P < 0.05$).
However, the values of $a^*$ and $b^*$ ranged among the treated quinoa biscuits (D1→D4 samples) were not significantly changed ($P > .05$). This reflected the absence of relationship between $a^*$ and $b^*$ values and the MD concentrations in quinoa biscuits.

**CONCLUSION**

Microbial dextran (MD) synthesized by *L. mesenteroides* NCIM-2198 was successfully used to mitigate acrylamide formation in quinoa biscuits. FT-IR and NMR studies confirmed that the synthesized MD is a linear dextran with α-(1-6) glycosidic bonds. The addition of 5% MD decreased significantly ($P < .05$) AA content to 233 ± 6 µg kg$^{-1}$ dry biscuit with 89.1% inhibition rate, which is lower than the European council benchmark level of AA in biscuits (350 µg kg$^{-1}$). This is the first time to achieve this high level of AA mitigation in quinoa baked product using a simple mitigation approach. The effect of MD fortification by improving the water-binding capacity as well as the presence of functional (OH) groups in MD polymer could be the effective reasons for mitigation of AA formation in quinoa biscuits. The current study could motivate the food researchers to find out the physicochemical characterization of microbial hydrocolloids for developing the quality of bakery products and the mitigation or prevention of toxic chemicals formation in food products.

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No potential conflict of interest was reported by the author(s).

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