Milk casein modification in response to dual oxidative enzymatic system

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ABSTRACT

Enzymatic cross-linking of proteins refers to the formation of intra- and inter-molecular cross-links by biocatalysts. Thus, the purpose of present work was to evaluate whether the dual oxidative enzymatic systems could be used for enhancing the functional properties of casein in order to use it as a texture improver. The results showed that peroxidase in the crude extract of moringa (Moringa oleifera) leaves exhibits 1342.19 specific activity (U/mg protein), while at 20% saturation of ammonium sulfate had specific activity of 1617.14 (U/mg protein) within 14.03% yield and 1.21 purification fold. Also, oxidative cross-linked casein clearly had higher relative di-tyrosine intensity than control casein. The control casein had the smallest particle size (272.121 μm) but other samples possessed enlarged particle size ~392.48-1294.60 μm which confirmed with the electrophoretic profile using SDS-PAGE. Moreover, the acid curd contains cross-linked casein had greater viscosity and lower syneresis than control. The total amino acids (TAA), essential amino acids (EAA), and TAA/EAA ratio of acid curd with enzymatic cross-linked casein were higher than control curd. Protein efficiency ratio and biological value of both CMII and CMIII treatments were higher than control curd. Also, CMIII treatment had the highest chemical score of valine, histidine, isoleucine, and leucine; while control acid curd had the highest chemical score of threonine only. It could be concluded that dual oxidative enzymatic system improved the functional properties of casein which could be used as texture improver without any negative impact on the nutritional value of such product.

Keywords: Casein; peroxidase; Glucose oxidase; Crosslinking; Functional property; Moringa.

INTRODUCTION

Cross-linked protein consequently experiences the property changes as a result of the altered spatial configuration. Enzyme-mediated cross-linking is one of the studied methods for cross-linking proteins, as well as it seems to be a promising approach for changing both spatial designs and physicochemical characteristics.

However, a few transferases and oxidoreductases are suitable for prompting protein cross-linking (Wu et al., 2016). The application of transglutaminase (TGase, EC 2.3.2.13) in protein cross-linking has received sufficient evaluation. Thus, TGase-mediated cross-linked milk proteins showed improving of yoghurt thickness (Ibrahim et al., 2017a; Chen et al., 2018). Then again, whey protein hydrolysate would do well to interfacial properties and less in vitro antigenicity (Yu et al., 2019). Also, oxidoreductases enzymes (e.g., laccase and peroxidase) could be cause proteins to change their properties and form covalent cross-links; For instance, according to Stuchell and Krochta (1994), enzymatic cross-linking approach of soybean protein isolate (SPI) using horseradish peroxidase (HRP, EC 1.11.1.7) might be used for enhancing their film strength. However, HRP, TGase, and laccase (EC 1.10.3.2) have potential impact for protein modification. HRP is a well-known enzyme among mainstream researchers, and it has generally been studied for its use in different fields such as wastewater treatment. HRP with H2O2 and hydrogen benefactors of low sub-atomic weight, could incite the cross-linking of α-lactalbumin (Dhayal et al., 2014). This kind of protein cross-linking is well-known to have a working mechanism. Di-tyrosine residues resulted from the oxidation of proteins' tyrosine residues in the presence of H2O2, which subsequently prompts covalent protein cross-linking (Dhayal et al., 2015). However, HRP is inactivated at a higher H2O2 concentration (Lan et al., 2006), and as a result, produces less dityrosine. The level of H2O2 in the protein cross-linking reaction system must be effectively controlled. D-glucose can be oxidized by glucose oxidase (EC 1.1.3.4) to produce H2O2 and gluconic acid. It is evident that HRP can simultaneously use the generated H2O2 to induce protein cross-linking through the formation of dityrosines (Heijnis et al., 2011). As a result, dual enzymes including HRP and glucose oxidase with D-glucose form a ternary system that has potential impact for protein cross-linking.

However, this ternary system had only altered a small number of protein components, such as SPI, and evaluated their property changes (Chang and Zhao, 2012; Jiang and Zhao, 2014); whether this dual oxidative enzymes (e.g., peroxidase and glucose oxidase with D-glucose) has potential approach for inducing changes of milk casein has not been investigated so far.

In the present work, milk casein was modified using dual oxidative enzymes including partial purified peroxidase from moringa (Moringa oleifera) leaves and glucose oxidase in the presence of glucose. The conformational alters including di-tyrosine content, free sulfhydryl groups assay, particle size determination, and electrophoretic profile were assessed. Also, functional and nutritional characteristics of enzymatic modified casein samples were investigated. Hence, the purpose of present work was to determine whether the dual oxidative enzymatic systems could be used for enhancing the functional properties of casein in order to use it as a texture improver for foods.

MATERIALS AND METHODS

Materials and chemicals

Casein was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Ammonium sulfate was obtained from SD fine-chem limited, Mumbai, India. Skim milk powder (SMP) was purchased from BIELMLEK Spoldzielnia mleczrska, Poland. Moringa (Moringa oleifera) leaves was obtained and identified from the Egyptian Scientific Society of Moringa, National Research Centre (NRC), Cairo, Egypt. Fresh cow and skim milk were obtained from Animals Production Research Institute, Giza, Egypt. Yoghurt starter culture consisted of the mixture of Streptococcus thermophilus and Lactobacillus bulgaricus were obtained from Egyptian microbial culture collection, (MIRCEN), Ain Shams University. Dye Coomassie brilliant blue G-250 was purchased from MP Biomedical, LLC, and Germany. Standard amino acids and polyvinylpyrrolidone (PVP) were obtained from Sigma-Aldrich, Steinheim, Germany. Other chemicals and reagents were used in this study were of analytical grade.

Methods

Extraction and purification of peroxidase

Extraction of peroxidase from the fresh leaves (0.5 g/ml) of moringa (Moringa oleifera) was performed according to Khatun et al. (2012) using 100 mM Tris-HCl pH 7.5 with 0.1% PVP as an extraction buffer. After macerating for 24 h/5 °C, the extract was filtrated through gauze and centrifugation (5000 rpm, 20 min, 5 °C), the recovered fractions were pooled and considered as a crude enzymatic extract, and then peroxidase activity and protein content were determined. Peroxidase of crude extract was partial purified using ammonium sulphate procedure according to Colowick and Kaplan (1955). The rich peroxidase active fraction was dialyzed against 100 mM Tris-HCl pH 7.5 for 24 h/ 5 °C. Peroxidase activity, and protein content of the recovered fractions were determined which served as a partial purified peroxidase.

Determination of peroxidase activity

The peroxidase enzyme assay was carried out using guaiacol as a substrate according to Sakharov et al. (2000) by spectrophotometer (UV 1201-vis spectrophotometer SHIMDZU, Japan) at OD 470 nm. One unit of peroxidase activity (U) was defined as the amount of enzyme that caused the oxidation of 1 μmole of guaiacol as a substrate per min under standard conditions.

Determination of protein content

Protein content of the crude and purified enzyme fractions were determined calorimetrically at Optical Density 595 nm using Coomassie brilliant blue G-250 dye according to Bradford (1976). The reaction mixture was composed of 10 μL of enzyme extract, 490 μl distilled water, and 500 μL of Coomassie brilliant blue G-250 dye was added. The developed color was measured at OD 595 nm, using spectrophotometer.

Peroxidase specific activity

The peroxidase specific activity was calculated and expressed as units (U) of peroxidase activity per mg of protein of each fraction.

Preparation of cross-linked casein

Casein was modified with dual oxidative enzymes including peroxidase extracted from moringa (Moringa oleifera) leaves, and glucose oxidase enzyme with glucose in one-step treatment as described by Chang and Zhao (2022). The dissolved casein was mixed with glucose oxidase (6 U) and glucose (0.05 mmol) per gram protein, respectively. Afterwards, the moringa peroxidase was added to the whole reaction system at the level of 100, 200, and 300 U/g protein, which namely CMI, CMII, CMIII, respectively, which kept at 37 °C for 3 h, and

then heated at 85 °C for 10 min. The resulted treated casein samples were lyophilized for further analysis.

Relative dityrosine, free sulfhydryl groups assay

The relative dityrosine contents of the casein samples were measured and expressed as described by Ke and Huang (2016). In brief, each casein samples (10 mg/ml) were suspended with 0.2 mM phosphate buffer pH 7.5 and measured at a fluorescence spectrometer (F-4500, Hitachi Co., Tokyo, Japan) using 320/420 mm of excitation/emission wavelengths. The value of fluorescence intensity was used to reflect relative dityrosine content.

The assay contents of free sulfhydryl groups as described by Qin et al. (2016), and Chen et al. (2019). To determine the content of free sulfhydryl groups, the casein sample of 30 mg/ml was suspended in buffer A (pH 8.0) containing 0.2 mol/L Tris-HCl, 8 mol/L urea, 10 mmol/L dinitrosalicilic acid, 3 mmol/L EDTA, and 1% sodium dodecyl sulfate (SDS). The whole system was kept at 5 °C for 2 h in dark and then centrifuged at 5000 rpm for 15 min to remove the particulates. Aliquot supernatant of 0.42 ml was dissolved with 5.58 ml buffer B (pH 8.0) containing urea, EDTA, SDS, and Tris-HCl with previous mentioned concentrations, and then the absorbance was read at 412 nm using a spectrophotometer.

Particle size determination of casein samples

Particle size of each casein sample was performed and expressed as intensity particle size distribution and average particle size (μm) at 20 °C using a particle size analyzer [Mastersizer 2000, Malvern Instruments Ltd., Malvern, UK].

Electrophoresis of casein samples

Molecular weight changes of casein samples were detected using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) procedure according to Laemmi (1970). The casein samples were loaded for electrophoresis at 50 V which performed in a vertical slab gel apparatus in 12.5% polyacrylamide separating gel, 4% stacking gel. Casein samples were visualized by Coomassie brilliant blue R–250. The pre-stained standard proteins in the range of 10–250 kDa were used as a molecular weight marker.

Functional and nutritional characteristics of casein samples

For functional and nutritional evaluation, the enzymatic cross-linked casein samples were acidified using yoghurt starter culture (2%) consisted of Streptococcus thermophilus and Lactobacillus bulgaricus and then incubated at 42 °C until the coagulum is formed, as well as the total solids was increased using 6% skim milk powder for all prepared samples. Three acid curd from enzymatic cross-linked casein samples (CMI, CMII, and CMIII) compared to control casein were prepared for further analysis.

Apparent viscosity

The apparent viscosity of acid curds samples was performed using Brookfield viscometer (LVT, Brookfield Engineering Inc., Stoughton MA) by spindle 5 at share rate 20-100 (sec-1) and 20 °C.

Syneresis

For syneresis determination, the acid curds samples were centrifuged at 1000 rpm for 20 min. The separated serum was determined, and syneresis (%) was calculated as the mass of released serum related to the total mass of the curd before centrifugation.

Amino acids profile

The amino acids profile of casein acid curds were determined using HPLC-Pico-Tag method according to Heinrikson and Meredith (1984), White et al. (1986), and Cohen et al. (1989). The Pico-Tag method was developed commercially by Waters Associates which was an integrated technique for amino acids analysis. Phenylisothiocyanate (PITC, or Edman's reagent) was used for pre-column derivatization, while reversed-phase gradient elution high-performance liquid chromatography (HPLC) separates the phenylthiocarbamyl (PTC) derivatives which were detected by their UV absorbance. The sample corresponding to the protein ratio was weighted into 25 × 150 mm hydrolyzed tube using 6 N HCl and placed in 110 °C oven for 24 h, then tube was removed from the oven and allow to cool. The tube contents were quantitatively transferred to volumetric flask and diluted with HPLC grade water. The diluted hydrolysate was filtered through 0.45 μm sample filter. Aliquots of hydrolysate, together with appropriate standards, were placed in disposable glass sample tubes from Waters Associates. The samples were placed into Pico-Tag amino acids Workstation (Waters, USA) for sample preparation (Drying, redrying and derivatization) using Waters reagents. The chromatographic analysis was

carried out using HPLC with Pico-Tag amino acids method. The Liquid chromatography apparatus was equipped with 600 E Multisolvent Delivery System and the following gradient of Pico-Tag solvent A and B (Waters Eluent A and B) at 38 °C, flow rate 1 ml/min. Twenty microliter of sample was injected and loaded on amino acids column Pico-Tag amino acids (150 x 3.9 mm, stainless steel) using linear gradient elution. Detection of the PTC derivatives is by ultraviolet absorption measurements using a fixed wavelength 254 nm (2489 UV/Vis Detector). Before injecting of the sample, the illustrated was calibrated by two injections of the lysine standards.

Calculation of chemical score, protein efficiency ratio and biological value

The chemical score of samples based on their amino acid content was calculated according to Bhanu et al., (1991), as follows: Chemical score = (mg of amino acid in 1g test protein/ mg of amino acid in 1g reference protein) × 100

The protein efficiency ratio (PER) of samples based on their amino acid content was calculated using the equation suggested by Alsmeyer et al. (1974), as follows: PER = – 0.468 + 0.454 (leucine) – 0.105 (tyrosine)

The biological value (BV) of samples based on their amino acid content was calculated using the equation suggested by Oser (1959), as follows: BV = 49.09 + 10.53 (PER)

RESULTS AND DISCUSSION

Extraction and purification of peroxidase

The peroxidase enzyme was detected in the crude extract of moringa (Moringa oleifera) leaves using guaiacol as a substrate with 1342.19 specific activity (U/mg protein) as presented in Table 1. Afterwards, the peroxidase was precipitated using ammonium sulfate precipitation (ASP) procedure in order to partial purify and concentrate. The ASP results showed that the peroxidase was detected in the fraction with 20% saturation of ammonium sulfate with specific activity of 1617.14 (U/mg protein). Also, Table 1 summarizes all purification steps which compared the crude extract with the rich fraction of peroxidase resulted from ASP procedure. The crude extract had total activity of peroxidase of 5766.50 U compared to 808.60 U for ASP fraction. Also, ASP fraction had 14.03% yield of peroxidase with 1.21 purification fold.

Relative dityrosine, free sulfhydryl groups assay

Relative dityrosine contents were detected in enzymatic cross-linked casein (CMI, CMII, and CMII) using dual oxidative enzymatic system (moringa peroxidase and glucose oxidase) compared to control casein. Fig. 1 shows that all enzymatic cross-linked casein clearly detected higher relative dityrosine intensity than control casein. It could be mainly due to the action of dual oxidative enzymatic system which induces higher protein cross-linking extent in dityrosine form, as well as the casein is suitable substrate for such oxidative enzymes due to their conformation form. Moreover, the treatment protocol as a one-step treatment is more efficient. However, one step enzymatic treatment resulted higher cross-linking in WPI and SPI which treated with ternary enzymatic system (Chang and Zhao, 2012; Jiang and Zhao, 2014; Yang and Zhao, 2022). Also, caseinate treated by one step procedure has greater dityrosine content than WPI which confirms the present results of casein as a good substrate for such oxidative enzymes with the one-step procedure (Chang and Zhao, 2012).

The obtained results also demonstrated that all enzymatic cross-linked casein had lower free sulfhydryl groups intensity than control casein (Fig. 2). It could be due to the heating and oxidizing condition by H2O2 formation during the dual oxidative enzymatic system (moringa peroxidase and glucose oxidase) treatment, which expedited the proteins cross-linking and oxidation of free sulfhydryl groups. Yang and Zhao (2022) reported that the conducted cross-linking using ternary enzymatic system led to decrease free sulfhydryl groups as well as increase disulfide bonds in modified WPI than control WPI using one-step protocol. Also, Jiang and Zhao (2014) reported that the ternary system-induced cross-linking of SPI increased the disulfide bonds formation as well as decreased free sulfhydryl groups in such protein.

Fig. 3 shows that the particle size of enzymatic cross-linked casein using dual oxidative enzymatic-induced casein in comparison of control casein. The findings showed that untreated casein has the smallest particle size (272.121 μm) but other cross-linked casein (CMI, CMII, and CMII) possessed enlarged average particle size (392.48, 1112.58, and 1294.60 μm, respectively). It could be due to the formation of protein polymers resulted from dual oxidative enzymes-induced cross-linking action on

casein as a substrate (Wen-qiong et al., 2017). This finding confirmed the detected intensity of dityrosine content as shown in Fig. 1. Liu et al. (2019) and Wang et al. (2020) reported that TGase-induced the cross-linking of whey proteins using TGase led to increase their molecular mass, as well as modified WPI using ternary enzymatic system had higher particle size values than control WPI (Yang and Zhao, 2022).

Electrophoresis of casein samples

Electrophoretic profile of enzymatic cross-linked casein using dual oxidative enzymatic system shows that protein polymers had greater molecular weight molecules (lanes 1, 2, and 3) than control casein (lane 4) (Fig. 4). Also, the aggregates proteins increased with the moringa peroxidase level increased (lane 3< lane 2< lane 1). It means that the enzymatic modification of casein using such dual oxidative enzymatic system led to aggregate the protein molecules which form protein polymers; it confirms the dityrosine intensity (Fig. 1) particle size distribution (Fig. 3) of oxidative-induced casein compared to control casein.

Functional and nutritional characteristics of casein samples

Enzymatic cross-linked casein using dual oxidative enzymatic system (moringa peroxidase and glucose oxidase) were used in the preparation of acid curd (Fig. 5) as described in materials and methods part in order to measure their apparent viscosity (Fig. 6) and syneresis (Fig. 7).

The results demonstrated that acid curd contains (all enzymatic cross-linked casein) had greater apparent viscosity than acid curd contains control casein. Also, the apparent viscosity increased with the moringa peroxidase level increased (CMI< CMII< CMIII). It could be due to the formation of protein polymers resulted from dual oxidative enzymes-induced cross-linking action. This result confirms the previous findings in the present study regarding to relative dityrosine contents (Fig. 1), particle size distribution (Fig. 3), and electrophoretic profile of casein (Fig. 4). However, Jiang et al. (2017) reported that SPI cross-linked by peroxidase showed increased apparent viscosity. Also, Truong et al. (2004) that the TGase-induced cross-linking of α-lactalbumin and β-lactoglobulin resulted for viscosity increasing. On the other hand, Fig. 7 shows that oxidative enzymatic-induced casein decreased the syneresis of the resulted acid curd compared to control which mainly due higher viscosity of such treated curd resulted from enzymatic modified casein were used during their preparation compared to control. These present findings showed that the dual oxidative enzymatic modification of casein considers efficiently improved rheological properties of casein in order to use it as modified casein (food additive) in dairy and non-dairy processing.

Table 2 shows that the amino acids profile of acid curd with casein treated by dual oxidative enzymatic system. The results showed that the total amino acids (TAA), essential amino acids (EAA), and the ratio of TAA/EAA of acid curd with casein treated by dual oxidative enzymatic system were higher than control curd. CMII treatment had the highest TAA, while CMIII treatment had the highest EAA and TAA/EAA ratio which close to CMII treatment, among other acid curd samples (Table 2). It could be mainly due to varied content of individual amino acids of such treatments. CMII treatment had the highest content of lysine, phenylalnine, methionine, serine, and aspartic acids.

It is well known fact that the liberation of individual amino acids in cheese is primarily controlled by starter peptidases and the degree of liberation of amino acids varies depending on the starter enzyme systems and degree of autolysis in cheese (Lane and Fox, 1996). In the present study, the same starter bacteria were used in the production of acid curd. Therefore, no difference was expected in terms of release of enzyme from the starter bacteria and/or degree of starter autolysis in acid curd; except for the oxidative enzymatic modification which affected the individual amino acids profile of the resulted acid curd to some extent. Similar findings were also reported by Di Pierro et al. (2010), Özer et al. (2013), and Ibrahim et al. (2017b) who used TGase in the modification of milk proteins in the cheese production.

Chemical score is a comparison of the amount of the limiting amino acid in a food with the amount of that same amino acid in a reference food. CMIII treatment had the highest chemical score of valine, histidine, isoleucine, and leucine; followed by CMII treatment which had the highest chemical score of methionine + cysteine, phenylalanine + tyrosine, and lysine, while control acid curd had the highest chemical score of threonine only as shown in Fig. 8.

Biological value (BV) measures the proportion of absorbed nitrogen which is

retained and presumably utilized for protein synthesis and therefore reflects true protein quality. PER (Fig. 9) and BV (Fig. 10) of both CMII and CMIII treatments were higher than control acid curd. It could be due to the amino acids content including leucine and tyrosine. It means the enzymatic treatment of casein which used to prepare this acid curd have no negative impact on the nutritional value of the resulted protein in such product.

CONCLUSION

It could be concluded that dual oxidative enzymatic system improved the functional properties of casein which could be used as enzymatic modified casein in order to improve the food texture without any negative impact on the nutritional value of such product.

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Table 1: Purification steps summary of peroxidase from *M. oleifera* leaves.

Purification step	Total peroxidase activity (U)	Total protein (mg)	Specific activity (U/mg protein)	Yield (%)	Purification fold
Crude extract	5766.50	4.30	1342.19	100	1.0
ASP	808.60	0.50	1617.14	14.03	1.21

Specific activity= Enzyme activity/Protein content; Yield= Total activity of purified enzyme/Total activity of crude enzyme X 100; Purification fold= Specific activity of purified enzyme/Specific activity of crude enzyme.

Table 2: Amino acids profile of acid curd with casein treated by dual oxidative enzymatic system.

Amino acids (mg/g protein)	Acid curd treatments			
	Control	CMI	CMII	CMIII
Aspartic acid	2.88	3.23	4.47	3.73
Glutamic acid	5.20	6.73	6.11	6.33
Serine	2.97	4.59	4.81	3.72
Glycine	1.73	2.64	2.34	2.52
Histidine	3.70	4.69	5.17	7.30
Arginine	3.11	3.71	3.41	3.73
Threonine	9.36	6.19	7.20	5.83
Alanine	4.45	3.66	3.42	4.63
Proline	4.58	5.01	4.75	2.79
Tyrosine	4.14	3.54	3.01	3.41
Valine	2.20	1.85	2.21	2.45
Methionine	5.98	5.99	6.61	6.38
Cysteine	0.72	0.46	0.45	0.58
Isoleucine	1.94	1.48	1.58	2.74
Leucine	2.85	2.28	2.65	2.91
Phenylalanine	8.20	9.31	9.96	7.25
Lysine	7.91	7.05	8.48	7.86
Total EAA	35.89	36.36	40.07	40.62
Total amino acids (TAA)	71.92	72.41	76.63	74.16
EAA/TAA ratio	0.49	0.50	0.52	0.55

Control: control acid curd; CMI: enzymatic modified acid curd with treated casein by peroxidase (100 U/g protein); CMII: enzymatic modified acid curd with treated casein by peroxidase (200 U/g protein); CMIII: enzymatic modified acid curd with treated casein by peroxidase (300 U/g protein). EAA, essential amino acids; TAA, total amino acids.

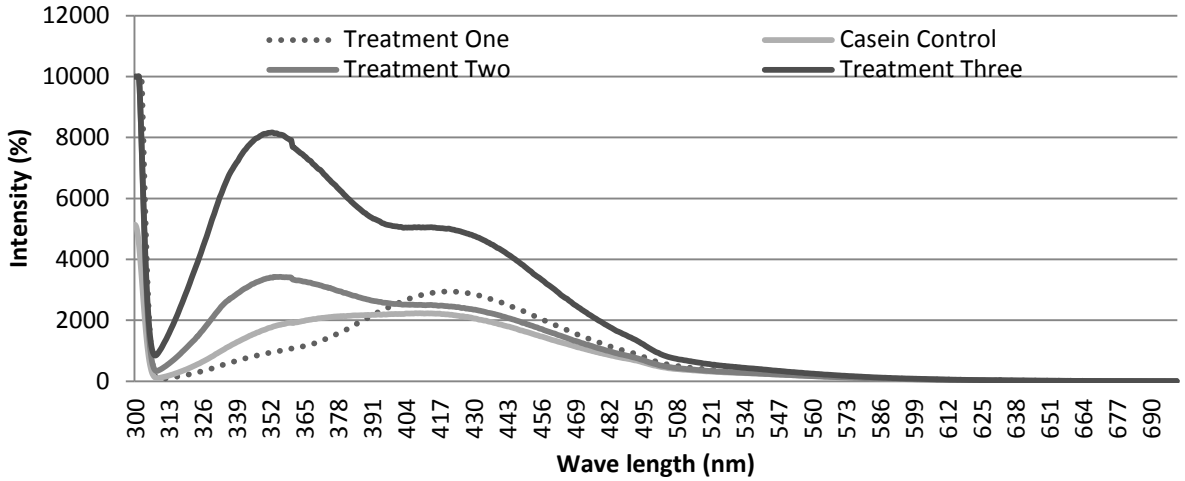


Figure 1: Relative dityrosine content of casein treated with dual oxidative enzymatic system.

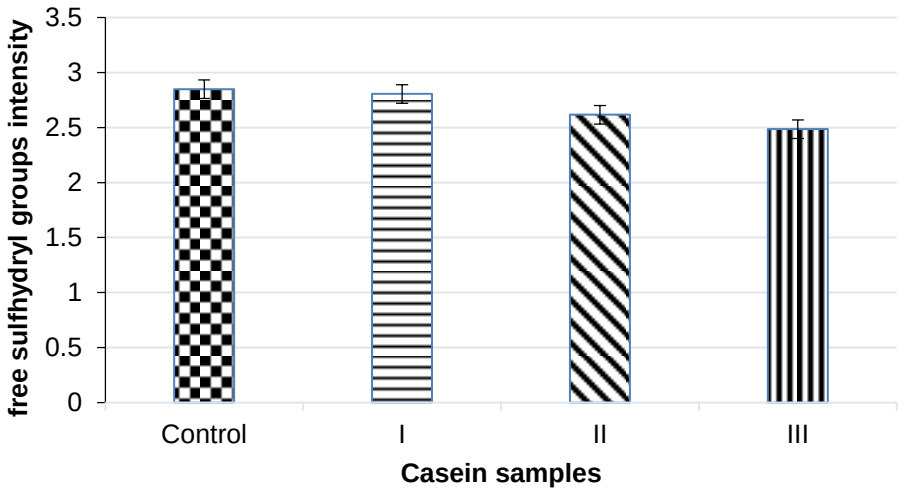
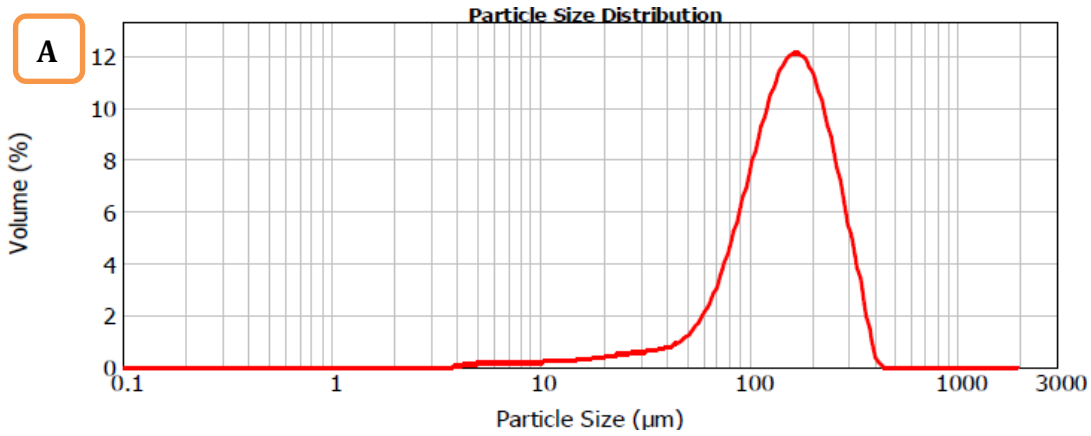


Figure 2: Free sulfhydryl of casein treated with dual oxidative enzymatic system.

Particle size of casein samples



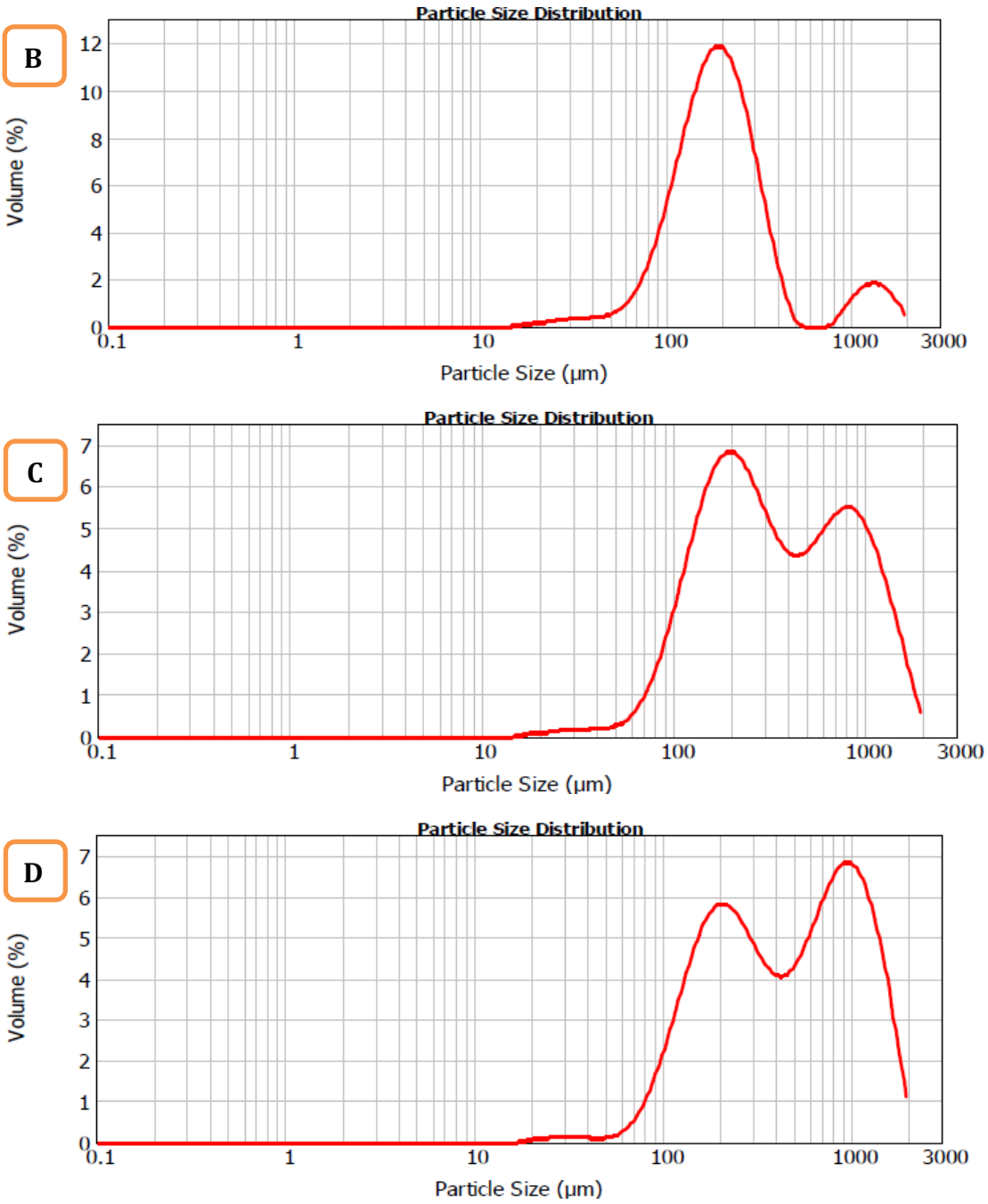


Figure 3: Particle size of casein treated with dual oxidative enzymatic system.
A: control casein; B: enzymatic modified casein with peroxidase (100 U/g protein); C: enzymatic modified casein with peroxidase (200 U/g protein); D: enzymatic modified casein with peroxidase (300 U/g protein).

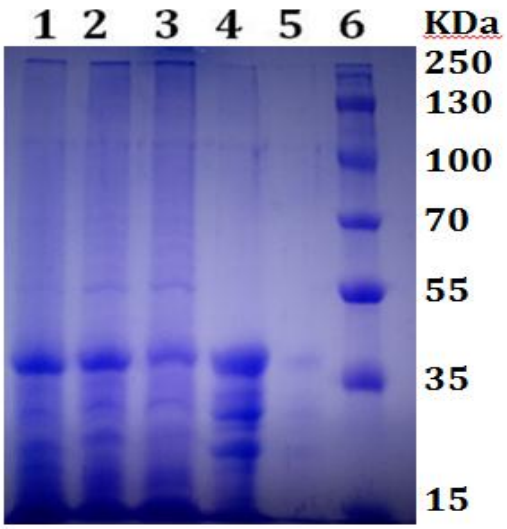


Figure. 4: Electrophoresis of casein treated with dual oxidative enzymatic system.

Lane 1: enzymatic modified casein with peroxidase (300 U/g protein); lane 2: enzymatic modified casein with peroxidase (200 U/g protein); lane 3: enzymatic modified casein with peroxidase (100 U/g protein); lane 4: control casein (positive control); lane 5: phosphate buffer (negative control); lane 6: molecular weight marker.

Figure 5: Photo of enzymatic treated casein powder (a) using dual oxidative system and resulted acid curd (b).

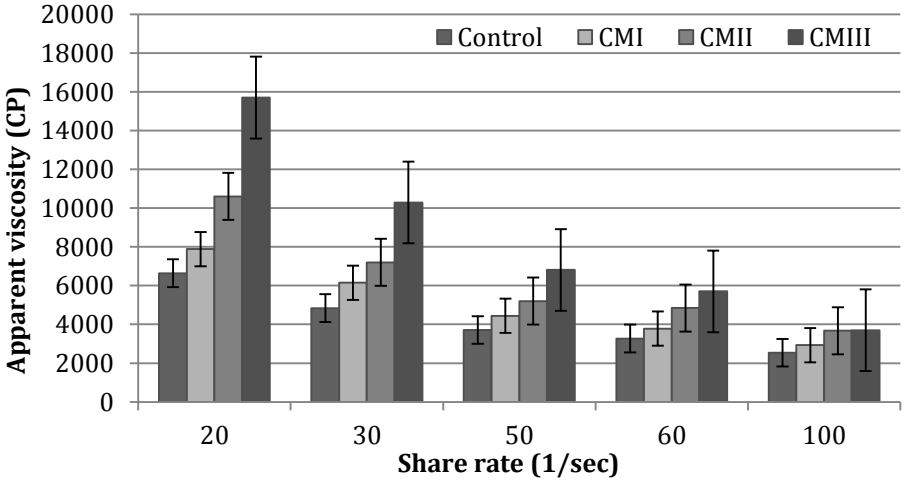


Figure 6: Apparent viscosity of acid curd with casein treated by dual oxidative enzymatic system.
Control: control acid curd; CMI: enzymatic modified acid curd with peroxidase (100 U/g protein); CMII: enzymatic modified acid curd with peroxidase (200 U/g protein); CMIII: enzymatic modified acid curd with peroxidase (300 U/g protein).

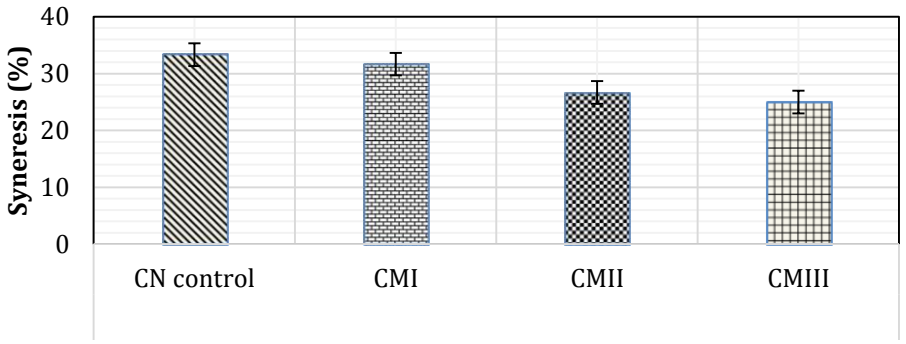


Figure 7: Syneresis of acid curd with casein treated by dual oxidative enzymatic system.
Control: control acid curd; CMI: enzymatic modified acid curd with peroxidase (100 U/g protein); CMII: enzymatic modified acid curd with peroxidase (200 U/g protein); CMIII: enzymatic modified acid curd with peroxidase (300 U/g protein).

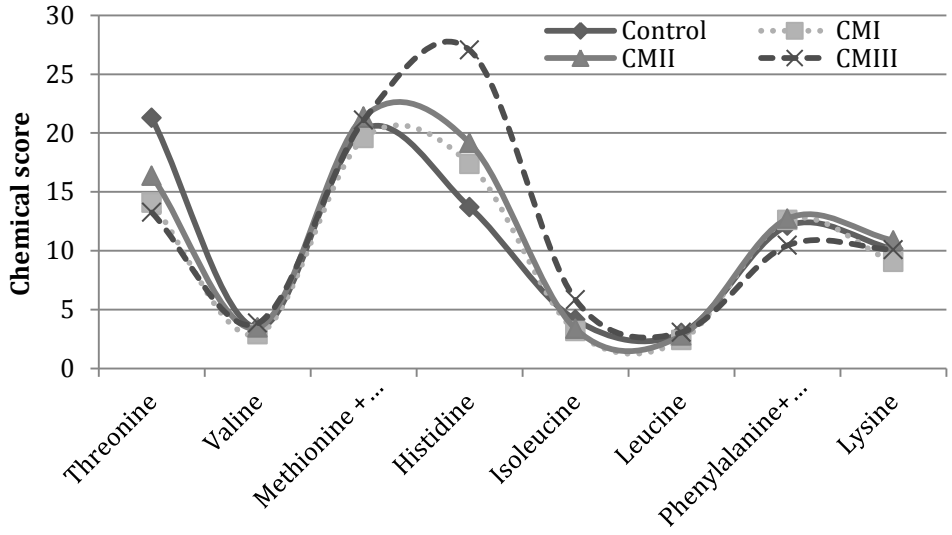


Figure 8: Chemical score of acid curd with casein treated by dual oxidative enzymatic system.
Control: control acid curd; CMI: enzymatic modified acid curd with treated casein by peroxidase (100 U/g protein); CMII: enzymatic modified acid curd with treated casein by peroxidase (200 U/g protein); CMIII: enzymatic modified acid curd with treated casein by peroxidase (300 U/g protein).

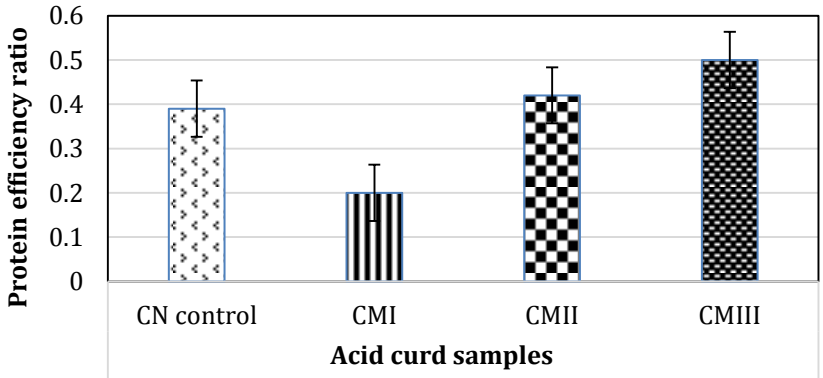


Figure 9: Protein efficiency ratio of acid curd with casein treated by dual oxidative enzymatic system.
CN control: control acid curd; CMI: enzymatic modified acid curd with treated casein by peroxidase (100 U/g protein); CMII: enzymatic modified acid curd with treated casein by peroxidase (200 U/g protein); CMIII: enzymatic modified acid curd with treated casein by peroxidase (300 U/g protein).

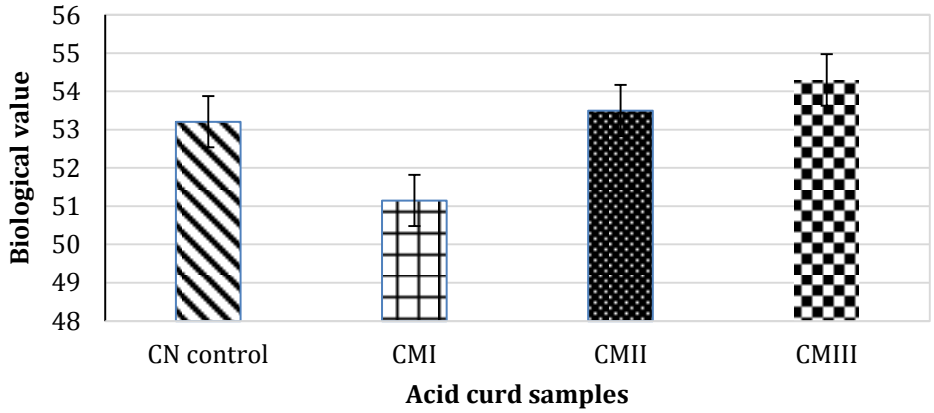


Figure 10: Biological value of acid curd with casein treated by dual oxidative enzymatic system.
CN control: control acid curd; CMI: enzymatic modified acid curd with treated casein by peroxidase (100 U/g protein); CMII: enzymatic modified acid curd with treated casein by peroxidase (200 U/g protein); CMIII: enzymatic modified acid curd with treated casein by peroxidase (300 U/g protein).

التعديل في كازين اللبن كاستجابة للنظام الإنزيمي المؤكسد المزدوج

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الملخص العربي

يشير الارتباط التشابكى الإنزيمي إلى تكوين روابط متشابكة داخل وبين جزيئات البروتينات بواسطة الإنزيمات. و تهدف الدراسة الحالية الي تقييم النظام الإنزيمي المؤكسد المزدوج (CMI و CMII و CMIII) في قدرته على تحسين الخصائص الوظيفية للكازين من أجل إستخدامه كمحسِّن للقوام. أظهرت النتائج أن النشاط النوعى للبيروكسيديز في المستخلص الخام لأوراق المورينجا (*Moringa oleifera*) 1342.19 وحدة/ملجم بروتين، بينما عند تشبع 20٪ من كبريتات الأمونيوم كان له نشاط نوعى 1617.14 وحدة/ملجم بروتين، تصافى 14.03٪ و 1.21 مرات التنقية. أيضًا، من الواضح أن الكازين المتشابك بالإنزيمات المؤكسدة أعلى نسبيًا فى شدة إمتصاص جزيئات الداى تيروزين الناتجة من الكازين المقارن. كذلك فإن الكازين المقارنة أصغر حجم جزيئى (272.121 ميكرومتر) لكن عينات الكازين المعملة إنزيمياً لديها حجم جزيئى أكبر يتراوح ما بين 392.48-1294.60 ميكرومتر والذي تم تأكيده باستخدام التفريد الكهربي باستخدام SDS-PAGE. كذلك, فإن الخثرة الحامضية التى تحتوى على على الكازين المتشابك إنزيمياً ذات لزوجة عالية وأقل إنفصال للشرش بالمقارنة بالخثرة التى تحتوى على الكازين الغير معامل إنزيمياً. كانت الأحماض الأمينية الإجمالية (TAA) والأحماض الأمينية الأساسية (EAA) ونسبة TAA / EAA للخثرة الحامضية مع الكازين المتشابك إنزيمياً أعلى من الخثرة المقارنة. كانت نسبة كفاءة البروتين والقيمة البيولوجية لكل من معاملات CMII و CMIII أعلى من خثرة المقارنة. أيضا، كان للمعاملة CMIII أعلى Chemical score من كل من الفالين، الهيستيدين، الآيزوليوسين، والليوسين. بينما الخثرة الحامضية المقارنة أعلى Chemical score من الثريونين فقط. وبذلك يمكن الاستنتاج أن النظام الإنزيمي المؤكسد المزدوج يمكن أن يحسن الخواص الوظيفية للكازين ولذلك يمكن إستخدامه كمحسّن للقوام دون أي تأثير سلبي على القيمة الغذائية للمنتج.

الكلمات الاسترشادية: الكازين، البيروكسيديز، أوكسيديز الجلوكوز، التشابك، المورينجا.