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HEAT STABILITY AND SOLUBILITY OF CAMEL WHEY PROTEIN ISOLATE CONJUGATED WITH GUM ARABIC UNDER WET HEATING CONDITION BY MAILLARD REACTION

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Abstract

The structure of the protein is usually enhanced with enzyme and heat treatment. Another way to improve the functional properties of the final product is a covalent protein with polysaccharide during the Maillard reaction. We aimed to investigate the impacts of pepsin enzyme on camel whey protein (CWP). To reach this goal, covalent linkage of 2% (w/v) camel whey protein isolated and 0.5%, 1% and 2% (w/v) Gum Arabic were analysed during wet heat treatment (15 min at 85°C). During the Maillard reaction, the grafting of CWPI to GA was confirmed by Fourier transformation infrared spectra (FT-IR) and UV-vis spectroscopy. The formation of Amadori and browning compounds was then measured. The solubility properties of CWP improved by adding pepsin enzyme (1:100) during 360 min. The solubility of all samples, therefore, were compared to CWPI over the wide range of pH. Grafting of CWPI and GA was then compared with CWPI. Indicated high heat stability, especially at the pH near isoelectric point (pI) when subjected to 95°C for 30 min. The results show that pepsin enzyme, wet heating conditions and GA, significantly boosted the properties of pure CWP. In conclusion, the optimal samples were related to conjugate of CWPI with 0.5% GA.

Keywords: Camel milk isolate, Conjugate, Gum arabic, Maillard reaction, Whey protein.

1. Introduction

Numerous nations consume camel milk for its diverse medicinal characteristics and broad use for human disease [1]. Based on the Statistical Centre of Iran, around 149,600 camels live in the Yazd, Kerman, Sistan-Baluchestan, Isfahan, and Semnan provinces [2]. Beneficial properties of camel milk are anti-carcinogenic, anti-diabetic, and anti-hypertensive [3]. The majority of these properties are attributed to proteins, particularly CWP [1].

Whey is one of the most valuable sources of functional proteins and consists of the water-soluble part of milk. The main proteins in whey which is named whey protein (WP), mostly are composed of globular proteins, α-lactalbumin (α-La), β-lactoglobulin (β-Lg), and serum albumin [4]. Bovine and CWP composition are a significant difference. In bovine milk α-La (20%) and β-Lg (>50%) are the main part of whey proteins. while, in the camel whey milk, it doesn't have β-Lg and α-La, lactophorin, Camel serum albumin (CSA), and immunoglobulins are the most plentiful proteins. Lack of β-Lg in camel milk affects the behaviour of these proteins [1].

The functional qualities of proteins (such as digestibility, hydrolysis, emulsifying, gel formation, distribution ability, and solubility) relate to their structure [4]. Various scientists have been trying to improve the characteristics of proteins by different methods (physical, chemical and enzymatic treatments) [5]. The first technique, physical adjustment, includes different mechanical forces such as high pressure or shear [6]. The second technique, chemical changes, is not good for food uses, because of health hazards [5]. The last technique is an enzymatic modification. It is a simple method to change the structure of proteins and enhance the functional properties [7, 8]. The main points of enzymatic modifications are safety, time-consumption of process, enzyme cost efficiency, and the effect of protein hydrolyses on the test, aroma, and colour of the final product [6].

The enzymatic method can be performed in two ways: dry and wet heating. From the industrial perspectives, Maillard reaction through dry heating is not attractive because it takes a long time (more than several days or weeks), so it is difficult to control the method which may produce intermediate or/and advanced Maillard reaction and the conjugates have the light yellow to brown colour. At the end of this process, it is necessary to remove insoluble conjugates by some techniques [9]. On the other hand, Zhu et al. [9] believe that the wet heating method requires less time to produce protein-polysaccharide conjugates. By controlling of the Maillard reaction in the initial stage, the formation of Schiff base with special functional properties in aqueous solution is still applicable. Therefore, researchers are aiming to advance the performance qualities of proteins, according to the Maillard reaction [6, 7, 10].

Gum Arabic (GA), also known as Acacia gum, is a natural hydrocolloid and is made from the branches of acacia species trees [6]. Three main fractions of GA are Arabinogalactan-peptide, arabinogalactan protein, and glycoprotein (contributing to about 85–90, 10, and 2% of total mass, correspondingly). Based on a diverse variety of GA, contains around 1–3% protein and 90–95% carbohydrate. The key amino acids of GA are hydroxyproline, serine, aspartic acid, threonine, and proline (in the ratio of ~4:2:1:1:1, respectively) [11]. GA is an expensive ingredient and is a suitable candidate for food systems, compared to other polysaccharides of similar molar mass. GA has a lower viscosity, based on its physicochemical behaviour [6]. Because it is an anionic polysaccharide, GA could interact at a wide pH range with

whey protein isolate (WPI) [12]. Hydrophilic heteropolysaccharide, the proteinaceous content of GA is around 2% [13].

Previous studies in the field of CWP are scarce. Most of the previous studies rely on structure of Camel milk protein (CMP), antioxidant and antimicrobial activities, heat stability of CWP, and the effect of dry heating on functional properties [14-18]. This research, therefore, aimed to enhance the whey protein application of camel milk by examining the modification of CWP isolates with GA under wet-heating conditions by a Maillard reaction.

2. Material and Method

2.1. Material

Camel milk was collected from the Khur and Biabanak –Isfahan province (in Iran) and mixed well. The samples were stored in a refrigerator for later processing. Pepsin enzyme (EC.3.4.23.1/activity≥400 units/mg protein (Sigma-Aldrich)), GA was obtained from Nexira Company (France) it contains low protein content (less than 1%). All other chemicals were of analytical grade.

2.2. Method

2.2.1. Camel milk preparation

Camel milk was skimmed by centrifugation at 5000 g for 15 in 50 mL centrifuge tubes sited in a Universal 320R centrifuge. Samples were cooled to 4ºC to eliminate any solid fat from the top of the milk.

2.2.2. Whey fraction preparation

The following method was modified from Salami et al. [18]. For the preparation of whey protein, the pH of skimmed milk was set to 4.3 by 1N HCl. Solutions were stirred for 30 min at 37ºC in a shaker incubator (model-PIT053RS), then centrifuged at 5860 g, for 60 min in 4ºC to separate caseins (CNs) and whey proteins. This process repeated three times. The supernatant, which constituted the whey, was lyophilized and put at -20ºC until further use.

2.2.3. Whey protein isolate preparation

Camel Whey Protein Isolate (CWPI) was prepared by the modified method applied in Salami et al. [19]. CWP was reconstituted (4% total solid) in 20 mM sodium acetate buffer and set pH 2 for optimum enzymatic pepsin action. The hydrolysis was carried out by incubation at 37ºC at an enzyme/substrate of 1:100 (w/w), up to 6 h. After each hydrolysis, the hydrolysed sample was heated in the water bath for 15 min at 85ºC to inactive the enzyme. Whey protein and the hydrolysates were centrifuged for 15 min, 7000 g at 4ºC. All samples lyophilized and stored at -20ºC until further use.

2.2.4. CWPI-Gum Arabic conjugates preparation

0.5, 1, 2% (w/v) GA dissolved with 2% (w/v) CWPI, in phosphate buffer solution (0.2 M pH=7). All samples were mixed during 2 h at room temperature with a magnetic stirrer. For the complete hydration, they were stirred overnight at 4ºC. The pH of all solutions was (pH=7) fixed by 0.1N HCl or 0.1N NaOH. All of the

samples were heated in water bath at 85ºC for 15 min then immediately cooled in an ice-water bath to stop the reaction. It should be mentioned that the mixture of CWPI-GA without heating and CWPI were used as control samples. The final products were centrifuged for 15 min, 7690 g at 4ºC. The supernatant was lyophilized and put at -20ºC until further use.

2.2.5. Amadori and browning compound formation measurement

Formation of Amadori and browning aggregates were measured using a UV spectrophotometer (model 320 spectra UV) and a visible spectrophotometer (model Unic 2100 spectrophotometer), by their absorbance at 304 nm and 420 nm respectively. Based on the method of Golkar et al. [13], 2% (wt.) total biopolymers of CWPI and CWPI-GA were mixed and conjugates centrifuged at 3000 g for 10 min before absorbance measurement.

2.2.6. UV-Vis spectroscopy

The UV-Vis spectroscopy was determined, by the spectrophotometer model (UV-Vis Array spectrophotometer-photo nix Ar2017). The method was modified from Liu et al. [20]. The protein solution (2 mg/mL) was made in 10 mM phosphate buffer (pH=7). The UV-vis absorbance was reported over the range of 200-500 nm, at 5 nm intervals.

2.2.7. Fourier transforms infrared (FT-IR) measurement

The infrared analysis was implemented, by using the FT-IR technique outlined in Pirestani et al. [21]. Whilst all the Fourier Transform Infrared (FTIR) spectrum of CWPI, CWPI-GA conjugate and mixtures were obtained using an FTIR spectrometer (model RAY leigh-WQF-510A). Samples were mixed with KBr before scanning was performed between 600 to 4200 cm^{-1} with a resolution of 4 cm^{-1}.

2.2.8. Solubility of protein

The solubility of protein was modified from the method of Jiménez-Castaño et al. [22] with modifications, by dissolving samples in distilled water (2 mg/mL) and changing the pH of the sample solutions from 3 to 9 by adding 1N NaOH or 1N HCl with gentle stirring. Then samples centrifuged at 7000 g for 15 min at 4ºC. The protein content was specified from the standard curve by measuring the absorbance at 280 nm of supernatants. The solubility uttered as the percentage of the initial CWPI concentration.

2.2.9. Heat stability

Heat stability testing was analysed indirectly by measuring the solubility, as outlined in Chevalier et al. [23] with some modification. Instead, the solution was made with distilled water (2 mg/mL) at pH 5 and 7. After solubilisation in the suitable buffer, samples were put in a water bath at temperature 55˚C to 95˚C for 30 min. Heated samples were cooled immediately at 4°C and centrifuged at 7000 g for 15 min. The UV absorption at 280 nm of the supernatant was measured using a UV spectrophotometer (model 320 spectra UV). To estimate the concentration of protein, the results were compared with the untreated sample.

3. Statistical Analysis

All data has been investigated, using a one-way ANOVA test. The data was shown significant differences ($P<0.05$) by Duncan's test with SAS software. All measurements were carried out three times.

4. Results and Discussion

4.1. Measurement of Amadori and browning compounds formation

During the primary stage of the Maillard reaction, the carbonyl group formed subsequently covalent bonds with a free amine group and forming a Schiff base. The Schiff base undergoes a non-returnable Amadori rearrangement to form the Amadori product [24, 25]. As shown in Table 1, the formation of Amadori and browning compounds was determined by the absorbance at 304 and 420 nm respectively [13]. The formation of Amadori compounds was considerable. All the unheated samples named as mixtures and heated as conjugates samples in each ration of CWPI/GA had significance ($p<0.05$). When the portion of GA increased from 0.5% to 2%, the formation of Amadori component increased. All samples had a significantly higher content of Amadori compounds, but the maximum Amadori component found was for unheated CWPI (0.690±0.002).

It may be related to the availability of residual lactose content of sample [26], and amino groups of CWPI. On the other hand, there were numerous reaction factors, such as inherent properties of sugar/protein, pH, the amino groups, reducing sugar ratio, water activity (aw), time, temperature, effects on the yields, and types of Maillard reaction products (MRPs) [24]. According to Table 1, the maximum browning component was related to conjugates of 2% GA with CWPI (0.061±0.001). Our results agree with Jiménez-Castaño et al. [27], who found that aggregation of β-Lg and dextran happened faster when the reaction was at the highest water activity (aw) and highest weight ratio 6:1 (polysaccharide: protein (conjugate)) for the dry heating condition. On the other hand, a little different variety of colour from white to light yellowish was observed. A visual change in colour related to increasing the amount of GA during heat reaction with CWPI represents the formation of some Maillard products. Change of the colour of the product during Maillard reaction was observed by Golkar et al. [13], who represented, the reaction of BLG and Angum Gum based on Angum content and heating time were yellow to brownish in terms of colour range. These results have shown that the wet-heating condition of these biopolymers had a specific effect on the creation of browning conjugates rather than the mixture compounds.

Table 1. Formation of Amadori and browning compounds monitored at 304 and 420 nm, respectively in CWPI, mixture and conjugate of CWPI with (0.5, 1 and 2%) GA.

Sample	Absorption at 304 nm	Absorption at 420 nm
CWPI	0.690±0.002^{a}	0.023±0.002^{d}
2% GA-CWPI(mixture)	0.453±0.003^{c}	0.027±0.001^{c}
1% GA-CWPI(mixture)	0.256±0.002^{d}	0.025±0.003^{cd}
0.5% GA-CWPI(mixture)	0.208±0.001^{g}	0.015±0.001^{e}
2% GA-CWPI(conjugate)	0.462±0.002^{b}	0.061±0.001^{a}
1% GA-CWPI(conjugate)	0.247±0.001^{e}	0.033±0.001^{b}
0.5% GA-CWPI(conjugate)	0.224±0.001^{f}	0.015±0.001^{e}

Data are means of three replicates ($n = 3$) ± standard deviation. Means within each row with different superscript letters are significantly ($p < 0.05$) different.

4.2. UV-Vis spectroscopy

The effect of CWPI and different levels of GA concentration during wet heating were examined by recording the UV-absorbance. Usually, UV-Vis spectra have been used to determine MRPs [20]. The absorption spectra were measured over the range of 200-500 nm for CWPI alone, CWPI-GA mixtures and CWPI-GA conjugates. In a different percentage of GA, illustrated in Fig. 1, a slight absorbance peak at around 280 nm observed for the CWPI alone. It suggests that CWPI had a consequence of some residual reducing saccharides [6]. As indicated in Fig. 1, the differences in UV-Vis absorbance were not significant in the mixture of CWPI-GA in a different range of GA. Conjugation of CWPI-GA had two types of picks, approximately 280 nm and 452 nm were observed. The first absorbance peaks at around 260-280 nm attributed to the formation of a Schiff base [6, 20]. The second type of peak about 452 nm was related to the final stage of melanoidin products through a Maillard reaction [28]. In line with our results, some researchers working on different types of amino acid with glucose indicated that the formation of MRPs absorbance peak for arginine- lysin- and methionine-glucose were around 265 nm and 215 nm, however, the absorbance peaks of xylose were approximately 300 nm and 215 nm [29]. At the end of MRPs absorption around 215 nm, related to the amino acid or protein, if at this wavelength the protein or amino acid showed the higher absorbance, it means that Maillard reaction induced modification to enhance that absorption [30].

4.3. Fourier transforms infrared (FT-IR) measurement

FTIR is a suitable and practical technique to study the difference in the secondary structure of protein conformation and interactions between mixed protein and polysaccharide systems analysis [8, 31, 32]. Variations in FTIR spectra were detected, such as changes in the intensity or location of absorption bands and the presence of new bands [32]. Unique spectral features for proteins were the amide I band at 1600–1700 cm^{-1} (C═O stretching), amide II bands in the range of 1500–1550 cm^{-1} (N–H deformation), amid III band at 1200–1300 cm^{-1} (C–N stretching and N–H deformation). On average, the amide I, II, III bands were strong, weak and moderate respectively [8, 33].

In Fig. 2, the bands of amide I are CWP (CWP), alone CWPI ,CWPI-GA mixtures and conjugates for different range of GA 0.5%, 1%, 2% and 2% CWPI located at 1649 cm^{-1}, 1658 cm^{-1}, 1652 cm^{-1}, 1650 cm^{-1}, 1651 cm^{-1}, 1651 cm^{-1}, 1651 cm^{-1}, 1650 cm^{-1} sequentially. The results illustrated that the amide I band of CWP was changed by adding the enzyme and GA. However, the effect of other conditions such as wet heating, different levels of GA was insignificant between CWPI-GA mixtures and the CWPI-GA conjugates. Meanwhile, CWPI-GA conjugates had the maximum peak compare with the CWPI-GA mixtures.

The results of Fig. 2 also point out that all samples may comprise the second structure of alpha-helical [8], having a highest record approximately 1660–1650 cm-1 [33]. The FTIR spectral features of CWP, alone CWPI, CWPI-GA mixtures and CWPI-GA conjugates in a different range of GA 0.5%, 1%, 2% and 2% CWPI showed a clear change between 1500 cm-1 and 1550 cm-1. The band intensity of CWP, CWPI, CWPI-GA conjugates with amounts of 2%, 1%, 0.5% GA, which were at 1570 cm-1, 1545 cm-1, 1522 cm-1, 1500 cm-1, 1500 cm-1, decreased dramatically while other samples did not have a sharp peak. Therefore, wet heating

conditions and adding GA incurred a hug conformation variation of the amide II band (N–H deformation). Although, there were fluctuations from the range of 1200 cm-1 to 1300 cm-1 region for all samples of amide III bands. The FT-IR spectroscopy is adequately indifferent to distinguish such minor variation [33].

Areas in the mid-infrared spectrum, including 1800 cm-1 to 800 cm-1 related to the amide group (C–O, N–H, and C–N), such as MRPs, Amadori compound, Schiff bases, and pyrazines, [20]. On the other hand, the area of 1180–953 cm-1, which is associated with "saccharide" bands. In fact, CWPI had a sharp peak, which implies the presence of saccharides and the formation of MRPs.

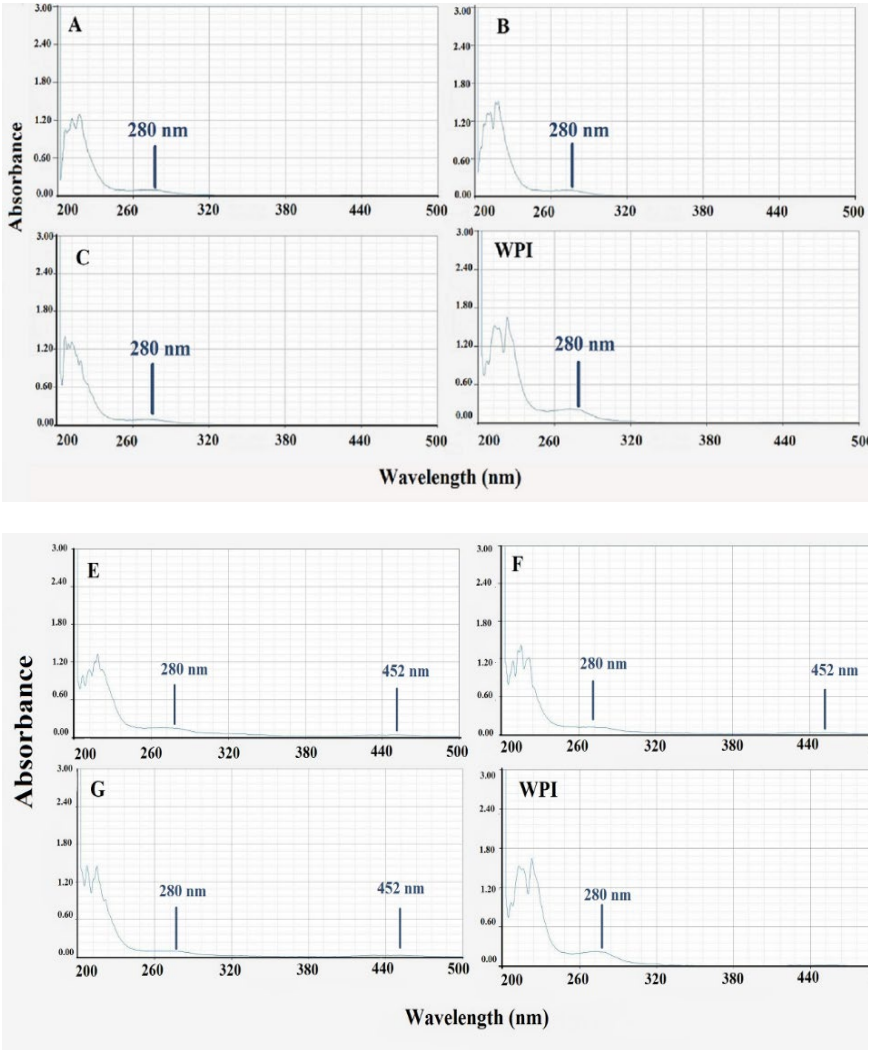

Fig. 1. Difference UV absorbance spectra the CWPI alone, CWPI- GA mixture alone: A (2% GA-CWPI), B (1%GA-CWPI), C (0.5% GA-CWPI) and CWPI-GA Conjugate alone: E (2% GA-CWPI), F (1%GA-CWPI), G (0.5% GA-CWPI).

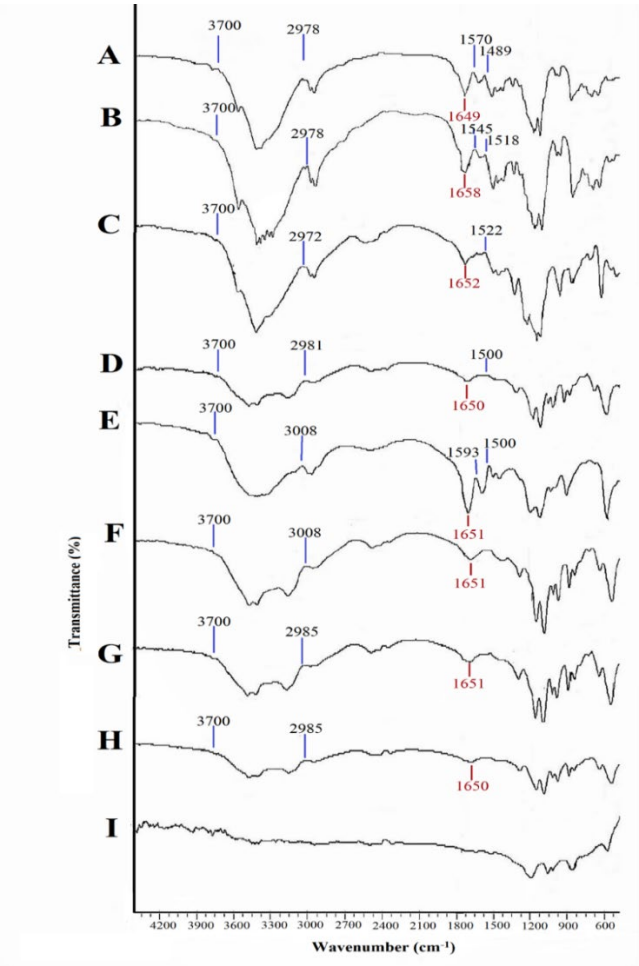

Fig. 2. FTIR spectra of CWP (A), CWPI (B), CWPI-conjugates(C: 2% GA-CWPI, D: 1% GA-CWPI, E: 0.5% GA-CWPI), CPI-GA mixtures (F: 2% GA-CWPI, G: 1% GA-CWPI, H: 0.5% GA-CWPI) and GA (I).

4.4. Protein solubility

One of the main functional characteristics of proteins is the solubility [34]. Figure 3 illustrates the differences in solubility of CWPI, CWPI-GA mixtures, and conjugates at several pH values. The outcomes confirmed that the lowest solubility of CWPI was at the isoelectric point which confirms earlier research findings [17, 35]. Moreover, CWPI had the highest solubility in comparison with CWP (data was not shown). This phenomenon could be attributed to CWP isolated that had the highest hydrophilic groups. Because enzymatic hydrolysis causes a decline in the molecular weight of the proteins, and soar in the number of smaller hydrophilic units [36, 37]. The solubility of CWPI-GA conjugates was more than the CWPI-GA mixture due to the technological processing such as heat treatment can be significantly affected the solubility of whey protein products [17]. Not only, raising the solubility of CWPI-GA conjugates but also the solubility of CWPI-GA mixtures

greatly enhanced when the level of concentrated protein increased or the amount of GA from 2% decreases until 0.5%.

The presence of GA resulted to improve the number of hydrophilic groups of CWPI. It was reported that whey protein from camel milk was less soluble than the bovine whey protein [37]. Camel and bovine whey proteins have different solubility when influenced by the temperature [17]. Therefore, it can be understood why the results of some research indicated that bovine whey protein glycosylation had more than 90% solubility [35]. However, our data has shown that the solubility of all samples was upheld ≥55% at all pH. At the isoelectric point, bovine whey proteins maintain good solubility because β-LG, as native globular whey protein, namely has low surface hydrophobicity [35]. On the other hand, the higher amount of α-LA and the lack of β-LG in camel milk whey, cause a higher sensitivity of camel whey solubility upon pH alteration and find out the differently behaves of camel milk whey protein [37].

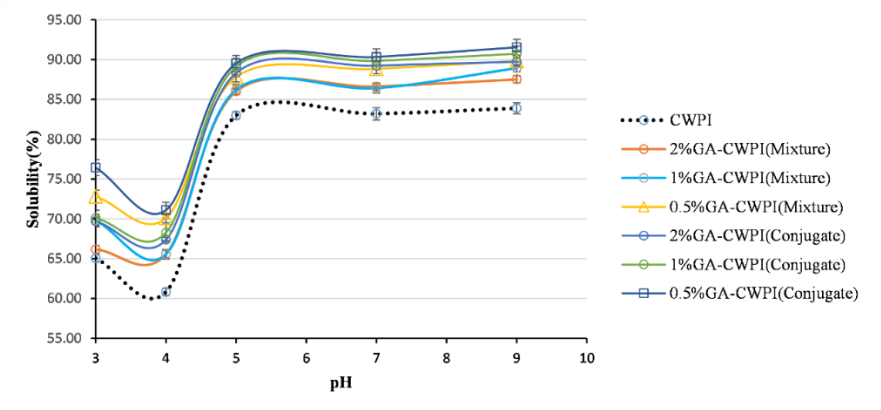

Fig. 3. Solubility at various pH values of CWPI, CWPI-GA mixtures and conjugates (Standard deviation is demonstrated by error bars from three separate tests).

4.5. Heat stability

The heat stability of CWPI, CWPI-GA mixtures, and CWPI-GA conjugates were indirectly evaluated by the solubility in different temperatures, from 55°C to 95°C for 30 min. The experiment has done in two different pH conditions (5 and 7). As it can be seen in Fig. 4, by increasing temperature, the solubility changed, and two different pH significantly affected the heat stability. At pH 7, the thermal treatment caused denaturation and made proteins less soluble [17]. Based on Fig. 4(a), all samples after being heated at 55˚C were almost soluble up to 80%. By increasing the temperature from 75˚C to 95˚C, the solubility decreased steadily but the curve of solubility remains flat. During the process of heating, the solubility of CWPI changed from 81.87% to 72.61% as the temperature changed from 55˚C to 95˚C. El-agamy found that the absence of β-Lg due to heat stability is an important parameter in chemical compositions of CWPs [38]. At this condition, the highest heat stability is related to CWPI-GA conjugates and then mixtures. These results represented that GA and wet heating during the Maillard reaction improve the heat stability of CWPI. Heat stability after glycation attributes to repulsive steric interactions rendered by an adequate quantity of the glycated GA [39]. Famelart et al. [26], also observed that hydrophilicity of proteins increases by dry heating of

bovine whey proteins in the presence of low amounts of lactose, due to hydrophilic carbohydrate residues grafting onto proteins and their polymerisation that in turn may enhance solubility.

The lowest heat stability was at pH 5 when measured at the highest temperature (the shape of the diagram illustrating the interaction of pH and temperature). Based on Fig. 4(b) solubility of proteins decreased around 16% and the range of solubility became up to 60% so the effect of both temperature (55˚C to 95˚C) and pH 5 caused a major change than pH 7. The heat stability of CWPI-GA conjugates was higher than CWPI at a greater temperature. From the results, it can be found out that GA is the main factor to improve the functional properties of glycated CWPI during the Maillard reaction. With comparing the effect of pH on the heat stability of all samples at the same temperature in Figs. 4(a) and 4(b), we observed that heat stability at pH 7 were greater than pH 5. In line with our results, other researchers found that CWP contains α-La (a major protein and susceptible to pH) that affects heat stability [17, 40]. The sensitivity to pH makes particles denaturation and increased tendency to aggregate. In other words, near the isoelectric point (pH 4.5) proteins are unstable and heat stability decreased [17].

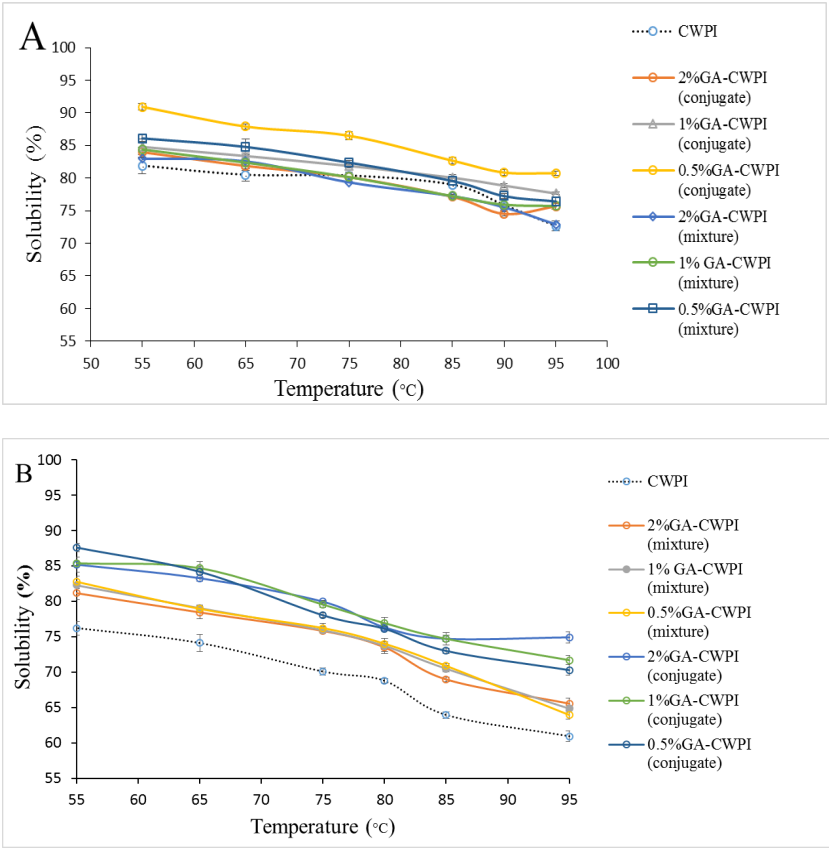

Fig. 4. Heat stability at pH 7 (a) and 5(b) during temperature (55-95 ˚C) of CWPI, CWPI-GA mixtures and conjugates (Standard deviation is demonstrated by error bars from three separate tests).

5. Conclusions

The current study demonstrated the improvement of numerous functional properties of CWP. CWPI was performed by adding hydrolysis pepsin enzyme 1:100 (w/w), up to 6h incubated at 37ºC to CWP. Different portions of GA (0.5, 1, 2 % (w/v)) were selected in vitro to compare the effect of wet heating on the performance of the final product. This research finds a series of interesting outcomes. First, it was found that GA had a specific effect on solubility in different range of pH. Second, pre-heat treatment could be introduced as a suitable way to boost solubility and heat stability at the highest temperature (95ºC) while the pH was close to the pI. Third, it's found that 0.5% is the best ration of GA related to 2% CWPI through the Maillard reaction in wet heating. Lastly, the CWPI aggregates can have great potential use in the food industry.

Nomenclatures

α-La	α-lactalbumin
β-Lg	β-lactoglobulin
pI	isoelectric point

Abbreviations

aw	water activity
CMP	Camel milk protein
CWP	Camel Whey Protein
CWPI	Camel Whey Protein Isolate
FT-IR	Fourier transforms infrared
GA	Gum Arabic
MRPs	Maillard reaction products

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