J. Food and Dairy Sci., Mansoura Univ., Vol. 10 (1): 1 - 8 , 2019

Characterization of Whey Protein Isolate Covalently Modified with Phenolic Compounds
2: Physiochemical and Emulsifying Properties

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ABSTRACT

This work was performed to study the effect of the covalent modifications of whey protein isolate (WP) by chlorogenic acid (CA) and rosmarinic acid (RA) on their physiochemical and emulsifying properties. Phenolic compounds, RA and CA, were covalently bound to WP as investigated by the decrease in free amino and thiol groups. Zetasizer apparatus and ANS (1-anilino-8-naphthalensulfonate) analyses showed some changes between modified and unmodified WP in zeta potential, particle size, isoelectric point, and surface hydrophobicity. Modified WP by CA had the increased surface hydrophobicity and isoelectric point, while modified WP by RA had the decreased isoelectric point compared with unmodified one. WP modified by RA and CA showed a change in emulsifying properties. Particle size and zeta potential of sunflower emulsion emulsified with 0.3% WP with and without modification were investigated using static light scattering (SLS). The results showed that WP modified by RA produced an emulsion with smaller particle size, while WP modified by CA showed the opposite. Moreover, both modified proteins formed emulsions with zeta potential higher than unmodified one. Finally, WP modified by RA improved the stability of emulsion against creaming compared with WP control and that modified by CA. Although the covalent interactions of the phenolic compound with whey protein isolate may decrease their nutritional properties, the phenolics – whey protein isolate conjugates are being considered as a way to develop functional foods due to their good emulsifying properties.

Keywords: covalent modification, whey protein isolate, rosmarinic acid, chlorogenic acid, zeta potential, physiochemical and emulsifying properties

INTRODUCTION

Whey, a by-product of the cheese industry, is the remaining watery and thin liquid after separating casein curd from the milk. Recently, it was recognized that whey is an excellent source for nutritional and functional proteins, which has helped to convert it from a waste to a valuable dairy product (Smithers 2008). Concerning such facts and their functional properties, whey proteins have been applied as ingredients in the food industry, for instance, bakery, meat, and dairy industries. Moreover, its high value led to use it in pharmaceutical and cosmetic industries (Arriaga 2011). The most of whey is produced into whey powder, such as whey protein isolate and whey protein concentrate. Functional properties of whey proteins, for example, foaming, emulsification, and gelation are related to their structure basically the beta-lactoglobulin, the main compound, in addition to their hydrophobic/hydrophilic ratio (Nakai and Li-Chan 1993, Ali *et al.* 2013, Keppler *et al.* 2017, Keppler and Schwarz 2017). Functional properties of proteins are defined as the physical and chemical properties, which effect on its behavior during processing, storage, and consumption of food (Sreerama *et al.* 2012). The most interest of such properties in the food industry are foaming, emulsification, water binding……etc. Proteins work as emulsifiers by creating a film around oil droplets spread in an aqueous phase, thus, avoiding structural changes. Emulsifying properties of food proteins could be studied by oil droplet size, zeta potential, and emulsion stability against creaming (Khalil *et al.* 2012, Ali *et al.* 2013, Keppler and Schwarz 2017).

Functional properties of proteins could be altered by the modifications of its structure through enzymatic, chemical, or physical treatments (Hudson *et al.* 2000, Ibanoglu and Karatas 2001, Herceg *et al.* 2005, Aewsiri *et al.* 2013). The conformation and functionality of whey proteins can be changed through the covalent interactions with plant-derived compounds, such as allyl isothiocyanate, chlorogenic acid, and rosmarinic acid (Rade-Kukic *et al.* 2011, Ali *et al.* 2013, Keppler *et al.* 2014, Keppler *et al.* 2017, Keppler and Schwarz 2017, Ali and Elsharkawy 2018). Where the interactions led to shift pH isoelectric point to a more acidic pH. As well as, a loosening of the protein structure in addition to an increase or decrease in the surface hydrophobicity. Emulsions prepared with modified whey proteins by allyl isothiocyanate and chlorogenic acid showed no significant changes in the emulsifying properties (Rade-Kukic *et al.* 2011, Ali *et al.* 2013, Keppler and Schwarz 2017). Other researchers have studied the covalent interactions between the plant-derived compounds and protein and they found that the formed conjugates may modify the main properties of protein (Aewsiri *et al.* 2009, You *et al.* 2012, Gan *et al.* 2016, Abd El-Maksoud *et al.* 2018, Karefyllakis *et al.* 2018). However, according to our research, no studies on the effect of rosmarinic acid on whey protein isolate emulsions were done, while the influence of the enzymatic and alkaline modifications on the antimicrobial, antioxidant, and antiviral properties of whey protein isolate were studied in our previous studies (Ali and Elsharkawy 2018, Ali *et al.* 2018).

Therefore, the main objective of this work was to study the effect of the covalent modification of whey protein isolate by rosmarinic and chlorogenic acids on the physiochemical and emulsifying properties of whey protein isolate.

MATERIALS AND METHODS

Materials

The whey protein isolate was purchased from Davisco Foods International, Inc., US. Chlorogenic acid (CA), rosmarinic acid (RA), and 8- anilino-1-naphthalenesulfonate (ANS) were obtained from Sigma, Germany. All other chemicals and solvents were in analytical grade.

Methods

Samples preparation

In order to modify WP by CA and RA, the method of Ali *et al.* (2018) was used. One gram of WP was dissolved in 95 mL distilled water and incubated with CA

and RA (60 mg/ 5 mL ethanol), after adjusting pH to 9 (using NaOH), at room temperature in the presence of air.

The mixtures were stirred for 24 h, and then they were dialyzed for 24 h at room temperature against distilled water. The mixtures were finally freeze-dried and stored in polyethylene bags at – 20 C till analysis. The unmodified WP was prepared with the same manner but without CA and RA.

Determination of free amino and thiol groups and tryptophan contents

Free amino groups, using trinitrobenzene sulfonic acid (TNBS), and thiol groups, using 5,5′- dithiobis(2-nitrobenzoic acid) (DTNB), and tryptophan content, using Varian Cary Eclipse fluorescence spectrophotometer (Varian Australia) were determined as described in Ali *et al.* (2018).

Determination of the surface hydrophobicity of proteins

The surface hydrophobicity of modified and unmodified WP, using 1-anilino-8-naphthalensulfonate (ANS), was investigated as mentioned in detail in Keppler *et al.* (2017). The initial slope (S0) of the highest fluorescence intensity versus protein concentrations was used to calculate the protein surface hydrophobicity.

Particle size and Zeta potential of proteins

Particle size and Zeta potential of WP modified by CA (WP-CA) and WP modified by RA (WP-RA) compared to unmodified WP (UWP) were studied by Malvern Zetasizer (Herrenberg, Germany) as mentioned by Ali *et al.* (2018).

Isoelectric point of proteins

The change in isoelectric point of modified and unmodified WP was determined by measuring the change in zeta potential of proteins at different pH values according to Keppler *et al.* (2017). One mg of each protein was dissolved in 100 mL of deionised water for at least 30 min, after that the pH of solution was measured. 0.1N of Hcl and NaOH solutions were used to change the pH of protein solutions to different pHs (3 - 7). Disposable zeta cell was filled with protein solution, at new pH, and zeta potential was determined by a Malvern Zetasizer (Herrenberg, Germany).

Determination of emulsifying properties of proteins

Preparation of emulsion

Modified and unmodified WP emulsions (oil in water), using 10% sunflower oil, were prepared by stirring the sunflower oil with proteins used (3mg/mL phosphate buffer, PBS) at 500 rpm for 15 min. Afterward, the emulsification was finished using an ultrasonic homogenizer (Bandelin electronic GmbH, Germany), 5 min in an ice bath and 70% energy input (Khalil *et al.* 2012).

Oil droplet size of emulsions

Oil droplet size of UWP, WP-CA, and WP-RA emulsions were studied by static light scattering (SLS, Retsch Technology GmbH, Germany) using Mie theory according to Keppler and Schwarz (2017). The volume weighted mean oil droplet size (d4,3) and surface weighted mean diameter of oil droplet (d3,2) was calculated by the software of equipment.

Emulsion stability against creaming

The stability of fresh emulsions against creaming was examined according to Khalil *et al.* (2012). One mL of each emulsion was centrifuged at 3000g for 70 min. After each 10 min of centrifugation, the absorbance of emulsions at 500 nm was measured using a Helios Gamma spectrophotometer, Germany.

Particle size and zeta potential of emulsions

Fresh emulsions were diluted 100 fold with PBS buffer pH 7.2, then filled in zeta cell. Measurement conditions were described in detail in Keppler and Schwarz (2017). Measurements were done in triplicates from two separately emulsions

Statistical Analysis

The differences between the samples were examined using an analysis of variance (ANOVA) and a post-hoc Tukey test using SPSS software (SPSS, version 18). The results of $p \leq 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Effect of the modification by CA and RA on physiochemical properties of proteins

The influence of the modification of WP by CA and RA, on the amount of free amino groups, thiol groups, and tryptophan content of proteins was studied and the results are outlined in Table (1). The results show that these interactions led to a significant decrease in the free amino groups of WP modified by CA and RA compared to unmodified WP. These results are in accordance with others (Aewsiri *et al.* 2009, Aewsiri *et al.* 2013, Ali *et al.* 2013, Keppler *et al.* 2014, Ali *et al.* 2018). They stated that the interaction between different phenolic compounds, for example, caffeic, ferulic, tannic, chlorogenic, rosmarinic acids, and different proteins, cuttlefish skin gelatin, beta-lactoglobulin (ß-Lg), and whey protein isolate, at different conditions, caused a decrease in the content of free amino groups. The percentage of the remaining in free amino groups of WP-CA was higher than that found for WP-RA, where the values were 77.12±2.17 and 71.89±0.66 %, respectively (Table 1). This is an indication that RA was more reactive than CA. The main compound in WP is β-Lg, which contains one free thiol group and two disulfide bonds. The thiol group in cysteine is sensitive and available to react with phenolic compounds. Therefore, the decrease in its content was used to characterize the modification of proteins with phenolic compounds. As given in Table 1, the thiol groups content significantly decreased upon the modification. The thiol groups are not detected at the modification by RA, while 11.07± 1.93 % of thiol groups was the remaining percentage when WP was incubated with CA (60 mg CA /g of protein). A similar phenomenon was observed in previous studies (Rawel *et al.* 2002a, Prigent *et al.* 2007, Ali *et al.* 2013, Keppler *et al.* 2014, Cao and Xiong 2017, Ali *et al.* 2018), who found the same results for the interactions between different phenolic compounds and proteins.

Table 1. The effect of modification by CA and RA on free amino (nM/mg protein), thiol (µM/mg protein) groups and tryptophan (nM/mg protein) contents of WP

Parameters	Proteins		
	UWP	WP-CA	WP-RA
Free amino groups:	543.00± 66.00[a]	418.75±11.76[b]	390.38±3.60[c]
% of the remaining	100.00	77.12±2.17	71.89±0.66
Free thiol groups:	71.42± 0.5[a]	7.90± 1.38[b]	ND
% of the remaining Tryptophan	100.00	11.07± 1.93	ND
content:	61.84± 4.63[a]	25.5±0.1[b]	9.3±0.2[c]
% of the remaining	100.00	41.28± 0.27	14.97±0.46

Where: UWP; unmodified WP, WP-CA; modified WP by chlorogenic acid and WP-RA; modified WP by rosmarinic acid, ND; not detected, the different letters represent significant differences

The highest tryptophan fluorescence intensities of unmodified and modified WP were used to calculate the change in its tryptophan content and the data are presented in Table (1). Significant differences between all proteins were found where unmodified WP exposed a higher tryptophan content, 61.84± 4.63 nM/mg of protein, while WP modified by CA and RA recorded 25.5±0.1 and 9.3±0.2 nM/mg of protein, respectively. This means that the contents of tryptophan were decreased by 58.7 and 85 % for WP-CA and WP-RA, respectively. These results are in line with the literatures (You *et al.* 2012, Ali *et al.* 2013, Gan *et al.* 2016, Ali *et al.* 2018). Moreover, the possibility of the interaction of different phenolics with tryptophan has been found (Rawel *et al.* 2001, Rawel *et al.* 2002b, Rawel *et al.* 2005, Rohn *et al.* 2005, Ali *et al.* 2013). Finally, the results of free amino and thiol groups and tryptophan contents in Table 1 showed that the reactivity of RA for interacting with WP was higher than CA. The higher reactivity of RA may be related to its two catechol groups compared to only one in CA. These results could be explained by the ability of reactive quinone, an oxidized form of phenolic compounds, to interact covalently with a nucleophilic amino acid side chains (Kroll *et al.* 2003, Ali *et al.* 2013, Ali and Schwarz 2018).

Effect of the modification by CA and RA on aggregation and charge of proteins

Particle size of proteins

The aggregation behavior, analyzed by dynamic light scattering, of the modified WP by CA and RA, compared to unmodified WP was investigated by measuring the change in the particle size of proteins and the data are listed in Figure 1 A. The results show that the average particle diameter of the main peak of UWP was 6.82±0.75 nm, with the percentage distribution 100%. The average particle diameter of unmodified whey protein isolate was 5.6±0.21 nm with the percentage distribution 99.8% as reported by Wilde *et al.* (2016). After 24 h interaction of WP by CA and RA, at pH 9, an increase in the average particle size of WP-CA conjugates was found, where the value was 7.49±0.62 nm. On the other hand, there is no change in the average particle size of WP-RA conjugates (Figure 1A). These results are in the line with the previous studies (Wilde *et al.* 2016, Keppler and Schwarz 2017, Ali *et al.* 2018), who found that there are no significant differences between the particle size of modified WP, with allicin, allyl isothiocyanate, and rosmarinic acid, and unmodified WP. The slight changes in the values of particle size can be explained by the presence of free CA or its degradation products in the solution that may be appeared to make the non-covalent aggregation of ß-Lg or the competition of them with water molecules in the solvation shell of the protein molecule (Wilde *et al.* 2016).

Zeta potential of proteins

Zeta potentials of UWP, WP-CA, and WP-RA proteins were determined to study its electrical charges, by their movement in an electrical field. The results were illustrated in Figure 1B. Data given in the Figure showed a slight increase in the values of zeta potential after modifications. Where, the recorded values were -10.97 ±1.31, -11.13 ±1.32, and -11.35 ±0.65 for UWP, WP-CA, and WP-RA, respectively. All values of zeta potential were negative because the proteins were dissolved in PBS buffer pH 7, this observation is in accordance with Gbassi *et al.* (2012) and Wilde *et al.* (2016). The increase in zeta potential of WP modified by RA was slightly higher than the WP modified by CA.

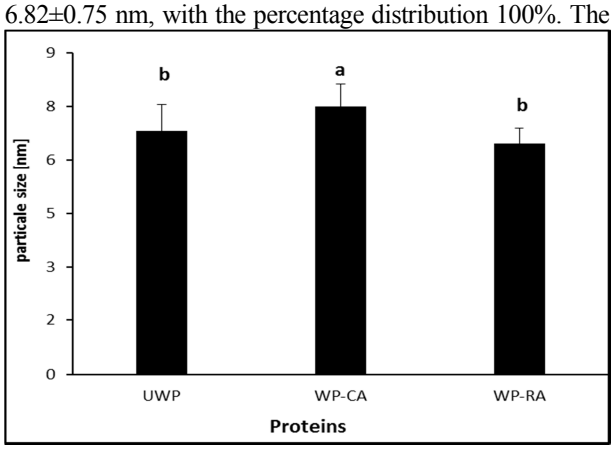

Figure 1A .Particle size of proteins

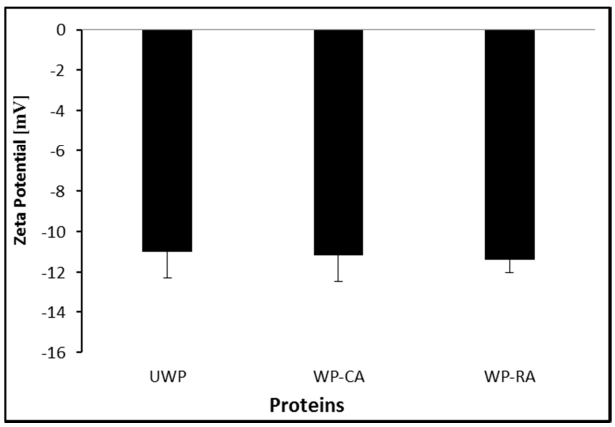

Figure 1B . Zeta potential of proteins

Where: UWP; unmodified WP, WP-CA; modified WP by chlorogenic acid and WP-RA; modified WP by rosmarinic acid, the different letters represent significant differences

Effect of the modification by CA and RA on the surface hydrophobicity of proteins

The surface hydrophobicity was studied to examine the non-polar character of proteins. The changes in surface hydrophobicity of WP modified by CA and RA, at alkaline conditions, are summarized in Figure (2). Generally, WP modified by CA (WP-CA) showed a significant increase in surface hydrophobicity, at $p \leq 0.05$, compared to WP modified by RA (WP-CA) and unmodified WP. The surface hydrophobicity values of UWP, WP-CA, and WP-RA, were 1082.50 ± 46.39, 1306.33 ± 79.02, and 1085.43 ± 39.74, respectively. This observation is in the line with Keppler *et al.*(2017). On the other hand, these results are not agree with Rawel *et al.* (2002a), Aewsiri *et al.* (2009), and Ali *et al.* (2013), who found that the interactions between soya, cuttlefish skin gelatin, and beta-lactoglobulin proteins with different phenolic compounds decreased the surface hydrophobicity. These differences may be related to the conditions of interactions, such as the ratio between protein⁄ phenolics. The mechanism of the used method depends on a fluorescence agent, ANS, has an ability to bind with hydrophobic pockets of amino acids on the surface of protein including an aromatic ring, for example, tryptophan and phenylalanine. Therefore, it can apply to measure the surface hydrophobicity of protein (Benjakul *et al.* 1997).

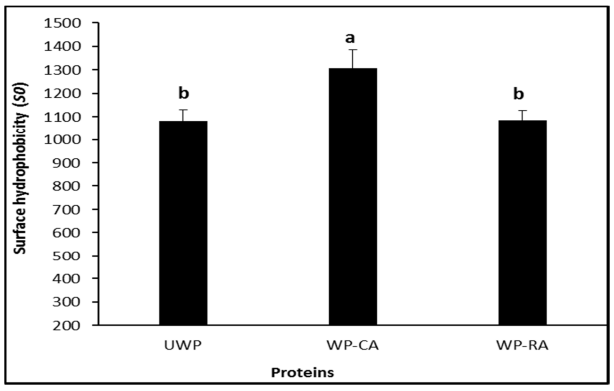

Figure 2. Surface hydrophobicity of WP modified by CA and RA

Where: UWP; unmodified WP, WP-CA; modified WP by chlorogenic acid and WP-RA; modified WP by rosmarinic acid, the different letters represent significant differences

The increase in the surface hydrophobicity of WP may be discussed with the covalent attachment of CA to the free thiol groups, as noticed above, possibly caused some liberating of the compact protein structure, because of steric hindrance and hydrophobicity of the hosted molecule. Such liberating made it possible for ANS to bind with some hydrophobic sites inside of the WP molecule. Thus, causing an increase in the ANS fluorescence intensity of WP modified CA.

Effect of the modification by CA and RA on the isoelectric point of proteins

The isoelectric point, zeta potential is ~0 mV, is strongly connected to protein solubility. As mentioned above, the zeta potential measures the protein surface charge, which is related to the proteins precipitate. The change in the isoelectric point of proteins using zeta potential measurements at different pH values was investigated. The values are mentioned in Figure (3). The zeta potential values of modified and unmodified WP at pH between 3 and 5 were positive, whereas the values reported at pH from 5 to 7 were negative. The results in Figure (3) show that the isoelectric point of unmodified WP was decreased from pH ~4.88 to pH ~4.66 for WP modified by RA, this means that the isoelectric point shifted to a more acidic pH value. In contrast, the isoelectric point of WP modified by CA was slightly increased to pH ~4.99. These results are in accordance with the results of Keppler *et al.* (2017), who reported that the isoelectric point of WP decreased after modification with allyl isothiocyanate. The shifting in the pH of isoelectric point, decrease of increase, can be discussed with the decrease or increase of the charged groups by adding the phenolic compounds to an amino chain of protein, as mentioned above, which leads to remove some protein charges (Kroll and Rawel 2001).

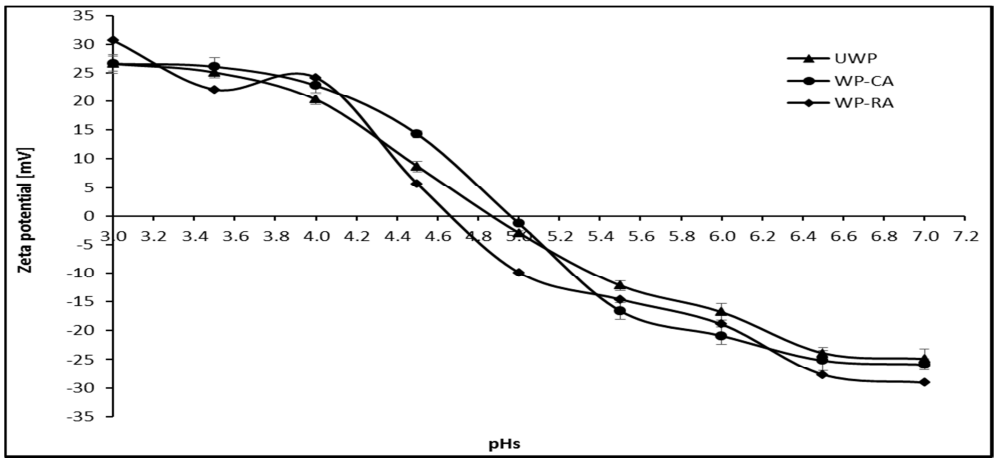

Figure 3. Zeta potential [mV] of WP modified by CA and RA at different pH values

Where: UWP; unmodified WP, WP-CA; modified WP by chlorogenic acid and WP-RA; modified WP by rosmarinic acid

Effect of the modification by CA and RA on the emulsifying properties of proteins

Oil droplets size of emulsions

Modified WP by CA and RA were used, as emulsifiers, to prepare emulsions (oil in water) and the oil droplets size of emulsions were measured directly after the emulsification (Figure 4). Volume weighted mean of oil droplet size (d4,3) values of UWP, WP-CA, and WP-RA were 1.385± 0.08, 1.813± 0.07, and 1.231± 0.03 µm, respectively. While, the values of the surface weighted

mean diameter of oil droplet (d3,2) were 1.473± 0.07, 1.899± 0.06, 1.310± 0.04 µm, respectively. It was observed from Figure (4) that the d4,3 and d3,2 values of the emulsion prepared using WP-CA was significantly increased compared to the emulsions prepared using UWP and WP-RA. On the other hand, d4,3 and d3,2 values of the emulsion prepared using WP-RA were significantly decreased (at $p \leq 0.05$) compared to the emulsions prepared using UWP and WP-CA. The large droplets size of WP-CA emulsion could be explained by the low solubility of WP-CA, which is reported by the increase in isoelectric point.

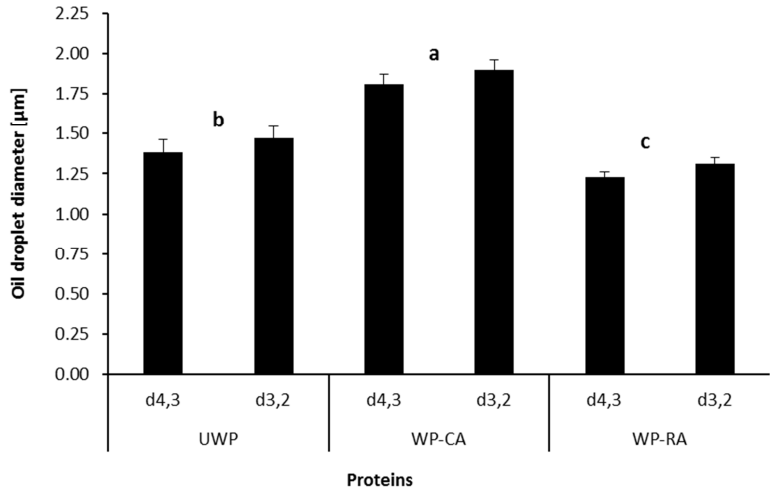

Figure 4. Oil droplet size of emulsions prepared using modified WP by CA and RA

Where: UWP; unmodified WP, WP-CA; modified WP by chlorogenic acid and WP-RA; modified WP by rosmarinic acid, the different letters represent significant differences

Stability of the emulsions against creaming

The emulsion stability normally reflects the ability of the proteins to impart strength to an emulsion for resistance to stress and changes and is therefore related to the consistency of the interfacial area over a defined period (Liu *et al.* 2008, Boye *et al.* 2010). Stability of UWP, WP-CA, and WP-RA emulsions against creaming was studied by centrifugation of fresh emulsions at 3000 rpm for 70 min and the data are presented in Figure (5). The data indicate that all emulsions seem to be stable against creaming at the first of the experiment, then the emulsion emulsified with WP-CA was started in losing its stability compared to another two emulsions. As also noticed in the Figure, significant differences were observed in the stability of UWP, WP-CA, and WP-RA emulsions at the end of centrifugation. The emulsion prepared with WP-CA showed the lowest stability while the emulsion prepared using WP-RA showed the highest stability. The emulsion stability values, as a percentage, for UWP, WP-CA, and WP-RA emulsions after 70 min were ~ 78.2, 73.5, and 88.8%, respectively. Ali *et al.* (2013) reported that the stability of emulsion emulsified with beta-lactoglobulin was increased after its interaction with chlorogenic acid at two different conditions, enzymatic and alkaline. Moreover, the covalent modification of whey protein isolate with allyl isothiocyanate improved the stability of emulsion compared to native one (Keppler and Schwarz 2017). These results support the oil droplet size results (Figure 4), where the large oil droplet size of an emulsion stabilised with WP-CA led to the weak emulsion.

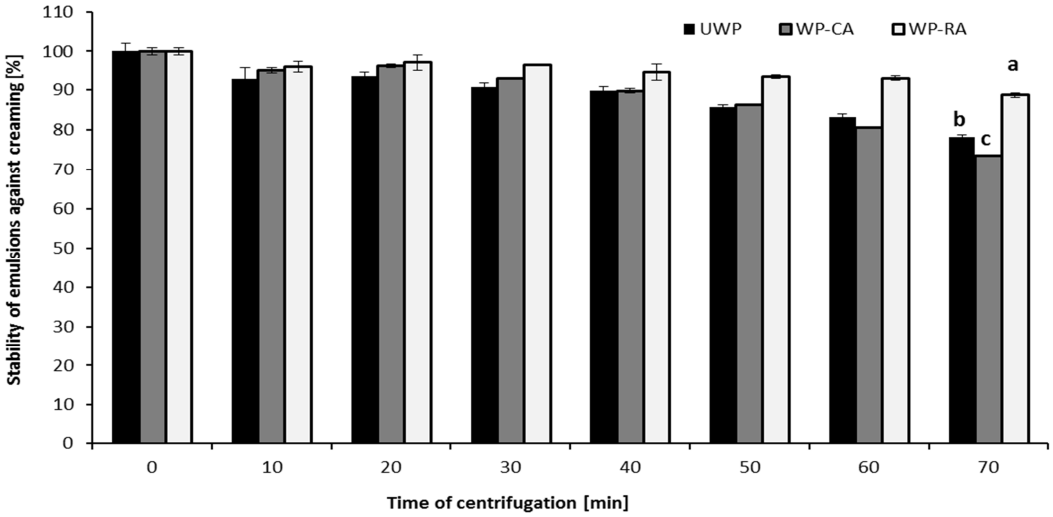

Figure 5. Stability of emulsions prepared with WP modified by CA and RA against creaming

Where: UWP; unmodified WP, WP-CA; modified WP by chlorogenic acid and WP-RA; modified WP by rosmarinic acid

Particle size and zeta potential of emulsions

The particle size of sunflower emulsion emulsified with unmodified and modified WP by CA, RA was determined using Zetasizer apparatus, and the results are shown in Figure (6A). The particle size of the emulsion prepared by unmodified WP was 1.41 ± 0.05 nm, whereas the values for emulsions stabilized with WP modified by CA and RA were 1.82 ± 0.05 and 1.25 ± 0.03 nm, respectively. The particle size of the emulsion markedly decreased after modification by RA, while significantly increased when the modification was done by CA. This can be explained with the change in the surface hydrophobicity of protein after the modification or the amount of protein covered the oil droplet might be not enough. Therefore, this led to the lower emulsifying property of the protein.

Zeta potential values, at pH 7, of the emulsions emulsified with WP (without modification), WP modified by CA, and WP modified by RA were -25.17 ± 0.35, -26.79 ± 1.1, and -31.2 ± 1.04 mV, respectively (Figure 6B). The negative charge of the emulsion droplets might be due to the negatively charged amino acid in the WP surrounding the oil droplet. The lower value of zeta potential was observed in emulsion emulsified with unmodified WP when compared with that of emulsions prepared by WP modified by CA and RA, while emulsion of WP modified RA showed the highest value. This increase could be explained by the incubation of WP with oxidised CA and RA might add the negative charge of phenolics to WP. The results of particle size and zeta potential are in agreement with the result of Keppler and Schwarz (2017), who reported that the particle size of emulsion stabilized with whey protein isolate modified with allyl isothiocyanate was lower than that emulsified with unmodified one while the value of zeta potential was higher.

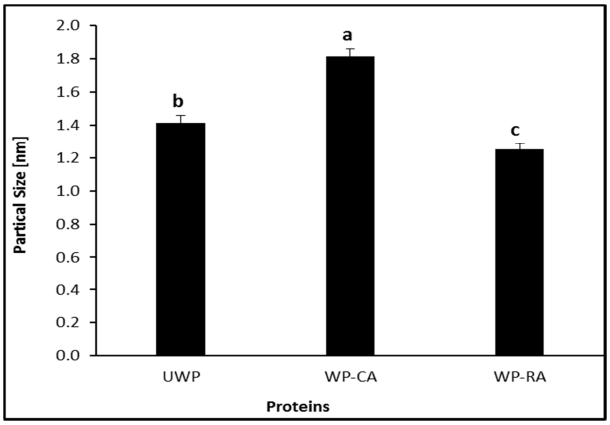

Figure 6A. Particle size of emulsions

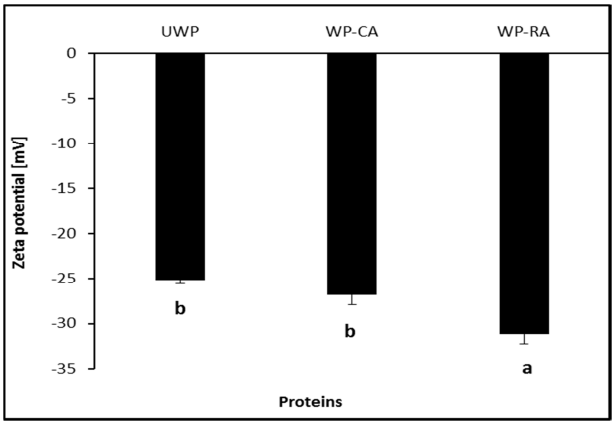

Figure 6B. Zeta potential of emulsions

Where: UWP; unmodified WP, WP-CA; modified WP by chlorogenic acid and WP-RA; modified WP by rosmarinic acid, the different letters represent significant differences

CONCLUSION

This study was conducted to increase the knowledge about the covalent interactions between RA and whey protein isolate compared with CA. The obtained results demonstrated that RA showed more reactivity for the covalent interactions with WP than CA. RA caused a significant decrease in oil droplet size of emulsion and an increase in zeta potential. Moreover, the emulsion emulsified with WP modified by RA was more stable against creaming than that emulsified with unmodified and modified WP by CA. It could be concluded that the consumers can benefit from a health-promoting effect of conjugated phenolic compounds. Also, the covalent interactions of WP by RA could be a possible technique to change the emulsifying properties of WP. Finally, these proteins could possibly be used in preparing functional foods.

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توصيف معزول بروتين الشرش المعدل تساهمياً بواسطة مركبات فينولية
2:- الخواص الفيزيوكيميائية والاستحلابية

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لقد تم هذا العمل بغرض دراسة تاثير التعديلات التساهمية لمعزول بروتين الشرش(WP) بواسطة حمض الكلوروجينيك (CA) وحمض الروزمارنك (RA) على خواصه الفيزيوكيميائية والاستحلابية. أظهرت النتائج أن هذه الأحماض ارتبطت بشكل تساهمي مع الـ WP، وقد تم التحقق من ذلك بالانخفاض في محتوى مجموعات الأمين والكبريت الحرة. كما أوضحت نتائج جهاز الـــ Zetasizer وتحليل (1-anilino-8-naphthalensulfonate, ANS) بعض التغييرات بين WP المعدل وغير المعدل في الجهد زيتا ، حجم الجسيمات ، نقطة التعادل الكهربية واللا قطبية السطح. وكان الـ WP المعدل بـ CA له تأثير زيادة فى اللا قطبية السطح ونقطة التعادل الكهربي ، في حين أن الـ WP المعدل بـ RA كان له نقص فى نقطة التعادل الكهربى. أظهرت نتائج التعديل أدى الى تغيير في خصائص الاستحلاب. تم فحص حجم الجسيمات والجهد زيتا لمستحلب عباد الشمس المحضر باستخدام 0.3٪ WP (المعدل والغير معدل) باستعمال جهازتشتت الضوء الساكن (SLS) وأظهرت النتائج أن الـــ WP المعدل بـــ RA انتج مستحلب بحجم جسيمات أصغر ، في حين أظهر WP المعدل بـ CA عكس ذلك. علاوة على ذلك ، البروتينات المعدلة شكلت مستحلبات بجهد زيتا أعلى من تلك الغير معدلة. وأخيرًا ، عمل الـ WP المُعدل بـ RA على تحسين ثبات المستحلب ضد تكون الكريمة بالمقارنة مع WP الكنترول و WP المعدل بـ CA .وعلى الرغم من أن التفاعلات التساهمية للمركبات الفينولية مع معزول الشرش قد تقلل من خواصها الغذائية ، إلا أن معقدات الفينولات مع بروتين معزول الشرش ، يُنظر إليها كطريقة لتحسين الخواص الأطعمة الوظيفية بسبب خصائصها الجيدة في الاستحلاب.