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Milk proteins consideration in some interactions

[1] Z.M.R. Hamdan,[2]Hala M. Ahkamanidan,[1]A.A. Hossein

[1]Food Science Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt
[2]Dairy Science & Technology Department, National Research Center, Dokki, Giza, Egypt

Abstract: Interaction between milk proteins (casein and/or whey protein isolate) and some of phenolic compounds were incubated and fractionated using Sephadex G-25 column chromatography. Fractions corresponding to the protein and the possible phenolic-protein complexes and fractions corresponding to the free phenolics were collected and their phenolic content was determined by Folin–Ciocalteu reagent. Among the selected commercial phenolic standards tested (Rosmarinic acid, Chlorogenic acid, Quercetin, Vanillin, Gallic acid, Caffic acid and Catechin), the strongest milk protein-binding affinity was with Caffic acid, whereas Chlorogenic acid and Vanillin less interact with milk protein. Interaction in the range wide of pH was determined by precipitating potential assay and the highest interaction was in pH 3 for (Rosmarinic acid, Quercetin, Gallic acid, Caffic acid and Catechin), while it was pH 5 for (Chlorogenic acid and Vanillin) and the lowest interaction for all phenolic compounds was at pH 7. The aim of the study was to further our understanding of the interaction evaluation between some phenolics and milk proteins.

Key words: Phenolics proteins interactions • Casein • Whey protein isolate

INTRODUCTION

Milk is one of the unique sources of nutrients and wildly recognized by the consumers as a source of compounds beneficial to growth and health in children and adults [1]. Recent interest in dietary phenolic compounds on focused mainly on their health benefits that are ascribed to their ability to act as antioxidant [2]. This attributed to the presence of electron donating as withdrawing groups at the aromatic system which has been shown to strongly influence the redox potential of phenols [3]. The bioavailability and nutraceutical effects of many phenolic compounds are modified in the presence of proteins. To obtain the maximum benefit of these phenolic compounds, it is of fundamental importance to understand the nature of the protein-phenolic interaction due to their importance in dairy products. Phenolic compounds that occur naturally with tannins in plants are responsible for the systemic effects of diets containing proanthocyanidins [4]. Interactions between proteins and polyphenols can be reversible associations (via hydrogenbonding, hydrophobic interactions and van der Waals forces) that alter the solution properties of proteins, or can be permanent modifications of proteins. Reversible associations may or may not result in protein precipitation, dependent on factors such as ionic composition of solution and pH. Protein precipitation that occurs at low ratios of protein to polyphenolic may be reversed as the ratio increases [5]. The phenolic-protein interactions are a matter of continuous research due to their importance in food. Nutritional effects of polyphenols are largely attributed to the capacity of phenolics to bind and precipitate proteins [6, 7]. The sensation of astringency is due to the interaction of polyphenols with proteins [8]. Polyphenols can also bind proteins in beer, wine and fruit juices, resulting in undesirable turbidity and colloidal haze [9, 10].

The objective of the study was to further our understanding of the interaction evaluation between some phenolics (Rosmarinic acid, Chlorogenic acid, Quercetin, Vanillin, Gallic acid, Caffic acid and Catechin) and milk proteins (casein and/or whey proteins), because this interaction may be reduce the nutritional value of the milk protein, so the aim of this study to know which phenolic compounds less interaction with milk protein.

Corresponding Author: ZMR Hamdan

MATERIALS AND METHODS

Materials: Fresh cow's skim milk was obtained from faculty of agriculture, Cairo University. Whey proteins isolate (WPI) was obtained from Davisco Foods International Inc., (Minnesota, USA). Sephadex G-25, pharmacies fine chemicals. Folin-Ciocalteu reagent and Catechin were purchased from Fluka Chemical Co. Gallic and Rosemarinic acids were obtained from MP Biomedical LLC. Caffic acid was purchased from Bio Basic INC. Vanillin and Chlorogenic acid, from Roth GmbH Co. Qurecetin, from ALDRICH. Ferric chloride, Sodium carbonate anhydrous, Sodium di basic and Hydrochloric acid (HCl): from Egyptian Co. for Chemicals & Pharmaceutical (ADWIA). Sodium mono basic and Sodium dodecyl sulfate: from MERCK (Darmstadt - Germany). Tri- ethanolamine, Ethanol and ether: from sd. fine- Chem. Limited.

Methods:

Preparation of Casein Powder: Casein powder was prepared from fresh cow's skim milk according to Morr [11].

Preparation of Standard Phenolic Compounds Solution: Phenolic compounds (Catechin, Gallic acid, Qurecetin, Caffic acid and Vanillin) was dissolved in 0.1M sodium phosphate buffer pH 7, (0.1% w/v) while Chlorogenic acid and Rosemarinic acid was dissolved in ethanol (0.1% w/v).

Preparation of Milk Proteins Solution: Casein and whey protein isolate solution were prepared by dissolving it in 0.1 M sodium phosphate buffer pH 7 (0.1% w/v) [12].

Separation of Milk Proteins and the Phenolics by Sephadex G-25 Chromatography: Two mixtures were prepared from protein/phenolics (1:1 v/v) and phenolics/ buffer (1:1, v/v) [12]. The procedure described by Bartolomé *et al*. [13] with some modifications. The mixtures were incubated at 37°C for 2h. Immediately after incubation, 5.0 ml of each mixture were fractionated through a Sephadex G-25, column (50 cm X 3 cm ID) contain 0.1 M sodium phosphate buffer pH 7.0 (200 ml). Mixtures were eluted with 0.1 M sodium phosphate buffer pH 7.0 at a flow rate of 1 ml/min. Fractions of 10 ml were collected for each tube. Fraction free phenolic was determined total phenolic compound by Folin-Ciocalteu Reagent. Calculation was performed to differentiate the behavior of different phenolic structure:

Protein-bound phenolics % = [{Σ (Phenolic / buffer) – Σ (free phenolics)} ÷ Σ (Phenolic/buffer)] x 100.

Precipitating Potential Assay: The ability of phenolic compounds to precipitate casein and whey protein isolate was investigated employing according to Hagerman and Butler [14].

Determination of Total Phenolic Compounds: The total phenolic content determined by Folin-Ciocalteu Reagent according to Zheng *et al.* [15] and calculated as Gallic acid

RESULTS AND DISCUSSION

Interactions Between Phenolic Compounds and Milk Proteins by Column Chromatography: Figure 1 to 8 evaluated the total phenolic compounds eluded from the column contain complex of casein and/or whey protein isolate with (Rosmarinic acid, Chlorogenic acid, Quercetin, Vanillin, Gallic acid, Caffic acid and Catechin), as phenolic compounds (1:1, v/v). As expected, the free phenolics from casein and or whey protein isolate /buffer mixture gave a single peak. The free caffic acid elution volume corresponded to the column void volume (110-230 ml) for casein and between 150 to 250 ml for whey protein isolate,

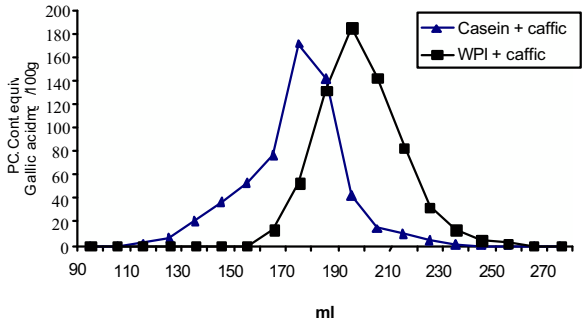

Fig: 1: Sephadex G-25 elution profiles for the mixture of caffic acid and casein/ WPI.

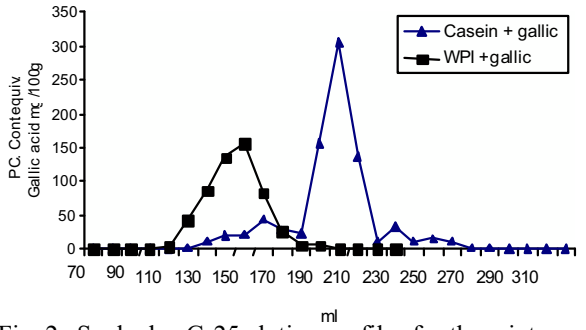

Fig. 2: Sephadex G-25 elution profiles for the mixture of gallic acid and casein /WPI.

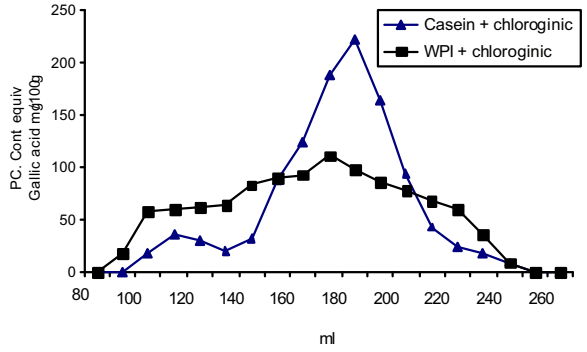

Fig. 3: Sephadex G-25 elution profiles for the mixture of chloroginic acid and casein /WPI.

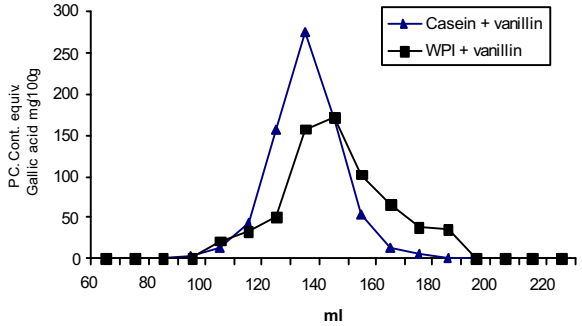

Fig: 4: Sephadex G-25 elution profiles for the mixture of vanillin and casein /WPI.

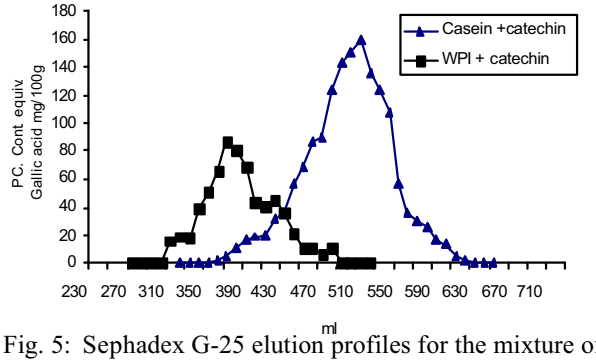

Fig. 5: Sephadex G-25 elution profiles for the mixture of catechin and casein/ WPI.

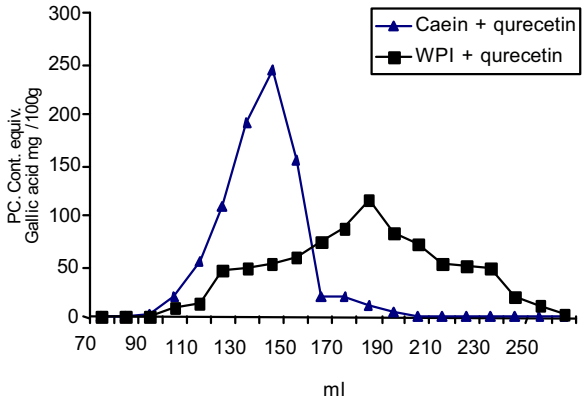

Fig. 6: Sephadex G-25 elution profiles for the mixture of qurcetin and casein/ WPI.

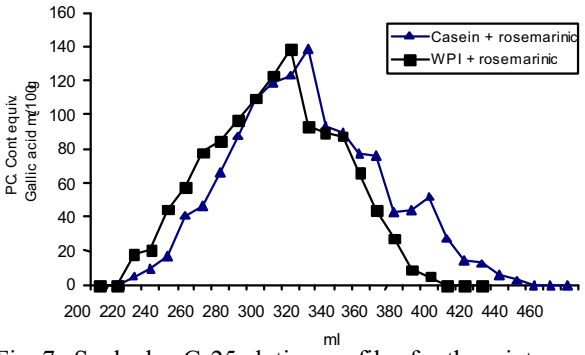

Fig. 7: Sephadex G-25 elution profiles for the mixture of rosemarinic acid and casein / WPI.

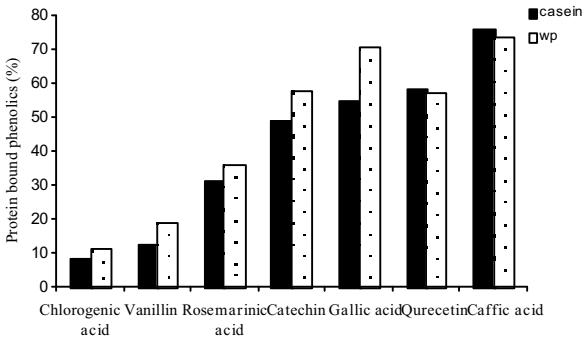

Fig. 8: Interaction evaluation of casein and WPI-bound phenolics

whereas free gallic acid eluted between 130 to 270 ml and 110 to 190 ml for casein and whey protein isolate respectively, while the free chlorogenic acid eluted between 90 to 250 ml for casein and from 80 to 250 ml for whey protein isolate, but free vanillin was eluted between 90 to 190 ml for both casein and whey protein isolate, whereas the free catechin eluted between 390 to 620 ml for casein and from 310 to 480 ml for whey protein isolate, in the same time the free qurecetin eluted between 90 to 190 ml for casein and between 90 to 470 ml for whey protein isolate, in the end the free rosemarinic acid eluted between 220 to 440 ml for casein and between 220 to 390 ml for whey protein isolate. Low molecular weight phenolic in the phenolic/buffer elutions of caffic acid, gallic acid, chlorogenic acid, vanillin, catechin, qurecitin and rosemarinic acid can be attributed to retention of phenolics in the Sephadex gel [16].

As shown in the absorbance profiles, elution of free phenolics was appeared for casein and or whey protein isolate/phenolic mixtures than for phenolics/buffer mixtures. The total phenolic compound of phenolics in the elutions of free phenolics from casein and or whey protein isolate/phenolic was smaller than in the corresponding elutions of phenolics/buffer (Table 1). The “protein-bound phenolics” corresponds to

Table 1: Interaction evaluation between phenolic compound and milk protein

	Total phenolic compound cont. Equiv. Gallic acid mg /100g				Interaction evaluation	
			Σ (free phenolics)		Protein bound phenolics (%)	
Phenolic compounds	Phenolic standard	Σ (Phenolic / buffer)	Casein	WPI	Casein	WPI
Chlorogenic acid	2337.4	1198.7	1103.8	1070.1	7.90	10.70
Vanillin	1088.7	835.7	734.0	682.0	12.10	18.40
Rosemarinic acid	2798.1	1868.1	1296.7	1192.9	30.60	36.10
Catechin	3068.1	1569.5	1534.0	667.6	48.80	57.50
Gallic acid	2936.1	1856.1	837.9	547.3	54.90	70.50
Qurecetin	3161.1	1994.1	836.2	854.4	58.00	57.15
Caffic acid	2786.1	2426.1	581.3	650.1	76.00	73.20

the percentage of phenolics that interacts with milk proteins (Table 1). Caffic acids exhibited the highest value for casein and or whey protein isolate-bound phenolics (76% and 73.2%) respectively. Whereas, chlorogenic acid exhibited the lowest for both casein and or whey protein isolate-bound phenolics (7.9% and 10.7%) respectively. The percentage of casein-bound phenolics arranged ascending as vanillin< rosemarinic acid < catechin < gallic acid < qurecetin, it was 12.1%, 30.6%, 48.8%, 54.9% and 58%. On other hand, whey protein isolate-bound phenolics arranged ascending as vanillin< rosemarinic acid < qurecetin < catechin < gallic acid (%) was 18.4%, 36.1%, 57.15%, 57.5% and 70.5% respectively. The interaction between phenolic compounds and protein depend on structure of phenolic, where some complexes exhibit hydrogen bonding and electrostatic interaction plays a dominant role in the stabilization of the peptide by phenolic compounds. The ð-OH type of interaction also observed in the peptide stabilization, Phenolics molecule has been placed appropriately near the side chain groups of the peptide. The functional groups para-OH (ñ-OH) meta-OH (m-OH) and COOH of phenolics have been assumed to act as a hydrogen bond donor/acceptor for different side chain groups of amino acid [17]. Moreover, it was noticed that the interaction between the studied phenolic compounds and whey protein was more than casein, Fig. (8). This may be due to the presence of more sulphur containing amino acid (cysteine) in whey protein rather than casein which forming covalent bonds via a quinone mediated mechanism including thiol group. The quinones on the rings react to form permanent covalent bonds with compounds such as proteins and sulphur-containing compounds [3]. These explain the higher interaction evaluation of caffic acid and gallic acid than other experimental phenolic compounds.

Caseins have a considerable amount of proline, the hydrophobic attraction between proline and the phenolic group is stabilized by H-bond formation between phenolic ring groups and the bis-alkyl substituted amide nitrogen of the proline amino group. The interaction is a complex one, with water also likely to play a part [5] and this interaction was non-covalent [18]. The hydrophobicity of the surface sites of milk proteins was decreased in the presence of green tea flavanoids. The decrease in protein surface hydrophobicity was explained by the hydrophobic binding between milk proteins and green tea flavanoids [18]. The caffic acid caused rapid reduction in the number of cysteine residues in the milk on heating, which will interfere with whey protein isolate interactions with the casein micelle that contribute to ê-casein depletion. Calcium chelation is responsible to some extent: caffic acid at 0.1% depleted Ca^{+2} in unheated milk by ~20%, but its effect on milk stability was only partly reversed on adjustment back to usual calcium levels and it had little effect on other calcium-dependent processes such as rennet coagulation [3].

Effect of pH Value on Interaction between Phenolic Compounds and Milk Proteins: It was noticed the existence of strong electrostatic attraction between positively charged milk proteins and negatively charged phenolic compounds at pH 3. On the other hand, the minimum adsorbed phenolic compounds and milk protein at pH 7 may arise from the electrostatic repulsion between negatively charged protein surfaces and negatively charged molecules [19]. The relationship between the amount of protein-phenolic complex precipitated (A_{510}) and the content of phenolic compound in the mixture was investigated at pH 3, 5, 7 and 9, the curves were depicted in Fig. 9 and 10. Concerning to the chlorogenic acid and vanillin was found that pH 5 was the maximum pH to form insoluble casein or whey protein-phenol complexes it was 0.447 and 0.19 with casein respectively but and 0.188 and 0.282 with whey protein isolate, respectively.

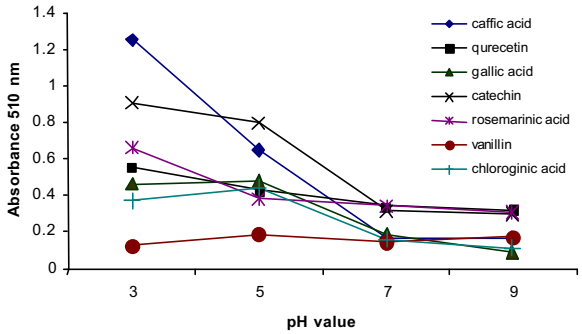

Fig. 9: Effect of pH on formation of insoluble complexes between phenolic compound and casein.

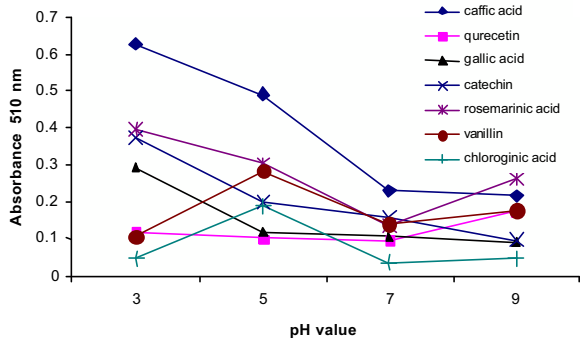

Fig. 10: Effect of pH on formation of insoluble complexes phenolic compound and WPI.

CONCLUSION

The aim of the present study was to investigate the binding type between phenolics compounds and casein or whey protein isolate, using different approaches. It may be assumed that the initial binding between phenolics and milk proteins yields soluble complexes which upon subsequent cross-linking are transformed into insoluble precipitates and determination this interaction based on fractionation by G-25 Sephadex chromatography, for evaluating the extent of the interaction between low molecular weight phenolic compounds and milk proteins that could affect the antioxidant capacity of phenolic compounds in food systems. Cross-linking of milk proteins is an important fact because of potential effects on textural properties of milk products, e.g., yogurt and cheese. The cross-linking effect of vanillin phenolic on milk proteins can be used for manufacturing of milk products (ice cream) with desired textural properties.

Therefore, interactions of vanillin phenolic with milk proteins must be considered in this regard. Nevertheless, they may be acceptable food additives because of their antioxidant and anticarcinogenic activities; so they also contribute to the functionality of the products. Displaying the interactions of phenolic compounds with milk proteins is a necessary subject, as they may be potential food additives.

REFERENCES

1. Rahimi, Y.S. and M. Corredig, 2012. Heating of milk alters the binding of curcumin to casein micelles. A fluorescence spectroscopy study. Food Chemistry, 132(3): 1143-1149.
2. Kosiñska, A., M. Karamaæ, K. Penkacik, A. Urbalewicz and R. Amarowicz, 2011. Interactions between tannins and proteins isolated from broad bean seeds (*Vicia faba* Major) yield soluble and non-soluble complexes. Eur. Food Res. Technol., 233: 231-222.
3. Singh, R., 2011. Interaction of phytochemicals with dairy ingredients. M.Sc. Thesis, the University of Auckland.
4. Jimenez-Ramsey, L.M., J.C. Rogler, T.L. Housley, L.G. Butler and R.G. Elkin, 1994. Absorption and distribution of ^{14}C-labeled condensed tannins and related sorghum phenolics in chickens. J Agric. Food Chem., 42: 693-967.
5. Luck, G., H. Liao, N.J. Murray, H.R. Grimmer, E.E. Warminski, M.P. Williamson, T.H. Lilley and E. Haslam, 1994. Polyphenols, astringency and proline-rich proteins. Phytochemistry, 37(2): 357-371.
6. Butler, L.G., 1989. Effect of Condensed Tannin on Animal Nutrition. Chemistry and Significance of Condensed Tannins. New York: Plenum Press, pp: 391-402.
7. Jansman, A.J.M., 1993. Tannins in feedstuffs for simple stomached animals. Nutrition Research Reviews, 6: 209-236.
8. Haslam, E., editor, 1998. Practical Polyphenolic: From Structure to Molecular Recognition and Physiological Action. Cambridge: University Press, pp: 178-225.
9. Yokotsuka, K. and V.L. Singleton, 1995. Interactive precipitation between phenolic fractions and peptides in wine-like model solutions: turbidity, particle size and residual content as influenced by pH, temperature and peptide concentration. Am. J. Enol. Vitic., 46: 329-338.
10. Siebert, K.J., 1999. Effects of protein-polyphenol interactions on beverage haze, stabilization and analysis. J. Agric. Food Chem., 47: 353-362.

11. Morr, C.V., 1985. Functionality of heated milk protein in dairy and relate foods. J. Dairy Sci., 68(10): 2773-2781.
12. Ali, H., 2002. Protein-phenolic interactions in food. M.Sc. Thesis, Department of Food Science and Agricultural Chemistry Macdonald campus, McGillb University Monteral, Québec.
13. Bartolomé, B., I. Estrella and T. Hernandez, 2000. Interaction of Low Molecular Weight Phenolics with Proteins (BSA). J of Food Science, 65(4): 617-621.
14. Hagerman, A.E. and L.G. Butler, 1978. Protein precipitation method for the quantitative determination of tannins. J. Agric. Food Chem., 26: 809-812.
15. Zheng, H.H., P.F. Tu, K. Zhou, H. Wang, B.H. Wang and J.F. Lu, 2001. Acta Pharmacol Sin., 12: 1094-1108.
16. Porath, J., 1979. Molecular-sieving and non-ionic adsorption in polysaccharide gels. Biochem Soc. Trans, 7: 1197-1149.
17. Madhan, B., P. Thanikaivelan, V. Subramanian, J. Raghava Rao, U.N. Balachandran and T. Ramasami, 2001. Molecular mechanics and dynamics studies on the interaction of gallic acid with collagen-like peptides. Chemical Physics Letters, 346: 334-340.
18. Yuksel, Z., E. Avci and Y.K. Erdem, 2010. Characterization of binding interactions between green tea flavanoids and milk proteins. Food Chemistry, 121: 450-456.
19. Haslam, E., 2003. Thoughts on thearubigins. Phytrochem., 64: 61-73.