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ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENTS OF RECONSTITUTED SKIM MILK – ROSEMARY WATER EXTRACT MIXTURE

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ABSTRACT

To evaluate the effect of supplementation of skim milk (SM) with Rosemary (Rosemarinus officinalis) water extract (RWE), the radical scavenging activity (RSA) using DPPH assay and total phenolic compounds (TPC) with Folin–Ciocalteau reagent were determined. Also, mixing periods and the total solids (TS) of the mixture were taken into consideration. Reducing the TS of reconstituted SM (3% to 0.35%) increased its RSA%, while its whey derivative showed high RSA% than its casein. RSA% of 10% RWE was 94%, it was high through the few min. of mixing and decreased upon elongated the mixing period until 60 min. Casein derivative showed the lowest value of RSA% and this value was not affected with mixing periods. Reducing the TS of SM—RWE mixture increased its RSA % at all mixing periods, whereas its casein derivative almost unaffected upon dilution its mixture.

Moreover, SM showed the highest TPC, reducing its TS decreased its TPC and also for its casein and whey derivatives. The TPC of RWE was 458mg / L gallic acid, reducing the concentration of RWE to 1.5 % decreased its TPC to 130 mg / L. gallic acid SM- RWE mixture had the highest content of PC after all mixing periods (few min to 60 min) for all TS conc. Conversely, its separated casein showed the lowest content of TPC, and diluted the mixture decreased the TPC of casein. This confirms that there was a part of antioxidant activity which masked by specific interactions between casein protein and rosemary phenolic compounds.

Furthermore, the correlation coefficient between RSA% and TPC of RWE was 0.992 and it was 0.990 and 0.884 for casein and whey derivatives respectively, while SM had negative correlation coefficient taking TS as a factor. There was a good correlation coefficient between RSA% and TPC for SM- RWE and its whey derivative through the few min. of mixing with reducing the TS of the mixture to 0.75 and 0.35 % SM-TS, whereas casein derivative had negative correlation coefficient at all different mixing periods and TS concentration of the mixture.

INTRODUCTION

Milk is a basic food for human development. Previous studies revealed that milk and its fractions have antioxidative properties (Zulueta *et al.*2007; Calligaris et al, Lo Scalzo *et al.*2004; Chen *et al.*2003; Rawel *et al.*2001; Steijns & Van Hooijdonk 2000 and Taylor & Richardson 1980), and some have been patented (Suetsuna *et al.*2000, Rival *et al.*2001). They demonstrated that several components of milk are active in preventing lipid oxidation and maintaining milk quality, and also pointed to their potential

usage as ingredients in food and pharmaceuticals to provide products for enhanced consumer health.

Milk casein has polar domains that contain phosphorylated serine residues, and their characteristic sequences are effective cation chelators that form complexes with calcium, iron and zinc, for that casein has been shown to provide antioxidant activities against TBAR assay (Pihlanto 2006). Moreover, Suetsuna *et al.*(2000) reported that milk casein has scavenging activity of hydroxyl radical by oxidation of amino cid residues.

Several power food antioxidants in whey have recently been discovered and examined (Wikiprojct whey protein Food and Drink 13 July 2007; Bartfay *et al.*2003 and Tong *et al.*2000 a,b), among these are proteins, enzymes (superoxide dismutase, catalase, glutathione peroixdase), peptides, amino acids, citrate, phosphate and ascorbic acid (Colbert & Decker 1991). Hernandez – ledesma *et al.*(2005) identified several peptides in β- lg, possessed high scavenging activing as a result of their content of specific amino acids. Sulfur-containing amino acid such as cystein and methionine, imidasol of histidine have antioxidant activity (Zommara *et al.*2002 and Taylor *et al.*1980) and tyrosine and tryptophan served as hydrogen doners (Pihlanto 2006).

Rosemary (Rosemarinus afficialis) is one of the most effective spices widely used in food processing. It is found that rosemary has been more extensively studied and its extracts are the first marketed natural antioxidants (Nedyalka *et al.*2006). Besides, it used as food flavouring, is also known medicinally for its powerful antioxidant activity, its antibacterial and antimuginic properties (Perez *et al.*2007).

Rosemary extracts provide a major source of natural antioxidants including; phenolic diterpens such as carnosic acid, carnosol rosemanol, epi- and iso-rosemanol, rosemadiol and methyl carnosate (Ibanez *et al.*2003), besides, other compounds such as rosmarinic acid, caffeic acid and flavonoides (Suhaj *et al.*2006 and Del Bano *et al.*2003). Furthermore, Kosaka & Yokoi 2003 found that carnosic and carnosol were able to promote markedly enhanced synthesis of nerve growth factor, because phenolic antioxidant are excellent hydrogen or electron doners and their radical intermediates are relatively stable due to resonance delocalization and lack of suitable sites for attack by molecular oxygen. Silvia *et al.*(2006) decided that rosemary extract is a good candidate for function foods as well as for pharmaceutical plant-based products.

The advantage of using rosemary extract that its polar hydrophilic components partitioned into the water phase and becomes more protective than other herbs (Zheng & Wang 2001 and Frankel 1999), this was preferable to approximate the levels of antioxidant in rosemary that might consumed and observed during normal dietary intake .

The objective of the current study is therefore to evaluate the DPPH radical scavenging activity (RSA %) and the amount of TPC in SM, RWE and the mixture of them, also the effect of mixing periods and TS of the mixtures on their content of RSA and PC. The study also aims whether there is a correlation between RSA % and TPC because there is a lack of information about this and in order to evaluate the possible synergistic or

antagonistic effect on the milk components and their contribution to the antioxidant activity and phenolic components of rosemary extract. Moreover the interaction between herbs supplementation and nutrient components of milk is now an area of future interest which might suggest their inclusion in function dairy products.

MATERIALS AND METHODS

Low heat skim milk powder (total protein 34%).Rosemary dried herb was purchased from local herbal store. DPPH and Folin–Ciocalteau reagents were from sigma chemical co.

Skim milk powder was reconstituted to 12 % TS, and then diluted to 3 % , 1.5 %, 0.75 % and 0.35 % TS casein and whey samples were made from the previous reconstituted skim milk by adjusting pH to 4.6 with 0.1 N HCL followed by centrifugation at 200 rpm at room temperature for 10 min . Whey separated by paper filtration (whatman 40) its pH was raised to 6.7 and the precipitated casein washed by distilled water several times to remove the traces of HCL , then 2.5 g dissolved in phosphate buffer pH 7 .

The rosemary dried herb was ground and 90 ml of distilled water 60^{o}C were added to 10 g powdered herb and stirred for 15 min in a dark place at room temp. The extract was allowed to stand for 15 min and then centrifuged at 200 rpm at 10 min to obtain free RWE. Skim milk rosemary water extracts mixture (SM –RWE) was prepared by adding 1ml of 10 % RWE to 12 %reconstituted SM, then the mixture diluted to one fourth with distilled water to obtain mixture of 3 % skim milk total solids , then to half (1.5 % SM total solids) ,….. etc, and then left for few minutes , 20, 40, 60 min. after mixing . Casein and whey derivatives were prepared from the previous mixtures of SM – RWE with the same method as described before.

Radical – scavenging activity of reconstituted skim milk RWE and their mixture was measured according to the method of Brand- Williams 1995. One tenth of sample was added to 2.9 ml of (DPPH) radical solution. The absorbance was measured at 514 nm after 20 min of reaction. The scavenging activity of the sample was expressed as percentage of inhibition of the DPPH radical, defined as

$RSA\ \% = A_{Control} - A_{Sample} / A_{Control} \times 100.$

Total phenolics was determined spectrophotometrically using the Folin–Ciocalteau reagent by Slinkard & Singleton 1977 using Gallic acid as a standard. The resulted were expressed as milligrams of one liter of gallic acid.

RSA% and total phenolic content experiments were run in triplicate and averages correlation coefficients between RSA% and TPC were calculated to estimate the effect of mixing periods and the reducing of total solids of the SM-RWE on their antioxidant activity and phenolic compounds.

RESULTS AND DISCUSSION

1-Evaluation of radical scavenging activity (RSA %)

1-1: Reconstituted skim milk

Antioxidant activity of reconstituted skim milk with different total solids concentration, and its prepared casein and whey was illustrated in fig 1. It is obvious that skim milk, whey and casein had antioxidant properties through the determination of RSA using DPPH-radical assay. This observation was confirmed by the previous investigators. The antioxidant activity of reconstituted skim milk increased with decreasing its total solids from 3%to 0.35%. This probably due to the dissociation as a result of dilution which made the antioxidant amino acids easy exposed; the role of the dilution of skim milk and its prepared fractions was not clear. Skim milk- derivative whey showed higher RSA than its casein. Several researches reported the powerful antioxidants in whey (Wikipedia WP food and drink 2007; Altern 2004; Sukkar &Bounous 2004; Medves et al. 2003; Tien *et al.*2001; Tong *et al.*2000 a,b and Zhang & Beynen.1993) .

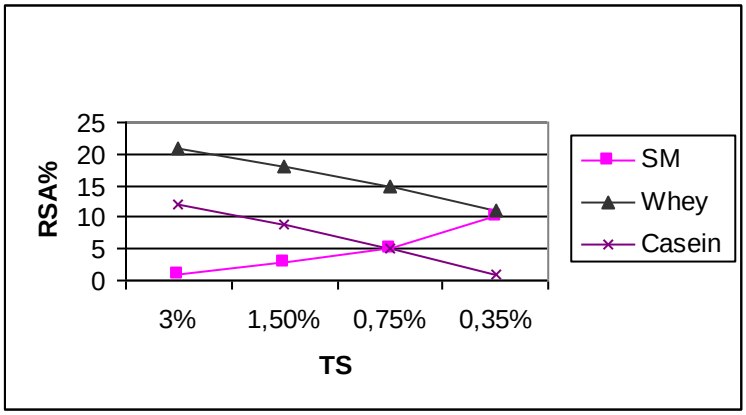

Fig (1): DPPH radical scavenging activity (RSA) of reconstituted skim milk and its whey and casein derivatives at pH 6.7

Conversely other studies suggested that casein had higher antioxidant activity than whey (Chen *et al.*2003 and Wong & Kitts 2003), these activities depended on several factors including pH, state of the sample, temperature and the experimental differences. Moreover, the antioxidant of casein was hypothesized to be mostly due to a physical binding of proxidants, while the antioxidant activity of whey thought to be chemical in nature (Tong *et al.*2000 a,b). Chen & Nawar 1991 showed that dissolution of some amino acids in water or 0.1 N HCL reduced its antioxidant activity. It was thought that in water the activity was lost due to possible hydrogen bonding of the antioxidant activity with water. The effect of the acid on activity was thought to be due to the state of - COOH group which is less effective than Coo^{-} group.

1-2: Rosemary water extract:

Rosemary water extract (RWE) showed high radical scavenging activity (92%) against stable radical DPPH. This value slightly lower than 10% rosemary methanol extract (94.82%) by Hamed 2003. Theretofore, Debersac *et al.*2001 reported that water – soluble extract of rosemary contained rosemarinic acid (molecule having catichol portions at the two ends), flavones (drevatives of flavonoids) and monoterpenes. Wada *et al.*2004 evaluated the quenching effects of non-water-soluble and water-soluble rosemary extract against active oxygen species by a chemiluminescent assay and reported that the two extracts might be available as antioxidant for foodstuffs. Silvia *et al.*(2006) mentioned that methanol rosemary extract containing 5% rosemarinic acid RA, while, water extract containing 15% RA. Furthermore, Perez *et al.*(2007) evaluated antioxidant activity of methanol, ethanol and water rosemary extract using DPPH-radical scavenging method. They found that IP (inhibition percentage) was constant at its maximum value and no significant differences were observed due to the effect of the solvent. Water extraction is safety as no organic solvents are used, therefore no residual solvents were present.

Upon gradual decrease the concentration of ground herb, the RSA% decreased to 29% for 1.5% concentration, this may be due to possible formation of hydrogen bonds with water (Pekkarinen *et al.*1999). Yanishlievia 2001 showed that the activity of carnosic acid and carnosol was attributed to the co-operation their ortho phenolic groups with their isopropyl group and their effectiveness was concentration dependent.

1-3 Skim milk rosemary extract mixture:

1-3-1 Effect of mixing period

Effect of supplementation reconstituted skim milk with 1 % RWE on its antioxidant activity (RSA %) at different mixing periods (few mints to 60 min.) was presented in fig 2. As general, it is clear that RSA % for all samples was high through the few minutes of mixing and then decreased upon prolonging the mixing period until 60 min. After 20 min of mixing, the RSA % of SM-RWE mixture and its prepared whey were dropped and then gradually decreased for all different total solids conc. However, casein showed the lowest value of RSA % and its antioxidant activity almost did not greatly affect with mixing periods. This indicated that a part of antioxidant activity was masked by specific interactions between casein protein and rosemary components lead to negative effect on the antioxidant activity. Arts *et al.*2002 attributed the masking of the total antioxidant capacity of milk green and black tea extracts mixture to the combination between certain flavonoids in tea and B-casein in milk. Moreover, Meda *et al.*2004 found high correlation between RSA and proline content in honey samples. On the basis of the results presented by Farrel *et al.*2004, B-casein contains the highest content of proline (35 residues).

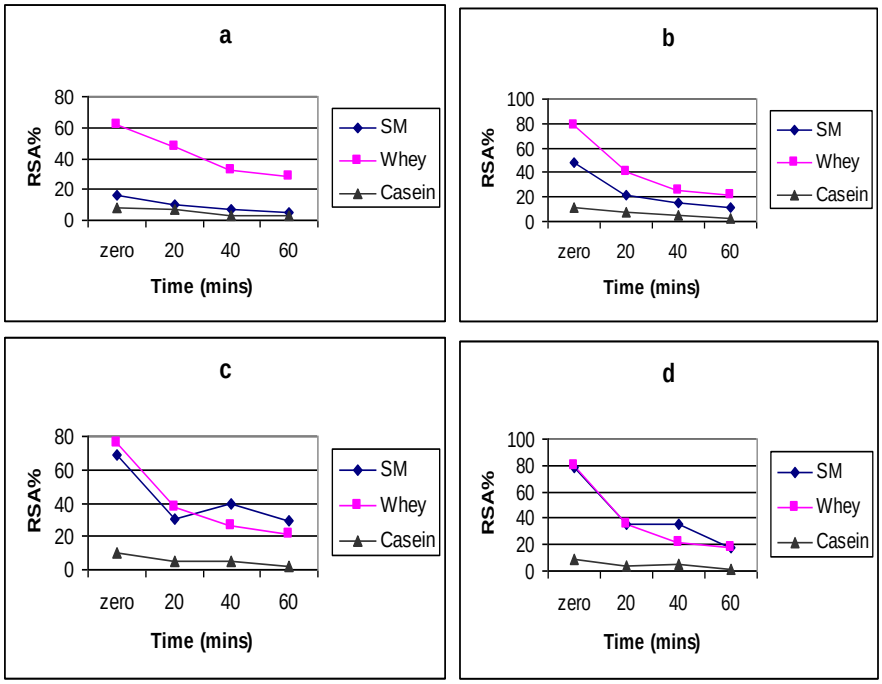

Fig (2): DPPH radical scavenging activity (RSA) of SM- RWA mixture with different SM-TS Conc.
a) 3% b) 1.5% c) 0.75% d) 0.35%

1-3-2: Effect of reducing the total solids

Fig 3, showed that reducing the total solids of SM-RWE mixture, clearly increased RSA % at all mixing periods. This probably due to the synergistic effect between skim milk antioxidant and rosemary components by regenerating their antioxidant properties and this has been observed as a result of dilution. Meanwhile, RSA % of prepared whey gradually decreased after 20 min of dilution the mixture. The mechanism behind the antagonistic effect may be attributed to hydrogen bonding between certain components in whey and hydroxyl group of rosemary extract phenolic compounds. Viljanen *et al.*2005 investigated the effect of raspberry and blackberry juice with different conc. of whey proteins on oil- in-water emulsion oxidative stability using TBARS assay, loss of natural tryptophan fluorescence and formation of protein carbonyl compounds. They found that the antioxidant activity increased with increasing the conc. of berry juices and whey proteins. RSA %.of prepared casein almost unaffected upon diluted the mixture and it was lower than corresponding skim milk and whey, this confirm the previous explanations.

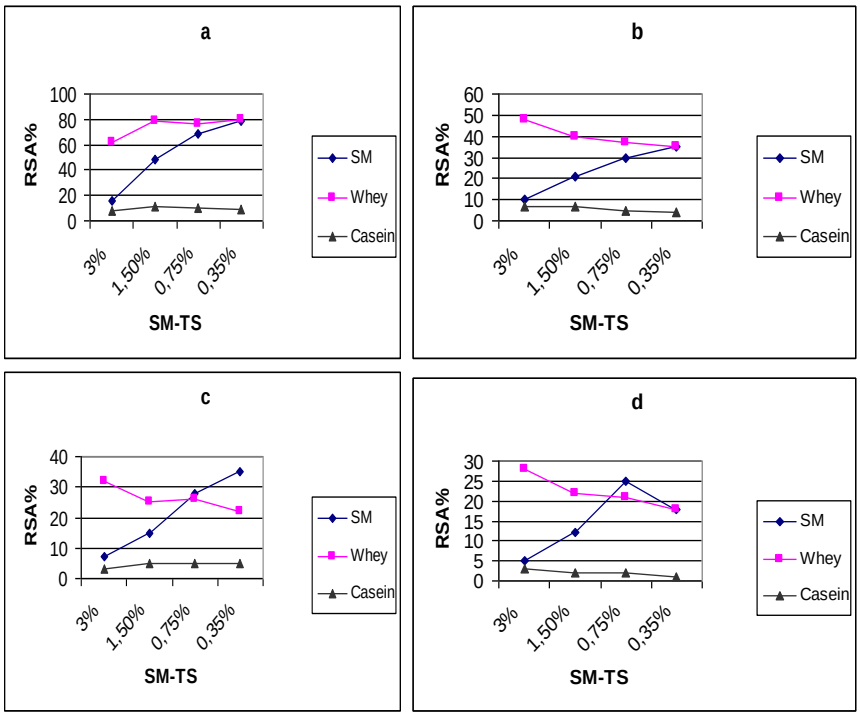

Fig (3): DPPH radical scavenging activity (RSA) of SM-RWE mixture at different mixing periods
a) few mins b) 20 mins c) 40 mins d) 60mins.

2- Evaluation of total phenolic content:

2-1 Reconstituted skim milk

Phenolic content of reconstituted skim milk and its prepared casein and whey was illustrated in fig4. It is obvious that skim milk showed the highest total phenolic content, this presumably due to the presence of one or more residues of amino acids tyrosine, tryprophan, histidine and proline in the antioxidative peptides of milk protein. Pihlanto 2006 mentioned that the phenoxyl and indolyl radicals are serving as hydrogen donors and much more stable. Reducing the total solids of reconstituted skim milk decreased the phenolic content for all samples.

2-2 Rosemary water extract (RWE):

As mentioned before, rosemary water extract contain different phenolic compounds. The present study showed that the total phenolic content of 10% RWE was 458 mg/L gallic acid. Dorman *et al.* 2003 determined the phenolic content of 3% dry RWE to be 185 mg gallic acid equivalent /100g of herbs using ultrasound technique in extraction. Moreover, Zheng & Wong 2001 evaluated the phenolic compounds of 13.3% rosemary phosphate buffer pH 7 - extract and showed that rosmanol is an active antioxidant and has more activity than α-tocopherol or BHT (commercial antioxidants).

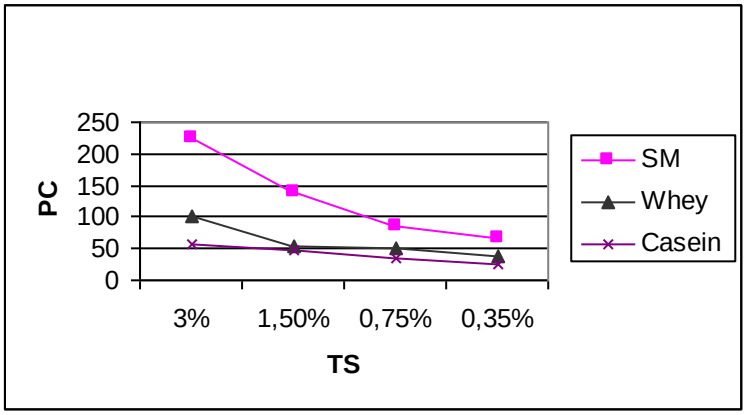

Fig (4): Total phenolic contents (mg/ L gallic acid) of reconstituted skim milk and its whey and casein derivatives at pH 6.7

Reducing the concentration of rosemary from 10% to 1.5% gradually from decreased its total phenolic content to reach 130 mg /L gallic acid at 1.5% rosemary conc., this attributed to the formation of hydrogen bonds between the oxygenated phenolic compounds of rosemary extract and water , thereby the activity , the strength of the hydrogen bonds which were likely more prevalent in water-system and their effectiveness were concentration dependent (Yanishlieva 2001, Zheng & Wang 2001 and Pekkarinen *et al.*1999).

2-3- Skim milk rosemary extract mixture:

2-3-1: Effect of mixing period

Fig(5) showed the effect of supplementation rosemary extract to the reconstituted skim milk with different total solids concentration on the phenolic content of the mixture after 20, 40, 60 min of mixing. It could be noticed that, SM-RWE mixture had the highest content of total phenolic content after all mixing periods for all total solids concentrations. Conversely, its separated casein showed the lowest phenolic content especially after 20, 40 min. of mixing. This suggested that there was casein protein- rosemary phenolic compounds interactions as discussed before. Baier *et al.*2001 hypothesized that the origin of the interaction between dairy proteins and Para –hydrobenzoic acid was partly hydrophobic and partly electrostatic which accounts for its dependence on protein surface area and surface chemistry.

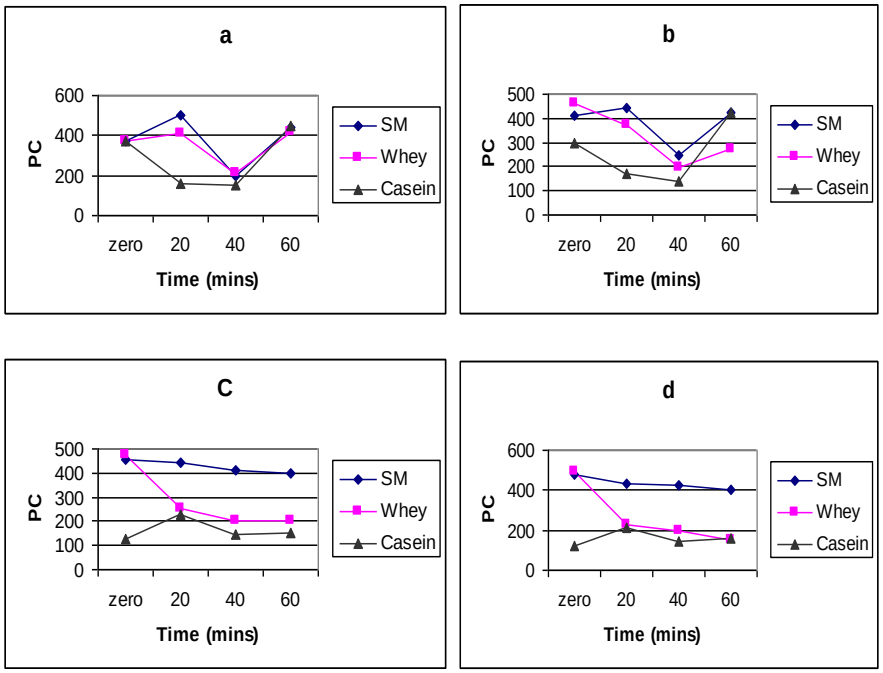

Fig (5): Total phenolic contents (mg/ L gallic acid) of SM- RWA mixture with different SM-TS Conc.
a) 3% b) 1.5% c) 0.75% d) 0.35%.

2-3-2: Effect of reducing the total solids of the mixture

Fig (6) demonstrated the effect of reducing the total solids of SM-RWE mixture on its content of phenolic compounds. Reconstituted skim milk 3% TS and its prepared fractions have nearly the same content of phenolic compounds after a few minutes of mixing, then upon diluted the mixture , the total PC of derivative casein decreased, this means that dilution of the mixture facilitated the interactions between rosemary PC and casein protein as mentioned before. By the time, the diluted mixture had no effect on its PC content, whereas, its whey and casein clearly affected. This indicated that there were also interactions between rosemary PC and whey components as well as casein especially after 60 min of mixing. Rawel *et al.*2001reacted whey protein with selected phenolic compounds (ferulic, chlorogenic, caffeic and gallic acid), and they found that the derivatization was accompanied by a reaction of lysine and tryptophan side chains whereby their content was decreased in comparison to that in control whey protein. Moreover, Bartolome *et al.*2000 fractionated mixture of bovine serum albumin and several low molecular weight phenolic compounds using G-50 Sephadex chromatography, they observed interactions among the commercial phenolic standers and BSA.

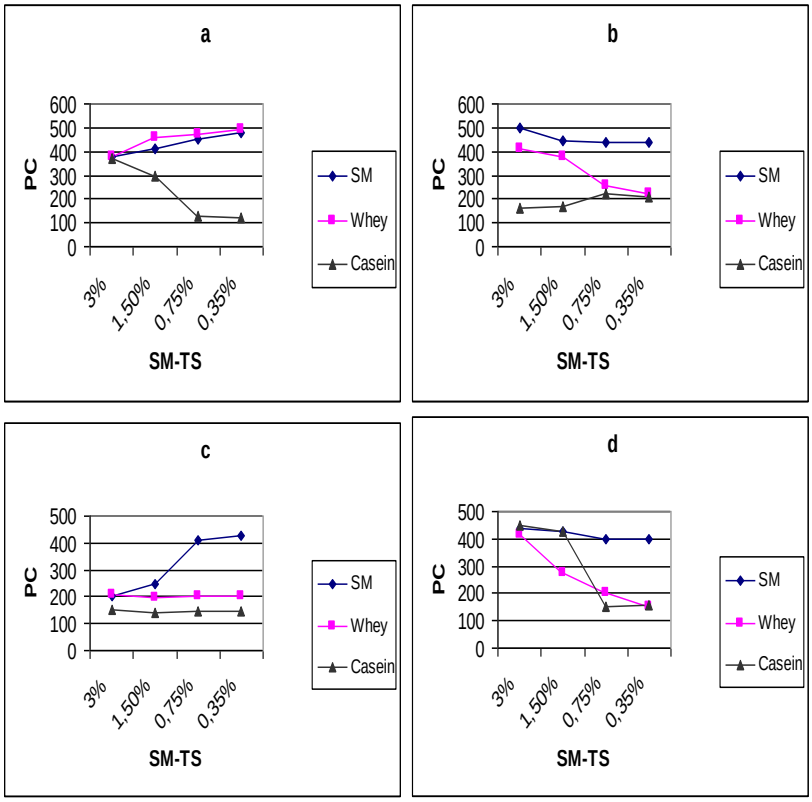

Fig (6): Total phenolic contents (mg/ L gallic acid) of SM- RWA mixture at different mixing periods
a) Few mins b) 20 mins c) 40 mins d) 60mins.

3- Relation between RSA % and phenolic content:

The correlation coefficient between DPPH-RSA and phenolic content of RWE with different concentration (10 % - 1.5%) was 0.992, this revealed that RWE is considered an effective potential source of natural antioxidant; therefore, supplementing a balanced diet (milk) with it may have beneficial health effects
(Perez *et al.*2007; Silvia *et al.*2006 and Zheng & Wang 2001).

On the other hand, there was a negative correlation between RSA% and PC for reconstituted skim milk with 3, 1.5, 0.75, and 0.35 % TS conc.). This indicated that the antioxidant activity of skim milk was not necessarily related to the phenolic amino acids content, there were other compounds acts as efficient antioxidants rather than contributing to total phenolics. Conversely, the corresponding casein and whey derivatives showed high positive correlations between their RSA% and total phenolic content r = 0.999 and 0.884 respectively.

Table (1) illustrated the relation between RSA % and PC for SM-RWE mixture as affected by mixing periods. It is clear that the rosemary phenolic contents synergistic in raising the phenolic content of the mixture.

Table (1): Correlation coefficients between RSA% and TPC of SM-RWE and its derivatives as affected by mixing periods.

Sample	% SM-TS			
	3%	1.5%	٠,٧٥%	0.35%
SM	0.111	0.303	0.805	0.984
Whey	0.297	0.892	0.994	0.993
Casein	- 0.120	- 0.289	- 0.338	- 0.562

The mixing periods affected positively of increasing the correlation between RSA % and PC of the mixture and this relation was observed at low concentration of total solids, it was 0.111 and 0.983 for 3 % and 0.35 SM total solids respectively. Moreover, the RSA % correlated better with PC of whey SM rosemary mixture derivative r = 0.297, 0.892, 0.994 and 0.993 for 3, 1.5, 0.75, 0.35 % SM- TS respectively using mixing periods as a factor. However, incase of casein –derivative it could find a reverse correlation between RSA % and PC, this finding was an illustraction of the complex influence of various factors and interactions which were explicated before .The effect of reducing the total solids of the SM – RWE mixture and its derivatives on the correlation coefficient between RSA % and their content of phenolic compounds was shown in table (2). A good correlation was observed for SM – RWE attributable their total solids concentration after few and 40 min. r = 0.983 and 0.981 respectively. On contrary there was an inversely proportion (negative correlation coefficient) after 20, 60, min of mixing. Whey prepared samples showed high correlation coefficient between RSA % and TPC using dilution as a factor for all mixing periods, this indicated that reducing TS of the mixture reduced the phenolic content of its whey derivative and thereby RSA % decreased.

Table (2): Correlation coefficients between RSA% and TPC of SM-RWE and its derivatives as affected by reducing TS of the mixture.

Sample	Time (min)			
	3%	1.5%	٠,٧٥%	0.35%
SM	0.983	- 0.913	0.981	- 0.941
Whey	0.963	0.900	0.805	0.985
Casein	- 0.210	- 0.882	- 817	0.724

Moreover, reducing the TS of the mixture showed negative (reversible) correlation coefficient between RSA % and PC of casein – derivative the best positive correlation was after 60 min of mixing , r = 0.424 . These results support the aforementioned hypothesis casein protein interactions with phenolic compounds mentioned by Zulueta *et al.*2007.

The study clearly indicated that , it is important to take both RSA and PC into account while evaluation the antioxidant potential in mixture of milk and any source of natural antioxidant (herbs , spices , vegetables and fruits)

because the matrix influences the efficacy of an antioxidant. Also, using various radical and oxidation systems to characterize the antioxidant activity of milk and milk –herb mixtures to get a good understanding on the antioxidant properties of the mixtures in order to realize the health benefits from potential milk – plant mixtures.

REFERENCE

Altern Med. Rev. (2004 Jun 9) (2) B6-156. Therapeutic Application of Whey Protein.

Arts, M.J.T.J; Haenen, G.R.M.M.; Wilms, L.C.; Beetstra, S.A.J.N.; Heijnen, C.G.M.; Voss, H.P. and Bast, A. 2002. Interactions between flavonoids and proteins: Effect on the total antioxidant capacity. American chemical society.

Baier, S.K.; McClements, D.J. and Shetty, K. (2001). Caracterization of binding interaction of model phenolic compound (para-Hydrobenzoic Acid) with dairy protein. IFT Annual Meeting – New Orleans, Louisiana.

Bartfay, W. J.; Davis, M.T.; Medves, J.M. and Lugouski, S. (2003). Milk whey protein decreases oxygen free radical production in a murine model of choronic iron – overlood. Can. J. Cardiol. 19 (10): 1163-1168. a

Bartolome, B; Estrella, I and Hernandex, M. T. (2000). Interaction of low molecular weight phenolics with protein (BSA). J. Food Sci. 65 (4): 617- 621.

Brand- Williams.; Cuvelier M. E.; Berest C., 1995. Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol., 28, 25-30.

Calligaris, S.; Manzocco, L.; Anese, M. and Nicoli, M.C. (2004). Effect of heat treatment on the antioxidant and pro-oxidant activity of milk. Inter.Dairy J. 14:421-427.

Chen. J.; lindmark- Mansson H.; Gorton L. and Akesson B. (2003). Antioxidant capacity of bovine milk as assayed by spectrophotometric and amperometric methods. Int. Dairy J. 13: 924-935.

Chen, Z. Y and Nawar, W.W. The role of antioxidants in the antioxidation of milk fat. JAOCS (1991) 68:47-50.

Colbert, L. B. and Decker, E. A. (1991). Antioxidant activity of an ultrafiltration permeate from acid whey. J. Food Scie. 56 (5): 1248-1250.

Debersac, P.; Vrnevant, M.F.; Amiot, M.J.; Suschelet, M. and Siess, M.H. (2001). Effect of water – soluble extract of rosemary and its purified component rosemarinic acid on xenobiotic metabolizing enzymes in rat liver. Food – Chem. Toxical 39(2):109-117.

Del Bano, M. J.; Lorente, J.; Castillo, J.; Benavente-Garcı´a, O.; Del Rıo, J. A.; Ortuno, A., et al. (2003). Phenolic diterpenes, flavones, and rosmarinic acid distribution during the development of leaves, flowers, stems, and roots of Rosmarinus officinalis. Antioxidant activity. Journal of Agricultural and Food Chemistry, 51, 4247–4253.

Dorman, H.J.D.; Peltaketo, A.; Hiltunen, R. and Titttkkanen, M.J. (2003). Characterisation of antioxidant properties of de-odourised aqueous extracts from selected Lamaceae herbs. Food Chemistry, 85:255-262.

Farrell, H. M.; Jimenez-Flores, R.; Bleck, G. T.; Brown, E. M.; Butler, J. E.; Creamer, L. K.; Hicks, C. L.; Hollar, C. M.; No-Kwai-Hang, K. F. and Swiasgood, H. E. (2004). Nomenclature of the proteins of cow's milk-sixth revision J. Dairy Sci. 87: 1641-1674.

Frankel, E. (1999) Natural phenoilc antioxidant and their impact on health. Antioxidant food supplements in human health Chap. 25: 385-392.

Hamed, S. F. (2003). antioxidative activities of some local herb extracts. International conf. NRC, Cairo, Egypt 117-123.

Hernandez-Ledesma; Davalos, A.; Bartolome, B. and Amigo, L (2005). Preparation of antioxidant enzymatic hydrolysates from α-lactalbumin and B- lactoglobulin: Identification of active peptides by HPLC. MS/MS.J. Agric. and Food Chemistry 53:588-593.

Ibanez, E.; Kuba´tova´, A.; Senora´ns, F. J.; Cavero, S.; Reglero, G.; & Hawthorne, S. B. (2003). Subcritical water extraction of antioxidant compounds from rosemary plants. Journal of Agricultural and Food Chemistry, 51, 375–382.

Kosaka, K. and Yokoi, T. (2003). Carnosic acid, a component of rosemary, promotes synthesis of nerve growth factor in T 98 G human glioblastoma cells. Biological & pharmaceutical Bulletin 26 (11): 1620.

Lo Scalzo, R.; Iannoccari, T.; Summa, C.; Morelli, R. and Rapisarda, P. (2004) Effect of thermal treatment on antioxidant and antiradical activity of blood orange juice. Food chem. 85, 1- 47.

Medves, J. M.; Barlfay, W. J.; Davis L., S. and Candian, J. (2003). J. Cardiology 19 (10): 1163-1170.

Nedyalka, V.; Yanrshlieva; Emma Marinova; Jan Pokorny (2006). Natural antioxidants from herbs and spics. Eu. J. lipid sci. and Tch. 108 (9): 776-793.

Pekkarinen, S. S., Stockmann, H.; Schwarz, K.; Heinonen, M. and Hopia A. I. (1999). Antioxidant activity partitioning of phenolic acids in Bulk and emulsified Methyl linoleate. J. Agric. Food chem. 47: 3036-3043.

Perez, M. B.; Calderon, N. L. and Croci, C.A. (2007). Radiation induced enhancement of antioxidant activity in extracts of rosemary. Food chem. 104: 585-592.

Pihlanto, A. (2006). Antioxidative peptides derived from milk protein. International Dairy J. 16 Issue 11: 1306-1314.

Rawel, H.M.; Kroll, J. and Hohl, U. C. (2001). Model studies on reactions of plant phenols with whey proteins. Nahrung 45 (2) : 72- 81

Silvia, M.; Tamara; Catalina, R.; Vojnov, S. and Adrian, A. (2006). Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. Free Radical Research 40 (2): 223- 231.

Slinkard, K.; Singleton, V. L. (1977). Total phenol analysis: Automation and comparison with manual methods. Am. J. Enol. Vitic. 28, 49-55.

Steijns, J.M., and Van Hooijdonk, A.C. (2000).Occurrence, structure biochemical properties and technological characteristics of lactofeerin . British J. of Nutrition, 84,511-517.

Suetsuna, K.; Ukeda, H and Ochi, H. (2000). Isolation and characterization of free radical scavenging activities peptides derived from casein. J. of Nutritional Biochemistry, 11:128-131.

Suhaj, M.; Ra´cova´, J.; Polovka, M.; & Brezova´, V. (2006). Effect of c-irradiation on antioxidant activity of black pepper (Piper nigrum L.). Food Chemistry, 97, 696–704.

Sukkar, S. G. and Bounous, G. (2004). The role of whey protein in antioxidant defense. Anno 22. (4): 193-200.

Taylor. J. and Richardosn J. (1980) Antioxidant activity of cysteine and protein sulfhydryls in a linoleate emulsion oxidized by hemoglobin. J Food Sci 45: 1223-1227.

Tien, C. L. E.; Vachen, C.; Maieescu, M. A. and lacrox, 2001 M. J. Food Sci. 66: 512-516.

Tong, L. M.; Sakai, S.; Meclememts, D. J. and Decker, E. A. (2000a).Mechanisms of the antioxidant activity of a high molecule weight fraction of whey. J. Agric. Food chem. 48: 1473-1478.

Tong, L. M.; Sakai, S.; Meclememts, D. J. and Decker,E. A. (2000b). Antioxidant activity of whey in a salmon oil emulsion. J. Food Scie. 65(8): 1325-1329.

Viljanen, K.; Halmos, A. L.; Sinclair, A. and Heinonen, M. (2005). Effect of blackberry and raspberry juice on whey protein emulsion stability. Eur. Food.Res. And tech: 602-609.

Wada, M.; Kido , H.; Ohyama, K.; Kishikawa, N.; Ohba, Y. ; Kuroda, N. and Nakashima, K. (2004). Evaluation of quenching effect of non-water – soluble and water-soluble rosemary extracts against active oxygen species by chemiluminescent assay. Food chemistry.

Wong, P. Y. Y. and Kitts, D. D. (2003). Chemistry of butter milk solid antioxidant activity. J. Dairy Sci. 86: 1541 – 1547.

Yanishlieva - Maslarova, N.V. (2001).Sources of natural antioxidants: vegetables, fruits, spices and teas. Antioxidant in food Practical Application Book, Ch.(10)210-263

Zheng, W. and Wang, S. Y. (2001). Antioxidant activity and phenolic compound in selected herbs. J Agri. Food chem. 49: 5165-5170.

Zommara, M. A; Toubo, H. and Imaizumi, K. (2002). Supplementing bovine milk Ig prevents rats fed on a vitamin E Deficient dief from developing peroxidation stress. Ann Nutr. Metab. 46: 97- 102.

Zulueta A.; Esteve, M. J.; Frasquet, I. and Frigola A. (2007). Vitamin C, vitamin A, phenolic compounds and total antioxidant capacity of new fruit juice and skim milk mixture beverages marketed in Spain. Food chemistry vol.103 (4): 1365-1374.

النشاط المضاد للأكسدة والمحتوى الكلى من المركبات الفينولية لخليط اللبن الفرز المسترجع والمستخلص المائى للروزمارى

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يهدف البحث إلى تقييم تأثير تدعيم اللبن الفرز المجفف المسترجع بالمستخلص المائى لنبات الروزمارى على نشاط المضاد للأكسدة والمحتوى من المركبات الفينولية لكل منهما.

وعلى ذلك تم فى هذة الدراسة تقدير كل من Radical scavenging activity (RSA) بطريقة DPPH radical وكذلك المركبات الفينولية الكلية TPC بطريقة Folin–Ciocalteau وذلك لكل من المستخلص المائى لنبات الروزمارى المطحون والمحضر بطريق ١٠% وكذلك للبن الفرز المسترجع بنسب ٣، ١,٥، ٠,٧٥ ، ٠,٣٥ % جوامد كلية. وكذلك للكازين والشرش المفصولين من هذا اللبن بالتحميض بعد رفع pH لكل منهما إلى ٦,٧ كما تم ايضا تقدير RSA, TPC للبن الفرز بعد تدعيمة بنسبة ١% للمستخلص المائى للروزمارى وذلك بعد الخلط مباشرا وبعد ٢٠، ٤٠، ٦٠ ق من الخلط وكذلك للشرش والكازين المفصولين من هذا الخليط بالطريقة السابقة.

وقد وجد أن إنخفاض الجوامد الكلية للبن الفرز أدى إلى زيادة RSA% كما أن الشرش الناتج منه أعطى نسبة RSA% أعلى من الكازين. وأن RSA% للمستخلص المائى للروزمارى بتركيز ١٠% كان ٩٤% وبتخفيف المستخلص إلى تركيز ١,٥% إنخفضت RSA% إلى ٢٩%. كما أن أقل نسبة RSA% كانت للكازين الناتج من تحميض مخلوط اللبن الفرز لمستخلص الروزمارى وهذة النسبة لم تتأثر بمدة الخلط كما زادت نسبة RSA% بإنخفاض الجوامد الكلية للمخلوط لكل مدد الخلط المستخدمة.

من جهة أخرى فإن إنخفاض الجوامد الكلية للبن الفرز المسترجع أدى إلى إنخفاض محتواة من المركبات الفينولية وكذلك بالنسبة للكازين والشرش المفصولين منه أما محتوى المستخلص المائى للروزمارى من المركبات الفينولية فقد كان ٤٥٨ ملجم / لتر جاليك أسيد وأن إنخفاض تركيز المستخلص إلى ١.٥% أدى إلى إنخفاض محتواة من المركبات الفينولية إلى ١٣٠ ملجم / لتر جاليك أسيد. اللبن الفرز المسترجع المدعم بالمستخلص المائى للروزمارى يحتوى على نسبة عالية من المركبات الفينولية فى خلال الدقائق الأولى من الخلط لكل من التركيزات المستخدمة من الجوامد الكلية كما أن تخفيف الخليط أدى إلى إنخفاض محتواة من المركبات الفينولية وخاصة للكازين المفصول منه. هذا يؤكد أنة يوجد جزء من نشاط مضادات الأكسدة سواء للبن أو لمستخلص الروزمارى يحدث له Masking وسط تفاعلات خاصة بين بروتين الكازين والمركبات الفينولية للروزمارى.

كما أن معامل الإرتباط بين RSA, TPC لمستخلص الروزمارى كان ٠,٩٩٢ وكان ٠,٩، ٠,٨٨٤ للكازين والشرش على الترتيب. أى أن اللبن الفرز المسترجع المفصولين منه كانت العلاقة سالبة (عكسية) إذا أخذت الجوامد الكلية فى الإعتبار. كما وجد ارتباط قوى بين RSA, TPC للخليط وكذلك للشرش المفصول منه وذلك خلال الدقائق الأولى من الخلط وكذلك عند انخفاض الجوامد الكلية للخليط من ٣%- ٠,٣٥% بينما الكازين المفصول أعطى علاقة عكسية بين RSA, TPC له خلال مدد الخلط والجوامد الكلية المستخدمة.