

EFFECT OF MODIFICATION WHEY ENVIRONMENTAL ON WHEY PROTEIN FUNCTIONALITY AS ANTI-OXIDANT

Gad, A.S. and Hala M. Fakhr El-Din

Department of Dairy Science, National Research Centre, Cairo, Egypt.

ABSTRACT

Whey protein is rich in cysteine residues that can be found in relatively high amounts, compared to other protein sources. Cysteine, one of three amino acids are delivered to the interior of each cell and there they are assembled into glutathione. Using whey in preparing functional diet will expose whey protein to different environmental conditions and may affect during heat processing on its stability (denaturation) that leads to loss of solubility and biological functionality. In this research, many modifications for sweet whey concentrate by UF had been carried out, after that exposed to different heat treatments and study the effect of modification and heat treatment on the resultant whey components. Modifications had been carried out with keeping fixed total solids (6.4%). Treatments were the original whey, concentrate sweet whey TS 8.4% and 10.5% were diluted to TS 6.4%. All samples had been exposed to different heat treatments (HT), 60, 70, 80, 90 and 100°C / 10 min. free lactose, whey protein nitrogen index, total and reactive sulfhydryl groups and major minerals (monovalent and divalent cations) were assayed. Results showed that, with increasing protein concentration, free lactose was decreased specially at 80°C. Modification played a role in retarding denaturation as obvious from WPNI. Total and reactive sulphhydryl groups have a positive correlation with protein concentration and they increased with increasing heating, whereas divalent cations decreased with increasing HT until 80°C and rapidly increased with increasing HT to 90°C.

Measurement of free sulphhydryl (SH) groups in both whey and modification whey showed that the liberation of free SH is highly correlated to the change of the heat degree. Modification of high protein concentrate with phosphate buffer gave the best result for sweet whey as antioxidant potential.

keywords: Whey protein, sulphhydryl groups, antioxidant potential, glutathione.

INTRODUCTION

The immune system is directly dependent upon Glutathione for proper function, and is considered the body's frontline defense against infection. Glutathione, a potent antioxidant, is important for the safe metabolism of the hydrogen peroxide free radical. Research has shown that high levels of intercellular glutathione can protect cells from destruction, detoxify pollutants, and elevate the body to produce new cells of all types to replace that died. Whey proteins, which are high in the amino acid cysteine, help enhance the body's immune system by raising glutathione levels (Counous, 2000).

Heat treatment such as pasteurization, preheating and sterilization causes denaturation of the whey proteins, that affect on its functionality as antioxidant. The functionality of whey protein is markedly influenced by the presence and reactivity of certain amino acids, like cystine and cysteine during and after heat treatment. This thermal denaturation is affected by environmental conditions, like lactose, pH, and salt. Modification of whey environmental may affect on functional behavior. The aim of this research is

how to keep maximum level of whey protein during heat process to be more benefit in diet as antioxidant, by modification of whey environment that may be changed the denaturation behaviour. Modification samples were carried out by preparing whey from concentrate whey with total solids 8.4% and 10.4% and then made partial replacement with distilled water and phosphate buffer to become sample with total solid 6.4%. Phosphate buffer was used because calcium ions tend to decrease the heat stability of whey protein, and phosphate has ability to chelate calcium, and making unavailable for interaction with the whey protein. The other factor playing into protein stability is heat processing, because whey proteins are heat sensitive. So, we would like to investigate the effect of changing whey protein environment on whey protein stability at different temperature. Samples were exposed to different heat treatments. The inevitable heat treatment used, that control of whey protein functional properties. The level of whey protein denaturation was estimated. Free lactose contents and major minerals were determined.

MATERIALS AND METHODS

Materials:

5,5 dithiobis-(2-nitrobenzoic acid) reagent, dissolving in buffer, pH 8.0 to assay sulphhydryl groups (Taylor, 1980 and Stapelfeldt, et 1997). L.cystein hydrochloride monohydrate (MW 157.65) used to make a standard cystein-HCl curve. 0.1M Tris-HCl buffer, 4mM EDTA, pH 8.0 and 0.2M Tris-HCl buffer, 0.02M EDTA, 8M urea, pH 8.0, were used to assay sulphhydryl groups. 0.025% W/V Amido Black 10B in 0.3M citric acid used in determination of whey protein nitrogen index. Phosphate buffer used (P), has a similar ionic strength and pH to whey. 10mM phosphate buffer, 60mM NaCl, pH 6,7 (Bauer, R. & et, 1999). Lactose monohydrate used to make a standard lactose curve. Other chemicals used were of analytical grade.

Methods of analysis:

1. Sample preparation

Sweet whey was ultrafiltered at 30 C using DDS-Lab. 20 ultrafiltration unit, to get whey with total solids 8.4% and 10.4%. As increase total solids, fat content increased. Clarification refers to a specific defatting process of whey, followed by UF. During the process, any previously denatured proteins are removed by fat separation step at pH 4.6 (de Wit, J.N. 1990). Partial replacement for both concentrate whey samples, were carried out with distilled water, and phosphate buffer (P) to give finally whey with total solids 6.4% equal total solid of whey before concentrate. The new samples were:

A: Original whey from factory, with total solid 6.4%.

B: Whey with total solid 6.4% from (whey concentrate T.S. 8.4%+ D.W)

C: Whey with total solid 6.4% from (whey concentrate T.S. 8.4%+ P)

D: Whey with total solid 6.4% from (whey concentrate T.S. 10.4% + D.W).

E: Whey with total solid 6.4% from (whey concentrate T.S. 10.4% + P)

Heat treatment

Whey samples were exposed to temperature that represent a range in which whey proteins are known to unfold, 60°C, 70°C, 80°C, 90°C and 100°C. With continuous stirring samples were heated to define temperature to 10 min then immediately cooled in an ice bath.

2.Determination of "free" lactose content:

This determination was used to indicate the trends of physical binding or chemical reaction of lactose by heating. The content of "free" lactose remaining after precipitation and removal of protein and fat was determined by spectrophotometrically method (Li- Chan, 1983).

3.Determination of whey protein solubility:

An aliquot of whey filtrate (0.5 ml, pH 4.6) was added to an Amido Black solution, mixed and held for 15 min. The samples were centrifuged for 5 min at 2200 rev/min. The absorbance of the supernatants was determined at 615 nm. A calibration curve was constructed from samples with known WPNI ranging from 0 to 6.2. The WPNI of all samples were determined from this calibration curve, (Anema and Lloyd, 1999).

4.Sulphydryl Assay:

Sulphydryl (SH) groups were determined using Ellman's reagent. For SH group determination, whey sample (5ml) was added to (0.1M Tris-HCl buffer, for determined RSH and 0.2M Tris-HCl for determined TSH, pH 8.0 was used and mixed. 0.3 ml Ellman's reagent was added to this solution. The color was allowed to develop for 10 min. The absorbance was measured at 412 nm. The concentration of SH groups was calculate from the cysteine-HCL standard curve,(Stapelfeldt, et al, 1997).

5.Determination of some major minerals:

Determinations include sodium, potassium and calcium ions in each individual heated whey samples, using photometric reference method. An important determinant for functional properties is the salt composition of whey protein products during heat treatments at temperature above 75°C (de Wit, J.N. 1990).

RESULTS AND DISCUSSION

Lactose content of whey

Lactose content of whey is important determination for the solubility of whey proteins after heat treatments (de Wit, 1986). Results showed that as in Fig.1.A, heated samples had "free" lactose content less than unheated samples, and with continuous heating, the "free" lactose content had dispersion. At 60°C, lactose concentration was decreased, as a result of association between parts of lactose with B-Lg molecules. This is early steps of the heat-induced denaturation/aggregation; like reported by (Morgan et al., 1999). Sample (D) did not glycosylated and sample (E) had less affect as

glycation reaction. At 70°C samples (C) and (E) had glycated whereas (B) and (D) had dissociated between lactose and whey proteins. At 80°C, most samples had dissociated and samples (E) had maximum dissociated. At 90°C samples were decreased in concentration of free lactose. At 100°C, association of lactose and whey protein increased and free lactose decreased. Sample (D) had less association at 90°C and 100°C. This suggests that at increasing heat treatment, lower extents of protein-lactose reactions may occur due to decreased exposure of reactive protein amino groups resulting from more extensive protein-protein association.

Whey protein nitrogen index

We used a pH 4.6 solubility index as the method to measure soluble or native undenatured protein. The WPNI was measured in all 5 samples, to assay the residual of native whey protein after define heating. The loss of solubility at pH 4.6 is commonly used to assay their extent of denaturation. Results showed that decreasing in solubility of whey protein with increasing heat treatment. That was obvious at 90°C and 100°C that was more decreased, means extent of denaturation. The last case owing to the formation of disulphide like protein aggregates. All samples took the same trends. Samples (D) and (E) had more soluble protein than other samples. Original sample without modification had less solubility of nitrogen protein, Fig.1.B. Modification played role in retard denaturation, that was obvious in samples made from concentrate whey had total solids 10.4%, that gave more solubility. Ultrafiltration procedure used, may have been encouraged solubilization of protein aggregates during heat treatment as a result of weaken intermolecular bonds.

Total sulphhydryl (TSH) groups

Total sulphhydryl (TSH) groups were apparently responsible for only part of the antioxidant activity of whey. Although all samples had the same total solids, TSH groups content were different. Samples (D) and (E) had more TSH groups than others, Fig.1, C. Positive correlation between TSH groups and whey total solids are used in preparing modified samples were shown. Decreasing sulphhydryl groups with increasing heat treatment may relate to the rupture of various intermolecular and intermolecular bonds stabilizing the native protein structure.

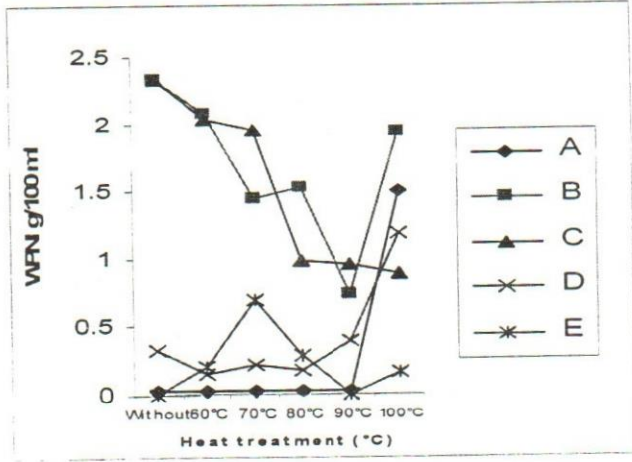
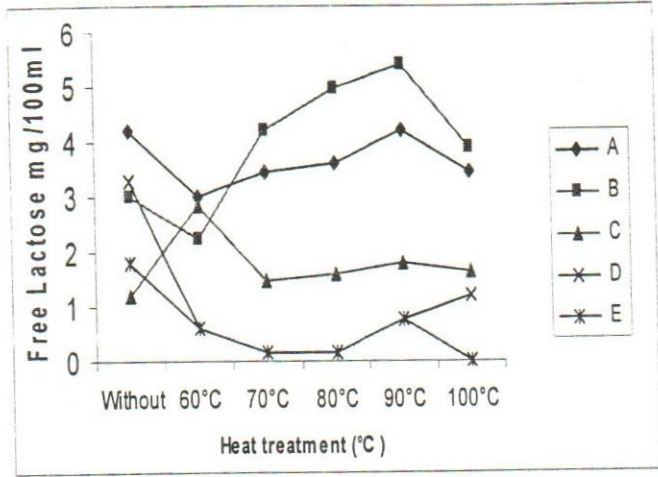
Reactive sulphhydryl (RSH) groups

The effect of various heating temperature on reactive sulphhydryl groups content resulted in modified samples is summarized in fig. 1.D. Generally RSH groups content increased with heating, but varied temperature degree and type of modification. Modified samples B,C,D and E as shown in Fig.1.D, had decreased RSH groups with heating at 60°C and increased slightly with increasing heat temperature, but when the treatment took place at 85°C their concentration increased significantly and arrive the maximum at 90°C. In original whey sample, RSH groups content was decreased at 70°C and increased with increasing heating to arrive the

maximum at 80°C. It is obvious that modification of whey was changed in thermal denaturation behavior. Samples had been partial replacement with distilled water gave more denaturation than had been partial replacement with phosphate buffer. That means finding phosphate anion able to slow down the rate of denaturation and also the rate of aggregation. This could be due to a specific interaction of the phosphate anion with a cation – rich region (calcium) of whey protein surface that inhibit or retard the denaturation of whey proteins.

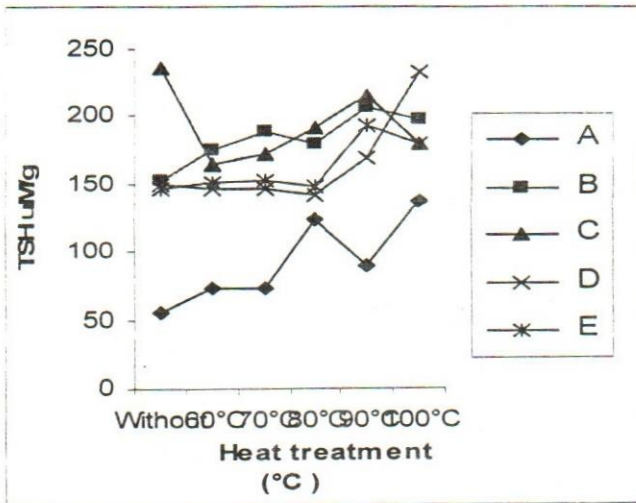
Soluble major minerals (Na⁺, K⁺ and Ca²⁺)

Sodium content in samples prepared by partial replacement with distilled water were nearly less than in original whey, whereas in samples prepared by partial replacement with sodium phosphate buffer were more than in original whey. Soluble sodium ions in modified samples were decreased with heating, Fig.2, A. This decrease was slowly in denaturation stage and increased with aggregation stage. Sodium in original whey decreased regularly to 90°C and then increased. Potassium content also, decreased with heating and increased when aggregation occurred at 100°C, Fig.2, B. Quantity of Ca concentrate showed decreased with raising temperature to 70°C, then increased rapidly as temperature raised to 80°C, followed by decreasing at 90°C little increase at 100°C, Fig.2, C. Results showed that monovalent cations took the same trend that decreasing in these cations accompanied with increasing with sulphhydryl groups and increasing was when aggregation had occurred. That obvious there is no association between these cations and sulphhydryl groups. In calcium, the divalent cation was also decreased with increasing heating to 70°C, but increased rapidly at 80°C then dropped at 90°C and 100°C, that may binding with B-Ig at step of irreversible denaturation step. This result was in agreement with Christophoros (1991).

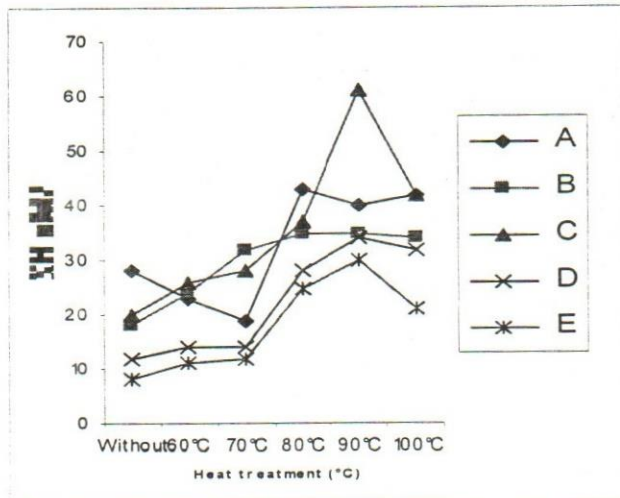


(A)

(B)

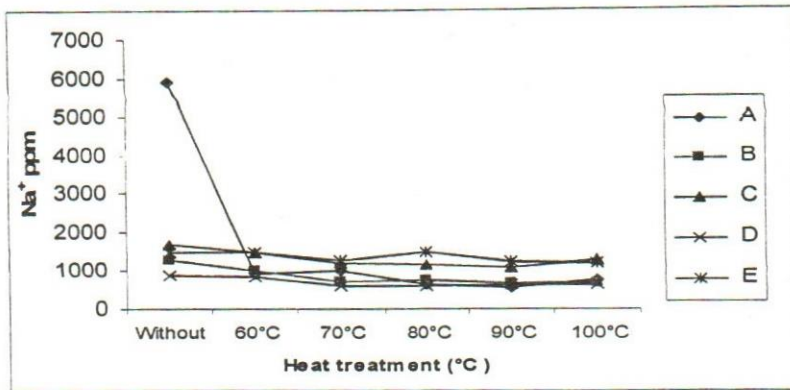


(C)

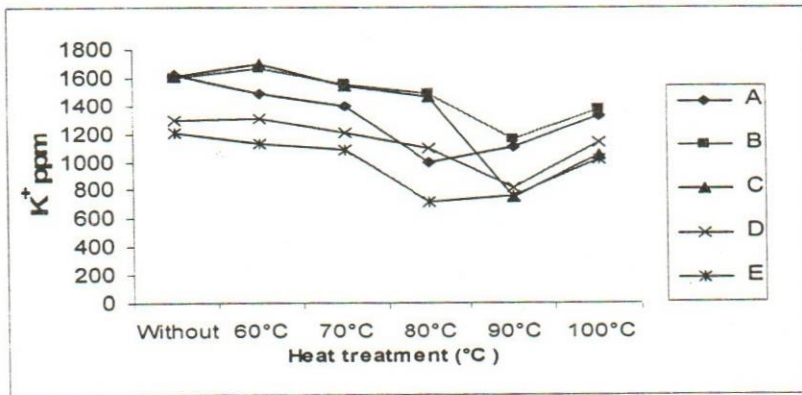


(D)

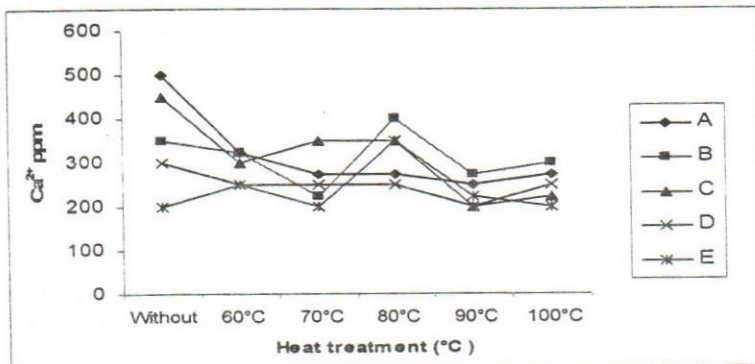
Figure (1): Influence of heat treatments on free lactose contents (A), whey protein solubility (B), total sulphydryl groups (C) and reactive sulphydryl groups (D) at different modify samples.



(A)



(B)



(C)

Figure (2): Influence of heat treatments on Na⁺ (A), K⁺(B) and Ca²⁺ (C)+ cations at different modify samples.

CONCLUSION

Whey protein denaturation starts at temperatures between 60 and 75°C and that the degree of denaturation increases with increasing the temperature, agreement with (Parris *et al.*, 1991). Heating whey increases its antioxidant activity, with corresponding decrease in the redox potential. The thermal labile amino acid cysteine destroyed by the heat, thus blocking the the body's ability to develop glutathione in every living cell. Using phosphate buffer retards denaturation of whey protein by chelate calcium, making it unavailable for interaction with the whey proteins that helps to elevate glutathione level in body cells. This modified improve whey protein heat stability. Also increasing whey protein content is carried out the same goal.

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دراسة تأثير تغير الوسط المحيط لبروتينات الشرش على كفاءتها كمضاد للأكسدة

أحمد سعد جاد - هاله محمد فخر الدين
المركز القومي للبحوث - القاهرة - الدقى - شارع البحوث

يمثل إنزيم الجلوتاثيون بيروكسيداز المركب المضاد للأكسدة الرئيسى والذى يتكون داخل كل خلية فى جسم الإنسان .. ويعتبر الحامض الأمينى السستين واحد من ثلاث أحماض تشارك فى تكوين هذا الإنزيم داخل الخلية. وزيادة حصول الإنسان عليه يساعد على زيادة تكوينه . ويعتبر بروتين الشرش مصدر بروتينى غنى بهذا الحامض مقارنة بمصادر البروتينيات الأخرى.

إلا أن حمض السستين يفد مع دنتره البروتين أثناء تعرضه للمعاملات الحرارية اتى يتعرض لها البن أثناء التصنيع .

ويهدف هذا البحث فى إجراء تحويل للوسط المحيط لهذه البروتينات للتعرف على أفضل الظروف التى يمكن من خلالها حدوث دنتره أقل . وشملت المعاملات تحضير مركزات من بروتين الشرش ٨,٤% ، ١٠,٤% ، كجوام كلية ثم تخفيفها إلى ٦,٤% كجوام صلبة .. مماثلة للشرش الناتج من الجبن . وتم التخفيف بالماء ، بمحلول منظم (محول الفوسفات) .. هذا بجانب محلول الشرش ٦,٤% . تم تعريض هذه المعاملات لدرجات حرارة مختلفة ٦٠ م° ، ٧٠ م° ، ٨٠ م° ، ٩٠ م° ، ١٠٠ م° . تم دراسة تركيب هذه المحاليل البروتينية بعد المعاملات المختلفة .

تم تقدير اللاكتوز الحر ، البروتين الذائب ، مجاميع السلفاهيدريل الكلية ، مجاميع السلفاهيدريل النشطة .. بالإضافة إلى تركيز الكاتيونات الأحادية كالصوديوم والبوتاسيوم والثنائية كالسيوم .

أوضحت الدراسة أنه استخدام المحلول المنظم للفوسفات ساعد على تأخير عملية الدنتره مع المعاملة التى تحتوى على ١٠,٤% جوام كلية حيث أعطت أقل دنتره وذلك عند استخدام الحرارة المنخفضة