

## EFFICACY OF SWEET WHEY CONTAINING FINAL DIPS IN REDUCING PROTEIN OXIDATION IN RETAIL-CUT CUBED BEEFSTEAK

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### Abstract

*Oxidative degradation results in extensive deterioration of shelf-life and quality of retail-cut muscle foods. Use of antioxidants, especially the ones of natural origin, can markedly reduce this process without adverse health consequences to the consumer. Sweet whey originating from Cheddar (CW) and Edam (EW) cheese manufacture possess remarkable antioxidative properties. The current study investigated the efficacy of CW and EW when used in edible coatings against oxidative degradation of retail-cut cubed beefsteak Steak samples, immersed for two minutes in the coatings were stored for 7 days at 4°C in Styrofoam polyover wrapped trays and their average carbonyl contents (CC) were analyzed. Results exhibited the marked efficacy of both types of sweet whey in reducing oxidative degradation as evidenced by significantly lower ( $P<0.05$ ) CC compared to the controls (immersed only in the buffer). Samples treated with CW exhibited numerically lower CC compared to EW for greater number of treatments. Best demonstration of the protective efficacy of CW was at a concentration of 2%, w/v throughout the entire storage period, which showed 35.6, 71.9, 59.3, 84.3 and 93.5% lower CC compared to the control respectively following storage for nil, 1, 3, 5 and 7 days. The same samples also exhibited 28.1, 48.3, 5.1, 28.7 and 39.6% lower CC relative to the EW treatments respectively for the same periods of storage. The study illustrated the potential for use of CW as a natural and relatively inexpensive constituent in edible coatings to protect and extend shelf life of retail-cut beef.*

**Keywords:** Antioxidant, carbonyl contents, Edam whey, Cheddar whey

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### 1. INTRODUCTION

Oxidative degradation results in a considerable amount of meat product wastage across the globe (Dave and Ghaly, 2011). Cubed beefsteak (also known as cubed or minute steak) is particularly susceptible to oxidation due to increased surface area caused by the metallic cubing machine, display lights and contact with metallic surface. This limits retail shelf-life of cubed steak (~1–2 days at 2–4°C) (Anon., 2011a), a period that is considerably less than that of retail steak that has not been subjected to the cubing process (~3–4 days) (Anon., 2011b). Various unfavorable consequences of oxidative degradation include: loss of color, flavor, texture etc. and deterioration of overall nutritive quality (Coronado et al. 2002; Thiansilakul et al., 2007).

Use of synthetic and natural antioxidants can considerably reduce oxidative degradation and extend the refrigerated storage life of a wide

range of food products from 3 to 5 days (Williams et al., 2013). Increase of display shelf-life for even one to two additional day results in a significant economic benefit for the retailer. Antioxidants of natural origin with GRAS status garner more interest than synthetic ones due to potential adverse health consequences of the later that may occur as a result of long term consumption (Pourmorad et al., 2006).

Sour whey, an inexpensive by-product of cottage cheese industry possess remarkable antioxidant properties and can potentially be used as an agent to preserve a diverse group of food products from oxidative degradation (Shon and Haque, 2007 a,b; Haque et al., 2009). Sweet whey, e.g., Cheddar (CW) and Edam (EW) also possess dramatic efficacy as antioxidants and have been seen to exhibit marked protective ability when use in edible coatings applied to

various types of foods (Weerasinghe et al., 2013). CW and EW are subjected to considerably different processing conditions in terms of starter culture and applied heat during the manufacture of the respective type of cheese. Both types of cheese manufacture, the curd is cut into cubes 'cooked' in the whey with constant agitation. Cheddar is subjected to cooking for a longer period at a higher temperature (~30 min at ~39°C) compared to Edam (~20 min at ~36°C) (Kosikowsky and Mistry, 1997). Thus, Cheddar has an enhanced surface area and copious microbial proliferation with concomitant increase in peptide content resulting from bacterial protease activity. Peptide rich in redox-reactive amino-groups react with the high content of reducing sugar (lactose) in whey to give Maillard reaction products (MRPs) that are released into the whey phase (von Smoluchowski, 1917; Meltretter et al., 2007). The MRPs bring about further augmentation of antioxidative properties of CW (Jayathilakan and Sharma, 2006; Haque and Mukherjee, 2014).

In the current study, we have investigated the efficacy of various concentrations of sweet whey used in edible coatings to preserve retail cut cubed steak from protein oxidation. We hypothesize that CW and EW have different inherent antioxidative properties with CW being more effective in thwarting the consequences of oxidative degradation.

## 2. MATERIAL AND METHODS

### Materials

Fresh CW and EW powders were obtained from the Mississippi State University (Mississippi State, MS, USA) dairy plant immediately after cheese manufacture using fresh milk from the MSU mixed herd. Fresh, retail cut cubed steak was purchased from a local grocery. Sodium phosphate dibasic, citric acid, hydrochloric acid, trichloroacetic acid (TCA), 2,4-dinitrophenylhydrazine (DNPH) and guanidine hydrochloride were from Sigma-Aldrich Co. (Milwaukee, WI, USA). All other chemical were analytical grade.

### Preparation of CW and EW powders

CW and EW were manufactured using standard commercially used and time honored methods (Kosikowski, 1982) under controlled conditions as described earlier (Shon and Haque, 2007) at the Mississippi State University Dairy Plant. Fresh CW and EW were immediately dehydrated as described earlier (Ji and Haque, 2003) at the Ammerman-Hearnsburger Pilot Plant, Mississippi State University, to obtain the respective type of whey powders.

### Sample preparation

Edible coatings containing different concentrations (0.25, 0.5, 1 and 2%, w/v) of CW and EW were prepared by dispersing the respective whey powder in McIlvaine's iso-ionic buffer (pH 7.0). Fresh cubed steak samples of equal weight (5 g) and uniform geometry were immersed for two min in the various coatings. Samples immersed in the buffer with no dispersed whey were used as controls. All experiments were conducted in triplicate dried, placed in styrofoam trays, wrapped with polythene film and stored at 4°C for nil (0), 1, 3, 5 and 7 days.

### Analysis of protein oxidation

Degree of protein oxidation was studied by analyzing the average carbonyl contents (CC) (the most ubiquitous product of protein oxidation) of the samples by the method described by Haque et al. (2009) with modifications. The samples were first homogenized in 50 mL McIlvaine's buffer using a commercial grinder (Oster Digital Blender, Sunbeam Products Inc., Boca Raton, FL, USA) for 30 sec at a speed attenuation of 18. A 0.5 mL aliquot of the homogenate was mixed with 2 mL of 20% (w/v) TCA and centrifuged at 3000×g for 10 min at 4°C using a Sorvall™ Legend™ Micro 21R centrifuge (Thermo Scientific, Waltham, MA, USA). The resulting pellet was treated with 2 mL DNPH reagent (10 mM DNPH in 2 N HCl) and incubated for 1 hour at 22°C. Next, 2 mL of 20% TCA was again added to the samples followed by centrifugation at 3000×g for 10

min., The resulting precipitate was washed twice with 2 mL of ethanol:ethyl acetate (1:1) to remove DNPH, dissolved in 1.5 mL of 6 M guanidine hydrochloride and centrifuged at  $3000\times g$  for 15 min. Absorbances of the samples were measured at 370 nm using a BioMate™ 3 UV-Vis. spectrophotometer (Thermo Electron Scientific Instruments Corporation, Middleton, WI, USA).

### Statistical analyses

Student's *t*-tests (Gosset, 1908) were performed to assess whether the CC of the various samples differed significantly ( $\alpha = 0.05$ ) compared to the control.

## 3. RESULTS AND DISCUSSION

Samples coated with either types of whey showed significantly lower ( $P < 0.05$ ) CC compared to the controls at 1, 3, 5 and 7 days of storage (Table 1). Notably, greater number

of samples coated with CW showed numerically lower CC compared to the ones immersed in the coatings containing EW. The best protective efficacy of CW was found at a concentration of 2%, w/v (Fig 1), where samples showed 27.9, 48.3, 5.1, 28.7 and 38.6% less CC at nil, 1, 3, 5 and 7 days of storage compared to the EW treatments after the same storage ones.

To further investigate the protective efficacy of either types of whey, slopes of CC for the treated samples were calculated for the different days of storage (Table 2). It was evident that the samples treated with CW (0.5 – 2%, w/v) had greater slopes compared the ones treated with EW. This conceivably expresses the marked reduction in CC of the samples induced by CW – thus depicting the better protective efficacy of CW against oxidative degradation compared to EW on a weight by weight basis.

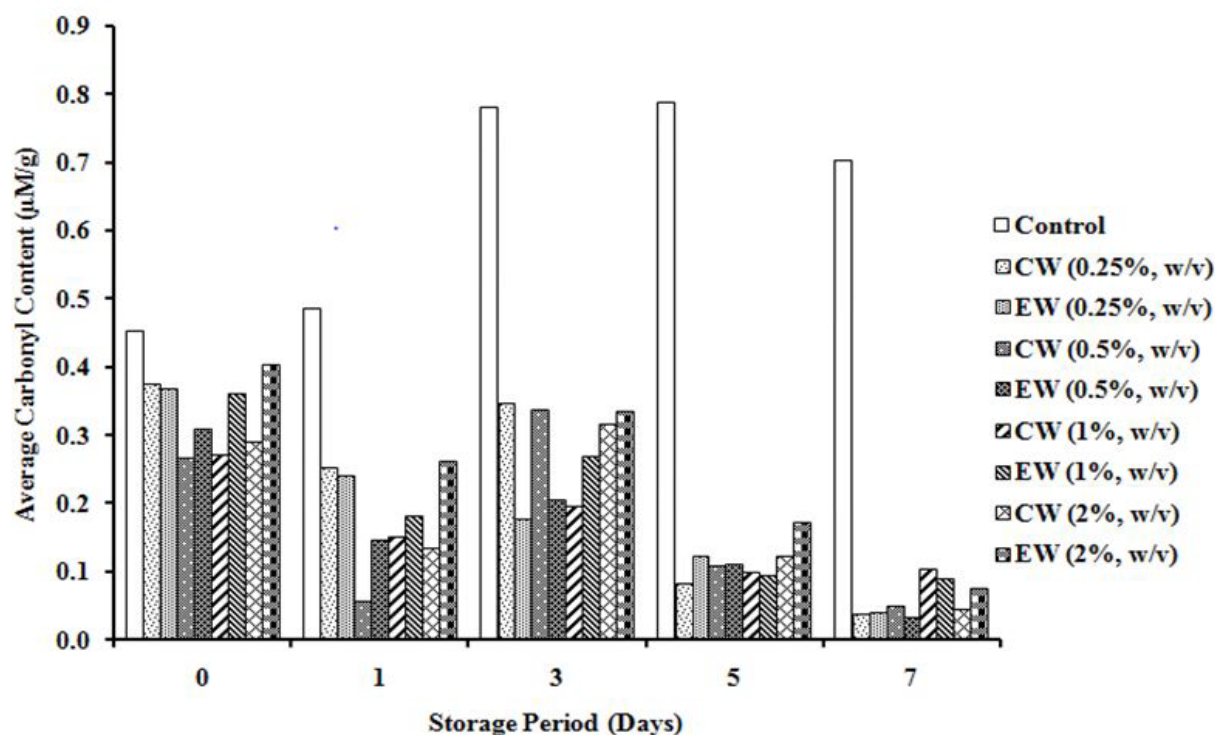


Fig. 1. Average carbonyl content of cubed steak samples immersed in coating dips containing various concentrations of sweet whey at different periods of refrigerated storage. The x axis represents storage period in days and y axis denotes average carbonyl content in micromole ( $\mu\text{M}$ ) per gram of sample following various periods of storage. Abbreviations are as follows: CW, Cheddar whey, EW, Edam whey

**Table 1. Reduction (in percent) in carbonyl content as an indicator of the degree of protein oxidation of cubed beefsteak immersed in coating dips containing different concentrations of sweet whey, compared to the controls (immersed only in the buffer) as determined during different periods of refrigerated storage.**

Whey Concentration (%, w/v)	CW				EW			
	0.25	0.5	1	2	0.25	0.5	1	2
<b>Storage Period (Days)</b>	<b>% Reduction in CC (compared to Control)</b>							
0	17.1	40.9	40.0	35.7	18.5	31.7	19.7	10.7
1	47.7	88.1	68.7	72.0	50.4	69.7	62.4	45.8
3	55.6	56.5	74.8	59.3	77.4	73.7	65.3	57.1
5	89.3	86.1	87.4	84.2	84.4	85.8	87.9	77.9
7	94.5	92.9	85.1	93.5	94.3	95.2	87.0	89.2

Abbreviations are as follows: CW: Cheddar whey; EW: Edam whey; w/v: weight/volume, CC: Carbonyl content.

**Table 2. Antioxidative efficacy of sweet whey, expressed as the slope of concentration (w/v) dependent changes in average carbonyl content for the various storage periods.**

Whey Concentration (%, w/v)	CW				EW			
	0.25	0.5	1	2	0.25	0.5	1	2
<b>Slope</b>	-0.084	-0.038	-0.039	-0.039	-0.077	-0.059	-0.063	-0.075

Abbreviations are as indicated in the legend for Table 1

The dramatic difference in the antioxidative properties between the two types of sweet whey used in the study evidently stems from the distinctive processing techniques they are subjected to. Incubation at a higher temperature for longer duration leads to the production of increased concentrations of peptides in CW. Higher temperature enhances Brownian motion (von Smoluchowski, 1917), which increases the probability of collision between peptides and lactose. Thus redox-reactive MRPs are formed, which further augment the antioxidative efficacy of CW. Therefore both incubation temperature and time are directly related with antioxidative and preservative properties of CW.

#### 4. CONCLUSIONS

The study exhibited a considerable enhancement in antioxidative properties of edible coatings containing sweet whey, with

CW showing an overall better efficacy compared to EW to preserve cubed beefsteak from protein oxidation. The investigation depicted potential use of CW as a natural and relatively inexpensive component of edible coatings to preserve muscle food products from the unfavorable effects of oxidative degradation.

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#### 6. REFERENCES

- [1] Anonymous. (2011a). Beef Cubed Steak. Beef Culinary Center, Minneapolis, MN.

- [http://www.beefandvealculinary.com/beef%20Cubed %20steak.aspx](http://www.beefandvealculinary.com/beef%20Cubed%20steak.aspx) (Accessed July 25, 2011).
- [2] Anonymous. (2011b). Steak. Beef Culinary Center, Minneapolis, MN. <http://www.beefandvealculinary.com/steaks.aspx> (Accessed July 25, 2011).
- [3] Coronado, S.A., Trout, G.R., Dunshea, F.R., & Shah, N.P. (2002). Antioxidant effects of osemmary extract and whey powder on the oxidative stability of wiener sausages during 10 months frozen storage. *Meat Science*, 62(2), 217-224.
- [4] Dave, D. & Ghaly, A.E. (2011). Meat spoilage mechanisms and preservation techniques: A critical review. *American Journal of Agricultural and Biological Sciences* 6 (4), 486-510.
- [5] Gosset, W.S. (1908). The probable error of a mean. *Biometrika*, 6 (1), 1-25.
- [6] Haque, Z.Z. and Mukherjee, D. (2014). Enhancement of radical quenching ability of sweet whey and casein hydrolyzate: mutual supplementation with thermally generated Maillard reaction products. *Journal of Dairy Science*, 97, (E-Suppl. 1), 134.
- [7] Haque, Z.Z., Mukherjee, D., & Chang, S. (2013). Effect of detergents on the antioxidative efficacy of sweet whey. *Journal of Dairy Science*, 96, (E-Suppl. 1), 626.
- [8] Haque, Z.U., Shon, J., & Williams, J.B. (2009). Efficacy of sour whey as a shelf-life enhancer: Use in antioxidative edible coatings of cut vegetables and fruit. *Journal of Food Quality*, 32(3), 381-397.
- [9] Ji, T. & Haque, Z.U. (2003). Cheddar whey processing and source: I. Effect on composition and functional properties of whey protein concentrates. *International Journal of Food Science & Technology*, 38(4), 453-461.
- [10] Kosikowski, F.V. (1982). Cheese and fermented milk foods, 1st Edition, (F. V. Kosikowski and Associates).
- [11] Kosikowski, F.V. & Mistry, V.V. (1997). Cheese and fermented milk food, 3rd Edition, (F.V. Kosikowski LLC).
- [12] Maillard, L.C. (1912). Action of amino acids on sugars. Formation of melanoidins in a methodical Way. *Comptes rendus de l'Académie des Sciences*, 154, 66-68.
- [13] Martinaud, A., Mercier, Y., Marinova, P., Tassy, C., Gatellier, P., & Renerre, M. (1997). Comparison of oxidative processes on myofibrillar proteins from beef during maturation and by different model oxidation systems. *Journal of Agricultural and Food Chemistry*, 45(7), 2481-2487.
- [14] Meltretter, J., Seeber S., Humeny, A., Becker, C.M. and Pischetsrieder, M. (2007). Site-specific formation of Maillard, oxidation, and condensation products from whey proteins during reaction with lactose. *Journal of Agricultural and Food Chemistry*, 55(15), 6096-6103.
- [15] Pourmorad, F., Hosseinimehr, S.J., & Shahabimajid, N. (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African Journal of Biotechnology*, 5, 1142-1145.
- [16] Shon, J. & Haque, Z.U. (2007a). Antioxidative ability of native and thermized sour whey in oxidation-catalyzed model systems. *International Journal of Dairy Technology*, 60(2), 143-148.
- [17] Shon, J. & Haque, Z.U. (2007b). Efficacy of sour whey as a shelf-life enhancer: Use in antioxidative edible coatings of cut vegetables and fruit. *Journal of Food Quality*, 30 (5), 581- 593.
- [18] Thiansilakul, Y., Benjakul, S., & Shahidi, F. (2007). Antioxidative activity of protein hydrolysate from round scad muscle using alcalase and flavourzyme. *Journal of Food Biochemistry*, 31(2), 266-287.
- [19] von Smoluchowski, M. (1917). Versuch eine mathematischen theorie der koagulations-kinetik kolloidaler losungen. *Zeitschrift für physikalische Chemie*, 92,129-168.
- [20] Weerasinghe, S., Williams, J.B., Mukherjee, D., Tidwell, D.K., Chang, S., & Haque, Z.Z. (2013). Quality and sensory characteristics of cubed beef steak dipped in edible protective solutions of thermized Cheddar whey. *Journal of Food Quality*, 36 (2), 77 - 90.
- [21] Williams, B., Haque, Z.Z., Mukherjee, D., Abessinio, H.B. & Chang, S. (2013). Efficacy of Cheddar and Edam Whey in reducing oxidative degradation of cubed beef steak (The 66th Reciprocal Meat Conference [RMC] of the American Meat Science Association [AMSA] [June 16, 2013], Auburn, AL, USA).