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CASEIN HYDROLYZATE AUGMENTS ANTIMICROBIAL AND ANTIOXIDATIVE PERSISTENCE OF CHEDDAR WHEY PROTEIN CONCENTRATE BASED EDIBLE COATINGS

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ABSTRACT
Hydrolysis of casein using chymotrypsin results in the formation of polypeptides (casein hydrolyzate, CH) with a hydrophobic aromatic amino acid on one end of the chain because the enzyme selectively cleaves the adjacent peptide-bond. Due to resonance of the aromatic micro-domain, thiols become redox-sensitive and actively participate in electron transfer. These types of amphipathic peptides also tend to be membrane-lytic. The two prong approach of this investigation was to, (1) assess antibacterial effect of the CH in beef steak, and (2), to determine its antioxidative efficacy as a constituent of Cheddar whey based edible coating mix. Coliform growth, using shoulder-cut beefsteaks as model, showed a significant (P<0.05) five log reduction to log CFU<1 even at its lowest concentration range (0.15-0.2 %) (w/v). Radical quenching ability reflected by increasedn time required for maximal alkoxyl- and peroxyl-radical accumulation in reaction mixtures without and with CH, by in vitro pyrolysis of 2, 2'-Azo-bis (2-amidinopropane), was six minutes even at its lowest trace concentration (0.1 %, w/v) indicating dramatic enhancement of long-term antioxidative persistence. At the same concentration, CH augmented 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging efficacy of the film by 28%. The study showed the potential for beneficial use of CH in dramatically enhancing efficacy of WPC based edible coatings to reduce microbial and oxidative degradation of muscle foods during retail-cut processing and display.

Keywords: peptide, coliform, sweet whey, free radical, muscle food

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1. INTRODUCTION

Post-harvest loss of foods due to microbial and oxidative degradations is of grave concern across the world. Change of global climate is potentially having a damaging effect on food safety due to elevated temperature causing microbiological deterioration in different food chains (Tirado et al. 2010). A number of deaths reported in the United States due to infections caused by Listeria monocytogenes contamination in 2011 (Hoelzer et al. 2012) raise concerns regarding food safety. Food-borne infections are major causes of death in developing countries, such as Middle East, North Africa and India. Therefore, food safety is an particularly important issue, both in terms of economics and human health. In addition to the microbial concerns, oxidative degradation due to reactive oxygen species (ROS) in food products is known to result in several detrimental health effects in consumers over time, including the etiology of cardiovascular diseases (Maxwell and Lip, 1997) and several types of cancers (Dreher and Junod, 1996). Like all chemical reactions, generation of ROS is elevated as the temperature increases.

Perishable food items, such as beefsteak can be contaminated by a wide range of microbial genera such as the coliforms. Consumption of food products contaminated with such microorganisms may result in life threatening consequences to the consumers (López-Expósito et al. 2007). Addition of antimicrobials and antioxidants can potentially reduce degradations which would be economically beneficial if the protection extends the refrigerated shelf life. It would be even more desirable if the preservatives are of natural origin due to increased consumer concerns of the potentially negative effects of long term consumption of synthetic preservatives such as butylated

hydroxyl anisole and butylated hydroxyl toluene (Pourmorad et al. 2006).

A number of natural agents have been identified with remarkable efficacy as preservatives. Significant extension of shelf-life of cubed beefsteak, vegetables and fruit using acid whey has been reported (Haque et al. 2009; Shon and Haque, 2007a). Noteworthy efficacy of sweet whey to preserve muscle food products from oxidative degradation was also been reported (Mukherjee and Haque, 2015; Weerasinghe et al. 2013). Enzymatic hydrolyzate of casein (CH) is known to possess marked antimicrobial and antioxidative properties. Antimicrobial efficacy of CH, primarily resulting from the membrane-lytic activity of amphipathic peptides (Darewicz et al. 2006), has been reported by several studies (Benkerroum, 2010; Biziulevičius et al. 2002; López-Expósito et al. 2007). Rabbit CH reportedly inhibited growth of a number of gram positive bacteria (Baranyi et al. 2003). Antioxidative properties of CH presumably results from redox-activity of thiol groups in the peptides (Brandes et al. 2009). These groups are particularly redox-sensitive when they are in the proximity of neighboring positively charged or aromatic residues (e.g., phenylalanine, tyrosine) (Antelmann and Helmann, 2011; Phelan et al. 2008).

In view of the justifiable public concern related to the safety of uncooked retail-cut meat and the potential for contamination through biofilms resistant to CIP practices (van der Veen and Abee, 2011), it imperative that natural antibacterials be developed to augment existing practices. Importantly, it is necessary to protect meat during the window of time between the cutting and packaging when it lies on processing surfaces that may harbor contaminating biofilm (Giaouris et al. 2014). And this protection has to be by edible means for ready to grill meats like steaks. With this in mind, we investigated the effectiveness of CH when used as an ingredient in WPC based edible coatings to protect beefsteak. Cheddar whey based WPC was produced and used for the study based on our earlier work related to its antioxidative efficacy (Haque et al., 2013; Weerasinghe et al., 2013). The intrinsic antioxidative efficacy and persistence of CH in the same coating dispersion was also investigated by real-time luminometry analysis.

2. MATERIALS AND METHODS

Materials

Calcium chloride, peptone, proteose-peptone, β-lactose, sodium chloride, crystal violet, neutral red, bile salts, bacteriological agar, 2, 2'-Azo-bis (2-amidinopropane) (ABAP), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were from Sigma-Aldrich (Milwaukee, WI). Black, clear bottom 96 well plates were procured from Fisher Scientific (Hanover Park, IL). WPC concentrate was produced as described earlier (Haque and Ji, 2003; Ji and Haque, 2003) in the Ammerman-Hearnsberger Pilot Food Processing Laboratory, Mississippi State University (Mississippi State, MS, US). Glucomannan was obtained from Konjac Food (Sunnyvale, CA). Sorbitol and carboxymethyl cellulose were procured from Archer-Daniels-Midland (Decatur, IL) and FMC-BioPolymer (Princeton, NJ), respectively. CH was prepared by chymotrypsin (EC 3.4.21.1) digestion of freshly prepared acid casein by the method described by (Haque et al. 1993). PetrifilmTM Coliform Count Plates were from 3M Microbiology Products (St. Paul, MN) and the Coliform Test Kits were obtained from LaMotte Co. (Chestertown, MD). Fresh shoulder-cut beef steak was procured with different batches from a local grocery.

Methods

Preparation of the Coating Dispersion.

The whey-based edible coating dispersion was prepared as described earlier (Shon and Haque, 2007b). WPC powder (5% w/v), sorbitol (2.5% w/v), glucomannan (0.25% w/v), calcium chloride (0.125% w/v), carboxymethyl cellulose (0.25% w/v) and different concentrations of CH (0.05 to 0.3%, w/v) were first dispersed in deionized water by gentle stirring for 30 min at 22˚C. The dispersion was

degassed using a vacuum aspirator, heated at 90oC for 30 min, homogenized for two min using a Vibra-CellTM sonicator (Sonics & Materials, Inc., Newtown, CT), filtered through five layers of cheese cloth, cooled to 22oC, degassed for a second time, the pH was adjusted to 7.0 with 1M HCl or NaOH and the mixture was finally cooled at 4oC for three to four hours until use for coating the test beefsteaks.

Assessment of Antimicrobial Activity

Beefsteak sample preparation

Fresh shoulder cut beefsteak (chuck) samples of equal weight (10 g) and uniform geometry were immersed for five min in the whey based edible coating with and without CH. Samples immersed in the coating dispersion containing no CH were used as controls. Three replicates were used for each treatment. Treated samples were air dried for five min, incubated at 37oC in a Thermolyne 5000 incubator (Thermolyne, Dubuque, IA) for 48 hours in sterile plastic petri-plates (Fisher Scientific, Hanover Park, IL), blended using a sterile Oster food processor (Sunbeam Products, Boca Raton, FL) in 90 mL of deionized water (leading to a final dilution of 10%, w/v) and extracted by filtration (Whatman 4).

Coliform Counts.

Each aqueous extract was used to produce three samples of different dilutions (10-2, 10-3 and 10-4, respectively) and plated on MacConkey agar plates (MacConkey, 1908). The plates were incubated at 37oC for 48 hours. Coliform counts for each sample were determined by the total plate count method. Presence of coliform was further confirmed using PetrifilmTM Coliform Count Plates (3M Microbiology Products, St. Paul, MN) and Coliform Test Kits (LaMotte Co., Chestertown, MD).

Assessment of Antioxidative Activity

Determination of peroxyl and alkoxyl radical quenching efficacy. Preparation of reaction mixture.

Black, clear bottom 96 well plates were used for running the assay. Coating dispersions with three different concentrations of CH (0.1, 0.2 and 0.3%, w/v) were prepared by the method described above. Each reaction mixture contained 100 µL of each of the whey-based edible coating dispersion (with no CH), 400 mM ABAP used as a source of free peroxyl and alkoxyl radicals, 10 mM luminol, that stoichiometrically chemiluminesces based of presence of unquenched radicals , and the coating dispersions with various concentrations of dissolved CH. McIlvaine's buffer (McIlvaine, 1921) and the same whey-based edible coating dispersion, neither containing dissolved CH were used as the blank and the control, respectively. Trolox (0.05%, w/v), a potent water soluble antioxidant, that is a derivative of vitamin E, was dissolved in the coating dispersion and was used as the positive standard (Hamad et al., 2010).

Measurement of Chemiluminescence maxima (Lmax)

Chemiluminescence was measured as described earlier by (Haque et al. 2002; Mukherjee et al. 2012). An IVIS 100 Imaging System (Caliper Life Sciences, Inc., Hopkinton, MA) was used to record the chemiluminescence at three min intervals, at 37oC for one hour. Total flux (number of photons emitted per second)(p/s) was a proportional measure the accumulation of unquenched radicals.

Determination of time required for maximal accumulation of radicals in reaction mixture (Max_T) and the change in MaxT (ΔMax_T) as a result of CH addition.

The Max_T was defined as the time required to attain maximal reaction mixture concentration of peroxyl and alkoxyl radical, based on chemiluminescence maxima (Lmax), from the point of initiation of pyrolysis. The increase in this time relative to the blank, i.e., ΔMax_T, was a direct reflection of the radical quenching persistence, i.e., antioxidative persistence (AP) of the coating. It was given by the expression (Mukherjee et al. 2012):

$$\Delta Max_T = Max_{TSample} - Max_{TBlank} \quad \text{---------- (1)}$$

2, 2-Diphenyl-1-picrylhydrazyl radical (DPPH) scavenging

Preparation of standards. The DPPH dispersion (0.1 mM) was prepared by dissolving 39.43 mg of DPPH in 1L of absolute ethanol. Trolox standard stock dispersion (10 mM) was prepared by dissolving 25.3 mg of Trolox in 10 mL of absolute ethanol. Next, 200, 150, 100, 75 and 37.5 µM serial dilutions of the Trolox stock dispersion were prepared (Alam et al. 2013) and a calibration curve with linear relationship between Trolox concentration and free radical scavenging rate was graphed by colorimetry according to Caillet et al. (2006).

Test samples. The same whey-based edible coating dispersion containing no CH was used as the control. Samples containing varying concentrations of CH (0.1, 0.2 and 0.3%, w/v) were prepared by dissolving CH in McIlvaine's iso-ionic buffer (pH 7.0).

Experimental Procedure. The radical scavenging capacity of the coating dispersions and CH samples were evaluated according to Xu and Chang (2007). A dose of 0.2 mL of the test sample (coating dispersions with different concentrations of CH) was added to 3.8 mL of DPPH-ethanol dispersion, the mixture was vortexed for 1 min, and left to stand in the dark for 30 min in an incubator at 22oC (Thermo-Scientific, Marietta, OH). The absorbance of the sample (A_{Sample}) was then measured using the Thermal BIOmate spectrophotometer (Thermo Electron Corp., Madison, WI) at 517 nm against the ethanol blank. A negative control ($A_{Control}$) was prepared by adding 3.8 mL DPPH dispersion to 0.2 mL of 100% ethanol. The percent of DPPH discoloration, i.e., free radical scavenging rate, of the test samples were calculated according to the following equation:

Percent discoloration =
$= [1 - (A_{Sample}/A_{Control})] \times 100$ ---------- (2)

The free radical scavenging activities of the samples were expressed as equivalents to that of Trolox. Three replicates were taken for each of the samples, and the results were calculated and expressed as micromoles (µmoles) of Trolox equivalents (TE) per gram of sample using the calibration curve representing the relationship of Trolox concentrations and free radical scavenging rates . Trolox equivalents of CH samples were calculated using the expression,

$y = 0.3906x + 0.1184$ ---------- (3)

where, x and y represented Trolox equivalents and radical scavenging rates, respectively.

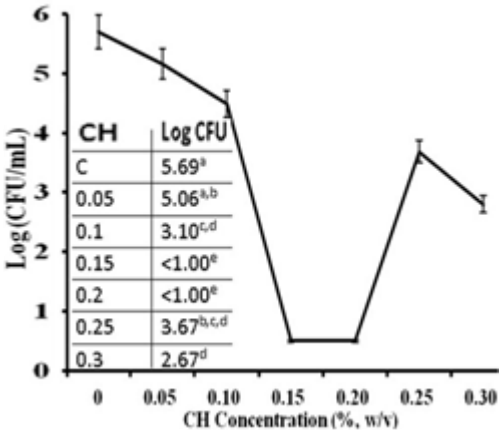
Data Analysis. A completely randomized design was used on three replicates and an analysis of variance (ANOVA) table was conducted to assess whether the increase in CH concentrations had any negative impact on coliform growth during the antimicrobial experiments. For the experiments related to radical scavenging, a series of t-tests were conducted to evaluate whether the total flux values for the control and the various samples (including the standards) were significantly different. Duncan's multiple range tests was carried out to test any significant differences between different CH concentrations ($\alpha = 0.05$). These analyses were conducted using the software program package Statistical Applicatory System (SAS) version 9.2 (SAS Institute Inc., Cary, NC).

3. RESULTS AND DISCUSSION

Antimicrobial Activity of CH

Coliform counts. The results showed a steady decrease in coliform counts in MacConkey agar with increasing CH concentration up to 0.2%, after which the number of colonies gradually increased (Fig. 1). This indicated a negative correlation between coliform growths at CH concentrations ≤0.2%. At the lowest concentration of CH (0.05%), no marked effect of CH on coliform growth was observed. The average number of colony forming units

(CFUs) recorded on the plates representing beef steak samples treated with 0.05 and 0.1% CH were 1.15×105 and 3.07 × 104 CFUs /mL, respectively.



CH	Log CFU
C	5.69[a]
0.05	5.06[a,b]
0.1	3.10[c,d]
0.15	<1.00[e]
0.2	<1.00[e]
0.25	3.67[b,c,d]
0.3	2.67[d]

Fig. 1. Coliform counts of extracts of beefsteak coated with different concentrations of casein hydrolyzate (CH) following 48 hours of incubation at 37˚C. The X-axis represents CH concentration (%, w/v) and the Y-axis showes coliform counts in log colony forming units (CFU/mL). The inset (table) exhibits log colony forming units per mL at the different CH concentrations (C is control without CH) with statistical notations where dissimilar letters indicate significant difference (P<0.05).

The most dramatic reductions in growth were observed at CH concentrations of 0.15 – 0.2%, which resulted in least number of colony proliferations (< 10 CFU/mL). These results were significantly lower (P< 0.05) than the control and all other CH concentrations. At concentrations above 0.2%, CH appeared to have lost its effectiveness in inhibiting coliform growth, evident by a gradual increase in the number of CFU at CH concentrations ranging from 0.25 – 0.3%. On an average, 4.83×103 and 6.30×102 CFU/mL were recorded from plates prepared from beef steak samples treated with the coating dispersions containing 0.25 and 0.3% CH, respectively (Fig. 1).

Casein hydrolyzate gives highly amphipathic peptides (Meisel, 1998). Moreover, in our experiments chymotrypsin used for hydrolysis cleaved the peptide bonds adjacent to an aromatic ring (Appel, 1986) resulting in peptides that are more hydrophobic on one side than the other and consequently amphipathic. This is reported to correlate to ability to form helical structures and partake in self-association. Self-association peptide has been associated with antimicrobial activity (Strahilevitz et al., 1994). Chymotryptic peptides of the major casein, αs1-casein, spontaneously self-associates to form large clusters (Haque and Kito, 1984).

Moreover, casein peptides are rich in lysine and arginine residues and thus cationic at the pH of our experiments (pH 7.0). Host defense cationic amphipathic peptides (AMPs) are ubiquitous in almost all biological forms (Zasloff, 2002). It has traditionally been thought that this activity of AMPs is due to their amino acid composition, amphipathicity, cationic charge and size that allow them to attach to and insert into microbial membrane bilayers to form pores by 'barrel-stave', 'carpet' or 'toroidal pore' mechanisms (Brogden, 2005). Moreover, in sharp contrast to humans, cytoplasmic membranes of bacteria (E. coli, S. aureus, or B. subtilis) and fungus (C. albicans) are generally much more electronegative with dramatically higher proportions of anionic phosphatidylglycerol (PG) and cardiolipin (CL) which are exceedingly low or entirely lacking in mammalian membranes (Yeaman and Yount, 2003). This accounts for selective action against microbes and not human cells. Koppelman et al. (2001) reported that CL is enriched specifically in the Escherichia coli minicell of the cell division complex (divisome) making it more susceptible to these types of peptides.

Antioxidative Activity of CH

Peroxyl and Alkoxyl Radical Quenching Efficacy. The changes in chemiluminescence of the control, test samples and Trolox standard over time compared to the blank are depicted in the Figs. 2A-B. The luminescence values of the individual CH samples were found to be superimposed on each other when their total flux values were compared to the control (Fig. 2A).

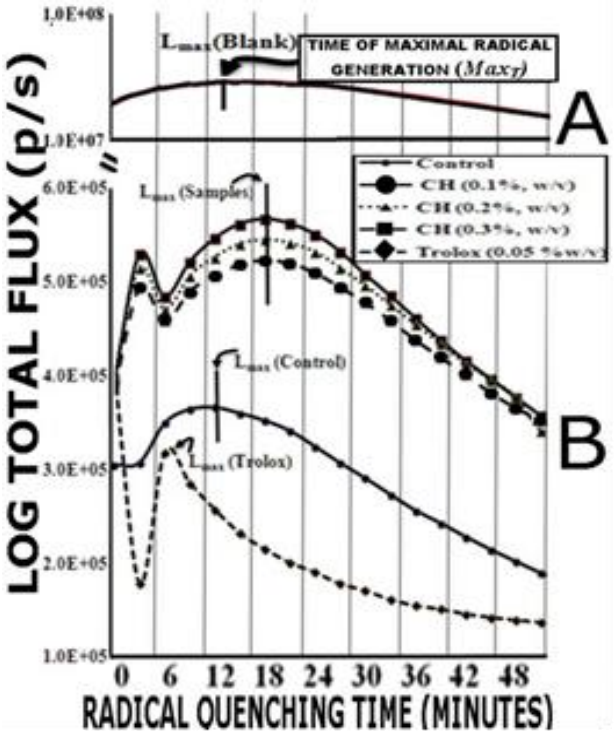


Fig. 2A. In vitro generation over time of peroxyl and alkoxyl radical by pyrolysis of 2,2'-Azo-bis (2-amidinopropane) in reaction mixture with buffer alone (blank).

Radical quantitation was by luminol emmited chemiluminescence resulting stoiciometrically from unquenched radicals generated by pyrolysis of 2,2'-Azo-bis (2-amidinopropane). The X-and Y-axes respectively represent time following initiation of pyrolysis and luminol emmited chemiluminescence in log total flux (photons emmitted per second)(p/s). Chemiluminescence maxima for the blank indicates the point of maximam accumulation of radicals in the reaction mixture and is identified as Lmax (Blank). Abbreviations are as follows: CH: Casein hydrolyzate; Photons/second: p/s). B. Peroxyl and alkoxyl radical quenching efficacy of the whey protein concentrate based edible coating dispersions without (control) and with different concentrations of casein hydrolyzate, compared to blank (buffer only) and Trolox standard (20 mM). The X-and Y-axes are as described for Figure 2A. Chemiluminescence maxima of the test samples, control and Trolox standards are designated by Lmax (Sample), Lmax (Control) and Lmax (Trolox), respectively. Abbreviations are the same as in figure 2A.

The parameters that were relevant in the chemiluminescence study were, (1) Lmax (p/s), (2) Max_T, and (3) ΔMax. These parameters were used to determine the two arms of antioxidative efficacy; antioxidative activity (AA), i.e., the ability of the test coating dispersions to neutralize radicals during the period of their maximal formation, reflected by Lmax and antioxidative persistence (AP) or the effectiveness over time was given by Max_T, and ΔMax. A sharp initial increase in luminescence (photon yield) following the initiation of ABAP pyrolysis was observed for all the samples represented a lag period before AA set in (Mukherjee et al. 2012). The prolonged increase in luminescence (photon yield) following significantly decreased chemiluminescence, representing the ability of the test coating dispersions to deplete the generated radicals through electron donation, represented Max_T (Fig. 2B). the subsequently radical depletion over time, evident by the gradual decrease of photon yield was the result of enervation of ABAP pyrolysis. The reduction in Lmax of control and the test samples compared to the blank were about two orders of magnitude in intensity (Table 1). Results of t test analyses indicated significantly greater ($P<0.05$) antioxidative efficacy. Though both the control and the Trolox standard showed lower photon yields at Lmax compared to the CH samples (Fig. 2B), the time required to reach it was significantly less for the CH containing test coating dispersions being 21 min compared to 18 min for the blank, 15 min) for control and 3 min for the Trolox standard (Fig. 3).

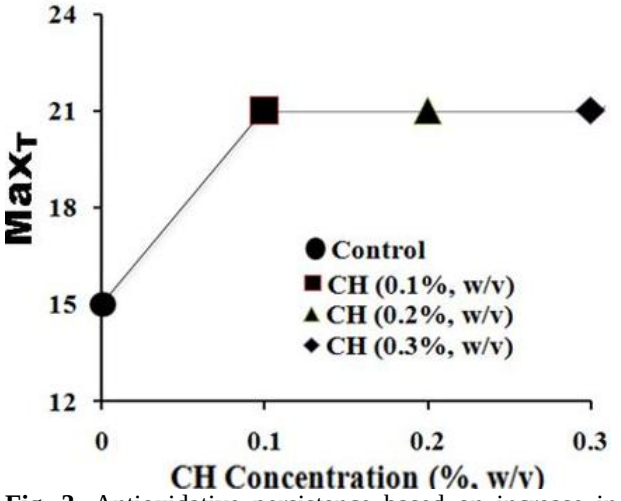


Fig. 3. Antioxidative persistence based on increase in time required for maximal alkoxyl and peroxyl radical accumulation (MaxT) in reaction mixture produced in vitro by pyrolysis of 2, 2'-azo-bis (2-amidinopropane). The X-axis represents different concentrations of casein hydrolyzate in the whey protein concentrate based edible coating and Y-axis represents Max_T. Abbreviations are as in Fig. 2A.

A comparison of the MaxT (Fig. 3) revealed dramatically better AP of the samples containing CH by increasing this parameter by 6 min and ΔMaxT was independent of CH concentration. This is dramatic in view of the harsh oxidative condition of the in vitro test that were conducted.

DPPH Assay. The samples containing CH significantly greater ($P<0.05$) DPPH radical scavenging activities compared to the control (Table 2), as was the case with the assay for determination of peroxyl and alkoxyl radicals. The assay also indicated a concentration dependent enhancement of DPPH radical scavenging activity of CH. The sample with 0.3% CH showed the highest antioxidative efficacy (evident from 35.4% inhibition of DPPH radical proliferation, which represented approximately 47% enhancement of radical scavenging activity of the sample compared to the control). The calculated Trolox equivalent of the various concentrations of CH is presented in the Table 3.

It is known that CH is a good source of antioxidant peptides (López-Expósito et al., 2007). We have recently reported that chymotryptic digestion of casein fraction α s2-casein produces highly antioxidative peptides (Haque and Zhang, 2015). This casein fraction is particularly rich in aromatic and positively charged amino acids (under our conditions) resulting in cationic peptides with greater tendency for association than the intact protein. Circular dichroism studied showed absence of restrictive secondary structure in these peptides allowing ease of juxtapositioning and stacking (Haque and Zhang, 2015). Chymotryptic digestion of aromatic residue rich protein, like αs2-casein, also assures the presence of an aromatic amino acid on the C-terminus due to enzyme specificity (Appel, 1986). Aromatic residues rich peptides can potentially participate in π-π or cation-π interactions (McGaughey et al., 1998). Such stacking cause overlapping of the electron clouds of the π orbitals above the plane of the aromatic rings allowing electronic continuity and the observed enhanced antioxidative efficacy of the CH containing coating dispersions. Such continuity would plausibly be paramount in the ability to effectively donate electrons to quench lone pairs on the radicals.

Contrary to expectations, under our conditions the control, i.e., coating dispersions with WPC alone, had the lowest Lmax indicating highest AA but this was short lived (Figs 2B and 3). Though we saw a CH concentration dependent non-significant increase in Lmax, apparently indicating an inverse relationship with electron donating efficiency, the Max_T, reflecting AP or electron donating persistence over time, increased dramatically and significantly by 74% compared to control but was CH concertation independent; Max_T was constant at all concentrations (Figure 3). It was conceivably related to the major antioxidative component in the edible coating mixture, WPC, the concentration of which was constant in all samples.

As indicated in the above discussion related to antimicrobial effects, casein peptides have a high tendency for self-association. They also dramatically increases the association tendency of co-dispersed whey proteins as observed earlier using quasielastic light scattering studies (Haque et al., 1993; Sharma et al., 1996). The apparent dose dependent increase in Lmax (Fig 2B) as a result of the addition of CH is ascribed to the formation of colloidal clusters through hetero-polymerization of the proteins and/or peptides resulting in drastic increase in antioxidant particle size and thus in surface area and thus reduction in number of side chain residues that can partake in electron donation. There is an inverse proportionality between surface area and particle size (Brandt et al., 2003). The observed lag period in the CH samples can also be attributed to this phenomenon where the clustered WPC proteins were unable to keep up with the profuse amount of radicals that were being generated until the reaction slowed down. This premise is substantiated by the fact that the intensity of this first peak was directly related to the CH concentration (Fig 2B).

Table 1. Alkoxyl and peroxyl radical scavenging ability[1] of coating solutions containing various concentrations of casein hydrolyzate compared to blank[2], control[3] and trolox standard[3]

Samples	Total flux (p/s) at L_{max}
Blank[2]	$(2.9\pm0.08) \times10^{7\ a}$
Control[4]	$(3.7\pm0.13)\times10^{5\ c}$
CH[5] (0.1%, w/v)	$(5.7\pm0.15)\times10^{5\ b}$
CH (0.2%, w/v)	$(5.4\pm0.12)\times10^{5\ b}$
CH (0.3%, w/v)	$(5.2\pm0.08)\times10^{5\ b}$
Trolox (0.05%, w/v)	$(4.0\pm0.10)\times10^{5\ c}$

Dissimilar superscripted letters indicate significant differences (P<0.05).
[1]Based on total flux as detected emitted photons per second (p/s) at the point of maximum pyrolytic generation of alkoxyl- and peroxyl-radical as reflected by luminol emitted chemiluminescence maxima (Lmax) of bank2.
[2] Buffer alone.
[3]Concentration of the Trolox standard was 20 mM.
[4]Whey protein concentrate based edible coating dispersion without casein hydrolyzate.
[5]CH is abbreviation of casein hydrolyzate.

Table 2. 2, 2-diphenyl-1-picrylhydrazyl radical scavenging ability of whey protein concentrate based coating solutions containing various concentrations of casen hydrolyzate compared to control[1]

CH Concentration (%,w/v)	DPPH[2] Radical Scavenging (%) activities(%)
Control[1]	24.1 ± 2.5^{a}
0.1	30.8 ± 1.5^{b}
0.2	31.1 ± 0.1^{b}
0.3	35.4 ± 1.3^{c}

[1] Whey protein concentrate based edible coating dispersion without CH
[2]DPPH is abbreviation for 2, 2-diphenyl-1-picrylhydrazyl
Other abbreviation and notations are as in the Table 1.

Table 3. 2, 2-diphenyl-1-picrylhydrazyl radical scavenging ability of whey protein concentrate based coating solutions containing various concentrations of casein hydrolyzate expressed as trolox equivalents

CH Concentration (%,w/v)	Trolox Equivalent (µM)
Control (0)	61.35
0.10	78.50
0.20	79.45
0.30	90.25

Abbreviations and notations are as in the Table 1.

The dramatic increase in the ΔMaxT is postulated to be due to the shielding of the electron-donors within the clusters from the radicals in the aqueous phase which resulted in reduced electron donor effect that continued longer (Fig. 2 B).

4. CONCLUSION

The current study showed significantly enhanced antimicrobial properties of WPC based edible coating used to coat beefsteak when CH was added to it. Addition of the CH at a concentration as low as 0.15 – 0.2% resulted in a five log reduction (CFU<1) of coliform growth, virtually eliminating it when all other conditions were uniform. Though real-time chemiluminescence studies showed using alkoxyl and peroxyl radical showed decreased AA compared to the control, the AP was dramatically enhanced by CH. The Max_T was increased 40% (from 15 min to 21 min) at all concentrations of CH. This reflected the outstanding persistence of the antioxidant combination as being significantly effective compared to the controls, and even, Trolox, the gold standard of antioxidants. The study depicts the potential of CH to be used in whey protein concentrate based edible coating to reduce oxidative and microbial degradation of cut beefsteak and other food items thus retail-cut display life.

5. ACKNOWLEDGEMENTS

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