Utilization of Agricultural By-products to Partially Replace Gelatin in Preparation of Products for Leather†

by

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Abstract

When polyphenolic-modified gelatin-products were used as fillers, improvements were seen in the subjective properties of the leather. When the treated samples were compared to control samples, there were no significant changes in mechanical properties. At the present time, gelatin is in short supply, costs are increasing, and there is an urgent need to find a substitute that could be combined with the gelatin, thereby, partially replacing and reducing the amount of gelatin required, with the goal that the new products would retain the desired characteristics of gelatin products. We have evaluated the potential of producing biopolymers from the reaction of polyphenols with gelatin in combination with other proteins (e.g. whey) or with carbohydrates (e.g. chitosan and pectin). Several researchers have recently demonstrated the feasibility of these reactions. These combinations would take advantage of the distinctive properties of both species and at the same time create products with improved functional properties. Recently, the preparation of polyphenolic-modified gelatin/whey biopolymer products was investigated, and the results of product characterization using physicochemical analyses indicated optimal products that could be used as fillers. In this continuing study, these products were applied to wet white, that was then finished, and subjective and mechanical properties were evaluated. At the same time a method was developed to determine the rate of uptake of the product. Results of the studies will be presented. These findings could further add to the knowledge of using renewable resources in production of unique products that may have leather processing application.

Introdction

Utilization of renewable or sustainable resources, for example, proteins (from leather or dairy industries) and carbohydrates (pectin, starch, or chitosan) could be employed to formulate products that could improve the quality of finished leather. These products could be applied as fillers to improve veiny hides, a continuing problem in the leather industry that results in lower quality finished leather. Or perhaps these products could provide better dye uptake or supplement more efficient utilization of fatliquoring agents. To improve the delivery of leather chemicals with less waste, they could also be used as encapsulating agents, as emulsions or as films or coatings for leather finishing.

The polyphenolic acids in vegetable tannins have been investigated at length for their ability to modify gelatin[1-7]. We have shown that vegetable tannin quebracho can be used to modify gelatin, and tara can be used to modify both gelatin and whey protein concentrate (WPC)[8-11]. The physical and chemical properties of both of the resulting products make them amenable to be used as fillers[8, 10]. The leather resulting from these treatments has improved subjective properties with no discernible differences in mechanical properties[9, 11].

Gelatins are in short supply and the costs are subsequently escalating[12]. Replacements for some of the gelatin with another substrate are being investigated. Recent research has established the possibility of producing biopolymers from the reaction of polyphenols with gelatin in combination with other

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proteins (e.g. whey) or with carbohydrates (e.g. chitosan and pectin). These combinations may well take advantage of the distinctive properties of both species and simultaneously create products with improved functional properties. Zhang, et al.[13] showed that a biopolymer, produced from tannic acid treated gelatin-gum arabic coacervate for the purpose of forming microspheres, allowed sustained release of allyl isothiocyanate, Strauss and Gibson[1] and Mathew and Abraham[14] have shown that biopolymers could be produced by reaction of polyphenols with gelatin and pectin as well as with starch and chitosan. Strauss and Gibson[1] also confirmed that plant-derived phenolic acids that have been used to cross-link gelatin–pectin coacervates could possibly result in microparticles for use as food ingredients and that these gels had superior mechanical strength, reduced swelling, and less free amino groups.

Whey also had been examined for its reactivity with polyphenols. Rawel, et al. reported that β-lactoglobulin, one of the components of whey, reacts with polyphenolic acids at pH 9.0 and a change is noted in the molecular weight distribution.[15] We initiated studies to see if there is potential for producing biopolymers from gelatin and another substrate, for example substituting WPC for some of the gelatin using the polyphenol reaction[16]. The resulting products were characterized with respect to their physical properties (gel strength, melting point and viscosity), molecular weight distribution (SDS-PAGE), hydrothermal stability (DSC), and fluorescent properties (epi-fluorescent imaging). The results showed that we have the potential for a product that could be used as a filler.

In this continuing study we treated wet white with the gelatin/WPC/tara product, described previously[16] and, after retanning, coloring and fatliquoring (RCF) we assessed the effect that these treatments had on subjective and mechanical properties. The product uptake was monitored using BCA protein determination assay. We further examined the products for changes in fluorescent properties and, if present, determined if this technique could be applied to monitoring product uptake. The resulting leather was also examined by scanning electron microscopy (SEM) to determine the effect of treatment on fiber structure. The results of these analyses will be presented.

Experimental

Materials

Commercial Type B gelatin from bovine skin, characterized in this laboratory as 175 grams Bloom, was obtained from Fisher (Fairlawn, NJ). Whey protein concentrate containing 80% protein, (Hilmar™ 8000) was generously supplied by Hilmar Ingredients (Hilmar, CA). Tara was obtained from Hermann Oak Leather Company (St. Louis, MO). Upholstery weight chrome-free stock or wet white (hides that may be tanned with glutaraldehyde, or combinations of synthetic tannins, vegetable tannins, and minerals, such as aluminium and zirconium) was purchased from local tanneries; area pieces were sampled from this stock. All other chemicals were analytical grade and used as received.

Application of Gelatin/WPC/Tara Product to Wet White Stock (Area Samples)

Gelatin/WPC/tara products, using 6% w/v gelatin (175 Bloom), 7% w/v WPC, and 4% tara (based on total weight of gelatin and WPC) at pH 9.5-10.0, 45°C for 4h, were prepared as described in a previous publication[16] and were applied to wet white stock as shown in Figure 1. Samples of wet white hides were taken for epi-fluorescent study. For wet white hides, six pieces, three tests and three controls, approximately 1 foot square, were cut sequentially from the butt, belly or neck area, and three pieces were added per drum. The wet white samples (tests and controls, ~650 g/drum) were placed in small Dose drums (Model PFI 300-34, Dose Maschinenbau GmbH, Lichtenau, Germany), washed (400% float based on hide weight) by drumming for 30 min at 45°C, drained and refloated in sodium bicarbonate (~2% on hide weight in 200% float). The samples were drummed at ambient temperature (25-28°C) until the pH stabilized (6.5-7.0). The floats were drained and the control samples set aside. To the test samples, the tannin product (6% gelatin, 7% WPC, modified with 4% tara) was added in 300% float. The samples were then drummed for 1 h at ambient temperature and then for 4 h at 45°C. The floats were drained and the samples were washed twice for 10 min at 45°C (400% float), drained, patted dry, and stored at 4°C. The tests and controls were retanned, colored and fatliquored (RCF) using upholstery formula (wet white) as described in prior publications[17-18]. When completed, the wet white samples were toggle stretched at ambient temperature and humidity, conditioned, staked and milled. The samples were subjectively evaluated. No finishing operations were done to the hide pieces and they were kept in a shelf in the conditioning room, at 23°C and 50% relative humidity for at least 3 days.

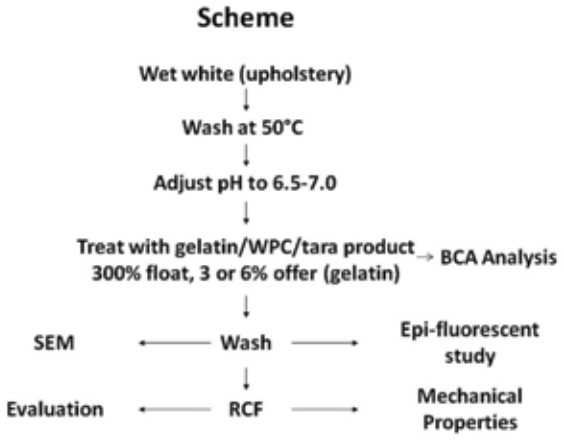

Figure 1. Flow diagram for treatment of wet white and protocol for sampling.

Analyses

Physical Properties, Mechanical Properties and Molecular Weight Distribution

Gel strength, melting point, and viscosity of the gel/WPC/tara-treated proteinaceous solutions were determined as described in a previous publication[19]. Mechanical properties (tensile strength, elongation, Young's Modulus, toughness index, tear strength, and thickness) were determined as described in a previous paper[20]. Protein molecular weights were estimated as described previously[21]. In summary, SDS-PAGE (polyacrylamide gel electrophoresis in sodium dodecyl sulfate) was run using precast 4-15 percent gradient gels. A broad range (BRS) calibration standard (Bio-Rad, Hercules, CA), which contains a mixture of nine proteins ranging in size from 6,500 to 200,000 Daltons, was used. Samples of lyophilized protein were dissolved in sample buffer (10 mM Tris-HCl at pH 8.0 containing 1 mM EDTA, 2.5% SDS, 5% β-mercaptoethanol and 0.01% bromophenol blue) and were then heated at 40°C for 4 h. Separation was achieved using a Phast-Gel System (Pharmacia Biotech Inc., Piscataway, NJ). Gels were stained with Coomassie Blue (Pharmacia).

Subjective Evaluation RCF Leather

Each treated and untreated sample of wet white hide was evaluated (one evaluator) with respect to handle, fullness, grain (break) and color. A rating value from 1 to 5 was allocated for each parameter, with 1 being the worst and 5 being the best. From these ratings, an overall evaluation was determined and this value (from 1 to 5) was reported.

Optical Microscopy (with Epi-fluorescent Attachment)

Control and treated samples of wet white were sectioned, using a razor (from grain to flesh) and mounted onto a glass slide. They were examined using an Eclipse E600 Polarizing Microscope (Nikon Instruments Company, Melville, NY), at 4X magnification, operating in optical mode. The instrument was equipped with a X-Cite™ 120 Fluorescence Illuminator System which was fitted with a metal halide lamp (EXFO Photonic Solutions, Inc., Mississauga, ON, Canada), with two filter cubes or optical blocks, containing epi-fluorescence interference and absorption filter combinations including an excitation filter, dichromatic beamsplitter (often referred to as a mirror), and a barrier (or emission) filter (515-555 nm or 600-660 nm), and with a digital camera (DS-Fi1)[22].

Scanning Electron Microscopy (SEM)

Wet white samples, after treatment, along with their respective control samples were cut into small strips (6.5 cm ´ 1 cm), placed in a test tube to which nano pure water was added (to cover strip) and freeze-dried. Two pieces (1.5 mm thick) were cut from each of the dry samples and were mounted onto the surfaces of carbon adhesive tabs with the help of Duco cement. After drying for 1 h, silver paint was applied to the exposed surface area around the samples. The samples were sputter-coated with a thin layer of gold using a Scancoat Six Sputter coater. Samples were viewed using a Quanta 200 FEG Environmental Scanning Electron microscope, FEI Company (Hillsboro, OR) in high vacuum-secondary electron imaging mode at an accelerating voltage of 10 kV (spot size 3.0, pressure 0.3 torr). Digital images were collected at 50, 250, 500, and 1000´ magnifications.

Protein Concentration Determination

Protein concentrations in the float, at different stages of the treatment, were determined using the bicinchoninic acid (BCA) assay[23] according to the directions supplied with the kit and with modification in which gelatin was used as standard as opposed to bovine serum albumin (BSA). Samples (1.5 ml) were centrifuged at 13,400 rpm for 30 min in a microcentrifuge (Eppendorf MiniSpin plus, Westbury, NY). One ml of protein supernatant was removed and typically a 1:25 (v/v) dilution was prepared in order to fall within the linear concentration range for the assay (200 to 1000 mg/ml protein). A 50 ml aliquot of the diluted solution was mixed with 1.0 ml of BCA reagent and incubated at 37°C for 30 min. The absorbance of a sample solution at 562 nm minus a reagent blank was compared with a standard curve using known concentrations of gelatin.

RESULTS AND DISCUSSION

In a previous study[16] we determined the appropriate combinations of gelatin and WPC, modified with tara that would give products whose properties would be similar to polyphenolic (vegetable tannin) modified gelatin. It was determined that 6% gelatin in combination with 7% WPC, modified with 3-4% tara, would give a product whose physical properties would make it a good contender to be used as a filler. When the physical properties of the products were compared to untreated control samples, the gel strength was 115.7 g vs 99.6 g, MP, 41.7°C vs, 34.0°C and viscosity, 6.52 cP @ 60°C vs 4.95 cP (16); all properties were better than the control samples and are characteristic of a product that could be used as a filler.

Treatment of Wet White and BCA Analysis

Wet white hides were treated with the gelatin/WPC tara product deemed to have optimal physicochemical properties necessary for application as a filler (6% gelatin, w/v, 7% WPC, w/v and 4% tara, based on weight of total protein, pH 9.5-10.0, 45°C, for 4 h). Products prepared for each trial were monitored for physical properties and molecular weight distribution (indicating the bands for both the gelatin and WPC had been diminished when compared to the control) and results were analogous to those reported earlier.[16]

We ran two sets of experiments in order to determine optimal amounts of filler that should be applied to wet white. In the first series, we added the biopolymer product using 6% gelatin, based on wet white weight, in a 300% float, and then the

leather was RCF, and evaluated. When the subjective properties were examined (Figure 2a), it was found that these properties were not improved, but were worse than the control. At the same time the samples were being treated with the biopolymer, aliquots of the float were taken so that the percent uptake could be monitored. Previously we tried to monitor uptake of a tara product by wet white[11] using a phenolics assay, but found that there were interferences in the analysis from the wet white. We decided to carry out a BCA analysis, a protein assay, using gelatin as a standard. In the 6% offer trial (Figure 3a) we found that the wet white samples had picked up a little less than 80% of the biopolymer that was offered and this literally swelled the leather (Figure 4) and possibly ruptured the fiber structure. Similar results were found in the duplicate trial that was run.

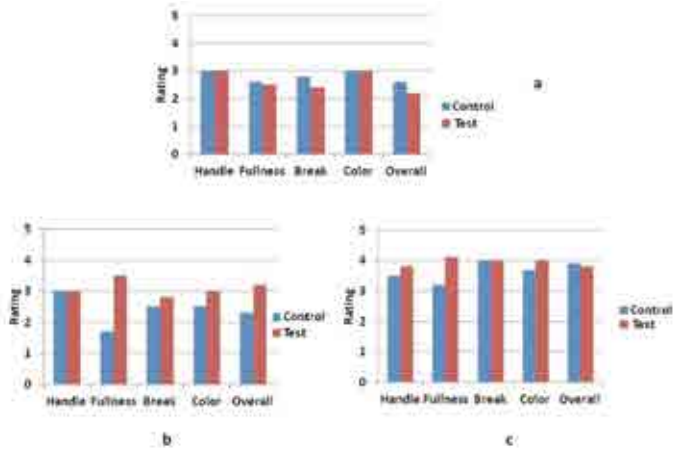

Figure 2. Subjective evaluation (handle, fullness, break, color, and overall) using rating scale of 1 = worst to 5 = best, of wet white (area pieces), treated with pH-adjusting agents (controls) and with tara-modified gelatin/WPC (tests); (a) using a 6% offer, 300% float, two trials, and (b) using a 3% offer, 300% float, 2 trials, old lot and (c) 2 trials, new lot.

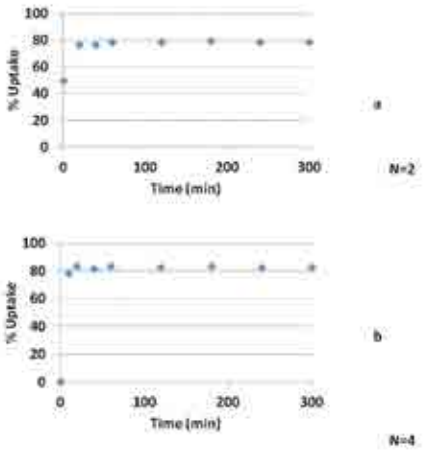

Figure 3. Comparison of percent uptake of gelatin/WPC/tara product by hide when using either a 6% (a) or 3% (b) offer based on wet white weight (carried out in a 300% float).

In the second set of experiments the amount of gelatin offered was cut in half to 3% based on the wet white weight. In this series of experiments, two trials were run using an older lot of wet white and two trials were run using a recently purchased new lot. When the subjective properties of the 3% offer were evaluated (Figures 2b and 2c), the results showed, essentially, an improvement in all parameters in both lots of hides, with the control parameters in the new lot of wet white (Figure 2c) indicating a higher rating in all instances. Mechanical properties were also run (Figure 5).

As indicated by the error bars, there were no significant differences in mechanical properties between the tests and the controls within a lot, however, there were differences in the tensile and tear strengths between the two lots. The BCA

Figure 4. Wet white hides that had been treated with a 6% offer of gelatin (based on wet weight of hides and carried out in a 300% float) and then were retanned colored and fatliquored. Six pieces on left are untreated control samples, six on right had been treated with product.

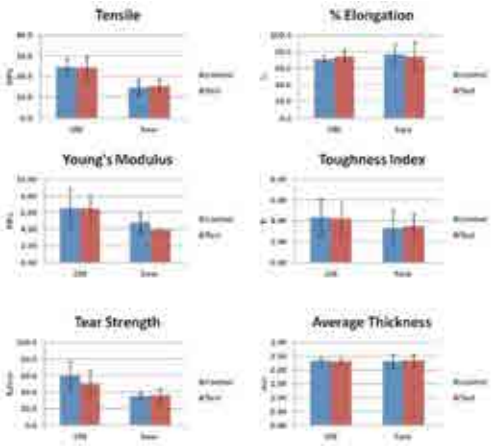

Figure 5. Mechanical properties (with error bars indicating STD) of area pieces of wet white treated with pH-adjusting agents (controls) and with tara-modified gelatin/WPC (tests), and then RCF; shown is the 3% offer (300% float) based on wet white weight (n=3 for old lot and n=2 for new lot); each mechanical property is an average of five determinations.

determination (Figure 3b) showed that more than 80% of the product offered was picked up by the hides. Four trials using the 3% offer were carried out, and when the percent uptake was calculated, along with standard deviation (STD), the results demonstrated the reproducibility of the method (as indicated by the error bars) (Figure 6).

SEM and Epi-fluorescent Imagery

Samples were taken for SEM analysis (Figure 7). Differences, at 1000X magnification, in the fiber structure of the test and the control can be seen, in that the control (Figure 7a) has a more distinct fiber separation than the test (Figure 7b) which appears less defined.

These observations are similar to what we observed in gelatin/quebracho treated blue stock[9] and gelatin/tara wet white studies[11].

Samples of the wet white, before and after treatment, were also examined using the epi-fluorescent microscope to determine if the leather was filled (Figure 8). The wet white auto-fluoresces strongly and we used a ND filter 4, which reduces illumination about 25% without changing the color balance of the light. The following images are representative of the numerous samples we examined and, because of filtering for high fluorescence, were auto-corrected.

The images at 515-555nm emission (control, Figure 8a, and test, Figure 8b) indicate that the treated wet white fluoresces with a more lime-green color as opposed to control which tends toward the bluish green. The images at 600-660nm emission (control, Figure 8c, and test, Figure 8d) show that the treated wet white fluoresces with a more orange-red color as opposed to control which tends toward the pinkish-red. This technique allows one to see if filler was taken up, but it is not possible to distinguish distribution because of the inherent high auto-fluorescence of wet white.

These series of experiments demonstrated that 3% gelatin, 3.5% WPC modified with tara, when applied as a filler, will give a leather product with good subjective and mechanical properties. These properties are similar to 10% gelatin/tara product alone being applied. As a consequence this biopolymer product reduces the amount of gelatin needed to improve the quality of leather to a third. This makes good economic sense given that gelatin is in short supply and increasingly more expensive.

CONCLUSIONS

Gelatin is in high demand and short supply, and there is an urgent need to augment it with new products that retain the desired characteristics of gelatin products. We have produced biopolymers from the reaction of polyphenols with gelatin in combination with WPC whose physicochemical properties suggest that it would be amenable to be used as a leather filler.

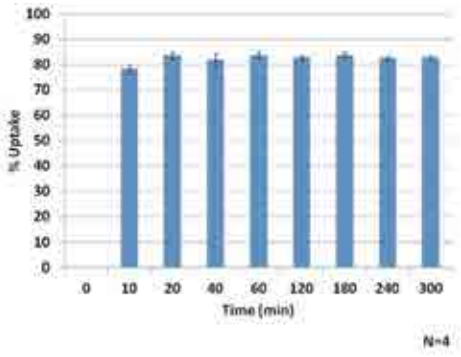

Figure 6. Percent uptake (using BCA assay with gelatin as standard) of gelatin/WPC/tara product by wet white (n=4), with error bars indicating STD.

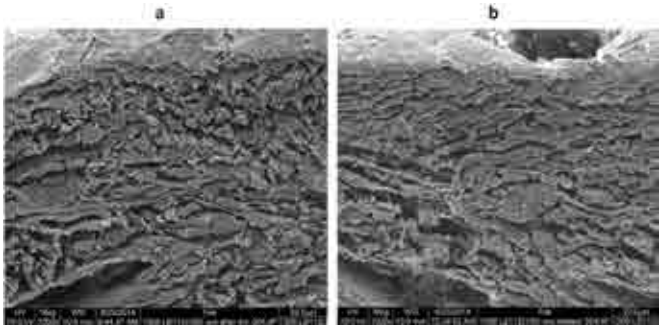

Figure 7. SEM micrographs (1000x) of wet white, untreated control sample after pH adjustment (a) and test sample, after treatment with tara-modified gelatin/WPC (b) (– = 20μm).

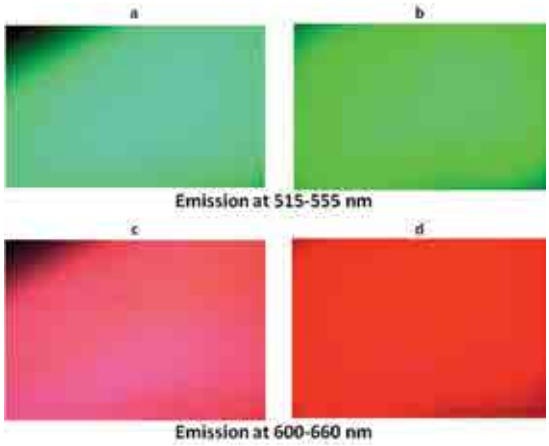

Figure 8. Epi-fluorescent micrographs of wet white stock; treated with pH-adjusting agents alone (control) (a and c), and test sample treated with tara-modified gelatin/WPC (b and d); two emission (barrier) filters, between 515-555 nm and 600-660 nm, were used.

These products were applied to wet white, and subjective and mechanical properties of the crust were evaluated. At the same time a method was developed to determine the rate of uptake of the product using a modified BCA analysis. In the wet white trials, improvements were seen in the subjective properties and at the same time there were no statistical differences observed in mechanical properties. SEM studies show that the fiber structure in treated wet white hides is not as organized as the more defined structure we see in the control samples. The gelatin/WPC/tara product fluoresces, however distribution of the product in the test sample was not seen because of high fluorescence of the wet white; changes were subtle in the colors at the different emissions. These trials demonstrated that gelatin can be supplemented using another waste agricultural product and as a result renewable resources could be used in production of unique products that may have leather processing applications.

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REFERENCES

1. Strauss, G., and Gibson, S.M.; Plant phenolics as cross-linkers of gelatin gels and gelatin-based coacervates for use as food ingredients. *Food Hydrocolloids* **18**, 81-89, 2004.
2. Cao, N., Fu,Y., and He, J.; Mechanical properties of gelatin films cross-linked, respectively, by ferulic acid and tannin acid. *Food Hydrocolloids* **21**, 575-584, 2007.
3. Balange, A. and Benjakul, S.; Enhancement of gel strength of bigeye snapper (*Priacanthus tayenus*) surimi using oxidised phenolic compounds. *Food Chemistry* **113**, 61-70, 2009.
4. Balange, A. and Benjakul, S.; Effect of oxidised phenolic compounds on the gel property of mackerel (*Rastrelliger kanagurta*) surimi. *LWT - Food Science and Technology* **42**, 1059-1064, 2009.
5. Balange, A. K. and Benjakul, S.; Effect of oxidised tannic acid on the gel properties of mackerel (*Rastrelliger kanagurta*) mince and surimi prepared by different washing processes. *Food Hydrocolloids* **23**, 1693-1701, 2009.
6. Kosaraju, S. L., Puvanenthiran, A., Lillford, P.; Naturally crosslinked gelatin gels with modified material properties. *Food Research International* **43**, 2385-2389, 2010.
7. Zhang, X., Do, M.D., Casey, P., Sulistio, A, Qiao, G.G., Lundin, L., Lillford, P., Kosaraju, S.; Chemical modification of gelatin by a natural phenolic cross-linker, tannic acid. *Journal of Agricultural and Food Chemistry* **58**, 6809-6815, 2010.
8. Taylor, M.M., Lee, J., Bumanlag, L.P., Latona, R., Brown, E.M., and Liu, C.K.; Preparation and characterization of polyphenol-modified gelatin products. *JALCA* **107**, 51-59, 2012.
9. Taylor, M.M., Medina, M., Lee, J., Bumanlag, L.P., Brown, E.M., and Liu, C.K.; Treatment of wet blue with quebracho-modified gelatin biopolymer products. *JALCA* **107**, 416-421, 2012.
10. Taylor, M.M., Lee, J., Bumanlag, L.P., Latona, R.J., Brown, E.M., and Liu, C.K.; Preparation and evaluation of tara-modified proteins. *JALCA* **108**, 16-22, 2013.
11. Taylor, M.M., Medina, M., Lee, J., Bumanlag, L.P., Latona, N.L., Brown, E.M., and Liu, C.K; Treatment of hides with tara-modified protein products. *JALCA* **108**, 438-444, 2013.
12. http://www.ft.com/intl/cms/s/0/1f01d088-4900-11e1-954a-00144feabdc0.html#axzz1xsCCKblO (accessed 07/16/14)
13. Zhang, Z.-Q., Pan, C.-H., Chung, D.; Tannic acid cross-linked gelatin–gum arabic coacervate microspheres for sustained release of allyl isothiocyanate: Characterization and in vitro release study. *Food Research International* **44**, 1000-1007, 2011.
14. Mathew, S., and Abraham, T.E.; Characterisation of ferulic acid incorporated starch–chitosan blend films. *Food Hydrocolloids* **22**: 826-835, 2008.
15. Rawel, H. M., Kroll. J., and Hohl, U. C.; Model studies on reactions of plant phenols with whey proteins. *Nahrung/ Food* **45**, 72-81, 2001.
16. Taylor, M.M, Lee, J., Bumanlag, L.P., Latona, R.J., Brown, E.M., and Liu, C.K.; Biopolymers produced from gelatin and whey protein concentrate using polyphenols. *JALCA* **109**, 82-88, 2014.
17. Taylor, M.M., Marmer, W.N., and Brown, E.M.; Effect of fillers from enzymatically modified proteins on mechanical properties of leather. *JALCA* **103**, 128-137, 2008.
18. Taylor, M.M., Lee, J., Bumanlag, L.P., Hernàndez-Balada, E., and Brown, E.M. Treatments to enhance properties of chrome-free (wet white) leather. *JALCA* **106**, 35-41, 2011.
19. Taylor, M.M., Cabeza, L.F., Marmer, W.N., and Brown, E.M.; Enzymatic modification of hydrolysis products from collagen using a microbial transglutaminase. I. Physical Properties. *JALCA* **96,** 319-332, 2001.
20. Liu, C.-K., and McClintick, M.D.; Measurements of the initial strain energy of leather. *JALCA* **92**, 157-171, 1997.
21. Taylor, M.M., Marmer, W N., and Brown, E M.; Molecular weight distribution and functional properties of enzymatically modified commercial and experimental gelatins. *JALCA* **99,** 129-140, 2004.
22. http://www.microscopyu.com/articles/fluorescence/filtercubes/filterindex.html (accessed 07/16/14)
23. Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J. and Klenk, D.C.; Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150**, 76-85, 1985.