Biopolymers Produced from Gelatin and Whey Protein Concentrate Using Polyphenols*

by

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Abstract

Several researchers have recently demonstrated the feasibility 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). These combinations would take advantage of the unique properties of both species and at the same time create products with enhanced functional properties. We have successfully demonstrated that the polyphenolic gallic acid and the vegetable tannins quebracho and tara could be used to modify gelatin and whey protein concentrate (WPC) resulting in a subsequent change in the physicochemical properties of each. When gelatin-polyphenol products were used as fillers, considerable improvements were seen in the subjective properties of the leather and when compared to control samples, there was no significant impact on mechanical properties. In this continuing research, we have begun to evaluate the potential of tara-modified gelatin/WPC biopolymers, specifically for their application as fillers. In this study, modification parameters for gelatin/WPC combinations will be explored, and the results of product characterization using physicochemical analyses will be presented. These studies could further contribute to the use of sustainable resources in production of unique products that may have leather processing applications.

Introduction

The utilization of renewable resources, such as, but not limited to, proteins (e.g. from leather or dairy industries) and carbohydrates (e.g. starch, pectin, and /or chitosan) could be employed to make products that can improve the quality of finished leather. For example, 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. Furthermore, these products provide better dye uptake or more efficient utilization of fatliquoring agents. They could also be used as encapsulating agents to improve the delivery of leather chemicals with less waste, as emulsions in finishing stages of leather processing and 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] 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.[8] With respect to gelatin modification by polyphenols, it has also been reported that some vegetable tannins could be applied to gelatin to give products with interesting physical properties.[9] 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).[10-13] The physical and chemical properties of both of the resulting products make them amenable to be used as fillers.[10,12] The leather resulting from these treatments has improved subjective properties with no discernible differences in mechanical properties.[11,13]

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However, it has been recently reported[14] that gelatins are becoming scarce and the costs are subsequently increasing. Substitutes for gelatin are being investigated, for the most part replacing some of the gelatin with another substrate. We have found that several researchers have recently established the feasibility 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). These combinations would take advantage of the unique properties of both species and simultaneously create products with enhanced functional properties. Zhang, et al.[15] produced biopolymers from tannic acid treated gelatin-gum arabic coacervate microspheres, Strauss and Gibson[1] and Mathew and Abraham[16] have shown that biopolymers could be produced by reaction of polyphenols with gelatin and pectins 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 greater mechanical strength, reduced swelling, and fewer free amino groups. Jones, et al.[17] prepared and characterized biopolymer particles based on thermal treatment of protein (ß-lactoglobulin)-polysaccharide electrostatic complexes and they conjectured that these products could be used in encapsulation.

Since the supply of gelatin, at the moment, is decreasing, we have initiated studies to see if there is potential for producing biopolymers from gelatin and another substrate, in particular, substituting WPC for some of the gelatin using the polyphenol reaction. The resulting products will be 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 of these analyses will be presented.

EXPERIMENTAL

Materials

Commercial Type B gelatin from bovine skin, characterized in this laboratory as 175g Bloom, was obtained from Fisher Scientific (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). All other chemicals were analytical grade and used as received.

Preparation of Tara-modified Gelatin and WPC Biopolymer Products

Gelatin (175g Bloom) (0-10g) and WPC (0-10g), were suspended in water (40 mL) held for 2 h at room temperature (25-28°C) and then stored overnight at 4°C. They were placed in a bath at 65ºC until dissolved. Control samples to which no tara was added, were run to monitor changes in physical properties. The pH was adjusted to 9.5-10.0 with 1 N NaOH. Tara (calculated to be 3-7% based on weight of total protein) solutions were prepared in 10 mL of water. The tara solutions were first heated to dissolve the product, centrifuged, and the supernatant added with stirring to the protein solutions to give final protein concentration of 10% w/v. Aliquots (10 mL) of all the reaction mixtures were added to test tubes for melting point determination and 30-mL aliquots were poured into appropriate containers (39-mm diameter jar) for determining gel strength. The samples were warmed to 45°C in a shaker bath and the reaction was carried out for 4 h. The samples were cooled to room temperature and then chilled for 17 h at 10ºC in a constant temperature bath. Physical analyses (gel strength, melting point and viscosity) were run on these samples. Aliquots of the samples were lyophilized and molecular weight distribution was determined. Sodium azide (70 µL of 1% solution) was added to the remaining treatment solution as a preservative; the samples were stored at 4°C.

Analyses

Physical Properties and Molecular Weight Distribution

Gel strength, melting point, and viscosity of the tara-treated proteinaceous solutions were determined as described in previous publications.[18] Protein molecular weights were estimated as described previously.[19] In summary, SDS-PAGE (polyacrylamide gel electrophoresis in sodium dodecyl sulfate) was run using precast 4-15 percent gradient gels. A broad range SDS-Standard (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° for 4 h. Separation was achieved using a Phast-Gel System (Pharmacia Biotech Inc., Piscataway, NJ). Gels were stained with Coomassie Blue (Pharmacia).

Hydrothermal Stability

Hydrothermal stability of tara -modified gelatin/WPC biopolymers and unmodified control samples were determined on a Multi-Cell Differential Scanning Calorimeter (DSC) (model CSC-4100) from Calorimetry Sciences Corporation, Lindon, UT, as previously described.[20] In preparation for DSC experiments, unmodified and modified-gelatin/WPC (100-150 mg) samples were weighed into ampoules and a small amount of distilled water (500 µL) was added; the ampoules were sealed and placed in the calorimeter. The calorimeter was programmed to record heat flow as µcal/°C while the temperature was increased from 10°C to 180°C at 1.0°C/min with an equilibration period of 600 s at the start. The temperature at the peak of the calorimetry trace, $T_{p,}$ was considered to be an apparent melting temperature.

Optical Microscopy (with Epi-fluorescent Attachment)
Samples of unmodified gelatin/WPC and gelatin/WPC modified with 0%, 4%, and 7% tara, in addition to samples of unmodified and 4% tara- modified gelatin and unmodified and 4% tara- modified WPC, were prepared and then checked for fluorescence using an epi-fluorescent microscope. 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).[21]

RESULTS AND DISCUSSION

In prior research, we investigated the reaction of gelatin and WPC with quebracho, tara, or gallic acid.[10,12] We found that when gelatin was treated with quebracho at approximately 2% of gelatin weight and at pH of 9.5 and temperature of 45°C, gave products whose physicochemical properties (melting point, viscosity and molecular weight) made them amenable to be used as fillers.[10] However we were not successful in modification of WPC to any discernible measure. In our continuing studies[12] when tara was reacted with gelatin it was found that gelatin products were again amenable to be used as fillers, and that tara modified WPC showed significant changes in viscosity and in molecular weight distribution. Both gelatin and WPC showed similar results when reacted with gallic acid. Based on the results with tara, we proceeded to design experiments where we could substitute WPC for gelatin which has recently become increasingly expensive.[14]

In the initial experiments, the WPC concentration remained constant, the gelatin concentration varied from 0 to 10%, and 4% tara, based on the weight of the protein (both gelatin and WPC), was added. The reaction was run at pH of 9.5-10.0, at 45°C, for 4 h. The physical properties were examined (Figure 1) and at the higher concentrations of gelatin, there was an increase in gel strength and melting point, but a decrease in viscosity, a phenomenon we have seen previously when WPC is reacted with polyphenols; this may be attributable to reactivity of β-lactoglobulin with the polyphenols[22].

Molecular weight distribution studies were carried out on these samples in which the WPC remained constant and the gelatin varied (Figure 2). Basically the gels are showing an increase in the intensity of the gelatin bands as the concentration is increased. This is seen more definitely in the control samples to which no tara was added (Figures 2a and c).

In the test samples, (Figures 2b and d), in Figure b, the gelatin bands are not as intense, indicating reaction with the tara, and at the same time, the bands for WPC are less intense. In Figure 2d, the changes are more dramatic, wherein the bands for both the gelatin and WPC have almost disappeared as one approaches the 7% gelatin concentration. These gels are correlating with the viscosity, in that it is lower than the controls, indicating that possibly the β-lactoglobulin, the protein that contributes to viscosity in WPC,[22] has reacted with the tara and the bands have disappeared.

Knowing that 10% WPC, with 4% tara and approximately 7% gelatin will show that the reaction is significantly affecting the viscosity and the molecular weight distribution of the product, we further optimized on the best WPC and gelatin concentration to give a product with desired viscosity (~5.0 to 7.0 cP) for fillers. In the next set of experiments the gelatin

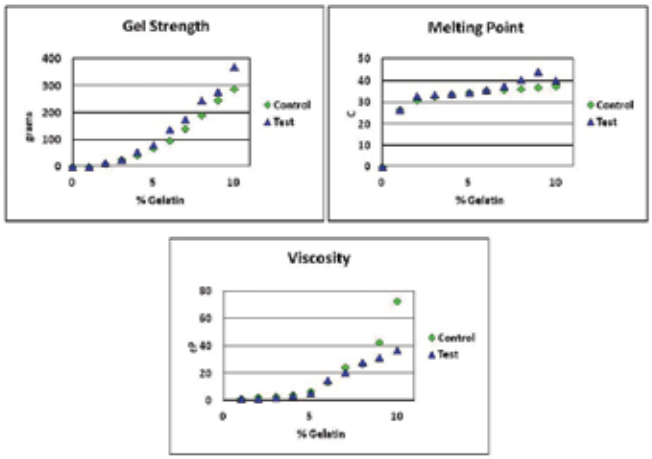


Figure 1. Gel strength, melting point, and viscosity (at 60°C) of WPC (10% w/v) and 175 Bloom gelatin (0-10% w/v) treated with 4% tara at pH 9.5-10.0, 45°C for 4 h.

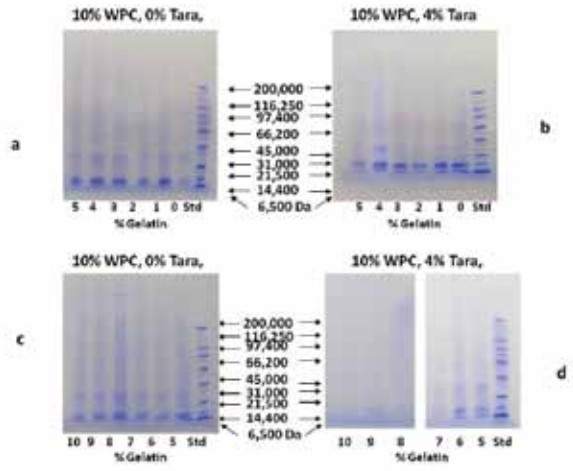


Figure 2. SDS-PAGE of WPC (10% w/v) and 175 Bloom gelatin (0-10% w/v) treated with 0% tara (a and c) and with 4% tara (b and d) at pH 9.5-10.0 at 45°C for 4 h; molecular weights are shown in Da.

concentrations were kept constant at 5, 6, and 7% while the WPC concentrations were varied. The physical properties (Figure 3) indicated that 6% gelatin with approximately 7-8% WPC gave a desired product. The 5% gelatin series did not show any improvements and the 7% gelatin, even though showing more reaction, might be hard to control reproducibly and of course would add to the expense. The molecular weight distribution studies (Figure 4) substantiate these observations in that the 5% gelatin is not showing significant changes until the 9% WPC and the 7% gelatin is demonstrating changes in all WPC concentrations. The 6% gel is indicating a change around the 7% WPC concentration.

The 6% gelatin with 7% WPC concentration was subjected to further studies in order to determine the optimal concentration of tara needed to obtain the desired product. In this series, the tara concentration was varied from 3 to 8% and the physical properties (Figure 5) were determined. In both the gel strength and viscosity we are seeing increases at the 4-5% tara concentration range; interestingly the viscosity falls off as the tara concentration increases, as we have seen previously. The molecular weight distribution (Figure 6) is correlating with the physical property data, in that all the bands after the 5% tara loading are disappearing.

We further investigated the properties of the 6% gelatin with 7% WPC and 3, 4, and 7% tara by analyzing the effect on the hydrothermal stability. In a previous study we looked at tara modified-gelatin and WPC by DSC.

When the 4% tara-modified gelatin (pH 10.0) and its control were examined by DSC (Figure 7), the endothermic or melting point of unmodified gelatin was about 31.9°C and the peak was sharp; the modified gelatin sample gave a broader, less distinct peak at about 31.4°C and an exothermic peak appeared at about 163.1°C.

DSC analyses for tara-modified WPC samples (Figure 8) (control, test A = 1% and test B = 5% tara) show that a peak for the control (WPC) was approximately 25°C and is not seen in the treated samples.

The hydrothermal stability of the biopolymer test products, 6% gelatin with 7% WPC and 3%, 4%, and 7% tara along with the control sample (0% tara) are shown in Figure 9. The endothermic peak is between the reported WPC and gelatin peaks (21°C and 31°C). In the control sample (0% tara) the peak is somewhat distinct. However in the 3, 4, and 7% tara offerings, the peaks for the 3% and 7% loading become more rounded and smaller, while the 4% loading is similar to the control. With respect to the exothermic peaks, at approximately 134°C, 148°C, and 156°C, peaks are seen, similar to those found individually with gelatin and WPC and tara, but not as distinct. These scans are basically showing slight changes in temperature from the scans of the individual tara treatments of

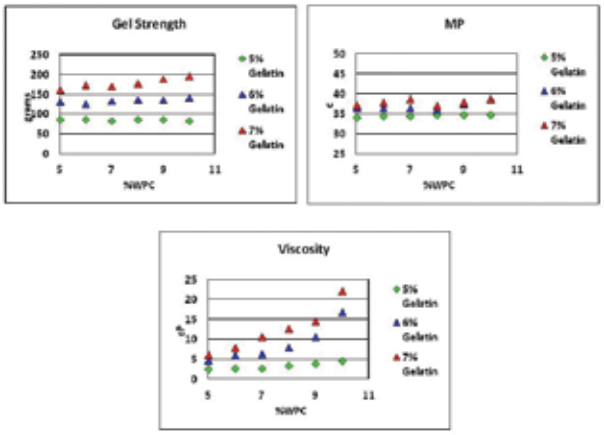


Figure 3. Gel strength, melting point, and viscosity (at 60°C) of WPC (5-10% w/v) and 175 Bloom gelatin (5-7% w/v) treated with 4% tara at pH 9.5-10.0, 45°C for 4 h.

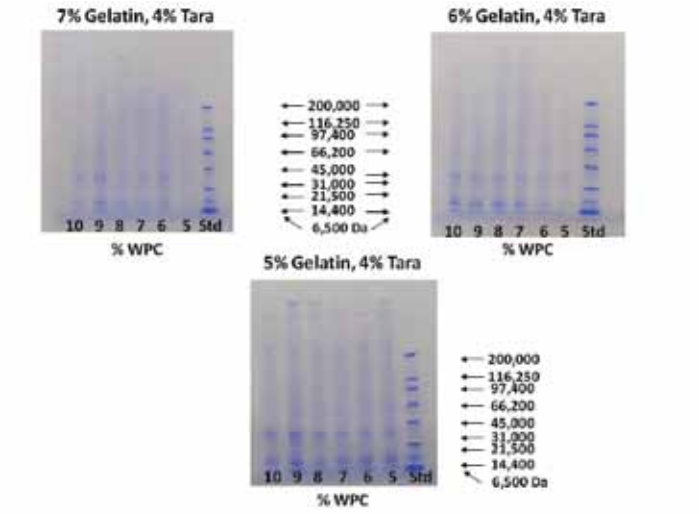


Figure 4. SDS-PAGE of WPC (5-10% w/v) and 175 Bloom gelatin (5% w/v, 6% w/v, and 7% w/v) treated with 4% tara at pH 9.5-10.0 at 45°C for 4 h; molecular weights are shown in Da.

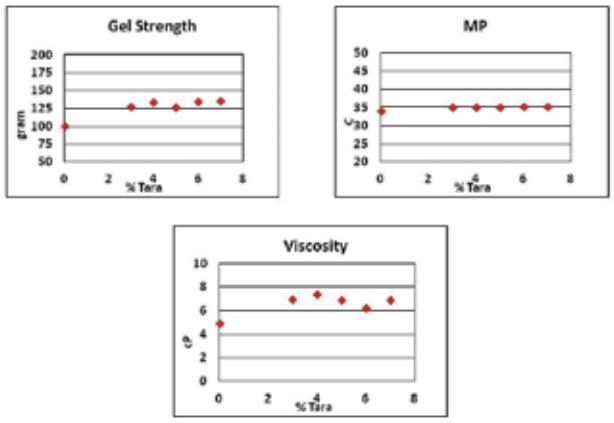


Figure 5. Gel strength, melting point, and viscosity (at 60°C) of WPC (7% w/v) and 175 Bloom gelatin (6% w/v) treated with 3-8% tara at pH 9.5-10.0, 45°C for 4 h.

gelatin and WPC. Modification has taken place and this is substantiated by the physical data, specifically viscosity, and corresponding molecular weight distribution studies. Physicochemical properties similar to those of products made with 10% gelatin and 4% tara were obtained while using only 6% gelatin.[13]

In prior studies[11,13] we found that both quebracho- and tara-treated gelatin products moderately fluoresced; with quebracho products fluorescing a little more intensely than tara products. We examined the biopolymer product to see if it has similar fluorescent properties. Three gel samples of 6% gelatin/7% WPC were prepared; to the control, no tara was added and to the test samples, 4% and 7% tara was added (Figure 10). The samples, run at pH 9.5-10.0, 45°C, for 4h, were examined using an epi-fluorescent microscope. The images (Figure 10) indicate that the tara-treated gelatin/WPC products have emissions between 515-555 nm (green) and 600-660 nm (red).

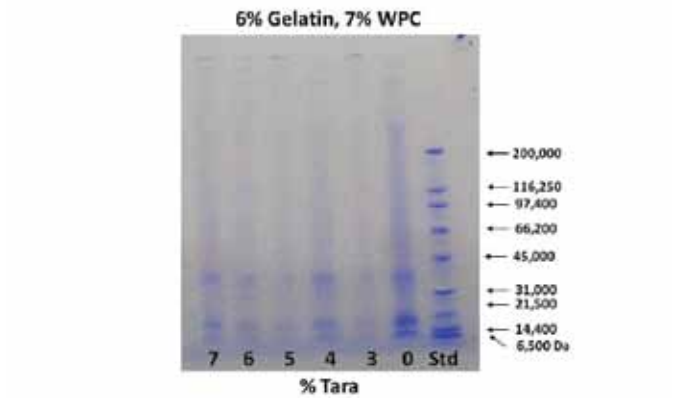
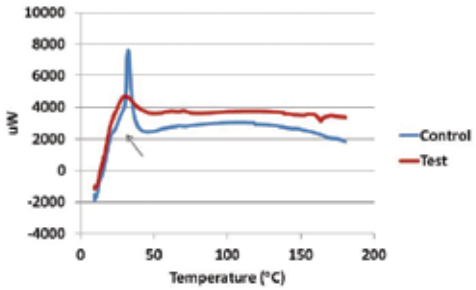


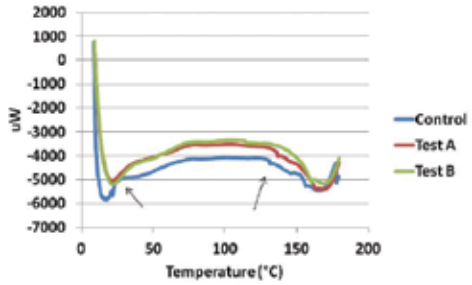
Figure 6. SDS-PAGE of WPC (7% w/v) and 175 Bloom gelatin (6% w/v) treated with 3-8% tara at pH 9.5-10.0 at 45°C for 4 h; molecular weights are shown in Da.



Sample	Endothermic Peak (°C)	Exothermic Peak (°C)
10% Gelatin/0% tara (Control)	31.9	-
10% Gelatin/4% tara (Test)	31.4	163.1

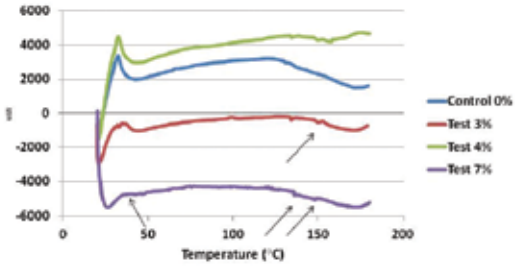
Figure 7. DSC analysis of 175 Bloom gelatin (10% w/v) treated with 0% tara at pH 10.0 (control), and with 4% tara at pH 10 (test), 45°C for 4h; table indicates melting (endothermic) and exothermic temperature peaks.

It also should be noted that, individually, gelatin and WPC, treated with 0% and with 4% tara, have some emission at these wavelengths with treated gelatin fluorescing a little more intensely at 515-555 nm and 600-660 nm and treated WPC at 515-550 nm (Figure 11). When 6% gelatin and 7% WPC are combined (Figure 10), the control sample (0% tara) is indicating less intense fluorescence than the treated samples (4 and 7% tara), but the control sample is showing a little more intensity in the 600-660 nm range than is seen in individual untreated and treated sample images. Furthermore it appears, in the treated combined sample, that the fluorescence is more intense in the 600-660 nm range, with highest amount of fluorescence seen in the sample treated with 7% tara (Figure 10). Thus, the combination of the two proteins treated with tara gives fluorescence properties superior to individual treated and untreated samples. This property will enable one to track distribution of the biopolymer if it is used as a filler.



Sample	Endothermic Peak (°C)	Exothermic Peak (°C)
10% WPC/0% Tara (Control)	21.9	133.2
10% WPC/1% Tara (Test A)	-	138.9
10% WPC/5% Tara (Test B)	-	-

Figure 8. DSC analysis of WPC (10% w/v), treated with 0, 1, and 5% tara at pH 9.0, 45°C for 4h.



Sample	Endothermic Peak (°C)	Exothermic Peak (°C)
6%Gel/7%WPC/0% Tara (Control)	29.0	-
6%Gel/7%WPC/3% Tara (Test)	35.0	99.5, 133.8, 147.0
6%Gel/7%WPC/4% Tara (Test)	32.3	134.0, 148.8, 156.3
6%Gel/7%WPC/7% Tara (Test)I	35.9	135.8, 147.7

Figure 9. DSC analysis of WPC (7% w/v) and 175 Bloom gelatin (6% w/v) treated with 0, 3, 4, 7% tara, pH 9.5-10.0 at 45°C for 4 h.

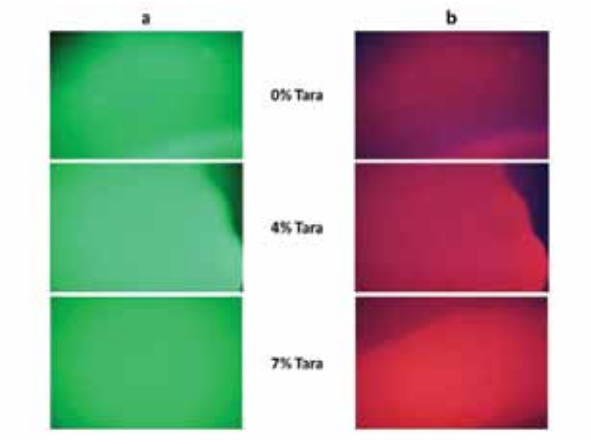


Figure 10. Epi-fluorescent micrographs of 6% gelatin/7% WPC gel treated with 0% tara (control) 4% tara (test) and 7% tara (test), pH 9.5-10.0 at 45°C for 4 h; two emission (barrier) filters, (a) between 515-555 nm (green) and (b) between 600-660 nm (red) were used.

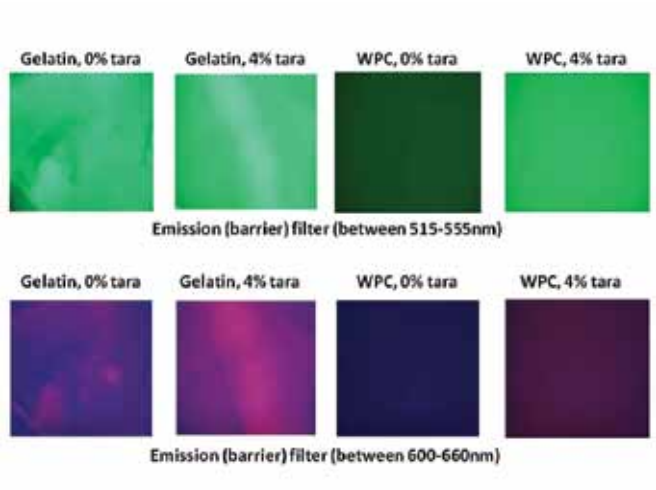


Figure 11. Epi-fluorescent micrographs of gelatin and WPC samples, treated with 0 and 4% tara (pH 9.5-10.0 at 45°C for 4 h); two emission (barrier) filters, between 515-555 nm (green) and 600-660 nm (red) were used.

CONCLUSIONS

Varying combinations of gelatin/WPC were treated with different concentrations of tara, resulting in changes to the physicochemical properties. When approximately 6% gelatin/7% WPC was modified with 4% tara, physical properties and molecular weight distribution studies indicated that a product with similar properties to that produced from 10% gelatin/4% tara was attained. The hydrothermal stability scans of the biopolymer showed endothermic peaks between 22°C and 32°C, a range near those of the individual proteins modified with tara, as well as small exothermic peaks (from 130°C-156°C) also similar to individual scans. The samples had fluorescent properties superior to tara-treated individual gelatin and WPC samples, and these properties became more intense as the concentration of tara was increased. The fluorescent property would be advantageous for observing the distribution of fillers in treated leather. These biopolymer products, produced from renewable resources, using a lesser amount of gelatin in conjunction with a whey product, would assist in reducing cost in view of the fact that the supply of gelatin has become limited and, as a result, is increasingly more expensive.

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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. 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.

9. Peña, C., de la Caba, K., Eceiza, A., Ruseckaite, R., Mondragon, I.; Enhancing water repellence and mechanical properties of gelatin films by tannin addition. *Bioresource Technology* **101,** 6836-6842, 2010.
10. 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.
11. 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.
12. 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.
13. 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.
14. http://www.ft.com/intl/cms/s/0/1f01d088-4900-11e1-954a-00144feabdc0.html#axzz1xsCCKblO
15. Zhang, Z.-Q., Cheol-Ho Pan, 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.
16. Mathew, S., and Abraham, T.E.; Characterisation of ferulic acid incorporated starch–chitosan blend films. *Food Hydrocolloids* **22**: 826-835, 2008.
17. Jones, O. G., Decker, E.A., and McClements, D.J.; Formation of biopolymer particles by thermal treatment of ß-lactoglobulin–pectin complexes. *Food Hydrocolloids* **23**, 1312-1321, 2009.
18. 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.
19. 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.
20. Ding, K., Taylor, M. M., and Brown, E. M.; Effect of genipin on the thermal stability of hide powder. *JALCA* **101**, 362-367, 2006.
21. http://www.microscopyu.com/articles/fluorescence/filtercubes/filterindex.html
22. Andersen, J. G., Ipsen, R., and Karlsson, A.O.; Relative influence of a-lactalbumin and b-lactoglobulin on the viscosity of whey protein solutions. *Annual Transactions of the Nordic Rheology Society* **16**, 175-178, 2008.