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Characteristics of gelatin from pig skin waste; effect of different organic acids used on pre-thermal treatment

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Abstract

Upcycled products prevent food waste and contribute to a sustainable solution to obtain novel by-products. This research paper presents the properties and characteristics of gelatin obtained from pig skin waste using different organic acids solutions as a pretreatment that have been studied. The pH, hydroxyproline content (Hyp), the thermal properties and the color of the product were evaluated. All acids used are food-grade acids. There were differences the gelatin yield showed among the food-grade acid solutions pretreatment, Acetic (AH), Latic (AL), Citric (AC) and Ascorbic acid (AA) (0.5M). AH and AA treatments resulted in higher gelatin Hyp content than AL and AC. The pH of each gelatin solution was 3.46, 3.10, 2.76 and 2.30 for AH, AA, AL and AC, respectively. The AH showed two gel-sol thermal transitions at 57.8°C and 127.5°C while treatments AL, AC, and AA showed only one thermal transition at 122.9°C, 121.4°C and 97.2°C, respectively. The DSC enthalpies energies result in differences between gelatin-films. The acid pretreatment affected the brightness of the gelatin-films and the color parameters. The lowest lightness resulted from AA, and the highest a* and b* color parameters were observed in the AC gelatin-film. The results showed that the use of food-grade acids to obtain gelatin from waste pig skin is promising. Moreover, a good Hyp content can be obtained from all such treatments. The food-grade acids used affect the gelatin thermal, gelation and color properties. The results suggest that the acid pretreatment causes substantive changes in protein functionality.

Statement Of Novelty

Meat industry waste valorisation opportunities are presenting relevant interest nowadays. Gelatin is a recognized biopolymer for its major property, form a three-dimensional gel. However, new properties for novel applications are presenting big interest. These are obtained by manufacturing procedure modifications. Traditionally, it uses acid in processing of waste-pigskin to obtain gelatin. Nevertheless, there are no studies on the effects of different food-grade acids applied to the manufacturing process of gelatin from pigskin. Also, these have antioxidant or antimicrobial functions, among others, that could improve the final products. This work evaluated the effects of different food-grade-acids in the extraction of pigskin-gelatin on their chemical, physical and thermal properties, in order to find new or different properties that broaden their potential applications.

1. Intruduction

The principal sources of gelatin are skin, bones and tendons, which are the main slaughter waste, especially from pigs and bovines. These meat-processing industry losses and wastes are rich in collagen proteins, a source of collagens and gelatins –their derivative product–, all of them used in numerous industries and processes.

Collagen Type-I is the type of collagen that animals naturally produce the most (90%). This polymer is formed by two α-1 chains and one α-2 chain [1]. The gelatin is a polypeptide product of the thermal denaturation-disintegration of collagen fibres. The process to convert insoluble collagen in soluble gelatin

requires a treatment to destroy the tertiary, secondary and partially primary structure by breaking noncovalent bonds [2]. According to Ockerman and Hansen [3] the noncovalent bonds disruption could cleaver inter- and intramolecular covalent crosslinks, without cleavage of any peptide bonds. This hydrolysis allows to convert collagen molecules (molecular weight ≈ 345,000-360,000) into gelatin molecules (molecular weight ≈ 10,000–65,000). The molecular weights and the isoionic points depend on the source of collagen and the process of recovery from collagen [4].

Many industries are interested in gelatin due to its property to form a threedimensional network or gel at concentrations and temperatures conducive to chain entanglement. However, new food implementations, like those used in drinks, require new properties, such as a higher glass transition temperature, soft gels, protein isoelectric point, etc.

The type of pretreatment used for gelatin production is important for the gelatin type to be manufactured, the acid pretreatment allows to obtain gelatin called type A and alkali pretreatment, gelatin called type B. Furthermore, the acid solutions used (concentration, type, time, temperature, etc.) have an effect on the noncovalent bonds disruption and inter-and intra-molecular covalent crosslinks cleavage [5]. The pretreatment mainly used for pig skin waste is acid pretreatment because these sources have a low concentration of intra- and intermolecular crosslinks [2].

Traditional gelatin industries use acid processing for pig skin to obtain gelatin. Sompie et al., [6] studied the effect of acetic acid concentration on the chemical and physical properties of pig skin gelatin. Furthermore, Liu, Lin and Chen [5] studied the effect of different organic acid solutions (acetic, citric and lactic) on the collagen content in chicken feet. More recently, Chakka, et al. [7] evaluated the gelatin extraction from chicken feet using different food-grade acids (acetic, citric and lactic acid) at different concentrations. In addition, Kanwate and Kudre [8] investigated the effect of various organic acid solutions (acetic, propionic and phosphoric) on the physicochemical properties of gelatin obtained from fish. Considering such prior research, this study has evaluated the effects of several food-grade acids on the extraction of gelatin from pig skin. The advantage of studying different acid solutions treatments is the possibility of finding new or different properties and/or a suitable of their gelatin by-products.

Even now, no reports or research results have been found on the organic foodgrade acids used in the process to obtain gelatin from pig skin. These acids also have other functions such as antioxidants or antimicrobials, among others, that could improve the final products.

The purpose of this study is to investigate how (food-grade) acid pretreatment could affect pig skin gelatin properties. To that end, chemical, physical and thermal assays were performed on the gelatin obtained.

2. Material And Methods

Material

The frozen skin from the pig carcass was the object of this study. Before treatment, the pig skin –free from fat– and ears (approximately 1x1cm) were washed with water (1:5 m:V; g:ml) during 2 hours at room temperature for globular protein extraction. Afterwards, the pig skin was filtered and, once clean, it was stored at 18°C.

Acid and Thermal treatments

The pig skin samples were treated with 0.5 M of different food-grade acid solutions –Acetic acid (AH), Lactic acid (AL), Citric Acid (AC) and Ascorbic acid (AA)–. The pig skins (wet base [w.b.]) were soaked in each acid solution with ration 1:10 (m:v; g:ml) during 24 h at 4-8°C with stirring. After the acid treatment, each soluble fraction (acid-soluble collagen) was filtered and neutralized to pH 5.0-5.5 with NaOH cc. and the acid-soluble collagen was precipitated at 4°C during 24 h The samples were centrifuged at 10,000 rpm during 10 mins. (Jouan®, BR 4i Centrifuge, Saint Herblain, France) and the neutralized liquid fractions were removed and thrown away. The acid insoluble pig skin was suspended in water (1:10 m:v) and heated at 85°C-90°C during 90 mins. (collagen thermal denaturation process). Each thermal-soluble fraction and gelatin solution was filtered. Finally, the insoluble pig skin residues were separated and dried at 37°C during 24 h. All treatments were done three times.

Analysis of pH

The pH of acid solution and gelatin solutions were measured using a pHmeter (HANNA Edge®; HI5222, made in Romania, Woonsocket, RI 02895 USA)

Chemical analysis, Hydroxyproline quantified.

The collagen content was evaluated in acid-soluble fraction, gelatin solution and dry insoluble fraction. The samples were hydrolyzed in HCl (6N) at 110°C for 16 h (m:V; 1:10; g:ml). After hydrolysis, samples were neutralized and the Hydroxyproline (Hyp) concentration was determined by spectrophotometry using the colorimetric method of Bergman and Loxley [9]. Colorimetric measures were assayed twice. The total collagen content was calculated by using a correction factor of 7.55. The values were expressed as mg Hyp and mg Collagen per gram of pig skin wet base (w.b.).

Gelatin Filmmaking procedure

The gelatin solution –obtained after each acid and thermal treatment– was used for the gelatin cast filming procedure. Glycerol was added for plasticization (0.7% p/p). The solution gelatin-glycerol was heated at 60°C with stirring (125 rpm) during 30 mins. Solutions were fractioned (15 ml) on silicone plates. The fractioned systems were dried during 48 h. at 37°C. Finally, the films were peeled from the polystyrene plates and stored in desiccators over blue silica gel (cobalt chloride, indicator) of proven moisture content at 25°C (20.0% HR). The films were used for DSC test.

Differential Scanning Calorimetry (DSC)

The thermal denaturation kinetics of gelatin-films samples were analyzed using a DSC Setaram Evo 131. Samples (~10 mg strips) were dried at 37°C for 24 h. Samples were encapsulated in a small aluminum sample pan. Non-isothermal DSC curves were obtained using heating rates of 10°C min-1, at temperatures ranging between 25°C and 300°C using Argon as sweeping gas. An empty pan was used as reference. After proper baseline correction using a polynomial function, enthalpies (ΔH) and mean transition temperatures (Td) were determined from the curves.

Gelatin Films color

The color of each gelatin film was assessed. The sample was placed on a white tile and CIE color space coordinates L* a* b* values were acquired three times, using a Minolta Chroma meter CR-400 (Minolta Co. Ltd., Osaka, Japan) with illuminate D65 and α:2° observer angle.

Statistical analysis

All experiments were performed at least three times. The results are reported as standard deviation (SD) and standard error of the mean (SEM). Comparisons among the results of each treatment were performed by one-way ANOVA with Post-hoc Tuckey´s post-test (α 0.05). A statistical analysis was carried out using Graph-Pad Prism version 5.00 for Windows, Graph-Pad Software, San Diego, California USA http://www.graphpad.com

3. Results And Discussion

pH and gel characteristics

The pH and pKa values of acid solutions are shown in **Table-1**. According to the analysis, acid pKa´s values using the same concentration for four acid solutions (0.5 M) are similar for AH and AA acid solutions and have a higher pH than AL and AA solutions. It is known that the gelatin process requires procedures that consist of a first pretreatment of the raw material with alkali or acid solution and a second thermal process. During the first step, the polypeptide chains and the cross-linkages are broken (hydrogen bonds are destroyed) and then, during the thermal treatment, denaturation of the collagen (triple helix) protein is produced [10]. According to Choe and Kin [11], for pig and chicken skin, acid processing is the most suitable treatment. Also, the acid process is applied in the industry to obtain gelatin Type A. During the acid treatment, the tissue is swollen and the electrostatic intra and intermolecular collagen interactions are weakened [12].

The four acid solutions –AH, AL, AC, and AA– have similar good swelling in pig skin after 24 h at 4°C. Choe and Kim [11] observed that the optimum swelling times are achieved when the soaking solution has a constant pH (1.68-1.88) during 24 h at 4°C.

Following acid pretreatment, all acid-soluble collagen fractions were separated and neutralized at the same pH (**Table-1**). The neutralized solutions were left during 24 h at 4°C, then they were centrifuged and, finally, the resulting pellets (acid-soluble collagen) residues were separated. Liu et al. [5] worked in the

acid conditions of extracting collagen from chicken feet and found that the percentage of pellet soaked was higher in citric acid and lactic acid than in acetic acid. However, it could be observed that the AC and AL acid-soluble collagen pellets turned out slightly bigger and gummier than AH and AA residues.

After acid pretreatment, the remaining swollen pig skin was suspended in water (1:10 m:v) and was thermal treated at 85°C-90°C during 90 mins. Heating the collagen solution at temperatures higher than 70°C results in an irreversible transition from a helical structure to a disordered structure [13]. Once the thermal treatment was completed, the pH of the gelatin solution showed significant differences (**Table 1**) AC<AL<AA<AH. Part of this difference may be that several acid molecules remain present, collagen helix-acid molecule interactions, after acid pretreatment.

Sompie et al. [6] explained that the gelatin pH is very important for the gelatin properties and determined the application of gelatin. Likewise, according to Kanwate & Kudre, [8] the collagen molecules cleave during the acid treatment, and the cleaves depend on the acid force.

When gelatin solutions with enough concentration of molecule chains (in general ≥ 1% w/v) reach a low temperature, the nucleation of triple helic regions occurs and the helixes overlap, resulting in gel formation [14]. Such results showed that all gelatin solutions contained a protein concentration over 1% w/v (2.3-2.6 g/100 ml, **Table-1**). However, it was observed that after 24 h at 4°C, not all gelatin solutions presented good gelling properties. After 24 h at 4°C, AH and AA turned into gel form (jellified), AL turned into weak gel and AC did not form a gel (cloudy solution). Differences in pH during treatments could affect the longest chain [15]. The hydrolysis that occurs during the acid treatment may produce different cleavages, resulting in differences in the molecular weights of the chain obtained. In addition, the acid could produce various disruptions of the inter-junction zone and collagen networks too, which could result in gels strength variability.

Kaewruang et al. [15] worked on gelatin extracted from unicorn leatherjacket skin and indicated that the gelatin with the lowest hydrolysis was more likely to present the longest chains and that the maintenance of chain length was a prerequisite for a better gelation. Moreover, Koli et al. [16] indicated that the differences in the pH treatment could modify the amphoteric nature and the hydrophobic zones on the peptide chain gelatin, limiting functional protein properties. Besides, the gelatin solution pH could affect the gelatin particle structure. Xu et al. [17] observed that the microstructure of gelatin particles played a key role in gelling properties and that the pH and the Ionic strength affect both gelatin at the particle structure and on a micrometric scale. In this work, the AC and AL gelatin solutions with the lowest pH corresponded to a poor gel formation, while solutions with the highest pH (AH and AA) presented a stable and firm gelation.

It is known that gel strength is a physical property of gelatin that the food industry highly values. Gelatin is usually used as a water-binding, thickening, gelling, foaming, emulsifying and film-forming agent [18]. However, according to Gudmundsson [19] the ability to form weak gels may find new applications as “non-gelling” gelatins and could be possibly used in refrigerated products and in products requiring low gelling temperatures.

Extraction yield and Hydroxyproline content

The yield data on the collagen protein extracted from pig skin after each treatment –acid pretreatment, thermal treatment– and the remain pig skin residues are shown in **Fig 1**. The thermal-soluble collagen (gelatin) product was the main upcycled food product from pig skin on all treatments (AH, AL, AC and AA). Moreover, collagen acid-soluble and acid-thermal resistant collagen remained after all treatments. See et al. [2] indicated that the pretreatment with acid induced some loss of collagenous proteins in the solution, while alkali pretreatment effectively removed non-collagenous proteins.

Even though the pig skin was treated with acid and high temperatures, the content of resistant collagen in pig skin could be a consequence of the higher iminoacid content of mammalian skins. According to Li et al. [20], the collagen thermostability is enhanced by hydroxylation of proline and lysine. Furthermore, the authors [20] indicated that the hydroxyproline is a key factor to stabilize the triple super-helix structure and that the hydroxylysine iminoacid promotes the formation and stabilization of the collagen cross-links.

Table 2 shows the Hyp content (mg Hyp/100 g pig skin [w.b.]) obtained in the acid-soluble, thermal-soluble, and resistant fractions from the different treatments. The acid-soluble fraction Hyp content was significantly different between treatments. AH and AA, showed lower Hyp content than AC and AL treatments. Furthermore, there were differences in the Hyp content in the gelatin fraction. AH and AA treatments showed higher Hyp content than AC and AL treatments. The final residues decreased significantly during the drying process. Part of the final pig skin residue was strongly attached to the filter paper and could not be fully recovered for the Hyp analysis. The Hyp was also quantified and the Hyp content in the obtained pig skin residues is shown in **Table-2**.

The results showed that the AC and AL treatments (with lower pH 1.8-1.9) resulted in higher acid-soluble Hyp content than AH and AA treatments. On the contrary, a low amount of Hyp in the acid-soluble fraction in AH and AA treatments resulted in the highest Hyp content in the thermal soluble fraction (gelatin). Kanwate and Kudre [8] studied the effect of acid on the characteristics of gelatin from fish (*Labeo rohita*).

The authors showed that the properties of the gelatin extracted differed depending on the acid pretreatment used. The gelatin extracted with propionic acid showed higher Hyp content as compared with acetic and phosphoric acid. On the other hand, Lui et al. [5] evaluated the optimum condition of extracting acid-soluble collagen from chicken feet. The authors [5] showed that the content of crude collagen pretreated with acetic acid and lactic acid was significantly higher than when pretreated with citric and hydrochloric acid. The differences observed in the Hyp content for each treatment could be linked to the gelatin stability. According to Sompie et al. [6] hydroxyproline in gelatin stabilizes the hydrogen bonds between free hydroxyl groups and water molecules. Moreover, Kaewruang et al. [15] proposed that the iminoacid (Hyp) determine the gel strength by introducing pyrrolidine rings for bridging between chains, apart from H-bonding.

Chakka et al. [7] studied the extraction of gelatin from chicken feet using different food-grade acids (acetic, citric, and lactic acid) and observed that high gelatin Hyp content results in better bloom strength.

On the other hand, the differences in the pH of gelatin solutions could also affect the gelation characteristics. According to Zandi et al. [21] the isoelectric point of gelatin ranges between 4.8 and 9.4 and the gelatin molecule net charge depends on the solution acidity; and it is probable that the pH value controls the tendency of the gelation process. The authors observed that the influence of amino acid interaction strongly depends on the polarity and the ability to form hydrogen bonds; furthermore, individual amino acids play a specific role during the gelation process. For that reason, any deviation from neutral pH conditions could cause an increase in molecular dynamics and affect the viscosity and/or the gelation process.

Gelatin films Differential Scanning Calorimetry (DSC)

DSC is commonly used to study thermal transitions of proteins, such as gelatin. According to Sobral and Habitante [22] crystalline gelatin presents two transitions: glass and helix-coil. However, gelatin in an aqueous solution presents a polypeptide chain with a considerable lack of internal order and a random configuration, and sol-gel transition occurs. In this study, the thermal transition temperatures were determined by DSC in order to evaluate the effect of acid pretreatments on the gelatin-film (ternary system: gelatin + glycerol + water). Physicochemical properties of edible films are known to be important in evaluating potential applications, the plasticizer concentration required for flexibility, etc. [23]. **Fig 2** shows the DSC curves of gelatin cast films treated with AH, AL, AC, and AA (**Fig-2 a, b, c, and d**, respectively) after conditioning at constant relative humidity (20%) at 25°C. Acetic acid is commonly used in traditional treatments to obtain commercial gelatin type A. The results showed that only the AH treated gelatin film exhibited two thermal transitions. A first thermal transition at 60°C and a second one at 127°C (**Fig 2a**). The remaining samples (treated with AL, AC, AA) showed only one thermal transition between 98°C and 125°C (**Fig 2b, c, d**). Chiellini et al. [24], indicated that gelatin cast film transition temperature at ≈ 60°C could be associated with gelatin rigid blocks composed of sequences mainly made up of the amino acids (proline, hydroxyproline and glycine). The authors observed that gelatin films presented a phase transition at ≈130°C and suggested that this transition correspond to several thermal events such as evaporation, structural reorganization of the rigid blocks, etc. It is known that crystalline quality could affect gel and solid states properties by increasing thermal stability. The history of acid and thermal treatments dominated the properties of the solid state and the crystallinity. These properties were also controlled by the remaining acidic molecules and by using a diluent, a plasticized and water.

The treatments with AL, AC, and AA presented a shift of the endothermic peak to lower temperatures and an increment in the enthalpy of the reaction, in relation to traditional acetic acid (AH) (**Table 3**).

According to Nishinari [25], this fact could be attributed to the following events:

(i) Degree of reaction between acid and proteins and the impact on thermal properties. For example, ascorbic acid-protein reaction products, protein ascorbylation, effect in covalent bonding of AA or

breakdown products [26]. All of these products experience changes such as browning, protein cross-linking, etc. This could affect the thermal properties.

(ii) Acid molecules or newly formed acid-protein products can lead to helix formation and aggregation.

On other hand, Tsereteli and Smirnova [27] studied the properties of the glass transitions in amorphous and crystalline gelatins with different melting heats. The authors observed that, unlike bound water, the free water in gelatins does not act as a plasticizer, but forms a rigid matrix inhibiting the glass transition.

In this study, non-traditional acid pre-treated gelatin films may have a different number of free and bonded water molecules. Unfortunately, this study did not assess water-related activities. This will be studied in detail in future investigations.

Table 3 shows the results of thermal transition temperature and total enthalpies from DSC. The second endothermic peak in the range 98°C-127°C was statistically analyzed and the results showed significant differences between treatments. The AH, AA, and AL presented higher Td than AC while, the DH values were higher for AC and AA > AL and lower for AH (J/g gelatin-film).

It could be thought that the two thermal events present in the AH treated gelatin film occurred in a single thermal event of rigid blocks at 97°C-123°C for other treatments. This thermal behavior could be attributed to the crosslinking of gelatin chains resulting from the effects of acid-protein reactions in AL, AC, and AA gelatin film. Fraga et al. [28] observed a broader temperature range of gelatin and indicated that it could be attributed to the devitrification of blocks rich in iminoacids. It was also observed that in mammalian gelatins rigid blocks prevail.

Moreover, this difference could be associated with changes in hydrogen bounds and hydrophobic bounds. Finch and Ledward [29] suggested that the collagen triple helix structure may be stabilized in both hydrogen bonds and hydrophobic bonds. Rochdi et al. [30] proposed that the hydrogen bond cleavages (endothermic process) decrease both Td and ΔH. However, if hydrophobic cleavage of the bond occurs (exothermic process), ΔH might increase whilst transition temperature might decrease.

On the other hand, Tseretely and Smirnova [27] studied gelatins in which free water did not act as a plasticizer and observed that depending on the type of gelatin (extraction process and/or nature gelatin) the “melting heat” presents a stringer relation with the number of cross-links present in the starting gelatin. Finally, the authors indicated that all gelatins (gel or crystalline state) form metastable collagen-like structures and that the resulting thermodynamic parameters depend on their production conditions.

Furthermore, the results of the acid-protein interaction could affect the thermal properties. Xu et al. [17] studied the use of citric acid to cross-link wheat-derived gliadin during 4 h at low temperatures (50°C-75°C). The study indicated that Citric acid is a tricarboxylic acid, and when more than one carboxyl group in the citric acid molecule participates in the reaction, protein inter- or intra-molecular cross-linking could occur. In addition, the effects of citric acid-mediated cross-linking under non-acidic conditions on

the surface hydrophobicity, on the solubility and on several properties of protein were studied by Li et al., [31].The authors observed that the cross-linking mediated by citric acid under non-acidic aqueous conditions produced changes in hydrophobicity and enhanced properties. Pischetsrieder, et al. [26] studied the reactions and the products formed by AA and proteins. The results showed that the protein ascorbylation, the covalent binding of AA or its degradation products produced various changes such as browning, protein cross-linking, etc.

The results of this study suggest that the solute-acid and the solution-pH conditions might affect the pig gelatin hydrogen and hydrophobic bonds. Therefore, different quantities of protein-protein and protein-water interactions could be present in each studied gelatin-film.

More studies will be required to understand the molecular interactions. Tests like DSC at different gelatin water activity (aw), FTIR, DMA, etc. on powders and /or film gelatins could allow a better understanding.

Gelatin-Films color

Gelatin color has proven to influence acceptability and food application. The color of gelatin films obtained from pig skin with different acid pretreatment is shown in **Table-4**. Results demonstrated that the different pretreatments used to obtain gelatin affected ($p < 0.05$) the film´s values of lightness (L*), redness (a*) and yellowness (b*). The AH-gelatin film presented the highest L* value and it was similar to the value of commercial gelatin. The AA-gelatin film resulted in the lowest L*value and the highest browning (<b*). During the thermal process, multiple reactions AA-proteins occur [26] and the Maillard reaction products reduce lightness. The L* values of AL and AC-gelatin films were lower as compared with AH treatment and higher than AA treatment. Moreover, significant differences between treatments were found in red (a*) and (b*) yellow hues. The AC-film a* value was higher as compared with gelatin films obtained by AL<AA< AH acids-treatment. However, the highest values of yellow hue, analyzed by b* parameter, were observed on AC-films and AA gelatin-films. AH-gelatin resulted to be the most similar one to a commercial sample. Both the colour hue (a* and b*) and the clarity (L*) of a gelatin are important aesthetic properties and affect its application. The results suggest that this effect could be perceived as commercially negative. However, the effect of these changes should be evaluated on the products per se.

4. Conclusion

The gelatin derived from pig skin is normally referred to as gelatin type A. This is obtained from acid-treated collagen and presents an isoelectric point of 6-9. The pH of all gelatin solutions pH was far from the gelatin isoelectric point, which would indicate that many proteins-water interactions and few protein-protein interactions occur. However, these results showed that AH and AA treatment (gelatin solution pH >3; 3.6 and 3.2, respectively) presented good gelation. This property diminished as pH decreased (AL pH 2.8) and was not present in AC treatment (pH=2.4). This could be because the protein net charge conditions lead to a reduction of the intra- and inter-molecular hydrogen bonds (proteins-water interaction) and a reduced gelation property.

There were significant differences in the Hyp content showed between treatments. AH and AA treated acid-soluble fractions showed lower Hyp content than AC and AL treated fractions. Moreover, there were differences in the Hyp content in the gelatin fraction. The Hyp content in AH and AA treatments was higher than in AC and AL treatments. There is a direct relation between the iminoacid (Hyp) present in the gelatin solution and the gelation characteristics.

The DSC results showed that the acid pretreatment modified the thermal transition characteristics of the gelatin films. The pretreatment with different acids could affect the remaining number of hydrophobic and hydrogen bonds present in the gelatin. These differences in the thermal stability of the gelatins may due to the different number of free and bound water molecules.

Gelatin-film color was affected by the different acid pretreatments, being AH-treated gelatin the one that resembled the commercial sample analyzed in this work the most.

One of the main aims of the developing modifications of the gelatin is to widen the field of their application. Obtaining different gelatin characteristics by different acid-food grade pretreatment conditions could be an option for the rendering industry. Furthermore, its nearness to new industry requirements could generate interest from food, biomedical or biomaterials industries. However, more studies are required to understand chemical differences between the gelatins obtained and to find new functions and/or applications.

Declarations

Declaration of Competing Interest

The authors report no declarations of interest.

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Tables

Table-1 Solution pH values, acid dissociation constants at 25°C and Gelatin solution concentration.

	AH	AL	AC	AA
pKa	4.75	3.86	3.08	4.01
pH Value*				
Acid Solution (0.5M)	2.55 ± 0.07	1.98 ± 0.04	1.70 ± 0.14	2.39 ± 0.13
Collagen Acid-Solution	5.40 ± 0.25	5.35 ± 0.30	5.25 ± 0.13	5.25 ± 0.22
Gelatin Solution	3.55 ± 0.15^{a}	2.80 ± 0.2^{bc}	2.35 ± 0.10^{c}	3.20 ± 0.25^{a}
g Gelatin**/100 ml	2.59 ± 0.21(0.10)	2.27 ± 0.12(0.06)	2.66 ± 0.06(0.03)	2.26 ± 0.57(0.28)

*Value are given as mean ± standard deviation (n=3). The different letters in the same column indicate significant differences (P<0.05) ANOVA One-way (Tukey's Multiple Comparison Test).

** Gelatin correspond to Hyp content (g/100 ml) x Conversion Factor (7.55). Value are given as mean ± standard deviation and (SE) (n=3).

Table 2- Hydroxyproline content (mg Hyp/ g pig skin wet base [w.b.]). Soluble fractions; acid-soluble collagen; thermal-soluble fraction (gelatin solution) and residual pellet (insoluble remained fraction). Raw pig skin monster content was 43.2%

Treatment	mg Hyp acid-soluble/ g Pig skin (w.b)*	mg Hyp gelatin solution/ g Pig skin (w.b)*	mgHyp insoluble remain fraction/ g Pig skin (w.b.)*
AH	0.48 ± 0.07(0.04)[a]	41.40 ± 5.19 (2.32)[a]	1.47 ± 0.02 (0.01)[a]
AL	0.91 ± 0.02(0.14)[b]	34.44 ± 2.73 (1.22)[b]	0.31 ± 0.02 (0.01)[b]
AC	0.82 ± 0.01(0.10)[b]	33.75 ± 2.62 (1.17)[b]	1.66 ± 0.15 (0.11)[a]
AA	0.48 ± 0.15(0.09)[a]	40.37 ± 2.57 (1.49)[a]	0.52 ± 0.04 (0.03)[b]
p-Value	0.005	0.0094	0.0002

*Value are given as mean ± standard deviation and (SE) (n=3). The different letters in the same column indicate significant differences (P<0.05) ANOVA One-way (Tukey's Multiple Comparison Test).

Table 3-Thermal gelatin-films properties after different acid pretreatments (J/g gelatin film) = total denaturation energy expressed as J per g Gelatin film (mean ± standard error, n = 3 for all points). Thermal transition temperature (Tt) is mean temperature (◦C) of the endothermic peak in DSC curves.*

	Tt_1 (°C)	ΔH_1 (J/g)	Tt_2 (°C)	ΔH_2 (J/g)
AH	57.8 ± 1.4(0.8)	123.1 ± 0.3(0.1)	127.5 ± 6.4 (3.7)[a]	76.8 ± 26.8 (15.5)[a]
AL			121.4 ± 1.8 (1.3)[a]	169.2 ± 9.1 (6.4)[b]
AC			97.2 ± 3.5 (2.5)[b]	244.2 ± 12.0 (8.5)[c]
AA			122.9 ± 0.5 (0.4)[a]	298.7 ± 13.4 (9.5)[c]

*Value are given as mean ± standard deviation and (SE) (n=3). The different letters in the same column indicate significant differences (P<0.05) ANOVA One-way (Tukey's Multiple Comparison Test).

Table 4- Gelatin films COLOR CIELab.

Acid Treatment**	g Gelatin* /100ml	L*	a*	b*
AH	2.59±0.21(0.10)	83.68 ± 1.09 (0.03)[a]	-0.83±0.05(0.03)[a]	7.40 ± 0.41 (0.24)[a]
AL	2.27±0.12(0.06)	69.52 ± 2.92 (1.69)[b]	4.18 ± 1.54 (0.89)[b]	25.01±3.07 (1.77)[b]
AC	2.66±0.06(0.03)	64.93 ± 0.80 (0.46)[c]	9.86 ± 1.36 (0.79)[c]	39.01 ± 2.52 (1.46)[c]
AA	2.26±0.57(0.28)	35.86 ± 1.16 (0.82)[d]	0.90 ± 0.21 (0.15)[d]	-0.30 ± 0.04 (0.03)[e]
Comercial Gelatin	2.50	80.59 ± 0.65 (0.38)	-1.13 ± 0.08 (0.04)	14.19 ± 0.79 (0.46)

**Value are given as mean ± standard deviation and (SE) (n=3). The different letters in the same column indicate significant differences (P<0.05) ANOVA One-way (Tukey's Multiple Comparison Test).

Figures

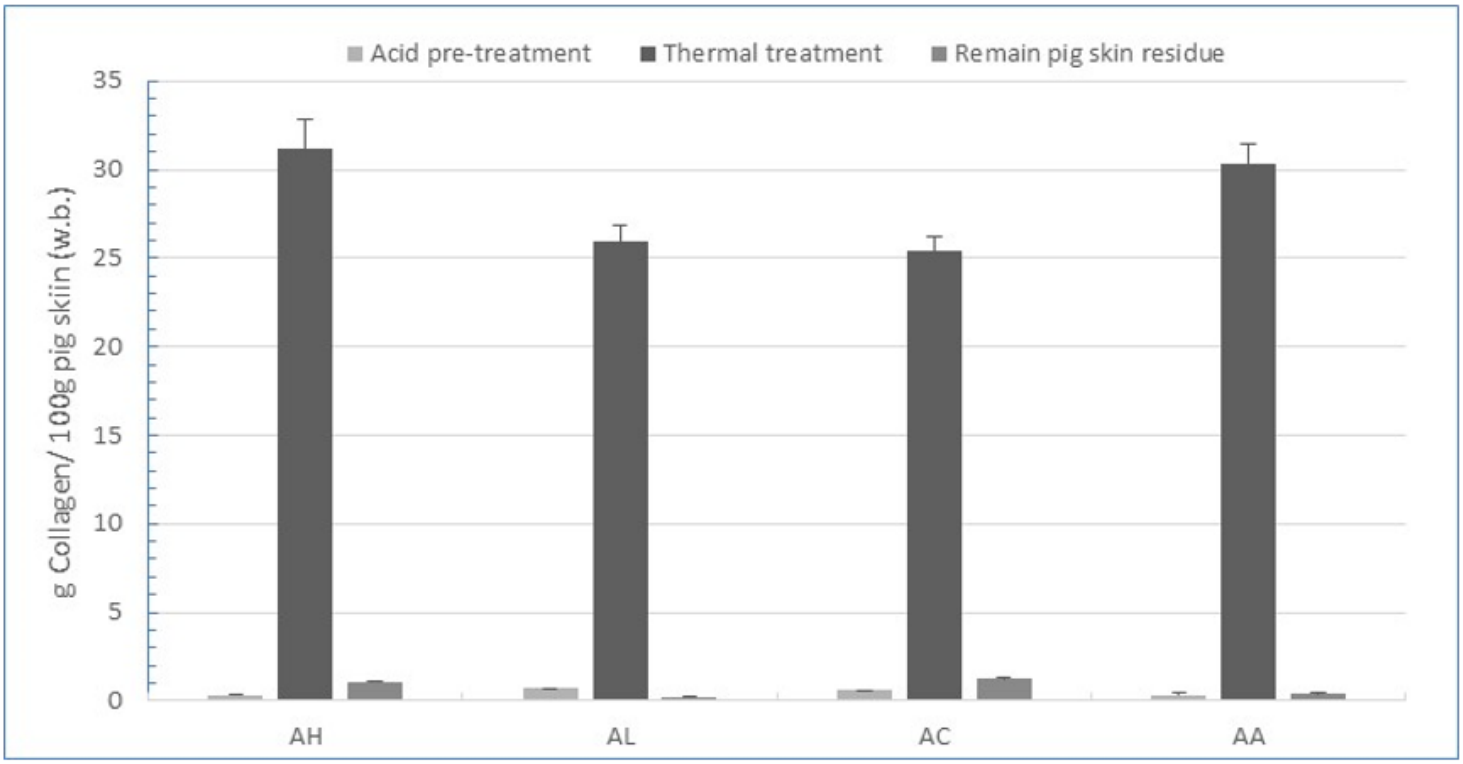

Figure 1

Yield of collagen extracted from pig skin after acid pretreatment; thermal treatment; remain pig skin residue.

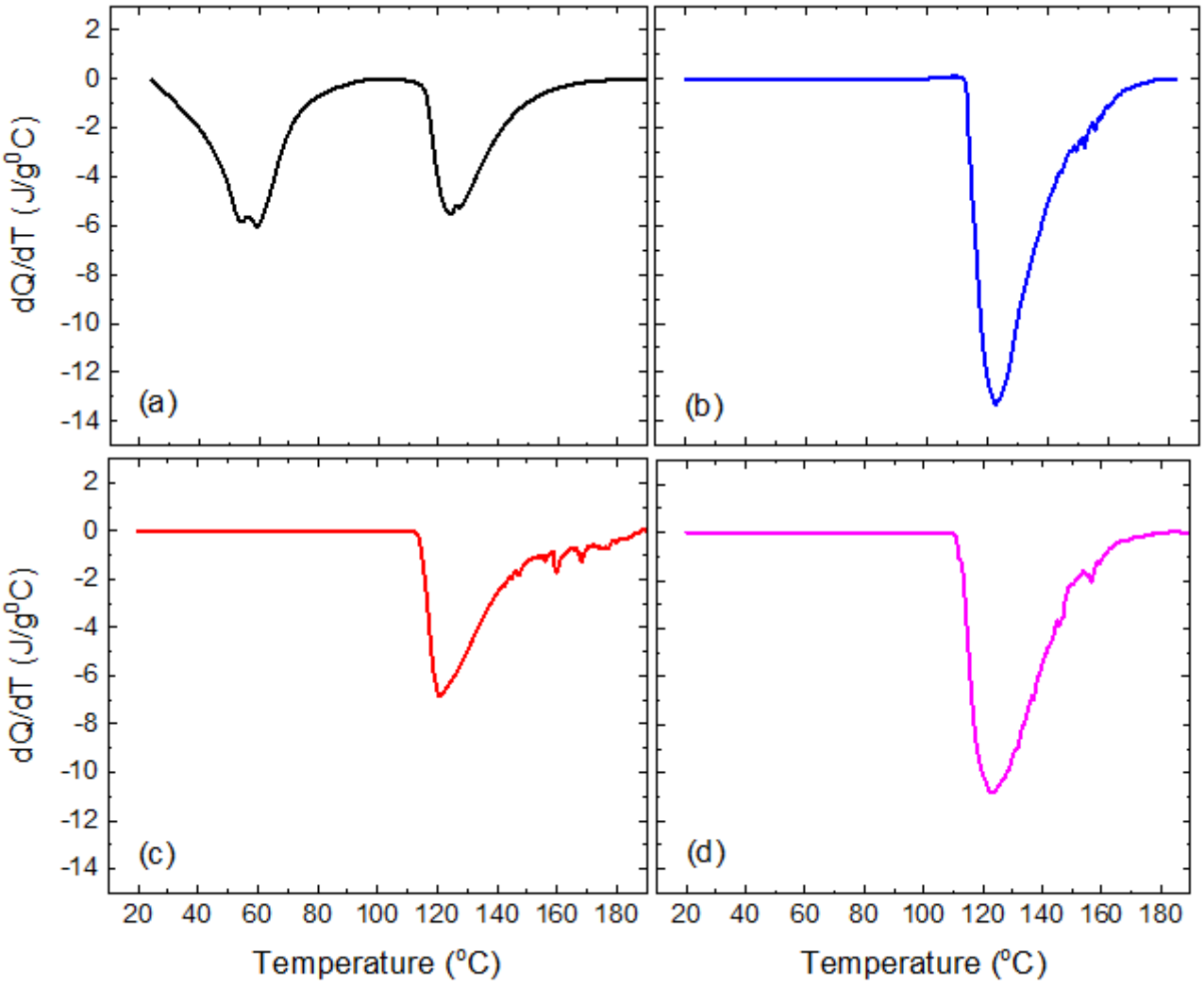

Figure 2

Normalized DSC curves recorded on heating at 10 ∘C min^{-1} on gelatin-films. Parameters calculated from the curves are shown in Table 3. (a) AH; (b)AL; (c) AC; (d)AA treatment, respectively).

Supplementary Files

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