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Pigskin Treatment Using Different Food-Grade-Acids: Effects on The Physicochemical Characteristics of The By-Products

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ABSTRACT

Upcycling foods contributes to reducing food loss and waste and provides sustainable solutions to novel products. In the present work, it was studied the use of food-grade acids (Acetic(AH), Latic(AL), Citric(AC), and Ascorbic(AA) acid) to obtain pigskin by-products, acid-soluble collagen (ASC), and gelatin (G). The aim was to evaluate the effect of the use of different food-grade acids on pigskin by-product characteristics. The physicochemical and thermal features, including Hydroxyproline (Hyp), pH, Differential Scanning Calorimetry (DSC), and color, were evaluated on by-products. The ASC and G solutions pH´s showed relation with the acid solution pH used. The AH and AA ASC fractions, showed lower Hyp content than AC and AL-treatments. By contrast, G Hyp-content was higher for AH and AA than AC and AL-treatments. The dried ASC-AH and -AA thermal transition temperatures (Td) resulted lower than AL and AC. The four dried-G samples showed an endothermic signal around 120 °C but with differences on enthalpy values. Current results suggest that the acid used and the pH of the solution during the thermal process would affect the physical-chemical properties of the by-products. The possibility to obtain different pigskin by-products using food grade acid could be an option for obtaining novel ASC and G use. Independently of the treatment, the G by-product was the main yield. Likewise, further studies are required to understand the by-products chemical differences and their potential uses.

Keywords: Biopolymers, DSC, Upcycling food products, Loss and Waste Food

INTRODUCTION

The principal sources of collagen 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 by-products–, all of them used in numerous industries and processes.

Collagen Type-I is the type of collagen that animals mostly produce naturally (90%). Its most prominent functional roles in the skin and bone, and to a lesser of other tissues. This polymer is formed by two α-1 chains and one α-2 chain, which are joined into a triple helix, heterotrimeric α1(I)2–α2(I) (Amirrah, et al., 2022). The gelatin is a polypeptide product of the hydrolysis and 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 non-covalent bonds (See et al., 2015). It is known that the non-covalent bonds disruption could cleave inter- and intra-molecular covalent crosslinks, without cleavage of any peptide bonds. This hydrolysis allows the conversion of collagen molecules (molecular weight ≈345,000-360,000) into small molecules (molecular weight ≈10,000-65,000).

The main methods used for collagen extraction are acid, enzymatic or alkali hydrolysis at low temperature (~4 °C) Matinong et al. (2022). The tissue treated in low concentration-acid allows the destabilization of the salt bonds between molecules and Schiff bases and causes collagen fibres to expand and dissolve Hua and Zibin (2014). Acetic, lactic, or citric acids (0.2-0.5M) have been used to study native collagen extractions of several tissues. For example, Liu et al. (2001) studied the effect of different organic acid solutions (acetic, citric and lactic) on the native collagen obtained from chicken feet. Kanwate &Kudre (2017) evaluated the influences of various acids (acetic, phosphoric, and propionic acids) used for gelatin extraction from the fish *Labeo rohita*, and observed that the acid affected the gelatin yields, the triple-helix loss and gelatin pH solubility. Moreover, Sompie et al. (2015) studied the influences of acetic acid concentration and extraction temperature on pigskin gelatin physical and chemical characteristics. The authors observed that the optimal gel strength, viscosity, protein concentration and pH of the gelatin solution were obtained using 4% acetic acid. More recently, Chakka et al. (2017) evaluated the gelatin extraction from chicken feet using different food-grade acids (acetic, citric and lactic acids) at different concentrations.

Nowadays, collagen and gelatin by-products present interest in food, pharmacology, cosmetic industries, and tissue bioengineering. Today, the by-products collagen and derived-peptide are considered as important components of innovative sustainable food systems. These animal by-products show novel impacts on the food system, processing, packaging, preservation, and functional foods (Changwei et al., 2021). Furthermore, several industries are interested in gelatin due to its property to form a three-dimensional network or gel at concentrations and temperatures conducive to chain entanglement. In addition, the new food implementations, like those used in bread, yogurts, drinks, etc., require new properties, such as a higher glass transition temperature, soft gels, etc. The yields and characteristics of the obtained by-products collagen and gelatin depend on the tissue source, extraction methods, and the extraction process conditions.

The steps to convert collagen to gelatin require hydrolysis as a necessary first step, following thermal denaturation. The denaturation temperature of pigskin collagen is ~65-67 °C (Li et al., 2020). The hydrolysis determines the gelatin types, the acid treatment allows to obtain Type-A gelatin, the alkali treatment, the Type-B gelatin. The acid treatment is widely used because the isoelectric point of range of gelatin type A is

pH 6-9, so presents a wide range of food, pharmaceutical and industrial uses Mariod, et al., 2013). The acid solutions used (concentration, time, temperature, etc.) have an effect on the non-covalent bond disruption and inter-and intra-molecular covalent crosslinks cleavage (Liu et al., 2001). The importance of this step is "opening up" the protein structure, breaking the intra- and intermolecular crosslinks (See et al., 2015), which is necessary for the following thermal collagen denaturation to obtain gelatin.

Thus, this research aimed to investigate the effect of the use of different food-grade acids to obtain pigskin by-products: i-acid soluble collagen and ii-gelatin. The by-products chemical and thermal characteristics were evaluated. Knowledge of the properties of by-products would allow assessment of potential uses in different and/or new applications.

MATERIALS AND METHODS

Material

The frozen skin from the pig carcass, was provided by slaughterhouse for pig habilitated. This was the object of this study. Before treatment, the pigskin –free from fat– and ears (approximately 5x5 mm) were washed with water (1:5 m:V; g:ml) during 2 hours at room temperature for globular protein extraction. Afterwards, the pigskin was filtered and, once clean, it was stored at 18 °C.

The pigskin tissue composition was analysed. Moisture content was determined using gravimetric method (drying in oven 80 °C during 48 h). Total Nitrogen by Kjeldahl method was performed according to method 992.15 of the AOAC International (2012), amount of total nitrogen in the raw materials were multiplied by a conversion factor of 6.25. Lipid content was evaluated using hexane:isopropanol extraction (3:2; v:v) (Saini et al., 2021) . Ash content was quantified as total dry matter residue obtained by burning in muffle at 550 ± 10 °C (20 h).

Acid and Thermal treatments

The pigskin frozen samples were treated with different food-grade acid solutions (0.5M) –Acetic acid (AH), Lactic Acid (AL), Citric Acid (AC) and Ascorbic Acid (AA)–. Figure 1 outlines the overall process employed for obtaining the by-products from the pigskin. The frozen tissues (wet base [w.b.]) were soaked in each acid solution at a ratio 1:5 (m:v; g:ml) during 24 h at 4-8 °C, with stirring. Acid soluble collagen (ASC) obtained after the acid treatment was filtered and separated. The liquid ASC was precipitated with NaCl at 4 °C during 48 h. ASC was centrifuged at 5,000 rpm during 10 min (Jouan®, BR 4i Centrifuge, Saint Herblain, France) and the pellet was separated and dried at 37°C 24 h. The swelling pigskin acid insoluble fraction (solid residue) was re-suspended in water (1:5 m:v) and heated at 85-90 °C during 90min (collagen thermal denaturation process) with the aim of obtaining gelatin (G) solutions. Each G solution was filtered at 45 °C and separated from the thermal insoluble fraction. Glycerol was added in each G solution (0.8% Glycerol w/v), previously to the drying process. The solution G-glycerol was heated at 60 °C with stirring (125 rpm) during 30 min. Finally, each solution was fractioned (15 ml) on silicone plates and dried during 48 h at 37 °C. The dried samples (dried G in Fig.1) were stored in desiccators 7 days at 25 °C with blue silica gel (cobalt chloride, indicator). The insoluble residue, resistant collagen (RC), was dried at 37 °C during 24h (dried RC in **Fig.1**). All treatments were performed three times. Figure 1 outlines the overall process employed to obtain the pigskin by-products, ASC and G, by physical-chemical treatment.

Previously to the drying process, the pH was measured in each solution (ASC and

G). Total Hydroxyproline (Hyp) was quantified in ASC and G solutions, dried RC and raw tissue (pigskin wet base (w.b.)).
Dried ASC and dried G samples were used for DSC analysis. The color was evaluated on dried G samples.

Analysis of pH

The pH of the acid solution and ASC and G solutions was measured using a pHmeter (HANNA Edge®; HI5222, made in Romania, Woonsocket, RI 02895 USA).

Chemical analysis. Quantification of Hydroxyproline.

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 according to Velazquez & Latorre (2019). 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 pigskin (w.b.). The yield of each fraction (ASC and G) was calculated by the following equation:

$$\text{Yield \%} = \frac{\text{Hyp content of fraction*}}{\text{Total Hyp content**}} \times 100$$

*Hyp content in each collagen fractions, ASC, G and RC. **Sum of ASC, G and RC Hyp content of each treatment.

Differential Scanning Calorimetry (DSC)

Dried ASC and dried G samples were analysed using a DSC Setaram Evo 131. Samples of ~10 mg mass and encapsulated in small aluminium sample pans were evaluated. Non-isothermal DSC curves were obtained using heating rates of 10 °C min-1, from 25 °C to 300 °C using Ar as sweeping gas, and an empty pan as reference. After proper baseline correction using a polynomial function, enthalpies (ΔH) and mean transition temperatures (Td) were determined from the curves.

Color

The dried G samples were placed on a white tile and CIE color space coordinates L*, a* and 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 mean, standard deviation (±sd) and standard error (SE). Comparisons among the results of each treatment were performed by one-way ANOVA with Post-hoc Tuckey´s post-test (α 0.05). The 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

RESULTS AND DISCUSSION

Pigskin tissue characteristics

The chemical composition of the pigskin raw tissue is shown in **Table 1.** The results showed high protein content (Total Nitrogen with a Factor 6.25). The total collagen content confirms that this protein is the major protein in pigskin tissue. The composition characteristics (55% water, 35% connective tissue (collagen), 5–10% fat) (Feiner, 2006) have allow to use as effective alternative ingredients and components the lower protein in non-protein ingredients (Alves et al., 2016; Olivera et al., 2017). The knowledge of the total lipid content shows the importance of a previous lipid extraction step for future studies. The lipid extraction allows obtaining purer collagen by-products.

Physicochemical characteristics of the by-products, collagen and gelatin.

The pH of the food-grade acid solution, ASC and G solutions, soluble fractions obtained from the acid and thermal

treatments, are shown in **Table 2.** The pH in the ASC solutions (soluble fraction) as expected, showed relation with the values of the acid solution used. The hydrolysis step (acid treatment or enzymatic) is necessary in the collagen extraction protocols (Xu, et al., 2021).

During this process the tissue is swollen and the electrostatic intra- and intermolecular collagen interactions are weakened and some collagen molecules (ASC) may be solubilized. The laboratory experiment showed that the four insoluble fractions after acid treatments AH, AL, AC and AA (24 hours at 4 °C) exhibited good and equal swellings. Choe & Kim (2018) indicated that the optimum swelling times were observed when the soaking solution had a constant pH (1.68-1.88) during 24 h at 4 °C. During this first step (hydrolysis) the polypeptide chains and the cross-linkages are broken (hydrogen bonds are destroyed) allowing to the denaturation of the collagen protein (triple helix) during heat treatment (Gorgieva and Kokol 2011). According to Choe and Kin (2018), for pig and chicken skin, acid processing is the most suitable treatment. Acid process is applied in the industry to obtain Type A-gelatin.

In this work, the neutralization step previous to the thermal process was not done because the interest was to evaluate the acid effect during the denaturation of proteins. The pH values of solution G ranged from 2.4 to 3.6 (**Table 2**). The pH values of G-solution would indicate the effects of the swelling processes (collagen structure and interactions of acid molecules) and the possible presence of acids molecules in the thermo-soluble fractions. Knowing the gelatin pH is important since it might affect other properties, such as gel strength, viscosity, etc., and its application.

The neutralization step is relevant and necessary to obtain normal gelatin according to the standards of Gelatin Manufacture Institute of American (GMIA) (2019) (gelatin powder reconstituted in water, pH values 4.5 to 6.6). According to Yudhistira et al. (2019) the neutral gelatin pH is commonly used in meat products, pharmaceuticals, photography, painting, etc., whereas low gelatine pH is used in products like juices, mayonnaise, soups.

According to Donald (2001), 1% w/v is the minimal concentration at which the nucleation of the triplex helix occurs. Thus, the helixes overlap resulting in gel formation. For all the thermo-soluble fractions, the G-solutions obtained (**Table 2,** not significant differences p=0.1934), resulted in higher concentration than the minimum required (1% w/v) for nucleation of the triplex helix. Before drying the G-solutions, the samples were kept 24h at 4 °C to observe the gelling properties. It was observed that AH and AA turned into a firm gel (jellified), AL turned into "weak" gel (poor gel) and AC did not jellify (cloudy solution). This difference could be due to the effect of the pH values on the hydrolysis of the collagen-chains s and/or on the proteins charges. Kaewruang et al. (2013) 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. (2013) indicated that the differences in the pH treatment could modify the amphoteric nature and the hydrophobic zones on the peptide chain of gelatin, limiting functional protein properties.

Hydroxyproline content and extraction yield

The Hyp content (mg Hyp/100 g pigskin [w.b.]) of ASC, G and RC fractions is shown in **Table 3.** The ASC Hyp content was significantly different between treatments ($p \leq 0.05$). The AH and AA ASC fractions,

showed lower Hyp content than AC and AL treatments.

The G Hyp content resulted higher in AH and AA than in AC and AL treatments. Several studies analysed the use of different acids to obtain native collagen (acid soluble collagen) and gelatin from different matrixes (waste tissue), but separately. The ASC collagen content results, calculated by using a correction factor of 7.55, were 3.9; 6.8, 6.2 and 3,6 mg Collagen/g pigskin (w.b.) to AH; AL, AC and AA treatment, respectively. acid soluble collagens (ASC) Oslan et al., (2022) studied the acid soluble collagens (ASC) from skin of the purple-spotted bigeye snapper (*Priacanthus tayenus*) using acetic, lactic and citric acid. In this study the highest ASC content were obtained by acetic (5.79%), then in citric acid (4.15%), the lowest by lactic (3.19%).

On the other hand, Kanwate& Kudre (2017) studied the gelatin characteristics from fish (*Labeo rohita*). The authors showed that the gelatin extracted with propionic acid showed higher Hyp content when compared with acetic and phosphoric acid.

It is known, that the Hyp contents of "pure" gelatins is variable according to the specie, race, age, et. and it suggest used as a valid criterion of purity for mammalian collagens and gelatins. According to Sompie et al. (2015), hydroxyproline in gelatin stabilizes the hydrogen bonds between free hydroxyl groups and water molecules. Moreover, Kaewruang et al. (2013) proposed that the iminoacid (Hyp) determine the gel strength by introducing pyrrolidine rings for bridging between chains, apart from H-bonding. This could explain the differences observed in the G-Hyp content and the jellified characteristic from AH, AA vs. AL and AC. However, other studies, including FTIR, amino acid profile, isoelectric point, etc., are required for further understanding.

RC fractions exhibited a number of losses in the drying process. In particular, the AL and AC samples resulted highly sticky, which affected the full recover of the dried CR. According to this and avoiding to infer into an error, the RC Hyp content has not been analyzed by ANOVA-analysis. This results are novel, because at the moment nor study reported the Hyp (collagen equivalent) content in the solid remained after gelatin obtaining process.

Nevertheless, the sum of ASC, G and RC Hyp content (Total Hyp content mg/g) allowed confirming these losses. The Total Hyp content resulted lower in AL and AC than in AH and AA treatments. This observation is supported by the Hyp content in the raw pigskin (43.6 mg/g pigskin w.b.) *vs* the total Hyp (sum of fractions) in AH and AA (43.4 and 41.4 mg/g pigskin w.b., respectively) and AL and AC (35.7 and 36.2 mg/g pigskin w.b., respectively). Likewise, estimated yields are presented in **Figure 2**. For all treatments, G is the main percentage of the by-product.

Differential Scanning Calorimetry (DSC)

DSC is widely used to study the thermal transitions of proteins. In this study, the thermal characteristics of dried ASC and G samples were studied by DSC. Thermal transition temperatures and enthalpy changes were determined in order to evaluate the effect of using different food-grade acids on the ASC and G protein by-products. The samples were measured after conditioning at 25 °C, as described in section 2.2.

The collagen thermal transition temperature (T_d) is the temperature at which collagen triple-helix is converted to randomized coil structures (corresponding to the irreversible unfolded-denatured step) (Latorre et al., 2018). The ASC-DSC results showed differences between treatment. The AH and AA presented similar T_d (59 and 48 °C, respectively) between them, whereas AL and AC showed similar T_d, 124 and 139 °C, respectively, both higher than AH and AA.

These differences could be due to an increase of inter and intra fibrillar interactions with higher Hyp content (**Table 3**).

Moreover, it is known that multi-processes are involved in collagen crosslinking. For example, the reaction between collagen and hydrolysed vegetable tannins, which confers moderate hydrothermal stability. According to Cass &Burg (2012), tannic acid functions as a collagen cross-linking agent through hydrogen-bonding mechanisms and hydrophobic effects. The authors observed that thermal denaturation temperatures of the cross-linked scaffolds (68 °C) resulted significantly higher than those of uncross-linked scaffolds (55 °C). Furthermore, collagen stabilization involves water molecules, hence the water activity resulting an important factor on the thermal characteristics.

The enthalpy results showed discrepancy between samples (data not shown). The drying procedure could be responsible for differences in the samples. This may be due to non-uniform distribution of the water in the samples (Latorre & Velazquez, 2020). Unfortunately, in the present work water activity was not measured.

Thus, based on these observations, more studies such as, water activity, scanning electron microscopy images, FTIR, etc., are necessary to explain collagen (ASC) structural characteristics obtained by the different acid treatments.

Table 4 shows the results of the glass thermal transition temperature (T_g) and total enthalpies corresponding to dried G samples determined from DSC curves. The endothermic signals resulted around 120 °C for all treatments. However, differences on ΔH results (**Table 4**) suggest that the process used affected the protein hydrophobic and/or hydrogen binding. The enthalpy values resulted higher for AA and AC than for AL and AH in dried G.

As mentioned before, DSC curves show a single endothermic signal around 120 ºC for all treatments. This could correspond to the superposition of more than one thermal process, such as evaporation, structural reorganization, non-equilibrium Tg of the rigid blocks, etc. In addition, polymers take time to crystallize because crystallization requires movement of the chains to order them in the crystalline phase (Vlasova 2019). Gelatin films can be considered as a semicrystalline polymer, with crystalline domains, which during drying could form triple helix structures that act as physical cross-links (Mosleh et al., 2020).

These structural changes could affect the thermal behaviour of the gelatins studied in this work. According to Mosleh et al. (2020), the glass transition is a time and temperature dependent transition (amorphous regions of the random coil string). For that reason, parameters such as thermal history (eg, drying time and temperature) and moisture content are relevant in investigations of gelatin glass transition.

For other side, Tsereteli & Smirnova (1992) observed that, depending on the type of gelatin (extraction process and/or nature of gelatin), the “melting heat” presents a stronger relation with the number of cross-links present in the starting gelatin. The authors indicated that gelatin (gel or crystalline state) forms metastable collagen-like structures and that the resulting thermodynamic parameters depend on the production conditions.

Additionally, in the present results the acid-protein interaction might be affecting the thermal properties. For example, Xu et al. (2013) studied the citric acid use to cross-link wheat-derived gliadin at low temperatures. The study reported that when more than one carboxyl group is involved in the reaction, further inter and/or intra-molecular crosslinking can be possible.

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The current ΔH results may also suggest that the process used to obtain the gelatin may affect protein interactions. In addition, despite all G samples were dried under the same conditions, water activities were not quantified in the present work. Therefore, different amounts of protein-protein and water-protein interactions could be present in each sample studied.

Unfortunately, this study did not assess water activities neither the FTIR study of the dried-G samples was performed to explain the differences observed. These studies will be included in detail in future research.

Gelatin color

Gelatin color has proven to influence acceptability and food application. The color of dried G obtained from pigskin by different acid pre-treatments is shown in **Table 5**. The results demonstrated that the different pre-treatments used to obtain gelatin influenced ($p < 0.05$) the values of the films: lightness (L*), redness (a*) and yellowness (b*). The AH-dried G presented the highest L* value, while the AA-dried G resulted in the lowest L*value and the highest browning (<b*). The L* values of AL and AC-dried G were lower as compared with AH treatment and higher than AA treatment. In the present work, the acids used at the same concentration during the pigskin hydrolysis don't present the same pH (data not sowed). Musso et al., (2016) work with commercial gelatin, adjusted different pH and the color of gelatin films (G) obtained resulted clear and colorless for all pHs tested. Significant differences between treatments were found in red (a*) and (b*) yellow hues (both $p < 0.0001$). The AC-dried G a* value obtained by AL<AA< AH acids-treatment was higher as compared with dried G. However, the highest values of yellow hue, analyzed by b* parameter, were observed on AC- and AA-dried G. This and lightness differences could be at different Mailliard and or (AA and AC) oxidation reaction during the thermal process.

More studies, like low acid concentration or acid mix, are necessary to improve the gelatin color.

CONCLUSION

Results indicated that ASC and G by-products of pigskin could be obtained using different food grade acids. The acid and thermal processes showed differences in Hyp content of the ASC and G by-products. The AC and AL treatments had a higher Hyp content than the AH and AA ASC fractions G Hyp content resulted higher in AH and AA than in AC and AL treatments. The DSC results showed that the acid treatment affected the thermal properties of the by-products. The current results suggest that the acid used and the pH of the solution during the thermal process affects the ASC and G protein interactions and/or their structures (cross-linking). Dried-G color was affected by the different acid treatments. Results suggest that obtaining different pigskin by-products using acid-food grade could be an option for the rendering industry. The results presented invite an opportunity to deepen future research.

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REFERENCES

Alves, L.A.A.S., Lorenzo, J.M., Gon¸calves, C.A.A., Santos, B.A., Heck, R.T., Cichoski, A.J. & Campagnol, P.C.B.(2016), Production of Healthier Bologna Type Sausages Using Pork Skin and Green Banana Flour as a Fat

Replacers, Meat Science, 121,73-78. https://doi.org/10.1016/j.meatsci.2016.06.001

Amirrah, I.N.; Lokanathan, Y.; Zulkiflee, I.; Wee, M.F.M.R.; Motta, A.; Fauzi, M.B. A (2022) Comprehensive Review on Collagen Type I Development of Biomaterials for Tissue Engineering: From Biosynthesis to Bioscaffold. *Biomedicines*, *10*,2307. https://doi.org/10.3390/biomedicines10092307.

AOAC Official Method, 19th Edition, Volume II, 2012, 992.15, Crude Protein in Meat and Meat Products including Pet Foods - Combustion Method, Chapter 39, 6-7.

Cass CA, Burg KJ. (2012) Tannic acid cross-linked collagen scaffolds and their anti-cancer potential in a tissue engineered breast implant. J Biomater Sci Polym Ed.23(1-4):281-98 doi: 10.1163/092050610X550331.

Chakka, A.K., Muhammed, A., Sakhare, P.Z. and Narayan Bhaskar (2017). Poultry Processing Waste as an Alternative Source for Mammalian Gelatin: Extraction and Characterization of Gelatin from Chicken Feet Using Food Grade Acids. Waste Biomass Valor 8:2583–2593 https://doi.org/10.1007/s12649-016-9756-1

Changwei Cao, Zhichao Xiao, Changrong Ge & Yinglong Wu. (2021) Animal by-products collagen and derived peptide, as important components of innovative sustainable food systems— a comprehensive review, Critical Reviews in Food Science and Nutrition, 62 (31): 8703-8727 https://doi.org/10.1080/10408398.2021.1931807

Choe, J and H Y Kim (2018) Effects of chicken feet gelatin extracted at different temperatures and wheat fiber with different particle sizes on the physicochemical properties of gels. Poultry Science, 97(3):1082-1088 https://doi.org/10.3382/ps/pex381

Donald, A. (2001). Food Gels. Encyclopedia of Materials: Science and Technology, 3231-3233 (2001) https://doi.org/10.1016/B0-08-043152-6/00575-1.

Feiner G. (2006): Meat Products Handbook: Practical Science and Technology. Boca Raton, CRC Press.

Gorgieva, Selestina & Kokol, Vanja. (2011). Collagen- vs. Gelatine-Based Biomaterials and Their Biocompatibility: Review and Perspectives. 10.5772/24118. In Biomaterials Applications for Nanomedicine, edited by Pignatello, R. Croatia: InTech.

Hua Yang and Zibin Shu J. (2014) The extraction of collagen protein from pigskin Chem. Pharm. Res., 6(2):683-687

Jing Xu, Tianduo Li, Furong Tao, Yuezhi Cui, Yongmei Xia. (2013). Structure Evolution of Gelatin Particles Induced by pH and Ionic Strength. Microscopy Research and Technique 76 (3):272-281 https://doi.org/10.1002/jemt.22164

Kaewruang P, Benjakul S, Prodpran T. (2013) Molecular and functional properties of gelatin from the skin of unicorn leatherjacket as affected by extracting temperatures. Food Chem 138:1431–1437. doi: 10.1016/j.foodchem.2012.09.114.

Kanwate BW, Kudre TG. (2017). Effect of various acids on physicochemical and functional characteristics of gelatin from swim bladder of rohu (*Labeo rohita*). J Food Sci Technol.54(8):2540-2550 https://doi:10.1007/s13197-017-2699-0

Koli, J. & Basu, Subhasis & Kannuchamy, Nagalakshmi & Gudipati, Venkateshwarlu. (2013) Effect of pH

and ionic strength on functional properties of fish gelatin compared to mammalian gelatin. Fishery Technology. 50. 1-8.

Latorre M.E, D.E. Velázquez, P.P. Purslow (2018) Differences in the energetics of collagen denaturation in connective tissue from two muscles. International Journal of Biological Macromolecules, 113, p1294-1301 https://doi.org/10.1016/j.ijbiomac.2018.02.132

Latorre M.E & Velazquez D.E (2020) Effects of thermal treatment on collagen present in bovine M. Semitendinosus intramuscular connective tissue. Analysis of the chemical, thermal and mechanical properties, Food Structure https://doi.org/10.1016/j.foostr.2020.100165

Li He and Lan, Wenting and Zhao, Yuqing and Chen, Shujuan and Liu, Shuliang and Cen, Liyuan and Cao, Shu and Dong, Lei and Jin, Ruoyun and Liu, Yaowen (2020). Characterization of biocompatible pig skin collagen and application of collagen-based films for enzyme immobilization. RSC Adv. The Royal Society of Chemistry,10(12): 7170-7180 http://dx.doi.org/10.1039/C9RA10794K

Mariod, Abdalbasit & Fadul, Hadia (2013). Review: Gelatin, source, extraction and industrial applications. ACTA Scientiarum Polonorum Technol. Aliment.,12:135-147

Matinong, A.M.E.; Chisti, Y.; Pickering, K.L.; Haverkamp, R.G. (2022). Collagen Extraction from Animal Skin. Biology, 11(6), 905; https://doi.org/10.3390/biology11060905

Mosleh, Y., de Zeeuw, W., Nijemesland, M., Bijleveld, J. C., van Duin, P., & Poulis, J. A. (2020). The Structure–Property Correlations in Dry Gelatin Adhesive Films. *Advanced Engineering Materials*, *23*(1), [2000716]. https://doi.org/10.1002/adem.202000716

Musso, Y.S., Salgado, P.R., Mauri, A.N., Gelatin based films capable of modifying its color against environmental pH changes, Food Hydrocolloids (2016), doi: 10.1016/j.foodhyd.2016.06.013

Official procedures of the Gelatin Manufacturers Institute of America, Inc. Revised: January 2019 http://www.gelatin-gmia.com

Oliveira, Fagundes & Dos Santos, Bibiana & Fagundes, Mariane & Heck, Rosane & Cichoski, Alexandre & Wagner, Roger & Campagnol, Paulo. (2017). Pork skin and canola oil as strategy to confer technological and nutritional advantages to burgers. Czech Journal of Food Sciences. 35. 352-359. 10.17221/67/2017-CJFS.

Oslan, S.N.H.; Shapawi, R.; Mokhtar, R.A.M.; Noordin, W.N.M.; Huda, N. Characterization of Acid and Pepsin Soluble Collagen Extracted from the Skin of Purple-Spotted Bigeye Snapper. Gels **2022**, 8, 665. https://doi.org/10.3390/gels8100665

See SF, Ghassem M, Mamot S, Babji AS (2015). Effect of different pretreatments on functional properties of African catfish (Clarias gariepinus) skin gelatin. J Food Sci Technol.52(2):753-762 https://doi:10.1007/s13197-013-1043-6

Saini RK, Prasad P, Shang X, Keum YS. (2021) Advances in Lipid Extraction Methods-A Review. Int J Mol Sci. 2021 Dec 20;22(24):13643. doi: 10.3390/ijms222413643. PMID: 34948437; PMCID: PMC8704327.

Sompie M., S.E. Surtijono, J.H.W. Pontoh, N.N. Lontaan (2015) The Effects of Acetic Acid Concentration and Extraction Temperature on Physical and

Chemical Properties of Pigskin Gelatin. Procedia Food Science, 3, 383-388 https://doi.org/10.1016/j.profoo.2015.01.042

Tsereteli, G.I. & Smirnova, Olga. (1992). DSC study of melting and glass transition in gelatins. Journal of Thermal Analysis and Calorimetry - J Therm Anal Calorim.,38:1189-1201. doi:10.1007/BF01979179

Velázquez D.E and M.E. Latorre, Physicochemical, thermal and mechanical characterization study of perimysial collagen of two bovine muscles, International Journal of Biological Macromolecules,(2019),136, 404-409. https://doi.org/10.1016/j.ijbiomac.2019.06.092

Vlasova Mariia (2019). Fast scanning calorimetry combined with millisecond time-resolved x-ray micro-diffraction to study the reorganization phenomena in poly (ether ether ketone). Thesis Université de Haute Alsace Université de Strasbourg. (open Access : https://www.fzu.cz/~sestak/yyx/Glassy%20state.pdf)

Xu Q, Torres JE, Hakim M, Babiak PM, Pal P, Battistoni CM, Nguyen M, Panitch A, Solorio L, Liu JC. Collagen- and hyaluronic acid-based hydrogels and their biomedical applications. Mater Sci Eng R Rep. 2021, 146:100641. doi: 10.1016/j.mser.2021.100641.

Yudhistira B, E Palupi, and W Atmaka (2019) The Effect of Acid Concentration and Duration of Submersion toward the Characteristics of Gelatin of Eel Fish Bone (*Anguilla bicolor*) Produced through Acid Process. 4th International Conference on Tropical and Coastal Region Eco Development IOP Conf. Series: Earth and Environmental Science 246. doi:10.1088/1755-1315/246/1/012046

Table 1- The chemical composition of Pigskin, raw tissue (g /100 g pigskin wet base (w.b.)).

Raw tissue (pigskin)	g/100 g (w.b.)*
Moisture	37.5 ± 0.68
Total Protein	45.4 ± 1.45
Total Lipid	12.0 ± 1.91
Total Ash	0.26 ± 0.10
Total Collagen	32.9 ± 5.41

*Value are given as mean ± standard deviation (n=3).

Table 2- pH solutions values; food grade acid solutions (0.5M), ASC and G solutions (soluble fractions obtained after the acid and thermal treatments, respectively). Total collagen protein content (Hyp quantification) in G soluble fraction after thermal treatment (90 °C-90 min).

	AH	**AL**	**AC**	**AA**
pH Value*				
Food-grade acid Solution	2.55 ± 0.07^{a}	1.98 ± 0.04^{b}	1.70 ± 0.14^{c}	2.39 ± 0.13^{a}
ASC solution	2.60 ± 0.10^{a}	2.00 ± 0.30^{b}	1.85 ± 0.13^{b}	2.40 ± 0.22^{a}
G solution	3.55 ± 0.15^{a}	2.80 ± 0.2^{bc}	2.35 ± 0.10^{c}	3.20 ± 0.25^{a}
G-solution**				
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). One-way ANOVA (Tukey's Multiple Comparison Test).
** Gelatin corresponds to Hyp content (g/100 ml) x Conversion Factor (7.55). Value are given as mean ± standard deviation and (SE) (n=3).

Table 3- Hydroxyproline content (mg Hyp/ g pigskin w.b.) in ASC, G and RC fractions by treatment.

	mg Hyp /g pigskin (w.b)*		
Treatment	ASC	G	RC
AH	0.48 ± 0.07(0.04)^{a}	41.40 ± 5.19 (2.32)^{a}	1.47 ± 0.02 (0.01)
AL	0.91 ± 0.02(0.14)^{b}	34.44 ± 2.73 (1.22)^{b}	0.31 ± 0.02 (0.01)
AC	0.82 ± 0.01(0.10)^{b}	33.75 ± 2.62 (1.17)^{b}	1.66 ± 0.15 (0.11)
AA	0.48 ± 0.15(0.09)^{a}	40.37 ± 2.57 (1.49)^{a}	0.52 ± 0.04 (0.03)
p-Value	0.005	0.0094	

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

Table 4- Dried G thermal transition temperatures (T; °C) and changes in enthalpy (ΔH; J/g)

	T_g (°C)*	ΔH (J/g)*
AH	122.4 ± 6.4 (3.7)	121.2 ± 26.8 (16)[a]
AL	120.1 ± 1.8 (1.3)	169.2 ± 9.1 (6.4)[b]
AC	119.9 ± 3.5 (2.5)	244.2 ± 12.0 (8.5)[c]
AA	120.3 ± 0.5 (0.4)	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) One-way ANOVA (Tukey's Multiple Comparison Test).

Table 5- Dried G CIELab parameters.

Acid Treatment*	L*	a*	b*
AH	83.68 ± 1.09 (0.03)[a]	-0.83±0.05(0.03)[a]	7.40 ± 0.41 (0.24)[a]
AL	69.52 ± 2.92 (1.69)[b]	4.18 ± 1.54 (0.89)[b]	25.01±3.07 (1.77)[b]
AC	64.93 ± 0.80 (0.46)[c]	9.86 ± 1.36 (0.79)[c]	39.01 ± 2.52 (1.46)[c]
AA	35.86 ± 1.16 (0.82)[d]	0.90 ± 0.21 (0.15)[d]	-0.30 ± 0.04 (0.03)[e]

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

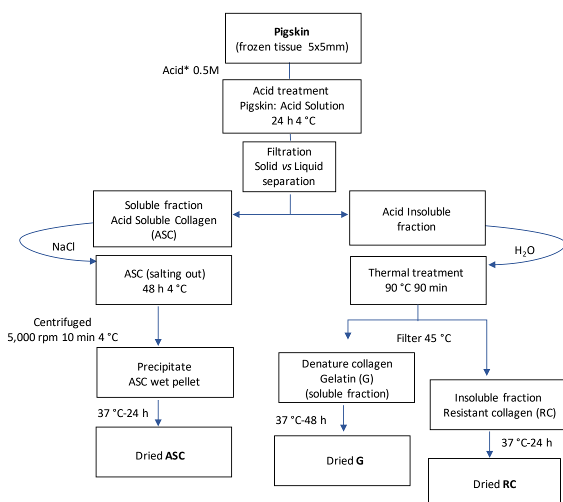

Fig 1- Schematic diagram of the process used to obtain the pigskin by-products. *Acid solutions Acetic acid (AH); Lactic acid (AL); Citric acid (AC) and Ascorbic acid (AA) 0.5M

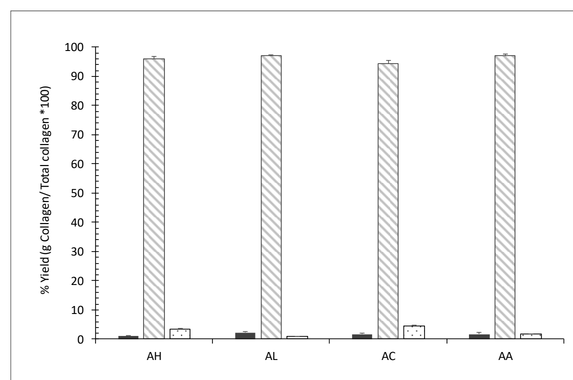

Fig 2- Yield of collagen extracted from pigskin after different acid-thermal treatments. Black: ASC-yield, striped: G-yield and spotted: RC-yield; respectively.

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