Comprehensive evaluation of the properties of camel bone gelatin: impact of the duration of pre-treatment and extraction

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Keywords

Camelids, gelatin, abattoir by-products, food technology, functional properties, Algeria

Submitted: 01 April 2024
Accepted: 19 November 2024
Online: 31 December 2024
DOI: 10.19182/remvt.37442

Summary

Background: The growing interest in innovative uses for slaughter by-products from camels could generate added value from this multipurpose animal. ***Aim***: This study investigates the extraction of gelatin from camel bones, discusses its potential as a novel protein source and assesses how its properties are affected by the duration of the pre-treatment and extraction processes. ***Methods***: Four bone samples from 4-4.5-year-old male Sahraoui camels were utilized. The gelatin extraction process comprised demineralization with hydrochloric acid, followed by pre-treatment with sodium hydroxide for 24 or 48 hours and subsequent chemo-thermal extraction in acetic acid for 6 or 12 hours. ***Results***: Physicochemical, microscopic and functional properties of the extracted gelatin were evaluated. Gelatin yields varied from 15.65% ± 0.15 to 21.85% ± 0.25. Variations were attributed to the combined duration of pre-treatment and chemo-thermal extraction. Extended processing times increased structural degradation. The elemental analysis revealed a stable carbon and oxygen content. The variable nitrogen levels revealed a positive correlation with extraction intensity. The gelatin pH values exhibited little variation, ranging from 4.66 to 4.91. The gelatin demonstrated interesting functional properties, including a high water holding capacity of 1080 ± 4.24%, a fat binding capacity of 880 ± 98.99%, and a Bloom value of 317.96 ± 8.51 g. These characteristics were predominantly influenced by the length of pre-treatment and extraction. Optimal results were obtained under moderate processing conditions. ***Conclusions***: Camel bone gelatin has physicochemical and functional characteristics, including a high water holding capacity, a high fat binding capacity and a favorable Bloom value, which make it a valuable candidate for various industrial applications.

■ How to cite this article: Imelhayene M., Adamou A., Becila S., Redjeb A., Saidj D., Senoussi A., Sarris D., Naziri E., 2024. Comprehensive evaluation of the properties of camel bone gelatin: impact of the duration of pre-treatment and extraction. *Rev. Elev. Med. Vet. Pays Trop*., 77: 37442, doi: 10.19182/remvt.37442

■ INTRODUCTION

Camels are an essential livestock resource in arid and semi-arid regions because of their exceptional capacity to adapt to harsh conditions and thrive on mediocre quality diets. Camels constitute a renewable biological resource of tremendous intellectual and cultural importance. In communities that depend on camels, they are symbols of identity and heritage (Faye et al., 2022).

Camels have multiple functions (Faye, 2022; Senoussi et al., 2023). In their native regions, they make an essential contribution to diverse aspects of life, particularly in three areas: economic, social and cultural, and ecological. On an economic level, they provide basic products, such as milk, meat, hides and lint (Senoussi et al., 2023). On a social and cultural level, they are used for transport and agriculture. In addition, their role in cultural events (folklore and racing) makes them an integral part of daily life, deeply embedded in community traditions (Dhawal et al., 2021). On an ecological level, camels contribute to environmental sustainability through seed dispersal (endozoochory), which helps regenerate vegetation (Trabelsi et al., 2017). Their selective feeding habits help preserve vegetation from overgrazing (Mahma et al., 2019).

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In recent years, the traditional role of camels has been undermined, particularly in agriculture and transport. To address this shift and make the most of camels' potential, researchers have begun exploring different innovative developments, including: the therapeutic and industrial applications of camel milk; and the processing of slaughterhouse by-products to produce functional materials, such as gelatin (Redjeb, 2022).

Camel gelatin is derived from both skin and bone, the by-products of meat production. It is a valuable source of halal protein with applications in the pharmaceutical, cosmetic, biotechnology and food sectors. Thus, camel gelatin could provide a key resource for various industries, which would consolidate the multifunctional role of camels (Mariod and Adam, 2013).

Recent research has identified camels as a significant renewable source of gelatin (Al-Kahtani et al., 2017; Jaswir et al., 2019; Al Hassan et al., 2021; Fawale et al., 2021). However, research on camel bone gelatin remains in its early stages.

Most extant studies on camel bone gelatin have focused on the effects of a single extraction stage, specifically the demineralization process (Al-Kahtani et al., 2017). Their limited scope has left major gaps in our understanding of the broader extraction process, such as the impact of various extraction conditions on the quantitative yield and qualitative properties of camel bone gelatin (Jaswir et al., 2019). Therefore, more extensive research in the field of camel bone gelatin extraction is required.

In this context, our study aims to investigate the potential of camel bones as a source of gelatin and to identify the physicochemical, microscopic and functional characteristics of camel gelatin. We examine how the duration of pre-treatment and extraction affects its different characteristics. Our comprehensive approach seeks to contribute to the literature, by providing a more holistic understanding of camel bone gelatin extraction.

■ MATERIAL AND METHODS

Biological material

Bone samples were obtained from male Sahraoui camels aged between 4 and 4.5 years. The age was estimated using dentition, as precise dates of birth were unavailable. This age range was selected for several reasons.

According to Al-Hassan et al. (2021), bones from camels between 4 and 4.5 years yield approximately 23.9% gelatin, which is significantly higher than the yield from camels of 2.5 or 7 years of age. Optimizing the gelatin yield aligns with the study's objectives.

We selected 4-year-old male camels in compliance with Algerian legislation, which prohibits the slaughter of female camels and animals under two years of age (Ramadan/Shul, 2017), except in the case of a medical emergency. This legal framework significantly influenced the study design and sample selection method.

Gelatin is a protein derived from the partial hydrolysis of collagen. The composition and molecular structure of collagen in bone tissue change significantly during the different stages of animal development. The changes are closely linked to the animal's growth and maturation process. In the early years of growth, collagen molecules aggregate into heterogeneous filamentous structures. Over time, the structures are subject to a process of degradation and remodelling. This leads to the formation of three tightly bonded specialized chains, which help form the slatted bones that are characteristic of adult camels (Buddhachat et al., 2016). Camels typically reach puberty between four and five years of age (Gherissi, 2020).

Although the use of dentition for age estimation is less precise than birth records, it provides a reasonable approximation of age. It is a widely accepted method in research, both in veterinary and animal science. In our study, it allowed us to ensure that the standard sample selection complied with the legal requirements and corresponded to biological optimization.

The bone samples were collected from the camel market in Ouargla, Algeria. Samples were frozen at a temperature of -20°C and transferred to the scientific research laboratory for the valuation and protection of desert resources (Faculty of Nature and Life Sciences, University of Kasdi Merbah Ouargla), where the experimental extraction was conducted.

Gelatin extraction

The extraction program was divided into four phases:

Sample preparation

The collected bones were washed with tap water to remove all the dirt. The marrow was extracted manually and then the bones were washed in hot water at 60°C to remove meat residues and associated lipids. The bones were cut into fragments of <2 cm in length. Samples were stored in the refrigerator until use.

Bone demineralization

To prepare the ossein, the bone samples were demineralized. This involves dissolving the bone's mineral composition, which is essentially calcic and covers the bone matrix. We used the demineralization method as recommended by Al-Kahtani et al. A 100 g sample of fragmented bones was immersed in a 5% HCl solution (v/v, with 37% purity, supplied by VWR International S.A.S., 201 Rue Carnot, Fontenay-Sous-Bois, France), at a bone-to-solution ratio of 1:10 (100 g bones to 1 l solution). The process lasted five days, during which the HCl solution was kept at room temperature and renewed daily to maintain its efficacy. Following this treatment, the resulting ossein was meticulously rinsed with tap water until a neutral pH was obtained.

Pre-treatment

Prior to extraction, demineralized bones were pre-treated to remove non-collagenous proteins and peptides and to denature intermolecular bonds. We followed the pre-treatment method described by Lassoued et al. (2014), using a 1.5% NaOH solution (w/v) at a bone-to-solution ratio of 1:10 (100 g of bones to 1 l of solution). The NaOH with a minimum chemical purity of 99.5% was supplied by BIOCHEM (Chemopharma, ZA Cosne sur Loire, France).

Chemo-thermal extraction

This involves the partial hydrolysis of collagen fibres in an acidic medium, enhanced by heat dissolution. An acetic acid solution was prepared at a 1:4 (v/v) ratio (acetic acid to water) for use in a bone-to-solution ratio of 1:5 (100 g of bones to 500 mL of solution). The solution was then heated in a water bath at 65°C with continuous stirring to promote collagen hydrolysis. The acetic acid had a minimum chemical purity of 99.5% and was supplied by BIOCHEM (Chemopharma, ZA Cosne sur Loire, France). The extraction conditions chosen in this study are shown in Table I.

The extraction protocol was applied to a set of four distinct camel bone samples and performed in triplicate. This yielded a total of twelve independent extraction procedures. The product of the chemo-thermal extraction was then subject to filtration, centrifugation, further filtration, freeze-drying, grinding (with a Moulinex), before being stored prior to analysis.

Table I: The conditions for extracting camel bone gelatin /// *Conditions d'extraction de la gélatine d'os de dromadaire*

Test N°	PTH		CTE	
	[NaOH] (%)	Time (h)	Temperature (°C)	Time (h)
01	1.5	24	65	6
02	1.5	24	65	12
03	1.5	48	65	6
04	1.5	48	65	12

PTH: Pre-treatment, CTE: Chemo-thermal extraction, [NaOH]: NaOH concentration.

Identifying camel bone gelatin properties

The characterization of camel bone gelatin was conducted at the technical platform of physico-chemical analysis (CRAPC, PTAPC Biskra), in Biskra, Algeria, and at the university research laboratories in the Food Science and Nutrition Department, School of the Environment, at the University of the Aegean, in Greece.

We studied the quantitative and qualitative characteristics of gelatin. The quantitative characteristics primarily relate to yield, while qualitative characteristics encompass physico-chemical features (color, pH and elemental composition), microscopic properties and functional properties (water holding and fat binding capacities, gel strength). This comprehensive classification provides a framework for understanding gelatin's diverse characteristics. Our analysis examines the different characteristics and the distinct factors that may influence them during the extraction process.

Gelatin yield

Gelatin yield was obtained as a function of the weight after drying and the dry weight of the raw material (Roy et al., 2017; Roy et al., 2021) as follows:

Yield in % = gelatin weight after drying / initial camel bone weight ×100.

Morphological analysis and elemental analysis - microstructure

The morphology of gelatin was analysed using scanning electron microscopy (SEM) at multiple magnification scales. Dried gelatin powder samples were mounted on rods using a two-sided carbon band and a gold-coated sputter for 20 s, using an Emitech K575X sputter coating unit to prevent surface charging by the electron beam. The samples were then examined using JSM-7610FPlus SEM, attached to an EDS (energy dispersive X-ray spectroscopy) detector with a 10 kV accelerating voltage. The elemental analysis of gelatin was conducted at the same time as the morphological analysis. A total of 512×340 points were analysed, with each point measuring 0.8 µm, corresponding to an area of 1.39 mm^2.

pH

We measured pH on the basis of a 1% (W/V) solution, following Alfaro et al. (2014), with a Hack pH meter (HQ411d, Spain).

Color

The color of gelatin powder was measured with a Minolta Color Reader CR-10 (Minolta Co. Ltd., Osaka, Japan), according to the method described by Mulyani et al. (2017). Color was expressed by CIE L* (whiteness or brightness), a* (redness/greenness), and b* (yellowness/blueness) coordinates.

Water holding capacity and fat binding capacity

The water holding capacity (WHC) and the fat binding capacity (FBC) of camel bone gelatin were measured following Roy et al. (2017). This involved preparing a 1% (W/V) gelatin solution, by adding DI water or sunflower oil. The mixture was left to stand for 1 hour, with agitation using a vortex mixer for 5 seconds every 15 minutes. The residue was collected by centrifugation at 4500 rpm for 20 min at 4°C. The tubes were left to drain for 30 minutes at a 45° angle. The following equation was used to compute the WHC and FBC:

WHC or FBC (%) = Weight of the contents of the tube after draining (g)/Weight of the lyophilized gelatin (g) × 100

Gel strength - Bloom Value

The gel strength (or Bloom value) of camel bone gelatin was estimated. Following the British Standard Institution (1975) for 6.67% gelatin solution by mixing 7.5 g of gelatin and 105 ml of distilled water in a Bloom jar. The mixtures were then left to stand for 3h at room temperature. The solution was moved into a water bath, kept at 45°C and subsequently cooled to room temperature. The samples were maintained at 9 ±1°C in the refrigerator for 17 hours before being examined for gel strength. The gel strength (g) was determined at a speed of 0.5 mm/sec throughout a 4 mm penetration distance, using a texture analyser equipped with a flat-faced cylindrical Teflon plunger with a 1.27 cm diameter.

Statistical analysis

We analysed the features of camel bone gelatin, in addition to the effects of the duration of pre-treatment and extraction on its properties. For normally distributed variables, an ANOVA test was used. For non-normal variables, we used non-parametric tests, including the Kruskal-Wallis test with Dunn-Bonferroni post-hoc, the Mann-Whitney U test and Welch's t-test. A significance level of $p < 0.05$ was used throughout the analysis. All analyses were conducted in November 2022, using R (version 4.2.2).

■ RESULTS AND DISCUSSION

Gelatin yield

In this study, we set out to optimize gelatin yield, by using a substantial extraction process lasting 8 days. The data on gelatin yield were analysed with respect to two factors: the pre-treatment phase, where the variable is the duration of the pre-treatment; and the chemo-thermal extraction phase, where the variable is the duration of extraction. This analytical framework allows us to determine how the variables affect the yield of camel bone gelatin. The primary level of analysis examines the individual effects of each factor, namely: the duration of pre-treatment, the temperature and the duration of extraction. The secondary level investigates the combined influence of the duration of pre-treatment and that of chemo-thermal extraction. Figures 1, 2 and 3 present the effects on gelatin yield.

The gelatin yield ranged from 15.65% ± 0.15 to 21.85% ± 0.25 (Figure 1). A progressive increase in yield was observed between the first and third tests, with values rising from 16.3% to 21.85%. This trend suggests that extending the duration of both pre-treatment and chemo-thermal extraction (CTE) increases yield within certain parameters.

Indeed, the gelatin yield is significantly influenced by extending the chemo-thermal extraction time to 12 hours. This protocol yielded 18.09% ± 0.21 compared to 16.30% ± 0.32 for a 6-hour extraction. This is primarily attributed to the synergistic effects of prolonged exposure to a high temperature (65°C) and acetic acid, which boost gelatin production.

This phenomenon aligns with observations reported by Charoenchokpanich et al. (2022), Ee et al. (2021), and Kusumawati et al. (2019), who noted a positive correlation between extended extraction time and increased gelatin yield. According to our

statistical analysis, the longer extraction process did not have a statistically significant effect (p = 0.16) on gelatin yield (Figure 2), although it does appear to increase yield by an estimated 1.79%.

Previous research has indicated that collagen solubilization in acidic environments is reduced by the presence of various cross-links in the telo-peptide region of collagen molecules (Zhang et al., 2010). It is interesting to note that the duration of the chemo-thermal extraction alone has a relatively modest impact on gelatin yield, compared to the combined effect of the duration of pre-treatment and the shorter 6-hour extraction period. Indeed, the combined effect of these parameters had a greater impact on the overall gelatin yield. Increasing the pre-treatment time to 48 hours increased gelatin yield. A longer reaction time was more efficient and may have enhanced the extraction of active substances.

This finding is consistent with the observations of Ismail et al. (2017) and Ahmed et al. (2017), who reported that extending the duration of pre-treatment significantly increases the yield of gelatin, particularly from Tilapia skins.

Although pre-treatment time alone (Figure 3) does not demonstrate a statistically significant effect on gelatin yield (p = 0.37), the interaction between pre-treatment time and extraction time significantly enhances yield (Figure 1), as shown by a highly significant p-value of 8.23×10^{-3}.

In addition, the pre-treatment duration may enhance the disruption of collagen structures and, thus, facilitate subsequent extraction. At the same time, the shorter chemo-thermal extraction period mitigates potential gelatin degradation and optimizes extraction efficiency.

However, the last test exhibited an unexpected decline in yield to 15.65%, despite an increased pre-treatment duration of 48 hours. This suggests that there is a critical threshold for the duration of chemo-thermal extraction, beyond which the gelatin yield may fall. When both the pre-treatment and chemo-thermal extraction times are increased, gelatin production declines. This can be attributed to several factors: inefficient processing, increased solubilization of non-gelatinous components, decreased selectivity, over-extraction of collagen fibres, and degradation and loss of collagen integrity.

The optimal results were obtained in Test 3, demonstrating that a 48-hour pre-treatment followed by a 6-hour chemo-thermal extraction produces the highest yield (21.85% ± 0.25). Extending these times led to suboptimal outcomes, as shown in Test 4. When the chemo-thermal extraction was increased to 12 hours, the extracted materials were degraded, sharply reducing yield compared to the other tests. Our results for the optimal yield of gelatin are similar to those presented by Al-Kahtani et al. (2017) and Al-Hassan et al. (2021), who reported 23.66% and 21.3%, respectively.

Gelatin yield is not only affected by the total extraction time. Other factors are involved, including the source of the raw material, temperature and pH (Alipal et al., 2021), as well as external factors related to the maturation of collagen fibres in bones and the animal's age (Al-Hassan et al., 2021).

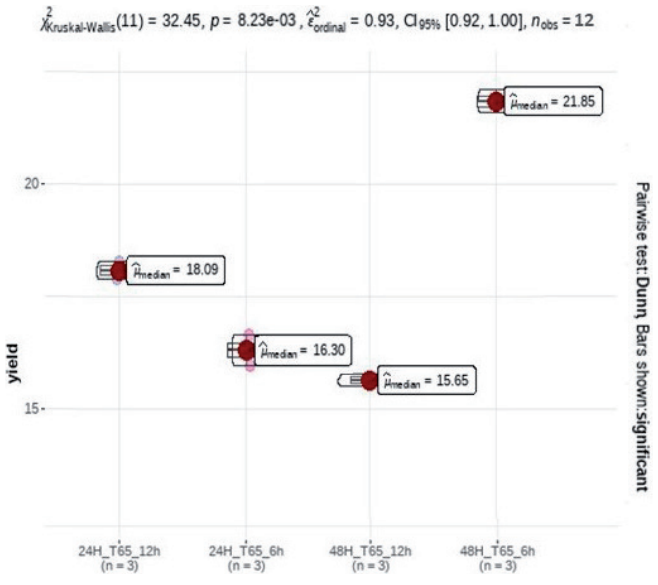

Figure 1: Combined effect of pre-treatment and extraction time on gelatin yield /// *Effet combiné du prétraitement et de la durée d'extraction sur le rendement en gélatine*

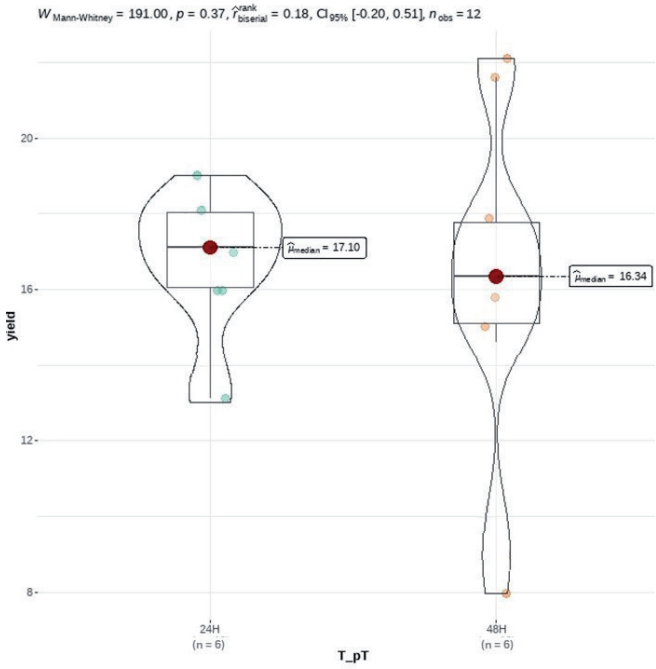

Figure 2: Effect of pre-treatment time on gelatin yield /// *Effet du temps de prétraitement sur le rendement en gélatine*

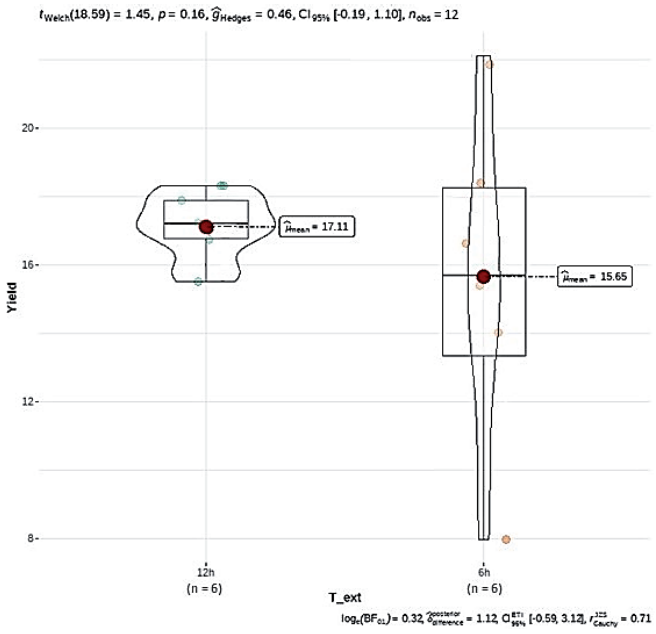

Figure 3: Effect of extraction time on gelatin yield /// *Effet de la durée d'extraction sur le rendement en gélatine*

Morphological and elemental analysis

The morphological analysis using the scanning electron microscope (SEM) provides valuable information. We studied the microstructure of camel bone gelatin, by selecting the most representative scale for each sample (Figure 4: a, b, c, d). Thus, we investigated the variations in gelatin microstructure under different experimental conditions.

The microstructure of gelatin is a good indication of the degree of consistency of gelatinous molecules. In Test 1 (a), with 24 hours of pre-treatment and 6 hours of extraction at 65°C, the structure shows moderate fragmentation, indicating partial denaturation. Test 2 (b), with 12 hours of extraction, exhibits a smoother and more degraded texture. Test 3 (c), with an extended 48-hour pre-treatment and a 6-hour extraction, shows more pronounced structural breakdown and a fragile uniform appearance. In Test 4 (d), with both extended pre-treatment (48 hours) and extraction (12 hours), the structure is highly denatured and appears loose and degraded.

With longer pre-treatment and extraction times, there is an increase in gelatin yield and collagen denaturation, as shown by the more fragmented and loose textures across the samples.

Microstructural changes indicate that higher PTH and longer extraction times progressively degrade the structure, which may increase the gelatin's solubility and denaturation. This visual observation aligns with the chemo-thermal extraction processes described. It shows that time significantly affects gelatin recovery and the integrity of its microstructure.

The elemental analysis shows the global composition of gelatin, by providing an overview of the biochemical structure and the degree of purity of our samples. Gelatin is a functional macromolecule composed of many amino acids, essentially glycine (21.13%), proline (10.05%), alanine (9.23%) and hydroxyproline (8.56%) (Al-Hassan et al., 2021). According to Susilowati et al. (2021), gelatin comprises carbon (50.5%), hydrogen (6.8%), nitrogen (17%), and oxygen (25.2%), represented by the molecular formula C102H151N31O39. Therefore, elemental analysis is expected to reveal the presence and distribution of nitrogen, carbon and oxygen in the sample.

The detection of elements with a low atomic number, particularly nitrogen, poses analytical challenges in EDS analysis due to instrumental limitations. While carbon and oxygen quantification is reliable, nitrogen analysis is complicated by spectral interference. Despite using modern EDS systems and optimizing sample preparation, our nitrogen measurements were less precise than was the case with the heavier elements.

The results of the elemental analysis of the extracted gelatin are shown in Figure 5 (a, b, c, d). They are consistent with the results obtained in the studies mentioned above. The elemental analysis reveals distinct spectral peaks corresponding to oxygen, carbon and nitrogen. We noted that the concentrations of carbon and oxygen demonstrate remarkable stability across varying extraction conditions and exhibit no significant fluctuations. In contrast, nitrogen levels show discernible variations, suggesting a differential response to alterations in the extraction parameters. The nitrogen content exhibits a positive correlation with the intensification of extraction conditions. It reaches its apex in the third test which is characterized by extended durations for the pre-treatment phases. This increase in nitrogen content is directly

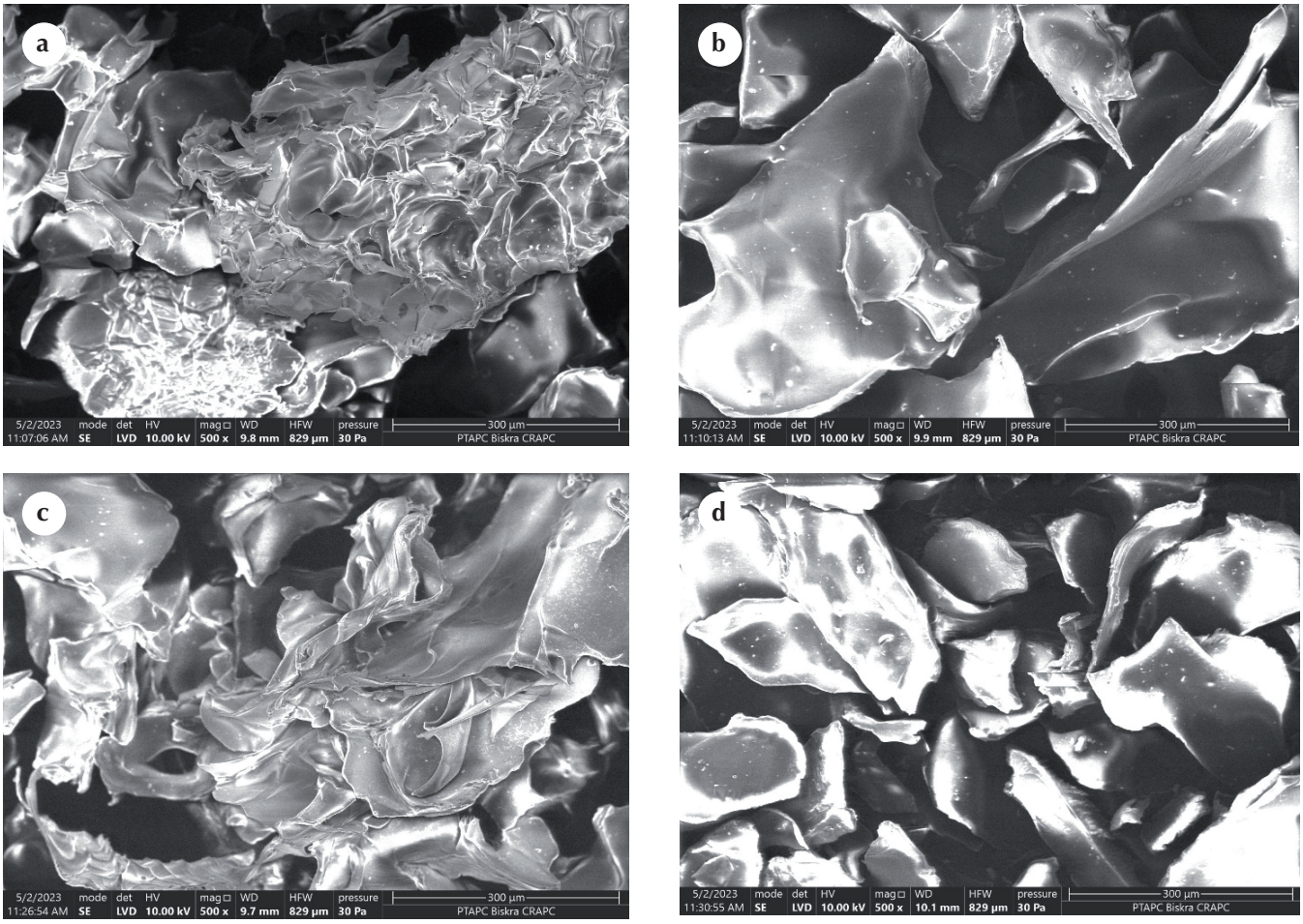

Figure 4: Microstructure of camel bones gelatin (a: test 1, b: test 2, c: test 3 d: test 4) /// *Microstructure de la gélatine d'os de chameau (a : test 1, b : test 2, c : test 3 d : test 4)*

proportional to the increase in the gelatin yield. This observation may be attributed to the higher degradation of the protein bonds in camel bones. Degradation facilitates the liberation of free amino acids and protein components, which are key indicators of gelatin quality.

Notwithstanding the improvements in nitrogen content and yield, our samples contained impurities, particularly sodium. The accumulation of sodium appears to correspond to the prolonged pre-treatment phase. Despite following a rigorous post-treatment washing protocol to neutralize pH and eliminate Na residues, a fraction of sodium persists and remains associated with the gelatin matrix.

The levels of sodium are inversely correlated to the duration of the chemo-thermal extraction, with longer extraction times corresponding to lower sodium concentrations. There are two possible explanations for this. First, the washing protocol may not be totally effective. Second and most likely, the sodium is naturally occurring and is extracted from the camel bone matrix during processing. This explanation corresponds to the pattern observed, as extended extraction times may alter the structural integrity of the bone matrix, thus, affecting its ability to retain sodium compounds that are naturally present.

Physical properties of gelatin

Gelatin's most important physical properties are pH and color. The pH has a vital role in determining its overall functional characteristics. It serves as a crucial standard that can influence the extent of the gelatin's transformation and its future application (Matulessy et al., 2021). Similarly, the whiter the gelatin, the greater its industrial appeal. Its degree of whiteness affects its interaction with various color additives and can enhance the reflection or absorption of color additives.

The pH of gelatin (Figure 6) is between 4.66 and 4.93, which falls within the normal range (between 4.2 and 5.6), according to the GMIA (2019) standards. The pH shows slight fluctuations across the tests. In Test 1, the pH is 4.93, reflecting a moderately acidic environment after 24 hours of pre-treatment and 6 hours of extraction. With a longer extraction time of 12 hours in Test 2, the pH decreases slightly to 4.66. This is probably due to increased protein breakdown, which may release acidic amino acids (glycine, alanine, valine, leucine, isoleucine, methionine, proline and phenylalanine) into the gelatin. In Test 3, where pre-treatment is extended to 48 hours, but extraction time is kept at 6 hours, the pH remains relatively stable at 4.70, suggesting that increasing the length of pre-treatment alone does not significantly alter acidity. Lastly, in Test 4, with the longest pre-treatment and extraction times, the pH almost returns to its original level (4.91). This suggests that prolonged chemo-thermal processing may neutralize or stabilize the acidity because collagen hydrolysis leads to a more balanced pH.

The pH of gelatin was not significantly influenced by pre-treatment time (PTH) or extraction time (CTE) individually, as shown by our

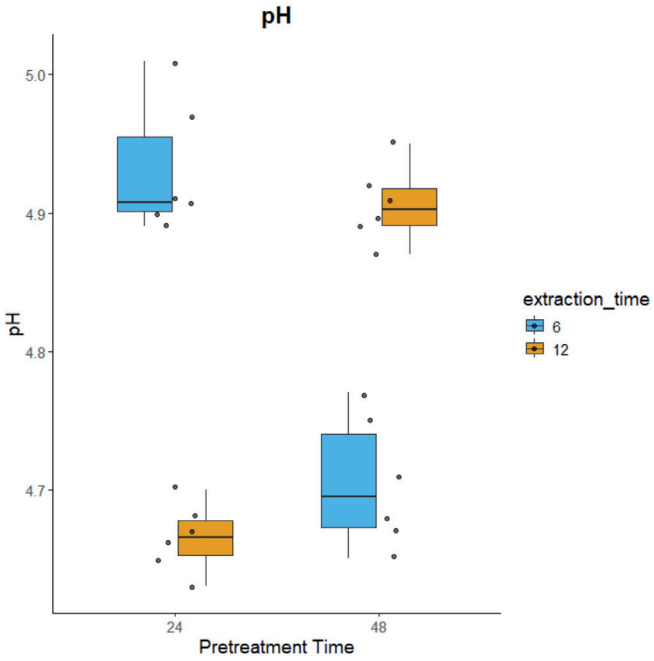

Figure 6: pH of camel bones gelatin /// *pH de la gélatine d'os de dromadaire*

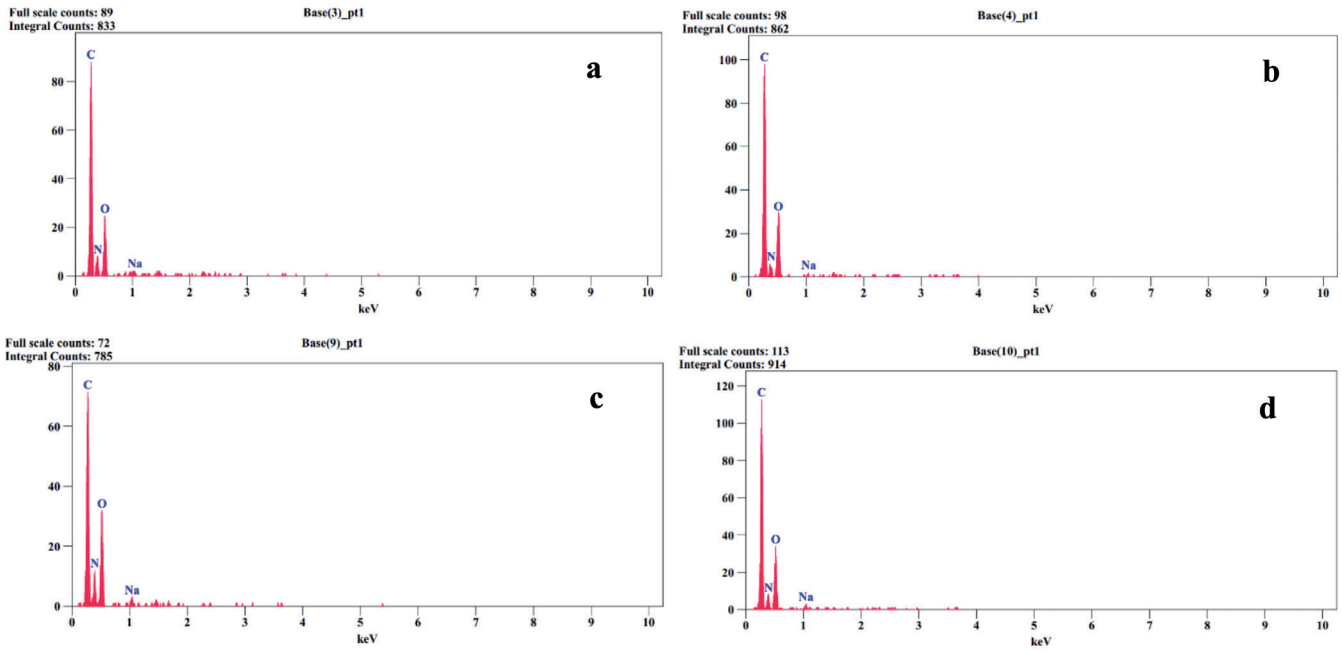

Figure 5: Elemental analysis results of camel bones gelatin (a: test 1, b: test 2, c: test 3 d: test 4) /// *Résultats de l'analyse élémentaire de la gélatine d'os de chameau (a: test 1, b: test 2, c: test 3 d: test 4)*

statistical analysis (P PTH = 0.72, P CTE = 0.132). However, the interaction between these factors demonstrated a significant effect on pH (P PTH*CTE = 3.91e-03). Our results show that optimum pH can be obtained by controlling two key variables: pre-treatment time and extraction time. The data suggest that an extended pre-treatment period of 48 hours, coupled with a prolonged extraction phase of 12 hours, may yield better outcomes in terms of pH adjustment to target levels. This protocol appears to offer a promising approach for fine-tuning the pH of the resultant solution.

These findings complement the work by Da Trindade Alfaro et al. (2015), who reported that gelatin pH is primarily influenced by the chemical agent employed during prolonged extraction. This observation aligns with Rabiatul Amirah et al. (2017), who validated that acidic chemical agents lower the pH of gelatin, while alkaline agents produce a neutral pH (approximately 7.2), irrespective of the solution concentrations used.

Gelatin is generally white or slightly yellowish. Its color is often influenced by the source of the raw material and the presence of impurities in the molecular structure of the final extract. In our experiment (Figure 7: a, b, c), the samples displayed a high degree of whiteness, ranging from 43.35 ± 5.02 to 50.41 ± 5.39, which is indicative of high purity. Color analysis revealed that the redness/greenness (a*) values were between 2.79 ± 2.48 and 7.20 ± 2.18, while the yellowness/blueness (b*) values ranged from 4.10 ± 0.79 to 5.78 ± 1.47.

Results demonstrate a positive relationship between the processing time and gelatin whiteness (L* values) (Figure 7, a). Test 1

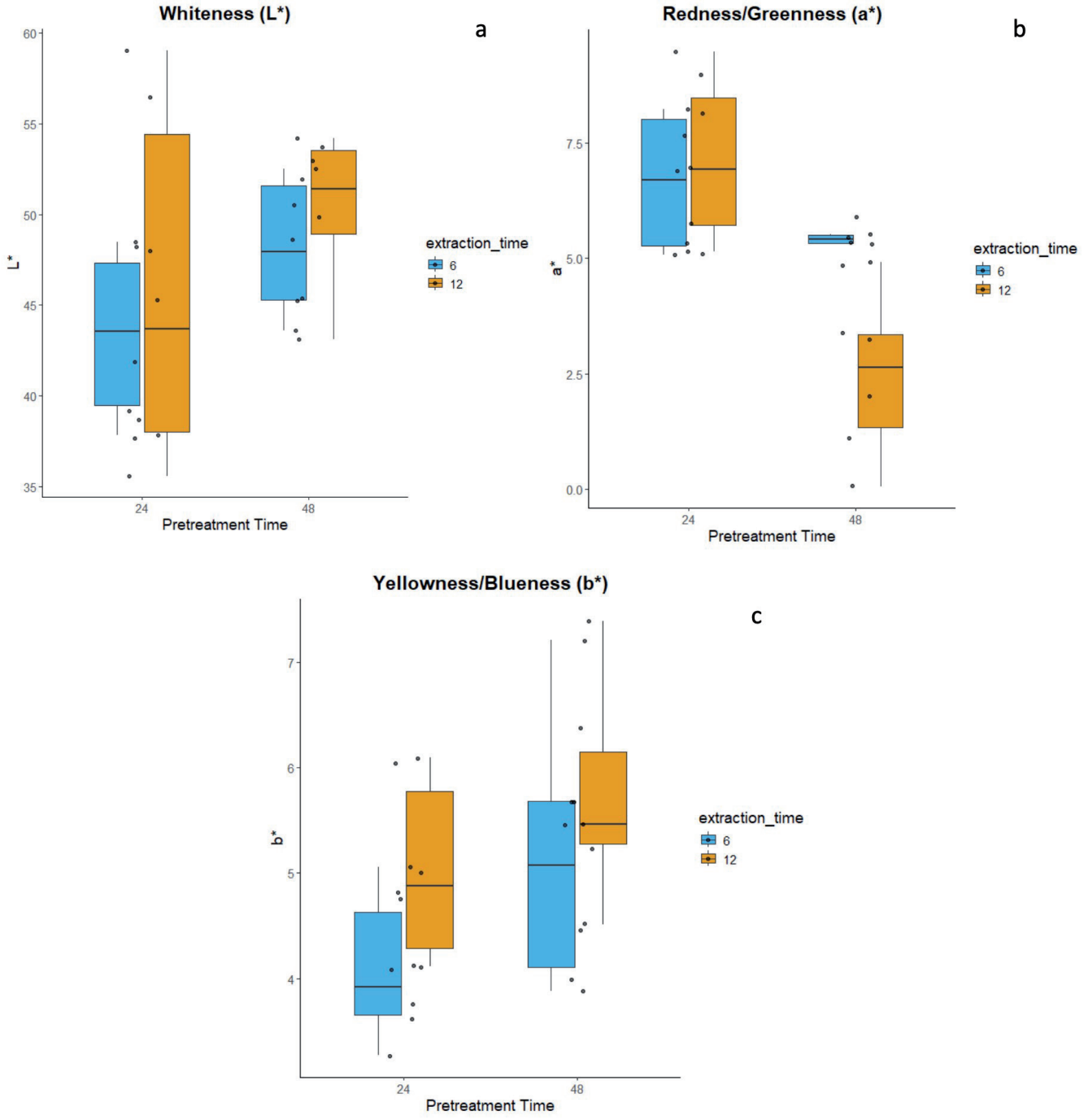

Figure 7: Colorimetric parameters of camel bones gelatin (a: whiteness, b: Redness/Greenness, c: Yellowness/Blueness) /// *Paramètres colorimétriques de la gélatine d'os de chameau (a: blancheur, b: rougeur/verdure, c: jaune/bleu)*

(24h pre-treatment, 6h extraction) yielded an L* value of 43.35, which marginally increased to 46.02 in Test 2 (24h pre-treatment, 12h extraction). A more substantial improvement was observed in Test 3 (48h pre-treatment, 6h extraction) with an L* value of 48.20, while Test 4 (48h pre-treatment, 12h extraction) produced the highest value for whiteness, at 50.41.

Although the study revealed that gelatin whiteness increased with longer pre-treatment and chemo-thermal extraction, our statistical analysis showed that the effect was not significant. Pre-treatment time had a p-value of 0.0852, suggesting a moderate but not statistically significant influence at the 0.05 significance level. Extraction time had a higher p-value of 0.3504, indicating no significant impact on whiteness. The interaction between pre-treatment and extraction times also showed no significant effect, with a p-value of 0.9273.

Previous research has demonstrated an inverse relationship between extraction temperature and duration and the resultant gelatin whiteness. Kittiphattanabawon et al. (2016) reported that gelatin extracted at high temperatures exhibits more pronounced yellowing compared to that obtained at moderate or low temperatures. However, this phenomenon appears to be species-specific and camel bone gelatin does not follow this pattern. Empirical observations and visual assessments of camel bone gelatin samples reveal negligible differences in brightness, with a consistently high degree of purity.

The exceptional whiteness of camel bone gelatin distinguishes it from gelatin derived from camel leather (L* = 22.8) (Redjeb, 2022). This unique characteristic may be linked to the intrinsic properties of camel bone or species-specific responses to the extraction process.

The impact of pre-treatment and extraction conditions on the redness/greenness (a*) values of camel bone gelatin shows varying effects (Figure 7, b). In Test 1 (24h pre-treatment, 6h extraction), the a* value was 6.66, indicating a red hue. Test 2 (24h pre-treatment, 12h extraction) increased slightly to 7.20, possibly due to the release of additional color compounds. However, Test 3 (48h pre-treatment, 6h extraction) led to a significant decrease to 5.36, suggesting the breakdown of red compounds with extended pre-treatment. Test 4 (48h pre-treatment, 12h extraction) showed the lowest a* value at 2.79, indicating that prolonged chemo-thermal processing effectively neutralizes red pigments. Statistical analysis revealed that the pre-treatment duration has a highly significant effect on redness/greenness ($p = 8.06\times 10^{-5}$, $\alpha < 0.001$), revealing its critical role with regard to gelatin's chromatic properties. The extraction time appeared to have a marginal, but non-significant effect ($p = 0.0530$) on the red-green balance. The interaction between pre-treatment and extraction times is statistically significant ($p = 0.0102$, $\alpha < 0.05$). Therefore, it may determine the gelatin's final red-green hue.

The yellowness/blueness (b*) values of camel bone gelatin show a consistent increase in yellowness with longer pre-treatment and extraction protocols (Figure 7, c). Test 1 yielded a b* value of 3.94. The value increased to 4.96 in Test 2, with a longer extraction time. In Test 3 (48-hour pre-treatment), the b* value rose to 5.59. In Test 4, which had the longest pre-treatment and extraction protocol, the b* value peaked at 5.78. This progression suggests that prolonged processing enhances the release of yellow pigments. Statistical analysis shows that the pre-treatment time significantly affects the yellowness/blueness ($p = 0.0410$). In contrast, extraction time appears to have a moderate effect on the b* value, but this is not statistically significant ($p = 0.0772$). The interaction between pre-treatment and extraction times is not significant ($p = 0.6817$), which suggests that there is no combined effect on the gelatin's yellowness/blueness.

Gelatin's chromatic properties are intrinsically linked to the nature of the raw material used in the extraction process. The importance of gelatin coloration varies depending on the intended application. While color does not directly impact the functional properties of gelatin, it can influence its interactions with other chromophores. This factor is particularly relevant in applications where gelatin serves as a component in complex multi-colored systems.

These findings underscore the importance of species-specific research in gelatin production and highlight the potential advantages of camel bone as a source material for bright high quality gelatin. Further investigation of the molecular basis for species-specific differences could provide valuable insights and help optimize gelatin extraction processes across various source materials.

Functional properties of gelatin

Many of gelatin's properties are linked to its water holding capacity and fat binding ability, both of which are influenced by the chemical interactions between gelatin proteins and other substances. Gelatin's ability to retain water is attributed to its polar nature. It contains hydrophilic bonds that facilitate the retention of water molecules within its protein matrix (Kudo and Nakashima, 2020).

Fat binding capacity is also a functional property closely related to texture and water/oil reactions. The structural integration between gelatin protein and lipid molecules promotes binding convergence, as proteins often feature specific binding sites capable of recognizing and binding certain types of fat molecules (Corey et al., 2019).

Water holding capacity is a critical functional property. Our gelatin samples exhibit significant variation for WHC across the tests, which confirms the impact of pre-treatment and chemo-thermal extraction protocols on WHC (Figure 8). Test 1 has the highest WHC at 1080%, demonstrating that a 24-hour pre-treatment combined with a 6-hour extraction effectively preserves the gelatin's water retaining ability.

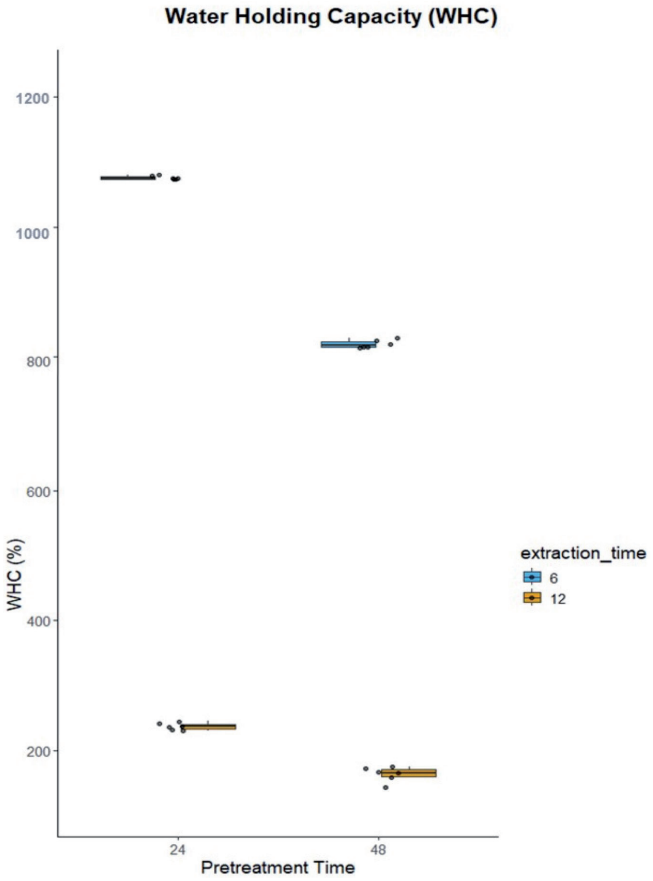

Figure 8: Water holding capacity of camel bones gelatin /// *Capacité de rétention d'eau de la gélatine d'os de dromadaire*

Conversely, in Test 2, which has a longer extraction time, the WHC drops sharply to 234%. This may be due to the over-degradation of the protein matrix, which undermines the WHC. Test 3, with a 48-hour pre-treatment and a 6-hour extraction, shows a partial recovery of WHC to 825%. This suggests that while longer pre-treatment may enhance water holding potential, it is crucial to limit extraction time to prevent excessive protein breakdown. However, in Test 4, with the longest pre-treatment and extraction times, there is a further decrease in WHC to 165%. This reveals the detrimental effects of prolonged processing.

There are significant differences in WHC across various pre-treatment and extraction conditions. With a 24-hour pre-treatment, a significant difference is observed between the 12-hour and 6-hour extraction times ($p = 0.0016$). Extending pre-treatment from 24 to 48 hours, while maintaining a 6-hour extraction, has an even greater impact on WHC, with a highly significant p-value of 0.0000. In addition, the difference between 12-hour and 6-hour extraction times after a 48-hour pre-treatment remains significant ($p = 0.0016$), indicating that extraction time influences WHC even after extended pre-treatment. However, other comparisons yield p-values of around 0.0708, which means that these differences are not significant.

The water holding capacity of camel bone gelatin generally increases under milder extraction conditions, since less aggressive processing helps preserve the protein structure, enhancing its ability to retain water.

The fat binding capacity (FBC) of the gelatin samples exhibits significant variability across the tests (Figure 9). Test 1 shows the highest FBC at 913%, indicating that a 6-hour extraction, combined with a 24-hour pre-treatment, provide the optimal conditions for enhancing fat retention. Conversely, in Test 2, which has a longer 12-hour extraction time, FBC decreases to 656.67%, suggesting that prolonged extraction negatively impacts the gelatin's FBC. In Test 3, the FBC drops to 556.5%, which suggests that extended pre-treatment and a shorter extraction time can diminish fat binding efficiency. However, Test 4 shows a rise in FBC to 748.83%, with both longer pre-treatment and extraction times. Therefore, certain combinations of pre-treatment and extraction protocols may partially restore fat binding capacity. Here, the increased FBC may stem from the removal of fat associated with proteins that have an affinity to collagen during the extended processes. This potentially frees up protein binding sites, enhancing interaction with added fat.

The pre-treatment time has a highly significant impact on FBC ($p = 5.23\times 10^{-7}$), underscoring its crucial role in enhancing fat retention. In contrast, the effect of chemo-thermal extraction time is not statistically significant ($p = 0.0948$), though it approaches the threshold of 0.05, suggesting a moderate influence on FBC. The interaction between pre-treatment and extraction times is highly significant ($p = 8.86\times 10^{-11}$). Therefore, the effects of these two factors appear to be interdependent, suggesting that a specific pre-treatment/extraction protocol is required to maximize FBC.

Gel strength, one of gelatin's critical functional properties, is essential for various industrial applications. GS refers to the gelatin's ability to form a stable, three-dimensional molecular network capable of absorbing and retaining solvent (Bkhairia et al., 2016). Gelatin's capacity for gel formation determines its structural integrity and functionality in different formulations, making it a key quality parameter in industrial applications.

Gelatin is typically categorized into three commercial gel strength ranges: low (50–125 g), medium (175–225 g), and high (225–325 g) (GMIA, 2019). However, certain types of gelatin can exhibit gel strengths that exceed these standard categories. High gel strength is generally preferred, particularly when gelatin is used as a structural stabilizer in various applications. Its excellent gel-forming capacity enhances the stability and integrity of products, making it highly sought after in industries that require robust gelation properties.

Camel bone gelatin exhibits a high bloom value, ranging from 232.69 to 318.96 g (Figure 10). This reveals notable variations in gel strength across the four tests due to the different pre-treatment and extraction times. Test 1, featuring a 24-hour pre-treatment and a 6-hour extraction, yielded the highest gel strength of 318.96 g. This suggests that shorter extraction times help preserve the gelatin's molecular integrity and enhance gel formation. In contrast, Test 2, with a 12-hour extraction and the same pre-treatment conditions, resulted in the lowest gel strength of 232.69 g, due to excessive

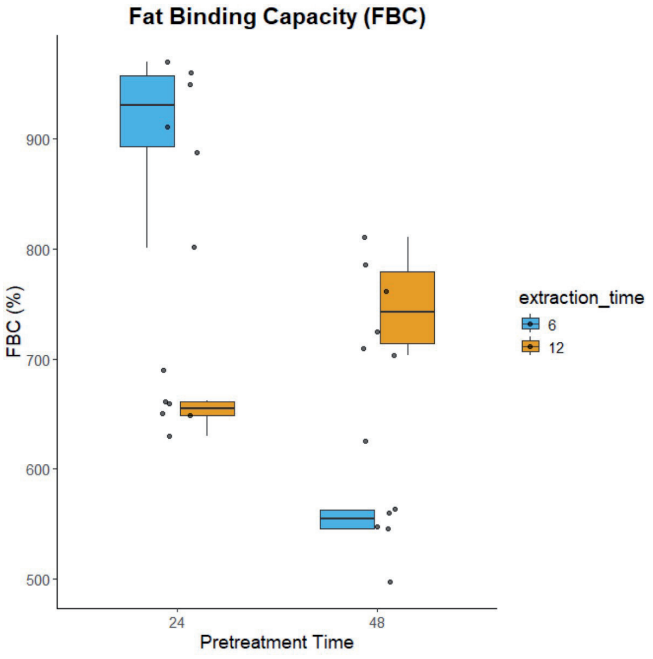

Figure 9: Fat binding capacity of camel bones gelatin /// *Capacité de fixation des graisses de la gélatine d'os de chameau*

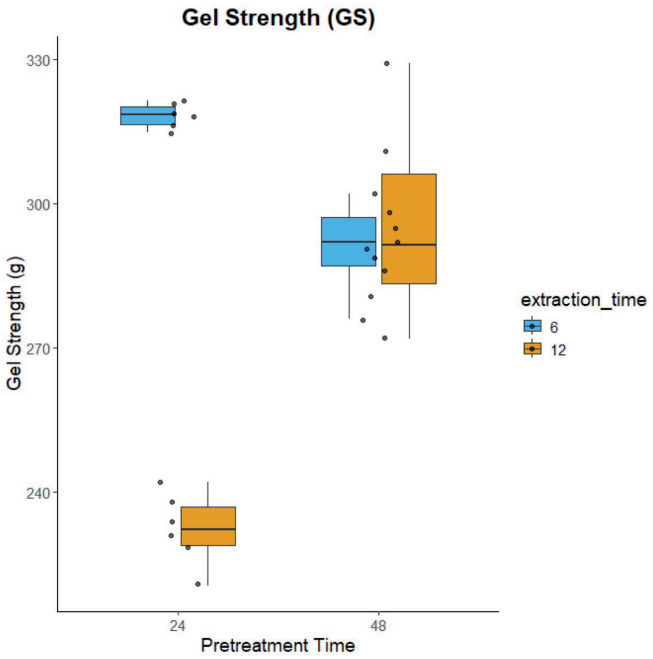

Figure 10: Gel strength (Bloom value) of camel bones gelatin /// *Résistance (indice de Bloom) de la gélatine d'os de dromadaire*

protein breakdown from prolonged extraction. Test 3, with a 48-hour pre-treatment and a 6-hour extraction, obtained a moderate gel strength of 291.03 g. This suggests that while longer pre-treatment can enhance gelatin properties, keeping extraction times within optimal limits is critical. Test 4, combining a 48-hour pre-treatment with a 12-hour extraction, generated a GS of 295.62 g. This suggests that while extended extraction may reduce GS, prolonged pre-treatment can partially offset the degradation.

The analysis underscores the significant impact of pre-treatment and extraction times on GS. Increasing extraction time from 6 to 12 hours with a 24-hour pre-treatment markedly decreases GS ($p = 0.0000$). Conversely, extending the pre-treatment to 48 hours while maintaining a 12-hour extraction substantially improves GS ($p = 0.0064$). A similar trend, showing significant differences ($p = 0.0152$), is observed when comparing a 48-hour pre-treatment with a 6-hour extraction with a 24-hour pre-treatment and a 12-hour extraction. Additionally, comparisons between a 48-hour pre-treatment with either extraction time reveal significant effects ($p = 0.0471$ and $p = 0.0227$). However, no significant difference is found between 6 and 12 hours of extraction after a 48-hour pre-treatment ($p = 0.3720$). Therefore, at this point, further changes in extraction time appear to have a minimal effect on GS.

Pre-treatment and chemo-thermal extraction times are limiting factors that affect the Bloom value, which increases considerably when extraction conditions are shorter. To optimize the Bloom value, the protocol combining a 24-hour pre-treatment and a 6-hour chemo-thermal extraction is preferable.

Camel bone gelatin typically exhibits a higher gel strength than many commercially available choices, including: pig gelatin, which has a Bloom value of 135.80 g (Roy et al., 2021); bovine gelatin with a Bloom value of 238.25 g (Alipal et al., 2021); and cow heart gelatin at 268.84 g (Roy et al., 2017). In our research, camel skin and bone gelatin extracted under various conditions showed gel strengths of 266.69 g (Fawale et al., 2021), 265.8 g (Redjeb, 2022), 226 g (Al Hassan et al., 2021), and 205.74 g (Al-Kahtani et al., 2017). These variations in gel strength are influenced by extraction conditions, which can lead to the dissolution of peptide bonds and an increase in collagen protein solubility. This ultimately reduces the molecular cohesion of gelatin, particularly in terms of proline and hydroxyproline bonds (Skopinska-Wisniewska et al., 2021).

The differences in GS values can be attributed to differences in molecular weight distribution rather than differences in amino acid composition. However, it is important to recognize that other factors may also affect these parameters. A high melting point for mammalian gelatin is an indication of its higher molecular weight (Netter et al., 2020). In gelatin, the hydrogen bonds between water molecules and the free hydroxyl groups of amino acids play a crucial role in determining GS. Gelatin's gel strength increases proportionally with the hydroxyproline content (Tumerkan et al., 2019). Indeed, the hydroxyproline component is important because it affects the gelatin's overall Bloom value.

■ CONCLUSION

The results of this study indicate that camel bones represent a significant source of gelatin with good characteristics, yielding a substantial 21.85%. The duration of pre-treatment and chemo-thermal extraction influences yield. Although yield generally increases with extended extraction protocols, the most pronounced effect on yield occurred with a 48-hour pre-treatment period and a 6-hour chemo-thermal extraction. This finding suggests that optimizing the pre-treatment phase may be crucial for maximizing the gelatin yield from camel bones.

Regarding the qualitative properties of camel bone gelatin, the critical roles of pre-treatment time, chemo-thermal extraction time and their interaction were as follows:

The extended pre-treatment time significantly enhances gelatin yield and solubility, although optimal gelatin characteristics (high GS, whiteness and WHC) are achieved with a 24-hour pre-treatment. This protocol strikes a balance, by maximizing the structural integrity of gelatin, while promoting efficient extraction.

Shorter extraction times yield higher gel strength and fat binding capacity, preserving the structure of the protein. Prolonged extraction negatively impacts these properties, indicating that shorter extraction conditions are preferable.

The interaction between pre-treatment and chemo-thermal extraction times significantly affects various gelatin properties, including pH, whiteness and water holding capacity. Optimizing both parameters is essential for obtaining the desired characteristics of camel bone gelatin, particularly in terms of its commercial application.

Overall, the study emphasizes the importance of fine-tuning extraction parameters to maximize gelatin yield and quality. It positions camel bone gelatin as a superior alternative for industrial applications.

This study acknowledges several limitations, which could orient future research. While the extraction conditions and their effects on camel bone gelatin properties were explored, further investigation into the deep components and molecular weights of the gelatin is needed. Specifically, analysing the molecular weight of amino acids and their composition using high-performance liquid chromatography (HPLC) could offer valuable insights into gelatin's functional properties. In addition, further research is required to improve our understanding of camel bone gelatin and to explore its potential for industrial applications.

Acknowledgments

The authors gratefully acknowledge the University of Kasdi Merbah, Ouargla, as well as the University of the Aegean and the food department, Lemnos, Greece, for supporting and sustaining this work.

Funding

Authors M. Imelhayene and A. Redjeb worked on this study at the University of Kasdi Merbah, Ouargla, Algeria, and the University of the Aegean, Lesvos, Greece. They received financial support from the Algerian Ministry of Higher Education and Scientific Research and the European Union, as part of the international project CAMEL-SHIELD "PRIMA", and from the Erasmus+ programme for the international traineeship. This research only reflects the authors' views and opinions. The European Union and the University of Kasdi Merbah cannot be considered responsible for the views expressed herein.

Conflict of interests

The authors declare that there is no conflict of interest.

Author contributions

IM and SD conceptualized the study; IM conducted the formal analysis; IM, BS, SD and NE investigated the data; IM and SD prepared the original draft of the manuscript; SD and NE contributed to reviewing and editing; AA and SD acquired funding; SD and NE provided resources; AA, RA and NE supervised the study; IM, SD and NE curated the data; SA and AA critically revised the manuscript; and all authors contributed to manuscript validation.

Ethical approval

This research did not involve any direct interaction with animals or human subjects, and thus did not require ethical approval.

Access to research data

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declaration of Generative AI in the writing process

Artificial intelligence (AI) tools were used for auxiliary editing, primarily to improve the linguistic content, logical connectivity and academic writing style. Additionally, they helped with translation tasks and grammatical accuracy. All the scientific content, data interpretation and conclusions presented in this study were independently developed by the authors. The use of AI was strictly limited to language and style improvements and did not contribute to the intellectual substance of the research. The authors assume full responsibility for all the scientific inputs, analyses and conclusions presented in this work.

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Résumé

Imelhayene M., Adamou A., Becila S., Redjeb A., Saidj D., Senoussi A., Sarris D., Naziri E. Évaluation complète des propriétés de la gélatine à partir d'os de dromadaire : impact du prétraitement et de la durée d'extraction

Contexte : Une utilisation innovante des sous-produits issus d'abattoir pourrait générer de la valeur ajoutée à l'élevage de dromadaire. ***Objectif*** : Cette étude explore l'extraction de gélatine à partir des os de dromadaires Sahraouis, âgés de 4 à 4,5 ans, pour maximiser la valeur ajoutée des sous-produits de l'abattage tout en préservant la polyfonctionnalité des dromadaires. ***Méthodes*** : Le processus d'extraction de la gélatine a impliqué la déminéralisation des os dans une solution d'acide chlorhydrique, suivie d'un prétraitement avec de l'hydroxyde de sodium pendant 24 ou 48 heures, puis d'une extraction chimio-thermique dans de l'acide acétique durant 6 ou 12 heures. ***Résultats*** : Le rendement en gélatine obtenu varie de 15,65 % ± 0,15 à 21,85 % ± 0,25, en fonction des durées combinées de prétraitement et d'extraction. Des temps de traitement prolongés augmentent la dégradation structurelle, mais l'analyse élémentaire révèle des niveaux stables de carbone et d'oxygène, avec une teneur en azote fluctuante, corrélée à l'intensité de l'extraction. Le pH de la gélatine se situe entre 4,66 et 4,91, sans grande variation. En termes de propriétés fonctionnelles, la gélatine obtenue affiche une capacité de rétention d'eau élevée de 1080 ± 4,24 % et une capacité de liaison des graisses de 880 ± 98,99 %. De plus, la valeur de Bloom est de 317,96 ± 8,51 g. Ces caractéristiques fonctionnelles sont influencées par les conditions de traitement et s'améliorent sous des paramètres d'extraction plus doux. ***Conclusions*** : La gélatine des os camelins présente des caractéristiques physicochimiques et fonctionnelles souhaitables, y compris des capacités de rétention d'eau et de liaison des graisses élevées ainsi qu'une valeur de bloom favorable, ce qui en fait un candidat précieux pour diverses applications.

Mots-clés : camélidés, gélatine, sous-produit d'abattage, technologie alimentaire, propriété fonctionnelle, Algérie

Resumen

Imelhayene M., Adamou A., Becila S., Redjeb A., Saidj D., Senoussi A., Sarris D., Naziri E. Evaluación exhaustiva de las propiedades de la gelatina de huesos de dromedario: efecto del pretratamiento y de la duración de la extracción

Contexto: Un uso innovador de los subproductos provenientes de los mataderos podría generar valor añadido en la cría de dromedario. ***Objetivo***: Este estudio explora la extracción de gelatina a partir de los huesos de dromedarios saharauis, con edades de 4 a 4,5 años, para maximizar el valor añadido de los subproductos de su sacrificio, conservando la polifuncionalidad de los dromedarios. ***Métodos***: El proceso de extracción de la gelatina implica la desmineralización de los huesos en una solución de ácido clorhídrico, seguida por un pretratamiento con hidróxido de sodio durante 24 o 48 horas y a continuación una extracción químicotérmica en ácido acético durante 6 o 12 horas. ***Resultados***: El rendimiento de gelatina obtenida varía del 15,65 ± 0,15 % al 21,85 ± 0,25 %, en función de las duraciones combinadas de pretratamiento y de extracción. Los tiempos de tratamiento prolongados aumentan la degradación estructural, pero el análisis elemental revela niveles estables de carbono y de oxígeno, con una proporción de nitrógeno fluctuante, correlacionada con la intensidad de la extracción. El pH de la gelatina se encuentra entre 4,66 y 4,91, sin gran variación. En términos de propiedades funcionales, la gelatina obtenida presenta una capacidad de retención de agua elevada, del 1080 ± 4,24 %, y una capacidad de enlace de grasas del 880 ± 98,99 %. Además, el valor de Bloom es de 317,96 ± 8,51 g. Estas características funcionales están influidas por las condiciones de tratamiento y se mejoran con parámetros de extracción menos intensos. ***Conclusiones***: La gelatina de los huesos de camélido presenta características fisicoquímicas y funcionales deseables, incluyendo capacidades de retención de agua y de enlace de grasas elevadas, así como un valor de Bloom favorable, lo que la convierte en una candidata valiosa para diversas aplicaciones.

Palabras clave : camélidos, gelatina, subproductos del matadero, tecnología de alimentos, propiedad funcional, Argelia