Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2017, 9(4):154-164

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Simplified Extraction and Characterization of Acetic Acid Solubilized Type I Collagen Derived from *Solea Solea* Skin and Wistar Rat Tails for Biomedical and Biotechnological Applications

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ABSTRACT

This research project presents an experimental plan to control the best possible processing parameters (acetic acid concentration, centrifugation speed and temperature) for the extraction of acetic acid soluble collagen (AASC) from the Wistar rat tails and the skin of Sole fish (Solea solea). The results showed that these three independent variables had significant effects on the yield and the physico-chemical proprieties of AASC. Moreover, the extraction efficiency was also affected by the interaction between these variables. Optimal conditions for a higher yield of AASC extracted from rat tails, as well as the extracted from Sole fish, were: an acetic acid concentration of 0.1%, a centrifugation speed of 3000 rpm and an extraction temperature between 4 and 20°C. The characterization of AASC shows an acceptable pH value (3 to 5), an electrical conductivity and resistance variable relatively to the temperature and exponentially with acetic acid concentration. Relative viscosity of AASC dissolved in different acetic acid concentrations (0.1, 2.9 and 10%) decrease continuously as the temperature increase from 4 to 50°C, indicating a satisfactory degree of thermal denaturation of AASC molecules. Therefore, a skin of Sole solea, as well as Wistar Rat tail, can be used as alternative collagenous sources.

Keywords: *Solea solea;* Biotechnological applications; Sole fish

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INTRODUCTION

The collagen protein, as popular biomaterial, can be derived from different animal models, as bovine hide, pig skin, and chicken wastes, but creating much anxiety among health-conscious consumers for the past decades due to the outbreaks of bovine spongiform encephalopathy (BSE), foot-and-mouth disease (FMD), and avian flu. More than these health risks, some kind of extraction are not chosen for social or religious reasons. Hindus do not eat cow-related foods and Muslims consider the pig-related products as non-halal which means being non consumable. Therefore, the marine sources have been paid increasing attention as a potential new collagen source for alimentary and biomedical applications as well as the rat tails for the collagen designated especially for research uses [1,2].
Many studies were done and all these studies demonstrate that fish and rat collagen is certainly able to serve as natural alternative sources to change the commercial collagen, and the most of them have utilized sources such as

skins, bones, and tendons during processing of fishes [3-11] or directly from laboratory rats [12] for collagen extraction. These new extraction methods are relatively difficult and need some high developed technologies to be achieved ; these technologies are not available, generally, in normal laboratories, hence the necessity to find some alternative simplified technique, especially that the fish industry in Morocco knows a considerable development these last years. Same, Wistar rat is very used and popular in the Moroccan laboratories for research applications.
The related literature to the extraction of collagen by different acid concentration and their influence on the yield is relatively limited. There is also neither simplified method on the extraction of native collagen from the skin of Moroccan Sole fish and Wistar rat nor any optimization relying on the centrifugation speed influence in the extraction results. Furthermore, it's known that is relatively expensive this process of extraction, and as consequence the high cost of extracted collagen and the collagen as biomaterial for biomedical and research uses.
The aims of this research are to simplify the type I solubilized collagen extraction from two different sources (Sole fish and Wistar rat) and to describe its physicochemical proprieties in different acetic acid concentrations using different centrifugation speeds.
All the preparative procedures were performed at 4 - 20°C. The methods for extraction of collagen from fish skin and rat tails consisted: 1) skin and tails preparation, 2) removing non-collagenous tissues, 3) solubilizing collagen, 4) centrifuging, 5) concentration and yield measurement before the characterizations of the extracted collagen.

EXPERIMENTAL SECTION

Animals

The study was conducted in accordance with the accepted principals outlined in the "Guide for the care and use laboratory animals" prepared by the National Academy of Sciences and published by the National Health Institute.

Collagen Extraction Preparation

The Sole fish and rat-tails were placed in a bag full of ice, then, at the laboratory, the white skin for Sole fish was cleaned by sharp knife from any attached muscle and scales. The cleaned white skin obtained was cut into fine small pieces (0.5 × 0.5 cm). Then it was placed in 500 ml flask. The rat-tails soaked in ETOH 70% to remove debris, after removing skin from rat tail; the tendon was detached by forceps, scalpel and special piler from rat-tails.

Histological Observation

The skins are cut into pieces (0.5 cm × 0.5 cm) and fixed in buffered formalin (5%) for 24 hours. The specimen is dehydrated in a series of graded ethanol solutions (70%, 80%, 90%, and 100%), toluene by Histokinette and finally embedded in paraffin. The perpendicularly sections (5 μm thick) through the skin surface were cut using a Microtome and mounted onto slides. The slides with the sections are treated with toluene to remove paraffin, rehydrated through graded ethanol solutions, in reverse order (100%, 90%, 80%, and 70%), and stained with Masson's trichrome stain. The stained sections are observed through light microscopy [Mono-Leica microscope] and using color digital camera [Fujifilm Finepix JV 16 MP] at an original magnification of 4, 10 and 40X. Finally the digital images are collected.

Removing Non-Collagenous Tissues

After preparing the Sole fish skin and in order to remove non collagenous substance, the skin was treated with 500 ml of NaOH (0.1 M, pH=12) for 24 hours. The skins become very loose.
The solution was placed on the skin and softly stirred for 5 minutes. The resulting solution was replaced to the fridge for a whole day. After that, the solution was removed by using double filter paper (size 125 mm), and the skin was washed carefully with cold distal water until its pH become neutral. During this step, the skin was washed carefully for four times, using of each wash, 600 ml of cold distaled water. pH measurement was taken after each wash. The skin was washing gently, treated with butyl alcohol (10%, Butanol) for 48 hours. The main reason for this treatment was to eliminate fat tissue from the Sole fish skin. After being placed in the fridge for 24 hours, the solution was removed by using the standard 125 mm double filter paper, and replaced by a new 500 ml of butyl alcohol (10%). The solution was placed in the fridge for another 24 hours.

Solubilizing Collagen

After 2 days of butyl alcohol buffer (Butanol), the solution with skin was filtered by using double filter paper (size 125 mm). The skin was washed in cold distilled water thoroughly and gently. In each time, the skin was washed for four times with 600ml of cold distilled water. After the washing, the skin was suspended in 462 ml (66 ml/g) of 0.1, 2.9 or 10% acetic acid with stirred magnetic bar agitation at ambient temperature degree for three days. The system

was composed especially in order to keep a permanent agitation changing the temperature by adding cold water to the closed system checking usually the temperature variation.

Centrifuging Collagen

After three days of acetic acid suspension, the solution was filtered by using 125 mm double filter papers. Then, the solution (11 ml of solubilized collagen/every 15 ml tube) was centrifuged by centrifuge machine [Jouan centrifuge] with speeds of 3000, 4000, or 5000 rpm for 2 hours (in the ambient temperature). The solution obtained, from each centrifuged 10 ml of supernatant was collected in a tube of 15 ml (Figure 1).

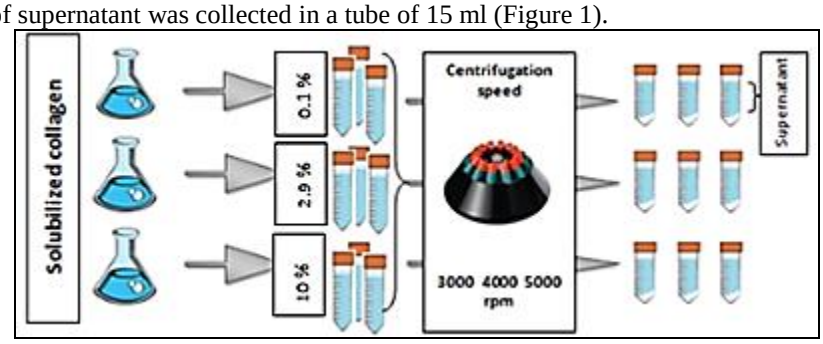


Figure 1: Centrifugation procedure

Yield Measurement

The whole low-cost extraction process was performed. The final sterile solution takes around 2–3 weeks. The result solutions were grouped aseptically condition according to collagen sources, acetic acid concentration and centrifugation speed. The Sole fish skin and rat-tails extracted collagen yields and concentrations were calculated by taking a sterile tissue culture dish 35 × 10 mm and measuring its weight. A small amount of extracted collagen was placed on the dish until the solution evaporated on ambient temperature. The weight of the collagen was taken, and the collagen concentration was deduced by dividing the weight of collagen on the volume of the solution used.

Collagen Yield Measurement

The yield of the resulted acid soluble collagen was deduced using the following equation:

$$\text{Yield of collagen (\%)} = [\text{weight of collagen (g)} / \text{weight of skin (g)}] \times 100\%$$

Checking Type I Efficiency

Preparing fibrillar collagen matrices using basic solution:

Firstly, all buffer used are frozen: 10X DPBS (pH 7.4), acid solubilized rat-tails, tendon and Sole fish skin collagen stock solution, $NaHCO_3$ (Sodium bicarbonate to neutralize the pH), distilled water and sterile tube (15 ml). The collagen gel and check efficiency was performed by mixing on sterile tube 50 µl of 10X DPBS, 250 µl of collagen stock solution and 200 µl of distilled water The mixture was vortexed [Vibrofix], pipetting into sterile wells and checked pH, adjusted by $NaHCO_3$. Tissue culture dish 35 × 10 mm was incubated in 37°C or 4°C. The gel will be removed from solution carelessly by sterile blue pipette. And before leaving the wells dries under the hood until being able to see a white haze (if fibril matrix has formed), which be observed under phase contrast microscopy [Nikon].

Collagen gelation: Ammonia hydroxide method:

In aseptic conditions, ammonia vapor chamber was prepared by taping a 2 inch sterile gauze sponge to the inside a 150 mm Petri dish. The gauze was saturated with ammonium hydroxide. Aseptically, 0.1 µl volume of type I AASC was added to each numerated 0.1 µl well of the polystyrene microtitre plate (12 well/ microtitre), spreading with sterile pipet to cover entire surface. AASC pH was neutralized (by volatile ammonia effect) by placing the microtitre plate inside the 150 mm dish for 24 hours, and observed before removed then dishes from chamber. Finally, the dishes were washed twice by PBS to eliminate the ammonium hydroxide.

Physico-chemical Proprieties of AASC

The physico-chemical characterization was effectuated for the AASC derived from the alternatives origins used for this research.

pH Calibration and Measurement Procedure

The pH meter [wtw pH-Meter pH 522] was adjusted by The two-point calibration at two different pH values. The second calibration point is selected on the basis of the type of solution (acidic in this case pH<7) to be measured. When measuring acidic solutions, acidic and neutral buffers were used (pH = 4.00 and pH=7 solution are common). pH was measured for different concentrations of acetic acid (0.1, 2.9 or 10) and at different degrees of temperature (8, 20 or 37).

Electrical Conductivity and Resistance

In this study, the electric conductivity values of the solubilized collagen were measured by a microprocessor conductivity meter [HydrocureME2673] for different concentrations of acetic acid (0.1, 2.9 or 10), at different degrees of temperature (8, 20 or 37), and with 3 centrifugation speeds (3000, 4000 or 5000 rpm). The electrical resistance was calculated by Amperemeter [Suns YX-2000A] from conductivity values.

Viscosity

A falling ball type viscometer - containing 25 ml of solubilized collagen prepared from different concentrations of acetic acid (0.1, 2.9, or 10) using rat-tails and Sole fish skin- was immersed in a water bath at 4 to 50°C. The viscosity of the AASC was measured for each temperature after 10 minutes of incubation. The fractional change was calculated using the relation [13] :

Relative viscosity= flow time of sample solution / flow time of acetic acid

The temperature of denaturation was determined by causing a 50% decrease in viscosity. Each point is the mean of triplicate determinations.

RESULTS AND DISCUSSION

The Sole fish skin was obtained from Moroccan Sole fish by a simplified method adapted from Hema method [14]. According to Prabjeet et al. [15], the fish skin contain high amount of collagen, this is the reason for chosen as alternative source in the present study. As well as for rat tails, collagen was extracted by an adapted way using a modified method of which used by Huang et al. [16] and Rajan et al. [12] methods. Non-collagenous tissues were removed before the solubilization by acetic acid in designed system capable to keep temperature in 4 to 20°C interval and maintain agitation speed. The aim of this step is to solublize the fibril collagen into tropocollagen.

For the centrifugation step, usually, to extract collagen we use an ultra-quick refrigerated centrifugation, but in this current study, we modified these methods and make an easier, simplified and optimizing collagen extraction for scientific research and biomedical uses. We analyze the impact of temperature and the centrifugation speed.

Histological Observation

Before obtained AASC from our animal sources, a histological observation was done. The Masson's stain colors collagen green and muscle red. This specific staining is a very valuable tool in determining the source of a number of pathologies. The Sole fish skin was stained by the Masson's stain. The results show that: in microscopic observation, clear red color for muscles but not very visible collagen (Figure 2). The same coloration was used for the Rat-tails and we obtained these results: clear red color for muscles and around them, green color for collagen fibers in (Figures 3 and 4). For the best results, we directly stained by Masson's coloration the tendon and the result showed a green zone wrapping around the rat-tail tendons keeping always the red color for a muscle.

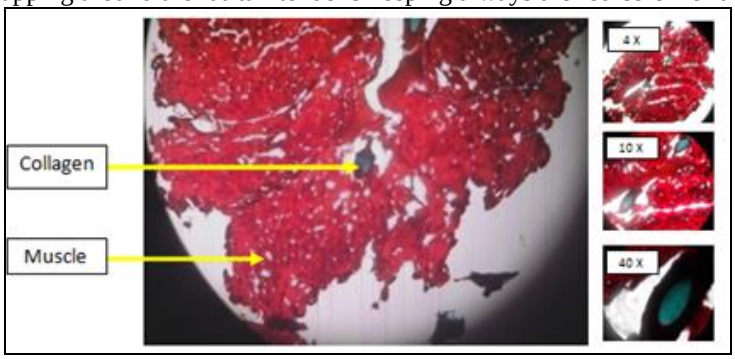


Figure 2: Histological observation of the Sole fish skin spreading (4X)

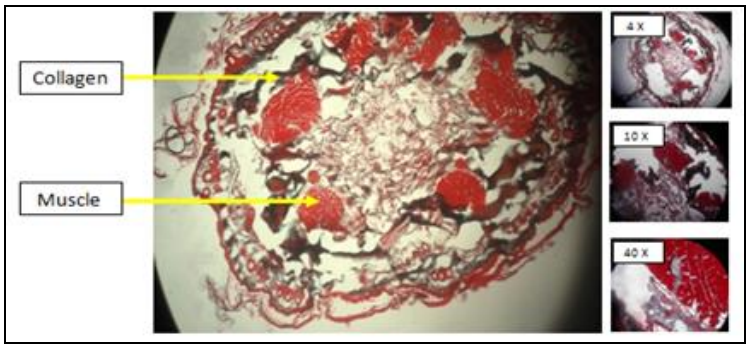


Figure 3: Rat-tails transversal histological observation (4X)

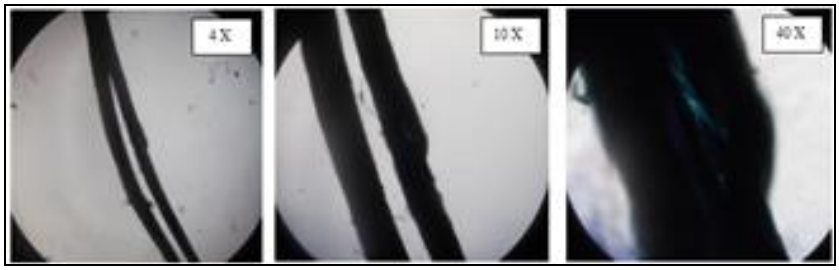


Figure 4: Spreading of rat tails tendons in different magnifications (4X, 10X and 40X)

The Insufficiency of visible green color in the histological observations of tendon and skin (especially for the s/Sole fish skin is probably due to the collagen dissolution, but the collagen traces remarked prove, at least, that collagen exist with more density in Rat-tails that in Sole fish skin. Therefore, the expected result was not very clear.

Collagen Extraction and Yield Measurement

All experiments were conducted in accordance with the Moroccan National Law (License XX-XX). All efforts were made to minimize the number of animals used and their suffering, and the study met the ethical standards used to extract collagen. The result for collagen yield measurement showed a difference between the rat-tails and the Sole fish skin yields. In addition, it was observed that mature female rats present the best yield relatively to male and non-mature rats. The final yield of extracted collagen was calculated and resumed in the following tables (Tables 1 and 2).

Table 1: Yields of sole fish skin AASC

Acetic acid Concentration	Centrifugation speed	Yields
0.10%	3000 rpm	0.175 (±0.046)
	4000 rpm	0.175 (±0.046)
	5000 rpm	0.175 (±0.046)
2.90%	3000 rpm	0.15 (±0.053)
	4000 rpm	0.15 (±0.053)
	5000 rpm	0.137 (±0.051)
10%	3000 rpm	0.112 (±0.035)
	4000 rpm	0.1 (±0.053)
	5000 rpm	0.075 (±0.046)

Table 2: Wistar tails AASC yields

Acetic acid Concentration	Centrifugation speed	Age		
		Less than 20 days	More than 20 days	
			Male	Female
0.10%	3000 rpm	0.312 (0.035)	0.362 (0.051)	0.387 (0.035)
	4000 rpm	0.287 (0.035)	0.337 (0.051)	0.375 (0.046)
	5000 rpm	0.312 (0.035)	0.337 (0.051)	0.35 (0.053)
2.90%	3000 rpm	0.25 (0.053)	0.25 (0.053)	0.287 (0.035)
	4000 rpm	0.187 (0.035)	0.187 (0.035)	0.187 (0.035)
	5000 rpm	0.125 (0.046)	0.25 (0.053)	0.175 (0.046)
10%	3000 rpm	0.087 (0.035)	0.125 (0.046)	0.175 (0.046)
	4000 rpm	0.087 (0.035)	0.125 (0.046)	0.175 (0.046)
	5000 rpm	0.087 (0.035)	0.087 (0.035)	0.125 (0.046)

For AASC solubilized with 0.1% and 3000 rpm , the concentration average of the result solution, was 3 mg/ml (from >20 days female Wistar) and 1.5 mg/ml (from *Sole solea*), but when it was raised to more than these conditions, improvement in the yield of AASC was no longer significant, especially for cell culture applications.
After solublizing collagen and centrifuging. The yields measurement equation, defined the best yields from Sole fish skin and Rat-tails respectively 17.5 and 38.7%. The concentration of acetic acid (and therefore its pH) influences clearly the extraction yields of the protein (solvent effect denaturation thereafter affect the solubility) (Figure 5).

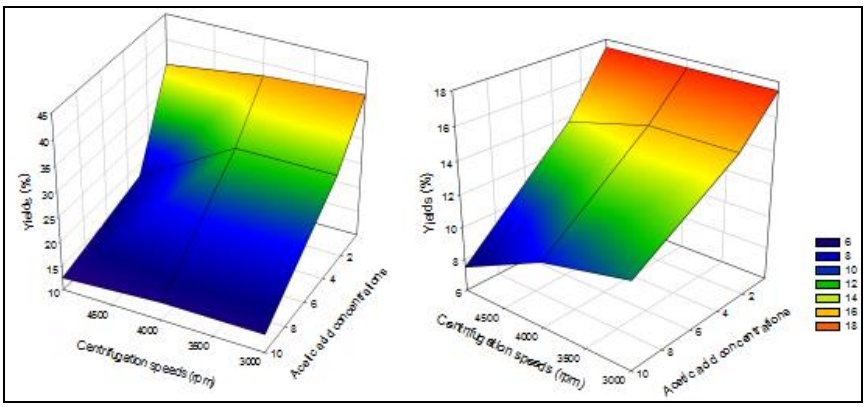


Figure 5: Response surface plot showing the effects of acetic acid concentration and centrifugation speed on the yield of AASC of Rat-tails (left) and Sole fish skin (right). The extraction temperature interval was constant at 4 to 20°C

More precision needs using more measurement tools and protocol optimizations. There are many ways to extract proteins and especially for collagen extraction, 3 major methods known : pepsin-solubilized collagen , salt-solubilized collagen, acid solubilized collagen [17]. In this study, only one method was used: acid-solubilized collagen method with the acetic acid. Therefore, an extraction of collagen using one of the other different methods or all the three methods comparing their results may give more positive findings, and it should be especially recommended using acid pepsin method which showed, in others scientific previous studies, a higher yield result of collagen from various collagen sources [18]. Using stander double layers filter papers in this study for collagen solution might allow a favored passage to collagen through its pore, and quantitative loss. In the future, a specific membrane should be used. For example, using a spectra/ pormwco 12-14,000RC membrane, which is very specific for Collagen type I [19]. This membrane is specific for collagen type I. Other methods for collagen extraction must be used to identify and check the purification of AASC, such as high-performance thin-layer chromatography (HPTLC), Sodium Dodecyl Sulphate Polyacrylamide Gel electrophoresis (SDS-PAGE) and/or modified Lowry's method [20].

Checking AASC Type I Efficiency

As we could not have a positive result for Hydroxyproline test, we tried to check AASC efficiency by gel formation using two methods: basic solution and ammonia method. The aim of these two ways was to neutralize the pH of AASC and induce the matrices formation.

Collagen Matrices Formation by a Basic Solution

A cloudy visual result was observed in the incubated dishes, and it was exanimated under a phase microscopy. The observations were resumed as following: (Tables 3 and 4) (Figures 6-13).
The combination of the results above determines the AASC extracted Wistar Rat-tails as best source of gel formation in the optimal found conditions (37°C and neutral pH). Acetic acid concentration and centrifugation speeds influence the gel formation. It's also noted that the female Wistar give the better result than male.
The black points seen on the observation, under contrast phase microscopy, are normally fibrils extending perpendicularly of the used wells surface. The best result expected for AASC of the Rat-tails are 0.1% Acetic acid concentration and centrifuged at 3000 rpm comparatively to the other used solution for ammonia collagen gelation.
From the observed result we could concludes that the collagen can polymerize at about pH 6.0 to 8.0 but the polymerization efficiency of collagen to gels at lower pH are weaker. In result of basic solution gel formation method: The temperature was also an influent factor of polymerization. The Incubated collagen at high temperatures (37°C) results more polymerized gels than at lower temperature (4°C) weaker and with thicker fibers. The use of more 10X DPBS results larger polymerized gels and leaving plates uncovered in laminar flow hood gives quick results but with a risk to dry if leaved for long duration.

Table 3: Visual estimation of gel formation

			Wistar rats						Sole fish	
			> 20 days				< 20 days			
			Male		Female					
% ac	Cen.sp	pH/T°	4	37	4	37	4	37	4	37
0.1	3000	6	++	++	+++	+++	-	-		
		8	++	++	+++	++++	+	+		
	4000	6	+	+	++	+++	-	-		
		8	+	++	++	+++	-	-		
	5000	6	+	+	+	++	-	-		
		8	+	+	++	++	-	-		
0.5	3000	6	+	+	+	++	-	-		
		8	+	+	+	++	-	-		
	4000	6	-	-	+	++	-	-		
		8	-	+	+	++	-	-		
	5000	6	-	-	+	+	-	-		
		8	-	+	-	+	-	-		
10	3000	6	-	-	-	-	-	-		
		8	-	-	-	-	-	-		
	4000	6	-	-	-	-	-	-		
		8	-	-	-	-	-	-		
	5000	6	-	-	-	-	-	-		
		8	-	-	-	-	-	-		

++++: Very good gel formation; +++: Good gel formation; ++: Weak gel formation; +: Very weak gel formation; -: No gel formation

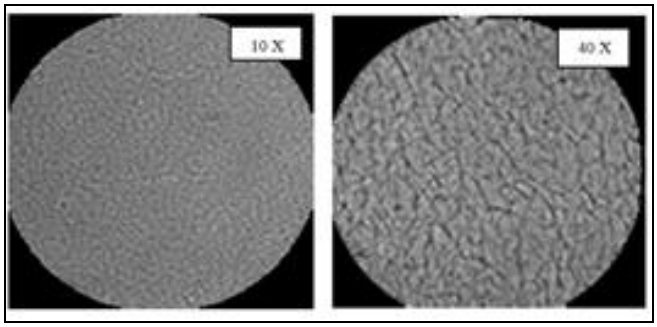
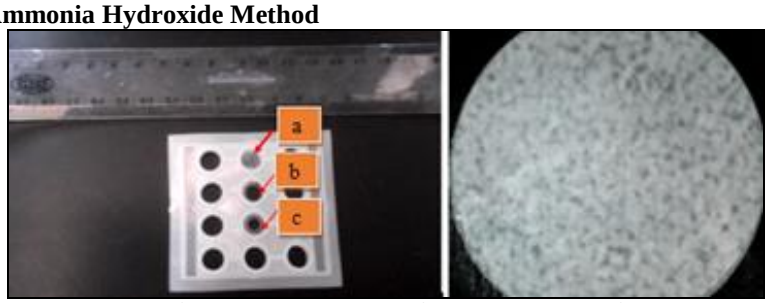


Figure 6: Phase microscopic observation of the formed collagen fibrils

Collagen Gelation: Ammonia Hydroxide Method



(a: Good gel formation, b: very weak gel formation, c: weak gel formation)

Figure 7: Visual and phase microscopic (10x) result of Ammonia method

It was noted, from the obtained results, that the gelation is more observed in Rat-tails extracted collagen, and that it's increases with the increase of incubation temperature, and with the decrease for both: the centrifugation speed and the acetic acid concentration. The same results were partly noted in Ammonia method: The combination of the results above determines the AASC extracted Wistar Rat-tails as best source of gel formation in the optimal founded conditions (37°C and neutral pH). It's also noted that the female Wistar give better result than the male and that acetic acid concentration and centrifugation speeds influence this gel formation. The two methods prove a remarkable efficiency of AASC extracted from the two sources, and the use of the second method (ammonia method) was done to avoid any effect of the AASC contact with neutralizing solution because of the ammonia nature as neutralizing volatile chemical product.

Table 4: Visual estimation of collagen gel formation

		Wistar rats			Sole fish
		> 20 days		< 20 days	
		Male	Female		
0.1	3000	+++	+++	++	++
	4000	++	+++	+	++
	5000	++	++	+	+
0.5	3000	++	+++	+	-
	4000	++	++	+	+
	5000	++	++	+	-
10	3000	-	+	-	-
	4000	-	-	-	-
	5000	-	-	-	-

+++: Good gel formation; ++: Weak gel formation; +: Very weak gel formation; -: No gel formation

Physico-chemical Proprieties of AASC

After checking AASC efficiency, its physic-chemical characterization was done and the results prove that the extracted AASC can be used in scientific and biomedical research applications.

pH Measurement

The acetic acid concentration of and the temperature changes were determinants controller factor of solubilized collagen pH.

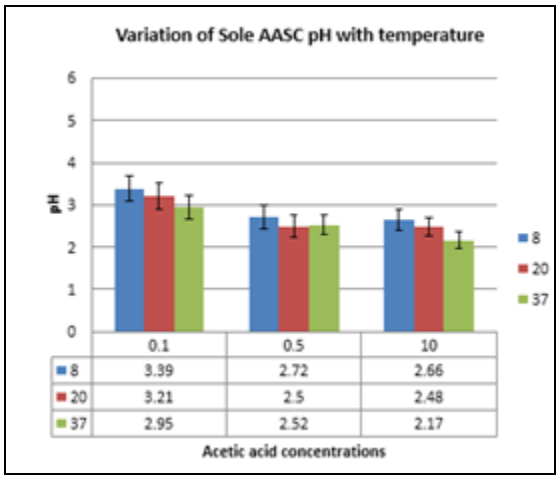


Figure 8: Presentations of the acid concentrations (M) and temperature influence on AASC extracted from sole fish skin

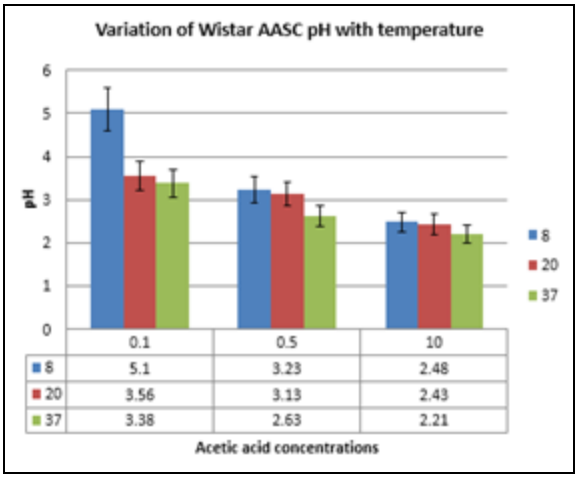


Figure 9: Presentations of the acid concentrations (M) and temperature influence on AASC extracted from Wistar Rat-tails

The influence of acid concentration and the temperature on the collagen extracted was noted, and the influence of centrifugation speed was not measured to eliminate confusions (large standard deviation values were noted), we need sophisticated materials for observation.

The pH of AASC of the two sources was acid due to the use of acetic acid for solubilization, and the best yields correspond respectively to the highest pH values. These pH values are more acid in the case of AASC extracted from Sole fish skin. In addition, and due to the low precision of the used pH meter and the large standard deviations in the results, the influence of centrifugation speed on the AASC pH wasn't measured. This pH variation study demonstrate that the collagen extraction should be done by solution of acetic acid diluted with a relatively high pH (>3) to avoid the collagen denaturation. But, comparatively to the pH of commercial solution of collagen type I (3 to 5), the results were tolerable.

Electrical Conductivity and Resistance

The conductivity of the AASC extracted from the Sole fish skin and Wistar rat-tails was influenced clearly by temperature, acid concentration and slightly by centrifugation speed. The lowest values of conductivity are observed in low values of variable conditions (except centrifugation speed). The results of the electrical conductivity showed expected results that we can discussed as following: the nature of ions and the viscosity of liquids influence the conduction phenomenon. In aqueous solutions the conduction is due to ionic movement and the behavior is totally different from metals. In the human tissues the specific conductivity is variable according to the nature of each tissue [21-24].

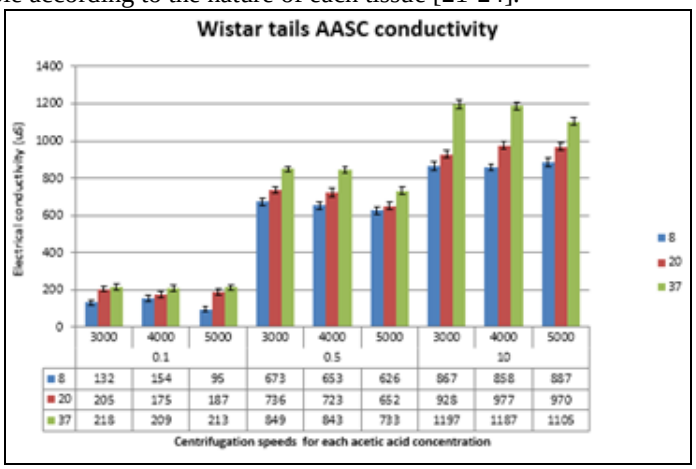


Figure 10: Presentation of the influence of centrifugation speed (rpm), the temperature (°C), and acid concentration (M) on the Wistar (female) rat-tails AASC electrical conductivity (uS)

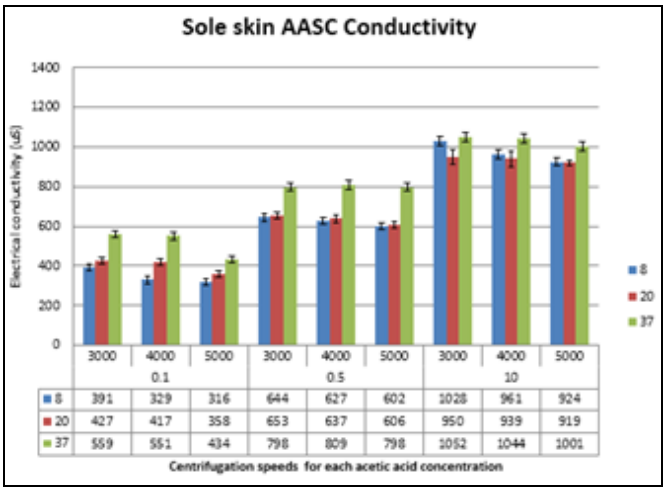


Figure 11: Presentation of the influence of centrifugation speed (rpm), the temperature (°C), and acid concentration (M) on the sole fish skin AASC electrical conductivity (uS)

The theoretical electric resistance values correspond closely to the values found by experimental verification (with an average standard deviation equals to 7 µS). The values of electrical resistance are inversely proportional to those of conductivity. Therefore, the collagen whose yield is highest matches to an electrical semi-conductor or bad conductor. Tomaselli [25] have been proved that the collagen acts as a semiconductor whose direct current conductivity increases exponentially with hydration. They show that everything happens as if, in the context of a semi-electronic conduction, the water was acting a "donor" of charge. This effect of water is reversible and does not involve any chemical alteration of the

collagen. And as known in regenerative medicine, nerves and skeletal muscles respond and communicate via electrical signals; because of this fact, there is recent emphasis on using conductive biomaterials to improve electrical conduction through tissue-engineered scaffolds, and as consequence increase cell differentiation and tissue regeneration.

As expected, the conductivity of the used animal sources rises with the temperature increase, which is similar of what happens in graphite but opposite to what happens in metals. Therefore, it's clearly observed that the electric conductivity of collagen depends on the temperature and increase exponentially with the augmentation of the used acetic acidity concentration. Moreover, the AASC whose yield is higher corresponds generally to low electrical conductivity (high electrical resistance) and vice-versa. That means that we will control cell responses in three-dimensional conductive collagen gel scaffolds, but also produce collagen scaffolds compatible to every tissue regarding its own specific conductivity. The influence of centrifugation speed was opposite to expected. As known, the molecular weight distribution of collagen could be changed by centrifugation. The results obtained can be understood as a possible mechanical degradation of collagen in small scale and in specific conditions of centrifugation favored by the acetic acid concentration which decreases the proportion of population with high molecular weight in favor of those of oligomers and small molecules. But due probably to the hypothesis that a high speed centrifugation for a long time can induce an agglomeration of these oligomers and make conductivity lower. This destruction mechanism depends on the acetic acid concentration because the protonation degree controls the conformation of macromolecules, but also the centrifugation speed which influences probably this molecular distribution and then the collagen conductivity and its energy activation point.

Viscosity

The denaturation temperature (Td) of Sole fish skin and Wistar Rat-tails collagen sample was calculated from the thermal denaturation curves and the Td of the collagen was noted as the temperature at which the fractional viscosity value was 0.5.

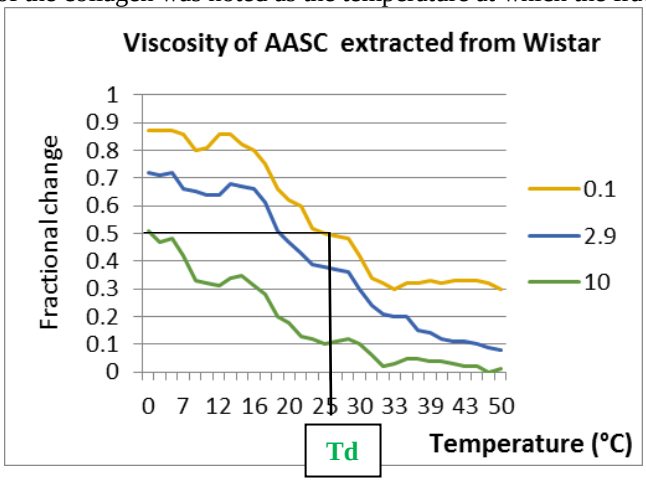


Figure 12: Thermal denaturation curves of Wistar (female) rat-tails collagen solution as measured by viscosity in 0.1, 2.9 and 10% of acetic acid

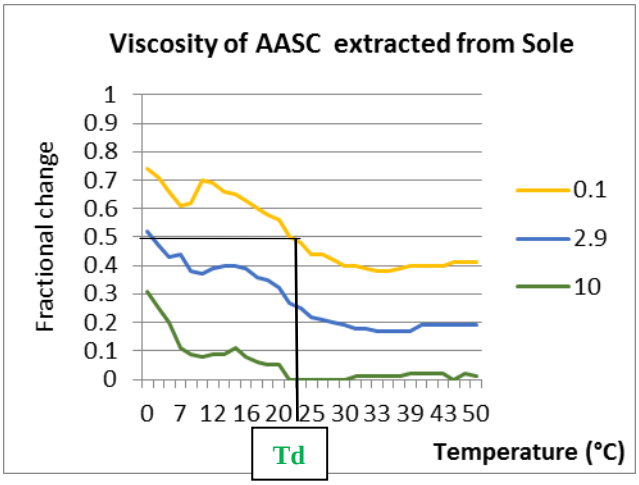


Figure 13: Thermal denaturation curves of sole fish skin collagen solution as measured by viscosity in 0.1, 2.9 and 10% of acetic acid

The result curves illustrate the inverse proportional variation of the AASC (extracted from Sole fish skin and Wistar Rat-tails) viscosity according to the temperature changes and acetic acid concentration. These results signify that the AASC whose the yield is higher, have consequently, a higher denaturation temperature, and it is more viscous.
The viscosity characterization results demonstrated that the best Td of the AASC from Wistar Rat-tails was about 26°C, but the Td of AASC from Sole fish skin was only about 22°C, which was mainly because of the hydrolysis action of enzyme. The Td of the AASC of Sole fish skin, was lower than of Wistar Rat-tails which also was lower or higher than the Td of many other sources of collagen measured under, approximately, the same conditions [26,27]. The earlier studies showed that the stability of collagen is correlated to environmental and body temperatures [28]. And this present studies reported that Hydroxyproline is important in main- taining the stability of trimmers in collagen.

CONCLUSION

This study confirmed that Wistar rat-tails and *Sole solea* fish skin are respectively alternative sources of collagen type I which can be used as natural biocompatible and biodegradable material (scaffolds), especially in 3D cell culture for biomedical applications and research uses. The optimal parameters to produce collagen from both alternatives were: 0.1% of acetic acid concentration; 3000 rpm centrifugation speed and 3 days of extraction time of solubilization in a temperature interval of 4 to 20°C.

ACKNOWLEDGEMENTS

This research was supported by the Faculty of medicine and pharmacy Authors thank Mr Mohammed FELLAHI of Histology – Embryology & Cytogenetics Laboratory (Mohammed V University, Faculty of Medecine and Pharmacy, Rabat, Morocco) for his help and availability during the research and analysis steps.

REFERENCES

[1] S Yamada; K Yamamoto; T Ikeda; K Yanagiguchi; Y Hayashi. *BioMed Res Int.* **2014**, 2014, 8.
[2] C Meena; S Mengi; SG Deshpandy. *Proc Indian Acad Sci*. **1999**, 111, 319-329.
[3] H Yuna; JR Ahn; JW Woo; CK Jung; SM Cho; YB Lee; SB Kim. *Fisheries Aquat Sci*. **2010**, 13, 102-111.
[4] B Gimenez; J Turnay; MA Lizarbe; P Montero; MC Gomez-Guillen. *Food Hydrocolloid.* **2005**, 19, 941-950.
[5] M Sadowska; I Kolodziejska; C Niecikowska. *Food Chem*, **2003**, 81, 257-262.
[6] JW Woo; SJ Yu; SM Cho; YB Lee; SB Kim. *Food Hydrocolloid.* **2008**, 22, 879-887.
[7] P Singh; S Benjakul; S Maqsood; H Kishimura. *Food Chem.* **2011**, 124, 97-105.
[8] LS Senaratne; PJ Park; SK Kim. *Bioresource Technol.* **2006**, 97, 191-197.
[9] JH Muyonga; CGB Cole; KG Duodu. *Food Chem*. **2004**, 85, 81-89.
[10] P Kittiphattanabawon; S Benjakul; W Visessanguan; T Nagai; M Tanaka. *Food Chem.* **2005**, 89, 363-372.
[11] JH Wang; S Mizuta; Y Yokoyama; R Yoshinaka. *Food Chem.* **2007**, 100, 921-925.
[12] N Rajan; J Habermehl; M Coté; CJ Doillon; D Mantovani. *Nat Protocols*. **2007**, 1, 2753-2758
[13] S Kimura; X Zhu; R Matsui; M Shijoh; S Takamizawa. *J Food Sci*. **1988**, 23, 1315-1316.
[14] GS Hema; K Shyni; M Suseela; R Anandan; G Ninan; PT Lakshmanan. *Ann Bio Res.* **2013**, 4, 271-278.
[15] S Prabjeet; B Soottawat; M Sajid; K Hideki. *Food Chem*. **2011**, 1, 97-105.
[16] YC Huang; TW Wang; JS Sun; FH Lin. *J Biomed Sci*. **2005**, 12, 855-67.
[17] AG Ward, A Courts. The science and technology of gelatin, Academic Press, London ; New York, **1977**, xvi, 564.
[18] E Skierka; M Sadowska. *Food Chem.* **2007**, 105, 1302-1306.
[19] S Cliché; J Amiot; C Avezard; C Garie´py. *Science*. **2003**, 82, 503-509
[20] MA Markwell; SM Haas; LL Bieber; NE Tolbert. *Anal Biochem*. **1978**, 87, 206-210.
[21] LA Geddes; LE Baker. *Med Biol Eng*. **1967**, 5, 271-293.
[22] BR Epstein; KR Foster. *Med Biol Eng Comput*. **1983**, 21, 51-55.
[23] S Rush; JA Abildskov; McFeer. *Circ Res*. **1963**, 12, 40-50.
[24] HP Schwan; CF Kay. *Ann NY Acad Sci*. **1957**, 65, 1007-13.
[25] VP Tomaselli. *Biopolymers*. **1974**, 13, 2423-2434.
[26] T Nagai; W Worawattanamateekul; N Suzuki; T Nakamura; T Ito; K Fujiki; M Nakao; T Yano. *Food Chem*, **2000**, 70, 205-208.
[27] T Nagai; Y Araki; N Suzuki. *Food Chem*. **2002**, 78, 137-177.
[28] BJ Rigby. *Nature*. **1968**, 219, 166-167.