The Effect of Gamma Irradiation on Shrinkage Activity of Collagen in Vegetable Tanned Leather

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The dose dependent effect of gamma irradiation on collagen in vegetable tanned leather was studied by the MHT method. Examination of the irradiated samples at 3 months after the irradiation treatment revealed variations of the main shrinkage temperatures and intervals. Up to 25 kGy, the shrinkage temperature does not vary while the total shrinkage interval decreases suggesting cross-linking as the main process. At higher doses, the decrease of shrinkage temperature is accompanied by the increase of the total shrinkage interval indicating that peptide chain scission becomes predominat. Quebracho-tanned sheep leather shows to be the less resistant to the deterioration effect of gamma radiations, while both mimosa-tanned goat leather and quebracho-tanned calf leather better withstand the destabilization effects.

Keywords: vegetable tanned leather, collagen, gamma irradiation, imageMHT, shrinkage activity

Sterilization by ionizing radiation, primarily by ^{60}Co gamma rays, is a low-temperature sterilization method that is routinely used for a number of medical products (e.g., tissue for transplantation, pharmaceuticals, medical devices), food and spices. Where irradiation facilieties were available, starting from 1970s its applications have been expanded to cultural heritage materials [1]. Even though it has high costs, its suitability for large-scale sterilization and volume processing led to considerable work on the application of gamma irradiation sterilization for wooden objects [2-3], other materials [4] and archival paper-based materials [5-8]. However, much less is known about the dose-dependent effect of gamma radiation on collagen-based heritage materials such as parchment, leather, gelatin glue and bone. Nunes *et al.* (2012) [9] tested parchment samples from the archives of University of Coimbra and found no significant decrease in their mechanical and optical properties up to 30 kGy. Lungu *et al.* [10] reported no significat changes in the mechanical properties (i.e. tensile stress, tensile strain and modulus of elasticity) of new vegetable leather and parchment up to 10 kGy. On the other hand, low-field NMR measurements showed a change in the relaxation times of vegetable tanned leather at 25 kGy [11]. It is therefore still necessary to increase knowledge on the effects of medium- to high-dose gamma irradiation on collagen-based materials to find out the limitations for this kind of processing and its suitability for treating historical and hence already damaged collagen-based objects and artefacts.

This paper concerns with the dose-dependent effect of gamma irradiation on shrinkage activity of collagen fibres from vegetable leather. The influence of both tannin type and collagen species on leather thermal stability are evaluated and discussed. Micro Hot Table (MHT) is a consolidated method for the analysis of collagenous materials such as historical and archaeological materials and specific qualitative markers for the evaluation of changes induced by external physical and/or chemical factors have been previously defined [12-22].

Experimental part

Materials, methods and equipments

New vegetable tanned leathers were prepared from calf, sheep and goat hides using commercial vegetable tannins and a method based on traditional recipes [23, 24] developed at the National Research and Development Institute for Textile and Leather, ICPI Division, Bucharest. Quebracho-wood extract was used for tanning calf and sheep hides, while the goat hide was tanned using mimosa-bark extract. The symbols used throughout the paper to identify the three leather types are: Cq for quebracho-tanned calf leather; Sq for quebracho-tanned sheep leather and Gm for mimosa-tanned goat leather. All leather samples were exposed to ^{60}Co gamma rays at the IRASM Center for Technological Irradiations of the Horia Hulubei National Institute for Physics and Nuclear Engineering (HH-IFIN), Bucharest. IRASM facility is a category IV gamma irradiator. The radiation doses applied were 10, 25, 50 and 100 kGy with a dose uniformity ratio (DUR) of 1.14.

ImageMHT method

The measurement of collagen fibres' shrinkage activity was carried out using new portable equipment called imageMHT developed within the project COLLAGE (www.collage.com.ro). Shrinkage motion was digitally recorded with an electronic microscope Dino-Lite® AD7013MZT. The sample (few fibres of about 0.1 – 0.2 mg) was placed on a microscope slide with a concavity, completely immersed in demineralised and degassed water to ensure thoroughly wetting. Then, the fibre bundles were well separated using fine needles and the microscope, covered with a cover glass, placed on the hot stage and heated at a rate of 2°C min^{-1} in the range 25 – 95°C. The shrinkage of the fibres was visualised on the PC

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screen and the shrinkage intervals [25] were automatically evaluated by the imageMHT software [26]. This software substitutes the visual evaluation of the collagen fibres motion by an operator and eliminates the drawbacks of the traditional MHT method, e.g. time-consuming, high errors and impossibility of the inter-laboratory comparison of data, due to subjective factors such as experience, skills, visual acuity and mood of the operator. All measurements were performed three times for each sample and the average values are reported with the standard errors.

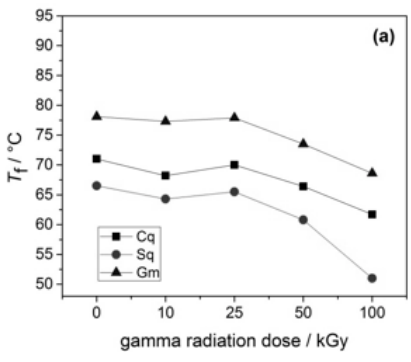
Results and discussions

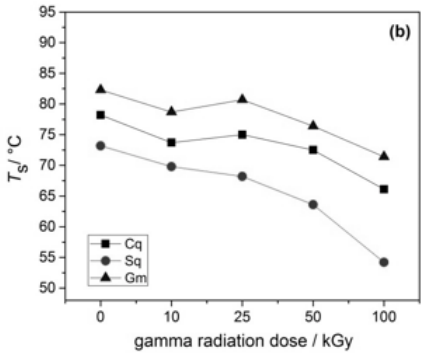
The effect of gamma irradiation on the hydrothermal behaviour of collagen fibres

When heated in water, the collagen triple-helical structure converts to random coil disordered structures over a defined temperature interval. The macroscopic manifestation of this process called thermal denaturation can be observed through a stereomicroscope as a shrinkage motion of the collagen fibres. Collagen shrinkage activity associated with its thermal denaturation is described by a sequence of temperature intervals: no activity - A1 - B1 - C - B2 - A2 - complete shrinkage [27]. In the first two intervals, A1 and B1, shrinkage discretely occurs in individual fibres and displays higher activity (namely higher amount of shrinkage per unit of time) in B1 interval. Then, the majority of the fibre mass shrinks in the main interval *C*. The starting temperature of this interval is the shrinkage temperature, T_s. Generally, the shrinkage activity levels off through B2 and A2 intervals. T_f is the temperature at which the very first motion is observed whereas T_l is the temperature of the very last observed motion. The total shrinkage interval is calculated as $\Delta T = T_l - T_f$.

Of these parameters, only T_s is measured for routine analysis of leather being used as a gross metric of damage in historical and archaelogical leathers. In fact, five categories of damage were defined based on the T_s value microscopic evaluation of collagen fibres coherence [28]. Although T_s is still considered a good marker for damage quantification, the evaluation of T_f and T_f together with shrinkage main and total intervals can provide important information on the leather heterogeneity and its respobse to various physical and chemical deterioration factors [25, 29, 30].

The variation of shrinkage main temperatures, i.e. T_s, T_f and T_l, and intervals, i.e. C and ΔT, in function of for the three leather types are illustrated in figures 1 and 2. The main observation that comes out from figures 1-2 is that collagen-tannin interaction (i.e animal species and tanin type) influence the response of leather to gamma irradiation treatment. Mimosa-tanned goat leather shows the highest thermal stability (i.e. the highest T_s, T_f and T_l values) and lowest structural heterogeneity (i.e. the lowest C and ΔT intervals), whereas quebracho-tanned sheep leather displays the lowest T_s, T_f values and the highest ΔT





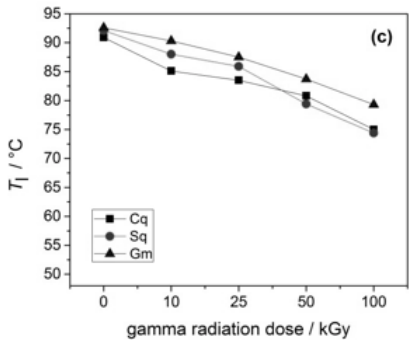


Fig. 1. Variation of the main shrinkage temperatures (a) T_f, (b) T_s and (c) Tlast for the three types of vegetable leather as a function of gamma irradiation dose. Cq – quebracho-tanned calf leather; Sq – quebracho-tanned sheep leather; Gm – mimosa-tanned goat leather

interval. Quebracho-tanned calf leather shows an intermediate thermal stability and structural heterogeneity but behaves similar to mimosa-tanned goat leather (i.e. Cq and Gm plots as a function of radiation dose behaves similarly). The differences between calf and sheep leathers should be mainly ascribed to the specific interaction between collagen from different animal species and tannin. In fact, we have previously reported higher T_s and lower ΔT values for quebracho-tanned calf leather compared to quebracho-tanned sheep leather [29]. It is likely that micro-morphology differences between calf and sheep collagen, especially collagen fibril diameter and orientation [30-32], determine a looser link between collagen and tannin matrix in sheep leather. This, in turn, leads to less resistance against deterioration as reported by some of us in a recent study on the effects of tannin and animal species on thermal stability of vegetable leather [33].

On the other hand, two distinct patterns are evident from the analysis of plots in figures 1 and 2, confirming the influence of dose on vegetable leather hydrothermal behaviour and indicating 25 kGy as a threshold. Specifically, the T_s, T_f and plots show a clear change of trend at 25 kGy, while ΔT plots display a further change of trend at 50 kGy.

At 50 kGy dose, T_s values (fig. 1b), which practically do not vary up to 25 kGy dose except for Sq leather, decreases by about 6°C for Cq and Gm leathers and by 10°C for Sq leather. The decrease doubles at 100 kGy dose. T_f (fig. 1a) behaves as T_s confirming the very good corelation between these two parameters [25, 28, 30]. The T_l variation as a function of gamma radiation dose (fig. 1c) clearly depends on the types of leather: it shows a rather liniar decrease for mimosa-tanned goat leather, while for quebracho-tanned sheep leather and quebracho-tanned calf leather the plots become steeper at 25 kGy and 50 kGy respectively, suggesting a sudden drop of T_l and hence less resistance to deterioration compared to Gm leather.

Yet, ΔT plots (fig. 2b) display a decrease trend up to 25 kGy for all leather types (its decrease reaches about 33% for Cq and Gm leathers and about 40% for Sq leather) followed by a very slight increase at 50 kGy doses except for Sq leather which shows a strong ΔT increase at 100 kGy. T_s, T_f and ΔT variations suggest the prevalence of cross-

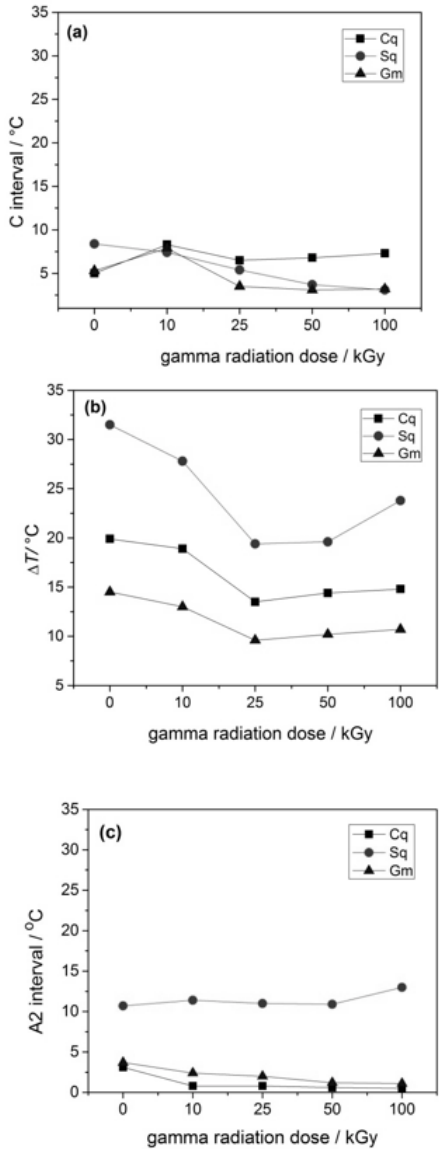


Fig. 2. Variation of the main shrinkage intervals (a) *C*, (b) ΔT and (c) A2 for the three types of vegetable leather as a function of gamma irradiation dose. Cq – quebracho-tanned calf leather; Sq – quebracho-tanned sheep leather; Gm – mimosa-tanned goat leather

linking process up to 25 kGy followed by progressive structural destabilization at higher doses. The sharp ΔT at 100 kGy indicates an increase of structural heterogeneity at 100 kGy for Sq leather. In addition, a monotonous decrease of the main shrinkage interval (fig. 2a) is observed for Sq leather indicating a progressive deterioration. The shrinkage behaviour of collagen in leather at 50 and 100 kGy suggests that polyppeptide chain scisions becomes prevalent and induce the formation of damaged intermediate states with progressively lower thermal stability (i.e. lower T_f and T_s) and occurence of multiple collagen populations with distinct thermal stability (i.e. higher ΔT). Formation of cross-links on human tendon collagen after low dose gamma irradiation was reported by Nikolaeva *et al.* (1988) [34]. For Maslennikova *et al.* [35], the formation of molecular cross-links appeared to be a principal mechanism of collagen damage and remodeling induced by *in vivo* low-dose gamma irradiation, with the cross-links number dependent on radiation dose. On the other hand, the formation of damaged intermediate states with progressively lower thermal stability and occurence of multiple collagen populations with distinct thermal stability was reported by some of us [33] for vegetable leathers exposed to dehydrothermal treatments for increasing times was explained by progressive cleavage of peptide bonds.

Unlike other shrinkage parameters, A2 interval shows only slight variations. Furthermore, much higher values were found for Sq leather than for Cq and Gm leathers that can partially explain the correpsonding higher ΔT values (fig. 2c). Interestingly, Sq leather displays a mirror baheviour compared to Cq and Gm leathers. Large A2 and ΔT intervals were related to the coexistance of collagen population with distinct thermal stability where the shrinkage of more stabilised collagen is mailnly detected in A2 [25]. We could thus infer an intrinsic heterogeneity of Sq leather due to the presence of collagen populations with distinct thermal stability. Most likely, this could be due to a less effective tanning process. More sophisticated techniques such as micro Differential Scanning Calorimetry can provide an unequivocal characterisation of the various collagen populations [15, 25, 36]. This method, however, requires expensive equipment, highly qualified personel and a higher amount of sample (e.g. 2 mg) than MHT method.

It is known that C interval decreases as deterioration increases. We found that it could be even absent in historical pachments [25]. Any relation between C interval value, collagen species and tannin type has been found as far. The plots in Figure 2c do no explicitely indicate a corelation between C interval and gamma radiation dose. More data are necessary to better understand how gamma irradiation influences C interval variation.

Conclusions

The effects of gamma irradiation dose on the thermal stability and structural heterogeneity of vegetable leather was studied by MHT method. The variations of shrinkage temperature T_s and total shrinkage interval ΔT well corelates with gamma radiation dose suggesting the formation of molecular cross-links as the principal process at doses up to 25 kGy and peptide, whereas peptide bonds cleavage prevails over 50 kGy. The collagen species and tannin type and their interaction determine the leather response to gamma irradiation. Quebracho-tanned sheep leather shows to be the less resistant to the deterioration effect of gamma radiations, while mimosa-tanned goat leather better withstand the destabilization effects. The differences between quebracho-tanned calf and sheep leathers were partially ascribed to the different micro-morphology of collagen in calf and sheep hides and partially to the tanning process effectiveness. The results are expected to be of use for choosing safe treatment doses for leather decontamination as well as for other clinical applications.

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