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Variability in pig skin gelatin properties related to production site: A near infrared and fluorescence spectroscopy study

Anne Duconseille [a], Donato Andueza [b], Fabienne Picard [b], Véronique Santé-Lhoutellier [a], Thierry Astruc [a, *]

[a] INRA Auvergne-Rhône-Alpes, Centre de Clermont-Ferrand-Theix, UR370 QuaPA, F-63122 Saint-Genès-Champanelle, France
[b] INRA, UMR Herbivores 1213, F-63122 Saint-Genes-Champanelle, France

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ABSTRACT:

The pharmaceutical industry requires narrow variability in the dissolution rate of hard gelatin capsules. To test this property, gelatin is aged in high temperature and humidity conditions to mimic gelatin shelf-life. These conditions induce cross-link formation in gelatin chains and change the properties of the capsule. Gelatin is produced worldwide in various environmental conditions. This study set out to evaluate the impact of geographic production origin on gelatin composition, before and after aging treatment, and on its dissolution properties. Non-aged and aged pig skin gelatins from three different production plants (A, B and C) were analyzed in raw granules and in powder by near infrared and fluorescence spectroscopy to identify the mechanisms of cross-link formation during aging. Gelatin composition (lipids, dityrosine, 3,4 dihydroxyphenylalanine (DOPA)) and oxidation level, before and after aging, varied according to production origin. The gelatin from C showed no variability in dissolution rate, while gelatins from A and B dissolved more slowly after aging. Near infrared spectroscopy results suggest that water was more strongly bound to the gelatin chains in the gelatins that yielded non-compliant dissolution test results. Non-compliant gelatin exhibited more CH_2, usually assigned to lipids, and more aldehydes. Even before aging we found that non-compliant gelatins tended to fluoresce more at 344 nm under 280 nm excitation. These results open new perspectives for designing tools to predict the dissolution quality of freshly produced gelatins.

1. Introduction

Type A gelatin extracted from the extracellular matrix of pig skins results from collagen solubilization. This industrial process consists in grinding and pre-treating the pig skins in acidic conditions to extract the collagen. Generally, diluted sulfuric or hydrochloric acid (2–4%) is used for up to 24 h at room temperature. During this pre-treatment, most of the fat floats to the surface, and so is easy to remove. The skins are then washed in water for 24 h to remove acid and salts, and immersed in a hot water bath with a temperature ranging from 50 to 100 °C for 18–36 h. In this step, the collagen is solubilized and extracted from the skins. The gelatin obtained is then filtered, demineralized, concentrated, sterilized, dried and ground into granules. During the extraction step, Maillard reactions can occur between the proteins and the traces of lipids, darkening the gelatin (Schrieber & Gareis, 2007). Bleaching agents such as hydrogen peroxide can be added to lighten the color (Schrieber & Gareis, 2007). The pharmaceutical industry uses gelatin to make hard capsules that must meet dissolution quality standards to release drugs homogeneously throughout their shelf-life (United States Pharmacopeial Convention, 2012). Gelatin film dissolution in water is assessed by the increase of absorbance in the dissolution medium at 218 nm. At least 50% of the gelatin has to dissolve in 9 min to meet the quality standard, throughout its lifetime. Gelatin dissolution is generally always good soon after it has been produced and variability in dissolution generally appears after aging (Duconseille, Andueza, Picard, Santé-Lhoutellier, & Astruc, 2016). Thus prior to the dissolution test the gelatin is artificially aged. This aging step consists in exposing gelatins to high temperature and humidity conditions for several weeks.

One explanation for this variability that occurs mainly after gelatin aging could be an effect of the pig breed differences and to the making process which could influence cross-link formation

* Corresponding author.
E-mail address: thierry.astruc@clermont.inra.fr (T. Astruc).

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between gelatin chains. Although not all the mechanisms of cross-link formation have been identified (Duconseille, Astruc, Quintana, Meersman, & Sante-Lhoutellier, 2015), Maillard reactions are known to be responsible for the formation of some cross-links in high temperature and humidity conditions (Digenis, Gold, & Shah, 1994).

Gelatin is produced worldwide at production sites with various climates (temperatures, humidity, etc.). The raw material (pig skins) is likely to differ in composition according to the country of production because of variability in genotypes, feeding and rearing conditions. Thus the manufacturers need to adapt their manufacturing process according to the raw material used in order to decrease variability in the final product.

During the manufacturing process, gelatin is submitted to pH and temperature variations that are known to change its structure (Digenis et al., 1994; Ofner, Zhang, Jobeck, & Bowman, 2001; Schrieber & Gareis, 2007; Yannas & Tobolsky, 1967). As increase in temperature increases cross-link formation (Digenis et al., 1994), it can be assumed that the process may influence this reaction. Gelatin is a polycrystalline polymer whose structure consists of helical regions composed of triple or double helices and a coil structure, also called amorphous phase. The triple helices, considered as physical bonds (Yang et al., 2016), reduce the mobility of the gelatin chains and thus should impair cross-link formation. During manufacturing, the drying step is particularly important because speed of drying influences gelatin structure (Dai, Chen, & Liu, 2006). In order to gain a better understanding of the impact of gelatin manufacturing on cross-link formation and on its dissolution in water, it is necessary to preserve the physical state of the gelatin, and avoid any sample transformations such as solubilization. Near infrared and fluorescence spectroscopy can be used to study raw gelatin, i.e. in its commercial form, with no prior sample solubilization. These two techniques have already been used to study the structure, composition and physico-chemical properties of gelatins (Hashim et al., 2010; Liu, Yao, Wang, & Li, 2000; Segtnan, Kvaal, Rukke, Schuller, & Isaksson, 2003; Segtnan & Isaksson, 2004). Segtnan et al. (2003) found that near infrared spectroscopy (NIRS) combined with a partial least square (PLS) regression could accurately predict the bloom, viscosity and moisture content of gelatin gels and dried gelatins, with a better prediction for the latter form.

Here we studied three production sites: A, B and C. The objective was to determine and explain the impact of production origin on the chemical composition and dissolution rate in water of fresh and aged pig skin gelatins and to suggest tools to predict the dissolution quality of gelatins. NIR and fluorescence spectroscopy combined with data processing were used to identify the key chemical functions involved in the possible changes.

2. Materials and methods

2.1. Gelatin processing

Pig skin gelatin batches from the three different production sites A, B and C were obtained from the same supplier. Sites A and C were located in EU countries (France and Belgium respectively) and site B was located in the USA. Irrespective of production site, pig skins were immersed in a sulfuric acid bath (pH = 1.3) at 18 °C for 8 h. The skins were washed in water and the gelatin extracted in a hot water bath. The gelatin was filtered, deionized, sterilized, dried and granulated. The main differences between the three processes are given in Table 1.

The final moisture content of the gelatins was around 12% for all three sites.

2.2. Dissolution level determination

The dissolution rate of gelatin was determined in six repeats per sample after the aging step following the USP convention, apparatus 2 (United States Pharmacopeial Convention, 2012). Gelatin films were prepared and aged for 4 weeks in a climatic chamber at 50 °C ± 2 °C and 80% ± 5% of relative humidity (RH). They were introduced in sinkers and immersed in the tanks of apparatus 2 described in the USP convention. The tanks contained deionized water at 37 °C stirred with paddles at 50 rpm. The absorbance at 218 nm of the dissolution medium was measured at 0, 3, 6, 9, 12 and 15 min. The dissolution rate was considered as corresponding to "compliant gelatin" when at least 50% of the gelatin had dissolved at 9 min. Otherwise, the dissolution rate was considered as indicating "non-compliant gelatin".

2.3. NIR spectroscopy analysis

Analysis was performed on all the samples in granule form provided by the company, i. e. 10 samples from A, 44 samples from B and 13 samples from C. In total 67 gelatin samples with "compliant" and "non-compliant" dissolution rates, before and after aging, in five repeats, using a Foss NIRSystems model 6500 scanning visible/NIR spectrometer (Foss NIRSystems, Silver Spring, MD, USA) under ISIscan software version 2.21 (Infrasoft International, Port Matilda, PA, USA). The aging conditions of the granules were the same than those previously described: 4 weeks in a climatic chamber (Sanyo, 294L, Osaka, Japan) at 50 °C ± 2 °C and 80% ±5% of relative humidity (RH).

Approximately 3 g of gelatin granules was placed in a 38 mm internal diameter ring cup. Five ring cups were prepared for each of the 134 samples. The ring cups were scanned in reflectance mode at 2 nm intervals from 400 nm to 2500 nm, and 16 scans per cup were applied with a beam width of approximately 1 cm using an automatic sampler. The ring cups were rotated during the scanning so that the whole surface of the sample was analyzed, and the mean spectrum was recorded.

2.4. Fluorescence spectroscopy analysis

Fluorescence spectroscopy was carried out on a subsample owing to acquisition time. A total of 30 non-aged and 30 aged samples (n = 60) were analyzed using a spectrofluorometer in the range 200–750 nm (JASCO model FP-8300, Tokyo, Japan). To reduce variability of the analysis due to granule size, all the granules were crushed into a fine powder. The grinding was done in liquid nitrogen to prevent overheating due to friction. Approximately 300 mg of each powdered gelatin sample was placed in a quartz cup and introduced into the spectrofluorometer. 3D spectra identified two relevant excitation/emission wavelengths; 280/305 nm and 355/420 nm. Each of the 60 samples was analyzed in triplicate using these two excitation wavelengths (280 nm and 355 nm). Emission spectra were acquired from 290 nm to 750 nm for the 280 nm excitation wavelength and from 365 nm to 750 nm for the 355 nm excitation wavelength. The excitation and emission slit widths were both set at 5 nm.

2.5. Spectra treatments and statistical analysis

The spectra obtained from the NIRS were plotted in Log (1/*R*) with *R* the reflectance and an extended multiplicative signal correction (EMSC) was applied under the Unscrambler software (v9.8, Camo Software AS, Norway). A linear baseline correction and normalization was applied to the fluorescence spectra under the same software. The water region was kept in this study because the