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# TOWARDS THE DISCRIMINATION OF GELATIN BY HRMAS: ROLE OF PROCESSING ON ITS CHEMICAL COMPOSITION

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**Abstract** – The gelatin is an animal protein that comes from collagen skin or bone. It is used in pharmaceutical industry to make hard capsules. The variations of environmental conditions of production applied to the collagen and therefore gelatin may have an impact on its chemical composition and, as a consequence, on the capsules properties. Indeed, previous studies revealed that the gelatin tends to form cross-links between its amino acids in high temperature and humidity conditions or in the presence of chemical compounds as aldehydes (sugars, lipids, oxidations). To understand the impact of the processing on the chemical composition of the gelatin, pig skins gelatin produced under different environmental conditions were analyzed in High-Resolution Magic Angle Spinning NMR (HRMAS). 1D proton NMR spectra were acquired and analyzed using PCA (Principal Components Analysis). The environmental conditions of production were discriminated and the spectral zones contributing to separate them showed differences on the amino acids composition and their structural arrangement. However, the abundance of amino acids masked the possible presence of other molecules. In our conditions, the HRMAS, alone, did not allow identifying all the molecules as the cross-links, sugars or lipids, but suggested the formation of Desmosine-type cross-link under one environmental condition of production.

**Key Words** –HRMAS, amino acids, Desmosine, cross-links

## I. INTRODUCTION

Meat industry generates wastes which are valued in by-products such as gelatin. Gelatin has a wide range of applications in food science and pharmaceutical industries use gelatin to coat drugs and make hard capsules. These capsules have to fit to strict quality specifications to be sold on the market. The properties of the capsules depend on

the gelatin properties. This is the reason why the gelatin used to make hard capsules is previously submitted to quality tests validated by the pharmacopeia [1]. Previous studies revealed that the gelatin properties were affected by the variations of the environmental conditions such as the temperature and the humidity [2, 3]. Indeed, under high humidity and/or temperature conditions, the gelatin tends to form cross-links between its amino acids in presence of other compounds as aldehydes (coming from sugars, lipids, protein oxidation or from drugs contained in the capsules) [3]. The structure of gelatin is very sensitive to various environmental parameters such as temperature of extraction during the making process, pH, type of stirring, speed and temperature of the drying step, storage humidity level and temperature [4-6]. NMR spectroscopy has been used to study the structure and composition and physico-chemical properties of gelatin [7] and high-resolution NMR has been applied to study the cross-links in gelatin [8, 9]. These studies showed that it was possible to access to the composition and deduce the structure of gelatin chains with <sup>1</sup>H NMR and to access to the cross-linking degree with High-Resolution Magic Angle Spinning NMR (HRMAS). On the basis of the literature, the HRMAS has been chosen to differentiate the samples according to their environmental conditions of production. Our objectives were to characterize the impact of the variations of environmental conditions of production on the chemical composition of gelatin, especially the cross-links, and to identify the key chemical functions involved in the cross-links formation in order to better understand the causes of variability in the quality of industrial gelatin.

## II. MATERIALS AND METHODS

### *Samples preparation*

3 groups of gelatin with different environmental conditions of production (6 samples per group), so in total, 18 samples have been used.

Approximately 100 mg of gelatin powder is introduced in a tube with 1 ml of D<sub>2</sub>O and swelled for 24h at 4°C.

### *Acquisitions*

<sup>1</sup>H NMR experiments were conducted at room temperature using a NMR BRUKER Avance NB at resonance frequency of 500MHz. High-resolution NMR spectra were measured using the probe HR-MAS 500SB BL4 HCD/ZG.

### *Spectra treatment and analysis*

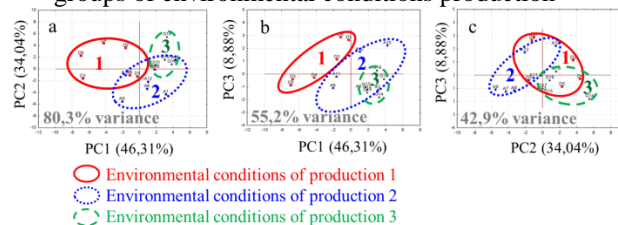
Spectra were divided into buckets. The buckets corresponding to the baseline (0) and the buckets corresponding to the exchangeable protons were removed. Indeed, the exchangeable protons were replaced by the deuterium and became partially invisible.

ANOVA on buckets have been made to identify the relevant areas of spectrum. Then, PCA have been applied on these relevant buckets using Statistica (v12, Statsoft Inc, Tulsa, USA).

## III. RESULTS AND DISCUSSION

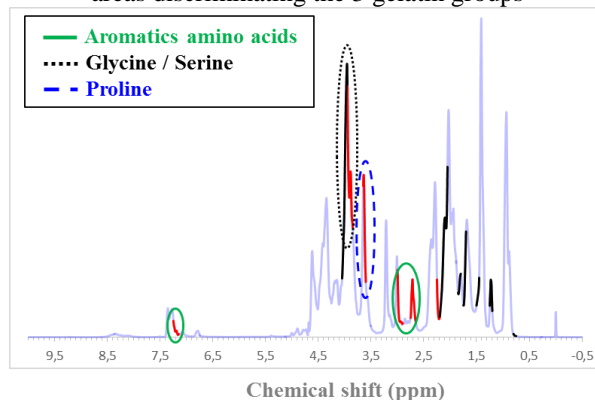
The three environmental conditions of production have been discriminated with HRMAS (Figure 1). The three first principal components separate the three different groups of gelatins with total variance from 42.9% to 80.3%.

Figure 1: Score plots of PCA discriminating the 3 groups of environmental conditions production



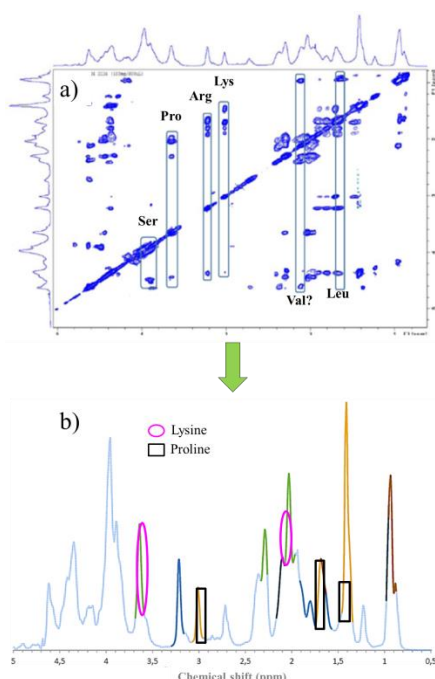
The spectral areas discriminating the groups have been identified and correspond to amino acids chemical shifts [10] (figure 2). PCA score plots (figure 1) and loadings (data not shown) allow identifying the amino acids the most reflective of each environmental condition of production groups. The aromatics amino acids are more visible in the group 3 than in the group 1. The latter has more Glycine/Serine signal than the groups 2 and 3. The couple Glycine/Serine cannot be separated in 1D. The CH<sub>2</sub> functions of Proline are also involved in the separation of the groups. Indeed, the βCH<sub>2</sub> is more reflective of the groups 1 and 2 and the δCH<sub>2</sub> is more visible in the group 3.

Figure 2: Representative spectrum and the relevant areas discriminating the 3 gelatin groups



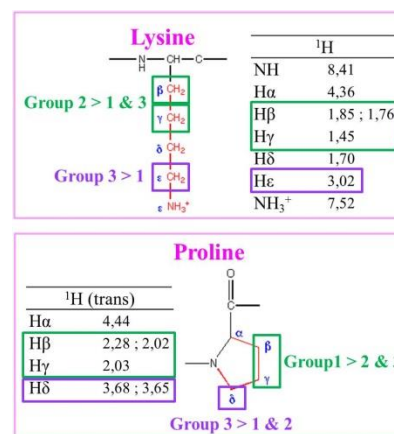
The 1D NMR thus reveals the impact of environmental conditions of production on the amino acids composition and on the structure of gelatin. However, it does not allow making any conclusion about the presence and the nature of cross-links in gelatin. In order to identify others discriminating molecules, a 2D TOCSY has been realized. Among all the amino acids identified (figure 3, a), only the Lysine and Proline are located in discriminating spectral areas (figure 3, b).

Figure 3: 2D TOCSY spectrum with the amino acids identified in 2D (a) and the representation of amino acids signals on 1D corresponding spectrum (b) with, in black squares, the Lysine areas and in pink squares, the Proline areas contributing to discriminate the groups of samples.



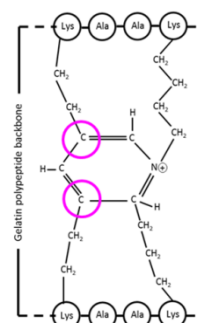
The 2D TOCSY thus allow pointing out the contribution of Lysine in the separation of the three groups of gelatins. The chemical functions of these two amino acids implied in the group discrimination have been identified and are illustrated by the figure 4.

Figure 4: The chemical functions of Lysine and Proline contributing to discriminate the groups of samples



The Lysine result supports the hypothesis of structural differences between the three groups. The  $\beta$  and  $\gamma$  CH<sub>2</sub> functions of Lysine are more reflective of the group 2 than the two other groups. And the  $\epsilon$ CH<sub>2</sub> is more visible in the group 1 than in the group 3. This suggests that the  $\epsilon$ CH<sub>2</sub> of the group 3 is involved in a chemical bond as a cross-link. Knowing that a desmosine-type cross-link implies two  $\epsilon$ CH<sub>2</sub> functions of two lysines (figure 5), this result lead to the hypothesis that the environmental conditions of group 1 would increase the desmosine-type cross-links formation compared to those of group 3 [3].

Figure 5: A Desmosine-type cross-link involving 2  $\epsilon$ CH<sub>2</sub> of Lysine (adapted from Digenis et al. 1994)



#### IV. CONCLUSION

The environmental conditions of gelatin production impact its amino acids composition and its structural arrangement. According to the literature, HRMAS allowed making the hypothesis that environmental conditions have also an impact on cross-links formation, particularly the desmosine-type cross-link.

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