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► **To cite this version:**

Stéphane Portanguen, Charlotte Dumoulin, Maïa Meurillon, Anne Duconseille, Jason Sicard, et al.. Meat-based functional foods - Variability of the glycation reaction in gelatin. 69th International Congress of Meat Science and Technology, Aug 2023, Padova, Italy. hal-04189168

HAL Id: hal-04189168

<https://hal.inrae.fr/hal-04189168>

Submitted on 28 Aug 2023

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MEAT BASED FUNCTIONAL FOOD - VARIABILITY OF THE GLYCATION REACTION IN GELATIN

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I. INTRODUCTION

Glycation is a non-enzymatic biochemical reaction that creates covalent bonds between the carbonyl groups (aldehyde or ketone) of reducing monosaccharides and the amino residues of the side chains of proteins [1, 2]. This reaction has the potential to modify the texture of food products [3] by directly changing the macromolecular structure [4]. It is therefore a possible method to help texturize meat products by 3D printing without adding any texturizing additive. Such an approach could be of great interest for developing meat products suitable for people with oral decline. In this study, using gelatin gels as model media, we developed a multi-scale approach to investigate the effect of glycation on gelatin structure, considering two different Blooms index (which reflect the gel strength) and two different initial water contents. The control of the reaction and its impact on the texture depend on the operating conditions and the gelatin used.

II. MATERIALS AND METHODS

The effect of gelatin Bloom index, initial water content and glycation reaction was characterized by the following methods: CIE L*a*b* colorimetry (highlighting the reaction), LC-MS/MS (Schiff base formation), Transmission Electron Microscopy - TEM (ultrastructural characterization), Rheology (Textural Profil Analysis - TPA and rheological properties). Gelatins at 125 and 200 Bloom index ("B125" and "B200") were conditioned to obtain dry - "D" ($2.68 \pm 0.04 \text{ kg}_{\text{water}}/\text{kg}_{\text{Dry Matter}}$) and wet - "W" ($3.74 \pm 0.11 \text{ kg}_{\text{w}}/\text{kg}_{\text{DM}}$) powders. The gels were prepared with a pH9 Tris buffer. Glycation was initiated by the addition of 7% *D*-ribose ("G" for glycated) versus 0% for control conditions ("UG" for unglycated). Operating conditions: 50 °C, 3h under stirring.

III. RESULTS AND DISCUSSION

Glycation has a significant impact on the color of gels. After glycation, a^* increases up to 8-20 times and b^* by a factor of 1.5-2 for W and D samples, respectively. This phenomenon is associated with an increase in gel cross-linking [5]. Preliminaries LC-MS/MS analyses showed the formation of conjugates between *D*-ribose and Serine/Threonine residues, which disagree with literature (Lysine/Arginine are preferentially mentioned) [1, 2]. This may be explained by glycation reactions during the gelatin manufacturing process where Lysine/Arginine could already be engaged in covalent bonds. Microstructural analysis (TEM) showed the appearance of network-like structures for B125 WG gel but not for B125 WUG gel (Fig. 1 A, B). These structures are attributed to triple-helices. Glycation does not appear to cause this effect for 200 Bloom index (D or W) (Fig. 1 C, D). This would reflect a steric hindrance limiting *D*-ribose access to reactive sites. We observe that the hardness and masticability of UG gels are significantly higher than G gels, indicating that glycation would tend to decrease the mechanical strength of gels.

Bloom index is the most influential parameter for the measurement of shear modulus - G' (higher for B200 D than for B200 W and B125 D/W). This was in line with literature [7]. The addition of *D*-ribose significantly decreases the value of G'_{max} under the same water content conditions. The correlation

between G' and the number of triple-helices was demonstrated by Joly-Duhamel et al. (2002) [6]. The higher the values of G' , the higher the number of triple-helices. Glycation is assumed to strengthen the structure of matrices through covalent bonds [4], resulting in firmer gels, but the opposite is observed here. Similar results have been shown [8], suggesting that glycation would tend to weaken the gel structure. However, it also reinforces the hypothesis that the main reactive sites of gelatin (Lysine and Arginine) are already involved in covalent bonds. The potentially available Serine or Threonine are present in smaller amounts in gelatin than Lysine and Arginine, they would therefore have less impact on structure and texture. These results are in agreement with our TEM observations. The present results suggest that adding *D*-ribose in porcine gelatin gels prevent the aggregation of triple-helices resulting in the weakening of gel strength. A possible explanation of mechanism is that *D*-ribose would prevent the formation of hydrogen bonds between the triple-helices of gelatin by forming hydrogen bonds between protein chains and monosaccharides [6].

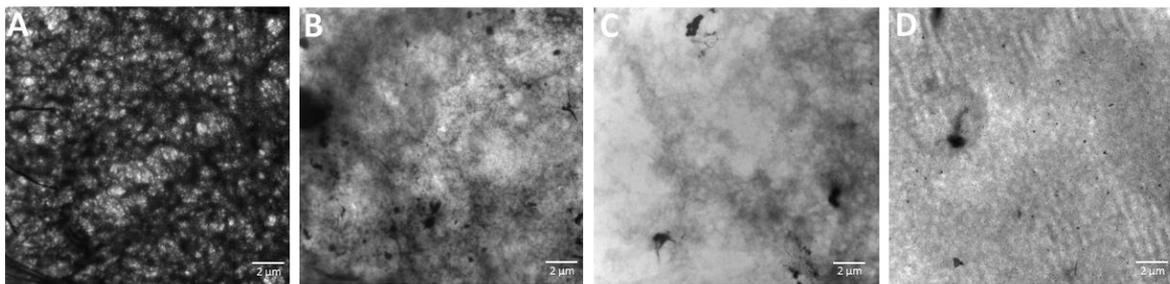


Figure 1. Observation of gelatin gels by TEM: **A** – Gelatin 125 Bloom index wet and glycated (B125 WG). **B** – Gelatin 125 Bloom index wet and unglycated (B125 WUG). **C** – Gelatin 200 Bloom index wet and glycated (B200 WG). **D** – Gelatin 200 Bloom index dried and glycated (B200 DG).

IV. CONCLUSION

The mechanisms of the glycation reaction in a gelatin gel are complex and sometimes contradictory in the literature. Our results suggest that *D*-ribose could: 1) not to bind to the reactive sites because of too much steric hindrance in a gelatin 200 Bloom index, 2) promote, in association with a high-water content, the formation of bonds hydrogen, 3) not to bind because of an insufficient number of reactive sites (previous glycation reactions during the gelatin manufacturing).

ACKNOWLEDGEMENTS

The authors thank the “Centre d’Imagerie Cellulaire Santé” - CICS (Clermont Auvergne University).

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Meat-based functional foods

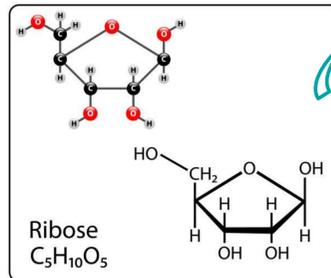
Variability of the glycation reaction in gelatin

Background

ORAL DECLINE: elderly or disabled people



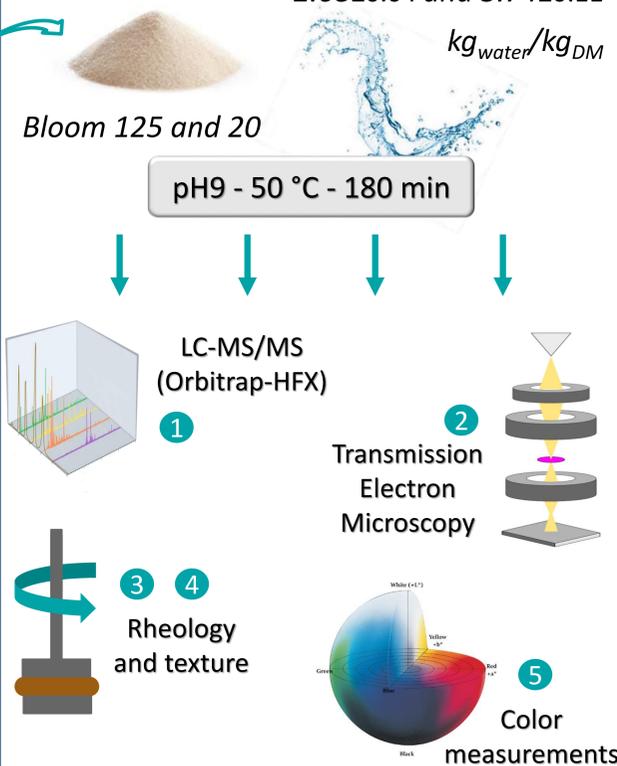
Suitable meat-based foods (gelatin model)



The glycation reaction for texturing

Glycation is a non-enzymatic biochemical reaction that creates covalent bonds between the carbonyl groups (aldehyde or ketone) of reducing saccharides and the amine or hydroxyl groups of the side chains of proteins [1, 2]. This reaction has the potential to modify the texture of foods [3] by directly changing the macromolecular structure [4].

Methods



Objectives

- Characterizing the effect of gelatin water content on its structure and on the glycation reaction;
- Understanding the underlying mechanisms involved

Results

1 Proteomic approach

- Glycation noted on Serine and Threonine
- Limited availability of reactive sites

2 Ultrastructural characterization

- Glycation modifies the ultrastructure of the gel
- Aggregates visible in high water content condition

3 Rheological properties

- Glycated conditions: less structured gels
- Initial water content of the gelatin: effect on the rheological properties of the gels
- More triple-helices for Dry and Unglycated conditions
- D-ribose would interfere with the reformation of triple-helices during cooling

4 Textural properties

- Visible Bloom Effect
- Glycated condition: hardness decreases but better cohesiveness of the gel

5 Color measurements

- Glycation observed
- Initial water content modulates the production of reaction intermediates

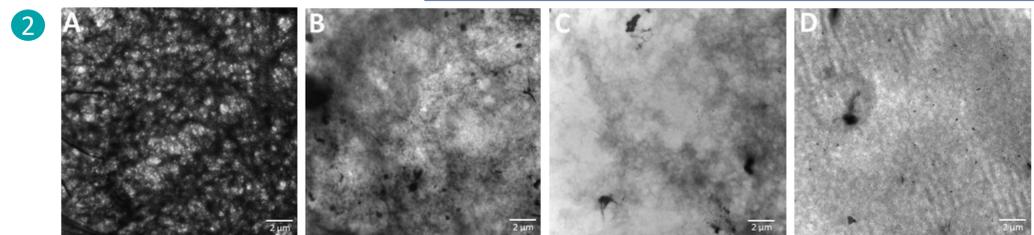


Fig. 1: Observation of gelatin gels by TEM: **A** – Gelatin 125 Bloom index wet and glycated (B125 WG). **B** – Gelatin 125 Bloom index wet and unglycated (B125 WUG). **C** – Gelatin 200 Bloom index wet and glycated (B200 WG). **D** – Gelatin 200 Bloom index dried and glycated (B200 DG).

	Hardness (N)	Chewiness (N)	Cohesiveness	Adhesiveness	Springness
B125	43.28±2.23 ^a	37.99±2.07 ^a	0.89±0.00 ^a	-0.16±0.01 ^a	0.98±0.00 ^a
B200	56.11±2.25 ^b	49.67±1.89 ^b	0.91±0.00 ^b	-0.16±0.01 ^a	0.98±0.00 ^a
Dry	62.29±1.47 ^a	55.08±1.21 ^a	0.90±0.00 ^a	-0.17±0.01 ^a	0.98±0.00 ^a
Wet	37.63±1.20 ^b	33.05±1.15 ^b	0.89±0.00 ^b	-0.15±0.01 ^a	0.98±0.00 ^a
Glycated	45.84±2.24 ^b	40.69±2.04 ^b	0.91±0.00 ^b	-0.15±0.01 ^a	0.98±0.00 ^a
Unglycated	53.84±2.54 ^a	47.22±2.24 ^a	0.89±0.00 ^a	-0.17±0.01 ^a	0.98±0.00 ^a

Table 1: Average values (n=18) of 5 texture variables (column name) of gelatin gels based on a "Texture Profile Analysis" test as function:

- > Bloom index (B125 vs. B200)
- > Initial water content (Dry vs. Wet)
- > Glycated Status (Glycated D-ribose + vs. Unglycated D-ribose -)

Tukey's multiple range test was used. Comparisons are made two by two within same-color rows:

- > **Green** variable show a significant difference ($P < 0.05$)
- > **Blue** variable show no significant difference ($P > 0.05$)

Conclusions

The mechanisms of the glycation reaction in a gelatin gel are complex and sometimes contradictory in the literature. Our results suggest that D-ribose could: 1) not bind to the reactive sites because of too much steric hindrance in a gelatin 200 Bloom index, 2) promote, in association with a high-water content, the formation of hydrogen bonds, 3) not be bound because of an insufficient number of reactive sites (previous glycation reactions during gelatin manufacturing).



References

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