Chemometric analysis of Near-Infrared spectra for determining molecular distribution of gelatin extracted from pork rinds

S. Duthen[1]; K. Jacq[1]; A. Ouali Alami[1]; D. Kleiber[2]; F. Violleau[1]; J. Dayde[2]; C. Raynaud[3]; C. Levasseur-Garcia[1]

[1] *Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRA, INPT, INP-EI PURPAN, Toulouse, France*

[2] *Physiologie, Pathologie et Génétique Végétales (PPGV), Université de Toulouse, INP-EI PURPAN, Toulouse, France*

[3] *Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRA, INPT, Toulouse, France*

Email: *simon.duthen@purpan.fr, cecile.levasseur@purpan.fr*

Introduction

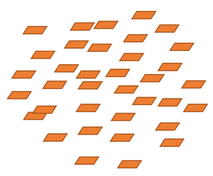
Gelatin is a natural biopolymer obtained by denaturation and partial hydrolysis of collagen, which is a fibrous protein present in the connective tissues of all animal species. Gelatin is used in many different industries, such as food, pharmaceutical, photographic, and cosmetics. Its widespread use is mainly due to its multifunctional properties. The characteristics of gelatin depend on its origins. For example, fish gelatin has a lower melting temperature than pork gelatin (≈ 35–37 °C) due to its amino acid composition. However, the origin of a gelatin is not the only factor that determines its properties. Extraction conditions (time, temperature, acid hydrolysis, and basic hydrolysis) also strongly influence the physicochemical properties by affecting the size of the final collagen fragments.

In this work, the molecular-weight distribution of pork gelatin is studied by Asymmetric flow field-flow fractionation (AF4) coupled with multi-angle light scattering. This technique allows the fractionation of proteins ranging from 1×10^4 to 1×10^7 g/mol.

The aim of the present study is to investigate the use NIR spectroscopy to determine the molecular distribution of various pork gelatins.

Material & Methods

Preparation of gelatins

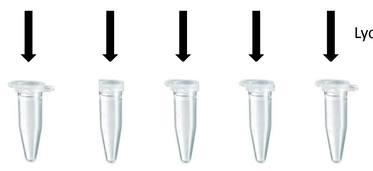


Pork rinds cut into 1 cm square

Acid pretreatment
pH 1,78; 1:10 (w:w)

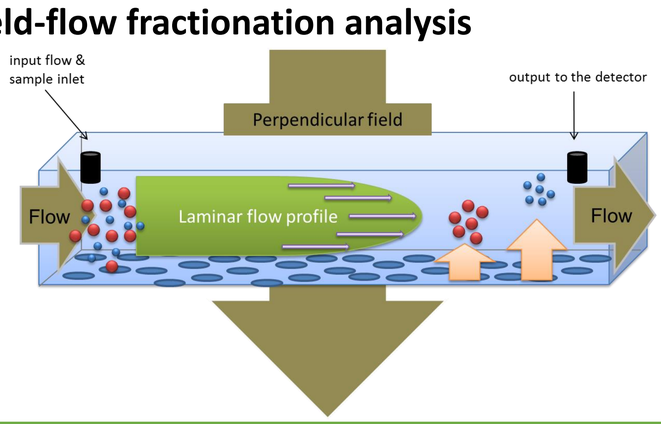
Gelatin hot extraction
60°C, 70°C and 80°C at different time
2, 4, 6, 8, 10, 12 and 14 hs
1:3 (w:w)

Lyophilization



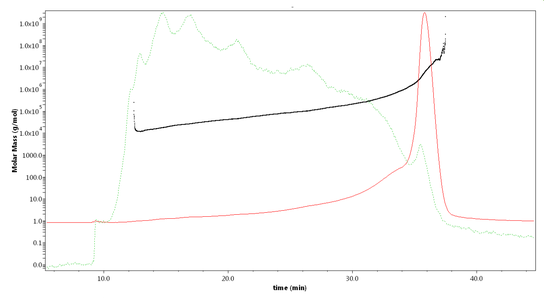
Asymmetric Flow field-flow fractionation analysis

Solubilization of the gelatin in the eluent
1:3 (w:w)



UV detector

MALS detector

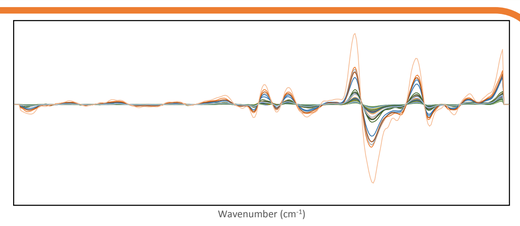


Fractogram from gelatin sample extracted at 60°C after 4 hours. Transmitted UV intensity (green dotted line) and light-scattering intensity (red solid line) are expressed in arbitrary units. The calculated molar mass is represented by the **black dots**.

Near-Infrared spectroscopic analysis

Acquisition of near infrared spectra in transmission (MPA-Bruker)

Transmission acquisition module



Second derivate and smoothing spectrums of lyophilized gelatin samples (6000-8500cm^{-1})

Reference value
Mn total
Total number-average molecular weight

Results

Asymmetric flow field-flow fractionation analysis

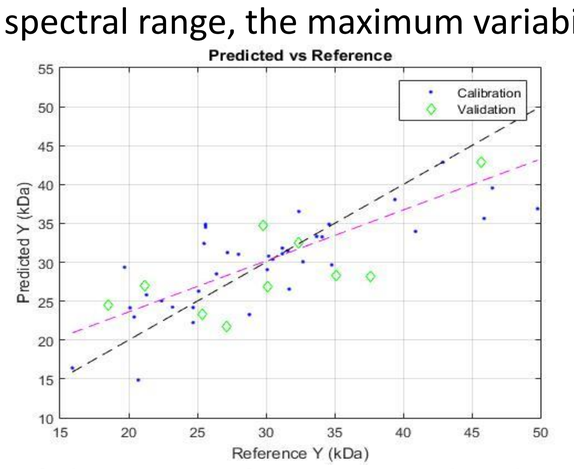
The effect of temperature and extraction time on the total number-average molecular weight (Mn) and the polydispersity highlights a decrease in Mn as a function of temperature and time, and a decrease in polydispersity as a function of time.

Time (h)	Temperature (°C)	Mn (Kda) total	SD	polydispersity
	60	Ø	Ø	Ø
2	70	54.63	5.6	5.5
	80	28.63	7.6	9.5
	60	45.50	6.5	6.2
4	70	45.10	1.6	6.4
	80	24.63	3.0	6.9
	60	33.75	1.5	5.4
6	70	30.35	6.7	8.6
	80	20.53	2.0	8.9
	60	27.55	0.6	5.3
8	70	29.60	0.7	5.5
	80	27.36	6.8	8.2
	60	31.20	Ø	6.4
10	70	25.53	0.9	6.1
	80	20.05	0.5	3.1
	60	31.27	0.7	8.1
12	70	24.40	1.7	4.2
	80	19.03	2.2	2.9
	60	33.70	Ø	3.7
14	70	29.20	3.7	4.4
	80	24.15	0.8	4.1

In addition, Mn decreases strongly at the highest temperature (80 °C). After 8 h, Mn and polydispersity become rather stable. The AF4 analysis thus reveals the differential distribution of molar mass from gelatin samples extracted under different conditions.

Near-Infrared determination of average molecular weight

The gelatin samples extracted from the same pork rinds have the same amino acid composition. The only difference that appears involves the molar distribution. Within this spectral range, the maximum variability is explained by seven PLS components.



Parameters	Calibration	Validation
Unit	kDa	kDa
N	39	12
Outliers	1	1
Min	15,9	18,5
Max	49,80	45,7
Mean	30,48	31,47
RMSEP	Ø	5,59
LVs	7	7
SD	8,47	8,53
SEP	Ø	5,57
SEC	4,91	Ø
RPD	1,70	1,53
R-square	0,76	0,53

The results indicate that the molar distribution may be determined by NIR spectroscopy. The root-mean-square error of prediction (RMSEP) is 5.59 kDa, which corresponds to 16.43% of the range of average molecular weight. The RPD cross validation (data not presented) is 2.06, which suggests that a good quantitative determination of Mn is possible. Both gelatin extraction and AF4 analysis strongly affect the molar distribution, which suggests that the model can be greatly improved with less analytical variability.

Conclusion and perspectives

The results indicate that the near-infrared transmission spectrum varies as a function of total number-average molecular weight. This means that differences other than in chemical composition may be detectable with NIR spectroscopy.

In forthcoming work, we shall study the molar distribution as a function of molecular weight and apply other types of spectroscopy, such as Raman spectroscopy. Although the present work only used gelatins from acid extraction on pork skin, gelatins from fish and beef with acidic or basic extraction should also be considered.

Références

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