Innovative beef protein co-products to substitute gelatine as gelling agents, and sodium caseinate as emulsifiers: Determination of optimal conditions using the response surface methodology (RSM)

Rozenn Le Foll, Francoise Nau, Pascaline Hamon, Catherine Guérin-Dubiard, Xavier Lambert, Amélie Deglaire, Valérie Lechevalier-Datin

LWT - Food Science and Technology 198 (2024) 115945

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Innovative beef protein co-products to substitute gelatine as gelling agents, and sodium caseinate as emulsifiers: Determination of optimal conditions using the response surface methodology (RSM)

Rozenn Le Foll [a], Françoise Nau [a,*], Pascaline Hamon [a], Catherine Guérin-Dubiard [a], Xavier Lambert [b], Amélie Deglaire [a], Valérie Lechevalier [a]

[a] INRAE, L'Institut Agro, STLO, 35042, Rennes, France
[b] CORNILLE sas, 35500, Cornillé, France

ARTICLE INFO

Keywords:
Beef co-product
Collagen
Optimisation
Gel
Emulsion

ABSTRACT

Meat co-products are a promising alternative for meeting the increasing demand for protein, especially for the formulation of meat products. The present study aims to determine the optimal conditions under which two innovative bovine co-products, resulting from the fat rendering process, can mimic the gelling and emulsifying properties of commercial gelatines and sodium caseinate (NaCas), respectively, using Response Surface Methodology (RSM). The desirability function was used to determine the values for protein concentration, pH and NaCl content that enable the two co-products to effectively mimic gelatine and NaCas. The co-product obtained from water recovered during the fat rendering process proved to be the most suitable to mimic commercial gelatines. Very high desirability scores were obtained with this ingredient on 4 criteria out of 7, and a high overall score as well, provided 90 g/L protein was used to mimic a 50 g/L gelatine 150 Bloom. Both co-products appeared as effective alternatives to NaCas as emulsifiers, especially regarding their capacity in stabilizing emulsions. The co-product made of greasy greaves can be even regarded as more effective than NaCas, as less proteins are needed to obtain the same performances (110 g/L vs 125 g/L, respectively).

1. Introduction

The acceleration of climate change, combined with global population growth, threatens worldwide food security (Nelson et al., 2009). In particular, the growing food demand requires to look for new protein sources. Meat co-products are a promising alternative, especially to meet the expected increase in demand for meat-like products, in that they are available protein resources poorly exploited in human food (Lynch, Mullen, O'Neill, Drummond, & Álvarez, 2018). Yet, meat co-products are an excellent source of protein with good nutritional value, which could help mitigate the growing global demand for proteins. However, one of the conditions for the use of such co-products is that, over and above their nutritional value, they express functionalities that enable the desired food structures and textures to be developed, comparable to those obtained with currently available ingredients. It is therefore essential to ensure their functionality under realistic conditions of use.

Fat rendering is a common process in the meat industry, whereby fatty materials are melted away from the solid portion of the animal tissue (Meeker & Hamilton, 2006; Prokop, 1985). This process makes it possible to obtain the fat (tallow), solid protein products in the form of greaves, and water recovered during the process which is generally considered as a waste product (Álvarez, Drummond, & Mullen, 2018). Dehydrated bovine proteins, derived from bovine co-products after fat rendering process, contain a complex mixture of proteins of different molecular weights, and the solubility of which depends on pH and ionic strength (Le Foll et al., 2024). We previously established that the two protein ingredients, obtained through an innovative process (Denis, 2009), may have good gelling properties, in particular due to the presence of collagen, and also good emulsifying properties (Le Foll et al., 2024).

In continuation of this previous study, the question arose as to whether these two dehydrated beef proteins could be effective substitutes for common functional ingredients. To address this question, we

* Corresponding author.
E-mail addresses: rozenn.lefoll@institut-agro.fr (R. Le Foll), francoise.nau@institut-agro.fr (F. Nau), pascaline.hamon@institut-agro.fr (P. Hamon), catherine.guerin@institut-agro.fr (C. Guérin-Dubiard), xavier.lambert@mousquetaires.com (X. Lambert), amelie.deglaire@institut-agro.fr (A. Deglaire), valerie.lechevalier@institut-agro.fr (V. Lechevalier).

https://doi.org/10.1016/j.lwt.2024.115945
Received 15 November 2023; Received in revised form 29 February 2024; Accepted 3 March 2024
Available online 8 March 2024

Abbreviations used

CCD	central composite design
GGRP	greasy greaves recovered proteins
NaCas	sodium caseinate
PCA	principal component analysis
PC	principal component
RSM	response surface methodology
TSI	Turbiscan stability index
WHC	water holding capacity
WRP	water recovered proteins

chose to compare them with gelatines and sodium caseinate (NaCas), chosen as references for gelation and emulsification, respectively. Replacing gelatine with these dehydrated bovine proteins may be interesting from an economic and environmental point of view, as the production process is simpler (no collagen-to-gelatine conversion stage). As for NaCas, it could be interesting to replace it in pH conditions where emulsifying properties of NaCas are impaired, i.e., close to the pI of caseins (pH 4.6) (Rasnani & Mirhosseini, 2011; Surh, 2009).

Gelatine is used in the food industry for a number of functionalities, one of which is its cold gelling power (Gómez-Guillén, Giménez, López-Caballero, & Montero, 2011). The critical concentration for gelling is between 5 and 10 g/kg of gelatine. NaCas is widely used in the food industry for its emulsifying properties, for example in ice creams and frozen desserts, where it contributes to creaminess and texture (Zayas, 1997). It contains approximately 900 g/kg protein and is completely soluble in water, forms viscous solutions, resists thermal denaturation and coagulation, and rapidly forms interfacial films (Cruijsen, 1996).

In the present study, the response surface methodology (RSM, Box & Wilson, 1951) was implemented to determine the optimal conditions (protein concentration, pH, NaCl content) that enable the bovine co-products to mimic the reference functional ingredients, i.e., gelatines for gelling properties and NaCas for emulsifying properties, using the desirability function (Derringer & Suich, 1980). The RSM was used to compare dehydrated bovine proteins with gelatines and NaCas as it is an efficient and accepted methodology in monitoring and optimisation of food manufacturing processes, due to its advantages over conventional methods (Yolmeh & Jafari, 2017). The basic principle of the RSM is to determine model equations that describe the interrelationships between independent and dependent variables (Edwards & Jutan, 1997). Its main advantage is the low number of experimental trials needed to generate enough information to provide a statistically acceptable result.

2. Materials and methods

2.1. Ingredients

The two dehydrated bovine proteins characterized in the present study, namely Greasy Greaves Recovered Proteins (GGRP) and Water Recovered Proteins (WRP), were produced by a local factory (CORNILLE sas, Cornillé, France) from bovine co-products (fat rendering process) (Denis, 2009) previously described (Le Foll et al., 2024). Briefly, GGRP correspond to the solid fraction (greasy greaves); WRP are obtained from water recovered during the fat rendering and bone degreasing processes. The composition of these two bovine ingredients has been detailed in Le Foll et al. (2024) (*Supplementary data*, Table S1).

The commercial ingredients regarded as functional references were two gelatines extracted from bovine skins, namely one of 150 Bloom (880 g/kg protein) purchased from Rousselot (Courbevoie, France) and another of 240 Bloom (900 g/kg protein) from Gelita (Ter Apel, Netherlands), as well as a sodium caseinate (NaCas, 960 g/kg protein) from Ingredia Dairy Experts (Arras, France).

All the ingredient solutions were prepared by dispersing the powders in water according to the experimental design conditions (Table 1), heating to 50 °C, and maintained at this temperature under magnetic stirring for 2 min.

2.2. Experimental design

The effect of protein concentration, pH, and ionic strength on the functional properties of the ingredients, was simultaneously studied using the response surface methodology (RSM, Box and Wilson, 1951). The conditions tested were determined according to a central composite design (CCD), with pH ranging from pH 4 to pH 7; protein concentration from 50 to 200 g/L; and NaCl added from 0 to 0.4 mol/L (Table 1). The experimental domain was selected based on preliminary experiments, and the CCD was designed using Statgraphics software (Statgraphics Technologies, Inc., The Plains, VA). The resulting experimental data were adjusted to a second-degree polynomial regression model that contained coefficients of linear, quadratic and interaction effects (Montgomery, 2013).

2.3. Protein solubility

The protein solubility index of each ingredient was measured in duplicate in each condition of the CCD. This is the percentage of soluble protein, i.e., measured in the supernatant after centrifugation at 160*g* for 5 min, from a solution prepared by mixing 10 g of powder (ingredient) with 100 mL of water (Le Foll et al., 2024).

2.4. Functional properties

All the measurements of functional properties were carried out in the same way as previously described in Le Foll et al. (2024), and summarized below.

2.4.1. Gelling properties

The gelling temperature was measured by monitoring the storage modulus (G′) and the loss modulus (G″) of the solutions during cooling from 50 °C to 1 °C at a rate of 1 °C/min, using an MCR 301 rheometer (Anton Paar, Les Ulis, France) equipped with a cone-plate system (diameter 49.96 mm, angle 1.996°, tcation 209 μm), at a deformation rate of 1% and a frequency of 1 Hz. The gelling temperature was

Table 1
Central Composite Design (CCD) for independent variables of protein solutions. C1: protein concentration (g/L) for gelling properties of WRP, and emulsifying properties of WRP and GGRP; C2: protein concentration (g/L) for gelling properties of GGRP; I: ionic strength (mol/L NaCl added).

	Experimental variables			
	pH	C1	C2	I
1	5.55	125	160	0.20
2	4.44	72	132	0.34
3	5.55	200	200	0.20
4	4.44	178	188	0.06
5	5.55	125	160	0.40
6	5.55	125	160	0.20
7	5.55	50	120	0.20
8	4.44	72	132	0.06
9	4.00	125	160	0.20
10	5.55	125	160	0.20
11	5.55	125	160	0.00
12	6.56	72	132	0.06
13	7.00	125	160	0.20
14	4.44	178	188	0.34
15	6.56	178	188	0.06
16	6.56	72	132	0.34
17	6.56	178	188	0.34
18	5.55	125	160	0.20

determined as the temperature at which G′ and G″ cross or, if they do not cross, when G' becomes greater than 1 Pa.

Gel samples were prepared after dissolving and heating for 30 min, either at 50 °C for WRP, or at 90 °C for GGRP. The solutions were then poured into 2 cm-diameter plastic tubes (Krehalon, Deventer, Netherlands), before storage at 4 °C for 72 h. Gelling properties were determined on 1.5 cm-height and 2 cm-diameter gel cylinders, using a TA-plus texture analyser (Lloyd Instruments, Elancourt, France). The strength and the deformation at the rupture were measured by applying a uniaxial compression at 1 mm/s with a flat 4 cm-diameter probe. A 33% deformation was applied and maintained for 3 min to determine the water content exuded and calculate the water holding capacity (WHC). Hardness, cohesiveness, and adhesiveness were determined from a double compression cycle test (TPA-type test) up to 33% deformation and at 1 mm/s, with a 1.2 cm-diameter cylindrical probe. Measurements were all performed in triplicate for each sample of the CCD.

2.4.2. Emulsifying properties

Emulsions were prepared by homogenizing sunflower oil and each protein solution (O:W 30:70, vol:w; 20,000 rpm for 3 min at 50 °C). The size distribution of the lipid droplets was determined at room temperature immediately after emulsification using a laser scattering particle size analyser (MasterSizer 2000; Malvern, Palaiseau, France), based on three parameters: the mean droplet volume-surface diameter ($d_{3,2}$), the droplet volume ($d_{4,3}$), and the droplet polydispersity index (span). The stability of the emulsions was estimated throughout a resting period of 24 h at 50 °C, using the stability analyser Turbiscan Lab Expert (Microtrac Formulaction SAS, Toulouse, France). The measurement is based on the dynamic backscattering of light at 880 nm to monitor the change in droplet volume fraction due to migration, or in their average size due to coalescence (Mengual, Meunier, Cayre, Puech, & Snabre, 1999). Stability was expressed by the Turbiscan Stability Index (TSI), which is actually an indicator of instability (the higher the TSI, the lower the stability).

2.5. Statistical analysis

2.5.1. Principal component analysis

Principal Component Analyses (PCA) were carried out on gelling properties data (except gelling temperature that was not measured for GGRP) on one hand, and emulsifying properties data on the other hand, to highlight the correlations between the variables, and to characterize the two protein ingredients with regard to these variables. Parameters describing gelling and emulsifying properties were used as active variables, while pH, protein concentration, ionic strength and protein solubility index were used as supplementary variables. The commercial beef gelatines and NaCas were included as supplementary individuals. The PCA analyses were performed using the FactoMineR package of the R software (version 4.0.3) (Lê, Josse, & Husson, 2008). The variables were automatically standardized (centred mean and scaled) by the software to give them all the same importance.

2.5.2. Modelling from CCD results

Statgraphics software was used to perform regression analyses of the experimental data resulting from the CCD, and to plot the corresponding response surfaces. For each experimental factor, the variance was divided into linear, quadratic, and interaction components to assess the suitability of the following second-order polynomial function (Eq. (1)), and the relative importance of these components:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i,j=1}^{3} \beta_{ij} X_i X_j \quad \text{[Eq.1]}$$

with Y the estimated response; β_0, a constant, β_i, β_{ii}, β_{ij} regression coefficients of the model; X_i, X_j, two independent variables among pH, protein concentration and NaCl added.

For each response variable, the significance of the equation parameters was estimated by a Fisher test, with a level of significance set at $p < 0.05$. The error assessment was derived from the four replications of the CCD central point.

After modelling each functional property studied for each protein ingredient as a function of pH, protein concentration and NaCl added, the optimum conditions to mimic the performances of the reference ingredients were determined; the reference ingredients were tested at pH 5.55, 0.2 mol/L NaCl added (central values of the CCD), and 50 g/L protein concentration for the gelatines, and 125 g/L for the NaCas. Next, a multi-response optimisation was performed, initially by sub-category of properties (gel rupture properties, gel texture properties, WHC, size distribution of the lipid droplets, and emulsion stability). Then in a second stage, an optimisation was carried out, grouping all the experimental results by functionality category (gelling, or emulsifying properties). In order to combine the various responses into a single function that can be optimized, a desirability function has been defined for each response (Polhemus, 2005). The function d(y) expresses the desirability of a response value equal to y on a scale of 0–1. For a response to be maintained at a given value, the desirability function is defined as follows:

$$d = \begin{cases} 0 & \hat{y} < \text{low} \\ \left(\dfrac{\hat{y} - low}{target - low}\right)^S & \text{low} \le \hat{y} \le \text{target} \\ \left(\dfrac{\hat{y} - high}{target - high}\right)^S & \text{target} \le \hat{y} \le \text{high} \\ 0 & \hat{y} > \text{high} \end{cases} \quad \text{[Eq.2]}$$

where $\hat{y}$ is the expected value of the response; *low* is a value below which the response is not acceptable; and *high* is a value above which desirability is at its maximum. The parameter *S* defines the form of the function; S = sensitivity of the response, defined as 1 for "medium". The desirability rises linearly from 0 at the low value to 1 at the high value.

To combine the desirability of *the m* responses, a single function *D* was created. Since all responses were considered to have the same importance, the composite function was defined as the geometric mean of separate desirability values:

$$D = \left\{ d_1 d_2 \ldots d_m \right\}^{1/m} \quad \text{[Eq.3]}$$

3. Results and discussion

3.1. WRP is able to form gels broadly similar to beef gelatine gels, unlike GGRP

As meat protein ingredients rich in collagen, WRP and GGRP may be potential candidates for substituting commercial beef gelatines as gelling agents. To assess this assumption, the gelling properties of WRP and GGRP were compared with those of the two commercial beef gelatines mentioned above, for which the difference in gel strength (240 and 150 Bloom, respectively) was probably related to the extraction process, since gelatine strength decreases as extraction temperature increases for example (Sha, Hu, Ye, Xu, & Tu, 2019). The commercial gelatines have been tested at pH 5.55, 50 g/L protein, and 0.2 mol/L NaCl added, while WRP and GGRP have been evaluated under all the conditions of the CCD (Table 1).

For an overview of all the gelling properties considered simultaneously, a PCA was first performed with all the properties as active variables, and physicochemical parameters (pH, ionic strength, protein concentration, and protein solubility) as supplementary variables (Fig. 1A). The first two principal components (PC) explain a cumulative

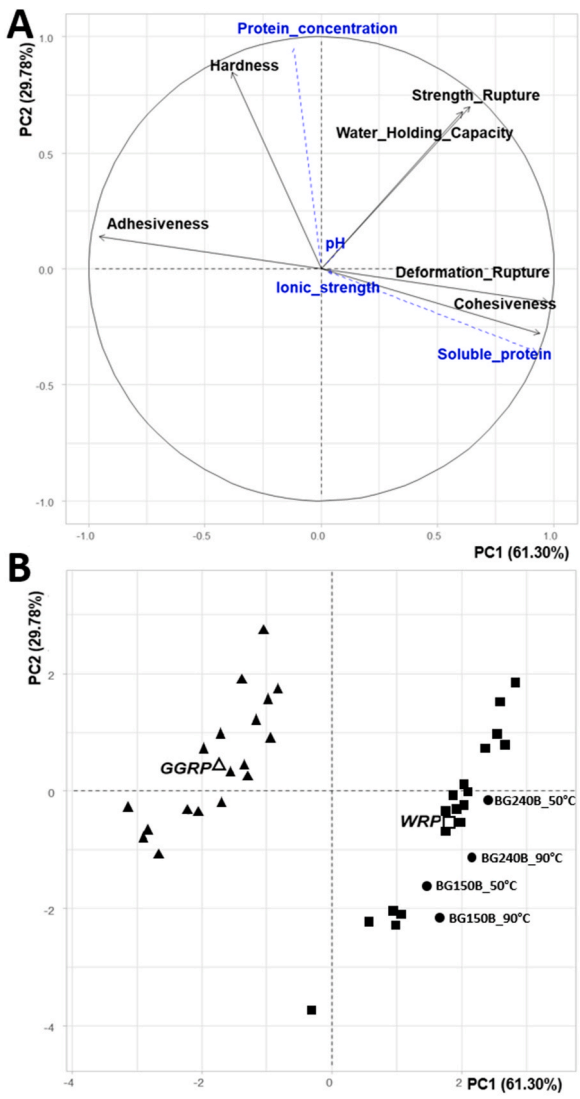

Fig. 1. Gelling properties: projection of the variables (A) and of the individuals (B) on the first two dimensions (PC1 and PC2) of the principal component analysis (PCA). (A) Active variables (black solid lines) correspond to the characteristics measured to describe gelling properties; pH, protein concentration, ionic strength and protein solubility were added as supplementary variables (blue dashed lines). (B) Individuals are represented by symbols according to the ingredients (triangles, Greasy Greaves Recovered Proteins (GGRP); squares, Water Recovered Proteins (WRP); circles, commercial beef gelatines (BG)). Empty symbols correspond to the barycentre of each of the two protein ingredients. The gels of commercial bovine gelatines, 150 Bloom (BG150B) and 240 Bloom (BG240B), have been prepared at 50 g/L protein, pH 5.55 and 0.2 mol/L NaCl, after dissolution at 50 °C or 90 °C; these samples have been added as supplementary individuals in the PCA.

variability of 91.08%, thus explaining a major part of the information provided by the dataset. PC1 (61.30% of the variability) is negatively correlated with gel adhesiveness (R^2 = - 0.96), and positively correlated with cohesiveness (0.94) and deformation at the gel rupture (0.98). Adhesiveness, cohesiveness and deformation at the gel rupture are strongly correlated with protein solubility (−0.92, 0.97, and 0.96, respectively) (*Supplementary data*, Table S2). PC2 (29.78% of the variability) is positively correlated with gel hardness (R^2 = 0.85), strength at the gel rupture (0.70) and WHC (0.68), which are all variables positively correlated with protein concentration (0.82, 0.63, and 0.57, respectively). However, pH and ionic strength are not properly represented in the PC1-PC2 plan, indicating the absence of correlation with the variables described above (*Supplementary data*, Table S2). The ionic strength and pH are neither correlated with PC3 nor PC4 (data not shown).

The graph of individuals clearly opposes the WRP gels to the GGRP gels along PC1 (Fig. 1B). The position of GGRP gels on the left side of the graph indicates higher adhesiveness, but lower cohesiveness and deformation at the gel rupture as compared with WRP gels. Moreover, it underlines the lower protein solubility of GGRP, previously reported (Le Foll et al., 2024). In addition, PC2 separates the gels according to protein concentration, with the more concentrated gels (upper part of the graph) having the highest hardness, strength at the rupture and WHC, regardless of the ingredient. Lastly, Fig. 1B highlights the proximity, in the PC1-PC2 plan, between WRP and the two commercial beef gelatines used as references, indicating that, unlike GGRP, WRP can form gels broadly similar to that of the two commercial beef gelatines, at least under some conditions of the experimental domain investigated. As expected, the 240 Bloom gelatine has higher coordinates on PC2 than the 150 Bloom gelatine, indicating higher gel hardness and strength at the gel rupture, but also higher WHC. However, both gelatines do not differ in adhesiveness, cohesiveness and deformation at the gel rupture (same coordinates on PC1). Moreover, for each commercial gelatines, it should be noted that dissolution and heating at 90 °C for 30 min before gelation, as compared to dissolution at 50 °C, decreases gel hardness, strength at the gel rupture and WHC. This suggests some gelatine hydrolysis during heat treatment at 90 °C, consistently with literature (Correra de Moraes & Lopes Cunha, 2013). As a reminder, two different processing temperatures were applied for GGRP (90 °C) and WRP (50 °C), in order to melt the insoluble collagen present in GGRP, unlike WRP where the collagen is already soluble (Le Foll et al., 2024). For this reason, commercial gelatines were evaluated according to both gel preparation methods, so as not bias comparisons with WRP on the one hand, and GGRP on the other occurs.

3.2. *Multi-response optimisations confirm the potentialities of WRP as an efficient substitute for beef gelatine*

In order to investigate in more detail, the potentialities of the two protein ingredients as beef gelatine substitutes for gelling properties, optimal conditions have been determined for each ingredient to mimic as closely as possible the functionalities of the two commercial beef gelatines, based on the regression analyses that assessed the effect of protein concentration, pH and ionic strength on each of the gelling properties (*Supplementary data*, Table S3). The corresponding desirability values were then calculated.

In a first step, optimisation has been carried out considering separately four subcategories of gelling properties, i.e., gelling temperature (for WRP only), gel rupture properties (strength and deformation together), gel texture (hardness, cohesiveness, and adhesiveness together), and WHC. The optimum pH, protein concentration and ionic strength values thus determined for WRP and GGRP to mimic 150 Bloom gelatine are summarized in Table 2. The corresponding maps of desirability in the experimental domain are presented in Fig. 2. Similarly, comparisons with the 240 Bloom gelatine are given in supplementary data (Table S4, Figs. S1–S2). The small differences obtained between the two commercial gelatines were in agreement with literature data, such as for the gelation temperature that increases with the Bloom degree (12.96 and 17.89 °C for 150 Bloom and 240 Bloom gelatines, respectively) (Osorio, Bilbao, Bustos, & Alvarez, 2007).

As expected from the PCA results presented above, GGRP did not really enable to form gels similar to gelatine gels, as indicated by the optimum desirability scores of 0.55 for gel rupture properties, and 0.49 for gel texture properties (Table 2A, Fig. 2). Even considering separately each characteristic constitutive of gel rupture and gel texture properties, the desirability scores are all lower than 0.60, except for gel rupture strength (0.88). However, the maximum score of desirability (1.00) could be obtained for WHC, indicating that GGRP gels can perfectly

Table 2
Optimal conditions for Water Recovered Proteins (WRP) and Greasy Greaves Recovered Proteins (GGRP) to mimic a commercial beef gelatin, considering four categories of gelling properties (A), or all properties taken together (B). Protein concentration (C), pH, and ionic strength (I) are given to mimic a 150 Bloom gelatin, tested at 50 g/L protein, pH 5.55, and 0.2 mol/L NaCl added, after heating for 30 min at 50 °C (when compared with WRP) or 90 °C (when compared with GGRP). d: desirability function of a response value equal to y on a scale of 0–1. D: all responses were combined for a single desirability score.

A	WRP	Reference	d	D	GGRP	Reference	d	D
C (g/L)	61.0	50.0						
pH	5.43	5.55						
I (mol/L NaCl added)	0.18	0.20						
Gelling temperature (°C)	**12.96**	**12.96**	**1**	**1**				
C (g/L)	97.9	50.0			120.0	50.0		
pH	6.04	5.55			6.99	5.55		
I (mol/L NaCl added)	0.016	0.20			0.39	0.20		
Gel rupture strength (N)	**23.12**	**23.12**	**1**	**1**	**13.47**	**9.18**	**0.88**	0.55
Deformation at the gel rupture (%)	**54.91**	**54.91**	**1**		42.44	56.83	0.34	
C (g/L)	113.1	50.0			157.0	50.0		
pH	4.00	5.55			7.00	5.55		
I (mol/L NaCl added)	0.39	0.20			0.18	0.20		
Gel hardness (N)	**0.97**	**0.97**	**0.99**	0.77	3.65	0.77	0.56	0.49
Gel cohesiveness	0.94	0.96	0.77		0.76	0.97	0.38	
Gel adhesiveness (J)	1.30	1.20	0.60		1.75	1.13	0.56	
C (g/L)	75.2	50.0			134.3	50.0		
pH	6.57	5.55			5.65	5.55		
I (mol/L NaCl added)	0.33	0.20			0.24	0.20		
WHC (%)	**99.63**	**99.63**	**1**	**1**	**99.65**	**99.65**	**1**	**1**
B	WRP	Reference	d	D	GGRP	Reference	d	D
C (g/L)	91.0	50.0			128.7	50.0		
pH	6.49	5.55			7.00	5.55		
I (mol/L NaCl added)	0.00	0.20			0.15	0.20		
Gelling temperature (°C)	**14.79**	**12.96**	**0.89**	0.79	nd	nd		
Gel rupture strength (N)	**18.90**	**23.12**	**0.85**		**13.60**	**9.18**	**0.87**	0.50
Deformation at the gel rupture (%)	**55.12**	**54.91**	**0.97**		40.32	56.83	0.24	
Gel hardness (N)	**0.81**	**0.97**	**0.85**		2.40	0.77	0.75	
Gel cohesiveness	0.94	0.96	0.79		0.73	0.97	0.29	
Gel adhesiveness (J)	1.32	1.20	0.52		1.94	1.13	0.42	
WHC (%)	99.70	99.63	0.73		**99.68**	**99.65**	**0.81**	

mimic beef gelatine gels with respect to this property. This implies similar pH (5.65 vs 5.55) and ionic strength (0.24 mol/L NaCl added *vs* 0.2 mol/L) than those applied for the commercial 150 Bloom gelatine, but much higher protein concentrations (134.3 g/L *vs* 50 g/L) (Table 2A). Finally, for GGRP, multi-criteria optimisation of all gelling properties failed to identify experimental conditions that perfectly met all the objectives at the same time, with a desirability score of 0.50 (Table 2B, Fig. 3).

Concerning WRP, Table 2A shows that the maximum score of desirability could be obtained for gelling temperature, gel rupture properties and WHC. Hence, with some adjustments to pH (5.43 *vs* 5.55), and to ionic strength (0.18 mol/L NaCl added *vs* 0.2 mol/L), the same gelling temperature as that of the 150 Bloom gelatine (12.96 °C) could be obtained with 61 g/L WRP protein, *vs* 50 g/L gelatine. With respect to WHC, strictly identical performance (99.63% WHC) could be obtained with WRP and the 150 Bloom gelatine, albeit with a higher protein concentration for WRP (75.2 g/L vs 50 g/L), higher pH (6.57 vs 5.55) and higher NaCl added content (0.33 vs 0.2 mol/L). However, there is actually a number of conditions in the experimental domain, which enable to obtain exactly the same gelling temperatures and WHC (desirability ~ 1.00) as reference gelatine (Fig. 2). As for gel rupture properties, the maximum score of desirability could be obtained so that the WRP mimicked the 150 Bloom gelatine with respect to the deformation and the strength at the gel rupture. This implies similar pH conditions to that applied for the gelatine (pH 6.04 vs 5.55), lower ionic strength (0.016 mol/L NaCl added vs 0.2 mol/L), but a higher protein concentration (97.9 g/L vs 50 g/L). The higher protein concentration required for WRP to mimic gelatine was to be expected, as WRP proteins do not consist solely of gelatine (Supplementary data, Table S1). Therefore, a higher amount of WRP proteins is required to obtain the same gelatine content as with pure gelatine. Lastly, it should be noted that optimal conditions for WRP to mimic 150 Bloom gelatine in terms of gelling temperature, WHC and gel rupture, exist throughout the pH range tested in the present study, i.e., from pH 4.0 to pH 7.0 (Fig. 2).

However, with respect to gel texture properties, only gel hardness of the 150 Bloom gelatine could be properly imitated with WRP, with a desirability score of 0.99, provided that much higher protein concentration is used (113.1 g/L *vs* 50 g/L; Table 2A), similarly to the optimisation of the gel rupture properties mentioned above. Finally, multi-response optimisation led to sub-optimal results, as indicated by a desirability score of 0.77 for WRP. Therefore, WRP cannot strictly mimic the commercial 150 Bloom gelatine in that all the gel texture properties cannot be fully and simultaneously mimicked (Fig. 2).

Once the optimisations for the four subcategories of gelling properties of WRP had been completed, they were supplemented by a further multi-response optimisation for all the gelling properties taken together (Table 2B; Fig. 3). This challenging multi-criteria optimisation underlines that, although some very high desirability scores were calculated when gelling properties were considered separately (see above), it is quite impossible to determine experimental conditions that met perfectly all objectives at the same time. However, a quite high desirability score (0.79) was calculated for WRP, when mimicking the 150 Bloom gelatine, indicating that this protein ingredient might efficiently replace this commercial gelatine as a gelling agent. For this, the protein concentration must be increased (91 g/L vs 50 g/L), the pH increased (6.49 *vs* 5.55) and the addition of NaCl decreased (0 *vs* 0.2 mol/L). In addition, it should be noted that WRP, but not GGRP, forms thermoreversible gels like commercial gelatines. Under the above optimal

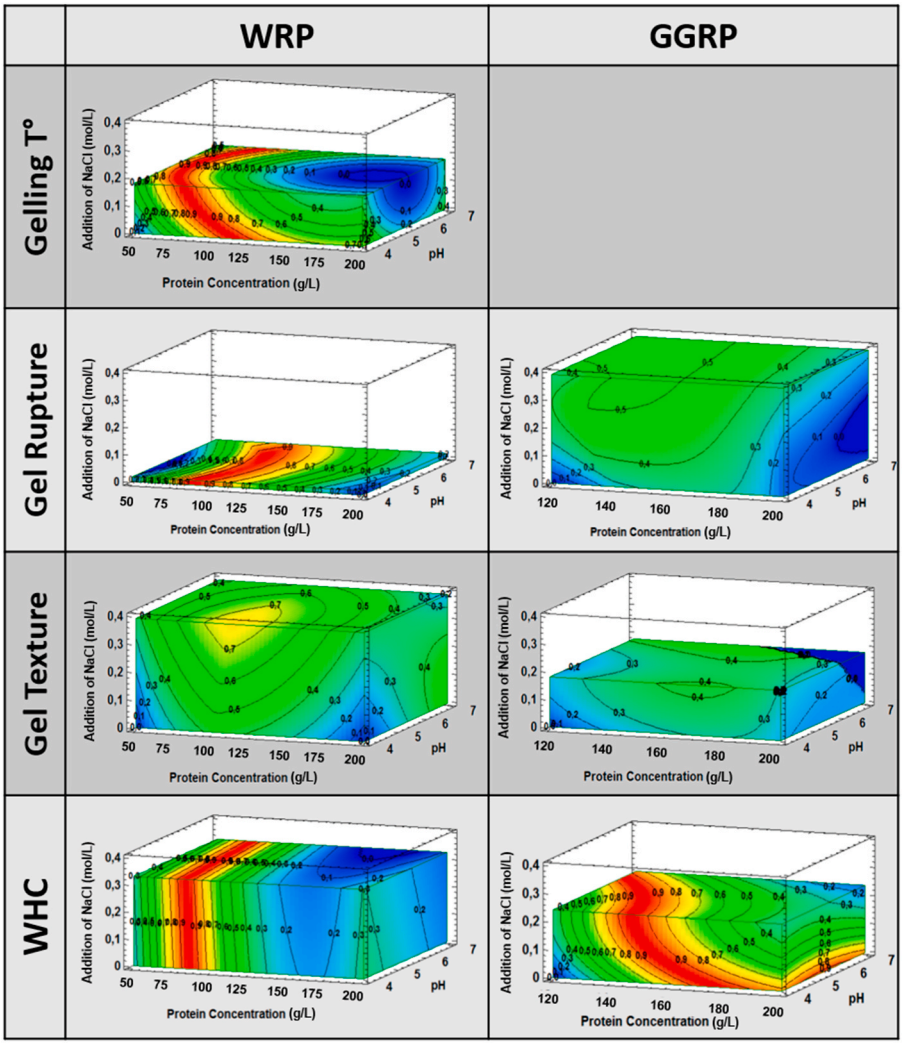

Fig. 2. Desirability mapping for the four categories of gelling properties of Water Recovered Proteins (WRP) and Greasy Greaves Recovered Proteins (GGRP). Desirability values indicate the capability of each ingredient to reproduce separately the gelling temperature, gel rupture, gel texture, or WHC of a 150 Bloom commercial beef gelatine. Gel rupture optimisation considers strength and deformation at the rupture of the gel simultaneously; gel texture optimisation considers gel hardness, cohesiveness, and adhesiveness simultaneously. Desirability is presented as a function of protein concentration and pH, the ionic strength (mol/L NaCl added) being set in each case for the maximum desirability. The value indicated on each level line corresponds to the desirability score, for which the colour code (11 levels) evolves from a desirability equal to 0 (dark blue) to 1 (red), passing through 0.3 (light blue), 0.5 (medium green) and 0.8 (orange-yellow).

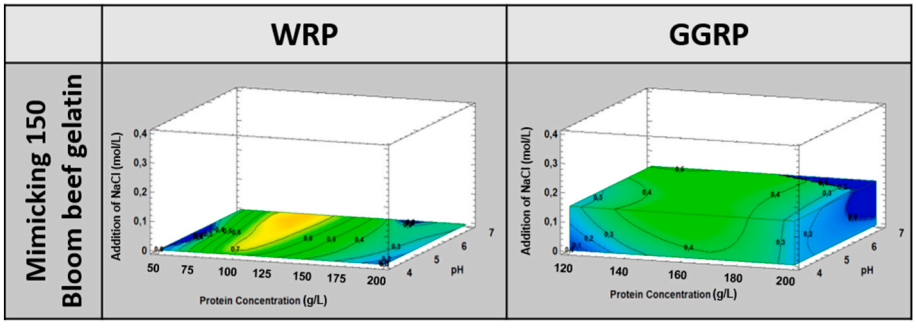

Fig. 3. Desirability mapping for all gelling properties of Water Recovered Proteins (WRP) and Greasy Greaves Recovered Proteins (GGRP) taken together. Desirability values indicate the capability of each ingredient to mimic a 150 Bloom beef gelatine in all dimensions of gelling properties (gelling temperature, gel rupture strength and deformation, gel hardness, cohesiveness, adhesiveness, and WHC). Desirability is presented as a function of protein concentration and pH, the ionic strength (mol/L NaCl added) being set in each case for the maximum desirability. The value indicated on each level line corresponds to the desirability score, for which the colour code (11 levels) evolves from a desirability equal to 0 (dark blue) to 1 (red), passing through 0.3 (light blue), 0.5 (medium green) and 0.8 (orange-yellow).

conditions for WRP to mimic the 150 Bloom gelatine (Table 2B), the difference between gelling and melting temperatures was 7.2 °C for WRP compared to 13.2 °C for the commercial bovine gelatine. WRP gels were therefore slightly less heat-stable than the commercial gelatine. However, the thermoreversibility of gels, which is an important property for food processors, does exist in WRP gels as it is in gelatine gels.

A number of co-products of animal origin have already been reported to have gel-forming properties comparable to those of gelatine. These include beef lungs, mechanically separated chicken meat and fish skin (Amiza, Shima, Nor Hayati, & Nizaha Juhaida, 2015; Mokrejš, Gál, Pavlačková, & Janáčová, 2021; Roy, Omana, Betti, & Bruce, 2017), albeit with different performances depending on the protein content and origin. For example, fish gelatine has been found to have lower gelling and melting temperatures and lower gel strength than pork or beef gelatines; this is particularly true for cold-water fish gelatines, which have gelling and melting temperatures around 10 °C lower and gel strength 10 times lower (Derkach, Voron'ko, Kuchina, & Kolotova, 2020; Rahman & Al-Mahrouqi, 2009). With regard to chicken gelatine, Abedinia et al. (2020) reported a gel strength that varied greatly depending on the origin of the gelatine and the extraction process, being either lower or higher than that of bovine gelatine. On the other hand, the gelling and melting temperatures of chicken gelatines (23–28 °C and 27–40 °C, respectively, under Bloom test conditions) are similar to those of mammalian gelatines (20–27 °C and 28–34 °C, respectively; Derkach et al. (2020). However, it is difficult to position WRP and GGRP in relation to these other co-products. Indeed, the way in which gels are formed has a major impact on their properties, and this varies systematically from one study to another. This is why we chose to compare WRP and GGRP with commercial gelatines, tested under the same conditions as the two co-products of interest. In this way, equivalences, particularly in terms of protein concentration, could be established to best mimic each of the gelling characteristics or groups of characteristics, depending on the objectives targeted during a food formulation stage, for example.

3.3. *WRP can produce emulsions broadly similar to a sodium caseinate emulsion*

Like many proteins, beef proteins have already been reported as efficient emulsifiers (Kurt & Zorba, 2007; Zorba & Kurt, 2006; Selmane, Vial, & Djelveh, 2010), and we could previously confirm that the two beef protein ingredients studied here actually offer this feature (Le Foll et al., 2024). With a view to proposing WRP and GGRP as new food emulsifiers, especially for meat products such as sausages or meatballs, these two ingredients were compared with NaCas, widely used by the food industry to stabilize emulsions (Huck-Iriart, Álvarez-Cerimedo, Candal, & Herrera, 2011; Ma & Chatterton, 2021). A commercial NaCas was tested as a reference at pH 5.55, 125 g/L protein, and 0.2 mol/L NaCl added, i.e., the middle of the experimental design used to test WRP and GGRP.

For a first overview of the emulsifying properties considered in a holistic way, a PCA was performed with all properties as active variables, and physicochemical parameters (pH, protein concentration, ionic strength, and soluble protein content) as supplementary variables (Fig. 4A). The first two PCs explain most of the information provided by the dataset, with a cumulative variability of 90.85%. PC1 (68.45% of the variability) is positively correlated with the characteristics of the droplet size distribution (R^2 = 0.94, 0.97, and 0.78 for $d_{3,2}$, $d_{4,3}$ and span, respectively). These variables are inversely correlated to protein solubility (−0.86, −0.95 and −0.86, respectively). PC2 (22.40% of the variability) is positively correlated with TSI 1 h (R^2 = 0.80), which is negatively correlated to protein concentration (−0.71). However, pH and ionic strength are not properly represented in the PC1-PC2 plan, indicating the absence of correlation with the variables described above (*Supplementary data*, Table S5). These two variables are neither correlated with PC3 nor PC4 (data not shown).

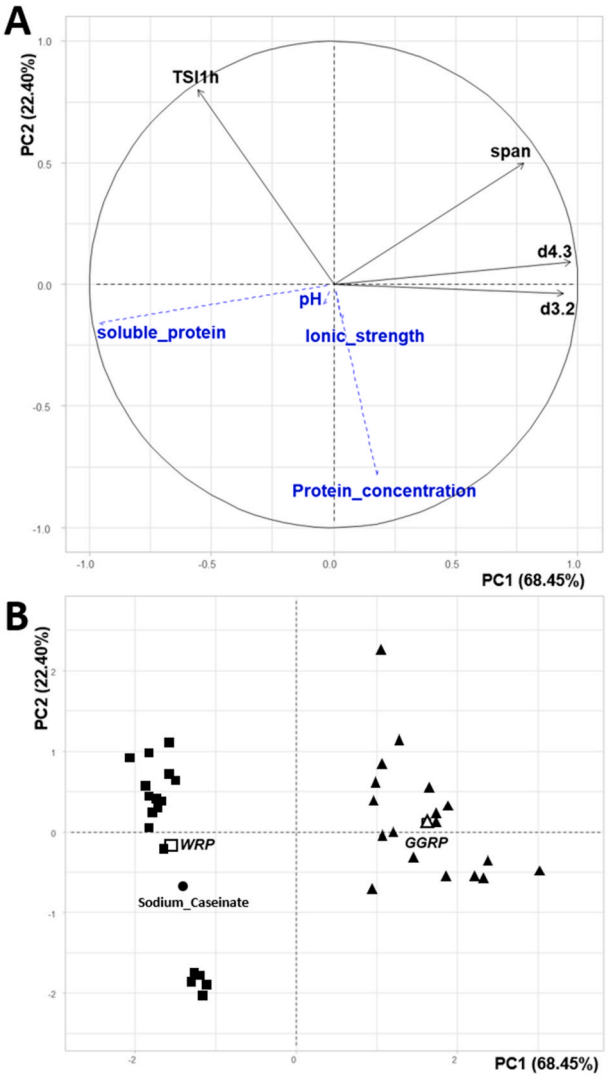

Fig. 4. Emulsifying properties: projection of the variables (A) and of the individuals (B) on the first two dimensions (PC1 and PC2) of the principal component analysis (PCA). (A) Active variables (black solid lines) correspond to the characteristics measured to describe emulsifying properties; pH, protein concentration, ionic strength, and protein solubility were added as supplementary variables (blue dashed lines). (B) Individuals are represented by symbols according to the ingredients (triangles, Greasy Greaves Recovered Proteins (GGRP); squares, Water Recovered Proteins (WRP); circle, commercial sodium caseinate). Empty symbols correspond to the barycentre of each of the two protein ingredients. The emulsion of sodium caseinate has been prepared at 125 g/L protein, pH 5.55 and 0.2 mol/L NaCl; this sample has been added as a supplementary individual in the PCA.

As with the gelling properties, the graph of individuals clearly contrasts the WRP-based emulsions with the GGRP-based ones along PC1 (Fig. 4B), indicating a broader lipid droplet size distribution and also a larger mean diameter in the GGRP emulsions, in relation to the lower solubility of GGRP proteins. However, as previously established in the case of the GGRP-based emulsions, there may be confusion between the lipid droplet size and the insoluble particle size due to the low solubility of this ingredient (Le Foll et al., 2024). No real difference seems to separate the WRP emulsions from the GGRP emulsions along PC2. Moreover, the WRP barycentre is close to the NaCas individual, suggesting that certain conditions may exist for a high degree of similarity between these two ingredients in terms of emulsifying properties.

3.4. Multi-response optimisations highlight the strong potential of WRP and GGRP as substitutes for NaCas in stabilizing emulsions

As in the optimisation approach presented above for gelling properties, optimal conditions were sought so that WRP and GGRP would offer emulsifying properties identical, or at least close to those of NaCas. Optimisation was first carried out considering lipid droplet size distribution, i.e., parameters $d_{3,2}$, $d_{4,3}$, and/or span taken together, and TSI 1 h. For WRP, $d_{3,2}$ was excluded from the optimisation because the regression model equation was not significant for this variable; for the same reason, the $d_{4,3}$ and span parameters were excluded for GGRP (*Supplementary data*, Table S6). The optimal protein concentration, pH, and ionic strength values thus determined for WRP and GGRP are summarized in Table 3A. The response surfaces of desirability values throughout the experimental domain investigated are presented in Fig. 5.

Table 3A shows the maximum desirability value for the polydispersity parameter (span) and a desirability of 0.99 for the droplet size ($d_{4,3}$) of WRP emulsions. As a result, the optimal desirability calculated for droplet size distribution is close to the maximum (0.99) for WRP, which can therefore efficiently mimic NaCas. To achieve this, the WRP emulsion must be prepared at a higher pH than NaCas emulsion (pH 6.96 *vs* pH 5.55), and almost without NaCl addition (0.065 mol/L *vs* 0.2 mol/L), probably because of the natural NaCl content of WRP (59.6 g/kg; Table S1). More interesting is that WRP requires a lower protein concentration than NaCas (53.2 g/L vs 125 g/L) to obtain a similar droplet size distribution, suggesting better emulsifying properties of WRP with respect to this feature. As for GGRP, only the $d_{3,2}$ parameter could be optimized, but the result is a much higher value compared with NaCas (27.7 μm *vs* 1.18 μm), and a moderate desirability value (0.80). However, as mentioned above, this may be an artefact due to the low solubility of the GGRP proteins (from 20% to 30%; Le Foll et al., 2024), making that particle size analysis could include indiscriminately both lipid droplets and insoluble particles.

Concerning TSI 1 h, maximum desirability was calculated for the two protein ingredients (Table 3A), indicating that some conditions exist in the experimental domain investigated, for WRP and GGRP to perfectly mimic the stability of NaCas emulsions. Compared with the reference NaCas emulsion tested, the pH must be either similar for GGRP (pH 5.78 *vs* pH 5.55), or higher for WRP (pH 6.62); NaCl addition should be the same for GGRP (0.2 mol/L), lower for WRP (0.08 mol/L); protein concentration should be lower for GGRP (77.5 g/L *vs* 125 g/L), higher for WRP (162.5 g/L). Therefore, it can be concluded that the WRP proteins are almost as effective as NaCas in stabilizing emulsions, and that the GGRP proteins are even more effective, bearing in mind that stability is a major criterion of emulsion quality. According to the literature, NaCas-stabilized emulsions are unstable at a pH close to the isoelectric point of caseins (pH~4.6), at high processing and storage temperatures, at high ionic strength, and at high caseinate concentration (Ma & Chatterton, 2021). These factors actually limit the use of NaCas in acidic or salt-rich foods, and the experimental conditions set in the present study to evaluate NaCas as a reference emulsifier (pH 5.55, 0.2 mol/L NaCl added, 125 g/L protein, and 50 °C) may not be optimal. Lastly, it should be noted that maximum desirability for emulsion stability is obtained in a wide range of experimental conditions in the experimental domain investigated, and this for the two ingredients (Fig. 5), suggesting a wide range of food applications for which WRP and GGRP may be efficient ingredients to stabilize emulsions.

To go further, multi-response optimisations were performed for both ingredients, including all emulsifying properties together. The optimal conditions thus defined, and corresponding desirability scores are summarized in Table 3B; the desirability maps are presented in Fig. 6. This exhibits that, although high desirability scores were calculated when emulsifying properties were considered separately (see above), it is quite impossible to determine experimental conditions that met perfectly all objectives at the same time. However, an almost maximal d score (0.99) was calculated for emulsion stability for both ingredients, and the overall desirability scores (D) were quite high for WRP and GGRP (0.77 and 0.87, respectively). For WRP, this optimum desirability score is obtained for a higher protein concentration compared to NaCas (149.7 g/L *vs* 125 g/L), a lower pH (4.00 *vs* 5.55) and a little more NaCl added (0.24 mol/L *vs* 0.2 mol/L). For GGRP, the optimal conditions are a lower protein concentration than that of the NaCas emulsion (109.2 g/L *vs* 125 g/L), a higher pH (6.01 *vs* 5.55) and a lower addition of NaCl (0.08 mol/L *vs* 0.2 mol/L). However, even under optimal conditions, the GGRP emulsion is much coarser than the NaCas emulsion, with a lipid droplet size ($d_{3,2}$) of 33.39 μm *vs* 1.18 μm, respectively.

Table 3
Optimal conditions for Water Recovered Proteins (WRP) and Greasy Greaves Recovered Proteins (GGRP) to mimic a commercial sodium caseinate (NaCas), considering the size distribution and emulsion stability separately (A), or all properties taken together (B). Protein concentration (C), pH, and ionic strength (I) are given to mimic a NaCas tested at 125 g/L protein, pH 5.55, and 0.2 mol/L NaCl added. d: desirability function of a response value equal to y on a scale of 0–1; D: all responses were combined for a single desirability score.

A	WRP	Reference	d	D	GGRP	Reference	d	D
C (g/L)	53.2	125.0			74.4	125.0		
pH	6.96	5.55			5.47	5.55		
I (mol/L NaCl added)	0.065	0.20			0.003	0.20		
$d_{3,2}$ (μm)	nd	1.18	nd	**0.99**	**27.7**	**1.18**	**0.80**	**0.80**
$d_{4,3}$ (μm)	**8.30**	**8.31**	**0.99**		nd	8.31	nd	
span	**1.97**	**1.97**	**1**		nd	1.97	nd	
C (g/L)	162.5	125.0			77.5	125.0		
pH	6.62	5.55			5.78	5.55		
I (mol/L NaCl added)	0.08	0.20			0.20	0.20		
TSI 1h	**10**	**10**	**1**	**1**	**10**	**10**	**1**	**1**
B	WRP	Reference	d	D	GGRP	Reference	d	D
C (g/L)	149.7	125.0			109.2	125.0		
pH	4.00	5.55			6.01	5.55		
I (mol/L NaCl added)	0.24	0.20			0.08	0.20		
$d_{3,2}$ (μm)	nd	1.18	nd	0.77	33.39	1.18	0.75	**0.87**
$d_{4,3}$ (μm)	15.17	8.31	0.72		nd	8.31	nd	
span	1.63	1.97	0.62		nd	1.97	nd	
TSI 1h	**10**	**10**	**0.99**		**10**	**10**	**0.99**	

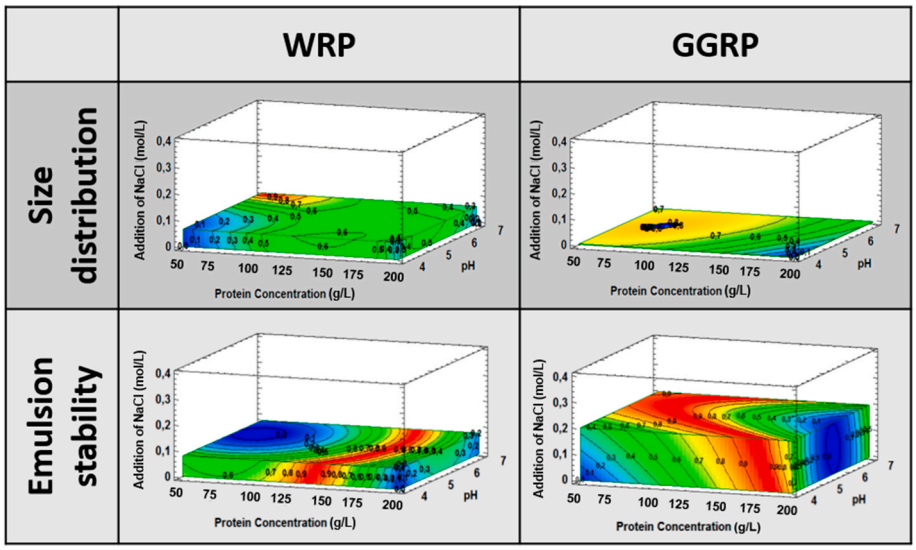

Fig. 5. Desirability mapping for the two categories of emulsifying properties of Water Recovered Proteins (WRP) and Greasy Greaves Recovered Proteins (GGRP). Desirability values indicate the capability of each ingredient to reproduce separately the droplet size distribution or emulsion stability of a sodium caseinate. The optimisation of droplet size distribution simultaneously considers $d_{4,3}$, and span for WRP, and $d_{3,2}$ only for GGRP. Desirability is presented as a function of protein concentration and pH, the ionic strength (mol/L NaCl added) being set in each case for the maximum desirability. The value indicated on each level line corresponds to the desirability score, for which the colour code (11 levels) evolves from a desirability equal to 0 (dark blue) to 1 (red), passing through 0.3 (light blue), 0.5 (medium green) and 0.8 (orange-yellow).

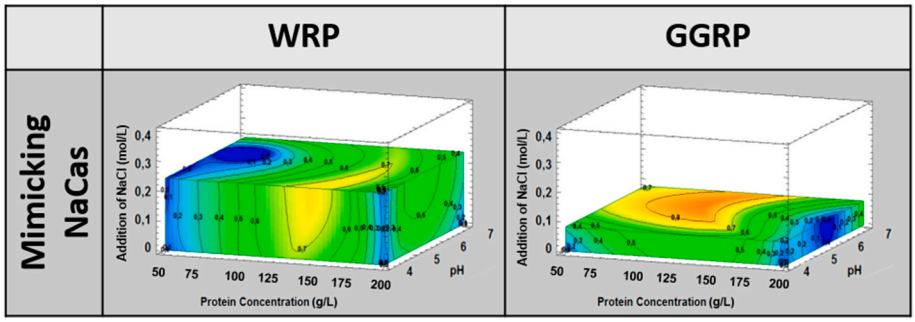

Fig. 6. Desirability mapping for all emulsifying properties of Water Recovered Proteins (WRP) and Greasy Greaves Recovered Proteins (GGRP) taken together. Desirability values indicate the capability of each ingredient to mimic a commercial sodium caseinate in all dimensions of emulsifying properties ($d_{3,2}$, $d_{4,3}$, span, stability). Desirability is presented as a function of protein concentration and pH, the ionic strength (mol/L NaCl added) being set in each case for the maximum desirability. The value indicated on each level line corresponds to the desirability score, for which the colour code (11 levels) evolves from a desirability equal to 0 (dark blue) to 1 (red), passing through 0.3 (light blue), 0.5 (medium green) and 0.8 (orange-yellow).

4. Conclusion

RSM was successfully used to determine the optimum conditions of pH, protein concentration and ionic strength for WRP and GGRP, two protein ingredients derived from bovine co-products, to mimic either bovine gelatine as a gelling agent, or NaCas as an emulsifier. These results pave the way for both products to be used as functional ingredients by the food industry, beyond their current use as “filler proteins” or “meat substitutes” without further specification, mainly in pet food. In particular, WRP proved to be an efficient potential gelatine substitute, mimicking most of the gelling properties satisfactorily, including the thermoreversibility of the gels formed. In terms of emulsifying properties, WRP and GGRP proved to be effective alternatives to NaCas, particularly due to their high ability to stabilize emulsions. It is also noteworthy that the functional properties of WRP and GGRP are little affected by pH and ionic strength, demonstrating their robustness to environmental conditions. This point is particularly interesting as it suggests a great versatility of both ingredients in different food applications. However, the animal origin of WRP and GGRP, as well as their colour, odour and taste, would suggest that both ingredients are primarily intended for use in meat products, or at least in salted products. Preliminary food development and sensory analysis with a trained panel of 9 people allowed us to identify maximum levels of GGRP in salted cake and carrot soup, above which the foods were considered unacceptable. These maximum levels were 6.7% GGRP in the soup and 9.9% in the cake. Obviously, sensory testing will need to be carried out for any proposed application, but these preliminary results are encouraging.

Funding sources

This study was funded by Fondation Institut Agro.

CRediT authorship contribution statement

Rozenn Le Foll: Formal analysis, Investigation, Visualization, Writing – original draft. **Françoise Nau:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. **Pascaline Hamon:** Investigation. **Catherine Guérin-Dubiard:** Writing – review & editing. **Xavier Lambert:** Resources, Writing –

review & editing. **Amélie Deglaire:** Writing – review & editing. **Valérie Lechevalier:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Françoise Nau reports financial support was provided by Fondation Institut Agro.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2024.115945.

References

Abedinia, A., Mohammadi Nafchi, A., Sharifi, M., Ghalambor, P., Oladzadabbasabadi, N., Ariffin, F., et al. (2020). Poultry gelatin : Characteristics, developments, challenges, and future outlooks as a sustainable alternative for mammalian gelatin. *Trends in Food Science & Technology, 104*, 14–26. https://doi.org/10.1016/j.tifs.2020.08.001

Álvarez, C., Drummond, L., & Mullen, A. M. (2018). Novel "gel demineralizing" method for protein recovery from fat rendering waste stream based on its gelling properties. *Food Hydrocolloids, 84*, 529–536. https://doi.org/10.1016/j.foodhyd.2018.06.033

Amiza, M. A., Shima, W. M., Nor Hayati, I., & Nizaha Juhaida, M. (2015). Optimization of gelatin extraction conditions from Cobia (Rachycentron canadum) skin and its physicochemical characteristics as compared to bovine gelatin. *International Food Research Journal, 22*, 213–224.

Box, G. E. P., & Wilson, K. B. (1951). On the experimental Attainment of optimum conditions. *Journal of the Royal Statistical Society: Series B, 13*(1), 1–38. https://doi.org/10.1111/j.2517-6161.1951.tb00067.x

Correra de Moraes, M., & Lopes Cunha, R. (2013). Gelation property and water holding capacity of heat-treated collagen at different temperature and pH values. *Food Research International, 50*(1), 213–223. https://doi.org/10.1016/j.foodres.2012.10.016

Cruijsen, J. (1996). Physical stability of caseinate stabilized emulsions during heating. https://www.semanticscholar.org/paper/Physical-stability-of-caseinate-stabilized-during-Cruijsen/c3f9667b19cabb06e41f5d17672840074b37d3ca.

Denis, A. (2009). *Method for obtaining proteins by treating adipose tissue* (Brevet EP2108266). https://www.freepatentsonline.com/EP2108266.html.

Derkach, S. R., Voron'ko, N. G., Kuchina, Y. A., & Kolotova, D. S. (2020). Modified fish gelatin as an alternative to mammalian gelatin in modern food Technologies. *Polymers, 12*(12), 3051. https://doi.org/10.3390/polym12123051

Derringer, G., & Suich, R. (1980). Simultaneous Optimization of Several response variables. *Journal of Quality Technology, 12*(4), 214–219. https://doi.org/10.1080/00224065.1980.11980968

Edwards, I. M., & Jutan, A. (1997). Optimization and control using response surface methods. *Computers & Chemical Engineering, 21*(4), 441–453. https://doi.org/10.1016/S0098-1354(96)00007-5

Gómez-Guillén, M. C., Giménez, B., López-Caballero, M. E., & Montero, M. P. (2011). Functional and bioactive properties of collagen and gelatin from alternative sources : A review. *Food Hydrocolloids, 25*(8), 1813–1827. https://doi.org/10.1016/j.foodhyd.2011.02.007

Huck-Iriart, C., Álvarez-Cerimedo, M. S., Candal, R. J., & Herrera, M. L. (2011). Structures and stability of lipid emulsions formulated with sodium caseinate. *Current Opinion in Colloid & Interface Science, 16*(5), 412–420. https://doi.org/10.1016/j.cocis.2011.06.003

Kurt, S., & Zorba, O. (2007). Emulsion characteristics of beef and Sheep offal. *Journal of Muscle Foods, 18*(2), 129–142. https://doi.org/10.1111/j.1745-4573.2007.00072.x

Le Foll, R., Lechevalier, V., Hamon, P., Guérin-Dubiard, C., Lambert, X., Deglaire, A., et al. (2024). Beef protein ingredients from fat rendering process are promising functional ingredients. *Food Chemistry, 433*, Article 137298. https://doi.org/10.1016/j.foodchem.2023.137298

Lê, S., Josse, J., & Husson, F. (2008). FactoMineR : An R package for Multivariate analysis. *Journal of Statistical Software, 25*, 1–18. https://doi.org/10.18637/jss.v025.i01

Lynch, S. A., Mullen, A. M., O'Neill, E., Drummond, L., & Álvarez, C. (2018). Opportunities and perspectives for utilisation of co-products in the meat industry. *Meat Science, 144*, 62–73. https://doi.org/10.1016/j.meatsci.2018.06.019

Ma, X., & Chatterton, D. E. W. (2021). Strategies to improve the physical stability of sodium caseinate stabilized emulsions : A literature review. *Food Hydrocolloids, 119*, Article 106853. https://doi.org/10.1016/j.foodhyd.2021.106853

Meeker, D. L., & Hamilton, C. R. (2006). *An overview of the rendering industry. Essential Rendering. Meeker* (Vol. 17). National Renderers Association.

Mengual, O., Meunier, G., Cayre, I., Puech, K., & Snabre, P. (1999). Characterisation of instability of concentrated dispersions by a new optical analyser : The TURBISCAN MA 1000. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 152*(1), 111–123. https://doi.org/10.1016/S0927-7757(98)00680-3

Mokrejš, P., Gál, R., Pavlačková, J., & Janáčová, D. (2021). Valorization of a by-product from the production of mechanically Deboned chicken meat for preparation of gelatins. *Molecules, 26*(2). https://doi.org/10.3390/molecules26020349. Article 2.

Montgomery, D. C. (2013). *Design and analysis of experiments* (8th ed.). John Wiley & Sons, Inc.

Nelson, G., Rosegrant, M., Jawoo, K., Richard, R., Timothy, S., Tingju, Z., et al. (2009). *Climate change : Impact on Agriculture and Costs of Adaptation*. International Food Policy Research Institute.

Osorio, F. A., Bilbao, E., Bustos, R., & Alvarez, F. (2007). Effects of concentration, Bloom degree, and pH on gelatin melting and gelling temperatures using small Amplitude Oscillatory Rheology. *International Journal of Food Properties, 10*(4), 841–851. https://doi.org/10.1080/10942910601128895

Polhemus, D. N. W. (2005). *How to : Perform an Optimization experiment using STATGRAPHICS Centurion*.

Prokop, W. H. (1985). Rendering systems for processing animal by-product materials. *Journal of the American Oil Chemists' Society, 62*(4), 805–811. https://doi.org/10.1007/BF03028757

Rahman, M. S., & Al-Mahrouqi, A. I. (2009). Instrumental texture profile analysis of gelatin gel extracted from grouper skin and commercial (bovine and porcine) gelatin gels. *International Journal of Food Sciences & Nutrition, 60*(sup7), 229–242. https://doi.org/10.1080/09637480902984414

Rasnani, N. M., & Mirhosseini, H. (2011). *Effect of pH on physicochemical properties and stability of sodium caseinate-pectin stabilized emulsion* (Vol. 8).

Roy, B. C., Omana, D. A., Betti, M., & Bruce, H. L. (2017). Extraction and Characterization of gelatin from bovine lung. *Food Science and Technology Research, 23*(2), 255–266. https://doi.org/10.3136/fstr.23.255

Selmane, D., Vial, C., & Djelveh, G. (2010). Production and functional properties of beef lung protein concentrates. *Meat Science, 84*(3), 315–322. https://doi.org/10.1016/j.meatsci.2009.03.007

Sha, X.-M., Hu, Z.-Z., Ye, Y.-H., Xu, H., & Tu, Z.-C. (2019). Effect of extraction temperature on the gelling properties and identification of porcine gelatin. *Food Hydrocolloids, 92*, 163–172. https://doi.org/10.1016/j.foodhyd.2019.01.059

Surh, J. (2009). Comparison of emulsion-stabilizing property between sodium caseinate and Whey protein concentrate : Susceptibility to changes in protein concentration and pH. *Milk protein*.

Yolmeh, M., & Jafari, S. M. (2017). Applications of response surface methodology in the food industry processes. *Food and Bioprocess Technology, 10*(3), 413–433. https://doi.org/10.1007/s11947-016-1855-2

Zayas, J. F. (1997). *Functionality of proteins in food*. Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-59116-7

Zorba, Ö., & Kurt, Ş. (2006). Optimization of emulsion characteristics of beef, chicken and Turkey meat mixtures in model system using mixture design. *Meat Science, 73*(4), 611–618. https://doi.org/10.1016/j.meatsci.2006.02.017