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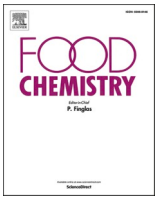

Beef protein ingredients from fat rendering process are promising functional ingredients

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

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ABSTRACT

The valorization of co-products may be a promising way to meet the dual challenge of increasing global food resources and sustainability of food systems. In particular, meat co-products may be nutritionally interesting protein resources, if they offer functional properties in accordance with food applications. In that aim, two bovine co-products, resulting from the fat rendering process, have been characterized, regarding the protein solubility, gelling, and emulsifying properties. The effect of protein concentration, pH variation and NaCl addition on these properties was tested. Despite an effect of the ionic strength on the protein solubility of the two ingredients, a little or no significant impact was observed on the functionalities. Similarly, the functional properties were scarcely affected by pH. In the end, the protein concentration has proven to be the only important parameter, which points to an easy utilization of these ingredients in many food conditions.

1. Introduction

The current demographic outlook predicts an increase in the world's population of two billion people over the next 30 years, reaching 9.7 billion in 2050 (United Nations et al., 2019). This outlook underscores the urgency of increasing food supply to combat hunger and malnutrition, and among the many nutritional issues, the availability of quality protein resources is a key point. In addition, environmental issues force us to imagine sustainable solutions to meet this growing demand for protein resources of high nutritional quality. In this context, it is essential to maximize the use and exploitation of all protein sources.

The valorization of co-products may be a promising way to meet this dual challenge, nutritional and environmental. The aim is to improve the extraction of proteins from raw materials and their use as food ingredients, the functionality of which has to meet food applications. In this way, a co-product can be changed into a valuable product, capable of covering all processing and disposal costs and reducing environmental damage (Toldrá et al., 2021). The development of techniques for the recovery and use of animal co-products has therefore attracted considerable interest in recent years. In particular, many studies have been conducted on proteins from meat co-products, such as blood, bones and organs (lung, liver, heart)(Lynch et al., 2018). However, protein ingredients such as GGRP (Greasy Greaves Recovered Proteins) and WRP (Water Recovered Proteins), on which this study focuses, have not yet been studied. These proteins are extracted during the process of fat recovering from beef fats and bones (primary co-products), by melting and degreasing, either as greaves (GGRP), or from process water (WRP) (Fig. 1). These two protein ingredients (secondary co-products) are innovative products currently marketed at a rate of 2,000 tons per year; this volume results from the valorization of 80,000 tons of slaughterhouse primary co-products (personal communication).

Meat proteins can exhibit a wide range of textural properties. Yet, texture plays a key role in food quality and sensory evaluation. In particular, muscle proteins are widely used as gelling agents (Xiong, 2018), and their gelling properties have been studied (Rahman & Al-Mahrouqi, 2009). Among meat proteins, myofibrillar proteins have

Abbreviations: AUC, area under the curve; DTT, dithiothreitol; GGRP, greasy greaves recovered proteins; WHC, water-holding capacity; WRP, water recovered proteins.

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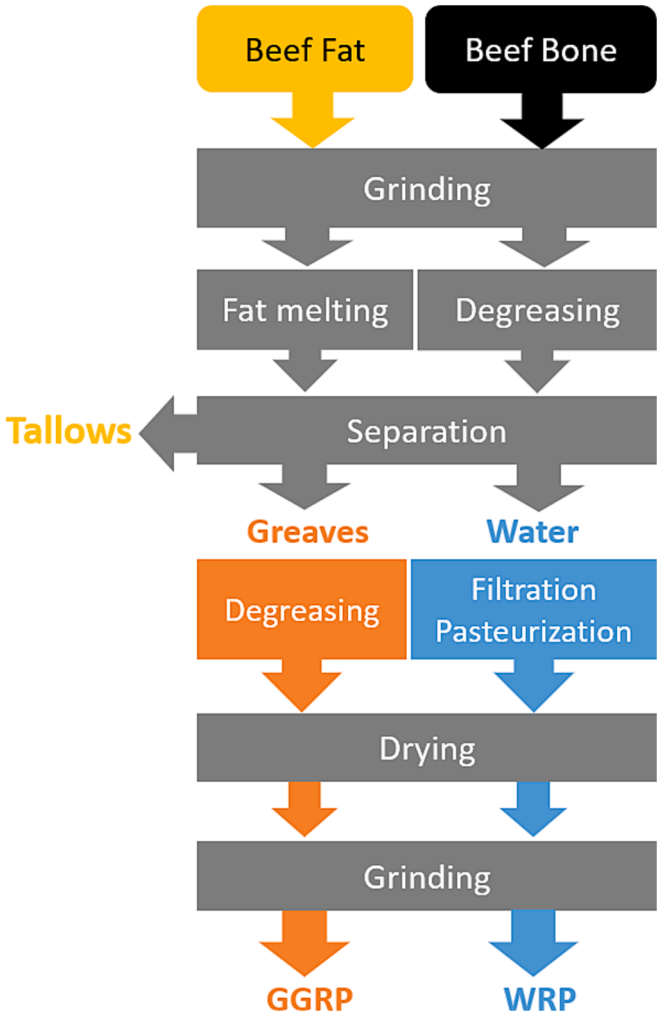

Fig. 1. Diagram of the production process for Greasy Greaves Recovered Proteins (GGRP) and Water Recovered Proteins (WRP).

high gelling properties due to myosin that coagulate upon heating (Sun & Holley, 2011). Stromal proteins such as collagen (connective tissue) are also used in industry as gelling agents (Gómez-Guillén et al., 2011). In addition, the heat treatment of collagen allows to obtain gelatin, which has amazing gelling properties with cooling (Gómez-Guillén et al., 2011). The emulsifying properties of beef proteins have also been studied. In particular, myofibrillar proteins are effective emulsifiers (Zorba & Kurt, 2006). However, no one has characterized the gelling and emulsifying properties of the two bovine co-products studied here. Although they are currently marketed as "filler proteins" or "meat substitutes" without further specification, and largely in pet food, these two ingredients could be interesting alternative and sustainable protein sources for human consumption. In particular, it is reasonable to assume that they can substitute texture agents, at a competitive selling price, as these ingredients are produced using a minimal and gentle process, *i.e.* without chemical reagents and without going as far as purified molecules as it is the case, for instance, in gelatin production.

In this context, more information on the functional properties of the two dehydrated beef proteins considered here, derived from fat rendering process and water recovery, could be useful for food industry. Indeed, these proteins are not currently valued at the height of their quality, since they are rarely used for human consumption, although they are fully compliant with European regulations in this area (Corrigendum to Regulation (EC) No 853/2004). Beyond their nutritional interest, these animal proteins can offer interesting functional solutions, without requiring specific modifications unlike many plant proteins (Damodaran, 2006). The present study aims to characterize as completely as possible the gelling and emulsifying properties of these industrial co-products, under physicochemical conditions relevant to usual food applications.

2. Materials and methods

2.1. Origin of the samples

The dehydrated beef proteins studied, namely the Greasy Greaves Recovered Proteins (GGRP) and Water Recovered Proteins (WRP), were produced by a local plant (CORNILLE sas, France) from bovine co-products. GGRP results from greasy greaves, obtained during the beef fat rendering process. WRP is produced from water recovered during the beef fat rendering process and during the bone degreasing process (Denis, 2009). The fat rendering process of edible fats by the wet process consists of a first step of grinding and sorting before the injection of steam (140 °C) into the product, causing the melting of the fat. A knitter then separates the solid fraction (proteins called greaves) from the melted animal fats and from an aqueous fraction (recovery water). The solid fraction is then defatted (at 90 °C), dried using a rotary dryer (at 102 °C for few minutes), and finally ground to obtain the GGRP. The aqueous fraction from the fat centrifugation is partly recovered for further processing, after mixing with the aqueous fraction from the centrifugation of beef bone fats, which is also recovered. These two fractions are then filtered and sterilized (118 °C), before being concentrated by vacuum evaporation (from 5% to 24% at 65 °C), dried on a roller dryer (at 157 °C for few seconds) and then ground to obtain the WRP (Fig. 1).

The powder samples were packed in vacuum bags before storage at 4 °C away from light until analysis. One production batch only was used for each protein ingredient. Protein solutions were prepared according to the experimental design presented in Table 1, heated to 50 °C, and maintained at this temperature under magnetic stirring for 2 min.

2.2. Experimental design

Response Surface Methodology (RSM, Box & Wilson, 1951) was used to simultaneously study the effect of pH, protein concentration, and added NaCl (Table 1), which are known to have a major effect on the functional properties of protein ingredients (Zayas, 1997). A Central Composite Design (CCD) was used, with pH values ranging from pH 4 to

Table 1
Central Composite Design (CCD) for independent variables of protein solutions. C: protein concentration (%) for WRP/GGRP; I: ionic strength (M added NaCl).

Run	Experimental variables		
	pH	C	I
1	5.55	12.5/16	0.2
2	4.44	7.2/13.2	0.34
3	5.55	20/20	0.2
4	4.44	17.8/18.8	0.06
5	5.55	12.5/16	0.4
6	5.55	12.5/16	0.2
7	5.55	5/12	0.2
8	4.44	7.2/13.2	0.06
9	4	12.5/16	0.2
10	5.55	12.5/16	0.2
11	5.55	12.5/16	0
12	6.56	7.2/13.2	0.06
13	7	12.5/16	0.2
14	4.44	17.8/18.8	0.34
15	6.56	17.8/18.8	0.06
16	6.56	7.2/13.2	0.34
17	6.56	17.8/18.8	0.34
18	5.55	12.5/16	0.2

pH 7; protein concentration ranging from 5% to 20% for WRP, and from 12% to 20% for GGRP; and NaCl addition ranging from 0 to 0.4 M NaCl. Considering account the NaCl content of each of the two ingredients, these quantities of NaCl added mean that the total effective NaCl content of the solutions tested varied between 0.05 and 0.60 M for WRP, and between 0.01 and 0.42 M for GGRP. The pH and NaCl ranges thus explored include conditions commonly applied in meat products, which are the most likely food applications for GGRP and WRP, and in particular the ionic strength value that favors myofibrillar protein solubilization (from 0.3 M NaCl according to Chen et al., 2017). The pH was adjusted with a few drops of NaOH (4 M) or HCl (6 M). The CCD resulted in 18 experiments that were performed randomly (Table 1), including four central points (pH 5.55, 12.5% protein for WRP *vs* 16% for GGRP, and 0.2 M NaCl). Experimental data were adjusted to a second-degree polynomial regression model that contained coefficients of linear, quadratic and interaction effects (Montgomery, 2013).

2.3. Physicochemical characterization

2.3.1. Composition

The dry matter content of the powders was determined gravimetrically after drying at 102 ± 2 °C for 5 h, and the ash content was obtained after incineration at 525 ± 25 °C, and then at 820 °C for 5 h in a muffle furnace. NaCl content was determined by coulometric titration-based chloride analysis (Corning 926), where 0.5 ml sample was added to a combined acid buffer solution (ReageconTM). Total nitrogen content was measured by the Kjeldahl method, and a nitrogen-to-protein conversion factor of 6.25 (industrial standard) was applied to determine protein content. Lipid content was determined gravimetrically after extraction with a chloroform:methanol:0.8%KCl (3:48:47) mixture, followed by evaporation under nitrogen, according to Folch (1957).

The total collagen content was calculated from the hydroxyproline content, determined by an amino acid assay method, and using a conversion factor of 8, knowing that collagen contains 12.5% hydroxyproline and is in a 3:1 collagen/elastin ratio. The method for amino acid determination consisted of acid hydrolysis of the proteins in 6 M HCl for 24 h at 110 °C under nitrogen according to Davies and Thomas (1973), followed by cation-exchange chromatography. Chromatography was performed using an automatic amino acid analyzer (Biochrom Ltd., Cambridge, UK) equipped with a 200 mm × 4.6 mm sulfonated polystyrene resin (Biochrom 30+; Serlabo technologies, Trappes, France). Samples were diluted in 0.2 M lithium citrate buffer, pH 2.2. Absorbance was measured at 440 nm after post-column derivatization with ninhydrin (EZ-NIN, Biochrom) according to Moore et al. (1958). Hydroxyproline was quantified using an external calibration curve previously established with a standard (*trans*-4-hydroxy-L-proline, Sigma-Aldrich).

All analyses were carried out in duplicate or triplicate. All values were calculated as a percentage (w:w).

2.3.2. Protein fractionation and fraction quantification

Protein fractionation was performed using an adaptation of the method that Malva et al. (2018) proposed to separate sarcoplasmic proteins (soluble at low ionic strength), myofibrillar proteins (soluble at high ionic strength), and stromal proteins (insoluble). Four grams of powder were homogenized (3 min, Ultraturax, IKA) in 40 ml of 50 mM phosphate buffer pH 7.5, followed by centrifugation (8,000*g*, 20 min, 4 °C) after which the supernatant was collected. The centrifugation pellet was then diluted in 10 volumes of KCl phosphate buffer pH 7.5 (0.45 M KCl, 15.6 mM Na_2HPO_4, 3.5 mM KH_2PO_4), homogenized, and then centrifuged (8,000*g*, 20 min, 4 °C), to recover the supernatant. The remaining pellet was recovered to quantify the insoluble proteins. Finally, each protein fraction was quantified by nitrogen determination using the Kjeldahl method, as described above. The analyses were performed in duplicate.

2.3.3. Protein analysis

Soluble proteins, the most likely involved in functional properties (Zayas, 1997), were analyzed by SDS-PAGE on acrylamide gels (4–20%) using a Mini-Protean TGX electrophoresis system® (Bio-Rad Laboratories Inc), and following protocols adapted from Parés et al. (2020) and Toldrà et al. (2019) in order to visualize myofibrillar and sarcoplasmic proteins. Protein extracts were previously diluted (1:4 ratio) in a reducing and denaturing solution-2x Laemmli Sample Buffer (2.1% SDS, BioRad) and 1 M dithiothreitol (DTT, Sigma-Aldrich) (95:5, w:w), then heated at 95 °C for 3 min before loading. Electrophoresis was performed at 200 V for 30 min. After migration, the gels were fixed (ethanol:acetic acid, 40:60) for 30 min, and then stained with Coomassie blue overnight, after which decoloring was performed in 20% ethanol solution. Protein molecular weights were estimated using molecular weight markers ranging from 10 to 250 kDa (Precision Plus Protein™ Kaleidoscope™ Prestained Protein Standards, Biorad).

2.3.4. Protein solubility index

Ten grams of powder were added into 100 ml water at 40 °C, mixed in a blender (Waring blender) for 90 *sec* at 3,800–4,000 rpm, and then centrifuged at 160*g* for 5 min at 20 °C. The protein solubility index (expressed as %) was calculated by the ratio of the protein concentration (Kjeldahl method) in the supernatant to that in the initial solution (before centrifugation). The analyses were performed in duplicate.

2.4. Gelling properties

2.4.1. Gelling temperature

The gelling temperature of the protein ingredients was determined by monitoring the viscoelastic properties of the solutions under a temperature gradient, following a protocol adapted from Xiao et al. (2020). The viscoelastic properties were evaluated using a MCR 301 rheometer (Anton Paar) equipped with a cone-plate system (diameter 49.96 mm, angle 1.996°, truncation 209 μm). The sample solution (1.5 ml) was deposited on the plate surface previously heated to 50 °C, and a fixed deformation rate of 1% and a constant frequency of 1 Hz were applied. The storage modulus (G') and the loss modulus (G'') were then recorded during cooling from 50 to 1 °C at 1 °C.min^{-1}. The gelling temperature was determined as the temperature at which G' and G'' cross or, if they do not cross, when G' becomes greater than 1 Pa. Only the WRP samples were analyzed, each in triplicate, because of insoluble solid particles present in GGRP samples.

2.4.2. Preparation of the gels

The gels were prepared by stirring (speed 3) the ingredient solutions in a Thermomix® food processor for 30 min at 50° C for WRP, and 90° C for GGRP. Then, the mixtures were cooled to 20 °C under stirring for about 30 min, before pouring the solutions into 2 cm-diameter plastic tubes (Krehalon, Deventer, Netherlands); pre-cooling was performed to avoid phase shift of the GGRP solutions during gel formation. The filled plastic tubes were then stored at 4 °C for 72 h before analysis.

2.4.3. Rupture strength and deformation

A uniaxial compression test was performed on gel cylinders of 1.5 cm height and 2 cm diameter until the gels broke, using a TA-plus texture analyzer (Lloyd Instruments, Elancourt, France), equipped with a flat cylindrical probe (4 cm diameter) and a 100 N load cell. The probe compression speed was 1 mm.s^{-1}. Data were recorded throughout the compression and analyzed using the Nexygen plus software (Lloyd Instruments, Elancourt, France). Force (N) and distance (cm) were measured at the time of the breakage; the distance measurement was used to calculate the deformation (expressed in %), dividing this value by the initial height of the gel sample. All experiments were triplicated.

2.4.4. Texture profile analysis (TPA test)

TPA-type tests were performed on gel cylinders 1.5 cm height and 2

cm diameter to access hardness, cohesiveness and adhesiveness (stickiness) parameters. The tests were performed using the aforementioned TA-plus texture analyzer, equipped with a 1.2 cm-diameter cylindrical probe and a 100 N load cell. A double compression cycle test was performed up to 33% deformation (5 mm) at a compression rate of 1 mm.s^{-1}. A rest period (5 *sec*) was applied between the two compressions. The previously mentioned Nexygen plus software was used to record compression strength and distance throughout the test.

The hardness value was determined as the maximum force (N) required to apply a 33% deformation during the first compression. Cohesiveness (dimensionless), corresponding to the ability of the gel sample to recover its original shape after the deformation stops (deformation memory), was calculated by dividing the area under the curve (AUC) of the second compression by the AUC of the first compression. Adhesiveness (J) was defined as the work required to lift the gel off the probe as its ascent after the first compression (negative area between the curve and the x-axis after the first compression) (Bourne et al., 1978). Three replicates were performed for each sample.

2.4.5. Water holding capacity

A uniaxial compression test was performed to measure the water holding capacity (WHC) of the gels. A 33% deformation (5 mm) of the gel cylinders (1.5 cm height and 2 cm diameter) was maintained for 3 min using the TA-plus texture analyzer equipped with a 4 cm-diameter plate and a 100 N load cell; the compression speed was 1 mm.s^{-1}. Each gel cylinder was previously placed on a filter paper that was weighed before and after compression. The WHC was calculated, according to a method adapted from Lechevalier et al. (2007), as the percentage of water retained in the gels, normalized by the initial weight of the gel cylinders, *i.e.* before compression:

$$\mathrm{WHC}(\%) = \frac{M_0 - M_1}{M_0} x\,100 \tag{1}$$

with M_0 the weight of the gel cylinder (g) before compression, and M_1 the amount of water released during compression (g). Three replicates were performed for each sample.

2.5. Emulsifying properties

2.5.1. Emulsion preparation

The oil/water emulsions were prepared by homogenizing the ingredient solutions and sunflower oil to a constant volume fraction (O:W 30:70, vol:w), using an Ultraturax rotor–stator system (IKA) at a speed of 20,000 rpm for 3 min at 50 °C.

2.5.2. Droplet size distribution

Lipid droplet size distribution was measured using a liquid laser scattering particle size analyzer (MasterSizer 2000, Malvern). The analyses were performed at room temperature immediately after the emulsions were prepared. A small amount (0.05–0.1 ml) of emulsion was introduced into the measuring cell of the apparatus containing ultrapure water (100 ml), in order to achieve 10% obscuration. The diluted emulsions were released at a constant, low flow rate of approximately 1 ml.min^{-1}. Typical droplet size distributions were related to three parameters, namely the mean droplet volume-surface diameter ($d_{3,2}$) (Eq. (2)), the droplet volume ($d_{4,3}$) (Eq. (3)), and the width of the droplet size distribution, *i.e.* the span (droplet polydispersity index) (Eq. (4)). The $d_{3,2}$ parameter is more sensitive to the presence of fine particles, in contrast to $d_{4,3}$, which is more sensitive to the presence of large particles.

$$d_{3,2} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \tag{2}$$

$$d_{4,3} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \tag{3}$$

$$Span = \frac{(D_{90} - D_{10})}{D_{50}} \tag{4}$$

with n_i the number of droplets of diameter d_i; D_{10}, D_{50} and D_{90} the diameters at 10, 50 and 90 % of the cumulative volume, respectively.

2.5.3. Emulsion stability

The stability of the emulsions was estimated using the TURBISCAN Lab Expert apparatus (Formulaction Company, France), based on dynamic light scattering using transmission and backscatter profiles. The emulsions were transferred into cylindrical flat-bottomed glass cells that were inserted into the device, and the measurement started immediately with a scan at 880 nm, at 50 °C, and at different times during 24 h (1 h, 2 h, 4 h, 6 h, 8 h, 24 h). The principle of this measurement is based on the variation of the volume fraction of droplets (migration) or of their average size (coalescence), which leads to a variation of the signals (Mengual et al., 1999). Since the emulsions are opaque, their stability was measured by backscattering. The signal variation was calculated as the difference between the signal at time 0 and at a given time. The stability of the emulsions was determined by the Turbiscan Stability Index (TSI) (Eq. (5)), using the Turbiscan software:

$$\mathrm{TSI} = \sum_i \frac{\sum_h |scan_i(h) - scan_{i-1}(h)|}{H} \tag{5}$$

with $scan_i(h)$ the light intensity of the sample height at the scan time i; $scan_{i-1}(h)$ the light intensity of the sample height at the previous scan time; and H the total sample height. The TSI aggregates all destabilization phenomena occurring in the sample during backscattering. The lower the TSI values, the higher the stability of the emulsion.

2.6. Statistical analysis

The data set for physicochemical characteristics and protein solubility index was subjected to multiple comparison of means (lsmeans adjust Tukey Contrast) with significance level set at $p < 0.05$, using R software (version 4.0.3). All results were expressed as mean ± SD.

Experimental design and statistical analyses were performed using Statgraphics software (Statgraphics Technologies, Inc.), including performing regression analysis on the experimental data and plotting response surfaces. For each experimental factor, the variance was divided into components (linear, quadratic, and interaction) to assess the relative importance of these components and the suitability of the following second-order polynomial function:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i,j=1}^{3} \beta_{ij} X_i X_j \tag{6}$$

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 added NaCl (ionic strength). The significant effect of the equation parameters for each response variable was assessed by Fisher's test, with significance level set at $p < 0.05$ for all tests. Error was assessed from replication of treatment conditions at the central point.

3. Results and discussion

3.1. Composition of the two dehydrated beef protein ingredients

The composition of the two protein ingredients is given in Table 2. The dry matter content is close to 95% for the two ingredients of which protein is the main component. The protein content (62.32 % in WRP *vs* 83.71% in GGRP) is high as compared with other meat co-products or processing streams, such as water recovered after freeze drying (30/40%) (Mullen & Álvarez, 2016). Between the two ingredients, GGRP has the highest protein content, and the lowest fat (9.07%) and ash content (2.48%) in comparison with WRP (13.23% fat; 9.17% ash). Moreover,

Table 2
Physicochemical characteristics of dehydrated Water Recovered Protein (WRP) and Greasy Greaves Recovered Protein (GGRP) ingredients (mean ± SD, n = 3). On a given lane, different letters and grey intensity indicate significant difference obtained from multiple comparison of means (Tukey test, $p < 0.05$).

	WRP	GGRP
Dry matter (%)	$94.90_a \pm 0.06$	$95.38_a \pm 0.32$
Protein (%) among which	$62.32_a \pm 0.00$	$83.71_b \pm 1.17$
% Soluble in 0.05 M phosphate buffer pH *7.5*	$80.27_a \pm 0.11$	$12.43_b \pm 0.04$
% Soluble in 0.45 M KCl phosphate buffer pH *7.5*	$2.37_a \pm 0.01$	$3.50_b \pm 0.22$
% Insoluble	$4.36_a \pm 0.03$	$70.46_b \pm 1.73$
Collagen (%)	$40.03_a \pm 1.69$	$36.83_a \pm 0.18$
Fat (%)	$13.23_a \pm 0.25$	$9.07_b \pm 0.38$
Ash (%) among which	$9.17_a \pm 0.04$	$2.48_b \pm 0.63$
NaCl (%)	$5.96_a \pm 0.00$	$0.60_b \pm 0.02$
pH	5.55	6.00

GGRP pH is slightly higher (6.00 *vs* 5.55). Actually, GGRP has a composition similar to beef greaves: 95.8% dry matter; 92.1% protein; 34% collagen; 9.6% fat; 1.7% ash; pH 6.75, as reported by Jobling (1994). However, GGRP and WRP are difficult to compare with other co-products described in literature, as the composition depends on each particular type of co-product and the animal species from which they originate (Toldrá et al., 2016).

Protein separation showed that 80.27% of total proteins in WRP are soluble at low ionic strength (hereafter named as "soluble"), while 2.37% were soluble at high salt concentration, and 4.36% were insoluble (Table 2). On the contrary, only 12.43% of total GGRP proteins were soluble, 3.50% were soluble at high ionic strength, and 70.46% were insoluble. Hence, very few proteins are soluble in GGRP unlike WRP, consistently with the processes applied to obtain these two ingredients. Indeed, as a reminder, WRP is obtained from water recovered during the fat rendering process of beef meat and during the bone degreasing process, whereas GGRP is derived from greasy greaves, which are solid protein products. Thus, it is assumed that the collagen present in WRP (40.03%) is mainly soluble collagen, unlike that contained in GGRP (36.83%). However, because of high temperatures and extreme conditions to which proteins have been subjected during the extraction process, it is not possible to identify with certainty the nature of the constituent proteins of each of the fractions separated based on solubility. It is therefore likely that the soluble fraction does not correspond solely to sarcoplasmic proteins. This fraction could also contain hydrolysis products of other proteins and, as mentioned above, soluble collagen. Similarly, the fraction soluble at high ionic strength probably contains more than just myofibrillar proteins.

These hypotheses are supported by the SDS-PAGE profiles of GGRP and WRP (Supplementary data, Fig. S1), which did not allow for a clear separation of proteins or profiles characteristic of muscle proteins, except a band at 15 kDa that could correspond to myoglobin (17 kDa) and/or hemoglobin (16–18 kDa) (Grujić et al., 2018). However, SDS-PAGE mainly reveals smears that suggest the presence of hydrolysis products in large quantities and of very variable size. In particular, low molecular weight components (8–12 kDa) may be small proteins and/or peptides of collagen, as suggested by Álvarez et al. (2018). Consistently

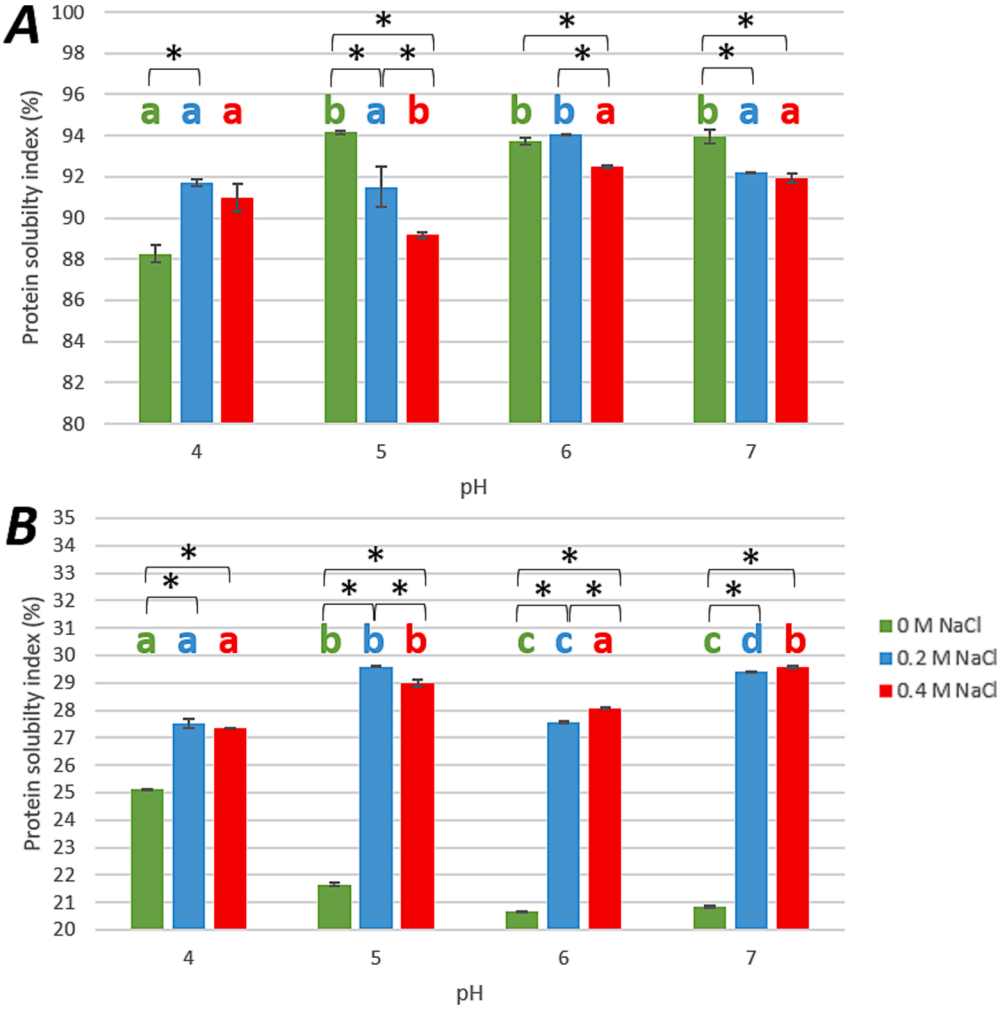

Fig. 2. Protein solubility index of Water Recovered Protein (WRP, A) and Greasy Greaves Recovered Protein (GGRP, B) depending on pH and concentration of added NaCl (mean ± SD, n = 2). For a given concentration of added NaCl (green bars, 0 M; blue bars, 0.2 M; red bars, 0.4 M), different letters indicate a significant difference ($p < 0.05$) between pH. For a given pH, * indicates a significant difference ($p < 0.05$) between added NaCl concentrations. The multiple comparisons of means were performed using the post-hoc Tukey test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with the overall composition (Table 2) and the production processes, it should be noted that the SDS-PAGE profiles of WRP proteins that are soluble at low or high ionic strength, and that of GGRP are not exactly the same. Such differences in the protein profiles may induce different functional properties.

3.2. Protein solubility is more sensitive to ionic strength than to pH

The protein solubility index of WRP varies between 88% and 94% regardless of the pH and the ionic strength (Fig. 2A). Such high values are reported in the literature for sarcoplasmic proteins, which are described as highly soluble in water and low ionic strength solutions (Xiong, 2018). In contrast, the protein solubility index of GGRP is much lower, ranging from 20 to 30% depending on the conditions (Fig. 2B). These low values are characteristic of stromal proteins, *i.e.* proteins that are substantially insoluble in neutral saline solutions of low or high ionic strength (<0.5 M) (Kijowski, 2001).

It is widely accepted that the solubility of a protein highly depends on pH and/or ionic strength. However, the effect of pH on protein solubility appears very limited for the two ingredients studied here, except at low ionic strength. In this condition (0 M NaCl added), the protein solubility significantly decreases at pH 4 for WRP (Fig. 2A), while that of GGRP increases at pH 4 (Fig. 2B). In contrast, the ionic strength has a significant impact on the protein solubility regardless of the pH, and that for the two ingredients.

Except at pH 4, WRP solubility tends to decrease when the ionic strength increases. The decrease is significant after adding 0.2 M and 0.4 M NaCl at pH 5 and pH 7, and only after adding 0.4 M NaCl at pH 6. Considering the NaCl content naturally present in WRP ingredient (5.96%; Table 2), the 10% solutions prepared for protein solubility measurement (see section 2.3.4.) contained initially about 0.6% NaCl, and up to 1.8% to 2.9% NaCl after 0.2 or 0.4 M addition, respectively. Yet, WRP ingredient mainly consists of soluble collagen (64.2% of total proteins; Table 2), the solubility of which decreases above 1% NaCl (Jeevithan et al., 2014). Thus, it is likely that after addition of 0.2 M NaCl, and even more 0.4 M NaCl, the WRP solution was in ionic strength conditions of lower protein solubility as compared with the solutions without NaCl addition.

On the opposite, whatever the pH, the protein solubility of GGRP increases with the ionic strength (Fig. 2B). The ten percentage points increase or approximately, measured from pH 5 to pH 7 after adding 0.2 M or 0.4 M NaCl could be related to sarcoplasmic and myofibrillar proteins (about 16% of GGRP proteins; Table 2), since these proteins are more soluble in salty conditions (Song et al., 2020). At pH 4, the protein solubility increase due to salt addition is lower (about 2.5 percentage points), but still significant, due to a much higher solubility at low ionic strength at this pH than at other pH values. This could be due to the presence of collagen, since beef collagen can be dissolved up to a few percent in a solution of neutral salts, or in acidic solutions (0.6% soluble with salt, 2.6% soluble with acid) (Kijowski, 2001).

3.3. Protein concentration is by far the most decisive factor in gelling properties, far more than pH or ionic strength

The formation of gel confers functional attributes (texture) important for the manufacture of many foods. A large number of proteins exhibit gelling capacity, which is influenced by pH, ionic strength, temperature, protein concentration, and of course by the nature of the proteins (Zayas, 1997). The ability to gelation is a key functional property of muscle proteins (Xiong, 2018), and in particular, bovine co-product proteins are considered as potential food ingredients when a gel-like texture is desired.

In the present study, the gelation is assumed to result from myofibrillar proteins and from gelatin. Myofibrillar proteins, namely myosin, coagulate with heat, and are widely known for their gelling properties (Sun & Holley, 2011). Gelatin, which is obtained by the heat treatment of collagen, is extensively used in food industry (Gómez-Guillén et al., 2011) as a gelling agent in the cold state. In addition, sarcoplasmic proteins may contribute to gelation when the ionic strength is low (<0.4 M), and after thermal coagulation (greater than 40 °C) (Zayas, 1997).

Gels can be defined by different characteristics, which require different analyses to study their rheological and textural properties, and their ability to immobilize a liquid (water retention). In the present study, gelling properties, *i.e.* gelling temperature, gel rupture strength and deformation, gel hardness, cohesiveness, adhesiveness and WHC, have been measured (Supplementary data, Table S1) on all samples from the experimental design described in Table 1. As a reminder, the experimental design carried out for GGRP is different from that of WRP, in that the protein concentrations tested were higher for GGRP (Table 1). Indeed, the two ingredients have different critical concentrations, *i.e.* minimum protein concentrations for gelation. Only 5% protein of WRP is enough to form a gel, while it is necessary to increase protein concentration up to 12% for GGRP. This difference may be related to their different protein composition (Table 2), leading to different protein networks.

In order to assess the relative impact of pH, protein concentration, and ionic strength on the gelling properties of WRP and GGRP, regression analyses of all experimental data have been carried out for all gel properties. Except the cohesiveness model for WRP, the resulting quadratic models (Table 3) reveal high R^2 values: 79.20% to 96.95% of the variation in the experimental data are explained by the estimated equations. However, it should be noted that the quality of the models is the poorest for gel cohesiveness and adhesiveness, with R^2 values ranging from 74.53 to 80.10%, and from 79.20 to 79.76%, respectively.

The most striking finding is the predominant effect of protein concentration that affects significantly all the gelling properties measured (Table 3). In particular, the WRP gelling temperature increases from 12.96 to 28.86 °C with protein concentration increase from 5 to 20% (while pH and added NaCl are 5.55 and 0.2 M, respectively), as well as the gel rupture strength (from 4.59 to 76.79 N) and deformation (from 51.99 to 59.69%), hardness (from 0.20 to 3.11 N), and WHC (from 99.29 to 99.89%) (Supplementary data, Table S1). Similarly for GGRP, protein concentration increase from 12 to 20%, at pH 5.55 and 0.2 M added NaCl, results in an increase of the gel rupture strength (from 7.43 to 45.51 N) and deformation (from 39.57 to 44.04%), hardness (from 1.37 to 7.47 N) and WHC (from 99.58 to 99.76%) (Supplementary data, Table S1). Regarding the gelling temperature, it is quite difficult to compare our results to literature data, since the cooling rate of the protein solutions, which was 1 °C.min^{-1} in the present study, can influence the value of the gelling point (Zayas, 1997). However, it seems reasonable to assume that the gelation occurs earlier during the cooling of the solutions when the protein concentration increases, because it enhances the probability of encountering between protein molecules, and consequently it favors protein–protein interactions. Likewise, since protein–protein and protein-solvent interactions are promoted when protein concentration increases, leading to a denser protein network able to retain water inside the gel (Zayas, 1997), it is therefore logical to see an increase in the gel rupture strength and deformation, hardness, and WHC with protein concentration.

On the opposite, gel cohesiveness and adhesiveness either increase or decrease as protein concentration increases, as indicated by the positive (gel cohesiveness of GGRP) *vs* negative (gel adhesiveness of WRP and GGRP, and gel cohesiveness of WRP) coefficients (Table 3). The texture of the gels prepared with WRP and GGRP ingredients is thus different. These differences could be due either to the composition and solubility of the proteins these ingredients are made of, and/or to a different organization of the proteins during gelation. Indeed, gelation is reported highly dependent on the type of meat proteins and the method by which these proteins are extracted (Zayas, 1997). Nonetheless, these results are overall consistent with the literature data dedicated to the gel texture of beef gelatin (Rahman & Al-Mahrouqi, 2009), which suggests that gelatin (or soluble collagen) plays a major role in the gelling properties of the

Table 3
Regression coefficients and analysis of variance of the regression models for the gelling properties of Water Recovered Protein (WRP) and Greasy Greaves Recovered Protein (GGRP) ingredients. C: protein concentration; I: ionic strength; ***p-value < 0.001, **p-value < 0.01, *p-value < 0.05 (exact p-values are given in Supplementary Table S2); SEE: standard error of estimation; MAE: mean absolute error.

	Gelling T° (°C)		Rupture strength (N)		Deformation at the rupture (%)		Hardness (N)		Cohesiveness		Adhesiveness (J)		Water holding capacity (%)	
	WRP		**WRP**	**GGRP**	**WRP**	**GGRP**	**WRP**	**GGRP**	**WRP**	**GGRP**	**WRP**	**GGRP**	**WRP**	**GGRP**
Constant	27.45		34.29	21.53	56.40	41.46	1.34	3.55	0.92	0.70	1.29	1.96	99.81	99.71
Linear														
pH	−0.15		−0.07	1.69	0.31	0.28	−0.04	0.24	0.002	0.016	−0.01	−0.07	0.02	**0.02** ***
C	**7.63** ***		**25.92** ***	**12.03** ***	**2.88** ***	**1.24****	**0.99** ***	**1.81** ***	**−0.01***	**0.03****	**−0.02***	**−0.19****	**0.15** ***	**0.06****
I	−0.49		−3.07	−0.31	0.40	**1.39** ***	−0.13	−0.13	−0.01	0.01	−0.01	−0.03	0.01	**0.03***
Interaction														
pH × C	0.23		−1.59	−0.35	−0.27	−0.23	−0.10	−0.06	0.01	−0.01	−0.01	0.02	−0.01	−0.01
pH × I	−1.21		2.10	0.54	0.11	0.07	0.05	0.08	**−0.01***	−0.01	0.01	0.02	0.01	0.01
C × I	−0.26		−2.47	−1.54	−0.05	−0.45	−0.12	−0.19	−0.01	0.01	0.01	−0.02	−0.01	0.01
Quadratic														
pH × pH	**−3.13***		−0.9	−0.01	0.80	0.08	−0.07	−0.10	−0.01	0.01	0.01	−0.05	0.01	0.01
C × C	**−3.61***		1.83	2.79	−0.14	0.10	0.09	0.45	−0.01	−0.01	**0.03****	0.30	**−0.1****	−0.01
I × I	−2.63		1.15	−1.71	0.3	−0.72	0.01	−0.27	0.01	−0.01	0.01	0.08	0.013	−0.02
R^2	90.95		96.95	94.52	92.76	88.20	96.62	93.80	74.53	80.10	79.20	79.76	92.28	89.89
R^2 adj	80.76		93.52	88.36	84.63	74.93	92.81	88.83	45.88	61.06	55.80	60.18	83.59	78.53
SEE	3.44		5.72	3.69	1.03	0.90	0.23	0.59	0.01	0.02	0.02	0.14	0.06	0.03
MAE	1.66		3.26	2.23	0.54	0.49	0.13	0.36	0.01	0.01	0.01	0.07	0.03	0.01
p-value	0.003		0.000	0.000	0.000	0.002	0.000	0.000	0.096	0.021	0.050	0.023	0.001	0.003

two ingredients studied here, even if it is not the main protein component, in particular in GGRP. Thus, it was reported that the hardness of beef gelatin increases significantly as protein concentration increases (Rahman & Al-Mahrouqi, 2009), as it is observed in the present study for the two ingredients. A significant decrease of the adhesiveness of beef gelatin gels as the protein concentration increases has been also reported, as it is observed here for GGRP and WRP. In terms of cohesiveness, WRP exhibits a different behavior than GGRP, with a cohesiveness value that decreases with increasing protein concentration. This could result from the quite high fat content in WRP (13.23%), as compared to GGRP (9.07%), by analogy with the negative effect of lipids on gel cohesiveness of beef ground patties, likely due to the weakening of the internal bonds of the protein matrix (Troutt et al., 1992).

Moreover, a quadratic effect of protein concentration is observed on the one hand on gelling temperature, gel adhesiveness and WHC of WRP (Table 3), which means there is a protein concentration from which the effect on these properties is reversed (Supplementary data, Fig. S2). Thus, in the experimental range of protein concentration, maximum values are measured for WRP for gelling temperature (31.5 °C at 18.1% protein, pH 5.53 and 0.17 M added NaCl), and WHC (99.91 % at 16.4% protein, pH 7, 0.33 M added NaCl), whereas a minimum value is measured for adhesiveness (1.27 J at 12.7% protein, pH 5.46, 0.4 M added NaCl).

On the opposite, the effect of pH and ionic strength on the gelling properties of WRP and GGRP is much more marginal. Table 3 indicates a significant impact of pH on the WHC of GGRP gels, which increases with pH, and a quadratic effect of pH on the gelling temperature of WRP (Table 3), with a maximum (31.5 °C) at pH 5.53 (and 18.1% protein, 0.17 M added NaCl) (Supplementary data, Fig. S2). The marginal role of pH can be explained by the fact that pH mainly modifies the solubility of myofibrillar proteins (Zayas, 1997) that can be assumed very minor proteins in the two ingredients (2.37 to 3.50% proteins soluble at high ionic strength; Table 2), compared to soluble (in WRP) or insoluble (in GGRP) proteins.

GGRP is the ingredient for which gelling properties are the most sensitive to ionic strength, as the WHC and the gel deformation at the rupture increase with ionic strength (Table 3). The positive effect of NaCl addition on the deformation at the rupture of the gels suggest that, despite their low concentration in GGRP, the proteins soluble at high ionic strength play a certain role in this property of the gels. These proteins may be myofibrillar proteins, the solubility of which is known to be increased in salty conditions (Song et al., 2020). Furthermore, the conformation of meat proteins is influenced by ionic strength, which is determining for gelling properties since gelation results from protein-protein and protein-solvent interactions (Zayas, 1997). Similarly, the WHC of the GGRP gels significantly increases with ionic strength (Table 3; Supplementary data, Fig. S2) probably because of the increase in protein solubility (Fig. 2B), since the literature mentioned that the WHC of muscle proteins highly depends on the NaCl concentration (Zayas, 1997). In addition, WHC of the GGRP gels also significantly increases with pH, consistently with the literature that reported a higher pH combined with the presence of collagen and other connective tissue proteins may be responsible for higher WHC (Devatkal et al., 2004).

It is interesting to note that the low impact of ionic strength and pH on the texture of the gels prepared with WRP and GGRP should be regarded as a positive point, as these ingredients can therefore be considered in many food applications, without constraint related to these two key physicochemical parameters in food science and food technology.

3.4. Emulsifying properties are mainly driven by protein concentration and pH

Many foods are oil/water emulsions, the sensory qualities of which (creaminess, smoothness…) are very appreciated by consumers. Unfortunately, these food systems are thermodynamically unstable, and

therefore require emulsifying agents to help emulsion formation, but more importantly, to stabilize the emulsions created. Emulsifying properties highly depend on the type and concentration of the emulsifier, pH, ionic strength and temperature, especially as the emulsifier is a protein (McClements, 2004). The emulsifying properties of WRP and GGRP may be due to the presence of collagen that is a good emulsifier (Gómez-Guillén et al., 2011). In addition, the presence of myofibrillar proteins (myosin) may also contribute to this functionality (Xiong, 2018).

In the present study, the emulsifying properties have been evaluated by measuring the size ($d_{3,2}$ and $d_{4,3}$ parameters) and polydispersity index (span) of the lipid droplets, and the destabilization phenomena during storage at 50 °C for 24 h (TSI index); the lower the TSI value, the more stable the emulsion. The temperature was chosen in order to avoid any gelation of the emulsions, which may bias the measurement of their stability. Moreover, emulsion stability was measured over a 24-hour period only, on the understanding that GGRP and WRP are mainly intended as emulsifying agents whose role would be to facilitate emulsion formation in meat products (such as sausages, liver mousse, etc.), while the subsequent cooking step sets the structure.

A regression analysis of all the experimental data, which are available in Supplementary Table S3, has been carried out in order to assess the relative impact of pH, protein concentration, and ionic strength on the emulsifying properties of WRP and GGRP (Table 4). In order to make the reading of Table 4 easier, the emulsion stability model is presented for TSI measurement after 1 h storage only; the TSI models corresponding to all storage durations are presented in Supplementary data (Table S5 and Fig. S4).

Except the $d_{3,2}$ model for WRP, and the $d_{4,3}$ and span models for GGRP, the quadratic models determined from the experimental results were all highly significant, with R^2 values ranging from 84.80% to 97.56% (Table 4), meaning that the variation in the experimental data is explained satisfactorily by the estimated models.

As for gelling properties, protein concentration appears as the main variable that significantly influences the emulsifying properties of the WRP and GGRP ingredients. Among the five significant regression models, protein concentration significantly affects all the parameters measured for the two ingredients (Table 4). For WRP, the coefficients of protein concentration in the droplet size and span models are negative, indicating that the increase in protein concentration induces smaller droplets with a lower polydispersity index. Specifically, as protein concentration increases from 5 to 20% (while pH and added NaCl are 5.55 and 0.2 M, respectively), $d_{4,3}$ decreases from 23.96 to 10.56 μm, and span index decreases from 2.66 to 1.41 (Supplementary data, Table S3). This can be explained by more proteins available to cover the surface of the droplets, consistently with Tcholakova et al. (2003). At the same time, the emulsion stability increases, as indicated by the negative coefficient of TSI, which is actually an instability index (Table 4). Thus, TSI-1 h decreases from 25.80 to 1.90 (Supplementary data, Table S3). This is in agreement with the known relationship between an increased protein concentration and a limited destabilization by coalescence (Delahaije et al., 2015). On the opposite, the droplet size ($d_{3,2}$) of GGRP emulsions increases (from 40.72 to 76.28 μm) with protein concentration increase from 5 to 20% (Supplementary data, Table S3). However, this may be an artefact due to the low solubility of GGRP proteins (from 20% to 30%; Fig. 2B), making that particle size analysis could include indiscriminately both lipid droplets and insoluble particles. Furthermore, as for WRP and as expected, the stability of GGRP emulsions increases (TSI-1 h decreases from 11.10 to 4.60) with protein concentration (from 5 to 20%) (Table 4; Supplementary data, Table S3). Indeed, in spite of the low protein solubility of GGRP, as the protein concentration increases, the quantity of soluble proteins also increases, thus likely contributing to the formation of a more viscoelastic interface (Bos & van Vliet, 2001). In addition, quadratic effects of protein concentration are significant (Table 4), indicating the existence of maximum or minimum values (Supplementary data, Fig. S3). Hence, for GGRP, $d_{3,2}$ is minimum (27.36 μm) when protein concentration is 7.74% (and pH 5.73, 0 M added NaCl), and for WRP, $d_{4,3}$ is maximum (33.08 μm) when protein concentration is 5% (and pH 4.25, 0 M added NaCl), while emulsion stability is minimum (maximum TSI-1 h of 28.94) when protein concentration is 6.74% (and pH 5.92, 0.16 M added NaCl).

In addition to the main influence of protein concentration presented above, it turns out that pH has also a significant effect on several characteristics of the emulsions (Table 4). Thus, the polydispersity index (span) of the WRP emulsions decreases (from 1.82 to 1.71) as pH increases (from pH 4 to pH 7, while protein concentration and added NaCl are 12.5% and 0.2 M, respectively) (Table 4; Supplementary data, Table S3). This is likely because of the trend towards an increased protein solubility at higher pH (Fig. 2A), since the more soluble proteins in solution, the more proteins available to emulsify the lipid fraction. Moreover, a decreased emulsifying capacity of proteins has been suggested at pH close to the isoelectric point (pI) (Cheftel et al., 1985). Yet, WRP is mainly composed of soluble proteins (Table 2), potentially including sarcoplasmic proteins, the pIs of which are close to pH 4. Then, in the experimental domain of the present study, pH increase could mean pH farther from pI. In addition, at a pH far away from pI,

Table 4
Regression coefficients and analysis of variance of the regression models for the emulsifying properties of Water Recovered Protein (WRP) and Greasy Greaves Recovered Protein (GGRP) ingredients. C: protein concentration; I: ionic strength; the shaded columns indicate not significant models; ***p-value < 0.001, **p-value < 0.01, *p-value < 0.05 (exact p-values are given in Supplementary Table S4); SEE: standard error of estimation; MAE: mean absolute error.

	$d_{3,2}$ (μm)		$d_{4,3}$ (μm)		Span		TSI 1 h	
	WRP	**GGRP**	**WRP**	**GGRP**	**WRP**	**GGRP**	**WRP**	**GGRP**
Constant	9.15	41.93	24.80	239.13	1.91	3.88	22.98	7.42
Linear								
pH	−0.11	−3.91	−1.73	−2.08	**−0.08***	−0.17	1.25	**−2.13****
C	−0.47	**16.49** ***	**−4.56** ***	27.17	**−0.41** ***	−0.34	**−10.57** ***	**−4.14** ***
I	−0.28	4.76	0.18	25.03	0.06	−0.40	−0.25	**−1.66***
Interaction								
pH × C	0.64	−7.54	**4.46****	0.86	0.07	0.14	−0.86	1.31
pH × I	0.29	3.58	0.87	−0.90	0.04	0.24	−0.48	0.41
C × I	−0.28	−4.50	−0.30	−35.06	−0.02	0.31	0.26	**1.56***
Quadratic								
pH × pH	0.28	5.37	**−2.78***	29.24	**−0.09***	−0.23	**−2.87****	**2.74****
C × C	−0.21	**11.93***	**−2.73***	13.98	0.04	−0.12	**−5.04** ***	−0.079
I × I	0.35	1.71	0.73	3.68	−0.03	0.28	−1.34	0.42
R^2	***55.89***	84.80	90.93	***63.65***	96.24	***64.31***	97.56	93.53
R^2 adj	6.26	73.03	81.61	22.75	92.3	24.17	94.82	86.25
SEE	1.01	11.42	2.68	49.82	0.10	0.66	2.27	1.83
MAE	*0.53*	6.03	1.34	*29.03*	0.05	*0.35*	1.25	0.94
p-value	*0.44*	0.01	0.003	*0.27*	0.00	*0.26*	0.000	0.0007

electrostatic repulsions between the lipid droplets increase, due to an increased net charge of the proteins surrounding the droplets, thus preventing flocculation (Delahaije et al., 2017). The pH also affects the stability of GGRP emulsions that increases with pH, as indicated by the TSI-1 h decrease from 16.70 to 10.30 when pH increases from 4 to 7 (Supplementary data, Table S3), but it does not affect that of WRP emulsions (Table 4). These contrasting behaviors could be related to the protein composition that differs between WRP and GGRP (Table 2), with in particular different pI ranges. As mentioned above, pH has been reported as a key factor for protein emulsion stability, with typically a lower stability at a pH close to the pI (Delahaije et al., 2017), despite some counter-examples such as gelatin and egg white proteins that have optimal emulsifying properties at their pI (Cheftel et al., 1985). Moreover, several quadratic effects of pH are also significant (Table 4), indicating minimum or maximum values in the experimental domain investigated here (Supplementary data, Fig. S3). In WRP emulsions, a maximum value of $d_{4,3}$ (33.08 μm) is observed at pH 4.25 (and 5% protein, 0 M added NaCl), a maximum value of span (2.7) at pH 5.01 (and 5% protein, 0.37 M added NaCl), but a minimum stability of emulsion (maximum TSI-1 h of 28.94) at pH 5.92 (and 6.74% protein, 0.16 M added NaCl). In GGRP emulsions, stability is maximum (minimum TSI-1 h of 1.24) at pH 5.59 (and 20% protein, 0.01 M added NaCl).

Interestingly, the ionic strength only affects the stability of GGRP emulsions (Table 4). The increased stability of GGRP emulsions as ionic strength increases may be a direct consequence of the much higher protein solubility at high ionic strength, especially at pH 5 to 7 (Fig. 2B). Therefore, more proteins are soluble and thus available to cover the surface of the droplets, promoting the stability of the emulsions. This hypothesis has already been raised to explain the behavior of emulsions of sausage meat type (pH 4 to pH 8, in the presence of 0.5 to 1 M NaCl), in which myofibrillar proteins solubilized better in moderate salty conditions (<1 M) by a "salting-in" effect (Cheftel et al., 1985).

4. Conclusions

The objective of this study was to better understand the solubility, gelling and emulsifying properties of two innovative protein ingredients derived from beef co-products that can offer a sustainable protein resource. These ingredients being logically more adapted to a use in meat products, or at least in salted foods, their functionalities have been studied in pH and NaCl ranges compatible with this type of food products, *i.e.* between pH 4 and pH 7, and without or with addition of NaCl up to 0.4 M.

The amount of NaCl added was found to have an impact on the protein solubility of both ingredients, but in opposite directions. Increasing the ionic strength tended to decrease the solubility of WRP proteins, extracted from water recovered during the fat rendering process, whereas the opposite was observed for GGRP proteins, extracted from the greasy greaves obtained during the same process. This is consistent with the protein composition of the two ingredients, since WRP is mainly composed of soluble proteins, whereas GGRP contains mainly insoluble proteins. This difference in protein composition also explains the much higher protein solubility of WRP (88–94%) compared to GGRP (20–30%). However, it should be noted that ionic strength had little or no impact on the functional properties studied. Regarding pH, the impact on protein solubility and functional properties is very limited, except for some parameters of emulsifying properties, with again different behaviors depending on the ingredient, probably due to the differences in protein composition, and therefore in average isoelectric point.

Overall, the most influential parameter on all functional properties of both ingredients is the protein concentration. The low impact of pH and ionic strength on their properties is finally a positive result for the use of both ingredients as functional agents, since it suggests they can express their functionalities regardless of pH and ionic strength conditions. The only parameter to be controlled would be their rate of incorporation in food.

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CRediT authorship contribution statement

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

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Francoise Nau reports financial support was provided by Fondation Institut Agro.].

Data availability

Data will be made available on request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.137298.

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