Controlled whey protein aggregates to modulate the texture of fat-free set-type yoghurts

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ABSTRACT

The impact of nanoparticulated whey protein aggregates on the texture of fat-free set-type yoghurts was investigated. Monodisperse (MFA) and polydisperse (PFA) fractal aggregates obtained from heated whey protein isolate (WPI) were added to skimmed milk for yoghurt manufacture at four different concentrations (0.2%–1.5%, w/w). The impact of the concentration and the polydispersity of the aggregates on fat-free set-type yoghurts were studied by instrumental measurements (rheology, penetrometry, syneresis and microscopy) and sensory analysis. Yoghurt gel strength and firmness increased with the concentration of WPI, MFA and PFA. However, yoghurts enriched with PFA clearly differed from the yoghurts enriched with WPI. Indeed, yoghurts enriched with PFA were characterised by a weak gel, a low firmness and a low-density of the protein network. Sensory analysis confirmed the results obtained by instrumental measurements. The whey protein aggregates studied are thus promising tools to modulate fat-free yoghurt texture while using milk-derived ingredients.

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1. Introduction

Yoghurt acceptance and consumption highly depends on texture properties (Huc, Michon, Bedoussac, & Bosc, 2016). Smoothness, creaminess and mouthfeel are considered as the most desirable properties by consumers (Nguyen, Kravchuk, Bhandari, & Prakash, 2017). To meet consumers' expectations of low-fat products and desirable texture, stabilisers (starch, gelatin, pectin) are used by yoghurt manufacturers to reduce the fat content (Cheng et al., 2017). However, over the last several years, special emphasis has been given to naturally made products with as few additives as possible (Thomas & Beeren, 2016). Another solution to improve yoghurt texture is to add milk ingredients considered natural and safe by consumers (skim milk powder, milk protein concentrate, whey protein isolate) (Matumoto-Pintro, Rabiey, Robitaille, & Britten, 2011). However, the amount of milk ingredients that can be added is limited because of a powdery taste, an excessive firmness or a higher whey release that may occur at high concentrations of added ingredients (Aziznia, Khosrowshahi, Madadlou, Rahimi, & Abbasi, 2009; Liu et al., 2016).

Whey proteins are mainly composed of β-lactoglobulin and α-lactalbumin and are heat-sensitive ingredients (Donato & Guyomarc'h, 2009; Nicolai, Britten, & Schmitt, 2011). Heating a solution of whey protein isolate (WPI) above 70 °C induces their unfolding and leads to the formation of whey protein aggregates (WPA) by covalent and non-covalent interactions (Lucey, Otter, & Horne, 2017). During yoghurt manufacture, the milk is heated prior to fermentation, leading to the formation of WPA, which are key elements for the gel formation. WPA interact with the casein micelles and enable the formation of a three-dimensional network during fermentation (Xu, Emmanouelidou, Raphaelides, & Antoniou, 2008). A means to optimise gel formation, and thus yoghurt texture, could be the control of the properties of WPA. This can be done by preprocessing native WPI in controlled conditions to obtain WPA that can then be incorporated in the milk during yoghurt manufacture.

In recent years, several studies focused on the addition of WPA with a size between 1 and 10 μm in dairy products (Ipsen, 2017). WPA with a size greater than to 1 μm (MicroWPA) have been used in an attempt to substitute fat in low-fat yoghurts (Sandoval-

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Castilla, Lobato-Calleros, Aguirre-Mandujano, & Vernon-Carter, 2004; Torres et al., 2018; Torres, Janhøj, Mikkelsen, & Ipsen, 2011). In these studies, the addition of MicroWPA had an impact on the structure of the protein network, leading to a less dense and more open structure. This raises the question of the way MicroWPA, and WPA in general, are integrated into the protein network.

It can be hypothesised that the impact of the addition of aggregates on the texture depends on the way they are integrated into the protein network (Torres, Mutaf, Larsen, & Ipsen, 2016). In this respect, the addition of WPA with a size less than 1 μm (NanoWPA) could be interesting to improve the texture of yoghurts. Indeed, NanoWPA are more able to interact with other proteins via covalent and non-covalent bindings because of their higher surface area (Liu, Jæger, Nielsen, Ray, & Ipsen, 2017). Gel enhancement and better whey retention have been reported in model systems enriched with NanoWPA (Andoyo, Guyomarc'h, Cauty, & Famelart, 2014). Moreover, a comparison between Nano and MicroWPA showed a higher firmness and viscosity as well as a lower syneresis and a denser microstructure in the systems containing NanoWPA (Liu et al., 2016). NanoWPA seem to be promising to improve texture in dairy products, but few studies have focused on targeted aggregates in this range of size (<1 μm) and on their impact on the texture of real food matrices.

Producing WPA with good texture functionality highly depends on the experimental conditions used for their production. In this regard, the most important factors are the protein concentration, the heating protocol, the type and concentration of added salts, and the pH (Nicolai et al., 2011). Optimal conditions for the production of WPA have been determined to produce nanoparticulated branched aggregates. These WPA are called fractal aggregates (FA) and are formed from a WPI solution (5%, w/w) heated at 80 °C for 2 h at neutral pH (Loiseleux et al., 2018).

FA structure and morphology have been characterised using innovative techniques by Loiseleux et al. (2018), but the textural properties of FA have not been studied yet in complex food matrices. The purpose of this study was to investigate how the presence of fractal WPA affects the textural properties of fat-free set type yoghurts. For that objective, yoghurts were prepared with low heat skimmed milk enriched either with native WPI, as usually performed in the food industry, or with pre-formed FA. The impact on texture was assessed by linking the rheological properties, the texture measurements and the yoghurt microstructure to the sensory characteristics of the yoghurts.

2. Materials and methods

2.1. Materials

Low-heat spray dried skimmed milk powder (34% proteins, <1.5% fat, 8.5% ash) and WPI (86.51% proteins including 1.98% caseins, 0.4% fat, 1.92% ash) were kindly supplied by local dairy companies (confidential origin). The composition of the powders is given according to the manufacturers information. Food grade sodium chloride (NaCl) and sodium hydroxide were bought at Sigma Aldrich (Saint-Louis, MO, USA). YFL-812 (Chr Hansen, Paris, France) was used as starter culture because of its low ability to produce exopolysaccharides. MilliQ (Merck Millipore, Burlington, VT, USA) water was used for the production of WPI solutions and FA. All ingredients were food-grade.

2.2. Experimental design

In the following text, the term "yoghurt" will be used to mean "fat-free set-type yoghurt".

The protein content in the yoghurts, the type of protein (WPI or FA) and the polydispersity of FA (MFA and PFA) were the three

Table 1
Overview of the experimental design used.[a]

Yoghurt sample codes	Protein content (%, w/w)		Type of protein added (%, w/w)	
	Total protein level	Total whey protein	Native WPI	Fractal aggregates
Control	3.40	0.68	—	—
WPI02	3.60	0.75	0.2	—
MFA02	3.60	0.75	—	0.2
PFA02	3.60	0.75	—	0.2
WPI05	3.90	1.02	0.5	—
MFA05	3.90	1.02	—	0.5
PFA05	3.90	1.02	—	0.5
WPI10	4.40	1.46	1.0	—
MFA10	4.40	1.46	—	1.0
PFA10	4.40	1.46	—	1.0
MFA15	4.90	1.90	—	1.5
PFA15	4.90	1.90	—	1.5

[a] Two factors were varied: the total protein content (theoretical values in %, w/w), and the type of protein (WPI, MFA and PFA) added to the yoghurts.

factors studied. The concentration of protein added in the yoghurts varied from 0.2% (w/w) to 1.5% (w/w) that led to yoghurts with a protein content between 3.6% and 4.9%. Yoghurts were enriched with native WPI, monodisperse fractal aggregates (MFA) or polydisperse fractal aggregates (PFA). The experimental design is shown in Table 1.

2.3. Preparation of protein solutions

WPI solutions were obtained by dissolving 50 g L^{-1} of WPI powder in Milli Q water under magnetic stirring. The solutions were stored overnight at 4 °C.

The protocol used for the production of FA was based on the protocol reported by Loiseleux et al. (2018). FA were produced from WPI solutions (50 g L^{-1}). To promote protein aggregation, 15 mM and 45 mM of NaCl was added to obtain respectively MFA and PFA. The solution was adjusted to pH 7 with 1 M sodium hydroxide and stored overnight at 4 °C to ensure complete solubilisation. A volume of 200 mL was held in a water bath at 80 °C for 2 h to obtain fractal aggregates. Due to the extensive heating time, a stable dispersion of aggregates is obtained and no native protein remains (Nicolai et al., 2011).

2.4. Characterisation of fractal aggregates

2.4.1. Dynamic light scattering

The particle size distribution of the FA was measured by dynamic light scattering (DLS) using a Zetasizer nano ZS (Malvern instruments Ltd, Malvern, England). Analysis was performed on the diluted sample in Milli Q water (1:100) to avoid multiple scattering effects. The diluted solutions were placed in a plastic cell and analysed at 20 °C in a backscattering configuration at 173° for 120 s. Each sample was run in triplicate to measure the hydrodynamic diameter. A refractive index of 1.45 was used for protein particles (Zhang, Arrighi, Campbell, Lonchamp, & Euston, 2016) and the % intensity was used for further analysis.

2.4.2. Transmission electron microscopy

Observations using transmission electron microscopy (TEM) were performed based on the method described in Loiseleux et al. (2018). Fractal solutions were diluted to reach a concentration of 0.05 g L^{-1}. A volume of 15 μL of diluted sample was deposited on a carbon grid (200 meshes) and stained with 2% uranyl acetate sodium during 2 min. Samples were rinsed twice with Milli Q water and dried for 20 min at 50 °C. Samples were observed using a

transmission electron microscope JEOL JEM-1230 (JEOL, Tokyo, Japan) operating at 80 kV.

2.5. Yoghurt manufacture

Skimmed milk was reconstituted to 100 g kg^{-1} milk solids using low-heat spray-dried skimmed milk powder. The reconstituted milk was stored overnight at 4 °C to allow hydration of the powders. After being heated at 90 °C for 5 min, the milk was cooled to the fermentation temperature (43 °C) and inoculated with the yoghurt starter culture. During yoghurt manufacture, the milk was enriched either with WPI or with FA that were added either before or after heat treatment of the milk (Table 1). The mix was conditioned in glass cups (40 mL for sensory analysis or 70 mL for instrumental analysis, except viscoelastic properties) and put in an incubator for fermentation at 43 °C during about 6 h until the pH reached 4.60. pH measurements were carried out at the end of fermentation with a pH 210 Microprocessor pH meter (Hanna Instruments, Woonsocket, RI, USA). Special cylindrical moulds were used for rheology measurements to prepare thin slices of yoghurt without damaging the texture of the gels. It was confirmed that the type of cup used had no influence on the texture properties of yoghurts. The samples were stored at 4 °C for 5 days before analysis.

2.6. Rheological measurements

Rheological analyses were performed using an AR1000 (TA Instruments, New castle, DE, USA) fitted with a sanded plate (diameter: 14 mm) to avoid slippage and a gap of 1 mm.

2.6.1. Viscoelastic properties

To define the linear viscoelastic zone, stress sweeps were performed at a frequency of 1 Hz between 0.1 and 1000 Pa. A stress yield (τ_0), defined as the intersection point between the storage modulus G′ and the loss modulus G″, was determined during the stress sweep. A strain of 1.0%, which was within the linear viscoelastic region for all samples, was selected for the frequency sweep experiments (0.1–10 Hz) that were performed on new intact yoghurt samples. For a comparative purpose, the value of G′, G″ and tan δ at 1 Hz were extracted from the frequency sweep results.

2.6.2. Flow properties

Flow curves were obtained by varying the shear rate logarithmically from 0.1 to 150 s^{-1}. The shear stress data were fitted to the Herschel Buckley model using a nonlinear regression on Excel software:

$$\sigma = \sigma_0 + K\dot{\gamma}^n \tag{1}$$

where σ is the shear stress (Pa), σ_0 is the yield stress (Pa), K is the consistency index (Pa s^n), $\dot{\gamma}$ is the shear rate (s^{-n}) and n is the dimensionless flow behaviour index, which indicates the proximity with the Newtonian behaviour of the fluid. Values of K, σ_0 and n were extracted from the equation.

2.7. Texture analysis

Firmness and mechanical properties of yoghurts were determined using a traction-compression device (INSTRON, Instron S.A., Boston, MA, USA). A cylindrical probe (12 mm diameter) was installed on the measurement cell (maximum capacity of 2 N). A 20 mm penetration was applied in the yoghurt at a speed of
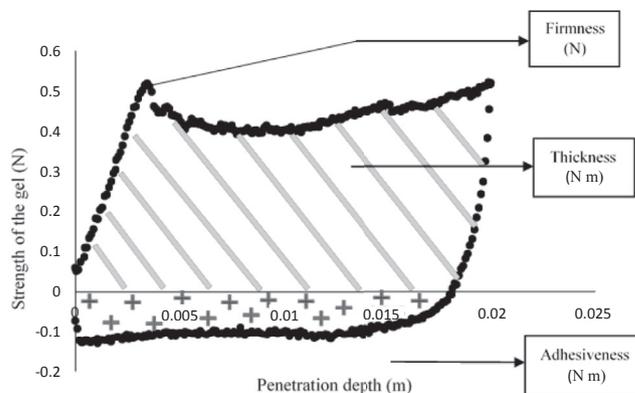

Fig. 1. Typical penetrometry profile obtained during a penetration test on low-fat set-type yoghurts: firmness, first force peak; stiffness, positive force area; adhesiveness, negative force area.

1 mm s^{-1} and returned to the surface of the yoghurt at the same speed. The measurements were done directly in the glass container (45 mm inner diameter) at 10 °C. Data were recorded using Merlin software. Three parameters were extracted from the penetrometry profiles: firmness, thickness and adhesiveness (Fig. 1).

Firmness (N) was defined as the first significant discontinuity on the force curve, produced when the probe penetrated into the yoghurt (Salvador & Fiszman, 2004). The area under the curve (N m) was extracted as it is related to the work necessary to break the product and it has been linked to thickness characteristics (Cayot, 2006; Seuvre, Turci, & Voilley, 2008). Adhesiveness (N m) was defined as the work necessary to pull the probe away from the sample (Pons & Fiszman, 1996) and corresponded to the area between the negative curve and the x-axis.

2.8. Syneresis

Syneresis was determined by centrifugation of a sample of yoghurt (10 g) at 222 g for 15 min at 4 °C as suggested by Saffon et al. (2013). After centrifugation, the clear supernatant was poured off and weighed. The percentage of syneresis (g of drained whey per g of yoghurt) was determined.

2.9. Confocal laser scanning microscopy

Rhodamine B isothiocyanate (RITC) was used to stain the proteins. Three hundred μL of a solution of RITC (2%, w/w) was mixed with 50 g of yoghurt just after inoculation of the starter culture. A few drops of the mixture were placed on a slide with a spacer, and a cover slip was placed over the sample. The slide was wrapped in aluminium foil and placed in an incubator at 43 °C for 6 h. The yoghurt samples were studied using a water objective of 40× on a Nikon A1 confocal scanning laser microscope (Nikon, Tokyo, Japan) with a laser emitting at 543 nm. Image analysis was done on ImageJ software and at least 5 representative images (256 × 256 pixels) of each yoghurt were chosen for image analysis processing to assess the porosity of the network. Porosity corresponds to the ratio between the aqueous phase area and the total area of the image. The aqueous phase was defined as the pixels in the image that do not contribute to the protein network. Images were transformed in grayscale images and a threshold was applied on them to differentiate between the water phase and the protein matrix. The particle analysis calculated the total area of the black holes (aqueous phase) of the image and the % area occupied.

2.10. Sensory analysis

2.10.1. Ethics

The sensory tests were conducted in accordance with the Declaration of Helsinki. All applicable institutional and governmental regulations concerning the ethical use of human volunteers were complied with during this research study. Panellists gave written consent after reading detailed information about the study. The sensory tests performed in this study were approved by the ethics evaluation committee of INSERM (IRB00003888, IORG0003254, FWA00005831).

2.10.2. Organisation

Panellists were recruited among students and university staff. No training was necessary for the sensory tests. The analysis took place in the sensory laboratory of Oniris, designed in accordance with international standards (ISO NF EN 8589). Paper forms were used.

2.10.3. Triangle tests

Triangle tests were performed to determine if differences were perceived among yoghurts enriched either with WPI or with FA. A triangle test consists of assessing which one of the three samples (2 similar and one different) is different from the others. At first, yoghurts enriched with WPI were compared with yoghurts enriched with MFA to determine if texture differences existed. Yoghurts enriched with MFA and PFA were also compared, bringing the overall number of triangle tests to five (Table 4). The tests were performed in 3 different sessions with 40 naïve participants each time. Samples were served straight from the refrigerator in coded glass containers in balanced random order to account for order effects. The six triads (AAB, ABA, BAA, BBA, BAB, ABB) were also counterbalanced between the participants. Panellists were instructed to taste the sample to determine the different one and to precise, which characteristic helped them choose (aspect, texture, flavour). They were encouraged to clean their mouth between samples by drinking water (Evian, France).

2.10.4. Ranking tests

The goal of the ranking test was to order 6 yoghurts with respect to firmness. Firmness was chosen for this study because it was easily understandable by naïve panellists and it could be related to instrumental measurements of texture in set yoghurts. The 44 panellists recruited for the test received 6 coded-yoghurt samples straight from the refrigerator in a balanced random order. They were instructed to rank the samples from the least firm to the firmest and were encouraged to clean their mouth between samples by drinking water (Evian).

2.11. Statistical analysis

One-way analysis of variance (ANOVA) was performed on the instrumental data to determine if significant differences exist between samples for texture, rheological parameters and syneresis. If significance was observed, the least significant difference (LSD) test was run to establish differences between means (StatgraphicsCenturion XVII, Statpoint Technologies Inc, Warrenton, VA, USA).

Regarding sensory analysis, the statistical analysis for the triangle test was based on the binomial law with a p-value of 5% (Type I error).

A Friedman test was performed on the ranking data obtained during the sensory analysis ($\alpha = 5\%$) using the software XLSTAT (Addinsoft, Andernacht, Germany, 2017). If significance was observed, the least significant difference (LSD) test was run to establish differences between means.

3. Results and discussion

3.1. Characteristics of fractal aggregates

Fig. 2 shows the size distribution (diameter) obtained for FA using DLS. Two types of FA were obtained depending on the NaCl concentration used during production. A thin and monodisperse population of fractal aggregates (MFA) with a Z-average size of 200 nm (±38 nm) was obtained on addition of 15 mM NaCl. The addition of a higher level of NaCl (45 mM) led to a much more polydisperse population of fractal aggregates (PFA) with two distinct sub populations: some aggregates with an average size of 220 nm (±15 nm) (identical to those obtained with the addition of 15 mM NaCl), and bigger aggregates with an average size of 1370 nm (±257 nm). It is already reported that the aggregation between whey proteins is facilitated by the addition of salt, which leads to a reduction of electrostatic interactions by increasing screening (Nicolai et al., 2011). During heating, the solutions changed progressively from a transparent yellow low viscosity solution to a creamier white solution. The solution with 45 mM NaCl was visually more viscous than the solution at 15 mM NaCl. The difference in viscosity between FA samples obtained with different quantities of NaCl could also indicate the presence of large whey protein aggregates formed during the heating process.

TEM images were useful to supplement the DLS results and gave more details about the structure of the different aggregates (Fig. 2a and b). These confirmed the size distributions observed in DLS with a homogeneous population of curved-strand aggregates for small amounts of NaCl (15 mM) and bigger aggregates when more NaCl was added (45 mM), as shown in Fig. 2a and b, respectively. The fractal structure of the bigger aggregates was confirmed by TEM. These were characterised by an expanded and branched structure, which is in accordance with the results of Baussay, Bon, Nicolai, Durand, and Busnel (2004). Loiseleux et al. (2018) showed that the apparent average density of the FA decreases when the size of FA increases. This is consistent with the branched morphology of large FA observed in our study. This expanded structure corresponds to a second step of aggregation where primary aggregates are linked by disulphide bonds and hydrophobic interactions to form FA (Nicolai et al., 2011).
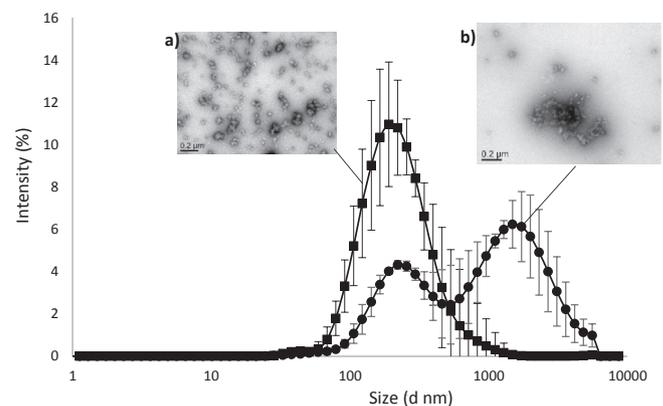

Fig. 2. Size distribution of MFA and PFA by intensity (DLS) coupled with TEM observation (scale bar = 200 nm): (a) filled squares, fractal aggregates (15 mM NaCl, pH 7, 2 h at 80 °C); (b) filled circles, fractal aggregates (45 mM NaCl, pH 7, 2 h at 80 °C).

Table 2
Rheological properties of yogurts.

Yogurt sample codes	Frequency sweep		Stress sweep	Fitting of power law model	
	G′ (Pa) at 1 Hz	G″ (Pa) at 1 Hz	τ_o (Pa)	K (Pa.s)	n
Control	342.8 ± 38[h]	91.5 ± 10.2[h]	56.5 ± 1.3[g]	46.7 ± 2.5[i]	0.12 ± 0.01[cba]
WPI02	572.7 ± 60.4[fed]	151.6 ± 15.0[def]	82.3 ± 8.7[def]	72.4 ± 7.7[fe]	0.11 ± 0.03[cb]
MFA02	443.2 ± 30.0[hg]	116.6 ± 7.9[gh]	65.7 ± 8.8[fg]	60.0 ± 6.0[hg]	0.15 ± 0.01[a]
PFA02	363.3 ± 32.5[h]	99.3 ± 6.3[h]	69.9 ± 11.0[fg]	54.0 ± 8.0[ih]	0.12 ± 0.02[ba]
WPI05	662.1 ± 95.5[d]	174.0 ± 22.7[d]	102.8 ± 10.9[cd]	84.0 ± 11.2[d]	0.07 ± 0.02[d]
MFA05	549.2 ± 46.8[fe]	143.3 ± 12.0[efg]	77.4 ± 14.9[ef]	68.3 ± 9.3[gf]	0.12 ± 0.02[cba]
PFA05	523.3 ± 100.3[gf]	134.6 ± 22.7[fg]	67.5 ± 18.0[fg]	63.2 ± 14.3[hgf]	0.14 ± 0.02[a]
WPI10	825.5 ± 56.9[c]	203.0 ± 16.8[c]	127.8 ± 6.7[b]	107.6 ± 6.9[cb]	0.04 ± 0.01[e]
MFA10	883.9 ± 93.9[c]	224.5 ± 32.1[b]	106.0 ± 23.1[c]	103.7 ± 11.6[c]	0.11 ± 0.02[c]
PFA10	642.5 ± 45.9[de]	163.2 ± 10.0[de]	90.7 ± 6.2[cde]	83.8 ± 13.1[ed]	0.13 ± 0.03[ba]
MFA15	1319.8 ± 167.1[a]	335.9 ± 49.9[a]	157.8 ± 17.0[a]	110.5 ± 13.2[a]	0.09 ± 0.02[d]
PFA15	989.6 ± 97.0[b]	245.4 ± 22.2[b]	146.3 ± 5.2[ab]	132.2 ± 6.3[b]	0.08 ± 0.02[d]

Values within a column not sharing a common superscript are significantly different (p < 0.05) according to the LSD method. The values of G′, G″ and tanδ are taken at 1 Hz. Control: yogurt without any protein addition; WPI: yogurt enriched with WPI; MFA: yogurt enriched with monodisperse fractal aggregate; PFA: yogurt enriched with polydisperse fractal aggregates.

Table 3
Results of the texture analysis from the penetrometry tests and the syneresis measurements of yoghurts enriched in different types of whey proteins.[a]

Yoghurt samples codes	Penetrometry test			Syneresis	CSLM
	Firmness (N)	Adhesiveness (N mm)	Thickness (N mm)	Syneresis (%)	Porosity (%)
Control	0.15 ± 0.01[h]	0.52 ± 0.07[f]	3.50 ± 0.14[h]	29.39 ± 0.2[ab]	–
WPI02	0.22 ± 0.03[f]	1.84 ± 0.24[cd]	4.83 ± 0.47[f]	29.85 ± 3.0[ab]	–
MFA02	0.16 ± 0.01[h]	1.19 ± 0.17[def]	4.01 ± 0.35[h]	30.98 ± 2.3[a]	–
PFA02	0.16 ± 0.02[gh]	0.81 ± 0.09[ef]	3.82 ± 0.34[gh]	30.61 ± 2.7[a]	–
WPI05	0.34 ± 0.04[cd]	2.14 ± 0.79[bc]	7.09 ± 0.99[cd]	26.05 ± 1.8[c]	–
MFA05	0.20 ± 0.02[fg]	1.44 ± 0.23[de]	4.77 ± 0.40[fg]	25.54 ± 0.6[c]	–
PFA05	0.17 ± 0.03[gh]	1.16 ± 0.41[def]	4.20 ± 0.81[gh]	27.07 ± 1.2[bc]	–
WPI10	0.40 ± 0.02[b]	2.58 ± 0.47[b]	8.35 ± 0.19[b]	18.83 ± 2.5[de]	12.86 ± 0.4[a]
MFA10	0.32 ± 0.02[d]	2.31 ± 0.40[b]	7.44 ± 0.65[d]	18.52 ± 2.0[de]	7.86 ± 2.4[b]
PFA10	0.26 ± 0.01[e]	1.43 ± 0.23[de]	6.12 ± 0.36[e]	21.49 ± 1.5[d]	14.39 ± 1.9[a]
MFA15	0.47 ± 0.03[a]	4.25 ± 1.21[a]	10.32 ± 0.85[a]	16.58 ± 0.7[e]	–
PFA15	0.35 ± 0.01[c]	2.33 ± 0.24[bc]	8.21 ± 0.41[c]	16.73 ± 0.4[e]	–

[a] Values within a column not sharing a common superscript are significantly different (p < 0.05) according to the LSD method. Sample codes are: Control, yoghurt without any protein addition; WPI, yoghurt enriched with WPI; MFA, yoghurt enriched with monodisperse fractal aggregate; PFA, yoghurt enriched with polydisperse fractal aggregates.

3.2. Effects of FA on the rheological properties of yoghurts

Rheological measurements of yoghurt gels were useful to determine the impact of the addition of WPI, MFA and PFA on the mechanical properties of the yoghurt protein network (Table 2). For all the yoghurts, enriched either with WPI, MFA or PFA, the storage modulus G′ was higher than the viscous modulus G″ over the whole frequency range studied (Fig. 3a and b). Moreover, the G′ value increased slightly with frequency. These results imply that the gels displayed a typical behaviour of weak gelled structures.

As expected, the viscoelastic parameters increased with the protein concentration for yoghurts enriched with WPI. The heat treatment of milk (5 min at 90 °C) ensured a high level of denaturation of the whey proteins (Kester & Richardson, 1984). As a consequence, strong interactions between denatured whey proteins and casein micelles appeared. Increasing whey protein concentration will lead to more interactions, enabling the formation of a denser and stronger network and thus leading to higher G′ values

(Chua, Deeth, Oh, & Bansal, 2017; Krzeminski, Großhable, & Hinrichs, 2011; Torres et al., 2011). The increase in G′ value was also noted for yoghurts enriched with MFA and PFA as shown in Fig. 3a for MFA. It can be noted that the rise of MFA concentration led to a sharp increase of gel strength (from 0.5% to 1.0%, G′ was increased by 61% for MFA versus 25% for WPI) (Table 2).

Storage modulus G′ and yield stress (τ_0) gave indications on gel strength (Table 2). G′ and τ_0 values of yoghurts enriched with PFA were the lowest, indicating that the addition of PFA led to weaker gels compared with WPI and MFA. Fig. 3b shows the differences in the viscoelastic properties of the yoghurts enriched with 1% of WPI, MFA or PFA. Yoghurts enriched with PFA had a 32% lower G′ compared with MFA-enriched yoghurts. These results confirmed and extended the conclusions of a previous study about the impact of whey protein aggregates in model WPI gels (Purwanti et al., 2011). At the same protein concentration (15%, w/w), the gel formed with larger whey protein aggregates (diameter of 62.7 nm) was weaker compared with the gel formed with smaller whey

Table 4
Results of the triangle tests performed on the yoghurts enriched with different protein types.

Test	Total number of panellists	Number of correct answers	Significant difference between samples
WPI02 versus MFA02	40	17	No
WPI05 versus MFA05	40	24	Yes (p = 0.00049)
MFA05 versus PFA05	36	14	No
MFA10 versus PFA10	36	16	No
MFA15 versus PFA15	36	20	Yes (p = 0.0047)

protein aggregates (diameter of 49.3 nm), while the gel formed with native WPI showed the strongest gel. The strength of the protein gel highly depends on surface interactions (e.g., hydrophobic interactions) between WPA (Andoyo, Guyomarc'h, & Famelart, 2016; Purwanti et al., 2011). Therefore, the size and the number of particles, as well as the number of connections and the distance between the particles, are key elements for the gel formation. These interactions have been suggested to be hydrophobic patches on the aggregates surface (Andoyo et al., 2016). In the current study, the larger particles in PFA have a more branched and expanded structure, as reported by Loiseleux et al. (2018) and Mahmoudi, Mehalebi, Nicolai, Durand, and Riaublanc (2007). The presence of these larger aggregates will lead to an increased surface, but probably with less hydrophobic patches, as the increased steric hindrance will restrict the access to reactive sites. This can substantially limit hydrophobic interactions, thus resulting in a lower gel strength.

The study of the yield stress (τ_0) allowed further characterisation of the yoghurts. As shown in Table 2, for the same protein concentration, yoghurts enriched with WPI exhibited a significantly higher yield stress than yoghurts enriched with both MFA and PFA. The yield stress is the point where the network structure starts to be disrupted, causing the yoghurt to flow. Therefore, it could be related to in-mouth sensations and texture perception. The model parameters calculated from the viscosity measurements (K and n) also indicated a difference between yoghurts enriched with WPI and yoghurts enriched with FA. Yoghurts with the lowest flow behaviour indexes corresponded to yoghurts enriched with WPI (WPI10 and WPI15) and to yoghurts enriched with high concentrations of MFA or PFA (MFA15 and PFA15) (Table 2). This means that, for yoghurts with added WPI, the higher the amount of added protein, the more shear-thinning the behaviour and the lower the viscosity at high shear. The other yoghurts enriched with MFA and PFA were grouped together regardless of the concentration added. A 2-way ANOVA on protein concentration and protein type confirmed these results by highlighting a significant difference between yoghurts enriched with WPI and yoghurts enriched with both MFA and PFA (results not shown). This significant difference in flow behaviour could explain differences in texture perception (Daget & Joerg, 1991).

3.3. Effect of fractal aggregates on the textural characteristics of yoghurts

The penetrometry test was used to assess the impact of FA and their polydispersity on mechanical properties of yoghurts at large deformations (Table 3). The aim was to relate the results with the sensory perception of texture.

Firstly, the results of the texture analysis confirmed the differences evidenced in rheology between yoghurts enriched with WPI and yoghurts enriched with FA. WPI-enriched yoghurts were always significantly firmer and thicker than the MFA enriched ones. At 1% added protein, firmness was 25% higher in yoghurts enriched with WPI than in yoghurts enriched with MFA. The same trend was found for thickness. Less work was necessary to disrupt the yoghurts enriched with FA. This could imply that, at the same protein content, the yoghurts enriched with MFA or PFA could be perceived as less stiff or less compact than yoghurts enriched with WPI. This property may be interesting to provide protein-enriched yoghurts with a softer texture, which could be more desirable for consumers.

The firmness of MFA-enriched yoghurts increased significantly with increasing concentrations of MFA, which indicated their texturising properties. MFA15 had the highest firmness, which was about 3 times higher than that of MFA02. The two other parameters, thickness and adhesiveness, also increased with the increase of MFA concentration. These results are consistent with rheological measurements which showed a gel reinforcement with MFA addition. A contribution of whey protein aggregates to gel hardness has been previously reported by Vasbinder, van de Velde, and de Kruif (2004), who showed that the addition of whey protein aggregates (diameter of 62 nm) to a whey protein-free skim milk led to an increase of gel hardness thanks to the formation of disulphide bonds between aggregates and casein micelles.

Finally, where 1.0% or 1.5% of FA was added to the yoghurts, a significant difference was found between MFA and PFA. Yoghurts enriched with MFA were significantly firmer and thicker than yoghurts enriched with PFA. Liu et al. (2017) compared micro-particulated and nanoparticulated and showed that the firmness of yoghurts enriched with nanoparticulated whey proteins was always higher than that of yoghurts enriched with microparticulated whey proteins. It is interesting to note that changing the polydispersity of whey protein aggregates in a smaller size range (≤ 1 μm) led to comparable results. It can be assumed that the reduction of the ability of large aggregates to interact with the protein network formed during acidification induced a reduction of firmness and thickness.

These results at high deformation suggested that it is possible to modulate texture perception of yoghurts by adding FA, and more precisely by adding MFA or PFA depending on the desired level of firmness and thickness.
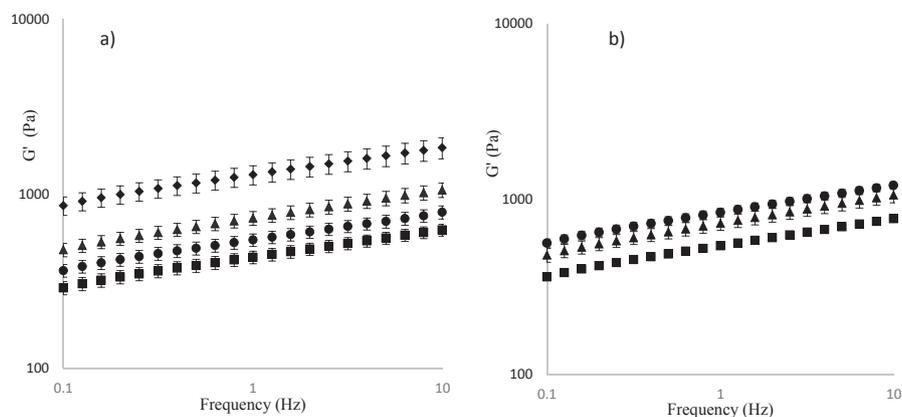

Fig. 3. Frequency sweep curves of yoghurts enriched with proteins: (a) impact of the concentration of monodisperse fractal aggregates on the storage modulus (G′) of the yoghurts (◆, MFA15; ▲, MFA10; ●, MFA05; ■, MFA02) and (b) impact of the type of protein added on the storage modulus of the yoghurts for 1% added proteins (●, WPI10; ▲, MFA10; ■, PFA10).

3.4. Syneresis

Syneresis measurements are shown in Table 3. As expected, the rise of protein concentration in yoghurts led to a reduction of whey separation. Syneresis showed an opposite tendency to firmness; the percentage of syneresis was 40% lower when the % of added proteins was increased from 0.2% to 1.0%. No more decrease of whey drainage was observed when more than 1% WPI and MFA were added to the yoghurts. This suggested that a minimum level of expelled whey had been reached by increasing the protein concentration. Interestingly, independently of their polydispersity, FA had the same impact on whey separation as native WPI for the same concentration. While the presence of a population of big aggregates in the FA led to yoghurts that were significantly less firm and less thick, this population has the same water-holding capacity as WPI and MFA. It can be hypothesised that, thanks to their large expanded structure, the population of large FA is able to bind more water than smaller FA. However, as seen before, these larger aggregates exhibit less interactions with the protein network, thus inducing a lower gel firmness. The protein concentration seems to be the only factor influencing whey separation. This result differs from previous studies where yoghurts with the lowest G' and the lowest firmness are usually linked to highest values of whey drainage (Andoyo et al., 2014). Liu et al. (2017) also reported that the addition of MicroWPA in a model gel significantly increased syneresis compared with NanoWPA.

3.5. Effect of FA on the microstructure of yoghurts

The confocal laser scanning microscopy (CLSM) images were used to visualise the protein microstructural network of the yoghurts enriched with different types of whey proteins (Fig. 4). The micrographs showed that the three samples (WPI10, MFA10 and PFA10) had a continuous and homogeneous protein network. However, MFA10 seemed to have a denser network in comparison with PFA10 and WPI10, which had a more open structure with larger pores. To confirm these small differences, image analysis was performed on a series of micrographs to quantify the porosity of the network. Porosity is defined as the division of the void area by the total image area and it is a way to characterise the protein network by quantifying the geometrical properties of the yoghurt structure (Silva, Legland, Cauty, Kolotuev, & Floury, 2015; Silva & O'Mahony, 2018). MFA10 had a significantly lower porosity than PFA10. But, surprisingly, WPI did not have a denser network than PFA (Table 3).

Although porosity was significantly different between MFA and PFA samples, it was difficult to make a visual difference at this scale of observation. However, the difference in porosity could explain the lower G', K, firmness and thickness obtained in the rheological and textural analysis. Previous studies reported the existence of large serum channels in the protein network of yoghurts enriched with MicroWPA (Silva & O'Mahony, 2018;

Torres et al., 2011). The MicroWPA belonged to a larger size range particle (from 6 to 60 μm), leading to clear differences between samples. According to Liu et al. (2016), the increase in the size of whey protein aggregates goes hand in hand with a lower connectivity and inability to be integrated in the protein network. The increased polydispersity of FA and the presence of a population of large FA could thus disturb the protein network formation, causing the development of larger pores. The open structure of the acid milk gels has been linked to a decreased water-holding capacity (Torres et al., 2011). The moderate impact of the change in the polydispersity of FA on the protein network structure might be the reason why no detrimental whey release was measured in yoghurts enriched with PFA.

3.6. Sensory analysis

Sensory tests were performed to determine if the observed impact of FA on mechanical properties, texture and microstructure were perceived by panellists.

Two sets of triangle tests were performed to determine if the differences between yoghurts enriched with MFA and WPI were perceived, and secondly to find out if the polydispersity of FA had an impact on texture perception (Table 4). For an enrichment of 0.2% of either WPI or MFA, the test was non-conclusive. For this low level of proteins, the panellists did not perceive differences between samples even if WPI02 had significantly higher G', K, firmness and thickness values than MFA02. However, differences were perceived by panellists for samples with higher added protein concentrations (MFA05 versus WPI05) (Table 4). This result showed the existence of a concentration threshold above which the addition of MFA has an impact on the sensory perception of yoghurts.

The impact of the polydispersity of the FA on texture perception was assessed by comparing yoghurts enriched with 0.5%, 1.0% and 1.5% of PFA and MFA. For yoghurts enriched with 0.5% or 1.0% of FA, no differences were perceived, whereas a significant difference was perceived between PFA15 and MFA15. The result for PFA05 and MFA05 was consistent with instrumental measurements, because no significant difference in firmness or thickness was detected. In contrast, for 1.0% of added protein, panellists did not perceive differences, even if significant differences were measured both in texture analysis and in the CLSM micrographs. Considering together the results of the triangle tests and the instrumental data, it can be hypothesised that yoghurts enriched with PFA were less firm than yoghurts enriched with MFA. Torres et al. (2011) studied the impact of two populations of MicroWPA using quantitative descriptive analysis (QDA) in low-fat yoghurts. For the same protein level, yoghurts enriched with the bigger WPA (30—58 μm) were characterised by a smoother but grainier texture than yoghurts enriched with smaller WPA. Using NanoWPA such as MFA and PFA could be relevant to tackle the problem of graininess while having a positive impact on the texture of yoghurts.
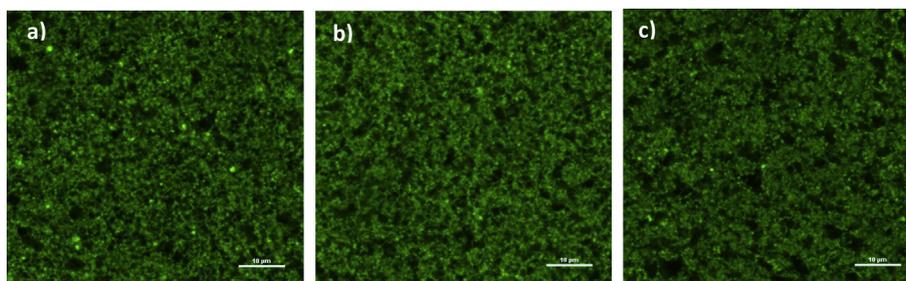

Fig. 4. CSLM images of the protein network of yoghurts enriched in (a) WPI10; (b) MFA10; (c) PFA10 (× 40 zoom 5).
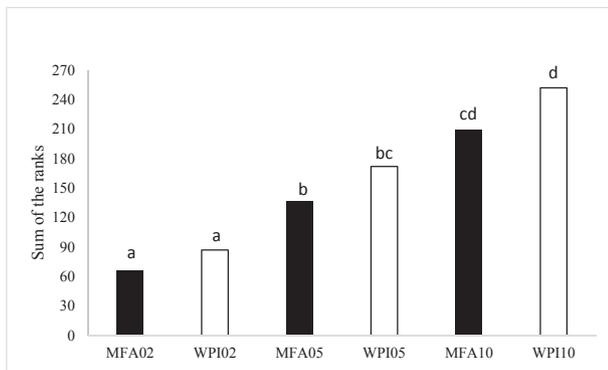
Fig. 5. Results of the ranking test on firmness of fat-free set yoghurts enriched in WPI (white bars) or MFA (black bars).

In the triangle tests, panellists were asked to choose the criteria (appearance, flavour, odour, texture) on which they based their choice to make a difference. For all the triangle tests, more than 80% of the panellists who gave the right answer based their choice on texture (results not shown). This suggests that the addition of native WP or FA was not responsible for the appearance of aromatic defaults or aftertastes.

Finally, a ranking test was performed to confirm the differences in texture perceived between yoghurts enriched with WPI and MFA by the panellists. Panellists were asked to order the yoghurts from least to firmest (Fig. 5). Firmness perceived by panellists increased with the quantity of added proteins, and the MFA-enriched yoghurts were always perceived slightly less firm than the native WPI-enriched yoghurts. These results corroborated the previous rheological and texture measurements.

Future work will focus on sensory descriptive tests to assess the impact of FA on other modalities. It would be interesting to evaluate if MFA, besides their texturising properties, have a positive impact on other desirable descriptors such as smoothness.

4. Conclusions

The results showed that the addition of FA modified the texture properties of fat-free set-type yoghurts compared with the conventionally used native WPI. The differences measured instrumentally were also perceived by panellists, who found yoghurts enriched with FA less firm than yoghurts enriched with WPI. These results obtained for fat-free yoghurts confirmed and expanded prior work done on NanoWPA in model systems (Andoyo et al., 2014; Purwanti et al., 2011).

Yoghurts enriched with PFA clearly differed from yoghurts enriched with MFA and WPI regarding the mechanical properties of the gel and the texture properties. The presence of a population of large aggregates might be the reason why yoghurts enriched with PFA had a lower storage modulus, yield stress, firmness and thickness. On the contrary, yoghurts enriched with MFA had a denser protein network, which helps to explain their higher firmness. Interestingly MFA and PFA samples had identical properties to WPI regarding whey retention. This study confirms previous results in a complex food matrix. By using both instrumental and sensory analyses, the impacts of structure and morphology modifications of WPA on texture properties and texture perception of fat-free set-type yoghurts were highlighted. These results are relevant to obtain desirable texture properties while developing fat-free dairy products using natural dairy ingredients.

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