On the use of the Couette Cell technology for large scale production of textured soy-based meat replacers

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ABSTRACT
We have demonstrated that application of simple shear flow and heat in a Couette Cell is a scalable process concept that can induce fibrous structural patterns to a granular mixture of plant proteins at mild process conditions. In particular, a Couette Cell device with 7-L capacity was employed for the production of structured soy-based meat replacers. A reduced factorial experimental design was used to find the optimum process conditions between two relevant process parameters (process time and rotation rate), while the process temperature remained constant at 120 °C. Fibre-structured products with high anisotropy indices were produced. Fibrousness is favoured at 30 ± 5 min and 25 ± 5 RPM. The up-scaled Couette Cell can be operated in higher industrial values and yield 30 mm thick meat replacers, which emulate meat. Besides, the study did not reveal any barriers for further upscaling of this concept. The flexibility in design allows production of meat alternative products with sizes that are currently not available, but could have advantages when aiming at replacement of complete muscular parts of animals, for instance, chicken breast or beef meat.

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1. Introduction

Plant protein-based products (i.e. meat replacers) form a more sustainable source for food compared to meat. As an example, the average water footprint for beef on world scale is 4235 l/60 g protein (Aldaya et al., 2012). This value is 6.6 times higher than the corresponding one for production of soybean proteins (Hoekstra and International Institute for Infrastructural Hydraulic and Environmental Engineering (IHE), 2003). On another example, contamination of water with NO3 due to poultry production is 21.9 mg/l; this value could be reduced to 6.2 mg/l if poultry production was to be substituted by a soybean production area (Hooda et al., 2000). Additionally, meat production is responsible for 18% of the annual global greenhouse gas emissions (Steinfield et al., 2006), higher than transportation emissions (13%) (Pachauri, 2008).

Consumers are interested in plant-based products that can replicate meat in terms of mouthfeel, texture, taste, colour and smell. In a recent survey however, consumers stated that these aspects were not at all recognized in current meat replacers (Hoek et al., 2011). Lack of fibrousness makes those products less appealing to the general public (Hoek et al., 2013). Even when in many cases the substitutes are healthier, this is still not enough to convince the consumers (Hoek et al., 2004). Currently, however, there is a clear growth in the meat alternatives market and higher consumer acceptance. Besides, there is a need for novel technologies, such as cultured meat (Post, 2012). The Couette Cell is a technology that might help expand the market even further.

Texturization processes currently available, such as extrusion, spinning and simple shear flow, can provide highly structured meat replacers. Presently, extrusion is the most widely applied technology for the production of meat replacers. A protein based mixture is subjected to intensive heating and mixing in the barrel screw region. Structure formation occurs only at the die region where the melted mixture is cooled and sheared (Riaz, 2000). During extrusion, the plant protein based mixtures are subjected to high...
temperatures, pressure and shear that can greatly influence the structural and chemical properties of the product (Ilo and Berghofer, 2003). Recent work in the field of extrusion cooking has been focussing on increased moisture content and various protein sources (Liu and Hsieh, 2008; Olsen et al., 2014).

In previous experimental projects, structured samples of a plant protein mixture (Soy Protein Isolate (SPI) and vital Wheat Gluten (WG)), were obtained using the concept of shear-induced structuring through simultaneous application of simple shear and heat (Grabowska et al., 2014; Krintiras et al., 2014, 2015). These experiments were performed either in a cone–cone device, called Shear Cell (SC), or in a lab-scaled Couette Cell (CC). With both devices, it was possible to obtain highly anisotropic fibrous samples at mild process conditions. After having obtained these promising results, the study has focused on process upscaling using the CC principle. The CC principle was chosen because it is amenable to up-scaling and can possibly be operated in a continuous mode in the future. Besides, we can obtain products with constant thickness. The SC seems better suitable for lab-scale operation due to its geometry (cone–cone), ease of filling and manual operation.

Previous studies with the lab-scaled CC (Krintiras et al., 2015) showed that by means of simple shear flow and heat, SPI-gluten mixtures could be texturized. Depending on the process conditions, variable structures were obtained and were classified as homogeneous, layered and fibrous. These experiments were performed to study the influence of temperature, rotational speed and time. The optimum process conditions (process time, rotational speed and temperature) for enhanced fibre structure formation were 15 min, 30 RPM and 95 °C, respectively. Additionally, fibrous samples had consistently higher AI values than the layered and homogeneous samples.

In this work, an up-scaled Couette Cell has been developed and tested. It was designed for a capacity of 7 l, which is 50 times higher than that of the lab-scale Couette Cell (Krintiras et al., 2015). The aim of the paper is therefore to demonstrate the possibility to upscale the concept of shear-induced structuring to make anisotropic structures. This concept allows for new opportunities, such as flexibility in product shape, via increased thickness. Product thickness in extrusion processes is typically limited to 5–10 mm (Thiebaut et al., 1996), whereas in the up-scaled CC, a product of 30 mm is obtained. A mixture of SPI and gluten was processed at 120 °C with variable process time and rotational speed, based on a reduced factorial experimental design, in order to obtain the optimum process conditions for structuring samples at large scale. The products were examined by means of scanning electron microscopy (SEM) and texture analysis. Texture analysis was performed to determine the global (whole sample) and local (across the thickness of the sample) Anisotropy Indices (AI) in order for quantitative characterization of the samples’ fibrousness.

2.2. Experimental set-up — Couette Cell

The up-scaled CC is based on the same principle as the lab-scaled CC (Krintiras et al., 2014, 2015; Peighambardoust et al., 2007), being the concentric cylinder rheometer concept. The up-scaled CC is shown in Figs. 1 and 2 and is composed of four main parts: the outer cylinder assembly, which consists of the housing and lid, the inner cylinder and the shaft. The inner cylinder can rotate via the shaft, while the outer cylinder remains stationary. The outer cylinder (housing) can be axially displaced and its removable lid grants access to half of the material. The material can be extracted from the device by slow rotation of the inner cylinder. The inner cylinder is connected by a shaft to a rhodrive unit (Haake PolyLab QC, Thermo Fisher Scientific, Karlsruhe, Germany). The main purpose of this unit is to control the angular velocity of the rotating inner cylinder. It is also used to record the pressure in the up-scaled CC, the torque response and the specific mechanical energy (SME). The sample material is placed in the space between the two cylinders; this space is called shearing zone and has a volume of ~7 L. The distance between the two cylinders is 30 mm. The device can be filled through a hole located in the middle of the lid. During the experiments, three temperature sensors (K-type) were fitted in the housing, along the axial direction to monitor temperature. Additionally, the housing is fitted with a pressure sensor and an air outlet port, which is used during the filling procedure only.

Both the inner and outer cylinders are heated by means of steam and cooled by means of air and/or water. The housing, lid and inner cylinder have their own separate heating jackets. A manifold is employed for this purpose to equally split the supply to the three heating jackets. The steam for heating up the device is at a constant pressure of 7 bar and is supplied by an industrial boiler unit. A flow regulation valve is used to control the temperature of the system. A controller (NL CRIO-9074, National Instruments, Austin, United States) is used to control all the valves and monitor and regulate temperature. Custom-made software programme (LabView, National Instruments, Austin, United States) is used to operate the device and vary the operating conditions.

2.3. Shear rate and velocity profile estimation

Couette Cells with inner to outer cylinder radius ratio, $0 < R_i/R_o < 0.97$, are described as “narrow-gap” cells (Chhabra and Richardson, 1999). The up-scaled Couette Cell, used in this study, has $b = 0.76$ and is called a “wide-gap” concentric-cylinder cell. This means that the shear stress will be dependent on the viscosity of the mixture. By means of a dynamic oscillatory test, the rheological properties of the SPI — gluten mixture has been characterized and can be described as a power-law fluid.

Dynamic oscillatory tests were carried out on a TA Instruments AR-G2 rheometer (TA Instruments, Delaware, USA) for the rheological study of the SPI — gluten mixture. For these tests, a plate—plate configuration was employed. The top rotating plate was oscillated from 0.1 to 100 rad/s and the material response was recorded. As a result of these tests, a consistency index $K$ [Pa·s$^n$] of 16,603 and a behaviour index $n$ of 0.13 were measured. These values indicate that the nature of the mixture is shear thinning. If the torque or the rotational speed is known, the shear rate and velocity profile in the CC can be calculated for given boundary conditions and fluid properties. The following boundary conditions are applied to the up-scaled CC: $v_{th} = 0$, $r = R_o$ and $v_{th} = 2\Omega R_o$, $r = R_i$, where $\Omega$ [rad/s] is the angular velocity, $v_{th}$ [m/s] is the azimuthal velocity of the rotating cylinder, and generally $R_i \leq r \leq R_o$.

Since only the inner cylinder is rotating, the shear stress can be defined as (Macosko, 1994):

2. Materials and methods

2.1. Materials

For the mixture used in this study, Soy Protein Isolate (SPI) (SUPRO EX37 HG IP, Solae, USA) and Vital Wheat Gluten (WG) (VITEN, Roquette, France) were used. The protein content of SPI was 90%, while gluten had a protein content of 81% based on a nitrogen-to-protein conversion factor of 6.25. These values were quantified using the Dumas method with an NA 2100 Nitrogen and Protein Analyser (ThermoQuest-CE Instruments, Rodeno, Italy). In addition, sodium chloride, referred to as salt hereafter, and demineralized water (demi-water) were used.
The shear stress is related to the viscosity and can be given by the following power-law model equation

$$\tau = \frac{T}{2\pi R_i^2 H}$$

where $\tau$ [Pa] is the shear stress; $T$ [Nm] is the torque applied to the inner cylinder and $H$ [m] is the height of the CC cylinders.

Since the mixture in this study is a Power-Law fluid, the shear stress is related to the viscosity and can be given by the following power-law model equation

$$\tau = K \left( \frac{d(\nu_r/r)}{dr} \right)^n = K\gamma^n$$

where $\gamma$ [s$^{-1}$] is the shear rate; $K$ [Pa·s$^n$] is the flow behaviour index. From Equations (1) and (2), the shear rate can be calculated as (Rao, 2007)

$$\dot{\gamma} = \left( \frac{T}{2\pi KH} \right)^{(1/n)} \frac{1}{r^{(2/n)}}$$

Integration of Equation (3) and application of the $\nu_0 = 0, r = R_o$ boundary conditions yields Equation (4) for the velocity profile

$$\nu_\theta = \frac{m}{2} \left( \frac{T}{2\pi KH} \right)^{(1/n)} \frac{1}{R_o^{(2/n)}} - \frac{1}{r^{(2/n)}}$$

Application of the $\nu_\theta = \Omega R_i, r = R_i$ boundary conditions to Equation (4) yields

$$\frac{2\Omega}{n} \left[ \frac{1}{R_o^{(2/n)}} - \frac{1}{R_i^{(2/n)}} \right] = \left( \frac{T}{2\pi KH} \right)^{(1/n)}$$

Substitution of the left hand side of Equation (5) into Equation (3) yields a relation between the rotational speed and the shear rate profile

$$\dot{\gamma} = \frac{2\Omega}{n} \frac{1}{r^{(2/n)}}$$

Substitution of the left hand side of Equation (5) into Equation (4) yields the following relation for the velocity profile

$$\nu_\theta = \frac{\Omega r}{K} \left( \frac{1}{R_o^{(2/n)}} - \frac{1}{r^{(2/n)}} \right)$$

Equations (6) and (7) can accurately predict the shear rate and velocity profiles, respectively, across the “shearing zone” for the Couette Cell as well as across the gap of “wide-gap” concentric cylinder devices. Equations (6) and (7) were used in this study to select the optimum inner/outer cylinders ratio and size that would determine the process conditions that allow for anisotropic structure formation. In addition, the shear rate and velocity profiles across the shearing zone, as calculated based on Equations (6) and (7) can help explain the structural patterns obtained from the experiments.

2.4. Sample preparation and filling procedure

During the mixture preparation step, 7.5 kg of SPI-gluten mixture was prepared likewise as in previous experiments with the lab-scaled Couette Cell (Krintiras et al., 2015). The mixture was prepared with 30% w/w SPI – gluten with ratio of 3.3:1. The rest of the mixture is demi-water (69%) and salt (1%). First, 5175 g of demi-water and 75 g of salt were manually mixed in a bucket. The solution was then added and mixed for 10 min with 1725 g of SPI in a Z-blade mixer (Winkworth Machinery Ltd., Basingstoke, UK). The mixture was left inside the mixer with its lid closed for 30 min in order to pre-humidify. Following this step, 525 g of gluten were added in the SPI demi-water-salt mixture and the content was mixed for 15 min. The SPI - gluten – demi-water – salt mixture, further referred to as mixture, was then ready to be processed.

The protein mixture consists of deformable granules. An in-house developed feeder was employed to insert the mixture into the device. It uses a pneumatic piston to press the material through
a silicon tube connected to the up-scaled Couette Cell’s custom-made feeding valve (Teessing BV., Rijswijk, the Netherlands), which is composed of a ball valve and a pin assembly. After tightly packing the mixture in the shearing zone so as no cavities are present, the ball valve and the pin of the feeding valve were closed. The housing air-outlet port was closed as soon as the measured pressure in the shearing zone was lower than 1 bar. Then the experiment was commenced.

At the end of each experiment, the steam heating of the jackets was switched to air/water for the cooling stage of the process. In order to extract the material, the lid was then removed. This could only be done when pressure reached values lower than 0.7 bar. The sample was extracted by cutting it axially at the lower part and by slowly rotating the inner cylinder. The samples were stored in a seal bag and placed inside a freezer at −20 °C.

2.5. Process conditions and mathematical regression

A reduced factorial experimental design was used to identify the optimum process conditions for the production of anisotropic meat replacers (Ferreira et al., 2007; Li et al., 2013). Two independent variables were used in this study namely, processing time (min) and rotational speed (RPM) of the inner cylinder. Temperature was fixed at 120 °C. Preliminary experiments revealed that temperatures >120 °C could not be consistently achieved due to excessive steam condensate accumulation in the heating jackets, while processing at temperatures >120 °C would result in severely burned and deformed samples when processed for 15–45 min in total. The processing temperature in the up-scaled CC is higher compared to the processing temperature in the lab-scaled CC, since higher temperatures are needed due to the increased material thickness. The process conditions selected for this study are shown in Table 1. This configuration was employed to avoid operation at extreme values of time and RPM at the same time, since preliminary experiments yielded undesired samples. Sample integrity and presence of anisotropic structures were the main criteria to define the maximum and minimum values of time and RPM. An extra experimental point (30 min, 20 RPM) was added to increase the accuracy of the area believed to host the optimum process conditions.

The $A_{lb}$ values obtained in the texture analysis were used to study the influence of time and RPM on the product structure. A second-order polynomial with interaction model (Equation (8)) was used to fit the experimental values of $A_{lb}$.

\[
Y = \beta_0 + \sum_{(l=1)}^{2} \beta_l X_l + \sum_{(l=1)}^{2} \beta_{12} X_1 X_2
\]  

(8)

where $\beta_0$, $\beta_l$, $\beta_{12}$ are the equation regression coefficients; $X_l$ and $X_2$ are the independent variables and $Y$ is the dependent variable. The software STATISTICA 12 (Statsoft Inc., Tulsa, U.S.A.) was used to fit the model to the experimental points; perform the statistical analysis and draw the surface plots. It was also used to compute the optimum conditions of the model. Later on, an experiment was performed at these conditions in order to compare and verify the model.

2.6. Texture analysis

The selection of the optimum process conditions requires a method, which allows for quantitative comparison of different product samples. The mechanical properties of each product sample may vary depending on the structures present in the bulk. In particular, samples with fibrous structures will show significant differences in tensile stress values between specimens obtained parallel and perpendicular to the formed fibres. For this reason, the tensile stress Anisotropy Index ($A_{ls}$) was devised to reveal the physical presence of anisotropic structures in the samples and their degree of fibrousness (Krintiras et al., 2014). Additionally, the tensile strain Anisotropy Index ($A_{ls}$) can quantify the textural and sensorial characteristics of the meat replacer.

The maximum values for tensile stress and strain were determined for each specimen. The maximum tensile strain was determined at the point of maximum tensile stress. The maximum tensile stress and strain per direction were averaged and the relevant Anisotropy Indices ($A_{ls}$) were calculated through Equations (9) and (10), respectively.

\[
A_{ls} = \frac{\sigma_{||}}{\sigma_{\perp}}
\]  

(9)

where, $A_{ls}$ is the stress anisotropy index; $\sigma_{||}$ is the normal stress for specimens cut parallel to the fibres and $\sigma_{\perp}$ is the normal stress for specimens cut perpendicular to the fibres

\[
A_{ls} = \frac{\varepsilon_{||}}{\varepsilon_{\perp}}
\]  

(10)

where, $A_{ls}$ is the strain anisotropy index, $\varepsilon_{||}$ is the normal strain for specimens cut parallel to the fibres and $\varepsilon_{\perp}$ is the normal strain for specimens cut perpendicular to the fibres.

Three specimens were cut for each sample created, both parallel and perpendicular to the formed fibres, resulting in a total of 108 tested specimens. The specimens were cut in a rectangular shape with a thickness of 30 mm. The cross-sectional area relevant for calculating the normal stress was manually measured each time. The tensile tests were performed on a Zwick Roell Z2005 (Zwick Roell AG., Ulm, Germany). The tensile tests were performed with a constant deformation rate of 0.5 mm $s^{-1}$. Two plain clamps with rough surfaces were employed to fixate the specimens in the tester. Additionally, for the study of the local thickness characteristics of the sample, roller clamps fitted with sandpaper were employed to fixate the specimens. In this case, smaller strips of $5 \times 5$ mm were cut from the bottom, centre and top of the total sample thickness (30 mm) in order to examine the local structure formation.

Table 1

<table>
<thead>
<tr>
<th>Test</th>
<th>Time [min]</th>
<th>Rotational speed [RPM]</th>
<th>$X_1$</th>
<th>$X_2$</th>
<th>Average $A_{ls}$</th>
<th>Average SME [kJ/kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>30</td>
<td>−1</td>
<td>0</td>
<td>1.18</td>
<td>11.3</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>10</td>
<td>0</td>
<td>−1</td>
<td>0.79</td>
<td>8.6</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>20</td>
<td>0</td>
<td>−0.5</td>
<td>1.67</td>
<td>18.5</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>1.70</td>
<td>32.6</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>50</td>
<td>0</td>
<td>1</td>
<td>1.05</td>
<td>63.1</td>
</tr>
<tr>
<td>6</td>
<td>45</td>
<td>30</td>
<td>1</td>
<td>0</td>
<td>0.81</td>
<td>59.9</td>
</tr>
</tbody>
</table>

Actual and coded values of the independent variables in the reduced factorial experimental design with the average experimental values for tensile stress Anisotropic Index ($A_{ls}$): Average SME values for each test performed.
2.7. Scanning electron microscopy

The microstructures of the samples were investigated with SEM (S-4800, Hitachi, Tokyo, Japan). The SEM at hand is a cold field emission scanning electron microscope, which features a maximum resolution of 1.0 nm at 15 kV. Inspection of the samples is possible with acceleration voltages of 0.5–30 kV without beam deceleration. The SEM bears a beam deceleration feature that can be used to inspect sensitive or charging samples. For these inspections, a low voltage of 2 kV was utilized. The microscope allowed for specimen imaging without gold or other coating. In SEM, the samples, in specimens of 5 × 5 × 5 mm, were cut parallel to the fibres at room temperature. The specimens were dried for 24 h in an oven set at 60 °C to reduce the moisture content. Both the parallel and perpendicular surfaces to the fibres were inspected by SEM.

3. Results and discussion

In this study, the SPI-gluten mixture was treated by means of simple shear flow and heat at variable process conditions. After each experiment, visual inspection of the product was performed. Fig. 3 (left) shows a typical sample obtained at 120 °C, 20 RPM and 30 min; it exhibits fibrous formations over the whole bulk of the sample when tearing it by hand. Fig. 3 (right) shows the cross-section of the same sample where fibre formation is also evident. Structure formation follows the flow direction from the inner rotating cylinder to the outer one, which is stationary. Fig. 3 (right), shows what is expected to be the structure formation mechanism for highly viscous systems, namely phase separation between SPI and gluten to form individual fibre structures (Krintiras et al., 2015). Specifically, (Manski et al., 2007), showed that highly viscus systems can favour structure formation when simple shear flow is applied. It can be seen in Fig. 3 (right) that long fibres are distributed over the bulk of the sample. The pale yellow (in the web version) coloured fibres are believed to be gluten fibres. It can also be seen that the shear rate profile, as calculated by Equation (6), across the shearing zone (sample thickness) matches the structural profile of the samples produced at 30-RPM rotational speed applied to the inner cylinder. The match between the shear rate and structural profiles has been reproducible for all the samples in this study except for those treated at 50 RPM and 45 min, respectively.

Fig. 4 shows a typical slab of the structured meat replacer. The striped and other patterns visible on the surface are due to the presence of corrugations at the inner and outer cylinder to help increase the surface contact and friction between wall and mixture. From such a slab, we cut the specimens needed for texture analysis and SEM inspections. To the best of our knowledge, this is the first time that fibrous meat replacers are produced in such characteristic dimensions as the slab depicted in Fig. 4 and with a thickness of 30 mm as shown in Fig. 3 (right). This characteristic thickness can help produce meat replacers that resemble complete muscular parts of animals, for instance, chicken breast or meat. Typical dimensions of a chicken breast are 10–15 cm long, 5–8 cm wide and 3–5 cm thick.

After each experiment, we would obtain, through the rheodrive unit, the measurement and recording of the specific mechanical energy (SME), which in our case is the response from the mixture, the seals and bearings in the up-scaled CC. The SME at the optimum process conditions for 120 °C, 30 min and 20 RPM was in average 18.5 kJ/kg and for 120 °C, 30 min and 30 RPM was in average 32.6 kJ/kg. The SME values reported from extrusion cooking vary between about 200 and 1200 kJ/kg depending on the mixture composition, the extruder set-up and the die shape (Fang et al., 2014; Jin et al., 1994; Lue et al., 1994; Osen et al., 2014). The SME values obtained while operating the up-scaled CC do not include the additional energy spent for the mixing step during the mixture preparation.

3.1. Texture analysis

Fig. 5 shows the tensile stress/strain and AI values obtained for samples treated at a constant process temperature of 120 °C and process time of 30 min at varying rotational speed. The tensile stress and strain values are shown for both perpendicular and parallel directions to the movement of the inner cylinder. Fig. 5 (left) shows that in the event of 10 and 50 RPM, the AI values are 0.79 and 1.05 respectively; AI values ~1 are typical for homogeneously or layered textured samples. During visual inspection, it was found that samples treated at 10 RPM were not sufficiently structured (homogeneous samples), whereas at 50 RPM the samples were damaged and deformed. On the other hand, samples processed at 20 and 30 RPM yielded high AI values, 1.67 and 1.7,
respectively, with an individual sample yielding an $A_{\text{I}}$ value of 3.6. Fig. 5 (right) shows the $A_{\text{I}}$ values for the same samples; the main observation is that the samples treated at 30 RPM are rigid compared to the more elastic samples obtained at 20 RPM. It is remarked that Fig. 5 reveals a local area of optimum process conditions; specifically, samples treated between 20 and 30 RPM will yield highly fibrous samples.

Fig. 6 shows the tensile stress, strain and $A_{\text{I}}$ values for samples processed at a constant process temperature of 120 °C and rotational speed of 30 RPM at varying processing times. In Fig. 6 (left), it can be seen that samples processed at 15 and 45 min yielded $A_{\text{I}}$ values of 1.18 and 0.81, respectively. At 15 min the samples did not exhibit any visual fibrous structures. Due to the increased thickness (30 mm) of the sample, thermal treatment for 15 min is not sufficient. On the other hand, samples treated for 45 min showed burned, deformed and plasticized areas due to excessive heating. The optimum process time that resulted in fibrous structured samples was 30 min, as can be seen in Fig. 6 (left). Fig. 6 (right) shows that samples treated at 15 and 45 min were more rigid and stiff compared to the more elastic ones produced at 30 min.

In the course of this study, additional analysis was performed to explore the local structure formation across the samples within their 30 mm thickness. Specimens were collected from the same samples as in the above described texture analysis. Fig. 7 presents a comparison between samples created at 120 °C, 30 min and varying RPM, for specimens collected from the bottom (close to the inner cylinder), centre and top (close to the outer cylinder) of these samples (see Fig. 3, right). The $A_{\text{I}}$ values for all cases suggest that the specimens obtained from samples processed at 20 RPM would yield the most anisotropic structures locally.

Fig. 8 shows the difference between the bottom, centre and top part of the samples processed at 120 °C, 30 min and 20 RPM. These samples were picked and further analysed due to the pronounced fibrous structures and the high $A_{\text{I}}$ values as shown in Fig. 7. No significant difference between the three positions was observed. It is therefore stated that the samples showed uniform strength over the sample thickness (Fig. 8). It is remarked that the global $A_{\text{I}}$ of a sample would then depend on the interconnection of layers and fibres, being similar at a local scale for all conditions. The averaged values of $A_{\text{I}}$ suggest that the middle part of the samples is the one with the highest density of fibres. This is also supported by Fig. 3 (right), where the top and bottom parts of the sample have slightly different textures than that in the centre. However, for both Figs. 9 and 10, due to the high deviation in the tensile stress values, and based on a t-test performed, the textural differences at local scale were not significant.

3.2. SEM analysis

SEM imaging allowed for identification of structure formation within the bulk of the treated samples. Fig. 9 (left) depicts a sample treated at 120 °C, 30 min and 20 RPM. Fibre structures of variable sizes are visible; micro-fibres of 1–5 μm in diameter bundle up to form fibres of 100–400 μm in diameter. The fibres are aligned along the flow direction, which is in accordance with Fig. 3. In Fig. 9 (right), the tips of fibres from the same specimen are shown and
the characteristic round cavities scattered in the domain might have contained air or water in its initial state. A closer look at the bottom left in Fig. 9 (right) reveals the presence of a single gluten fibre with its characteristic needle-like structure (Abang Zaidel et al., 2008; Krintiras et al., 2014, 2015).

3.3. Model fitting and statistical analysis

The obtained AI values from the first part of the texture analysis (see Figs. 5 and 6) were fitted in a quadratic model. The experiments performed at the edges (15, 45 min and 10, 50 RPM) of the experimental design (see Table 1) yielded the lowest AI values. Additionally, the highest averaged AI was found when processing for 30 min and 30 RPM, in the middle of the experimental design. Therefore, the proposed region of study and the experimental design were acceptable for this study and their results were used to adjust the coefficients of Equation (8). The objective of this model was to gain deeper insight into the system behaviour and to estimate the best conditions to obtain fibrous structures. High standard deviation values for AI were obtained due to the samples’ and specimens’ size, thickness and unpredictable values of AI. That is why the predictions were used as a tool to confirm the experimental study and highlight a region of confidence in which processing will result in fibrous structures. Fig. 10 (left) shows the surface plot obtained from the regression function (Equation (8)) for the ranges of 10–50 RPM and 15–45 min. As seen in Fig. 10 (right), the surface plot has a circular shape over the whole domain. In this case, the interaction term was not used. As reported in a previous study (Krintiras et al., 2015), the process time and rotational speed have similar effects.

The optimum process conditions extracted from the model were 30.3 min and 31 RPM and yielded an AI of 1.8, which is in agreement with the optimal experimental AI of 1.7 obtained at the conditions of 30 RPM and 30 min (Table 1). Due to the deviance of the experimental points, the R² had a low value of 0.2. The statistical analysis of the regression coefficients is summarized in Table 2.
It was found that 4 out of 6 coefficients were statistically significant, having p-values lower than 0.05. Although lower values were obtained while fitting, the visual inspection of the samples agreed with the model behaviour. A comparison between the averaged AIs and the predicted value by the model can also be seen in Table 1. Higher relevance is given to the qualitative results obtained during the inspection of the samples than the specific quantitative AI values. In order to confirm the results obtained from the model, a verification experiment was performed at the optimal process conditions predicted by the model. The average AI at the optimal process conditions was lower (AI of 1.4) than the predicted value (AI of 1.8). However, the obtained samples showed well-defined fibrous structures all over the domain as can be seen in Fig. 11. The results of the model, together with the verification experiment, suggest that working in a range between 25 and 35 min and 20 to 30 RPM will always lead to fibrous structured products.

4. Conclusions

We have demonstrated that application of simple shear flow and heat in a Couette Cell is a scalable process concept that can induce fibrous structural patterns to a granular mixture of plant proteins at mild process conditions. In particular, after processing the protein blend at 120 °C and variable rotational speeds and process times, a structured SPI-gluten product was formed. A reduced factorial experimental design was used to search for the optimum process conditions. It was found that the optimum area of operation is located at 30 ± 5 min process time and 25 ± 5 RPM rotational speed of the inner cylinder. In particular, samples created at 120 °C, 30 min and 20 RPM exhibited highly fibrous structures and yielded high average AIs values of 1.67 with individual samples yielding values up to 3.6. The fibres formed were clearly observed both visually and with SEM imaging. In the event of a lab-scale Couette Cell with 6 times smaller gap between the two cylinders (5 mm vs. 30 mm) and 50 times lower capacity (0.14 l vs. 7 l), a product with the same fibrous structural patterns was obtained at 95 °C, 30 RPM and 15 min. The increase in temperature and process time in the up-scaled Couette Cell, compared to the lab-scale counterpart, is necessary due to the increased product thickness. A study of the local structure formation across the thickness of the product (bottom, centre and top) was conducted and no significant differences were observed due to the high variations in the results. The energy input (SME) for the production of highly fibrous meat replacers with the up-scaled Couette Cell was ranging between 8.6 and 63.1 kJ/kg. The Couette Cell can be further scaled up linearly (increased length) and is amenable to continuous operation.

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