Research Article

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Rheological properties of pea protein melts used for producing meat analogues

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Abstract: Understanding the rheological properties of protein melts is critical in the design of meat analogues and the formation of texturised products from plant-based proteins. This study investigated the influence of temperature, moisture content (MC) and protein concentration on the rheological properties of pea protein isolate and pea fibre blends. The blends were chosen as an experimental space where it is possible to extrude fibrous meat analogues using high-moisture extrusion. Mechanical spectra by small amplitude oscillatory shear were determined using conventional rheometry and were compared to closed cavity rheometry (CCR) to extend the available temperature range. All blends behaved as polymer melts in the rubbery region with moduli increasing with frequency, and storage modulus larger than loss modulus for temperature 40–90°C, MC 54–63%, protein concentration 75–85%. Complex viscosity was strongly shear thinning. The relative influence of the parameters from additive and linear mixed models showed an influence of temperature > MC > concentration. The increase of modulus with concentration was quite weak and not statistically significant. The behaviour of the complex modulus was explained well with an Arrhenius-type log-linear mixed model. Conventional rheometry agreed well with CCR, showing an exponential decrease of moduli between 40 and 130°C.

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1 Introduction

Global meat consumption per person doubled between 1961 and 2009 and continues to increase [1]. The food chain contributes 25% of the total greenhouse gas emissions, with meat production alone contributing a staggering 14.5% [2]. The increase in meat consumption is alarming also from the nutritional perspective, as excessive consumption has been linked to health problems, such as coronary heart disease and certain cancers [3,4].

The food industry is experiencing a protein shift as plant-based meat analogues are gaining popularity. This is due to the mentioned concerns among consumers about sustainability and health, but also regarding animal welfare [5,6]. Among the alternatives, plant-based meat analogues are at the top to substitute animal meats mainly due to acceptable price, availability and cultural acceptance [7]. To further increase consumer liking, it is desirable that meat analogues mimic the texture, taste, sensory and nutritional profiles of animal meats. The high moisture extrusion (HME) process is the most common method to create a fibrous, meat-like structure. Fibrous analogues are today commercially produced from soy and pea protein and wheat gluten by HME, utilising the extruder to form a protein melt at high moisture content, high temperature and pressure with subsequent active cooling on exit [8–10]. Process parameters such as extrusion temperature, die geometry and flow rates, as well as the rheological properties, of the protein melt affect the flow behaviour. The formation of fibrous structures with varying degrees of anisotropy is influenced by the flow behaviour in the extruder and especially in the cooling die [11–14].

The exact mechanisms responsible for fibre formation are not fully understood. Recent work suggests that flow as affected by heat transfer, combined with phase separation are the most important mechanisms [11–13,15,16]. Guan

and coworkers observed protein nano aggregations but rather attributed fibre formation to flow instabilities [17]. All are dependent on melt rheology and composition which highlights the importance of a thorough characterisation of protein melt rheology and its dependence on temperature and composition.

Previous results indicate that properties such as shear thinning behaviour, viscoelasticity, and cross-linking are vital in the formulation and processing of plant-based proteins. Osen and coworkers highlighted the importance of controlling the extrusion conditions, while Dekkers and coworkers emphasised that the rheological properties of soy protein isolate-pectin blend meat analogues are crucial for mimicking the fibrous texture of meat [15,18]. The application of simple shear and heat in shear cells to produce structured meat analogues has been developed and studied in-depth by the van der Goot group [15,19–22]. Optimising process parameters and tuning the rheological properties will allow us to develop meat analogues with predictable and desirable properties. However, measuring the rheological properties of meat analogues at conditions relevant for extrusion is a challenge due to the inabilities of conventional rheometers to measure at high temperature and pressure, typically up to 150°C and 5–10 bar, and to avoid moisture loss at elevated temperatures. Recent findings show that a closed cavity rheometer (CCR) can determine rheological properties of plant-based proteins, at least in small-amplitude oscillatory shear (SAOS) [14,23,24], and it may potentially be capable of performing steady shear measurements in the future [25]. There are also recent publications where ultrasonic flow profiling [26] and high-pressure cells [11] have been utilised.

The aim of the present work was to understand the rheology of pea protein melts, especially the effect of protein concentration, temperature and moisture content (MC). The ranges of protein concentration, temperature and moisture were chosen as a parameter space where it would be possible to form fibrous structures by HME. However, here extrusion was used to form melts of the ingredients for further rheological analysis using conventional rheometry as well as CCR.

2 Materials and methods

2.1 Materials and methods

Pea protein isolate (Pisane™ M9) with a protein concentration on dry matter basis of 86% and pea fibre (Swelite™) with MC of about 5% was purchased from Cosucra (Lestrem, France and Warcoing, Belgium, respectively). Paraffin oil (>95% purity) was purchased from ThermoFisher Scientific, Belgium. The protein isolates and pea fibre powders were stored at 14°C and 30% RH.

2.2 Extrusion and sample preparation

A double screw extruder, model Brabender TwinLab-F 20/40 D (Duisburg, Germany) with a screw length to diameter ratio (L/D) of 40:1 was used. The extruder is equipped with four heating zones and separate die-heating elements. A 13-mm-diameter cylindrical die at the extruder outlet was used to prepare samples for rheological measurements. The extruder was fed at the rate of 4 kg/h, and all zones were heated to 100°C except for the feeding and mixing zones where it was set at 60°C. Different formulations of pea protein isolate, and pea fibre were thoroughly mixed immediately before extrusion. Pea fibre was included in the formulations as it is commonly included for HME extrusion of meat analogues. Mixtures tested consisted of 75:25, 80:20, 85:15 (w/w) pea protein isolate to pea fibre with various water content added in the extruder, 60, 62, 64, 66 and 68% (w/w) of the dry powder weight of isolate + fibre. The water content was compensated for the moisture adsorbed in the pea protein and pea fibre powders. The MC of the mixed powder was analysed gravimetrically by drying at 105°C over night. The powder mixture had a MC of 5%. The extrudates were stored at −40°C in sealed plastic bags. They were defrosted in a refrigerator at 4°C followed by rheological and moisture analysis the next day.

The extruder was used to blend and melt the ingredients into an as homogenous system as possible for further analysis at different temperatures in the rheometers used. The system is referred to as a melt throughout the paper. The procedure was not the same as utilised for producing fibrous structures in HMA, but rather used to form an homogenous melt.

2.3 Conventional rheometry

An HR-30 rheometer equipped with temperature-controlled 25-mm parallel plates (TA instrument, New Castle, USA) was used for SAOS tests where storage modulus G' and loss modulus G'' were determined. Flat plates were used rather than serrated ones as the samples were thin. No slip was observed. Samples were prepared from the 13-mm diameter extrudates

with a thickness of 2 mm using a vacuum holder [27]. The samples were then placed in the measurement gap, and the axial compression force was set to 5 N while heating the samples from 25 to 40°C and reduced to 1 N at 40°C and kept throughout the measurements. Mechanical spectra were recorded from 0.1 to 30 Hz at constant temperatures of 40, 50, 60, 70, 80, and 90°C with a heating rate of 10°C/min between the temperature steps. Paraffin oil was applied to the edges of all samples during measurements to reduce the impact of evaporation at elevated temperatures. All measurements were performed in the linear region and in triplicates to ensure reproducibility.

2.4 Closed cavity rheometry

A CCR, a rubber process analyser (RPA flex, TA Instruments, New Castle, DE, USA), was used to determine linear region and mechanical spectra in SAOS of pea protein melts. A detailed description of CCR can be found elsewhere [11,20,21]. About 5 g of extrudate samples was placed between the cones, and the temperature of the cones was controlled by electric heating and forced air cooling. The cones were sealed with a closing pressure of 4.5 bar to prevent water evaporation. The upper cone remains fixed while the lower cone applies oscillatory movement on the sample at a set frequency and strain. The linear viscoelastic region of the samples was determined by oscillatory strain sweeps at 110 and 130°C and constant frequency of 1, 10 and 50 Hz and for 0.01–1,000% strain. The temperature gradients were performed in the linear region (5% strain at 5 Hz) and in triplicates to ensure reproducibility.

2.5 Statistical analysis

Generalised additive/linear mixed models [28–30] are a class of flexible regression models, which encompass traditional statistical models like linear regression and ANOVA as special cases [31]. Advantages over traditional statistical models are that this broader class of models can capture more complex relationships within a dataset, e.g. by relating the response variable to parameters using non-linear smooth functions. Here we use additive and linear mixed models to correlate complex modulus, $G^* = (G'^2 + G''^2)^{1/2}$, with processing parameters and to adjust for the experimental design.

To recap the structure of the data and introduce model notation, three experiments were performed for each protein concentration PC = 75, 80, 85%, and for each of five levels of added water 60, 62, 64, 66 and 68%. For each experiment, a MC was recorded, and the moduli G' and G'' were measured at temperatures T = 40, 50, 60, 70, 80, 90°C. The experiments with PC = 75% and the lowest MC were excluded from the analysis as they did not form a melt structure and thus were regarded as an outlier. In total, 42 such experiments and 252 measurements are included in the dataset.

We formulate the following additive mixed model for the log-transformed complex modulus

$$\log(G_i^*) = f_T(1/T_i) + f_{\mathrm{MC}}(1/\mathrm{MC}_{E_i}) + \mu_{\mathrm{PC}_i} + \mu_{E_i} + \epsilon_i. \quad (1)$$

Here, i = 1, … , 252, indicates each separate measurement. To each measurement i, there is associated the measured complex modulus G_i^*, the temperature T_i at which the measurement was taken, an indicator $E_i \in (1, \ldots, 42)$ specifying which experiment the measurement belongs to, and the MC MC_{E_i} and protein concentration PC_{E_i} of that experiment. f_T and f_{MC} in the model are smooth functions of inverted temperature and inverted MC, respectively. μ_{PC_i} and μ_{E_i} are so-called random effects, meaning that a separate intercept is fitted for each protein concentration and for each experiment ϵ_i are normally distributed residuals.

The smooth functions f_T and f_{MC}, and the random effect μ_{PC_i} capture the relationship between the parameters and G^*. μ_{E_i} and ϵ_i capture variations between experiments and between measurements that are not otherwise explained by T, MC, and PC. Note that without the smooth functions f_T and f_{MC}, model (M1) would be a classic ANOVA model.

To compare with a model limited to an Arrhenius-type dependence, we also formulate the linear mixed model

$$\log(G_i^*) = \beta_T/T_i + \beta_{\mathrm{MC}}/\mathrm{MC}_{E_i} + \mu_{\mathrm{PC}_i} + \mu_{E_i} + \epsilon_i, \quad (2)$$

where β_T and β_{MC} are model coefficients.

The models are fitted using the R package *mgcv* [32,33]. The adjusted R^2_{adj} is used as a measure of model fit. The adjusted R^2_{adj} measures the proportion of the variance that is explained by the regression, while also adjusting for the number of predictors in the regression. To quantify the contribution from each predictor, a model with (full model) and without (sub-model) the predictor is fitted, and the difference in R^2_{adj} between the two models is used to quantify the contribution from the predictor. Denoting the difference $\delta R^2_{\mathrm{adj}}$, the models used to compute $\delta R^2_{\mathrm{adj}}$, sub-models of model (M2) are given in Table 1. When computing $\delta R^2_{\mathrm{adj}}$ for the parameters T, MC, and PC, the full model excludes the random effect μ_{E_i}. This is because μ_{E_i} can itself compensate for the exclusion of the parameter in the sub-model, which would make $\delta R^2_{\mathrm{adj}}$ more difficult to interpret.

3 Results and discussions

3.1 Moisture content of extrudates vs added water

The amount of water added to the extruder for blending pea protein and pea fibre, along with the resulting MC of the extrudates, is presented in Table 2. The flow in the extruder is not totally stable over time resulting in some occasional blowout of steam, especially for the extremes in the sample space. This results in MCs in the extrudates lower than expected from the added water, as well as some variation between the samples.

Blends with 15 and 20% fibre had comparable moisture levels, whereas the extrudate with 25% fibre had higher water retention capacity. Higher fibre levels typically enhance water-holding capacity due to fibre's hydrophilic nature. Pea fibre improves structural integrity and modifies melt viscosity, but large amounts may reduce available water for the pea protein isolate and affect the desired fibrous texture of the extrudates to meat analogues. Studies have previously shown that increasing the concentration of carrageenan, potato starch, and curdlan in pea protein isolate blend enhanced fibre formation [34].

Table 2: Total water content added to the extruder and final MC of the extrudates

Pea protein isolates to pea fibre (w/w)	Water content added into extruder (% of dry powder)	Moisture content of extrudates (% ± standard deviation)
75:25	60	56.4 ± 0.21
	62	55.2 ± 1.10
	64	56.2 ± 0.36
	66	58.5 ± 0.27
	68	61.1 ± 0.81
80:20	60	52.0 ± 0.26
	62	53.9 ± 1.15
	64	56.1 ± 0.89
	66	58.7 ± 0.10
	68	60.0 ± 1.19
85:15	60	51.5 ± 0.28
	62	51.5 ± 0.67
	64	55.4 ± 0.25
	66	57.6 ± 1.35
	68	57.5 ± 0.10

3.2 Influence of temperature, MC, and protein concentration on melt rheology

The sample space chosen was one set of protein concentrations and MCs where it is possible to extrude fibrous meat analogues. At lower MC and protein concentration, the extrudates did not behave as melts and were crumblier and more fragile. At higher MC and protein concentrations, the extrudates become rubbery and not suitable as meat analogues (results of pre-tests, not presented). Figure 1 is an attempt to show an overview of all rheological data measured and demonstrates the variation with temperature, MC and protein concentration for mechanical spectra of the melts. The data are reduced to show two levels of MC (low and high) as expressed by added water of 62 and 66%. A full set of data is available as supplementary material.

Figure 1 shows that G' is always considerably larger than G'' corresponding to phase angles of 6–20° (tan delta 0.11–0.35). The behaviour with moduli increasing with frequency and decreasing with temperature is typical for polymer melts.

Previously published studies of extrudates of pea protein isolate and soy protein isolate showed a similar increase in moduli with increasing frequency [35]. The magnitudes of the moduli observed in Figure 1 are in the same range as we previously have presented [12] and as extrudates from pea protein isolate and blends with wheat gluten and pea protein isolate blended with rice protein [13,36].

The data in Figure 1 can also be expressed as complex viscosity using $\eta^* = G^*/\omega$ where η^* is the complex viscosity and ω is the angular frequency. The complex viscosity then shows a Power law-behaviour with a flow behaviour index (n) in the range of 0.05–0.12. This is consistent with our

Table 1: Models used to compute δR^2_{adj}

Model component	Full model	Sub-model
T	$\beta_T/T_i + \beta_{\mathrm{MC}}/\mathrm{MC}_{E_i} + \mu_{\mathrm{PC}_i} + \epsilon_i$	$\beta_{\mathrm{MC}}/\mathrm{MC}_{E_i} + \mu_{\mathrm{PC}_i} + \epsilon_i$
MC	$\beta_T/T_i + \beta_{\mathrm{MC}}/\mathrm{MC}_{E_i} + \mu_{\mathrm{PC}_i} + \epsilon_i$	$\beta_T/T_i + \mu_{\mathrm{PC}_i} + \epsilon_i$
PC	$\beta_T/T_i + \beta_{\mathrm{MC}}/\mathrm{MC}_{E_i} + \mu_{\mathrm{PC}_i} + \epsilon_i$	$\beta_T/T_i + \beta_{\mathrm{MC}}/\mathrm{MC}_{E_i} + \epsilon_i$
Experiment	$\beta_T/T_i + \beta_{\mathrm{MC}}/\mathrm{MC}_{E_i} + \mu_{\mathrm{PC}_i} + \mu_{E_i} + \epsilon_i$	$\beta_T/T_i + \beta_{\mathrm{MC}}/\mathrm{MC}_{E_i} + \mu_{\mathrm{PC}_i} + \epsilon_i$

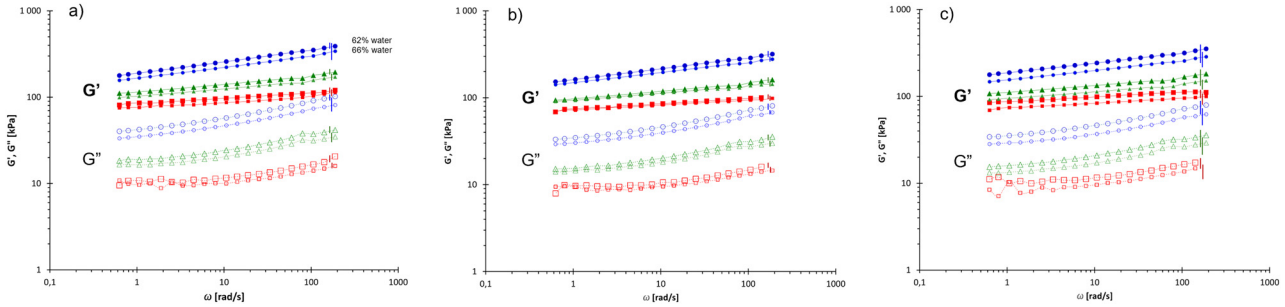

Figure 1: Mechanical spectra from conventional rheometry for pea protein melt for all variables: protein concentration (an 85% protein isolate:15% pea protein fibre, (b) 80:20, (c) 75:25), temperature (40°C blue circles, 60°C green triangles, 80°C red squares). *G′* have filled symbols and *G″* open symbols. The top data for each variable have 62% added water (larger symbols) and the bottom 66% (smaller symbols). Error bars denote standard deviation.

previous study where an n-value was observed at 60°C of $n = 0.17$ for MC = 64% and n = 0.22 for MC = 60% for 85% pea protein isolate and 15% pea fibre blends [12]. It is also similar to what has been found for extruded soy protein isolate samples [37].

Figure 2 illustrates how the moduli are influenced by temperature, MC and protein concentration at a specific angular frequency of 10 rad/s picked at the centre of the scale. The effects are visualised for a sub-set of the results presented in Figure 1 for MC, protein concentration and temperature in the middle of the ranges investigated. Figure 2a as expected shows decreasing moduli with temperature. The shape could be a result of an Arrhenius-type dependence which will be discussed in connection with the master curve below. There could also be an influence of moisture loss at higher temperatures despite the sample being immersed in oil, which will be discussed further for the influence of temperature below. Similar behaviour of pea-protein blends has been observed by others [38,39].

Figure 2b shows decreasing moduli for increasing moisture content as expected as water commonly acts as a plasticizer of biopolymers. Within the ranges of temperature and MC investigated, MC has a smaller effect than temperature on melt moduli. The dependence of moduli on MC will be further discussed below.

For protein concentrations ranging from 75 to 80%, the concentration had an insignificant influence on moduli. In general, higher polymer concentrations lead to higher moduli due to increased chain interactions and entanglements in melts, and a small increase of moduli with protein concentration was observed (Figure 2c). The blend of pea protein and pea fibre forms a complex fluid with more possible interactions than in a single polymer melt. The polymer concentration range is quite limited which also could explain the small influence of protein concentration.

The overall conclusion from Figure 2 is that the influence of the parameters on the moduli follows temperature > MC > concentration. This is further highlighted below.

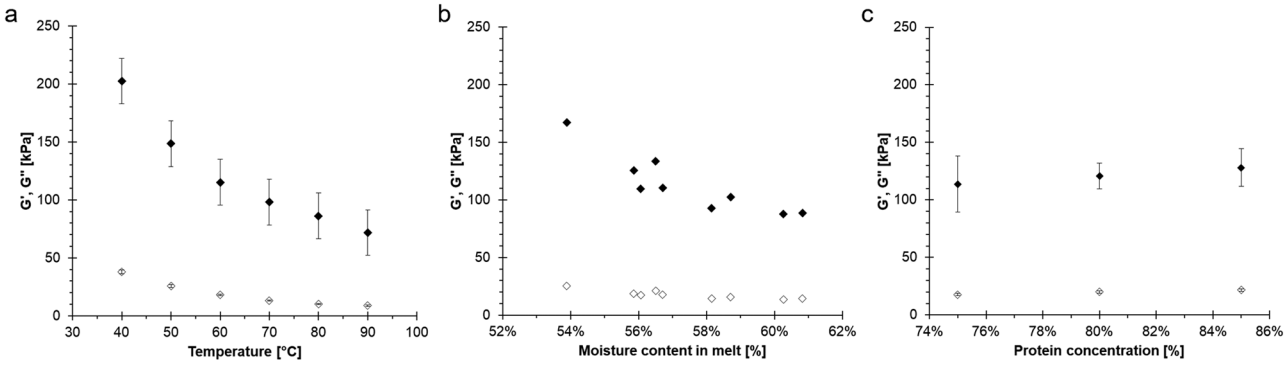

Figure 2: Dependence *G′* and *G″* at 10 rad/s on (a) temperature, (b) melt moisture content and (c) protein concentration. In (a) protein concentration is 75% and added water is 64%. In (b) protein concentration is 75% and temperature is 60°C. In (c) added water is 64% and temperature is 60°C. *G′* have filled symbols and *G″* open symbols.

3.3 Influence of extended temperature on the rheological properties

The thermomechanical mixing in an extruder that is used to produce meat analogues is normally operated above 100°C and at pressures around 5–10 bar. It is therefore relevant to determine rheological behaviour under these conditions which in practice is difficult. There are recent studies where ultrasonic flow profiling and high-pressure cells have been utilised [11,26]. A further option is to utilise a CCR, which encloses the melt thus preventing moisture loss at high temperature and pressure.

Figure 3 shows results obtained with conventional rheometry overlayed with those from CCR for two of the samples, 85% protein and MC = 52% and MC = 56%. The exponential decrease of moduli with temperature was to be expected and has been observed for plant protein melts by several studies [24,40,41]. The experiments for pea protein melts showed values within a similar range, with some variability due to differences in experimental setup and composition variations between batches [12,13].

Each method has good reproducibility and there is qualitative agreement. The measured moduli do not perfectly overlap, and the discrepancy could have several causes. There is a greater risk of moisture loss in conventional rheometry leading to overestimated moduli as the melt sample is surrounded by oil which is not a perfect barrier to moisture loss compared to the enclosure in the CCR. The geometry in the conventional rheometry (parallel plates) gives a simpler shear field than the grooved surfaces of the double-cone geometry of the CCR. Further, the torque sensor originally designed for rubber does not have the required sensitivity for low-moduli melts at high temperature.

3.4 Master curve of all data

All data from conventional rheometry were fitted using additive/linear mixed models, which are extensions of ANOVA to include a mix of categorical and continuous variables. The additive model (M1) fits exceptionally well (R^2_{adj} = 99.2%)(Figure 4a). The non-linear smooth function f_T only shows a slight deviation from a linear model. The same is true for the non-linear function f_{MC} of MC (see the Supplementary data for plots of f_T and f_{MC}). It is also relevant to compare model (M1) with the linearised model (M2). The quality of the model fit for model (M2) (R^2_{adj} = 98.3%) is only slightly lower than for model (M1) despite limiting it to Arrhenius-type relations (Figure 4b). This shows that almost all data can be explained by exponential relationships or equivalently by linear relationships on a logarithmic scale, as in model (M2). There is a possibility of more complex relationships as the functions f_T and f_{MC} in model (M1) are only approximately linear. Still, the fit for model (M1) is close to that of model (M2), and so we can say that the Arrhenius-type relations are a good fit for the data.

Comparing the model components, the effect of temperature, MC and experiment are statistically significant, whereas protein concentration is not. The *p*-values for each

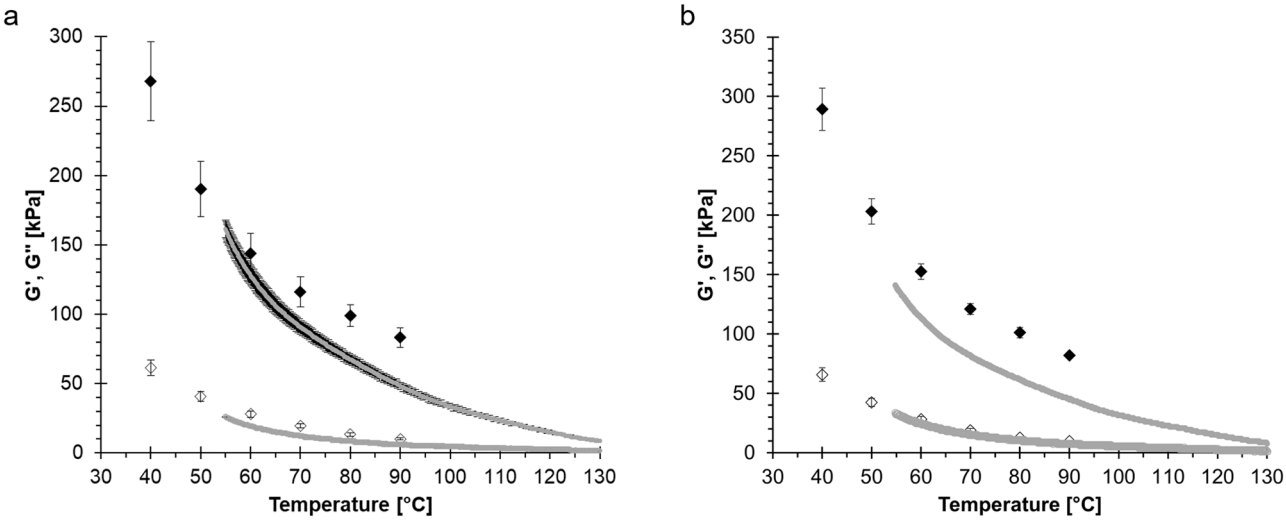

Figure 3: *G′* and *G″* for the protein melts with 85% pea protein at 5 Hz (53 rad/s) as a function of temperature. Black symbols are conventional rheometry and grey symbols are CCR. *G′* have filled symbols and *G″* open symbols. (a) melts with 52% MC, (b) melts with 56% MC.

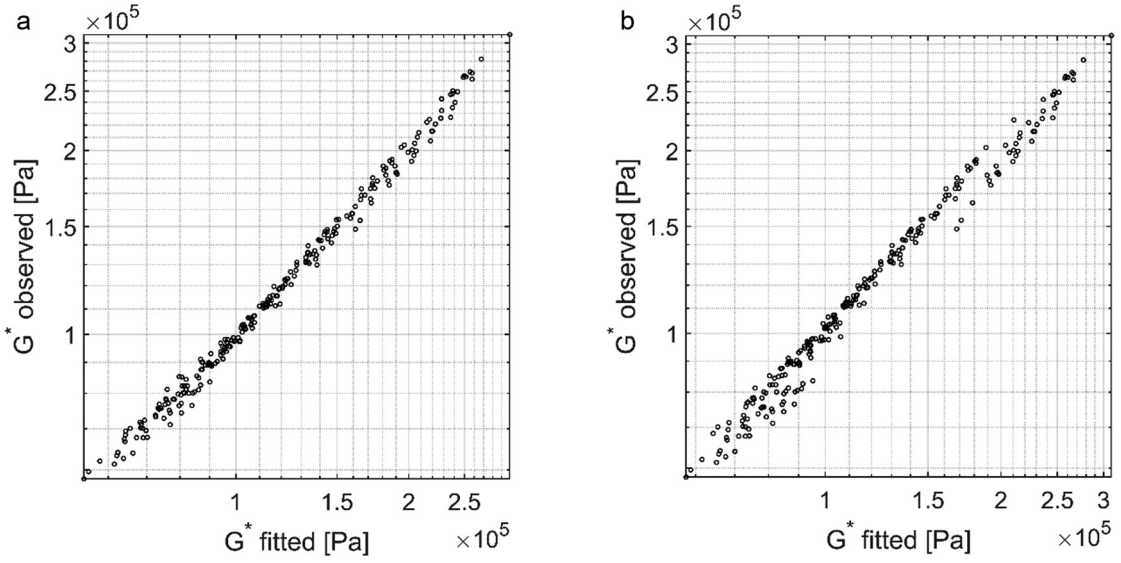

Figure 4: All data of G^* at 10 s^{-1} plotted against the (a) model M1, and (b) model M2.

Table 3: Results for the linear mixed models. δR^2_{adj} are computed for the pairs of models in Table 1. *p*-values are given for model (M2)

Model component	δR^2_{adj} (%)	*p*-value
Temperature	87.9	$<10^{-16}$
Moisture content	3.6	0.00005
Protein concentration	0.1	0.82
Experiment	9.4	$<10^{-16}$

component of the model (M2) are given in Table 3. The same result holds for model (M1), see Tables S1 and S2.

The proportion of explained variation by each model component is examined using the difference δR^2_{adj}, also given in Table 3. Temperature explains the majority of the variation. Moisture content explains a relatively small part, but still a larger part than protein concentration does. Thus, the influence of the parameters clearly follows the previously mentioned temperature > MC > concentration exemplified in Figure 2. The inclusion of the random effect of the experiment also improves model fit, and the variation explained by this model component is larger than that of both MC and protein concentration.

4 Conclusions

The rheological behaviour of melts of pea protein isolate and pea fibre normally utilised to extrude meat analogues was determined. The melt rheology is one of the determining parameters for fibre formation during HME processing and as such important for understanding the process and the mechanisms involved. Protein concentration, MC and temperature were varied, and the mechanical spectra of the melts were measured. All melts showed moduli increasing with frequency and storage modulus larger than loss modulus ($G' > G''$) for all samples as expected for a polymer melt in the rubbery region. The response can also be expressed as complex shear viscosity which showed a strong shear thinning response with flow behaviour index (n) in the range 0.05–0.12.

The relative influence of the parameters varied within the experimental space showed that the influence of temperature > MC > protein concentration. The increase of modulus with protein concentration was quite weak and not statistically significant, likely depending on the small concentration range and the complex structure of protein melts.

The moduli determined by conventional rheometry agreed with those determined using CCR, both showing an exponential decrease of G' and G'' between 40 and 130°C.

The statistical analysis showed that a linear mixed model with an Arrhenius-type relationship between parameters and complex modulus explains the results remarkably well (R^2_{adj} = 98.3%). Only a small increase in model fit was obtained when using an additive mixed model which allows for a more general, non-linear relationship.

Overall, conventional rheometry together with CCR and additive/linear mixed models was a powerful combination to analyse the rheology of protein melts relevant for meat-analogues.

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