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A novel approach to determine optimal protein texturization conditions - A critical moisture level with increased effect of temperature on viscosity reduction in the rubbery state

Åge Oterhals[a,*], Rafiq Ahmad[b], Tor Andreas Samuelsen[a]

[a] Nofima, P.O. Box 1425 Oasen, N-5844, Bergen, Norway
[b] Cargill, Dirdalsstranda 51, N-4335, Dirdal, Norway

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ABSTRACT

The effect of moisture on reduction of the flow starting temperature (T_f, i.e. start of flow through a capillary die at constant pressure conditions) in major protein ingredients used in fish feed has been assessed using a Phase Transition Analyzer. All proteins showed an initial linear reduction in T_f followed by a sharp sigmoidal reduction at higher moisture content. A critical moisture level (M_{cr}) could be defined at the onset of this transition region. Fishmeal and wheat gluten show the lowest M_{cr} (138 and 160 g kg^{-1}, respectively) and highest reduction in T_f above M_{cr}. Sunflower meal and soy protein concentrate demand more moisture, show higher variability in M_{cr} (210–262 and 233–306 g kg^{-1}, respectively) and less reduction in T_f at moisture levels above M_{cr}. The T_f level corresponds to an apparent viscosity of 10^5 Pa s and can be combined with the William-Landel-Ferry equation to establish moisture contour plots for the effect of temperature on viscosity reduction in the rubbery state. The protein specific M_{cr} levels and rheological profiles can be applied to determine optimal moisture and temperature conditions for plasticization and texturization of extruded food and feed products.

1. Introduction

The fish feed extrusion process involves several subsequent processing steps to transform the recipe dry powder mix to an expanded, porous and durable composite material shaped into pellets (Mitchell and Arêas, 1992; Samuelsen et al., 2014; Sørensen, 2012). To obtain the target pellet quality, the biopolymers are moistened and heated above the glass transition temperature (T_g) by water and steam addition in the preconditioner, and further heated in the extruder barrel by use of intense mechanical mixing and energy dissipation. By heating in the rubbery state, the viscosity will reduce and enable the unfolding and alignment of high molecular weight protein and starch molecules with formation of new inter- and intra-molecular hydrogen bonds, hydrophobic, van der Waals and electrostatic interactions, and covalent disulphide bonds (Verbeek and van der Berg, 2010) in the texturized product. In present industrial practise, the process operator has to rely on experience to define the right combination of moisture, and thermal and mechanical (i.e. shear strain) heat to obtain the target expansion rate (i.e. pellet porosity and density) and durability. To support the process operators, a new approach is needed to better characterize the technofunctional properties of the individual protein ingredients with relevance to the extrusion process.

Food protein ingredients can be characterized as amorphous solids. Upon heating, they undergo a softening around the T_g. The glass transition can be considered as a kinetic phenomenon or described by thermodynamic relationships (Abiad et al., 2009) and does not represent a specific temperature but rather the midpoint of a temperature range reported to be up to 100 °C in food polymers (Yildiz and Kokini, 2001). The transition temperature depends on the chemical composition of the foods and may be reduced by the addition of moisture or other plasticizers (Adeodato Vieira et al., 2001; Ahmad et al., 2018; Oterhals and Samuelsen, 2011). The viscosity at T_g is normally given as a universal value of 10^{12} Pa s (Roos, 1995) and is dramatically reduced by increasing temperature. The temperature dependency of viscosity at

Abbreviations: *a*, constant depicting the steepness at the sigmoid curve inflection point; FM, fishmeal; *m*, slope of the tangent line; M_c, characteristic moisture content; M_{cr}, critical moisture content; M_{eff}, effective moisture range; M_m, maximum effective moisture content; M_{ref}, reference moisture content; RT_f, relative flow starting temperature; SFM, sunflower meal; SPC, soy protein concentrate; T_f, flow starting temperature; $T_{f\text{-}m}$, T_f at maximum effective moisture content (M_m); $T_{f\text{-}cr}$, T_f at critical moisture content (M_{cr}); $T_{f\text{-}eff}$, effective flow starting temperature range; T_g, glass transition temperature; WG, wheat gluten

* Corresponding author.
E-mail address: aage.oterhals@nofima.no (Å. Oterhals).

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T_g + 100 K can be described by the William-Landel-Ferry (WLF) equation (Williams, 1955):

$$log\left[\frac{\eta(T)}{\eta(T_g)}\right] = \frac{-C_1(T - T_g)}{C_2 + (T - T_g)} \quad (1)$$

where $\eta(T)$ and $\eta(T_g)$ are viscosities at temperature T and reference temperature T_g, respectively, and C_1 and C_2 are system parameters. The parameters C_1 and C_2 are not universal for food systems and highly depend on the type of biopolymer and content of moisture and other plasticizers (Abiad et al., 2009; Matveev et al., 1999; Oterhals and Samuelsen, 2015; Yildiz and Kokini, 2001).

The viscosity of plasticized biopolymers can be measured by use of pressure capillary rheometry (Bengoechea et al., 2007; Hayashi et al., 1993; Igura et al., 1997; Samuelsen and Oterhals, 2016). The technique may also be used to measure a flow starting temperature (T_f) defined as the start of flow through the capillary at constant pressure conditions (Fujio et al., 1991; Igura et al., 1997). The Phase Transition Analyser (PTA; Strahm et al., 2000) used in this study is based on this principle. Earlier studies by Oterhals and Samuelsen (2015) have shown that the rubbery material starts to flow through the capillary at an apparent viscosity of approx. 10^5 Pa s.

Fish feed used in intensive aquaculture operations are formulated based on a blend of different protein, starch and lipid ingredients (Ytrestøyl et al., 2015). Micro ingredients like vitamins, minerals, essential amino acids and pigments are added to meet the nutritional requirements of the specific fish species and growth phase (Cho et al., 1993). Most of the lipids are added post extrusion and drying in a vacuum coating operation, i.e. the performance of the extrusion process is mainly influenced by the choice of protein and starch ingredients (Samuelsen et al., 2018). The food proteins assessed in this study represent the major commodity protein ingredients applied in Atlantic salmon feed formulations. Reported inclusion levels (of total diets) are for soy protein concentrate (SPC) 21.3%, sunflower meal (SFM) 6.0%, wheat gluten (WG) 5.8%, and fishmeal (FM) 19.5% (Ytrestøyl et al., 2015); however, this may vary depending on growth phase.

The moisture and temperature conditions for the gelatinization (cooking) and degradation of different starch sources like wheat, pea and tapioca have been documented (Xie et al., 2009). In comparison, less information is available for the physicochemical and rheological properties and variability of applied protein ingredients like fishmeal (Samuelsen et al., 2014), soy protein concentrate (Chajuss, 2011), sunflower meal (Gonzales-Perez and Vereijken, 2007), wheat and maize gluten (Belitz et al., 1986), and pea protein (Stone et al., 2015). This gives the feed manufacturing industry challenges related to obtaining a consistent target physical feed quality (Draganovic et al., 2011; Kraugerud et al., 2011; Samuelsen et al., 2018; Tyapkova et al., 2016).

The objective of this study was to establish a novel and systematic approach to determine optimal moisture and temperature conditions for texturization of protein ingredients. The approach is based on measurement of a flow starting temperature (T_f) and defining of a critical moisture level (M_{cr}) characterized by a sudden drop in T_f upon further increase in moisture level. The drop in T_f reflects an increased effect of temperature on viscosity reduction in the rubbery state. A second objective was to compare the M_{cr} level between the major food proteins applied in Atlantic salmon feed formulations and assess possible variation within SFM and SPC samples. The information can be used to better understand and define the moisture and temperature conditions needed to obtain a satisfactory plasticization and texturization of protein ingredients applied in extruded food and feed products.

2. Materials and methods

2.1. Materials

Fourteen protein samples (Table 1) from four different sources were used in this investigation. Fishmeal (FM) and wheat gluten (WG) were obtained from Norsildmel AS (Bergen, Norway) and Cargill Plc (Manchester, UK). Sunflower meal (SFM) was obtained from Bunge (Budapest, Hungary) and Linas Agro (Panevezys, Lithuania). Soy protein concentrate (SPC) was purchased from Sojaprotein (Bečej, Serbia), Sements Selecta (Goiania, Brazil), Caramuru Alimentos (Itumbiara, Brazil), and Imcopa Food Ingredients (Araucaria, Brazil). Numbers behind the individual samples in Tables and Figures represents our internal registration numbers.

2.2. Sample preparation

All proteins ingredients (Table 1) were ground in a Retch SR-3 centrifugal mill (Retsch GmbH, Haan, Germany) with a ring sieve aperture of 0.5 mm. The moisture content of samples were measured and adjusted to a predefined level (Tables 2 and 3) by addition of finely crushed ice cooled with liquid nitrogen according to Oterhals and Samuelsen (2015). The lowest applied moisture level was defined for each sample to give a T_f < 180 °C (i.e. the maximum recommended instrument operation temperature). The highest applicable moisture level was limited by the stickiness of the material; i.e. the moisture level giving tendency to form lumps and uneven distribution of added moisture. The samples were conditioned in a closed bottle at 4–5 °C overnight, and treated in a Waring MC3 mixer (Warring, Torrington, USA) to break down possible agglomerates and homogenize before final measurement of moisture content. Prepared samples were stored in closed bottles at minus 20 °C until use.

2.3. Chemical analysis

Moisture level was determined gravimetrically after drying of samples at 104 °C (ISO 6496, 1999). Crude protein (N x 6.25) was analysed by Dumas method (ISO 16634-1; 2008) and water-soluble protein by hot water extraction using Kjeldahl method (ISO 5983-2, 2009). Total ash content was determined according to ISO 5984, (2002) and salt (NaCl) content based on water-soluble chloride using AOAC (2000) method 937.09. The fat content was determined according to Bligh and Dyer (1959). The starch content was determined using a glucoamylase method (Chiang and Johnson, 1977). The amino acids composition was determined after hydrolysis with hydrochloric acid using the Waters Accq-Tag method as described in Oterhals and Samuelsen (2015). Non-starch polysaccharides were estimated on dry matter basis by subtracting the crude protein, fat, ash, and starch contents. The chemical analyses were carried out at Nofima BioLab (Bergen, Norway; accredited according to ISO 17025) based on duplicate measurements. Moisture analyses were performed in triplicate.

2.4. Measurement of T_g, T_f, and apparent viscosity at T_f

A Phase Transition Analyzer (PTA; Wenger Manufacturing, Sabetha, KS) (Strahm et al., 2000) was used to measure the T_g and T_f as described by Oterhals and Samuelsen (2015). The principle is based on the measurement of change in height (sample volume) with respect to temperature increase (8 °C/min) at constant pressure (100 bars). After T_g measurement, the blank insert (no capillary opening) is replaced with a 1.75 mm capillary opening. The temperature is further increased at the same rate and a T_f defined as the temperature level initiating start of flow through the capillary die.

The apparent wall shear stress (τ_{app}) and shear rate ($\dot{\gamma}_{app}$) in the capillary was calculated based on (Oterhals and Samuelsen, 2015):

$$\tau_{app} = \Delta P\frac{R}{2L} \quad (2)$$

$$\dot{\gamma}_{app} = Q\frac{4}{\pi R^3} \quad (3)$$

Table 1
Proximate chemical composition (g kg^{-1} DM) and amino acids composition (g kg^{-1} crude protein) of the assessed protein ingredients. Applied moisture levels in tested samples are given in Tables 2 and 3

Component	FM #5/14	WG #12/13	WG #13/13	SFM #23/14	SFM #2/13	SFM #35/14	SPC #2/14	SPC #75/14	SPC #5/13	SPC #56/14	SPC #60/14	SPC #7/13	SPC #5/14	SPC #58/14
Crude Protein (CP)	721.0	803.4	838.3	382.2	516.0	411.6	704.4	666.3	640.4	655.3	686.1	684.6	672.0	653.3
Water-soluble proteins (WSP)	128.3	61.9	50.3	34.4	40.2	36.2	90.2	96.6	81.3	78.6	68.6	53.4	75.9	82.3
Non-soluble proteins (Non-SP)	592.7	741.5	788.0	347.8	475.7	375.4	614.2	569.7	559.1	576.7	617.5	631.2	596.1	571.0
Ash	160.8	66.6	8.5	70.5	103.6	65.9	63.0	65.5	67.3	67.0	63.6	60.8	63.4	65.9
Fat	137.9	58.1	80.3	45.2	32.0	39.5	3.2	3.2	16.8	6.4	6.5	5.7	6.5	8.6
Salt	42.7	1.1	1.1	1.1	1.1	2.2	1.1	1.1	1.1	1.1	2.2	< 1.1	< 1.1	< 1.1
Starch	NA	102.5	95.1	12.1	10.0	13.2	21.3	14.8	10.5	12.8	10.8	13.8	11.8	10.8
Non-starch polyscharides (NSPs)	NA	NA	NA	490.1	338.4	469.8	208.1	250.3	265.0	258.5	233	235.1	246.2	261.3
In Crude protein DM														
Glycine (Gly)	74.4	35.5	35.2	59.9	62.0	60.0	45.9	45.8	44.3	46.4	45.9	47.4	45.9	45.8
Alanine (Ala)	66.2	26.2	25.3	42.7	41.9	44.3	44.1	44.2	43.7	45.1	44.7	46.6	45.0	44.5
Valine (Val)	52.7	43.4	42.4	54.8	55.6	57.1	53.5	53.4	52.5	54.4	53.1	54.9	53.3	53.4
Leucine (Leu)	74.5	71.3	70.1	62.8	63.7	67.5	79.7	79.1	79.6	81.8	81.4	84.1	81.4	81.8
Isoleucine (Ile)	42.6	38.0	37.1	42.7	43.6	45.3	48.2	48.2	48.4	49.4	49.4	50.9	49.0	48.9
Proline (Pro)	48.1	130.3	127.4	45.8	46.2	49.1	52.9	52.1	52.7	53.7	53.5	54.6	53.6	53.1
Methionine (Met)	28.5	15.9	16.0	18.2	22.6	22.7	12.4	15.8	13.6	14.0	14.2	14.4	12.0	11.2
Phenylalanine (Phe)	37.5	55.1	54.6	47.6	48.3	50.7	53.8	52.8	54.5	55.8	56.0	57.6	55.7	55.9
Glutamic acid (Glu)	121.1	364.5	356.9	188.5	194.9	204.0	184.8	182.3	185.6	188.3	187.1	196.0	188.8	185.1
Aspartic acid (Asp)	82.9	32.6	30.5	96.0	93.2	100.0	119.7	117.6	120.5	122.4	121.5	127.3	125.9	123.8
Lysine (Lys)	74.1	17.1	16.4	38.3	36.5	40.5	69.1	68.6	64.0	68.8	66.8	66.5	66.9	67.1
Arginine (Arg)	74.1	37.5	37.5	83.9	87.6	91.5	83.5	81.1	80.8	83.1	81.3	82.4	81.6	79.5
Histidine (His)	21.2	22.0	21.4	25.9	26.5	26.7	28.0	28.4	27.3	28.6	27.5	27.8	28.2	28.1
Tyrosine (Tyr)	32.6	35.0	38.0	23.6	24.8	28.0	32.9	34.7	33.2	34.3	35.1	35.2	33.6	32.7
Hydroxyproline (Hyd)	11.8	1.3	1.3	2.9	2.1	2.7	1.5	1.6	1.6	1.6	1.6	1.7	1.6	1.7
Serine (Ser)	41.7	51.1	50.4	44.7	44.4	47.5	54.8	53.9	53.7	55.7	56.1	57.8	56.2	55.7
Threonine (Thr)	41.6	24.7	25.1	33.4	34.2	34.9	41.1	41.4	41.4	43.0	41.7	43.6	41.6	41.2
Polar neutral amino acids (Polar-N)	424.4	415.8	408.1	374.4	383.8	396.5	390.5	391.4	389.5	400.6	398.1	410.6	395.8	394.5
Acidic amino acids (Acidic)	203.9	397.1	387.4	284.4	288.0	304.0	304.5	299.8	306.1	310.7	308.6	323.3	314.7	308.9
Basic amino acids (Basic)	169.3	76.6	75.3	148.1	150.6	158.7	180.6	178.1	172.1	180.5	175.6	176.7	176.6	174.7
Polar amino acids (Polar)	127.8	112.1	114.8	104.6	105.6	113.1	130.3	131.5	129.9	134.6	134.4	138.2	133.0	131.2
Free amino acids	30.2	1.1	1.4	11.0	12.0	8.5	3.4	1.4	7.0	4.7	3.1	2.3	3.4	1.7

FM, fishmeal; SFM, sunflower meal; WG, wheat gluten; SPC, soy protein concentrate; NA, not applicable.

where ΔP is the pressure drop along the capillary length L (Pa), R = capillary radius (0.000875 m), L = capillary length (0.025 m) and Q is the volume flow rate ($m^3\ s^{-1}$) measured at the initial displacement speed.

Apparent viscosity (η_{app}) at T_f could be estimated by:

$$\eta_{app} = \frac{\tau_{app}}{\dot{\gamma}_{app}} \tag{4}$$

2.5. Modelling of the effect of moisture on flow starting temperature

The observed relationship between the flow starting temperature and moisture content in the studied range has a sigmoidal shape as shown in Fig. 1. Relationship of this kind can be described by a general mathematical expression suggested by Peleg (1994) for the change in any mechanical property around the glass transition temperature:

$$Y(M) = \frac{Y_s}{1 + exp((M - M_c)/a)} \tag{5}$$

Y(M) is the magnitude of the mechanical property as a function of moisture (M), Y_s the magnitude of this parameter in a reference state (the glassy state), M_c a characteristic moisture content representing the location of the inflection point of *Y(M)*, and a an empirical constant depicting the steepness of the mechanical integrity loss. The curve will approach that of a step function when $a \rightarrow 0$, and be more flat with increasing values. In our study, the measured T_f *(M)* showed a minor linear negative behaviour above the concave transition range and a constant reference state could not be defined. Eq. (5) was modified by introducing a linear regression term for the Y_s parameter; i.e. T_f *(M)*:

$$Y_s\ (M) = T_f\ (M) = c - b \times M \tag{6}$$

where c is a constant and b the regression coefficient. Before modelling, the experimental T_f was converted to relative values (RT_f; Tables 2 and 3) by subtracting the T_f at a reference moisture content (M_{ref}) defined as the experimental moisture level above the lower upward concavity of the sigmoid curve. In case of several experimental points in this region, the moisture level giving the best fit to experimental data was chosen. Combing Eqs. (5) and (6) gives:

$$RT_f(M) = \frac{c - b \times M}{1 + Exp((M - M_c)/a)} \tag{7}$$

Substitution of the constant reference state Y_s in Eq. (5) with a moisture dependent level will change the curvature of the sigmoidal curve to a minor degree. A critical moisture level (M_{cr}) was defined as the intersection between the linear initial phase and the tangent at steepest slope defined by the first derivative of Eq. (7):

$$\frac{d}{dM}RT_f(M) = \left((bM - C - ab) \times Exp\left(\frac{M - M_c}{a}\right) - ab\right) / \left(a \times \left(Exp\left(\frac{M - M_c}{a}\right) + 1\right)^2\right) \tag{8}$$

The moisture level at the inflection point were found by setting the second derivative of Eq. (7) equal to zero. The point-slope form defines the tangent line:

$$RT_f - RT_{f1} = m \times (M - M_1) \tag{9}$$

Where RT_{f1} and M_1 is the relative flow starting temperature and moisture, respectively, and m the slope at the inflection point. The critical moisture content (M_{cr}) was found as the intersection between Eqs. (6) and (9):

$$m \times (M_{cr} - M_1) + RT_{f1} = c - b \times M_{cr} \tag{10}$$

The extrapolation of the tangent line (Eq. (8)) to zero RT_f gives the

Table 2
Moisture contents (M, g kg^{-1} wet basis) and measured phase transition temperatures (°C)[1] in FM, WG and SFM samples.

M	T_g	$T_{g\ pred}$	T_{gEnd}	T_f	T_f-T_{gpred}	C_2	RT_f	M	T_g	$T_{g\ pred}$	T_{gEnd}	T_f	T_f-T_{gpred}	C_2	RT_f
FM #5/14								WG #12/13							
84	23.0	22.3	55.0	179.7	157.4	234.7	141.4	115	15.5	15.5	59.0	174.1	158.6	236.6	142.0
113	17.1	18.3	38.3	173.4	155.1	231.3	135.2	130	13.4	13.9	41.3	173.6	159.7	238.2	141.5
144	14.4	14.1	29.9	152.3	138.2	206.1	114.1	154	11.8	11.4	39.8	164.3	152.9	228.0	132.2
172	10.5	10.3	23.0	69.2	58.9	87.8	30.9	167	10.8	10.1	26.6	53.8	43.7	65.2	21.7
201		6.3	18.9	52.3	46.0	68.5	14.1	189	7.2	7.8	16.5	43.3	35.5	53.0	11.2
231		2.3		38.3	36.0	53.7	0.0	205		6.1		32.1	26.0	38.7	0.0
252		−0.6		31.1	31.7	47.2									
SFM #23/14								SFM #2/13							
151	12.2	12.0	51.1	168.4	156.4	233.2	105.7	92	28.6	24.3	93.8	169.2	144.9	216.1	103.5
179	9.8	10.2	39.0	165.6	155.4	231.8	103.0	152	9.6	11.8	45.2	166.1	154.2	230.0	100.4
219	7.7	7.6	28.6	156.1	148.5	221.5	93.4	201	7.2	6.6	21.1	148.8	142.2	212.1	83.1
248		5.7	27.5	147.7	142.1	211.9	85.1	222	6.0	5.1	18.3	125.8	120.6	179.9	60.1
267		4.4	30.5	132.3	127.8	190.7	69.6	245		3.9	11.0	78.7	74.8	111.5	13.0
291		2.8		84.3	81.5	121.5	21.7	271		2.8		65.7	62.9	93.8	0.0
331		0.2		62.7	62.4	93.1	0.0	288		2.3		54.7	52.4	78.1	
WG #13/13															
112	16.5	16.9	51.2	178.0	161.1	240.3	141.0								
152	13.2	11.9	32.3	166.0	154.1	229.8	129.0								
155	11.1	11.5	30.2	163.0	151.5	225.9	126.0								
177	8.3	8.8	20.3	49.0	40.2	59.9	12.0								
199		6.1	12.8	37.0	30.9	46.1	0.0								
202		5.7	12.7	44.0	38.3	57.1	7.0								
SFM #35/14															
151	12.6	12.4	50.3	167.1	154.7	230.8	91.9								
182	9.9	10.3	36.9	167.1	156.8	233.8	91.8								
222	7.8	7.6	25.9	161.1	153.4	228.8	85.8								
251		5.7	24.2	156.8	151.1	225.3	81.5								
281		3.7	18.6	89.9	86.2	128.6	14.7								
302		2.3		75.3	72.9	108.8	0.0								

1) The average standard deviation for T_g, T_{gEnd} and T_f was calculated to be 0.7, 2.9, and 4.3 °C, respectively.
T_g - glass transition temperature; T_{gpred} - Predicted glass transition using linear relationship between T_g and Moisture (M); T_{gEnd} - End point of glass transition range. T_f – flow starting temperature; C2 - WLF parameter estimated based on eq. (1); RT_f - relative flow starting temperature; FM - fishmeal; SFM - sunflower meal; WG - wheat gluten meal.

maximum effective moisture level (M_m) and the effective moisture range ($M_{eff} = M_{cr} - M_m$) (Fig. 1).

2.6. Statistical analyses

RT_f data were fitted to a nonlinear model using the user-specified nonlinear regression module in Statistica 13.2 (StatSoft, Tulsa, OK, USA). The iteration was based on least squares regression and the quality of the models were assessed by the coefficient of multiple determinations (R^2). Principal component analysis (PCA) was applied to visualize the main data structure (Martens and Martens, 2001) using Unscrambler X 10.4.1 (Camo, Oslo, Norway). Each variable was mean centered and standardized prior to analysis.

3. Results and discussion

3.1. Effect of moisture content on glass transition and flow starting temperature

The measured T_f were in all cases above T_{gEnd} (Tables 2 and 3), i.e. representing a material in rubbery phase. The obtained extrudate after measurement of T_f was a continuous and homogenous string. The residual plug of material in the heating chamber after end of measurement and cooldown was of similar appearance as the extrudate. Some darkening of the test materials were observed at Tf > 150 °C, corresponding to moisture content below M_{cr}. This might be attributed to Maillard reactions, however, as the assessed proteins contain very low levels of free reducing sugars, other possible browning reactions (Mohammed et al., 2000) might also contribute.

All the assessed protein ingredients (Table 1) showed a minor linear reduction in T_g and an initial minor linear reduction in T_f with increasing moisture level (Fig. 2a–d) as reported by others (Bengoechea et al., 2007; Oterhals and Samuelsen, 2015). The linear T_f-region was followed by a sharp reduction at a critical moisture level characteristic for the major type of ingredient and by further increase in moisture level, a sigmoidal relationship was observed (Fig. 2a–d). The observed T_f varied from 31.1 to 179.7 °C (Tables 2 and 3). Due to limitation in the PTA measuring range (5–180 °C), the T_g values could not be measured at all the studied moisture levels. A close to linear decrease in T_g at comparable moisture range has been reported for wheat gluten, soy protein isolate and casein (Bengoechea et al., 2007), fishmeal (Oterhals and Samuelsen, 2015), and defatted soy protein (Yildiz and Kokini, 2001). The observed T_g varied from 28.6 to 6 °C within the moisture range 84–333 g kg^{-1} (Tables 2 and 3). To enable an approximate prediction of the difference between T_f and T_g at higher moisture levels, we have assumed a linear relationship for T_g with extrapolation to higher moisture levels.

The difference T_f minus $T_{g\ predicted}$ represents a transition in apparent viscosity of the material from approx. 10^{12} Pa s in the glassy state to approx. 10^5 Pa s at the flow starting temperature (Oterhals and Samuelsen, 2015). The initial PTA displacement speed at T_f (data not shown) was equivalent to a shear rate of 0.3–4.1 s^{-1}, giving an apparent viscosity in the range of 0.4–6 × 10^5 Pa s. This is in good agreement with values reported for fishmeal (3 - 8 × 10^5 Pa s; Oterhals and Samuelsen, 2015) and soy protein concentrate (0.2 - 4 × 10^5 Pa s; Ahmad et al., 2018).

The difference between T_f and $T_{g\ predicted}$ showed a relative low variance between the assessed samples below M_{cr} and was within the range of 153.5–162.6 °C (Tables 2 and 3). The deviating difference for SFM #2/13 (144.9 °C) was caused by a high T_g at the lowest moisture level (Table 2), however, the applied second lowest moisture level (154.2 °C) was within the range. In comparison, above M_m (Fig. 1) the difference between T_f and $T_{g\ predicted}$ showed higher variance (Fig. 2). FM and WG samples showed low temperature differences in the 26–38 °C range, and SFM and SPC samples considerable higher in the 52–90 °C range (Tables 2 and 3). For all samples the T_{gEnd} approached

Table 3
Moisture contents (M, g kg^{-1} wet basis) and measured phase transition temperatures (°C)[1] in SPC samples.

M	T_g	$T_{g\ pred}$	T_{gEnd}	T_f	T_f-T_{gpred}	C_2	RT_f	M	T_g	$T_{g\ pred}$	T_{gEnd}	T_f	T_f-T_{gpred}	C_2	RT_f	M	T_g	$T_{g\ pred}$	T_{gEnd}	T_f	T_f-T_{gpred}	C_2	RT_f
SPC #2/14								SPC #75/14								SPC #5/13							
164	17.1	16.8	75.6	174.1	157.3	234.5	83.5	153	14.4	14.9	78.73	173.8	158.9	237.0	87.8	170	17.7	17.3	53.5	170.8	153.5	229.0	65.0
249	11.5	12.1	38.5	169.2	157.1	234.3	78.6	203	13.6	12.6	54.15	173.8	161.2	240.5	87.8	201	14.3	15.0	40.7	169.9	154.9	231.1	64.1
296	9.8	9.4	22.8	155.5	146.0	217.8	64.9	250	9.6	10.4	36.68	170.1	159.7	238.2	84.1	240	12.4	12.1	29.6	155.9	143.8	214.5	50.1
333	7.4	7.4	16.5	110.2	102.8	153.4	19.6	304	8.0	7.9	19.35	165.1	157.2	234.5	79.1	277	9.3	9.3	23.5	112.4	103.1	153.7	6.6
365		5.6	7.3	102.2	96.6	144.0	11.6	330		6.7		132.7	126.0	188.0	46.7	320		6.1	8.1	105.8	99.7	148.7	0.0
388		4.3		90.6	86.3	128.7	0.0	354		5.6		101.1	95.5	142.5	15.1	349		4.0		94.2	90.2	134.6	
								359		5.3		100.1	94.8	141.4	14.1	383		1.4		81.7	80.2	119.6	
								400		3.4		86.0	82.6	123.2	0.0								
								429		2.1		69.2	67.1	100.1									
SPC #56/14								SPC #60/14								SPC #7/13							
152	14.3	15.0	71.5	176.2	161.2	240.5	82.8	198	14.1	14.2	44.7	170.0	155.8	232.3	66.2	166	12.9	13.4	72.0	176.0	162.6	242.5	74.1
202	13.4	12.8	44.6	170.8	158.0	235.7	77.4	255	11.8	11.5	30.6	165.3	153.8	229.3	61.5	191	12.9	12.4	56.0	172.4	160.0	238.6	70.4
253	11.3	10.5	28.7	157.2	146.7	218.7	63.8	282	10.4	10.2	23.7	160.7	150.5	224.4	61.4	252	10.2	9.9	35.4	166.5	156.6	233.5	64.6
303	7.6	8.4	17.4	143.9	135.5	202.2	50.5	301	9.0	9.3	20.7	158.0	148.6	221.7	49.6	293	8.0	8.3	19.1	159.7	151.4	225.8	57.7
351		6.3		104.2	97.9	146.0	10.8	332		7.9	13.0	108.4	100.5	149.9	4.6	321		7.1	16.9	128.8	121.6	181.4	26.8
383		4.8		93.4	88.6	132.1	0.0	361		6.5		103.8	97.3	145.1	0.0	332		6.7	11.0	110.9	104.2	155.4	9.0
402		4.0		85.6	81.6	121.7		362		6.5		104.2	97.7	145.7	0.3	353		5.9		108.1	102.2	152.4	6.1
								400		4.7		93.4	88.8	132.4		386		4.5		102.0	97.4	145.3	0.0
																424		3.0		87.5	84.5	126.0	
SPC #5/14								SPC #58/14															
150	14.3	15.4	73.2	171.3	155.9	232.4	86.1	202	14.6	14.5	47.5	170.8	156.3	233.1	74.7								
205	14.7	13.2	46.0	169.8	156.6	233.6	84.8	242	12.5	12.7	35.9	166.2	153.5	228.9	70.1								
254	11.7	11.2	30.8	164.0	152.8	227.9	79.0	282	11.0	10.9	23.2	158.0	147.1	219.4	61.9								
303	8.2	9.1	18.1	143.1	133.9	199.7	58.0	311		9.6	16.7	125.5	115.8	172.7	29.4								
332		7.9	12.4	107.4	99.5	148.3	22.4	351		7.8		104.3	96.5	143.9	8.2								
372		6.3		97.8	91.5	136.4	12.7	381		6.5		96.1	89.6	133.6	0.0								
381		5.9		88.3	82.4	122.9	3.3																
403		5.0		85.1	80.0	119.4	0.0																

1) The average standard deviation for T_g, T_{gEnd} and T_f was calculated to be 0.7, 2.9, and 4.3 °C, respectively.
T_g - glass transition temperature; T_{gpred} - Predicted glass transition using linear relationship between T_g and Moisture (M); T_{gEnd} - End point of glass transition range. T_f – flow starting temperature; C2 - WLF parameter estimated based on eq. (1); RT_f - relative flow starting temperature; SPC - soy protein concentrate.

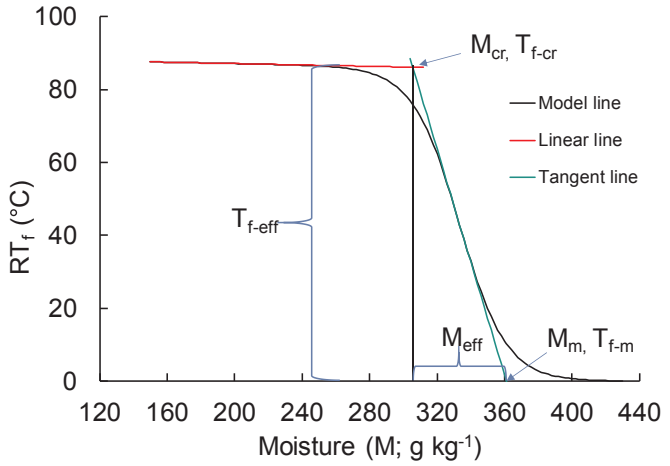


Fig. 1. Overview of parameters derived from modelling of the relative flow starting temperature (RT_f) vs moisture (Table 4). Critical moisture content (M_{cr}) and critical flow starting temperature ($T_{f\text{-}cr}$): moisture and temperature at onset of downward concavity. Moisture (M_m) and temperature ($T_{f\text{-}m}$) at maximum effective moisture level: moisture and temperature at onset of upward concavity. Effective moisture ($M_{c\text{-}eff}$) and temperature ($T_{f\text{-}eff}$) range: moisture and temperature range between onset of downward and upward concavity.

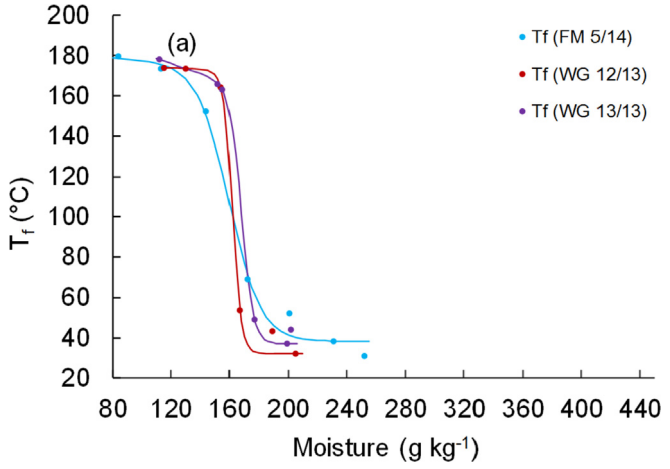


Fig. 2. Experimental T_f data vs moisture content. Experimental data (Tables 2 and 3) are given as points and continuous lines correspond to the respective T_f models given in Table 4. (a) Fishmeal (FM) and wheat gluten (WG), (b) Sunflower meal (SFM), (c) and (d) Soy protein concentrate (SPC).

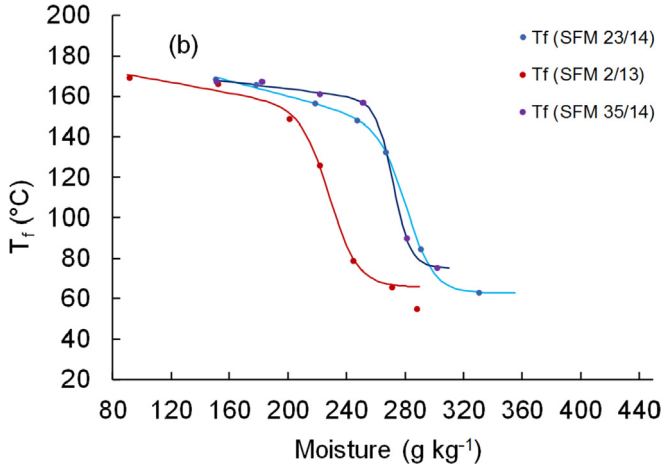
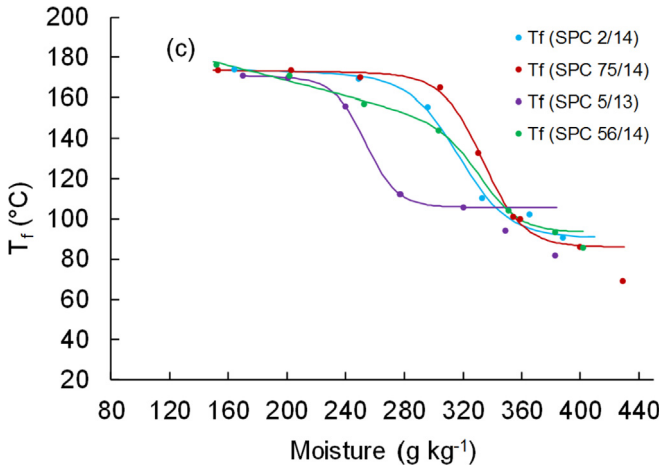


Fig. 2. (*continued*)



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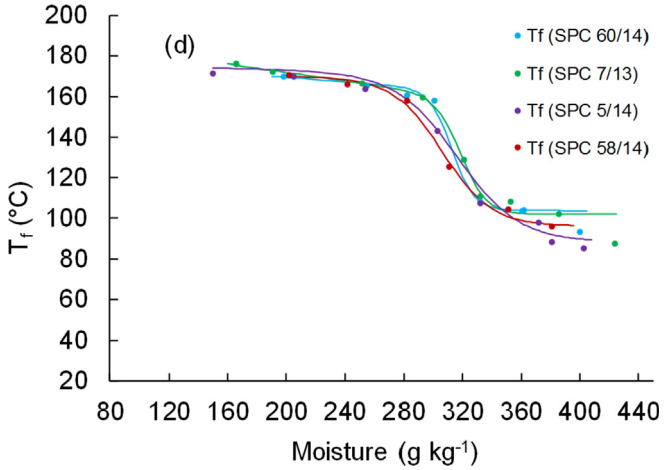

Fig. 2. (*continued*)

ambient temperature or below at the highest moisture levels (7–30 °C; Tables 2 and 3). This may reflect the stickiness, and agglomeration and lumping tendency observed during preparation of these samples in agreement with Roos (1995).

3.2. Modelling of flow starting temperature and viscosity reduction

Significant models ($p < 0.01$) could be established for the respective food proteins based on Eq. (7) with $R^2 \geq 0.99$ (Table 4; Fig. 2a–d). Several parameters were extracted based on the obtained empirical models (Fig. 1): A critical moisture level (M_{cr}) and flow starting temperature ($T_{f\text{-}cr}$), a maximum effective moisture level (M_m) and flow starting temperature ($T_{f\text{-}m}$), and an effective moisture (M_{eff}) and flow starting temperature range ($T_{f\text{-}eff}$) (Table 4). Graphs showing experimental data and model curves are given in Fig. 2a–d.

The apparent viscosity at T_f (10^5 Pa s) can be used to predict the temperature effect on viscosity reduction above T_g based on the WLF equation (Eq. (1); Williams, 1955).Where $\eta(T)$ and $\eta(T_g)$ are viscosity at temperature T and reference temperature T_g, respectively, and C_1 and C_2 are system parameters. C_1 is defined as the reciprocal of fractional free volume at T_g and C_2 is ratio of fractional free volume at T_g to the difference in thermal expansion coefficient in rubbery and glassy state. Yildiz and Kokini (2001) found C_1 values for soy proteins close to universal value, however, C_2 deviated greatly with water activity and moisture content. As a first approximation, setting C_1 equal to the universal value (−17.44) and the apparent viscosity at T_g and T_f equal to 10^{12} and 10^5 Pa s, respectively, the constant C_2 can be estimated based on Eq. (1) for any of the experimental moisture levels (Tables 2

Table 4
Regression parameters for T_f (M) model (Eq. 7), coefficient of multiple determination (R^2), and derived model parameters. Explanation of the used abbreviations are given in footnote on first page and in Fig. 1.

Sample	c	b	a	M_c	R^2	M_{cr}	$T_{f\text{-}cr}$	M_m	$T_{f\text{-}m}$	M_{eff}	$T_{f\text{-}eff}$
FM #5/14	144.00	0.042	10.80	159.90	0.993	138.0	160.3	181.0	55.2	43.0	105.1
WG #12/13	145.23	0.029	2.94	162.00	0.995	156.0	156.7	168.0	48.2	12.0	108.5
WG #13/13	167.80	0.239	3.99	168.04	0.998	160.0	151.3	177.0	49.0	17.0	102.3
SFM #23/14	135.79	0.192	9.53	282.00	0.999	262.0	138.4	300.0	72.6	39.0	66.8
SFM #2/13	116.94	0.131	9.76	228.92	0.993	210.0	143.9	248.0	76.2	39.0	68.8
SFM #35/14	106.37	0.090	5.91	272.00	0.999	260.0	148.7	284.0	84.7	24.0	64.0
SPC #2/14	85.98	0.018	17.08	317.44	0.990	283.0	162.0	351.0	100.4	66.0	60.6
SPC #75/14	88.99	0.009	13.88	333.42	0.998	306.0	161.6	361.0	96.3	56.0	66.0
SPC #5/13	67.55	0.015	10.75	253.79	0.999	233.0	161.8	275.0	113.5	42.0	48.3
SPC #56/14	112.70	0.188	13.68	334.08	0.998	304.0	143.7	359.0	98.7	56.0	44.3
SPC #60/14	76.32	0.053	7.66	312.83	0.999	298.0	156.7	328.0	111.0	30.0	45.7
SPC #7/13	90.76	0.104	8.70	319.40	0.996	301.0	155.1	336.0	109.2	36.0	46.6
SPC #5/14	88.15	0.015	21.02	316.69	0.991	275.0	162.2	358.0	98.5	83.0	63.7
SPC #58/14	74.64	0.003	16.77	306.14	0.995	274.0	160.3	340.0	105.2	68.0	56.6

FM, fishmeal; SFM, sunflower meal; WG, wheat gluten; SPC, soy protein concentrate.

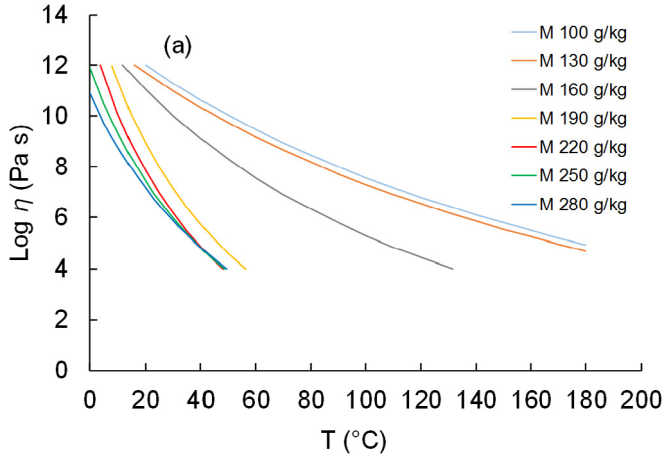

Fig. 3. Moisture contour lines showing the effect of temperature on viscosity reduction above the glass transition temperature. The contours are based on T_f models (Table 4) and estimated C_2 values. (a) Fishmeal (FM #5/14), (b) wheat gluten (WG #13/13), (c) Sunflower meal (SFM #2/13, (d) Soy protein concentrate (SPC #5/14).

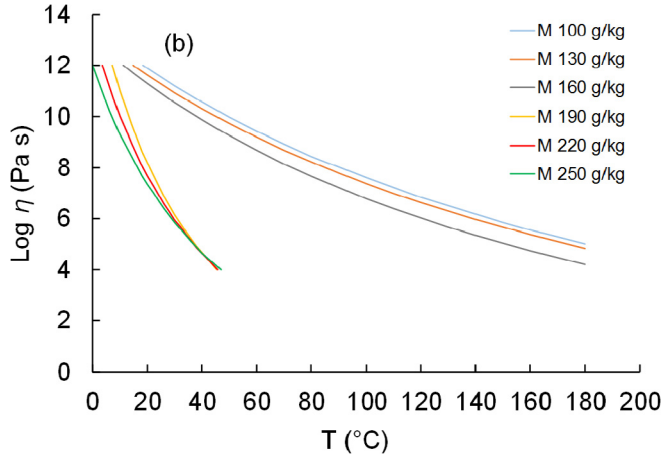

Fig. 3. (*continued*)

and 3). Combined, the model describing the effect of moisture on T_f (Eq. (7); Table 4) and the WLF equation (Eq. (1)) can be used to establish moisture contour plots representing the effect of temperature on apparent viscosity for the respective food proteins. Plots representing a sample within each of the studied groups FM, WG, SFM and SPC are given in Fig. 3a–d. In contrast to the T_f models, the WLF-models give an upward concavity of the viscosity reduction. As for the T_f-curves (Fig. 2a–d), at a critical moisture range a shift in the effect of temperature on viscosity reduction can be observed.

3.3. Industrial applications

The fish feed extrusion process is based on moistening and thermomechanical processing of protein and starch ingredients to form a high viscous fluid behind the die. The obtained models cannot account for the effect of specific mechanical energy (SME) input in the extrusion process, however, gives a map of the effect of the main operating parameters, moisture and temperature, on the apparent viscosity of the individual protein ingredients. Initially, each protein ingredient can be defined as a separate powder phase needing different moisture and thermomechanical conditions to reduce the viscosity and unfold the high molecular weight biopolymers with formation of new intra- and intermolecular binding networks. Achieving these conditions for all recipe ingredients is crucial for obtaining a satisfactory plasticization and acceptable physical product quality. Based on our own experience (unpublished results and Samuelsen et al., 2018) and other studies (Draganovic et al., 2011; Gonzalez-Perez and Vereijken, 2007; Kraugerud et al., 2011; Tyapkova et al., 2016), the assessed protein ingredients have different moisture requirements and show different behaviour in the extrusion process: SPC demands high moisture level and temperature to obtain a satisfactory transformation and durable pellets. SFM is applied in lower inclusion levels and shows less influence on the process. WG need less moisture, act as a binder and reduce

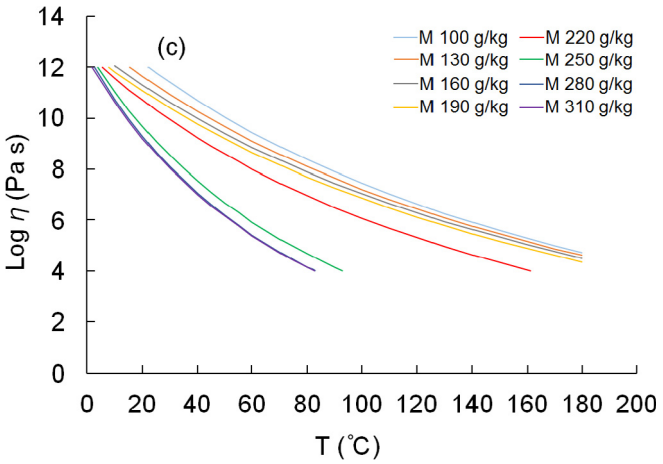

Fig. 3. (*continued*)

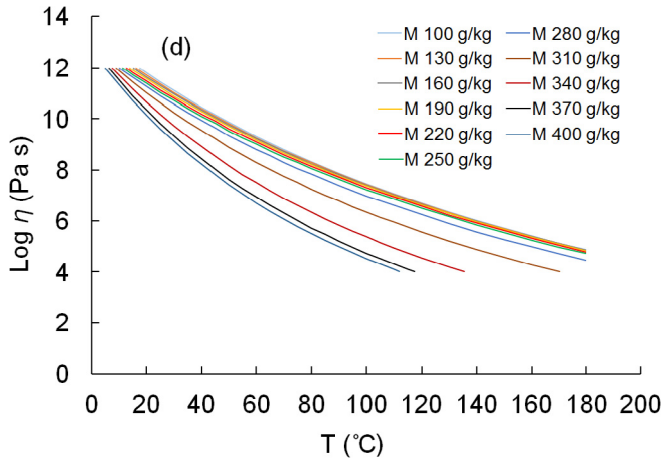

Fig. 3. (*continued*)

the expansion rate. FM needs less moisture, however, shows large variability and is perceived as a demanding ingredient. We hypothesize that the optimal moisture conditions for transformation of proteins in the extrusion process is at or above the M_{cr} defined in this study. In a feed mix, the protein ingredients with the highest M_{cr} level will define the conditions needed to exploit the binding properties of the proteins. When processing diets with high inclusion levels of SPC or FM, the respective M_{cr} observed in this study is in good agreement with reported moisture levels needed to obtain a good pellet quality (Draganovic et al., 2011; Samuelsen et al., 2014, 2018).

Use of the WLF equation and establishment of moisture contour plots for the effect of temperature on viscosity reduction (Fig. 3) may be a first approach for defining of the conditions required for obtaining new intra and intermolecular network formation and the desired physical properties of texturized products. More research is needed to define this operation region for mixtures of powders with different rheological properties. The two types of diagrams presented in this study may be looked upon as a type of state diagrams with applications within industry (Fig. 2) and research (Fig. 3), respectively.

3.4. Impact of physicochemical properties on the flow starting temperature

The studied food proteins represents products manufactured based on raw materials with a large span in physicochemical properties. In addition, differences in species, cultivars, and annual climatic conditions or seasonal variations might add to the variability of the respective products. The applied industrial manufacturing conditions also differ, both between and within the major groups and can be expected to influence the final properties. Fishmeal is manufactured from fish or fish by-products. The process involves heating to above 90 °C to denature the proteins followed by mechanical dewatering, oil separation, and thermal dewatering steps (Schmidtsdorff, 1995). Both type of fish species and level of water-soluble proteins have been shown to influence the thermomechanical properties (Oterhals and Samuelsen, 2015; Samuelsen et al., 2014; Samuelsen and Oterhals, 2016). Wheat gluten is removed from wheat flour by low temperature water assisted separation of the starch fraction. The recovered native gluten protein is dried using gentle conditions in order to preserve its functional properties (Lasztity, 1980). Sunflower meal is manufactured from sunflower seeds after removal of hull. The dehulled seeds are heat treated before oil extraction by use of expeller press and/or solvent (hexane) extraction (Gonzalez-Perez and Vereijken, 2007). In the latter case, the residual cake is desolventised/toasted before milling to the final sunflower meal. Soy protein concentrate is manufactured from solvent extracted soybean meal by use of an additional ethanol extraction step to remove soluble carbohydrates and other antinutritional factors (Chajuss, 2011).

A large variation in the chemical composition of the assessed food proteins was observed (Table 1). The crude protein content varied from 382 g kg^{-1} in SFM to 838 g kg^{-1} in WG. Major variance were found in the crude water soluble and insoluble protein (N x 6.25), ash, salt, starch and non-starch polysaccharide (NSP) levels, and in the amino acid composition of the crude proteins. Principal component analysis (PCA) was applied as a first approach to extract the major variance with impact on the estimated responses M_{cr}, M_m, M_{eff}, $T_{f\text{-}cr}$, $T_{f\text{-}m}$, $T_{f\text{-}eff}$ and a (Table 4). The fishmeal sample, characterized by high level of free amino acids, water-soluble protein, ash and salt (Table 1), was identified as an outlier and removed from the analysis. The PCA loading plot (Fig. 4a) depicts the association between chemical composition and estimated responses with principal component 1 (PC1), PC2 and PC3 (not shown) explaining 50%, 31%, and 7% of the variance, respectively. The score plot (Fig. 4b) showed a clear grouping of the samples with WG samples located to the upper right side, SFM samples in the lower left, and SPC samples in the upper left. Spread in scores for the individual SFM and SPC samples indicated some variability among these groups. The WG samples were associated with a high $T_{f\text{-}eff}$ (a feature in common with FM (Fig. 2a)), starch and fat content. The

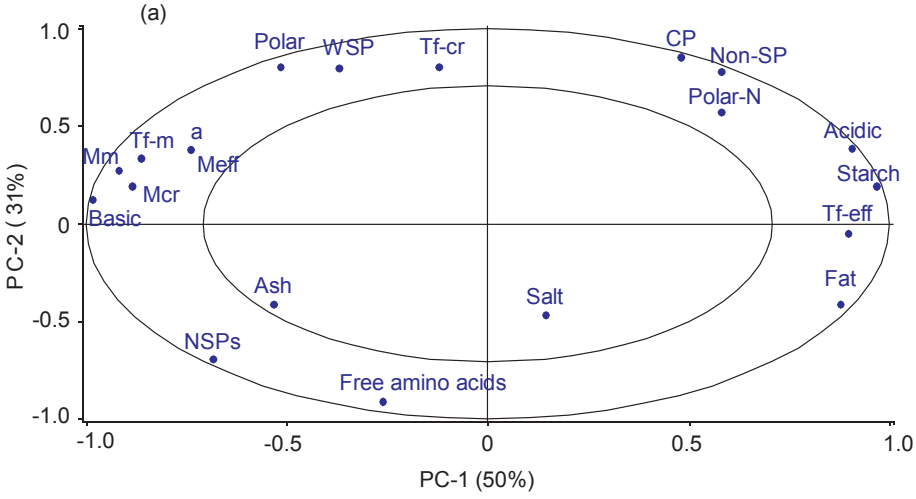

Fig. 4. (a) PCA loading plot based on independent variables and regression parameters given in Tables 1 and 4, respectively. (b) Score plot showing similarities among soy protein concentrate (SPC), sunflower meal (SFM), wheat gluten (WG) and fishmeal (FM) samples. M_{cr} - critical moisture content, M_m - maximum effective moisture content, M_{eff} - effective moisture range, $T_{f\text{-}cr}$, $T_{f\text{-}m}$ and $T_{f\text{-}eff}$ corresponding flow starting temperatures, a - constant depicting the steepness at the sigmoid curve inflection point.

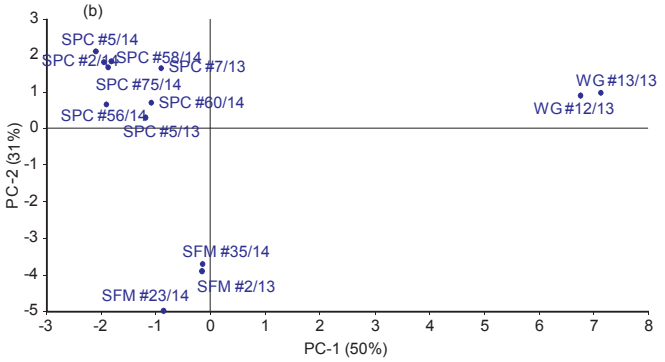

Fig. 4. (*continued*)

protein fraction was associated with higher level of amino acids with acidic and to some degree with neutral-polar side chains. WG samples also had a higher level of crude and non-soluble proteins. Among the above chemical features, the FM sample shares the high level of crude protein and fat content, however, contains higher level of ash, salt, water-soluble protein and free amino acids (Table 1). The two latter constituents have been shown to act as plasticizers and reduce the T_g and T_f in fishmeal (Ahmad et al., 2018; Oterhals and Samuelsen, 2015).

The SPC samples were associated with a high M_{cr}, M_{eff}, $T_{f\text{-}eff}$, and a value, reflecting the need for higher moisture content in the extrusion process and a less steep effect of moisture on the reduction of T_f. A high level of basic amino acids, and to some extent polar amino acids and water-soluble protein, characterized the protein fraction. The SFM samples were associated with the same response parameters along PC1, however, differentiated by a higher level of ash, non-starch polysaccharides and free amino acid level along PC2. The $T_{f\text{-}cr}$ parameter showed none association to the studied food proteins, reflecting that they shared a common T_f level at moisture levels above the sigmoidal range. The variability of ash and salt content was less explained by the PCA-model (< 50%), reflecting the low levels in the included plant proteins.

The need for less moisture to obtain a plasticized and low viscose rubbery phase in FM and WG (i.e low M_{cr}) compared to SPC and SFM samples is a favourable technofunctional property with respect to the extrusion process. The former ingredients also share a high T_{eff}. The chemical nature of these differences could be explained to a minor degree based the parameters included in this study. The results suggest that plant proteins with more fat and starch and less non-starch polysaccharides content would give favourable physical properties and more easily transform during the process at comparable moisture contents. Soy and sunflower proteins contain globular proteins while wheat gluten consists of insoluble gliadin and glutenin (Kokini et al., 1994; Sun, 2005). Globular proteins need more energy to unfold the secondary and tertiary structure before they are accessible to water or other plasticizers.

The differences in increased effect on viscosity reduction above a critical moisture level observed in this study might be linked to differences in bulk moisture sorption at high water activity (a_w) for the assessed food proteins. Food polymers normally show a sigmoid or type II moisture isotherm with a sharp and exponential increase in moisture sorption at water activity above 0.7–0.8 (Ertugay and Certel, 2000; Heinevetter et al., 1987). The increased moisture sorption reflects the formation of hydrogen bonds between water and polymers and is accompanied with swelling of the sample. When heating proteins at surplus water conditions (a_w = 1), this gives an intermediate increased viscosity followed by a reduction due to disintegration and unfolding of globular structures (Whalen et al., 1997). Water sorption isotherms of food polymers are normally reported at ambient temperature and up to a_w 0.90 due to measurement constraints. Increasing the temperature gives an isotherm shift to the right, i.e. a higher a_w at constant moisture level (Ertugay and Certel, 2000). Most of the measured T_f values in this study were above 60 °C (Fig. 2; Tables 2 and 3), corresponding to the maximum temperature level possible to use in conventional dynamic vapour sorption instruments. Only the highest moisture levels for WG and FM gave T_f levels below this threshold (Fig. 2a). Based on published moisture isotherms and taken into account possible temperature effects, the observed M_{cr} levels correspond to a_w level of approx. 0.9. Hayashi et al. (1993) have studied the influence of moisture on T_f in soy protein isolate and hypothesized that the reduced viscosity and T_f might be linked to the existing of free water at high moisture content (351–412 g kg^{-1}) acting as a lubricant. This corresponds to the highest moisture levels in this study and is associated with the upward concavity and flattening of the response curve for SPC (Fig. 2c–d). More studies are needed to evaluate any relationship between bulk moisture sorption at high a_w and temperature, and the effect on food protein hydration and reduction of T_f and viscosity in the rubbery state.

3.5. SFM and SPC variability

Sunflower meal and soy protein concentrate are perceived as relative standardized commodity products. However, some variability between the commercial samples included in this study was observed (Figs. 4 and 5). Only in case of SPC the number of samples enabled to assess this further based on PCA analysis. The loading plot revealed that M_{cr} was positive associated with the level of crude and non-soluble protein level, and negatively associated with the level of fat, free amino acids, non-starch polysaccharides and ash. $T_{f\text{-}m}$ was negatively associated with a high level of starch, basic amino acids and M_m (Fig. 5). The a value (i.e. response to moisture on T_f reduction), M_{eff}, and $T_{f\text{-}eff}$ did not show any clear association with the chemical composition. Based on the score plot, SPC #5/13 had the most favourable rheological properties (i.e. low M_{cr}) associated with a low crude and non-soluble protein level, and a high level of non-starch polysaccharides, fat, and free amino acids among the SPC samples. The sample has a deviating T_f curve compared to the other SPC samples (Fig. 2c). More studies are needed to elucidate the possible combined effect of raw material and process conditions giving these favourable properties. The individual manufacturers could be grouped to some extent based on the score plot (Fig. 5b) and confirms a variability in rheological properties both within and between the producers.

4. Conclusions

All the assessed protein ingredients followed a similar response to increased moisture level with respect to reduction of T_f, i.e. an initial

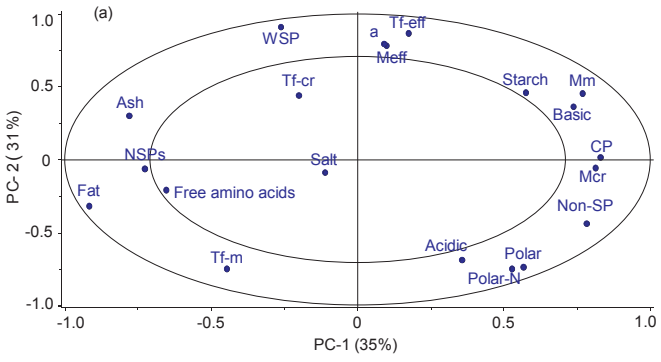

Fig. 5. (a) PCA loading plot based on independent variables and regression parameters for soy protein concentrate (SPC) samples given in Tables 1 and 4, respectively. (b) Score plot showing similarities among SPC samples. Circles are representing samples from the same supplier. M_{cr} - critical moisture content, M_m - maximum effective moisture content, M_{eff} - effective moisture range, $T_{f\text{-}cr}$, $T_{f\text{-}m}$ and $T_{f\text{-}eff}$ corresponding flow starting temperatures, a - constant depicting the steepness at the sigmoid curve inflection point.

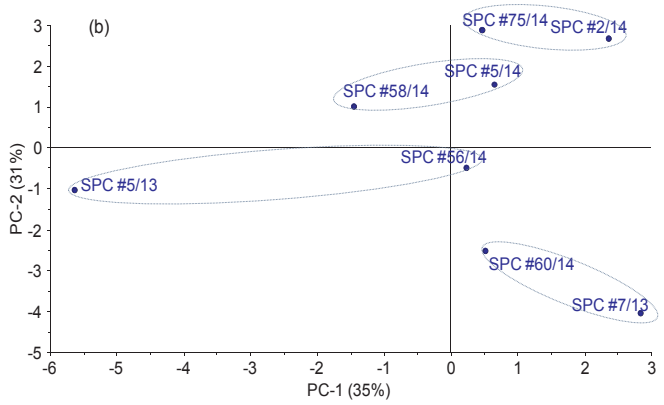

Fig. 5. (*continued*)

minor linear reduction followed by a sharp sigmoidal reduction above a critical moisture level (M_{cr}). Below M_{cr}, all proteins shared a T_f in the temperature range of 154–163 °C above T_g. FM and WG showed a lower M_{cr} (138 and 160 g kg^{-1}, respectively) compared to SFM and SPC (210–262 and 233–306 g kg^{-1}, respectively). SFM and SPC are perceived as standardized commodity ingredients, however, showed variability in the assessed properties. Compared to FM and WG, they have a higher M_{cr} and lower dynamic range with respect to viscosity reduction above M_{cr}. Combined with the WLF-equation, the T_f-models could be used to establish moisture contour plots for the effect of temperature on viscosity reduction above T_g. The observed differences in rheological properties could only partly be explained based on amino acid and proximate chemical composition. The reported approach for defining of a protein specific M_{cr} level and rheological profile will be of industrial and academic significance with respect to optimization of process conditions, and improved control in the manufacture of texturized food and feed products.

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