Amplitude Sweep Measurements in Heat-Induced Gel of Spray-Dried and Freeze-Dried Whey Protein Concentrate

Even Gausemel[1], Elling-Olav Rukke[1], Sigrid Svanborg[1,2] and Reidar Barfod Schüller[1]

[1] Dep. of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås, Norway
[2] TINE Research and Development Kalbakken, TINE SA, P.O. Box 7, N-0902 Oslo, Norway

ABSTRACT

Whey protein is known for its gel forming properties. This work employs amplitude sweep to evaluate the rheological properties of gels aggregated in a MCR 301 rheometer (Anton Paar, Graz, Austria). The aim was to detect significant differences between gels aggregated with different ingredients and process parameters. Gels where made from spray dried whey concentrate (SDWC) or freeze dried whey concentrate (FDWC).

Gels were aggregated at two different aggregation temperatures and holding times. The effects of these experimental factors on rheological responses were evaluated. LVE-range measuring points for storage modulus G’ and strain were registered for amplitude sweep after in situ aggregation. This work presents significantly different response values for storage modulus G’ and strain in the different gels. Information about differences in storage modulus G’ and strain may be of special importance for development of consumer products.

INTRODUCTION

Insight into the use of whey protein as a functional ingredient is still increasing in the dairy industry, and the effect of whey ingredients on rheological responses are of current interest in dairy research. The most common ingredients for whey protein application are whey protein concentrates (WPCs). These powder ingredients are used for several applications, one being heat induced formation of milk protein gel. The functional properties of the gels depend on ingredient composition and process parameters during aggregation. Earlier studies relate stiffness of gel to its storage modulus G’ and gumminess to its strain response[1-3]. Several studies describe storage modulus G’ responses in whey gels[1, 3-5] and in some cases also strain responses[3-5].

Storage modulus G’ increases when whey protein is denatured and forms a continuous network of proteins, crosslinking with covalent and non-covalent interactions[1, 3]. Elasticity is directly proportional to the density of crosslinking in the network where higher rate of intermolecular crosslinking increases storage modulus G’[1, 4]. Higher rate of covalent crosslinking increases response values for strain, which in whey protein is related to density of intermolecular disulphide bonds[3].

Whey protein gel is often dominated by a high number of non-covalent hydrophobic interactions. These interactions are formed early during denaturation and unfolding of whey protein and exposing of hydrophobic areas in the molecules. Covalent disulphide bonds are also formed early at denaturation phase unfolding and also affects storage modulus G’ response[1, 3, 4]. Spray dried protein ingredients have had a higher heat load during processing, which is associated with aggregation and with fewer exposable, reactive groups for covalent, intermolecular crosslinking during gel aggregation. Lower reactivity may yield lower storage modulus G’ response for gels of these ingredients[6, 7]. In this work the main objective focused on differences between gels from commercially spray dried and small scale freeze dried whey protein ingredients.

MATERIALS AND METHODS

Ingredients:

Commercial spray dried whey concentrate (WPC-80) was acquired from TINE SA Dairy (TINE SA, Verdal, Norway). Whey protein concentrates in liquid form was concentrated in the same dairy for both spray dried and freeze dried ingredients. Cheese whey was pasteurized at 72 °C for 15 seconds and concentrated by ultrafiltration to dry matter ~30 %.

The commercial spray dried whey protein ingredients was subjected to further heat treatment with preheating at 69 °C for 30-60 seconds and spray drying in air temperatures falling from 185-220 °C to ~71.5 °C in exhaust air at the TINE SA Dairy (TINE SA, Verdal, Norway). These ingredients were delivered in two sacks.

Liquid whey concentrate for freeze drying was delivered to Norwegian University of Life Sciences pilot plant in two containers. This concentrate came from the same dairy and same type of cheese making process. The liquid concentrate was stored at -20 °C before freeze drying. The liquid concentrate was freeze dried in a pilot freeze drying rig (Heto DryWinner, FD 6-85, S.nr. HE008807, Allerød, Denmark). Ingredients where produced in two batches for spray dried and freeze dried ingredients. Equal amounts of samples were based on each of these batches (n=8)

Solutions:

Protein solutions from each of the powder ingredients were prepared with 10 % total protein (TP). Total protein (TP) in powder ingredients was quantified by the use of Kjeldahl method [8, 9]. Solutions were prepared with distilled water and 40 % (wt%) 0.1 M sodium citrate buffer. The pH in solution at aggregation was 6.2.

Rheological measurements:

Protein solution was aggregated by in situ heating in the rheometer. Heating at rate of 10 °C/ min. up to either 80 °C or 90 °C and holding at this temperature for 10 or 20 minutes was followed by quick cooling to 4 °C and equilibration for 5 minutes. During heating, holding, cooling and equilibration the rheometer assessed the storage modulus G’ and loss modulus G’’. This was done by applying dynamic, small amplitude, oscillatory measurements with constant frequency (10 rad/s) and strain (0.02 %).

Due to water loss from surface at temperature increase, all samples were covered with a thin layer of sunflower oil. Due to shrinkage of gel during aggregation the normal force was set to compensate for shrinkage with constant normal force N = 0.

Storage modulus G’ and strain were measured in gels using rheometer (MCR 301, Anton Paar GmbH, Graz, Austria) with bob and cup geometry (C-CC27/T200/TI, Anton Paar GmbH; CC27/Ti Titanium probe, Anton Paar GmbH). Data was processed with Rheoplus software (Rheoplus/32 v3.40, Anton Paar GmbH)

After equilibration the gels were exposed to oscillatory amplitude sweep 0.01-100 % strain at 4 °C with constant frequency (10 rad/s). Linear ViscoElastic- range (LVE-range) measuring point was used to measure storage modulus G’ and strain at the point of quick reduction of structure integrity caused by permanent deformation. This measuring point shows strain (%) relative to gap and storage modulus G’ (Pa) at the point of increasing permanent structure deformation.

Statistical analysis:

Anova General Linear Model (GLM) in Minitab® 16.2.2. (Minitab Inc., Coventry, Great Britain) was used to evaluate the importance of the three different factors for the two response values. The model was also subjected to analysis of factor interaction by reverse elimination. The effects of experimental factors were compared with main effect plots, residual plots and R^2 values. The chosen confidence was P <0.05 for analysis of variance.

The results presented were based on a dataset of responses from 16 gels of 8 gel factor combinations. The sequence of sample evaluation was randomized. The gels may be organized into blocks by experimental factors: Ingredient (n=8), temperature (n=8) and temperature holding time (n=8). R^2 for the General Linear Model (GLM) was 95.78 for storage modulus G' and 52.87 for strain.

RESULTS

Figure 1. presents average storage modulus G' and strain for samples grouped by ingredients: Spray Dried Whey Concentrate (SDWC) (n=8) and Freeze Dried Whey Concentrate (FDWC) (n=8). Standard deviation average of standard deviations for groups: SDWC (n=8) and FDWC (n=8).

Fig 1. illustrates that the response values for Freeze-Dried Whey Concentrate (FDWC) was higher than the response values for Spray Dried Whey Concentrate (SDWC) for storage modulus G' response. The standard deviations overlap. Columns for strain responses were similar. Letter groups indicate significant difference between storage modulus responses.

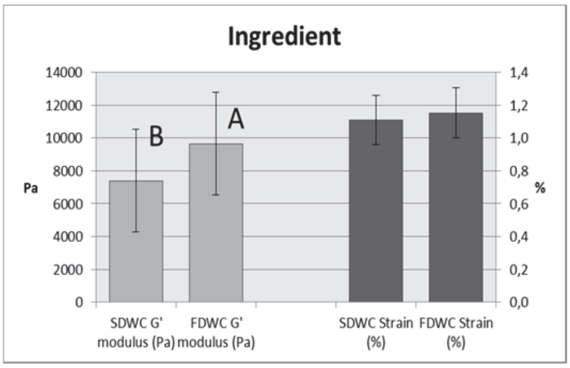

Figure 1. Average storage modulus G' and strain response values for the evaluated ingredients: Spray Dried Whey Concentrate (SDWC) and Freeze Dried Whey Concentrate (FDWC). Significant difference (p<0.05) indicated by letter groups A>B.

Figure 2. presents average storage modulus G' and strain for samples grouped by ingredient and temperature: Spray Dried Whey Concentrate (SDWC) 80 °C / 90 °C and Freeze Dried Whey Concentrate (FDWC) 80 °C / 90 °C (n=4). Standard deviation average of standard deviations for groups: 80 °C (n=8) and 90 °C (n=8).

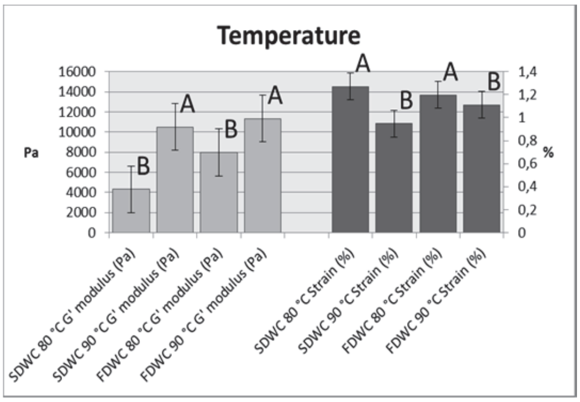

Figure 2. Average storage modulus G' and strain response values at 80 °C and 90 °C temperature separated into columns for: Spray Dried Whey Concentrate (SDWC) and Freeze Dried Whey Concentrate (FDWC) Significant differences (p<0.05) indicated by letter groups A>B

Figure 2. illustrates that the response values for 90 °C were higher than the response values for 80 °C for storage modulus G' response. Freeze Dried Whey Concentrate (FDWC) response values where

higher than Spray Dried Whey Concentrate (SDWC) response values for storage modulus G’. Opposite trend can be seen for strain response where columns for 80 °C show higher response values than columns for 90 °C. Letter groups indicate significant differences between storage modulus and strain responses.

Figure 3. presents average storage modulus G’ and strain response for samples grouped by ingredient and holding time: Spray Dried Whey Concentrate (SDWC) 10 min. / 20 min. and Freeze Dried Whey Concentrate (FDWC) 10 min. / 20 min. Standard deviation average of standard deviations for groups: 10 minutes (n=8) and 20 minutes (n=8).

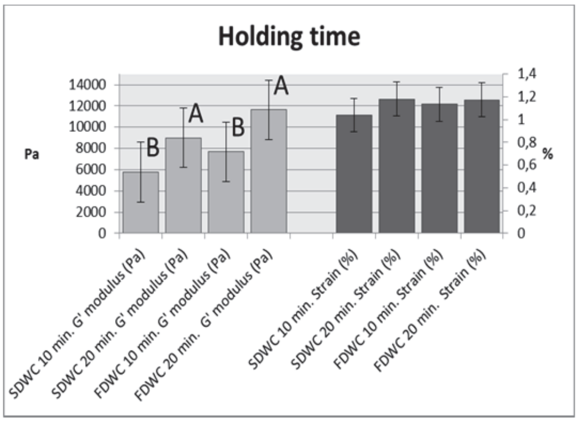

Figure 3. Average storage modulus G’ and strain response values for 10 and 20 min holding time. separated into columns for: Spray Dried Whey Concentrate (SDWC) and Freeze Dried Whey Concentrate (FDWC) Significant differences (p<0.05) indicated by letter groups A>B.

Figure 3. illustrates that the response values for 20 minutes holding time were higher than the response values for 10 minutes holding time for storage modulus G’. Columns for strain response values were similar. Letter groups indicate significant difference between storage modulus responses.

The differences seen in these column diagrams were confirmed by Anova General Linear Model (GLM) and Tukey pairwise comparison. Table 1. presents results showing that there were significant differences in storage modulus G’ responses from each of the three experimental factors and from factor interactions between ingredient and temperature factors.

Table 1. Results from Anova General Linear Model (GLM) presenting p-values for the significance of three factors and one significant factor interaction.

Significant factors and interactions		
Response	**Factor/Interaction**	**P-value**
Strain (%)	Ingredient	>0.05
	Temperature	<0.05
	Holding time	>0.05
G' (Pa)	Ingredient	<<0.05
	Temperature	<<0.05
	Holding time	<<0.05
	Ingredient + Temp.	<0.05

The average value for storage modulus G’ was significantly different with higher response values for FDWC gels aggregated at 90 °C temperature and 20 minutes holding time. We found significant effect of temperature on strain response, where 80 °C temperature had significantly higher response value.

DISCUSSION

The Freeze Dried Whey Concentrate (FDWC) produced gels with significantly higher storage modulus G’ when heat induced to aggregation at pH 6.2. This ingredient was expected to have a higher reactivity relative to spray dried ingredient because of lower heat load. Lower heat load in processing yields increased levels of covalent and non-covalent bonds and interactions forming continuous protein network. Lower heat load may consequently result in higher storage modulus G’ and a stiffer gel.

Aggregation at 90 °C temperature provided gels with significantly higher response values for storage modulus G’ relative to gels aggregated at 80 °C temperature. 20 minutes holding time at aggregation temperatures also gave significantly higher response values relative to 10 minutes holding time. As heat induced increasing amount of denaturation the exposing of hydrophobic regions also increased. Rate of hydrophobic interaction increased as the gel was subjected to higher heat load. The storage modulus G' response increased because there was an increase in crosslinking in the continuous protein network both at higher temperature and with longer holding time.

80 °C temperature provided gels with higher gel strain than 90 °C temperature. Strain is related to number of covalent bonds in the continuous protein network. The rate between covalent and non-covalent interactions is largely established early in the aggregation. Holding time is therefore less significant for the strain response value relative to temperature. 80 °C temperature induces a higher density of covalent disulphide crosslinks which gives significantly higher strain response values and a more rubbery gel. At 90 °C the denaturation and aggregation process resulted in fewer disulphide crosslinks and a high density of weaker interactions. Fewer covalent bonds results in a mobile structure where more non-covalent crosslinks may be formed.

CONCLUSION

The rheological measurements in this study showed that temperature, holding time and ingredient have significant effects on the rheological properties of whey gel.

- Freeze dried cheese whey concentrate had higher storage modulus G' response values compared to spray dried cheese whey concentrate
- Freeze dried ingredient yields stiffer gels than spray dried ingredient
- Aggregation temperatures at 90 °C gave gels with higher storage modulus G' compared to 80 °C and the longest holding time gave higher G' compared to the shortest holding time
- Higher storage modulus G' is associated with more crosslinking in continuous protein network during aggregation
- Aggregation temperature showed significant effect on strain response values, with higher strain response at 80 °C relative to 90 °C.
- Higher strain response value is associated with higher amount of covalent crosslinks in the continuous protein structure

ACKNOWLEDGMENTS

We acknowledge TINE SA for financial support and researchers at TINE SA R&D for help in the understanding of whey protein gel. We also wish to thank TINE SA for supplying whey concentrates and commercial whey ingredients for this study.

REFERENCES

1. Dissanayake, M., A.L. Kelly, and T. Vasiljevic, (2010), "Gelling properties of microparticulated whey proteins", *J Agric Food Chem*, **58**(11): p. 6825-32.

2. Dissanayake, M., S. Liyanaarachchi, and T. Vasiljevic, (2012), "Functional properties of whey proteins microparticulated at low pH", *Journal of Dairy Science*, **95**(4): p. 1667-1679.

3. Havea, P., P. Watkinson, and B. Kuhn-Sherlock, (2009), "Heat-Induced Whey Protein Gels: Protein−Protein Interactions and Functional Properties", *Journal of Agricultural and Food Chemistry*, **57**(4): p. 1506-1512.

4. Nguyen, N.H.A., M. Wong, F. Guyomarc'h, P. Havea, and S.G. Anema, (2014), "Effects of non-covalent interactions

between the milk proteins on the rheological properties of acid gels", *International Dairy Journal*, **37**(2): p. 57-63.

5. Vardhanabhuti, B., E.A. Foegeding, M.K. McGuffey, C.R. Daubert, and H.E. Swaisgood, (2001), "Gelation properties of dispersions containing polymerized and native whey protein isolate", *Food Hydrocolloids*, **15**(2): p. 165-175.

6. Heino, A.T., J.O. Uusi-Rauva, P.R. Rantamaki, and O. Tossavainen, (2007), "Functional properties of native and cheese whey protein concentrate powders", *International Journal of Dairy Technology*, **60**(4): p. 277-285.

7. Purwanti, N., M. Smiddy, A. Jan van der Goot, R. de Vries, A. Alting, and R. Boom, (2011), "Modulation of rheological properties by heat-induced aggregation of whey protein solution", *Food Hydrocolloids*, **25**(6): p. 1482-1489.

8. IDF, *Determination of nitrogen content Part 1: Kjeldahl method.*, in *20-1: Milk: 2001*. 2001, International Dairy Federation: Brüssel, Belgium.

9. IDF, *20-5 Milk - Determination of nitrogen content - Part 5: Determination of protein-nitrogen content.*, in *20-5: 2005*. 2001, International Dairy Federation: Brüssel, Belgium.