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TEXTURE AS A REFLECTION OF MICROSTRUCTURE

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ABSTRACT

The perception of texture has been correlated to the microstructure of particulate whey protein gels. A full, two-level, factorial experimental design was used in which the processing conditions, pH, heating rate and addition of salt were used as design factors. The texture of the gels was analysed by a sensory panel, and the microstructure was analysed by light and electron microscopy. The microstructure was quantified by using different types of image analysis.

In this study of particulate whey protein gels, the test principles of analysing texture were divided into two groups: destructive tests and non-destructive tests. The microstructural parameters can also be divided into two groups: overall network dimensions (pore size and particle size) and strand characteristics. The texture as measured with destructive methods was sensitive to overall network dimensions, whereas texture as measured with non-destructive methods was sensitive to the strand characteristics of particulate protein gels. Copyright © 1996 Elsevier Science Ltd

Keywords: texture; microstructure; sensory–microstructure relations; particulate protein gels.

INTRODUCTION

Flavour and texture influence the acceptance of a food product. Translating the sensation of texture into structural properties that can be measured with instruments can provide vital information on the perception of texture. The microstructure of particulate gels can explain texture as measured instrumentally (Stading *et al.*, 1993). Our aim in this paper is to test whether the microstructure of particulate gels can also be correlated with texture, as perceived sensorily. The knowledge about the relationship between microstructure and texture can be used to optimise food production processes as well as to develop new products with the desired sensory quality.

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The microstructure of particulate gels can be described as more or less spherical particles joined together forming strands in a three-dimensional network. The particulate network of a milk protein gel is illustrated in Fig. 1, which shows a micrograph taken under a scanning electron microscope, (SEM). In SEM a fracture surface is visualised, which gives a three-dimensional impression. The particles can join together in different strand formations, thus forming bunches, clusters, conglomerates or strings-of-beads. Figure 2 shows a schematic drawing of a) a regular network formed of strings of beads and b) an irregular network of clusters. The textural properties are affected by the strand formation as well as the particle and pore size. Both particle and pore size can be estimated on sections of the sample. The vast difference in size between particles and pores makes it necessary to use different magnifications during analysis. The small particles, of around one micron, were imaged by using transmission electron microscopy (TEM) while the larger pores were analysed on light micrographs, LM, at a lower magnification. In this study, whey protein gels were used as the model system. Whey protein forms particulate non-transparent gels when heated around the isoelectrical point. The microstructure of particulate whey protein gels is dependent on pH, heating rate, salt addition, etc. (Harwalker & Kaláb, 1985*a*,*b*; Hermansson, 1986; Mulvihill & Kinsella, 1988; Mulvihill *et al.*, 1990; Langton & Hermansson, 1992; Renard & Lefebvre, 1992). In our experiments, pH, heating rate and salt addition were used as design variables in a full, two-level, factorial design.

The perception of texture is sensitive to the test principle used (Bourne, 1983). This means that using different instruments, a fork, spoon or knife, as well as variable amounts of force during examination has an impact on the analysed texture descriptor. When making correlations with other parameters, we need to realise that not only sensorial parameters but also rheological and microstructural parameters can be measured differently. When the relationship is unknown, it is necessary to widen the space described by sensorial descriptors and microstructural parameters as much as possible. While it is essential to vary the samples used as much as possible, they should still resemble each other, i.e. samples should form the same type of product.

Previous work correlating microstructure with the perception of texture has been done on, for example, commercial products. The morphology of the fat was shown to influence the texture of different commercial cheese products (Kaláb *et al.*, 1981; Marshall, 1990). Crippen *et al.* (1989) used a full, 3-level factorial design to find out the effect of grind size, sucrose concentration and salt concentration on peanut butter texture. They found that an increase in grind size decreased sensory smoothness, spreadability, adhesiveness and preference rating, but increased instrumental hardness. The texture was quantified both by a consumer panel and instrumentally. At present, there seem to be few published results showing good correlation between microstructure and perceived texture. One reason could be that many researchers have used commercial products, instead of using a controlled experimental design.

This paper will present how the perceived texture of whey protein gels is correlated with and may be predicted by the microstructure.

MATERIALS AND METHODS

Material

Whey protein powder purchased from Denmark Protein A/S, as specified in Langton, 1995 and Langton & Hermansson, 1996 were used to form 13.5% gels. The whey protein powder was dissolved in distilled water or 0.1 mol/dm^3 NaCl and adjusted to pH 4.6 or 5.4. The gels were then set while heating at a controlled rate (1°C/min or 5°C/min) and kept at 90°C for 60 minutes. The samples were cooled and stored overnight (7°C) before examination.

Experimental design and statistics

The samples were prepared by using a full, two-level, factorial design, where pH, salt content and heating rate were used as design factors (Box *et al.*, 1978; Carlson, 1992). The design cube is illustrated in Fig. 3. The design generated eight samples, and all eight samples were produced and analysed three times.

The results were evaluated by using a response surface model, and analysis of variance (MANOVA) was performed by using the SYSTAT software package. Multivariate techniques were used to create models to describe groups of the sensory descriptors by some of the microstructural parameters, using the UNSCRAMBLE software package.

Sensory analysis

The sensory evaluation was performed by eight specially selected and trained panellists from SIK's external panel, who developed a partial sensory profile using Quantitative Descriptive Analysis (Langton *et al.*, 1996). The gels were assessed using standard procedure for test design (Amerine *et al.*, 1965; Meilgaard *et al.*, 1987). The descriptive test included several training sessions, during which the sensory panel identified and described the most appropriate attributes by evaluating repre-

FIG. 1. SEM micrograph of a particulate milk protein gel.

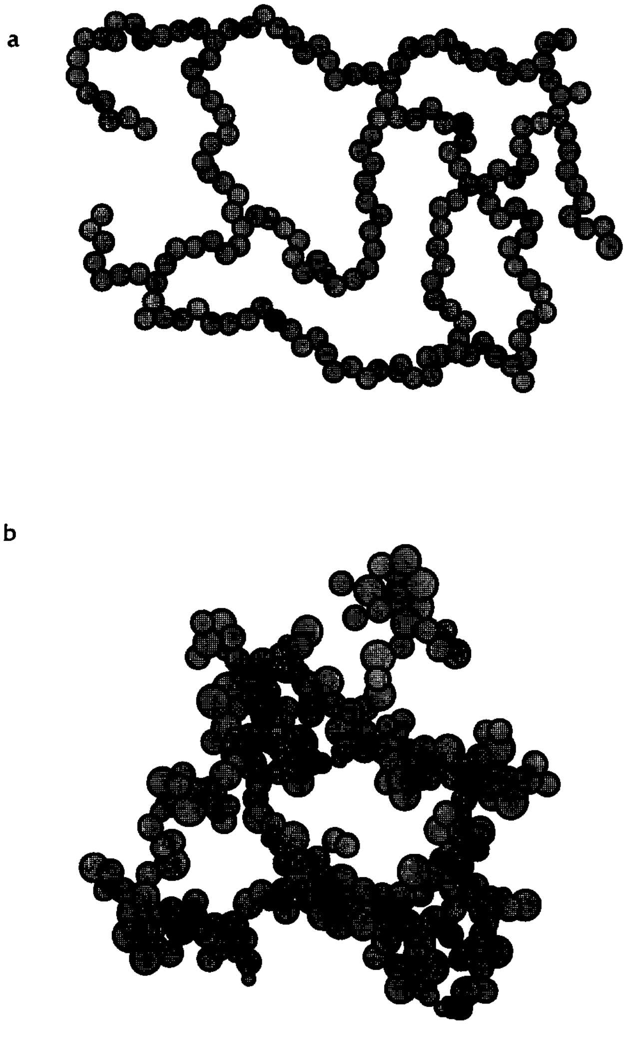

FIG. 2. Schematic illustration of strand characteristics: a) strings of beads and b) clusters, modified from Langton 1995.

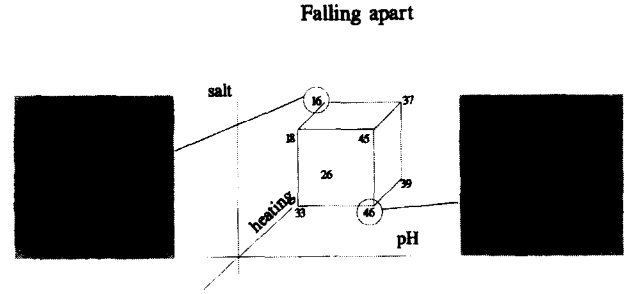

FIG. 3. The tendency to fall apart as related to experimental conditions, and thus the microstructure formed under various conditions. The corners of the cube are marked with the mean values of 'falling apart'. The cube represents the two-level factorial experimental design, with the design factors pH, heating rate and salt addition. The levels chosen were pH 4.6 and pH 5.4, no added salt and an addition of 0.1 mol/dm^3, and heating rates of 1°C/min and 5°C/min.

sentative whey protein gels. Assessors developed descriptors individually and took part in a round-table discussion to reach a consensus. During the training sessions the panel also discussed in what way the samples should be evaluated, and a standard procedure was developed. The sensory characterisation of the whey protein gels was focused on the properties related to consistency (see Table 1) though the perception of acid and salty taste was also included in the assessment of the products. The descriptors were evaluated in the same order as in Table 1. Each descriptor was evaluated by marking a 90 mm unstructured scale anchored 10 mm from each end with the terms 'slight' and 'pronounced'. Samples were served at room temperature in disposable plastic cups coded with three-digit random numbers. All gels were presented to the panel in fully randomised and balanced order. The profiling data were collected in three replicate judgements from each assessor.

Microstructure and image analysis

The microstructure was characterised at different microstructural levels using different microscopy techniques as well as different magnifications. Light microscopy (LM) was used to visualise the pores, while transmission electron microscopy (TEM) was used to evaluate the particle size. Scanning electron microscopy (SEM) was used to detect how the particles were linked together, as strings or in bunches and conglomerates. All eight samples were analysed by LM, TEM and SEM. The samples were first double-fixed in 2% v/v glutaraldehyde with 0.1% w/v ruthenium red, rinsed, and secondly fixed in 2% w/v OsO_4. The samples were then dehydrated in a graded ethanol series.

LM samples were embedded in Technovit 7100. Semi-thin sections, ~1 µm, were cut on glass-knife, stained with toluidine blue and examined under a Nikon microphoto fxa or fx, light microscope.

After dehydration TEM samples were transferred to propylene oxide and then embedded in Polybed 812. Around 80 nm thin-sections were cut on a diamond-knife. Sections were double-stained with uranyl acetate and lead citrate, and examined in a TEM, 100 cx II Jeol at an acceleration voltage of 80 kV.

The particle size was estimated by analysing 256 TEM images, and 144 (= 2 × 72) LM-images were used to estimate the pore size at two magnifications. (20× and 40×). The pore size and particle size were estimated by stereological methods. The star volume (V*) is an estimate of the volume-weighted mean volume and was used as an estimate of the size. It was estimated unbiasedly as

$$v* = \frac{\pi}{3} \bar{l}_0^{\,-3} \qquad (1)$$

where l_0 is the line intercept length and the average is $\bar{l}_0^{\,-3}$ (Cruz-Orive, 1976; Russ, 1990; Gundersen *et al.*, 1988).

Estimates were made on isotropic, uniform sections using arbitrary directions independent of the particles on the sections. This was done by using five lines parallel to one of the delineations of the independently positioned field of vision. The measuring probe (five horizontal lines) was laid on the binary image thus forming the line intercept length l_0.

SEM samples were critical point-dried through CO_2. Dried samples were fractured, and the fractured surface was coated with Au/Pd by diode sputter coating. The

TABLE 1. Descriptors used for sensory profiling of whey protein gels, modified from Langton *et al.* 1996

Attributes	Descriptors	Explanation
Manual Texture	Soft	how much the sample resists light pressure with forefinger
(touching) measured by a light pressure with forefinger	Springy	how rapidly the sample regains its initial shape after light pressure with the forefinger
Visual Texture	Surface moisture	how much water is released from the newly cut surface
(visualisation) observations of a newly cut surface	Grainy appearance	how grainy the newly cut surface appears
Oral Texture (mouthfeel)	Gritty	how gritty the sample is perceived during chewing
perception during the manipulation of	Creamy	how creamy and smooth the sample is perceived during chewing
the sample in the mouth	Sticky	how much the sample adheres to the teeth after chewing
	Falling apart	how easily the sample falls apart or disintegrates during chewing

samples were analysed in an SEM (Leica) at acceleration voltages between 1 and 5 kV.

The features in the SEM images were evaluated by a group of eight experienced microscopists, who assessed the microstructural parameters using a visual analogue scale. The microstructural panel formulated appropriate descriptors by evaluating relevant SEM images during several training sessions. The panellists evaluated five descriptors, porosity, clusters (many particles attached to each other like bunches of grapes), conglomerates (as if particles were joined together in non-linear, irregular, inhomogeneous order), strings of beads (as if the particles were attached to each other in linear order forming strings of beads) and hairiness (as if small threads were attached to the surface of the particles and their outline is indistinct.). The eight microscopists were also trained to use the five descriptors on an unstructured 90 mm line scale labelled 10 mm from each end with the terms 'slight' and 'pronounced'.

Three separate occasions were used for the final judgement, when all micrographs of the gels were presented to the microstructural panel in randomised and balanced order. All micrographs were coded with a three-digit random number. Each sample was evaluated three times. All eight samples were evaluated at each session.

RESULTS AND DISCUSSION

Perception of texture

Figure 3 shows how the preparation conditions pH, salt and heating rate affect the sensory descriptor 'falling apart' and how this is related to microstructure, i.e. the pore size. The use of an experimental design facilitates the creation of various microstructures, which can, in turn, affect the desired sensory quality.

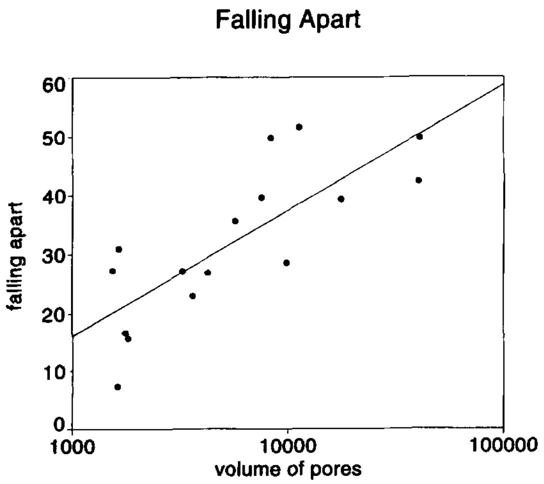

FIG. 4. The perceived tendency to fall apart of 13.5% whey protein gels as a function of the estimated star volume of pores in μm^3. Langton *et al.* 1996.

A response surface model was used to evaluate the effects of preparation conditions. Main effects and interaction effects were included in the model. Table 2 summarises the measured parameters, both the microstructural and the sensorial ones. The large β_{pH}-coefficients show that the pH has a large impact on the pore and particle size, as well as on the perception of grainy, gritty, falling apart and creamy.

The perception of texture is sensitive to the test principle used. Thus, the various attributes and descriptors are influenced by specific microstructural parameters. When the texture of particulate whey protein gels was evaluated, the two groups of attributes found were seen to be connected with the amount of force used during analysis. The first group is related to the use of larger forces during examination (large deformations) while the second group is related to the use of less force during examination (small deformations). This is also in agreement with instrumental measurement (Stading *et al.*, 1993), where the differences between non-destructive small deformations and large deformations are even more pronounced.

Large deformations, destructive tests

The first group was formed of descriptors connected with the use of considerable force during analysis, namely, cutting or chewing. This group consisted of four descriptors: grainy appearance, falling apart, gritty and creamy texture. The first three were positively related to each other, and the last one, creamy, was negatively related to the others. This group was sensitive to the variation in the overall particulate network dimensions, such as the pore and particle size. The sample which had a grainy appearance also had a gritty texture, a high tendency to fall apart and was less creamy. The perception of texture was explained by the microstructure, i.e. these samples had large pores and large particles. A logarithmic dependence was found between the microstructural parameters — volume of small and large pores and volume of particles — and the sensory parameters — grainy appearance, gritty, creamy and the degree of falling apart. As an example, the relationship between the perceived texture and the microstructural parameters is given below in Table 3. Figure 4 shows how the perception of falling apart is dependent on pore size. The logarithmic dependence indicates that the human being's perception of textural properties is very sensitive to fine deviations in small particles. The size of pores and particles was estimated by a mean volume and, assuming a spherical shape, the diameters of the pores were found to vary between 10 and 40 microns and of the particles between 0.8 and 1.4 microns.

The perception of falling apart, as well as gritty, grainy and creamy, might not depend on one microstructual parameter, but on combinations of microstructural parameters. In the case of particulate whey protein gels the pore size

TABLE 2. The β-coefficients in the response surface model for the microstructural parameters and sensory descriptors ***$p \leq 0.001$; **$p \leq 0.01$; *$p \leq 0.05$SE is the standard error of coefficients and s is the sample standard deviation, modified from Langton and Hermansson 1996 and Langton *et al.* 1996

		mean value β_0	main effect			interaction effect				SE	s
			β_{heat}	β_{salt}	β_{pH}	$\beta_{heat*salt}$	$\beta_{heat*pH}$	$\beta_{salt*pH}$	$\beta_{heat*salt*pH}$		
Digital image analysis	pore size 20×	8200***	−3700*	1800?	6300***		−4000*			1484	7270
	pore size 40×	2800***		−650*	1800***					290	1422
	particle size	0.76***	−0.087*		0.34***					0.036	0.144
	amount of threads	0.71***		0.35*					−0.41*	0.13	0.52
Mode of aggregation	porosity	45.5***	−4.3?	6.6**	13.2***		−4.6*			2.1	10.2
	clusters	44.1***	−4.4**	9.0***	9.3***		−3.1*		3.5*	1.3	6.6
	conglomerates	43.5***		7.8***			−5.4**	−4.2*		1.7	8.4
	string of beads	38.2***		−11.5***						1.7	8.2
	hairiness	38.4***		4.2*	−4.4*		−4.1*	−5.2**		1.7	8.2
Sensory descriptors	soft	38.3***	6.1*	9.2**				−9.2**		2.8	13.5
	springy	37.0***	−3.4*	−8.3***				6.2**		1.8	8.8
	grainy appearance	31.7***	−5.4**		6.8***			4.6**		1.6	7.7
	gritty	32.7***	−4.3**	−3.7**	14.9***			5.2***		1.2	6.0
	creamy	33.7***	5.1*	8.2***	−11.7***			−6.9**		2.0	9.7
	falling apart	32.9***	−3.1*	−3.5*	9.5***			2.9*		1.4	6.7

TABLE 3. The non-linear correlation between microstructure and sensory quality as described by Y= A + B *log(X), *n* = 16, modified from Langton *et al.* 1996

Y	X	A	B	r^2
gritty	star volume of particles	43	29	0.95
gritty	star volume of large pores	−85	13	0.91
falling apart	star volume of particles	40	19	0.96
falling apart	star volume of large pores	−48	9	0.95
creamy	star volume of particles	23	−28	0.91
creamy	star volume of large pores	· 139	−12	0.86

and the particle size were affected by the same sample preparation conditions; thus, the samples with the large pores also contained the large particles. A multivariate PLS model using three microstructural parameters, particle size, and pore size of small and large pores, gave a model with an overall correlation $R^2 \sim 0.9$.

Small deformations, non-destructive tests

The second group of descriptors was connected with the use of less force during analysis, i.e. light pressure with the forefinger. This second group of sensory descriptors consisted of two descriptors, soft and springy, which were negatively correlated. The perception of soft and springy was related to the strand characteristics of particulate gels. Figure 5 shows the loading plot of the PLS model used for correlating soft and springy texture to the microstructural parameters. The first component portrays the largest variation. The loadings of the variables which are plotted close to soft and springy, respectively, are

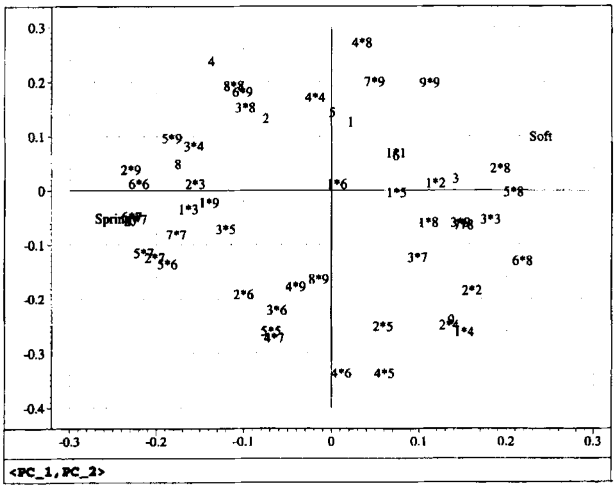

FIG. 5. Loading plot of the PLS-model used for correlating soft and springy texture to the microstructural parameters of whey protein gels. The loadings of the first and second PLS components are plotted against each other. The microstructure parameters have been assigned numbers as follows: 1) mean volume of large pores, 20×, 2) mean volume of particles, 3) proportions of threads within the pores, 4) mean volume of small pores 40×, 5) porosity, 6) clusters, 7) conglomerates 8) string of beads and 9) hairiness (Langton *et al.*, 1996).

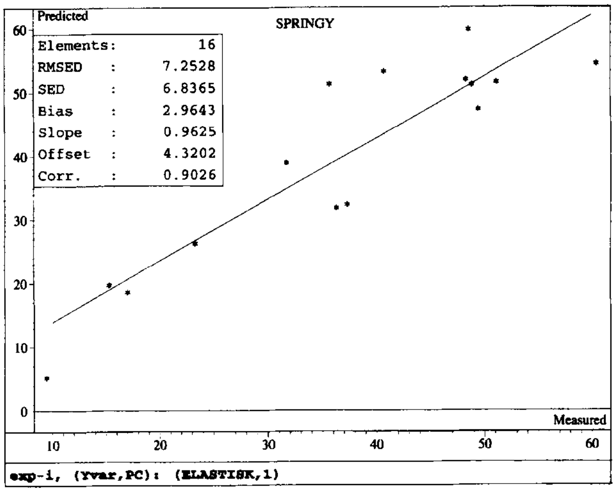

FIG. 6. The same PLS model as used in Fig. 5 was here used to predict the perceived springy texture. One replicate has been used to form the model and the other to predict. The measured values are plotted against the predicted values (Langton *et al.*, 1996).

correlated with these variables. The loading plot showed that one variable, string of beads, is located close to springy, and combinations including the terms string of beads, conglomerates and clusters also seem to have an impact on the soft and springy texture. Figure 6 shows that the PLS model could be used to predict springy texture from the microstructural variables with a correlation coefficient of 0.90. Gels formed of many strands in the shape of strings of beads seemed to be more springy. In contrast, it seems that gels formed of particles which were associated in clusters or in conglomerates were perceived as soft. To fully explain the soft and springy texture by microstructural parameters, a more complex relationship seemed to be needed. Many microstructural parameters, as well as interaction between microstructural parameters, had an impact on the perceived springy and soft texture.

CONCLUSIONS

When making correlations between microstructure and texture, it is vital to analyse the appropriate properties of both the microstructure and the texture. For example, when analysing particulate whey protein gels, we can divide the test principles of analysing texture into two groups: destructive tests and non-destructive tests. The microstructural parameters can also be divided into two groups: overall network dimensions (pore size and particle size) and strand characteristics. The texture of particulate gels as measured with destructive methods was sensitive to overall network dimensions, whereas texture evaluated with non-destructive methods was sensitive to the strand characteristics of particulate protein gels.

REFERENCES

Amerine, M. A., Pangborn, R. M. & Roessler, E. B. (1965) *Principles of Sensory Evaluation of Food.* Academic Press, New York, USA.

Bourne, M. C. (1983) Chapter 3.3: Correlating instrumental measurements with sensory evaluation of texture. In *Sensory quality in foods and beverages: definition, measurement and control,* ed. A. A. Williams & R. K. Atkin. Chichester, West-Sussex, UK, Ellis Horwood Ltd., ISBN 0-85312-480-9, pp. 55–173.

Box, G. E. P., Hunter, W. G. & Hunter, J. S. (1978) Statistics for Experimenters; An Introduction to Design, Data Analysis, and Model Building, John Wiley and Sons, Inc, ISBN 0-471-09315-7.

Carlson, R. (1992) *Design and optimisation in organic synthesis,* Advisory eds. B. G. M. Vandeginste & O. M. Kvalheim, Elsevier Amsterdam, ISBN 0-444-89201-X.

Crippen, K. L., Hamann, D. D. & Young, C. T. (1989). *Journal of Textural Studies,* **20**, 29.

Cruz-Orive, L.-M. (1976). Particle size-shape distributions: the general spheroid problem. *Journal of Microscopy,* **107**, 235.

Gundersen, H. J. G., Bendtsen, T. F., Korbo, L., Marcussen, N., Moller, A., Nielsen, K., Nyengaard, J. R., Pakkenberg, B., Sörensen, F. B.Vesterby, A. & West, M. J. (1988). Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. *APMIS,* **96**, 379–394.

Harwalker, V. R. & Kaláb, M. (1985*a*). *Milchwissenschaft,* **40**(2), 65.

Harwalker, V. R. & Kaláb, M. (1985*b*). *Milchwissenschaft,* **40**(11), 665.

Hermansson, A.-M. (1986) *Functional Properties of Food Macromolecules,* ed. J. R. Mitchell & D. A. Ledward, Elsevier Applied Science, London and New York, Chapter Water and fatholdning, pp. 273–314.

Kaláb, M., Sargent, A. G. and Froechlich, D.A. (1981) *Scanning Electron Microscopy* III, pp. 473–482, +514.

Langton, M. (1995) Correlating Microstructure with Texture of Particulate Biopolymer Gels, Thesis, Chalmers University of Technology, Göteborg, Sweden.

Langton, M. & Hermansson, A.-M. (1992). *Food Hydrocolloids,* **5**, 523.

Langton, M. & Hermansson, A.-M. (1996) Image analysis of particulate whey protein gels, *Food Hydrocolloids,* **10**(2).

Langton, M., Åström, A. & Hermansson, A.-M. (1995). Influence of the microstructure on sensory quality of whey protein gels. Accepted for publication in Food Hydrocolloids.

Marshall, R. J. (1990) Combined instrumental and sensory measurement of the role of fat in food texture. *Food Quality and Preference,* 2, pp. 117–124.

Meilgaard, M., Civille, G. V. & Carr, T. B. (1987) *Sensory Evaluation Techniques.* CRC Press, Boca Raton, Fl.

Mulvihill, D. M. & Kinsella, J. E. (1988). *Journal of Food Science,* **53**, 231.

Mulvihill, D. M., Rector, D. & Kinsella, J. E. (1990). *Food Hydrocolloids,* **4**, 231.

Renard, D. & Lefebvre, J. (1992). *Int. J. Macromol.,* **14**, 287.

Russ, J. C. (1990) *Computer-Assisted Microscopy: The Measurements and Analysis of Images.* ISBN 0-306-43410-5 Plenum Press, New York.

Stading, M., Langton, M. & Hermansson, A.-M. (1993). Microstructure and rheological behaviour of particulate β-lactoglobulin gels. *Food Hydrocolloids,* **7**(3), 195–212.