Food Structure

Volume 6 | Number 1

Article 6

1987

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Ruegg, M. and Moor, U. (1987) "The Size Distribution and Shape of Curd Granules in Traditional Swiss Hard and Semi-Hard Cheeses," *Food Structure*: Vol. 6 : No. 1, Article 6. Available at: https://digitalcommons.usu.edu/foodmicrostructure/vol6/iss1/6

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FOOD MICROSTRUCTURE, Vol. 6 (1987), pp. 35-46 Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0730-5419/87\$3.00+.00

THE SIZE DISTRIBUTION AND SHAPE OF CURD GRANULES IN TRADITIONAL SWISS HARD AND SEMI-HARD CHEESES

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Abstract

Curd granule junction patterns in hard (Emmentaler, Gruyere, Sbrinz) and semi-hard cheeses (Appenzeller, Tilsiter, Raclette) were visualized on slices and examined using light microscopy and digital image analysis. Horizontal and vertical sections were cut in different zones of the loaves, in order to obtain information on the orientation of the flattened curd granules.

The frequency histograms of the cross section areas could in most cases adequately be described as a log-normal distribution. The median values ranged from 0.97 to 1.15 mm² and, from 1.31 to 1.68 mm² for hard and semi-hard cheeses, respectively.

An elliptical form factor was used as a measure of the deformation of the granules. The average ratio of the elliptical axes was in the range of 0.41 to 0.56 in horizontal and 0.33 to 0.48 in vertical sections. The difference between the form factors in the orthogonal sections was less pronounced in the Appenzeller and Tilsiter cheeses than in the other varieties. Significantly different junction patterns were observed in regions of the edges and sides of the original billets of curd. The micrographs revealed interesting features around the eves and in the cheese rind.

Semi-mechanized and traditionally manufactured Appenzeller and Tilsiter cheeses had different curd granule junction patterns, mainly because of different moulding and pressing arrangements.

Initial paper received December 12, 1986 Manuscript received March 26, 1987 Direct inquiries to M.W. Ruegg Telephone number: 41-31-598 166

<u>Key words:</u> Cheese, curd granules, light microscopy, digital image analysis, size distribution, sample preparation technique, microstructure.

Introduction

The importance of both the size and uniform size distribution of curd granules for cheese quality has always been emphasized, in old and modern text books on cheese (e.g., Steinegger, 1904; Fleischmann and Weigmann, 1932; Mair-Waldburg et al., 1974; Scott, 1981). The approximate size of the granules is given in the recipe of each cheese variety. It depends on the way in which the coagulum is cut and on the subsequent thermal and mechanical treatment in the vat and press. Stirring and heating causes the protein matrix to shrink and expel whey (syneresis). Syneresis continues to some extent during pressing. Several studies assessed the significance of the size of the granules and the curd dust for factors such as syneresis (Kammerlehner, 1974), eye formation (Clark, 1918; Koestler, 1933; Hostettler, 1943; Schulz, 1953; Bolliger and Burkhalter, 1957; Flüeler and Kaufmann, 1985), cheese yield (Schwarz and Mumm, 1951; Bolliger and Burkhalter, 1957), moisture, fat content and acidification (Koestler, 1933; Bolliger and Burkhalter, 1957; Flüeler and Kaufmann, 1985). The separation of differently sized particles before pressing and its influence on the qua-lity of Emmental cheese has been studied by Dorner and Ritter (1942) and Bolliger and Burkhalter (1957). Bohac (1970) used frozen sections to study the orientation of the flattened curd granules in hard cheese after pressing. The grain boundaries in curd and cheese. which are known to have a low fat content (King, 1958; Mulder et al., 1966; Hansson et al., 1966), have been studied by various authors and with different techniques including microscopy of frozen or embedded thin sections (e.g., review by Heinrich, 1968; Fricker and Meyer, 1960), sanded preparations of dehydrated slices (Kalab, 1977; Kalab et al., 1982; Rüegg et al., 1985) and electron microscopy (Fricker and Meyer, 1960; Hostettler, 1961; Annibaldi and Nanni, 1979; Rüegg et al., 1980). Effects of various manufacturing processes and equipment on junction patterns in cheddar cheese have been investigated by Emmons et al. (1980), Kalab et al. (1982) and Lowrie et al. (1982).

With very few exceptions, the work on size

and size distribution conducted in the past was of a qualitative nature. Very few quantitative data on the shape and size of granules after cutting or within the cheese body after pressing are available. Dorner and Ritter (1942) most probably were the first who studied systematically the size and shape of curd granules throughout loaves of Emmental cheeses. They took into account the elongation of the curd granules after pressing and measured the diameters on differently oriented cross sections (horizontal, vertical, 45° angle). The small size of frozen sections and the manual measurements permitted the collection of only a limited number of data. Using techniques similar to those introduced by Kalab (1977) and Kalab et al. (1982) together with digital image analyzers, greater surface areas can be observed and the acquisition of a large number of data for stereological and statistical analyses becomes possible (Rüegg et al., 1985).

In the present work, the size-distribution, shape and orientation of curd granules in some important Swiss hard- and semi-hard type cheeses were examined by means of light microscopy and digital image analyses. The primary aim was to obtain reference data for normal first quality cheeses and to investigate the extent to which these were affected by different manufacturing equipment. The micrographs also revealed useful information about the fine structure around the eyes and in the cheese rind.

Materials and Methods

Cheese samples

Mature Emmentaler, Gruyere, Sbrinz, Appen-zeller, Tilsiter and Raclette cheeses were obtained directly from different Swiss factories. Raclette cheese was produced from pasteur-ized milk. The other cheese varieties were manufactured traditionally from raw milk according to procedures described in various textbooks (Muggli et al., 1959, Peter and Zollikofer, 1966, Mair-Waldburg et al., 1974, Steffen et al., 1987). The size distribution of the freshly cut curd particles was as described in standard recipes and varied between approxiwheat grains (2-4 mm; e.g., Mair-Waldburg et al., 1974). Loaves of each variety were purchased from 5 to 8 different manufacturers and samples were taken from the outer and central zone as indicated in Fig. 1. Vertical (nr. 1 to 6) and horizontal sections (nr. 7 to 12) were cut to obtain information about the orientation of the curd granules inside the cheese body. The curds of Appenzeller, Tilsiter and Raclette cheeses were prepressed in rectangular blocks before filling into the regular round hoops. Sections were therefore taken in these cheeses in the region of the original edge and side part of the billets of curd as shown in Fig. 2. For survey purposes near the rind, slabs were cut through the whole loaf near the hoop side and prepared for microscopical observation as described in the next paragraph. Typical diameters and heights of cheese loaves were: Emmentaler 85/20, Gruyere 55/12, Sbrinz 55/15, Tilsiter 25/7, Appenzeller 33/9 and Raclette 33/7 cm.







Fig. 2. Sampling zones for semi-hard cheeses in the region of the former edge (e) and side (s) part of the billets of curd.

Preparation of specimens and microscopy

A procedure similar to that proposed by Kalab et al. (1982) was used. The horizontal and vertical sections, $35 \times 25 \times 2 \text{ mm}$, were fixed, stained, dehydrated and defatted successively with the following solutions and solvents:

- glutaraldehyde/acrolein (6%, 3%), 2-4 d at 5° C
- citrate/phosphate buffer pH = 5.4 (0.005, 0.011 mol/1), 2 x 30 min - ethanol (95%), 2 x 1h

- methyleneblue (0.05% in ethanol), 5-10 min

- ethanol (95%), 2 x 30 min - diethylether, 2 x 4h

- n-hexane, 2 x 2 h

The solutions were stirred during each treatment. Overlapping of the sections was prevented by means of specially developed glass holders.

The prepared sections were dried at room temperature overnight between filter paper and glass plates to prevent deformation. One surface was finally sanded with carborundum paper. grade P320, using a sanding disk rotated by an electric stirrer (Heidolph, model 741.00, Kelheim, West Germany). Cheese samples shrunk by about 10% in all directions after drying. A mean factor was determined for each variety together with the magnification factor as described below.

Sections were photographed using a Wild-Leitz Fotomakroskop M400, equipped with a reducing lens (0.5 times) and an automatic 35-mm camera MPS55 (Wild-Leitz AG, Heerbrugg, Switzerland). Illumination was from one side at an angle of 30° by means of a low voltage microscopy lamp. Final magnification on the prints used for image analysis was 4.5-5.0 times. The exact magnification was determined for each variety by measuring cheese samples of known original dimensions on the final prints. The effect of shrinkage was thus included.

Digital image analysis

The visualized curd granule junctions were traced on the digitizer tablet of a Mop-Videoplan image analysis system (Kontron Bildanalyse GmbH, München, West-Germany). About 100 connected granules were measured on each photomicrograph. If granules were completely folded to spherical or elliptical particles only the outer contours were traced. The system was programmed to calculate the area (A), the major and minor diameters (a,b), the elliptical form factor or elongation factor (b/a) and the center of mass coordinates. To approximate the axis a and b the data acquisition program calculated an ellipse with the same moment of inertia as the traced structure.

The non-parametric U-test as available in the standard Mop-Videoplan statistical software was applied for testing the significance of differences between the measured distributions within a cheese and between different cheeses. Because of the asymmetry of the distributions of most of the measured parameters the medianvalue (50%-value) and the interguartil-range (lower and upper 25% values) were used to characterize the data. The shape of the distribution curves was compared with Gauss and log-normal distributions by means of the Kolmogoroff-Smirnow-test included in the Mop-Videoplan program for particle size analysis (TGA program).

The estimation of the number, size and shape of particles by using information only from two-dimensional sections is a well known problem (Weibel, 1979). The section profile distribution is more or less distorted when

compared to the true particle size distribution. Only under the strict assumption that all particles have the same known simple shape it is possible to correct the biased distribution of cross-sections and to estimate precisely, the particle number and size (Wicksell, 1925). The apparent diameters are usually smaller than the true diameters and random sections actually contain a relatively greater number of large particles than small ones. The two causes work in opposite directions thus having a chance to balance each other. The average curd granule diameters derived from cross-sections probably slightly underestimate the true dimensions. For comparison, spheres with a size distribution similar to that of curd granules would show in cross sections an average diameter which is about 5% smaller than the true mean diameter. (This difference was estimated by the method of Goldsmith (1967)) . Nevertheless, the principle of Dellesse shows that the volume density of particles is equal to the areal density of the profiles on sections (Weibel, 1979). In this study the approximate elliptical axes have mainly been considered to be a measure of the deformation of the curd granules.

Results and Discussion

Hard cheeses

Curd granule junction patterns in vertical sections typical for Emmentaler, Gruyere and Sbrinz cheese are shown in Figs. 3, 4 and 5, respectively. These sections were cut through the whole loaves near the hoop side including the rinds. Pressing of the fresh cheese, pressure from the gas inside the eyes and to some extent plastic deformation during ripening, leads to characteristic deformation and orientation of the granules. A region around an eye in an Emmentaler cheese is shown at higher magnification in Fig. 6. The regions near the rind and eyes were not considered for the determination of the size distributions of the curd granules. As an example of the increasing deformation in the rind, Fig. 7 shows the elliptical form factor as a function of the distance from the bottom rind of an Emmentaler cheese. The slope of the regression line indicates the increasing elongation of the granules near the rind. Only at a distance of about 10 -15 mm from the rind do the form factors remain constant within a certain bandwidth.

A pronounced difference could be observed between the patterns on vertical and horizontal sections. Pressing of the cheese loaves and some plastic deformation during ripening flattened the granules. In vertical sections the curd granule boundaries therefore appeared elongated and the maximum diameter was oriented preferentially in the horizontal direction. The difference between the extreme diameters was less pronounced in horizontal sections and there was no preferential orientation. In Fig. 8 are examples of vertical (8a) and horizontal (8b) sections from Sbrinz cheese. The diameters of the cross sections of the curd granules ranged from about 0.5 to 5.0 mm. Typical frequency

3 e hs 5mm bs 5mm

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Symbols in photographs: a, artifact from sanding paper; bs, bottom of loaf after filling; e, eyes; hs, hoop side of loaf; i, intensively stained, protein rich zones or curd dust (particles smaller than about 1 mm after cutting of coagulum); r, rind; s, slits.

distributions of the elliptical axes on horizontal and vertical sections are shown in Fig. 9. The median values for the major axis were similar for horizontal and vertical sections in the three types of hard cheeses (1.6 - 1.7 mm. Table 1). The median values for the minor axis ranged from 0.72 to 0.77 mm in vertical and from 0.84 to 0.93 in horizontal sections. It should be remembered that these axes do not correspond to the extreme diameters of the curd granules but represent the calculated axes of an ellipse which has the same moment of inertia as the cross section of the granule. The aver-age form factors determined from the ratio of the elliptical axes on horizontal and vertical sections differed significantly. Fig. 10 shows the frequency distributions of the form factors obtained for Gruyere cheese. The shape of the histogram for the horizontal sections is indicated by the dotted distribution curve. The



Fig. 7. Deformation of curd granules near the rind in Emmentaler cheese. Elliptical form factor in vertical sections as a function of the distance from the bottom rind of the cheese.

Fig. 3. Vertical section near the hoop side of Emmentaler cheese showing orientation of flat-tened granules near the rind.

Fig. 4. Vertical section through the loaf of a Gruyere cheese. Dark spots probably indicate zones of incomplete fusion of curd particles or curd dust. Note smear on rind.

Fig. 5. Curd granule junction pattern in vertical section through the loaf of a Sbrinz cheese. Pressure from bottom and hoop side leads to characteristic orientation of flattened granules.

Fig. 6. Deformation of curd granules around eyes in Emmental cheese.



Fig. 8. Typical curd granule junction patterns in vertical (8a) and horizontal (8b) section of Sbrinz cheese indicating deformation due to pressing.

dotted curve and the continuous line correspond to the calculated normal distributions. The actual distributions differ somewhat from the ideal Gauss distribution. Similar results were obtained for the other hard cheeses. The average f-values ranged from 0.557 to 0.561 and 0.436 to 0.475 in horizontal and vertical sections, respectively (Table 1). Sbrinz cheese had higher average form factors than Emmentaler and Gruyere cheese. The lower degree of deformation in Sbrinz cheese can partially be explained by the lower moisture content of this variety, which increases the viscosity of the cheese body and decreases its deformability. Lower temperature during the curing process also decreases the flattening of the cheese loaves.

As outlined in the experimental section the best measure of the size of the curd granules is the area of their cross section. The



Fig. 9. Frequency distributions of the calculated elliptical axes of cross sections of curd granules in Gruyere cheese. Each histogram is based on 720 values (6 cheeses and 6 zones according to Fig. 1).



Fig. 10. Frequency histograms of elliptical form factors in vertical and horizontal sections of Gruyere cheese. 720 data from 6 cheeses. The bars for the horizontal sections are not shown. The curves indicate the shape of a normal distribution. The actual distributions differ from an ideal Gauss distribution.

area values revealed an asymmetric distribution. Fig. 11 shows the distribution pattern obtained for Gruyere cheese. Similar histograms of the area frequencies were obtained for Emmentaler and Sbrinz cheese. The shape of the histograms can adequately be described by a loq-normal distribution. In Fig. 11, nineteen of the total of 720 values measured in horizontal sections were greater than 6 mm². In Emmentaler and Sbrinz cheese, 5 and 3% respectively of the surface areas in horizontal sections were greater than 6 mm². As summarized in Table 1 the median values of the area ranged from 0.9 to 1.2 mm². The interquartil range, which covers 50% of the values, was from about 0.5 to 2.2 mm². The differences between the 3 varieties were small. The only statistically significant_difference was between Emmentaler (1.15 mm²) and Gruyere (0.97 mm²).



Fig. 11. Size distribution of curd granules in Gruyere cheese. Cross section area frequencies on horizontal and vertical sections through the loaf according to the sampling scheme in Fig. 1.

Within the cheese loaves, small but significant differences could be observed between the size distribution profiles and average form factors. However, the differences between the parameters determined on the sections according to Fig. 1 were not systematic and did not indicate separation of differently sized particles. Only in Gruyere cheese were the form factors near the hoop side (sections nr. 1 + 2 + 3) systematically larger than in the central part of the loaf (sections nr. 4 + 5 + 6), indicating a different deformation in the middle of the loaf.

Different moulding and pressing arrangements were used by some manufacturers. However, the differences between the cheeses of a particular variety were in most cases of the same order of magnitude as the differences within the cheese loaves. A greater number of samples from each manufacturer would therefore be needed for an evaluation of the effect of equipment on the junction patterns. Emmentaler cheese had the largest and Gruyere cheese the smallest dispersity (see interquartil ranges in Table 1).

Semi-hard cheeses

Appenzeller, Tilsiter and Raclette cheeses are prepressed in rectangular curd billets before filling into round hoops. The extra mass of curd near the edges led to different deformations and thus granule junction patterns near the former edges and sides of the billets. Fig. 12 shows a horizontal cross section near the edge of an Appenzeller

Curd granules in cheese



Fig. 12. Horizontal section through loaf of Appenzeller cheese in the zone of the former edge of the billet. Arrows symbolize the pressure from the hoop side in this region.

cheese. The junction pattern looks like that of a vertical section of a hard cheese because of the flattening of the granules. The arrows indicate the radial pressure from the hoop side. This radial pressure, together with the vertical pressure in the cheese press (Fig. 13). leads to rod-shaped granules. As shown in Figs. 12 and 14 the granules therefore also appear flattened in horizontal sections. Fig. 14 shows typical patterns observed near the edges and sides of the billets of Appenzeller and Tilsiter cheese. A schematic drawing of the situation is given in Fig. 15. The relatively small values of the form factors in horizontal sections as well as the smaller surface areas in vertical sections, observed for Appenzeller and Tilsiter cheese, can be explained by the preferential orientation of the elongated curd granules (Table 1). In Raclette cheese the difference between the junction patterns near the edges and sides of the curd billets were less pronounced. However, the difference between the vertical and horizontal sections was again similar to that observed in hard cheese, as can be seen from Fig. 16. Comparison of Figs. 14 and 16 with corresponding micrographs of the other varieties also shows that Raclette cheese was manufactured with larger curd granules. The average granule size as well as the width of the size distribution in semi-hard cheeses were generally greater than those in the hard cheeses. It follows from the data in



Fig. 13. Typical pattern of curd granule junctions in a vertical section through the loaf of an Appenzeller cheese in the region of side of the former curd billet. Arrows symbolize the pressure from the upper and lower side.

Table 1 that Raclette cheese had the largest granules and the broadest size distribution of all the varieties tested.

As illustrated in Fig. 17, semi-hard cheeses sometimes showed special junction patterns in the edge zones. The example shows dark areas which represent regions of incomplete fusion of curd granules. The interstices were most probably filled with whey and possibly curd dust. Inhomogeneous "whirling" structures can be caused by uneven filling of the cheese moulds and nonuniform addition of curd remnants.

Some of the Appenzeller and Tilster cheeses were manufactured using cheesemaking machines, pumping and automatic pressing equipment. These cheeses differed significantly in their curd granule junction pattern from those manufactured traditionally using smaller vats, cloths and manual presses. The vertical sections in Fig. 18 show that flattening of curd granules was less pronounced in cheese loaves which were manufactured in a traditional





Fig. 14. Comparison of the junction patterns in Tilsiter cheese near the edge (14a) and side (14b) of the original curd billet. Horizontal sections.

Fig. 16. Typical patterns on vertical (16a) and horizontal (16b) sections of Raclette cheese.



Fig. 15. Schematic model showing cross-sections of curd granules in the region of the original edges of the curd billets in Appenzeller and Tilsiter cheese. Radial and vertical pressure leads to rod-shaped curd granules.



16a

Fig. 17. Vertical section through Tilsiter cheese in the region of an edge of the former curd billet. Larger arrows point to regions of abnormal orientation, incomplete fusion and inclusion of curd dust or whey.

Fig. 18. Difference between curd granule junction patterns of Appenzeller cheese manufactured traditionally (18a; vat, cloth) and cheesemaking machine (18b; pump, automatic pressing equipment). Curd granules in cheese



Table 1: Average size and elongation of curd granules in horizontal and vertica] sections of some traditional Swiss hard and semi-hard cheeses (median values, x, and interquartil ranges $r_{\chi}^{(1)}$

Parameter		Emmentaler	Gruyere	Sbrinz	Appenzeller	Tilsiter	Raclette	
a) horizontal								
Area, mm ²	A	1.15	0.97	1.07	1.31	1.42	1.68	
Major axis, mm	rAa	0.56-1.27 1.68	0.48-2.04 1.56	0.46-2.18 1.62	0.65-2.67 1.95	0.69-2.75 2.22	0.73-4.01 2.22	
Minor axis, mm	rab	1.23-2.38	1.05-2.35	1.09-2.35	1.33-2.85 0.93	1.45-3.28	1.39-3.38 1.05	
Form factor,b/a	rb f re	0.62-1.32 0.557 0.44-0.69	0.55-1.23 0.559 0.43-0.67	0.561 0.561 0.45-0.69	0.61-1.33 0.497 0.37-0.62	0.405	0.66-1.74 0.509 0.38-0.65	
b) vertical								
Area, mm∠	Α	1.00	0.89	0.93	1.02	1.06	1.34	
Major axis, mm	rAa	0.54-1.61 1.69	0.54-1.46 1.62	0.49-1.66 1.62	0.53-1.91 1.78	0.53-1.93 1.73	0.66-2.34 2.28	
Minor axis, mm	rab	1.21-2.40 0.77	1.19-2.26 0.72	1.10-2.25 0.77	1.22-2.68 0.76	1.22-2.58 0.80	1.53-3.45 0.77	
Form factor,b/a	rb f	0.57-1.02 0.448	0.54-0.93 0.436	0.56-1.02 0.475	0.54-1.04 0.436	0.56-1.10 0.459	0.57-1.01 0.334	
	rf	0.34-0.60	0.34-0.58	0.37-0.62	0.32-0.60	0.34-0.60	0.23-0.48	

 800-1000 measurements for each variety (5 to 8 different loaves, 6 (hard cheeses) or 12 zones (semi-hard cheeses) per loaf as shown in Figs. 1 and 2, and 10-20 granules per zone) M. Rüegg and U. Moor



Fig. 19. Different types of eyes in Raclette cheese (see text for explanation).

manual fashion. The higher pressure used in the automatic pressing equipment is probably the main reason for the the different junction patterns in the vertical sections.

The micrographs of the cheese sections also revealed structural features around holes. In Raclette cheese, for example, three different types of holes could be distinguished on the basis of the deformation of adjacent granules, inner surface and shape (Fig. 19). A first group consisted of eyeholes having smooth surfaces and circular contours. The diameters ranged from about 1 to 3 mm. On the inner surface, the curd granule junctions appeared as a network of dark lines. In the section plane. the contours did not follow the junction lines. It could not be deduced from the micrographs whether the inital germ at the beginning of eye formation was located between granules or inside a granule. On the basis of their shape we may conclude that the eyes of this first group were formed by gas pressure in homogeneous zones, where the curd granules were completely fused together. Examples are in Fig. 19a and the left side of Fig. 19b.

Openings with contours following the original curd granule borders could be considered as a second group of eyes. The largest diameters of the openings were in the approximate range of 1 - 8 mm. This type of opening most probably was formed between unfused granules, where whey inclusion favoured growth of microorganisms (Fig. 19b, right half and Fig. 19d).

Slits or cracks following not only the original curd granule borders but also cutting through granules formed a third group of eyes (Fig. 19c). In the Raclette cheeses tested the slits were usually 2 to 5 mm long. They occurred less frequently than the other types of openings. The slits must have been formed at a relatively late stage of maturation, when the cheese texture was less elastic ("short") and broke during gas development.

In conclusion, the technique used to visualize junction patterns over large areas of cheese is valuable for characterizing the type and quality of a cheese, for studying cheese defects and eye formation. From the quantitative analysis of the size, size distribution, deformation and orientation of the curd granules, data characteristic for manufacturing processes and equipment are obtained.

Acknowledgments

The authors wish to thank J. Schnider, A. Kessler, F. Rentsch and H. Schär for providing the cheese samples and to Dr. M. Casey for his linguistic assistance.

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Editor's Note: All of the reviewers' concerns were appropriately addressed by text changes, hence there is no Discussion with Reviewers.