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A FLUORESCENCE MICROSCOPIC STUDY OF CHEESE

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Abstract

Fluorescence techniques were used to study the microstructure of several varieties of cheese. The size and distribution of fat globules in various cheeses were demonstrated by fluorescent staining using Nile Blue A as a marker. Acridine Orange was used as a dye to detect structural differences between casein matrices of various cheese varieties. Differences between the ripe and the less-ripe zones of Camembert cheese were detected by staining the cheese sections with Acridine Orange and Nile Blue A separately, and then comparing the results of the stainings. The Nile Blue A staining showed that fat globules were absent from the ripe zone immediately below the surface molds, but were present in other areas of the cheese. Results from the Acridine Orange staining indicated that the protein matrix in the same area was different, both structurally and chemically, from the protein matrix in other locations. The findings indicated the presence of degraded proteins and fats in the ripe zone of the Camembert cheese. In another experiment, the presence of small crystalline inclusions, 10-30 μm in diameter, and their distribution in cheese were detected by various staining methods including the Von Kossa technique. Acridine Orange was also shown by the present study to be useful as a detecting reagent for revealing the crystalline structures. Two fluorochromes, Acridine Orange and Acriflavine, were used separately to reveal the presence and distribution of the microflora that was used in the ripening and flavor development of several cheese varieties.

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Key Words: Fluorescence microscopy, cheese, fat globules, casein networks, crystalline inclusions, microflora.

Introduction

The microstructure of cheese is closely related to its texture and flavor development. For example, freshly cheddared curd and stretched Mozzarella cheese have textures which are characterized by fibrous casein matrices whereas the protein matrix is unorientated in cheeses such as Edam and Gouda (Kalab, 1977 and Taranto et al., 1979). In mold-ripened cheeses, fungi such as Penicillium camemberti and Penicillium roqueforti, which are required for developing the special flavors, are part of the superficial (Camembert and Brie cheeses) or internal (Blue cheese) microstructures (Babel, 1953; Rousseau, 1984). Lactic acid-producing bacteria, mainly streptococci and lactobacilli, are components that play an important role in the ripening and flavor development of Cheddar cheese (Reiter et al., 1967). Crystalline inclusions, characteristic microstructures of hard cheeses, are formed during cheese ripening (Brooker et al., 1975). Most of the above structures have been revealed by transmission or scanning electron microscopy (TEM or SEM, respectively). The high resolution provided by these techniques made it possible to study some ultrastructures such as casein submicelles. However, the preparation steps used in these methods require that the sample must be dehydrated (unless it is examined frozen by cold-stage SEM). The use of organic solvents for dehydrating cheese samples can result in the loss of some constituents, e.g. fat globules which form one of the major structural components of cheese. Although both TEM and SEM can reveal detailed structural information, they can only provide limited chemical data. Furthermore, the limitation in sample size and the relatively long preparation time are factors that lower the efficiency of the above techniques, making them less convenient for cheese sample analysis.

Fat globules, the protein matrix, the microflora and crystalline inclusions can all be examined by bright-field microscopy (Dean et al., 1959; Rammell, 1960; Brooker et al., 1975 and Green et al., 1981). However, bright-field microscopy often does not provide sufficient contrast in thin sections and frequently allows only poor resolution in thick sections. In

contrast, epi-fluorescence microscopy employing relatively rapid and simple procedures can, at the same time, achieve considerably higher resolution. Most fluorescence microscopic methods use very sensitive and specific markers which are capable of yielding both structural and chemical information. Furthermore, samples that are prepared by fluorescence microscopic procedures can also be examined by other techniques such as bright-field and polarizing microscopy. The flexibility of the preparation procedures is thus another advantage of fluorescence microscopy.

Information on the use of fluorescence microscopic techniques in cheese studies is scattered and their use was limited to investigations of the structural relationship between fat and protein in various dairy products (King, 1958; Mulder et al., 1966 and Shimmin, 1982). The fact that these techniques are capable of revealing other microstructures in addition to fat and protein was not exploited, nor have they been used to investigate the effects of different processing techniques on the microstructure of cheese. The objective of the present study is to demonstrate the capability and ease of fluorescence microscopy as used for analyzing cheese microstructures.

Materials and Methods

Cheese samples

Cheese samples were obtained from commercially available sources and included the following varieties: mild, medium and extra-old Cheddar cheese, Mozzarella, Blue cheese, Camembert, Brie and process cheese. Samples (1-2 mm cubes) were taken from the surface and the centre of each cheese block. The cubes were fixed in 2% glutaraldehyde in 0.01 M phosphate buffer (pH 6.8) (PBS) at 4° C for 4 h. The fixed samples were washed with two changes of the buffer for 12 h prior to sectioning.

Frozen sections

The fixed cheese samples were immersed in a drop of the embedding medium for frozen tissues (Histo Prep, Fisher Scientific Co., Fair Lawn, New Jersey) on a cold object disc which was then placed immediately under a heat extractor. The sample block was frozen within 1 min and was ready to be sectioned at -20° C using a Cryo-Cut E (Reichert-Jung Scientific Instruments, Belleville, Ontario) microtome. Sections 3-6 µm thick were flattened in a drop of 0.25% glycerin on a glass slide. After drying at room temperature, the sections were ready to be stained.

Glycol methacrylate (GMA)-embedded sections

To study crystalline inclusions, cheese sections were embedded in GMA according to previously described procedures (Yiu et al., 1983). Fixed samples were dehydrated through methyl cellosolve, ethanol, n-propanol and n-butanol and infiltrated with GMA monomer for 3-5 days prior to polymerization at 60° C in gelatin capsules. Sections were cut 2-4 µm thick using an ultramicrotome (Sorvall Inc., Newtown, Connecticut) equipped with a glass knife and were affixed to glass slides for subsequent examination.

Staining procedures

Acridine Orange. Sections were stained for 1-2 min in 0.1% (w/v) aqueous Acridine Orange (AO) (BDH Chem. Ltd., Poole, England), modified as suggested by Pomeranz and Shellenberger (1961) by mixing with an equal volume of 1% acetic acid at a final pH of 2.9-3.0. After rinsing and drying, sections were mounted in non-fluorescent immersion oil under a cover slip and examined microscopically using filter system FC II (refer to Microscopic examinations for details).

Acriflavine. Sections were stained for 1-2 min in 0.1% (w/v) aqueous Acriflavine-HCl (BDH Chem. Ltd., Poole, England), rinsed in distilled water, air-dried, mounted in oil, and examined microscopically using filter system FC II.

Calcofluor White. Sections were stained for 1-2 min in 0.01% (w/v) Calcofluor White M2R (American Cyanamid Co., Bound Brook, NJ). After rinsing and drying, they were examined microscopically using filter system FC I (refer to Microscopic examinations for details).

Nile Blue A. Sections were stained for 1-2 min in 0.01% (w/v) aqueous Nile Blue A (Eastman Kodak Co., Rochester, N.Y.), rinsed, mounted in water under a cover slip and examined microscopically using filter system FC II.

The Von Kossa Method. The method is similar to the one described by Thompson (1966). Sections were immersed in 5% (w/v) aqueous silver nitrate inside a Coplin jar placed in front of a light source for 30 min. The sections were rinsed thoroughly with distilled water and then immersed for 3 min in 5% (w/v) sodium thiosulfate. After rinsing and drying, the sections were mounted in oil and examined under a light microscope.

Microscopic examinations

Sections were examined using a Zeiss Universal Research Photomicroscope (Carl Zeiss Ltd., Montreal) equipped with both a conventional brightfield illuminating system and a III RS epi-illuminating condenser combined with an HBO 100 W mercury-arc illuminator for fluorescence analysis. The III RS condenser contains two fluorescence filter combinations with a dichromatic beam splitter and an exciter/barrier filter set for maximum transmission at 365 nm/>418 nm (FC I) and 450-490 nm/>520 nm (FC II). All stained sections were examined for fluorescence except the ones stained by the Von Kossa method, which were examined under either brightfield or polarized-light illumination (via polarizing filters). Photomicrographs were taken with Ektachrome 400 Daylight film.

Results and Discussion

Fixation was a necessary step for the sample preparation as it hardened the cheese cubes and facilitated their subsequent sectioning. Fixation also stabilized the fat globules within the protein matrix and thus enabled the structures to be examined microscopically. Because hand-cutting tended to produce thick sections of uneven thickness and the GMA-embedding procedures were tedious (requiring more than 8 days for completion) and involved the use of organic

solvents which would dissolve the fat, frozen sections were used for most of the study. These sections were cut to uniform thickness (3-6 μm) and could be obtained within 2-3 days from the beginning of fixation to the end of sectioning.

Fat globules

Fixed and subsequently frozen sections were prepared from cheese samples for microscopic studies of the size and distribution of fat globules. Various cheeses were examined. Nile Blue A, a common fluorochrome used for staining fat-containing structures in fluorescence microscopy (Fulcher and Wong, 1980; Yiu et al., 1982 & 1983), was used as a stain to study the fat globules in the cheese sections. The relatively simple and rapid procedure was found most useful for studying the distribution of fat in cheese, especially in surface-ripened varieties such as Camembert and Brie. For example, the ripe zone in Camembert cheese immediately below the white surface layer of molds is known to be subjected to degradation by enzymes released from the molds (Tittsler, 1965). Microscopic examination of Camembert cheese sections stained with Nile Blue A revealed that this zone, which consisted of a compact protein matrix (details will be discussed later), was entirely devoid of fat globules (Fig. 1). However, relatively large fat globules were found in the layer immediately below. The numbers of the fat globules increased and their size decreased gradually in layers farther away from the surface. Similar results were obtained from microscopic examinations of samples taken from a Blue cheese variety. Relatively large fat aggregates were observed in the vicinity of the molds (Fig. 2a) while the fat globules further away from the molds were smaller (Fig. 2b). The distribution of fat globules within the protein networks of Mozzarella and Cheddar (mild, medium, extra-old) cheeses was also studied. The fat globules were evenly distributed throughout the entire protein matrix except in the curd granule junctions (Fig. 3), where, in agreement with SEM studies (Kalab, 1977), they were less numerous or absent.

Casein micelle structures

Most protein-staining fluorochromes such as Acid Fuchsin and 1-anilino-8-naphthalene sulfonic acid (Fulcher and Wong, 1980) were found suitable for staining the protein network in cheese. Previously published studies described the use of an aqueous 0.1% solution of Acridine Orange (AO), a metachromatic fluorochrome, to stain the protein matrix structures in Cheddar (King, 1958; Shimmin, 1982), Gouda and Edam (Mulder et. al., 1966) and Meshanger (de Jong, 1978) cheeses. The cationic Acridine Orange is a metachromatic dye with a strong affinity for anionic substances. Fluorescence changes from green through yellow and orange to red may be observed upon binding. The metachromatic binding is best achieved at low dye concentrations (0.01% - 0.1%) and at pH below 3.8 (Thompson, 1966). The pH of a 0.1% AO solution is about 5.1-5.2, and is not optimal for metachromatic binding of the dye. In the present study, a modified AO solution at pH 2.9-3.0 was used to stain the sections from different varieties of cheeses including Camembert. The results obtained were compared with those derived from duplicate sections stained with the 0.1% AO solution at pH 5.2. While the latter solution stained the protein matrices bright orange, the former solution (pH 3.0) stained them green. The green fluorescent structures could be examined microscopically for a longer period of time without causing eye-fatigue. Moreover, the modified AO solution was found more useful for distinguishing structural and chemical differences in the ripe and the less-ripe zones of Camembert cheese. The ripe zone below the white surface mold (Fig. 4) was stained to reveal the structure of a highly aggregated protein matrix intertwined with a few hyphae penetrated from the layer above. Unlike the protein networks present elsewhere, the protein matrix in this area was stained orange-red by the modified AO solution (Figs. 4 & 5a). The protein matrix in areas farther away from the ripe zone had an appearance of a reticulate network (Fig. 5b). These observations on the structural differences in the protein matrix between the ripe and the less-ripened zones in Camembert cheese are similar to those mentioned by Brooker (1980). Changes in fluorescence of AO on binding are believed to arise from different states of aggregation of the dye (Thompson, 1966). Factors affecting these in cheese may be complex, but a change in the dye-binding property of the protein network in the ripe zone of the cheese is evidence of a change in its chemical environment. This may reflect a change in the protein conformation or composition. However, it is known that during the process of surface-ripening of Camembert cheese, proteolytic enzymes are released by the molds to digest proteins in the adjacent area (Tittsler, 1965). Hence, the degraded proteins in the ripe zone could cause a change in its affinity for the metachromatic cations of the dye. The difference in staining could also be due to a change of pH in this area resulting from the presence of the proteolytic products (Tittsler, 1965) or the absence of fat globules. The latter was observed on sections taken from the same sample but stained with Nile Blue A (Fig. 1).

Acridine Orange can be used as a probe to study the effects of different processing methods on the microstructure of cheese. For example, fusion of individual curd granules occurs during pressing of milk curds and leads to the formation of so-called curd junctions which are basically compressed protein masses (Rammell, 1960 and Kalab, 1977). They can be easily detected by staining cheese sections with the same modified AO solution as the one used in this study (Fig. 6). Such junctions do not exist in process cheese, where the protein matrix has a relatively smooth, non-granular appearance (Fig. 7) due to the effects of melting and emulsifying the original cheeses. The simplicity of the AO staining method is an advantage because the dye can be used conveniently as a marker for detecting curd granule junctions and their patterns in cheeses made from different processing techniques.

Crystalline inclusions

The preceding microscopical examinations also revealed the presence of round or kidney-shaped structures in Cheddar and Mozzarella cheese sections stained with the modified AO solution. Each of these structures had a yellowish fluorescent periphery and an inner core which consisted of small clumps of reddish brown crystals (Fig. 6). Because they were detected in sections which had been subjected to fixation in an aqueous medium and to embedding in GMA, the crystals are assumed to be only slightly soluble or insoluble in water or organic solvents. They ranged from about 10 to 30 μm in diameter and were abundant near the curd junctions. The crystalline structures were stained red by Alizarin Red S, a dye specific for detecting the presence of calcium, and were stained blue by Toluidine Blue under brightfield microscopic examination (results not shown). These structures were also stained positively by the Von Kossa method which has been used for detecting the presence of calcium phosphate crystals in Cheddar cheese (Brooker et al., 1975). Furthermore, all the stained structures exhibited birefringence under polarized light (Fig. 8). Based on the above observations and on a comparison with previously published data (Brooker et al., 1975), the results strongly indicated that some of these crystalline structures contained calcium phosphate. The present study did not detect any large crystalline structures, such as calcium lactate, the presence of which had been reported in Cheddar cheese (Pearce et al., 1973 and Brooker et al., 1975). It is possible that calcium lactate, being very soluble in water, was removed during fixation of the cheese samples or that the above staining methods were not specific enough to detect its presence. More studies will be required in order to investigate the above possibilities.

Microflora

Only two groups of microorganisms were investigated by the present study. They were the molds found in surface-ripened cheeses and the lactic-acid producing bacteria found in hard cheeses. Most of these microfloras can be detected by staining the cheese sample with fluorochromes that have an affinity for microorganisms. Acridine Orange which has been used as a dye to differentiate dead and living yeast cells and fungi in other studies (Schwartz et al., 1977 and Chick and Durham, 1961), can be used as a probe for detecting surface microflora. For example, P. camemberti, a mold used for the ripening of Camembert and Brie cheeses and for flavor development, was stained either green or orange by the dye. Most of the surface-bound mycellium stained orange (Fig. 9) while the hyphae that penetrated the casein network below stained green (Fig. 5a). Details of the ultrastructure of the surface flora of Camembert cheese had already been described by Rousseau (1984) and therefore will not be covered by the present study. The above microorganisms can also be detected by using Calcofluor White, a fluorescent compound that has a strong affinity for cell walls (Holley et al., 1983). A Brie cheese section was stained by this compound to reveal the structure of its surface layer which was composed of a network of branching hyphae of

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Fig. 1. A Camembert cheese section stained with Nile Blue A. The distribution of fat globules (yellow fluorescence) is shown in an area away from the ripe zone (RZ) which is located below the surface mold. Photographed using FC II.

Fig. 2. A Blue cheese section stained with Nile Blue A showing the various sizes of fat aggregates (yellow fluorescence) present in (a) the vicinity of the molds (arrows) and (b) areas away from the molds. Photographed using FC II.

Fig. 3. A Cheddar cheese section stained with Nile Blue A demonstrating the difference in the distribution of fat globules (yellow fluorescence) between the interior of the curd granules (*) and the curd granule junctions (arrows). Photographed using FC II.

Fig. 4. A Camembert cheese section stained with Acridine Orange revealing the structures of the surface molds (arrows) and the protein matrix in the ripe zone (RZ) and in the less ripe zone (LZ) of the cheese. Photographed using FC II.

Fig. 5. At a higher magnification, the same section as the one presented in Fig. 4 shows different binding properties between AO and (a) the protein matrix (orange-red fluorescence) and the hyphae (large arrows) in the ripe zone and (b) the protein matrix (green fluorescence) and starter bacteria (small arrows) in the less ripe zone. Phographed using FC II.

Fig. 6. A Mozzarella cheese section stained with Acridine Orange demonstrating the presence of crystalline inclusions (large arrows), starter bacteria (small arrows) and the protein matrix (green fluorescence) within a curd granule junction (between arrows). Photographed using FC II.

Fig. 7. A process cheese section stained with Acridine Orange showing the non-granular appearance of its protein matrix. Photographed using FC II.

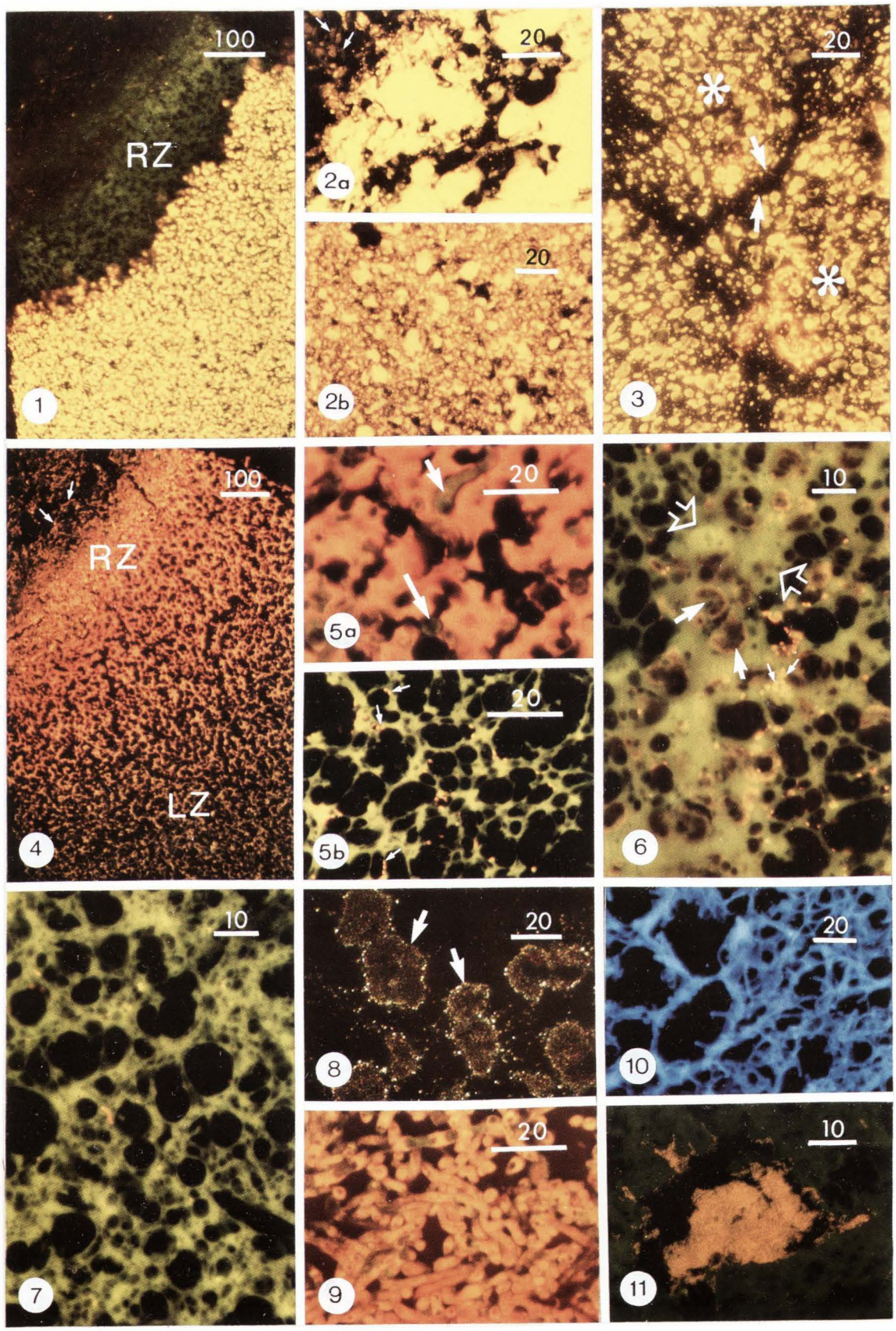
Fig. 8. A Cheddar cheese (GMA-embedded) section stained with the Von Kossa method and examined under polarized light to show the presence of calcium-containing crystalline inclusions (arrows).

Fig. 9. An Acridine Orange-stained Camembert cheese section showing the presence of surface molds. Photographed using FC II.

Fig. 10. A Brie cheese section stained with Calcofluor White to show the structural composition of the surface layer which consists of a network of branching hyphae. Photographed using FC I.

Fig. 11. An Acriflavine-stained Cheddar cheese section demonstrating a colony of lactobacilli. Photographed using FC II.

(Scale bars on the micrographs represent μm).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P. camemberti (Fig. 10). The presence of the lactic acid-producing bacteria in hard cheeses can be demonstrated by staining the sample with the modified AO solution or Acriflavine. The streptococci were seen as orange-red bead-like structures (Fig. 6) sparingly distributed between the fat globules and the casein network. The lactobacilli, another lactic-acid producing bacteria, having rod-like structures that form large well-defined colonies, can be located within the cracks of the casein matrix (Fig. 11).

Conclusion

The present study has shown that a detailed analysis of cheese structures by fluorescence microscopy can be accomplished within a relatively short period of time (2-3 days). Under optimal conditions (concentration and pH of the medium), Acridine Orange was shown by this study to be most suitable for simultaneously detecting the various cheese structures. These included the curd granule junctions, the microflora and the crystalline inclusions present in hard cheeses (Fig. 6) as well as the surface molds and the protein matrices in surface-ripened cheeses (Figs. 5a, 5b & 9). Acridine Orange also revealed the differences in structural organization of the protein networks resulting from various cheese manufacturing methods (Figs. 4, 5, 6 & 7) and can be used to detect microchemical changes that occur during the surface-ripening of cheeses (Figs. 5a & 5b). Further studies using various fluorescent dyes, e.g. Nile Blue A and Acridine Orange, might allow better understanding of the microchemical organization of cheeses. The study of Camembert cheese is an example of the possible application of fluorescence microscopic techniques to cheese quality analysis. The texture and flavor development of Camembert cheese, or the degree of ripeness, was reflected in microchemical and structural organizations of the cheese detected by fluorescence microscopy (Figs. 1 & 4). The above studies have demonstrated that fluorescence microscopy coupled with cryomicrotomy is indeed a convenient tool to be used for monitoring cheese quality.

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References

Babel FJ. (1953). The role of fungi in cheese ripening. Econ. Botany 7:27-42.

Brooker BE, Hobbs DG, Turvey A. (1975). Observations on the microscopic crystalline inclusions in Cheddar cheese. J. Dairy Sci. 42: 341-348.

Brooker BE. (1980). Milk and its products. In "Food Microscopy" (Vaughan JG ed.), Acad. Press, London. P. 273-311.

Chick EW, Durham NC. (1961). Acridine Orange fluorescent stain for fungi. Arch. Dermatol. 83: 305-309.

Dean MR, Berridge NJ, Mabbitt LA. (1959). Microscopical observation on Cheddar cheese and curd. J. Dairy Res. 26: 77-82.

de Jong L. (1978). Protein breakdown in soft cheese and its relation to consistency. 3. The micellar structure of Meshanger cheese. Neth. Milk Dairy J. 32: 15-25.

Fulcher RG, Wong SI. (1980). Inside cereals - a fluorescence microchemical view. In "Cereals for Food and Beverages" (Inglett GE, Munk L. eds.). Recent Advances in Chemistry and Technology. Acad. Press, New York, P. 1-25.

Green ML, Turvey A, Hobbs DG. (1981). Development of structure and texture in Cheddar cheese. J. Dairy Res. 48: 343-355.

Holley RA, Phipps-Todd BE, Yiu SH. (1983). Infection of oriental mustard by nematospora: a fluorescence and scanning electron microscope study. Food Microstruc. 2: 143-151.

Kalab M. (1977). Milk gel structure. VI. Cheese texture and microstructure. Milchwissenschaft 32: 449-458.

King N. (1958). Observations by fluorescence microscopy of casein in milk, curd and cheese. J. Dairy Res. 25: 312-319.

Mulder H, de Graaf JJ, Walstra P. (1966). Microscopical observations on the structure of curd and cheese. Proc. 17 Int. Dairy Congress, (published by the International Dairy Congress), Section D, P. 413-420.

Pearce KN, Creamer LK, Gilles J. (1973). Calcium lactate deposits on rindless Cheddar cheese. N. Z. J. of Dairy Sci. and Technol. 8: 3-7.

Pomeranz Y, Shellenberger JA. (1961). Histochemical characterization of wheat and wheat products. IV. Mapping the free fatty acids in germinating wheat. Cereal Chem. 38: 122-128.

Rammell CG. (1960). The distribution of bacteria in New Zealand Cheddar cheese. J. Dairy Sci. 27: 341-351.

Reiter B, Fryer TF, Pickering A, Chapman HR, Lawrence RC, Sharpe ME. (1967). The effect of the microbial flora on the flavour and free fatty acid composition of Cheddar cheese. J. Dairy Res. 1967. 34: 257-272.

Rousseau M. (1984). Study of the surface flora of traditional Camembert cheese by scanning electron microscopy. Milchwissenschaft 39: 129-135.

Schwartz D, Larsh HW, Bartels PA. (1977). Enumerative fluorescent vital staining of live and dead pathogenic yeast cells. Stain Technol. 52: 203-210.

Shimmin PD. (1982). Observations of fat distribution in cheese by incident light fluorescence microscopy. Aust. J. of Dairy Technol. 37: 33-34.

Taranto MV, Wan PJ, Chen SL, Rhee KC. (1979). Morphological, ultrastructural and rheological characterization of Cheddar and Mozzarella cheese. Scanning Electron Microsc.; 1979;III: 273-278.

Thompson SW. (1966). In "Selected Histochemical and Histopathological Methods." Thomas CC Pub. Springfield, Ill. P. 269, 581 & 1039.

Tittsler RP. (1965). Cheese chemistry. In "Fundamentals of Dairy Chemistry. (Webb BH and Johnson AH eds.), AVI Pub. Co., Inc., Westport, Connecticut, P.672.

Yiu SH, Poon H, Fulcher RG, Altosaar I. (1982). The microscopic structure and chemistry of rapeseed and its products. Food Microstruc. 1: 135-143.

Yiu SH, Altosaar I, Fulcher RG. (1983). The effects of commercial processing on the structure and microchemical organization of rapeseed. Food Microstruc. 2: 165-173.

Discussion with Reviewers

B.E. Brooker: Would the author like to comment on the stability of the fluorescent dyes in the stained sections? How quickly do they fade?
Author: Although the fading of fluorescence occurs in most stained sections in general, the fluorescent dyes used for this study remain stable enough so that fluorescent structures can be studied within a reasonable time limit. However Nile Blue A fades rapidly under blue light illumination.

B.E. Brooker: This method appears to produce exquisite differential staining of cheese components e.g. Fig. 3. The use of fluorescent dyes would appear therefore to offer great potential in image analysis of cheese sections. Have you any experience of the usefulness of these techniques in image analysis?
Author: No, I have not yet used image analysis to interpret results obtained from fluorescence microscopy. However, I may attempt to use both techniques in the near future as results obtained from these studies may provide semi-quantitative information.

M.L.Green: I presume that the orange fluorescence with acidified Acridine Orange in the ripe zone of Camembert cheese as opposed to the green fluorescence elsewhere is due to the higher pH in the ripe zone (Noomen, Neth. Milk Dairy J. 37: 229-232). Does the author think that the change in the colour of the fluorescence could be used to probe the distribution of pH in biological materials?
Author: The fluorescence emission and absorption spectra of Acridine Orange are greatly affected by environmental changes in concentration, pH, ionic strength and its substrate complex. Hence, the change in the colour of the fluorecence could not be used as an indicator for the change in pH alone.