THE TEXTURE AND MICROSTRUCTURE OF MOZZARELLA CHEESE AS AFFECTED BY FAT CONTENT, EPS PRODUCING STARTER CULTURE AND FAT REPLACERS

A thesis submitted for the degree of Master of Science in Food Technology

By

Raman K. Bhaskaracharya
B. Technology (Dairy Technology)

2000

School of Life Sciences and Technology
Victoria University of Technology
Werribee Campus
Victoria, Australia

DEDICATED

TO

MY PARENTS AND BROTHERS

ABSTRACT

The main goal of this study was to manufacture low fat mozzarella cheeses using fat replacers having similar texture and microstructure characteristics as full fat mozzarella. To achieve this goal it was essential to analyse the texture characteristics including hardness, cohesiveness, springiness, chewiness and gumminess of commercial mozzarella cheeses and to study the effects of moisture, fat and protein on these characteristics. The texture characteristics were analysed using an Instron Universal Testing Machine. A non-stretching cheddar cheese was used as a standard. The hardness and springiness were significantly higher with increased compression, while cohesiveness reduced. Chewiness and gumminess initially increased followed by a decrease. The cheddar cheese exhibited higher hardness compared to mozzarella cheeses and slightly lower springiness. There was a drastic decrease in chewiness and gumminess with increased compression in all the mozzarella cheeses. The cheddar cheese showed significantly lower cohesiveness, which decreased with increased compression. In general, the hardness decreased with increase in moisture content. The springiness increased with increase in fat content while the cohesiveness increased with increase in protein content. One of the mozzarella cheeses showed the texture characteristics of a typical full fat mozzarella cheese and these characteristics were used as a reference for further studies in developing a low fat mozzarella cheese made with fat replacers.

The microstructure of mozzarella cheeses was also studied using a simplified method of specimen preparation for imaging. Three batches of mozzarella cheeses were prepared each with or without exopolysaccharide producing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. Samples were obtained

from all the six batches to study their microstructure. Specimens were cut from the cheese samples, fixed in 2% glutaraldehyde, dehydrated and impregnated using 1.5% osmium tetroxide. The specimens were dried in a critical point drying equipment, fractured at room temperature (~20°C), sputter coated with gold and images of the cheese specimens were taken using a scanning electron microscope. The images showed clear internal structures of the specimens. The compact protein structure of cheeses interspersed with small and large voids representing location of the fat and serum phases, respectively, was seen. The exopolysaccharide produced by the streptococci appeared to be delicate and filamentous. The lactobacilli seemed to produce the exopolysaccharide in meagre amounts. The microorganisms were found propagating in serum channels.

The six cheese samples were also examined for their microstructure and exopolysaccharide produced by the starter cultures. Specimens measuring 2 x 2 x 10 mm size were cut and fixed in 2% glutaraldehyde solution, washed in cacodylate buffer, gradually dehydrated using ethanol and cryoprotected in 2.3 M sucrose solution. The specimens were cryofractured in liquid nitrogen, dehydrated using absolute ethanol and acetone solutions, mounted on aluminium stubs, sputter coated with gold and observed under a scanning electron microscope followed by recording of the images. Mozzarella cheese matrix interspersed with small and large voids representing location of fat and serum phases, respectively, was observed. The starter cultures were located in the serum channels. Exopolysaccharide seemed to be primarily produced by *S. thermophilus*. The exopolysaccharide appeared to be delicate in the form of thin, filamentous strands. Thick strands of stretched protein appear to be surrounded with large serum voids. This method of specimen preparation using

sucrose as a cryoprotectant was suitable to obtain details of internal structure and exopolysaccharide produced by the starter organisms.

Mozzarella cheeses were made using milk with 4.0, 3.5, 2.5, 1.5% fat, and skim milk. Exopolysaccharide producing *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* were used at the rate of 0.5% of each starter culture. The cheeses were analysed for their texture characteristics using an Instron Universal Testing Machine and the data collected using a Merlin software. Cheeses with higher fat contents showed lower values of hardness, cohesiveness, springiness, gumminess and chewiness. Adhesiveness values were higher for cheeses with higher fat content. The cheese made with skim milk showed similar characteristics to that made with 1.5% fat milk after 2 weeks of storage at 4°C. These observations were similar at both levels of compressions.

Three batches of skim milk mozzarella cheeses were prepared each with exopolysaccharide or non-exopolysaccharide producing starter cultures. The moisture, protein and fat contents were analysed. Texture characteristics such as hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness were measured at 5, 12, 19 and 26 d of manufacture using Instron Universal Testing Machine by compressing the samples to 50 and 70% of their original heights with a 500 N load cell flat plunger attached to the Instron. The microstructure of the cheese samples was examined using a scanning electron microscope at 28 d of manufacture. The exopolysaccharide cheeses showed 3.25% higher moisture content than non-exopolysaccharide cheeses. Both types of cheeses had similar protein content (~43%). The hardness, cohesiveness, springiness, gumminess and chewiness

decreased during storage for both types of cheeses. However, adhesiveness at 50% compression increased during storage. Both types of cheeses showed similar hardness and springiness values during storage, although exopolysaccharide cheeses had higher moisture content than non-exopolysaccharide cheeses. The exopolysaccharide cheeses showed lower values of cohesiveness and adhesiveness during storage. The microstructure of the cheeses showed large and small voids representing the location of the serum and fat phases. The starter bacteria were located in serum channels. The exopolysaccharide was in the form of filaments, which extended from the protein matrix. The exopolysaccharide was primarily produced by *S. thermophilus*, which was found in abundance. *L. delbrueckii* ssp. *bulgaricus* were few in numbers and seemed to produce meager amount of exopolysaccharide. The exopolysaccharide cheeses were more open and porous compared to the non-exopolysaccharide cheeses. The decrease in cohesiveness and adhesiveness of the exopolysaccharide cheeses could be partly due to the increased porosity of the protein matrix.

Skim milk mozzarella cheeses (<3% fat) were made using two maltodextrin based (Maltrin® M040 and M100) and a modified potato starch based (StaSlim® 143) fat replacers. A control batch was made without any fat replacers. The texture characteristics of the cheeses were measured using an Instron Universal Testing Machine. The location of the fat replacers in the cheese structure was examined using a scanning electron microscope. The moisture contents of the skim milk and Maltrin® M040 and M100 based cheeses were similar, while StaSlim® 143 based cheeses showed lower moisture levels. The protein contents of the cheeses made with fat replacers were lower than those of control cheeses. The hardness values of mozzarella cheeses with Maltrin® M040 and M100 were lower, while StaSlim® 143 based

cheeses showed slightly higher values compared to control cheeses. The cohesiveness values were lower for cheeses made using fat replacers especially those with Maltrin® M040 and M100 compared to control cheeses, while adhesiveness values were higher. Springiness values for cheeses made using fat replacers were lower than those of control cheeses. Gumminess and chewiness values were lower for cheeses added with Maltrin® M040 and M100, while StaSlim® 143 cheeses showed slightly higher values compared to the control cheeses. During storage Maltrin® M040, M100 and control cheeses showed decreased hardness, cohesiveness, springiness, gumminess and chewiness values, while adhesiveness values increased. The cheeses made with StaSlim® 143 showed increased hardness, gumminess and chewiness values during storage. The distribution of the fat replacers within the cheeses was influenced by the rate of addition, the extent of microparticulation of the fat replacer and the size of individual particles. Maltrin® M040 and M100 was present as a gel without any microparticulation while StaSlim® 143 formed smooth particles (about 0.05mm diameter) within the cheese matrix. Incorporation of Maltrin® M040, M100 and StaSlim® 143 resulted in an increased openness in cheeses, and large serum channels (upto 0.1mm diameter) were seen. There was less openness of the cheese structure with StaSlim® 143 compared with Maltrin® M040 and M100. The freeze-dried aqueous dispersion of StaSlim® 143 appeared as a continuous flowing gel; however, when incorporated in cheeses, they appeared as solid spheroidal particles, possibly due to shrinkage. These particles were found embedded within the protein matrix and were also present in the serum channels. No discrete Maltrin® M100 particles were found, although Maltrin® M040 seemed to exist as coalescing particles, which seemed to form a gel upon further hydration.

Dr. Nagendra P. Shah
(M. Sc., Ph. D)
Associate Professor of Food Science
School of Life Sciences and Technology
Victoria University of Technology
Werribee Campus, Werribee
Victoria, Australia

CERTIFICATE

This is to certify that the thesis entitled "THE TEXTURE AND MICROSTRUCTURE OF MOZZARELLA CHEESE AS AFFECTED BY FAT CONTENT, EPS PRODUCING STARTER CULTURE AND FAT REPLACERS" submitted by Raman K. Bhaskaracharya in partial fulfilment of the requirements for the award of the degree of Master of Science in Food Technology at the Victoria University of Technology is a record of bonafide research work carried out by him under my personal guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma or other similar title.

[redacted]

Werribee, Australia

Date: 22-05-00

(Dr. N. P. Shah)

Thesis Supervisor

ACKNOWLEDGEMENT

It is a great privilege and with immense pleasure I express my profound gratitude to my supervisor Dr. Nagendra Shah, Associate Professor, School of Life Sciences and Technology, Victoria University of Technology, Werribee Campus, Werribee for his inspiring and noble guidance, constructive criticism and valuable suggestions throughout the course of this study and in preparation of manuscripts.

I express my gratitude to Mr. Dale Tomlinson (Laboratory Manager), Mr. Vilnis Ezernieks, Mrs. Stacey Lloyd, Ms. Mai Giang, Mrs. Marzena Walkiewicz, Mrs. Joanne Gatt, Mr. Michael Rogerson, Ms. Kathy Janusko and Mr. Nikola Popovich for their support in procuring chemicals and allowing me to use the instruments and other facilities required for the successful operation of my research work. I am thankful to Dr. Roderick Williams (Research Biochemist-Ingredient Innovation), Food Science Australia, Sneydes Road, Werribee, Victoria, Mr. John Near (Factory Manager), Melbourne University, Sneydes Road, Werribee, Victoria, Mrs. Janetta Culvenor (Research Officer), Mrs. Anna Friedhuber and Mr. Cong Ho (Technical Officers), Pathology Department, Melbourne University, Parkville Campus, Melbourne, Victoria, for their active involvement and their personal contribution in providing technical assistance.

I express my sincere thanks and appreciation to my friends Dr. Rajiv Dave (Assistant Professor), Mr. Harshul Vora, Dr. Warnakulasuriya Lankaputhra, Mr. Ramakanth Ravula and their families for their help in some aspects of my research. I am highly indebted and I appreciate the friendship of Ms. Sandra McKenzie, Ms. Amal Shihata,

Mr. Aslam Khan, Mr. Mihir Sainani, Mr. Kiran Gummadi, Ms. Archana Sharma and Mr. Dishonavi Pohatu.

I would like to recall the love and affection and financial support of my parents, brothers, and other family members; without their blessings and wishes this study would not have been a reality. Last but not the least, I cannot forget the moral support and inspiration provided by Mrs. Maria Lettieri Noto, Mrs. Margaret Hadley, Mr. Kulen Vyravipillai and their families especially in pressing times when they stood by me.

Werribee, Australia

Date: 22-05-00

RAMAN KUMAR BHASKARACHARYA

List of Publications

REFEREED RESEARCH PAPERS

(1) Bhaskaracharya, R.K. and Shah, N.P. 1999. Texture evaluation of commercial mozzarella cheeses. *Aust. J. Dairy Technol.* 54 (1): 36-40.

(2) Bhaskaracharya, R.K. and Shah, N.P. 2000. A simplified method for examination of microstructure of mozzarella cheeses with scanning electron microscopy. *Aust. J. Dairy Technol.* 55(1): 28-32.

(3) Bhaskaracharya, R.K. and Shah, N.P. 2000. Texture Characteristics and microstructure of skim milk mozzarella cheese made using exopolysaccharide or non-exopolysaccharide producing starter cultures. *Aust. J. Dairy Technol.* (under review).

(4) Bhaskaracharya, R.K. and Shah, N.P. 2000. Texture and microstructure of skim milk mozzarella cheeses made using fat replacers. *Aust. J. Dairy Technol.* (under review).

CONFERENCE PRESENTATIONS

(1) Bhaskaracharya, R.K. and Shah, N.P. 1998. Texture evaluation of some commercial mozzarella cheeses. A paper presented at the 31st Australian Institute of Food Science and Technology Annual Convention, Melbourne, Victoria, Australia, April 26-29, 1998 (Paper No. A20).

(2) Bhaskaracharya, R.K. and Shah, N.P. 1998. Microstructure of full fat mozzarella cheese. A poster presented at the Cheese Science'98 Conference, Melbourne, Victoria, Australia, July 1-3, 1998 (*Aust. J. Dairy Technol.* 53(2): 129).

(3) Bhaskaracharya, R.K. and Shah, N.P. 1998. Texture analysis of mozzarella cheese. A paper presented at the 93rd Joint Annual Meeting of the American Dairy

Science Association and American Society of Animal Science, Denver, Colorado, USA, July 27-31, 1998 (*J. Dairy Sci.* 81(Suppl. 1): 11).

(4) Bhaskaracharya, R.K. and Shah, N.P. 1998. Ultrastructure of a full fat mozzarella cheese made with EPS producing starter culture. A paper presented at the 93rd Joint Annual Meeting of the American Dairy Science Association and American Society of Animal Science, Denver, Colorado, USA, July 27-31, 1998 (*J. Dairy Sci.* 81(Suppl. 1): 12).

(5) Bhaskaracharya, R.K. and Shah, N.P. 1999. Microstructure and texture characteristics of low fat mozzarella cheeses made using carbohydrate based fat replacers. A paper presented at the 94th Joint Annual Meeting of the American Dairy Science Association held in Memphis, Tennessee, USA, June 20-24, 1999 (*J. Dairy Sci.* 82(Suppl. 1): 15-16).

(6) Bhaskaracharya, R.K. and Shah, N.P. 1999. Ultrastructure of mozzarella cheese made using fat replacers. A poster presented at the 10th World Congress of the International Union of Food Science and Technology held in Sydney, NSW, Australia, October 3-8, 1999. (10th World Congress of Food Science and Technology Abstracts P13/20: 84).

(7) Bhaskaracharya, R.K. and Shah, N.P. 2000. Texture and microstructure characteristics of skim milk mozzarella cheeses made using carbohydrate based fat replacers. A paper presented at the International Dairy Federation Symposium on Cheese Ripening and Technology held in Banff, Canada, March 13-16, 2000. (*IDF Symposium Abstr.* 2000 VII-03: 38-39).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

USDA	United States Department of Agriculture
FDM	Fat in dry matter
FDW	Fat by dry weight
SNF	Solid not fat
TPA	Texture profile analysis
GRAS	Generally recognised as safe
SEM	Scanning electron microscope
EPS	Exopolysaccharide
HTST	High temperature short time
CPD	Critical point drying
TEM	Transmission electron microscope
STEM	Scanning transmission electron microscope
MNFS	Moisture in non-fat solids

1.0. INTRODUCTION

1.1. Background

Mozzarella cheese is a semi hard (semi-soft) variety of cheese. Mozzarella cheeses have gained popularity as a pizza cheese over the past 20-30 years. The per capita consumption of fluid milk in Australia has remained relatively static, whereas the amount of manufacturing milk increased from 5.2 billion litres to 7.1 billion litres in 1997 (Sutherland, 1998). Further it is reported that expansion in manufacturing milk supply is expected at a similar rate thereby more milk would be available for the manufacture of cheeses. Among the major milk products, cheese and whey products accounted for 40% of total manufacturing milk in 1996-97. Presently 60% of the manufactured products are exported (Sutherland, 1998).

In the past 10 years the proportion of cheeses manufactured in Australia has increased steadily and the amount in 1997 was 290,000 tonnes/year. The production of mozzarella cheeses increased from 13,708 tonnes in 1992/93 to 27,431 tonnes in 1997/98. The non-cheddar cheeses being exported have shown an increase from 14% to nearly 40% of total cheese exports between 1988 and 1997. The domestic cheese market has shown a shift towards consumption of pizza/shredding types and fresh cheese types within the non-cheddar sector. The domestic mozzarella and pizza cheese market is estimated to be 31,300 tonnes in 1997/98. The major growth in the cheese market is through the fast food outlets as an ingredient in pizzas and burgers. The non-retail sector accounts for 80% of mozzarella cheese market while that of cheddar is only 18%. The trend in production of mozzarella cheese in Australia is shown in Figure 1.1.1

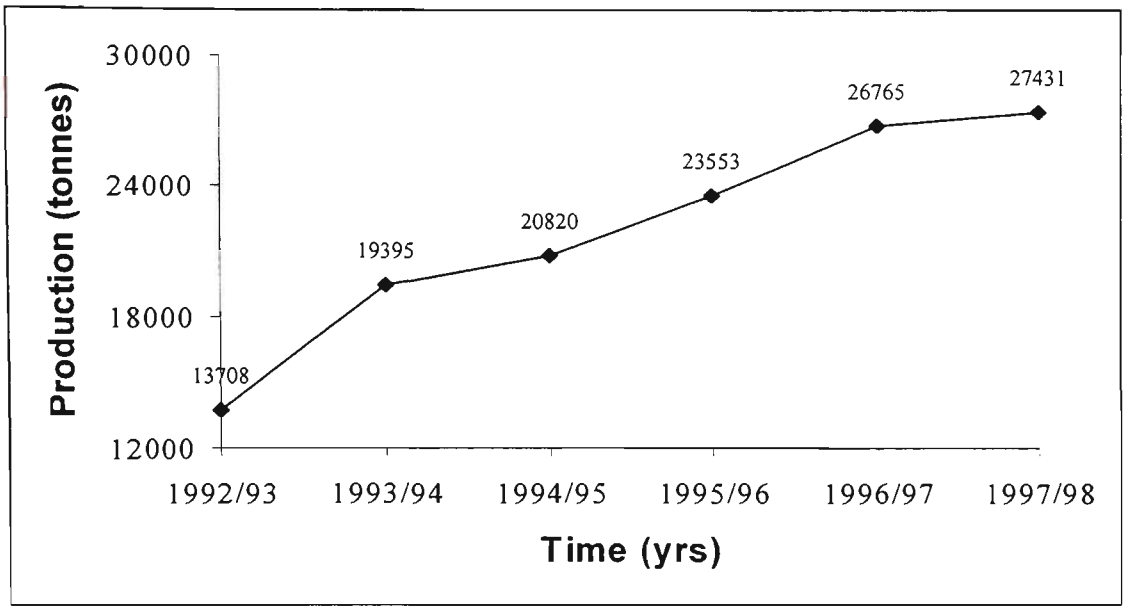

Figure 1.1.1. Production statistics of mozzarella cheeses in Australia (Sutherland, 1998).

Mozzarella and pizza cheeses account for about 16% of the total Australian cheese market (Australian Dairy Corporation, 1996) while in Italy these cheeses account for 78% of the total Italian cheese produced (Merrill *et al.*, 1994).

The present trend is to reduce the fat content in cheeses. This has led to manufacture of cheeses with low fat or reduced fat contents. In recent years demand for cheeses containing low fat has increased (Shepherd and Stockley, 1987; Anonymous, 1992; Anonymous, 1995).

Recent surveys have shown that consumers are becoming more aware of consumption of fat from cheese and other dairy products (USDA, 1991; Tunick *et al.*, 1993a, b; Australian Dairy Corporation, 1996). In a survey, 23% of the respondents reported that they had stopped eating dairy products because of their high fat content (Barr, 1990). Thus low fat cheeses are becoming increasingly popular among consumers. As a result, sales of reduced fat cheeses have increased by 9% in 1995/96 (Australian Dairy Corporation, 1996). The US dietary guidelines include a recommendation to choose a

diet low in fat and cholesterol. Suggested goals for fat in diets include total fat that provides 30% or less of calories, and saturated fat that provides less than 10% of calories (McGinley, 1994; Anonymous, 1994). According to Food and Nutrition Service of the USDA, the school lunch program provided 38% calories from unsaturated fat and 15% from saturated fat and only about 1% of the 92,000 schools in the program met these guidelines (McGinley, 1994; Tunick *et al.*, 1995 a, b, c). A significant correlation between dairy fat intake and mortality rates from coronary heart disease has been reported (Renaud and deLorgeril, 1992). There is apprehension by many consumers about dietary fat which has led to the development of mozzarella cheeses containing 9-11% fat. This is less than half the fat of normal full fat mozzarella, which could be labelled in the US as 'lite' according to new regulations (Tunick *et al.,* 1991; 1993a, b; 1995a, b, c).

But reduction in fat changes the flavour and texture profiles of mozzarella cheeses. A few varieties of reduced fat cheddar cheeses are reported to have characteristic cheddar flavour, but there are no reduced fat mozzarella cheeses that resemble delicately flavoured whole milk mozzarella. The flavour and texture characteristics are due to fat content and reduced fat cheeses have a plastic body (Burros, 1992). The texture characteristics of low fat mozzarella cheeses are adversely affected by reduction in fat level or substitution of vegetable oil for milk fat (Malin *et al.*, 1993). Mozzarella cheeses with fat content lower than that of partly skim cheeses have potential of finding an important market among light dairy products (Tunick *et al.*, 1993a, b). In mozzarella cheeses the flavour is of secondary importance, while stretchability and melting characteristics are of primary importance. There is a requirement for minimal fat leakage during cooking. Traditionally mozzarella is made at about 45% fat in dry matter

(FDM), but pizza cheese and mozzarella are now successfully made with substantially lower fat contents; as low as 19% FDM (Jameson, 1990). A few workers have studied manufacture of imitation cheese, low fat high moisture mozzarella cheeses and fat substituted mozzarella cheeses without much success. Previous studies using protein based (Simplesse D100 and Dairy lo), and carbohydrate based (Stellar 100X and Novagel RCN-15), fat replacers have been partly successful, especially when protein based fat replacer was used (Lucey and Gorry, 1993, Desai and Nolting, 1995, McMahon *et al.*, 1996). In the present investigation, maltodextrins, tapioca dextrins, corn syrup solids and polysaccharide based fat replacers were used for making low fat mozzarella cheeses. Also exopolysaccharide producing microorganisms were used to improve springiness characteristic in mozzarella cheeses.

The objectives of this study were to examine texture characteristics of four commercial cheeses, and to study the microstructure of low fat cheeses, and location and distribution of fat replacers in skim milk cheeses. The specific objectives of this project were:

(1) to assess the commercial mozzarella cheeses for their texture characteristics,

(2) to compare the texture characteristics of full fat mozzarella cheeses with low fat cheeses,

(3) to investigate methods of specimen preparation for microstructure study,

(4) to examine the effects of exopolysaccharide producing starter cultures on texture and microstructure characteristics of mozzarella cheeses, and

(5) to manufacture skim milk mozzarella cheeses using fat replacers and study characteristics of cheeses.

Chapters 1 and 2 of this thesis contain introduction, the review of literature and chapter 3 deals with the evaluation of commercial mozzarella cheeses. Chapter 4 focuses on the simplified method of specimen preparation of mozzarella cheeses for microstructure study while chapter 5 examines the microstructure of mozzarella cheeses made with or without exopolysaccharide producing starter cultures using sucrose as a cryoprotectant. Chapter 6 deals with examining the texture characteristics of skim milk mozzarella cheeses made using exopolysaccharide or non-exopolysaccharide producing starter cultures and chapter 7 includes the texture and microstructure study carried out on skim milk mozzarella cheeses made using fat replacers. Chapter 8 gives overall conclusions. Chapter 9 gives an outlook on the future research direction.

1.2. References

Anonymous. (1992). Soft options. German survey. *Dairy Ind. Int.* 57(6): 29-30.

Anonymous. (1994). School lunches will meet dietary guidelines by 1998 under new USDA proposal. *Cheese Reporter* 118(10):1.

Anonymous. (1995). Cheese manufacturers see market for healthy cheese having most potential. *Cheese Reporter* 120(15): 16.

Australian Dairy Corporation (1996). W*ritten communication.*

Barr, A. (1990). Consumer motivational forces affecting the sale of light dairy products. *Food Technol.* 44:97-8.

Burros, M. (1992). If butter fat is cut, cheese doesn't cut it. *Newyork Times* June 3, pC1.

Desai, N. and Nolting, J. (1995). Microstructure studies of reduced fat cheeses containing fat substitute. In Chemistry of structure- function relationships in cheese. eds. Malin, E. L. and Tunick, M. H., Plenum press, NY. 295-302.

Lucey, J. A. and Gorry, C. (1993). Effect of Simplesse®100 on the manufacture of low fat cheddar cheese, In Cheese yield and factors affecting its control. *Int. Dairy Fed. Sem.*, Cork, Ireland. 439-447.

Malin, E. L., Banks, J. M., Tunick, M. H., Law, A. J. R., Leaver, J. and Holsinger, V. H. (1993). Texture enhancement of low fat mozzarella cheese by refrigerated storage. *Int. Dairy J.* 3(4-6) 548 poster A4.

McMahon, D. J., Alleyne, M. C., Fife, R. L. and Oberg, C. J. (1996). Use of fat replacers in low fat mozzarella cheese. *J. Dairy Sci.* 79: 1911-1921.

McGinley, L. (1994). Planned new school lunch guidelines prompt food sellers to think nutrition. *Wall St. J. (Westn.)* June 6 pB1.

Merrill, R. K., Oberg, C. J. and McMahon, D. J. (1994). A method for manufacturing reduced fat mozzarella cheese. *J. Dairy Sci.* 77(7): 1783-9.

Renaud, S. and M. deLorgeril 1992. Wine, alcohol platelets and the french paradox for coronary heart disease. *Lancet* 339:1523.

Shepherd, R. and Stockley, L. (1987). Nutrition knowledge, attitudes, and fat consumption. *J. Amer. Diet. Asso.* 87(5): 615-9.

Sutherland, B. J. (1998). Developments in cheese science and technology in Australia. *Aust. J. Dairy Technol.* 53(1):63-65.

Tunick, M. H., Holsinger, V. H., Mackey, K. L. and Smith, P. W. (1991). Effects of composition and storage on the texture of mozzarella cheese. *Neth. Milk Dairy J.* 45:117-25.

Tunick, M. H., Mackey, K. L., Shieh, J. J., Smith, P. W., Cooke, P. and Malin, E. L. (1993a). Rheology and microstructure of low fat mozzarella cheese. *Int. Dairy J.* 3: 649-62.

Tunick, M. H., Malin, E. L., Shieh, J. J., Smith, P. W., Sullivan, B. C., Mackey, K. L. and Holsinger, V. H. (1993b). Proteolysis and rheology of low fat and full fat mozzarella cheeses prepared from homogenised milk. *J. Dairy Sci.* 76(12): 3621-8.

Tunick, M. H., Malin, E. L., Smith, P. W. and Holsinger, V. H. (1995a). School lunch pizzas topped with low fat mozzarella cheese. *Cult. Dairy Prod. J.* 30(2):6-9.

Tunick, M. H., Malin, E. L., Smith, P. W. and Holsinger, V. H. (1995b). Effects of skim milk homogenisation on proteolysis and rheology of mozzarella cheese. *Int. Dairy J.* 5: 483-91.

Tunick, M. H., Malin, E. L., Smith, P. W., Cooke, P. H. and Holsinger, V. H. (1995c). Influence of rennet source on proteolysis, rheology and microstructure of low fat mozzarella cheese. *J. Dairy Sci.* 78(suppl.1): 101.

USDA (1991). Dairy situation and outlook yearbook. US government printing office. Washington DC, USA, 17.

2.0. LITERATURE REVIEW

2.1. Mozzarella cheeses

Mozzarella cheeses are produced from whole or partly skimmed milk to which small amounts of starter or organic acids are added, followed by a milk coagulator, rennet extract. The curd formed is cut, and allowed to matt. Exposure of the drained curd to warm temperatures permits a mild acid ripening to pH 5.2; at this pH the curd is heated in hot water, stretched or mixed and moulded. The cheese is then slightly salted.

The physico-chemical and microstructural changes (Figure 2.1.1) taking place during refrigerated storage in the first two weeks along with proteolysis have been reported to be the driving forces behind the functional changes in mozzarella cheese (Kindstedt and Guo, 1997).

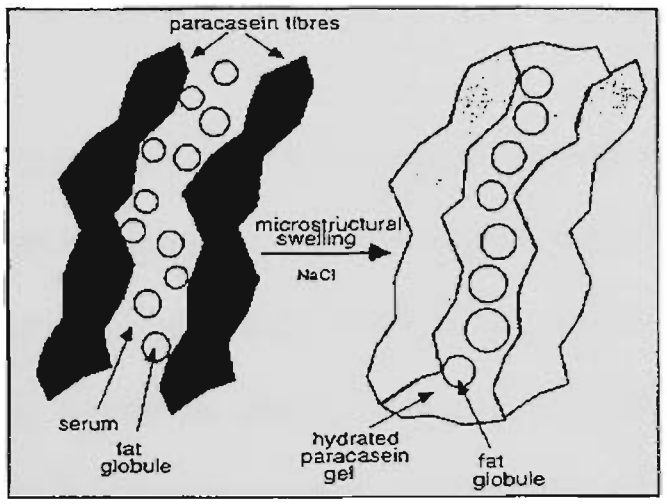

Figure 1: Proposed model for physico-chemical and microstructural changes in pizza cheese during short-term refrigerated ageing.

Figure 2.1.1. A model proposed for physico-chemical and microstructural changes in mozzarella cheeses (Kindstedt and Guo, 1997).

The aim of cheese making in 'Pasta Filata' is achieving the smooth texture and grain in cheese through a skilful stretching of curd in hot water (Jana and Upadhyay, 1991). The characteristics of mozzarella cheese are because of the action of lactic acid on dicalcium

paracaseinate which is converted to monocalcium paracaseinate at pH between 5.2 and 5.4. The latter provides the strings and sheen to the cheese (Kosikowski, 1982).

2.1.1. Types of mozzarella cheeses

Mozzarella cheeses can be classified as shreddable or non-shreddable varieties. The shreddable variety usually tends to have lower moisture content than the non-shreddable variety. The mozzarella cheeses are also classified on the basis of the method of acidification of milk ie. direct acidified or through addition of starter cultures. Several organic acids are used to cause coagulation of the milk including lactic acid, citric acid and acetic acid. Among these lactic acid is the widely used coagulant. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* are the commonly used starter cultures. *Lactobacillus helveticus* and *Lactobacillus casei* are also used to increase proteolysis in the mozzarella cheeses during storage; this enhances functional characteristics including melt, stretch and flow of the cheeses.

In the USA there are four categories in which mozzarella cheeses can be classified: mozzarella cheese, low moisture mozzarella cheese, part skim mozzarella cheese and low moisture part skim mozzarella cheese. These varieties differ in their fat and moisture content. Mozzarella cheese must have 45% fat (FDW) and 52 to 60% moisture. Most of the mozzarella cheeses produced in the US falls into the pizza cheese category ie. low moisture part skim mozzarella cheese with a fat content of 30 to 45% FDW and a moisture content of 45 to 52%. In compiling statistics all categories are usually combined and referred to as "mozzarella" cheese which is equivalent to low moisture part skim mozzarella cheese. The shreddable type mozzarella cheese must contain 50% moisture and not less than 40% FDW (Oberg *et al.*, 1993).

2.1.2. Starter cultures used for the manufacture of mozzarella cheeses

Several manufacturers use *Streptococcus thermophilus* only for manufacturing mozzarella cheeses. Details of the different types of cultures and their characteristics are shown in Table 2.1.1. (Sigsgaard, 1994). Normally the starter cultures consists of a combination of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* or *Lactobacillus helveticus* organisms.

Table 2.1.1. Chr. Hansen's culture programme for pizza cheese production (Sigsgaard, 1994).

Thermophilic lactic cultures	Acid production	Galactose fermentation	Proteolytic activity	Salt sensitivity (50%/100% inhibition)
Type DVS defined strains *Streptococcus thermophilus*				
TH-3	fast	negative	medium	2.1/4.0
TH-4	fast	negative	Medium	1.9/3.0
St-36	fast	negative	High	2.5/3.0
St-37	fast	negative	High	2.5/3.0
St-75	fast	negative	High	2.5/3.0
St-B 01	slow	negative	Medium	2.0/3.0
Type DVS defined strains *Lactobacillus delbrueckii* ssp. *bulgaricus*				
Lb-9	slow	positive	Medium	2.1/3.0
LB-12	fast	negative	Medium	1.7/3.0
Type DVS defined strains *Lactobacillus helveticus*				
Lh-B 01	slow	positive	Low	3.1/4.0
Lh-B 02	fast	positive	Low	2.5/3.0
Type DVS multiple strain, yoghurt *Streptococcus thermophilus/Lactobacillus delbrueckii* ssp. *bulgaricus*				
CH- 1	fast	negative	Medium	2.1/3.0
B-3	fast	negative	Medium	1.9/3.0
Type DVS composed cheese cultures *Streptococcus thermophilus/Lactobacillus delbrueckii* ssp. *bulgaricus*				
TCC-1	fast	negative	Medium	2.7/3.5
TCC-2	fast	negative	Medium	3.1/4.0
TCC-3	fast	negative	Medium	2.8/3.5
TCC-4	fast	negative	Medium	2.8/3.5
Type DVS composed cheese cultures *Streptococcus thermophilus/Lactobacillus helveticus*				
TCC-20	fast	positive	Low	2.8/3.5
Type DVS composed cheese cultures *Streptococcus thermophilus/Lactobacillus helveticus /Lactococcu lactis* ssp. *lactis or cremoris/Lactococcus lactis* ssp. *diacetylactis or cremoris*				
TCC-21	fast	positive	Low	3.7/>4
Type DVS composed cheese cultures *Streptococcus thermophilus/Lactococcus lactis* ssp. *lactis or cremoris*				
TCC-22	fast	positive	low	3.7/>4

The cultures are selected based on their acid production, galactose fermentation, proteolytic activity and salt sensitivity. Previous studies reported by Jana and Upadhyay (1991) have indicated the use of *Lactococcus lactis*, *Streptococcus durans* and *Streptococcus faecalis* for the manufacture of high moisture mozzarella cheese while the effect of *Pseudomonas cerevisiae*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Streptococcus faecalis* and *Streptococcus durans* along with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* to avoid blistering or burning of cheese during baking of pizzas was also reported to have been studied. These adjunct starter cultures mainly utilise the galactose accumulated in the cheese thereby reducing Maillard browning. The accumulation of galactose is due to the activity of *S. thermophilus*, which utilises lactose and glucose from milk, but does not have galactase enzyme (McMahon *et al.* 1993).

Hassan *et al.* (1995a, 1995b), measured the size of capsule formed and its effect on acid production by EPS producing starter bacteria. Further studies were carried out (Hassan *et al.*, 1996a) on the viscosity characteristics of yogurt manufactured using EPS producing starter cultures. Similar studies (Hassan *et al.*, 1996b) on yogurt made using exopolysaccharide producing starter cultures including encapsulated non-ropy or unencapsulated ropy strains of lactobacilli showed an increase in moisture retained in the yogurt gel. Hassan and Frank (1997) also observed a reduction in curd tension and firmness of rennet curd due to increased open casein network with large pores when made using EPS producing starter cultures compared to non-EPS producing starter cultures.

2.2. Factors affecting quality of mozzarella cheese

The major factors affecting the physical properties of mozzarella cheese such as pH, protein, moisture, calcium, salt, type of enzymes used, culture used, age (storage conditions) and fat are described below.

2.2.1. Cheese pH

Acid production is the key to the manufacture of good quality cheeses. It affects the state of milk solids. It has been observed that at pH 5.2 of cheese curd before hot stretching gives optimum stretchability for starter added cheese manufacture whereas for direct acidification the pH can be 5.3 or 5.4. At this pH the colloidal calcium phosphate attached to casein micelles is solubilised and a portion of calcium ions is lost in the whey. The native protein, casein is transformed chemically to another form of protein highly sensitive to coagulation in the presence of calcium. This precipitates out at room temperature as dicalcium paracaseinate, entrapping fat, insoluble salts, lactose and some serum. This precipitate is incapable of producing a smooth plastic mass when exposed to hot water or steam. By acid development some of the calcium of dicalcium paracaseinate is dissolved by the acid and monocalcium paracaseinate is formed. This caseinate has the unique property of forming smooth, pliable and stringy curd when heated to 54°C or higher. Further acid development during ripening causes more calcium to be lost and paracasein is formed which does not retain fat (Kosikowski, 1982). During stretching the free casein is available for stabilising newly created fat surfaces as fat globules are disrupted.

The moisture content of cheese is dependent upon the acidity or pH during curd formation (Oberg *et al.*, 1993). Excessive acid production causes more syneresis and the cheese becomes crumbly, whereas less acid production gives pasty or sticky cheese due to excess moisture retained. Also by controlling the moisture content the bacterial growth and enzyme activity are regulated which in turn influence the rate and extent of ripening. The decrease in calcium content enhances the susceptibility of casein during ripening to proteolysis and thus the textural and rheological properties of cheese (Holsinger *et al.*, 1995). Also the rennet activity is pH dependent and lowering the pH (Holsinger *et al.*, 1995) controls the activity of non-starter bacteria.

2.2.2. Protein

Protein plays a major role in the manufacture of cheeses. It is the major component of cheese body and gives the desired firmness, stretch and meltability, which are the essential characteristics of mozzarella cheese. Although cheese firmness is affected by the relative amounts of water, protein and fat, the dominant factor is the amount and ratio of water and protein which have the greatest effect (Prentice, 1987; Walstra *et al.*, 1987). The hardness of cheese varieties with diverse composition correlated most closely with protein content but not related to fat content (Chen *et al.*, 1979). Also when the ratio of fat to SNF increased there was a decrease in elasticity more so because of SNF part (Masi and Addeo, 1986). Similar effect due to moisture changes has been reportedly reconstructed (Olson and Johnson, 1990).

2.2.3. Moisture

The moisture content in cheese is affected by the rate and extent of syneresis which in turn is controlled by milk composition especially the calcium ion concentration, pH of whey, temperature of cooking, rate at which curd is stirred with the whey and time (Holsinger *et al.*, 1995). Moisture content affects various characteristics of mozzarella cheese such as hardness, springiness, chewiness, meltability and crumbliness. Decrease in moisture content of mozzarella cheese caused decrease in meltability, cohesiveness and an increase in hardness, springiness, chewiness and crumbliness (Tunick *et al.*, 1991). It has also been reported that reduction in moisture levels in mozzarella cheese resulted in higher values for hardness and springiness (because of increase in elastic modulus and viscous modulus) and lower values for cohesiveness, complex viscosity and meltability (Tunick *et al.*, 1993a).

2.2.4. Calcium

Calcium exists in milk in the form of bound calcium phosphate with casein and keeps the casein in a colloidal phase. The removal of calcium causes dissociation of casein which becomes available for emulsification of fat and there will be less oiling off upon melting. Excess of calcium has been linked with excessive curd firmness. So part of calcium is destabilised and expelled along with whey due to acid production by starter culture in milk or direct acidification during manufacture. It has also been reported that a pH of 5.6 obtained by acidification using citric acid gives similar curd as with lowering of pH to 5.2 by other acids because calcium gets removed because of chelating effect of citric acid (Oberg *et al.*, 1993).

2.2.5. Salt

Salt is incorporated into mozzarella cheese by either brining alone or along with dry salting. This step is also necessary to cool the hot stretched cheese. Moisture loss during salting depends on brine temperature, with greater loss as brine temperature increases. But the salt uptake is affected by the brine temperature and brine strength (Kindstedt, 1993). This step also has positive effects on the product.

Salting also improves emulsification of fat because of calcium exchange in protein and is independent of the moisture content of the product. So there are less chances of oiling off . This effect has been proven to be better in case of dry salting of cheese curd before it goes to the stretcher (Oberg *et al.*, 1993, Tunick, 1994).

Salting inhibits non-starter organisms from growing and causing spoilage during storage. Excessive salting may also inhibit the activity of bacteria which come as post manufacture contaminants and grow during storage. So there would be more unfermented sugars leading to browning of mozzarella cheese during pizza making (Tunick *et al.*, 1991). Also a study by Tunick *et al.* (1995c) has shown that it can cause decrease in melt.

2.2.6. Enzymes

The coagulants used in cheese making are enzyme preparations belonging to the family of aspartic proteinases (Harboe, 1998). Generally, rennet (an enzyme extracted from the stomachs of young ruminants) is used as the coagulant in cheese making. The enzyme used in manufacture of mozzarella cheese is of vital importance because each enzyme

gives different characteristics to the final product. The main desired characteristics (Harboe, 1998) of coagulants are:

(i) high milk clotting and low general proteolytic activity, thereby reducing casein fragment losses.

(ii) good curd forming properties, which will retain most of the fat globules and large peptides within the curd.

(iii) low pH dependency during milk clotting for better control and consistency.

(iv) complete inactivation of enzymatic activity with normal pasteurisation to get whey free of active enzymes that can be used for manufacture of products such as ricotta cheese.

(v) high purity especially free from starch degrading enzymes.

(vi) good stability during handling and storage.

Tunick *et al.* (1995c) studied characteristics of microbial rennet, using *Mucor meihei* and *Clostridium parasitica*. With calf rennet, the curd showed 25- 39 % decrease in hardness and 23- 26 % decrease in springiness in 6 week period. During the same time with cloned calf chymosin, the moisture retention in mozzarella cheese decreased and meltability increased. Control of moisture in non-fat substance is more important than the rennet source in manufacturing mozzarella cheese (Tunick *et al.*, 1995c). In another study by Oberg *et al.* (1993) pepsin produced more open texture curd, while chymosin gave better meltability to the mozzarella cheese. There was 19- 27 % increase in meltability in 6 week period when *Mucor meihei* enzyme was used (Tunick *et al.*, 1995c).

It has been reported that rennet retains some activity during manufacture of mozzarella cheese (DiMatteo *et al.*, 1982; Tunick *et al.*, 1991, 1993b). Table 2.2.1. shows the heat stability of some of the coagulants studied during pasteurisation of whey.

Rennet cleaves αs1-casein to form αs1-I- casein and a smaller peptide (McSweeney *et al.*, 1993a). The αs2-casein and β-casein were also found to be degraded which could be due to plasmin, a native milk enzyme, which is heat stable (Farkye *et al.*, 1991; Farkye and Fox, 1992). Yun *et al.* (1993) showed that αs1-casein only degrades due to action of rennet and not αs2-casein or β-casein.

Table 2.2.1. Heat stability of some of the coagulants at pasteurisation temperatures for whey (Harboe, 1998).

Type of Coagulant	Percent of residual activity after 5 min at 60°C (corresponds to 72°C for 15 sec.)		
	pH 5.0	pH 5.5	pH 6.0
Rennet containing chymosin and pepsin	6	40	>98
Microbial coagulant native type	1	2	3
Microbial coagulant destabilised	14	41	82
Microbial coagulant extra destabilised	17	68	99

Visser *et al.* (1989) in their studies on hydrolysis of αs2-casein by plasmin, showed that the largest peptide formed could have mobility similar to that of αs1-I-casein, αs1-casein or β-casein and therefore would not be detected. Rennet enzyme is most commonly used for the manufacture of cheeses.

2.2.7. Starter culture

The selection of a particular culture is very important to obtain the desired characteristics of the product. The parameters to be considered while selecting culture are acidification rate, proteolytic activity, lactose fermentation and salt sensitivity. Normally for pizza cheese *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus* and/or *Lactobacillus helveticus* are used. *Streptococcus thermophilus* are mainly used for acid production whereas *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Lactobacillus helveticus* are used for their proteolytic activity. *Lactobacillus helveticus* has a wider spectrum of proteolysis compared to *Lactobacillus delbrueckii* ssp. *bulgaricus* and almost all strains of *Lactobacillus helveticus* are galactose fermenting unlike *Lactobacillus delbrueckii* ssp. *bulgaricus* (Per Sigsgaard, 1994). Galactose fermentation is important because most strains of *Streptococcus thermophilus* cannot hydrolyse galactose and this may cause Maillard browning in the final cheese when baked on the pizza. To avoid this *Lactobacillus delbrueckii* ssp. *bulgaricus* or *Lactobacillus helveticus* are used.

Thus a proper starter culture must be used which may be a single strain of Streptococcus thermophilus or mixed strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* and/or *Lactobacillus helveticus*.

2.2.8. Storage

A specific ripening pathway during storage of mozzarella cheese has been indicated in the study by Malin *et al.* (1993). During storage various biochemical changes occur in cheese including glycolysis, lipolysis and proteolysis and secondary catabolic changes such as deamination, decarboxylation, β-oxidation and ester formation in cheddar cheese (Holsinger *et al.*, 1995). In case of mozzarella cheese, the principal biochemical change is due to proteolysis. Although there is very limited proteolysis in mozzarella cheese when compared to ripened cheese varieties, it affects the functional and rheological characteristics of mozzarella cheeses. Various methods of assessing proteolysis in cheeses have been reported (Fox *et al.*, 1995). Kindstedt *et al.* (1997) have suggested a model for physico-chemical and microstructural changes in pizza cheese during short time refrigerated ageing.

Tunick *et al.* (1991, 1993a, 1993b, 1995c) reported that during refrigerated storage meltability increases with proteolysis, whereas hardness, springiness, gumminess, chewiness decreased. Oberg *et al.* (1993) observed an increase in free oil formation after first two weeks of storage. Diefes *et al.* (1993) showed that refrigerated samples were less elastic and less viscous than frozen and thawed samples of same age. They also observed that during freezing local dehydration takes place causing structural changes in proteins and upon thawing these proteins are unable to rebind water and thus lose elasticity. Further, the fat granules were formed at freezing temperatures causing hardness. However, Tunick *et al.* (1995c) did not observe any textural defects in 1-week frozen and immediately thawed mozzarella cheese samples. It was observed that during

storage proteolysis by plasmin and residual coagulant caused cheese softening (Diefes *et al.*, 1993).

Reports (Tunick *et al.*, 1991; 1995b; Anonymous, 1995; Dryer, 1994) suggest that 6 weeks old low fat high moisture mozzarella cheese had similar textural properties as fresh 1 week old high fat low moisture mozzarella cheese. Tunick *et al.* (1991) observed frozen mozzarella cheese samples of 48 days to show poor cohesiveness and meltability when evaluated after thawing. Thawing at 4.4°C for 21 days produced optimum values of cohesiveness and meltability.

2.2.9. Fat

A linear decrease in elasticity of mozzarella cheeses was observed as the ratio of fat to solids-not-fat increased (Masi and Addeo, 1986). The effect of milk fat on cheese elasticity may be related to the interaction between the fat globule surface membrane and the cheese protein matrix. Globules that were washed to remove the surface membrane did not contribute to the elasticity of the acid milk gels. They did not react with the protein matrix, and the gels behaved as if the voids were filled with particles possessing the rheological properties of water. Fat globules coated with protein during homogenisation contributed substantially to the gel elasticity (Olson and Johnson, 1990).

Chen *et al.* (1979) observed that protein levels play dominant role affecting elasticity of cheese varieties of varying composition. However, fat plays a more dominant role in this rheological factor than in any other. Low fat high moisture cheeses exhibit

stickiness but effect of fat without change in protein level is unknown (Olson and Johnson, 1990).

The firmness of cheese is affected by the melting point of milk fat (Prentice, 1987). An indirect linear relationship was observed between penetrometer readings of cheese and iodine number of milk fat; the latter is a measurement of saturation and an indication of meltability. The proportion of solid to liquid fat at different temperatures might affect rheological properties of cheese. The impact of fat on temperature induced softening may be questionable because changes in relative firmness were linear and similar between 5°C and 30°C for cheese of varying composition (Prentice, 1987) including low fat (30% fat reduction) cheeses. In Scandinavia vegetable oils including soybean oil, sunflower oil and cotton-seed oil are used to replace milk fat. The typical fat contents of these products are about 50% FDM and the products are being marketed as "Lochol" and "Minichol" (Tunick *et al.*, 1991).

The effect of polyunsaturated: saturated fat ratio was not studied in much detail but cheddar cheese has been made successfully using Alta milk (milk produced from cows fed with protected polyunsaturated fat supplements) but in this process the starter cultures had to be changed (Tunick *et al.*, 1991).

It has been reported that when the fat content of the mozzarella cheese is decreased, the moisture retention also decreases giving the cheese a poor melt and stretch characteristic. The cheese is said to have a rubbery texture (Fecera *et al.*, 1995) and there is an increase in hardness, springiness and chewiness (Tunick *et al.*, 1991). Tunick *et al.* (1995b) concluded from their study that proteolysis and rheology of cheese are

affected more by fat content and storage rather than by fat globule size or homogenisation of fat. A study by Merrill *et al.* (1994) showed that different casein: fat ratios (1.2 ; 1.6 ; 2.0 & 2.4) to make low fat mozzarella cheese had no effect on the melt and stretch characteristics. It is also well known that low fat high moisture cheeses exhibit stickiness when masticated, especially when their fat content is 15 % or less (Olson and Johnson, 1990).

2.2.9.1. Free oil formation

A detailed study by Tunick *et al.* (1994) of the effect of different manufacturing conditions on the free oil formation has shown that:

(i) free oil formation is dependent on FDM. Above 37 % FDM there is more significant amount of free oil formed which increases with increase in fat.

(ii) free oil increases during refrigerated storage of mozzarella cheese due to more breakdown of casein which destabilises fat from its emulsified state.

(iii) free oil is greatly decreased if homogenisation is done to cheese milk prior to cheese manufacture.

(iv) addition of emulsifying salts or increase in pH tends to decrease free oil formation. However, increase in pH adversely affects the stretchability of mozzarella cheese.

(v) decrease in cooking temperature causes increase in free oil due to increase in proteolysis.

Studies carried out by Tunick *et al.*, 1991; 1995b; 1995c and Tunick (1994) showed that increase in FDM does not affect melting point of fat in homogenised cheese free oil. The melting point of fat in the cheeses was greater than the melting point of

corresponding free oil. The storage time did not affect the melting point of cheese fat or free oil formation. Melting point of free oil from non-homogenised and homogenised skim cheeses were not different but they were reported to be higher than that for melting point of fat in homogenised skim milk cheese. The fatty acid composition did not play a role in formation or thermal properties of free oil and the heat of fusion of free oil and cheeses were found not to be different. They have also suggested that when fat is not homogenised, the free oil formation is dependent on proteolysis.

2.2.9.2. Effects of reducing fat

Less fat globules in cheese results in more extensive syneresis and denser structural matrix causing firmness, dryness and improper melt. An increase in moisture retention in low fat cheese helps in decreasing hardness, (Tunick *et al.*, 1995b). Cheeses tend to be softer when the amount of liquid fat, which is not bound to the protein matrix, increases (Green *et al.*, 1985, Luyten, 1988). Hardness and meltability in mozzarella cheese is affected by manner of moisture distribution in protein matrix (Merill *et al.*, 1994).

Theoretically removal of fat, which exists in between the columns of protein fibres, causes narrower columns and makes cheese harder and less meltable (Merill *et al.*, 1994). Flavour compounds from fat lipolysis are not produced. Fat-soluble flavour compounds are not dissolved and their later release during consumption may be affected. Although this is not very serious as the flavouring added during pizza making masks this blandness (Tunick *et al.*, 1995a). FDM less than 30% caused an increase in the values of each texture profile analysis (TPA) parameter and decreased meltability

values. Reduction in fat or moisture levels and storage time also caused similar changes in the TPA values (Tunick *et al.*, 1995b).

2.2.10. Fat replacers

2.2.10.1. Classification

Summerkamp and Hisser (1990) have listed (see Table 2.2.2.) a few replacers which have been cleared by FDA to be of GRAS status whereas a few more are in the various stages of development.

Fat replacers, such as stated above are being developed to decrease the calories in the food products to the desired levels for the consumers. A wide variety of fat replacers are available which may be classified as protein based, carbohydrate based and synthetic.

Table 2.2.2. List of fat replacers available (Summerkamp and Hisser, 1990).

Name/Type	Uses/Applications
Simplesse® (Protein)	Yoghurt, cheese spread, cream cheese, sour cream, salad dressing, mayonnaise, margarine, etc.
Trail Blazer® (Protein)	Frozen desserts, etc.
Olestra® (Synthetic-Sucrose poltester)	Frozen desserts, table spreads, salad dressings, cheese, bakery items, shortenings, cooking oils, etc.
EPG (Synthetic- Esterified propoxylated glycerol)	Frozen desserts, table spreads, salad dressings, bakery items, etc.
DDM (Synthetic-Dialkyl dihexadecymalonate)	Mayonnaise, margarine, cooking oils, etc.
TATCA (Synthetic- Tri alkoxytricarbollate)	Mayonnaise, margarine, cooking oils, etc.
Gums (Carbohydrate-Hydrocolloids)	Salad dressings, formulated foods, etc.
Polydextrose (Carbohydrate)	Candy, chewing gum, candy coatings, dry cakes/ cookie mixes, frozen dairy products, icings, nutritional bars, puddings, frostings, etc.
Maltrin® M040 and M100 Carbohydrate- Maltodextrins)	Frozen desserts, table spreads, salad dressings, margarine, imitation sour cream, etc.
Tapioca Dextrins® (Carbohydrate)	Frozen desserts, table spreads, margarine, salad dressings, imitation sour cream, puddings, micro-waveable cheese sauce, etc.
Paselli SA2 (Carbohydrate-Potato Starch Maltodextrin)	Salad dressings, frostings, frozen desserts, dips, bakery products, mayonnaise, table spreads, meat products, confections, etc.
StaSlim® 143 (Carbohydrate- Modified Potato Starch)	Pourable and spoonable salad dressings, soups, cheese cakes, imitation cream cheese
Prolestra® (Sucrose polyester), Nutrifat® (hydrolysed starch), Finesse® (Piezoproteins), Colestra® (low calorie Olestra) Carbohydrate	Icecream, salad oils, mayonnaise, sauces, snacks, table spreads, baked products, etc.

2.2.10.2. Functionality of fat replacers in low fat mozzarella cheeses

Various fat replacers have been tried (Fife *et al.*, 1995) in mozzarella cheese with high moisture of about 56% and a casein:fat ratio of 4.2. A higher temperature of pasteurisation at 80°C for 29 sec was used. Mozzarella cheese manufactured with Stellar 100X (mixture of modified cornstarch and xanthan gum) showed greater melt and by 28 days the cheese became sticky and difficult to handle, whereas with Simplesse® 100 (microparticulate whey proteins) the cheese had greater melt. Mozzarella cheese had more open texture and it melted the least when added with Novagel® RCN-15 (mixture of microcrystalline cellulose and guar gum) but when made with Dairy lo® (35% whey protein concentrate) the cheeses showed similar moisture content as control. In the microstructure studies Stellar® and Novagel® (both carbohydrate based fat replacers) were observed in the pockets of fat and serum between strands of casein matrix while Simplesse® and Dairy lo® (protein based) were observed embedded in casein matrix as well as in serum pockets (Lucey and Gorry, 1993, Desai and Nolting, 1995, McMahon *et al.*, 1996).

Mann (1992) suggested an addition of 3 % gum arabic to give ideal characteristics for the manufacture of high fibre mozzarella cheese. Inulin a vegetable fibre, was used as a fat replacer by Pagliarini and Beatrice (1994). Low fat mozzarella cheese was made with a polysaccharide substitute (brand name fibrulin) added to make up fat plus fat substitute equal to fat in full fat mozzarella cheese using direct acidification to 5.8 pH with citric acid and stretched in brine solution. The product had better moisture retention and showed less hardness, more glossiness, more whiteness and was similar to the full fat mozzarella cheese.

2.3. Low fat mozzarella cheese

An acceptable mozzarella cheese with a fat content lower than that of the part skim cheese has the potential of finding an important market among light dairy products (Tunick *et al.*, 1993a). Mistry and Anderson (1993) have reported use of condensed milk for the manufacture of reduced fat cheddar cheese to overcome the problem of bitterness due to growth of non-starter organisms and their enzyme activities during cheese ripening. They have also reported that homogenisation would help to overcome the problem of hardness in cheeses because the surface area of fat within the cheese matrix would be increased. A lot of research work carried out on mozzarella has been reported on fat reduction and modifications in manufacture (Mann, 1992).

2.4. References

Anon. (1977). *Dairy Science Abstract.* 39:4379.

Anon. (1994). School lunches will meet dietary guidelines by 1998 under new USDA proposal. *Cheese Reporter* 118(10):1.

Anon. (1995). Cheese manufacturers see market for healthy cheese having most potential. *Cheese Reporter* 120(15): 16.

Chen, A. H., Larkin, J. W., Clark, C. J. and Irwin, W. E. (1979). Textural analysis of cheese *J. Dairy Sci.* 62(6): 901-7.

Christensen, V. W. (1966). *American Dairy Review.* 28:38, 92-96.

Desai, N. and Nolting, J. (1995). Microstructure studies of reduced fat cheeses containing fat substitute. In Chemistry of structure- function relationships in cheese. eds. Malin, E. L. and Tunick, M. H., Plenum press, NY. 295-302.

Diefes, H. A., Rizvi, S. S. H. and Bartsch, J. A. (1993). Rheological behaviour of frozen and thawed low moisture, part skim mozzarella cheese. *J. Food Sci.* 58(4):764-9.

DiMatteo, M., Chiovitti, G. and Addeo, F. (1982). Changes in mozzarella cheese composition during storage. *Sci. Tech. Latte. Cas.*33:197.

Dryer. (1994). Good news, bad news about light cheeses. Dairy foods® 95(6): 20.

Farkye, N. Y. and Fox, P. F. (1992). Contribution of plasmin to cheddar cheese ripening:effect of added plasmin. *J. Dairy Res.* 59:209-16.

Farkye, N. Y., Kiely, L. J., Allshouse, R. D. and Kindstedt, P. S.(1991). Proteolysis in mozzarella cheese during refrigerated storage. *J. Dairy Sci.*74:1433-8.

Fecera, R. M., McMahon, D. J. and Oberg, C. J. (1995). Effect of exopolysaccharide producing cultures on moisture retention in low fat mozzarella cheese. *IFT Annual Meeting*-Technical Session. p134.

Fife, R. L., McMahon, D. J., Oberg, C. J. and Alleyne, M. C. (1995). Functionality of low fat mozzarella cheese made with commercial fat replacers. *IFT Annual Meeting*-Technical Session. p133.

Fox, P. F., McSweeney, P. L. H. and Singh T. K. (1995). Methods for assessing proteolysis in cheese during maturation. *Chemistry Of Structure Function Relationships In Cheese* eds. Malin, E. L., Tunick, M. H., Plenum press, New York 367: 161- 94.

Green, M. L., Marshall, R. J. and Brooker, B. E.(1985). Instrumental and sensory texture measurement and fracture mechanisms of cheddar and cheshire cheeses. *J. Texture Stud.* 16:351.

Harboe, M. (1998). Coagulants. Research News, Publ. Chr. Hansen, 3:1.

Hassan, A. N., Frank, J. F., Farmer, M. A., Schmidt, K. A. and Shalabi, S. I. (1995a). Observation of Encapsulated Lactic acid bacteria using confocal scanning laser microscopy. *J. Dairy Sci.* 78: 2624-2628.

Hassan, A. N., Frank, J. F., Farmer, M. A., Schmidt, K. A. and Shalabi, S. I. (1995b). Formation of the yogurt microstructure and three dimensional visualization as determined by confocal scanning laser microscopy. *J. Dairy Sci.* 78: 2629-2636.

Hassan, A. N., Frank, J. F., Farmer, M. A., Schmidt, K. A. and Shalabi, S. I. (1996a). Rheological properties of yogurt made with encapsulated non-ropy lactic cultures. *J. Dairy Sci.* 79: 2091-2097.

Hassan, A. N., Frank, J. F., Farmer, M. A., Schmidt, K. A. and Shalabi, S. I. (1996b). Textural properties of yogurt made with encapsulated non-ropy lactic cultures. *J. Dairy Sci.* 79: 2098-2103.

Hassan, A. N. and Frank, J. F. (1997). Modification of microstructure and texture of rennet curd by using a capsule forming non-ropy lactic culture. *J. Dairy Res.* 64: 115-121.

Holsinger, V. H., Smith, P. W. and Tunick, M. H. (1995). Overview: Cheese chemistry and rheology. In *Chemistry Of Structure Function Relationships In Cheese.* Malin, E. L., Tunick, M. H. Plenum press, New York. 367: 1-6.

Jana, A. H., and Upadhyay, K. G. (1991). Mozzarella cheese- a review. *Indian J. Dairy Sci.* 44(2): 167-175.

Kindstedt, P. S. (1993). Effect of manufacturing factors, composition, and proteolysis on the functional characteristics of mozzarella cheese. *Crit. Revs. Food Sci. Nutr.* 33(2):167-87.

Kindstedt, P. S. and Guo, M. R. (1997). Recent Developments in the science and technology of pizza cheese. *Aust. J. Dairy Technol.* 52(1): 41-3.

Kosikowski, F. V.(1982). Cheese and fermented milk foods. Second ed. Publ. Kosikowski, F. V. and Associates, Brooktondale, N.Y.

Lucey, J. A. and Gorry, C. (1993). Effect of Simplesse®100 on the manufacture of low fat cheddar cheese, In Cheese yield and factors affecting its control. *Int. Dairy Fed. Sem.*, Cork, Ireland. 439-447.

Luyten, H. (1988). The rheological and fracture properties of gouda cheese. PhD thesis, Wageningen Agricultural University, The Netherlands.

Malin, E. L., Banks, J. M., Tunick, M. H., Law, A. J. R., Leaver, J. and Holsinger, V. H. (1993). Texture enhancement of low fat mozzarella cheese by refrigerated storage. *Int. Dairy J.* 3(4-6) 548 poster A4.

Mann, E. J. (1992). Mozzarella cheese. *Dairy Ind. Int.* 57(9): 17-8.

Masi, P. and Addeo, F. (1986). An examination of some mechanical properties of a group of Italian cheeses and their relationship to structure and conditions of manufacture. *J. Food Eng.* 5:217-29.

McGinley. (1994). Planned new school lunch guidelines prompt food sellers to think nutrition. *Wall St. J. (Westn.)* June 6 pB1.

McMahon, D. J., Oberg, C. J. and McManus, W. (1993). Functionality of mozzarella cheese. *Aust. J. Dairy Technol.* 48:99-104.

McMahon, D. J., Alleyne, M. C., Fife, R. L. and Oberg, C. J. (1996). Use of fat replacers in low fat mozzarella cheese. *J. Dairy Sci.* 79: 1911-1921.

McSweeney, P. L. H., Olson, N. F., Fox, P. F., Healy, A. and HΦjrup, P. (1993a). Proteolytic specificity of chymosin on bovine αs1-casein. *J. Dairy Res.* 60:401-12.

Merrill, R. K., Oberg, C. J. and McMahon, D. J. (1994). A method for manufacturing reduced fat mozzarella cheese. *J. Dairy Sci.* 77(7): 1783-9.

Mistry, V. V. and Anderson, D. L. (1993a). Composition and microstructure of commercial full fat and low fat cheeses. *Food Struct.* 12: 259-66.

Mistry, V.V. and Anderson, D. L. (1993b). Improving the sensory characteristics of reduced fat cheese. *Amer. Chem. Soc. Nat. Meet. Abst.* 208 part no AGFD 93.

Oberg, C. J., McManus, W. R. and McMahon, D. J. (1993). Microstructure of mozzarella cheese during manufacture. *Food Struct.* 12: 251-8.

Olson, N. F. and Johnson, M. E. (1990). Light cheese products: characteristics and economics. *Food Technol.* 44(10): 93-6.

Pagliarini, E. and Beatrice, N. (1994). Sensory and rheological properties of low fat filled 'Pasta Filata' cheese. *J. Dairy Res.* 61: 299-304.

Prentice, J. H. (1987). Cheese: Chemistry,Physics and Microbiology. vol.1., Fox, P. F. ed., Elsevier applied science publishers, London, 299-344.

Sigsgaard, P. (1994). Defined cultures for pizza cheese. *Scand. Dairy Info.* 8(3): 36-8.

Summerkamp, B. and Hisser, M. (1990). Fat substitute update. *Food Technol.* (3): 92-7.

Tunick, M. H. (1994) Effects of homogenisation and proteolysis on free oil in mozzarella cheese. *J. Dairy Sci.* 77(9): 2487-93.

Tunick, M. H., Holsinger, V. H., Mackey, K. L. and Smith, P. W. (1991). Effects of composition and storage on the texture of mozzarella cheese. *Neth. Milk Dairy J.* 45:117-25.

Tunick, M. H., Mackey, K. L., Shieh, J. J., Smith, P. W., Cooke, P. and Malin, E. L. (1993a). Rheology and microstructure of low fat mozzarella cheese. *Int. Dairy J.* 3: 649-62.

Tunick, M. H., Malin, E. L., Shieh, J. J., Smith, P. W., Sullivan, B. C., Mackey, K. L. and Holsinger, V. H. (1993b). Proteolysis and rheology of low fat and full fat mozzarella cheeses prepared from homogenised milk. *J. Dairy Sci.* 76(12): 3621-8.

Tunick, M. H., Malin, E. L., Smith, P. W. and Holsinger, V. H. (1995a). School lunch pizzas topped with low fat mozzarella cheese. *Cult. Dairy Prod. J.* 30(2):6-9.

Tunick, M. H., Malin, E. L., Smith, P. W. and Holsinger, V. H. (1995b). Effects of skim milk homogenisation on proteolysis and rheology of mozzarella cheese. *Int. Dairy J.* 5: 483-91.

Tunick, M. H., Malin, E. L., Smith, P. W., Cooke, P. H. and Holsinger, V. H. (1995c). Influence of rennet source on proteolysis, rheology and microstructure of low fat mozzarella cheese. *J. Dairy Sci.* 78(suppl.1): 101.

Visser, S., Slangen, K. J., Alting, A. C. and Vreeman, H. J. (1989). Specificity of bovine plasmin in its action on bovine αs2-casein. *Milchwissenschaft.* 44:335-9.

Walstra, P., Noomen, A. and Guerts, T. J. (1987). Cheese:Chemistry, Physics and Microbiology, vol. 2, Fox, P. F. ed. *Elsevier applied science publishers*, London, 45-92.

Yun, J. J., Barbano, D. M. and Kindstedt, P. S. (1993). Mozzarella cheese: impact of coagulant type on chemical composition and proteolysis. *J. Dairy Sci.*76:3648-56.

3.0 TEXTURE EVALUATION OF COMMERCIAL MOZZARELLA CHEESES

3.1 Introduction

Stretched curd cheese such as pizza and mozzarella are widely used on pizzas. Meltability, stretchability and elasticity are the main physical and textural characteristics of mozzarella cheeses which are of importance to pizza functionality while colour, blister size, coverage of blisters, flavour, aroma and free oil liberation during baking are secondary.

Texture profile analysis (TPA) was developed by Szczesniak (1963, 1968). Early studies on texture evaluation were carried out using the General Foods Texturometer by Szczesniak *et al.* (1963) and Kramer and Szczesniak (1973). Nowadays, texture analysis such as hardness, springiness, cohesiveness, chewiness and gumminess of cheeses is carried out using the Instron Universal Testing Machine (Lucisano *et al.*, 1987; Halmos, 1997).

Chen *et al.* (1979) demonstrated a multi-dimensional system of evaluation of hardness, springiness, cohesiveness, chewiness and gumminess based on the composition and pH. Yun *et al.* (1995) observed lower values of hardness, springiness and apparent viscosity during storage when lower draining pH was used. Sunder and Upadhyay (1991) observed a direct relationship between the casein/fat ratio in milk used for cheese manufacture to its textural characteristics. The effects of homogenisation of milk, cooking temperature and time and storage conditions for the finished cheese on the stretch and melt characteristics of mozzarella cheeses have been studied (Cervantes *et al.*, 1983; Jana and Upadhyay, 1991; Tunick *et al.*, 1991; Oberg *et al.*, 1992, 1993; McManus *et al.*, 1993).

A version of this chapter has been published (Bhaskaracharya, R. K. and Shah, N. P. (1999). Aust. J. Dairy Technol. 54:36-40).

Jana and Upadhyay (1991) studied the effect of homogenisation of buffalo milk on the textural characteristics of mozzarella cheese. Homogenisation had a significant effect on hardness, cohesiveness and springiness similar to that observed by Tunick *et al.* (1993).

Low fat mozzarella cheese is becoming increasingly popular. However, reducing fat content in mozzarella cheeses affects their textural and functional characteristics (McGinley, 1994). It was important to ascertain the textural characteristics of the currently available commercial mozzarella cheeses in the context of developing low fat or reduced fat mozzarella cheese using fat replacers with similar textural characteristics as full fat mozzarella cheese. The aim of this study was to examine the textural characteristics including hardness, springiness, cohesiveness, chewiness and gumminess of four commercial mozzarella cheeses in order to understand the variability of such characteristics in relation to their composition.

3.2 Materials and methods

3.2.1 Cheese samples

Four commercial mozzarella cheeses and a cheddar cheese were obtained from the dairy department of a local supermarket and were coded as M1, M2, M3 and M4 for mozzarella cheeses and C1 for the cheddar cheese.

3.2.2 Composition analysis

The fat content was determined for each cheese in triplicate by the modified Babcock method (Kosikowski and Mistry, 1997). The protein content was estimated by the Kjeldahl method in triplicate (Barbano *et al.*, 1990). The moisture content of the cheeses was determined by the oven drying method in triplicate (Egan *et al.*, 1987).

3.2.3 Texture analysis

The cheese blocks were cut into cylindrical specimens measuring 25 x 20 mm at about 20°C using a cheese corer. The specimens were obtained in triplicate from each of the cheese brands and were immediately analysed for their textural characteristics such as hardness, springiness, cohesiveness, chewiness and gumminess. Instron Universal Testing Machine (Model 5564; Instron Ltd., London, England) was used to analyse the textural characteristics of the five brands of cheeses. The samples were compressed to 50, 60 and 80% of their heights using a 500 N load cell with a flat plunger (see Figure 3.2.1) and the crosshead movement was adjusted to 50 mm per minute. Samples were compressed twice while data was collected using a Merlin software. All the analysis was carried out three times.

3.2.4 Statistical analysis

The effects of fat, protein and moisture contents on the textural characteristics of mozzarella and cheddar cheeses were assessed using the multivariate and tests of between-subjects effects analysis and regression equations were formulated using the parameter estimates. The effect of the cheese composition on the textural characteristics was analysed using the one-way analysis of variance. The SPSS version 8.0 was used for statistical analysis (Kirkpatrick and Feeney, 1997). The significance was determined at $P<0.05$. The dependent variables, degrees of freedom and the significance of each factor were measured.

3.3 Results and discussion

3.3.1 Composition

Table 3.3.1 shows the composition of the commercial mozzarella and cheddar cheeses. The fat content of the cheeses varied between 21.6% (M3) to 26.4% (M1) for the mozzarella cheeses and 34% (C1) for the cheddar cheese. The fat in dry matter (FDM) is an important

compositional parameter. The FDM ranged from 46.9% (M4) to 39.6% (M3). According to the Australian Food Standards code (Standard H9) the FDM should be ≥ 40%. The M3 cheese falls slightly below the standard of identity requirement. M1 cheese showed the highest protein content of 32.7%, while M4 cheese had the lowest protein content of 26%. M2 and M3 brands showed similar protein contents. The moisture content varied significantly from 41.6% to 46% for the mozzarella cheeses.

3.3.2 Texture

Compression curves for mozzarella and cheddar cheeses of force plotted against distance obtained using the Instron Universal Testing Machine were used to analyse the textural characteristics of the cheeses. Figure 3.2.1 shows a sample of cheese compressed using Instron Universal testing machine. The sample shown was not compressed as per experimental parameters and hence shows fractures. The hardness, springiness, cohesiveness, chewiness and gumminess of the cheeses were measured and the values compared with those of the cheddar cheese. The hardness, adhesiveness and springiness are directly obtained from the curves. Hardness is the peak value of force exerted by the sample during the first compression for the given set of conditions of compression while adhesiveness is the area of the curve (A3) below the X-axis following the first compression. Hardness and adhesiveness can be measured using a single compression. Springiness is the length by which the sample recovers after the first compression. Cohesiveness is measured as the ratio of area A2 to area A1 (Figures 3.3.1 and 3.3.2). Chewiness is calculated as the product of hardness and cohesiveness while gumminess is the product of chewiness and springiness.

3.3.2.1 Hardness

The hardness of the mozzarella cheeses plotted against three levels of compression is shown in Figure 3.3.3. The hardness of each of the mozzarella cheeses was significantly ($P<0.001$) higher with increased compression from 50 to 80% (Table 3.3.2). At 50% compression, the C1 cheese showed highest hardness followed by M1, M4, M2 and M3 mozzarella cheeses. At 60% compression, M1 showed slightly higher hardness than C1. At 80% compression the cheeses followed a similar trend as that at 50% compression. The cheeses showed a decrease in hardness values as moisture content increased.

C1 cheese had the lowest moisture content (Table 3.3.1) and the highest hardness. Except for M4 cheese, all the other cheeses showed a similar correlation between hardness characteristic and moisture content. Thus increase in moisture for mozzarella cheeses resulted in decreased hardness. These results are comparable to the findings of Olson and Johnson (1990) and Tunick *et al.* (1991).

3.3.2.2Springiness

Figure 3.3.4 shows the springiness of the cheeses plotted against three levels of compression. All the four mozzarella and the cheddar cheeses showed a significantly ($P<0.001$) higher springiness with increased compression from 50 to 80%. At 50% compression, M1 cheese showed highest springiness followed by M4, M2, M3 cheeses and C1 showed the least springiness. At 60% compression, all the cheeses except C1 which showed higher springiness than M3, followed similar order of springiness values as at 50% compression. At 80% compression the M1 cheese showed the highest springiness followed by M2, M4, C1 and M3 cheeses. M3 cheese showed the least springiness at 80% compression.

The springiness can be correlated with the stretchability of mozzarella cheeses. The protein network in mozzarella cheeses gives resilience to cheese structure when external forces are applied. Three of the four mozzarella cheeses showed higher springiness as compared to the cheddar at the three levels of compression. M3 cheese showed the lowest springiness at 60 and 80% compression levels, possibly due to its lower protein and higher moisture contents (Table 3.3.1).

The fat content of the mozzarella cheeses affected the springiness significantly (P=0.001, Table 3.3.2). The M1 cheese had the highest fat content (on wet basis) and exhibited the highest springiness, followed by M4, M2 and M3. Thus fat seemed to play a major role in determining springiness than any other textural characteristic (Olson and Johnson, 1990). Although C1 had the highest fat content (Table 3.3.1), it showed very low springiness.

The effects of protein and moisture were significant and possibly played a role in determining springiness of the cheeses. At 80% compression, M2 cheese with higher protein content than M4 or M3 showed higher springiness.

3.3.2.3 Cohesiveness

The cohesiveness of the mozzarella cheeses plotted against three levels of compression is shown in Figure 3.3.5. Cohesiveness was significantly (P<0.001) affected by compression. At 50% compression M1 cheese showed highest cohesiveness followed by M3, M2, M4 and C1 cheeses. The C1 cheese continued to show very low values of cohesiveness at 60 and 80% compressions. Among the mozzarella cheeses M3 showed the highest cohesiveness at 60% which was followed by M1, M2, and M4 cheeses. At 80% compression the M3 cheese showed the highest cohesiveness followed by M2, M1 and M4 cheeses. Thus among the

mozzarella cheeses M4 consistently showed low values of cohesiveness at 50, 60 and 80% compressions. The mozzarella cheeses showed higher cohesiveness at the three levels of compression than the cheddar cheese. This may be due to the ripening process, which results in breakdown of proteins in cheddar cheeses. In contrast the mozzarella cheeses with an intact protein network show a greater resistance when external force is applied. This results in increased cohesiveness.

The protein and fat contents significantly ($P<0.05$) affected the cohesiveness of cheeses (Table 3.3.2). The effect of moisture on cohesiveness was non-significant. The cheeses with higher protein content showed higher cohesiveness. The M1 cheese had the highest protein content (Table 3.3.1) and cohesiveness, while the M4 cheese had the lowest protein content and cohesiveness at 50% compression. The M2 and M3 cheeses had similar protein contents and cohesiveness; however, the M3 cheese with lower protein content than the M2 cheese showed higher cohesiveness at the three levels of compression. The cheddar cheese had the highest fat content and least cohesiveness at the three levels of compression. Hence the fat content of the cheeses along with the protein content appeared to play an important role in determining the cohesiveness characteristic of cheeses.

3.3.2.4 Chewiness

Figure 3.3.6 shows the chewiness plotted against three levels of compression. Among the five cheeses, M1 showed highest chewiness at 50, 60 and 80% compressions. At 50% compression M1 cheese was followed by M4, C1, M2 and M3. At 60% compression, M1 cheese showed the highest chewiness followed by M4, M2, M3 and C1. At 80% compression, M1 cheese showed the highest chewiness followed by M2, M3 and M4 cheeses. C1 cheese showed the lowest chewiness at 80% compression.

Although chewiness showed a significant (P=0.001) effect due to the protein content of the cheeses (Table 3.3.2), a clear correlation between chewiness and the composition of the cheeses could not be made.

3.3.2.5 Gumminess

Figure 3.3.7 shows gumminess plotted against three levels of compression. Among the five cheeses M1 showed the highest gumminess at 50, 60 and 80% compression levels. At 50% compression, M1 cheese showed the highest gumminess followed by C1, M4, M2 and M3. At 60% compression, the gumminess was in the following order: M1, M2, M4, M3 and C1. At 80% compression, M1 showed the highest gumminess followed by M2, M3, M4 and C1. Gumminess showed a significant effect (P<0.001) due to protein but correlation between them could not be made.

3.4 Conclusions

The composition of the cheeses influenced the textural characteristics. The increase in moisture content decreased hardness while the increase in fat content increased springiness. The increase in protein content increased cohesiveness. The M1 cheese showed the highest hardness and springiness among the four mozzarella cheeses. The M3 cheese had the highest cohesiveness and the lowest springiness due to its low fat content which is below the Food Standards code requirements for a full fat mozzarella cheese.

3.5 References

Barbano, D.M., Clark, J.L., Dunham, C.E. and Fleming, J.R. (1990). Kjeldahl method for determination of total nitrogen content of milk: collaborative study. *J. Assoc. Anal. Chem.* 73(6):849-859.

Cervantes, M.A., Lund, D.B. and Olson, N.F. (1983). Effects of salt concentration and freezing on mozzarella cheese texture. *J. Dairy Sci.* 66:1219-27.

Chen, A.H., Larkin, J.W., Clark, C.J. and Irwin, W.E. (1979). Textural analysis of cheese. *J Dairy Sci.* 62(6):901-907.

Egan, H., Kirk, R.S. and Sawyer, R. (1987). Pearson's Chemical Analysis of Foods. Eighth ed., Harlow: Longman Scientific and Technical Publications.

Halmos, A.L. (1997). Food texture and sensory properties of dairy ingredients. *Food Aust.* 49(4):169-173.

Jana, A.H. and Upadhyay, K.G. (1991). The effects of homogenisation conditions on the textural and baking characteristics of buffalo milk mozzarella cheese. *Aust. J. Dairy Technol.* 46(1):27-30.

Kirkpatrick, L.A. and Feeney, B.C. (1997). A simple guide to SPSS for windows: versions 6.0, 6.1, 7.0 and 7.5. Intl. Thomson Publ. Co., California, USA.

Kosikowski, F.V. and Mistry, V.V. (1997). Cheese and Fermented Milk Foods. Third edition, AVI Publishing Company, Westport, CT.

Kramer, A. and Szczesniak, A.S. (1973). Psychological, fundamental, sensory, mechanical and chemical procedures and their interrelationships In Texture measurements of foods, Reidel Dordrecht, Holland.

Lucisano, M., Pompei, C. and Casiraghi, E. (1987). Texture evaluation of some Italian cheeses by instrumental texture profile analysis. *J. Food Quality* 10:73-89.

McGinley, L. (1994). Planned new school lunch guidelines prompt food sellers to think nutrition. *Wall Street J. (Western)* June 6 pB1.

McManus, W., Oberg, C.J. and McMahon, D.J. (1993). Functionality of mozzarella cheese. *Aust. J. Dairy Technol.* 48(2):99-104.

Oberg, C.J., McManus, W.R. and McMahon, D.J. (1993). Microstructure of mozzarella cheese during manufacture. *Food Structure* 12:251-8.

Oberg, C.J., Merrill, R. K., Brown, R.J. and Richardson, G.H. (1992). Effects of freezing, thawing and shredding on low moisture part skim mozzarella cheese. *J. Dairy Sci.* 75:1161-6.

Olson, N.F. and Johnson, M.E. (1990). Light cheese products: Characteristics and economics. *J. Food Technol.* 44:93-96.

Sundar, M. R. and Upadhyay, K. G. (1991). Influence of casein/fat ratio of milk on baking, rheological and sensory characteristics of buffalo milk mozzarella cheese. *J. Food Sci. Technol.* 28(2):98-100.

Szczesniak, A.S. (1963). Classification of textural characteristics. *J. Food Sci.* 28:385-389.

Szczesniak, A.S. (1968). Correlations between objective and sensory texture measurements. *Food Technol.* 22:49-54.

Szczesniak, A.S., Brandt, M.A. and Friedman, H.H. (1963). Development of standard rating scales for mechanical parameters of texture and correlation between the objective and the sensory methods of texture evaluation. *J. Food Sci.* 28:397-403.

Tunick, M.H., Mackey, K.L., Smith, P.W. and Holsinger, V.H. (1991). Effects of composition and storage on the texture of mozzarella cheese. *Neth. Milk Dairy J.* 45:117-125.

Tunick, M.H., Malin, E.L., Smith, P.W., Sheih, J.J., Sullivan, B.C., Mackey, K.L. and Holsinger, V.H. (1993). Proteolysis and rheology of low fat and full fat mozzarella cheeses prepared from homogenized milk. *J. Dairy Sci.* 76(12):3621-3628.

Yun, J. J., Barbano, D.M. and Kindstedt, P.S. (1995). Mozzarella cheese: impact of whey pH at draining on chemical composition, proteolysis and functional properties. *J. Dairy Sci.*78:1-7.

Table 3.3.1. The means and standard deviation (n=3) of fat, protein and moisture contents in commercial mozzarella and cheddar cheeses.

Sample	Fat		Protein		Moisture	
	Mean (%)	SD	Mean (%)	SD	Mean (%)	SD
Mozzarella						
M1	26.40	0.42	32.70	0.71	41.60	0.96
M2	23.70	0.99	27.85	0.35	44.20	0.80
M3	21.60	0.28	27.20	0.57	45.43	1.48
M4	25.30	0.42	26.00	0.28	46.00	0.62
Cheddar						
C1	34.00	0.33	24.00	1.56	33.20	0.36

Table 3.3.2. The effects of fat, protein, moisture and compression on hardness, springiness, cohesiveness, chewiness and gumminess

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	HARDNESS	245.147[a]	5	49.029	36.409	.000
	SPRINGIN	26.846[b]	5	5.369	35.525	.000
	COHESIVE	.515[c]	5	.103	44.008	.000
	CHEWINES	20.530[d]	5	4.106	4.581	.017
	GUMMINES	2475.521[e]	5	495.104	7.558	.003
Intercept	HARDNESS	8.637	1	8.637	6.414	.028
	SPRINGIN	1.898	1	1.898	12.555	.005
	COHESIVE	1.941E-03	1	1.941E-03	.829	.382
	CHEWINES	3.840	1	3.840	4.284	.063
	GUMMINES	505.642	1	505.642	7.719	.018
FAT	HARDNESS	25.845	1	25.845	19.193	.001
	SPRINGIN	2.993	1	2.993	19.801	.001
	COHESIVE	1.319E-02	1	1.319E-02	5.632	.037
	CHEWINES	2.849	1	2.849	3.179	.102
	GUMMINES	346.103	1	346.103	5.284	.042
PROTEIN	HARDNESS	13.658	1	13.658	10.142	.009
	SPRINGIN	7.022	1	7.022	46.459	.000
	COHESIVE	3.632E-02	1	3.632E-02	15.510	.002
	CHEWINES	16.125	1	16.125	17.990	.001
	GUMMINES	1985.556	1	1985.556	30.311	.000
MOISTURE	HARDNESS	2.958	1	2.958	2.197	.166
	SPRINGIN	2.261	1	2.261	14.957	.003
	COHESIVE	1.976E-05	1	1.976E-05	.008	.928
	CHEWINES	1.812	1	1.812	2.022	.183
	GUMMINES	227.129	1	227.129	3.467	.090
COMPRESS	HARDNESS	136.527	2	68.264	50.693	.000
	SPRINGIN	18.427	2	9.214	60.961	.000
	COHESIVE	.290	2	.145	61.858	.000
	CHEWINES	2.584	2	1.292	1.441	.278
	GUMMINES	286.634	2	143.317	2.188	.159
Error	HARDNESS	14.813	11	1.347		
	SPRINGIN	1.663	11	.151		
	COHESIVE	2.576E-02	11	2.342E-03		
	CHEWINES	9.860	11	.896		
	GUMMINES	720.562	11	65.506		
Total	HARDNESS	1705.749	17			
	SPRINGIN	1213.229	17			
	COHESIVE	3.706	17			
	CHEWINES	227.494	17			
	GUMMINES	17460.924	17			
Corrected Total	HARDNESS	259.960	16			
	SPRINGIN	28.508	16			
	COHESIVE	.541	16			
	CHEWINES	30.390	16			
	GUMMINES	3196.082	16			

a. R Squared = .943 (Adjusted R Squared = .917)

b. R Squared = .942 (Adjusted R Squared = .915)

c. R Squared = .952 (Adjusted R Squared = .931)

d. R Squared = .676 (Adjusted R Squared = .528)

e. R Squared = .775 (Adjusted R Squared = .672)

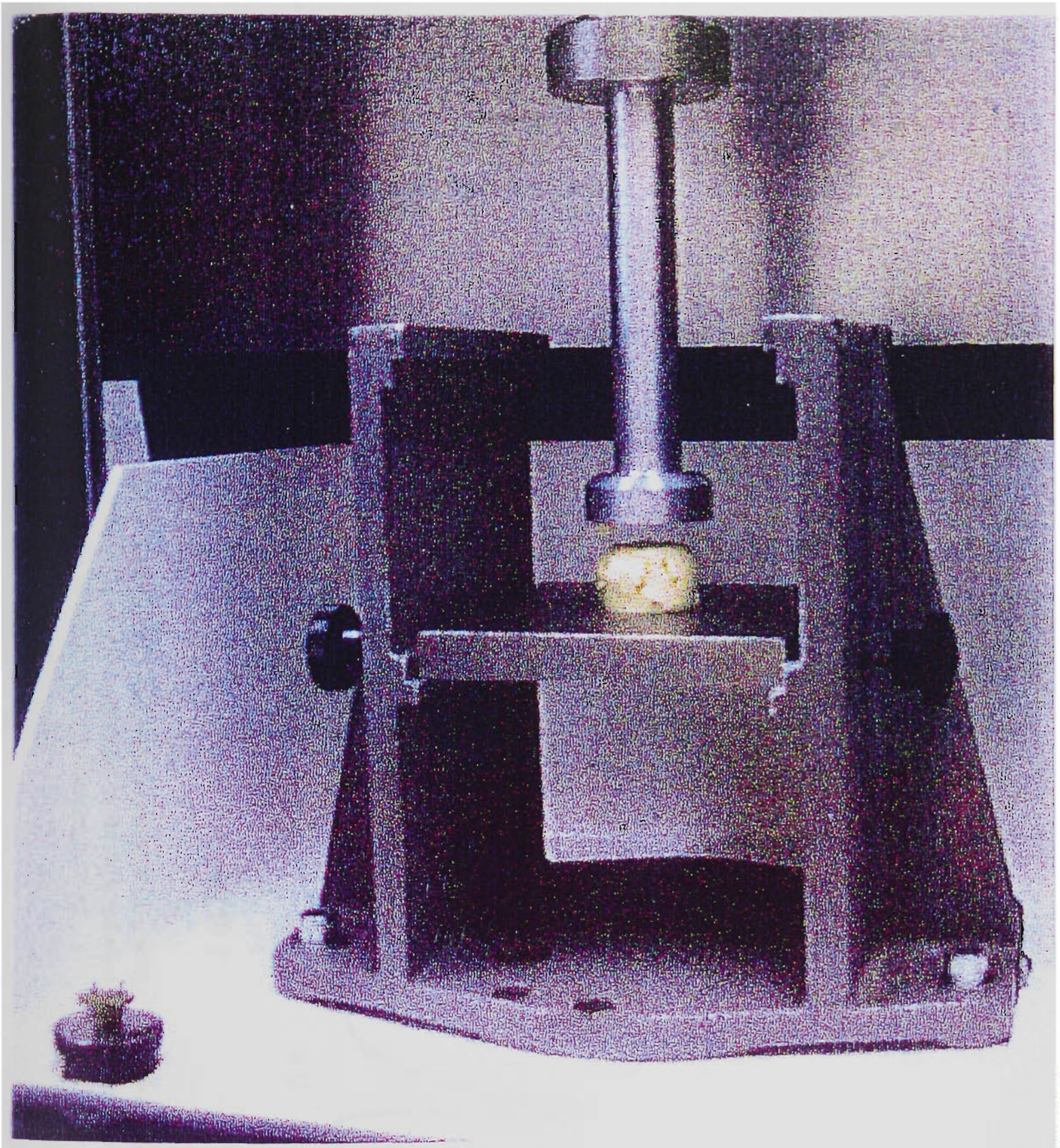

Figure 3.2.1: A mozzarella cheese specimen being compressed under a flat plunger using Instron Universal Testing Machine.

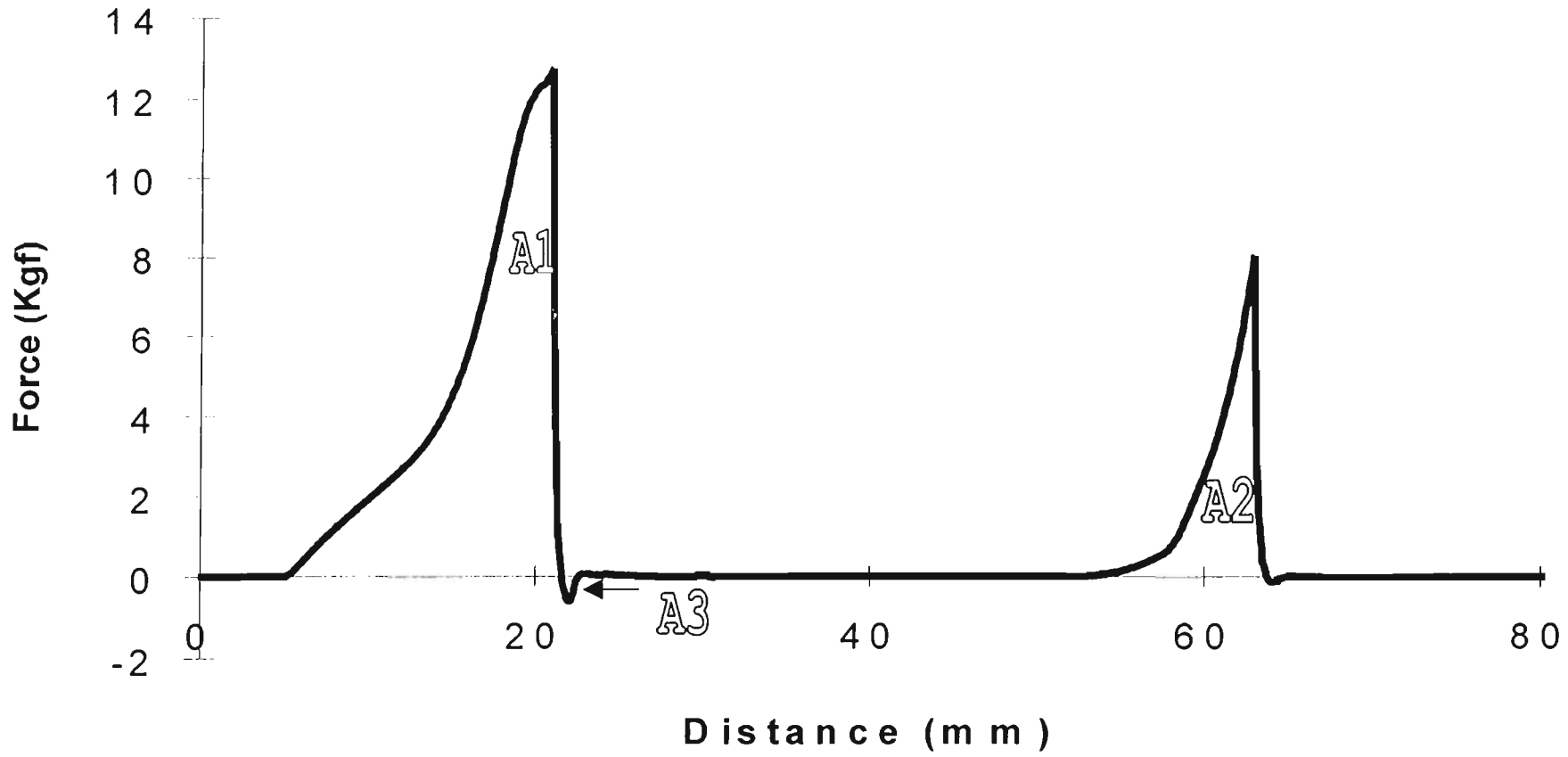

Figure 3.3.1. A typical texture profile curve obtained by double compression of mozzarella cheese specimen and plotting force (Y-axis) and distance (X-axis). A1 and A2 are the areas of curves obtained by the first and second compression, respectively, while A3 is the area of curve obtained during relaxation of the specimen after the first compression.

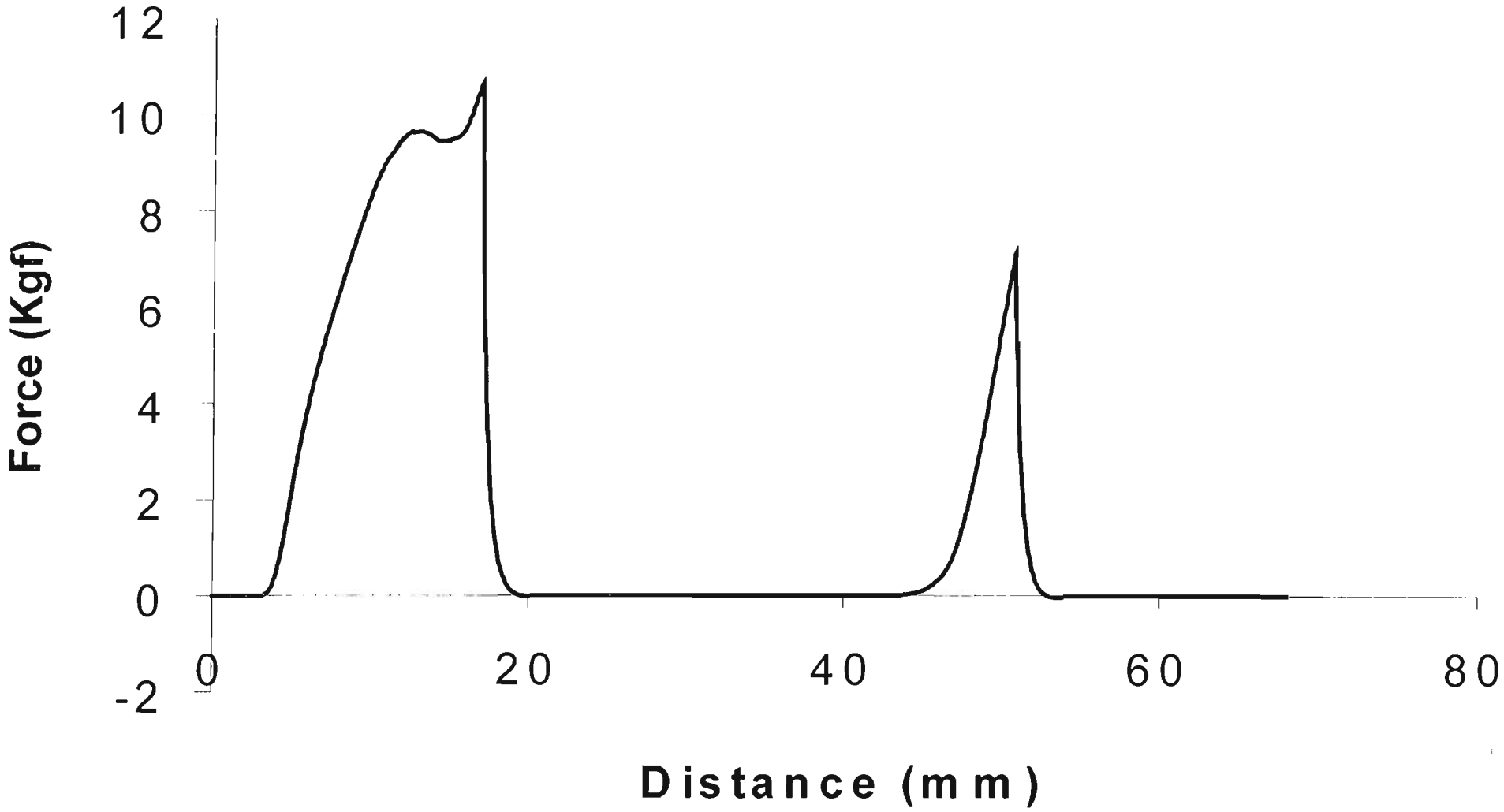

Figure 3.3.2. A typical texture profile curve obtained by double compression of cheddar cheese specimen and plotting force (Y-axis) and distance (X-axis). A1 and A2 are the areas of curves obtained by the first and second compression, respectively.

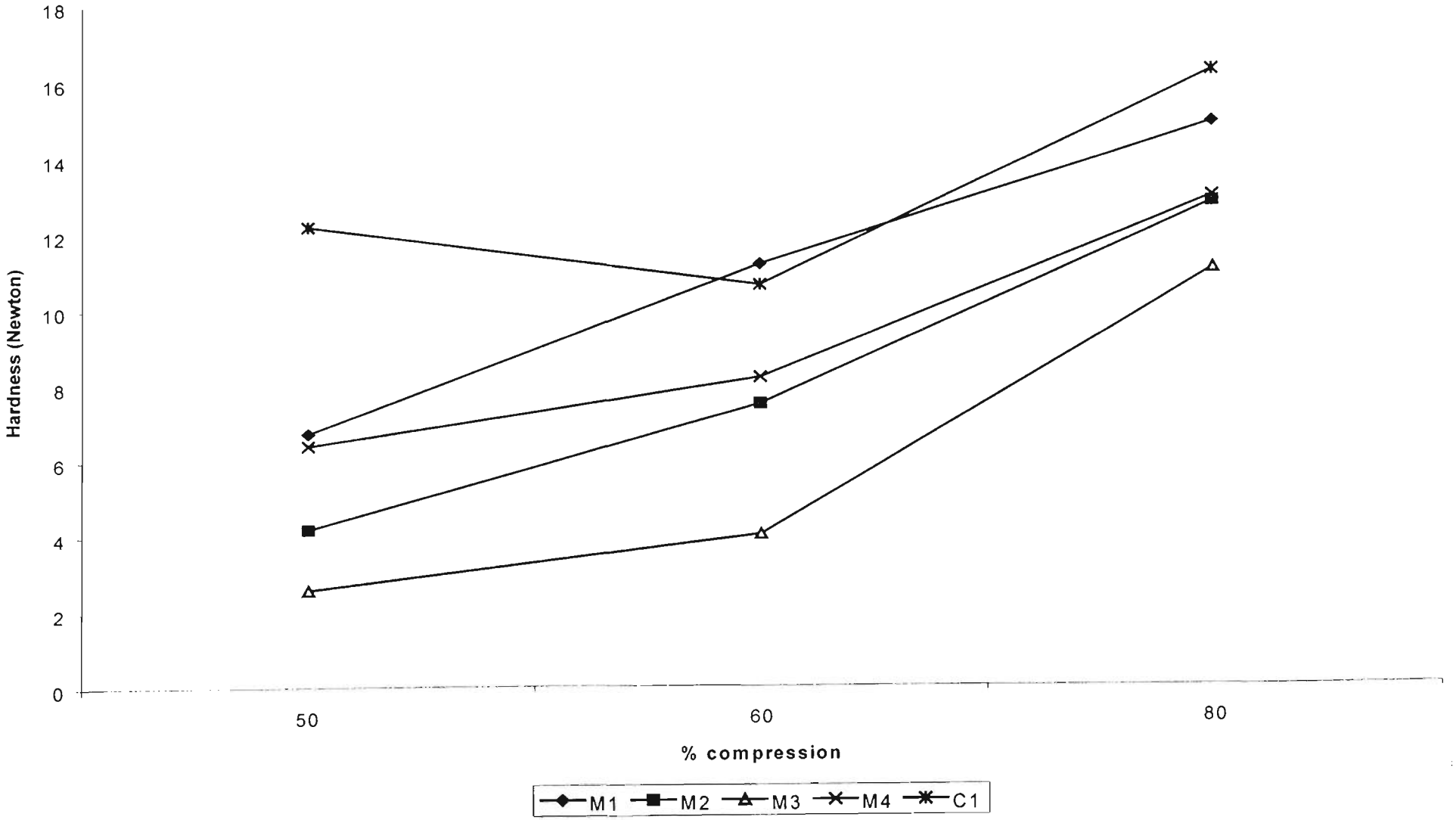

Figure 3.3.3. Effect of level of compression of the commercial mozzarella and cheddar cheeses on hardness.

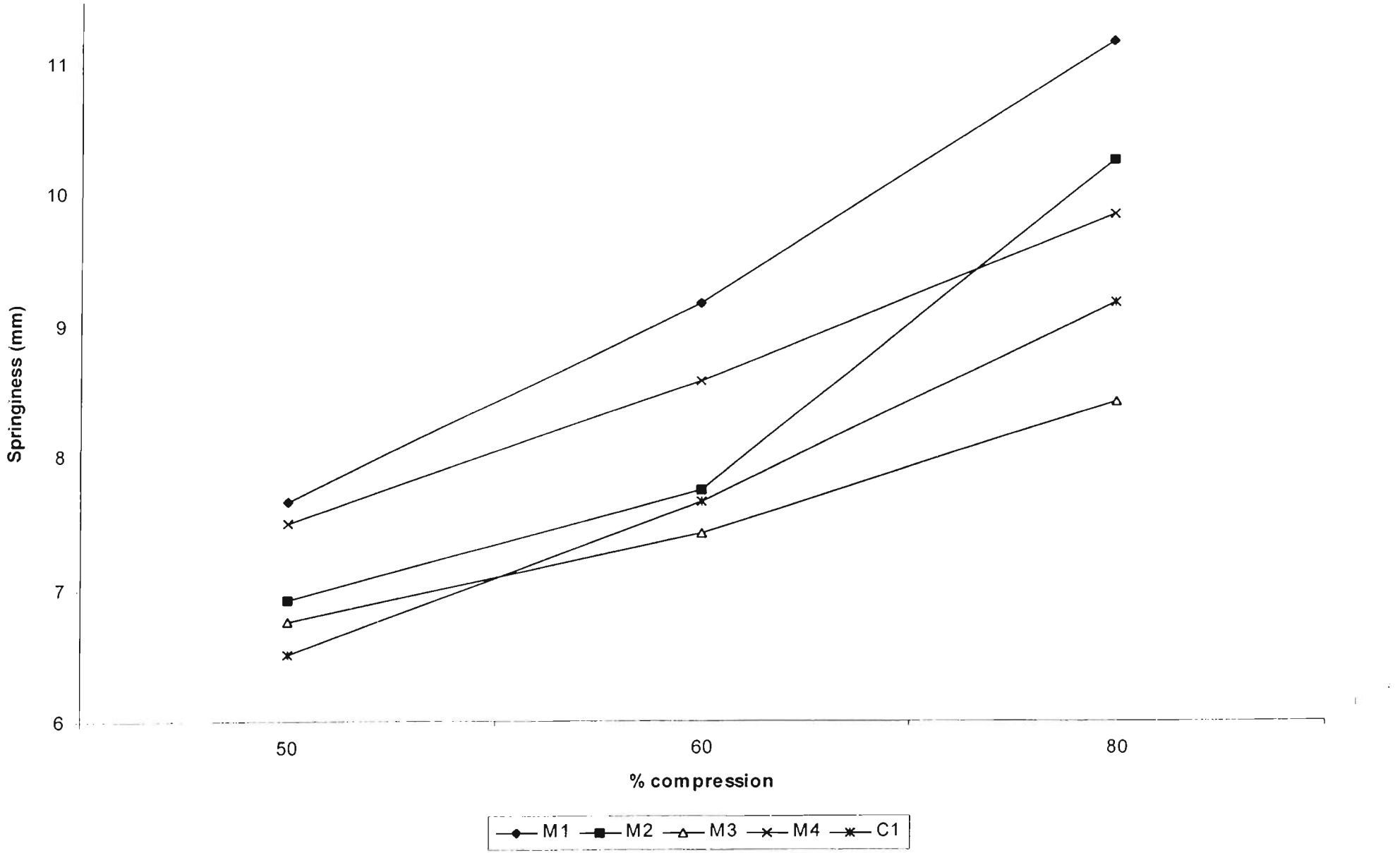

Figure 3.3.4. Effect of level of compression of the commercial mozzarella and cheddar cheeses on springiness.

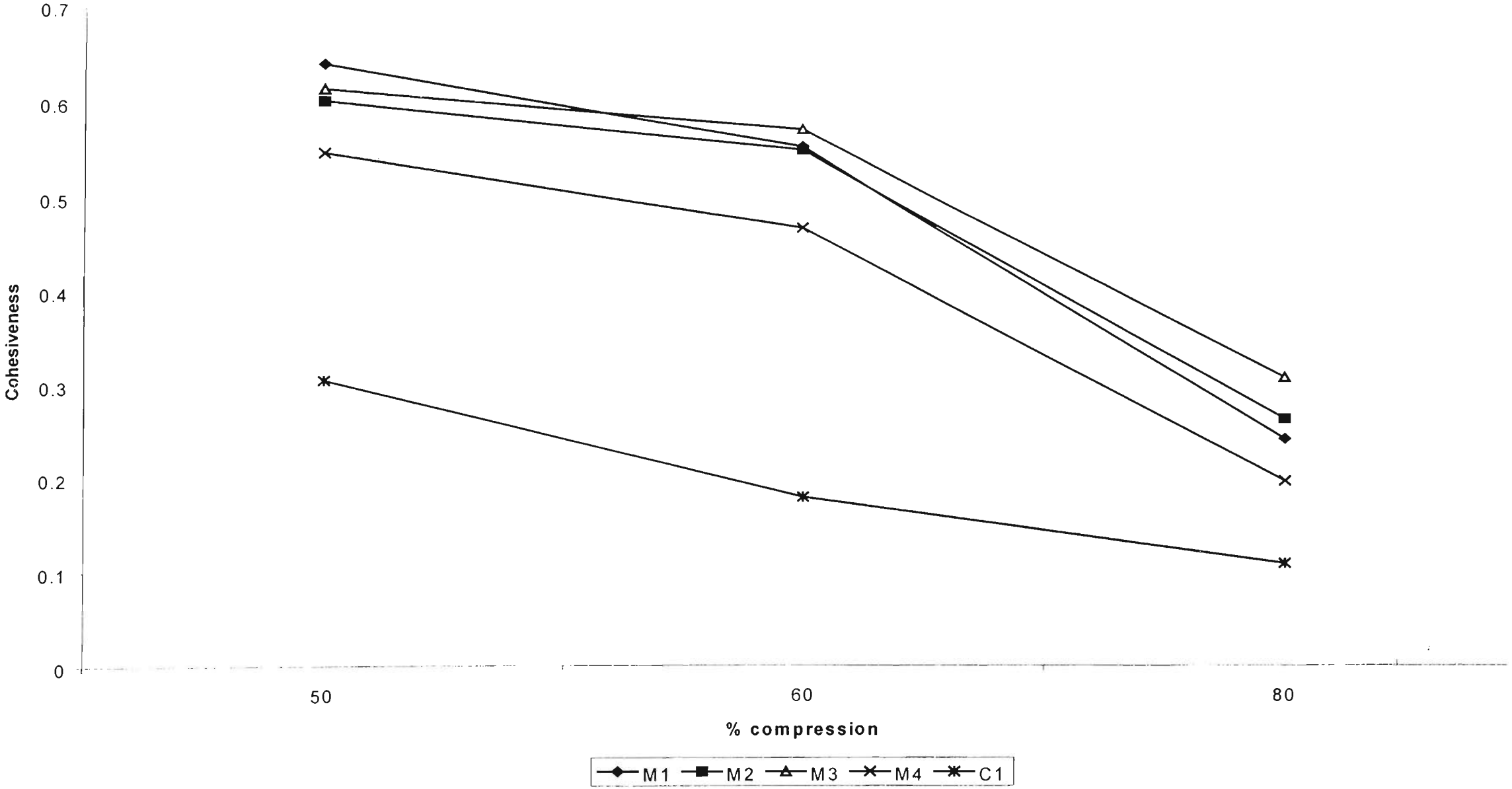

Figure 3.3.5. Effect of level of compression of the commercial mozzarella and cheddar cheeses on cohesiveness.

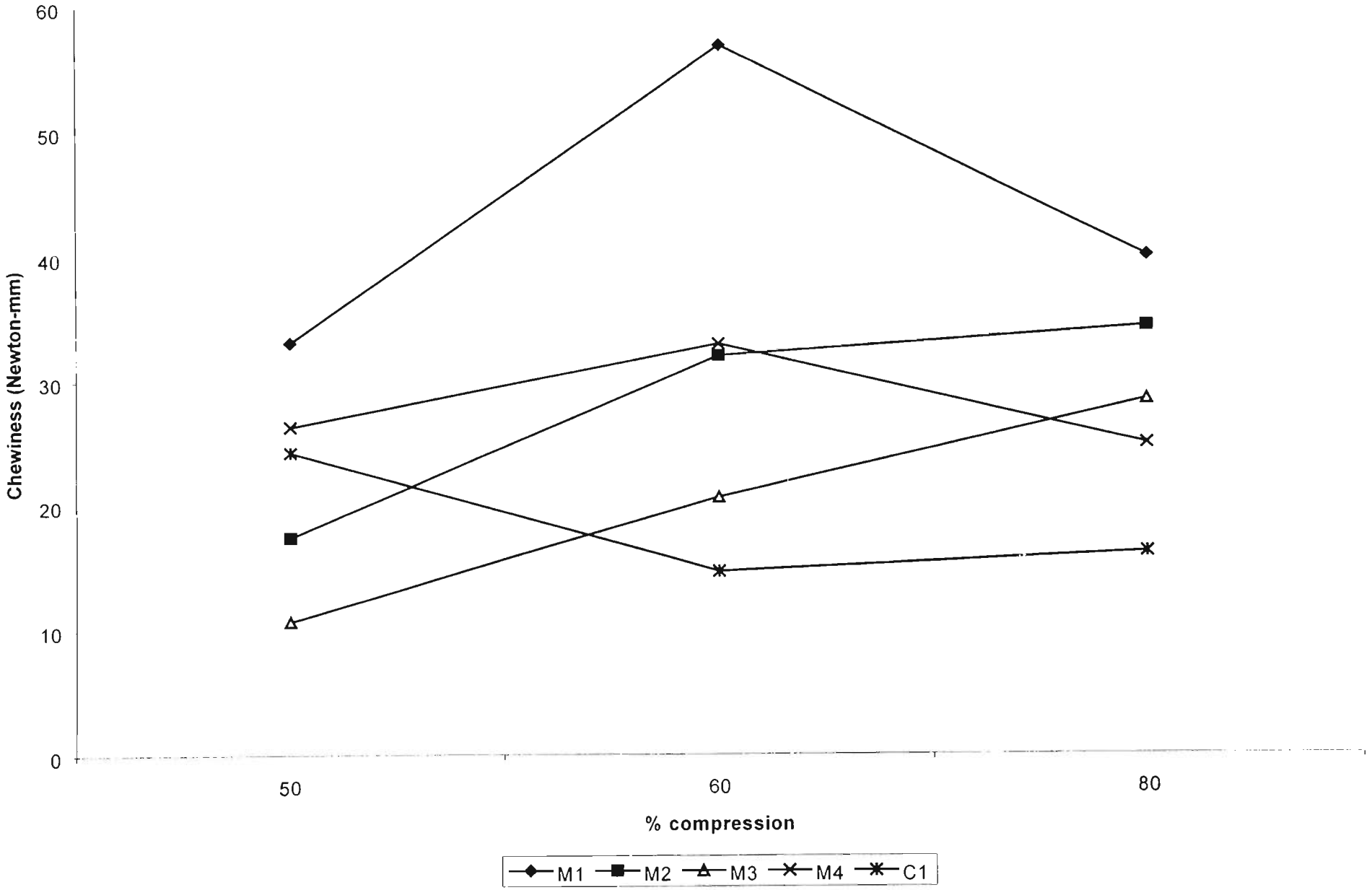

Figure 3.3.6. Effect of level of compression of the commercial mozzarella and cheddar cheeses on chewiness.

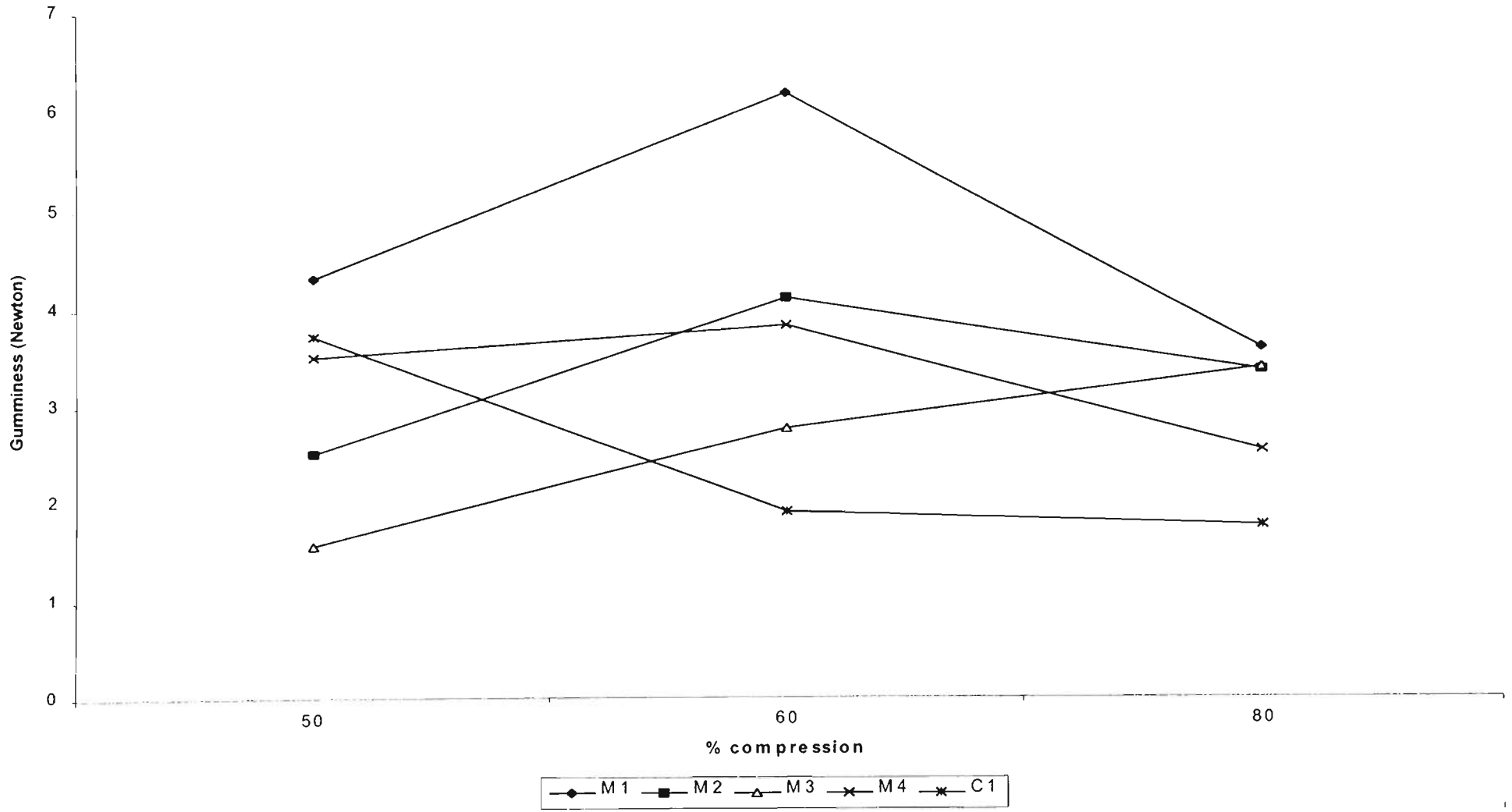

Figure 3.3.7. Effect of level of compression of the commercial mozzarella and cheddar cheeses on gumminess.

4.0 MICROSTRUCTURE OF MOZZARELLA CHEESES MADE WITH OR WITHOUT EXOPOLYSACCHARIDE PRODUCING STARTER CULTURES

4.1 Introduction

Mozzarella cheese is a semi-soft and fresh variety of stretched curd cheese (Fox and Guinee, 1987). McManus *et al.* (1993), Kalab (1995), McMahon (1995) and several others have studied microstructure of mozzarella cheeses. Carroll *et al.* (1968) observed, for the first time, that glutaraldehyde preserved the micelle structure in milk better than osmium tetroxide and formaldehyde. Taranto *et al.* (1979) studied the milk fat globule distribution and structure of protein matrix in cheddar and mozzarella cheeses. Schmidt and Buchheim (1992) used cryo-fixation techniques such as freeze fracturing and cryo-microscopy in order to study the sub-microscopic structures and physical properties of several dairy products. McManus *et al.* (1993) developed a specimen treatment protocol to study the microstructure of mozzarella cheese. The specimens were cryofractured, treated with potassium ferrocyanide, tannic acid and hydroquinone. Using this method of specimen preparation, good images of the internal structures of the cheeses could be obtained.

Kalab (1993) compared the structures of several cheeses including hard, mould-ripened, cream and process cheeses along with other dairy products such as cream and milk powders and reported the causes of various defects and aberrations formed during and after the specimen preparation. Mozzarella cheeses exhibited a compact protein matrix unlike an open fibrous protein matrix in cheddar cheese. Kalab (1995) studied the microstructure details of curd

---

A version of this chapter has been published (Bhaskaracharya, R. K. and Shah, N. P. (2000) Aust. J. Dairy Technol. 55(1): 28-32.

components and orientation of protein strands in mozzarella cheeses using Scanning Electron Microscope (SEM). McMahon (1993) used a similar method of specimen preparation as McManus *et al.* (1993) to study the microstructure of mozzarella cheese at various stages of its manufacture in order to correlate with the changes in the functional properties. Cooke *et al.* (1995) studied the electron density patterns of mozzarella cheeses and postulated a means to differentiate between fresh and aged cheeses based upon the size and distribution of fat globules in the cheese matrix.

Fecera *et al.* (1995) used exopolysaccharide (EPS) producing starter cultures to manufacture mozzarella cheeses and reported an increase in moisture retained in these cheeses. Similar studies have been carried out using EPS producing starter cultures by Low *et al.* (1997) and Perry *et al.* (1997, 1998). In these studies capsular EPS producing starter cultures were used in manufacturing mozzarella cheeses and the effect of EPS on the functional characteristics of the cheese including melt and stretch were examined. But the effect of EPS producing starter cultures on the microstructure of the mozzarella cheeses has not been studied. Due to this reason the changes in microstructure of full fat mozzarella cheeses made using EPS or non-EPS producing starter cultures have been examined in this study.

The methods used for specimen preparation by McManus *et al.* (1993) and McMahon (1995) have repetitive steps of dehydration and re-hydration along with a lengthy procedure using Freon to de-fat the specimens, and tannic acid and hydroquinone solution for metal impregnation. In this study such steps have been eliminated to make specimen preparation less tedious and time consuming. A simplified method of specimen preparation has been developed

based on the method of McManus *et al.* (1993) to examine the microstructure of mozzarella cheeses made with or without exopolysaccharide producing starter cultures.

4.2 Materials and methods

4.2.1 Preparation of full fat mozzarella cheese

Full cream milk was obtained from a local cheese factory and the cream was separated using a batch type cream separator (Model 107 AE, Alfa Laval, Sweden). The skim milk contained 0.1 to 0.2% fat, and the cream 35%. The skim milk was pasteurised at 72°C for 15 sec using a pilot scale HTST pasteuriser (Alfa Laval, Sweden) and the cream at 65°C for 15 min using batch method. The milk was standardised to 4% fat by mixing skim milk and cream in appropriate proportions. The standardised milk was tempered to 30°C in a 30 litre capacity cheese vat. The EPS producing *Streptococcus thermophilus* (St 2010) and *Lactobacillus delbruckeii* ssp. *bulgaricus* (Lb 2515) obtained from the VUT Culture Collection (Victoria University of Technology, Werribee, Australia) were added at the rate of 1% each. The milk was warmed to 35°C and this temperature was maintained throughout the cheese making process until stretching of the curd. When the pH dropped by 0.1 unit, rennet enzyme (Chymosin, 145 IMCU, Chr. Hansen, Bayswater, Australia) was added at the rate of 2.5mL per 10L of milk after diluting in 100 mL of distilled water. The milk coagulated in 35 min. The curd was cut using cheese knives to 1 cubic cm size and the curd held in the whey till the pH reached 5.6 followed by draining of the whey. The curd was piled and re-piled every 15 min until the pH 5.2 was reached. The curd was stretched in 70°C hot water, moulded, cooled under running tap water and placed in a saturated brine solution at 4°C for 2 h. The finished cheese was packed under vacuum and stored at 4°C. The experiment was repeated three times. Similarly three batches of cheeses were made using non-EPS producing *Streptococcus thermophilus* and

Lactobacillus delbruckeii ssp. *bulgaricus* (Chr. Hansen, Bayswater, Australia). The microstructure was examined at 28 d of storage.

4.2.2 Specimen preparation for microstructure

Specimens were prepared from mozzarella cheeses using a simplified method specifically developed for this study by modifying and eliminating the following steps from the method described by McManus *et al.* (1993) without detrimental effect on images obtained:

1. Specimens have been fractured at room temperature (~20°C) instead of cryofracturing.
2. Specimens have not been defatted using Freon.
3. The repetitive steps of dehydrating and re-hydrating have been avoided.
4. Metal impregnation with tannic acid and hydroquinone solution has not been used.
5. Sputter coating of the specimens has been carried out using gold instead of iridium.

The finished cheeses were allowed to equilibrate at room temperature (~20°C) and specimens of approximately 2 x 2 x 10 mm size were cut from several parts of the cheeses. In this study, the specimens were fixed using 2% glutaraldehyde made in 0.1M cacodylate buffer solution for 1 h at room temperature (~20°C) and for 2 days at 4°C, similar to the method described by McManus *et al.* (1993). However, the specimens were not defatted with Freon 113 and the steps involving dehydrating and rehydrating the specimens as reported by McManus *et al.* (1993) were avoided. The specimens fixed in glutaraldehyde were then washed in 0.1M cacodylate buffer (pH 7.3) for 5 min, dehydrated gradually using ethanol at 30, 50, 70, 95 and 100%, each for 5 min, washed in cacodylate buffer for 5 min and post-fixed overnight in 1.5% osmium tetroxide solution made in distilled water. These steps are similar to those used in the study of Rousseau (1988). The fixed specimens were washed in distilled water twice each for 10 min, and further dehydrated with 30, 50, 70, 95 and 100% ethanol, each for 5 min and in 100% acetone for 5 min. The dehydrated specimens were dried in Critical Point Drying (CPD)

equipment (custom made, Melbourne University, Melbourne, Australia) using liquid carbon dioxide. The dried specimens were fractured at room temperature (~20°C), mounted on aluminium stubbs, sputter coated with gold using Edwards Sputter Coater and observed under Philips SEM515 scanning electron microscope at 20kV.

4.3 Results and discussion

The procedure used in this study is suitable to prepare specimens from mozzarella cheese and probably other cheeses as well to examine their internal structures at the microscopic level. Although the method is simple and less time consuming no detrimental effect is seen in the SEM images of the cheese specimens and the fine details including surface of microorganisms have good clarity. The images obtained from the triplicate batches of mozzarella cheeses made using EPS or non-EPS producing starter cultures showed similar microstructure and typical ones among them which exhibited most of the attributes are shown.

Figure 4.3.1 shows the internal microstructure of the cheese made using non-EPS producing starter bacteria at about 9500 times magnification. The compact protein structures are clearly seen. Large voids (Figure 4.3.1a) possibly formed due to removal of serum are also seen. The smooth surfaced small voids measuring 1-2 μm are possibly formed due to removal of fat (Figure 4.3.1b), similar to those obtained by Schmidt and Buchheim (1992). The image is similar to those reported in the study of Oberg *et al.* (1993).

Figure 4.3.2 shows the details of the internal structure of the mozzarella cheese made using non-EPS producing starter cultures. Large voids (Figure 4.3.2a) of about 15 μm in diameter were possibly formed due to removal of the serum portion of the cheese. A long void running

almost through the specimen showing extensions of the protein matrix is seen. The starter bacteria (Figure 4.3.2b) are seen to propagate in the voids.

The EPS produced in the mozzarella cheese (Figure 4.3.3) is observed as filaments extending from the microorganisms to the protein matrix. These filaments seem delicate and thin measuring about 0.01-0.05 μm in diameter (Figures 4.3.3a and 4.3.4a). Such filamentous EPS was found primarily associated with the streptococci (Figures 4.3.4b and 4.3.5b) in the cheeses made using EPS producing starter cultures. Studies by Fecera *et al.* (1997), Low *et al.* (1997) and Perry *et al.* (1997, 1998) have not found such filamentous EPS because the cultures used in those studies produced capsules rather than loosely bound EPS. Such filamentous structures were absent in the control cheeses (cheeses made using non-EPS producing starter cultures) as expected.

The streptococci appeared to occur in chains and were found more abundantly (Figure 4.3.3b) than the lactobacilli (Figures 4.3.5a and 4.3.6d), although the initial levels of addition for both the organisms were similar. Both the starter bacteria seem to propagate in the serum channels (Figure 4.3.5c). The cheeses made using EPS producing starter cultures showed an open body which was interspersed with large and small voids possibly causing the mozzarella cheeses to have improved meltability (Fecera *et al.*, 1995). Similar improvements in functionality of mozzarella cheeses reported by Perry *et al.* (1997) could be due to the changes in microstructure caused by the EPS producing starter cultures. The increase in moisture retained in the mozzarella cheeses made using EPS producing starter cultures (Low *et al.*, 1997) is possibly due to the EPS produced in the cheeses that has higher retention capacity for moisture. The mozzarella cheese made using non-EPS producing streptococci and lactobacilli, showed fewer voids (Figure 4.3.6b) and the streptococci are seen to propagate closer to the fat globules

(Figure 4.3.6c). The surfaces of non-EPS producing streptococci (Figure 4.3.6a) appear smooth. The lactobacilli were seen in fewer numbers in the cheeses made using non-EPS producing starter cultures.

These results show that with a simple method of specimen preparation as adopted in this study, equally good images of mozzarella cheeses were obtained. The procedure used in this study is short and simple and does not cause any artifacts or aberrations in the specimen (Kalab, 1984). The procedure uses only the basic steps of fixing in glutaraldehyde, dehydrating, post fixation using osmium tetroxide followed by further dehydration, critical point drying and sputter coating of the specimens. There are no steps, which have been repeated, and thus it makes it easy to process the specimens and obtain clear images of mozzarella cheese internal structure.

4.4 Conclusions

The simplified method of specimen preparation described in this study was found to be suitable for examination of microstructure and provided good images of the mozzarella cheese microstructure. The minute details of the internal structure and the delicate structures of exopolysaccharide within the mozzarella cheeses were found to be well preserved. The compact protein matrix and voids of serum and fat phase were clearly observed. The exopolysaccharide producing streptococci and lactobacilli seemed to propagate in the serum channels. The streptococci seemed to produce large amounts of delicate, filamentous exopolysaccharide as compared with the lactobacilli. The non-exopolysaccharide producing streptococci had smooth surfaces and appeared to propagate closer to the fat globule surfaces than the serum phase.

4.5 References

Carroll, R.J., Thompson, M.P. and Nutting, G.C. (1968). Glutaraldehyde fixation of casein micelles for electron microscopy. *J. Dairy Sci.* 51(12):1903-8.

Cooke, P.H., Tunick, M.H., Malin, E.L., Smith, P.W. and Holsinger, V.H. (1995). Electron-density patterns in low-fat mozzarella cheeses during refrigerated storage. In Chemistry of Structure- Function relationships in cheese (ed.) E.L. Malin and M.H. Tunick, Plenum Press, NY, p. 311-320.

Fox, P.F. and Guinee, T.P. (1987). Italian cheeses. In Cheese: Chemistry, Physics and Microbiology. Vol.2- major cheese groups. P.F. Fox (ed.) Elsevier Applied Science Ltd., New York.

Fecera, R.M., McMahon, D.J. and Oberg, C.J. (1995). Effect of EPS producing cultures on moisture retention in low fat mozzarella cheese. *Int. Food Technol.* Annual Meeting- Technical session, 134.

Kalab, M. (1984). Artefacts in conventional scanning electron microscopy of some milk products. *Food Microstructure* 3:95-111.

Kalab, M. (1993). Practical aspects of electron microscopy in dairy research. *Food Structure* 12:95-114.

Kalab, M. (1995). Practical aspects of electron microscopy in cheese research. In Chemistry of Structure- Function relationships in cheese (ed.) E.L. Malin and M.H. Tunick, Plenum Press, NY, p. 247- 276.

Low, D., McMahon, D.J., Oberg, C.J., Hornel, D. and Broadbent, J.R. (1997). Influence of *Streptococcus thermophilus* 1 OJC EPS on the moisture content of low fat mozzarella cheese. *J. Dairy Sci.* 80(Suppl.1):107.

McMahon, D.J. (1995). How the microstructure of mozzarella cheeses is related to functional properties. In Proceedings of 32nd Annual Marschall Italian and Speciality cheese seminar. Rhone Poulenc, Madison, Wisconsin, USA, p. 18- 26.

McMahon, D.J., Oberg, C.J. and McManus, W.R. (1993). Functionality of mozzarella cheese. *Aust. J. Dairy Technol.* 48: 99-104.

McManus, W.R., McMahon, D.J. and Oberg, C.J. (1993). High resolution scanning electron microscopy of milk products: A new sample preparation procedure. *Food Structure* 12:475-82.

Oberg, C.J., McManus, W.R. and McMahon, D.J. (1993). Microstructure of mozzarella cheese during manufacture. *Food Structure* 12:251-8.

Perry, D.B., McMahon, D.J. and Oberg, C.J. (1997). Effect of EPS producing cultures on moisture retention in low fat mozzarella cheese. J. Dairy Sci. 80:799-805.

Perry, D.B., McMahon, D.J. and Oberg, C.J. (1998). Manufacture of low fat mozzarella cheese using EPS producing starter cultures. *J. Dairy Sci.* 81:563-566.

Rousseau, M. (1988). Changes in the microstructure of Saint Pauline cheese during manufacture studied by scanning electron microscopy. *Food Microstructure* 7:105-113.

Schmidt, D.G. and Buchheim, W. (1992). The application of electron microscopy in dairy research. *J. Microscopy* 167 (1):105-121.

Taranto, M.V., Wan, P.J., Chen, S.L. and Rhee, K.C. (1979). Morphological, ultrastructural and rheological characterisation of cheddar and mozzarella cheese. *Scanning Electron Microscopy* III: 273-278.

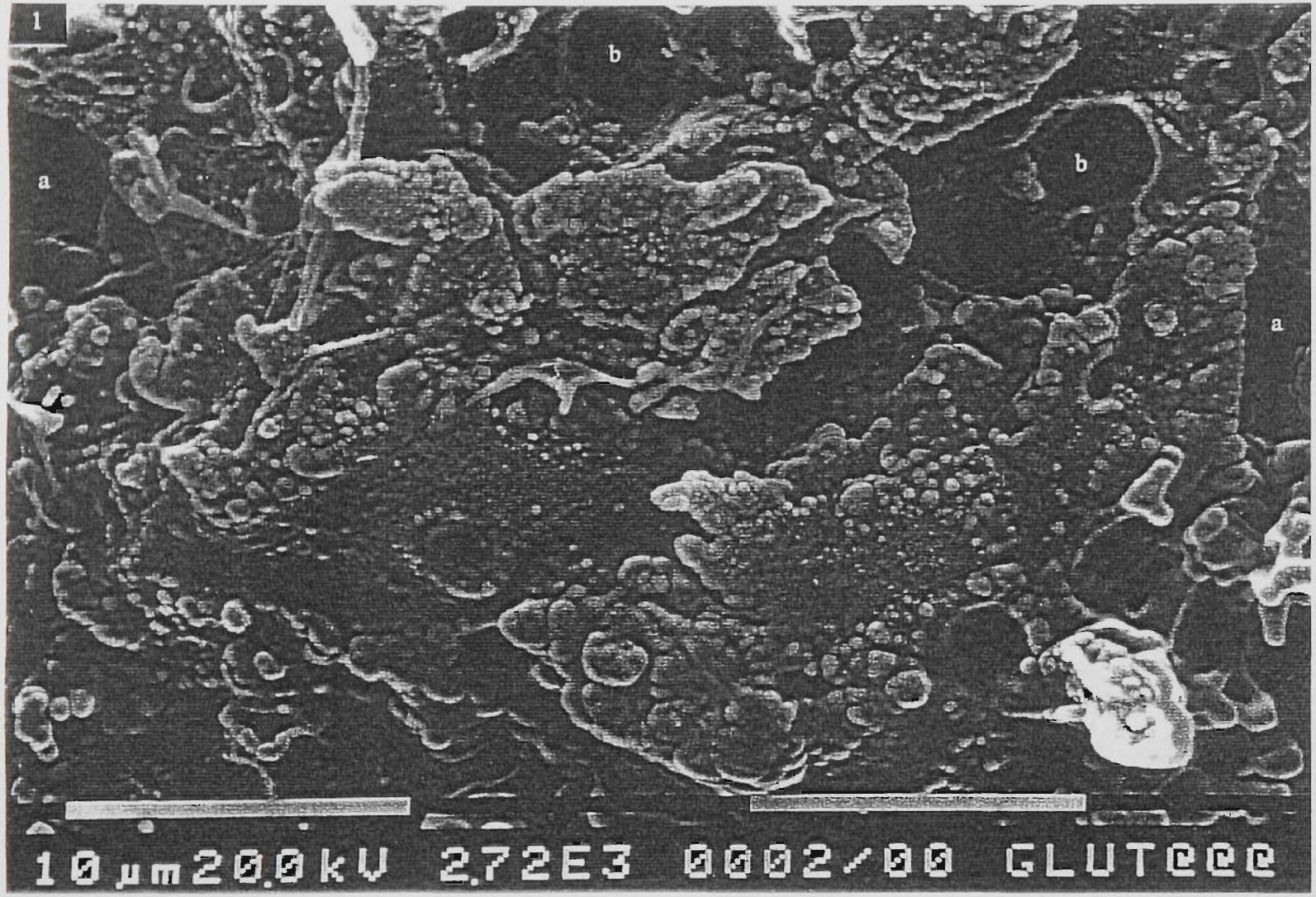

Figure 4.3.1 Microstructure of 28 d old full fat mozzarella cheese fixed using glutaraldehyde and osmium tetroxide. The compact protein matrix is seen interspersed with large serum voids (4.3.1a) and the smooth surfaced cavities (4.3.1b) formed due to removal of fat.

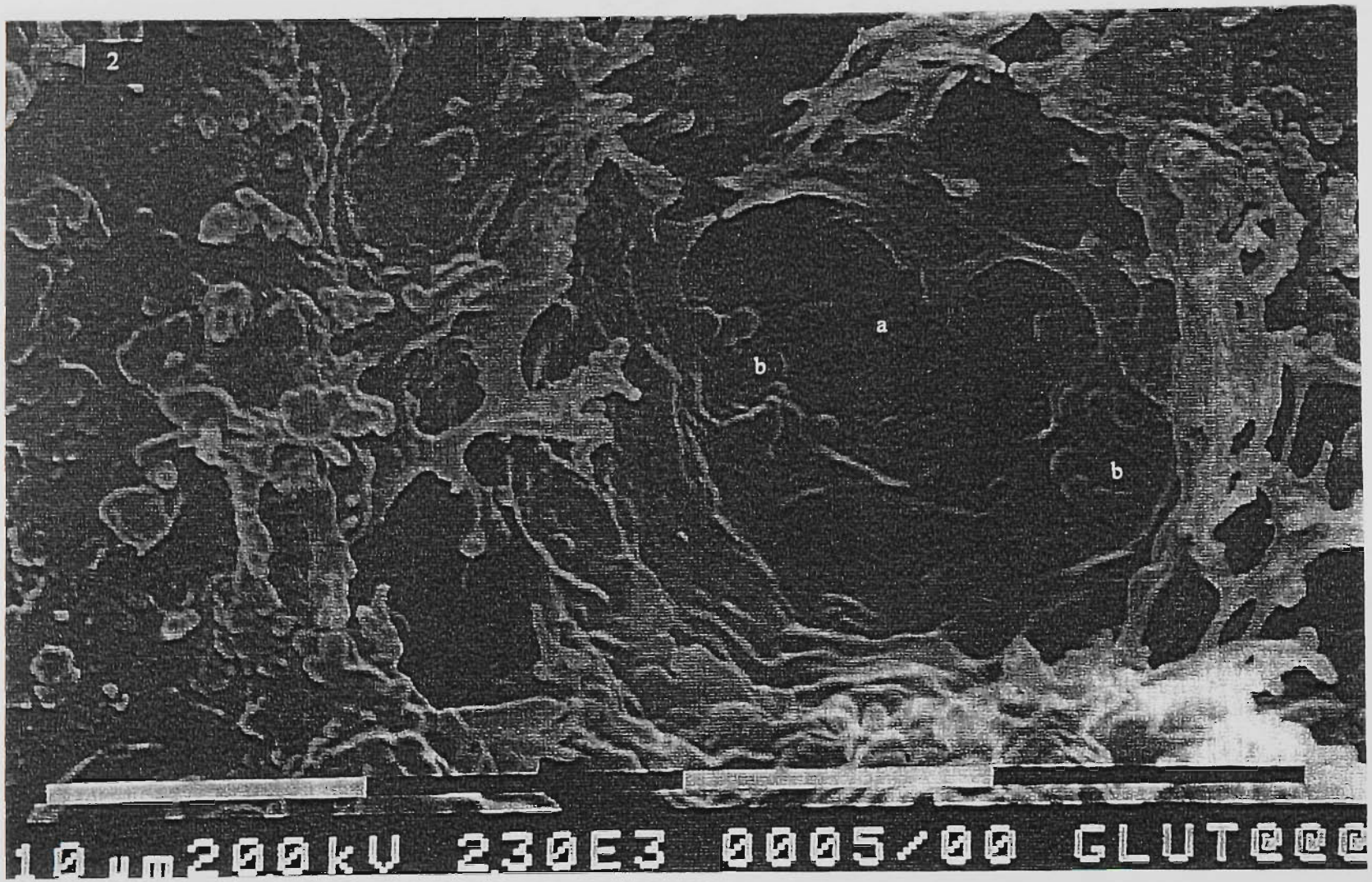

Figure 4.3.2. Microstructure of 28 d old full fat mozzarella cheese fixed using glutaraldehyde and osmium tetroxide. Large serum voids (4.3.2a) are seen with the starter bacteria (4.3.2b) propagating.

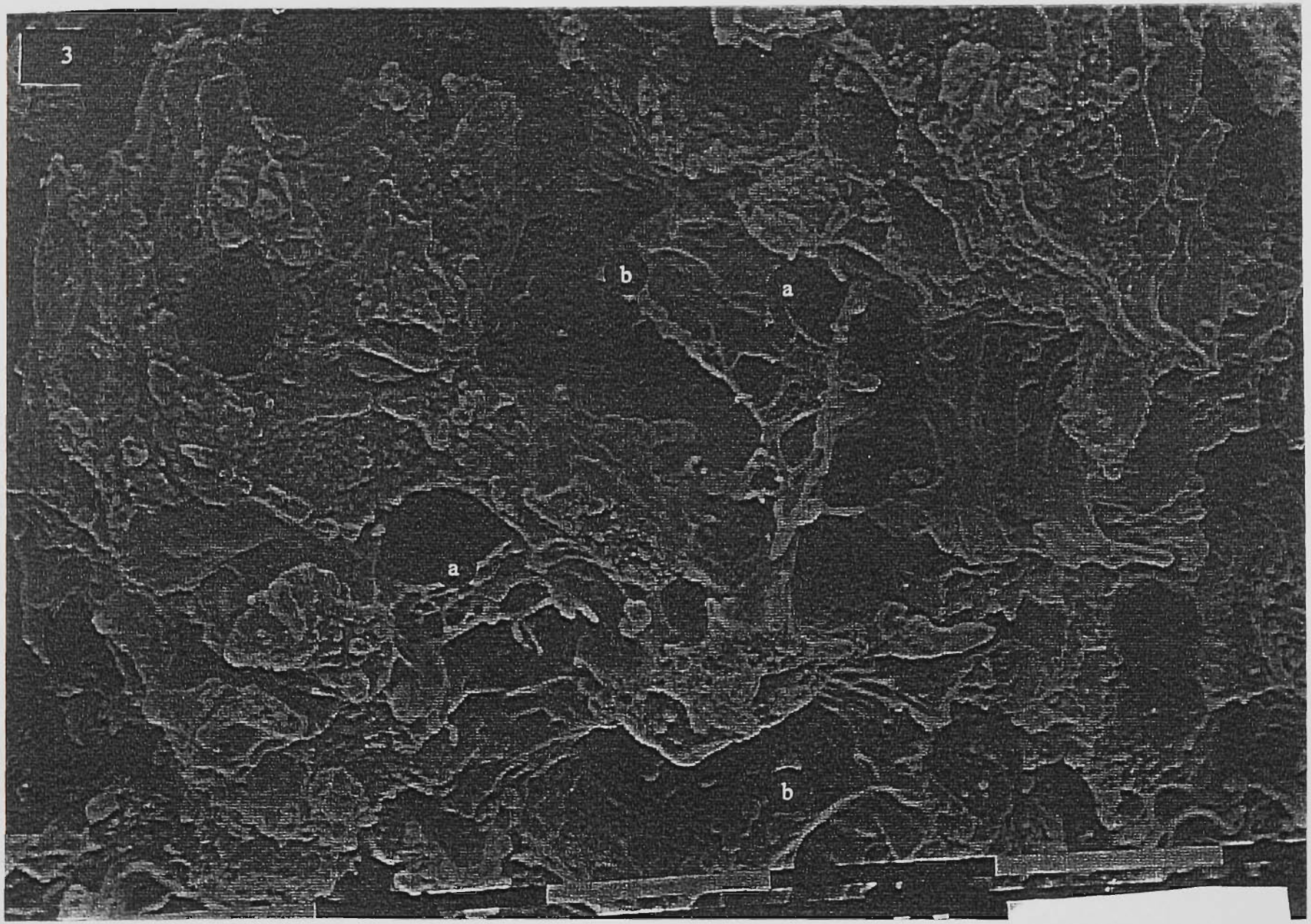

Figure 4.3.3. Microstructure of mozzarella cheese made using exopolysaccharide producing starter cultures. The filamentous exopolysaccharide (4.3.3a) and the streptococci (4.3.3b) are seen.

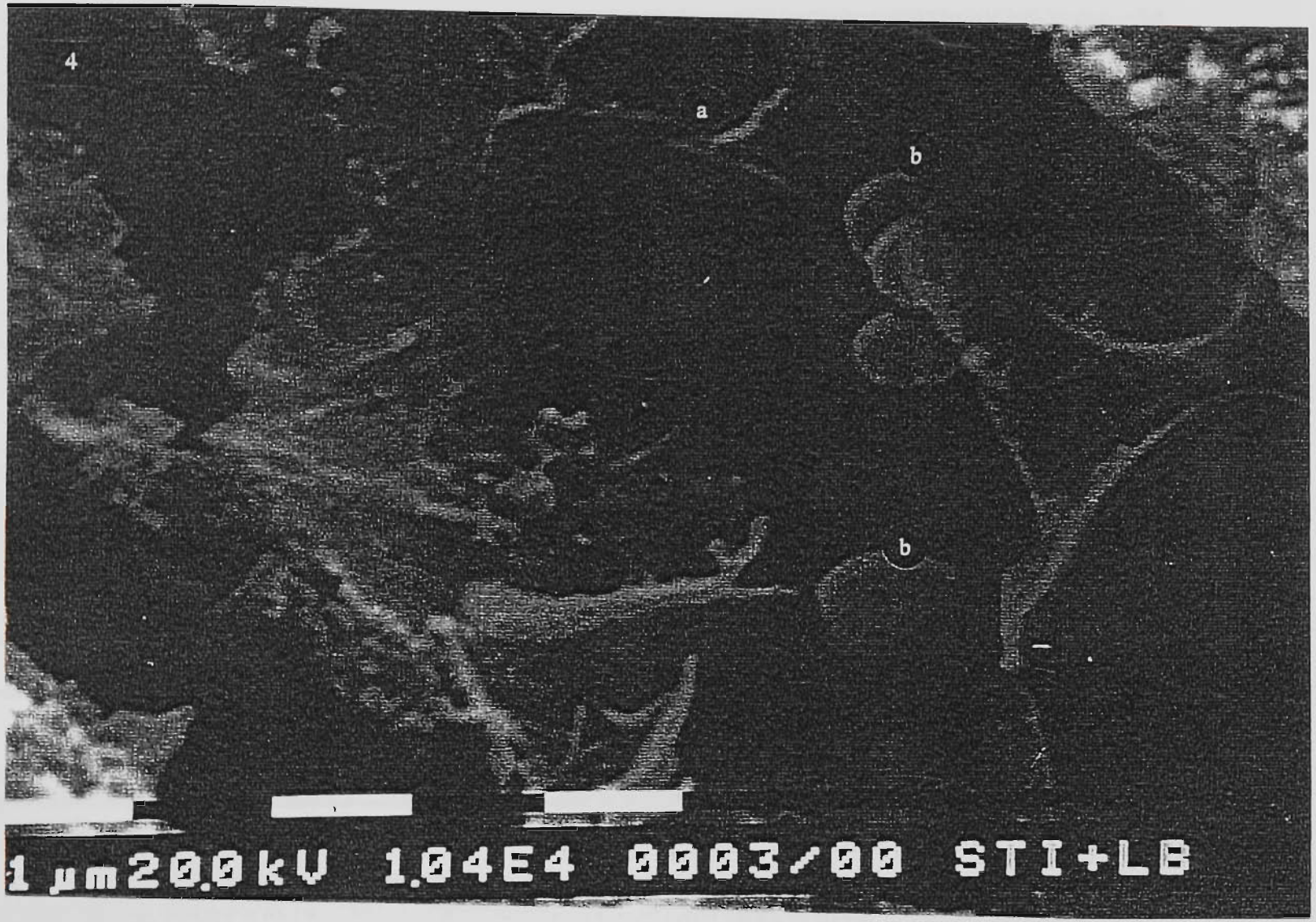

Figure 4.3.4. High magnification scanning electron microscope image of mozzarella cheese made using exopolysaccharide producing starter cultures showing exopolysaccharide (4.3.4a) produced by *S. thermophilus* (4.3.4b).

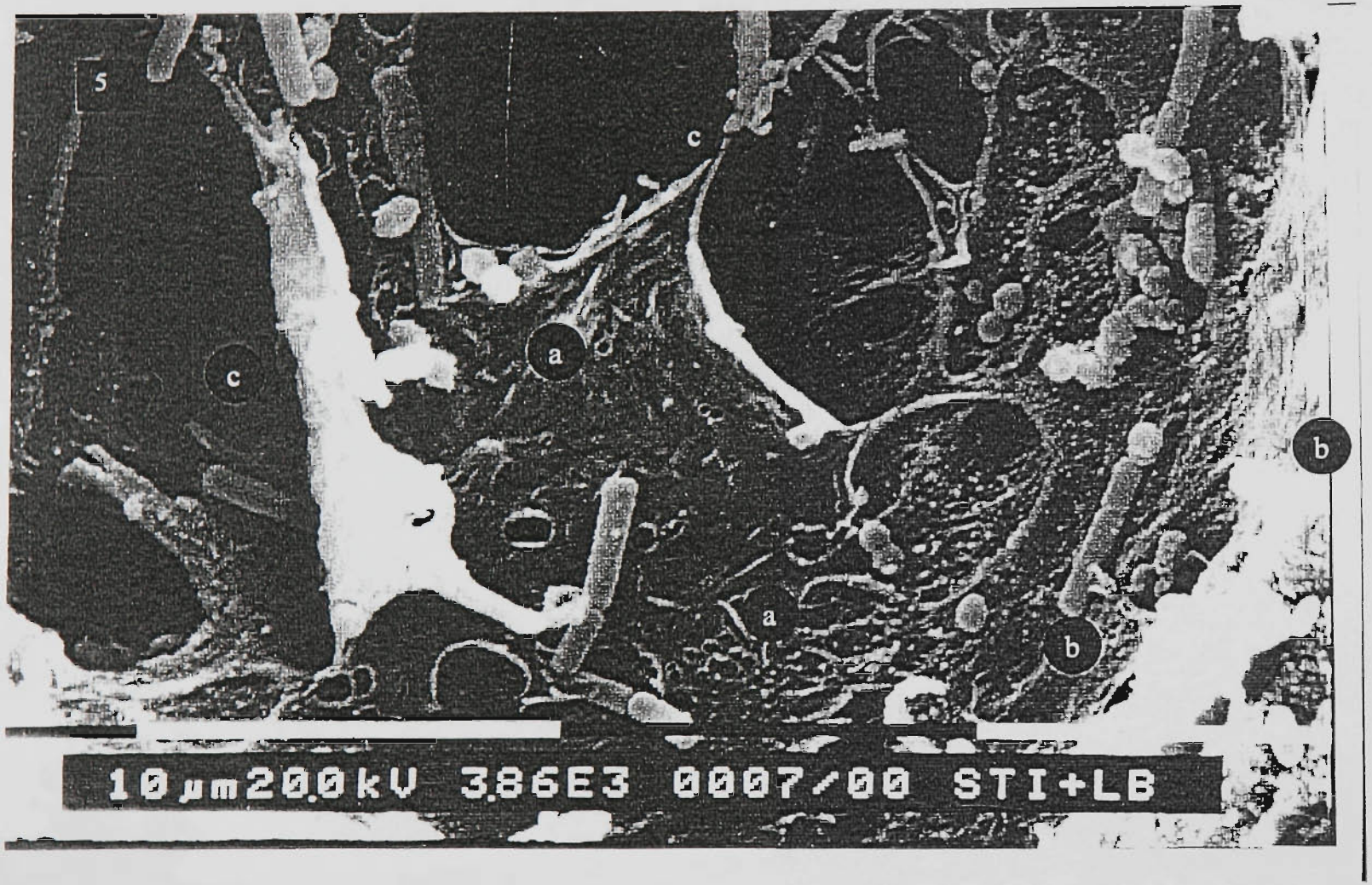

Figure 4.3.5. SEM image at high magnification of mozzarella cheese made using exopolysaccharide producing starter cultures showing *L. delbrueckii* ssp. *bulgaricus* (4.3.5a), *S. thermophilus* (4.3.5b) and large void (4.3.5c) formed due to removal of serum from the cheese.

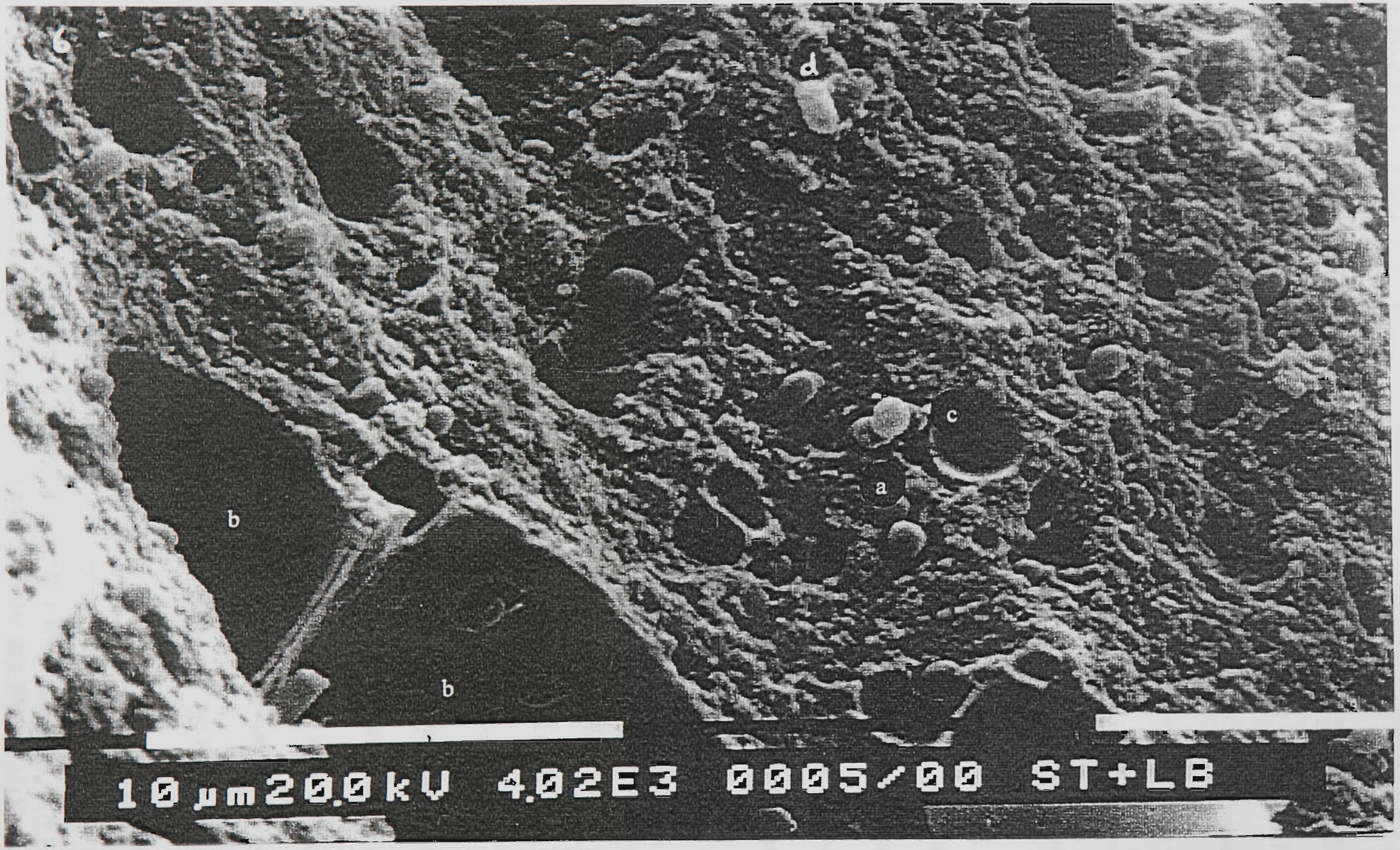

Figure 4.3.6. Microstructure of mozzarella cheese made using non- exopolysaccharide producing starter cultures showing *S. thermophilus* (4.3.6a), Serum channels (4.3.6b), smooth surfaced small voids (4.3.6c) and *L. delbrueckii* ssp. *bulgaricus* (4.3.6d).

5.0 MICROSTRUCTURE OF MOZZARELLA CHEESE: SPECIMEN PREPARATION USING SUCROSE AS A CRYOPROTECTANT

5.1 Introduction

Microstructure of cheeses has been studied using scanning electron microscope (SEM), transmission electron microscope (TEM), confocal, and scanning transmission electron microscope (STEM). SEM offers a simple means to study the microstructure of cheeses at a comparatively high magnification similar to TEM and allows observation of various constituents, their location and arrangement in cheeses. Studies by McManus *et al.* (1993), Kalab (1995) and McMahon (1995) correlating characteristics of cheeses with microstructure have added a new dimension for studying functionality of mozzarella type cheeses. Perry *et al.* (1997, 1998) studied the effect of exopolysaccharide producing organisms on moisture retention and melting properties in low fat mozzarella cheeses. They observed a 3% increase in the moisture retention and enhanced melt characteristic of low fat mozzarella cheeses. Confocal scanning electron microscopy was used to measure the capsule structure of the exopolysaccharide (EPS) produced by the starter bacteria. There is a need to reduce browning caused by Maillard reaction during pizza baking (Kindstedt, 1993).

Electron microscopy of milk products is difficult due to the low melting point fat and the high moisture content of the specimens (Schmidt and Buchheim, 1992). There is a need for a simple and easy method of specimen preparation for observing the microstructure of mozzarella cheeses (Bhaskaracharya and Shah, 1999). Scala *et al.* (1968) and Robards (1968) reported that sucrose could be used as a vehicle for fixation of biological

A version of this chapter has been published (Bhaskaracharya, R. K. and Shah, N. P. (2000). Aust. J. Dairy Technol. 55(1): 28-32).

specimens to obtain clear images. Glauert (1975) reported usage of sucrose as an agent for adjusting the osmolarity of the solutions that are used in preparation of specimens. In the present investigation, sucrose was used as a cryoprotectant in preparing the mozzarella cheese specimens to examine their microstructure and EPS produced by the starter bacteria.

5.2 Materials and methods

5.2.1 Preparation of full fat mozzarella cheese

Full cream milk was obtained from a local cheese factory and the cream was separated using a batch type cream separator (Model 107 AE; Alfa Laval, Sweden). The skim milk contained 0.1 to 0.2% fat and the cream 35%. The skim milk was pasteurised at 72°C for 15 sec using a pilot scale HTST pasteuriser (Alfa Laval, Sweden) and the cream using a batch method (65°C for 15 min). The milk was standardised to 4% fat by mixing skim milk and cream in appropriate proportions. The standardised milk was tempered to 30°C in a 30 litre capacity cheese vat. The exopolysaccharide (EPS) producing *Streptococcus thermophilus* (St 2010) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Lb 2515) obtained from the VUT Culture Collection (Victoria University of Technology, Werribee, Australia) were added at the rate of 1% of each. The milk was warmed to 35°C and this temperature was maintained throughout the cheese making process until the stretching of the curd. After the pH dropped by 0.1 unit, rennet enzyme (Chymosin, 145 IMCU, Chr. Hansen, Bayswater, Australia) was added at the rate of 2.5mL per 10L of milk after diluting in 100 mL of distilled water. The milk coagulated in 35 min. The curd was cut using cheese knives to 1 cubic cm size and the curd held in the whey until the pH reached

5.6 followed by draining of the whey. The curd was piled and re-piled every 15 min until pH 5.2 was reached. The curd was stretched in 70°C hot water, moulded, cooled under running tap water and placed in a saturated brine solution at 4°C for 2 h. The finished cheese was packed under vacuum and stored at 4°C. The experiment was repeated three times. The microstructure was examined at 28 d of storage.

5.2.2 Specimen preparation for microstructure

The method of Bhaskaracharya and Shah (1999) was used with some modification for preparation of cheese specimens. In the present study sucrose solution was used as a cryoprotectant. Similarly, osmium tetroxide was not used and the specimens were cryofractured.

The finished cheeses were allowed to equilibrate at room temperature (~20°C) and specimens of approximately 2 x 2 x 10 mm size were cut from several parts of the cheeses. In this study, the specimens were fixed using 2% glutaraldehyde in distilled water for 1 h at room temperature (~20°C) and for 2 days at 4°C, then washed in cacodylate buffer for 5 min, dehydrated gradually using ethanol at 30, 50, 70, 95 and 100%, each for 5 min, similar to the methods of Rousseau (1988) and Bhaskaracharya and Shah (1999). However, the specimens were cryoprotected by holding in 2.3 M sucrose solution until completely submerged (2 h to overnight). Further, specimens were cryofractured in liquid nitrogen, dehydrated using absolute solutions of ethanol and acetone, each for 5 min, and dried in a critical point drying apparatus. The dried specimens were mounted on stubs, sputter coated with gold and observed under a scanning electron microscope (SEM).

5.3 Results and discussion

The images of mozzarella cheese specimens obtained seem clear and include fine details of the surface of protein strands and the exopolysaccharide produced by the starter organisms. The specimens were cryoprotected with sucrose solution to enable observation of microstructure of the cheeses. Sucrose solution aids in balancing the osmotic pressure within the cheese specimens, thereby the original structures in the cheeses might be better preserved. Figure 5.3.1 shows the internal microstructure of the mozzarella cheese made using exopolysaccharide producing starter cultures. The three dimensional network structure of the protein strands can be observed. The protein strands are seen to be indented with small smooth surfaced voids (Figure 5.3.1a), which are possibly formed due to removal of fat during specimen preparation. Moreover, as the full fat cheeses were made from unhomogenised milk, several closely located voids caused by removal of fat are found, as expected, in the cheese matrix. Large voids (Figure 5.3.1b), which are possibly formed due to removal of serum during dehydration, are also seen. These figures are similar to the ones observed with the cheese specimens prepared using the simplified method as stated in chapter 4.0.

Figure 5.3.2 shows the microstructure of mozzarella cheese made using exopolysaccharide producing starter cultures magnified at about 10,000 times. Protein strands (Figure 5.3.2a) measuring 10 to 20 μm in length and 3 to 5 μm in diameter are seen. The compact protein matrix formed during the manufacture of mozzarella cheese is stretched giving such thick strands of protein. Such orientation of cheese matrix causes displacement of the moisture and fat trapped within the protein, which forms a layer surrounding the strands of protein. The serum extracted during the specimen preparation

may have caused formation of large voids (Figure 5.3.2b) measuring 10-15 μm in diameter; the voids seem to be extending behind the protein strands. Streptococci attached to strands are also seen (Figure 5.3.2c).

Figure 5.3.3 shows mainly the exopolysaccharide attached to *S. thermophilus* organisms, possibly produced by this organism. Each bacterium measures about 0.6-0.8 μm in diameter. The exopolysaccharide (Figure 5.3.3a) appeared to be delicate and filamentous, although not organised into any specific pattern. These filaments of EPS measure 0.8 μm in diameter with a length ranging from 2 to 4 μm. The streptococci (Figure 5.3.3b) seem to be attached to the exopolysaccharide and located in the serum voids. The protein strand (Figure 5.3.3c) appears to be covered with small (<1μm) particles (Figure 5.3.3d), possibly minor proteins extracted during dehydration of the specimens. This could be due to the use of sucrose as a cryoprotectant as reported by Glauert (1975).

5.4 Conclusions

The specimens prepared using sucrose as a cryoprotectant showed good internal structure of mozzarella cheeses, although the surface of the cheese appeared to be coated with minute deposits, possibly of proteins. Mozzarella cheeses showed large voids formed due to removal of serum and small smooth surfaced voids formed by the removal of fat. The streptococci were seen close to the serum voids. The exopolysaccharide appeared to be primarily produced by the streptococci. The exopolysaccharide was thin, filamentous and delicate. Thus, the method of specimen treatment using sucrose as a cryoprotectant was found to be suitable for observing the internal structure of the mozzarella cheese made using EPS producing starter cultures.

5.5 References

Bhaskaracharya, R.K. and Shah, N.P. (1999). Microstructure of mozzarella cheeses made with or without exopolysaccharide producing starter cultures. *Austr. J. Dairy Technol.* (accepted for publication).

Glauert, A.M. (1975). Fixation methods, Fixation, dehydration and embedding of biological specimens, North Holland Publishing Co.-Amsterdam Oxford American Elsevier Publishing Co. Inc. NewYork, USA.

Kalab, M. (1995). Practical aspects of electron microscopy in cheese research. In Chemistry of Structure- Function relationships in cheese (ed) E.L. Malin and M.H. Tunick, Plenum Press, NY. p. 247- 276.

Kindstedt, P.S. (1993). Effect of manufacturing factors, composition and proteolysis on the functional characteristics of mozzarella cheese. *Crit. Reviews Food Sci. Nutr.* **33**(2):167-187.

McMahon, D.J. (1995). How the microstructure of mozzarrella cheese is related to functional properties. In Proceedings of 32nd Annual Marschall Italian and Speciality cheese seminar. Rhone Poulenc, Madison, Wisconsin, USA. p. 18-26.

McManus, W.R., McMahon, D.J. and Oberg, C.J. (1993). High resolution scanning electron microscopy of milk products: A new sample preparation procedure. *Food Struct.* 12:475-82.

Perry, D.B., McMahon, D.J. and Oberg, C.J. (1997). Effect of exopolysaccharide producing cultures on moisture retention in low fat mozzarella cheese. *J. Dairy Sci.* 80(5):799-805.

Perry, D.B., McMahon, D.J. and Oberg, C.J. (1998). Manufacture of low fat mozzarella cheese using exopolysaccharide producing starter cultures. *J. Dairy Sci.* 81(2):563-566.

Robards, A.W. (1968). On the ultrastructure of differentiating secondary xylem in willow. *Protoplasma* 65: 449.

Rousseau, M. (1988). Changes in the microstructure of Saint Pauline cheese during manufacture studied by scanning electron microscopy. *Food Microstructure* 7:105-113.

Scala, J., Schwab, D. and Simmons, E. (1968). The fine structure of the digestive gland of venus's flytrap. *Am. J. Bot.* 55: 649.

Schmidt, D.G., Buchheim, W. (1992). The application of electron microscopy in dairy research. *J. Microscopy* 167(1):105-121.

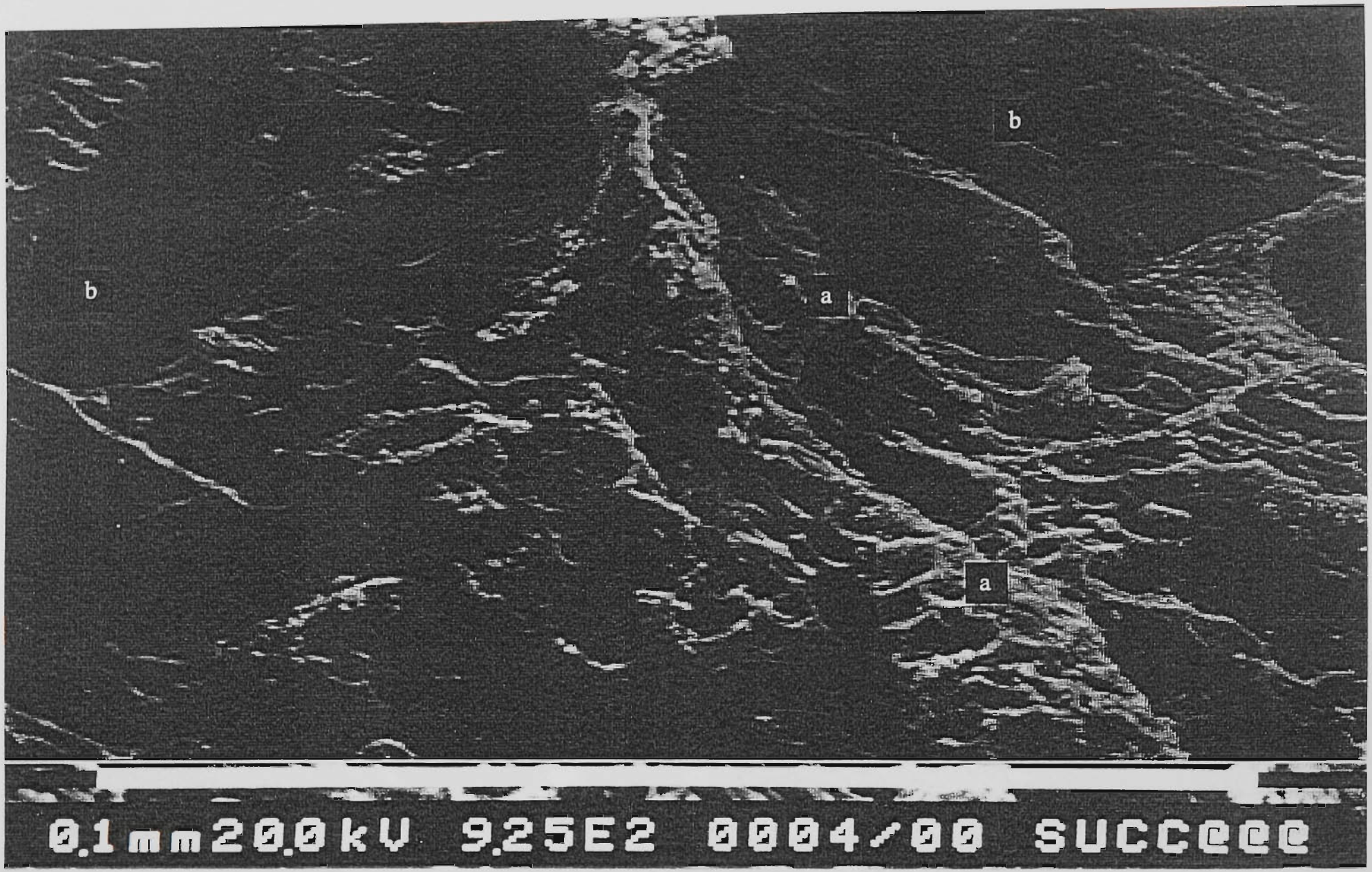

Figure 5.3.1. Microstructure of 28 d old full fat mozzarella cheese fixed using glutaraldehyde and osmium tetroxide and cryoprotected with sucrose. The protein strands are seen to be covered with numerous small smooth surfaced voids due to removal of fat (a). Large serum voids (b) are also seen.

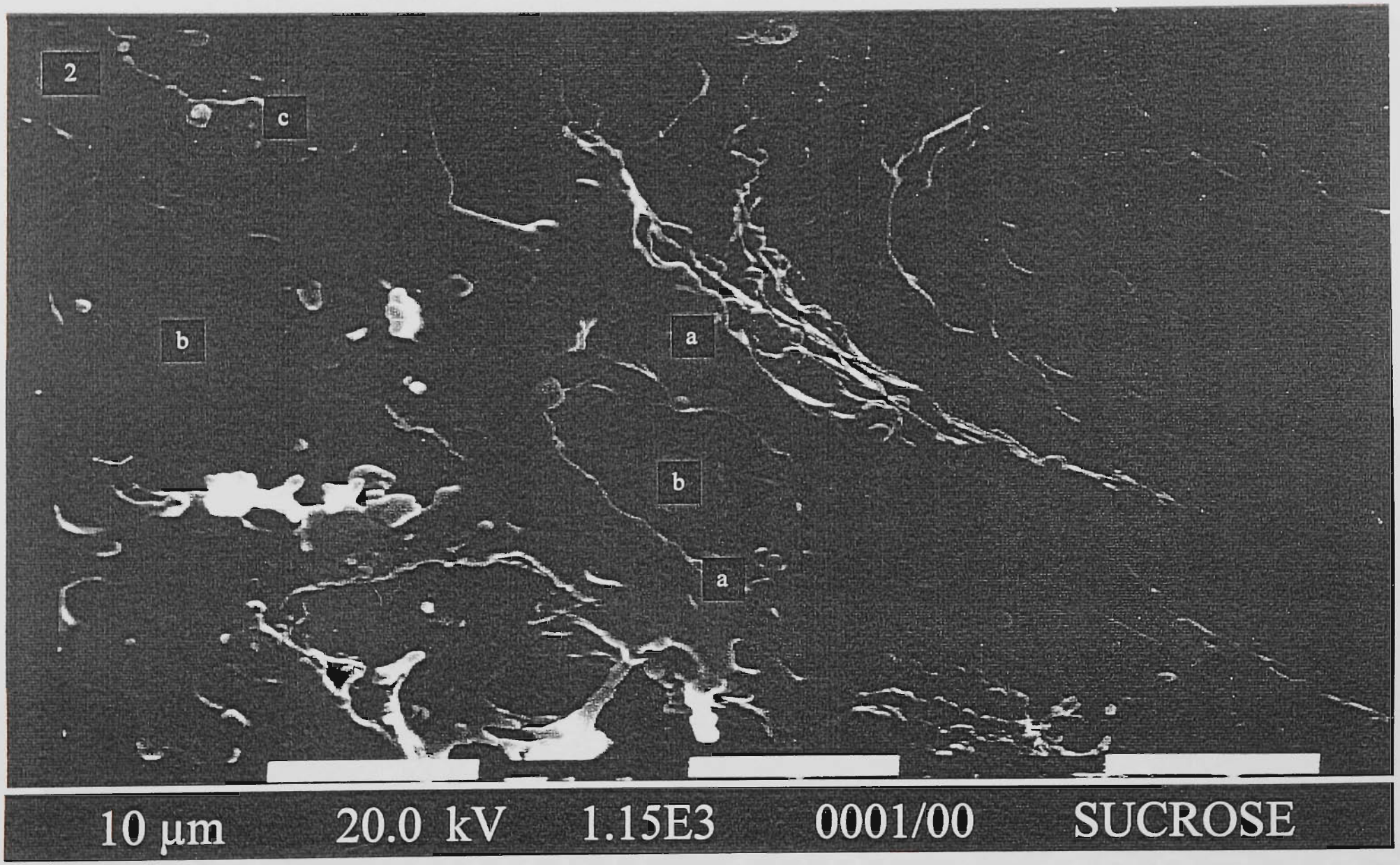

Figure 5.3.2. Microstructure of 28 d old full fat mozzarella cheese fixed using glutaraldehyde and osmium tetroxide and cryoprotected with sucrose. The protein strands (a) are seen surrounded by large serum voids (b). *Streptococcus thermophilus* (c) are seen attached to protein strands.

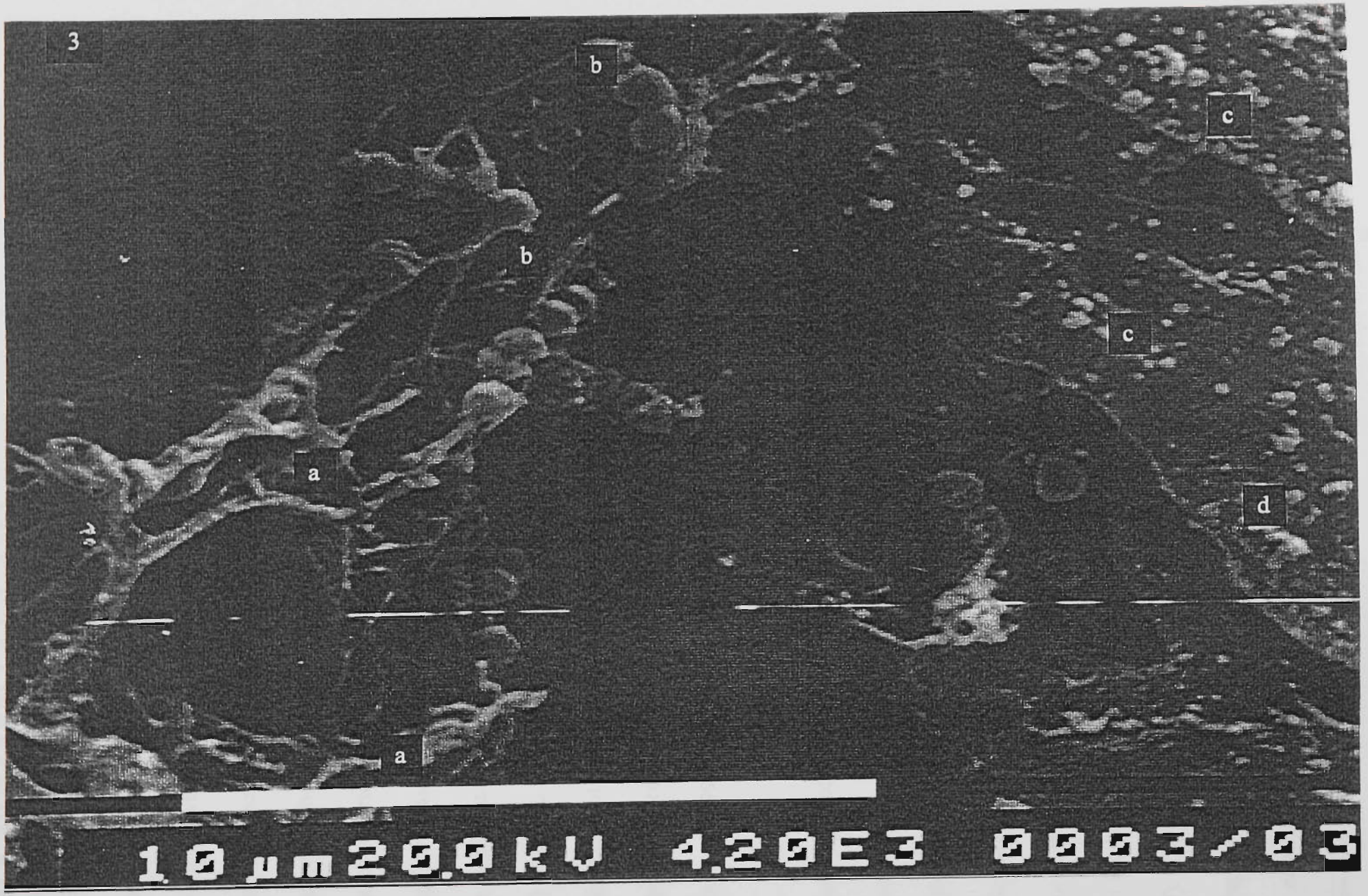

Figure 5.3.3. Microstructure of mozzarella cheeses made using exopolysaccharide producing starter cultures. The exopolysaccharide (a) produced by streptococci (b) is seen. The protein strand (c) seems to be covered with loose particles (d).

6.0 TEXTURE CHARACTERISTICS AND MICROSTRUCTURE OF SKIM MILK MOZZARELLA CHEESES MADE USING EXOPOLYSACCHARIDE OR NON-EXOPOLYSACCHARIDE PRODUCING STARTER CULTURES

6.1 Introduction

There is a growing interest in developing mozzarella cheeses with reduced fat content having characteristics similar to their full-fat counterpart. However, the quality of mozzarella cheeses is affected when the fat content is reduced. Several texture defects due to reduction in fat content have been reported in mozzarella cheeses including increased firmness or hardness, springiness and cohesiveness (Emmons *et al.*, 1980). A synergistic effect due to fat and moisture contents causes significant changes in gumminess and chewiness characteristics. Sunder and Upadhyay (1991) observed a direct relationship between the casein to fat ratio in milk used for cheese manufacture to its texture characteristics. Modifications in the manufacturing process have been reported to improve the texture and rheology of low fat mozzarella cheeses (Hong *et al.*, 1998; Merrill *et al.*, 1994; Patel *et al.*, 1998; Perry *et al.*, 1995; Rudan *et al.*, 1998).

The texture defects of low fat mozzarella cheeses are generally overcome by increasing moisture content of the cheeses, but the moisture content adversely affects the moisture-in-non-fat-solids (**MNFS**) level (Rodriguez, 1998). Low fat mozzarella cheeses (<25% fat-in-dry-matter) with similar MNFS levels as full fat cheese and comparable texture characteristics have been produced (Fife *et al.*, 1996; Tunick *et al.*, 1993b). An increase in moisture content and a decrease in pH increased meltability and decreased chewiness in low fat mozzarella cheeses (Chen *et al.*, 1997). The moisture in mozzarella cheeses initially is loosely bound within the protein matrix. During storage the moisture gets adsorbed leading to a decrease in expressible serum content. Fife *et al.* (1997) observed a decrease in amount of expressible serum during storage.

A version of this chapter is under review (Bhaskaracharya, R. K. and Shah, N. P. (1999) Aust. J. Dairy Technol.).

This was attributed to the movement of moisture from the fat or serum channels into the protein matrix. The interaction of the protein matrix with the serum phase in mozzarella cheeses has been shown to improve functional characteristics of the cheeses (Kindstedt and Guo, 1997; 1998). There was found to be a dramatic increase in the water holding capacity during the first weeks of ageing. During this same time there is a shift in the distribution of expressible serum from the insoluble to the soluble state. They have also reported a progressive migration of intact caseins and casein associated minerals from the cheese matrix to the serum phase.

Low fat mozzarella cheeses with similar sensory and rheological characteristics to those of full fat mozzarella have been made using a polysaccharide substitute (Pagliarini and Beatrice, 1994). Low fat mozzarella cheeses with improved melting characteristics could be made using exopolysaccharide (**EPS**) producing starter cultures; EPS increases moisture retention (Fecera *et al.*, 1995; Low *et al.*, 1997; Perry *et al.*, 1997, 1998).

The texture analysis such as hardness, springiness, cohesiveness, chewiness and gumminess of cheeses is carried out using the Instron Universal Testing Machine (Halmos 1997; Lucisano *et al.*, 1987). Low moisture content in cheeses has been reported to cause increased hardness, gumminess, springiness and chewiness while the cohesiveness and meltability are reported to decrease. Similarly, hardness, gumminess, springiness and chewiness decrease during storage while meltability increases (Chen *et al.*, 1979; Tunick *et al.*, 1991). Hardness and springiness decrease due to proteolysis during storage and changes in microstructure of mozzarella cheeses become evident (Tunick *et al.*, 1993a; 1993b; 1995b). Yun et al. (1995) also observed decreased hardness, springiness and apparent viscosity of mozzarella cheeses during storage. Several reports are available on the microstructure of mozzarella cheeses as observed under scanning

electron microscope (**SEM**) (Kalab, 1977; Kiely *et al.*, 1992; 1993; Masi and Addeo, 1986; Oberg *et al.*, 1993; Paquet and Kalab, 1988; Taranto *et al.*, 1979; Tunick *et al.*, 1993a; 1995a). More recently several studies report microstructure of low fat mozzarella cheeses (Kindstedt and Guo, 1998; McMahon, 1995; Paulson *et al.*, 1998; Poduval and Mistry, 1999; Rudan *et al.*, 1998). Fecera *et al.* (1995), Low *et al.* (1997) and Perry *et al.* (1997) reported improved characteristics of low fat mozzarella cheeses by incorporating EPS producing starter cultures. Earlier studies from this laboratory by Bhaskaracharya and Shah (2000) reported microstructure of full fat EPS and non-EPS mozzarella cheeses.

The aims of this study were (i) to examine the changes in texture characteristics including hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness of skim milk mozzarella cheeses made using EPS or non-EPS producing starter cultures over a 26 d storage period, and (ii) to observe the microstructure of the two types of cheeses at end of the storage period.

6.2 Materials and Methods

6.2.1 Preparation of Mozzarella Cheese

Full cream milk was obtained from a local cheese factory and the cream was separated using a batch type cream separator (Model 107 AK; Alfa Laval, Lund, Sweden). The skim milk contained less than 0.2% fat. The skim milk (10 L) was pasteurized (72°C for 15 sec) using a pilot scale HTST pasteurizer (Alfa Laval, Lund, Sweden) tempered to 30°C in a 30 L capacity cheese vat, and starter cultures consisting of EPS producing *Streptococcus thermophilus* (St 2010) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Lb 2515) (VUT Culture Collection, Victoria University of Technology, Werribee, Australia) were added at the rate of 1% of each.

After the pH dropped by 0.1 unit, rennet enzyme (2.5 mL/10 L; Chymosin, 145 IMCU, Chr. Hansen, Bayswater, Australia) was added to milk after diluting in 100 mL of distilled water. The milk was warmed to 35°C and this temperature was maintained throughout the cheese making process until stretching of the curd. The milk coagulated in 35 min. The curd was cut to 1 cubic cm size and the curd held in the whey until the pH dropped to 5.6, followed by draining of the whey. The curd was piled and re-piled every 15 min until the pH of 5.2 was reached. The curd was stretched in 70°C hot water, molded, cooled under running tap water, and placed in saturated brine solution at 4°C for 2 h, similar to the method used by Jana and Upadhyay (1997). The finished cheese was packed under vacuum and stored at 4°C. The experiment was repeated three times. Similarly, three batches of skim milk mozzarella cheeses were made using commercial non-EPS producing *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* (Chr. Hansen, Bayswater, Australia).

6.2.2 Composition Analyses

The moisture content of the cheeses was determined in triplicate by the oven drying method (Egan *et al.*, 1987). The protein content was estimated in triplicate by the Kjeldahl method (Barbano *et al.*, 1990). The fat content of the cheeses was analysed by the method described by Bhaskaracharya and Shah (1999).

6.2.3 Texture Analyses

The cheese blocks were left at 20°C for 1 h and cylindrical specimens measuring 25 mm x 20 mm were cut using a cheese corer. Three specimens were obtained from each cheese block and were analyzed for texture characteristics such as hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness. The Instron Universal Testing Machine (Model 5564;

Instron Ltd., London, England) was used to analyze the texture characteristics of all the cheeses according to the method described by Bhaskaracharya and Shah (1999). The samples were compressed to 50 and 70% of their heights using a 500 N load cell with a flat plunger and the crosshead movement was adjusted to 50 mm/min. A double compression was achieved and the data was collected using a Merlin software. All the analyses were carried out three times.

6.2.4 Microstructure

The specimens were prepared from mozzarella cheeses according to the method described by Bhaskaracharya and Shah (2000) and Tunick *et al.* (1997). The finished cheeses were allowed to equilibrate at room temperature (~20°C) for 1 h and specimens of approximately 2 x 2 x 10 mm size were cut from several parts of the cheeses. The specimens were fixed for 1 h at room temperature (~20°C) and for 2 d at 4°C in 2% glutaraldehyde in 0.1 M cacodylate buffer solution, similar to the method described by McManus *et al.* (1993). The specimens were then washed in 0.1 M cacodylate buffer (pH 7.3) for 5 min, gradually dehydrated using ethanol at 30, 50, 70, 95 and 100% concentration, each for 5 min, washed in cacodylate buffer for 5 min and post-fixed overnight in 1.5% osmium tetroxide solution. These steps are as reported earlier (Rousseau, 1988). The fixed specimens were washed in distilled water twice each for 10 min, and further dehydrated with 30, 50, 70, 95 and 100% ethanol, each for 5 min and in 100% acetone for 5 min. The dehydrated specimens were dried in critical point drying equipment (custom made, Melbourne University, Melbourne, Australia) using liquid carbon dioxide. The dried specimens were fractured at room temperature (~20°C), mounted on aluminium stubs, sputter coated with gold using Edwards Sputter Coater and observed under Philips SEM515 scanning electron microscope at 20 kV.

6.2.5 Statistical Analyses

The effects of age, compression and starter culture and their combined effects on the texture characteristics of mozzarella cheeses made using EPS and non-EPS starter cultures were assessed using the multivariate and tests of between-subjects effects analysis. The SPSS version 8.0 was used for statistical analysis (Kirkpatrick and Feeney, 1997) and the significance was determined at $P < 0.05$. The dependent variables, degrees of freedom and the significance of each factor were measured.

6.3 Results and Discussion

6.3.1 Composition

Table 6.3.1 shows the moisture, protein and fat contents of the EPS and non-EPS cheeses. The EPS cheeses showed 1.71% higher moisture content than the non-EPS cheeses. The EPS is reported to have better moisture binding ability and decreased syneresis than the protein matrix from milk gels (Fecera *et al.*, 1995). Similar observations were also made by Hassan *et al.* (1995a, 1995b, 1996a, 1996b) in yogurt made using EPS producing starter cultures. Both cheeses had similar protein contents of about 43%. EPS production did not affect the protein content of the cheeses. The skim milk cheeses contained negligible amount of fat (<3%).

6.3.2 Texture

Texture profile measurements were carried out for hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness. These measurements were taken based on the formulae reported by Bhaskaracharya and Shah (1999).

6.3.2.1 Hardness

Figure 6.3.1 shows the hardness values for the EPS and the non-EPS cheeses measured at 5, 12, 19 and 26 d of storage. In general the cheeses showed a decrease in hardness during storage, similar to that reported by Chen et al. (1979). This trend is apparent at 70% compression than at 50%. The hardness decreased uniformly during the storage period of 26 d for the EPS cheeses, while the non-EPS cheeses showed a slight decrease initially followed by a sharp decrease. Similar observations of decreased firmness in yogurt made using EPS producing starter cultures were reported (Hassan *et al.*, 1996b). The higher moisture content in EPS cheeses may have been responsible for the decrease in hardness characteristic. Bhaskaracharya and Shah (1999) reported a similar trend. The cheeses when compressed to 50%, also showed this trend. The decreased hardness of the EPS cheeses was found to be significantly ($P < 0.001$) affected by the age of the cheese, possibly due to the proteolysis (Tunick *et al.*, 1997). The extent of compression of the samples increases the hardness values measured with the Instron (Bhaskaracharya and Shah, 1999). This effect of compression on hardness was significant ($P < 0.001$). Moreover a combined effect due to age and compression on hardness characteristic was also found to be significant ($P < 0.001$).

6.3.2.2 Cohesiveness

Figure 6.3.2 shows the cohesiveness for the EPS and the non-EPS cheeses measured at 50 and 70% compression levels during 26 d storage. In general, both types of cheeses showed a decrease in cohesiveness during storage at both compression levels. The decrease in cohesiveness is apparent after 12 d of storage, especially at 70% compression. The EPS cheeses showed a greater decrease in cohesiveness than the non-EPS cheeses. In the study of Tunick *et al.* (1991) low moisture mozzarella cheeses showed decreased cohesiveness. The EPS cheeses with higher

moisture content than the non-EPS cheeses, showed lower cohesiveness at 19 d and 26 d. The decreased cohesiveness of the EPS cheeses was significantly ($P < 0.01$) affected by the EPS or non-EPS producing starter cultures. Although proteolysis was not measured, differing rates of proteolysis could be responsible for this characteristic. In the study of Bhaskaracharya and Shah (1999) the protein and fat contents affected cohesiveness while moisture had negligible effect. Thus in the present study, the decrease in cohesiveness of the cheeses was probably caused by the type of starter cultures used as the protein and fat contents were similar. The decreasing trend in cohesiveness of the cheeses was significantly ($P < 0.001$) affected by the age of the cheeses, which again suggests that increased proteolysis during storage caused decrease in cohesiveness. The level of compression ($P < 0.001$) of the cheese samples also had a negative effect on this characteristic. The opposite effects of age and compression levels on cohesiveness of cheeses caused a decrease in significance ($P < 0.05$) of their combined effect when compared to their individual effects.

6.3.2.3 Adhesiveness

Figure 6.3.3 shows the adhesiveness for the EPS and the non-EPS cheeses during a storage period of 26 d. There was no definite trend for this characteristic. Both type of cheeses showed an increase in adhesiveness during storage at 50% compression, but a variable effect at 70% compression. This unusual trend is possibly due to small values for adhesiveness. The age of the cheeses significantly ($P < 0.01$) affected the adhesiveness. This could again be due to an increase in small peptides in the serum channels resulting from proteolysis. These small peptides may increase the binding forces within the cheese matrix by increased absorption of moisture (Tunick *et al.*, 1997).

6.3.2.4 Springiness

Figure 6.3.4 shows the springiness for the EPS and the non-EPS cheeses measured at 5, 12, 19 and 26 d of storage. In general, the springiness values decreased during storage in both type of cheeses at both compression levels. These results are similar to those reported earlier (Tunick *et al.*, 1993a; 1993b). There was no change in springiness in EPS cheeses between 12 d and 19 d, while the non-EPS cheeses showed a further decrease during the same period. This is possibly due to the stabilising effect of absorption of serum within the protein matrix. The exact changes occurring within the cheeses need to be ascertained by examination of the microstructure. The EPS cheeses showed a decrease in springiness between 19 d and 26 d, while the non-EPS cheeses showed a slight increase. Unlike the EPS cheeses, the non-EPS cheeses showed a uniform decrease in springiness at 50% compression. Both type of cheeses showed decreased values of springiness during storage at 50% compression. Overall the EPS cheeses exhibited higher springiness than the non-EPS cheeses; although the former had higher moisture content and slightly lower protein content (Tunick *et al.*, 1991). Springiness was significantly ($P < 0.001$) affected by the age and compression as reported earlier (Bhaskaracharya and Shah, 1999), while the combined effects of type of starter cultures used and the age, and the combined effects of age and compression, were also significant ($P < 0.01$) for this characteristic.

6.3.2.5 Gumminess

Figure 6.3.5 shows the gumminess for the EPS and the non-EPS cheeses measured at 5, 12, 19 and 26 d of storage. In general, the gumminess values decreased for both type of cheeses during storage. These results are in agreement with those of Chen *et al.* (1979). The EPS cheeses showed lower gumminess than the non-EPS cheeses at 12, 19 and 26 d at 70% compression, whereas the non-EPS cheeses showed lower gumminess at 12 d and 19 d at 50% compression.

Although there was a marked difference between the gumminess values-of EPS and non-EPS cheeses during storage, the trend is not very clear. These results show that there was a significant ($P < 0.001$) effect due to age, compression and combined effects due to age and compression on both type of cheeses. The type of starter cultures used and the moisture content of the cheeses appeared to play some role, although not significant, in determining the gumminess values of the cheeses. Gumminess is a product of hardness and cohesiveness. The effects of age, compression, starter cultures and their combined effects on the hardness and cohesiveness individually during storage varied. These combined effects may be responsible for the unusual trend in gumminess values.

6.3.2.6 Chewiness

Figure 6.3.6 shows the chewiness for the EPS and non-EPS cheeses measured at 5, 12, 19 and 26 d of storage. Chewiness decreased for both the types of cheeses during storage similar to the gumminess values. The EPS cheeses showed similar chewiness as non-EPS cheeses at 5 d, and chewiness decreased for both types of cheeses at 12 d, 19 d and 26 d of storage. The chewiness decreased by approximately 80% for the EPS cheeses in 21-d, between 5 and 26 d, while the non-EPS cheeses showed approximately 68% decrease during the same period at 70% compression. Both types of cheeses showed similar chewiness on 5 d and 12 d but the EPS cheeses showed higher values than the non-EPS cheeses on 19 d at 50% compression. The non--EPS cheeses showed slightly higher values of chewiness than the EPS cheeses on 26 d at 50% compression. The chewiness of the skim milk cheeses was significantly ($P < 0.001$) affected by the age, level of compression and their combined effect but not due to the type of starter cultures that were used. There might also be an effect due to the moisture content on the chewiness characteristic of the cheeses.

6.3.3 Statistics

The texture characteristics including hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness were analysed for the EPS and non-EPS cheeses for the effect of age, compression, age x compression, starter culture, compression x starter culture and age x starter culture. The hardness of the cheeses showed a significant effect ($P < 0.001$) due to age, compression and age x compression. Cohesiveness was significantly affected due to the age ($P < 0.001$); compression ($P < 0.001$) and age x compression ($P < 0.05$). Adhesiveness was significantly ($P < 0.01$) affected due to age of the cheeses. Springiness was significantly ($P < 0.001$) affected by age and compression with the combined effect ($P < 0.01$) due to age x compression also being a factor for the difference in this characteristic between the EPS and non-EPS cheeses. The gumminess and chewiness characteristics were significantly ($P < 0.001$) affected by age, compression and their combined effect.

The effect of starter culture was found to be significant ($P < 0.0l$) for cohesiveness characteristic and there was also a combined effect ($P < 0.0l$) due to age x starter culture on the springiness characteristic.

6.3.4 Microstructure

Figure 6.3.7 shows the microstructure of a 28 d old, skim milk mozzarella cheese made using non-EPS producing starter cultures. The cheese showed a compact protein matrix (Figure 6.3.7a), which was sparingly indented with serum and fat voids (Figure 6.3.7b) located on the exposed surface. The voids are small and fewer in number, due to lack of fat in the cheese as reported earlier (Cooke *et al.*, 1995; Mistry and Anderson, 1993). *S. thermophilus* (Figure 6.3.7c) and *L.*

delbrueckii ssp. *bulgaricus* (Figure 6.3.7d) are visible on the surface. The cheese surface was found to be rugged and grainy. Stringiness was absent in the cheese microstructure.

Figure 6.3.8 shows the microstructure of a 28 d old, skim milk mozzarella cheese made using EPS producing starter cultures. Protein strands (Figure 6.3.8a) are seen separated by large and numerous voids (Figure 6.3.8b) that were formed during cheese making. The cheese appeared smooth and stringy and the protein strands were found elongated and thin. The protein strands also lack fat indentations. The indentations would be expected in a conventionally made full fat mozzarella cheese specimen. Similar stretchable structures produced by slime polysaccharide unlike capsular polysaccharide has been reported (Hassan *et al.*, 1996a).

Figure 6.3.9 shows the microstructure of a 28 d old, skim milk mozzarella cheese made using EPS producing starter cultures. The mozzarella cheese was porous and had an open texture with numerous voids (Figure 6.3.9a) unlike that of non-EPS cheese (Figure 6.3.7). Such voids were probably formed from dehydration of the EPS (secreted by the starter cultures) which might have formed globs and such globs of EPS when extracted from the fractured surface of the cheese during SEM preparation leave behind voids. This effect of the EPS on the cheese matrix could lead to an increased porosity. The voids in the cheeses were similar to the ones observed by Hassan and Frank (1997) for rennet curd made using EPS or non-EPS producing starter cultures. *L. delbrueckii* ssp. *bulgaricus* (Figure 6.3.9b) and *S. thermophilus* (Figure 6.3.9c) could be seen located on the cheese matrix and the organisms appeared to be covered with EPS material. These results are similar to those reported by Bhaskaracharya and Shah (2000). Minute particles probably of proteins, which were extracted during specimen preparation, are also seen on the cheese surface.

Figure 6.3.10 shows the micrograph of a 28 d old skim milk mozzarella cheese made using EPS producing starter cultures magnified about 2000 times. The cheese surface shows numerous small voids (Figure 6.3.10a) having depth suggesting that these belong to serum portion. The cheese matrix (Figure 6.3.10b) appears compact with a coarse or grainy surface. Some streptococci were also visible.

Figure 6.3.11 shows the micrograph of a 28 d old skim milk mozzarella cheese made using EPS producing starter cultures magnified 7700 times. *L. delbrueckii* ssp. *bulgaricus* (Figure 6.3.11a) and *S. thermophilus* (Figure 6.3.11c) are seen. The rods of *L. delbrueckii* ssp. *bulgaricus* seem to covered by some material, which could be EPS (Figure 6.3.11b), produced by the starter culture similar to that reported in an earlier study (Bhaskaracharya and Shah, 2000). The bacteria seem to be located in the serum void (Figure 6.3.11d).

The microstructure studies showed the EPS cheeses had a porous and open texture. The bacteria were located in the serum voids of the EPS cheeses and produced sufficient quantities of EPS, which could be seen under SEM. The EPS appeared in the form of filaments probably due to dehydration of the EPS during SEM preparation. In the natural form the EPS in the cheeses is expected to be in the form of a gel which would be constituted atleast to 95% water level. When this water is removed, the gel would shrink into filaments (in fully hydrated specimens EPS would not be observed as filaments). The amount of observed filaments or their thickness as observed under SEM do not necessarily correlate with the amount of EPS actually produced by the culture, but may in fact reflect effects of dehydration. Mistry and Anderson (1993) and Hassan *et al.*, (1995) observed that capsular/ ropy EPS produced a spongy structure in casein matrix thus mimicking the milk fat. The spongy texture of the milk gels observed could be a

result of decreased dominance of the structural matrix and increased water holding in such low fat products. The non-EPS cheeses were made from skim milk, and showed fewer voids of fat and a compact protein matrix.

The EPS cheeses showed a more open and porous body which could cause lower values of texture profile parameters. Although a definite relation between textural parameters and microstructure could not be established from this study it would give a better insight to understand how the texture and functionality changes with change in microstructure of the mozzarella cheese.

6.4 Conclusions

The EPS cheeses had 1.71% higher moisture content than the non-EPS cheeses. The EPS produced by the starter organisms probably caused an increase in moisture content of the mozzarella cheeses. In general, the EPS cheeses showed better texture characteristics than the non-EPS cheeses. EPS cheeses showed decreased hardness, cohesiveness and adhesiveness while springiness was higher compared to the non-EPS cheeses. There was no definite trend in the gumminess and chewiness for the two types of cheeses. The EPS cheeses showed more voids in the cheese matrix, which resulted in an increased porosity and openness of the cheeses. The EPS produced by the starter organisms appeared to be dehydrated during SEM preparation and could be thus seen as filaments extending from the protein matrix. Thus use of EPS producing starter cultures improved the texture and microstructure characteristics in skim milk mozzarella cheeses. Further work is required to study the actual changes in microstructure that are occurring during storage to correlate microstructure with the texture changes.

6.5 References

Barbano, D. M., Clark, J. L., Dunham, C. E., and Fleming, J. R. (1990). Kjeldahl method for determination of total nitrogen content of milk: collaborative study. *J. Assoc. Anal. Chem.* 73:849-859.

Bhaskaracharya, R. K., and Shah, N. P. (1999). Texture evaluation of commercial mozzarella cheeses. *Aust. J. Dairy Technol.* 54:36-40.

Bhaskaracharya, R. K., and Shah, N. P. (2000). A simplified method for examination of microstructure of mozzarella cheeses with scanning electron microscopy. *Aust. J. Dairy Technol.* 55: 28-32.

Chen, A. H., Larkin, J. W., Clark, C. J., and Irwin, W. E. (1979). Textural analysis of cheese. *J. Dairy Sci.* 62:901-907.

Chen, C. M., Jaeggi, J. J, and Johnson, M. E. (1997). Manufacturing lower fat mozzarella cheese by adding milk coagulant at different pH values. *J. Dairy Sci.* 80 (Suppl.1):105.

Cooke, P. H., Tunick, M. H., Malin, E. L., Smith, P. W., and Holsinger, V. H. (1995). Electron-density patterns in low fat mozzarella cheeses during refrigerated storage. p.311-320 *in* Chemistry of Structure-Function Relationships in Cheese. Plenum Press Publ., New York.

Egan, H., Kirk, R. S., and Sawyer, R. (1987). Pearson's chemical analysis of foods. 8 th ed., Harlow: Longman Scientific and Technical Publications.

Emmons, D. B., Kalab, M., Larmond, E., and Lowrie, R. J. (1980). Milk gel structure. X. Texture and microstructure in cheddar cheese made from whole milk and from homogenised low-fat milk. *J. Texture Studies* 11:15-34.

Fecera, R. M., McMahon, D. J., and Oberg, C. J. (1995). Effect of EPS producing cultures on moisture retention in low fat mozzarella cheese. *IFT Annual meeting*-Tech. session, 134.

Fife, R. L., McMahon, D. J., and Oberg, C. J. (1996). Functionality of low fat mozzarella cheese. *J. Dairy Sci.* 79:903-1910.

Fife, R. L., McMahon, D. J., and Oberg, C. J. (1997). Moisture partitioning in low fat mozzarella cheese and its relationship to cheese microstructure. *J. Dairy Sci.* 79:(Suppl. 1):112.

Halmos, A. L. (1997). Food texture and sensory properties of dairy ingredients. *Food Aust.* 49:169-173.

Hassan, A. N., Frank, J. F., Farmer, M. A., Schmidt, K. A. and Shalabi, S. I. (1995a). Observation of Encapsulated Lactic acid bacteria using confocal scanning laser microscopy. *J. Dairy Sci.* 78: 2624-2628.

Hassan, A. N., Frank, J. F., Farmer, M. A., Schmidt, K. A. and Shalabi, S. I. (1995b). Formation of the yogurt microstructure and three dimensional visualization as determined by confocal scanning laser microscopy. *J. Dairy Sci.* 78: 2629-2636.

Hassan, A. N., Frank, J. F., Farmer, M. A., Schmidt, K. A. and Shalabi, S. I. (1996a). Rheological properties of yogurt made with encapsulated non-ropy lactic cultures. *J. Dairy Sci.* 79: 2091-2097.

Hassan, A. N., Frank, J. F., Farmer, M. A., Schmidt, K. A. and Shalabi, S. I. (1996b). Textural properties of yogurt made with encapsulated non-ropy lactic cultures. *J. Dairy Sci.* 79: 2098-2103.

Hassan, A. N. and Frank, J. F. (1997). Modification of microstructure and texture of rennet curd by using a capsule forming non-ropy lactic culture. *J. Dairy Res.* 64: 115-121.

Hong, Y. H., Yun, J. J., Barbano, D. M., Larose, K. L., and Kindstedt, P. S. (1998). Mozzarella cheese: Impact of three commercial culture strains on composition, proteolysis and functional properties. *Aust. J. Dairy Technol.* 53:163-169.

Jana, A. H., and Upadhyay, K. G. (1997). Comparative appraisal of quality of buffalo-milk mozzarella cheese manufactured by two different methods. *J. Food Sci. Technol.* 34:381-385.

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

Kiely, L. J., Kindstedt, P. S., Hendricks, G. M., Levis, J. E., Yun, J. J., and Barbano, D. M. (1992). Effect of draw pH on the development of curd structure during the manufacture of mozzarella cheese. *Food Struct.* 11:217-224.

Kiely, L. J., Kindstedt, P. S., Hendricks, G. M, Levis, J. E., Yun, J. J., and Barbano, D. M. (1993). Age related changes in the microstructure of mozzarella cheese. *Food Struct.* 12:13-20.

Kindstedt, P. S., and Guo, M. R. (1997). Recent developments in the science and technology of pizza cheese. *Aust. J. Dairy Technol.* 52:41-43.

Kindstedt, P. S., and Guo, M. R. (1998). A physico-chemical approach to the structure and function of mozzarella cheese. *Aust. J. Dairy Technol.* 53:70-73.

Kirkpatrick, L. A., and Feeney, B. C. (1997). A simple guide to SPSS for windows: versions 6.0, 6.1, 7.0 and 7.5. Intl. Thomson Publ. Co., California, USA.

Low, D., McMahon, D. J., Oberg, C. J., Hornel, D., and Broadbent, J. R. (1997). Influence of *Streptococcus thermophilus* 1 OJC EPS on the moisture content of low fat mozzarella cheese. *J. Dairy Sci.* 80 (Suppl.1):107.

Lucisano, M., Pompei, C., and Casiraghi, E. (1987). Texture evaluation of some Italian cheeses by instrumental texture profile analysis. *J. Food Quality* 10:73-89.

Masi, P. and Addeo, F. (1986). An examination of some mechanical properties of a group of Italian cheeses and their relation to structure and conditions of manufacture. *J. Food Eng.* 5:217-229.

McMahon, D. J. (1995). How the microstructure of mozzarella cheese is related to functional properties. Proceedings 32nd Annual Marschall Italian and Speciality Cheese Seminar. Rhone-Poulenc, Madison, WI. p.18-26.

McManus, W. R., McMahon, D. J., and Oberg, C. J. (1993). High resolution scanning electron microscopy of milk products: A new sample preparation procedure. *Food Struct.* 12:475-482.

Merrill, R. K., Oberg, C. J., and McMahon, D. J. (1994). A method for manufacturing reduced fat mozzarella cheese. *J. Dairy Sci.* 77:1783-1789.

Mistry, V. V., and Anderson, D. L. (1993). Composition and microstructure of commercial full fat and low fat cheeses. *Food Struct.* 12:259-

Oberg, C. J., McManus, W. R., and McMahon, D. J. (1993). Microstructure of mozzarella cheese during manufacture. *Food Struct.* 12:251-258.
Pagliarini, E., and Beatrice. N. (1994). Sensory and rheological properties of low fat filled 'Pasta filata' cheese. *J. Dairy Res.* 61:299-304.

Pagliarini, E. and Beatrice, N. (1994). Sensory and rheological properties of low fat filled 'pasta filata' cheese. *J. Dairy Res.* 61: 299-304.

Paquet, A., and Kalab, M. (1988). Amino acid composition and structure of cheese baked as a pizza ingredient in conventional and microwave ovens. *Food Microstruct.* 7:93-103.

Patel, G. C., Vyas, S. H., and Upadhyay, K. G. (1998). Influence of fat content and heat treatment of milk on quality of mozzarella cheese made from buffalo milk by starter culture technique. *Indian J. Animal Sci.* 68:978-981.

Paulson, B. M., McMahon, D. J., and Oberg, C. J. (1998). Influence of sodium chloride on appearance, functionality, and protein arrangements in non-fat mozzarella cheese. *J. Dairy Sci.* 81:2053-2064.

Perry, D. B., McMahon, D. J., and Oberg, C. J. (1995). Effect of proteolytic adjunct cultures on the physical properties of low fat mozzarella cheese. *IFT Annual meeting*-Technical session, 134.

Perry, D. B., McMahon, D. J., and Oberg, C. J. (1997). Effect of EPS producing cultures on moisture retention in low fat mozzarella cheese. *J. Dairy Sci.* 80:799-805.

Perry, D. B., McMahon, D. J., and Oberg, C. J. (1998). Manufacture of low fat mozzarella cheese using EPS producing starter cultures. *J. Dairy Sci.* 81:563-566.

Poduval, V. S., and Mistry, V. V. (1999). Manufacture of reduced fat mozzarella cheese using ultrafiltered sweet buttermilk and homogenised cream. *J. Dairy Sci.* 82:1-9.

Rodriguez, J. (1998). Recent advances in the development of low fat cheeses. *Trends Food Sci Technol.* 9:249-254.

Rousseau. M. (1988). Changes in the microstructure of Saint Paulin cheese during manufacture studied by scanning electron microscopy. *Food Microstruct.* 7:105-113.

Rudan, M. A., Barbano, D. M., Guo, M. R., and Kindstedt, P. S. (1998). Effect of the modification of fat particle size by homogenization on composition, proteolysis, functionality and appearance of reduced fat mozzarella cheese. *J. Dairy Sci.* 81:2065-2076.

Sunder, M. R., and Upadhyay, K. G. (1991). Influence of casein/fat ratio of milk on baking, rheological and sensory characteristics of buffalo milk mozzarella cheese. *J. Food Sci. Technol.* 28:98-100.

Taranto, M. V., Wan, P. J., Chen, S. L., and Rhee, K. C. (1979). Morphological, ultrastructural and rheological characterization of cheddar and mozzarella cheese. *Scanning Electron Microscopy.* p. 273-278.

Tunick, M. H., Malin, E. L., Smith, P. W., and Holsinger, V. H. (1995a). School lunch pizzas topped with low fat mozzarella cheese. *Cult. Dairy Products J.* 30:6-9.

Tunick, M. H., Malin, E. L., Smith, P. W., and Holsinger, V. H. (1995b). Effects of skim milk homogenization on proteolysis and rheology of mozzarella cheese. *Int. Dairy J.* 4:483-491.

Tunick, M. H., Malin, E. L., Smith, P. W., Shieh, J. J., Sullivan, B. C., Mackey, K. L., and Holsinger, V. H. (1993b). Proteolysis and rheology of low fat and full fat mozzarella cheeses prepared from homogenised milk. *J. Dairy Sci.* 76:3621-3628.

Tunick, M. H., Mackey, K. L., Shieh, J. J., Smith, P. W., Cooke, P., and Malin, E. L. (1993a). Rheology and microstructure of low fat mozzarella cheese. *Int. Dairy J.* 3:649-662.

Tunick, M. H., Mackey, K. L., Smith, P. W., and V. H. Holsinger. (1991). Effects of composition and storage on the texture of mozzarella cheese. *Neth. Milk Dairy J.* 45:117-125.

Tunick, M. H., Cooke, P. H., Malin, E. L., Smith, P. W., and Holsinger, V. H. (1997). Reorganisation of casein submicelles in mozzarella cheese during storage. *Int. Dairy J.* 7:149-155.

Yun, J. J., D. M. Barbano, and Kindstedt, P. S. (1995). Mozzarella cheese: impact of whey pH at draining on chemical composition, proteolysis and functional properties. *J. Dairy Sci.* 78:1-7.

Table 6.3.1. Selected composition of mozzarella cheeses.

Type	Moisture (%) Mean	S.D.	Protein (%) Mean	S.D.	Fat (%)
EPS Cheese	54.38[a]	2.58	42.47[a]	0.86	< 3.0
Non-EPS Cheese	52.67[a]	3.75	43.48[a]	0.25	< 3.0

S.D. = Standard deviation
[a] Not significant

Table 6.3.2. Effect of age, compression, age x compression, culture, age x culture on the hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness of the EPS and non-EPS cheeses.

Texture Characteristics	Age	Compression	Age x Compression	Culture	Age x Culture
Hardness	***	***	***		
Cohesiveness	***	***	*	**	
Adhesiveness	**				
Springiness	***	***	**		**
Gumminess	***	***	***		
Chewiness	***	***	***		

*** $P < 0.001$

** $P < 0.01$

* $P < 0.05$

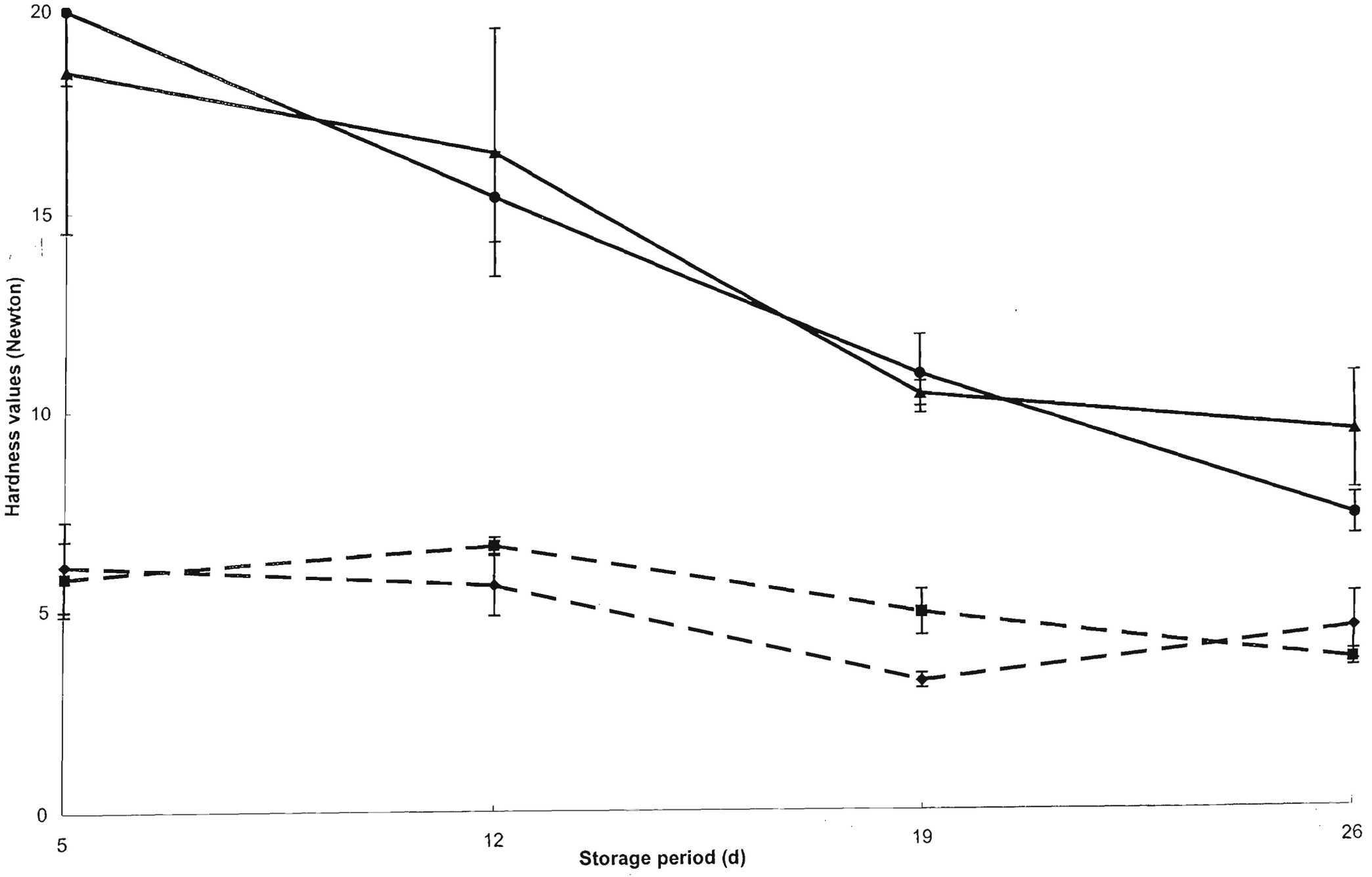

Figure 6.3.1. Hardness values of EPS and non-EPS cheese samples over a 26 d storage period measured at 50 (EPS = ■; non-EPS = ♦) and 70% compression (EPS = ● ; non-EPS = ▲).

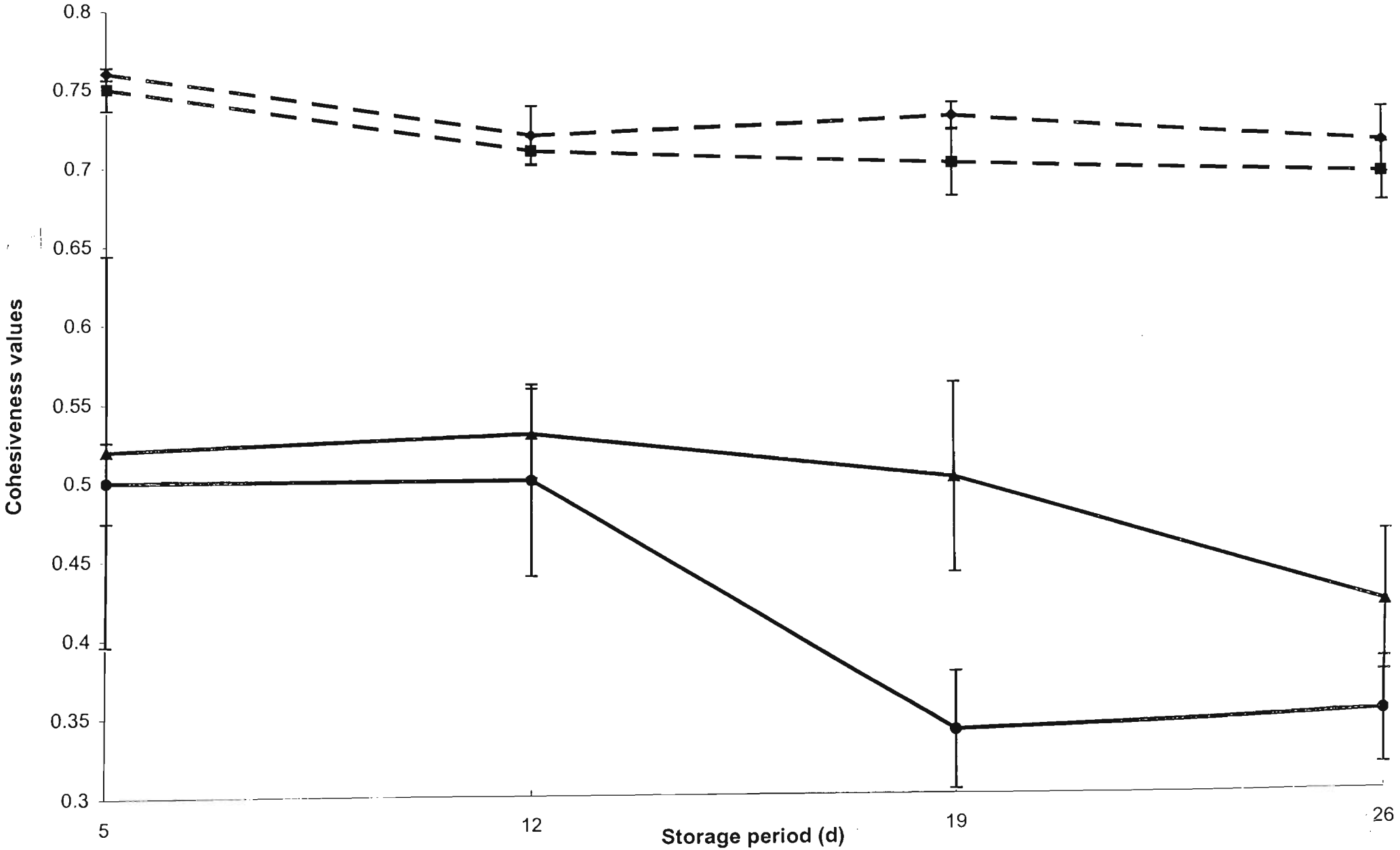

Figure 6.3.2. Cohesiveness values of EPS and non-EPS cheese samples over a 26 d storage period measured at 50 (EPS = ■; non-EPS = ♦) and 70% compression (EPS = ● ; non-EPS = ▲).

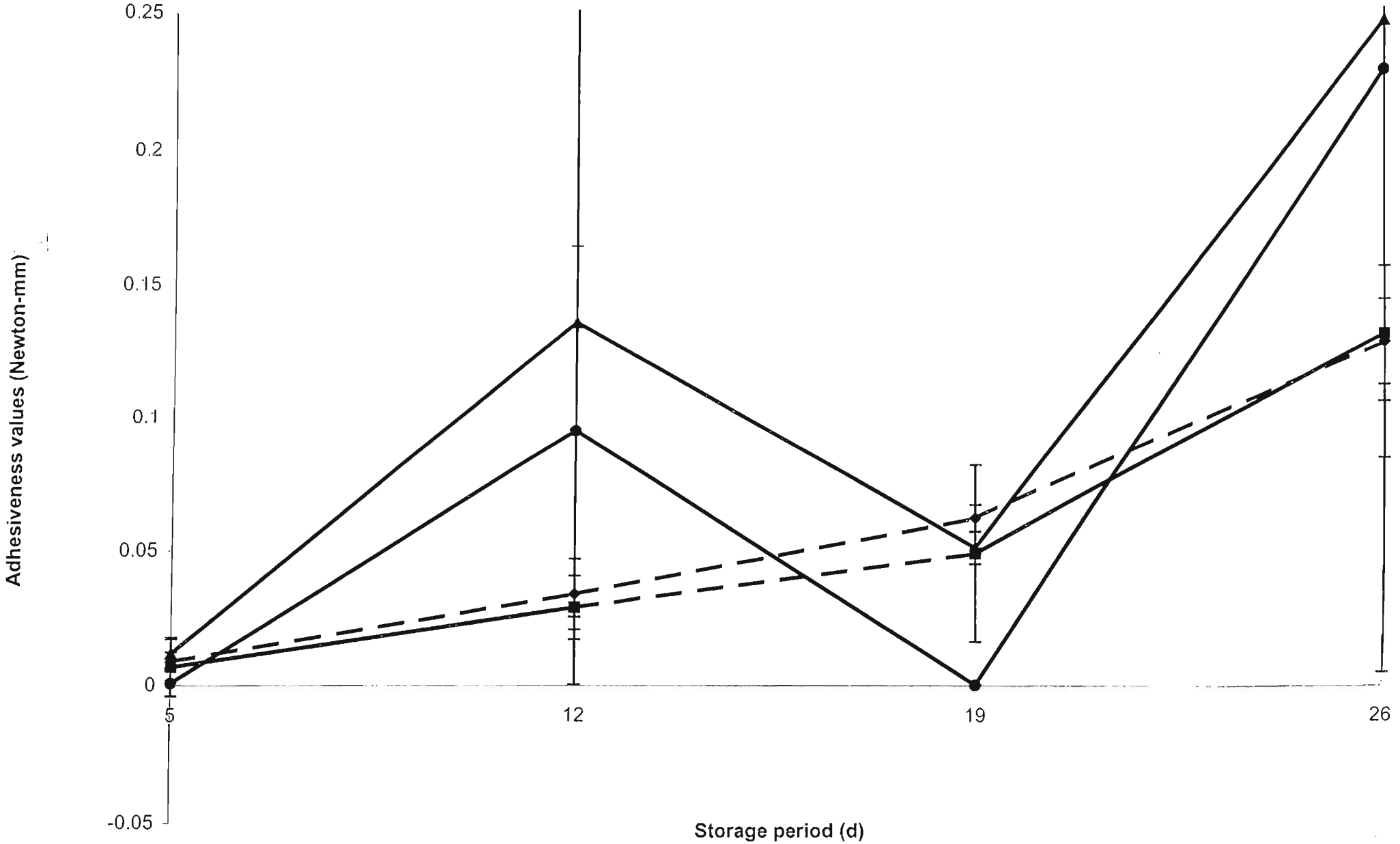

Figure 6.3.3. Adhesiveness of EPS and non-EPS cheese samples over a 26 d storage period measured at 50 (EPS = ■; non-EPS = ♦) and 70% compression (EPS = ● ; non-EPS = ▲).

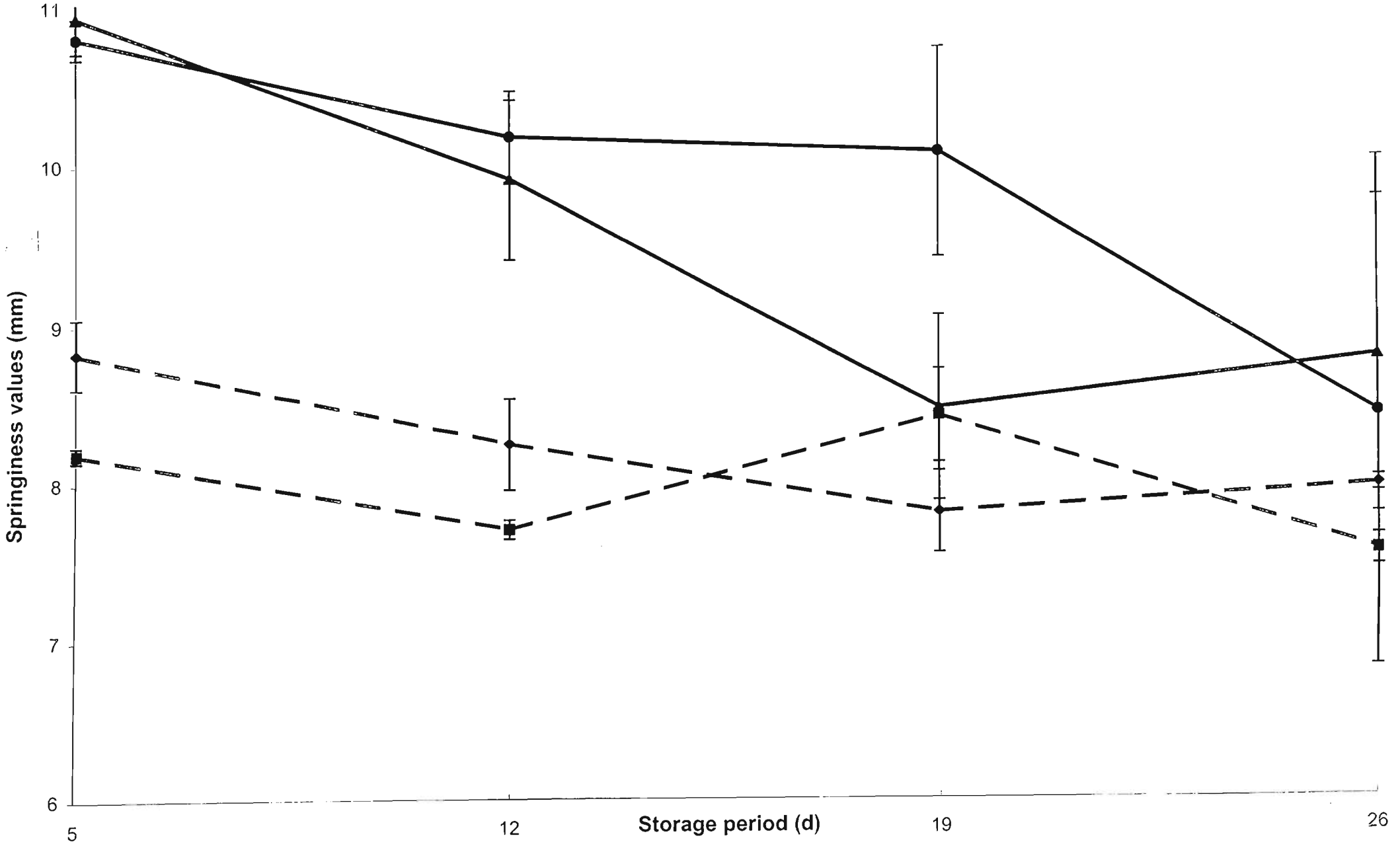

Figure 6.3.4. Springiness values of EPS and non-EPS cheese samples over a 26 d storage period measured at 50 (EPS = ■; non-EPS = ♦) and 70% compression (EPS = ● ; non-EPS = ▲).

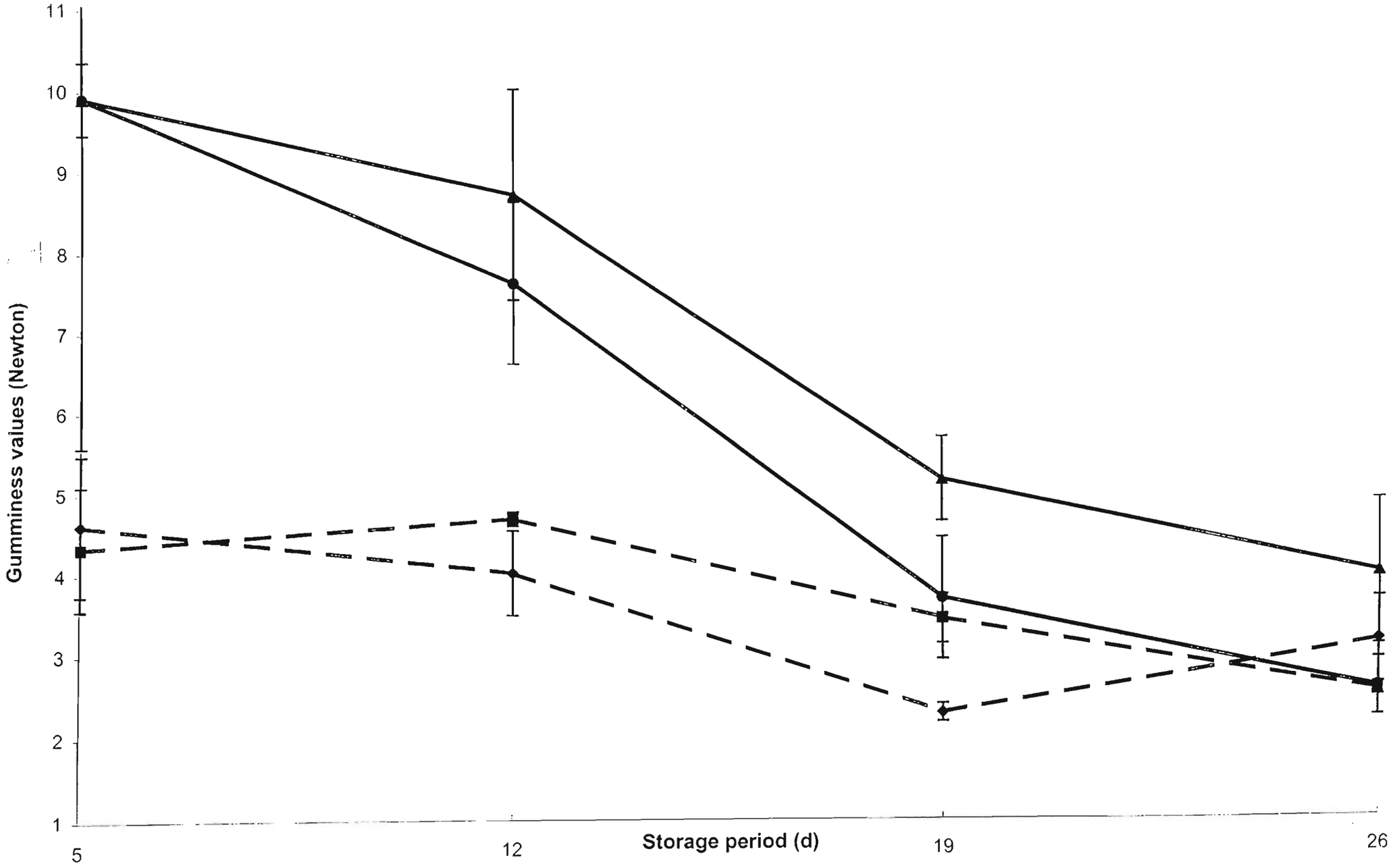

Figure 6.3.5. Gumminess of EPS and non-EPS cheese samples over a 26 d storage period measured at 50 (EPS = ■; non-EPS = ♦) and 70% compression (EPS = ● ; non-EPS = ▲).

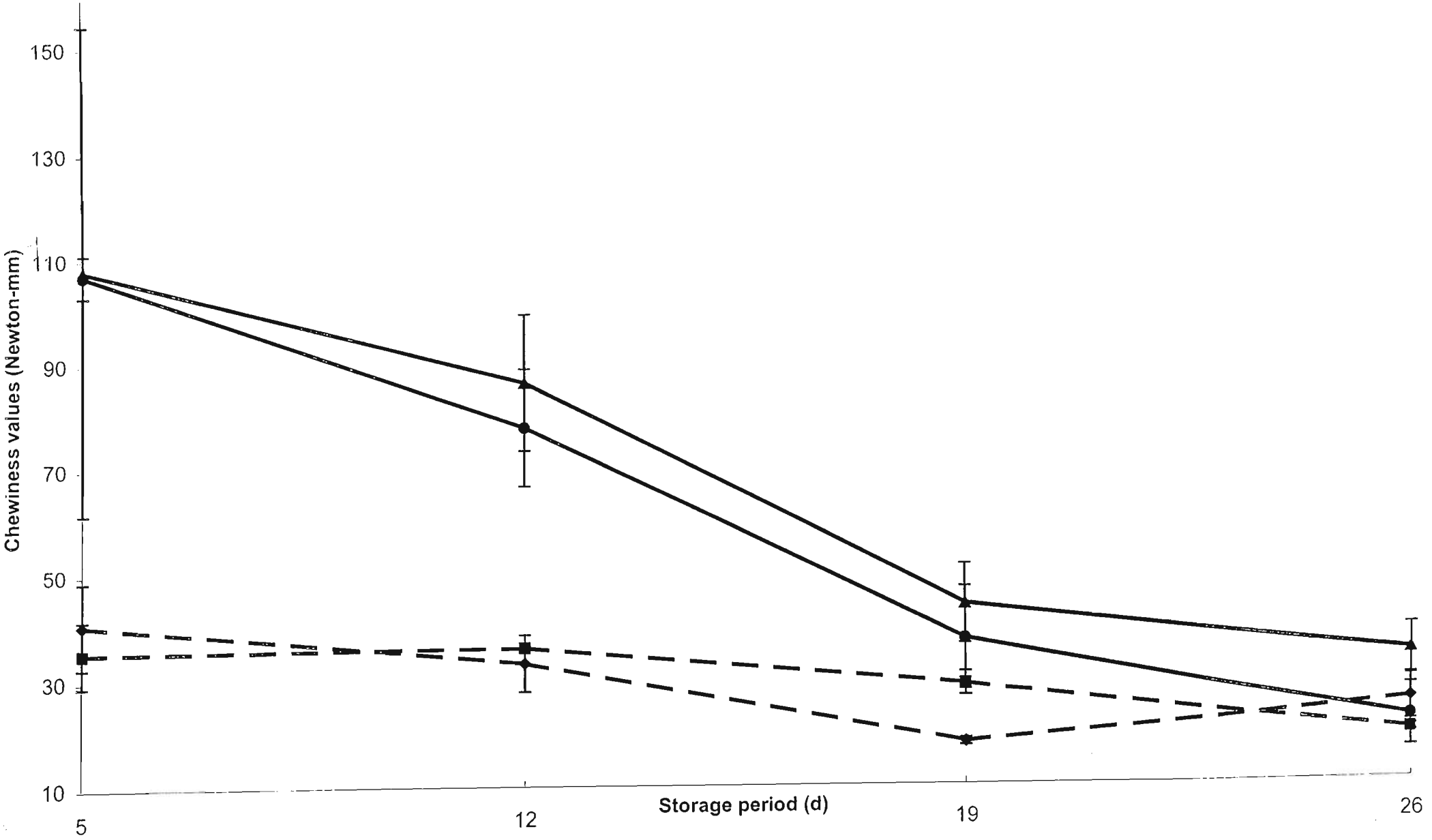

Figure 6.3.6. Chewiness of EPS and non-EPS cheese samples over a 26 d storage period measured at 50 (EPS = ■; non-EPS = ♦) and 70% compression (EPS = ● ; non-EPS = ▲).

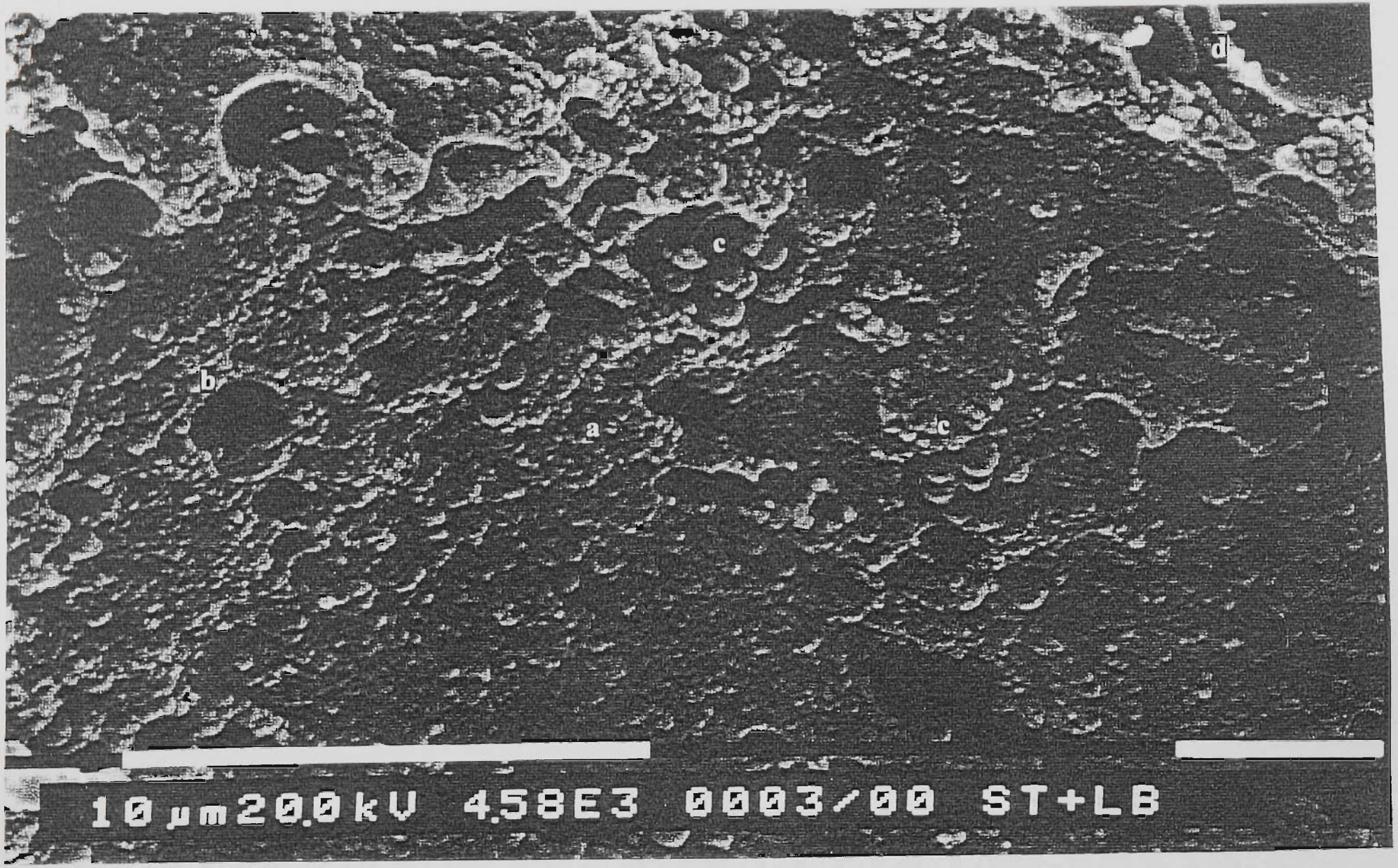

Figure 6.3.7. A scanning electron micrograph of 28 d old skim milk mozzarella cheese made using non-EPS producing starter cultures. The protein matrix (a) is seen having serum voids (b) and *S. thermophilus* (c) and *L. delbrueckii* ssp. *bulgaricus* (d) are found located in these voids.

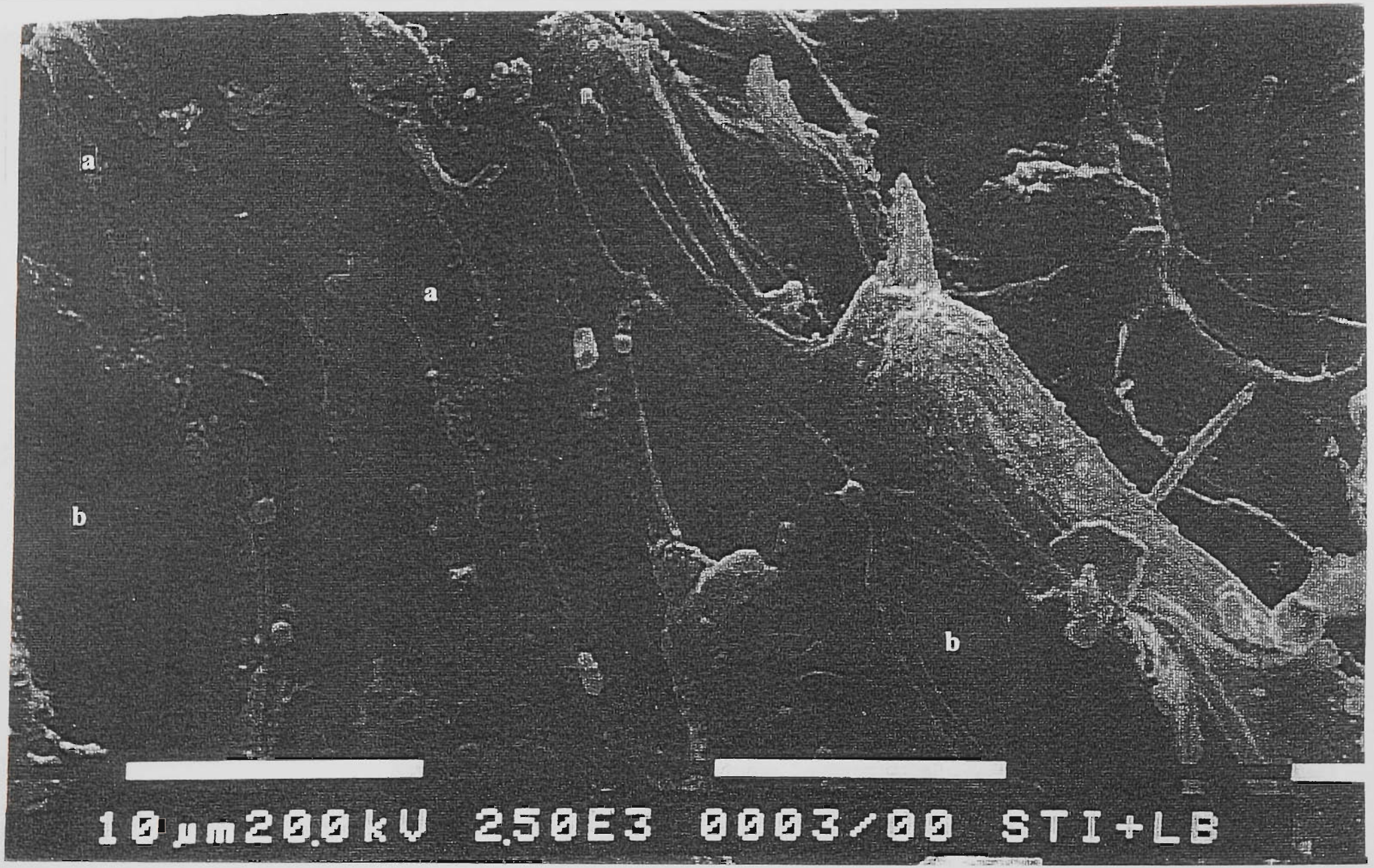

Figure 6.3.8. A scanning electron micrograph of 28 d old skim milk mozzarella cheese made using EPS producing starter cultures. The elongated protein strands (a) and large serum voids (b) are seen.

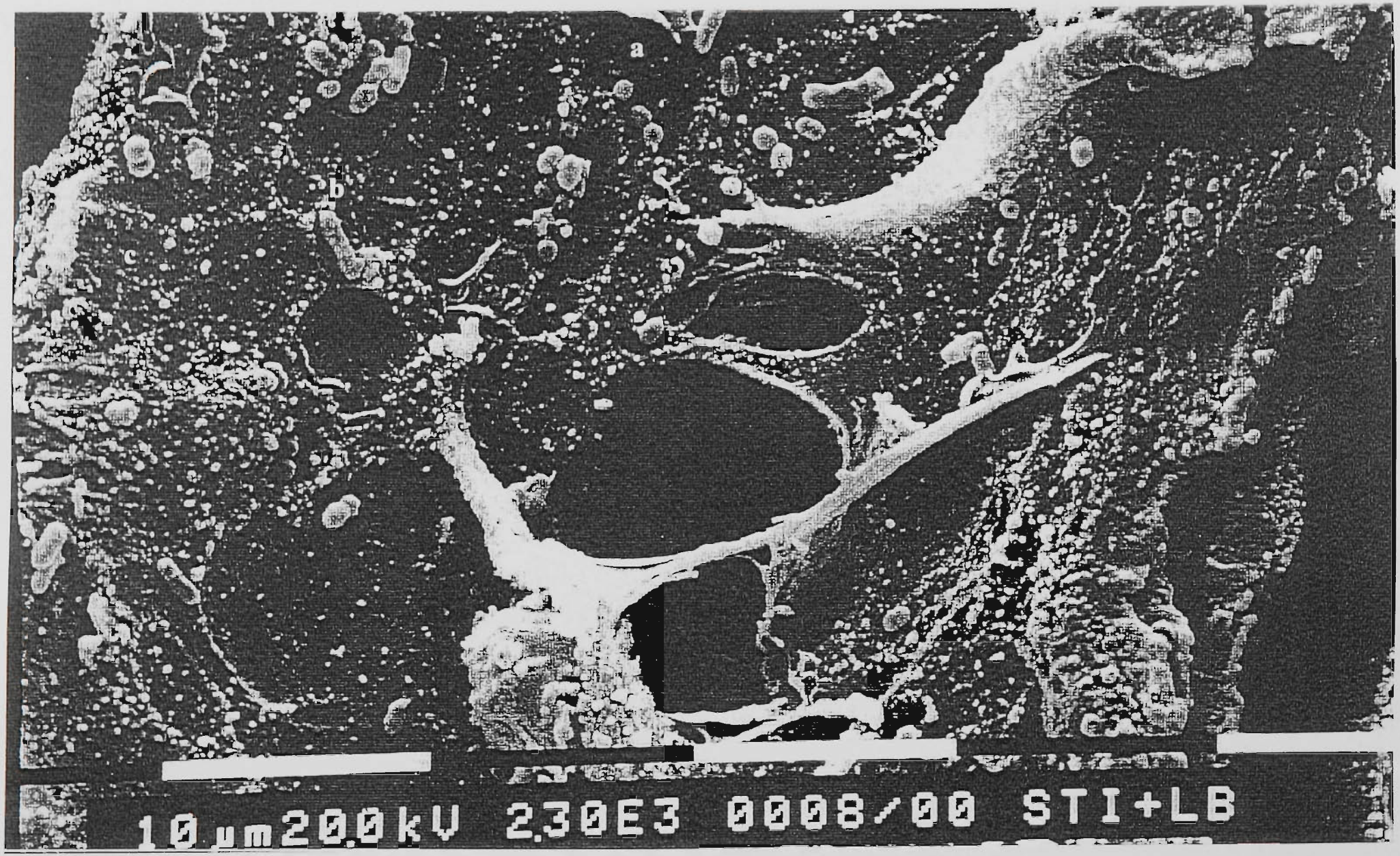

Figure 6.3.9. A scanning electron micrograph of 28 d old skim milk mozzarella cheese made using EPS producing starter cultures. *S. thermophilus* (a) and *L. delbrueckii* ssp. *bulgaricus* (b) are seen covered with EPS. Minute particles (c) of proteins are visible on the surface of the cheese matrix.

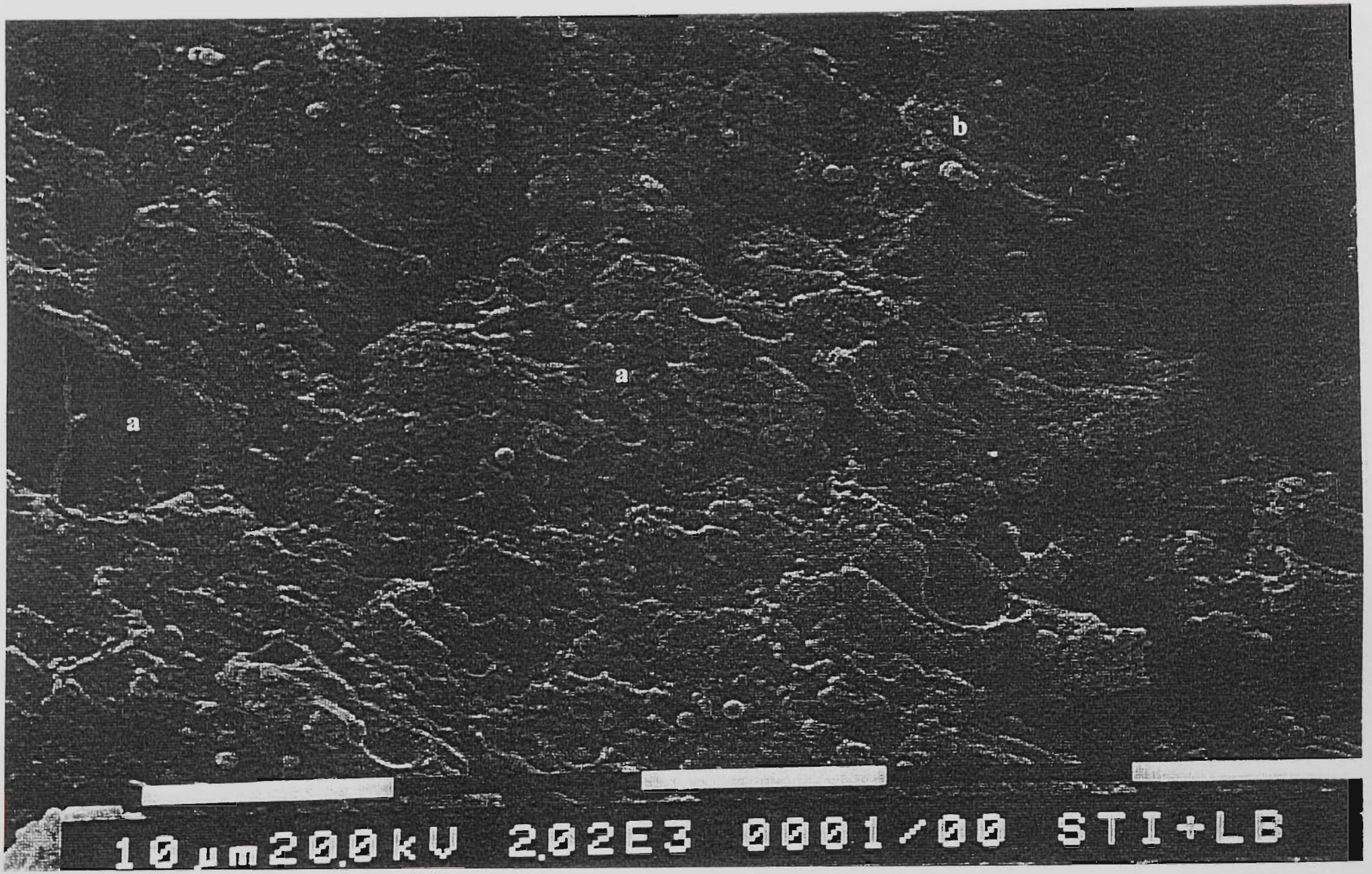

Figure 6.3.10. A scanning electron micrograph of 28 d old skim milk mozzarella cheese made using EPS producing starter cultures. The cheese specimen shows numerous small voids (a), and *S. thermophilus* (b) are visible on the surface.

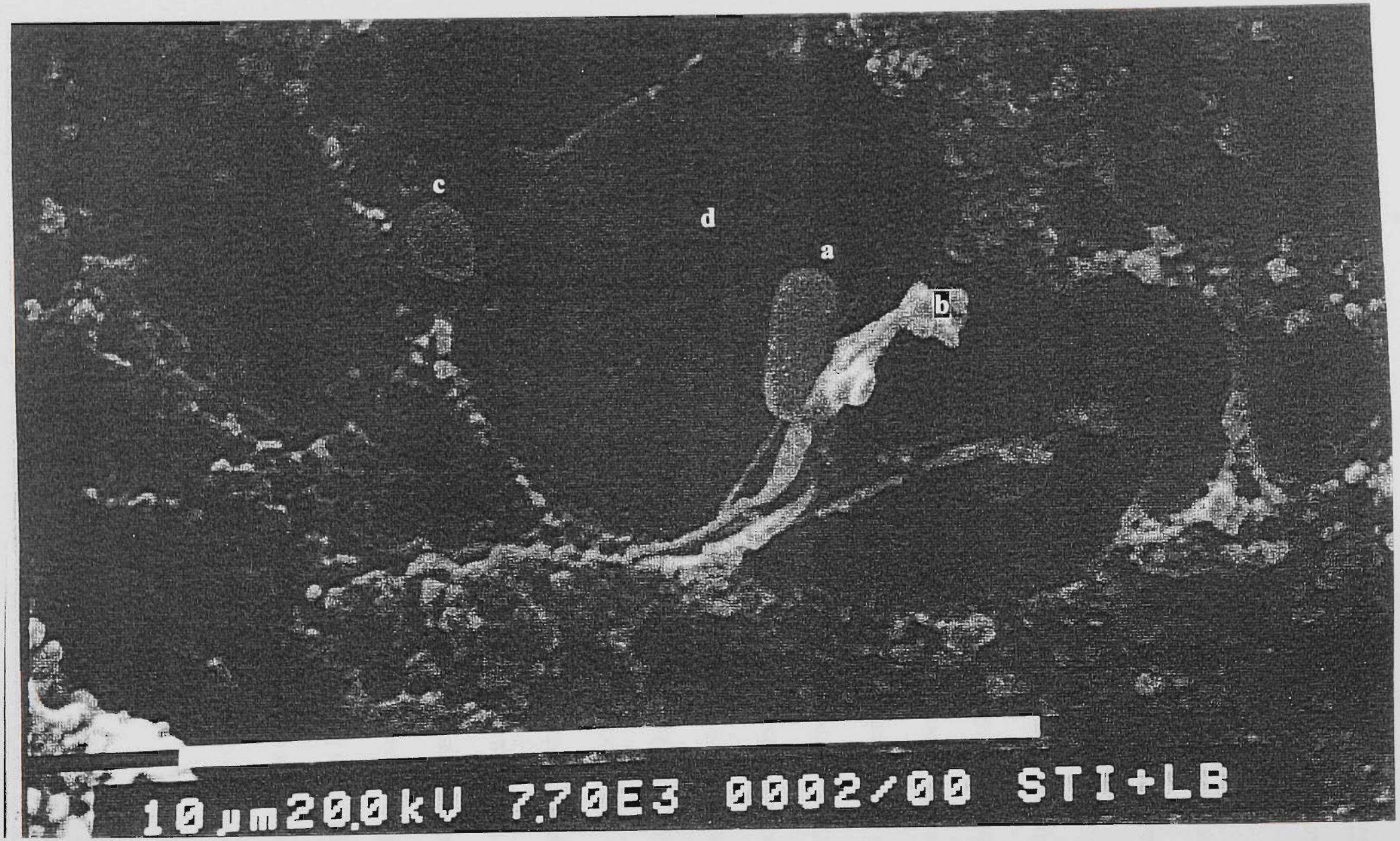

Figure 6.3.11. A scanning electron micrograph of 28 d old skim milk mozzarella cheese made using EPS producing starter cultures. *L. delbrueckii* ssp. *bulgaricus* (a), EPS (b) produced and *S. thermophilus* (c) are visible in the serum void (d).

7.0. TEXTURE AND MICROSTRUCTURE CHARACTERISTICS OF SKIM MILK MOZZARELLA CHEESES MADE USING FAT REPLACERS

7.1 Introduction

The manufacture of reduced fat and low fat mozzarella cheeses with organoleptic properties similar to full fat cheeses poses a challenge to the cheese manufacturers. The main texture defect ascribed to low fat cheese is an excessive firmness and a crumbly or chewy mouthfeel. The problems associated with reduction in fat content such as reduced melt, stretch and texture defects can be overcome by the addition of ingredients or by inducing changes through modifications in processing. To overcome these problems addition of fat replacers including Simplesse® in cheddar cheese manufacture (Lucey and Gorry, 1993; Desai and Nolting, 1995) and Stellar®, Dairy Lo®, Novagel® and Simlesse® in mozzarella cheese manufacture (McMahon *et al.*, 1996) have been studied. Addition of Simplesse®, a microparticulated whey protein, caused weakening of curd matrix in cheddar cheese; this was attributed to prevention of excessive cross-linking in cheese. Fat globules in cheese serve as pliable filler and interact with the casein matrix to reinforce or weaken the curd structure (Walstra and Jenness, 1984). The presence or absence of fat impacts the salt to moisture ratio, texture of the cheese (Lawrence et al., 1987), microstructure and functionality of the cheese (McMahon et al., 1996). Mozzarella cheeses made using fat replacers are reported to show a change in meltability. Reduction in fat content causes excessive coalescing of protein strands causing reduction in water retention thereby decreased melt and hard or rubbery body (McMahon, 1995). These problems in cheeses could possibly be overcome by addition of fat replacers.

The microstructure of full fat and reduced fat mozzarella, cheddar, swiss and processed

A version of this chapter is under review (Bhaskaracharya, R. K. and Shah, N. P. (1999). Aust. J. Dairy Technol.).

cheeses has been studied by Mistry and Anderson (1993) using scanning electron microscopy. The structure of low fat cheeses was dominated by the protein network, which had fewer and smaller fat globules.

The aims of this investigation were to study the effects on texture characteristics such as hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness over 18 d storage period and examine microstructure of mozzarella cheeses made using fat replacers using scanning electron microscopy.

7.2 Materials and Methods

7.2.1 Cultures, Rennet and Milk

Full cream milk was obtained from a local cheese factory and the cream was separated using a batch type cream separator (Model 107 AK; Alfa Laval, Lund, Sweden). The skim milk contained less than 0.2% fat. The skim milk (10 L) was pasteurised (72°C for 15 sec) using a pilot scale HTST pasteuriser (Alfa Laval, Lund, Sweden) tempered to 30°C in a 30 L capacity cheese vat. Liquid rennet (Chymosin, 145 IMCU) and starter cultures consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* were obtained from Chr. Hansen (Bayswater, Australia). The starter cultures were propagated in reconstituted skim milk supplemented with yeast extract and glucose.

7.2.2 Fat Replacers

The fat replacers Maltrin® M040 and M100 are maltodextrins while Staslim® 143 is a modified potato starch. The quantity of fat replacer used in making cheese was based on usage levels recommended by the manufacturer. The levels were 10g for Maltrin® M040 and M100, each and 20g for Staslim® 143 per kg of milk. Dispersion of fat replacers in milk was

also according to manufacturers recommendations. Each fat replacer was added to milk at 22°C and dispersed for 3 to 4 minutes using a high speed mixer (Kolloid Technik, Probst and Class GmbH, Rastatt, Germany); warmed to 35°C, passed through a two stage homogeniser (Manton-Gaulin, APV Co. Ltd., England) and pasteurised similar to control skim milk.

7.2.3 Preparation of Mozzarella Cheese

Three batches of mozzarella cheeses (10 L batch size) were made with fat replacers according to the methods of Bhaskaracharya and Shah (1999c). A control batch was made without fat replacers. Milk was pasteurised in a HTST pasteuriser, added with 1% of each starter culture, and allowed to ripen for approximately 1h. When the pH dropped by 0.1 unit, rennet enzyme (2.5 mL diluted in 100mL distilled water/10 kg) was added to milk. The temperature of milk was maintained at 35°C throughout the cheese making process until stretching of the curd. The milk coagulated in 35 min. The curd was cut to 1 cubic cm size and allowed to heal in the whey until the pH dropped to 5.6, followed by draining of the whey. The curd was cheddared (piled and re-piled every 15 min) until the pH 5.2 was reached. The cheddared curd was cut and hand stretched in hot water at 85°C until elastic and smooth, hand molded, cooled under running tap water, and placed in saturated brine solution at 4°C for 2 h, similar to the method used by Jana and Upadhyay (1997). The finished cheese was vacuum-packaged and stored at 4°C. The experiment was repeated three times.

7.2.4 Composition Analyses

The moisture content of the cheeses was determined in triplicate by the oven drying method (Egan *et al.*, 1987). The protein content was estimated in triplicate by the Kjeldahl method (Barbano *et al.*, 1990). The fat content of the cheeses was analysed by the method described by Bhaskaracharya and Shah (1999a).

7.2.5 Texture Analyses

The cheese blocks were left at 20°C for 1 h and cylindrical specimens measuring 25 mm x 20 mm were cut using a cheese corer. Three specimens were obtained from each cheese block and were analysed for texture characteristics such as hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness. The Instron Universal Testing Machine (Model 5564; Instron Ltd., London, England) was used to analyse the texture characteristics of all the cheeses according to the method described by Bhaskaracharya and Shah (1999a). The samples were compressed to 50 and 70% of their heights using a 500 N load cell with a flat plunger and the crosshead movement was adjusted to 50 mm per min. A double compression was achieved and the data was collected using a Merlin software. Samples of all the cheeses were analysed on 5, 10 and 18 d of storage.

7.2.6 Statistical Analyses

The effects of moisture content, age, compression and type of fat replacer and their combined effects on the texture characteristics of mozzarella cheeses made using fat replacers were assessed using the multivariate and tests of between-subjects effects analysis. The SPSS version 8.0 was used for statistical analysis (Kirkpatrick and Feeney, 1997) and the significance was determined at $P < 0.05$. The dependent variables, degrees of freedom and the significance of each factor were measured.

7.2.7 Microstructure

The specimens were prepared from mozzarella cheeses according to the method described by Bhaskaracharya and Shah (1999b) and Tunick *et al.* (1997). The finished cheeses were allowed to equilibrate at room temperature (~20°C) for 1 h and specimens of approximately 2 x 2 x 10

mm size were cut from several parts of the cheeses. The specimens were fixed for 1 h at room temperature (~20°C) and for 2 d at 4°C in 2% glutaraldehyde in 0.1 M cacodylate buffer solution, similar to the method described by McManus *et al.* (1993). The specimens were then washed in 0.1 M cacodylate buffer (pH 7.3) for 5 min, gradually dehydrated using ethanol at 30, 50, 70, 95 and 100% concentration, each for 5 min, washed in cacodylate buffer for 5 min and post-fixed overnight in 1.5% osmium tetroxide solution. These steps are as reported earlier (Rousseau, 1988). The fixed specimens were washed in distilled water twice each for 10 min, and further dehydrated with 30, 50, 70, 95 and 100% ethanol, each for 5 min and in 100% acetone for 5 min. The dehydrated specimens were dried in critical point drying equipment (custom made, Melbourne University, Melbourne, Australia) using liquid carbon dioxide. The dried specimens were fractured at room temperature (~20°C), mounted on aluminium stubs, sputter coated with gold using Edwards Sputter Coater and observed under Philips SEM515 scanning electron microscope at 20 kV.

7.3 Results and Discussion

7.3.1 Composition

The fat contents of the low fat cheeses were all below 3%. There were no differences ($P>0.05$) in the fat content among the cheeses (data not shown). Low fat cheeses made using Maltrin® M040 and M100 had similar moisture contents (Table 7.3.1) as the control cheese, while the protein contents were 8.7 and 11.6% lower. The low fat cheeses containing StaSlim® 143 had 10 and 11.9% lower moisture and protein contents respectively than the control cheeses. The differences in moisture and protein contents may reflect the quantity of fat replacers used in the cheese manufacture. The composition analyses (Table 7.3.1) shows that the cheeses made using fat replacers had low protein content but due to addition of fat

replacer the moisture content remained similar to the control cheeses. Addition of StaSlim® 143 lowered the moisture content unlike cheeses added with Maltrin® M040 and M100. Although StaSlim® 143 was added at a higher rate (2%) the moisture content of the cheese did not increase. The low fat, low moisture cheese was smooth and stretched in 85°C water similar to a full fat mozzarella cheese. The StaSlim® 143 particles possibly localised between the protein strands giving ease of stretching and behaved similar to the fat globules.

7.3.2 Texture characteristics

Addition of fat replacers had an effect on texture characteristics of the cheeses. Hardness values (Figure 7.3.1 and 7.3.2) were lower for cheeses added with Maltrin® M040 and M100 fat replacers, in particular with the latter compared to the control cheeses. The StaSlim® 143 based cheeses showed higher hardness values than the control cheeses throughout storage. This observation was consistent with lower moisture content of cheeses made with StaSlim.® 143. The hardness of cheeses made with StaSlim® 143 increased during storage. The increase in hardness in StaSlim® 143 cheeses over time could be attributed to the adsorption of water by the fat replacer particles. Water is taken up by the StaSlim® 143 particles as well as by the protein matrix. This could have led to the dramatic increase in hardness of StaSlim® 143 cheeses during storage.

It is interesting to note that both Maltrin M040 and M100 cheeses had similar moisture contents, but Maltrin M100 cheeses were less hard (or softer). The effect of addition of fat replacer and the type of fat replacer used had a significant ($P < 0.066$) effect on the hardness characteristic. Increased level of compression also accentuated to similar trends in the hardness values for all the cheeses although the scale for Y-axis was higher at 70% compression ($P < 0.001$).

Cohesiveness values (Figure 7.3.3, 7.3.4) of the fat replaced cheeses were significantly lower ($P < 0.001$) than the control cheeses throughout the storage. This trend is observed at 50 and 70% levels of compressions. The fat replaced cheeses showed similar values of cohesiveness characteristic at 50% compression while StaSlim® 143 based cheeses showed slightly higher values than the Maltrin® M040 and M100 based cheeses at 70% compression. The differences in the cohesiveness values among the cheeses was significant ($P <0.001$) when compression was increased from 50 to 70% levels.

Adhesiveness characteristic (Figure 7.3.5, 7.3.6) seemed to increase with the addition of fat replacer. The fat replaced cheeses showed increased adhesiveness values compared to the control cheeses throughout storage. Although adhesiveness characteristic was significantly affected ($P < 0.001$) by storage period the cheeses showed inconsistency in adhesiveness values during storage. The increased compression from 50 to 70% also significantly affected ($P < 0.05$) this characteristic of the cheeses.

Springiness values (Figure 7.3.7, 7.3.8) of fat replaced cheeses were lower than the control cheeses. Maltrin® M040 and M100 cheeses showed the least springiness through out storage at 50 and 70% compressions. StaSlim® 143 based cheeses showed higher springiness values compared to control cheeses at 70% compression. The effect of compression and storage period also had a significant ($P < 0.001$) effect on springiness characteristic of the cheeses similar to that reported by Bhaskaracharya and Shah (1999a). The type of fat replacer used and the rate of addition seemed to have a significant effect ($P < 0.068$) on the springiness values of the cheeses.

Gumminess (Figure 7.3.9, 7.3.10) and chewiness (Figure 7.3.11, 7.3.12) characteristics of control, Maltrin® M040 ad M100 cheeses decreased during storage while StaSlim® 143 based cheeses showed an increase. Maltrin® M040 and M100 cheeses showed lower values of gumminess and chewiness than control cheeses throughout storage. Both gumminess and chewiness characteristics showed a significant effect ($P < 0.001$) due to compression and the latter also showed an effect due to moisture ($P < 0.063$) and storage period ($P < 0.05$). These results are comparable to those reported by Bhaskaracharya and Shah (1999a).

7.3.3 Microstructure

7.3.3.1 Control cheeses.

Skim milk mozzarella cheeses made without the addition of fat replacer had the typical microstructure of mozzarella cheese; a continuous protein matrix interspersed with serum channels (Figure 7.13, 7.3.14). These hand stretched cheeses were less uni-directionally oriented compared to the mechanically stretched cheeses (Oberg *et al.*, 1993). The cheese samples were fractured perpendicular to the protein fibres so that the serum channels would be seen in cross section. The serum channels were about 5-10 μm in width. Compared with the low moisture, part skim mozzarella cheese (McMahon, 1995), the serum channels between the protein strands were fewer in the experimental cheeses. This phenomenon was expected due to the cheeses containing less than 3% fat.

In mozzarella cheese, the serum channels are the location of the fat globules and most of the bacteria (Bhaskaracharya and Shah 1999c, 1999d). The bacteria are lost from the serum channels after the sample is fractured during SEM preparation. Fat is also extracted during dehydration with ethanol. Although some bacteria were observed in the serum channel, most of them would have been washed from the fracture zone. Occasionally, bacteria and other

debris were observed on the fracture surface of the samples, probably as a result of an electrostatic attraction after being displaced from the serum channels during sample preparation (McMahon, 1995).

7.3.3.2 Maltrin® M040.

Addition of Maltrin® M040 increased the openness of the cheese structure (Figure 7.3.15, 7.3.16, 7.3.17) compared with that of the control cheese. Maltrin® M040 formed thick hydrated particles (0.01-0.05 mm diameter) which were embedded in the protein matrix and also were lying on the fracture surface. When the cheese samples were fractured during SEM preparation, the contents of exposed serum channels showed a continuous mass of Maltrin® M040 fat replacer which had formed a gel upon hydration and adhered to the protein matrix structure (Figure 7.3.15, 7.3.16, 7.3.17). Maltrin® M040 consists of particulated material which forms a fine network upon heat induced hydration (Figure 7.3.18) similar to heat induced whey protein gels (Langton and Hermansson, 1992). The same material upon freeze-drying (Figure 7.3.19) shows particles (0.5-1 μm diameter) coalescing into clumps. Although it would seem indistinguishable when Maltrin® M040 is intermixed with proteins with in the cheese structure, both the hydrated particles (Figure 7.3.15) and the gel (Figure 7.3.16) were observed. The shape and morphology of the fat replacer depended upon the location ie. within the protein matrix or in the serum channel. The morphology of Maltrin® M040 to exist as particulated material or as a fine stranded network possibly depends on the localised effects of pH, ionic strength and heating conditions (Desai and Nolting, 1995). The bacteria could also be embedded within the fat replacer particles and the latter could be being used as a carbohydrate source after degradation with the bacterial enzymes.

7.3.3.3 Matrin® M100.

Addition of Maltrin® M100 to the skim milk cheese increased the openness of the cheese structure (Figure 7.3.20, 7.3.21). These cheeses contained thick amorphous fat replacer gel embedded within the protein matrix. Due to binding forces and coalescing of fat replacer deep and large channels were formed which could be seen on the fractured surface during SEM preparation. A fractured surface parallel to the protein fibres shows the extent of indentation (Figure 7.3.22) but the displacement of the fat replacer from the exposed surface during SEM preparation caused only the debris to be seen. The fat replacer within the cheese matrix caused an expansion in the serum channels (0.1-0.2 mm diameter) thereby increasing the openness of the cheese structure (Figure 7.3.21).

The smooth amorphous fat replacer extruding from the protein matrix (Figure 7.3.20) in various locations can be observed on the surface of the cheese fractured perpendicular to the protein fibres. The extrusion of the fat replacer from the cheese could be a result of SEM preparation wherein the protein fibres were compressed together during dehydration of the specimens. During the SEM preparation the bacteria also seem to be displaced from the cheese surface thereby very few starter culture organisms could be seen similar to that reported by Bhaskaracharya and Shah (1999d). Maltrin® M100 seemed to more readily form an amorphous gel compared to Maltrin® M040. The air dried, aqueous dispersion of Maltrin® M100 powder, had the appearance of a continuous network of flakes (Figure 7.3.23). The network seemed to be quite stable to the dehydrating forces, although <0.01 mm in thickness. The high degree of porosity could cause an increased hydration or bonding of water molecules. When the aqueous Maltrin® M100 dispersion was freeze dried (Figure 7.3.24), a completely different structure was apparent, and the Maltrin® M100 particles were seen to exhibit a well defined boundary although coalescing could be seen. Such a difference

in structures could be expected due to the nature of the starch material. It was also apparent that Maltrin® M100 particles occupied the entire serum channel within the cheese and dehydration process during SEM preparation did not collapse this structure.

7.3.3.4 Staslim® 143.

Addition of StaSlim® 143 increased the openness of skim milk mozzarella cheese because the number and size of serum channels (Figure 7.3.25, 7.3.26) compared to those of control cheese (Figure 7.3.13, 7.3.14) were greater. At a higher magnification, spherical particles of approximately 0.01mm diameter were observed embedded within the protein matrix and also lying on the fracture surface. The size of StaSlim® 143 particles observed in the cheese (Figure 7.3.27) show that hydration caused swelling of these particles. These exhibited a smooth surface similar to that of fat globules and upon displacement during SEM preparation left behind small and large voids in the protein matrix.

The StaSlim® 143 particles observed on the fracture surface would originally have been contained in the serum channels, indicating that StaSlim® 143 was distributed between the protein matrix and the serum with no coalescence among the StaSlim® 143 particles themselves. However, unlike Maltrin® M040 and M100, which seemed to exist as a continuous flowing mass taking up the entire serum channels, the StaSlim® 143 particles seemed to exist separately within the serum channels (Figure 7.3.28) and possibly during storage increased in size, thereby increasing the diameter of the serum channels (0.01 mm to 0.2 mm diameter) while at the same time due to their indentations on the protein matrix probably caused a breakdown on the protein fibres. The air-dried, aqueous dispersion of StaSlim® 143 (Figure 7.3.29) showed particulation of the fat replacer but at the same time the freeze-dried specimen showed lack of particulation (Figure 7.3.30).

7.4 Conclusions

Strong correlations were observed between the moisture retained and texture characteristics of the cheeses made using fat replacers. Although no apparent increase in moisture content was observed the effect of fat replacer addition on the texture characteristics was observed. The size and extent of fat replacer microparticulation, as well as interaction between the fat replacer and caseins effected location of the fat replacer in the cheese structure. The small particles of the fat replacer (StaSlim® 143) could be seen distributed within the protein matrix while the larger particles were located in the serum channels. The former could have little effect on the openness of the cheese microstructure while the ones located in the serum channels could cause an increase in diameter of the serum voids thereby increasing the openness of the cheese. Heat induced gelation of the fat replacers (Maltrin® M040 and M100) changed the microstructure of the cheese.

7.5 References

Armbruster, B. L., Chastain, S., and Desai, N. (1995) Immunolocalization and microstructure of milk proteins and fat mimetics in reduced fat cheese in *Chemistry of structure-Function relationships in cheese* ed. E.L. Malin and M.H. Tunick, Plenum press, NY. 295-302.

Barbano, D. M., Clark, J. L., Dunham, C. E. and Fleming, J. R. (1990). Kjeldahl method for determination of total nitrogen content of milk: collaborative study. *J. Assoc. Anal. Chem.* 73:849-859.

Bhaskaracharya, R. K. and Shah, N. P. (1999a). Texture evaluation of commercial mozzarella cheeses. *Aust. J. Dairy Technol.* 54:36-40.

Bhaskaracharya, R. K., and Shah, N. P. (1999b). Microstructure of mozzarella cheeses made with or without EPS producing starter cultures. *Aust. J. Dairy Technol.* (accepted for publication).

Bhaskaracharya, R. K., and Shah, N. P. (1999c). Microstructure of mozzarella cheese: specimen preparation using sucrose as a cryoprotectant. *Aust. J. Dairy Technol.* (under review).

Bhaskaracharya, R. K., and Shah, N. P. (1999d). Texture characteristics and microstructure of skim milk mozzarella cheeses made using exopolysaccharide or non-exopolysaccharide producing starter cultures. *J. Dairy Sci.* (under review).

Desai, N. and Nolting, J. (1995). Microstructure studies of reduced fat cheeses containing fat substitute in *Chemistry of structure-Function relationships in cheese* ed. Malin, E. L. and Tunick, M. H., Plenum press, NY. 295-302.

Egan, H., Kirk, R. S. and Sawyer, R. 1987. Pearson's chemical analysis of foods. 8th ed., Harlow: Longman Scientific and Technical Publications.

Jana, A. H. and Upadhyay, K. G. 1997. Comparative appraisal of quality of buffalo-milk mozzarella cheese manufactured by two different methods. *J. Food Sci. Technol.* 34:381-385.

Kirkpatrick, L. A., and B. C. Feeney. (1997). A simple guide to SPSS for windows: versions 6.0, 6.1, 7.0 and 7.5. Intl. Thomson Publ. Co., California, USA.

Langton, M. and Hermansson, A. M. (1992). Fine stranded particulate gels of β-lactoglobulin and whey protein at varying pH. *Food Hydrocolloids.* 5:523.

Lawrence, R. C., Creamer, L. K. and Gilles, J. (1987). Texture development during cheese ripening. *J. Dairy Sci.* 70: 1748.

Lucey, J. A. and Gorry, C. (1993). Effect of Simplesse® 100 on the manufacture of low fat cheddar cheese in *Cheese yield and factors affecting its control.* Int. Dairy Fed. Seminar, Cork, Ireland. 439-447.

McMahon, D. J. (1995). How the microstructure of mozzarella cheese is related to functional properties in *Proceedings 32nd Annual Marschall Italian and Speciality cheese seminar*, Rhone-Poulenc, Madison, WI, 18-26.

McMahon, D. J., Alleyne, M. C., Fife, R. L. and Oberg, C. J. (1996). Use of fat replacers in low fat mozzarella cheese. *J. Dairy Sci.* 79:1911-1921.

McManus, W. R., McMahon, D. J. and Oberg, C. J. (1993). High resolution scanning electron microscopy of milk products: A new sample preparation procedure. *Food Struct.* 12:475-482.

Mistry, V. V. and Anderson, D. L. 1993. Composition and microstructure of commercial full fat and low fat cheeses. *Food Struct.* 12: 259.

Oberg, C. J., McManus, W. R. and McMahon, D. J. 1993. Microstructure of mozzarella cheese during manufacture. *Food Struct.* 12:251-258.

Rousseau. M. (1988). Changes in the microstructure of Saint Paulin cheese during manufacture studied by scanning electron microscopy. *Food Microstruct.* 7:105-113.

Tunick, M. H., Cooke, P. H., Malin, E. L., Smith, P. W. and Holsinger, V. H. (1997). Reorganisation of casein submicelles in mozzarella cheese during storage. *Int. Dairy J.* 7:149-155.

Walstra, P. and Jenness, R. 1984. Casein micelles in *Dairy Chemistry and Physics*, John Wiley and Sons, Inc., NY.

Table 7.3.1. Selected composition of cheeses made using fat replacers showing the means and standard deviations for moisture and protein contents.

Types of cheeses	Moisture		Protein	
	Mean	Std. Dev.	Mean	Std. Dev.
Control cheese	53.61	1.25	43.48	0.25
Maltrin® M040 cheese	52.84	0.06	34.73	0.23
Maltrin® M100 cheese	53.26	0.07	31.87	0.08
StaSlim® 143 cheese	43.55	1.55	31.54	0.32

Table 7.3.2. Effect of moisture content, compression, age and type of fat replacer on the hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness of fat-replaced cheeses.

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	HARDNESS	2390.159[a]	6	398.360	32.251	0.000
	COHESIVENESS	1.439[b]	6	.240	121.935	0.000
	ADHESIVENESS	.203[c]	6	3.376E-02	6.561	0.000
	SPRINGINESS	127.235[d]	6	21.206	35.250	0.000
	GUMMINESS	493.266[e]	6	82.211	26.213	0.000
	CHEWINESS	61368.015[f]	6	10228.003	26.763	0.000
Intercept	HARDNESS	7.487	1	7.487	.606	0.439
	COHESIVENESS	.159	1	.159	80.923	0.000
	ADHESIVENESS	1.686E-03	1	1.686E-03	.328	0.569
	SPRINGINESS	2.453	1	2.453	4.077	0.047
	GUMMINESS	3.243E-03	1	3.243E-03	.001	0.974
	CHEWINESS	1.400	1	1.400	.004	0.952
MOISTURE	HARDNESS	24.349	1	24.349	1.971	0.165
	COHESIVENESS	1.287E-03	1	1.287E-03	.654	0.421
	ADHESIVENESS	1.864E-03	1	1.864E-03	.362	0.549
	SPRINGINESS	.483	1	.483	.802	0.373
	GUMMINESS	10.337	1	10.337	3.296	0.074
	CHEWINESS	1367.945	1	1367.945	3.579	0.063
COMPRESS	HARDNESS	1132.642	1	1132.642	91.699	0.000
	COHESIVENESS	1.263	1	1.263	642.107	0.000
	ADHESIVENESS	2.631E-02	1	2.631E-02	5.113	0.027
	SPRINGINESS	48.044	1	48.044	79.864	0.000
	GUMMINESS	104.012	1	104.012	33.165	0.000
	CHEWINESS	16430.228	1	16430.228	42.992	0.000
DAYS	HARDNESS	.654	1	.654	.053	0.819
	COHESIVENESS	4.948E-02	1	4.948E-02	25.164	0.000
	ADHESIVENESS	.116	1	.116	22.465	0.000
	SPRINGINESS	19.290	1	19.290	32.066	0.000
	GUMMINESS	5.427	1	5.427	1.730	0.193
	CHEWINESS	1575.255	1	1575.255	4.122	0.046
TYPE	HARDNESS	69.854	2	34.927	2.828	0.066
	COHESIVENESS	9.278E-03	2	4.639E-03	2.359	0.102
	ADHESIVENESS	3.043E-03	2	1.522E-03	.296	0.745
	SPRINGINESS	3.361	2	1.680	2.793	0.068
	GUMMINESS	17.179	2	8.589	2.739	0.072
	CHEWINESS	1804.229	2	902.115	2.360	0.102
Error	HARDNESS	864.620	70	12.352		
	COHESIVENESS	.138	70	1.966E-03		
	ADHESIVENESS	.360	70	5.145E-03		
	SPRINGINESS	42.110	70	.602		
	GUMMINESS	219.537	70	3.136		
	CHEWINESS	26752.076	70	382.173		
Total	HARDNESS	8578.158	77			
	COHESIVENESS	24.873	77			
	ADHESIVENESS	1.049	77			
	SPRINGINESS	5749.739	77			
	GUMMINESS	2080.989	77			
	CHEWINESS	206262.084	77			
Corrected Total	HARDNESS	3254.779	76			
	COHESIVENESS	1.576	76			
	ADHESIVENESS	.563	76			
	SPRINGINESS	169.345	76			
	GUMMINESS	712.803	76			
	CHEWINESS	88120.091	76			

a R Squared = .734 (Adjusted R Squared = .712)
b R Squared = .913 (Adjusted R Squared = .905)
c R Squared = .360 (Adjusted R Squared = .305)
d R Squared = .751 (Adjusted R Squared = .730)
e R Squared = .692 (Adjusted R Squared = .666)
f R Squared = .696 (Adjusted R Squared = .670)

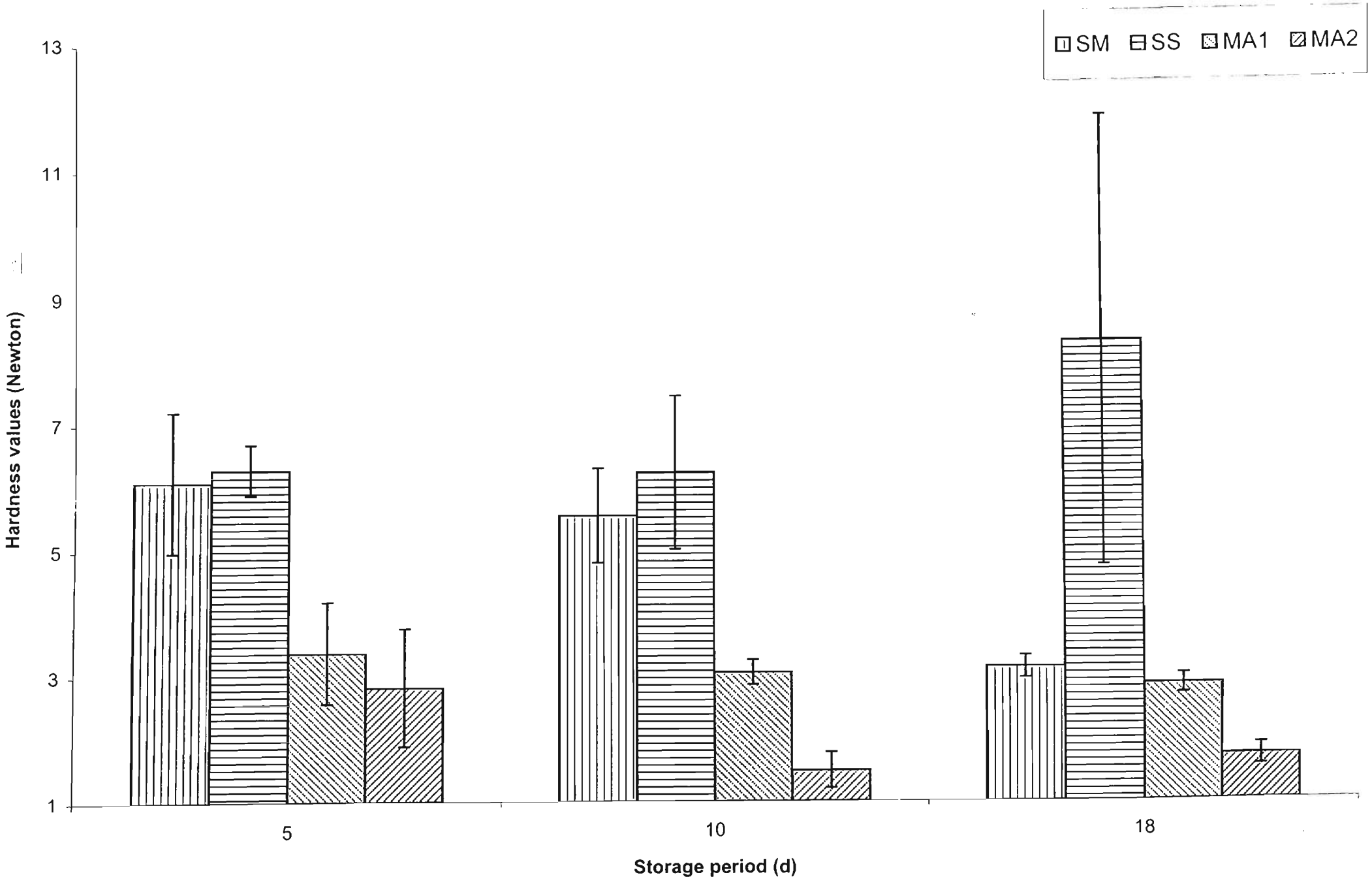

Figure 7.3.1 Hardness values of control cheese without any fat replacers and StaSlim® 143, Maltrin® M040 and M100 based cheeses over 18 d storage period measured at 50% compression.

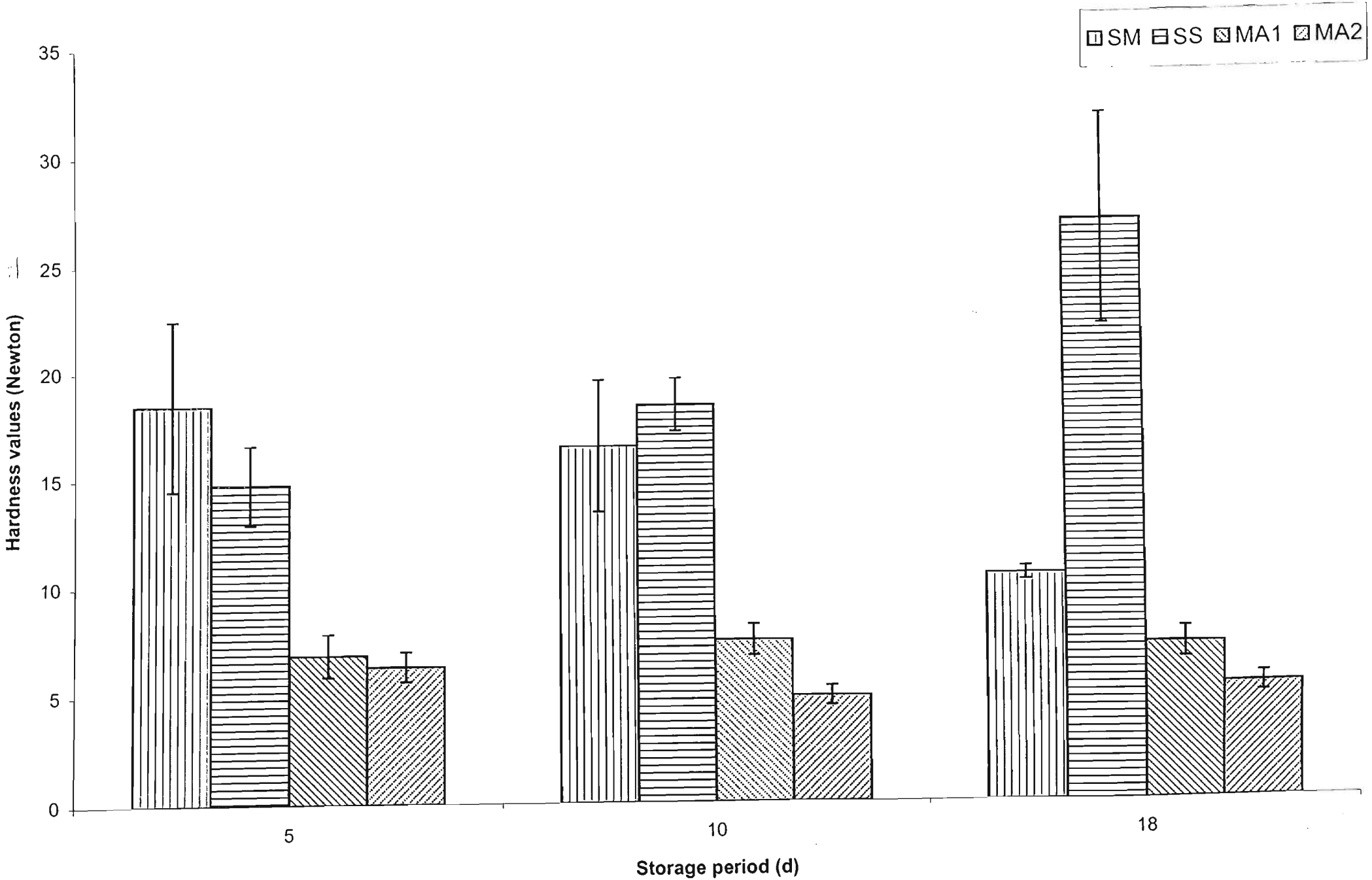

Figure 7.3.2 Hardness values of control cheese without any fat replacers and StaSlim® 143, Maltrin® M040 and M100 based cheeses over 18 d storage period measured at 70% compression.

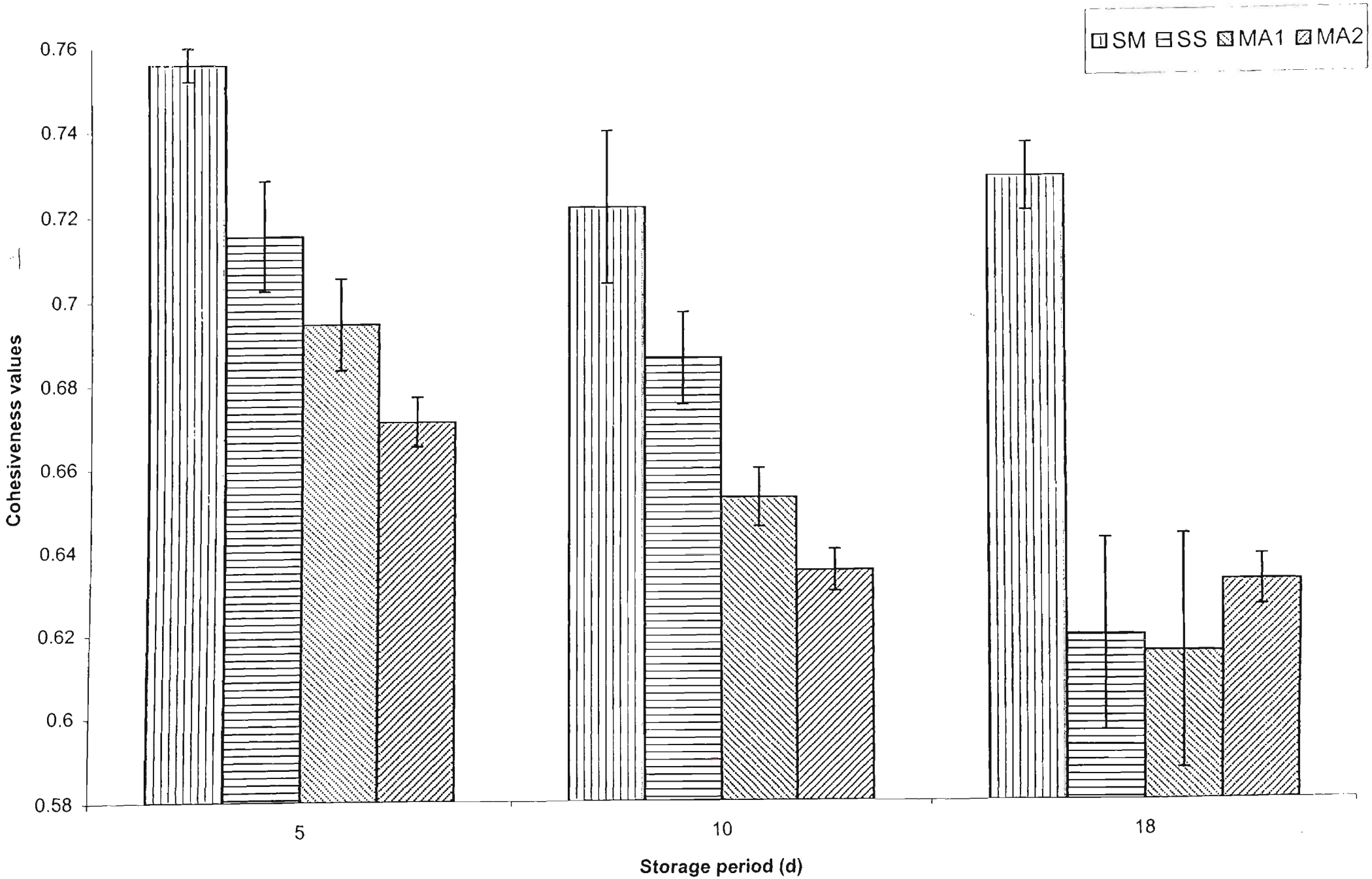

Figure 7.3.3 Cohesiveness values of control cheese without any fat replacers and StaSlim® 143, Maltrin® M040 and M100 based cheeses over 18 d storage period measured at 50% compression.

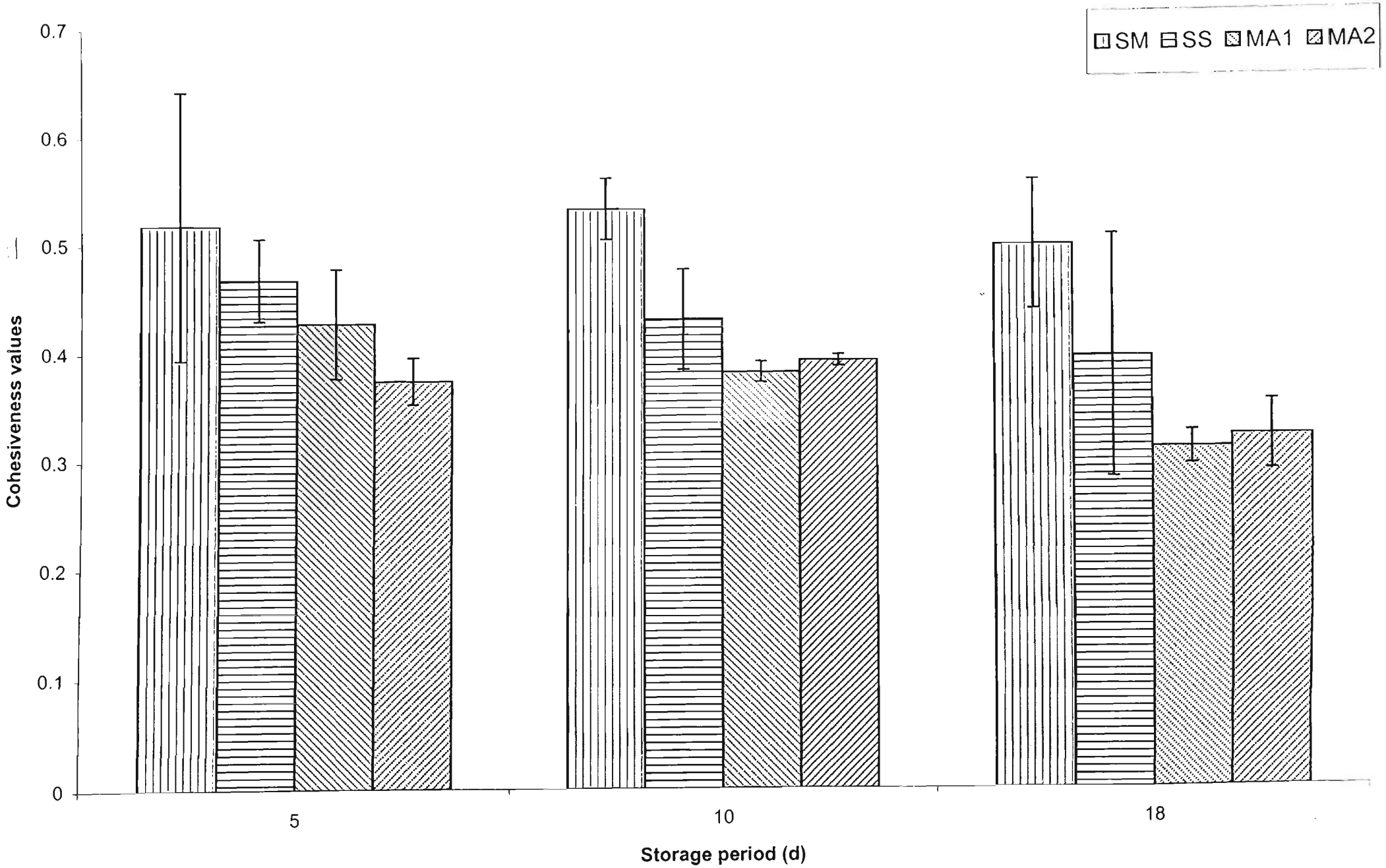

Figure 7.3.4 Cohesiveness values of control cheese without any fat replacers and StaSlim® 143, Maltrin® M040 and M100 based cheeses over 18 d storage period measured at 70% compression.

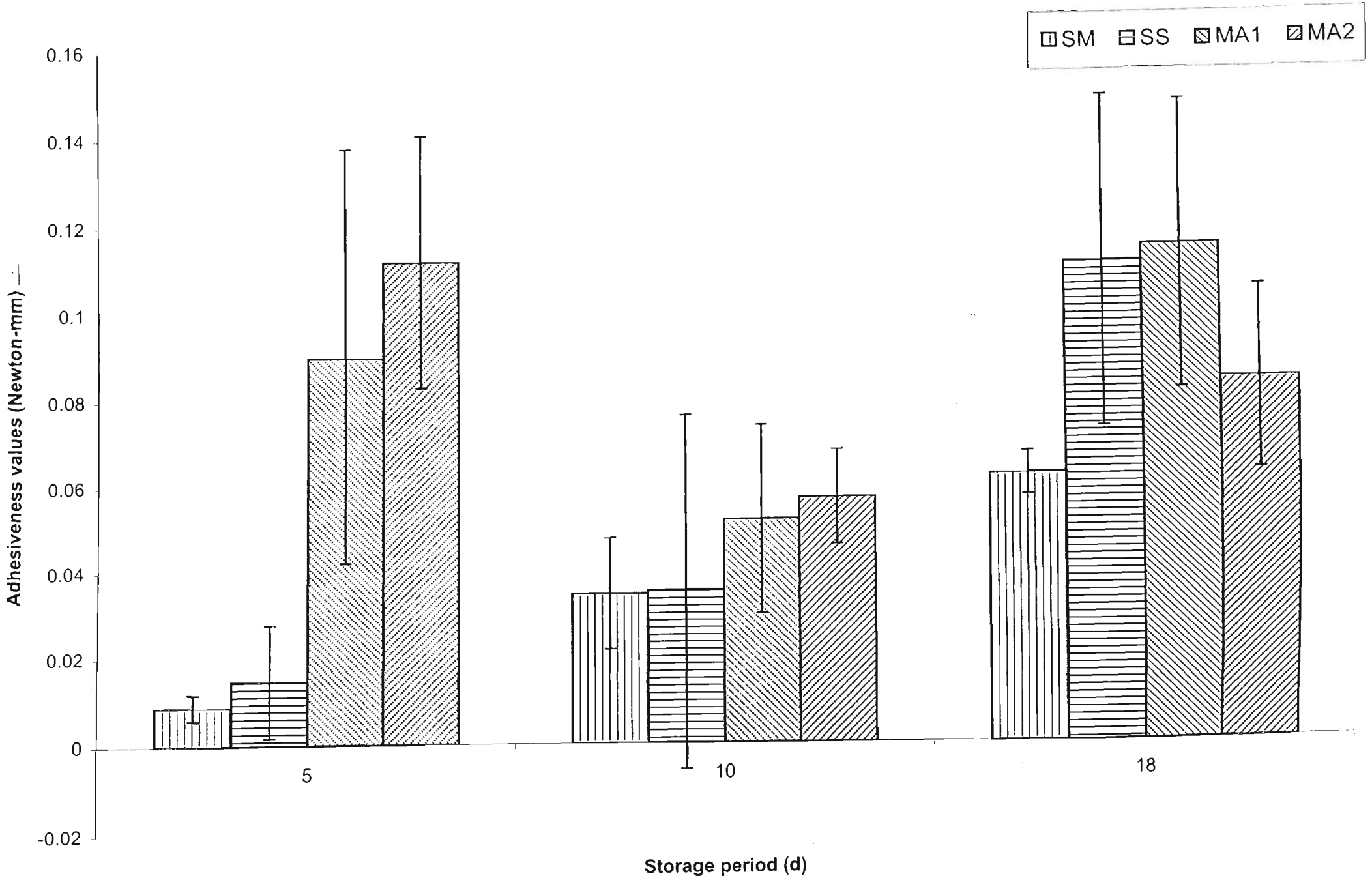

Figure 7.3.5 Adhesiveness values of control cheese without any fat replacers and StaSlim® 143, Maltrin® M040 and M100 based cheeses over 18 d storage period measured at 50% compression.

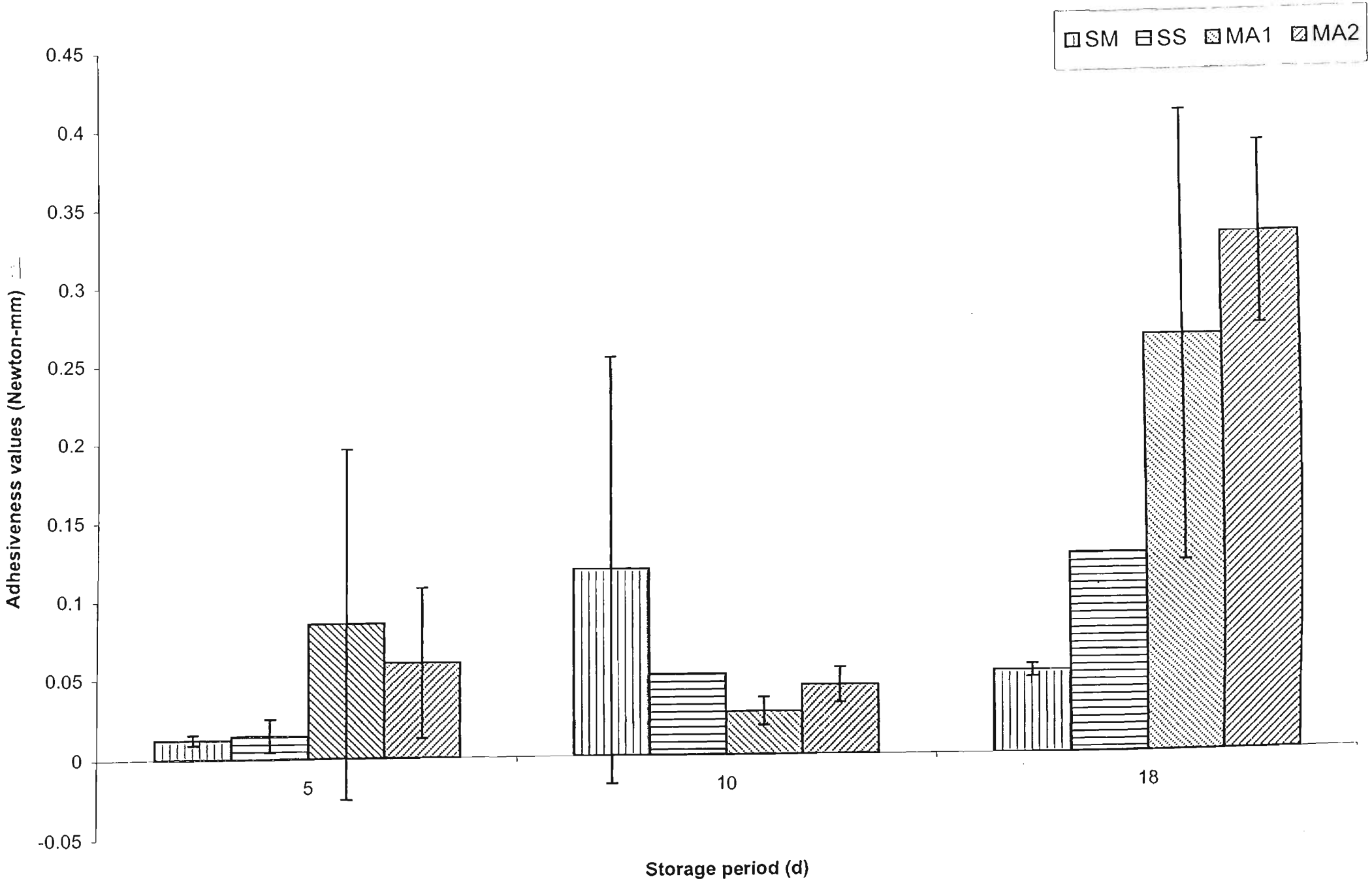

Figure 7.3.6 Adhesiveness values of control cheese without any fat replacers and StaSlim® 143, Maltrin® M040 and M100 based cheeses over 18 d storage period measured at 70% compression.

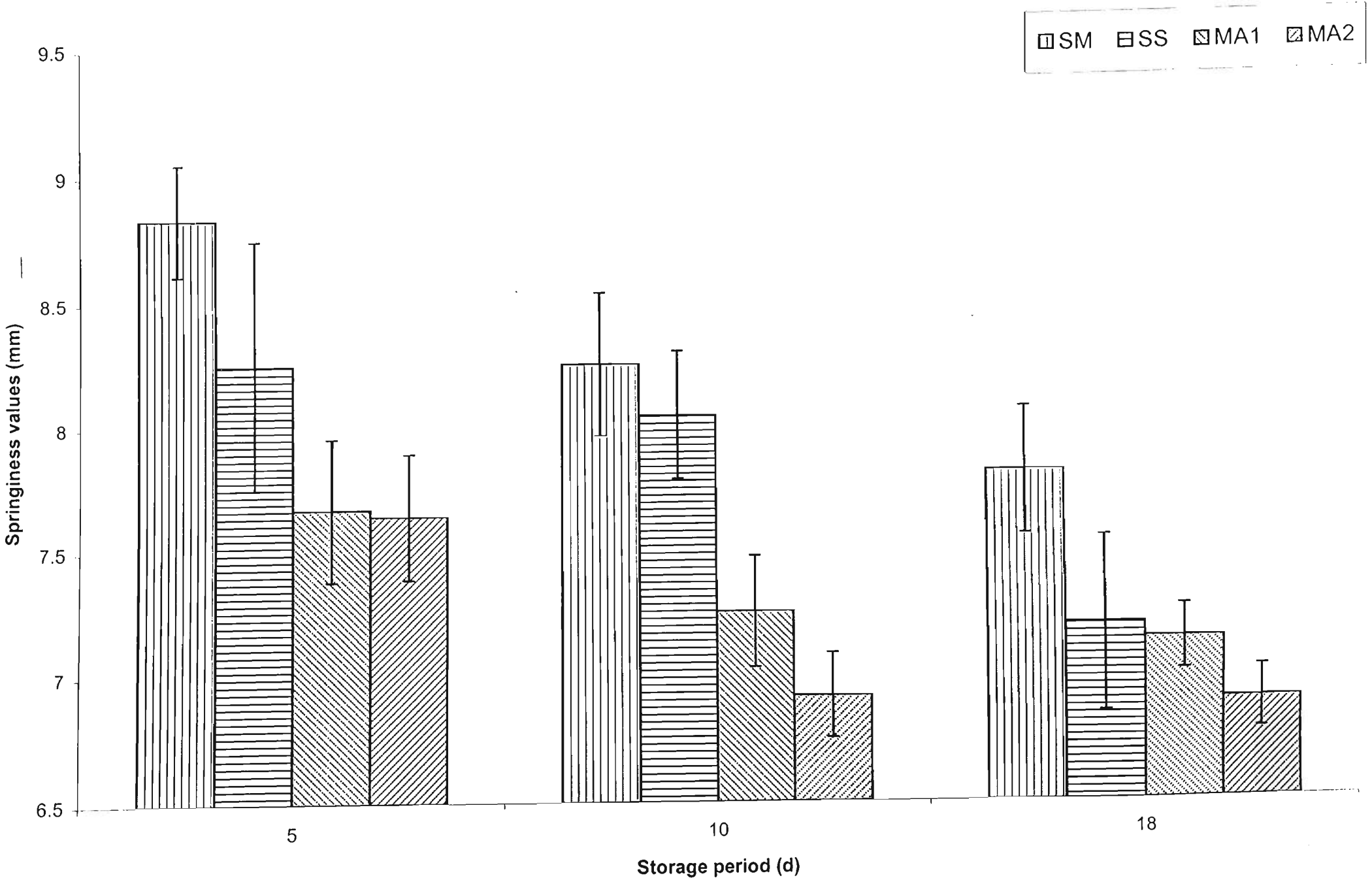

Figure 7.3.7 Springiness values of control cheese without any fat replacers and StaSlim® 143, Maltrin® M040 and M100 based cheeses over 18 d storage period measured at 50% compression.

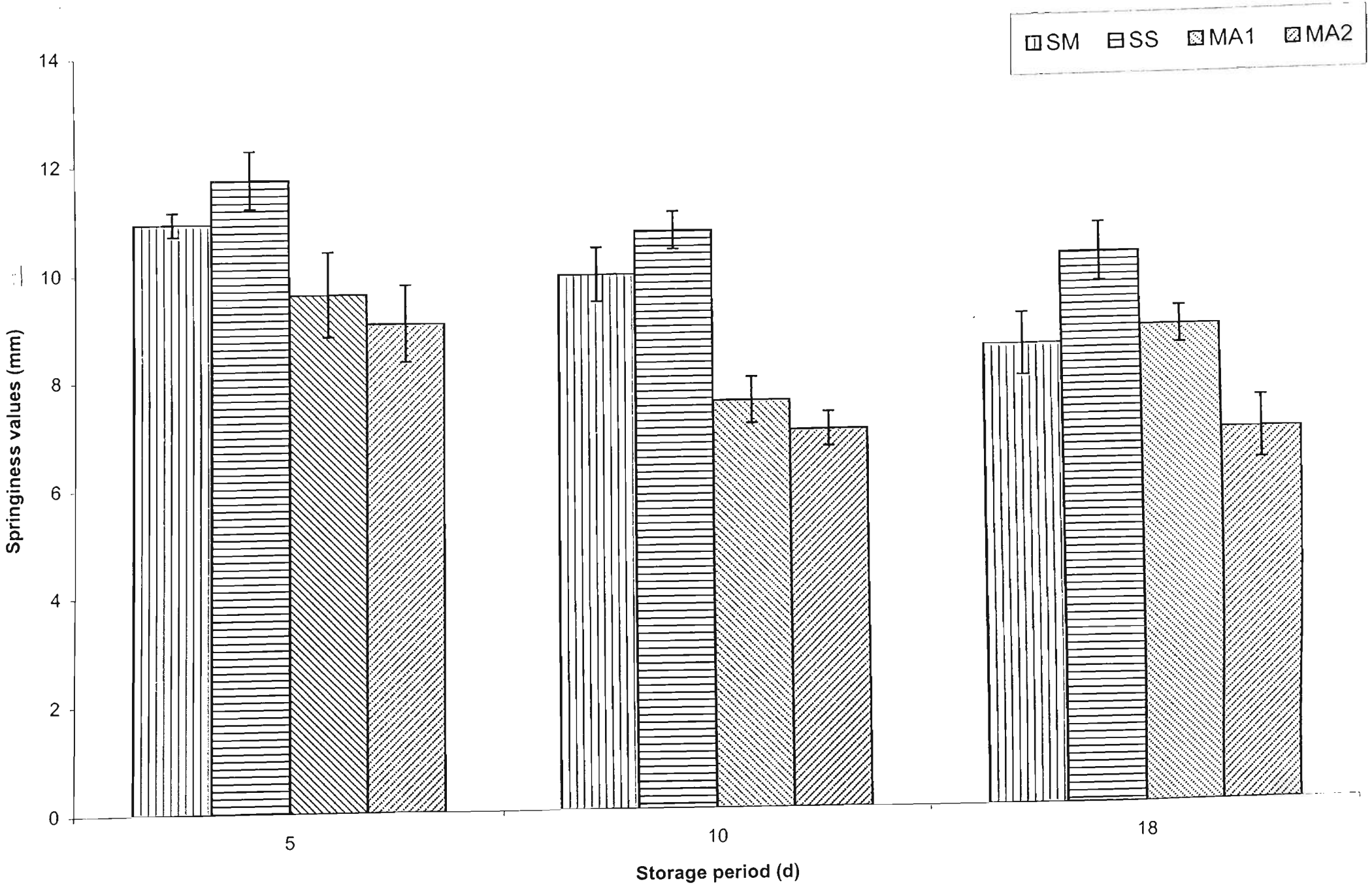

Figure 7.3.8 Springiness values of control cheese without any fat replacers and StaSlim® 143, Maltrin® M040 and M100 based cheeses over 18 d storage period measured at 70% compression.

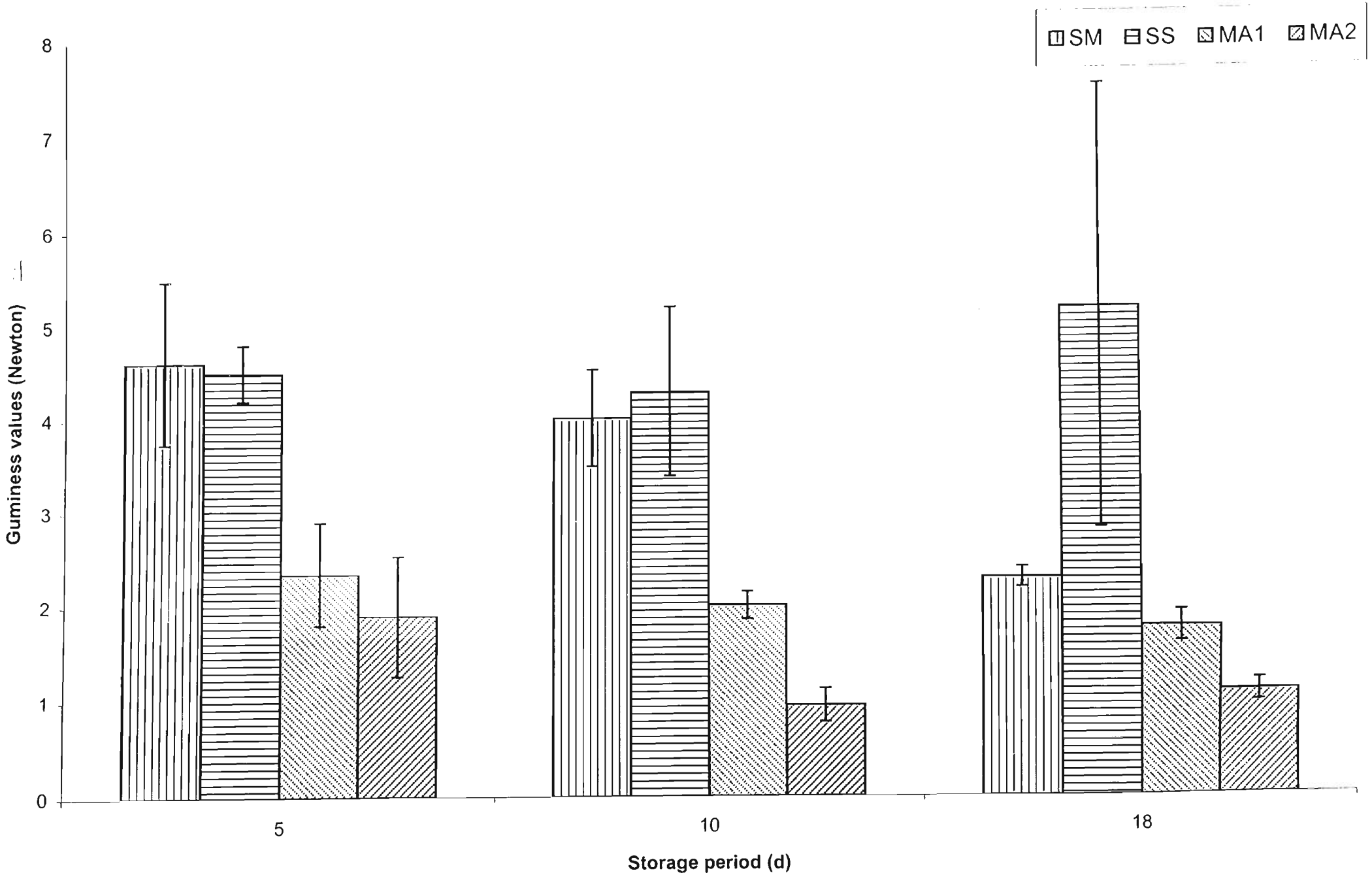

Figure 7.3.9 Gumminess values of control cheese without any fat replacers and StaSlim® 143, Maltrin® M040 and M100 based cheeses over 18 d storage period measured at 50% compression.

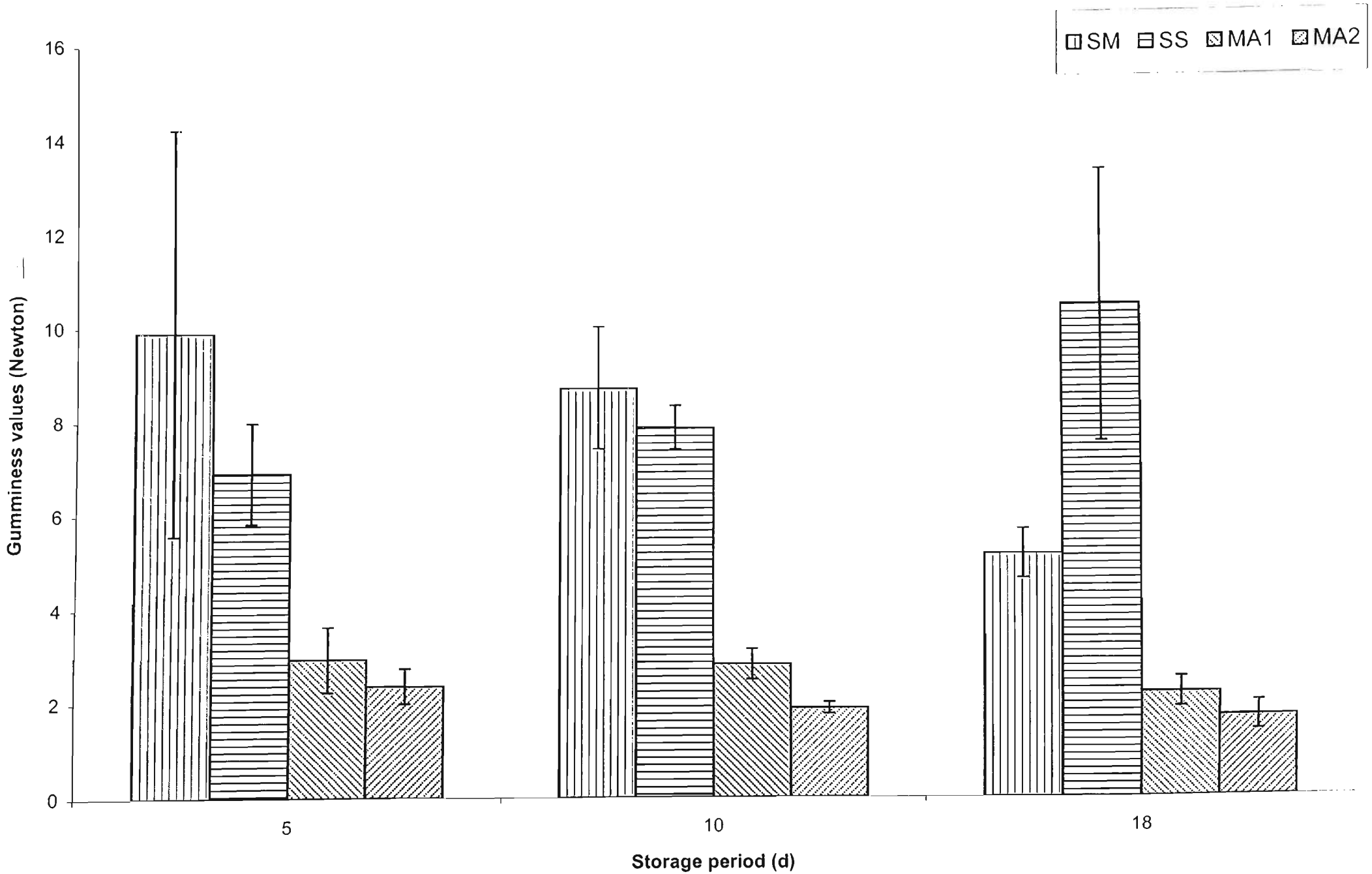

Figure 7.3.10 Gumminess values of control cheese without any fat replacers and StaSlim® 143, Maltrin® M040 and M100 based cheeses over 18 d storage period measured at 70% compression.

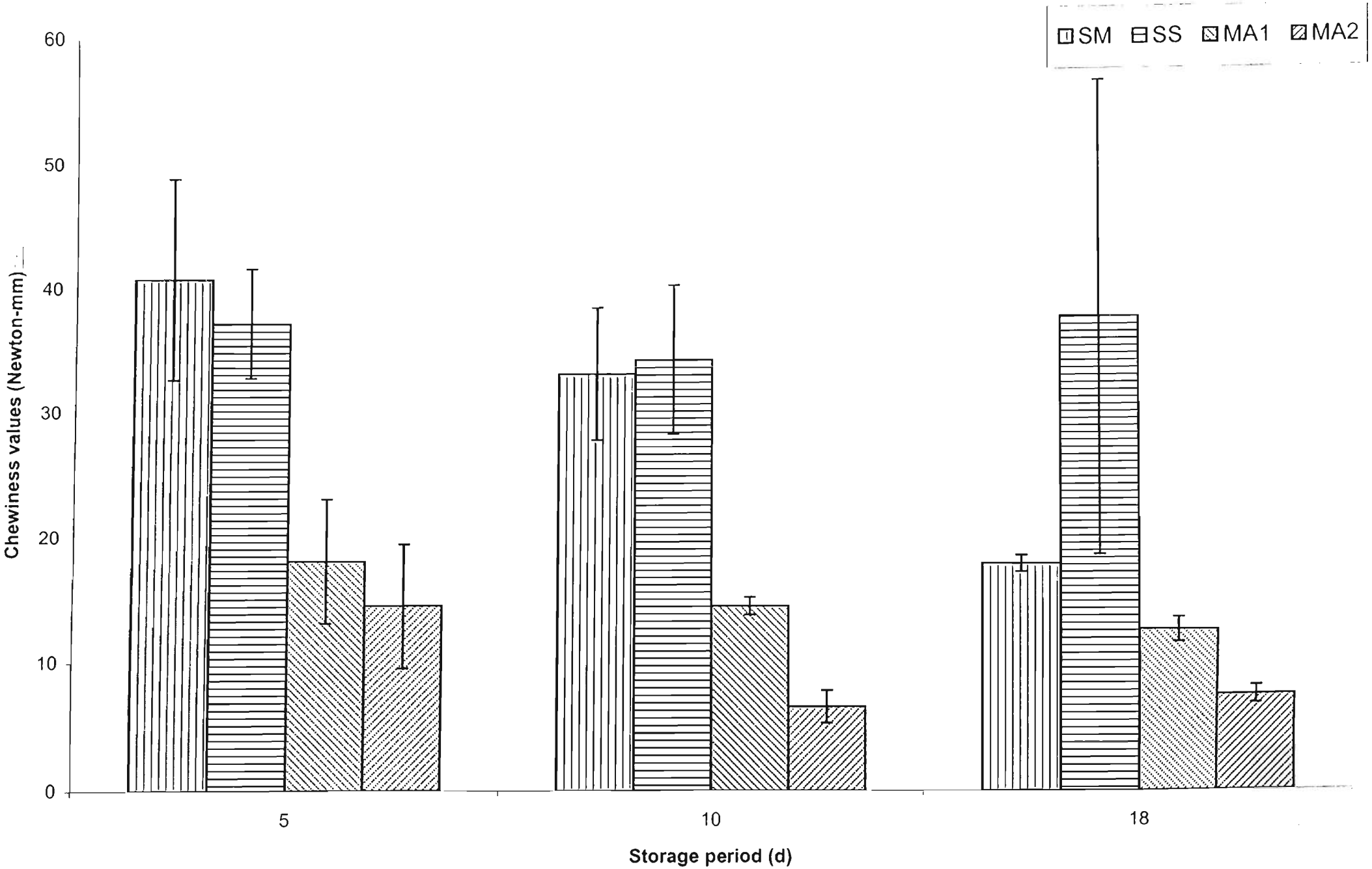

Figure 7.3.11 Chewiness values of control cheese without any fat replacers and StaSlim® 143, Maltrin® M040 and M100 based cheeses over 18 d storage period measured at 50% compression.

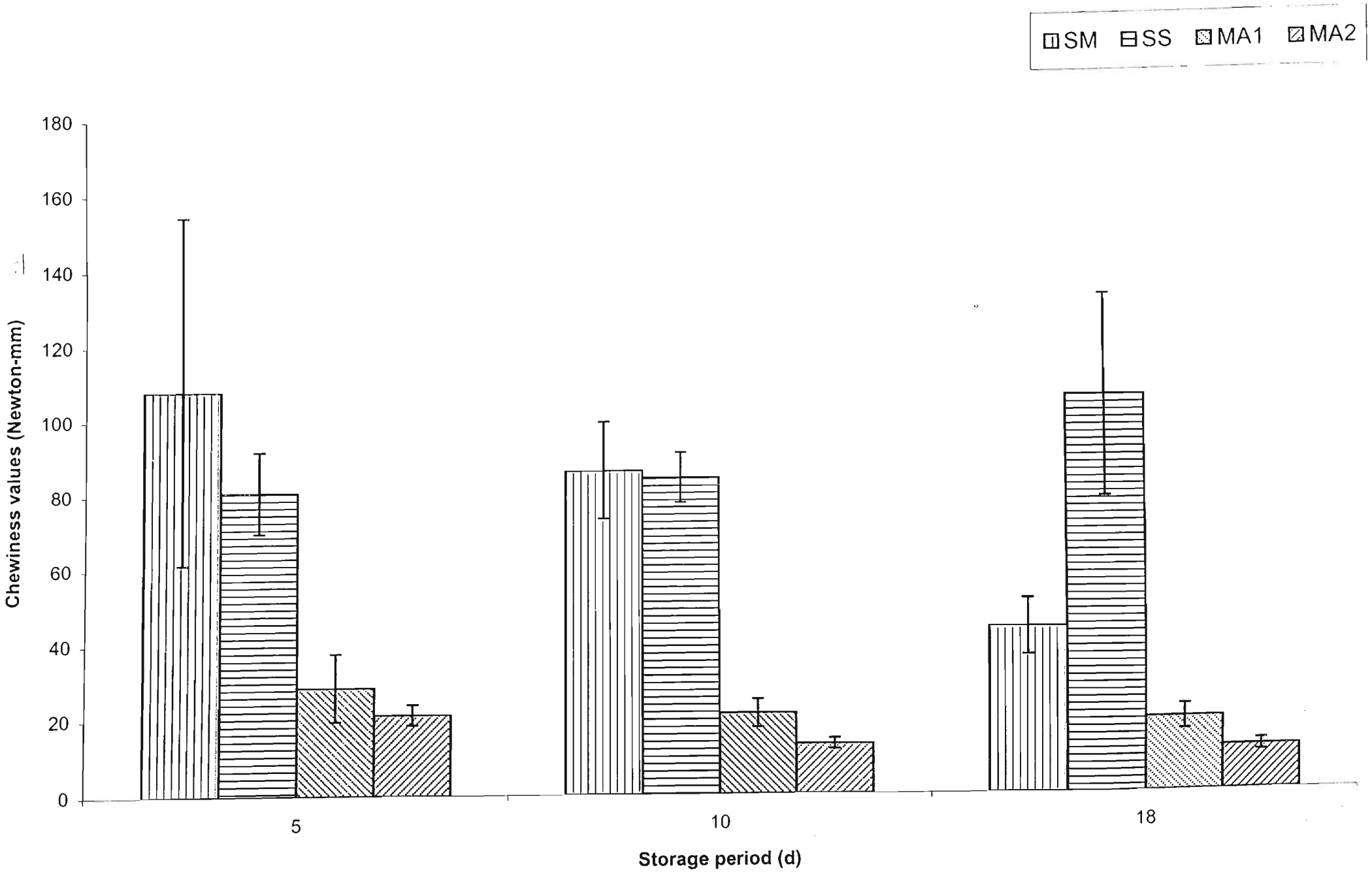

Figure 7.3.12 Chewiness values of control cheese without any fat replacers and StaSlim® 143, Maltrin® M040 and M100 based cheeses over 18 d storage period measured at 70% compression.

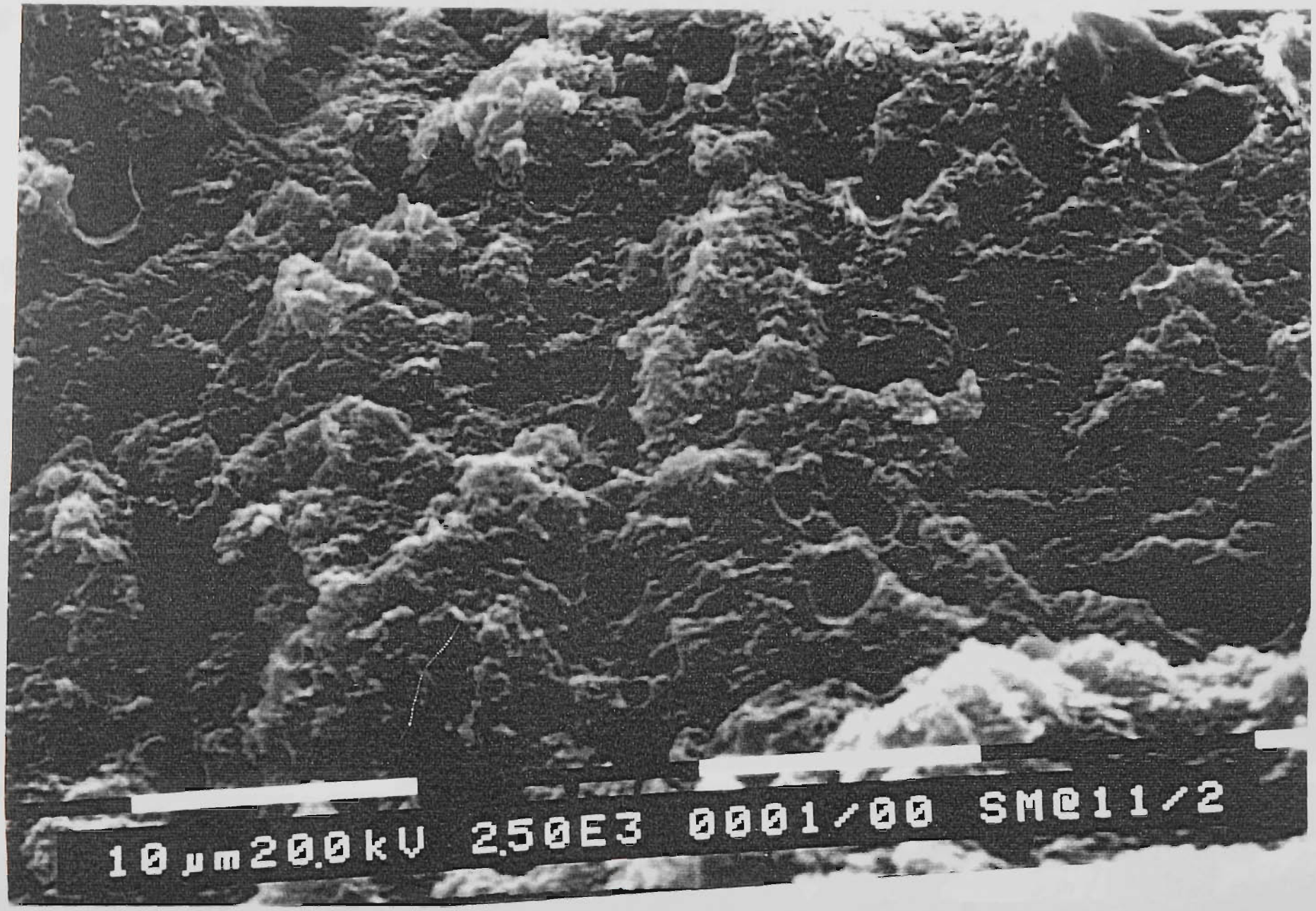

Figure 7.3.13. A scanning electron micrograph of 28 d old skim milk mozzarella cheese made without addition of fat replacers.

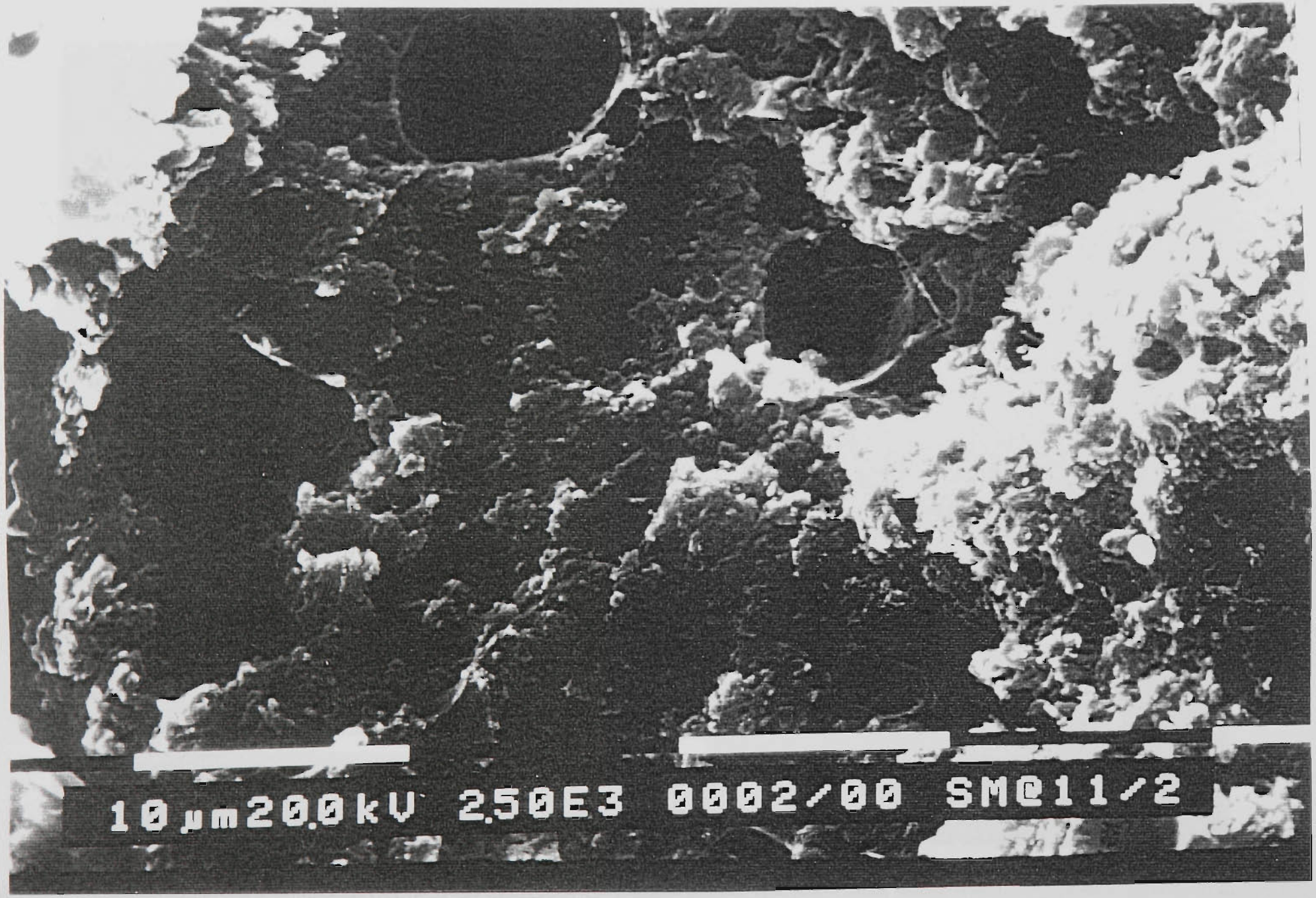

Figure 7.3.14. A scanning electron micrograph of 28 d old skim milk mozzarella cheese made without addition of fat replacers

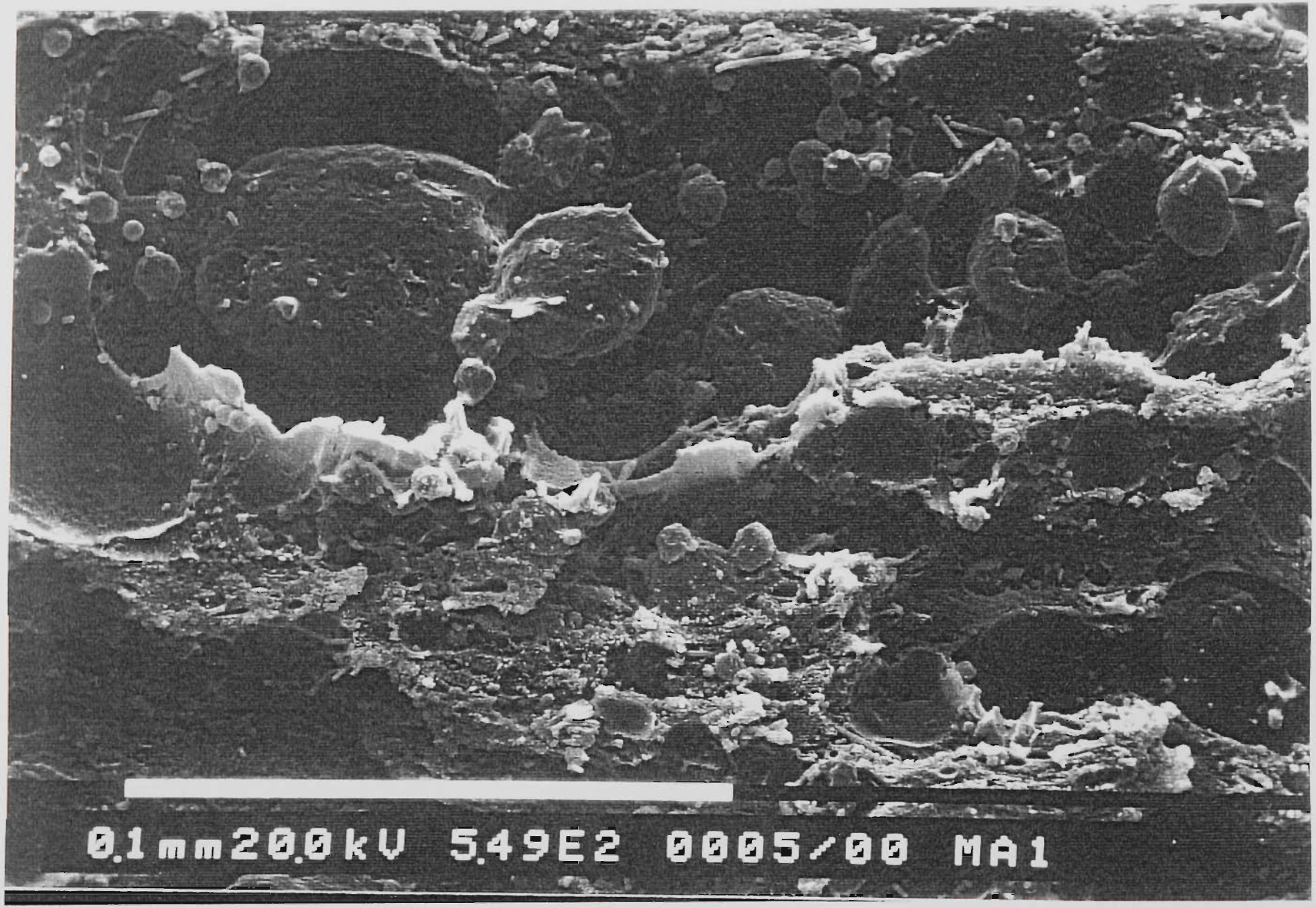

Figure 7.3.15. A scanning electron micrograph of 28 d old skim milk mozzarella cheese specimen made using Maltrin® M040 a carbohydrate based fat replacers, showing the cross section of cheese cut parallel to the protein strands

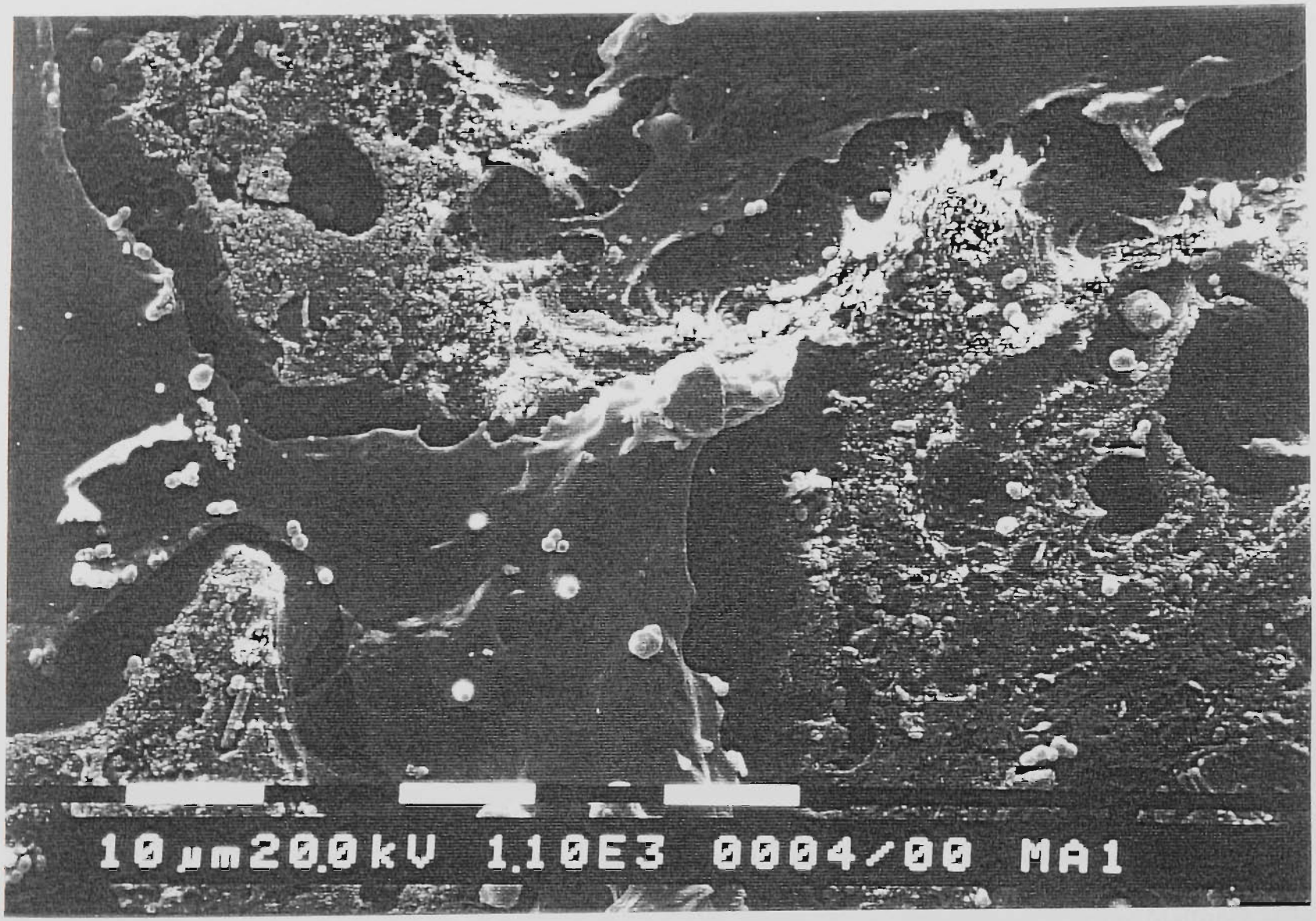

Figure 7.3.16. A scanning electron micrograph of 28 d old skim milk mozzarella cheese specimen made using Maltrin® M040 a carbohydrate based fat replacers, showing the gel like behaviour of the fat replacer. Note the cheese shows protein strands elongated in various directions due to manual stretching.

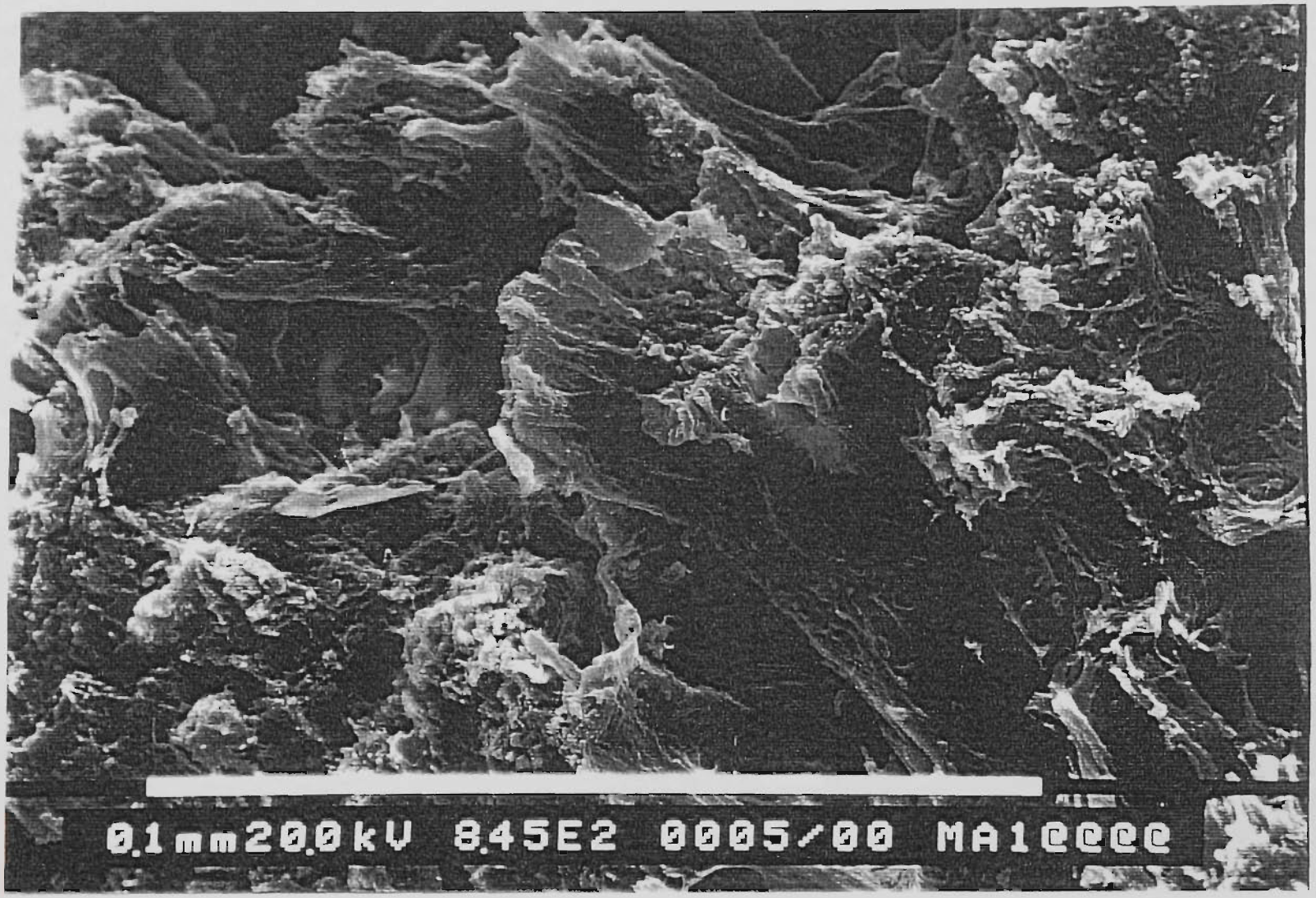

Figure 7.3.17 A scanning electron micrograph of 28 d old skim milk mozzarella cheese specimen made using Maltrin® M040 a carbohydrate based fat replacers, showing the gel like behaviour of the fat replacer. Note the cheese shows protein strands elongated in various directions due to manual stretching

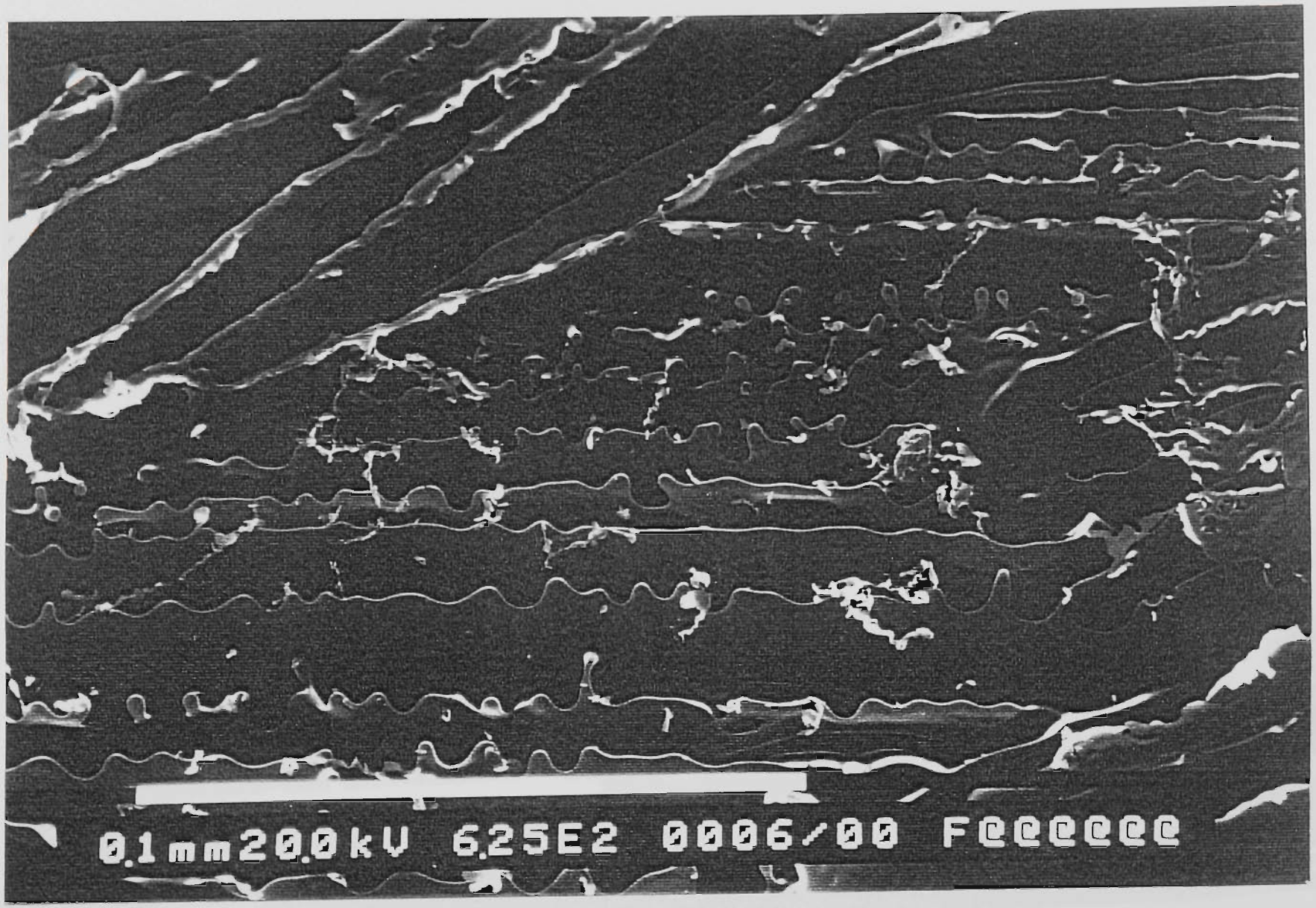

Figure 7.3.18. A scanning electron micrograph of air dried, aqueous dispersion of Maltrin® M040 a carbohydrate based fat replacer, showing the gel like behaviour of the fat replacer

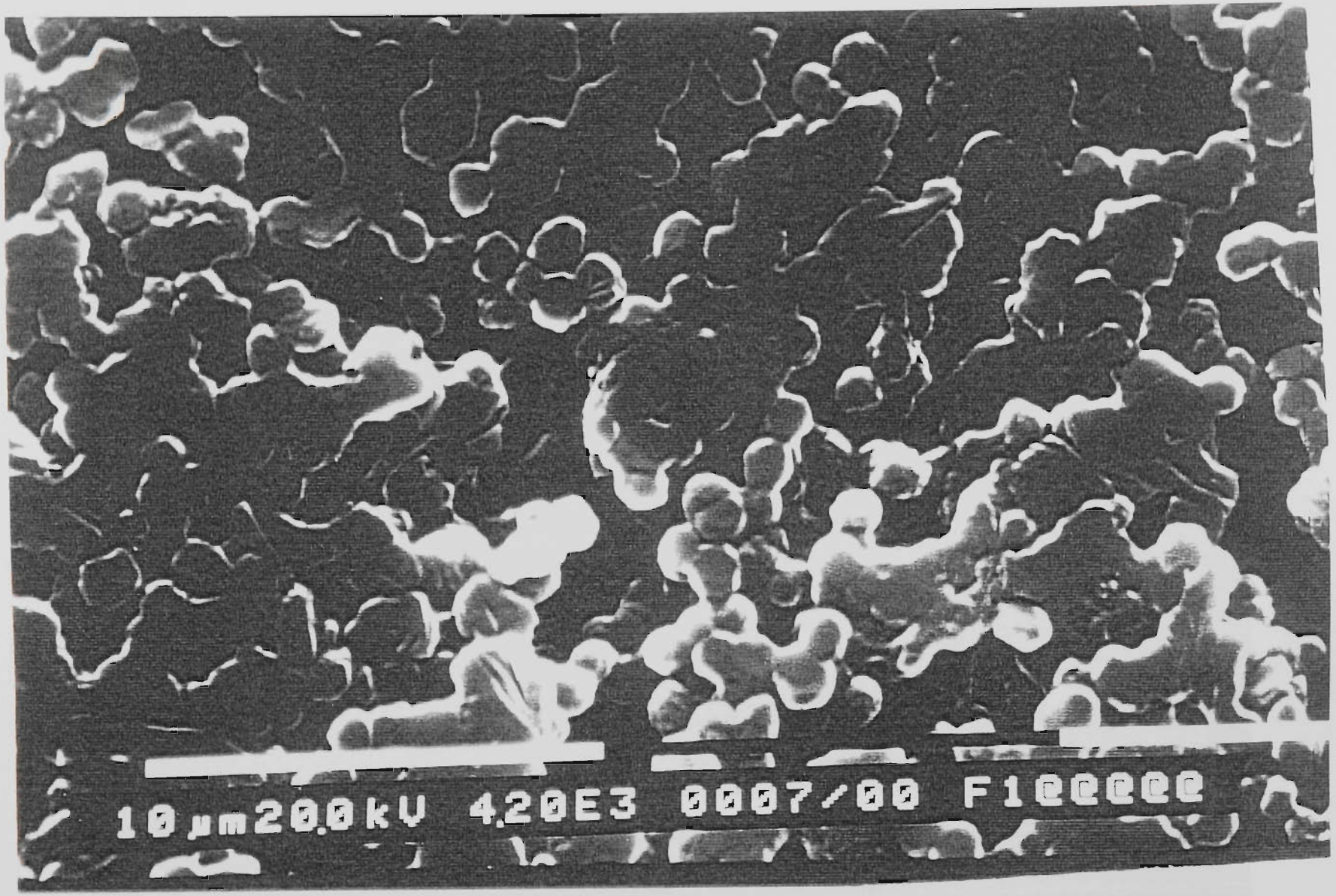

Figure 7.3.19. A scanning electron micrograph of freeze dried, aqueous dispersion of Maltrin® M040 a carbohydrate based fat replacer, showing the coalescing of fat replacer particles.

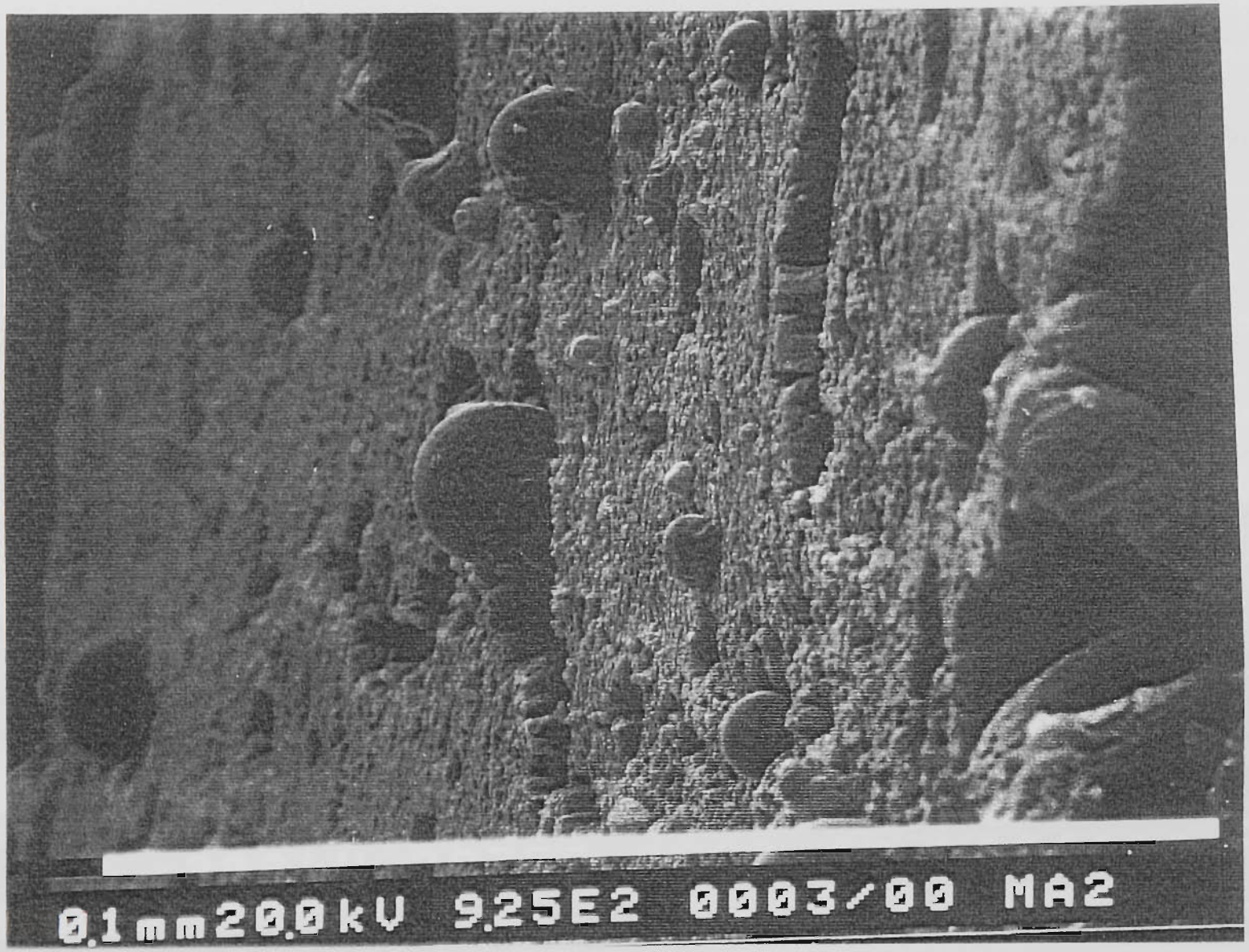

Figure 7.3.20. A scanning electron micrograph of 28 d old skim milk mozzarella cheese specimen made using Maltrin® M100 a carbohydrate based fat replacers, showing the gel like behaviour of the fat replacer

Figure 7.3.21 A scanning electron micrograph of 28 d old skim milk mozzarella cheese specimen made using Maltrin® M100 a carbohydrate based fat replacers, showing the voids formed and enlarged due to hydration of fat replacer and the protein fibres.

Figure 7.3.22. A scanning electron micrograph of 28 d old skim milk mozzarella cheese specimen made using Maltrin® M100 a carbohydrate based fat replacer. The cheese shows indentations made by the fat replacer on the protein matrix. The specimen shows the cross section of the cheese parallel to the protein strands

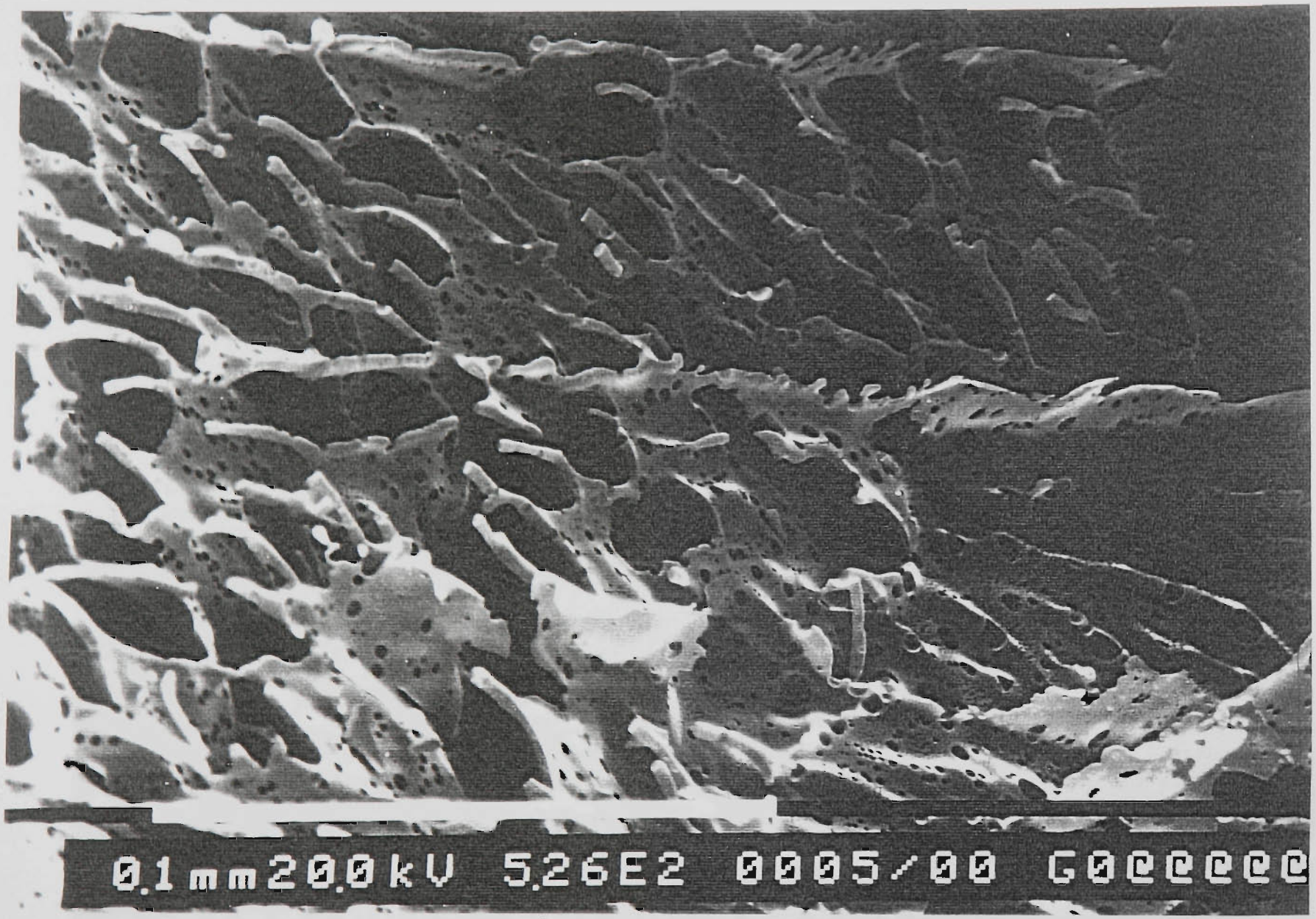

Figure 7.3.23. A scanning electron micrograph of air dried, aqueous dispersion of Maltrin® M100 a carbohydrate based fat replacer, showing the gel like behaviour of the fat replacer

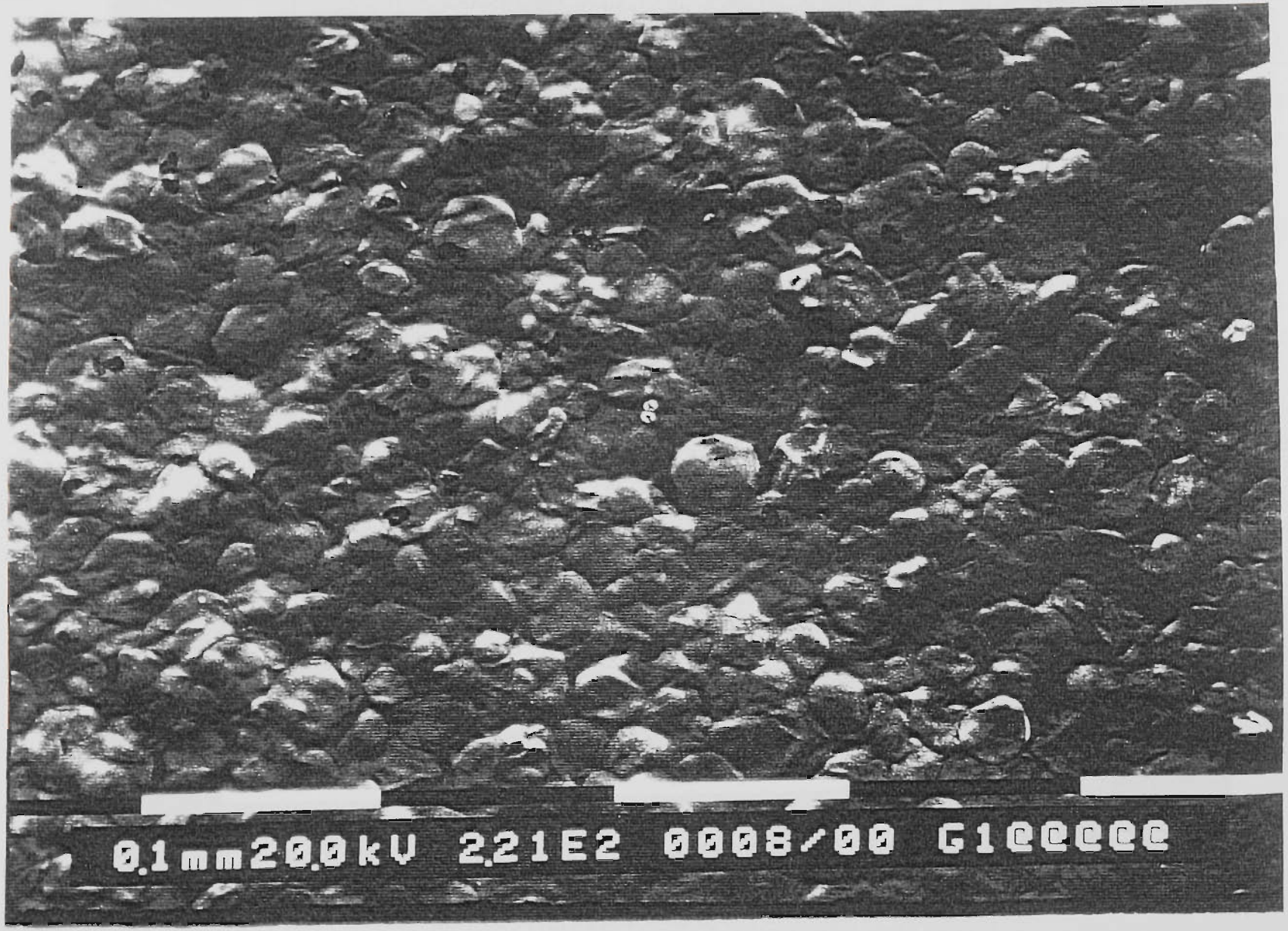

Figure 7.3.24. A scanning electron micrograph of freeze dried, aqueous dispersion of Maltrin® M100 a carbohydrate based fat replacer, showing the coalescing of fat replacer particles.

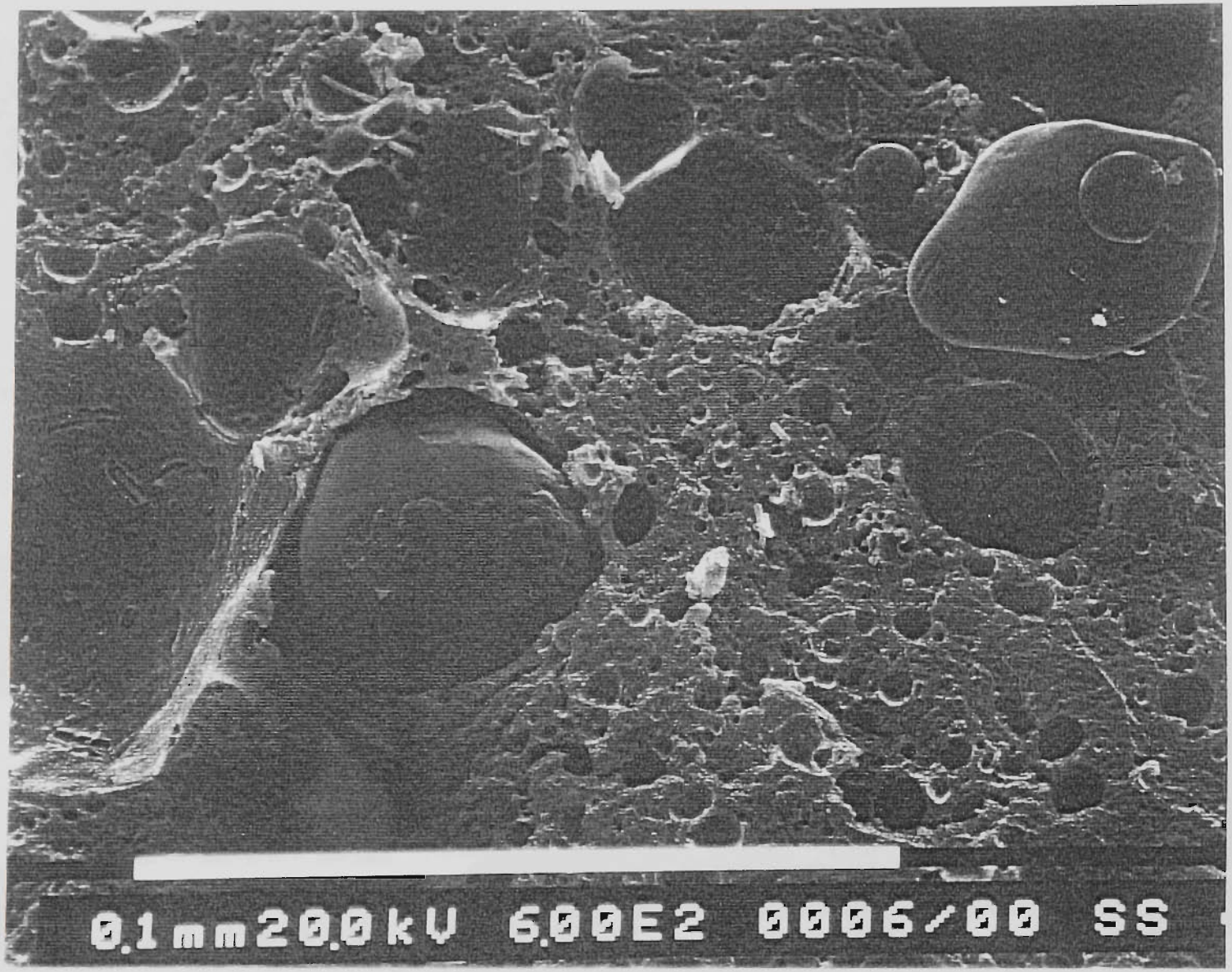

Figure 7.3.25. A scanning electron micrograph of 28 d old skim milk mozzarella cheese specimen made using StaSlim® 143 a carbohydrate based fat replacer. The cheese shows indentations made by the fat replacer on the protein matrix. The specimen also shows the particulate nature of the fat replacer.

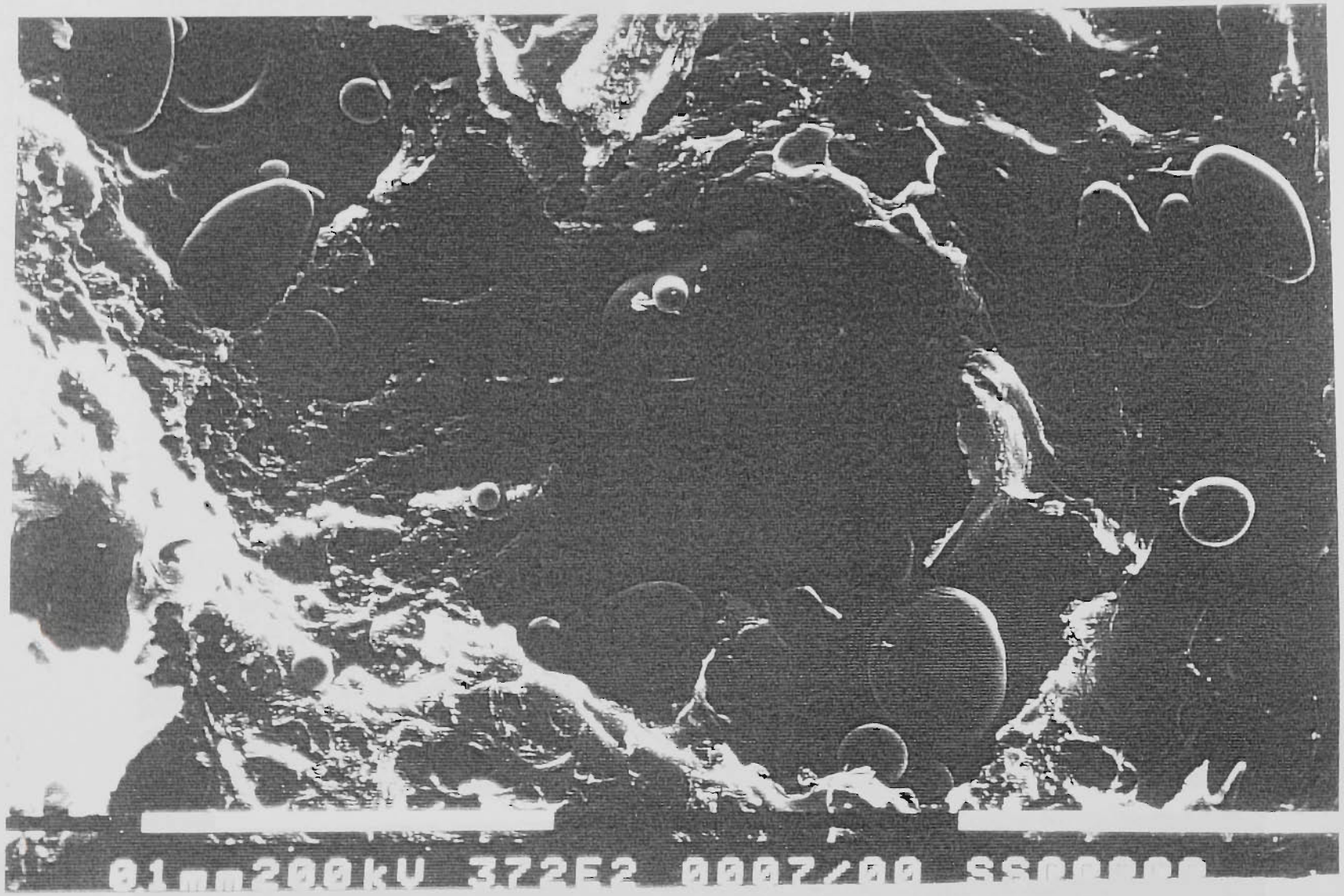

Figure 7.3.26. A scanning electron micrograph of 28 d old skim milk mozzarella cheese specimen made using StaSlim® 143 a carbohydrate based fat replacer. The cheese shows the fat replacer located within the serum channels. The specimen also shows the particulate nature of the fat replacer.

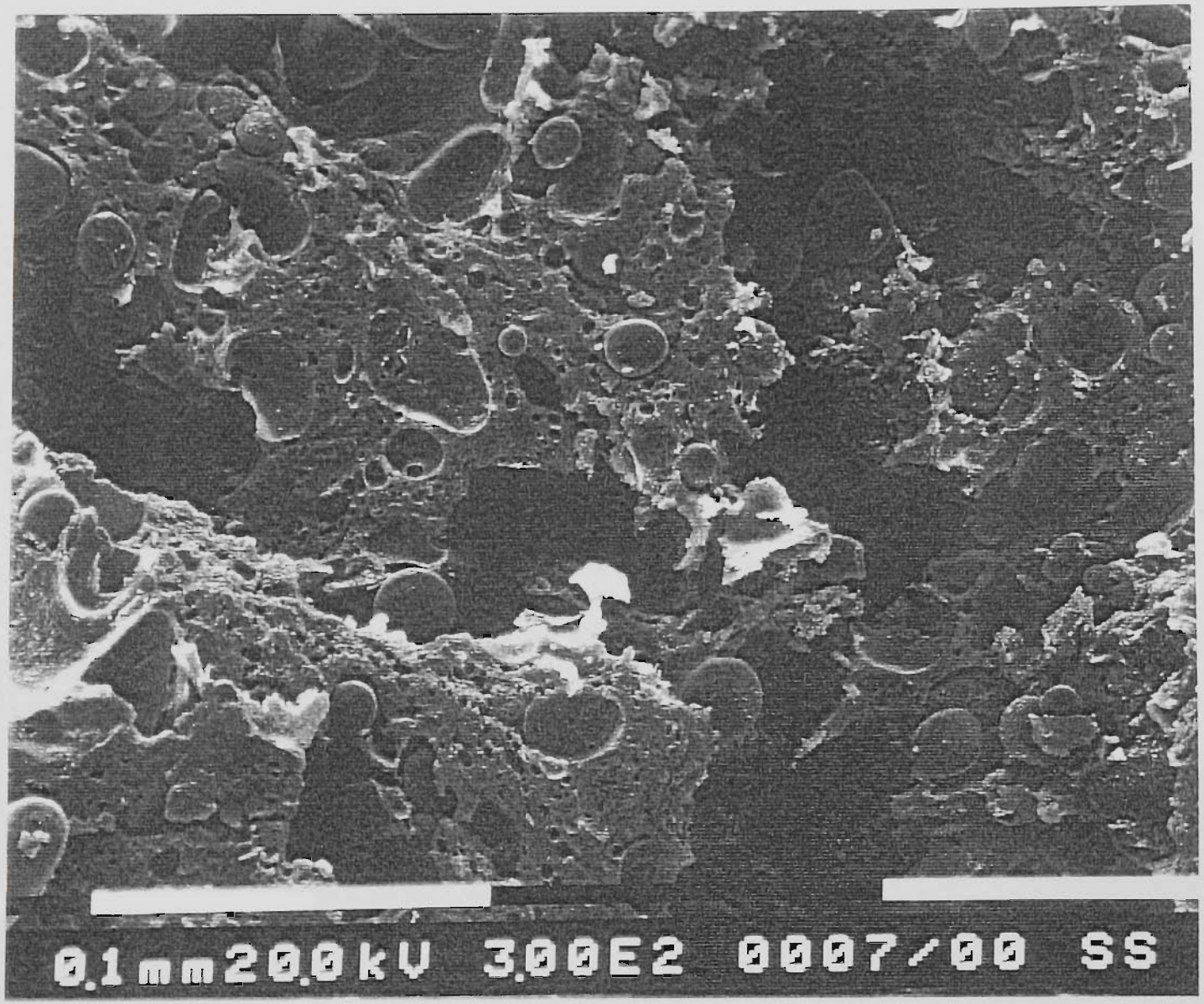

Figure 7.3.27 A scanning electron micrograph of 28 d old skim milk mozzarella cheese specimen made using StaSlim® 143 a carbohydrate based fat replacer. The cheese shows indentations made by the fat replacer on the protein matrix. These indentations look similar to the ones made by fat globules. Also the fat replacer shows a smooth surface indicating probably soft hydrated particles of the fat replacer.

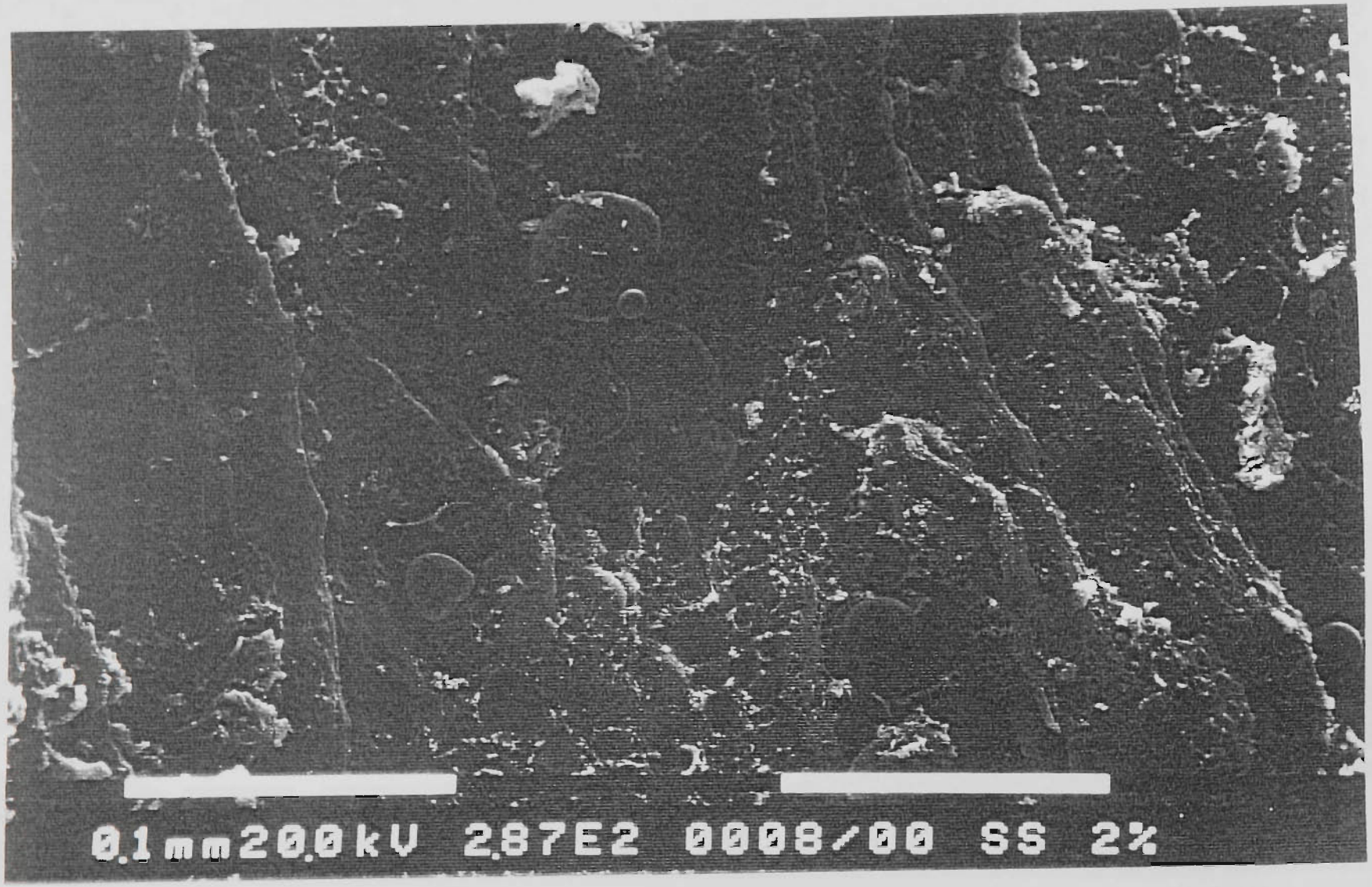

Figure 7.3.28. A scanning electron micrograph of 28 d old skim milk mozzarella cheese specimen made using StaSlim® 143 a carbohydrate based fat replacer. The cheese shows the fat replacer located between the protein strands, which could be the reason fro. the softness and pliable texture of the cheese.

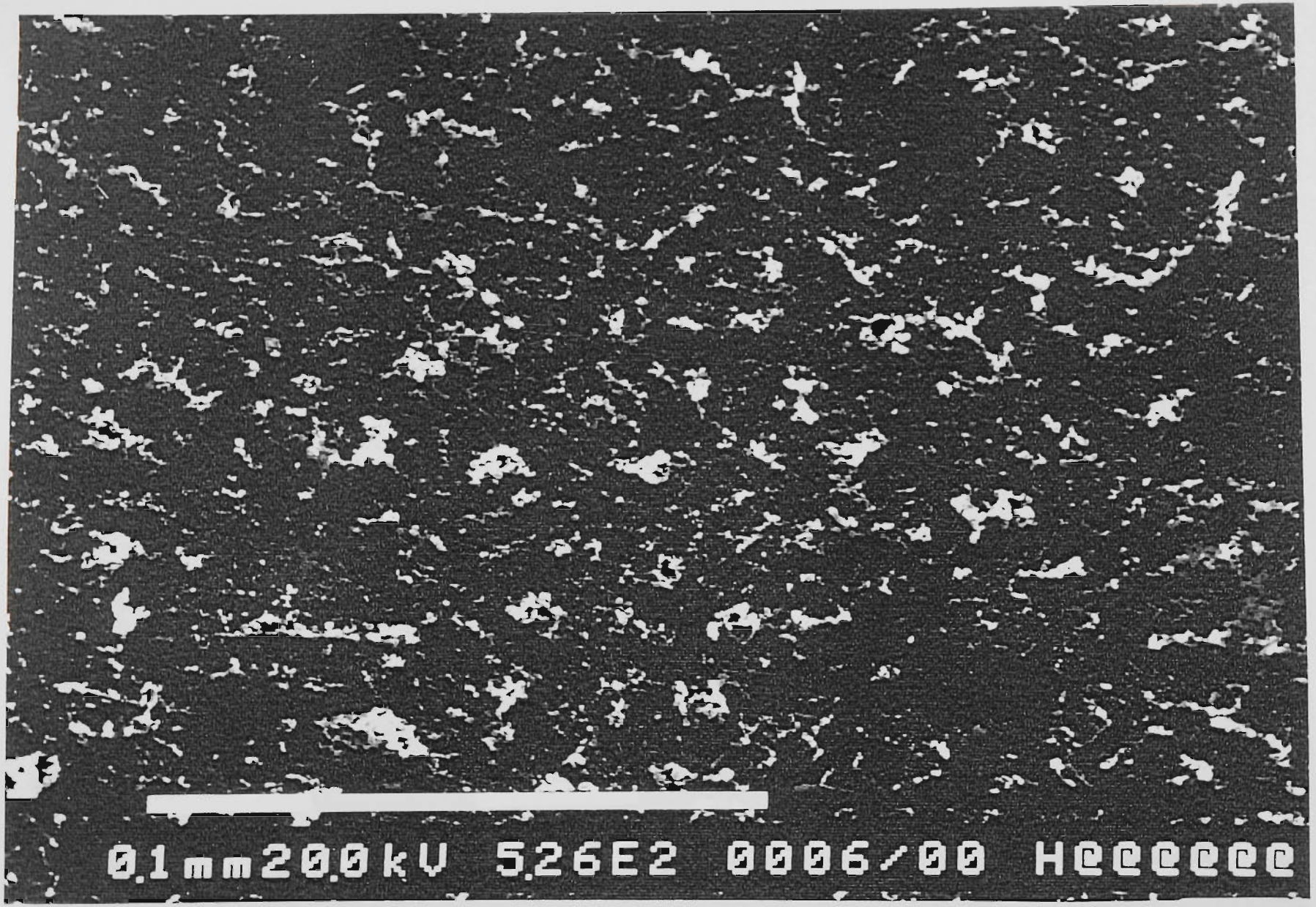

Figure 7.3.29. A scanning electron micrograph of air-dried, aqueous dispersion of StaSlim® 143 a carbohydrate based fat replacer, showing the particulate behaviour of the fat replacer.

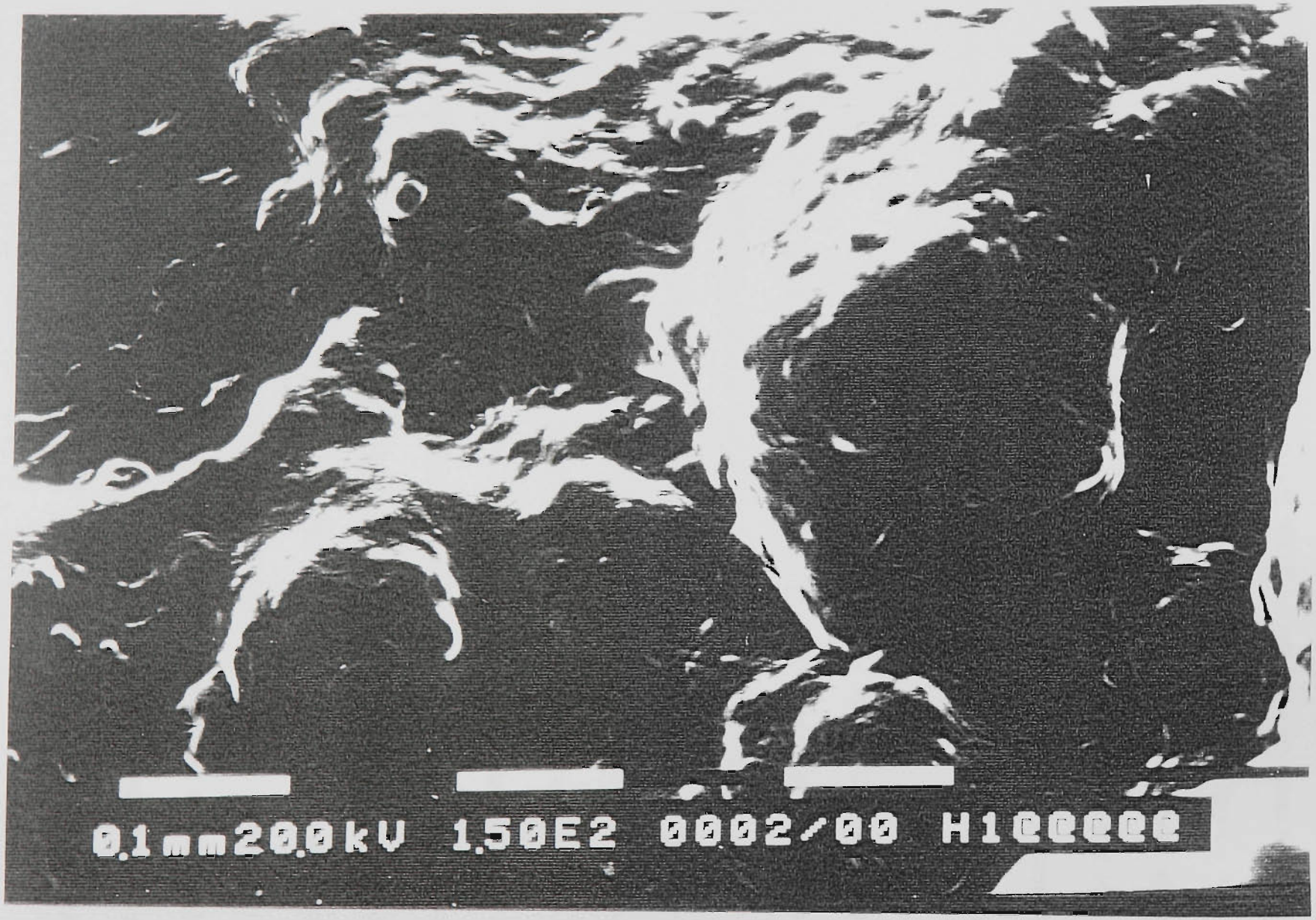

Figure 7.3.30. A scanning electron micrograph of freeze dried, aqueous dispersion of StaSlim® 143 a carbohydrate based fat replacer, showing the coalescing of fat replacer particles. The fat replacer seems to be a free flowing hydrated gel.

8.0. OVERALL CONCLUSIONS

Four commercial mozzarella cheeses and a cheddar cheese were studied for texture characteristics including hardness, springiness, cohesiveness, chewiness and gumminess. The mozzarella cheeses showed similar texture characteristics. An increase in moisture content of cheeses decreased the hardness. Similarly, an increase in fat content caused a decrease in hardness. There was an increase in springiness with the increase in fat content of cheeses. The protein content of the cheeses had a positive effect; an increase in protein content caused an increase in springiness. Cohesiveness was found to be the highest in mozzarella cheeses with the least fat content. The mozzarella cheeses showed lower hardness values and higher springiness, cohesiveness, chewiness and gumminess values compared to the cheddar cheese. The results suggest that there are chemical, physical and structural differences between the mozzarella cheeses and the cheddar cheese that could cause variations in their texture characteristics. The moisture and protein contents play a major role in enhancing these texture characteristics in the absence of fat although the effect of processing, storage and ripening or proteolysis needs to be taken into account.

Mozzarella cheeses were made using exopolysaccharide or non-exopolysaccharide producing starter cultures consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* and the cheeses microstructure was examined. A simplified method of specimen preparation was developed wherein the specimens were initially fixed in glutaraldehyde, washed in cacodylate buffer, post-fixed in osmium tetroxide, dehydrated gradually using ethanol and acetone, dried at critical point, coated with gold and observed under a scanning electron microscope. The cheeses showed a normal protein structure interspersed with large and small voids belonging to serum and fat phases that were extracted during specimen

preparation indicating that the specimen preparation method was suitable for examining microstructure of mozzarella cheeses. The cheeses made using exopolysaccharide producing starter cultures showed more open body with large serum voids and the starter bacteria were located in such voids. The delicate structure of exopolysaccharide produced by the microorganisms was evident from the micrographs and was well preserved. The streptococci were the primary producers of exopolysaccharide due to their quicker propagation compared to lactobacilli. Such streptococci were seen attached to the protein strands in the cheese through the exopolysaccharide. The non-exopolysaccharide producing organisms were seen located near the voids of fat probably indicating their need for the fat globule membrane proteins. Moreover the surfaces of such bacteria were smooth and thus they could be differentiated from the exopolysaccharide producing organisms.

In a similar study mozzarella cheeses were made using exopolysaccharide and non-exopolysaccharide producing starter cultures and their microstructure was examined using a modified method of cheese specimen preparation. In this method the cheese specimens were fixed in glutaraldehyde, washed in cacodylate buffer, dehydrated gradually in ethanol, cryoprotected in 2.3 M sucrose solution, cryofractured in liquid nitrogen, dehydrated using absolute solutions of ethanol and acetone, critical point dried, coated with gold and observed under a scanning electron microscope. In using the modified method of specimen preparation the osmotic balance in the cheese specimens were maintained by the sucrose solution. Thus the native structure of the proteins were better preserved in the cheeses and observations could be made on the three dimensional structure of the protein. The cheese matrix showed small voids, which were possibly formed due to removal of fat during specimen preparation. The large voids showing the location of serum phase in the cheeses were also observed. The delicate and thin filaments of the exopolysaccharide were better observed using this technique

as they were well preserved. The multi-directional orientation of the proteins showed that manual stretching of the cheeses during manufacture causes changes in the structure and direction of the protein strands unlike the mechanical stretching. The small voids on the protein strands showed the location of fat, which was lost during specimen preparation.

Skim milk mozzarella cheeses made using exopolysaccharide or non-exopolysaccharide producing starter cultures were analysed for composition, texture characteristics including hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness and microstructure. The cheese specimens were prepared using the simplified method and were observed under a scanning electron microscope. The skim milk cheeses made using exopolysaccharide producing starter cultures showed 3.25% higher moisture although all the cheeses had similar protein contents. The fat content of the cheeses was less than 3%. The increase in moisture content of the exopolysaccharide cheeses was possibly due to the exopolysaccharide produced by the starter bacteria. The exopolysaccharide cheeses showed decreased hardness, cohesiveness, and adhesiveness and increased springiness when compared to non-exopolysaccharide cheeses. The gumminess and chewiness characteristics of the cheeses did not show a definite trend. The exopolysaccharide cheeses showed increase in number and diameter of the voids, which caused an increase in openness of the cheese. The protein matrix, voids of fat and voids of serum phases were identified in the cheeses. Reduction in fat content of the cheeses caused a more compact protein matrix, fewer voids of fat phase and decreased number and size of serum voids compared to the full fat mozzarella cheeses. The exopolysaccharide produced by the starter cultures was observed to be smaller and less pronounced than in the full fat cheeses.

Skim milk mozzarella cheeses were made using fat replacers and the composition including moisture, protein and fat contents were analysed. The texture characteristics including hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness were measured over 18 d storage period and the distribution of fat replacers, extent of microparticulation, size of individual particles and interactions of the fat replacers with the caseins of the milk were observed using a scanning electron microscope. Maltrin® M040 and M100 cheeses showed similar moisture content as control cheeses made without the addition of any fat replacer, while StaSlim® 143 cheeses showed decreased moisture content. All the fat replaced cheeses showed lower protein content than the control cheeses. Hardness values of the mozzarella cheeses containing Maltrin® M040 and M100 were least while StaSlim® 143 cheeses showed slightly higher values compared to the control cheeses. Cohesiveness and springiness values of all the fat replaced cheeses were lower than the control cheeses, while adhesiveness values were higher. Maltrin® M040 and M100 cheeses showed lower gumminess and chewiness values compared to the control cheeses. All the texture characteristics except adhesiveness decreased during storage for the fat replaced and control cheeses. However, StaSlim® 143 cheeses showed increase in hardness, gumminess and chewiness values during storage. The fat replacers were located on the protein matrix and some of them in the serum channels. Depending on their location in the cheese their functionality either decreased or increased the hardness values as seen from the StaSlim® 143 particles located in the protein matrix and had higher hardness values. Maltrin® M040 and M100 mainly existed as a gel although the former was also found as particles due to incomplete hydration. Addition of fat replacers resulted in increased openness of the cheese body especially if the fat replacer were located in the serum channels.

9.0 FUTURE RESEARCH DIRECTIONS

The demand for low fat cheeses, in particular mozzarella cheeses with similar characteristics as their full fat counterparts, is increasing. The manufacturers are looking at several alternatives for manufacturing low fat cheeses, especially by use of fat replacers. The present investigation has shown that several cheeses currently available on market do not meet the desired composition and texture requirements. Due to the variations in mozzarella cheeses available on market, standardisation of cheeses by the mozzarella cheese manufacturers is recommended. There is a need to develop new methods for analysis of mozzarella cheeses that can differentiate them from the other cheeses such as cheddar and Italian varieties of cheeses. There is also a need to standardise the analytical methods and simplify the procedures especially for the microstructure study so that such methods may be adopted by the manufacturers for quality control purposes.

This study demonstrated that the texture characteristics of low fat mozzarella cheeses could be improved by using exopolysaccharide producing starter cultures. Similarly, starter organisms as *Lactobacillus casei*, *Lactobacillus plantarum* and *Lactobacillus helveticus* could be used for the manufacture of mozzarella cheeses. Several of these starter cultures have been utilised to manufacture mozzarella and other cheeses but the studies have been limited to changes that occur during manufacture. The starter cultures that may have potential in mozzarella cheese manufacture may be galactose positive, fast acid producers and proteolytic strains.

In this project microstructure of mozzarella cheeses was observed and components such as protein, fat and moisture phases were identified. But this elementary status of understanding needs to be further developed to include measures of minor components including calcium lactate, minerals and salt based on the observations under a scanning electron microscope.

Similarly, the use of confocal microscope is not fully exploited for the examination of cheese components with different staining techniques. There is a need to select and identify stains for observing the various components of the mozzarella cheese specimen. New techniques for microstructure study need to be developed in order to eliminate artefacts in micrographs obtained of the cheeses. Presently there is little information on the correlation between the microstructure and texture or functional characteristics of the mozzarella cheeses. There is a need for improvement in microstructure analysis and its correlation with the texture characteristics such as hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness. Similarly, the microstructure observations can be correlated with the functional characteristics of melt, stretch and browning. The use of sucrose solution in this study for cryo-protection of specimens gave better specimens wherein the protein strands, voids belonging to the fat and serum phases of the cheese could be observed in a great detail.