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Microstructure and Rheology of Process Cheese

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MICROSTRUCTURE AND RHEOLOGY OF PROCESS CHEESE

by

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ABSTRACT

Microstructure and Rheology of Process Cheese

by

Abdelaziz Hassan Rayan, Doctor of Philosophy

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Department: Nutrition and Food Science

Four batches of pasteurized process cheese were prepared from the same Cheddar cheese by cooking to 82C in the presence of sodium citrate, disodium phosphate, tetrasodium pyrophosphate or sodium aluminum phosphate. Each batch contained the same moisture (40.6%) and emulsifying salt concentration (2.5%). The process cheese was sampled for microstructural and rheological examination after 0, 5, 10, 20 and 40 min in the cooker at 82C.

Even though each emulsifying salt affected the physical properties of the process cheese differently, the cheese generally became firmer, stiffer, more elastic and less meltable as cooking time increased from 0 to 40 min. These changes were accompanied by a decrease in the dimension of fat masses and an increase in the degree of emulsification as evidenced by scanning electron microscopy and transmission electron microscopy. The degree of emulsification (fineness of fat particles) seemed directly related to firmness,

poor meltability, toughness, breaking force, apparent stiffness modulus, degree of elasticity, apparent ultimate stress and inversely related to hysteresis and apparent ultimate strain. Tetrasodium pyrophosphate produced the most rapid emulsification of the fat in the cheese and sodium aluminum phosphate the slowest. The effect of the other salts was intermediate. The softest most meltable cheese was poorly emulsified while the firmest most sliceable was well emulsified.

Sodium citrate and tetrasodium pyrophosphate crystals remained undissolved in the cheese after 40 min in the cooker while sodium aluminum phosphate crystals were still undissolved after 10 min.

There was a close statistical relationship among several of the rheological measurements viz. meltability and firmness, toughness and breaking force, and meltability and breaking force. Future rheological studies on process cheese should not require all of the above measurements. An increase in the fineness of the fat emulsion as determined by scanning electron microscopy was generally accompanied by increased firmness, poorer meltability and increased toughness.

INTRODUCTION

Process cheese is regarded as a popular and valued food. It is more uniform in quality and flavor than natural cheese and since its invention in 1911 different types of process cheese products have been marketed in the United States and other parts of the world.

Pasteurized Process Cheese, Cheese Foods, and Cheese Spreads constitute about 60% of the sales of American Cheese in the United States (383, 384). In 1978 this amounted to 1,599,743,000 pounds.

An essential part of process cheese manufacutre is the incorporation of emulsifying salts into ground natural cheese while cooking to at least 65.5C but more commonly to about 85C (371). The emulsifying agents such as trisodium citrate, disodium phosphate, tetrasodium pyrophosphate and potassium or sodium aluminum phosphate do not function as true fat emulsifiers. Instead they react with and change the physical properties of the protein so that it forms a very smooth uniform film about each fat droplet. This prevents the separation and bleeding of the fat from the cheese (91). In addition to improving emulsification of the fat, these salts help solubilize the casein. This is accomplished by raising the pH and sequestering part of the calcium that is associated with the casein (111). One of the most important problems in the processed cheese industry is associated with controlling the body and texture. In some instances cheese with a high melting index (198) suitable for cooking is desired. In others a more firm, sliceable product is needed. Cheese that is too firm

may have an undesirable hardness when eaten and consequently have poor meltability (91).

Another common problem encountered in process cheese making is known as the rework defect. It is manifest by the formation of a stiff hard body even while in the cheese cooker. It is precipitated by the addition of some previously processed cheese to a new batch. It also occurs when the process cheese is held in the cooker too long at a high temperature (37, 91, 211, 362). Lauck (211) and Tatter (361) reported that the adverse effects of rework (salvage) cheese and loss of melting properties when process cheese is reworked into a new blend may be overcome by the use of a combination of lipid-type surface active agents like phosphorylated stearyl monoglyceride to supplement the emulsifier. However, this application has not been adopted by the industry. While a number of factors affect the stiffness of processed cheese, (19, 31) little is known about the physical structure of the cheese and its relationship to body characteristics (169, 258, 340).

Some microstructural studies on Cheddar and other natural cheese varieties have been made, (56, 67, 87, 96, 118, 124, 169, 174, 178, 179, 182, 340, 359) but these do not represent the structure of processed cheese because the fat emulsion is completely reformed during processing.

The aim of this research was to examine the effect of certain emulsifying agents, and processing treatments on the microstructure of process cheese by scanning electron microscopy and transmission electron microscopy and to see if there is a relationship between the microstructure and specific rheological properties of the cheese.

REVIEW OF LITERATURE

The compounding of process cheese developed first in the United States as an art described in the original patents (10, 14, 15, 23, 24, 90, 113, 127, 128, 143, 157, 160, 165, 189, 203, 204, 205, 207, 211, 264, 265, 267, 303, 304, 317, 375). The objective was to produce a cheese product with sensory characteristics approximating those of natural cheese, but with extended keeping quality and capable of being delivered in a convenient form to please the consumer (17, 20).

Manufacturing Procedure

Process cheese is the result of blending natural cheese of different ages, degrees of maturity and sources; adding water, coloring and emulsifying salts and using heat and agitation to produce a homogeneous mixture. The final product is a semisolid with a consistency suitable for packaging and which can be stored at or near room temperature for prolonged periods (20). Cheddar and Swiss were the basic varieties of cheese used for processing when the industry was first established in the United States. Blends of other cheese varieties soon followed in which Limburger and brick cheese were used (234).

The effect of blending several lots of cheese of various ages, physical properties and compositions opened new possibilities for composition and quality (18, 19, 58, 163, 235, 258, 363). Low fat and skim milk cheeses have been used to make low-fat products desired by some consumers and to decrease cost (92, 134, 135, 162, 221). Skim

milk cheese, probably more than any other ingredient, aroused intense antagonism against process cheese manufacture before standards were established (221, 286).

Processing techniques varied according to the manufacturer's idea of what was economical and acceptable. Irvine et al. (162) and others (32, 130, 186) introduced the technique of using curd from whole milk cheese by conditioning it with treatments such as mixing finely divided curd with an alkaline solution to prepare it for immediate processing and treating granular or cheddared curd with acid (32, 130). The hard rind of Swiss cheese, after grinding and milling into small particles, was recommended for processing by Brickner (55).

Cheese made slightly rancid by a special lipolytic enzyme imparted an improved flavor in the processed product (13). High flavor for example, which were important to some manufacturers, were rejected by others with preference for a milder product (13, 17, 18, 20, 23).

Recently, Ernstrom et al. (99, 100) introduced a technique for conversion of ultrafiltered whole milk retentates into curd for making process cheese with a substantial increase in yield. An effective method for producing process Cheddar cheese from enzyme treated retentates also has been outlined (210, 337).

As demand developed for products to serve special diet needs, or to provide different physical characteristics, new and different food materials or combinations of them were introduced. All have been examined by regulatory officials as well as by the industry and, when acceptable to all interests have been included in definitions and

standards of identity (286). Standards established by law in Wisconsin and later by the Federal Government imposed limitations on composition, combination of cheese varieties and additives (92, 382).

Process cheese foods and spreads were developed in the 1920's. Body characteristics were modified in the development of cheese foods and spreads by adding non-fat milk solids or whey solids plus sweeteners (12, 364, 366).

The earliest published studies in the United States on composition and characteristics of process cheese and cheese spreads began in 1927, and dealt with emulsifiers, cooking temperatures, moisture variation and age of cheese (363, 366). By 1947 the production of cheese foods and cheese spreads was well established, and industry was cooperating with the FDA to develop definitions and standards for these and other cheese products. The definitions recognized that cream and concentrates of milk, skim milk, and whey were sometimes desirable in process cheese products. These additives were approved when the moisture did not exceed 44% and fat was not less than 23%. Such mixtures were designated "Pasteurized Process Cheese Foods". "Pasteurized Process Cheese Spreads" were defined to contain more than 44% but not more than 60% moisture and not less than 20% milk fat. Melting properties, firmness, plasticity and fluidity have been as important as flavor in defining process cheese quality. These characteristics have been controlled to a certain extent, but not entirely, by a careful choice of the natural cheese and emulsifiers (136, 258, 259).

Melting Properties

Various methods for measuring the melting properties of process cheese products are used in commercial laboratories. They usually require the rather difficult estimate of areas covered by the cheese before and after some prescribed heat treatment usually expressed as a "Melting index" (210). Uniform melting of process cheese is a desirable property. The fact that process cheese appears on the market with irregular melting behavior indicates that control of this property has not been mastered (11). Most methods are based on a visual examination of cylinders of cheese placed on a heat source for a given period of time (86). Eckberg and Mykleby (86) used visual observation of process cheese plugs heated on a steam bath. They reported a relationship between cheese pH and melting quality, a low pH showing a tendency toward good melting quality, and a high pH a tendency towards poor melting quality. These authors theorized that high-acid cheese, with the degree of protein breakdown being related to melting quality. They also reported that cheese less than 25 days of age might harm the melting qualities of process cheese.

Another method for measuring melting properties of process cheese was described by Arnot et al. (11). A standard cylinder of cheese is exposed to 100C for 15 min. Measurements of cylinder height before and after treatment are used as a basis of comparison and a tripoid micrometer was used for making the measurement. A cork borer (size 13) and cutting block were used to cut a cylinder of cheese approximately 17 x 17 mm. All cheese samples remained first at 4.4C for 24 hours then, the cylinder of cheese was positioned on a glass tray and

returned for 15 minutes to a refrigerator maintained at 4.4C. Then measurements of sample height were made with a depth micrometer. The samples were immediately placed in an oven at 100 ± 2C for 15 min. After which the sample trays were removed and allowed to stand at room temperature for 15 min then placed in a refrigerator at 7.2C for 30 min. The center of the cheese cylinders was selected for the final measurement regardless of the surface slope or depression. The test was repeated on samples that exhibited marked irregularity on the upper surface. Melting quality was expressed in terms of percent decrease in cylinder height after heat treatment.

Methods which expose the cheese to hot air during melting tend to cause film formation on the surface and uneven flowing of the melting spread. A melting test described by Olson and Price (257) for pasteurized cheese spreads with results expressed as cheese flow values appears to be a satisfactory measure of melting properties. The test is applicable with comparable precision to samples with varied melting properties. The coefficient of variation from three series of tests ranged from 5.0 to 6.5%. Dried surfaces and film formation were eliminated and the melting properties were expressed by a simple linear measurement.

Effect of Natural Cheese

The importance of natural cheese to the properties of process cheese cannot be over emphasized. Shultz and Morwetz (319) introduced the term 'Relative casein content' which expresses the ratio of insoluble casein nitrogen to the total nitrogen. The higher the relative

casein content of the natural cheese, the better it is for the production of a stable process cheese. Young rennet cheese, a few days old, would have a relative casein content of 90 - 95% which would decrease during curing. A high intact casein content results in process cheese with a long filament-like structure whereas a low content usually produces a short structure (36). Meyer (228) explained this phenomenon by stating that the sol produced during the processing of young cheese, retaines its long structure for a considerable time, and is extremely stable against chemical, thermal or mechanical influences. It is also hydrophobic, i.e., it absorbs moisture very slowly and in limited quantities. The same author (228) reported that shortening of this long structure can be achieved through intensive agitation. A short bodied structure with a high relative casein content is desirable for cheese spread. Medium ripened cheese with a relatively short structure and a relative casein content of 60 - 75% must, therefore, be handled carefully in order to aboid changing structure. The same is true for all ripe cheeses (231). Block processed cheese of desirable slicing qualities and elasticity demands raw materials with long structure and between 70 - 90% relative casein content. For spreadable process cheese and cheese spreads 60 - 80% relative casein content in the raw material is desirable. Schmelzpak (229, 232) is curd with an intact casein content of 90 to 100 percent which can be blended with various types of cheese of different maturities for the manufacture of process cheese.

Blending is a normal step in the manufacture of process cheese, and has been described by several workers (19, 23, 101, 122, 235, 258, 342, 362, 363, 371, 385, 417). Sommer and Templeton (336) suggested

that the cheese blend should contain about 75% of cheese up to 3 months old and 25% of well ripened cheese 6 to 12 months old. They pointed out that relatively young cheese produced a smooth texture, firm body and good slicing properties, while, old cheese tended toward higher flavor, grainy texture, weak body and poor slicing properties. Vakaleris et al. (385) and Olson and Price (258) showed that the body of process cheese is markedly affected by the acidity as well as age of the natural cheese from which it is made. Sweet or young natural cheese makes a firm-bodied process cheese, while, acid or aged natural cheese makes a soft-bodied product (291). Barker (16, 23) described a good blend as that consisting of four to five classes divided approximately as follows: 15% acid cheese, 15% current cheese, 45 - 50% short-held 2 - 3 months old cheese (on acid side) but not acid, and 20 - 25% sweet and open cheese. Six-month old cheese could replace the short-held cheese in whole or in part. These figures merely suggest approximate amounts to be selected from each group. Young cheese has a slight tendency toward fat separation but this tendency disappears when the cheese is 8 days old (362). The same authors (363) indicated that resistance to crushing decreased as the age of the cheese in the blend increased. Davel and Retief (74) reported that aged cheese produced a soft spreadable body. Palmer and Sly (261) stated that old cheese frequently gave a loose, grainy and oiled-off process cheese, while fresh cheese produced a stable emulsion. Templeton and Sommer (362) advised that the average age of the cheese in the blend should be 4 - 7 months. Such a blend, they indicated, might consist of 2 - 3 parts of two-month old cheese combined with one

part aged cheese. However, Barker (19), advised blends of cheese averaging from 1 - 4 months and 4 - 6 months of age, and warned against the use of large amounts of tough, fresh cheese and crumbly acid cheese. Dimove and Minerva (81) showed that process cheese of good quality could be produced from six-month old kachkaval cheese with or without the addition of 20 - 25% white pickled cheese or up to 30% fresh curd. This combination with 20% curd proved economical. Hayter et al. (135) reported that a low fat sliceable processed cheese product with optimum flavor and texture was produced from a blend containing 40% 10 day old skim milk Dariworld cheese, 20% aged Cheddar cheese, 4% NDM and 4% sweet dried whey. Templeton and Sommer (363) modified the body of process cheese by the addition of alkali or acid. They stated that the results varied with cheeses of different ages and lots. Process cheese with suitable slicing properties was generally attained in the pH range of 5.1 to 5.6. Barker (23) indicated that Cheddar cheese with a titratable acidity of 0.6% or higher should be used cautiously in process blends. He suggested that it not exceed 12 - 15% of the raw material.

As in other foods, flavor is a highly important quality in process cheese. Flavor contributed by the blend components is largely responsible for the flavor in the final product. Babel and Hammer (13) found that when aged cheese, showing varying degrees of rancidity was used at the rate of 25% of the blend, the finished product was rancid. Actually process cheese made in this way was considered to have more cheese flavor than process cheese containing the same amounts of identically aged, but not rancid cheese.

Emulsifying Salts

Plasticizers or emulsifying salts play an important role in the process cheese industry. The use of emulsifying salts is still something of a mystery with new salts and new combinations appearing almost annually. Some show great success and others produce alarming and costly failures. Because emulsification is not the sole function of these salts, the term 'melting salts' has been suggested (291). Processing cheese without emulsifying salts, but with heat and agitation, will result in the separation of cheese constituents. The curd will shrink and allow fat and moisture to escape. However, process cheese can be made without emulsifiers if the blend of natural cheese is carefully selected, heated and stirred (91, 286). Before emulsifying salts were generally used, process cheese defects such as fat separation, mealy body, and faulty consistency were common.

Use of emulsifying salts for process cheese in the United States began with the early work of Eldrege and Carpenter (89, 59). Subsequent studies and patents mentioned different salts and advanced explanations of their varied actions.

In the presence of emulsifying salts, the fat that separates at the beginning of the process will be reincorporated into a smooth homogeneous mixture which solidifies after packaging to give the semi-solid product characteristic of processed cheese (371).

The physiochemical changes during cheese processing in the presence of emulsifying salts were described by Bonell (40) as removal of the calcium from the protein system, protein solubilization or

peptization and dispersion, swelling and/or dehydration, pH stabilization and structure formation during cooling.

Templeton and Sommer (362, 363, 367, 369) found that emulsifying salts vary in their effect on the body of process cheese. They preferred the body characteristics of cheese emulsified with sodium citrate over that of other emulsifiers. They reported that disodium phosphate produced a weak bodied cheese which they thought was undesirable. Tetrasodium pyrophosphate gave an extremely hard cheese (31, 263). Sommer and Templeton et al. (335, 369) noted that fat globules, as observed by the light microscope, in cheese processed with polyphosphates, remained large and irregular in shape, while cheese emulsified with sodium citrate and tetrasodium pyrophosphate had small fat globules and a fine structure.

The effectiveness of emulsifiers at first was attributed to their emulsifying ability, and to their solvent action on cheese proteins (40). Processing also increases the water soluble nitrogen in the cheese (86, 91, 101, 122, 123, 137, 146, 151, 157, 235, 281, 287). Emulsifying salts possess a distinct affinity for calcium and combine with calcium ions by either precipitation or by sequestering. All of these changes contribute to changing natural cheese curd from its coagulated semisolid state to a more soluble state (5, 30, 31, 35, 40, 91, 101, 122, 137, 146, 263, 314, 333, 345, 367, 418, 425).

Electrophoretic studies of protein in natural cheese and process cheese showed no significant differences (123). This suggests that protein hydrolysis is not involved in solubilization of the protein. Habicht (122) states that an ideal emulsifying salt contains a mono-

valent alkaline cation with a polyvalent anion. The salts of monovalent cations are more capable of dispersing than those with multivalent cations (5). Some emulsifying salts e.g. trisodium phosphate disperse fat and therefore considered to be good emulsifier, but do not always give the best body characteristics in the finished cheese (82, 291). Other salts such as sodium potassium tartarate produce desirable body characteristics but do not act effectively during heating of the blended cheese (34, 371). In fact they do not form stable emulsions at processing temperatures above 65C (371).

Studies of emulsifying salts (5, 30, 31, 40, 82, 91, 101, 117, 122, 123, 137, 146, 151, 157, 159, 163, 184, 223, 233, 264, 281, 287, 291, 301, 314, 318, 333, 345, 362, 363, 367, 379, 418) have considered changes in water-soluble nitrogen, fat leakage, compressibility, knit and flavor of the finished product. In general, satisfactory emulsifiers all have trivalent anions, form alkaline solutions and precipitate or sequester calcium.

It has been suggested (122, 367) that process cheese is an oil-in water emulsion protected and stabilized by an emulsoid sol of hydrated casein and emulsifying salts. Meyer (235) summarized the function of melting salts in blended cheese as acting as a protein solvent, promoting the emulsification of the fat and water, acting as a protective film around the fat globule to stabilize the emulsion, controlling the final pH and increasing the water binding capacity of the protein. However, since all such compounds do not perform satisfactorily the choice of salts or combinations of salts has generally been determined by their ability to disperse fat, to promote

uniform melting and blending during processing and to give the desired flavor, consistency and melting properties to the finished cheese. In addition, the choice of salts, has been influenced by the type of natural cheese chosen for processing.

The process cheese emulsion should remain stable during processing and storage. Separation of fat or water or both, followed by bacterial decomposition, has been experienced by several compnaies (234). Bacterial degradation of some emulsifying salts reduces the quality of the cheese and causes a poor body and texture, and in extreme cases, water and oil separation, possibly followed by bacterial proteolysis (234, 371).

The first salts used as emulsifiers were citric acid and Rochell salts ($NaKC_4H_4O_6$) (234, 367). Later sodium salts of phosphoric acid, polyphosphoric acid, tartaric acid, lactic acid, mucic acid, malic acid, gluconic acid and glutamic acid were suggested (59, 157). Ammonium salts of these acids (122, 375) along with gamma and delta glucono lactone (235), injection of carbon dioxide and hydrogen (113, 235) and the use of proteolytic enzymes (186, 210, 303, 304) have been proposed. A novel complex of sodium aluminum orthophosphate (188, 212, 213) was recently introduced. However, only the citrate and the phosphate family have been given widespread consideration in the process cheese industry.

Citrates

Citric acid is a tricarboxylic acid with three dissociable protons whose pK values are 3.08, 4.74, and 5.4. Its dissolved sodium salts in distilled water have a pH of 3.8 for monosodium citrate, 5.1

for disodium citrate and 8.2 for trisodium citrate. Trisodium citrate is the only one used in cheese processing. Palmer and Sly (262) and Swiatek (345) stated that no other melting salt preserves the same delicacy of flavor after processing. Templeton and Sommer (367) and Prodanski (287) found that trisdoium citrate was a more powerful melting agent than some other emulsifying salts. It is very soluble and exhibits fairly good protein dissolving power. Cheese processed with trisodium citrate had a little tendency to absorb moisture, but the structure remained firm and heavy (31, 229). Templeton and Sommer (362, 363) observed that cheese made with sodium citrate retained air cells and that cheese without these air cells had a coarse body. With cheese blends of average maturity, sodium citrate produced a characteristic silky emulsion which solidified into a firm-bodied, smooth-textured cheese with good slicing properties and natural flavor (82, 229). With some cheese the texture was slightly gummy (183, 184, 262). Because of this effect, its use is limited to block cheese and to portions which need to be firm and non-spreading. It should not be used in the manufacture of spreadable process cheese unless an over-riped, very short bodied cheese is used as raw material or when citric acid is added to reduce the pH (262). Templeton and Sommer (363) devised an ingenious method for estimating fat leakage from process cheese. Their results demonstrated clearly the superior emulsifying power of trisodium citrate. If the correct cheese and conditions of processing are used with respect to pH, maturity and processing temperature are observed, sodium citrate makes a processed cheese of very high stability and long keeping quality.

Palmer and Sly (262) reported that the high solubility and chemical stability of sodium citrate precludes the possibility of recrystalization or decomposition even when the process cheese is stored for several years. The use of sodium citrate as an emulsifying salt allows the processing of properly blended cheese at very high temperatures without the emulsion breaking down (31, 101, 117, 122, 231, 234, 262, 264). This is especially important when process cheese is to be stored for periods under fluctuating temperatures as is often the case with canned cheese. Citrate on the other hand, has poor resistance to bacterial attack (235). Ruf and Kehrer (306) and Becker and Ney (31) found that citrate had the disadvantage of readily undergoing bacterial decomposition. Lack of creaming action and tendency toward mottling in the cheese structure have been reported as disadvantages of citrate (228, 234). Morris et al. (245) noted that calcium citrate crystals on packaged cheese was most apparent on areas exposed to pressure and eliminated it by avoiding the use of citrate as an emulsifier. They explained that the salt failed to dissolve in the liquid cheese in the kettle or it recrystalized after cooling. Other factors that cause crystal formation may be excessive amounts of emulsifying salts, low storage temperature, and a high calcium content in the natural cheese (234).

Monosodium dihydrogen citrate and disodium monohydrogen citrate were studied as melting salts (371), and found to be unsatisfactory when used alone because of their high acidity (234).

In general trisodium citrate is a good emulsifier when compared to disodium phosphate (30, 183, 184, 367). Unfortunately, trisodium

citrate is more expensive than polyphosphates since only 50% of the less expensive polyphosphates will give the same effect as citrate (235). For this reason the industry has moved away from the original and almost universal use of sodium citrate to phosphates which include disodium phosphate, trisodium phosphate, sodium metaphosphate, tetrasodium pyrophosphate and sodium polyphosphates (91, 234, 314, 371, 387, 414).

Recently there seems to be a trend back toward the use of trisodium citrate to improve body characteristics. However, it is used in combination with other emulsifiers. The recommended rate is nine parts of citrate to one part disodium phosphate (262). Such use in some of the newer sliced products however, causes a defect known as citrate haze. This appears as a fine precipitation of calcium citrate on the cheese surface and is probably related to physical abuse of the product as it is being processed and packaged (245).

Phosphates

The emulsifying agents derived from the phosphate family may be divided into the following groups (235):

a. Monophosphates (salts of orthophosphoric acid)
b. Condensed phosphates
    1. polyphosphates (oligophosphates and high molecular polyphosphates in chains)
    2. metaphosphates (rings)
c. Other condensed phosphates (rings with chains and branched chains).

Monophosphates are salts of orthophosphoric acid, a tri-basic acid. By neutralizing with soda ash, three different salts are produced. Solutions of the sodium salts gave the following pH values (235, 370):

Monosodium dihydrogen phosphate, NaH_2PO_4 pH 4.5

Disodium monohydrogen phosphate, Na_2HPO_4 pH 9.0

Trisodium phosphate, Na_3PO_4 pH 11.5

Disodium monohydrogen phosphate in the anhydrous state or containing water of crystalization are used most commonly for cheese emulsification. Therefore, the water of crystalization must be accounted for.

Monosodium orthophosphate. The acidic monosodium salt and the alkaline trisodium salt are generally used as pH corrective salts (234). Faiare (101) reported that the buffering effects of disodium phosphate occurs between pH 6.5 and pH 7.5 and is greatest at pH 7.1. Owing to its acid reaction, the use of monosodium orthophosphate as a cheese emulsifier leads to a lowering in the pH of the blend thus giving rise to a very coarse, open-textured process cheese product with an acid flavor (262). For this reason it is seldom used and should not be used as the only emulsifying salt. Thomas (371) reported that 3% monosodium dihydrogen phosphate broke up the cheese emulsion into its main constituents of fat, curd and water and that the cheese did not solidify after packaging. Even 2% salt was unsatisfactory (371). The same results were reported when used with Egyptian Ras cheese as a raw material (291). It has, however, been used to some extent to emulsify low-fat cheese with a high casein/fat ratio (Gouda type), for which a highly acid melting salt is often required (30, 234, 371).

Di- and Trisodium Orthophosphate. Thomas (371) claimed that disodium orthophosphate is not regarded as a strong emulsifying agent and is now seldom used alone. This is a false statement because Ellinger (91) showed that most of the sliced pasteurized process cheese now being marketed in the United States is prepared with either disodium phosphate or alkaline sodium aluminum phosphates. These salts, in proper levels, and sometimes combined with trisodium phosphate or sodium citrate, create the most acceptable melt and texture. Meyer (234) claimed that although disodium phosphate is very cheap, 5.8% of the weight of the natural cheese is necessary to obtain optimum results. However, the Federal Standard of Identity for pasteurized process cheese (91, 103) allow the addition of only 3% of approved emulsifying salts or any combination based on the weight of the process cheese. Noznick and Bernardoni (254) used Na_2HPO_4 in a new processed cheese product and reported that this salt performed as an excellent water soluble emulsifier.

Owing to its alkaline nature, the use of too much disodium orthophosphate may lead to undesirably high pH in the cheese (371). Hayter et al. (136) reported that the average pH values obtained with 2.0 and 2.5 percent disodium phosphate were 5.38 and 5.57 respectively. Barkan and Minkina (15) reported that high pH of process cheese in a Moscow factory (average pH 6.25 for 171 samples) caused rapid deterioration of the product. The pH increased with increasing amounts of disodium phosphate in the mixture. Replacement of over 50 percent of this salt by sodium dihydrogen phosphate decreased the pH of the cheese blend. The same authors reported that the addition of up to 25

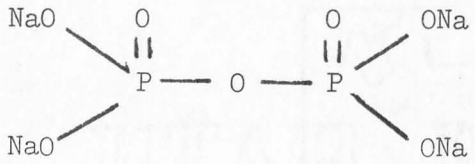
percent of fresh curd or non fat young cheese to old cheese during the melting process lowered the pH and improved the quality. Swiatek (345) (354) reported that the addition of 2, 2.5 and 3 percent disodium phosphate and trisodium phosphate gave the softest cheese when compared to the same cheese emulsified with either pyrophosphate or polyphosphate. Hayter et al. (136) reported that using only 1.5% disodium phosphate gave a mealy gummy body. In general disodium phosphate produced a weaker body than trisodium citrate (362, 367) and 3% trisodium phosphate resulted in a process cheese that was soft and sticky, inelastic and poor in general quality. Therefore, it is not recommended for use on its own (371). Khemelev (187) suggested that in the absence of pH control of microbial growth (high pH) resulting from the use of di and trisodium salts, the addition of these salts can be expected to result in the development of bitter taste. After long storage recrystalization of the melting salts is also expected particularly if this cheese is stored at low temperature. The same author (187) reported that Na_2HPO_4 caused corrosion of the wrapping foil. Palmer and Sly (263) deduced that recrystalization may occur in cheese processed with disodium phosphate when stored at low temperature (about 4.4C). These authors found two instances of sodium phosphate crystals in cheese emulsified with 3% disodium phosphate. The pH values were 6.7 and 6.8 respectively when the cheese was examined. This was obviously well above the normal pH for process cheese. In the first instance the cheese had been stored for 2 - 3 months in winter; the exact storage temperatures were uncertain. In the other case the cheese had been stored for 5 months at 10 - 15.5C. In each case the

crystals were about the size of small peas, and on analysis were found to be $Na_2HPO_4 \cdot 12H_2O$. Scharpf and Kichline (313) examined the type and extent of crystal formation in processed American cheese by x-ray diffraction in the pH range of 4.82 to 9.85 and found the predominant crystalline species to be the α-form of $Na_2HPO_4 \cdot 12H_2O$. Rank (289) and Meyer (233) reported the development of sandiness due to the crystallization of calcium phosphate.

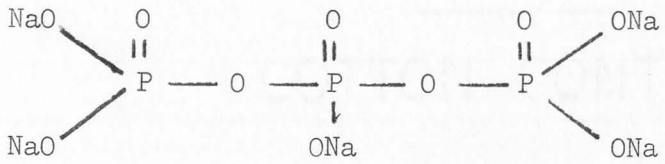
Reported references to studies on orthophosphates are quite confusing with respect to their success as emulsifying agents. When the properties of cheese emulsified with disodium phosphate and those prepared with sodium citrate were compared, Templeton and Sommer (363, 367) concluded that the additional cost of the citrate was not warranted.

The polyphosphates. We must not assume that developments in the United States proceeded unilaterally (91, 223, 286, 387). The Germans particularly have had much success with the higher polyphosphates. A leader in this field seems to the the Joha-Benckiser-Knapsack GMBH at Ludwigshafen-Rhein under the supervision of Professor Albert Meyer.

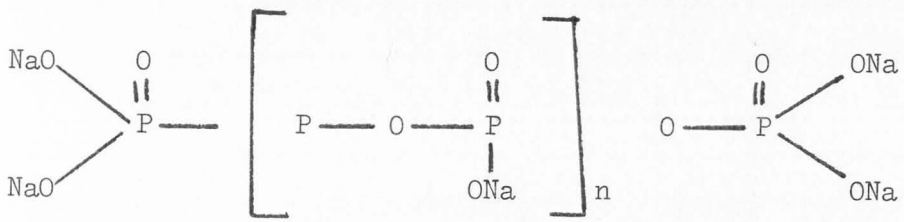
The polyphosphates are linear condensed phosphates (370, 371). The first ten members of the series extend from diphosphate (formerly called pyrophosphate) through triphosphate (tripolyphosphate). Tetra, penta, hexa, hepta, octa, nona and deca-phosphates are called oligophosphates.



Alkaline pyrophosphate



Sodium tripolyphosphate



General polyphosphate formula

In high molecular weight Graham's salt, the value of (n) is between 30 and 300 (227). The diphosphate molecule may exist in the mono, di, tri, or tetra sodium form. Solutions of these salts vary in their pH value from 2.7 for mono-diphosphate, 4.2 for disodium diphosphate, 7.0 for trisodium diphosphate to 10.2 for tetra sodium diphosphate. The pK values for the four protons of pyrophosphate are 0.85, 1.49, 5.77 and 8.22 respectively. The polyphosphates extending from diphosphate to decaphosphate perform well as melting salts due to their excellent properties as ion exchangers. They also show a very wide buffering capacity depending on the degree of condensation (301, 306). While the di- and triphosphates are obtainable in pure form, tetra- and higher phosphates cannot be obtained as single compounds. However,

by selecting the conditions of manufacture polyphosphate mixtures with the desired properties can be produced (235, 414, 416). Although the pH and buffering capacity of natural cheeses are quite variable, the pH can be brought to the desired level by use of blends of polyphosphates. Becker and Ney (30, 31) demonstrated the superiority of polyphosphates as melting salts. They did an especially good job when used in the manufacture of spreadable high fat content.

The removal of calcium from natural cheese is a primary prerequisite for a successful emulsifying salt. Bivalent calcium ions and univalent sodium ions have antagonistic effects on cheese protein (122, 167). Calcium dehydrates cheese protein and inhibits swelling. Sodium on the other hand acts as a dispersant and dissolves and hydrates the protein (122, 159). Bonell (40) points out that polyphosphates are good emulsifying salts because they form both soluble and colloidally bonded stable calcium complexes.

Several workers have proposed different values for the calcium binding capacity of specific emulsifying salts. Albonico and Gianani (5) added 0.75, 1.50 and 3.0% polyphosphate; 2.3, 4.6 and 9.2% sodium orthophosphate and 3% sodium citrate to the blend. Analysis of the cheese indicated that orthophosphate and polyphosphates were identical in their calcium complexing action. On the other hand Becker and Ney (30) reported that orthophosphates have very poor calcium binding capacity. Pyrophosphates are somewhat better, but are greatly surpassed by polyphosphates. Ring structured (meta) phosphates do not form calcium complexes to an appreciable extent (387). The calcium binding capacity of pryophosphate decreases considerably at acid pH

values (30). High molecular weight polyphosphates show the least decline in calcium binding ability with decreasing pH (159).

The complexing capacity of a polyphosphate chain is approximately proportional to the number of phosphorus atoms in the chain (151). According to Irani and Gallis (159) the calcium sequestering action of mixtures of phosphates is additive.

Sodium paracaseinate is more soluble than the calcium salt. The increase in soluble protein (usually expressed as "water soluble nitrogen") is due to the decalcifying properties of the emulsifying salt during the melting process (301). Soluble proteins provide a protective film around the fat globules of the cheese, preserving the fat and water in emulsion form (235, 380). Heide (137) determined the capacity of various types of phosphates to solubilize fat-free rennet casein under conditions employed in the industry. He reported that the solubilization of casein averaged about 30%, 45% and 85% for orthophosphate, pyrophosphate and polyphosphate respectively, and appeared to be additive for mixture of those salts. The casein solubilizing capacity of phosphate paralls its capacity to bind calcium. Of all polyphosphates, the higher molecular weight Graham's salt has outstanding ion exchange characteristics and protein solubilizing properties (380, 414). This salt is therefore, particularly suitable for the manufacture of block process cheese, and firm textured portions. Swiatek (345) reported that such firmness also was obtained by other polyphosphates. Hayter et al. (135, 136) indicated that firmness increased without loss of quality by combining sodium hexametaphosphate with citrate for use with cheese that tends to absorb moisture too readily. Degredation of polyphosphate salts usually takes place by

hydrolysis i.e. rupture of P-O-P linkages (151). The pH of the phosphate solutions generally decreases as hydrolysis continues due to the formation of phosphoric acids. Roesler (301) indicated that polyphosphates added as emulsifiers were rapidly hydrolyzed to mono and di- phosphates and that about half of the polyphosphate was hydrolyzed during the melting process. By the end of about seven weeks storage for spreadable cheese and ten weeks for the sliceable produce, the remainder was hydrolyzed. The end product in the spreadable cheese was predominantly monophosphate. Watzel (416) indicated that this change depended on processing time, processing temperature, type of cheese and some other factors. The same results were published by Horst (151) one year later. Studies of the hydrolysis of polyphosphates in process cheese showed that polyphosphate hydrolyzed first to pyrophosphate then to orthophosphate. The rate of hydrolysis depended on processing temperature, pH value, water content, storage temperature and storage time (151, 388). At neutral pH and room temperature polyphosphates are very stable, their half life being in the order of years (151). Glandrof (117) and Roesler (301), discussed this subject and indicated that polyphosphate hydrolysis does not justify the use of mono-phosphates as a melting salt. They stated that, "whereas fragmentation of the casein molecule is possible with polyphosphate, casein hydrolysis will not occur with mono-phosphate." Von Der Heide (414) and Polzhofer and Ney (281) in model experiments studied the formation of peptides in the presence of linear and cyclic condensed phosphates and reported that up to 4% dipeptides formed when these salts were used, but not with monophos-

phate under any condition. Among all polyphosphates, the diphosphates have the strongest moisture absorbing action. Palmer and Sly (263), reported that sodium pyrophosphate gave better results that sodium orthophosphate, especially for very mature cheese. Two or three percent of the anhydrous diphosphate gave a smooth, glossy emulsion that cooled to a fiarly firm bodied cheese with a close, brittle texture and flat flavor. Szabo (346) showed that both the viscosity of the melted blends and elasticity of the product were reduced by increasing the moisture, fat and processing temperature. The values of both properties increases as the percentage of melting salt was raised. The salt which yielded cheese with the most satisfactory consistency was sodium pyrophosphate. Dimov and Velev (82) reported that the best cheese as regards both flavor and consistency was obtained with sodium pyrophosphate. This salt tends to recrystalize after processing due to its low solubility (5 percent at 20C). The use of diphosphate can also cause sandiness due to the formation of calcium diphosphate crystals (233, 289, 313). Two types of sodium pyrophosphate are used in cheese making: alkaline pyrophosphate ($Na_2P_2O_7$) and acid pyrophosphate ($Na_2H_2P_2O_7$). Trials with 3% alkaline pyrophosphate increased the pH of the cheese during processing by 0.4 to 0.75 (371). Palmer and Sly (263) and Becker and Ney (31) demonstrated that pyrophosphates were not very satisfactory emulsifying salts because of high pH values caused by their alkalinity. pH reduction by the addition of organic acids caused a corresponding improvement in flavor but the texture became more coarse and grainy. Palmer and Sly (263) found that better results can be obtained by using 2 - 3%

sodium pyrophosphate together with a small proportion of citric acid to reduce the pH of the cheese. The low solubility of pyrophosphate, even at high temperatures, makes it necessary to add it in solution. At low storage temperatures there is a tendency for the melting salt to recrystalize from the cheese. Sommer and Templeton (336) reported the formation of lumps (white salty material) in cheese processed with 3% or more of tetrasodium pyrophosphate. However, they did not specify whether this material was pyrophosphate or orthophosphate.

In most cases, process cheese made with pryophosphate, even in small quantities has a very low melting index (371). Sodium pyrophosphate, on the other hand, is among the cheaper melting salts, and is effective at much lower concentrations than orthophosphate (235).

Generally, the versatility of phosphate salts has been indicated in numerous industrial patents which claim that certain salts can be used to control consistency (317, 425). Sodium metaphosphate, for example, has been indicated for making non-melting cheese for novelty meat loaf (380). Other combinations of phosphate and protein peptizing agents have been proposed for preparing emulsified cheese for drying (235).

In recent years the use of a complex sodium aluminum orthophosphate (Ka sal) has been developed for rapid heating with less fat leakage and less tendency for formation of surface crystals in the package (188, 212, 213, 371). Kichline et al. (180) claimed that optimum alkalinity is obtained in the preparation of process cheese by the use of sodium aluminum phosphate salt having the following

emperical formula: X Na_2 O. Y $Al_2O_3 \cdot 8P_2O_5$. Z H_2O, in which X is a number between 15 and 24; Y between 1.0 and 3.9; and Z between 0 and 50.

Rework Defect

A common problem encountered in process cheese is known as the rework defect. It is manifest by the formation of a stiff hard body even while in the cheese cooker. It is precipitated by the addition of previously processed cheese to a new batch. It also occurs when process cheese is held in the cooker too long at high temperatures (362). Loss of melting properties when process cheese is reworked into a new blend might be corrected by use of a small amount of a surface active agent such as phosphorylated stearyl monoglycerides to supplement the emulsifiers (211, 361). However, the phosphorylated monoglycerides have not been given widespread consideration in the process cheese industry.

Microstructure of Cheese

Interest of scientists as well as manufacturers in the microstructure of cheese and other dairy products has steadily increased and it has been suggested that until we have a better understanding of microstructure we cannot really understand the physical properties of the products. Baron and Scott Blair (26) stated that "Rheological work (on cheese) has been, hitherto, of an essentially emperical kind and this situation is only likely to be improved when other physical methods, capable of elucidating something of the very complex molecular structures, have been applied to cheese." Until 1920, many

workers had speculated concerning the fine structure of natural cheese, but little definite evidence in the form of photographs had been published. The few pictures which were available do not show the detailed structure of the curd very clearly.

Although fat globules in milk, which range in diameter from 0.5 to 10 μ, are readily observed under the microscopy, casein particles are too small to be seen by optical microscopy. An appreciable percentage of casein particles is less than 0.1 μ in diameter. For this reason the electron microscope, which can resolve down to 2 μm was used extensively in the early days in studies on the size and shape of native casein particles from milk (141, 156, 302), but little was done with cheese. There are, however, disadvantages to electron microscopy; artifacts may arise during fixation, dehydration, shadowing, ultra-secrioning, exposure to high vacuum or examination under the electron beam. The picture is a static one, and the effect of agents producing change in structure such as coagulation cannot be seen as a continuous process. In dairy products the fat cannot be seen in relation to the other constituents. Polarization effects cannot be studied, and the cost of the equipment is high (193). Optical microscopy has advantages in these aspects, and even though it permits observation of only the large aggregates, it is of considerable value in studying the size, shape and behavior of some casein particles. It may be used alone or as an adjunct to electron microscopy. With indirect physical methods such as flow birefringence (69), each technique provides evidence of value, giving a partial view of the constitution and structure of the specimen. The research worker must

correlate all the clues to deduce a coherent picture (193). In 1941 Koestler (197) showed that curd granule boundaries in Emmental cheese were visible at low magnification. Similar granule boundaries were observed in Gouda and Edam cheese by Hekma (139). Dean et al., (78) stated that a cheese mass consists of curd particles with a discrete boundary around them. They noted variations in size and intensity of staining of fat and other details in different types of cheese. King (192) applied fluorescence microscopy to the examination of casein in milk, curd and different types of cheese including Cheddar, Emmental, Edam and Process Cheddar cheese. He (192) found that, the grain boundaries in curd and cheese (Cheddar, Emmental, Edam) were almost free of fat, but were absent in process cheese. The boundary layers generally contained few fat globuls and exhibited the intense fluorescence color of protein. Abundant fat globules were slightly coalesced inside the curd. In the same year King and Czulak (193) showed a fibrous structure in freshly made Cheddar cheese curd. They also showed that curd cut parallel to the direction of flow possessed a network of long casein filaments that varied in thickness down to 0.1 μ. Transmission electron microscopy at about 1,400 magnification revealed images similar to coagulated blood fibrin (316). Fibrils of separate casein strands were attached more or less end to end. The author indicated that the flow of the curd mass may have contributed to this structure.

Light microscopy by Dean et al. (78) revealed that the fine protein structure in Cheddar curd tended to disappear as ripening

proceeded. They also noted that the distribution of lactic bacteria in cheese curd was markedly uneven. Rammell (290) confirmed this finding and specified that bacterial colonies were usually associated with curd junctions, particularly milled curd junctions. In addition to bacteria, light micrographs of stained cheese sections showed two different kinds of dark lines. Fine lines of curd granule junctions arose from pressed and cheddared curd, and strong lines of milled curd junctions were noted between pressed and salted curds. These junctions were characterized by a fat content lower than in the interior parts of the curd. Another interesting condition in Cheddar curd is where the junctions between milled curds are visible after pressing. This is a defect known as "seaminess". In extreme cases it persists after the cheese has matured. The phonomenon is regarded as a defect because of uneven color and because the junctions are usually weak due to incomplete fusion. This often leads to crumbling when the cheese is sliced or cut into small blocks for packaging. This defect was studied by Conochie and Sutherland (68). Under polarized light normal Cheddar cheese sections showed an even distribution of crystals through the body of the cheese, while seamy cheese showed a heavy agglomeration of crystals along the entire length of the seam. Microcrystals located in the "seams" were identified from x-ray diffraction patterns and refractive index as calcium orthophosphate dihydrate ($CaHPO_4 \cdot 2H_2O$). Washing the curd after milling and before salting the milled curd at as low pH as possible reduced the incidence of the above defect (68). Brooker et al. (56) examined microcrystalline inclusions in Cheddar cheese and distinguished two types of

birefringent crystals: irregularly shaped large aggregates of needle-like crystals of calcium lactate, and smaller, more numerous aggregates probably composed of calcium phosphate. The high incidence of spaces between the fat and casein phases of the cheese indicated that they crystalized from pockets of residual whey.

Using light microscopy and renneted whole milk, Mulder et al. (246) demonstrated the formation of a kind of "skin" around the curd granules of Dutch cottage cheese after the curd had been heated to 36C for an hour. When the curd granules were treated in a normal way (stirring at 32C), the formation of a skin was hardly observable. Repina and Krasheninin (297) studied the effect of melting temperature on dispersion of fat in high fat (70% in dry matter) process cheese. The cheese was heated for 20 min. at 70C, 80C, 85C, 90C and 95C then cooled and the fat globule size measure microscopically. The highest and thus optimal degree of fat dispersion accompanied by the lowest proportion of large fat globules (average diameter 5.9 nm and maximum diameter 21.0 nm) was obtained by melting the cheese at 85C. Sommer et al. (335) used differential staining on frozen microtome sections of process cheese emulsified with polyphosphates. The fat globules appeared large and irregular in shape when examined under the light microscope, while cheese emulsified with sodium citrate and tetra-sodium pyrophosphate had small fat globules and a fine structure.

Every cheese variety has its characteristic structural features (7, 56, 68, 78, 87, 97, 118, 124, 139, 156, 169, 172, 173, 174, 178, 191, 192, 193, 197, 202, 246, 293, 298, 359) which reflect the chemical

and biological conditions in the cheese. However, both the external and internal structure of cheese varieties remained largely unexplored until 1958 due to limited methods of examination (280).

Most cheese are composed of both protein and fat, and hence require special preparative procedures for electron microscopy. Methods such as thin sectioning, negative staining and freeze fracturing are invaluable in supporting electron microscopy (174).

Kalab (174) reported that scanning electron microscopy (SEM) is better suited for studies of more advanced stages of cheese making than the initial stages. It is considerably easier to examine the more compact structures produced after the removal of excessive water.

Curd granule junctions reveal the way in which the curd has been treated during cheese making, e.g., Brick, Edam, Mozzarella and Provalone (169). Kalab (172) recently reported work in progress to establish whether there is a relationship between the existence of curd granule junctions in full-fat cheese and the hypothetical "skin" on cottage cheese granules.

The development of structure in Cheddar cheese from adding starter to ripening the cheese was studied by Kimber et al. (190). Electron microscopic examination indicated that in spite of the coalescence of fat in the curd, most fat globules retained their individuality. Frequently intact fat globule membranes persisted in ripened cheese. Starter bacteria were seen entrapped in the casein near the fat-casein interface, which was shown to be the region of highest water content in mature cheese. Extending these studies, Brooker (57) examined the filaments through which starter bacteria of

Streptococcus cremoris were attached to casein micelles in cheese curd. He found that this extracellular material stained with colloidal iron hydroxide, ruthenium red, and periodic acid-thiosemizarbazide-silver proteinate indicating that it was largely composed of an acidic carbohydrate. This carbohydrate facilitates the adhesion of starter bacteria to the cheese-curd matrix and prevents their expulsion from the curd with the whey during the initial stages of syneresis.

The development of the so-called "chicken breast muscle" structure in freshly cheddared curd was studied by Kalab and Emmons (178). A model was designed to explain the loss of sub-microscopic orientation in milled and pressed curd. An important role in this process was attributed to the fat globules. The authors discussed their preference of TEM over SEM as reported by Eino et al. (88). They found it more instructive in showing gradual fusion of casein fibers into a coarser structure. In the development of Cheddar curd microstructure, bundles of casein micelle chains partly fused to each other during scalding became uniformly oriented during cheddaring. Spaces between protein fibers were filled with clusters of fat globules. The elongated inclusions of fat may exist only under conditions of flow while the entire mass of curd is under stress. As soon as these cheddaring forces cease to exist, the molten fat has a tendency to occupy a spherical shape similar to all other liquids. In this way it exercises positive as well as negative pressure on the surrounding protein until the final stage is reached.

Kalab and Emmons (178) reported that in freshly cheddared curd, the macrostructure of stretched granules is reflected at the micro scale by the development of protein fibers which may be observed by both SEM, and TEM. They noted that following the removal of tensile forces, the nature of the fibrous microstructure was rapidly changed as the protein fibers in the matrix contracted, whereas the parallel orientation of the curd granule junctions was preserved. On the basis of light and electron microscopy Kalab and Emmons (178) developed a model designed to explain the development of curd texture and microstructure during cheddaring and cheese ripening. In addition to protein, the authors, revealed the importance of fat as another major structural component of cheese. The exclusive use of scanning electron microscopy for examining cheese microstructure is of limited value. Nevertheless, Eino et al. (87, 88), Stanley and Emmons (340) used SEM alone in studying the effect of calf rennet and rennet substitutes on the microstructure of Cheddar cheese. They used three different techniques to study the microstructure. Freeze drying, modified trypsin etching and modified critical point drying. They also applied the critical point drying method alone. Traditional calf rennet cheese was softer but was more compact during the first six months of ripening than bovine pepsin cheese. SEM micrographs of curd made with rennet showed that aggregated protein materials were fused into large compact masses to form a sponge like structural network. The protein materials in curd produced with both bovine pepsin and procine pepsin formed a fibrous and more open framework which showed incomplete gel formation. They (87) indicated that "the loose

and fibrous structure produced by bovine and procine pepsin was difficult to explain except by differences in the nature and proteolytic specificities of these enzymes compared to chymosin." Ruegg et al. (305) examined the microstructure of curd and rind in Camembert, Emmental, Romadur and Tilsit cheese by SEM. Unlike some other studies, Hall and Creamer (124) used SEM and freeze-fracturing to compare the microstructure of Cheddar, Cheshire and Gouda cheese. Reed (293) suggested that it is fairly easy to distinguish the type of cheese with the freeze-etch technique, which was developed and applied by Peters and Hansen in 1958 (279). However, Hall and Creamer (124) found that a prior study wtih the SEM is necessary to facilitate interpretation. Their (124) cheese specimens were etched with trypsin enzyme in a phosphate buffer at pH 9.0 to remove the surface protein between fat globules, freeze dried at -30C, and vacuum coated with a 20 nm layer of carbon followed by two layers of gold-palladium to provide thermal protection. On the basis of both techniques, Gouda cheese consisted of globular units 10 to 15 nm in diameter. The protein matrix in Cheshire cheese was less uniformly organized and strands and globules 3 - 5 nm in diameter were observed.

The microstructue of curd granule junctions in cheese was studied by Kalab (169). These junctions which appeared as dark veins under transmitted light microscopy, and were found by SEM to contain considerably less fat and more protein than the interior portions. This was evident from the high number of empty cavities in the interior areas in contrast to very few cavities in the curd granule junctions. Consequently, the areas rich in cavities scattered light and appeared

lighter than the compact junctions. Kalab (174) cautioned that differences in the microstructure of the junction and the interior portions of the granules should be taken into consideration when cheeses are subjected to electron microscopical examination. Following fat removal, residues of fat globule membranes, lactic bacteria, and microcrystalline inclusions of calcium phosphate (56) became available for examination under the scanning electron microscope. Correlation between SEM and TEM of such components as well as the protein matrices in Cheddar, Gouda, Mozzarella and Provalone cheeses showed the way in which the two different electron microscopical techniques complement each other (169).

Another correlative study involving the light microscope, TEM and the SEM was reported by Taranto et al. (359). Sequential examination of specifically selected areas on the same specimen block with three microscope systems permitted analysis of morphology and the internal structure of the specimen. Because all milk products and their components are transparent to electrons and because only electron-dense objects may be distinguished, the specimen must be specially treated.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy uses scattered electrons reflected from the illuminated specimen and has become a powerful tool for providing information concerning a specimen's shape and surface topography. It produces a vivid seemingly three-dimensional picture of the specimen's surface over a wide range of magnifications. External

and internal surfaces of the specimen may be studied, and additional accessories make it possible to analyze the elemental composition of the specimen (133). The versatility of SEM becomes apparent when one compares the three most common microscope systems (4). Scanning electron micrographs can be used in conjunction with pictures of the same areas taken with either the light microscope of the TEM (91, 104, 124, 133, 169, 170, 172, 174, 178, 179, 181, 202, 247, 250, 305, 340, 359). Oatley (255) indicated advantages of the SEM over the TEM. The specimen need not be cut into thin sections before examination and relatively large areas ($\sim$1 cm^2) of the specimen can be examined. This allows reliable estimation of morphological variations in a given specimen. Finally, specimen preparation for SEM is simpler and more rapid than for TEM (250).

If the specimen is soft and hydrated (e.g. cheese) it may have to undergo fixation, dehydration, defatting and critical point drying before it is mounted on the stub (172).

Specimen Preparation for Electron Microscopy

In 1979, Kalab (174) stated that to study the microstructure of curd by SEM it is necessary to remove the fat unless a special cold stage attachment is used. One of the advantages of SEM is the simplicity of the preparation of the specimen. Kalab (170, 173) prepared two intensive reviews on the preparation of dairy products for SEM (173, 182). General preparation of biological specimens for SEM consists of fixation, dehydration, mounting on metal stubs with conductive cement such as carbon or gold.

Chemical Fixation

Newbury and Yakowitz (271) summarized the aims of fixation as preservation of specimen structure, minimizing alteration from the original state, protection against disruption during dehydration and preparation for fracturing and exposure to the electron beam. Samples high in fat must also be defatted (174). Fixation should minimize the alteration of chemical reactivity of various substances within the tissue. It is usually of selective rather than general effectiveness in the sense that the objective of the study determines the type of fixation to use. Ideal fixatives for SEM of biological specimens do not differ significantly from those used for TEM (39, 174) except with respect to osmolarity. The ideal osmolarity of fixatives for the former is lower than the recommended for the latter (250). The so called hypertonic fixatives that are used today for TEM are actually either isotonic or hypertonic because it is not the total osmolarity but the vehicle osmolarity that is primarily responsible for the osmotic effect of the fixative (39, 51, 119, 132). The importance of osmolarity in fixatives for non-biological specimens has not been reported. In general, cheese is fixed in a glutaraldehyde solution and post-fixed with OsO_4 to preserve fat globule membranes composed of lipoproteins (247). Among all aldehydes tested, glutaraldehyde has proven to be the most effective for preserving fine structure (150, 307, 308). The successful use of glutaraldehyde in fixing dairy product has been reported (170, 171, 174, 175, 179, 181). Glutaraldehyde not only stabilizes the fine structure and prevents gross distortion during embedding, but it also increases permeability of the

tissue to the embedding media and causes the least protein conformational changes. Kalab (170) reported that although glutaraldehyde preserves the fine structure better than any other known fixative and is capable of preventing breakdown of continuous membrane structure, post-fixation with osmium tetroxide is necessary not only to add contrast and develop membrane sharpness, but also to stabilize the fine structure already maintained by glutaraldehyde (150). It can then withstand embedding in plastic. Glutaraldehyde is incapable alone of rendering lipids insoluble in an organic solvent, but a specimen can be left in this fixative for many hours without apparent deterioration or protein conformational change (307). The rate of penetration of glutaraldehyde is slightly greater than that of Osmium tetroxide. However, the rate of penetration is dependent primarily upon the type of tissue and embient temperature (250). Glutaraldehyde ($C_5H_8O_2$) on reacting with proteins introduces both intra- and inter-molecular crosslinks. Since the molecule consists of a 5-carbon chain with aldehyde groups on both sides it could form two types of crosslinking bridges involving either one or both of the aldehyde groups (150). These aldehyde groups also may play a part in subsequent reactions during fixation, dehydration and in crosslinking polypeptide chains which would result in an increase in molecular size (150). However, the reaction products of glutaraldehyde with protein have been neither isolated nor identified. Sabatini (307, 308) states that available data are sufficient to conclude that bifunctional glutaraldehyde is capable of rapidly crosslinking proteins with which it reacts, especially those with active hydrogen, amino and

imino groups. It is assumed that the aldehyde firmly stabilizes tissue proteins against dehydration (119). Generally pH is the most important factor in obtaining the maximum binding of aldehyde groups to protein. An increase in pH increases the binding capacity of glutaraldehyde (150). However, the optimum pH to obtain maximum binding depends primarily on the type of tissue (308). Kalab and Harwalkar (179) and others (88, 181, 190, 305, 315) reported the best general preservation of ultrastructure at pH 7.0 to 7.6. The phenomenon of crosslinking of proteins is affected by the concentration of the glutaraldehyde solution (150). Studies on curd and cheese by Kimber et al. (190) led to excellent preservation of ultrastructure with 3% glutaraldehyde at pH 7.0 - 7.4 for 4 hours then postfixed at room temperature in 1% osmium tetroxide buffered at pH 7.2 with 0.1 M cacodylate buffer for 30 min at room temperature. There is no general aggreement on the importance of the contribution of glutaraldehyde to the effective osmotic pressure of the fixing solution. The aldehyde concentration can be changed over a wide range with relatively little change in the morphology of the structure (119, 150). Fixation of cheese for studying microstructure have reported concentrations of glutaraldehyde and osmium tetroxide of 0.7 to 3% glutaraldehyde and 1 to 4% osmium tetroxide (1, 87, 88, 97, 118, 156, 162, 170, 171, 172, 174, 175, 178, 181, 190, 191, 202, 247, 279, 280, 305, 340, 359).

Generally glutaraldehyde is supplied as a 25% solution with a slightly acid pH near 4.0 to 5.0. The aldehyde should be stored in the dark under moderately acidic conditions at low temperature. However, as long as the color stays very light yellow, there is not much polymerization or loss of aldehyde groups (119).

Osmium tetroxide or osmic acid acts not only as a fixative but as an electron stain, and this is its major advantage over most other fixatives (296). Reduced osmium imparts a high contrast to the osmophilic structures in the specimen. It is a noncoagulant type of fixative, and is able to stabilize some proteins by transforming them into clear gels without destroying many of their structural features (250). Kalab (168) studied the microstructure of low-methoxy pectin milk gels using thin sectioning of embedded specimens. Improper fixation of the gels resulted in various artifacts, e.g. alcohol maintained the gels in an insoluble form but aggregated and distorted the casein micelles imterspersed in a pectate network. However, the quality of gel preservation obtained through fixation with a mixture of glutaraldehyde and osmium tetroxide has not been surpassed by any other combination of fixatives. The rate of penetration of osmium tetroxide generally increased with an increase in temperature (273) and with an increase in the concentration of osmium tetroxide. However, this relationship was not linear (296). It is advisable therefore, to increase the fixation time by increasing the concentration of osmium tetroxide in the presence of $CaCl_2$. The more osmotically balanced the fixation mixture the less the swelling of the tissue, and the slower the rate of penetration (39).

It is obvious, that the smaller the size of the specimen, the more completely will be the rate of penetration (171). Unlike most polar oxidizing agents, osmium tetroxide is able to penetrate hydrophobic lipid material (296) with a moderate speed so that specimens larger than a millimeter or so in diameter often are not fixed uniformly (3). Kalab (170) recommended 4 hours for the best results.

Mechanical Fixation

For many years freeze drying was considered to be the most useful dehydration technique for a variety of biological specimens (250). However, it is difficult to apply to cheese since cheese loses maisture very slowly during freeze drying compared to other types of biological materials (171). Specimens, fixed in glutaraldehyde and post-fixed in an osmium tetroxide solution are rapidly frozen in liquid Freon 12 and freeze-dried in vacuo at -80C (179). Small particles of the specimen are immersed in the liquid freon at approximately -150C which causes water to freeze into submicroscopic ice crystals. Larger ice crystals develop if the cooling is too slow (175), particularly in the 0C to -40C range. In spite of its low temperature of -193C, liquid nitrogen is not used as a freezing agent because it vaporizes rapidly on contact with warm particles and covers it with a thin insulating layer of gas. This reduces the rate of freezing. Liquid Freon 13 is preferred because it does not vaporize readily. Crystalization of ice may deform and displace the fine structure of the specimen and produce artifacts. Effects of ice crystals on casein micelles in frozen skim milk were shown earlier (175).

Critical Point Drying

A relatively new technique of critical-point drying aboids freezing and prevents the formation of artifacts associated with it (6, 7, 27, 133, 154). It is based on the dehydration of the specimen in organic solvents miscible with water such as alcohol or acetone.

The replacement of these with liquid gases of low critical temperatures and pressures has been suggested (65, 66). Water is not completely miscible with commonly used transition fluids (a fluid that makes the transition from liquid to gas through the critical point) e.g., fluorocarbons and Liquid CO_2 (65, 250). Therefore, a dehydration fluid (ethanol or acetone) miscible with water as well as with the transition fluids is employed. This dehydration is similar to that employed for routine TEM (170). Fixed tissue specimens are dehydrated through a graded series of aqueous ethanol (or acetone) solutions, and terminated with one or two brief immersions in absolute ethanol (or acetone). A typical dehydration sequence has been recommended by Eino et al., (88) for specimens less than 1 mm thick. Specimens are most susceptible to distortion and shrinkage during the transition from 80 - 100% ethanol (or acetone) and seem to shrink $\sim$10% during this phase of dehydration (154). After dehydration the specimen should be defated in chloroform, then transferred to absolute alcohol and critical-point dried in either Freon 13 or liquid CO_2. Kalab (169) reported that the robust protein matrix in cheese was not affected by the chloroform or graded alcohol extraction; in fact fat globule membranes seemed better preserved in the freeze-dried cheese when consecutively extracted. Other methods were reported (88) where cheese specimens were extracted in a Sozhlet apparatus with 50:50 light petroleum ether for 4 hours to remove the fat and then freeze-dried for 2 hours. In my opinion and others (174), cheese specimens should be defated in chloroform after chemical dehydration. Fat extraction is much better when the specimen is dry. Soxhlet extraction

of wet samples, and heat distillation might cause distortion of the microstructure. After chemical dehydration cheese specimens are not completely dry. Water is not completely miscible with Freon 13 or CO_2. Therefore, an intermediate fluid that is miscible with both the dehydration fluid and the transition fluid may perform both the dehydration and intermediate functions (64, 66). In some cases it may be desirable to interpose an intermediate fluid even though the dehydration fluid is completely miscible with the transition fluid (154). The most common intermediate fluids used in the preparation of biological specimens for SEM are Freon TF (F-113) and amyl acetate. Most of the intermediate fluid must be removed before approaching the critical point of the transition fluid, otherwise the intermediate fluid may change the effective critical point (i.e., raise the effective critical temperature and pressure). This is not very critical if Freon TF is used as an intermediate (66). A typical procedure for gradual replacement of ethanol by Freon TF has been described (250). The specimens should be completely soaked in the intermediate fluid, then rapidly introduced into the critical point drying apparatus. It is desirable to use specimen holders to keep the specimens beneath the surface, so that they are not bathed in the less dense dehydration fluid which rises to the surface (66). The time needed for ethanol replacement by amyl acetate is slightly longer than that required for dehydration. The main advantage of Freon TF as an intermediate fluid is that all traces of it need not to be flushed out. The critical point drying process is quite tolerant of residual Freon TF (64). Also, Freon TF is readily miscible with liquid CO_2, Freon 116,

and Freon 13. Freon TF is non-inflammable and available in high purity, but its toxicity remains in doubt. It is volatile and requires caution in order to aboid evaporative drying during specimen transfer (38, 219, 220).

Air drying is the least desirable method used for soft biological specimens for SEM (171). The most common undesirable effects of air drying are excessive flattening, shrinkage, twisting and collapsing of the specimen (173). These distortions are related to surface tension forces associated with air drying. These forces develop as the receding surface of the evaporating water passes through the specimen. Surface tension forces may set up enormous stresses. Forces exerted by surface tension during water evaporation are of the order of 13789.5 Kpa (250). Anderson (7) calculated that the force exerted on a bacterial flagellum can be as high as 4.48 Mpa as the water-air interface passes it. The aim of drying is to leave the solids in their original location (104). At certain temperature and pressure ranges fluids exist as two discrete phases (vapor in equilibrium with liquid) separated by a sharp boundary (the surface of the liquid) (27). At higher temperatures or lower pressures, the rate of evaporation will exceed the rate of condensation. As a result, the liquid boundary will recede until only vapor remains. This receding liquid phase is held together by forces that manifest themselves in the form of surface tension. If the liquid boundary recedes to within the surface of a wettable porous solid these forces tend to pull the surface of the solid along with the receding liquid surface. The

effect of these forces is distortion of the specimen surface (27). If the two-phase system is placed in a sealed container and the container is heated the vapor pressure and therefore the vapor density will be increased (28). Concomitantly, the density of the liquid phase will be decreased (thermal expansion). There comes a point (the critical point) in terms of ambient temperature and pressure above which the liquid phase will become indistinguishable from the vapor phase. The state where these properties of saturated liquid are equal to the properties of a saturated vapor is called the critical state (28). A device which is extremely helpful in visualizing the behavior of a pure substance is the thermodynamic surface. This is simply a three-dimensional diagram on which equilibrium values of pressure, volume, and temperature are plotted on mutually perpendicular coordinates. For a system in equilibrium, these properties must be somewhere on the surface of this diagram. Hence it is often called an equilibrium surface (28, 219, 392). In other words above the critical point the density of the liquid becomes equal to that of the vapor phase, and the phase boundary disappears. At temperatures and pressures beyond this point only a single phase exists, and the surface tension is zero. The pressure and temperature at which the distinction between liquid and vapor disappears are called critical pressure and critical temperature (28). Above the critical temperature and pressure, the pressure can be reduced to about zero (with maintained temperature) without the appearance of surface tension forces (27). The critical point drying method therefore removes the interface, and thus the surface tension forces before the drying of the specimen is

begun (7, 64). Water has a critical temperature of 473C, and a surface tension of 73 x 10^{-4} Kpa at room temperatures. At its critical temperature super-heated water vapor will not only disintegrate the microstructure of the specimen but also the aluminum specimen holder (7). This actually means that solvent substitution is inevitable for successful critical point drying.

Solvent substitution is accomplished by using liquids with suitable critical temperatures and pressures. These liquids are fluorocarbons (freons) (66), Cohen (62) and liquid CO_2 (6, 7). The critical temperature and pressure of liquid CO_2 and 31.0C and 7,446.3 Kpa. However Freon 13 ($CClF_3$) exhibits a considerably lower critical pressure of 3867.5 Kpa and comparable critical temperature of 28.9C. Carbon dioxide and Freon 13 do not seem to damage biological specimens significantly (219) at their critical temperatures.

Both liquid CO_2 and Freon 13 exhibit advantages and disadvantages. They are both miscible with ethanol, acetone, and Freon TF (CCl_2F - $CClF_2$) (also known as Freon 113, and often employed as an intermediate solvent). Drying with Freon 113, allows a lower pressure than that used for drying with CO_2. The highest pressure of the Freon dryer is $\sim$ 5515.8 Kpa (27), while that of CO_2 is $\sim$8273.7 Kpa. After solvent substitution has been completed, drying with Freon 13 or CO_2 requires $\sim$ 15 min (272). The drying process with Freon 13 should not be applied rapidly. Best results are achieved in 45 min. However, CO_2 has some advantages in that it is less expensive than Freon 13 and is readily available. Hayat (133) reported that at relatively low resolutions there seems to be no perceptible difference between drying from

liquid CO_2 and Freon 13 with respect to their effects on the specimen. However, there is no available information about their effects on the ultrastructure of dairy products in general.

Recently a rapid method for chemical dehydration of biological specimens for thin-section electron microscopy was introduced (248). It is based on the use of slightly acidified 2,2 dimethoxypropane (2,2 - DMP), which is instantly hydrolyzed into methanol and acetone on contact with water. The method was initially designed for the dehydration of single cell algae and plant and animal tissues to be embedded in resin prior to sectioning by an ultramicrotome. Kalab (171) reported that this dehydration method should be equally suitable for SEM of some dairy products. Chemically dehydrated specimens can be critical-point dried in the same way as those dehydrated in organic solvents.

Fracturing, Mounting and Coating

Specimens should be mounted as soon as possible, particularly small fragile specimens such as process cheese, smears and cultures. As soon as is convenient small specimens should be placed either on a substrate which can subsequently be mounted on a stub, or directly on a stub. Once a specimen has been mounted it is less susceptible to damage and contamination and is easier to handle. Kalab (171) described a very suitable mounting procedure for dry specimens of the dairy products in which aluminum stubs were cleaned with a fine carborandum paper and immediately coated with a layer of conductive silver cement. Dry specimens of dairy products under study were

fragmented into particles less than 0.5 mm. He (171) cautioned that improper handling of the samples resulted in the production of artifacts. The dry cheese specimens were fragmented before mounting on metal stubs for SEM. Otherwise, the surface affected by the fixation and dehydration procedures would be scanned instead of the true internal structure. The same results were reported by Eino et al. (87, 88) and others (147, 148). Proper orientation of the fragmented specimen on the metal stub makes it possible to inspect either the cavities or terminals of the broken protein fibers. Fragmentation perpendicular to the fibers exposed only the cavities which were initially filled with fat (171). Kalab (171) showed that a different picture arose when a smooth surface was obtained by cutting a specimen wet or dry with a blade or scalpel. Damage to the microstructure was evident. Touching the surfaces destined for SEM with a needle or a pair of tweezers produced another type of artifact. Although a pair of tweezers and a needle or a blade are usually needed to fracture the dry specimen (111). Kalab (171) advised that the fragments should be mounted with their touched surface placed facing down on the stub. It is not advisable to scan speciments under the SEM too close to their edges painted with conductive cement even though such areas are usually free from "charging" artifacts. The conductive coat may sometimes be mistaken for the diary product under study (171). There is even another danger that the solvent from the cement may have penetrated the border layer and collapsed the adhering protein network. The latter artifacts may not be recognized in spite of examining a large number of specimens if an improper mounting

technique has been used or a small amount of thin cement was applied to all the specimens (169, 171).

Generally, specimens are attached to the stub with the aid of an adhesive prior to coating. Dried cheese specimens are usually exceedingly light and brittle, and the utmost care should be used while mounting them on the stub. Normally the specimen should be coated after adhesion to the stub, then any vapor present is lost in the vacuum coating unit.

The objective of coating is to prevent or appreciably reduce the build-up of electric charge on the specimen surface. The cheese specimen when dry is a very poor electrical conductor (124). Therefore, the conductivity should be strong enough that the net specimen current (primary current minus secondary current) can be carried away with very small potential differences between the top and bottom of the specimen. If this is not accomplished image distortion will occur (249, 271). Other advantages of metallic coating are an increase in the emission of low-energy secondary electrons which are responsible for contrast in the image, and reduction in specimen damage caused by the electron beam. Because elements of low atomic number have poor stopping power for the primary electron beam, and thus are inefficient in the production of secondary electrons, deposition of a heavy metal filter alleviates this problem (250, 253). An ideal coating consists of a uniform film of conductive material on the surface of the specimen so that the surface of the film is almost an exact replica of the underlying surface. To accomplish this the entire surface of the specimen is exposed to the source of conductive material for the same

duration. A reasonably flat specimen presents no problem when it is rotated about its own axis in one place at an appropriate angle to the source. Irregular surfaced specimens such as fractured cheese on the other hand present a problem (87, 88). It is difficult to be certain that the deposited film is uniform. This type of specimen requires rotation about multiple axes, but even this may not provide a completely uniform coating. Coating with carbon (50 Å) followed by a 200 or 500 Å coating with gold or gold-palladium by vacuum evaporation was preferred because a better contrast was obtained under the SEM (87, 88, 97, 156, 170, 171, 175, 178, 179, 181, 340, 359). An ideal coating procedure for a given specimen has to be determined by trial and error. In our study, dry process cheese specimen were coated with carbon and three times with gold-palladium. A 15 cm length of 0.8 mm diameter gold palladium wire (60:40) (by weight) was wound evenly on a tungsten filament for each coating. The rotary specimen table was situated 10 cm from the gold-palladium source and inclined at an angle of 30°. For certain complex specimens such as dry cheese it may be desirable to coat the specimen first with carbon (not more than 20 nm thick) and then by gold palladium (182). Carbon atoms have the advantage of bouncing off the sides of a vacuum chamber and approaching the specimen from all sides. Thus, minute spaces under surfaces of the specimen are coated more completely. Carbon coating produces better coverage of edges and crannies than metal coating alone. In addition, a carbon film is tough, stabilizes the specimen and provides a clean smooth substrate to which a subsequent metal coat firmly adheres (145). Furthermore, carbon is easy to evaporate and

causes negligible specimen heating. In general, application of carbon before metal coating is desirable for critical-point dried cheese specimens (169). Films of gold-palladium alloy are more uniform and show less graininess than pure gold. However, this alloy must be evaporated at relatively high temperatures. This requirement is apparently not desirable for coating frozen specimens. It also tends to alloy with the tungsten heater filament. When the evaporating metal alloys with the tungsten filament it becomes extremely brittle and needs to be replaced after each coating cycle (250).

In 1976 Eino et al. (87, 88) reported that little information had been published on techniques for examining the microstructure of cheese. This may be because of serious technical difficulties in preparing cheese specimens for examination in high resolution instruments (i.e., SEM and TEM). Cheese specimens have to be prepared with extra care to establish conditions which produce the least damage to structure. It has been common practice to fragment any dried specimens before mounting on SEM stubs (111). In this way the true microstructure is observed rather than the surfaces which may have been contaminated during the preparative steps.

Most cheese reveals two kinds of structures (169) 1) the macrostructure arising from the way in which curd granules are fused, e.g., the fusion in cheese such as Brick, Edam, Gouda etc. or from the stretching of fused curd granules by various processes such as in Cheddar, Mozzarella or Provalone (169, 359), and 2) the microstructure of the protein matrix inside the curd granlues. Taranto (359) and Kalab (172) reported that these differences can be observed in fixed, dehydrated

and defated cheese sections under the light microscope at a very low magnification. Different patterns were observed with various cheese (169).

Rheology of Cheese

There has been an increasing trend in recent years to use the word rheology to include that group of physical characteristics of foods that describe the physical properties sensed in the mouth during mastication (46). Rheology was originally defined by Bingham (33) as the study of the deformation and flow of metter. This definition has remained essentially unchanged to the present time. A recent statement by two long-time workers in this field defined rheology as "the study of deformation of materials, including flow" (295). Some workers are attempting to broaden this definition. One such attempt is to define rheology as the reaction of materials to stress. A definition restricted to foods was given by White (420) as the study of the deformation and flow of materials, intermediate products, and final products of the food industry. In each of these definitions the key words are deformation and flow.

There is no question that rheological properties of foods are important to the food technologist. For example, they describe the manner in which an article of food deforms when squeezed in the hand or when subjected to pressure at the bottom of a filled box or bin, and the manner in which a fluid flows over the plate or behaves when pumped through a pipe. They constitute part of the initial sensation during the first bite when it deforms under the forces applied by the teeth or tongue.

Mastication is a process in which lumps of food are ground into a fine state, mixed with saliva and converted to a liquid slurry at approximately body temperature ready for swallowing. The process of size reduction, wetting with saliva, melting, release of moisture or fat are not rheological processes. The perception of moistness, size, shape, and roughness of food particles are important factors in texture sensation that are not rheological and yet are important aspects of the acceptability of a food. Rheological measurements constitute an important part of food texture measurements, but they do not cover all the factors that make up the texture of a food. Food texture measurement lies partly within and partly outside the field of conventional rheology. When rheology was emerging as a field of science, Bingham (33) stated: "In spite of all early advantages, the flow of matter is still not understood and since it is mysterious like electricity, it does not attract the attention of the curious. The properties are ill defined and are imperfectly measured if at all. They are in no way organized into a systematic body of knowledge which can be called a science. The old description is no longer applicable to modern rheology but it accurately describes the state of food texture measurement several years ago. It is hoped that food texture will reach the stage where its properties are no longer "ill defined and imperfectly measured if at all." The attitude that food texture measurement lies entirely within the realm of rheology will only delay its emergence as a bonafide scientific discipline.

Among the limitations in the application of rheology to food texture measurement is the problem of calibration (47). Rheology instruments are calibrated in units that eventually are based upon the fundamental, exactly defined, and completely reproducible units of mass, time and distance (e.g., force, poise). Rheological instruments used for food can be calibrated in fundamental scientific units and this is usually done (47). However, the ultimate calibration of the instruments must be against the human mouth, which poses many problems. There is also an enormous range of variability in rheological properties among foods. Foods exhibit rheological properties that range from an ideal Hookean solid all the way to an ideal Newtonian fluid with almost every combination of plasticity, elasticity, recoverability and time effect. Unfortunately, few foods are ideal Hookean or Newtonian systems and the majority are very complex (420).

Several authors have reviewed the rheology of foods (131, 269, 321, 322, 331, 420). Most of their publications have stressed rheological principles and rheometric measurements and were aimed at food scientists, but were usually influenced by the specific interest and experience of the author. Thus, Scott Blair (321, 322) treated in depth dairy products, especially cheese. Rheology includes very important attributes of food that affect consumer acceptance, and in some products may be more important than flavor (351, 356). Good textural quality is associated with excellence in cooking (355). Much of the current rheological work on food products deals directly or indirectly with generating an understanding of food texture and with methods of measuring it. Such methods fall into the categories of

fundamental rheological or emperical and imitative tests. The latter imitate conditions to which the food is subjected during consumption (353).

With solid-foods, it is an open question whether the sensed parameter is a reflection of the rate of force application for a constant deformation rate, or a rate of deformation for a constant force application (396). It may be that the situation shifts depending on the degree of a product's firmness.

In addition to a poor understanding of forces acting on the food during sensory evaluation, there are two other paramount problems in this area. One is concerned with relating the fundamental rheological concepts (e.g., elasticity, relaxation time, etc.) to sensory texture terms (e.g., firmness, gumminess, chewiness), and the other with the question of whether a rheological approach can provide a full description of food texture (274, 396). It should be emphasized that most fundamental rheological measurements are made at low stresses and strains, and for all practical purposes are non destructive. In the mouth, however, foods are subjected to high stresses and strains and the evaluation process is highly destructive to the test material. Szczesniak (353), reported that rheological problems in the food industry are challenging and exciting because they deal with essential consumer products. They have practical and social connotations, and because so much needs doing, they offer much opportunity for creative and satisfactory work.

Sensory Evaluation

Measurement of food texture plays a significant role in the control of manufacturing processes and in the evaluation of the quality of finished products. Many parameters must be measured with precision. There has been increasing interest during the past decade in developing sensory and objective techniques for evaluating quality in relation to consumer reaction. Sensory analysis is evolving into a sophisticated measuring tool as indicated by recent work (52, 328, 353, 354, 357). The technique, however, is time consuming and requires the use of considerable amounts of trained labor. Thus, in recent years interest has accelerated toward objective measurements of textural characteristics to provide efficient and precise quantitative descriptions.

Texture Measurement

The kinesthetic characteristics of foods are related to the physical properties sensed by the eyes before eating (except color); the sense of touch in handling the food, and the feel in the mouth during consumption. Thus, the consumer is aware of factors such as size and shape, particle size, moisture content, fat content structure and mechanical properties. These properties are interrelated and together create the complex food quality termed texture. Because food texture is composed of so many variables, it is not possible to obtain an overall index in a single measurement. In general, only those properties which have the greatest influence on consumer acceptance

are measured (359). Voisey (394) reviewed the recent advances in the instrumentation used to measure these properties and pointed out the advantages of employing more sophisticated apparatus. Bourne (42) proposed a classification of objective methods into force, distance, and energy measuring instruments and there have been several recent reviews of instruments in use (107, 348, 350).

There are common problems in objective texture measurements which can be solved with readily available equipment. The mechanical properties of food encompass the reaction of the material to applied forces. Most materials, and especially foods, are neither entirely elastic or truly viscous, but possess rheological properties associated with both states of matter. Hence, they may be referred to as viscoelastic materials (42, 107, 242, 310). Both time-dependent and time-independent measurements are required (394). The increasing interest of engineers in the field of texture measurement and physical properties of biological materials related to agriculture has been reviewed by Mohsenin et al. (238, 239, 240). This has led to intensive studies of the mechanical and rheological aspects of foods, but the relation between these measurements and consumer reaction is still not fully understood.

Measurements of texture in terms of elastic and viscoelastic parameters require the product to be deformed in some arbitrarily selected but defined manner. The force and time required to produce a given deformation or flow are recorded (110, 218, 394).

Standard Units of Measurement

In measuring the force and deformation of test samples, the data must be expressed in a rational set of units. This is obvious for either fundamental or emperical tests (33). Units used in testing engineering and other materials are therefore being adopted for food texture studies. This can, however, lead to errors. When a material is subjected to a tensile, compression or shearing force F, its original length L will deform by an amount ΔL. The force will distirbute over an area A, depending on the homogeneity of the material and the uniformity of contact between the material and the loading surfaces. The result can be recorded in two ways: (a) force and deformation and (b) stress and strain. If either of these methods are used, it is implied that all the dimensions of the specimen are known throughout the test. Obviously, the area supporting the load will influence the results, and if materials subjected to stress change dimensions either along the axis of the applied force or along the axis perpendicular to the applied force a problem is created. This is the "Poisson effect" which has been demonstrated in several biological materials (61). A technique has been developed for measuring this parameter in biological materials (126). Segars et al. (311) described a device for measuring the transverse deformation of cylindrical samples during axial compression. The device was fitted to an Instron Universal Testing Instrument and required only standard Instron electronics and data recording systems. The transverse deflection gave a good estimate of Poisson's ratio and was valid for

both isotropic anc anisotropic materials. Correlation coefficients for a magnitude estimate of three sensory texture attributes in a military "beef-roll" increased from 0.5 to 0.9 when Poisson's ratio was substituted for the uniaxial "modulus of elasticity." Poisson's ratio was defined as the ratio of transverse strain to axial strain (310, 311). Theoretically, it is restricted to ideal deformations that lie within the elastic limits of a material. In practice, it is applied to deformations which approximate ideal conditions, i.e. small, not always totally elastic, and nearly constant deformation throughout the sample. In this study (311) the deformation obtained even with an invented sample holder did not approximate ideal conditions. Barreling was evident as soon as compression began. Measurement of various foam rubbers showed that volume reduction of nearly 50% occurred before a significant transverse deformation was observed. This indicated a Poisson's ratio near zero over this range of compression (126). The other extremes occur when isotropic materials, and probably others, become incompressible (i.e. bulk modulus equals infinity). Standard uniaxial compression parameters derived from tests made at a compression rate of 2 cm/min with a maximum compression of 20% failed to characterize adequately the mechanical texture properties of beef (309). These dimentional changes are difficult to measure and the Poisson effect is not often considered in testing foods (394).

If only force and deformation are recorded, the samples tested must have identical dimensions to make meaningful comparisons (61).

If the relationship between force and deformation is linear during loading, the ratio of force to deformation (F/D) or stiffness can be used as an index of product properties. The maximum force provides an index of breaking strength which is related to cohesiveness (derives from the strength of internal bonds holding the body of substance together (270, 394).

The fundamental concepts involved in rheological studies of food-stuffs have been surveyed recently in some detail by Mohsenin (239) and Voisey (394). If the area A over which the force is distributed is known, then the data can be recorded as stress and strain where stress is the force per unit area (F/A), and strain is the change in length per unit length of $\Delta L/L$. However, this implies that the stress and the strain are uniformly distributed through out the sample. This can only be true if the material is perfectly homogeneous which is most unlikely in biological materials. Also A is varying by an unknown amount as the specimen is loaded. For example, considerable wasting occurs in a tensile test in the region where the material breaks and the area A is reduced to a when fracture occurs. Thus F/a should be used to precisely express failure stress. It is becoming customary to use elastic moduli to quantify the properties of food. A review of these conditions was published by Breene (54). These moduli are the ratio of stress to strain (tensile, compressive or shear). This concept was developed to describe the behavior of metals and uses the basic assumption that stress is directly proportional to strain and that all the deformation is recoverable upon the removal of force. Since most foods are viscoelastic, this behavior is seldom exhibited

and the term modified elastic constant has been adopted to reflect this deviation from the ideal state (394).

Theoretical stress analysis is a highly developed subject and several workers (106, 376) have attempted to adopt it to food analysis. There is, however, great danger in adopting the simple classical elastic stress theory to test biological materials because such analyses are based on two assumptions: (a) the material is homogeneous and (b) the deformations are small. These two assumptions do not usually apply to food. Sherman (328) has pointed out that "classical elastic theory deals with very small strains so that when considering the much larger strains involved in mastication the accepted definition of strain may not be valid."

Compressive deformation of foods has been one of the main measurements in many texture evaluations. The force-deformation relationship in such tests provide objective parameters that might be correlated with sensory parameters (349). The conditions under which the tests have been performed are very diverse with regard to the geometry of the systems, the deformation level and the deformation rate (54).

The level of deformation reached in food tests is quite large. This has two main outcomes that frequently have been pointed out (241, 328, 395): (a) the true strain is different from the apparent strain because of the decreasing length of the specimen, (b) the strain rate is increasing progressively with the advance of deformation. Peleg (276) discussed the effect of these factors and their implication in deformation tests. He demonstrated that the true rheological properties are not and cannot be fully revealed by a single curve of the stress-

strain relationship. The reason was the difficulty in separating the factors that regulate the stress level, i.e. the characteristic rheological properties and the conditional response to the strain history. Since the true strain increases progressively with the strain (and in most real materials this process is accompanied by a cross-sectional area expansion), the apparent stress-strain curve will almost appear as concave. Only when internal fractures overwhelming outweigh these effects would the curve be convex. He indicated that a better insight into the deformation pattern can be obtained from a three-dimensional representation of the true stress-true strain-true strain rate relationship. Even in such a case, some difficulties will still remain. The stress level is determined by the combined contribution of various physical mechanisms. In rheological model terminology these are represented as elastic and Maxwellian components. Even if the latter responses were independent of each other, there would be no way to separate their contributions from a single curve, not to mention identifying the specific elements. Also, tests that include several stress-strain curves obtained at various deformation rates also suffer from the same problem because as long as the specific elements contributions are not identified the rate effect cannot be calculated. Furthermore, if the internal fracture mechanism was also involved in the deformation pattern, the ratio between the elastic and Maxwellian components will also depend on the strain and the strain rate history. This will obviously complicate meaningful interpretation of stress-strain curves to a much further extent (276). In general the situation may be cleared, at

least partly, if relaxation data are added to the stress-strain relationship. Shama and Sherman (355) suggested that stress-relaxation precesses in cheese may involve intermolecular Van der Waals' attraction forces between protein chains in the three dimensional network. However, it can offer a general indication about the true rheological nature of a material, and about gross structural changes that may have occurred due to deformation. Obviously, the relaxation tests also can be repeated after various straining conditions, thus revealing whether the material has rheological memory and to what extent its rheological characteristics are influenced by its past deformation history (277).

Two approaches can be used for the selection of specimens: (a) make all the specimens the same size and report either force and deformation or stress and strain, (b) use specimens of a defined shape (but not necessarily the same size). Measure the size of each sample and report stress and strain.

The method selected depends primarily on practical considerations in preparing samples of each specific product (394). If, however, the distribution of stress throughout the sample is not known, as for example in the General Foods Texturometer, only force and deformation can be reported.

Texture Measuring Instruments

A variety of instruments have been developed for measuring food texture and several have been adopted. Examples such as the Kramer shear press (208) and most recently the General Foods Texturometer (114)

have appeared extensively in the literature. However, all these systems have common concepts and requirements. Samples of food are subjected to compression, tension, or shear (in combination or as a single operation) and force deformation. Therefore, a texture measuring system consists of five components: (a) a mechanical device for deforming the sample (b) a means of recording the force (c) a means of recording the deformation (d) a method of recording the time during deformation, and (e) a test cell to hold the sample. The first four components are readily available since these items have been developed for other purposes. The test cell should be considered as the most critical component of the system. The cell may be a single or a multiblade shearing device such as is used in the Kramer shear press, a pair of grips to apply tension, flat surfaces to apply compression, a probe to puncture the sample, or a container and paddle used in a viscometer. The design of the cell determines the manner in which the forces are applied, how they are distributed throughout the specimen and which type (tensile, compressive or shearing) predominates in the resulting reaction. A vast array of equipment has become available that can be adopted to measuring food texture provided an appropriate test cell can be produced and the proper loading of the sample is available. The development of such a cell has been described by Voisey (400).

Deformation mechanisms. There are three basic design philosophies which can be considered in designing mechanical systems to deform foods during texture tests (240, 394): (a) a universal machine that will handle a majority of tests; (b) a general purpose machine that

will accomodate a specific range of test cells; (c) a special purpose machine that will test only one sample type in a specific manner. There are advantages and disadvantages to each, but there are common requirements for all.

The speed at which the test material is deformed must be considered as critical because foods are viscoelastic and their reaction to force is time dependent. Also, the speed of deformation governs the rate of force application which must not exceed the response rate of the recorder (238, 240, 274, 394, 396). Shama and Sherman (326) stated that the load capacity of the mechanism must be adequate to handle the resistance forces generated in the deformed samples. Control of deformation speed is affected if this is not the case. Universal testing machines offer a wide range of test conditions with particular reference to the magnitude of the applied load and the rate at which it is applied. Both of these variables will influence the instrumental evaluation of texture properties (107, 396). A perusal of the published literature indicates that this important fact has been not fully appreciated (396). Furthermore, even if the correct test conditions are fortuitously selected for one food it does not follow that the same test conditions can be applied to another food having different properties. Dental researchers have shown quite clearly that the same mechanical force is not used during mastication of foods with different textural properties (396). For example, larger forces are required to chew hard foods than to chew soft foods. The rate at which force is applied in the mouth depends on the nature of the food. The teeth move together more slowly for hard foods than

for soft foods. This suggests that when an objective comparison is being made between foods with different textural properties, or even between different grades of the same food, different instrumental test conditions may be necessary. Studies with gouda and white stilton cheese illustrate the importance of this point (396). Members of a texture profile panel always rated gouda as being harder than white stilton. However, a large number of force-distance curves were derived for the two cheeses at various loading rates, and from the collective data a three dimensional plot was prepared of force, compression and rate of loading values. The curve for white stilton was alsays higher than that for gouda cheese at a crosshead speed of 5 cm min^{-1} irrespective of the percent compression that was selected. However, at a crosshead speed of 20 cm min^{-1} the two curves intersected at two points on the three dimensional space so that within the range of 38 - 62% compression the curve for gouda was always higher than that for white stilton. Voisey (395) reported that if high deformation rates (e.g. 150 cm min^{-1}) are needed to test foods, instrument complexity will be increased due to the need for controlling deformation and recording reactions at these speeds. Recent emphasis on the importance of texture test conditions points to the desirability of using much higher deformation rates (45) that will require more sophisticated recording instrumentation than is presently available in many laboratories. The use of slow deformation speeds to predict oral reaction should be viewed with caution. However, considerable research will be required to establish the optimum instrumental test speeds to maximize correlation with consumer reaction. This is becoming an

increasingly important objective of texture testing. Texture test instruments must be designed with this in mind so that proper simulative test conditions can be achieved (395). The most reliable and simple method of achieving constant deformation speeds is to use a synchronous electric motor to drive a screw carrying the deforming member of the test cell.

Universal testing machines. A universal testing machine is a most useful apparatus because its basic mechanism can be employed to conduct most texture tests that have been reported in the literature (48, 107, 240). All that is required is to manufacture the attachments for holding the specimen or for mounting the test cells. Zachringer (423) illustrated special attachments for testing apple slices. Universal testing machines can be used for compression, tension and shear and can be employed with special test cells (394).

There are commercially available universal testing machines that have been developed for different industries such as paper, textile, plastic and steel (394). They range from simple hand powered units with a spring scale to complex fully automatic test systems. In general, the forces required in texture tests depend on the type and size of the sample as well as the nature of the test cell (48).

Machines for food measurements are usually restricted to those developed for products such as the textiles, paper and similar products (48). The choice should be made on the basis of operating flexibility i.e. the range and number of compression speeds and sensitivities of the force recording system (270).

One series of machines that has become popular for texture tests is manufactured by Instron Ltd. (Canton, Massachusetts). These machines have been used for testing a number of biological materials, e.g., egg shells (155). Following the pioneering work of Bourne et al., (48) the Instron Universal testing machine has gained widespread use for the evaluation of solid foods (48, 107, 396). This is a screw drive machine and its principle components were described by Hindman and Burr (144). A motor drives two vertical screws via a series of gears to move the crosshead up and down. The length of the down and up strokes and reversal of motion (i.e., deformation) are automatically controlled at preselected positions by switches which are adjusted by calibrated dials. The up and down speeds can be selected independently from an extremely wide range (e.g., 0.2 - 2.4 cm min^{-1}) (326) by changing gears in the drive mechanism. A load cell is mounted on the frame of the machine above or below the crosshead to detect the force applied to the test specimen. There is a wide range of load cells available having maximum capacities upwards from 5 N (217) in tension or compression. In addition, the sensitivity of each load cell can be adjusted within its capacity by electronic controls.

Force is recorded on a potentiometric type strip-chart recorder. The chart is driven by an independent motor at pre-selected speeds ranging from 5 - 500 mm/min (217) so that different crosshead chart speed ratios can be used to record force-time curves. However, the lower range normally is used (326). Optional plug-in attachments allow the automatic control or cycling of the crosshead according to either the force on the specimen or its deformation (i.e., extension).

Provision also is made to automatically start, stop and reverse the recorder chart in synchronization with the crosshead so that either force-deformation or force-time curves can be plotted. The recording system may be built into the loading frame or into a separate chassis. If desired, other load cells built into different test machines can be conveniently connected to utilize the same recording system.

The most suitable machines for texture tests from the available series are the table models with 250 Kg maximum capacity. The 500 Kg capacities come in ranges and provide alternative degrees of operating flexibility. Manufacturers supply these machines complete with load cells and force recording systems, and have a range of test accessories that can be added to the basic machine (360).

While the Instron and other universal testing machines are intended for up and down linear motion they can be adapted to perform a levered motion as in the General Food Texturometer. This is not the most convenient or economic method to accomplish these tests. Instron Ltd., supplies a torsion testing attachment for one of the machines, and points out the operational flexability (394).

The Instron is discussed here because it has been widely adopted for testing foods following the pioneering work of Bourne et al., (48). However, other similar units are available and have been used by different researchers. Another series of machines that has become competitive to Instron for texture tests is the MTS tensile testing machine (J.J. Lloyd Instruments Ltd., Southhampton, England), which have the same capabilities and operate in the same principle as Instron.

Curtis and Hendrick (71) described several inexpensive machines that are available for testing at loads up to 5 Kg (e.g., Model VTM-11 Imass Inc., Accord, Massachusetts 02018). Machines that are simpler or more sophisticated than Instron and that have different features also are available (394). The choice may depend on economy, availability of service, range of available accessories and so on. Considerable economies can be achieved in some cases by combining components from different manufacturers. The load cells and electronic recording apparatus may be purchased separately. This depends greatly on the technical support available to the researcher to modify and assemble the equipmetn (240). The table models of MTS or Instron systems are primarily tensile testing machines i.e. force is applied to the drive screws in the direction of crosshead pulling downwards. This is because the drive screws are spring loaded against the upper thrust bearings. If the crosshead is used to pull upward the screws can then move axially. This introduces an error in determining the deformation of the specimen (238, 394). Thus, tensile testing must be done above the crosshead pulling against a load cell mounted at the top of the frame. Compression testing, particularly at high loads, should be done in the same position using a cage attachment to convert the tensile pull to compression. In general, compression tests can be accomplished below the crosshead against a load cell mounted on the machine base if the loads are small (394). Testing in this manner places the long drive screws in compression, and under these conditions the strength of the screws is reduced. Also, at high loads e.g. in using Kramer Shear cell accurate force deformation

records cannot be obtained for both entry and withdrawal of the blades because the screws may move axially as the force reverses. The position of the blades cannot be determined accurately at this point. This may not be important in practice, but the technique might be used to estimate adhesive properties of foods (393, 406). The problem does not arise when the test forces are small such as in testing gels under tension (140). Voisey (394) reported that the limiting force when the crosshead is pulling upward can easily be measured with an Instron load cell to record the force required to overcome the springs at the top of the screws. The springs are adjustable, but care should be taken that the machine is not damaged.

There is also the problem of making fixtures to attach to the machine (394). Access to a machine shop solves this problem and various researchers have developed many attachments and fixtures (164).

The selection of a universal testing machine is primarily dictated by economic consideration or by having access to a machine for other purposes. For example Bourne et al., (48) and Bourne and Monday (49), used an Instron floor model with a capacity of 1000 Kg which is more expensive than a table model (e.g. 2500 Kg $11,000 or 1000 Kg $16,000 in 1971). As previously noted, a maximum capacity of 250 Kg is probably sufficient for testing most foods (394). The Kramer Shear Press has a capacity of 2500 Kg which is seldom required except when testing materials such as rehydrated dried beans which produce forces of 750 - 1250 Kg (Food Technology Corp., Reston, Virginia 22070). Forces up to 500 Kg are required for testing meat and 1500 Kg for peanuts (358). Thus, before making the selection it must be decided

which test cells, sample size, and type of products are to be tested. The load capacity can then be estimated from the published data. The lower the capacity, the lower the cost. However, if the budget allows it is wise to purchase a greater load capacity to handle future unforseen tests (394).

Components for modernization of texture instruments. Several different principles are used to convert the applied force to an electric signal: the piezioelectric effect of certain crystals, photoelectric detectors, variable reluctance and resistance systems. An excellent review of these devices and sources of supply was prepared by Minnar (237). Strain guages are currently the most popular elements used to construct force transducers and it is recommended that they be used for texture measurements. They are reliable and available in many shapes, load capacities and prices. The electronic equipment for their operation is supplied by numerous manufacturers. These transducers may be designed to measure tension, compression or bulk.

Deformation of the specimen can be automatically recorded by driving the recorder chart from the deformation mechanism. This is used in the Kramer Shear Press, the MTS and Instron (Models T5002 and 1130). However, it may not be satisfactory for many applications because the length of the chart used during each test is fixed by the gear ratio between the deformation mechanism and the recorder. This can be changed, but the flexibility of operation is restricted (237). It is often desired to expand or contract the deformation scale on the chart during testing to accentuate specific features on the force-deformation curve for easier or more accurate observation.

Driving the chart from the deformation mechanism is a definite advantage since the recorder deformation is directly proportional to the movement of the deformation mechanism, and is independent of the deformation speed (394).

The most common method of recording deformation is the relationship between deformation and recorder chart speed. Providing this is constant, the recorder chart can be calibrated in both time and deformation by using s conversion factor (237). This has been demonstrated with an Instron by Bourne (43), who used this approach to develop simple models to describe the rheological behavior of several types of food (56). The principle of the method is that the higher the chart speed the greater the resolution of the deformation measurement. Accuracy depends on how accurately the chart and the deformation speeds can be measured with a ruler and stop watch.

There is another problem with this technique caused by force transducers which must deflect in order to measure load. In testing foods where sample deformations are large, these errors may be insignificant but this cannot be assumed (44). Deformation is generally linearly related to the applied force and the errors involved can be calculated and corrected. Voisey and Hunt (402, 403) demonstrated this in testing the strength of egg shells. One method of reducing the error is to use a force transducer with a higher capacity than the maximum forces to be recorded, and to use electronic amplification to increase the sensitivity (394). The transducer structure must be stiffer at higher capacity and, therefore deflects less at a given load.

To record deformation precisely, particularly at small deflections (< 0.02 cm), a separate transducer can be used to record deformation vs time (or force) directly. This implies either a two channel or an X - Y recorder to record both force and deformation. This obviously increases costs (62). Deformation transducers are available in many types. One of the simplest types is a variable resistance or potentiometer. Linear potentiometers are also available and have been used by various workers (292). One of the most popular types is the differential transformer (62, 63). These devices are generally called linear velocity displacement transducers (LVDT) (196, 377).

Time can be recorded by standard laboratory clocks and stop watches operated manually or automatically by electric switches on the deformation mechanism at selected points in the test (394). The conventional method is to rely on the speed of the recorder chart which is driven at a constant speed by a synchronous electric motor. It is, however, recommended that the chart speed be checked with a stop watch to ensure that the correct gears, etc., have been installed in the drive mechanism (394).

Modern electronic force deformation recording systems are capable of giving accurate and reproducible data only if accurate calibration methods have been used. The cost of the electronic instrumentation required to do this has decreased rapidly to the point where the major cost is in the deformation mechanism (394).

The terminology used by manufacturers to specify the performance of different instruments can be confusing (243). It is recommended

that this be clearly understood before selecting a particular instrument. Glossaries of instrument terms are available from various sources (8). Many instruments for texture measurement have been recently redesigned or new ones constructed to take advantage of the above described modern components. An early example was the addition of a force transducer and a recording system to the Kramer Shear Press by Decker et al., (79). The following examples are given to indicate the way transducers and recording systems are used to improve accuracy and flexibility of texture tests. They should also serve to indicate how the existing apparatus can be modified to take advantage of the developments in the field of electronics.

Puncture test. The puncture test was one of the first measurements used to detect textural characteristics of food (421). Early instruments were manually operated and used the deflection of a spring to indicate the force applied to the probe. There are several variations in available designs (238, 372, 373). The puncture test has the advantage that a specimen can be tested with a minimum of preparation and that under certain conditions physical constants of the test material can be derived from the data. For example, the relationship between punch area and force has been described by De Man (80) in a simple relationship: $F = K \cdot A$, where F = force, K = coefficient, and A = punch area. Bourne (42) reported that the nature of the coefficient K is related to the rheological properties of the material. In products such as butter and margarine it is suggested that the deformation does not involve compression and shear, but flow (80). However, De Man's experiments indicate that flow patterns

exist in the area under and near the punch. Therefore, the coefficient K is a coefficient of flow K_f. The relationship between punch perimeter at constant area and force was a straight line for process cheese. The same results were reported by Bourne (42). Both authors reported that penetration tests involve three possible factors viz. shear, compression and flow. Which of these will apply depends on the physical structure of the food under study. In foods with cellular structure, e.g. fruits, shear and compression may be the major factors. Foods such as process cheese contain a strongly bound continuous structure in which the main factors are shear and flow. In products with weakly bound network structures such as plastic fats, flow is the only major factor involved. The usefulness of this simple instrument is indicated by its application in extensive work with different varieties of food (394). Kalab (181) reported the application of a penetrometer in measuring the firmness of induced milk gels.

Deformation and creep testing. It is often more convenient to record deformation only, particularly when using a constant test force. For example, in an elastic material where the force is proportional to deformation, elasticity or stiffness can be determined by a non-destructuve technique as with egg shells (401, 403). Creep and stress relations are two important properties of viscoelastic materials. Both properties are functions of time. When a force is applied to a viscoelastic material the deformation may not only be proportional to force, but may increase continuously (usually at a decreasing rate), with time (107). This behavior is called 'creep'. On the other hand, if the material is subjected to a constant deformation, then the force

necessary to maintain this specified deformation will decrease with time. This response is designated as 'stress-relaxation' (48). Nearly all biological and food materials exhibit creep and relaxation properties. These properties are particularly important in studies on flour dough quality (48, 125, 218).

Tensile and compression testing. Tensile and compression can be measured conveniently by commercial universal testing machines, and many such applications have been reported (402). There are, however, several points which must be considered. In tensile tests the clamps used to grip the specimen always present a problem, particularly with biological materials (394). Any damage due to the pressure of the clamps may cause premature failure of the specimen. This can be overcome by careful clamping arrangements. A more satisfactory method is to reduce the area of the specimen over the portion along the strain axis. The specimen should then fail in this zone and the effect of the clamps is reduced to negligible proportions (402). In compression testing the finish of the compression surfaces can influence the results under certain conditions since it has a great bearing on contact stresses that are developed. This is particularly important in testing hard brittle materials (394). In 1976 Culioli and Sherman (70) reported that the behavior of gouda cheese in compression tests with the Instron Universal Testing Machine was quite complex. It depended on maturity, sample dimensions, temperature and the nature of the contact surface between the cheese and the instrument. Introducing mineral oil or emery paper between the sample's surfaces and the compression plates affected the shape of the compressed samples.

Cine film records indicated that deformation was barrel-shaped (convex) with emery paper and concave with mineral oil. In the first test situation friction prevented the cheese surfaces from spreading to the same extent during sample compression as when using mineral oil. The best policy is to select a material such as commercially available stainless steel ground to a specified surface finish, and make all compression surfaces of this material (402). Another problem reported with compression tests is the heterogenity of the cheese specimen. Emmons et al., (97) indicated that effort was made to obtain candle cheese plugs free of openness. This was not always possible, and forces required for initial compression were unrealistically low in such cases. Finally, the action of teeth for repeated compression of food can be simulated mechanically and the forces recorded. An example of this imitative type machine is the General Foods Texturometer which is now in commercial production (Zenken Co., Hackensack, N.J.). Available speed motors allow testing at different deformation rates where the forces vary sinusoidally with time (404).

Shear tests. The most widely used shear measurements are based on the principle suggested by Warner (415) and Bratzler (53). The technique has been adopted widely for testing meat and other foods, and the shearing blade has been made to fit the Kramer Shear Press (129) and the Instron (153) so that force deformation records can be obtained.

The routine Warner-Bratzler shear test does not require a sophisticated deformation mechanism because the test conditions are particularly constant. However, the versatility of the machine can

be increased by using additional blades. This can increase the precision of measurements under certain conditions since a better estimate of the average shear force is obtained. The instrument has been adapted for testing meat (214) and other products such as strawberries, french fries and cheese (94, 95, 357, 394). Another popular shear test employs a circular cutter or wire to cut through soft foods such as cheese curd. The original apparatus used by Emmons and Price (93) to estimate curd firmness in cottage cheese was based on a Cherry Burrell tension meter which indicated the cutting force. The apparatus was recently modified by Voisey and Emmons (399); Voisey et al., (405) and Emmons et al. (94, 95). Reliable estimates of firmness of cheese were obtained using 2 or 3 wires. The same apparatus was also used to estimate firmness of milk puddings and custards using the Cherry-Burrell circular cutter instead of the wires (96). Another example of a wire shear apparatus was a small universal deformation machine adopted to estimate the spreadability of butter (328). A single wire was sheared through a container full of butter conditioned at a preselected temperature. The container had four slots in the sides so that it could be rotated 90 degrees and a second measurement obtained on the same sample. The error introduced by the first passage of the wire was negligible and the technique was found to reflect the consistency of the butter (395).

The wire shear apparatus is convenient and economical and has the advantage that the most critical component, the wire, is inexpensive and easily replaced (399). However, the most critical point in any of these apparatus is the shape of the cutting edge since it has a

pronounced effect on the shearing force. This is true for any device including the Kramer Shear Press, the pea tenderometer and the Warner-Bratzler Shear. However, Warner (415) and Bratzler (53) used radius edges on their blade whereas other workers have used flat sharp edges, and the data cannot be compared (394). The shape of the cutting edge determines the distribution of stresses particularly at the point of contact where compression, tension or shear may be generated depending on the shape of the cutting edge (395). For a blade type test cell a sharp edge is easier to manufacture but it is more prone to wear and damage than a radius edge. For this reason the wire shear test technique has a distinct advantage because wire is manufactured to close tolerances and yet is inexpensive (394). Recently attention has been paid to the effect of sample size on force readings with shear-type instruments. Pool and Klose (282) and Davey and Gilbert (75) showed a very significant effect of small differences in sample diameter on forces recorded by the Warner-Bratzler Shear Press. This point has been overlooked by many workers and, undoubtedly contributed to many inaccuracies (394). Samples have been prepared by using coring tools which makes difficult a precise control of the diameter, particularly in meat. Recently, Kastner and Henrickson (185) developed a method of producing uniform cores. However, Szczesniak et al., (358) have shown that when the compression-shear cell of the Kramer Shear Press is filled beyond a certain level, the maximum force is independent of sample weight for some foods whereas there is a pronounced relationship for others. With some foods under certain conditions shearing by wires may take place at an almost constant force (399).

In such cases, the sample size may not be critical with wire type cells providing sufficient material is used to achieve this 'steady state' condition.

Other instruments and testing techniques. Many devices have been developed for the measurement of food texture but none have advanced the art of mechanical testing as practiced by engineers and physicists for centuries (395). The field of stress analysis has evolved far beyond what is being applied to foods. There are numerous test techniques as reviewed by several workers (72, 83, 84, 85, 142, 378) which have not been applied to foods or have yet to be fully investigated in food technology applications.

A logical goal in texture measurement is to develop instantaneous non-destructive techniques preferably using inexpensive instruments. The tools available for this purpose cover a wide range. The entire electro-magnetic spectrum is available for investigation. Currently vibration is the only thing being investigated for measuring elastic properties of foods (1, 107, 108) according to techniques developed for other materials (9). Only recently has suitable equipment become commercially available (e.g. Model PPM - SR-H.M. Morgan Co. Inc. Cambridge, Massachusetts; Model VI, Nametre Acoustic Spectrometer, Abbott et al. 170). Nuclear techniques is another area which may find more widespread future application. It is used to estimate egg shell thickness and density (412). Also it could be used to a distinct advantage if the texture of a product could be monitored continuously during a production process. Viscosity can be measured in this manner, but the techniques for solid and semi-solid products have yet to be

achieved. Different examples of existing possibilities was given by Boisey et al. (409) and Richardson et al. (299) who indicated that the viscometer (Brookfield LVT-Helipath) has been adopted for studying curd formation by direct acidification. Szabo (346) measured the viscosity of process cheese at 80C during processing, but his results were never correlated with the physical properties of the process cheese at 18C.

There is limited evidence to support the use of nondestructive test techniques for measuring food texture. Finney (109) found that the Magness Taylor pressure tester (a destructive test) correlated better with taste panel evaluation of apple texture than acoustic measurements (a nondestructive test). Nondestructive techniques and other methods are used to determine physical properties of engineering materials (394). The determination is, however, indirect and is based on theoretical analysis. There is no doubt that successful measurements are accomplished in this way for a range of materials and there is a distinct possibility that it can be done with foods (395). The major problem in applying the technique to foods is the complex shape and structure involved and the high water content of many products.

Standards

Sensory standards have been developed so that taste panels can be trained to recognize and evaluate different textural characteristics (348). This is accomplished by using a series of foods of different types, which allow the establishment of scales for each specific characteristic.

In objective tests, force and deformation recording systems can be calibrated mechanically so that these data are recorded precisely. The question is often raised, however, as to how accurately the instrument measures textural properties. This depends on many factors including the accuracy of the recording system (which can be verified), the control of sample size, the environmental cinditions, the operating techniques, the sampling techniques, the sample heterogeneity, the sample orientation and the condition of the test cells (395). All these factors can be controlled within experimental limits. The test cells present the greatest problem. Its condition can be determined by measuring its dimensions and checking the sharpness of the cutting edges etc. (240). This is difficult, time consuming and requires expensive meteorological instruments. Thus, the only reliable information available is that the force required to compress, pull or shear the sample under the test conditions at any given time is measured accurately.

There is a definite need for standard materials that can be used to monitor changes taking place within the test cell. Relatively limited work has been done in this respect and to date the most successful material has been various waxes. Warner (415) used beeswax to test the meat shear and Staley (339) used a microcrystalline wax to standardize the pea tendrometer. There are, however, problems associated with the manufacture of wax samples of uniform size, weight, and density and the properties of this material are greatly affected by temperature. If allowance is made for these factors, meaningful compressions can be made (339). Voisey and Nonneck (411) compared 31 pea tendero-

meters to grade green peas. Tests with microcrystalline wax indicated that there were serious differences between machines. Much research is needed on standardizing techniques.

If careful techniques are used it is feasible to reduce the variation of mechanical measurements within a given food sample. For example, in testing dough it was possible to reduce variation to less than 5%, and since this included natural biological variations it was concluded that the variation attributed to instrument error was of a lower order (408, 410).

Sensory analysis is still the final yardstick of how well an instrument reflects textural parameters (348). However, this can only be considered as a crude means of calibrating the instrument which, in theory should have greater accuracy and resolution of measurement than a trained taste panel. While the great majority of objective texture tests can be considered only as emperical, it is realistic and desirable to take any measurement as accurately as possible. Electronic instruments are the most efficient way to accomplish this.

MATERIALS AND METHODS

Natural Cheese

Thirty-five day old Cheddar cheese was obtained from the Utah State University Dairy Products Laboratory. It was minced in a Hobart vertical cutter model VCM 25 (Hobart Mfg. Co., Troy, Ohio).

Emulsifying Salts

Emulsifying salts were food-grade, and legally permissable for use in pasteurized process cheese. The emulsifying salts were:

1. Trisodium Citrate (CIT) Pfizer Inc. New York, New York 10017.
2. Disodium Orthophosphate (DSP) Stauffer Chemical Co. Westport, Connecticut 06880.
3. Tetrasodium Pyrophosphate (TSPP) Stauffer Chemical Co. Westport, Connecticut 06880.
4. Sodium Aluminum Phosphate (SALP) Stauffer Chemical Co. Westport, Connecticut 06880.

Processing the Cheese

The comminuted cheese, emulsifying salts (2.5%), and sodium chloride (0.5%) were placed in the cooker along with the required amount of water for standardization to 40% moisture. The cheese was processed in a pilot size Damrow horizontal cooker. (Damrow Brothers Co.

Fon du Lac, Wisconsin, U.S.A.). The cooker had a capacity of approximately 22.7 Kg of cheese and was heated by indirect heating. The cheese mixture was brought to a cooking temperature of 82C within 5 - 6 min., and the contents kept at this temperature for 0, 5, 10, 20 and 40 min respectively before being discharged. Sitrring was maintained throughout the heating period.

Packaging

Process cheese was discharged into 0.45 Kg round plastic containers. The cheese samples were then placed in a vacuum chamber at 25C where a vacuum of 26 in (658 mm) was applied momentarily to eliminate air bubbles. Samples were stored at 4.4C then analyzed for moisture, fat and pH.

Cheese Analysis

Chemical analyses were carried out on the natural cheese and also on the finished process cheese samples.

Moisture

Cheese moisture was determined according to Price et al. (285). All samples were analyzed in duplicate (256).

Fat

Cheese fat was determined in duplicate by a modified Babcock method described by Van Slyke and Price (386).

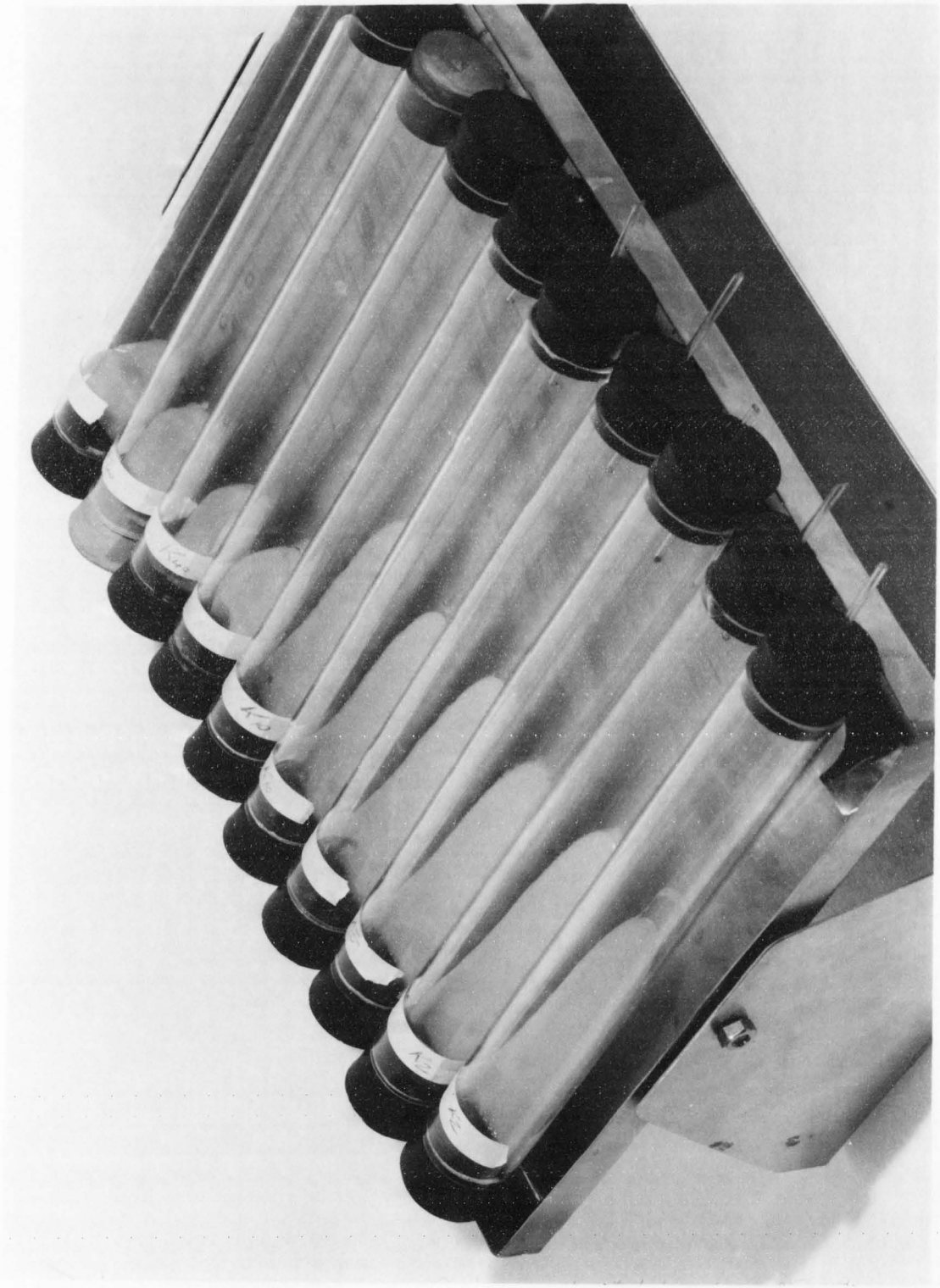
pH

pH was measured in duplicate directly on 50 g of well comminuted cheese as described by Meyer (234) and Thomas (371) using a Corning Digital 110 pH meter and a single reference combination glass electrode (Corning Model 476051 Corning Scientific Instruments, Medfield, Massachusetts 02052, U.S.A.).

Meltability

Pasteurized Process Cheese was tested for meltability by a modification of the method described by Olson and Price (257). A pyrex glass tube 30 mm. I.D. and 250 mm long was used to hold the cheese during the melting test. One end of the tube was closed with a rubber stopper perforated by a 1 mm I.D. glass tube to act as a vent. A reference line was etched on the glass 27.5 mm from the opposite end of the melting tube. This end of the tube was also closed with a rubber stopper after a solid 15g cheese cylinder had been placed in the tube with its front edge aligned with the etched reference line. Ten melting tubes were placed in a vertical position on a rack for 40 min at 4.4C, then in a horizontal position in an oven at 110C for 30 min. Flow of the hot cheese was stopped instantly for measurement with a tilt-control rack (Figure 1). Thedistance of flow from the reference line to the leading edge of the melted cheese was quickly measured and recorded in millimeters as "cheese flow". All tests were run in duplicate.

Figure 1. Test apparatus for measuring the meltability of process cheese (257).



Rheology Measurements

A table model universal testing machine (MTS type T5002), (J.J. Lloyd Instruments Ltd., Brooke Avenue, Warsash, Southhampton 503 6HP England) was used for all rheological measurements (Figure 2), and was coupled with a two channel X - Y automatic recording system that was built in a separate chassis. Provision was made to automatically start, stop and reverse the recorder chart pen in synchronization with the crosshead on the testing machine so that either force-deformation or force-time curves could be plotted. The recorder chart pen speed was related to the crosshead speed by a conversion factor. The MTS machine contained a plug-in attachment to allow automatic control or cycling of the crosshead in response to either the force on the specimen or its deformation. The testing machine was operated at a crosshead speed of 30mm/min with a 500 Newton load cell. The recorder crosshead speed ratio was 10/1 unless otherwise stated. Cheese samples were tempered for 48 h at 15.5C prior to making rheological measurements.

Firmness

The cheese was cored into cylinders 19 mm in diameter and 20 mm high with a special coring device (Figure 3). Firmness was measured in quadruplicate by a modification of the procedure described by Emmons et al. (95) in which resistance to the passage of a 0.04 cm stainless steel wire through the cheese was measured as shows in Figure 4.

Figure 2. The MTS model T5002 Universal Testing Machine.

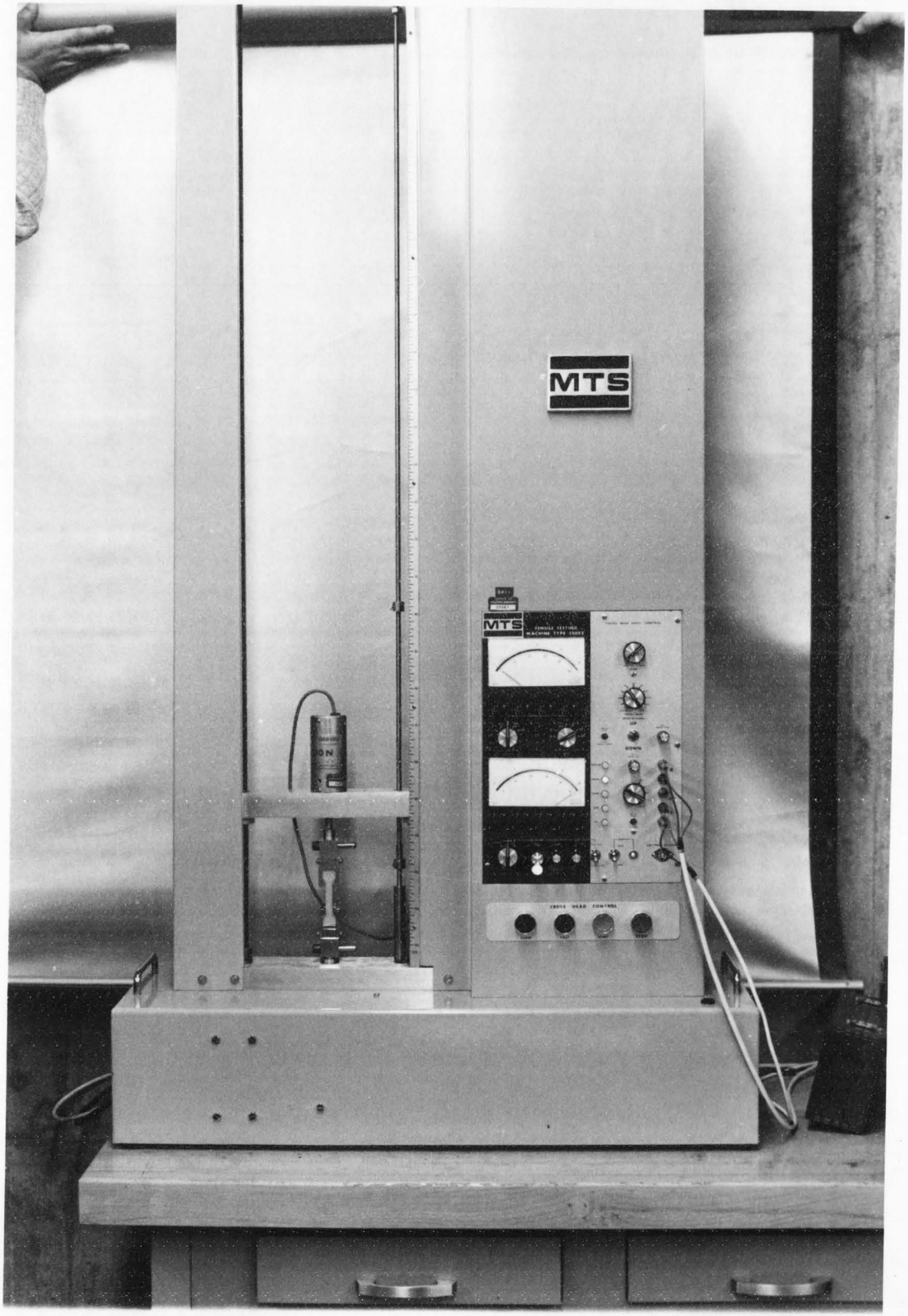

MTS
MTS
CROSS HEAD CONTROL

Figure 3. Left - Stainless steel coring device for cutting cheese cylinders. Center - Stainless steel cylinder I.D. 20 mm and 20 mm high. Right - Special stainless steel cutter to obtain a slice of process cheese 12.7 mm in thickness for tensile strength testing.

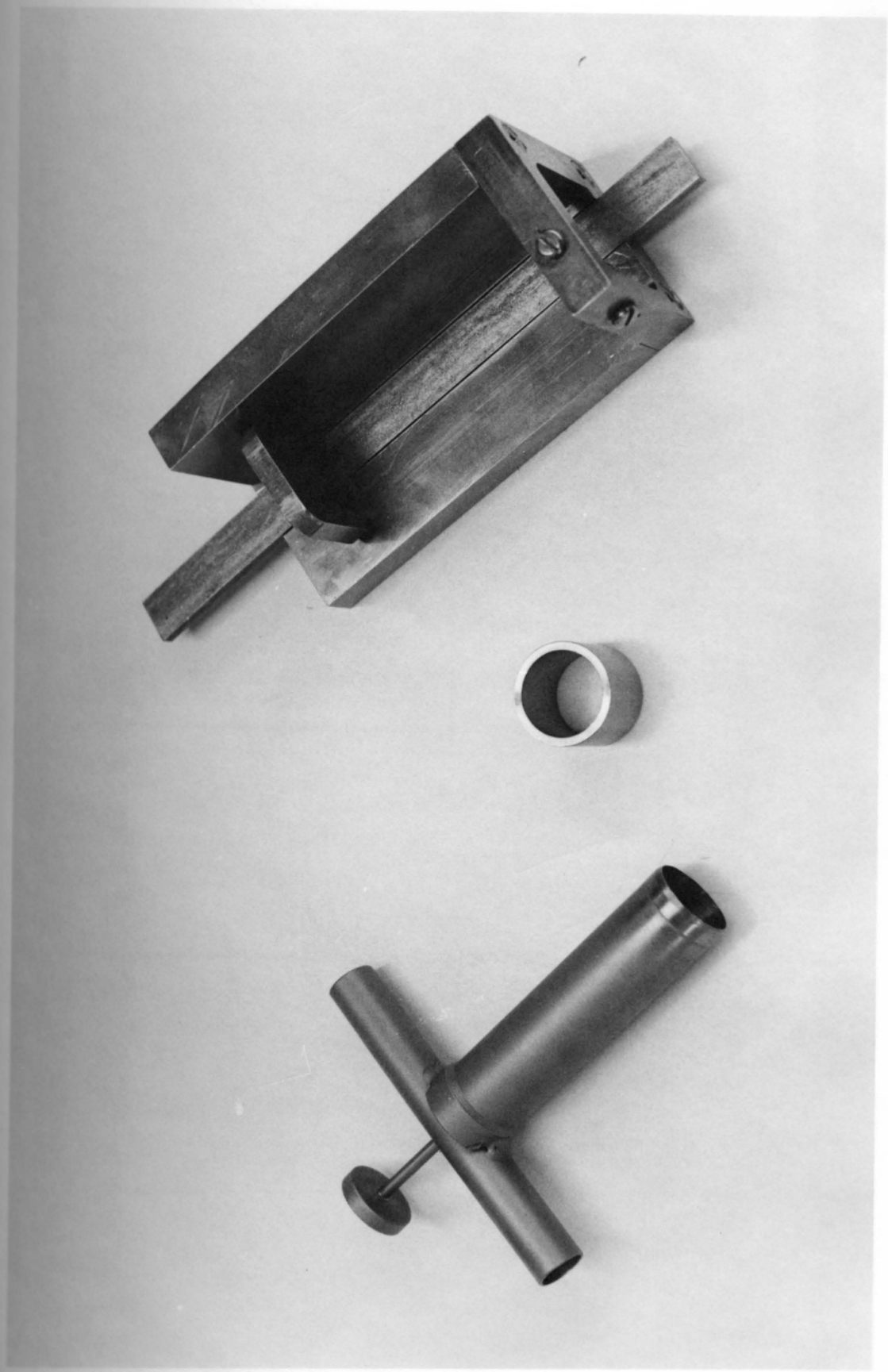
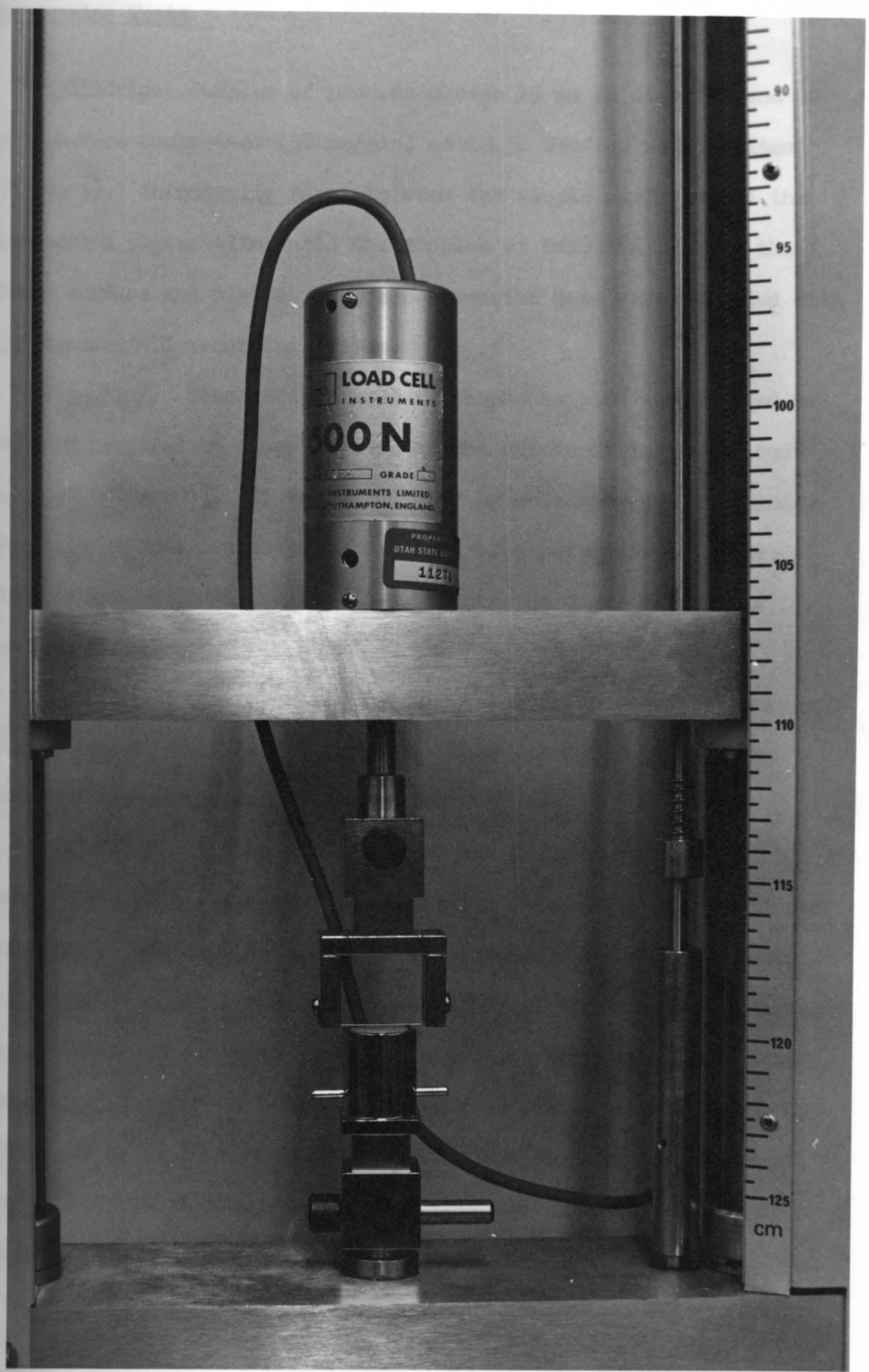


Figure 4. Device used for measuring cheese firmness, showing wire cutter and slotted cheese holder.


LOAD CELL
INSTRUMENTS
500 N
GRADE
INSTRUMENTS LIMITED
SOUTHAMPTON, ENGLAND
UTAH STATE
90
95
100
105
110
115
120
125
cm

Compression Tests

Cylindrical samples of process cheese 19 mm in diameter and 20 mm high were compressed (30 mm/min) at 15.5C between metal plates (Figure 5). Introducing paper between the sample surfaces and the compression plates eliminated the problem of friction between the cheese surface and plates. Force-compression data were recorded with the standard MTS recording system.

Toughness. Toughness of process cheese samples was defined as the work required to cause rupture of the cheese cylinders by compression (Figure 6). It was calculated by measuring the area under the force-deformation curve to the left of a perpendicular line drawn to the abscissa from the point where the curve attained a negative slope.

Breaking Force. Breaking force was the force required to rupture the cheese cylinder (Figure 6). This was determined by the maximum force on the force-deformation curve.

Hysteresis. Hysteresis of cheese was defined by Weaver et al. (419) as the energy absorbed by a material in a cycle of loading and unloading or the damping capacity. The standard cheese cylinders were compressed until the force reached 67% of the measured breaking force for the same cheese sample. Relaxation upon unloading of the cheese cylinder was an indication of its memory of elastic recovery. Hysteresis was a loss of that memory and was measured as area A in Figure 7, where Δ L represented the vertical cylinder deformation at 67% of the breaking force, ΔL_1 represended the vertical cylinder

Figure 5. An illustration of a cheese cylinder during compression between parallel plates.

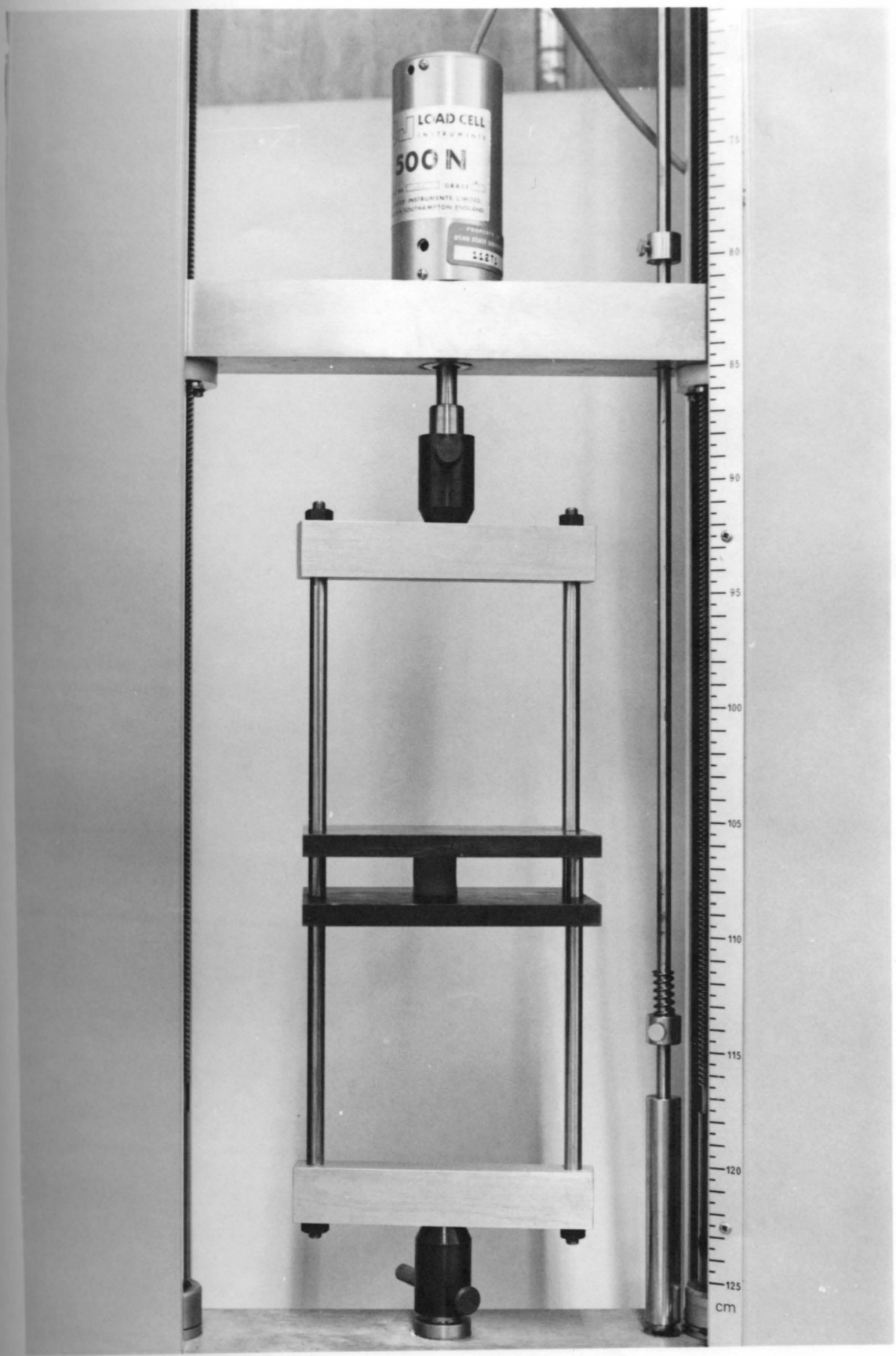

LOAD CELL
500 N
75
80
85
90
95
100
105
110
115
120
125
cm

Figure 6. Cheese cylinders before and after measurement of breaking strength.

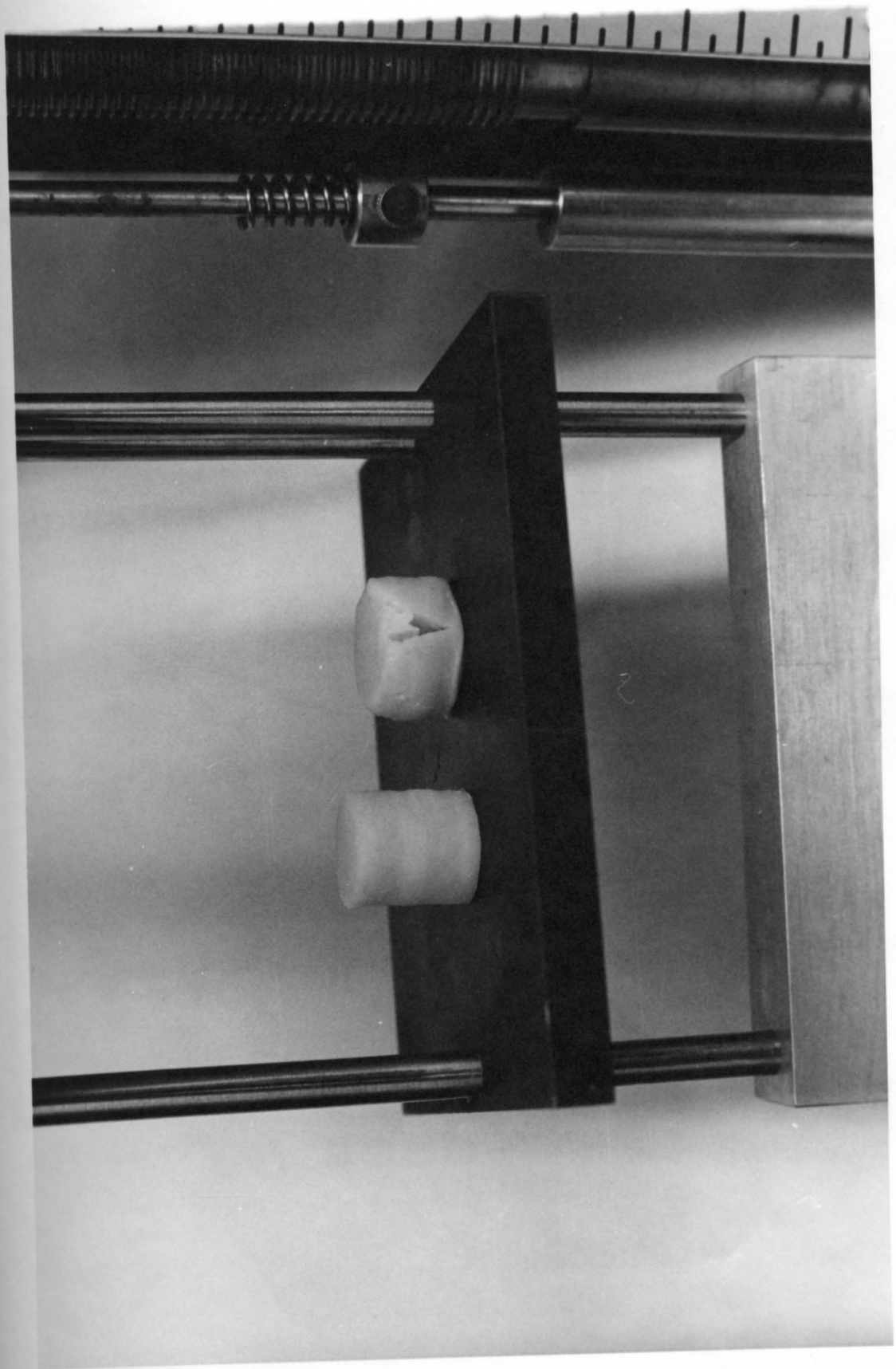
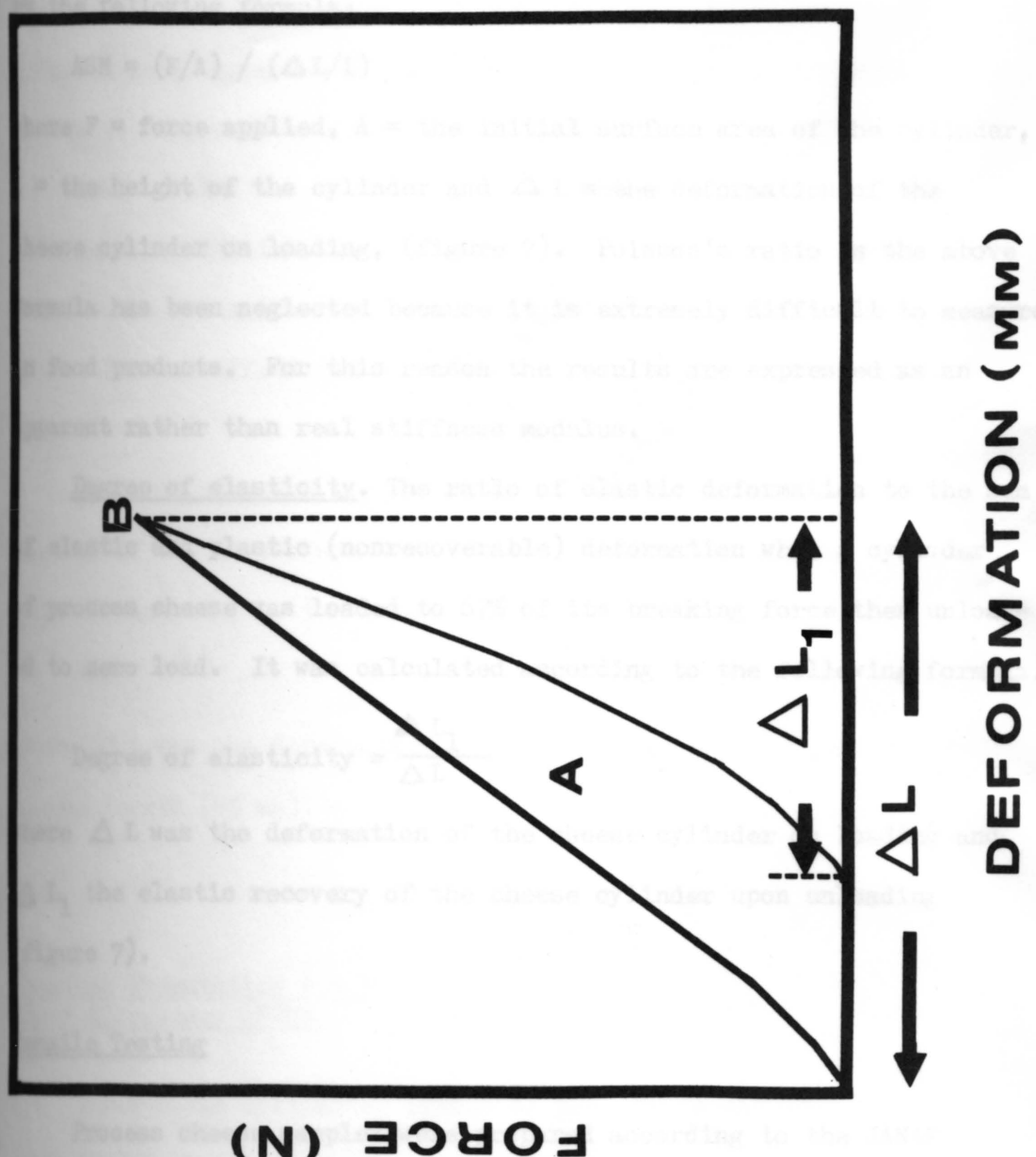


Figure 7. Idealized force deformation curve from the MTS Universal Testing Machine. Δ L = vertical deformation of the cheese cylinder, ΔL_1 = vertical recovery of the cheese cylinder after removal of the compression force N, B = maximum force applied during compression (67% of breaking force), A = area corresponding to hysteresis or loss of recovery memory.


B
A
ΔL_1
ΔL
DEFORMATION (MM)
FORCE (N)

recovery after removal of the force, and B represented a force equal to 67% of the breaking force.

Apparent stiffness modulus (ASM). The ratio of compression stress to strain within the elastic limit of a cheese cylinder was calculated by the following formula:

$$ASM = (F/A) / (\Delta L/L)$$

where F = force applied, A = the initial surface area of the cylinder, L = the height of the cylinder and ΔL = the deformation of the cheese cylinder on loading, (figure 7). Poisson's ratio in the above formula has been neglected because it is extremely difficult to measure in food products. For this reason the results are expressed as an apparent rather than real stiffness modulus.

Degree of elasticity. The ratio of elastic deformation to the sum of elastic and plastic (nonrecoverable) deformation when a cylinder of process cheese was loaded to 67% of its breaking force then unloaded to zero load. It was calculated according to the following formula:

$$\text{Degree of elasticity} = \frac{\Delta L_1}{\Delta L}$$

where ΔL was the deformation of the cheese cylinder on loading and ΔL_1 the elastic recovery of the cheese cylinder upon unloading (figure 7).

Tensile Testing

Process cheese samples were prepared according to the JANAF specimen model (244). The cheese was cut into slices 1.26 cm thick by a special cutter (Figure 3). The JANAF specimens were then cut

from the slices by means of a die and wire cheese cutter (figure 8). Stainless steel clamps were used to grip the specimen. Figure 9 illustrates the way that tensile testing was applied.

Apparent ultimate stress (AUST). The force required to stretch the cheese along the strain axis until the structure failed was referred to as apparent ultimate stress. The test is illustrated in figure 9. Apparent ultimate stress was calculated as follows:

$$AUST = F/A$$

where A = the initial cross sectional area of the cheese (94.5 mm^2). The results are again expressed as apparent to avoid the complications of the Poisson effect due to a constantly changing cross sectional area.

Apparent ultimate strain (AUS). This is the ratio of maximum stretch of the cheese specimen to the original length, and was calculated according to the formula:

$$AUS = \frac{\Delta L}{L}$$

where ΔL was the deformation upon stretching and L = the original cheese length (45 mm).

Light Microscopy

Specimen Preparation for Transmitted Light Microscopy (TLM)

Three cubic millimeter pieces of process cheese were fixed in a 2% buffered glutaraldehyde solution for 4 h at 4C. Fixed samples were embedded in glycol methacrylate according to the procedure of

Figure 8. Dies used for cutting and mounting cheese samples for tensile strength measurements. The center portion of the cheese was 12.7 mm thick and 94.5 mm long. Upper left - Grips for holding during tensile testing. Lower center - Die for cutting a uniform cheese section. Right - Wire cutter for cutting the cheese section in the die.

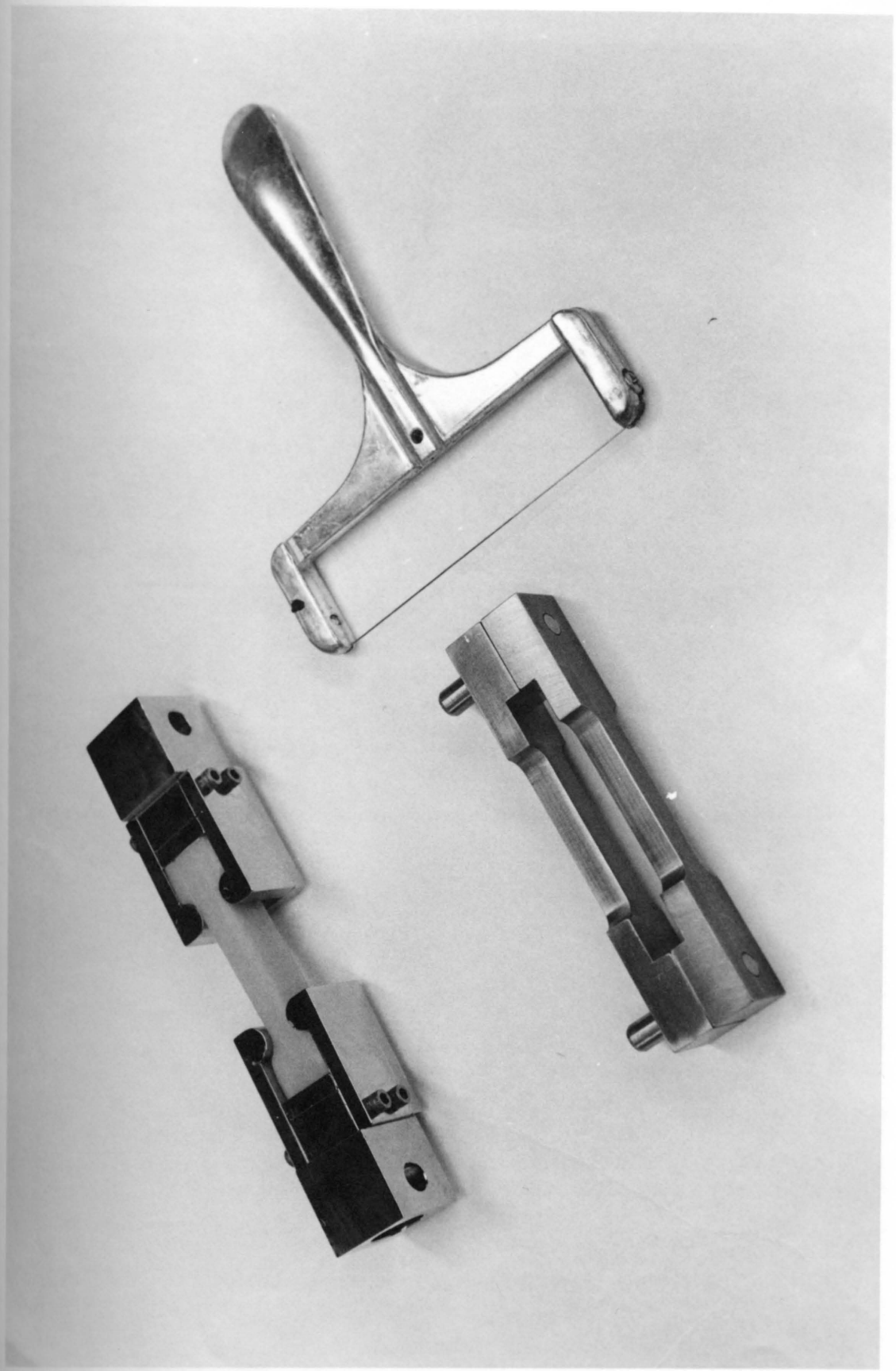
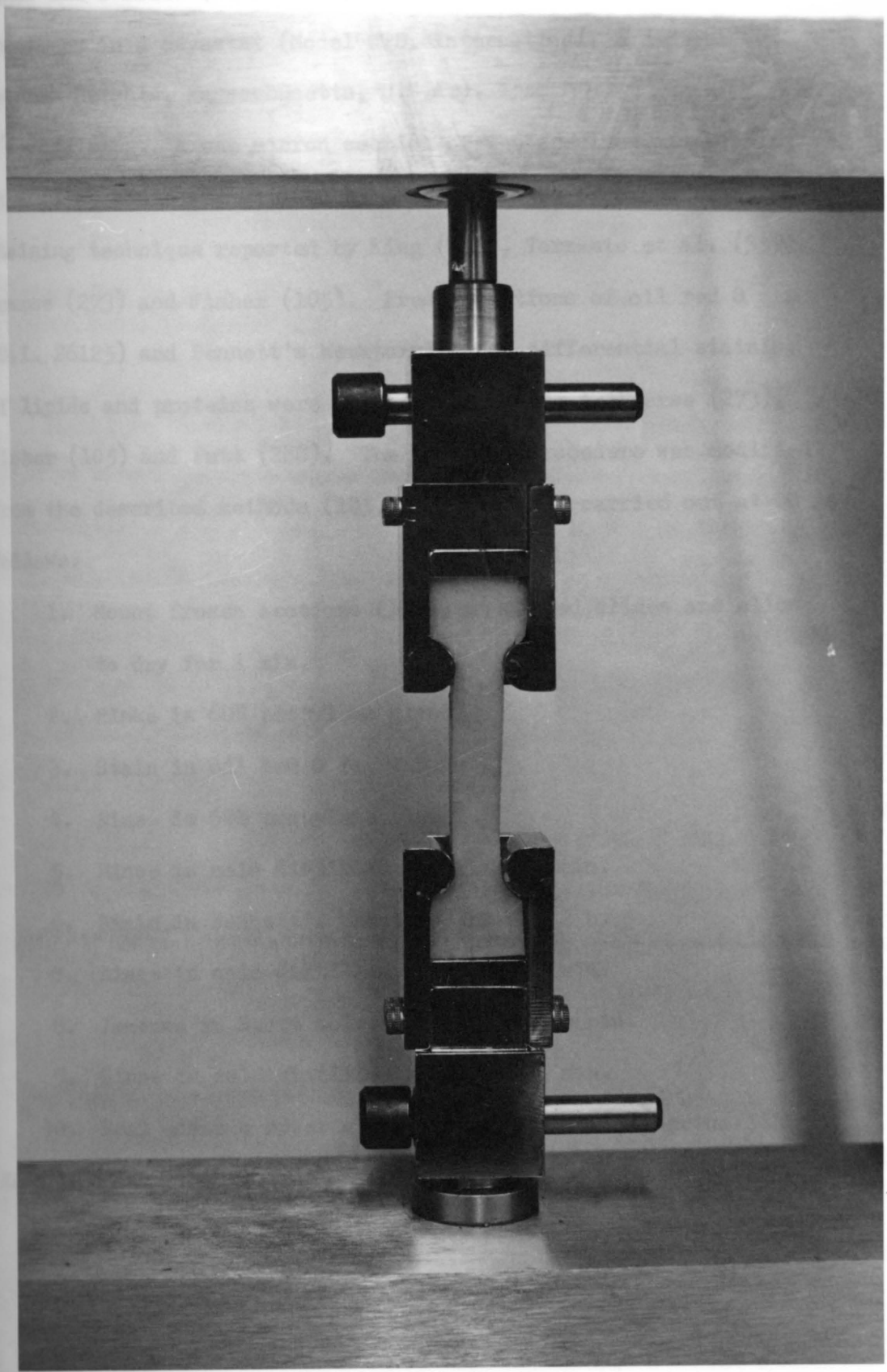


Figure 9. Cheese sample mounted in tensile testing device.



Feder and O'Brien (102). Sectioning was carried out on frozen specimens in a cryostat (Model CTD, International Equipment Co. Needham Heights, Massachusetts, U.S.A.).

Staining. A one micron section of the specimen was mounted on a glass slide and stained by a modification of a differential staining technique reported by King (192), Torranto et al. (359), Pearse (273) and Fisher (105). Fresh solutions of oil red O (C.I. 26125) and Bennett's hematoxylin for differential staining of lipids and proteins were prepared according to Pearse (273), Fisher (105) and Putt (288). The staining procedure was modified from the described methods (105, 273, 288) and carried out at 4C as follows:

1. Mount frozen sections (1μ) on subbed slides and allow to dry for 1 min.
2. Rinse in 60% propylene glycol.
3. Stain in oil red O for 2 h.
4. Rinse in 60% propylene glycol.
5. Rinse in cold distilled water for 2 min.
6. Stain in Bennett's hematoxyline for 2 h.
7. Rinse in cold distilled water for 3 min.
8. Immerse in Scott solution (288) for 3 min.
9. Rinse in cold distilled water for 3 min.
10. Seal under a cover glass with a drop of glycerine jelly.

Keep at 4C all the time.

Microscopic Examination

Cheese sections were examined by a Zeiss photomicroscopy and photographed by high contrast Kodachrome film 40 type A, KPA 135-36, positive film 5070 (Eastman Kodak Co., Rochester, N.Y.).

Electron Microscopy

Specimen Preparation for Scanning Electron Microscopy (SEM)

Fixation. A sharp scalpel wetted with a glutaraldehyde solution was used to cut representative samples of process cheese into particles approximately 1 x 2 x 2 mm. They were fixed for 4 h in fresh aqueous-2% glutaraldehyde solution buffered at pH 7.2 as reported by Kalab (171). The samples were further cut into 0.5 mm slices to achieve good penetration of OsO_4 (133, 178). They were then post-fixed in uncontaminated fresh 4% OsO_4 solution EM, (Ted Pella, Inc. Tustin, California 92680 U.S.A.) for 4 h (179).

Dehydration. Fixed cheese particles were wrapped placed in perforated stainless steel mesh baskets and dehydrated through a graded series of aqueous ethanol solutions (88, 170) beginning with 2% ethanol and increasing by 10% increments to 100% ethanol. Specimens were exposed to each concentration for 15 min.

Defating. Dehydrated cheese specimens were defatted through a graded series of chloroform in absolute alcohol, then terminated with two treatments in absolute alcohol (168).

Intermediate fluids. Freon TF (F-113) was used as an intermediate fluid to replace the ethanol by a procedure described by Hayat (133).

Critical point drying. Process cheese samples were critical point dried in a Bomar apparatus (Model SPC-900) (The Bomar Co., Tacoma, Wa., 98401, U.S.A.) Freon 13 was used as a transition fluid to replace the freon F-113 (133).

Specimen mounting and coating. Cheese particles were fragmented under a low-magnification microscope with the help of a fine needle and needle-sharp tweezers. Particles were less than 0.5 mm in diameter. Static electricity generated in a fine hair brush by friction on a glass plate was used to lift the particles and place them on the wet surface of a freshly applied coat of silver cement (173). Samples were mounted on a special mount (AMR 1000 aluminum stub, 12.7 mm in diameter) which was cleaned with fine carborandum paper. The sides of the particles were carefully painted with conductive cement using another fine brush. The cement was maintained at a proper consistency with n-butyl acetate as a diluent. The fragments with their fractured surfaces facing up were coated with carbon in a Varian vacuum evaporator at a pressure of 10^{-5} torr followed by coating with gold-palladium three times at pressures of 10^{-5} torr to achieve thermal protection. A 15 cm length of 8 mill gold-palladium wire was wound evenly on a tungsten filament for each vacuum coating. The rotary specimen table was situated 10 cm from the alloy source and inclined at an angle of $\sim 30^{\circ}$ (173).

Scanning Electron Microscopy (SEM)

The microstructure of process cheese was examined by a scanning electron microscope (AMR Model 1000), (Advanced Metals Research

Corporation, 160 Mddlessex, Turnpike, Bedford, Massachusetts 01730), operating at 20 Kv. Pictures were taken on Kodak Graphic Arts film (10.2 x 12.7 cm)., 4127 thick (Eastman Kodak Co., Rochester, N.Y.).

Transmission Electron Microscopy (TEM)

Process cheese samples fixed in 2% glutaraldehyde at 25C were sent to Dr. M. Kalab, Food Research Institute, Research Branch, Agriculture Ottawa, Ontario, Canada K1A for transmission as well as scanning electron microscopy. The same technique for sample preparation was applied as for SEM except CO_2 was used for crytical point drying and the fragments were coated in Vacuo with carbon (50Å) and gold (200 Å) (173). A Cambridge Stereoscan Electron Microscope operating at 20 Kv was used for SEM and pictures were taken on Kodak plus-x 35 mm film. TEM was carried out on cheese samples fixed in glutaraldehyde solution and post fixed in buffered OsO_4. They were progressively dehydrated through a series of increasing ethanol concentrations, and embedded in Spurr's resin (low-viscosity) as reported by Kalab (169). Thin sections were obtained using an OM U_2 microtome (Reichert Optische Werke AG, Vienna 17, Austria) and were stained with 5% uranyl acetate in methanol and with lead acetate. They were observed under an EM 300 electron microscope (N.V. Philips, Eindhoven, The Netherlands) operated at 40 or 60 Kv (168).

Statistical Treatment of Rheological Data

The effect of cooking time with each emulsifying salt was compared statistically with each rheological measurement. Statistical

comparisons also were made between each pair of rheological measurements in all combinations. All correlations were made by determining pearson correlation coefficients (252) with significance indicated by the alpha values.

Analysis of variance (260) was used to determine the significance of cooking time and type of emulsifying salt on each rheological measurement.

RESULTS

Cheddar Cheese

The Cheddar cheese used for processing contained 40.3% moisture, 31.4% fat and 52.6% fat in the dry matter.

Process Cheese

Composition

The moisture content of the process cheese made with each emulsifying salt and at each stage of cooking is given in table 1. All four batches of process cheese contained 40.6% moisture and 31.0% fat when removed from the cooker after 0 and 5 min at 82C. Further cooking resulted in a slight reduction in moisture. After 40 min the moisture had decreased to 40.2% in CIT cheese and 40.3% in cheese processed with the other three salts.

The percent fat in the cheese and percent fat in the dry matter are shown in tables 2 and 3 respectively. The slight decrease in fat in all the samples during cooking and consequent decrease in fat in the dry matter is difficult to explain in view of slight moisture losses during the same period. The modified Babcock test is capable of reading accurately only to the nearest 0.5%, and it is possible that the slight decrease in fat that was observed was not significant. The values given are averages of duplicate determinations with readings estimated between the 0.5% marks on the Babcock bottles. However,

Table 1. Moisture content of process cheese during cooking at 82C with four different emulsifying salts.

Emulsifying salt	Cooking Time (min)				
	0	5	10	20	40
	(%)	(%)	(%)	(%)	(%)
CIT[1]	40.6	40.6	40.6	40.5	40.2
DSP[2]	40.6	40.6	40.5	40.5	40.3
TSPP[3]	40.6	40.6	40.5	40.4	40.3
SALP[4]	40.6	40.6	40.5	40.5	40.3

[1]sodium citrate

[2]disodium phosphate

[3]tetrasodium pyrophosphate

[4]sodium aluminum phosphate

Table 2. Fat content of process cheese during cooking at 82C with four different emulsifying salts.

Emulsifying salt	Cooking Time (min)				
	0	5	10	20	40
	(%)	(%)	(%)	(%)	(%)
CIT[1]	31.0	31.0	30.8	30.8	30.7
DSP[2]	31.0	31.0	30.8	30.8	30.6
TSPP[3]	31.0	31.0	30.9	30.8	30.6
SALP[4]	31.0	31.0	30.8	30.7	30.7

[1]sodium citrate

[2]disodium phosphate

[3]tetrasodium pyrophosphate

[4]sodium aluminum phosphate

Table 3. Fat in the dry matter of process cheese during cooking at 82C with four different emulsifying salts.

Emulsifying salt	Cooking Time (min)				
	0	5	10	20	40
	(%)	(%)	(%)	(%)	(%)
CIT[1]	52.2	52.2	51.9	51.8	51.3
DSP[2]	52.2	52.2	51.8	51.8	51.3
TSPP[3]	52.2	52.2	51.9	51.7	51.3
SALP[4]	52.2	52.2	51.8	51.6	51.4

[1] sodium citrate

[2] disodium phosphate

[3] tetrasodium pyrophosphate

[4] sodium aluminum phosphate

the consistency of decreasing fat during cooking suggests that it may not have been a random error. During cooking the fat droplets in the cheese became smaller, and it is known that very tiny fat globules are not all incorporated into the fat column in the Babcock test for fat in milk (334). A similar effect may have slightly reduced the recovery of finely emulsified fat from process cheese. This observation certainly warrants further investigation.

The pH of the process cheese was 5.4 in CIT samples and 5.8 in the DSP, TSPP and SALP samples (Table 4). The composition of all process cheese samples was quite uniform. Therefore, it is unlikely that differences in rheological properties could be attributed to variation in the moisture or fat content of the cheese. The range of moisture values was from 40.2% to 40.6% and the fat content varied from 30.6% to 31.0%.

Meltability

Figure 10 shows the effect of cooking time on the meltability of cheese processed at 82C with four different emulsifying salts. Analysis of variance (table 9) revealed a highly significant ($\alpha < 0.001$) overall effect of cooking time on meltability. It also showed no significant differences among replicates. The overall differences between the effects of emulsifying salts on meltability were also highly significant ($\alpha < 0.001$). Analysis of variance of the effect of the salt on meltability at each cooking time was determined and is shown in table 5. The results of Duncan's (260) multiple range test for significance of differences between the salts at each stage

Table 4. pH of cheese processed for varying times at 82C with four different emulsifying salts.

Emulsifying salt	Cooking Time (min)				
	0	5	10	20	40
CIT[1]	5.4	5.4	5.4	5.4	5.3
DSP[2]	5.8	5.8	5.8	5.8	5.8
TSPP[3]	5.8	5.8	5.8	5.8	5.8
SALP[4]	5.8	5.8	5.8	5.8	5.8

[1]sodium citrate

[2]disodium phosphate

[3]tetrasodium pyrophosphate

[4]sodium aluminum phosphate

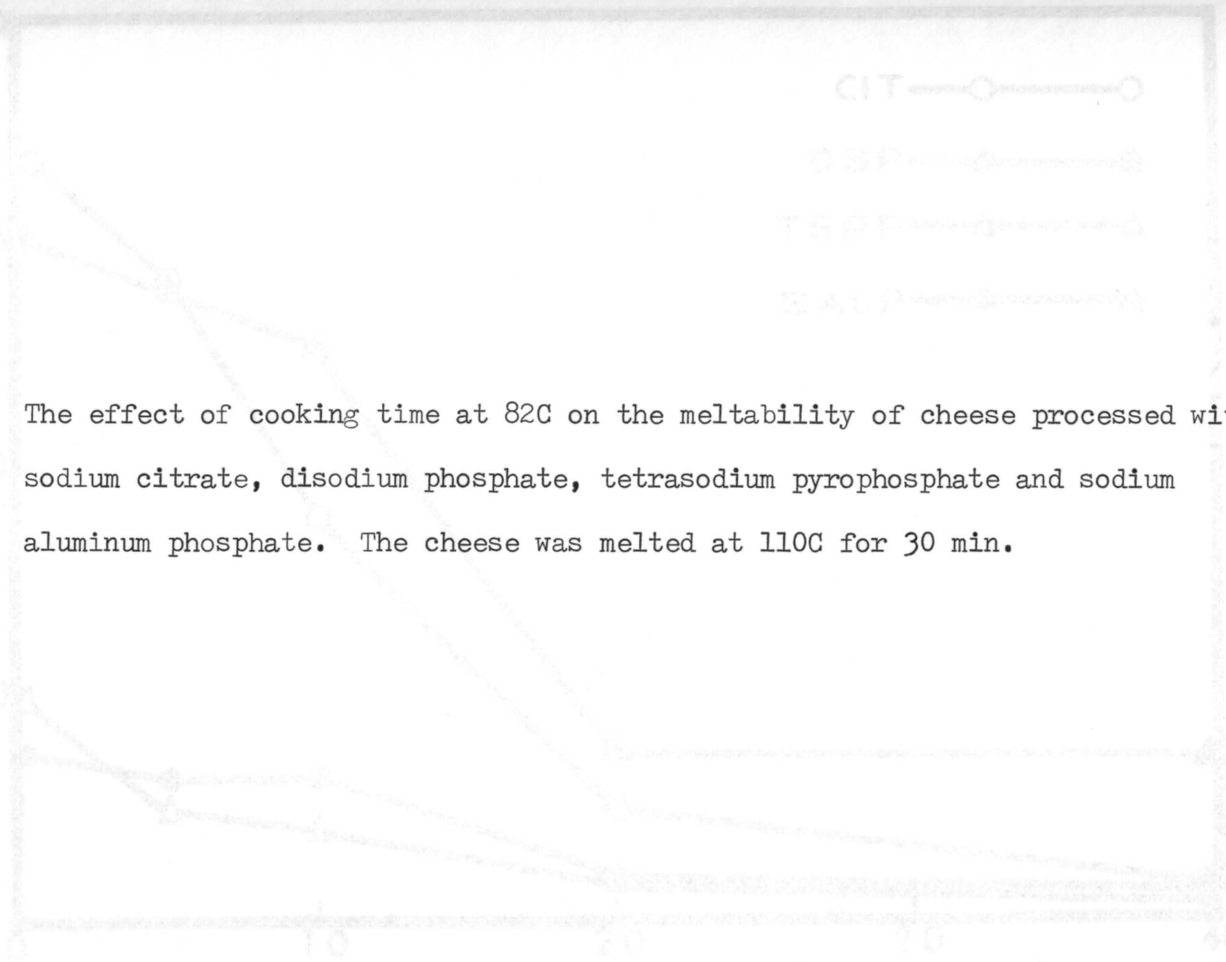


Figure 10. The effect of cooking time at 82C on the meltability of cheese processed with sodium citrate, disodium phosphate, tetrasodium pyrophosphate and sodium aluminum phosphate. The cheese was melted at 110C for 30 min.

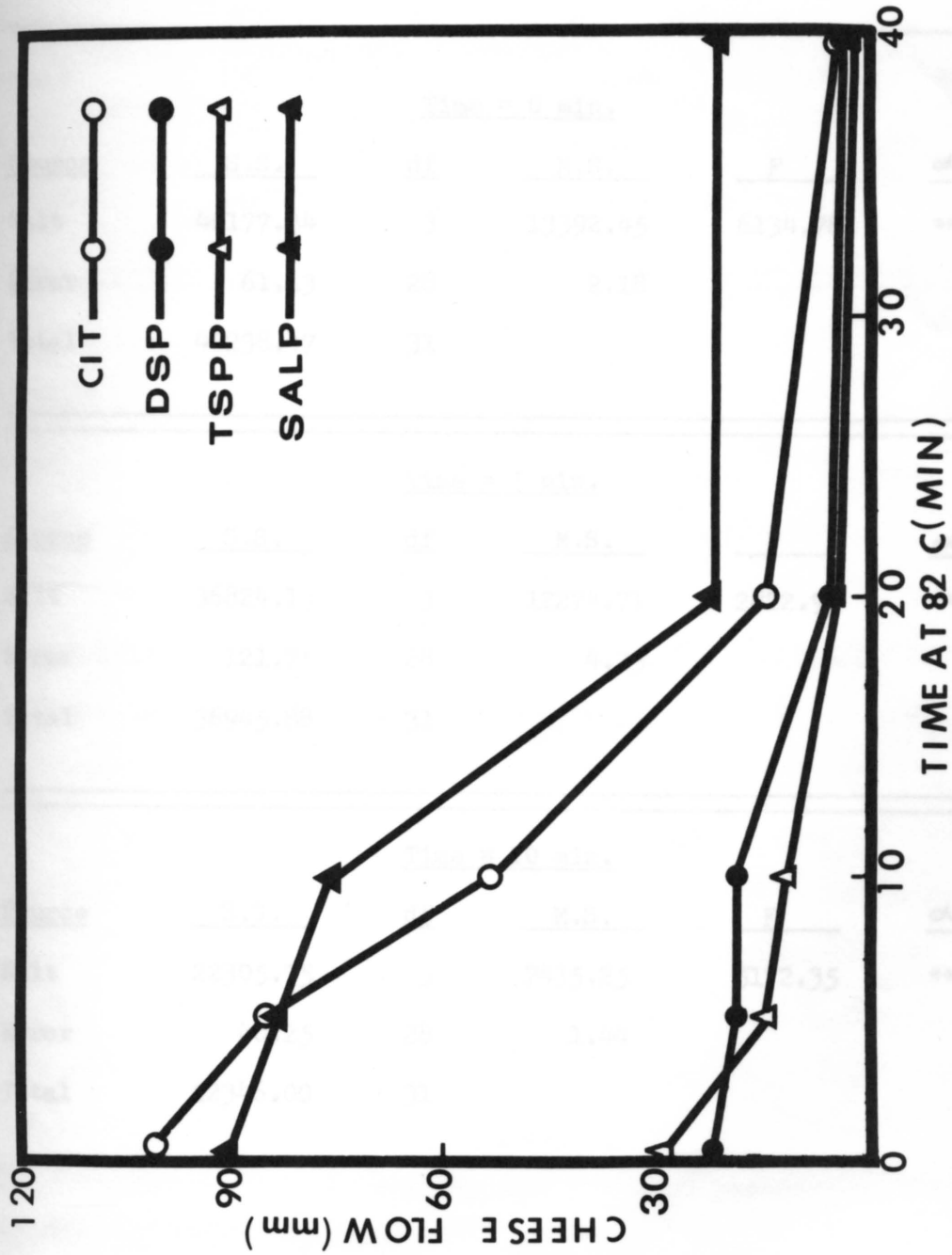

CIT
DSP
TSPP
SALP
CHEESE FLOW (mm)
120
90
60
30
0
10
20
30
40
TIME AT 82 C(MIN)

Table 5. Analysis of variance of the effects of emulsifying salts on meltability of process cheese after four different cooking times at 82C.

Time = 0 min.

Source	S.S.	df	M.S.	F	α
Salt	40177.34	3	13392.45	6134.78	**
Error	61.13	28	2.18		
Total	40238.47	31			

Time = 5 min.

Source	S.S.	df	M.S.	F	α
Salt	36824.13	3	12274.71	2822.93	**
Error	121.75	28	4.35		
Total	36945.88	31			

Time = 10 min.

Source	S.S.	df	M.S.	F	α
Salt	22305.75	3	7435.25	5172.35	**
Error	40.25	28	1.44		
Total	22346.00	31			

Table 5. (Continued)

Time = 20 min.

Source	S.S.	df	M.S.	F	α
Salt	1360.75	3	453.58	215.40	**
Error	54.75	26	2.11		
Total	1415.50	29			

Time = 40 min.

Source	S.S.	df	M.S.	F	α
Salt	1563.28	3	521.09	749.22	**
Error	18.088	26	0.70		
Total	1581.37	29			

**Indicates significance at 0.01% level

of cooking are shown in table 6. The only melting values that were not different were found with CIT and SALP cheese after 5 min and DSP and TSPP cheese after 20 min in the cooker.

Upon discharge from the cooker at 0 time the CIT cheese was the most meltable followed closely by SALP cheese. Both of these were much more meltable than TSPP or DSP cheese. Extending the cooking time to 40 min. reduced the meltability of all process cheese samples, but during the first 20 min in the cooker, loss of meltability was greatest for CIT and SALP cheese. DSP and TSPP cheese did not melt well initially and became even worse with increased cooking time.

Pearson correlation coefficients (tables 10, 11, 12 and 13) between cooking time and meltability were -0.9834 for CIT cheese -0.9308 for DSP cheese, -0.9382 for TSPP cheese and -0.9182 for SALP cheese.

The meltability test proposed by Olson and Price (257) for use with Pasteurized Process Cheese Spreads proved useful and acceptable for use on process cheese when the heating time was extended to 30 min. Table 6 gives the mean values and standard deviations for 8 replications of the melting test for cheese made with each salt. It is evident that the relative error with this test increased as the meltability of the cheese decreased. However, melting characteristics of cheese samples that exhibited reasonably good meltability were easily measured with good reproducability.

Firmness

The firmness of all process cheese samples increased with increased cooking time (Figure 11), but cheese emulsified with

Table 6. Duncan's multiple range test of mean melting values of cheese processed with four different emulsifying salts for 0, 5, 10, 20 and 40 min at 82C. ($\alpha = 0.01$)

Time = 0 min.

Salt	Mean		S.D.	Difference*
CIT	100.63	±	1.51	A
SALP	90.25	±	1.49	B
TSPP	28.63	±	1.41	C
DSP	21.63	±	1.51	D

Time = 5 min.

Salt	Mean		S.D.	Difference*
CIT	84.88	±	2.53	A
SALP	82.75	±	2.87	A
DSP	18.38	±	1.30	B
TSPP	13.75	±	1.04	C

Time = 10 min.

Salt	Mean		S.D.	Difference*
SALP	75.75	±	1.58	A
CIT	53.38	±	1.19	B
DSP	18.00	±	0.76	C
TSPP	10.88	±	1.13	D

Table 6. (Continued)

Time = 20 min.

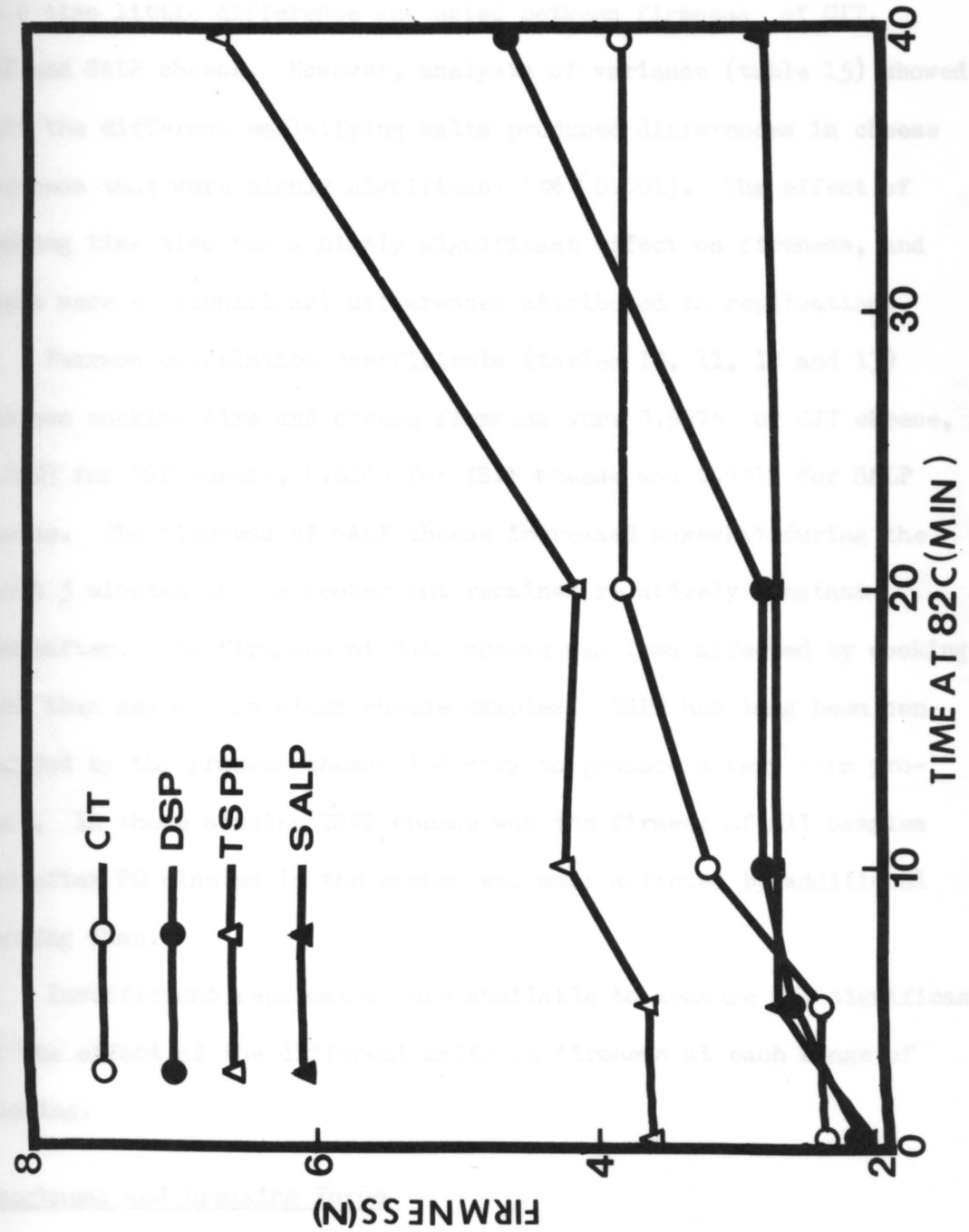
Salt	Mean		S.D.	Difference*
SALP	21.50	±	1.87	A
CIT	13.88	±	1.96	B
DSP	5.13	±	0.99	C
TSPP	4.25	±	0.71	C

Time = 40 min.

Salt	Mean		S.D.	Difference*
SALP	20.83	±	1.47	A
CIT	3.63	±	.52	B
DSP	2.63	±	.74	C
TSPP	2.25	±	.46	D

*same letters indicate no significant difference at $\alpha = .01$

Figure 11. The effect of cooking time at 82C on the firmness of cheese processed with sodium citrate, disodium phosphate, tetrasodium pyrophosphate and sodium aluminum phosphate. Firmness measured at 15.5C.


CIT
DSP
TSPP
SALP
FIRMNESS(N)
TIME AT 82C(MIN)
0
10
20
30
40
2
4
6
8

TSPP was the firmest at all stages of cooking and that made with SALP remained softest. DSP and CIT cheeses were soft initially but increased in firmness during cooking more rapidly than SALP cheese. At 0 time little difference was noted between firmness of CIT, DSP and SALP cheese. However, analysis of variance (table 15) showed that the different emulsifying salts produced differences in cheese firmness that were highly significant ($\alpha < 0.001$). The effect of cooking time also had a highly significant effect on firmness, and there were no significant differences attributed to replications.

Pearson correlation coefficients (tables 10, 11, 12 and 13) between cooking time and cheese firmness were 0.9375 for CIT cheese, 0.8293 for DSP cheese, 0.8266 for TSPP cheese and 0.7512 for SALP cheese. The firmness of SALP cheese increased somewhat during the first 5 minutes in the cooker but remained relatively constant thereafter. The firmness of SALP cheese was less affected by cooking time than any of the other cheese samples. TSPP has long been considered by the process cheese industry to produce a very firm product. In these studies TSPP cheese was the firmest of all samples and after 20 minutes in the cooker was most affected by additional cooking time.

Insufficient replicates were available to measure the significance of the effect of the different salts on firmness at each stage of cooking.

Toughness and Breaking Force

All process cheese samples increased in toughness and breaking force during 40 min in the cooker at 82C (Figures 12 and 13).

Figure 12. Effect of cooking time at 82C on the toughness of cheese processed with sodium citrate, disodium phosphate, tetrasodium pyrophosphate and sodium aluminum phosphate. Toughness was measured at 15.5C. No toughness measurements were possible of SALP cheese at cooking times of 0, 5 and 10 min., because the cheese was too soft to register a deflection on the force-deformation curve.

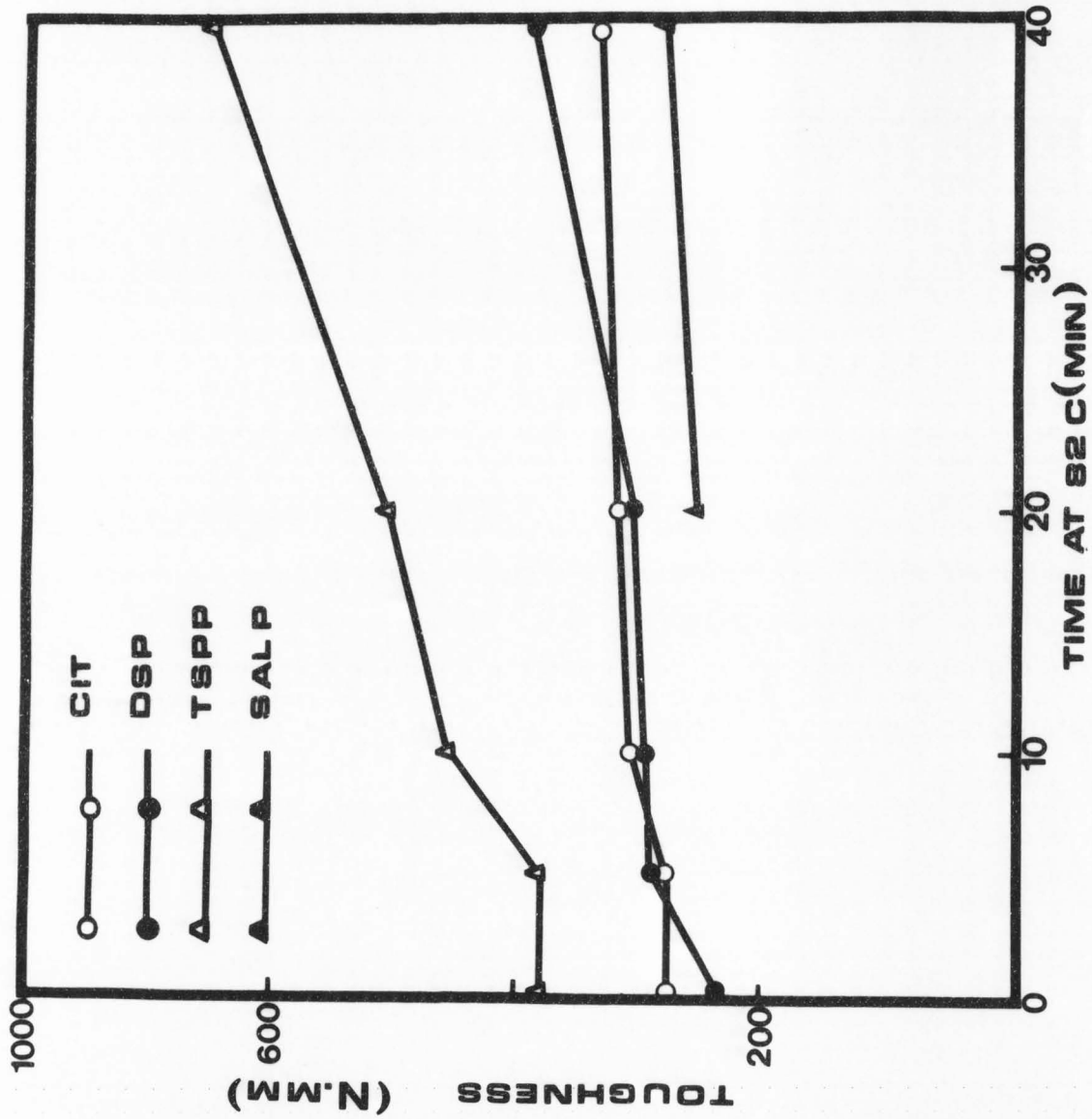
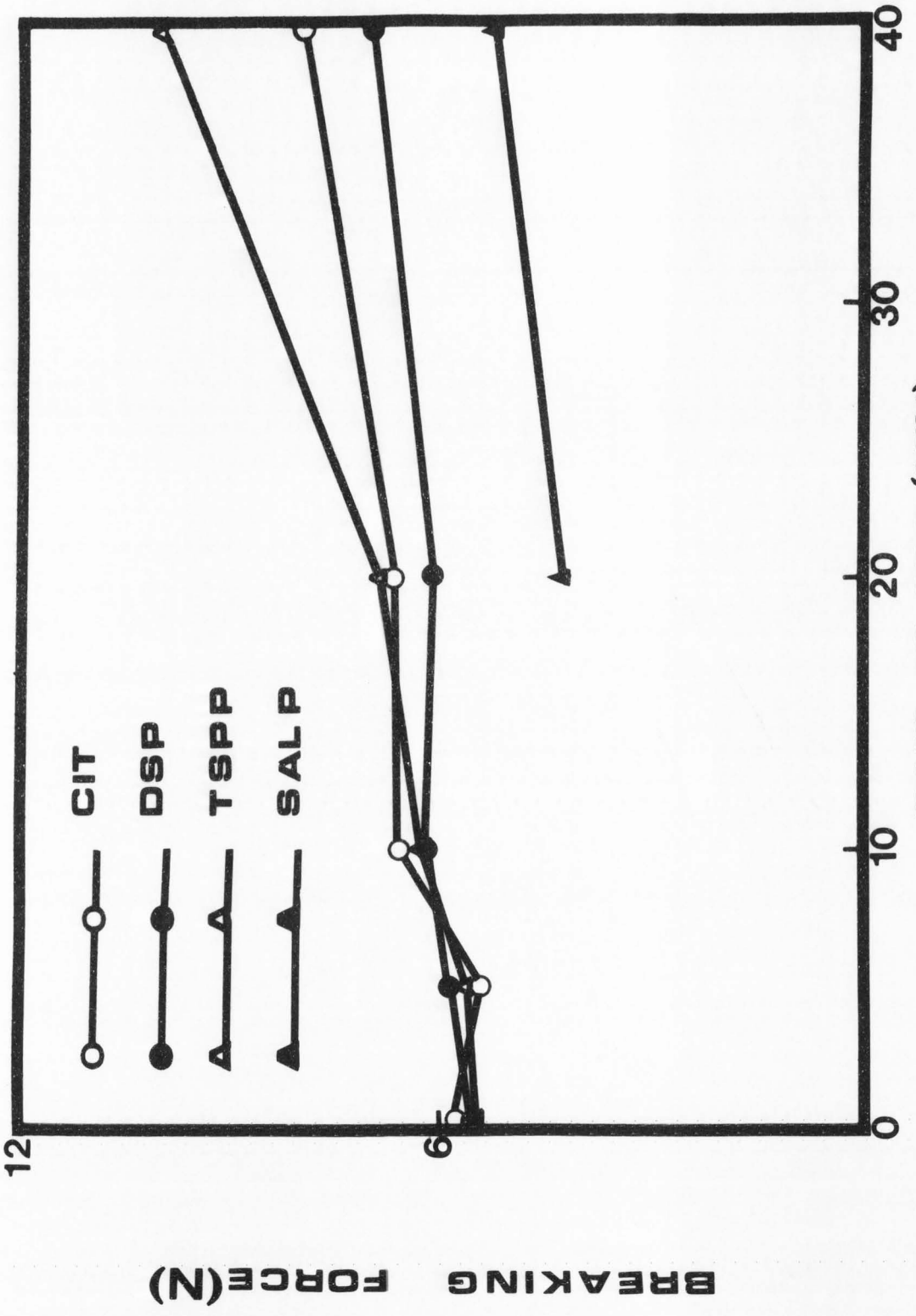

CIT
DSP
TSPP
SALP
TOUGHNESS (N.MM)
1000
600
200
0
10
20
30
40
TIME AT 82 C(MIN)

Figure 13. Effect of cooking time at 82C on the force required to rupture (breaking force) cheese processed with sodium citrate, disodium phosphate, tetrasodium pyrophosphate and sodium aluminum phosphate. Breaking force measured at 15.5C. No breaking force measurements were possible on SALP cheese cooked for 0, 5 or 10 minutes because the cheese was soft enough that no rupturing was detectable.

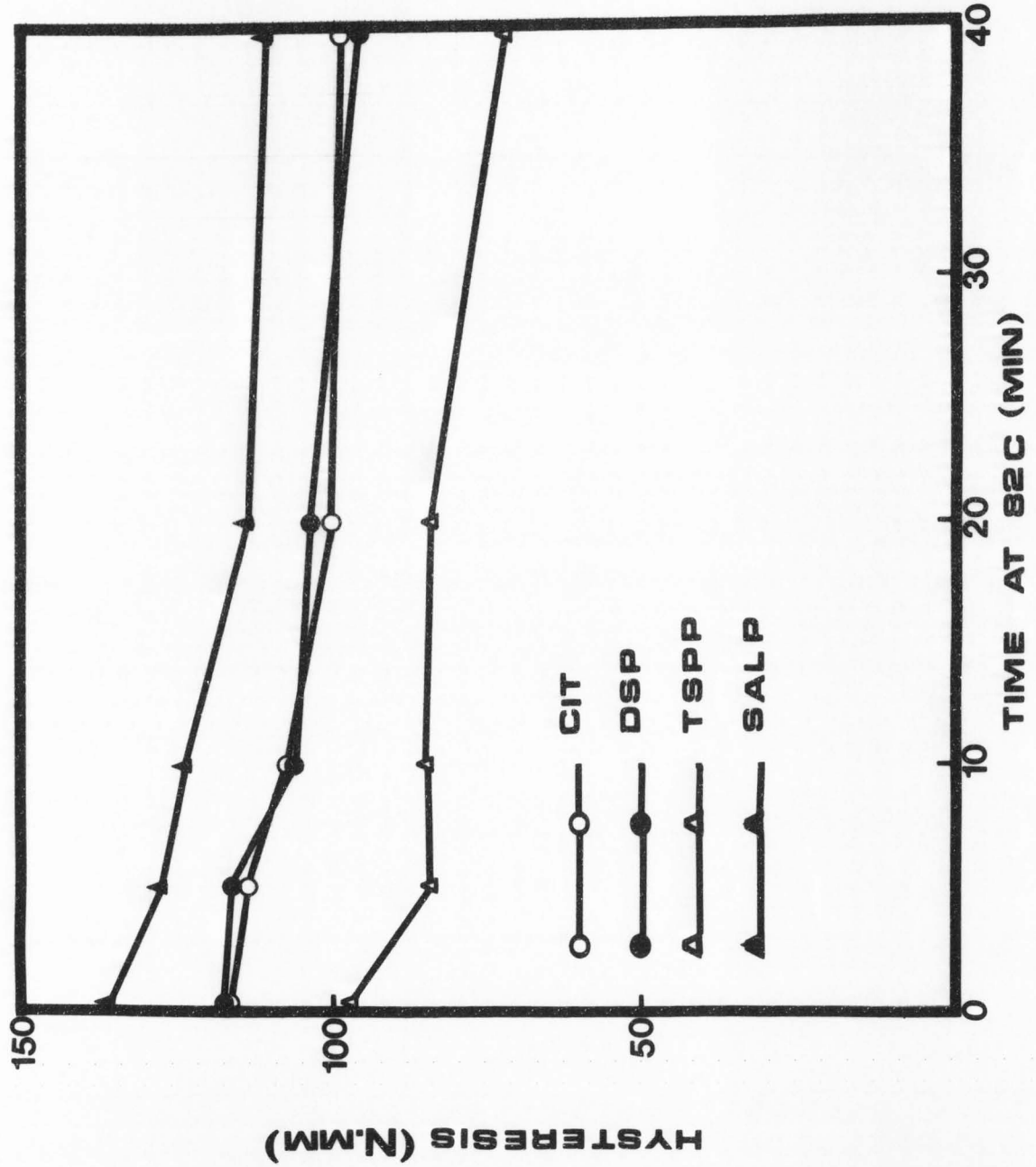

BREAKING FORCE(N)
12
6.5
0
10
20
30
40
TIME AT 82C (MIN)
CIT
DSP
TSPP
SALP

It was impossible to measure toughness or breaking force on cheese processed with SALP for 0, 5 or 10 min at 82C. Therefore, no values for these samples are shown in figures 12 and 13. They were so tender that they flattened out like putty when compressed, and registered no deflection on the force-deformation curve. However, after 20 min in the cooker, the cheese structure became rigid enough to show measureable toughness and breaking force. Analysis of variance (tables 17 and 19) showed that cooking time had a highly significant ($\alpha < .001$) effect on the toughness and breaking force of process cheese and that differences between replications were not significant. Pearson correlation coefficients (tables 10, 11, 12 and 13) for relationships between cooking time and toughness were 0.9656 for CIT cheese, 0.9423 for DSP cheese, 0.9380 for TSPP cheese and 1.0000 for SALP cheese. All of these correlations were highly significant. Overall differences in the effects of different emulsifying salts on toughness and breaking force also were highly significant ($\alpha < 0.001$) (See Table 20). TSPP cheese was consistently tougher than cheese made with the other salts at all stages of cooking. The same cheese also exhibited a greater breaking force than the other samples although it was not particularly evident until after 20 min cooking at 82C. Insufficient replicate measurements were available to determine the statistical significance of salts on toughness or breaking force at each cooking interval.

Hysteresis

Hysteresis (loss of memory of elastic recovery) during the cooking of process cheese is illustrated in figure 14. The different

Figure 14. Effect of cooking time at 82C on the hysteresis of cheese processed with sodium citrate disodium phosphate, tetrasodium pyrophosphate and sodium aluminum phosphate. Hysteresis was measured at 15.5C.

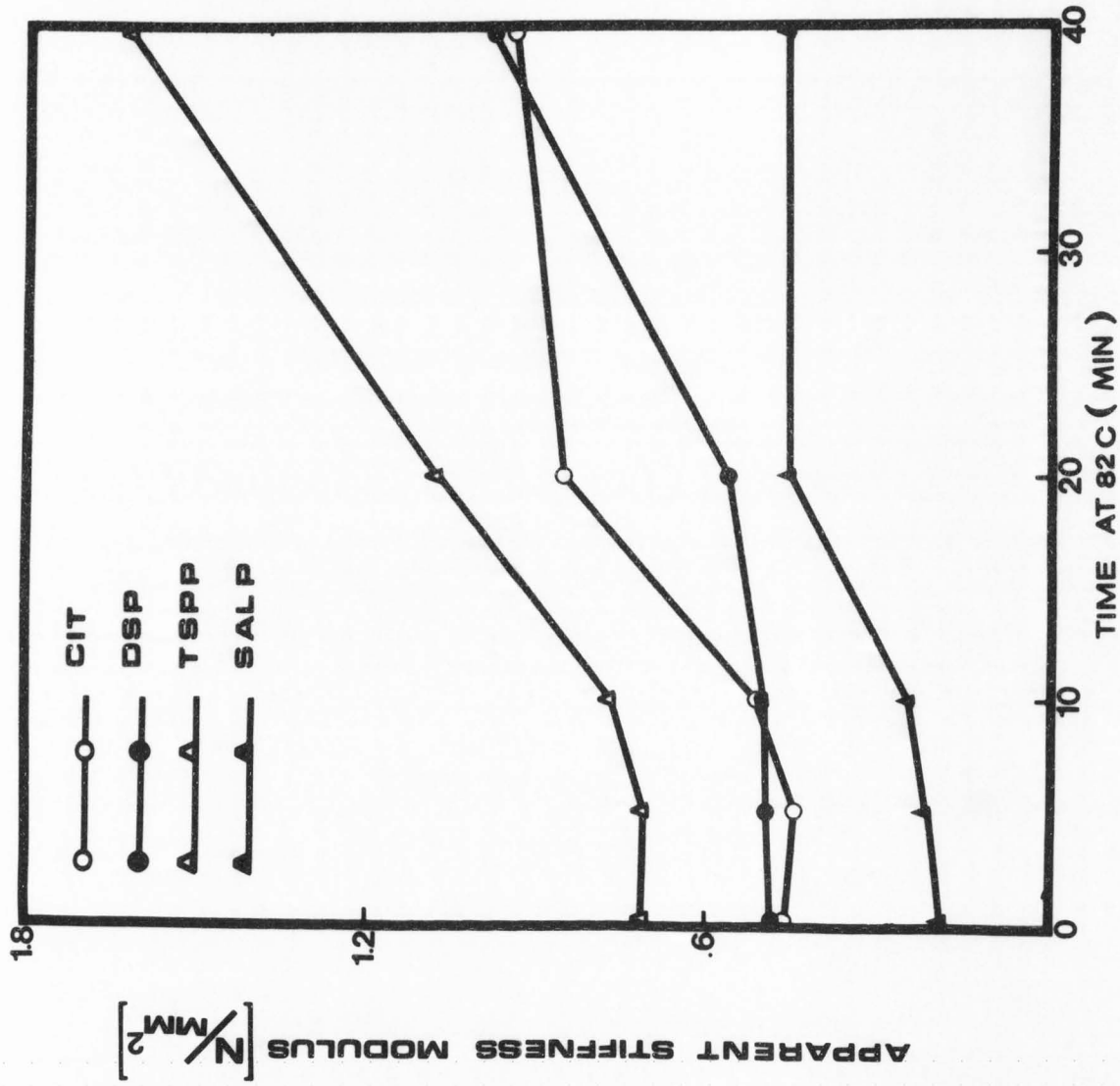

HYSTERESIS (N.MM)
150
100
50
0
10
20
30
40
TIME AT 82C (MIN)
CIT
DSP
TSPP
SALP

emulsifying salts had significantly ($\alpha < .05$) different effects on the hysteresis of the cheese. According to the analysis of variance (table 22) and overall Pearson correlation coefficient (table 20) there was no significant relationship between hysteresis and cooking time. However the correlation coefficients for each individual salt (tables 10, 11, 12 and 13) were significant or highly significant. They were -0.9582 for CIT cheese, -0.9624 for DSP cheese, -0.8927 for DSP cheese and 0.6354 for SALP cheese. It is noteworthy that all these values were negative and highly significant ($\alpha < 0.001$) except for SALP cheese which had a significantly positive ($\alpha < 0.05$) value. The fact that one salt had a positive correlation value and three had negative values could explain why the overall analysis on variance and Pearson's correlation coefficient showed no significance. Hysteresis which, in a way, is a measure of lack of elastic properties was greatest for SALP cheese. During most of the cooking process SALP cheese remained soft and pliable which ruggested high hysteresis values. TSPP cheese exhibited the least hysteresis. DSP and CIT cheese had similar hysteresis properties that were intermediate between those of cheese made with the other two salts.

Apparent Stiffness Modulus (ASM)

Figure 15 illustrates the ratio of stress to strain obtained from force-deformation curves. The changing surface area of the sample and resulting changing force per unit of surface area (Poisson's ratio) were neglected in these calculations. A true modulus of stiffness determination would require a constant force per unit of surface area. Therefore the moduli expressed here are apparent rather than real.

Figure 15. Effect of cooking time at 82C on the apparent stiffness modulus of cheese processed with sodium citrate, disodium phosphate, tetrasodium pyrophosphate and sodium aluminum phosphate. Measurements were made at 15.5C.

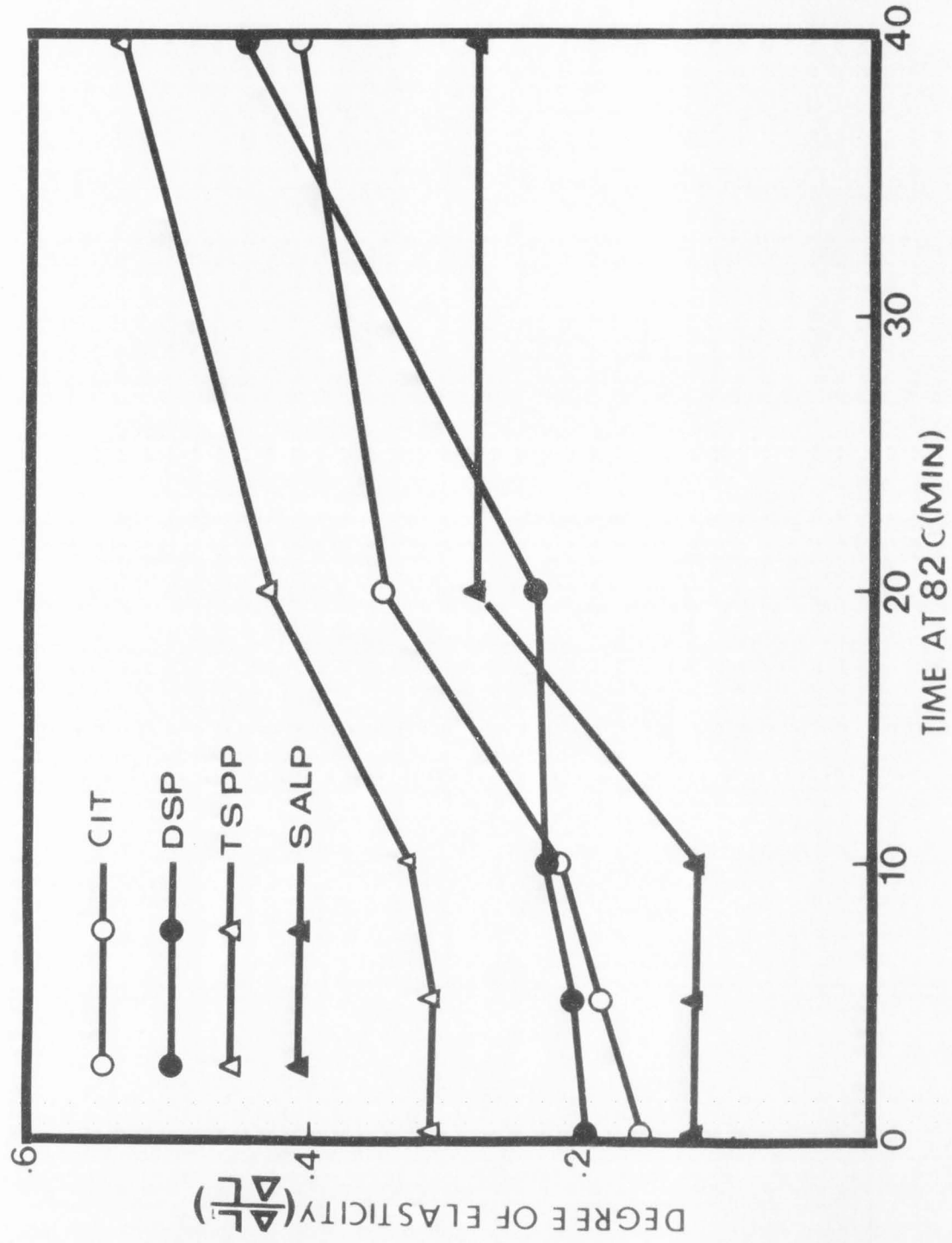

CIT
DSP
TSPP
SALP
1.8
1.2
.6
0
10
20
30
40
TIME AT 82C (MIN)
APPARENT STIFFNESS MODULUS [N/MM²]

The overall analysis of variance (table 24) and overall Pearson's correlation coefficient (table 20) indicated no significant relationship between cooking time and ASM or between cooking time and emulsifying salt. Neither were the replicates significantly different. Correlation coefficients between cooking time and ASM for each individual salt indicated significance ($\alpha < 0.001$) only for cheese processed with CIT. The overall relationship between ASM and type of emulsifying salt was not significant on the basis of analysis of variance (table 19). Because of a changing structure in cheese when it is put under stress, it is questionable whether ASM can be a useful rheological measurement. Other measurements seem to relate more to the processing variables used in the study.

Degree of Elasticity

This was a measure of springiness or ability of a cheese cylinder to recover after partial deformation by compression. The greatest degree of elasticity was found in cheese processed with TSPP (figure 16) at all stages of cooking while the least elastic behavior was shown by SALP cheese. Again CIT and DSP cheese showed similar degrees of elasticity that were intermediate between cheese processed with the other two salts. Analysis of variance (table 26) revealed a highly significant ($\alpha < 0.001$) overall relationship between emulsifying salt and elasticity, but no significance between cooking time and elasticity or between replications. The overall Pearson correlation coefficient imdicated no significant relationship between cooking time and elasticity. However, correlation coefficinets between cooking time and elasticity for cheese made with individual emulsifying salts

Figure 16. Effect of cooking time at 82C on the degree of elasticity of cheese processed with sodium citrate, disodium phosphate, tetrasodium pyrophosphate and sodium aluminum phosphate. Elasticity was measured at 15.5C.

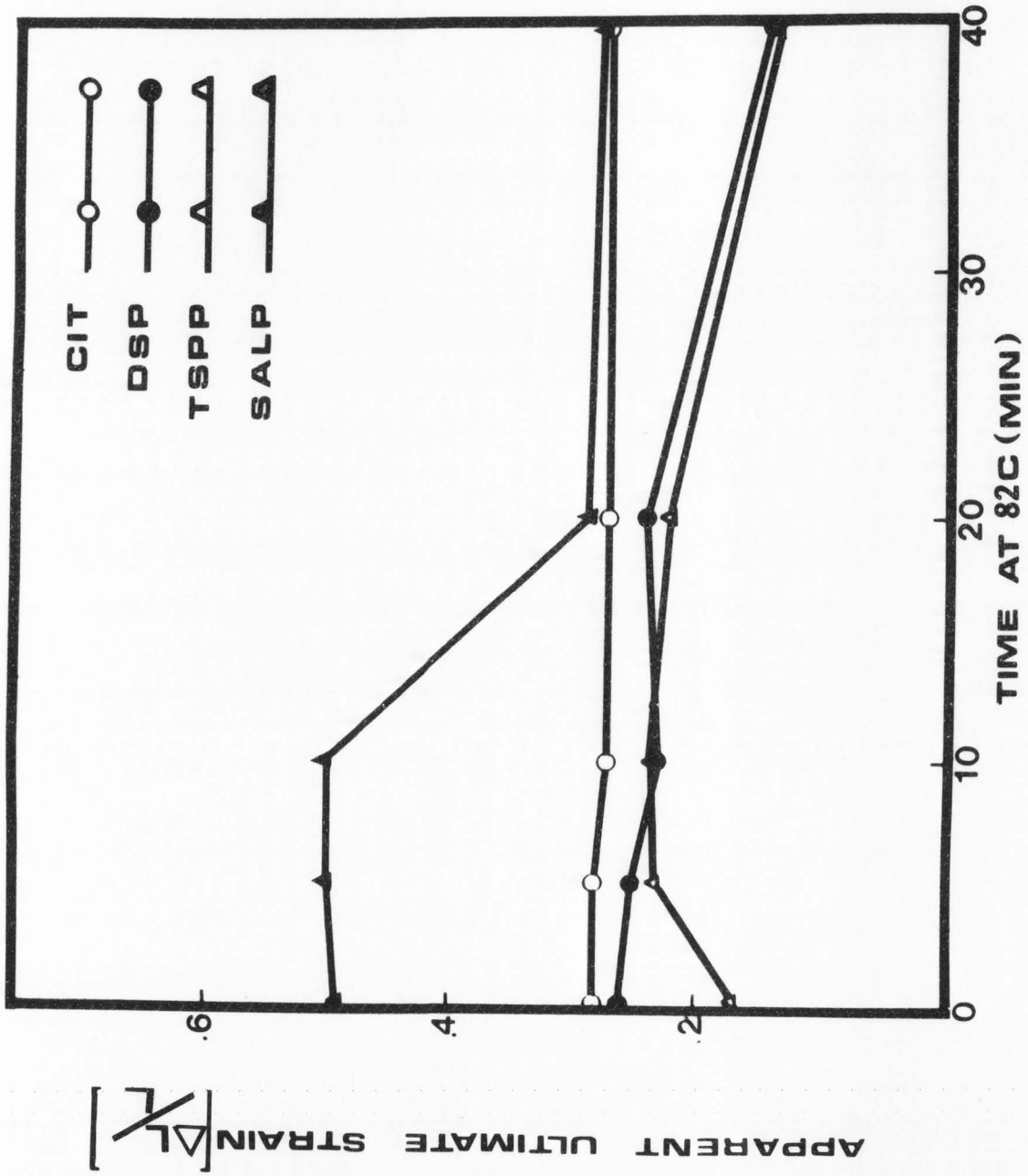

CIT
DSP
TSPP
SALP
.6
.4
.2
0
10
20
30
40
TIME AT 82 C(MIN)
DEGREE OF ELASTICITY $(\frac{\Delta L}{\Delta L})$

were 0.9714 for CIT, 0.7970 for DSP, 0.9067 for TSPP and 0.5534 for SALP. Highly significant relationships ($\alpha < 0.001$) were evident for the first three salts, but no significance was found between elasticity and cooking time for SALP cheese. The soft putty-like consistency of cheese processed with SALP and its ability to resist physical change during cooking was again illustrated by these elasticity measurements (figure 16).

Apparent Ultimate Strain (AUS)

The stretchability of process cheese was measured as apparent ultimate strain because there was a constantly decreasing cross sectional area of the strip of cheese as it was stretched from length L to length L + Δ L (Poisson's effect). True ultimate strain would have required a knowledge of the cross sectional area of the cheese strip at the time it broke. Figure 17 shows that the SALP cheese exhibited very much greater AUS than all the other samples up to 10 min in the cooker. After 20 min cooking it became somewhat comparable to that of the other samples and did not change much during the last 20 min. The AUS of CIT cheese remained relatively constant throughout cooking while that of DSP and TSPP cheese was quite constant up to 20 min cooking, but decreased between 20 and 40 min cooking. Overall analysis of variance (table 28) indicated highly significant ($\alpha < 0.001$) effects of the different emulsifying salts on AUS and a significant ($\alpha < 0.05$) relationship between cooking time and AUS. There was no significance between replicates. The overall Pearson correlation coefficient between cooking time and AUS was 0.2126 and

Figure 17. Effect of cooking time at 82C on the apparent ultimate strain of cheese processed with sodium citrate, disodium phosphate, tetrasodium pyrophosphate and sodium aluminum phosphate. Apparent ultimate strain was measured at 15.5C.


CIT
DSP
TSPP
SALP
.6
.4
.2
0
10
20
30
40
TIME AT 82C (MIN)
APPARENT ULTIMATE STRAIN [ΔL/L]

not significant. Correlation coefficients between cooking time and AUS for individual emulsifying salts were -0.3555 for CIT, -0.7782 for DSP, -0.2858 for TSPP and 0.6386 for SALP. The correlation between cooking time and AUS for DSP cheese was highly significant ($\alpha < 0.05$). The same relationships for the other two salts were not significant.

Apparent Ultimate Stress (AUST)

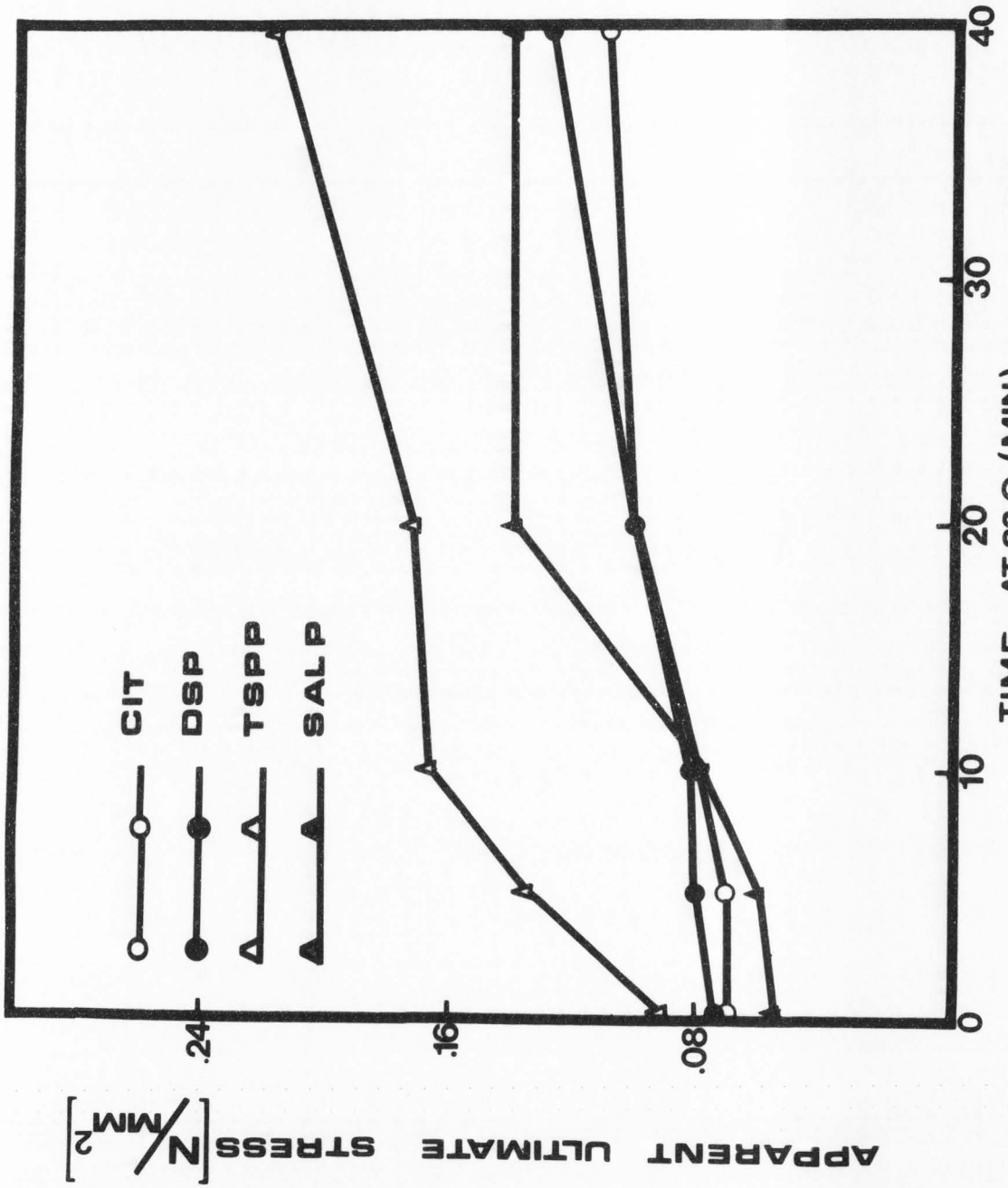
Cheese processed with TSPP required the greatest force to break the test strip during tensile testing. Figure 18 shows the relationship among the cheese samples with respect to AUST. In general AUST increased in all samples as cooking time increased.

Analysis of variance (table 30) indicated a highly significant ($\alpha < 0.001$) relationship between the type of emulsifying salt used and AUST. It also indicated a significant ($\alpha < 0.05$) overall relationship between cooking time and AUST, and no significant differences between replicates. The overall Pearson correlation coefficient (table 20) between cooking time and AUST was not significant. Similar correlations between cooking time and AUST for individual salts were 0.7177 for CIT, 0.8716 for DSP, 0.9672 for TSPP and 0.1595 for SALP. The significant correlations were CIT ($\alpha < 0.05$), DSP ($\alpha < 0.01$) and TSPP ($\alpha < 0.01$).

Comparison of Rheological Measurements

Some of the rheological measurements used in this study appeared to reflect similar or nearly similar physical properties. Future

Figure 18. Effect of cooking time at 82C on the apparent ultimate stress of cheese processed with sodium citrate, disodium phosphate, tetrasodium pyrophosphate and sodium aluminum phosphate. Apparent ultimate stress was measured at 15.5C.


APPARENT ULTIMATE STRESS [N/MM²]
.24
.16
.08
0
10
20
30
40
TIME AT 82 C (MIN)
CIT
DSP
TSPP
SAL P

experiments on the rheology of process cheese could benefit from knowing which measurements reflect similar properties. Some could then be eliminated.

Statistical correlations between each pair of rheological measurements were examined. Those with significant relationships are recorded in table 7. Correlations between meltability and firmness and between toughness and breaking force were highly significant ($\alpha < .01$) on the basis of the overall Pearson correlation coefficient (table 20) plus all four Pearson correlation coefficients for cheese made with the individual emulsifying salts (tables 10, 11, 12 and 13). The correlation between meltability and firmness was negative while that between toughness and breaking force was positive.

A significant negative correlation ($\alpha < .05$) was found between meltability and breaking force on the basis of overall Pearson correlation coefficient plus correlation coefficients for cheese made with each individual emulsifying salt.

It may be unnecessary to make both meltability and firmness measurements or toughness and breaking force measurements in future rheological studies on process cheese. It is also possible that meltability and breaking force are sufficiently correlated to make it unnecessary to run both tests.

The significance of other correlations involving the overall Pearson correlation coefficients plus similar coefficients for cheese made with three of the four emulsifying salts is shown in table 7. There is a possibility that some of these measurements may be sufficiently related to justify the elimination of additional

Table 7. Significant correlations between pairs of rheological measurements on process cheese.

Rheological pair	*Significance of overall correlation plus correlations of four salts	**Significance of overall correlations plus correlations of three salts
	***	***
Meltability - Firmness	$\alpha < .01$ (-)	
Toughness - Breaking Force	$\alpha < .01$ (+)	
Meltability - Breaking Force	$\alpha < .05$ (-)	
Meltability - Toughness		$\alpha < .05$ (-)
Firmness - Toughness		$\alpha < .01$ (+)
Firmness - Breaking Force		$\alpha < .01$ (+)
Elasticity - Breaking Force		$\alpha < .01$ (-)
AUST - Breaking Force		$\alpha < .05$ (-)
Hysteresis - Stiffness		$\alpha < .01$ (+)
Hysteresis - Elasticity		$\alpha < .01$ (+)
Hysteresis - Breaking Force		$\alpha < .05$ (-)
Firmness - AUST		$\alpha < .05$ (-)
Stiffness - Breaking Force		$\alpha < .05$ (-)

*Significance level for overall correlation and correlations for cheese made with all four salts.

**Significance level for overall correlation and correlations for cheese made with three of the four salts.

***Indicates positive or negative correlation.

measurements. However, it is suggested that more work is needed before such a recommendation is made.

Microstructure

CIT cheese. Figure 19 represents SEM micrographs of fractured process cheese heated to 82C for 0, 5, 10, 20 and 40 min (A, B, C, D and E). The micrographs show a continuous reduction in the dimension of fat masses during cooking. The same change is seen by comparing A (0 min) with B (40 min) in figure 20. At 0 time the fat particles, seen as dark empty cavities, vary greatly in size from very large ones to small ones the size of bacteria. This image is confirmed by TEM at 0 time (Figure 20 C) where large fat masses may be seen along with very small fat particles. During cooking the large fat particles disintegrated; the process has been captured in TEM micrographs D (10 min) and E (40 min) in figure 20. Constriction associated with the large fat particles are sites where they will disintegrate into smaller particles. Figure 20 C, D and E represent TEM micrographs showing needle-like spaces that had been occupied by undissolved CIT crystals. Crystals at 0 time are in C. Their dimensions gradually decreased during cooking D (10 min) and E (40 min), but even after 40 min in the cooker some small CIT crystals still remained undissolved. Figure 20 D also shows a crystal of calcium phosphate that was probably originally present in the cheese (56, 68).

Figure 19. SEM of fractured process cheese emulsified with CIT and heated to 82C for 0, 5, 10, 20 and 40 min. The micrographs show a progressive change from coarse to fine emulsion with increased cooking time.

A = SEM at 0 time showing large and small fat cavities. Crystals of CIT shown in the lower right corner.

B = SEM after 5 min cooking. Note reduction in size of fat cavities from A.

C = SEM after 10 min cooking are smaller and more numerous than in B.

D = SEM after 20 min cooking. The protein matrix is more dense than in C. Fat cavities are about the same size as in C.

E = SEM at 40 min cooking. Fat cavities are much smaller than in D. Note the very fine fat emulsion compared to all other samples.

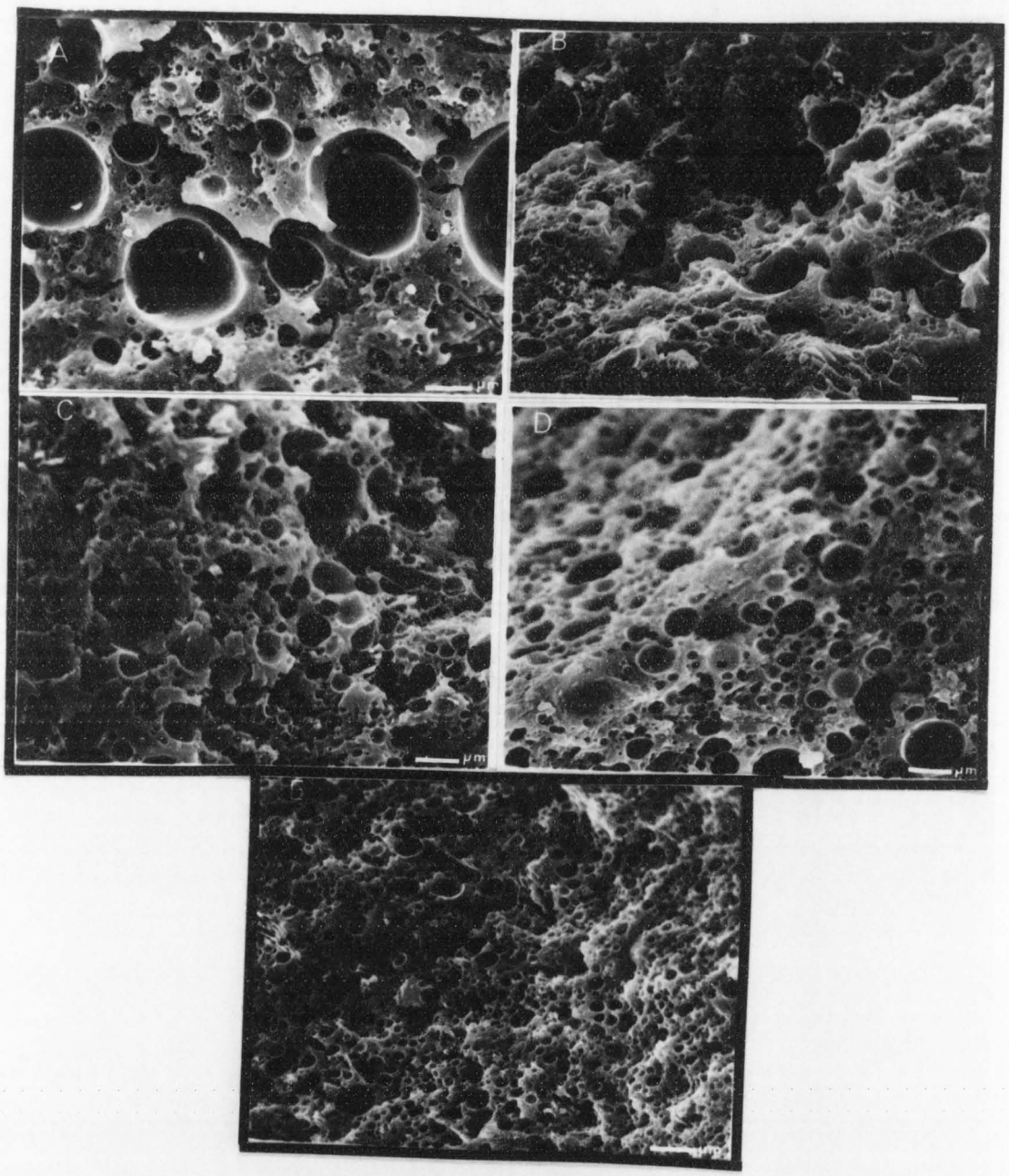
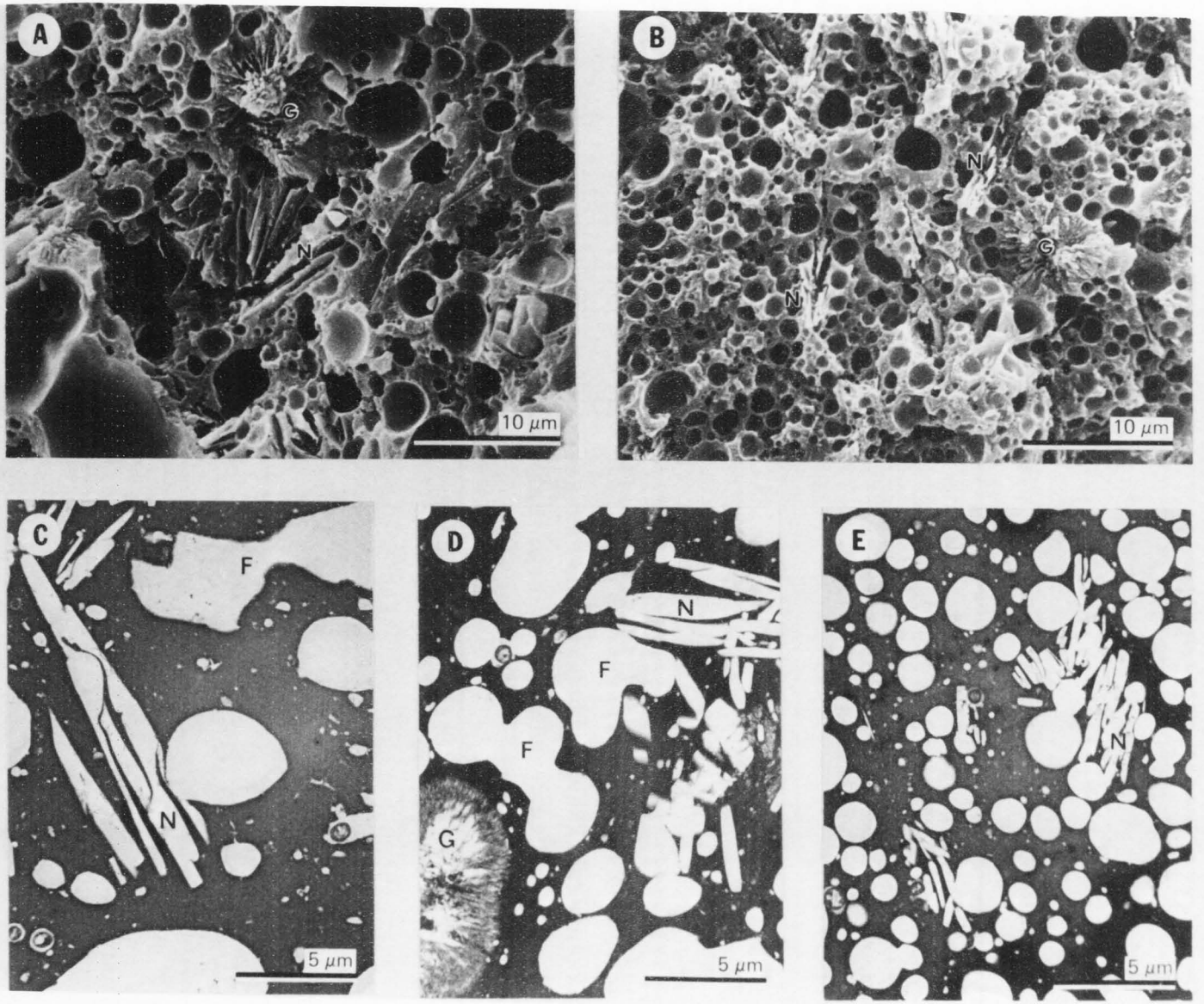

A
B
C
D
E
μm

Figure 20. Development of microstructure in process cheese in the presence of sodium citrate (CIT) (Courtesy M. Kalab). A = SEM at 0 time. G = Initially present calcium phosphate crystal. N = Crystals of added sodium citrate. B = SEM after 40 min in the cooker. G and N same as in A. C = TEM at 0 time. Dark areas are the protein matrix. F = Fat particles undergoing emulsification. N = Crystals of added sodium citrate. D = TEM after 10 min in the cooker. F = Fat particles being divided into smaller particles at the constrictions. G = Initially present crystal of calcium phosphate. N = Needle-like crystals of added sodium citrate. E = TEM after 40 min in the cooker. Dimensions of the fat particles (round light areas) and crystals of added sodium citrate (N) have been reduced during the 40 min process.


A
G
N
10 μm
B
N
N
G
10 μm
C
F
N
5 μm
D
N
F
F
G
5 μm
E
G
N
5 μm

DSP Cheese. Figures 21 and 22 represent SEM and TEM micrographs of cheese processed with DSP. As with CIT samples, there was a reduction in the size of the fat particles with increasing time in the cooker (Figure 21 A, B, C, D and E). Compare SEM micrographs in figure 21 A and E with figure 22 A and B (0 time and 40 min respectively). The same disintegration of irregularly sized fat masses as seen with other salts can be found in TEM figure 22 C (0 time) and 22 E (40 min). A crystal of calcium phosphate with a hairy structure as reported by Brooker et al. (56) is shown under high magnification in TEM, figure 22 D. The hairy structure suggests that the crystal was growing. A similar crystal under lower magnification is in figure 22 E. Cheese processed with DSP generally contained larger pockets of fat than CIT cheese at 0 time and the emulsion was not as fine after 40 min in the cooker. Compare figure 19 E with 21 E and figure 20 B with 22 B.

TSPP Cheese. Figures 23 and 24 illustrate the reduction in the fat size from a very coarse to a very fine emulsion during cooking of cheese processed with TSPP. Compare SEM micrographs in figure 23 A and E with those in figures 24 A and B in which SEM micrographs A in both figures represent 0 time and micrographs E and B represent 40 min in the cooker at 82C. Also compare TEM micrographs in figure 24 F (0 time) and G (40 min). "Torch like" TSPP crystals are evident in TEM micrographs in figure 24 D and in SEM micrograph C which also suggest that the crystals are growing. Another rather common but unidentified crystalline form found in TSPP cheese is shown in figure 24 E.

Figure 21. SEM of fractured process cheese emulsified with DSP and heated to 82C for 0, 5, 10, 20 and 40 min. The micrographs show a progressive change from coarse to fine emulsion with increased cooking time.

A = SEM at 0 time showing large fat particles embedded in a heavy protein matrix compared to citrate cheese at 0 time. Large fat cavities have started to be emulsified into smaller particles.

B = SEM after 5 min cooking. Note reduction in size of fat cavities from A. Crystals of calcium phosphate which were already present in the initial cheese (56) or had grown in the process cheese are shown in the upper left corner and in the lower right center of the micrograph.

C = SEM after 10 min cooking. Fat cavities are smaller and more numerous than in B but some large ones are still present. Note the calcium phosphate crystals in the right center.

D = SEM after 20 min cooking. The protein matrix is more dense than in C. Note the reduction in size of fat cavities from C.

E = SEM after 40 min in the cooker. Fat has undergone considerable emulsification and is represented by very small cavities compared to all other samples.

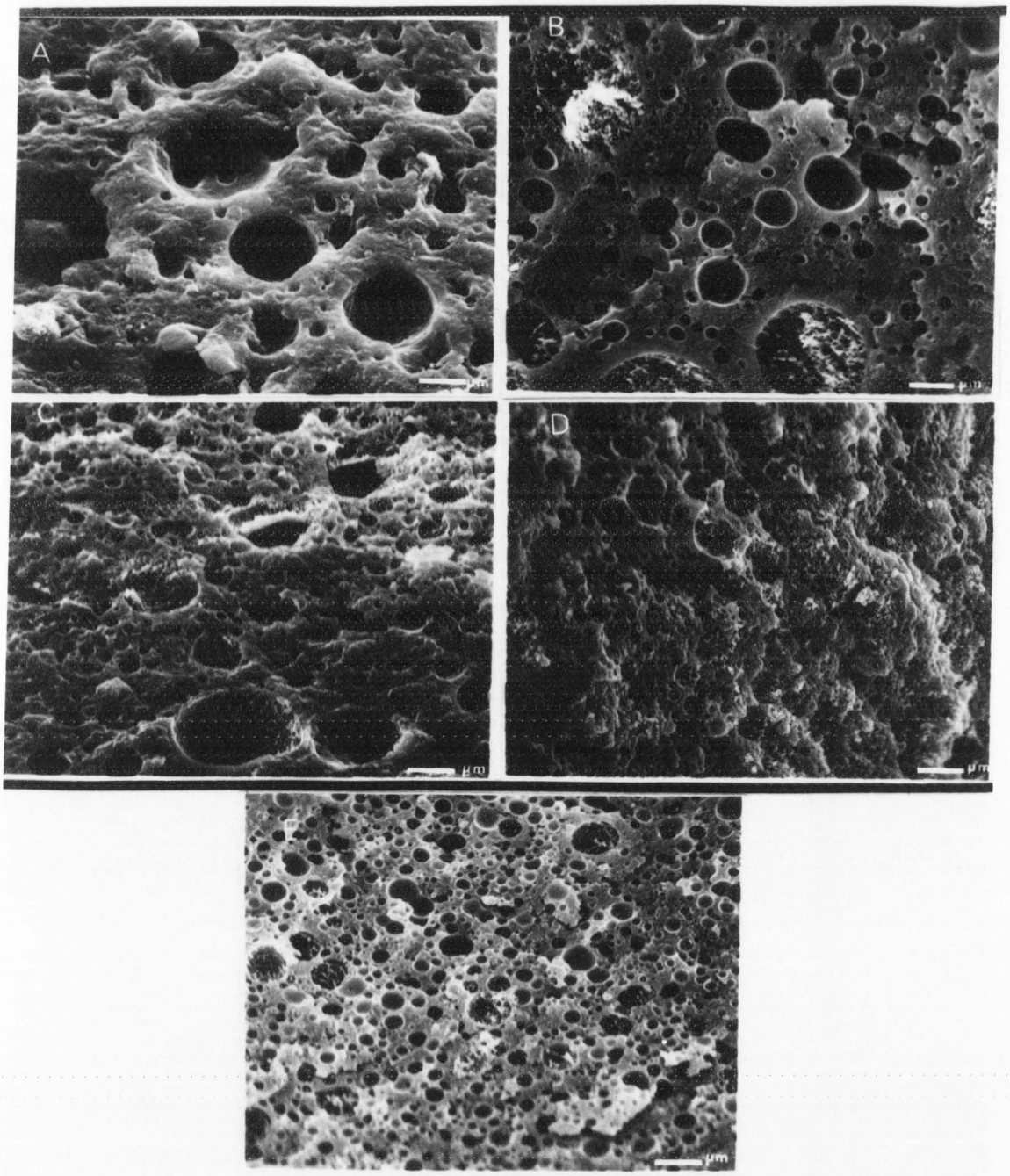

A
B
C
D

Figure 22. Development of microstructure in process cheese in the presence of disodium phosphate (DSP) (Courtesy M. Kalab). A = SEM at 0 time. Large fat particles (F) have started to be emulsified into smaller particles. B = SEM after 40 min in the cooker. Fat has undergone emulsification and is present in the form of small globular particles. Arrows show calcium phosphate crystals which had been already present in the initial cheese. C = TEM at 0 time. Dark areas are protein, light areas were occupied by fat. Grey areas indicate residual fat that had been removed during preparation of the specimen for TEM. D = TEM detail of an initially present calcium phosphate crystal showing additional growth of fine spikes at the perimeter. E = TEM after 40 min in the cooker. G - An initially present calcium phosphate crystal showing signs of additional growth. F = Fat particles undergoing emulsification.

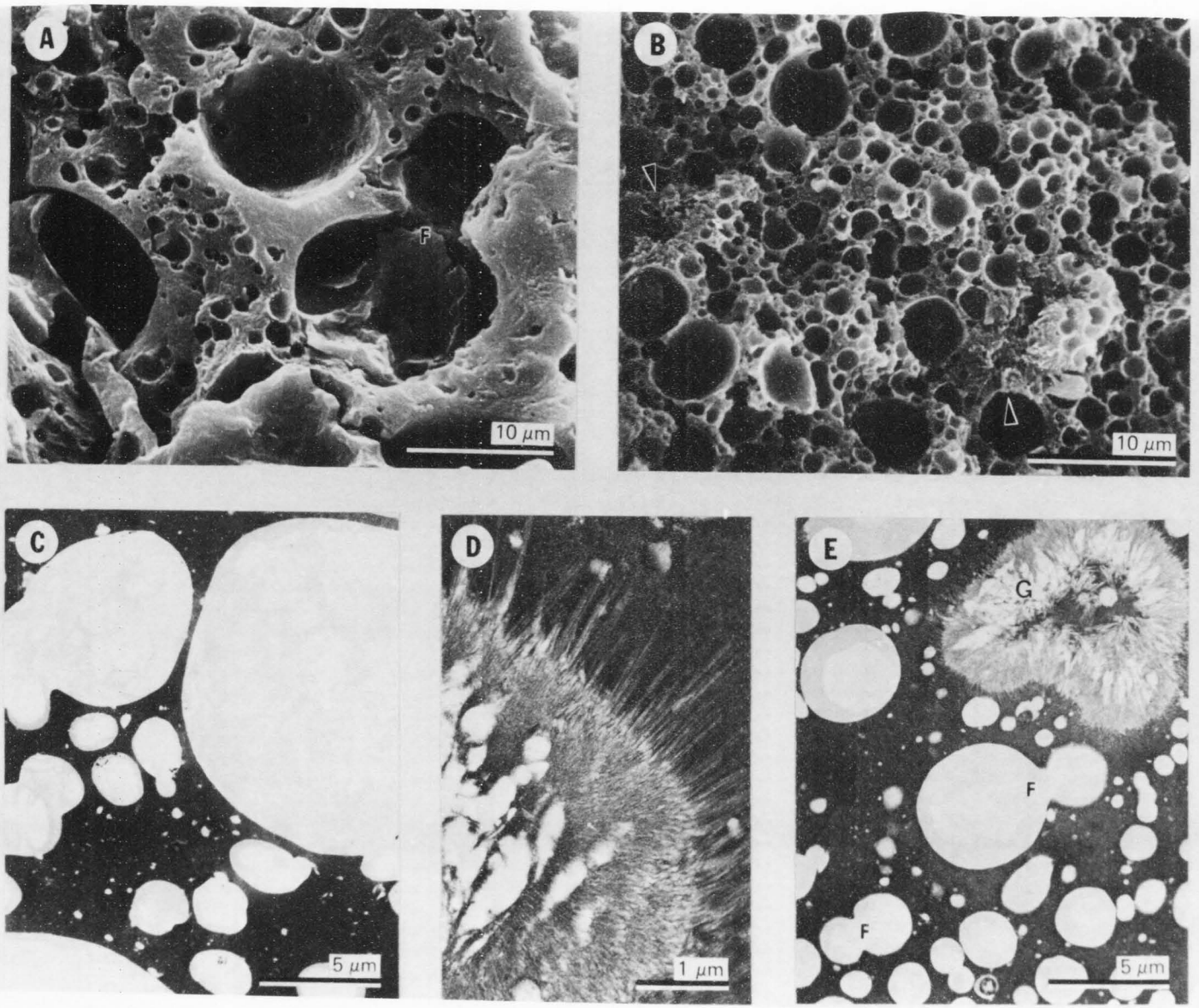

A
F
10 μm
B
10 μm
C
5 μm
D
1 μm
E
G
F
F
5 μm

Figure 23. SEM of fractured process cheese emulsified with TSPP and heated to 82C for 0, 5, 10, 20 and 40 min. The micrographs show a heavier initial protein matrix than those processed with the other salts; also the rate of emulsification seemed more rapid. Even so a progressive change to a fine emulsion with increased cooking was evident.

A = SEM at 0 time showing one large cavity initially occupied by fat and relatively small ones indicating the rapid emulsifying power of TSPP.

B = SEM after 5 min cooking. Note the disintegration of large cavities to small ones. An unidentified crystal is shown in the upper left center of the micrograph.

C = SEM after 10 min cooking. Fat cavities are smaller and more numerous than in B. Salt crystals are shown in the upper left center.

D = SEM after 20 min cooking. The protein matrix is more dense than in C and fat cavities are smaller and more numerous.

E = SEM after 40 min cooking. Fat cavities are much smaller than in D. Note the very fine fat emulsion compared to all other samples. The final emulsion formed with TSPP was finer than that formed with any other emulsifying salt.

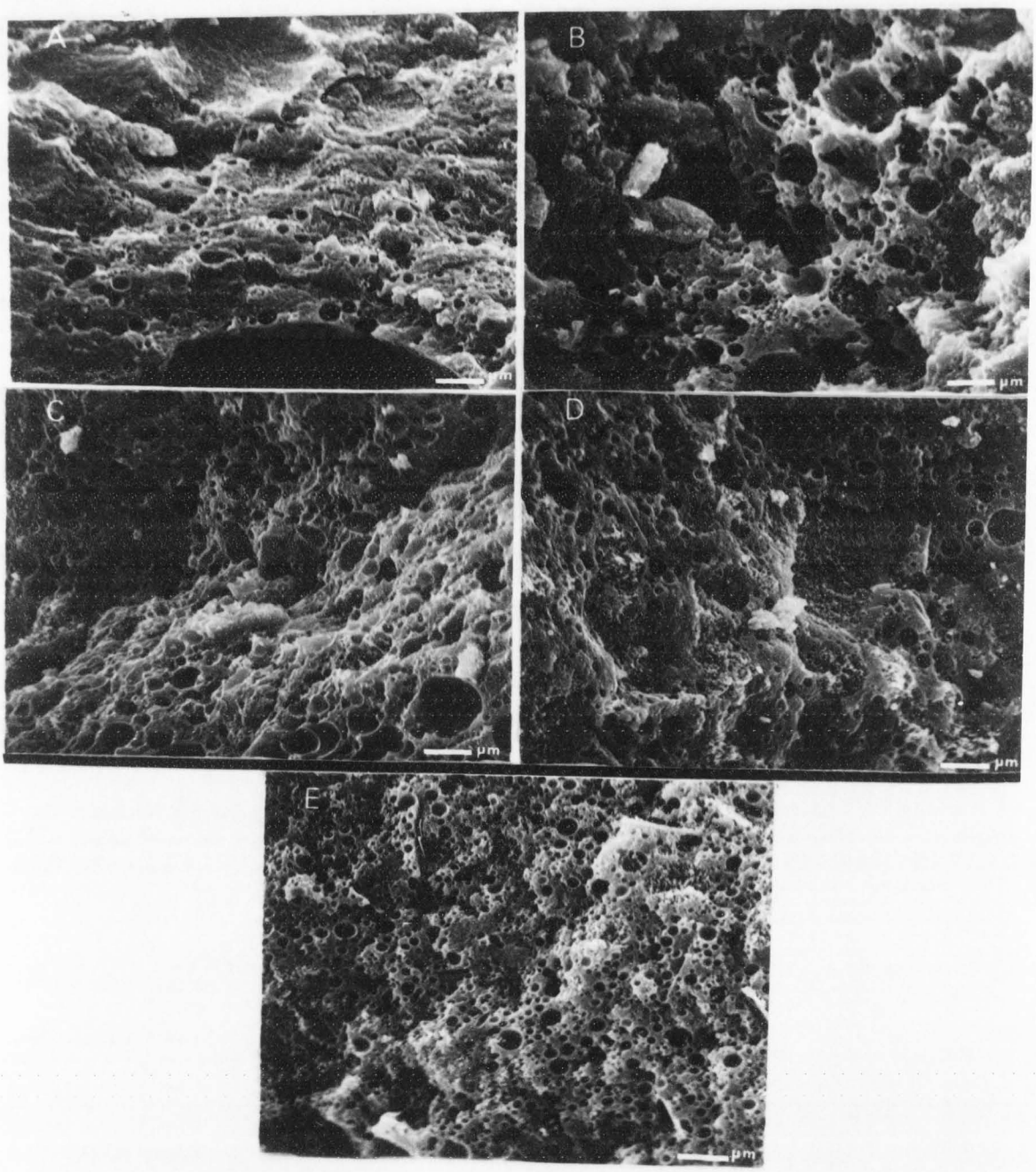

A
B
C
D
E
μm

Figure 24. Development of microstructure in process cheese in the presence of tetrasodium pyrophosphate (TSPP) (Courtesy M. Kalab).

A = SEM at 0 time. F - Two cavities initially occupied by fat.

B = SEM after 40 min in the cooker. Arrows show crystals of added TSPP.

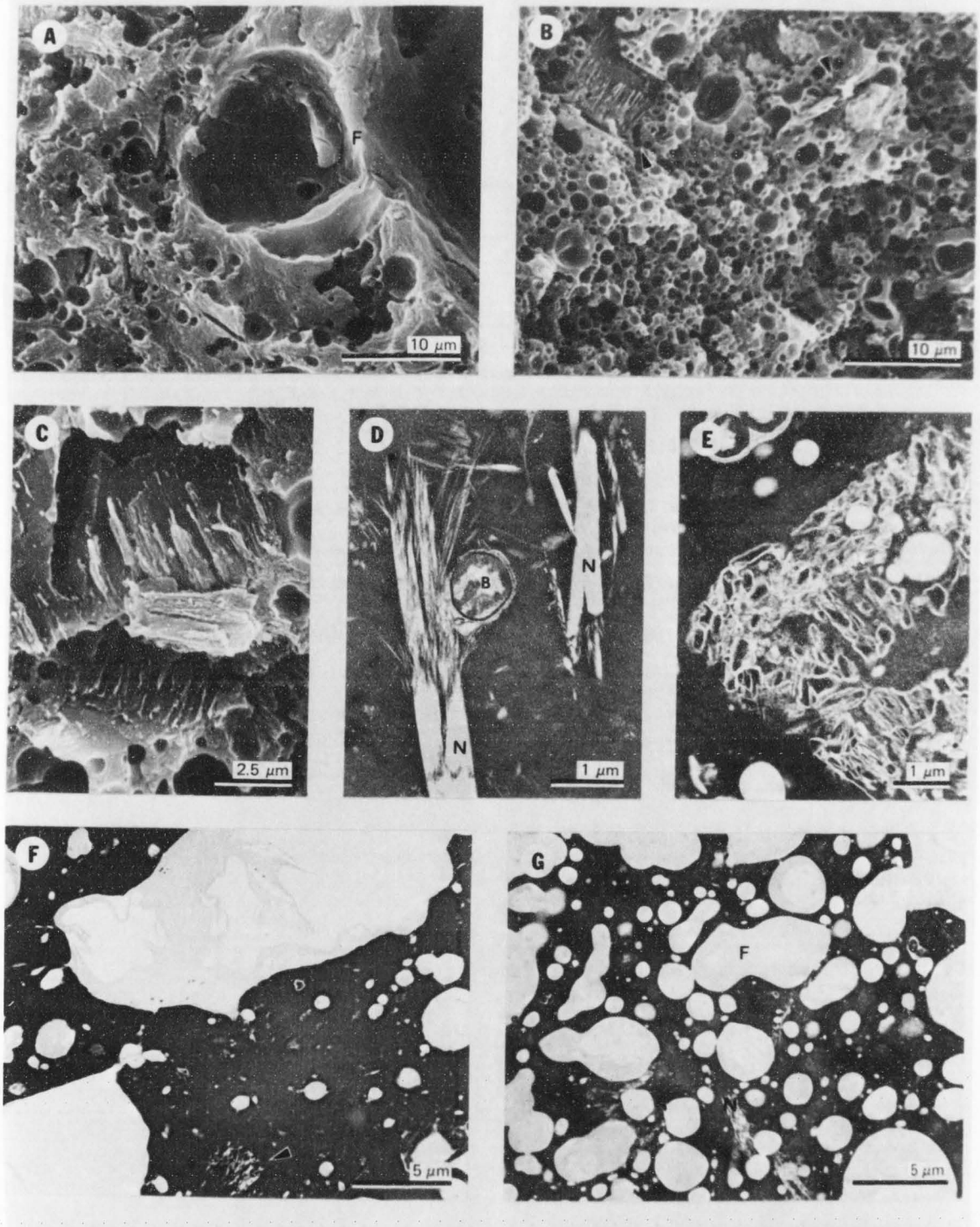
C = An SEM detail of TSPP crystal in the cheese protein matrix.

D = TEM detail of TSPP crystals (N); long thin spikes extending from the compact bodies of the crystals give them a torch-like appearance, which is a sign of recrystallization.

E = TEM detail of abundant yet unidentified crystal-like structures.

F = TEM of the cheese at 0 time. Lace-like structure (arrow) is a tip of an identified crystal.

G = TEM after 40 min in the cooker. Fat still undergoes emulsification (F). N = Spikes of torch-like TSPP.


A
F
10 μm
B
10 μm
C
2.5 μm
D
B
N
N
1 μm
E
1 μm
F
5 μm
G
F
5 μm

TSPP cheese appeared to emulsify more rapidly and to a greater extent than cheese made with any of the other emulsifying salts. The final emulsion also was much finer than in the other cheeses.

SALP Cheese. Figures 25 and 26 show that the emulsification process with SALP proceeded more slowly than with the other salts as judged by SEM figure 25A, B, C, D and E. The emulsion in figure 26 B (10 min) has not progressed much beyond that shown in 26 A (0 time). SEM and TEM micrographs in figure 26 C and E (40 min) indicate that emulsification is still progressing. Very large fat masses at 0 time are shown in the TEM micrograph 26 D. Undissolved SALP crystals were found in abundance during the initial 10 min in the cooker as shown in TEM micrograph 26 F.

Light microscopy. The cavities shown in SEM micrographs were identified as areas occupied by fat prior to fixing, dehydration and defatting the specimens. While this was verified by TEM, (see shadows of unextracted fat in TEM micrograph in figure 24 F), an effort also was made to examine a sample of process cheese under the light microscope after subjecting the specimen to differential staining for fat and protein. In figure 27, a stained frozen microtome section (one micron) of CIT cheese processed at 82C for 0 min shows lipid material stained with oil red O (red and orange) and protein material stained with hematoxylin (blue). Appearance of the fat is similar to that shown in electron microscopy with discontinuous fat masses dispersed in a continuous protein phase. This lends additional support to substantiate the identity of fat cavities in the SEM micrographs.

Figure 25. SEM of fractured process cheese emulsified with SALP and heated to 82C for 0, 5, 10, 20 and 40 min. The micrographs show a progressive change from coarse to relatively medium size fat emulsion with increased cooking time. Emulsification was slower than with other emulsifying salts.

A = SEM at 0 time showing large fat cavities along with medium sized ones.

B = SEM after 5 min cooking. Note the relatively large fat cavities still remaining. They are only slightly smaller than in A. This suggests that SALP has poorer emulsifying power than the other salts.

C = SEM after 10 min cooking. Fat cavities are smaller and more numerous than in B but some large ones are still present. The protein matrix is denser than B.

D = SEM after 20 min cooking. The protein matrix is more dense than in C. Most fat cavities are about the same size as in C.

E = SEM after 40 min cooking. Most fat cavities are smaller than in D but is still progressing. Fat cavities seem to lack the depth noted in the other cheese samples with comparable treatment.

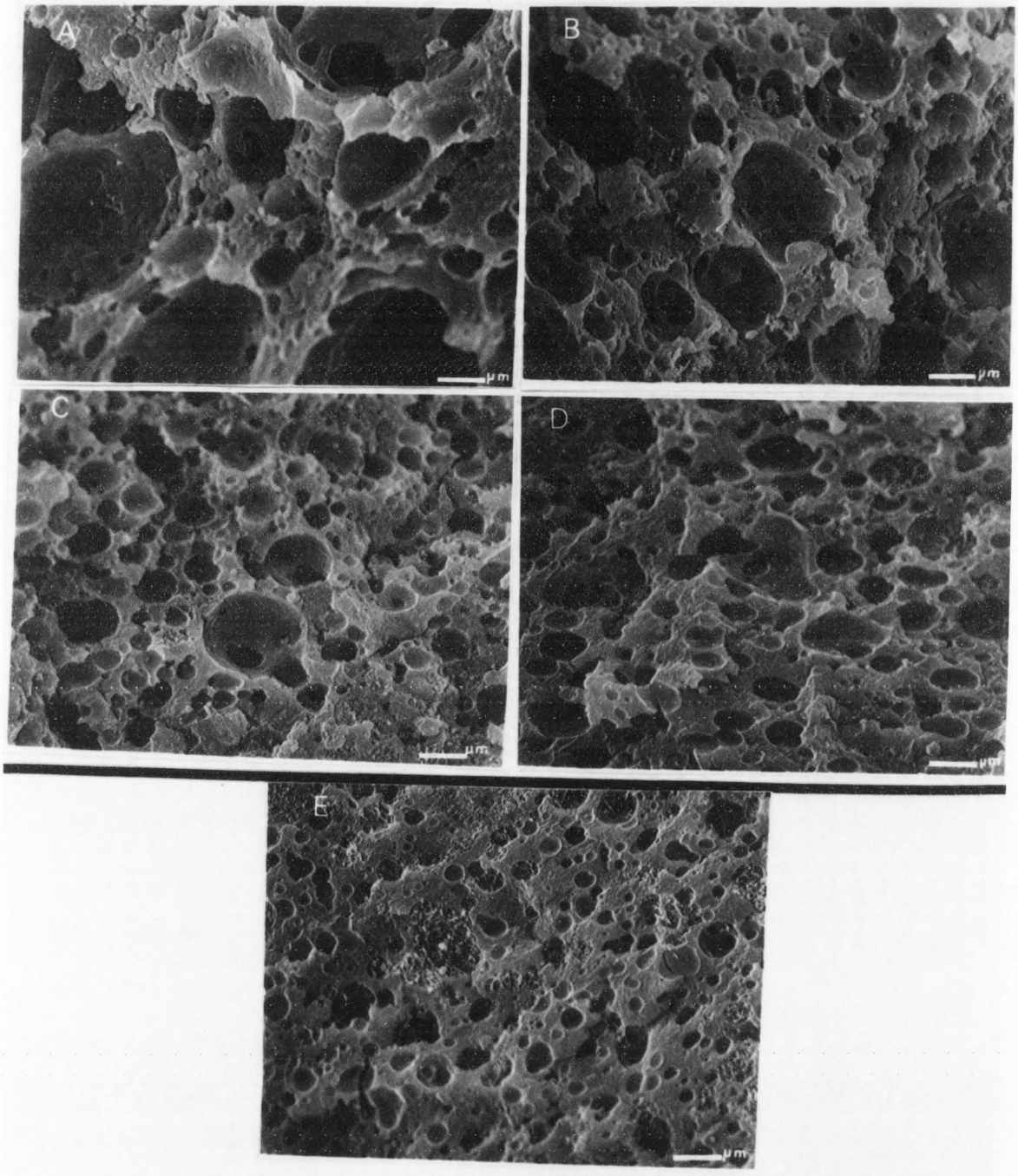

A
μm
B
μm
C
μm
D
μm
E
μm

Figure 26. Development of microstructure in process cheese in the presence of sodium aluminum phosphate (SALP) (Courtesy M. Kalab).

A = SEM at 0 time. Large fat particles (F) started to be degraded into smaller particles. G = Fragmented of an initially present calcium phosphate crystal.

B = SEM after 10 min in the cooker. Fat is still in the form of large particles, many of which are being emulsified (F) into smaller particles.

C = SEM after 40 min in the cooker. Some fat particles (F) are still undergoing emulsification.

D = TEM at 0 time. Dark areas are the cheese protein matrix, light areas indicate fat.

E = TEM after 40 min in the cooker. The emulsification process has not been completed and fat particles (F) are still undergoing emulsification.

F = TEM detail of one of the added SALP crystals found in abundance during the initial 10 min in the cooker.

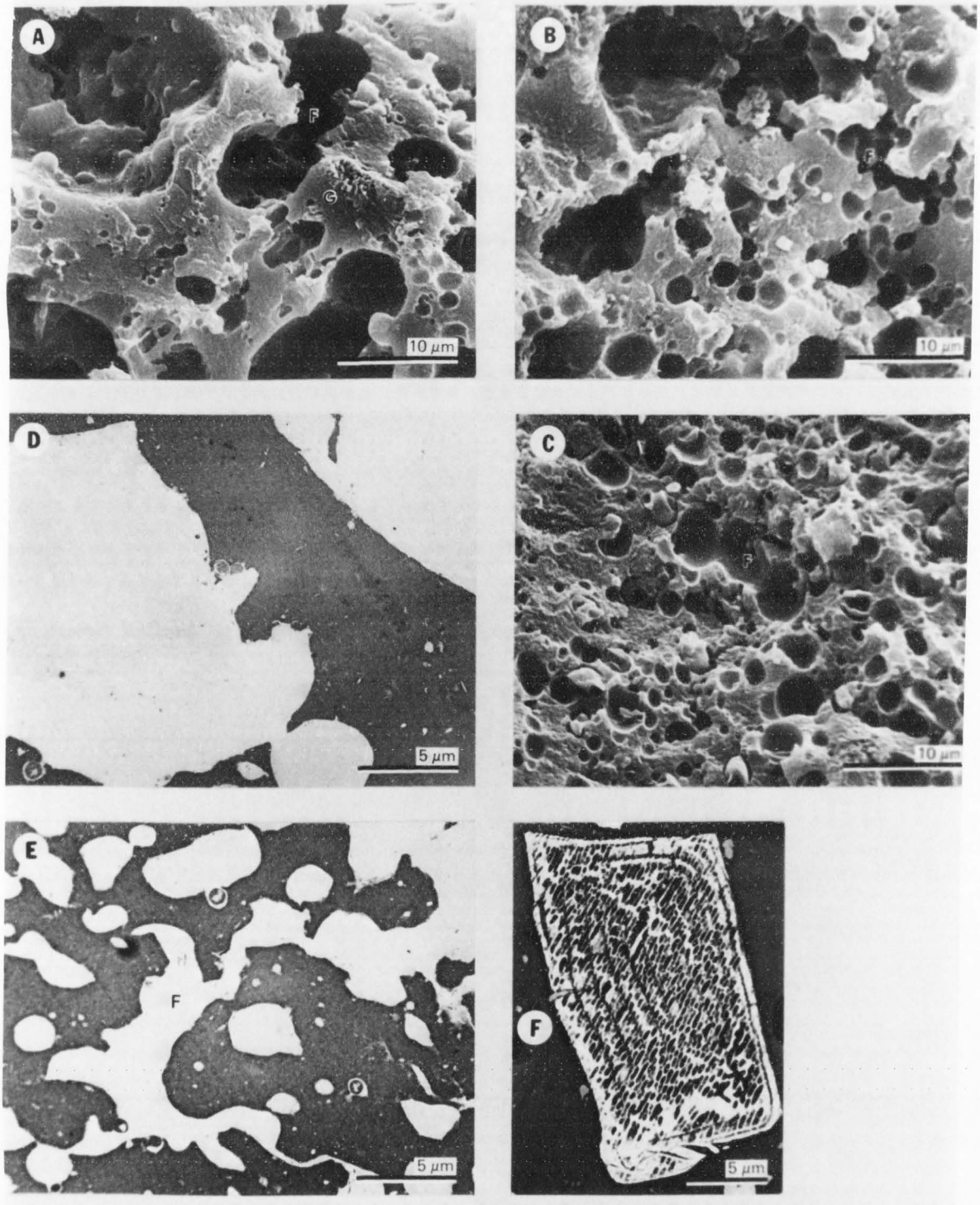
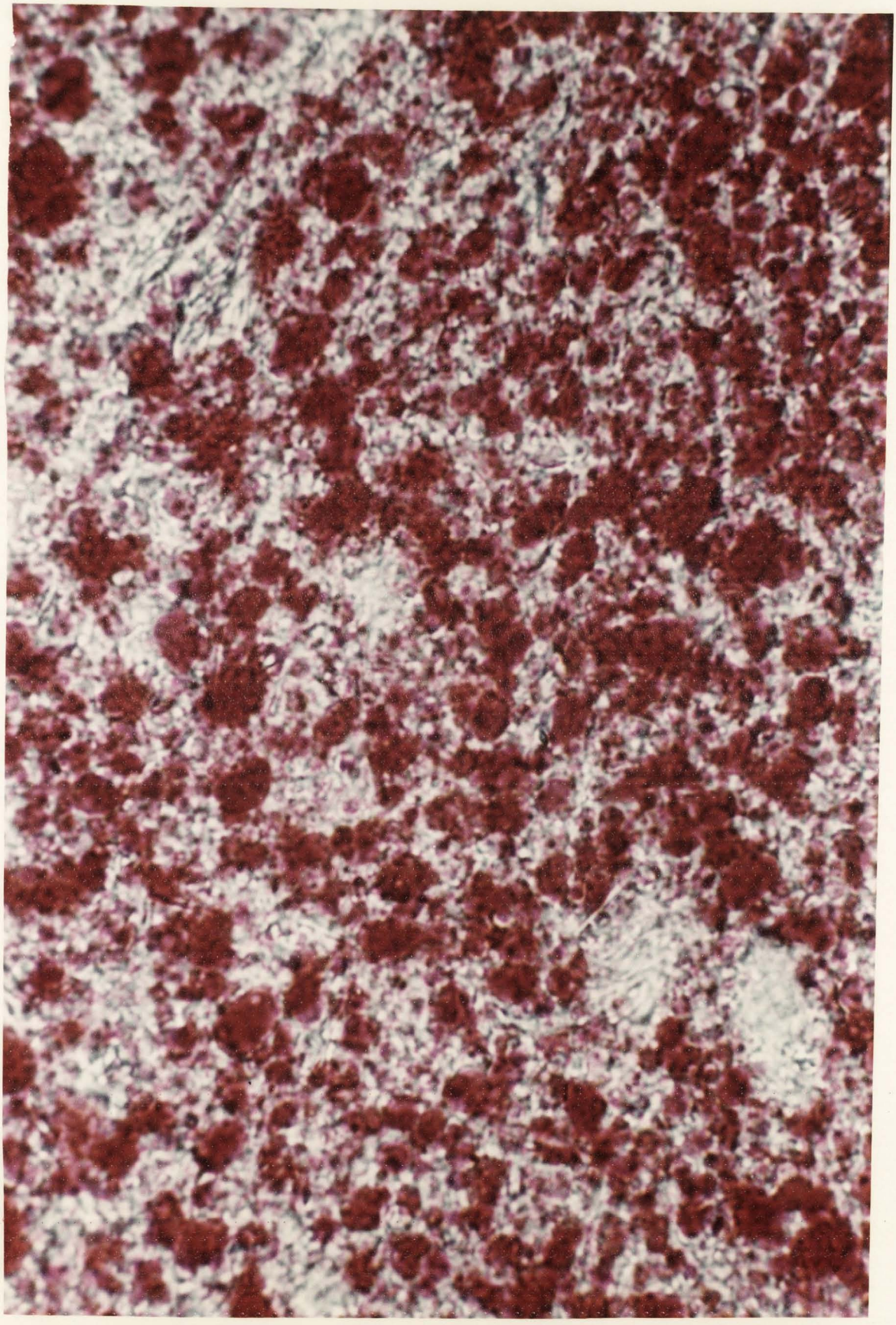

A
F
G
10 μm
B
F
10 μm
D
5 μm
C
F
10 μm
E
F
5 μm
F
5 μm

Figure 27. Light microscope micrograph of a stained frozen section of CIT cheese processed at 82C for 0 time. Lipid material is stained with oil red O and the protein matrix is stained with hematoxylin (blue).



DISCUSSION

Emulsifying salts used in the manufacture of process cheese, process cheese food and process cheese spreads function under the influence of heat to solubilize the cheese proteins, raise the pH of the cheese and form a stable fat emulsion (234). All of the approved emulsifying salts also are good calcium sequestering agents (91) which make them partly responsible for increasing protein solubility.

When the cheese used in this study was being processed, and before its temperature reached 60C the fat emulsion of the natural cheese was completely destabilized. A mass of partially melted cheese was literally swimming in a sea of free fat inside the cooker. As the temperature continued to increase the fat was re-emulsified back into the cheese mass. Without the emulsifying salts the fat never would have been reincorporated into the cheese. If heat is applied to process cheese more rapidly than was possible with the cooker used in these studies, the initial de-emulsification of the cheese fat is less dramatic. Nevertheless, the formation of a stable fat emulsion in process cheese is one of the main functions of the emulsifying salts (40).

These salts cannot be looked upon as functioning in the same way as traditional dipolar emulsifying agents that are normally used in preparing food emulsions (234). It is postulated that they react in a rather poorly defined way with the cheese proteins to increase

the emulsifying properties of the proteins themselves (91). This explanation would require that the modified proteins would then function as emulsifying agents. Such a process has been recognized in stabilizing the fat emulsions in processed meat products (91).

Bosy et al. (41) reported that it required only half as much emulsifying salt to satisfactorily process cheese if the salt was added to the cooker in the dissolved rather than the dry state. This suggests that the salts must be in solution before they can react with the protein, and could account for the slow rate of emulsification encountered in these studies where all the salts were added in the dry form. Furthermore, it was noteworthy that even after 40 minutes at a cooking temperature of 82C some of the emulsifying salts still remained undissolved.

The emulsifying salts were added at a concentration of 2.5% of the finished product. This was 0.5% less than the maximum concentration allowed by the Federal Standards of Identity for process cheese (103). It also represented an emulsifying salt concentration of 5.8% in the aqueous phase. The solubility of these salts in aqueous solution is 42% at 25C and 62.5% at 100C for sodium citrate, 44% at 50C and 48% at 80C for disodium phosphate and 3.1% at 0C and 28.7% at 100C for tetrasodium pyrophosphate (144A). The solubility of sodium aluminum phosphate was not given because it varies substantially in composition (212). It is unlikely that the solubility of any of these salts was exceeded in the cheese with the possible exception of tetrasodium pyrophosphate during low temperature storage. The presence of other substances dissolved

in the water phase of the cheese no doubt reduced the solubility of the salts. There are also other factors such as immobilization of the aqueous phase by strong association of the water in they pydrated protein. This could reduce the solvent power of the water. Even so it is difficult to understand why the sodium citrate did not dissolve completely during cooking. There is always the possibility that it did, but recrystalized during subsequent storage of the cheese at refrigerated temperatures. This seems unlikely however, because the sodium citrate crystals illustrated in the TEM micrographs (figure 20) became progressively smaller with increased time in the cooker. Had these crystals resulted from recrystallization this pattern probably would not be so evident.

Additional work is needed to determine the factors affecting the solubility of emulsifying salts in process cheese and how this solubility affects the amount of salt needed as well as the properties and structure of the cheese.

There is always the question of identity of the salt crystals seen under the electron microscope. Brooker et al. (56) was successful in identifying certain types of crystalline inclusions in cheese. Calcium phosphate crystals were identified in figures 20 D, 21 B, and 22 D of the basis of micrographs and descriptions provided by the above authors. These crystals probably were already present in the natural cheese before processing (M. Kalab, personal communication 1980). However, the hairy outer structure of the crystals suggest that they had been growing during storage of the process cheese. Ellinger (91) indicated that calcium phosphate crystalli-

zation can be a problem during storage of process cheese, particularly when the concentration of emulsifying salt is too high. Crystals other than calcium phosphate were assumed to represent the original emulsifying salt since the crystals in the cheese made with each salt were not identified in any of the cheese samples made with any of the other salts.

The SEM micrographs produced in this study clearly show that the state of the fat emulsion in process cheese progresses from coarse to fine with increased time in the cooker. This was observed with each of the four emulsifying salts. However, it also was evident that the rate of emulsification varied with the species of salt. SALP appeared to act slower in forming a fine emulsion during cooking than the other three salts. TSPP acted quite rapidly and easily produced the finest of all the emulsions at the end of the 40 minute cooking period. The other two salts were intermediate in this regard. This may mean that SALP reacts more slowly with the proteins than TSPP under cheese processing conditions, or it could mean that SALP - modified cheese protein simply has poorer emulsifying properties than TSPP - modified cheese protein.

There was an apparent relationship between some of the physical properties of the process cheese and the state of the fat emulsion in the cheese. Cheese with a fine emulsion was generally characterized as having poor meltability and body that was firm and tough with a high apparent stiffness modulus, high breaking force and high degree of elasticity. It was further characterized as having a low degree of hysteresis and a low value for apparent ultimate strain.

Conversely, the cheese with a coarse emulsion exhibited good meltability and other physical properties opposite to those just mentioned.

This does not suggest that there is a cause and effect relationship between the state of emulsion and the physical properties. They both may have resulted from some other less obvious changes during cooking. The effect of heat on the salt modified proteins, for example, could have contributed to both effects.

The meltability of cheese might be expected to decrease with the formation of a finer emulsion as the fat globules became disintegrated and dispersed (M.V. Taranto, personal communication 1980). This could prevent them from coalescing. As cooking time increased there developed a more uniform protein network. This was probably due to an interaction of the proteins with the emulsifying salts under the influence of heat and mechanical work imparted during the cooking process. The more uniform network of modified cheese protein would be expected to have fewer "weak links" and allow for a more uniform distribution of stress. This could then result in greater firmness, more elasticity, greater toughness, greater breaking force and perhaps poorer meltability.

The question is still unanswered as to whether these rheological changes can be attributed to the observed changes in the state of the emulsion or attributed to the effect of heat on the salt modified proteins or both. Heat could make the proteins tougher and less capable of disintegrating upon subsequent melting. This problem could only be answered by conducting experiments on model systems of isolated cheese proteins and emulsifying salts.

The rework defect encountered in commercial cheese processing occurs when too much process cheese is reworked back into a new batch. It is manifest by the cheese becoming very stiff and firm, even while in the cooker. If salt-modified cheese proteins are essential to the formation of suitably emulsified process cheese, and if there is a direct relationship between emulsification and firmness, one could argue that cheese that has been processed contains proteins that are already salt modified and are ready to function immediately when added to a new batch of natural cheese. If a normal charge of emulsifying salts also are added to such a batch, it is conceivable that it could very quickly become over emulsified and exhibit the characteristics of the rework defect.

Stauffer et al. (342) theorized that the rework defect resulted from hydrophobic areas on cheese proteins which interacted during reprocessing. These crosslinked hydrophobic bonds were considered to decrease melting while increasing the firmness of the cheese. They did not really establish this experimentally, but reported that surface active agents such as monoglycerides successfully eliminated the rework defect. They assumed that the surface active agent was able to break the hydrophobic protein bonds. This procedure has not been widely adopted by the industry and raises the question why cheese proteins that have been modified by emulsifying salts should exhibit more hydrophobic properties when the process cheese is known to become more hydrophyllic and water soluble during processing.

Further work on the rework defect should include experiments in which process cheese is mixed in varying proportions into new batches with and without additional emulsifying salts. The cheese should then be examined by SEM to determine the state of the emulsion. Its rheological properties also should be assessed. This would clarify the point as to whether salt-modified cheese proteins are capable of functioning as emulsifiers in the absence of additional salts.

Two major factors affected the melting quality of process cheese in this study: selection of the type of emulsifying salt and cooking time. They also had a marked effect on the other physical properties of the cheese.

This study included a large number of rheological measurements, many of which were statistically related to the type of emulsifying salt or to the time in the cooker or both. There were adequate replications (8 for all cheese except the SALP cheese which had 6) to enable good statistical evaluation of the effect of the emulsifying salts and cooking time on the meltability of the cheese.

The emulsifying salts had a highly significant effect on the meltability of the cheese at all stages of cooking with the exception of CIT and SALP cheese whose meltability was the same after 5 min in the cooker, and DSP and TSPP cheese whose meltability was the same after 20 min cooking. At 0 time the SALP and CIT cheese were both very meltable. However as cooking time increased their meltability decreased rapidly up to 20 min after which there was little change. DSP and TSPP cheese posessed very poor melting characteris-

tics at 0 time and this property became progressively worse with increased time in the cooker.

The changes in meltability during cooking for cheese processed with each emulsifying salt were highly significant. The changes shown in figure 10 can be attributed to cooking time and emulsifying salts since there were no significant differences attributed to replicate measurements.

It is suggested that if process cheese is desired with good meltability, and a soft tender body, processing conditions should be selected that will give only sufficient emulsification to prevent fat leakage. On the other hand, if meltability is not important and a firm non-sticky sliceable cheese is desired, processing conditions should be selected to give rapid and complete emulsification.

Most of the rheological measurements other than meltability were made in duplicate on cheese processed with each salt at each cooking time. This did not provide sufficient replicate samples to determine the statistical significance of differences between salts at each cooking time. However it was possible to determine the overall significance of cooking time and the overall significance of the effect of emulsifying salts. It also was possible to show the overall significance of the effect of cooking time on each rheological measurement for cheese emulsified with each salt.

Emulsifying salts had highly significant ($\alpha < .01$) effects on firmness, toughness, breaking force, degree of elasticity, apparent ultimate stress; and a significant ($\alpha < .05$) effect on hysteresis.

In none of these measurements were there significant differences among replicate measurements. Therefore the changes illustrated in the figures representing these attributes are assumed to be real.

Cooking time had a highly significant ($\alpha < .01$) effect on firmness, toughness and breaking force; and a significant ($\alpha < .05$) effect on apparent ultimate strain and apparent ultimate stress. Cooking time had no significant effect on the other rheological measurements. However the relationship between cooking time and hysteresis was either significant or highly significant for cheese processed with each individual salt. Correlations were positive for SALP cheese and negative for cheese processed with the other salts. This, no doubt, resulted in no significance for the overall effect. One could say therefore that the relationship between cooking time and hysteresis was significant for cheese processed with any given salt.

The application of Pearson correlation coefficients (260) to all the apirs of rheological measurements revealed a highly significant ($\alpha < .01$) negative correlation between meltability and firmness and a highly significant positive correlation between toughness and breaking force. A significant ($\alpha < .05$) negative correlation was also found between meltability and breaking force. These results were based on overall correlation coefficients as well as correlation coefficients for cheese processed with each of the four emulsifying salts. It is probably safe to assume that all of these rheological measurements need not be run in future experiments since each one in the pair probably reflects properties closely related to the other.

Other significant and highly significant relationships are shown in table 7, but these are based on overall correlation coefficients plus those correlation coefficients from only three out of the four salts. Therefore additional work should be done before concluding that any of these measurements duplicate each other.

It is interesting that SALP cheese was the one that did not correlate with the others in all the "three cheese" relationships except hysteresis-stiffness and hysteresis-AUS. This suggests that SALP is an emulsifying salt that produces effects that are quite different from cheese processed with other salts. An examination of the microstructures of SALP cheese (figure 25 and 26) reveals rather slow and incomplete emulsification. It did not develop enough structure during 10 minutes in the cooker at 82C to register a break in the force-deformation curve that was used to determine breaking strength and toughness. It also remained softer (less firm) and more meltable after extended cooking than cheese emulsified with any of the other salts.

CONCLUSIONS

1. The state of the fat emulsion in process cheese became progressively finer as the cheese was held from 0 to 40 min in the cooker at 82C.

2. SEM micrographs revealed excellent views of the state of the fat emulsion in process cheese.

3. SEM micrographs revealed that TSPP produced the most rapid, and SALP the slowest emulsification during cheese processing, while CIT and DSP were intermediate.

4. There was a general direct relationship between the fineness of the fat emulsion in process cheese and its firmness, toughness, breaking force, apparent stiffness modulus, degree of elasticity and poor meltability. There was a general inverse relationship between the fineness of the emulsion and apparent ultimate strain and hysteresis.

5. TEM micrographs revealed that some CIT and TSPP crystals remained undissolved in process cheese even after 40 min in the cooker. Undissolved SALP crystals were still present after 10 min. No DSP crystals were found in the micrographs.

6. It was possible to show vividly by TEM the emulsification of fat during the processing of Cheddar cheese.

7. Some calcium phosphate crystals were identified in process cheese. These may have been present in the original natural cheese, but showed evidence of some growth in the process cheese.

8. There were highly significant relationships between the effect of different species of emulsifying salt and all rheological measurements except hysteresis which was only significant ($\alpha < 0.05$).

9. Cooking time had a highly significant ($\alpha < .01$) effect on firmness, toughness, breaking force, and a significant ($\alpha < 0.05$) effect on apparent ultimate strain and apparent ultimate stress of process cheese.

10. There were no significant differences among replicate rheological measurements.

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APPENDIX

Table 8. The effect of cooking time at 82C and four different emulsifying salts on melting properties of process cheese expressed as 'cheese flow'.

Emulsifying salt	Cooking Time (min)				
	0	5	10	20	40
	(mm)	(mm)	(mm)	(mm)	(mm)
CIT[1]	101	85	53	14	4
DSP[2]	22	18	18	5	3
TSPP[3]	29	14	11	4	2
SALP[4]	91	83	76	22	21

[1]sodium citrate

[2]disodium phosphate

[3]tetrasodium pyrophosphate

[4]sodium aluminum phosphate

Table 9. Analysis of variance of the effects of emulsifying salt and cooking time on meltability.

```
                MELT
             BY SALT
                TIME
                REP
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
                                    SUM OF                    MEAN          SIGNIF
SOURCE OF VARIATION                 SQUARES      DF           SQUARE     F  OF F

MAIN EFFECTS                      139688.911      14      9977.779 7007.048 0.000
   SALT                            65826.392       3     21942.131******** 0.000 ***
   TIME                            63028.681       4     15757.170******** 0.000 ***
   REP                                20.802       7         2.972    2.087 0.055 n.s.

2-WAY INTERACTIONS                  29285.033      61       480.083  337.145 0.000
   SALT      TIME                   28267.479      12      2355.623 1654.272 0.000
   SALT      REP                      107.205      21         5.105    3.585 0.000
   TIME      REP                       51.736      28         1.848    1.298 0.184

EXPLAINED                         168320.126      75      2244.268 1576.072 0.000

RESIDUAL                             112.493      79         1.424

TOTAL                             168432.619     154      1093.718
    161 CASES WERE PROCESSED.
      6 CASES (  3.7 PCT) WERE MISSING.
DUE TO EMPTY CELLS OR A SINGULAR MATRIX,
HIGHER ORDER INTERACTIONS HAVE BEEN SUPPRESSED.
STATISTICAL PACKAGE FOR THE SOCIAL SCIENCES

FILE   NONAME   (CREATION DATE = 03/28/80)
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Table 10. Correlation between each pair of rheological measurements for all cheese processed with sodium citrate at 82C.

PEARSON CORRELATION COEFFICIENTS

VARIABLE PAIR		VARIABLE PAIR		VARIABLE PAIR		VARIABLE PAIR		VARIABLE PAIR		VARIABLE PAIR	
TIME WITH MELT	-0.9834 N(39) SIG .001	TIME WITH FIRM	0.9375 N(19) SIG .001	TIME WITH TOUGH	0.9656 N(14) SIG .001	TIME WITH BREAK	0.8589 N(9) SIG .003	TIME WITH HYST	-0.9582 N(9) SIG .001	TIME WITH STIFF	0.9099 N(9) SIG .001
TIME WITH ELAST	0.9714 N(9) SIG .001	TIME WITH STRAIN	-0.3555 N(9) SIG .348	TIME WITH STRESS	0.7172 N(9) SIG .030	MELT WITH FIRM	-0.9770 N(20) SIG .001	MELT WITH TOUGH	-0.9769 N(15) SIG .001	MELT WITH BREAK	-0.8577 N(10) SIG .002
MELT WITH HYST	0.9794 N(10) SIG .001	MELT WITH STIFF	-0.9377 N(10) SIG .001	MELT WITH ELAST	-0.9648 N(10) SIG .001	MELT WITH STRAIN	0.2150 N(10) SIG .551	MELT WITH STRESS	-0.7883 N(10) SIG .007	FIRM WITH TOUGH	0.9623 N(15) SIG .001
FIRM WITH BREAK	0.8429 N(10) SIG .002	FIRM WITH HYST	-0.9518 N(10) SIG .001	FIRM WITH STIFF	0.9144 N(10) SIG .001	FIRM WITH ELAST	0.9220 N(10) SIG .001	FIRM WITH STRAIN	-0.2920 N(10) SIG .413	FIRM WITH STRESS	0.8111 N(10) SIG .004
TOUGH WITH BREAK	0.9036 N(10) SIG .001	TOUGH WITH HYST	-0.9571 N(10) SIG .001	TOUGH WITH STIFF	0.8884 N(10) SIG .001	TOUGH WITH ELAST	0.9184 N(10) SIG .001	TOUGH WITH STRAIN	-0.2186 N(10) SIG .544	TOUGH WITH STRESS	0.7639 N(10) SIG .010
BREAK WITH HYST	-0.7766 N(10) SIG .008	BREAK WITH STIFF	0.8358 N(10) SIG .003	BREAK WITH ELAST	0.8517 N(10) SIG .002	BREAK WITH STRAIN	-0.2938 N(10) SIG .410	BREAK WITH STRESS	0.8071 N(10) SIG .005	HYST WITH STIFF	-0.9054 N(10) SIG .001
HYST WITH ELAST	-0.9335 N(10) SIG .001	HYST WITH STRAIN	0.1095 N(10) SIG .763	HYST WITH STRESS	-0.6796 N(10) SIG .031	STIFF WITH ELAST	0.9767 N(10) SIG .001	STIFF WITH STRAIN	-0.1827 N(10) SIG .613	STIFF WITH STRESS	0.8550 N(10) SIG .002
ELAST WITH STRAIN	-0.2356 N(10) SIG .512	ELAST WITH STRESS	0.8084 N(10) SIG .005	STRAIN WITH STRESS	-0.3872 N(10) SIG .269						

Table 11. Correlation between each pair of rheological measurements for all cheese processed with disodium phosphate.

PEARSON CORRELATION COEFFICIENTS											
VARIABLE PAIR		VARIABLE PAIR		VARIABLE PAIR		VARIABLE PAIR		VARIABLE PAIR		VARIABLE PAIR	
TIME WITH MELT	-0.9308 N(40) SIG .001	TIME WITH FIRM	0.8293 N(20) SIG .001	TIME WITH TOUGH	0.9423 N(15) SIG .001	TIME WITH BREAK	0.8557 N(10) SIG .002	TIME WITH HYST	-0.9624 N(10) SIG .001	TIME WITH STIFF	0.8001 N(10) SIG .005
TIME WITH ELAST	0.7970 N(10) SIG .006	TIME WITH STRAIN	-0.7782 N(10) SIG .008	TIME WITH STRESS	0.8716 N(10) SIG .001	MELT WITH FIRM	-0.7796 N(20) SIG .001	MELT WITH TOUGH	-0.9022 N(15) SIG .001	MELT WITH BREAK	-0.7996 N(10) SIG .005
MELT WITH HYST	0.9064 N(10) SIG .001	MELT WITH STIFF	-0.8065 N(10) SIG .005	MELT WITH ELAST	-0.7838 N(10) SIG .007	MELT WITH STRAIN	0.7369 N(10) SIG .015	MELT WITH STRESS	-0.8819 N(10) SIG .001	FIRM WITH TOUGH	0.9343 N(15) SIG .001
FIRM WITH BREAK	0.9121 N(10) SIG .001	FIRM WITH HYST	-0.8143 N(10) SIG .004	FIRM WITH STIFF	0.9459 N(10) SIG .001	FIRM WITH ELAST	0.9615 N(10) SIG .001	FIRM WITH STRAIN	-0.9414 N(10) SIG .001	FIRM WITH STRESS	0.8016 N(10) SIG .005
TOUGH WITH BREAK	0.9402 N(10) SIG .001	TOUGH WITH HYST	-0.8918 N(10) SIG .001	TOUGH WITH STIFF	0.9010 N(10) SIG .001	TOUGH WITH ELAST	0.9017 N(10) SIG .001	TOUGH WITH STRAIN	-0.8773 N(10) SIG .001	TOUGH WITH STRESS	0.9030 N(10) SIG .001
BREAK WITH HYST	-0.8669 N(10) SIG .001	BREAK WITH STIFF	0.9412 N(10) SIG .001	BREAK WITH ELAST	0.9441 N(10) SIG .001	BREAK WITH STRAIN	-0.8959 N(10) SIG .001	BREAK WITH STRESS	0.9197 N(10) SIG .001	HYST WITH STIFF	-0.8215 N(10) SIG .004
HYST WITH ELAST	-0.8092 N(10) SIG .005	HYST WITH STRAIN	0.8308 N(10) SIG .003	HYST WITH STRESS	-0.8623 N(10) SIG .001	STIFF WITH ELAST	0.9846 N(10) SIG .001	STIFF WITH STRAIN	-0.9312 N(10) SIG .001	STIFF WITH STRESS	0.8975 N(10) SIG .001
ELAST WITH STRAIN	-0.9091 N(10) SIG .001	ELAST WITH STRESS	0.8463 N(10) SIG .002	STRAIN WITH STRESS	-0.8023 N(10) SIG .005						

Table 12. Correlation between each pair of rheological measurements for all cheese processed with tetrasodium pyrophosphate.

PEARSON CORRELATION COEFFICIENTS											
VARIABLE PAIR		VARIABLE PAIR		VARIABLE PAIR		VARIABLE PAIR		VARIABLE PAIR		VARIABLE PAIR	
TIME WITH MELT	-0.9382 N(40) SIG .001	TIME WITH FIRM	0.8261 N(20) SIG .001	TIME WITH TOUGH	0.9380 N(15) SIG .001	TIME WITH BREAK	0.8628 N(10) SIG .001	TIME WITH HYST	-0.8921 N(10) SIG .001	TIME WITH STIFF	0.3458 N(10) SIG .328
TIME WITH ELAST	0.9067 N(10) SIG .001	TIME WITH STRAIN	-0.2858 N(10) SIG .424	TIME WITH STRESS	0.9672 N(10) SIG .001	MELT WITH FIRM	-0.6411 N(20) SIG .002	MELT WITH TOUGH	-0.7830 N(15) SIG .001	MELT WITH BREAK	-0.7071 N(10) SIG .022
MELT WITH HYST	0.8996 N(10) SIG .001	MELT WITH STIFF	-0.1964 N(10) SIG .587	MELT WITH ELAST	-0.7509 N(10) SIG .012	MELT WITH STRAIN	0.0254 N(10) SIG .945	MELT WITH STRESS	-0.9442 N(10) SIG .001	FIRM WITH TOUGH	0.9488 N(15) SIG .001
FIRM WITH BREAK	0.9735 N(10) SIG .001	FIRM WITH HYST	-0.8097 N(10) SIG .005	FIRM WITH STIFF	0.7667 N(10) SIG .010	FIRM WITH ELAST	0.9213 N(10) SIG .001	FIRM WITH STRAIN	-0.6297 N(10) SIG .051	FIRM WITH STRESS	0.8321 N(10) SIG .003
TOUGH WITH BREAK	0.9699 N(10) SIG .001	TOUGH WITH HYST	-0.8394 N(10) SIG .002	TOUGH WITH STIFF	0.5771 N(10) SIG .081	TOUGH WITH ELAST	0.9702 N(10) SIG .001	TOUGH WITH STRAIN	-0.5591 N(10) SIG .093	TOUGH WITH STRESS	0.9013 N(10) SIG .001
BREAK WITH HYST	-0.8397 N(10) SIG .002	BREAK WITH STIFF	0.7060 N(10) SIG .022	BREAK WITH ELAST	0.9498 N(10) SIG .001	BREAK WITH STRAIN	-0.6244 N(10) SIG .054	BREAK WITH STRESS	0.8399 N(10) SIG .002	HYST WITH STIFF	-0.5407 N(10) SIG .107
HYST WITH ELAST	-0.7814 N(10) SIG .008	HYST WITH STRAIN	0.2550 N(10) SIG .477	HYST WITH STRESS	-0.9011 N(10) SIG .001	STIFF WITH ELAST	0.5012 N(10) SIG .140	STIFF WITH STRAIN	-0.6642 N(10) SIG .036	STIFF WITH STRESS	0.4245 N(10) SIG .221
ELAST WITH STRAIN	-0.5619 N(10) SIG .091	ELAST WITH STRESS	0.8511 N(10) SIG .002	STRAIN WITH STRESS	-0.2040 N(10) SIG .572						

Table 13. Correlation between each pair of rheological measurements for all cheese processed with sodium aluminum phosphate at 82C.

PEARSON CORRELATION COEFFICIENTS

VARIABLE PAIR		VARIABLE PAIR		VARIABLE PAIR		VARIABLE PAIR		VARIABLE PAIR		VARIABLE PAIR	
TIME WITH MELT	-0.9182 N(36) SIG .001	TIME WITH FIRM	0.7512 N(20) SIG .001	TIME WITH TOUGH	0.9977 N(6) SIG .001	TIME WITH BREAK	1.0000 N(4) SIG .001	TIME WITH HYST	0.6354 N(10) SIG .048	TIME WITH STIFF	0.2167 N(10) SIG .548
TIME WITH ELAST	0.5534 N(10) SIG .097	TIME WITH STRAIN	0.6386 N(10) SIG .047	TIME WITH STRESS	0.1595 N(10) SIG .660	MELT WITH FIRM	-0.5795 N(20) SIG .007	MELT WITH TOUGH	-0.0711 N(6) SIG .894	MELT WITH BREAK	0.0000 N(4) SIG ****
MELT WITH HYST	-0.7101 N(10) SIG .021	MELT WITH STIFF	-0.3748 N(10) SIG .286	MELT WITH ELAST	-0.5372 N(10) SIG .109	MELT WITH STRAIN	-0.5740 N(10) SIG .083	MELT WITH STRESS	-0.3077 N(10) SIG .387	FIRM WITH TOUGH	0.4258 N(6) SIG .400
FIRM WITH BREAK	0.3333 N(4) SIG .667	FIRM WITH HYST	0.3287 N(10) SIG .354	FIRM WITH STIFF	0.1427 N(10) SIG .694	FIRM WITH ELAST	0.1730 N(10) SIG .633	FIRM WITH STRAIN	0.4017 N(10) SIG .250	FIRM WITH STRESS	-0.4455 N(10) SIG .197
TOUGH WITH BREAK	0.9991 N(4) SIG .001	TOUGH WITH HYST	-0.0152 N(4) SIG .985	TOUGH WITH STIFF	-0.2964 N(4) SIG .704	TOUGH WITH ELAST	0.2939 N(4) SIG .706	TOUGH WITH STRAIN	0.7526 N(4) SIG .247	TOUGH WITH STRESS	-0.3707 N(4) SIG .629
BREAK WITH HYST	-0.0009 N(4) SIG .999	BREAK WITH STIFF	-0.3015 N(4) SIG .698	BREAK WITH ELAST	0.3318 N(4) SIG .668	BREAK WITH STRAIN	0.7625 N(4) SIG .238	BREAK WITH STRESS	-0.3333 N(4) SIG .667	HYST WITH STIFF	0.7767 N(10) SIG .008
HYST WITH ELAST	0.3993 N(10) SIG .253	HYST WITH STRAIN	0.7546 N(10) SIG .012	HYST WITH STRESS	0.2883 N(10) SIG .419	STIFF WITH ELAST	0.2841 N(10) SIG .426	STIFF WITH STRAIN	0.5469 N(10) SIG .102	STIFF WITH STRESS	0.2882 N(10) SIG .419
ELAST WITH STRAIN	0.5807 N(10) SIG .078	ELAST WITH STRESS	0.6302 N(10) SIG .051	STRAIN WITH STRESS	0.1817 N(10) SIG .615						

Table 14. Effect of emulsifying salts and cooking time at 82C on the firmness of process cheese.

Emulsifying salt	Cooking Time (min)				
	0	5	10	20	40
	(N)	(N)	(N)	(N)	(N)
CIT[1]	2.4	2.4	3.2	3.8	3.8
DSP[2]	2.2	2.7	2.8	2.8	4.6
TSPP[3]	3.6	3.6	4.2	4.1	6.6
SALP[4]	2.1	2.7	2.7	2.7	2.8

[1] sodium citrate

[2] disodium phosphate

[3] tetrasodium pyrophosphate

[4] sodium aluminum phosphate

Table 15. Analysis of variance of the overall effect of emulsifying salt and cooking time on firmness.

FIRM
BY SALT
TIME
REP

* *

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
MAIN EFFECTS	68.744	10	6.874	333.543	0.000
SALT	36.708	3	12.236	593.678	0.000***
TIME	32.399	4	8.100	392.996	0.000***
REP	0.013	3	0.004	0.204	0.893n.s.
2-WAY INTERACTIONS	15.176	33	0.460	22.314	0.000
SALT TIME	14.805	12	1.234	59.859	0.000
SALT REP	0.137	9	0.015	0.739	0.671
TIME REP	0.278	12	0.023	1.124	0.373
EXPLAINED	83.989	43	1.953	94.769	0.000
RESIDUAL	0.721	35	0.021		
TOTAL	84.710	78	1.086		

161 CASES WERE PROCESSED.
82 CASES (50.9 PCT) WERE MISSING.
DUE TO EMPTY CELLS OR A SINGULAR MATRIX,
HIGHER ORDER INTERACTIONS HAVE BEEN SUPPRESSED.
STATISTICAL PACKAGE FOR THE SOCIAL SCIENCES

FILE NONAME (CREATION DATE = 03/28/80)

Table 16. Effect of emulsifying salts and cooking time at 82C on the toughness of process cheese.

Emulsifying salt	Cooking Time (min)				
	0	5	10	20	40
	($N.mm^2$)	($N.mm^2$)	($N.mm^2$)	($N.mm^2$)	($N.mm^2$)
CIT[1]	275.1	275.9	302.9	310.8	322.9
DSP[2]	239.1	285.7	290.4	305.4	370.4
TSPP[3]	375.3	375.6	448.1	500.0	648.9
SALP[4]	---	---	---	250.0	275.2

[1]sodium citrate

[2]disodium phosphate

[3]tetrasodium pyrophosphate

[4]sodium aluminum phosphate

Table 17. Analysis of variance of the overall effect of emulsifying salt and cooking time on toughness.

```
                TOUGH
             BY SALT
                TIME
                REP
```

* *

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
MAIN EFFECTS	462209.377	9	51356.597	34.103	0.000
SALT	393384.664	3	131128.221	87.076	0.000***
TIME	126339.354	4	31584.839	20.974	0.000***
REP	77.282	2	38.641	0.026	0.975n.s.
EXPLAINED	462209.377	9	51356.597	34.103	0.000
RESIDUAL	60236.463	40	1505.912		
TOTAL	522445.840	49	10662.160		

161 CASES WERE PROCESSED.
111 CASES (68.9 PCT) WERE MISSING.
DUE TO EMPTY CELLS OR A SINGULAR MATRIX,
HIGHER ORDER INTERACTIONS HAVE BEEN SUPPRESSED.
STATISTICAL PACKAGE FOR THE SOCIAL SCIENCES

FILE NONAME (CREATION DATE = 03/28/80)

Table 18. Effect of emulsifying salts and cooking time at 82C on the breaking force of process cheese.

Emulsifying salt	Cooking Time (min)				
	0	5	10	20	40
	(N)	(N)	(N)	(N)	(N)
CIT[1]	5.8	5.4	6.6	6.7	8.0
DSP[2]	5.6	5.9	6.2	6.1	7.4
TSPP[3]	5.5	5.7	6.2	6.8	10.0
SALP[4]	---	---	---	4.2	5.2

[1]sodium citrate

[2]disodium phosphate

[3]tetrasodium pyrophosphate

[4]sodium aluminum phosphate

Table 19. Analysis of variance of the effect of emulsifying salt and cooking time on breaking force.

```
        BREAK
     BY SALT
        TIME
        REP
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
```

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
MAIN EFFECTS	48.431	8	6.054	16.802	0.000
SALT	28.821	3	9.607	26.664	0.000 ***
TIME	31.754	4	7.939	22.033	0.000 ***
REP	0.091	1	0.091	0.252	0.620 n.s.
EXPLAINED	48.431	8	6.054	16.802	0.000
RESIDUAL	8.647	24	0.360		
TOTAL	57.079	32	1.784		

161 CASES WERE PROCESSED.
128 CASES (79.5 PCT) WERE MISSING.
DUE TO EMPTY CELLS OR A SINGULAR MATRIX,
HIGHER ORDER INTERACTIONS HAVE BEEN SUPPRESSED.
STATISTICAL PACKAGE FOR THE SOCIAL SCIENCES

Table 20. Overall correlation between each pair of rheological measurements for all cheese processed with four different emulsifying salts.

PEARSON CORRELATION COEFFICIENTS

VARIABLE PAIR		VARIABLE PAIR		VARIABLE PAIR		VARIABLE PAIR		VARIABLE PAIR		VARIABLE PAIR	
TIME WITH MELT	-0.6153 N(155) SIG .001	TIME WITH FIRM	0.5768 N(79) SIG .001	TIME WITH TOUGH	0.3348 N(50) SIG .017	TIME WITH BREAK	0.4656 N(33) SIG .006	TIME WITH HYST	0.2704 N(39) SIG .096	TIME WITH STIFF	0.0978 N(39) SIG .554
TIME WITH ELAST	0.1614 N(39) SIG .326	TIME WITH STRAIN	0.2126 N(39) SIG .194	TIME WITH STRESS	0.0306 N(39) SIG .853	MELT WITH FIRM	-0.5741 N(80) SIG .001	MELT WITH TOUGH	-0.4119 N(51) SIG .003	MELT WITH BREAK	-0.3433 N(34) SIG .047
MELT WITH HYST	-0.0925 N(40) SIG .570	MELT WITH STIFF	0.0204 N(40) SIG .900	MELT WITH ELAST	0.1585 N(40) SIG .329	MELT WITH STRAIN	0.1024 N(40) SIG .530	MELT WITH STRESS	0.2416 N(40) SIG .133	FIRM WITH TOUGH	0.9103 N(51) SIG .001
FIRM WITH BREAK	0.8136 N(34) SIG .001	FIRM WITH HYST	-0.1573 N(40) SIG .332	FIRM WITH STIFF	-0.1417 N(40) SIG .383	FIRM WITH ELAST	-0.2792 N(40) SIG .081	FIRM WITH STRAIN	-0.2387 N(40) SIG .138	FIRM WITH STRESS	-0.3337 N(40) SIG .035
TOUGH WITH BREAK	0.7259 N(34) SIG .001	TOUGH WITH HYST	-0.2501 N(34) SIG .154	TOUGH WITH STIFF	-0.1884 N(34) SIG .286	TOUGH WITH ELAST	-0.2754 N(34) SIG .115	TOUGH WITH STRAIN	-0.2335 N(34) SIG .184	TOUGH WITH STRESS	-0.2902 N(34) SIG .096
BREAK WITH HYST	-0.4102 N(34) SIG .016	BREAK WITH STIFF	-0.3401 N(34) SIG .049	BREAK WITH ELAST	-0.4664 N(34) SIG .005	BREAK WITH STRAIN	-0.3711 N(34) SIG .031	BREAK WITH STRESS	-0.4996 N(34) SIG .003	HYST WITH STIFF	0.8241 N(40) SIG .001
HYST WITH ELAST	0.5902 N(40) SIG .001	HYST WITH STRAIN	0.8030 N(40) SIG .001	HYST WITH STRESS	0.5337 N(40) SIG .001	STIFF WITH ELAST	0.5130 N(40) SIG .001	STIFF WITH STRAIN	0.6646 N(40) SIG .001	STIFF WITH STRESS	0.5181 N(40) SIG .001
ELAST WITH STRAIN	0.8146 N(40) SIG .001	ELAST WITH STRESS	0.8668 N(40) SIG .001	STRAIN WITH STRESS	0.6583 N(40) SIG .001						

Table 21. Effect of emulsifying salts and cooking time at 82C on the hysteresis of process cheese.

Emulsifying salt	Cooking Time (min)				
	0	5	10	20	40
	(N mm)	(N mm)	(N mm)	(N mm)	(N mm)
CIT[1]	117.4	114.7	108.1	100.8	99.2
DSP[2]	117.8	117.1	107.8	104.0	96.8
TSPP[3]	97.9	84.8	84.9	84.3	72.6
SALP[4]	137.2	128.0	124.0	113.9	111.2

[1]sodium citrate

[2]disodium phosphate

[3]tetrasodium pyrophosphate

[4]sodium aluminum phosphate

Table 22. Analysis of variance of the overall effect of emulsifying salt and cooking time on hysteresis.

HYST
BY SALT
TIME
REP

* *

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F	
MAIN EFFECTS	7094110.383	8	886763.798	2.044	0.135	
SALT	4870181.769	3	1623393.923	3.742	0.045	*
TIME	2095162.123	4	523790.531	1.208	0.362	n.s.
REP	316932.844	1	316932.844	0.731	0.411	n.s.
2-WAY INTERACTIONS	9876152.785	19	519797.515	1.198	0.389	
SALT TIME	7182726.888	12	598560.574	1.380	0.301	
SALT REP	1229488.315	3	409829.438	0.945	0.452	
TIME REP	1628489.086	4	407122.271	0.939	0.477	
EXPLAINED	17704512.091	27	655722.670	1.512	0.239	
RESIDUAL	4771531.734	11	433775.612			
TOTAL	22476043.825	38	591474.837			

161 CASES WERE PROCESSED.
122 CASES (75.8 PCT) WERE MISSING.
DUE TO EMPTY CELLS OR A SINGULAR MATRIX,
HIGHER ORDER INTERACTIONS HAVE BEEN SUPPRESSED.
STATISTICAL PACKAGE FOR THE SOCIAL SCIENCES

FILE NONAME (CREATION DATE = 03/28/80)

Table 23. Effect of emulsifying salt and cooking time at 82C on apparent stiffness modulus of process cheese.

Emulsifying salt	Cooking Time (min)				
	0	5	10	20	40
	(N/mm^2)	(N/mm^2)	(N/mm^2)	(N/mm^2)	(N/mm^2)
CIT[1]	0.46	0.44	0.51	0.85	0.94
DSP[2]	0.48	0.49	0.50	0.56	0.98
TSPP[3]	0.70	0.70	0.76	1.07	1.62
SALP[4]	0.18	0.21	0.24	0.45	0.46

[1]sodium citrate

[2]disodium phosphate

[3]tetrasodium pyrophosphate

[4]sodium aluminum phosphate

Table 24. Analysis of variance of the overall effect of emulsifying salt and cooking time on apparent stiffness modulus.

STIFF
BY SALT
TIME
REP

* *

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
MAIN EFFECTS	2576.051	8	322.006	1.147	0.406
SALT	1852.480	3	617.493	2.199	0.146n.s.
TIME	561.629	4	140.407	0.500	0.737n.s.
REP	187.366	1	187.366	0.667	0.431n.s.
2-WAY INTERACTIONS	3241.947	19	170.629	0.608	0.836
SALT TIME	1719.338	12	143.278	0.510	0.868
SALT REP	441.578	3	147.193	0.524	0.674
TIME REP	1069.443	4	267.361	0.952	0.471
EXPLAINED	5780.357	27	214.087	0.763	0.729
RESIDUAL	3088.168	11	280.743		
TOTAL	8868.526	38	233.382		

161 CASES WERE PROCESSED.
122 CASES (75.8 PCT) WERE MISSING.
DUE TO EMPTY CELLS OR A SINGULAR MATRIX,
HIGHER ORDER INTERACTIONS HAVE BEEN SUPPRESSED.
STATISTICAL PACKAGE FOR THE SOCIAL SCIENCES

FILE NONAME (CREATION DATE = 03/28/80)

Table 25. Effect of emulsifying salt and cooking time at 82C on the degree of elasticity of process cheese.

Emulsifying salt	Cooking Time (min)				
	0	5	10	20	40
	$(\Delta L_1/\Delta L)$	$(\Delta L_1/\Delta L)$	$(\Delta L_1/\Delta L)$	$(\Delta L_1/\Delta L)$	$(\Delta L_1/\Delta L)$
CIT[1]	0.16	0.19	0.22	0.35	0.41
DSP[2]	0.20	0.21	0.23	0.24	0.45
TSPP[3]	0.31	0.31	0.33	0.43	0.54
SALP[4]	0.12	0.12	0.12	0.28	0.28

[1] sodium citrate

[2] disodium phosphate

[3] tetrasodium pyrophosphate

[4] sodium aluminum phosphate

Table 26. Analysis of variance of the overall effect of emulsifying salt and cooking time on degree of elasticity.

ELAST
BY SALT
TIME
REP

* *

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
MAIN EFFECTS	11637.283	8	1454.660	7.553	0.002
SALT	10801.253	3	3600.418	18.694	0.000**
TIME	835.566	4	208.892	1.085	0.411n.s.
REP	223.315	1	223.315	1.160	0.305n.s.
2-WAY INTERACTIONS	3513.793	19	184.936	0.960	0.549
SALT TIME	2220.843	12	185.070	0.961	0.530
SALT REP	429.357	3	143.119	0.743	0.548
TIME REP	843.058	4	210.765	1.094	0.406
EXPLAINED	15105.238	27	559.453	2.905	0.033
RESIDUAL	2118.549	11	192.595		
TOTAL	17223.787	38	453.258		

161 CASES WERE PROCESSED.
122 CASES (75.8 PCT) WERE MISSING.
DUE TO EMPTY CELLS OR A SINGULAR MATRIX,
HIGHER ORDER INTERACTIONS HAVE BEEN SUPPRESSED.
STATISTICAL PACKAGE FOR THE SOCIAL SCIENCES

FILE NONAME (CREATION DATE = 03/28/80)

Table 27. Effect of emulsifying salt and cooking time at 82C on apparent ultimate strain of process cheese.

Emulsifying salt	Cooking Time (min)				
	0	5	10	20	40
	(ΔL/L)	(ΔL/L)	(ΔL/L)	(ΔL/L)	(ΔL/L)
CIT[1]	0.28	0.28	0.27	0.27	0.27
DSP[2]	0.26	0.25	0.23	0.24	0.14
TSPP[3]	0.17	0.23	0.23	0.22	0.13
SALP[4]	0.49	0.50	0.50	0.28	0.27

[1]sodium citrate

[2]disodium phosphate

[3]tetrasodium pyrophosphate

[4]sodium aluminum phosphate

Table 28. Analysis of variance of the overall effect of emulsifying salt and cooking time on apparent ultimate strain.

STRAIN
BY SALT
TIME
REP

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
MAIN EFFECTS	10106.806	8	1263.351	13.813	0.000
SALT	8630.312	3	2876.771	31.453	0.000**
TIME	1347.907	4	336.977	3.684	0.039*
REP	370.364	1	370.364	4.049	0.069n.s.
2-WAY INTERACTIONS	5436.626	19	286.138	3.128	0.028
SALT TIME	4020.749	12	335.062	3.663	0.020
SALT REP	1028.412	3	342.804	3.748	0.045
TIME REP	351.509	4	87.877	0.961	0.466
EXPLAINED	15848.647	27	586.987	6.418	0.001
RESIDUAL	1006.100	11	91.464		
TOTAL	16854.747	38	443.546		

161 CASES WERE PROCESSED.
122 CASES (75.8 PCT) WERE MISSING.
DUE TO EMPTY CELLS OR A SINGULAR MATRIX,
HIGHER ORDER INTERACTIONS HAVE BEEN SUPPRESSED.
STATISTICAL PACKAGE FOR THE SOCIAL SCIENCES

FILE NONAME (CREATION DATE = 03/28/80)

Table 29. Effect of emulsifying salt and cooking time at 82C on apparent ultimate stress.

Emulsifying salt	Cooking Time (min) 0	5	10	20	40
	(N/mm^2)	(N/mm^2)	(N/mm^2)	(N/mm^2)	(N/mm^2)
CIT[1]	0.07	0.07	0.08	0.10	0.11
DSP[2]	0.07	0.08	0.08	0.10	0.13
TSPP[3]	0.09	0.13	0.16	0.17	0.21
SALP[4]	0.05	0.06	0.08	0.14	0.14

[1] sodium citrate

[2] disodium phosphate

[3] tetrasodium pyrophosphate

[4] sodium aluminum phosphate

Table 30. Analysis of variance of the overall effect of emulsifying salt and cooking time on apparent ultimate stress.

STRESS
BY SALT
TIME
REP

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F	
MAIN EFFECTS	27663.787	8	3457.973	19.822	0.000	
SALT	24646.689	3	8215.563	47.093	0.000	**
TIME	2367.307	4	591.827	3.392	0.049	*
REP	7.610	1	7.610	0.044	0.838	n.s.
2-WAY INTERACTIONS	8206.369	19	431.914	2.476	0.063	
SALT TIME	7535.612	12	627.968	3.600	0.021	
SALT REP	31.007	3	10.336	0.059	0.980	
TIME REP	641.007	4	160.252	0.919	0.487	
EXPLAINED	35807.210	27	1326.193	7.602	0.001	
RESIDUAL	1918.994	11	174.454			
TOTAL	37726.205	38	992.795			

161 CASES WERE PROCESSED.
122 CASES (75.8 PCT) WERE MISSING.
DUE TO EMPTY CELLS OR A SINGULAR MATRIX,
HIGHER ORDER INTERACTIONS HAVE BEEN SUPPRESSED.