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# AMINO ACID COMPOSITION AND STRUCTURE OF CHEESE BAKED AS A PIZZA INGREDIENT IN CONVENTIONAL AND MICROWAVE OVENS

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## Abstract

Amino acid compositions of stirred-curd Mozzarella, stretched Mozzarella, and process Cheddar cheeses were similar and did not change as the result of baking in a conventional oven. D-glutamic acid (D-Glu) and D-phenylalanine (D-Phe) were present at low concentrations in all cheese samples, the lowest concentrations having been found in unbaked stirred-curd Mozzarella cheese (2.7% D-Glu of total Glu present and <1.0% D-Phe of total Phe present). The highest concentrations were detected in unbaked stretched Mozzarella cheese (5.6% and 1.2%, respectively). The changes were not significant and were not the result of baking, indicating that the heat treatment during baking did not cause racemization of the amino acids.

Each cheese had a characteristic structure before baking. The structures of the Mozzarella cheeses were altered by baking in the conventional oven and also in a microwave oven and their original features such as curd granule junctions and fat globule membranes vanished. Stirred-curd Mozzarella cheese melted most rapidly and partly flowed down from the pizza dough over the edge. Electron microscopy revealed aggregation of the fat globules and a laminar orientation of the protein matrix as the result of the flow. Stretched Mozzarella cheese melted easily but did not flow away. Process Cheddar cheese melted slowly. Fat particles in this cheese aggregated only slightly during baking.

The effects of microwave baking were comparable to those produced in the conventional oven.

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## Introduction

Severe heating is known to induce chemical changes in proteins, e.g., losses of lysine (Lys) [12, 31], serine (Ser), and threonine (Thr) [11, 12, A. Paquet: unpublished results] and racemization, i.e., the conversion of amino acids from the L-form into the D-form [9, 11, 18, 19, 21]. Ser, aspartic acid (Asp), glutamic acid (Glu), and phenylalanine (Phe) have been reported [11] to be particularly susceptible to racemization in dietary proteins upon heat and/or alkali treatments.

Cheese is heated during processing and also in culinary practice, where heating by baking is quite common. Pizza, which is topped with shredded cheese [4, 7] is an example. In a conventional oven, the pizza surface is exposed to overheating which results in the formation of a crust on the dough until the interior of the pizza is baked. Browning of the pizza dough has been associated with a loss of Lys present in wheat proteins [31]. Heating causes the cheese to melt and the fat to separate from the protein, thus altering the original structure of the cheese. In a microwave oven, heating is generated throughout the body of the moist material [22] by oscillating water, carbohydrate, and fat molecules. The food is heated from the interior towards the surface which may be the last place to bake [1]. Thus, no crust would be formed on a pizza unless a crust-forming infrared heater is used or the dough is prepared by a newly developed process [23]. Depending on the microwave energy used, high temperature is achieved considerably more rapidly than in the conventional oven.

It has been well established in the literature [21] that protein-bound amino acids are susceptible to racemization when exposed to elevated pH or temperature [10-12, 21]. Natural dietary proteins are composed almost exclusively of L-amino acids. During the treatment of these proteins, e.g., by high temperature or by alkali, some of the L-isomers tend to partially change into the D-isomers, i.e., they racemize. Racemization is a process during which a proton is abstracted from the α-carbon of an amino acid and changes the α-carbon into a negatively charged planar carbanion. As the proton returns, it has an equal chance of rejoining the planar carbanion either from the same side, thus regenerating the original L-form, or from the opposite side of the molecule, thus forming the isomer of an opposite configuration, i.e., the D-amino acid. Racemization of amino acids in proteins has detrimental nutritional consequences because it leads to the formation of nutritionally unavailable D-amino acids as well as unhydrolyzable D-L- and L-D-peptide bonds which are generated

around the racemized amino acid residue [24-26, 29].

Earlier work on the racemization of amino acids in proteins was done using samples treated under severe conditions, some of which never occur in food processing [10, 18, 21]. One of the objectives of this study was to determine whether regular culinary treatment such as baking can cause changes in the amino acid composition and configuration of milk proteins. The effects of baking in a conventional oven on the amino acid composition and amino acid racemization were studied using 2 types of natural cheese and a process cheese. The other objective was to examine the effects of baking in the conventional oven and in a microwave oven on the microstructure of the cheeses.

## Materials and Methods

### Baking the cheese.

Natural cheeses (Italian-style "stretched" Mozzarella and American-style "stirred-curd" Mozzarella) and process cheese (processed Cheddar cheese) of commercial origin were used. The cheeses were shredded using a shredder with openings 3 mm in diameter. Shredded cheese was spread on a wet cotton cloth circle placed on commercially produced pizza dough discs that were, on an average, 18 cm in diameter and 1.2 cm high. All baking was done in duplicate. The cloth was used to separate the pizza dough from the cheese in order to isolate the cheese during baking for subsequent amino acid analysis and for electron microscopy. In the first run, the cloth was 16 cm in diameter to make observation of the pizza dough possible. Because the cheese flowed during baking, in the second run the cloth size was increased to 20 cm in diameter making it larger than the pizza dough. Each pizza contained 65 g of the shredded cheese in a layer 0.5-1.0 cm thick.

The temperature of the conventional oven was set at 205°C. The temperature of the pizzas was monitored using a contact thermometer inserted into the dough below the cotton cloth. The pizzas were baked for 12 min and the final temperature of the cheese was measured at several points using a contact thermometer immediately after each pizza was taken out of the oven.

Based on a preliminary run in a microwave oven (Panasonic, The Genius II model with a turntable), each pizza was baked for 4 min at the medium energy setting. The temperature of the cheese was measured only in the preliminary test. Experimental baking was carried out without interruption.

### Nitrogen determination.

Cheese samples (approximately 5 g each) taken before and after baking were freeze-dried at -20°C for 24 h and were pulverized. The cheese powders were analyzed for the content of nitrogen by the automated Dumas method [2] using a Coleman Model 29 nitrogen analyzer. The values found were used to calculate the protein content by multiplication with the factor of 6.38 in order to express the contents of the individual amino acids (amino acid composition).

### Amino acid analysis.

Freeze-dried cheese samples (approximately 80 mg) were each hydrolyzed in 5 mL of 6 N HCl at 110°C for 23 h. The hydrolyzate was cooled and filtered through a sintered glass disc and brought up to 10.0 mL with distilled water. Aliquots (200 µL) were brought up to 1.0 mL with a 2 M sodium citrate buffer, pH 2.2. These solutions were analyzed in a Beckman Model 120 B amino acid analyzer.

### Determination of amino acid enantiomers.

The method for the separation of enantiomeric amino acids [3], recently modified and extended for the determination of enantiomers in dietary proteins [A. Paquet: unpublished results] was used as follows:

The cheese protein hydrolyzates obtained for the amino acid analysis were divided into two aliquots and evaporated *in vacuo* at 50°C. One of the two dry residues was reacted with ethoxycarbonylphenylalanine N-hydroxysuccinimide ester (Eoc-Phe-ONSu) and the other residue was reacted with ethoxycarbonylvaline N-hydroxysuccinimide ester (Eoc-Val-ONSu) in the presence of a 10% sodium bicarbonate solution (pH 7.5-8.0) in aqueous acetone (acetone and water, 1:1, v/v). The resulting ethoxycarbonylvaline dipeptides (Eoc-Val-Xx) and ethoxycarbonylphenylalanine dipeptides (Eoc-Phe-Xx) (where Xx is the amino acid under study) were isolated and analyzed by reverse phase high-pressure liquid chromatography (HPLC) (Vista Series 5000, Varian) on a $C_{18}$-column using aqueous acetonitrile as the solvent. Eoc-Phe-Xx was used to determine the D-isomers of polar amino acids (such as Asp, Glu, Ser, etc.) in the protein hydrolyzates as Eoc-Phe-L-Xx and Eoc-Phe-D-Xx. The enantiomers of Phe in the hydrolyzates were separated in the form of Eoc-Val-L-Phe and Eoc-Val-D-Phe. The Eoc-Phe-ONSu and Eoc-Val-ONSu reagents used in this analysis were synthesized from Eoc-Phe and Eoc-Val, respectively, that had been prepared by a standard condensation of ethoxycarbonyl chloride with Phe or with Val [8]. Esterification with N-hydroxysuccinimide was carried out as shown previously [25]. Synthetic HPLC standards were prepared using the same reactions of Eoc-Val-ONSu and Eoc-Phe-ONSu with the corresponding amino acids purchased from the Sigma Co., St. Louis, MO.

### Electron microscopy.

Cheese samples taken before and after baking were examined by scanning electron microscopy (SEM) and by transmission electron microscopy (TEM). For SEM, samples 1 x 1 x 15 mm were fixed in a 2.8% aqueous glutaraldehyde solution for 48 h at 6°C, dehydrated in a graded ethanol series, defatted in chloroform, impregnated with absolute ethanol, and freeze-fractured under liquid nitrogen [16]. The fragments were critical point-dried from carbon dioxide, mounted on SEM stubs using a silver-based cement, coated with gold, and examined in an ISI DS-130 electron microscope operated at 20 kV. Micrographs were taken on 35-mm film. For TEM, 1 $mm^3$ samples were fixed in a 2.8% aqueous glutaraldehyde solution for 48 h at 6°C, washed with water, postfixed with a buffered (0.05 M veronal-acetate buffer, pH 6.75) 2% osmium tetroxide solution for 6 h, dehydrated in a graded ethanol series, and embedded in a low-viscosity Spurr's resin (J. B. EM Service, Inc., Pointe Claire-Dorval, Quebec, Canada). Sections (approximately 90 nm thick) were stained with uranyl acetate and lead citrate solutions and were examined in a Philips EM-300 electron microscope operated at 60 kV [14].

## Results and Discussion

### Amino acid composition and enantiomeric analysis of unbaked and baked cheeses.

All the three cheeses under study had similar amino acid compositions before baking that were in agreement with the data published in the literature [18, 31]. Baking increased the temperature of the cheeses as shown in Fig. 1. In the conventional oven, the increase was more gradual than in the microwave oven. Heating above

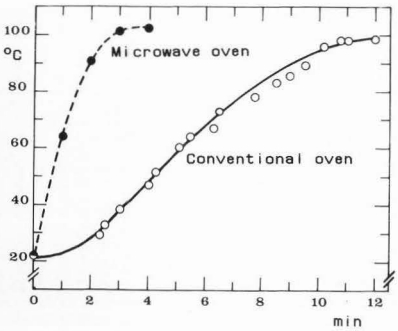



*Fig. 1. Temperature of the pizzas during baking in microwave and conventional ovens. Ordinate: time in minutes. Abscissa: temperature in degrees Celsius.*

80°C, that has been shown to induce changes in the protein structure of process cheese [16], lasted for 4 min in the conventional oven and for only 2.5 min in the microwave oven. However, baking in the conventional oven did not alter the amino acid composition of any of the cheeses under study and no decrease in the Ser or Thr concentrations was observed. This is interesting in view of our earlier analyses of evaporated cow and goat milks (A. Paquet: unpublished observation), where slightly decreased levels of both hydroxyamino acids were found. The loss is caused by the elimination of the hydroxy groups from the two amino acid residues in proteins treated with alkali and heated at high temperature, which leads to the formation of dehydroamino acids. The dehydroamino acids may then react (cross-link) with the terminal amino group of Lys in the protein molecule and form lysinoalanine [12]. Apparently, the effects of the heat treatment, to which the cheeses were exposed during baking in the conventional oven, were not severe enough to cause dehydration of Ser and Thr.

As baking in the conventional oven produced no significant changes in the amino acid composition of the cheese proteins, it was of interest to examine whether some of the amino acids underwent racemization. Masters and Friedman [21] determined that the rate of racemization of protein-bound amino acids decreases in the order of Asp>Phe>Glu>Ala>Pro>Val>Leu, where Asp, Phe, and Glu are racemized at a rate approximately one order higher than the other amino acids. However, Kemp [17] found that Ser was racemized even more rapidly than Asp. Recently, Liardon and Ledermann [19] also showed that Ser was most sensitive to racemization under moderate alkaline treatment of proteins. Thus it may be considered that Ser, Asp, Phe, and Glu are the most sensitive amino acids in dietary proteins toward racemization occurring at elevated pH or temperature. The side chains of these amino acids have electron-withdrawing capacity which greatly facilitates the proton abstraction from the $\alpha$-carbon causing easy racemization [17, 21]. Although racemization occurs most readily as the result of the alkali treatment, it may also proceed to a lesser extent during the cooking of proteins at high temperature, particularly in the presence of lipids and/or reducing sugars [13]. Because the proteins in the cheese during baking are exposed to high temperature as well as to other effects such as the presence of salts and carbohydrates in the cheese, it was of interest to determine whether such conditions may cause racemization of the amino acids. The four amino acids mentioned above were used as indicators of the overall racemization.

Two types of diastereomeric dipeptides (Eoc-Phe-Xx and Eoc-Val-Xx) served for the detection of D-amino acids in baked cheeses. Fig. 2 shows the separation of L- and D-Phe standards as Eoc-Val-L-Phe and Eoc-Val-D-Phe. The separation of the corresponding amino acid enantiomers in baked cheeses is shown using process cheese as an example (Fig. 3). The relative concentration of D-Phe in this sample was less than 2% of the total concentration of L- and D-Phe. The stirred-curd Mozzarella and the stretched Mozzarella cheeses contained comparable concentrations of D-Phe both before and after baking (Table 1). Polar amino acids in the cheese samples were separated in the form of Eoc-Phe dipeptides, exemplified in Fig. 4. D-Asp and D-Ser were not found in any of the samples, but D-Glu was found in small quantities in all samples. The concentrations of D-Glu ranged from 2.4% of the total L- and D-Glu concentration in fresh stirred-curd Mozzarella to 6.2% in baked stretched Mozzarella. The differences between the concentrations of the D-amino acids in unbaked and baked cheeses are statistically not significant as the standard deviations in Table 1 indicate. These D-amino acids were already present in the unbaked cheeses. D-enantiomers of unpolar amino acids (Val, Leu, and isoleucine) were not detected. Potential racemization during acid hydrolysis was not considered in this study. It was shown by Manning [20] that methionine (Met) is the amino acid most susceptible

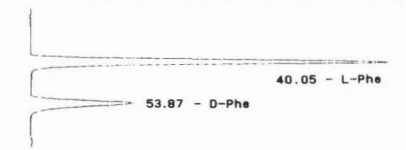



*Fig. 2. High-pressure liquid chromatography separation of a standard mixture of L-Phe (retention time: 40.05 min) and D-Phe (retention time: 53.87 min) as Eoc-Val-L-Phe and Eoc-Val-D-Phe. Solvent: 83% water (containing 0.1% $H_3PO_4$) and 17% acetonitrile. Flow rate: 2.0 mL/min. UV detector (208 nm).*


*Fig. 3. High-pressure liquid chromatography separation of L-Phe (retention time: 39.87 min) and D-Phe (retention time: 53.91 min) as Eoc-Val-Xx dipeptides in baked process cheese. Solvent: 83% water (containing 0.1% $H_3PO_4$) and 17% acetonitrile. Flow rate: 2.0 mL/min. UV detector (208 nm).*

Table 1.
Contents of D-glutamic acid and D-phenylalanine in the proteins of unbaked cheeses and cheeses baked in a conventional oven

| Amino acid[a] | Stirred-curd Mozzarella | | Stretched Mozzarella | | Process Cheddar cheese | |
|---|---|---|---|---|---|---|
| | fresh | baked | fresh | baked | fresh | baked |
| D-Glu | 2.7 ± 0.4 | 3.6 ± 0.5 | 5.6 ± 0.4 | 5.9 ± 0.5 | <1 | <1 |
| D-Phe | <1 | <1 | 1.2 ± 0.6 | 2.2 ± 0.2 | <1 | 1.6 ± 0.2 |

[a]The values listed [100D/(D+L)%] represent the means from two HPLC determinations.

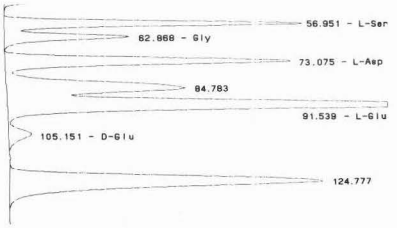



*Fig. 4. High-pressure liquid chromatography separation of polar amino acids in unbaked stretched Mozzarella cheese as Eoc-Phe-Xx dipeptides. D-Glu (retention time: 105.15 min) was detected as the only D-enantiomer. Solvent: 94% water (containing 0.1% $H_3PO_4$) and 6% acetonitrile. Flow rate: 3.0 mL/min. UV detector (208 nm).*

to racemization under acid hydrolysis conditions. Since only traces of D-Met (<1%) were detected in the cheeses under study, it is assumed that no D-epimers were formed during acid hydrolysis.

It may thus be concluded that baking of cheese in the conventional oven did not alter the nutritional quality of the cheese proteins that could be attributed to changes in the amino acid composition and configuration. The temperature and the duration of baking were apparently insufficient to induce such changes. As the temperature treatment in the microwave oven was even more moderate than that in the conventional oven (Fig. 1), it is highly improbable that measurable racemization would have occurred in the cheeses baked in the microwave oven.

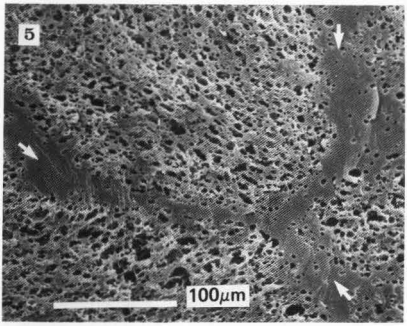
Microstructure.

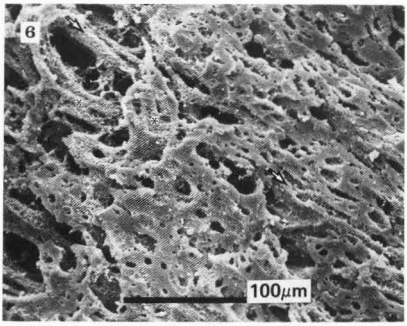
Control cheese samples. SEM revealed considerable differences in the original microstructure of the three cheeses used in this study. Before baking, the stirred-curd Mozzarella cheese (28% fat, 42% moisture) had the structure similar to other stirred-curd cheeses with the curd granule junctions [15, 28, 30] clearly visible as areas depleted of fat (Fig. 5). Lactic acid bacteria were associated with the curd granule junctions more frequently than with the interior areas of the granules. Fat in the form of fat globule clusters was distributed relatively evenly throughout the granules. The stretched Mozzarella cheese (15% fat, 52% moisture) revealed an oriented structure under the SEM provided that the samples were fractured along the protein fibres (Fig. 6). This orientation was not noticeable in cross fractures (Fig. 7). Fat particles consisted of clustered fat globules. Fat globule membrane residues and lactic acid bacteria were clearly noticeable (Fig. 8).

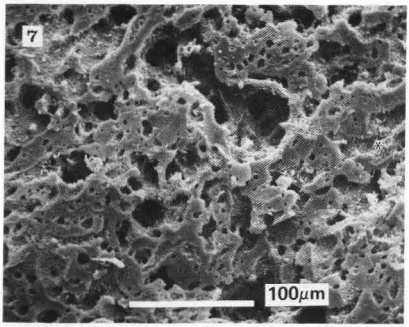
The process Cheddar cheese (28% fat, 44% moisture) contained uniformly emulsified fine fat particles in a protein matrix free of melting salt residues (Fig. 9). Although the process Cheddar cheese had approximately the same fat content as stirred-curd Mozzarella and almost twice as much fat as the stretched Mozzarella, the small dimensions of the fat particles made the process cheese appear under the microscope as having a considerably lower fat content. The fat particles in process Cheddar cheese contained no membranes as the latter had been disrupted during cheese processing. In contrast to the uniform structure of the Mozzarella cheeses, the process Cheddar cheese used in this study was found to contain osmiophilic areas (Figs. 10 and 11) already in the original state before baking. This is an interesting finding because such areas have not been reported [5, 6, 27] to exist in laboratory-made or commercially produced process cheese but were found in process cheese that had been heated excessively for 5 h at 82°C or in process cheese, in which such excessively heated cheese was used as so-called "rework" [16]. It may thus be anticipated (as direct information on the manufacture of the cheese was not available) that the process Cheddar cheese used in this study contained an excessively heated cheese as one of the ingredients.

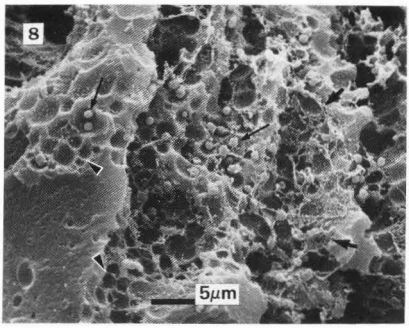
Cheeses baked in the conventional oven. The rise of temperature in the pizza dough during baking in the conventional oven is shown in Fig. 1. After 12 min, the pizza dough acquired a baked appearance and the cheeses melted to a varying extent. Although the process cheese and the stirred Mozzarella cheese had similar fat and moisture contents, the latter cheese melted rapidly and flowed down over the edge of the pizza dough whereas the process Cheddar cheese melted without flowing. The behaviour of stretched Mozzarella was closer to that of the process cheese. However, brown blisters up to 10 mm in diameter developed on the cheese surface, probably as the result of the low fat content.

The microstructure of the natural cheeses was affected by baking to a considerably greater extent than that of the process cheeses. Agglomeration of the fat particles in the natural cheeses was noticeable under a low-magnification dissecting microscope. To visualize these changes by SEM, magnifications lower than those used with the unbaked cheeses had to be used. The



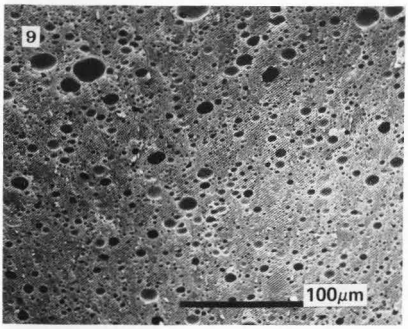




*Fig. 5. Curd granule junctions (arrows) in stirred-curd Mozzarella cheese.*

*Fig. 6. Parallel orientation of protein fibres (arrows) in stretched Mozzarella cheese is evident from a longitudinal freeze-fracture. Fat globules have been removed during the preparation of the samples for SEM from places marked with asterisks.*

*Fig. 7. Cross freeze-fracturing of stretched Mozzarella cheese reveals no orientation of the protein matrix. Fat globules were aggregated in clusters (asterisk).*

*Fig. 8. Fat globules (arrowheads) clustered in stretched Mozzarella cheese. Large arrows point to fat globule membrane fragments. Lactic acid bacteria (small arrows) are also noticeable.*

*Fig. 9. In the process Cheddar cheese, fine fat globules are relatively uniformly distributed in the protein matrix. There is no evidence of melting salt crystals in this sample.*

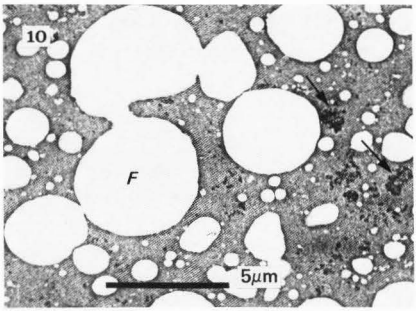



*Fig. 10. The distribution of fat (light circles - F) and dark osmiophilic areas (arrows) in a thin section of process Cheddar cheese.*

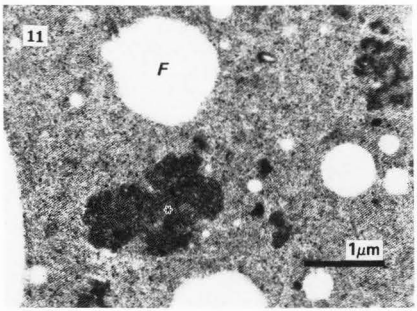
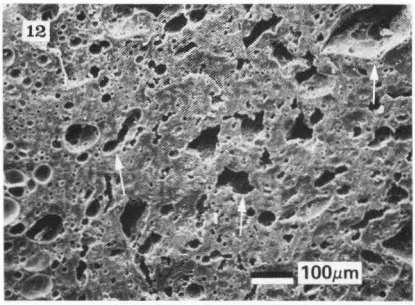
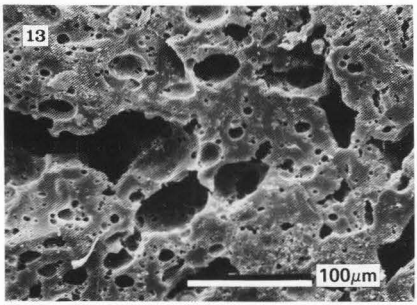
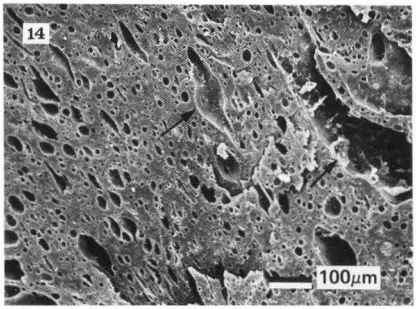


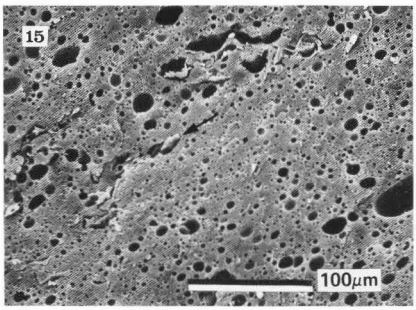

*Fig. 11. Detail of an osmiophilic area (asterisk) in process Cheddar cheese. (F = fat).*

*Figs. 12 to 15: See next page for legends.*



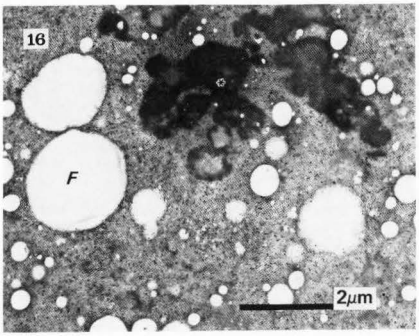



*Fig. 16. TEM of a thin section of process Cheddar cheese that had been baked in a conventional oven reveals the presence of dark osmiophilic areas (asterisk) in the protein matrix.*

general view of the coarse laminar structure of the stirred-curd Mozzarella cheese shows that fat globules agglomerated into larger particles. Distortion of the fat particle shapes shown in this figure was apparently the result of the collapse of the original structure which led to the flow of the fat out from the cheese (Fig. 12). Interestingly, salt crystals similar to those of calcium phosphate reported elsewhere [5, 14, 27] developed in this cheese as the result of baking. The same magnification in Figs. 5 and 13 makes it possible to compare the structures of unbaked and baked cheese. The agglomeration of the fat particles also took place in the stretched Mozzarella cheese (Fig. 14) with occasional occurrence of large fat particles. Fat globule membrane fragments were displaced by this process in both Mozzarella-type cheeses. This development is particularly noticeable in the stretched Mozzarella cheese by comparing Fig. 14 with Figs. 7 and 8 obtained at the same magnification. The agglomeration of the fat particles was least advanced in the process Cheddar cheese (Fig. 15).

*Fig. 12. Microstructure of stirred-curd Mozzarella cheese that had been baked in a conventional oven. Fat globules are aggregated in large fat particles which acquired irregular shapes (arrows).*

*Fig. 13. Detail of the microstructure of stirred-curd Mozzarella cheese that had been baked in a conventional oven.*

*Fig. 14. Microstructure of stretched Mozzarella cheese that had been baked in a conventional oven. The orientation of the protein matrix is apparently due to the flow in the oven rather than due to the original stretching. Fat globules are aggregated in large fat particles which acquired irregular shapes (arrows).*

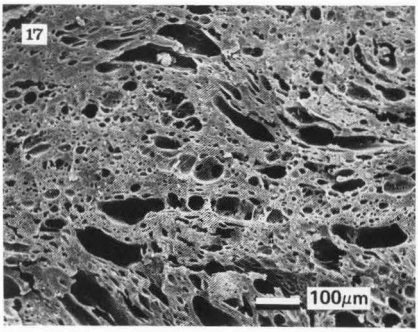
*Fig. 15. Microstructure of process Cheddar cheese was only slightly altered by baking in a conventional oven. Arrow points to a disturbance in the structure.*

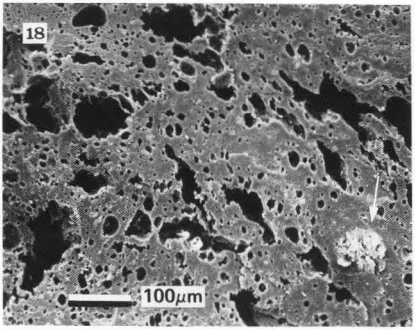
The changes, which took place in the cheeses during baking including the loss of oriented structure in stretched Mozzarella cheese, made them more difficult to prepare for TEM than the control cheeses. The aggregation of fat produced relatively larger fat particles. The protein matrix depleted of the fat became considerably more compact. This compaction, particularly severe in the low-fat stretched Mozzarella cheese and the blisters, led to problems when impregnating the samples with the resin and resulted in poor micrographs suffering from several artefacts. Visual examination of the cheese protein matrices failed to reveal changes other than those associated with the increased structural heterogeneity of the baked cheeses. In process Cheddar cheese, the dark osmiophilic areas found before baking were noticeable in the protein matrix after baking (Fig. 16).

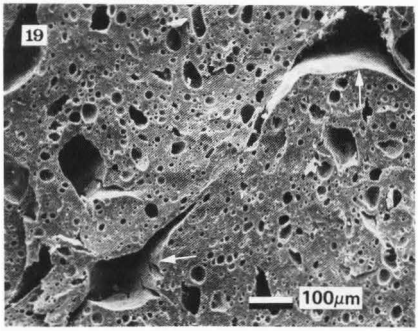
Cheeses baked in the microwave oven. Although the duration of heating was considerably shorter in the microwave oven than in the conventional oven, the cheeses were heated at or above 90°C for similar periods (2 to 2.5 min) as is evident from Fig. 1. The effects of microwave baking on the microstructure of the cheeses were similar to the effects caused by baking in the conventional oven but were not as severe. However, the characteristic features found in the natural Mozzarella cheeses vanished (Figs. 17 to 19). Fat globules and their clusters aggregated into larger particles and became distorted by the flattening of the cheese or its flow. Compared to the baking in the conventional oven (Figs. 12 to 14), where the flow of the cheeses was almost completed, the cheeses baked in the microwave oven were sampled while they were still flowing. This is particularly clearly seen in Figs. 17 and 19. Although heating of the cheese in the microwave oven lasted only shortly, this time was sufficient for some salt crystals to develop (Fig. 18). The structure of baked process Cheddar cheese is shown in Figs. 20 and 21, where fat particles widely ranging in dimensions are noticeable. The process cheese contained compact areas (Fig. 20) as well as areas having fat particles larger than 50 μm in diameter (Fig. 21). Similar to the cheeses baked in the conventional oven, TEM showed that the Mozzarella cheeses consisted of uniform protein matrices (Fig. 22) and that osmiophilic areas were present in the process Cheddar cheese (Fig. 23).

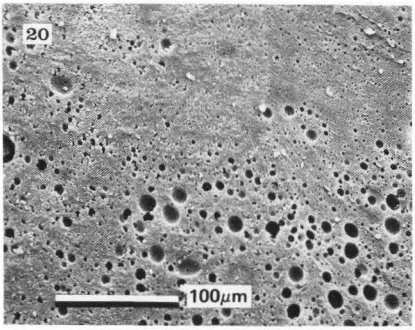
In conclusion, monitoring of the temperature in pizzas during baking showed that the cheese received only a small amount of heat compared to the heat to which process cheese may be exposed during manufacture (71-95°C [5]). The temperature in the cheeses baked in the conventional oven did not exceed 100°C and the maximum temperature found in the cheese baked in a microwave oven was 103°C. The cheeses were exposed to these high temperatures for only a few minutes. This exposure changed the microstructure of the cheeses but did not affect their amino acid composition. Low concentrations of D-Phe and D-Glu were found in the unbaked cheeses and no additional formation of these or any other D-amino acids by racemization was observed as the result of baking the cheeses in the conventional oven. By not having altered the amino acid composition of the cheese proteins, the short-time baking apparently has not affected the nutritional quality of the cheeses.

Process cheese was subjected to the least structural changes with fat globules agglomerating to a small extent. No major release of the fat from the cheese was observed. Fat particles agglomerated to a greater extent



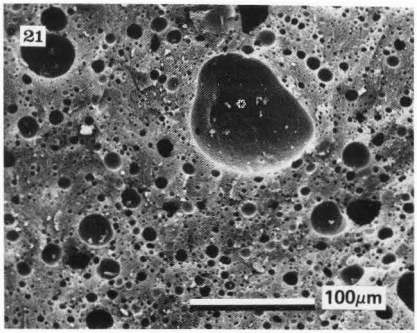




*Fig. 17. Microstructure of stirred-curd Mozzarella cheese that had been baked in a microwave oven. Parallel orientation of the protein matrix suggests that the cheese was subjected to flow before cooling and fixation.*

*Fig. 18. A salt crystal (arrow) (probably calcium phosphate) developed in stirred-curd Mozzarella cheese baked in a microwave oven.*

*Fig. 19. Microstructure of stretched Mozzarella cheese that had been baked in a microwave oven. The dimensions of the fat particles fluctuate within a wide range. Large aggregations of fat have acquired irregular shapes (arrows).*

*Figs. 20 and 21. Microstructure of process Cheddar cheese that had been baked in a microwave oven. Fig. 20 shows a more compact area (asterisk) than Fig. 21, where fat globules larger than 50 μm in diameter (asterisk) may be seen.*

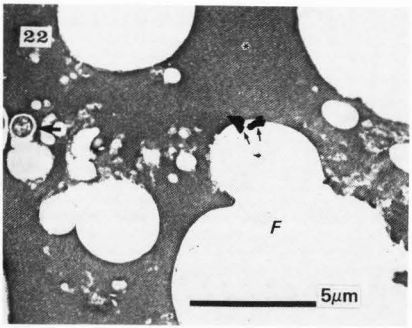



*Fig. 22. TEM of a thin section of stirred-curd Mozzarella cheese that had been baked in a microwave oven. Asterisk: protein matrix; large arrow points to a bacterium and small arrows point to contaminating particles (artefact).*

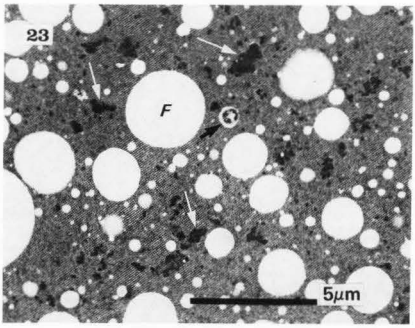



*Fig. 23. TEM of a thin section of process Cheddar cheese that had been baked in a microwave oven reveals the presence of dark osmiophilic areas (white arrows). Black arrow points to a bacterium.*

in low-fat stretched Mozzarella cheese and there were signs of fat release from the cheese. The most severe changes took place in high-fat stirred Mozzarella cheese. The cheese melted during pizza baking very rapidly, had a tendency to flow away from the dough, and released a considerable part of the fat.

The protein matrices of all three cheeses under study, examined by TEM, showed no alterations due to baking.

## Acknowledgments

The authors thank Mrs. Vivian Agar for baking the pizzas, Mrs. Paula Allan-Wojtas and Mr. John Emery for assistance with electron microscopy, and Mr. George Morris for amino acid analysis and nitrogen determination. Appreciation is expressed to Dr. H. W. Modler and Dr. L. Lloyd for useful suggestions. Electron Microscope Unit, Research Branch, Agriculture Canada in Ottawa provided facilities. Contribution 754 from the Food Research Centre.

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## Discussion with Reviewers

D. N. Holcomb: The authors have stated that "in a microwave oven ... the food is heated from the interior towards the surface..." Is this really correct? Don't some foods remain cold in the center with a warmer surrounding region, after microwaving? Isn't microwave radiation limited in its penetration depth because it is absorbed before it reaches the center of the food, i.e., the energy is absorbed first nearer the outside? Thus, in a microwave oven as in a conventional oven, frozen products thaw from the "outside in". In some other instances where there is a lot of surface moisture evaporation to cool the outside of the food, one can get the impression that the inside is heated preferentially, but I think that it is incorrect to state that the "food is heated from the interior..."

Authors: According to the literature [1, 2, 33], microwave energy penetrates to a depth limited to 5-7 cm in foods and the heat generated is then conducted farther. The surface is cooled by the evaporation of moisture from it. Consequently, the surface layer is heated by both the absorption of the microwave energy and by heat conduction from deeper areas. Thus, the heat travels from the subsurface areas to the surface and the food is heated from the "inside". Compared to baking in a conventional oven, the surface of the food is not overheated as would happen in the case where the microwave energy would all be absorbed by the superficial layer.

D. N. Holcomb: Does mentioning a "contact thermometer" mean that the "surface" temperature was measured or, as seems to be indicated, was the thermometer inserted into the interior of the product for temperature measurement? It would be interesting to compare interior and surface temperatures; in a conventional air convection oven, the surface may be hotter than the interior, while in a microwave oven, the surface may be colder than the interior, because of surface evaporation. Thus, one would expect more protein damage on the surface of the food heated in a conventional oven than on the surface of the food heated in a microwave oven. Did the authors distinguish between surface and interior areas when performing the amino acid analyses and microstructural studies?

Authors: The layer of shredded cheese on the pizza dough before baking was 0.5 to 1.0 cm thick and the total mass of the cheese was 65 g per pizza, which was 18 cm in diameter. Baking caused the cheese to melt and spread over the entire pizza surface. Thus, each $cm^2$ of the pizza surface contained ~0.25 g of the cheese, i.e., a layer ~2 mm thick. Taking the effects of bubbling, flow, and partial absorption of some cheese fat in the underlying cloth into consideration, it was virtually impossible to separate superficial and interior cheese areas for amino acid analysis and electron microscopy.

The contact thermometer was inserted into the thin cheese layer and touched the underlying cloth barrier.

D. N. Holcomb: Were the moisture contents the same in all of the cheese samples and at all locations within a given baked sample? Did the moisture content change upon

baking? One might expect the surface of a product baked in a conventional oven to be drier than the interior of that product and just the opposite in the case of a microwaved product, if there were moisture migration toward the surface of the product in the microwave oven. If the moisture contents varied, then the amino acid content might also vary.
Authors: All cheese samples had been freeze-dried for amino acid analysis and the results listed in Table 1 relate to the dry matter content. The moisture contents in the baked cheeses were as follows:

| Cheese: | Moisture content (%) in the cheeses unbaked (fresh): | baked in an oven convent.: | microwave: |
|---|---|---|---|
| Stirred-curd Mozzarella | 44.4 ± 0.9 | 33.7 ± 0.7 | 37.4 ± 2.1 |
| Stretched Mozzarella | 39.2 ± 0.1 | 29.2 ± 1.5 | 29.2 ± 0.5 |
| Process Cheddar cheese | 41.5 ± 0.1 | 29.5 ± 0.3 | 30.0 ± 0.2 |

E. Parnell-Clunies: The authors should indicate the pH of each cheese since it will affect texture.
Authors: Regrettably, pH measurements were not included in the tests.

E. Parnell-Clunies: Is it appropriate to use the term "fibre" in describing a continuous protein network such as that shown in Figs. 6 and 7? Don't the differences in these figures arise from fracturing areas of differing degree of compactness?
Author: These figures show stretched (Italian-style) Mozzarella in which the protein is oriented in one direction in the form of fibres. The differences arise from freeze-fracturing the protein network either along the fibres (Fig. 6) or across them (Fig. 7).

E. Parnell-Clunies: Osmiophilic areas in the unbaked process Cheddar cheese and the process cheese baked in a conventional oven occur as localized bodies. When this cheese is baked in a microwave oven, osmiophilic areas are more widely distributed. Do the authors have any suggestions for this redistribution?
Authors: Osmiophilic areas are shown by TEM in unbaked process Cheddar cheese (Figs. 10 and 11 - detail), in process cheese baked in the conventional oven (Fig. 16 - detail), and in process cheese baked in the microwave oven (Fig. 23). Using these micrographs as well as the unpublished ones, we have been unable to confirm the above suggestion that the osmiophilic areas are more widely distributed in the cheese baked in the microwave oven than in the other process Cheddar cheese samples.

E. Parnell-Clunies: Do the authors have any data on relative quantities of osmiophilic bodies? If so, are these proportions in keeping with normal usage levels of "rework" commercially manufactured processed cheese?
Authors: We have no such data. In fact, the presence of the osmiophilic areas in commercial process cheese selected for these experiments is quite surprising in view of an earlier finding [16] that the development of such areas is related to an excessive heat treatment of process cheese rework, where the osmiophilic areas were observed for the first time.

E. Parnell-Clunies: Given that a microwaveable crust has been developed [23], would the authors recommend microwave heating of cheese to the pizza industry?
Authors: Yes. We would also suggest that process cheese be considered for this purpose.

G. Sarwar: What are the nutritional and/or organoleptic implications of the changes in the microstructure or natural cheeses caused by baking?
Authors: The amino acid composition and configuration in the three cheeses studied was not affected by microwave or conventional baking. Thus, the nutritional values of the cheeses remained unaltered as far as the amino acid composition is concerned. Organoleptic properties (sensory attributes) of the cheeses were markedly affected by baking as the cheeses melted. In our opinion, the changes would be acceptable with the process Cheddar and low-fat stretched Mozzarella cheeses but would not be acceptable with stirred-curd Mozzarella because this latter cheese oiled off severely during baking. The preservation of small fat globules in the baked process Cheddar cheese was associated with its resistance to excessive melting. In contrast, the microstructural studies of the two Mozzarella cheeses as carried out in our experiments before and after baking would not be sufficient to characterize the suitability of the cheeses as pizza ingredients. However, this study showed for the first time the structural changes which take place in various cheeses during baking.

## Additional References

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