

Chapter 6

Principles and Applications of Chemical Markers of Sterility in High-Temperature-Short-Time Processing of Particulate Foods

Hie-Joon Kim¹, Irwin A. Taub¹, Yang-Mun Choi²,
and Anuradha Prakash^{3,4}

¹U.S. Army Natick Research, Development and Engineering Center,
Kansas Street, Natick, MA 01760-5018

²Institute of Biotechnology, Korea University, 1 Anam-dong,
Sungbuk-ku, Seoul 136-701, Korea

³Department of Food Science and Technology, Ohio State University,
Columbus, OH 43210

Continuous sterilization and aseptic packaging technologies have a great deal of potential to produce shelf-stable foods in convenient packages. A direct measurement of time-temperature history within food particulates is not practical in continuous, high temperature/short time (HTST) processes. The yield of thermally produced compounds offers an alternative as a time temperature integrator and as a chemical marker of sterility. One such a compound, 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (M-1), is formed at sterilizing temperatures from D-glucose or D-fructose and amines through 2,3-enolization under weakly acidic or neutral conditions. Another marker, 4-hydroxy-5-methyl-3(2H)-furanone (M-2), is formed similarly from D-ribose or D-ribose-5-phosphate. Application of these compounds to mapping lethality distribution within food particulates in two volumetric heating processes, ohmic heating and microwave sterilization, is demonstrated.

Conventional thermal processing, such as retorting, relies on heat transfer from the surrounding heat source, often through a liquid medium, to the center of particulate foods. Therefore, when producing shelf-stable foods, a certain amount of overprocessing takes place by the time commercial sterility is achieved at the cold spot of the food particulates. Such overprocessing could be avoided if the particulates are sterilized by heat generation throughout the volume.

⁴Current address: Department of Food Science and Nutrition, Chapman University, Orange, CA 92666

Ohmic heating and microwave sterilization are two volumetric heating technologies available to food processors. In ohmic heating, the electrical conductivities of the fluid and the particulates are important parameters (1,2). In microwave sterilization, heat generation depends on the dielectric loss factor of the food materials (3,4). For industrial applications, both ohmic and microwave processes are carried out in a continuous mode. In ohmic heating, foods are continuously pumped through sets of electrodes under high voltage, holding tubes, and cooling tubes, and then aseptically packaged (5). In microwave processing, prepackaged foods are sterilized, under high pressure, with microwaves from magnetrons above and below the foods moving on a conveyor belt (6).

In either case, the time-temperature measurement within the moving food particulates is difficult, and consequently assuring commercial sterility without overprocessing is not a straightforward matter. In this paper, we will discuss how thermally produced compounds can be used as chemical markers of sterility in ohmic heating and microwave sterilization.

Selection of the Chemical Markers

Destruction vs. Formation. When looking for chemical markers of sterility, one is tempted to look for compounds that are destroyed at sterilizing temperatures for the simple reason that the chemical identity and the assay method is already known to the investigator. Several examples were listed by Kim and Taub (7). This approach has a limitation, because a typical chemical reaction in foods is much slower than bacterial destruction at high temperatures and one has to be able to measure a small loss of the compound. For example, the D-value (time required to reduce the concentration by 90%) for destruction of thiamin is 244 min at 122°C (8). The D-value for destruction of *B. stearothermophilus* is about 1 min at the same temperature. The D-value and k, the rate constant for a first-order reaction, are related by eq. (1).

$$k = 2.303/D \quad (1)$$

Thus, the rate constant for destruction of thiamin is 0.0094 min^{-1} at 122°C. For commercial sterility, 5-7 min heating at 121°C is usually required (9). After 5 min heating at 122°C, e^{-kt} equals 0.954 and only 5% loss of thiamin will take place, which is difficult to measure accurately. On the other hand, some reactions such as enzyme inactivation are too fast at sterilization temperatures and would be useful only as markers for pasteurization.

However, if one were to turn attention to the products of such slow reactions, accurate determination becomes much easier, because one starts with a zero baseline. The product (marker) concentration approaching a limiting value exponentially can be expressed as follows:

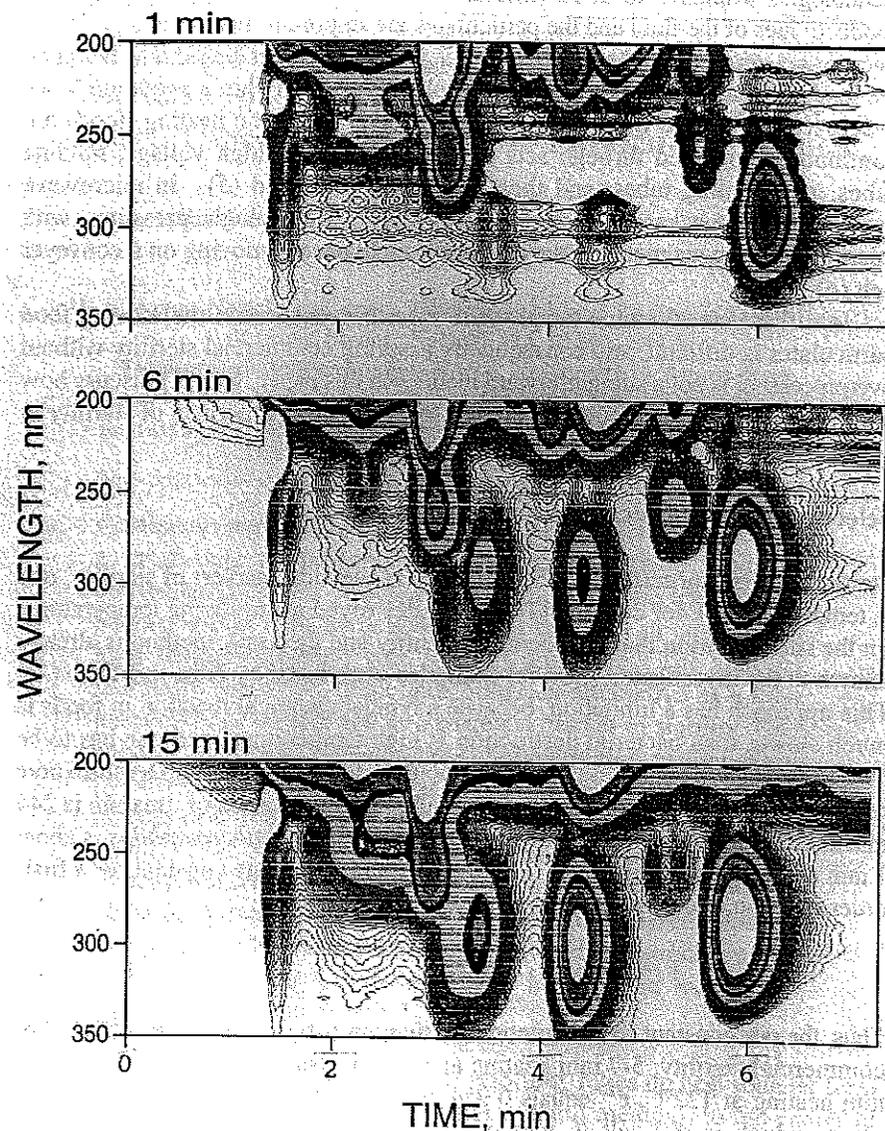


Figure 1 Contour diagram for water-extractable compounds in beef heated for 1, 6, and 15 min at 121°C. The x-axis is chromatographic retention time, the y-axis is uv wavelength, and the z-axis is absorbance.

$$M(t)/M_{\infty} = 1 - e^{-kt} \quad (2)$$

For $kt \ll 1$, e^{-kt} can be approximated as $1 - kt$ and eq. (2) becomes

$$M(t)/M_{\infty} = kt \quad (3)$$

which indicates that the marker concentration is directly proportional to the heating time at a given temperature. Particularly interesting possibilities exist in the case where two markers are formed with different rates and activation energies (10).

Ease of Detection and Stability. Numerous compounds are thermally produced in foods, but not all are suitable as chemical markers of sterility. Some of the compounds that need to be ruled out include volatiles and unstable intermediates that rapidly undergo subsequent reactions. Preferably, the marker compound should be easily extracted with an aqueous solvent and easily determined without many additional operations. The marker should also be stable during analysis. In situ analysis would be ideal; however, accurate quantitation by simple in situ methods, such as surface fluorescence or near infrared measurements, is questionable.

Figure 1 shows contour diagrams of spectrochromatograms of water-soluble compounds from beef heated for 1, 6, and 15 min at 121°C using pressurized steam. The three-dimensional spectrochromatogram was obtained using anion exclusion chromatographic separation and photodiode array detection (7). It is clear that the compound with elution time of 5.8 min and absorption maximum of 285 nm (M-2)(7) is formed quite rapidly and approaches a limiting value after 15 min heating. The formation of another compound with elution time of 4.2 min and absorption maximum of 298 nm (M-1)(7) is slower and still ongoing after 15 min. These compounds are easily extracted with water and determined by liquid chromatography (7). Spectrophotometric detection at a fixed uv wavelength (285 or 298 nm) could be used for simultaneous determination of both M-1 and M-2 without any interference. The heated sample or the extract could be frozen and stored for several days without affecting the analysis.

Identification. Purification and identification of M-1 and M-2 as 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one and 4-hydroxy-5-methyl-3(2H)-furanone, respectively, by mass spectrometry have been published (7,11). Analysis of different types of foods heated similarly at sterilizing temperatures revealed that M-1 is formed in meats and vegetables and M-2 is formed in meats only. Another compound, 5-hydroxymethylfurfural, appears to be a useful marker in heating fruits and fruit juices (M-3)(7).

Earlier Work and Precursor-Marker Relationship

Both 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (referred to as M-1 for convenience) and 4-hydroxy-5-methyl-3(2H)-furanone (referred to as M-2) have been known for almost 30 years. They were observed in heated foods and synthesized in simple model systems. We were interested in identifying the natural precursors of M-1 and M-2 in real foods.

M-1. Formation of M-1 was first noticed by Shaw et al. in 1967 in acid-catalyzed dehydration of D-fructose (12). In fact, they identified the compound as 4-hydroxy-2-(hydroxymethyl)-5-methyl-3(2H)-furanone. In the same year, the same authors observed the compound in stored dehydrated orange powder (13), which represents the first detection of M-1 from real foods. In 1968, Severin and Seilmeier reported a new compound formed from D-glucose, acetic acid, and methylamine and assigned an incorrect structure (14). The correct structure assignment was reported by Mills et al. in 1970 (15). In the following year, Shaw et al. confirmed the correct structure (16).

In 1976, Ledl et al. demonstrated that M-1 is formed in heated carrots, onions, tomatoes, cabbage and meat as well as in caramelized sugars and bread crust (17). Takei detected M-1 as an aroma compound in roasted sesame seed (18). In 1990, Nishibori and Kawakishi noted that M-1 is a major flavor component in baked cookies (19). They also reported that reaction between fructose and protein yield more M-1 than that between glucose and protein after 10 min baking at 150°C. They extended this work to fructose and β -alanine and again observed that a slightly higher yield of M-1 is obtained with fructose than with glucose (20).

M-1 was also produced from glucose and piperidine (21), lactose and lysine (22), glucose and proline (23), 1-deoxy-D-erythro-2,3-hexodiulose and piperidine (24), and glucose and propylamine (25). In 1976, Mills and Hodge showed that 1-deoxy-(L-proline)-D-fructose is converted to M-1 upon pyrolysis (26). In 1987, Njoroge et al. detected the formation of M-1 from glucose and neopentylamine under physiological conditions (27).

From these results, it appears that both glucose and fructose will react with proteins and amino acids in foods to form M-1. In mammalian muscle after rigor mortis, approximately 0.17% is glucose-6-phosphate and 0.01% is glucose by wet weight (28). Ribose-6-phosphate was implicated in the formation of M-2 (29-31). We performed spiking experiments to determine whether glucose-6-phosphate or glucose is the natural precursor of M-1 in meats. One % D-glucose, D-glucose-6-phosphate, D-fructose, or D-ribose was added to a meat extract, and the time course of M-1 formation in the meat extract at 121°C was monitored up to 100 min. Figure 2 shows that the control meat extract and the ribose-added

meat extract yield the same amount of M-1 from the precursors already present in the meat. Thus ribose served as another control. Addition of fructose increased the M-1 yield by approximately 70% over the control. Addition of the same amount of glucose increased the M-1 yield approximately three-fold. On the other hand, glucose-6-phosphate seemed to slightly decrease the M-1 yield. Glucose-6-phosphate might compete with glucose for reaction with the amines, but does not lead to the formation of M-1. It is not clear why fructose shows higher reactivity than glucose toward M-1 formation in baking and the reverse is true in heating meat. The concentration of fructose is much lower than that of glucose in meats; therefore, we believe that glucose, not glucose-6-phosphate, is the natural precursor of M-1 in meats. Fructose might play a more important role in M-1 formation in vegetables.

M-2. Severin and Seilmeier first synthesized M-2 from pentoses and primary amine salts in 1967 (32). In the following year, Peer et al. synthesized M-2 from D-xylose or D-ribose and secondary amine salts (33), and Peer and van den Ouweland synthesized M-2 similarly from D-ribose-5-phosphate (29). In the same year, Tonsbeek et al. published identification of M-2 from beef broth (34). Subsequently, Tonsbeek et al. identified ribose-5-phosphate and pyrrolidone carboxylic acid/taurine as natural precursors of M-2 in beef (30).

In 1974, Anderegg and Neukom showed that M-2 can be formed from purinnucleosides and -nucleotides as well as from pentoses upon heating without amines (31). Hicks et al. prepared M-2 from D-glucuronic acid and amine (35). In 1979, Nunomura et al. identified M-2 as an important flavor component in soy sauce, which is rich in pentoses such as arabinose, ribose, and xylose as well as amino acids (36). In 1980, Honkanen et al. identified M-2 in fresh wild raspberries (37). Knowles et al. observed M-2 resulting from addition of spinach chloroplast ribosephosphate isomerase to ribose-5-phosphate (38). Nursten and O'Reilly produced M-2 from glycine and xylose (39). Idstein and Schreier identified M-2 from guava fruit (40). M-2 was also identified from roasted sesame seed (18).

Above results indicate that M-2 can be formed from pentoses and their phosphates (29,31,38). Results of our spiking experiment (Figure 3) also show that both ribose and ribose-5-phosphate participate in the formation of M-2 in meats. It appears that the phosphate group is readily hydrolyzed from ribose-5-phosphate and the ribose participates in the formation of M-2, whereas glucose-6-phosphate is not hydrolyzed and does not participate in the formation of M-1. The relative concentrations of ribose, ribose-5-phosphate, and other pentoses in meats are not well known. Presumably, both ribose and ribose-5-phosphate are the primary precursors of M-2 in meats.

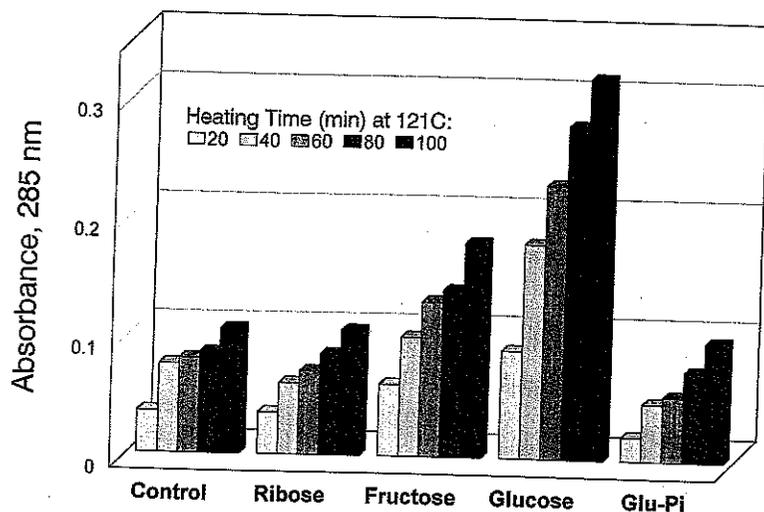


Figure 2 Results of spiking experiment showing change in M-1 yield upon heating beef extract with added ribose, fructose, glucose, and glucose-6-phosphate.

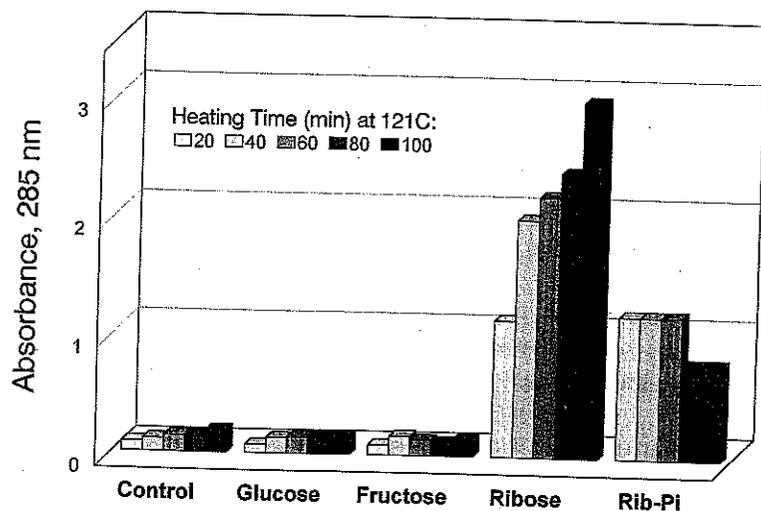


Figure 3 Results of spiking experiment showing change in M-2 yield upon heating beef extract with added glucose, fructose, ribose, and ribose-5-phosphate.

Mechanism of the Marker Formation

In 1964, Anet described a dehydration pathway of Amadori compounds involving enolization and formation of dicarbonyl intermediates (41). It is believed that, in general, 1,2-enolization is favored in strongly acidic media, where the nitrogen atom of the Amadori compound is protonated, and subsequent dehydration leads to 2-furaldehyde from pentoses and 5-hydroxymethylfurfural from hexoses. On the other hand, under weakly acidic or alkaline conditions 2,3-enolization is favored leading to furanones and pyranones. In fact, Hicks and Feather showed that Amadori compounds form 2-furaldehyde in 2 N sulfuric acid and M-2 at pH 7 (42). Tressl et al. also showed that 5-hydroxymethylfurfural is a predominant product below pH 4 and M-1 is a major product above pH 5 from the proline/glucose model system (43). Feather stated that production of furaldehyde indicates a 1,2-enolization pathway and furanone a 2,3-enolization pathway (44). The 1,2- and 2,3-dicarbonyl intermediates are formed during dehydration of sugars (12) as well as of Amadori compounds.

We tested this reaction scheme in meat. Ten percent D-glucose was added to a beef extract and the pH was adjusted with a 6 N hydrochloric acid or sodium hydroxide solution. Figure 4 shows the pH-dependence of M-1 and M-3 after 15 min heating at 121°C. As expected, 1,2-enolization and M-3 formation predominates below pH 4. Above pH 5 there is little M-3 formation, which explains why we never observed M-3 from heated meats (pH 5.4). There is a sharp decline in the M-1 yield above pH 10. This observation is consistent with the base-catalyzed fructose dehydration Shaw et al. investigated at pH 11.5 (45). They reported that M-1 was not formed at pH 11.5; however, if the alkalinity was not constantly maintained, M-1 was formed as the pH decreased. Similarly, when 1% D-ribose was added to the beef extract, formation of 2-furaldehyde dominated at low pH and M-2 formation became more important at pH above 4.5, which again explains why 2-furaldehyde was never observed in heated meats. The general reaction pathways for formation of the markers are summarized in Figure 5.

Applications to Ohmic Heating and Microwave Sterilization

In general, a given chemical marker concentration can be arrived at through many different time-temperature histories. The possibilities are drastically reduced when two markers are used. It may even be possible to determine a unique time-temperature history based on computer simulations if a practical range of time and temperature were given for the yield of two markers for which the reaction rate constants and activation energies are known. We are currently investigating this interesting aspect of the chemical marker application. In this paper, we will demonstrate how the markers, with certain limitations, can be used to provide

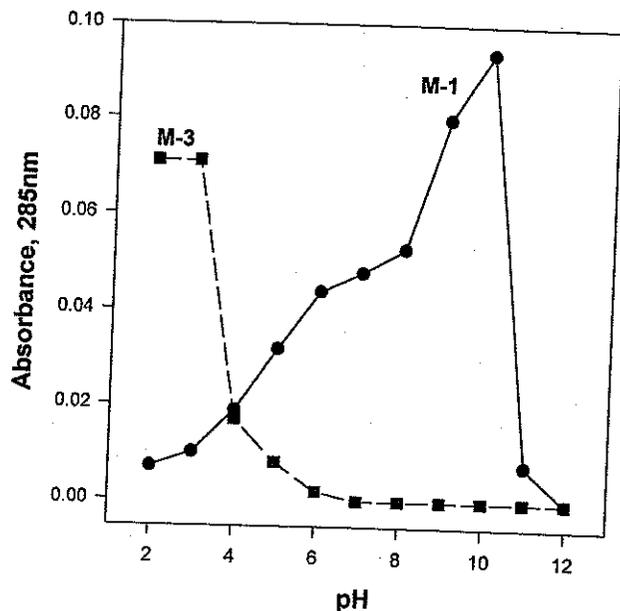


Figure 4 pH-dependence of M-1 and M-3 yield from beef extract with 1% glucose added.

Ohmic Heating. In ohmic heating, foods are sterilized by electroresistive heat generation throughout the volume. A key advantage of ohmic heating is the possibility of faster heating in the particulates than in the fluid (2,46-47). If a range of product and process parameters (such as the fluid and particulate electrical and thermal conductivities, solids content, applied voltage, and flow rate) can be selected that will assure faster heating of the particulates, the fluid temperature can be measured and used to guarantee sterility of the entire food product. The chemical markers turned out to be extremely useful for demonstrating faster heating of the particulate center than the particulate surface or the fluid in a continuous ohmic heating system, where a direct temperature measurement within moving food particulates is nearly impossible.

Figure 6 shows that the concentrations of both chemical markers, M-1 and M-2, are higher at the center than near the surface of a meatball ohmically processed using a 5 kW system (48). At a given temperature, there is a linear relationship between log-reduction in bacterial population and the marker yield (49). Clearly, a higher lethality was achieved at the particulate center. Results in Table I, summarizing bacterial destruction predicted from the chemical marker yield and observed directly, also show higher lethality at the center at three operating temperatures.

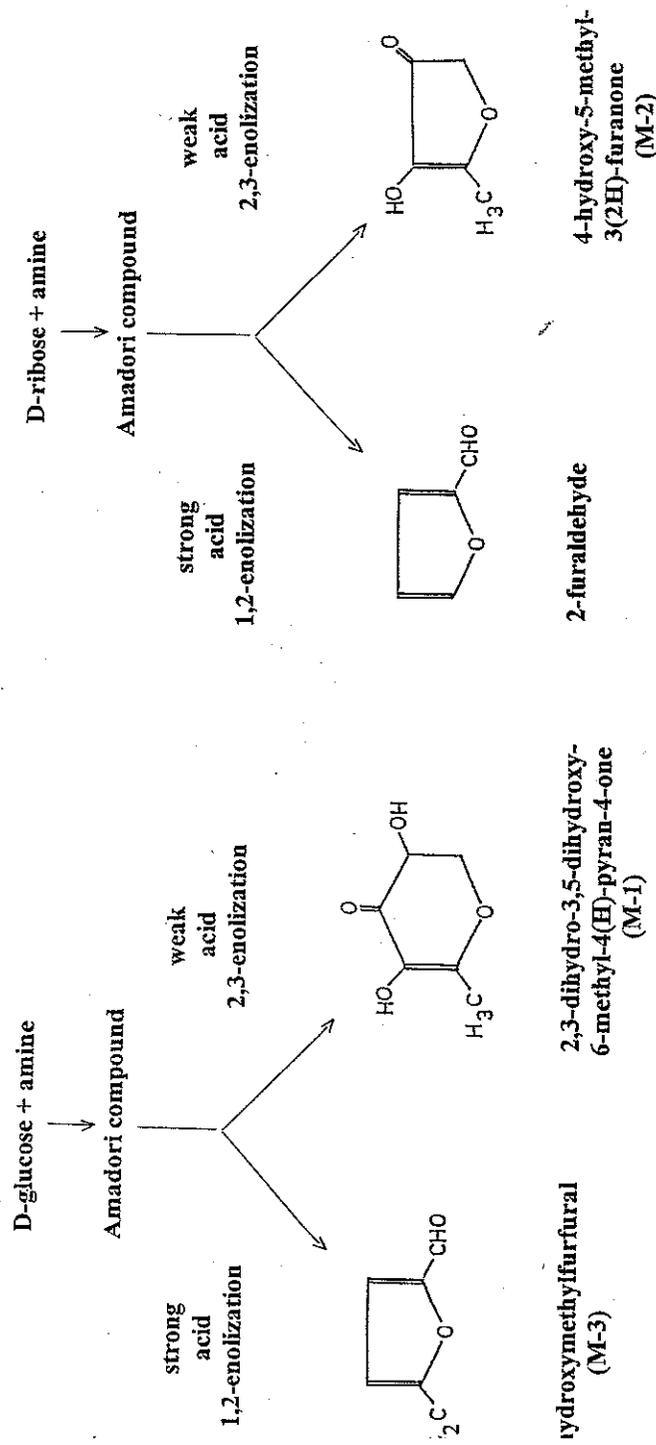


Figure 5 Summary of reaction pathways leading to the chemical marker formation.

This remarkable inversion in temperature is believed to be due to both faster heating and lower specific heat of the particulate than of the fluid. Faster heating of the particulates was also demonstrated indirectly by observation of a temperature rise in the fluid as the fluid-particulate mixture flows through the holding tubes of the ohmic processing system (H.-J. Kim et al., 1995, unpublished data). Such volumetric heating makes ohmic heating an attractive technology for producing high quality, shelf-stable foods (50).

Microwave Sterilization. In microwave heating of foods, heat generation is primarily due to the dipolar water molecules trying to align to the oscillating electrical field component of the microwave. The microwave energy is needed to break the hydrogen bonds in water to allow the dipoles to align. As the water molecules reform hydrogen bonds, the stored energy is dissipated as thermal energy (51). Several parameters, such as dielectric properties, shape, and size of the food, play important roles in microwave heating in generating the temperature profile in the food. At a low electrolyte concentration, the microwave penetration depth is significant and center heating can occur through a focusing effect when the object has a cylindrical or a spherical shape (4,52,53). As the electrolyte concentration is increased, the conductivity loss becomes important and more microwave power is dissipated at the surface creating a sharp temperature gradient (surface heating)(4,54). These observations imply that in principle it should be possible to select the salt concentration and the shape of the food that would provide a uniform heating throughout the food.

To test this idea, cylindrically shaped ham (3 cm diameter, 6 cm height) was prepared with 0.5, 1.0, 2.6, and 3.5% salt in addition to other usual ingredients. Microwave heating was performed in a cylindrical, pressurized container using a CEM microwave sample preparation system (MDS-2000, CEM Corporation, Matthews, NC). After heating for 2 min after the temperature of 121°C has been reached at the center of the cylinder (come-up time about 45 sec), the ham sample was cut into three equal sections along the height and the middle section was again cut radially into three portions (outer, middle, and the core portion). Figure 7 shows the M-2 yield in different portions of the ham. For the ham with 0.5% salt, the core showed higher M-2 yield than the outer ring probably due to a focusing effect. At 2.6 and 3.5% salt concentration, the outer ring showed much higher M-2 yield than the core. Clearly, the penetration depth is decreased as salt concentration is increased as observed previously by Mudgett (4) and Anantheswaran (54). It is interesting that the marker yield at the core is higher for the ham with 3.5% salt than that with 2.6% salt, even though the penetration depth should be smaller. It appears that, for the microwave that has penetrated, the conductivity loss is greater with the higher salt concentration.

At 1.0% salt concentration, the M-2 yield showed no gradient suggesting a uniform heating. This situation represents a balance between surface heating and focusing. Obviously, the salt concentration for achieving a uniform heating will

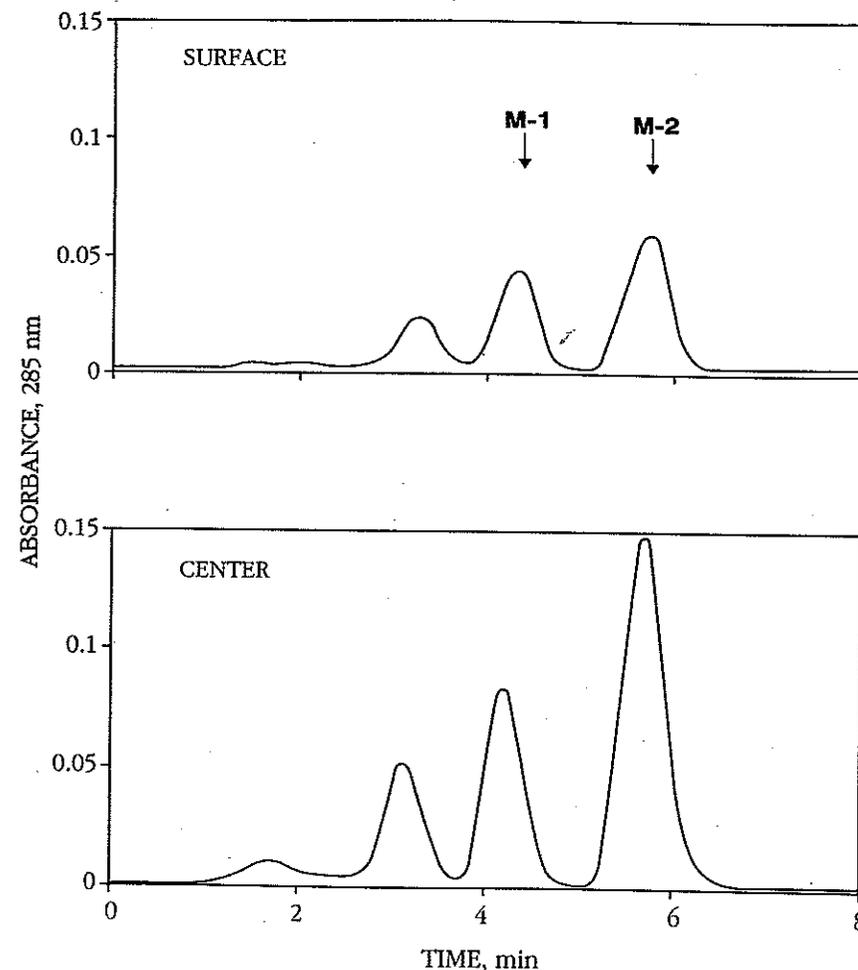


Figure 6 Chromatograms showing higher yield of M-1 and M-2 at the center than at the surface of an ohmically processed meatball. Reproduced with permission from Ref. 48.

depend on the size of the food. This example illustrates that the marker yield can be used to map the temperature distribution within a solid food and to optimize the product formula for uniform heating. The technique is also expected to be useful in verifying the thermal contour in microwave sterilized meals in various package geometries (55).

Table I. Bacterial Destruction in Ohmically Heated Meatballs Predicted from M-1 Yield and Observed Microbiologically

Temperature (°C)	Flow rate (L/min)	Location	log(N ₀ /N)	
			Predicted	Observed
125	1.1	Center	7.8	> 5
		Surface	3.2	4.1
128	0.9	Center	13.9	> 5
		Surface	5.0	> 5
132	0.9	Center	23.8	> 5
		Surface	13.5	> 5

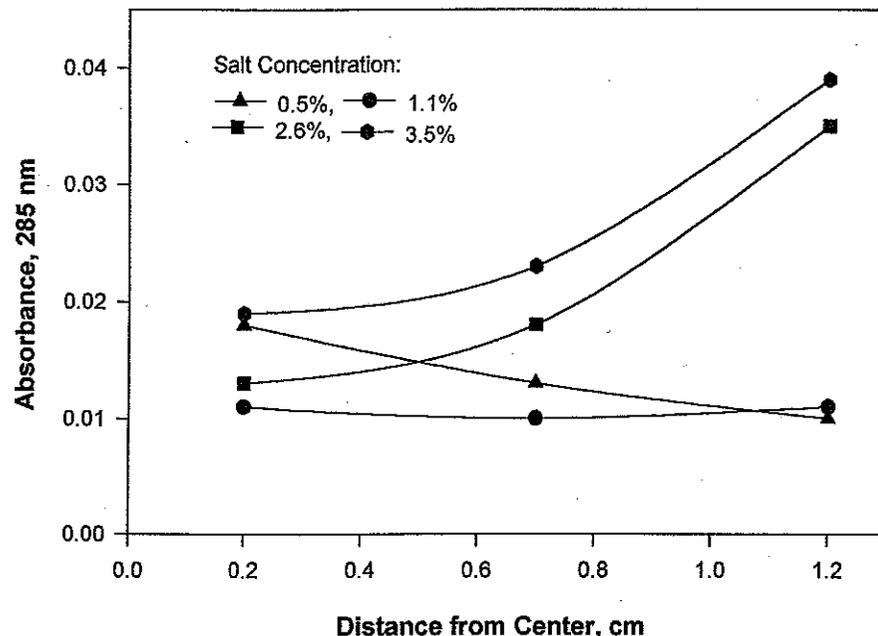


Figure 7. Yield of M-2 in the core, middle, and outer portion of ham containing different amounts of salt heated at 121°C for 5 min with microwave.

Conclusion

The reported chemical markers are useful markers of sterility, which is an important quality index in shelf-stable foods. The use of the markers to map lethality distribution in particulate foods has been demonstrated. The markers can be used for validating and optimizing new thermal processing technologies such as ohmic heating and microwave sterilization.

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