A CHEMICAL MARKER (M-2) BASED COMPUTER VISION METHOD TO LOCATE THE COLD SPOT IN MICROWAVE STERILIZATION PROCESS

By

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The members of the committee appointed to examine the dissertation of RAM BHUWAN PANDIT find it satisfactory and recommended that it be accepted.

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ABSTRACT

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The single-mode 915 MHz microwave sterilization system developed at Washington State University, Pullman has the capability to produce high quality shelf stable foods. In order for this technology to receive FDA approval there is a need for a rapid and reliable method to determine the location of cold spots in food products of different chemical composition, and size. My dissertation overcomes the limitations of the single point temperature sensor with a special focus on the development of a novel approach for determining heating patterns using chemical marker M-2 based computer vision method.

Kinetic of chemical marker M-2 formation in mashed potato has been studied to develop a method to locate the cold spots in microwave sterilization processes. Formation of chemical marker M-2 with 1.5% D-ribose was found to be suitable over the time-temperature range of the microwave sterilization process. Factors for chemical marker formation and kinetic parameters, including the order of reaction, reaction rate constant and energy of activation, were determined in this study. The results demonstrated that formation of chemical marker M-2 in mashed potato is a first order reaction.

A computer vision method based on the yield of chemical marker M-2 was developed to determine the heating patterns in a model food, mashed potato. Through interactive programming an IMAQ Vision Builder script was designed to locate the cold spot in foods during thermal processing. Sensitivities to the heating patterns were tested at different levels of salt and for different tray sizes. Results indicated that salt significantly influenced the dielectric loss but microwave heating patterns were repeatable for model foods. The location of the cold spot predicted by the model was validated using fiber optic temperature probes and microbial inoculation studies.

The developed method here was further improved to facilitate the comparison of the heating patterns for multiple trays. To do this, a new visual scale which adjusted the brightness of the scale for samples was developed. A new image system independent of the lighting position was designed as part of this study. Relationships among computer vision parameters, color value, thermal lethality (F_o), and M-2 yield for mashed potatoes were established for two different pathes of heating. Validation tests confirmed that the method based on chemical marker M-2 yield can accurately determine the cold spot location in pre-package model food processed by microwave.

To evaluate this method in a food product, salmon in Alfredo was used to determine the efficacy of this computer vision method. For these studies, a different model food based upon whey protein gels were used to simulate the heating patterns in salmon with Alfredo sauce. The dielectric properties of the whey protein gel were matched as closely as possible to the target food with addition of 0.3% salt. To predict the heating patterns in salmon with Alfredo sauce, relationship among color value in terms of grayscale value, thermal lethality to C. *botulinum* (F_o), and M-2 yield were studied with

whey protein gels. Matching the time-temperature profile between whey protein gel and salmon during microwave sterilization process confirmed that whey protein gel can be used to emulate the heating patterns in real foods. The microbiological study was conducted in 10 oz polymeric trays to validate the cold spot location in auto processed salmon with Alfredo sauce. Results showed that whey protein gels in combination with a computer vision method can predict the cold spot in real food system.

The developed computer vision method in this study is effective in locating the cold spots in model and real food systems. Because microwave sterilization process is a promising alternative to conventional retorting methods for producing high quality shelf stable foods, methods are needed to ensure that these foods can be made safely and that processes can be reliably validated. The developed method and protocol can be used to prepare documentation for FDA approval.

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DISSERTATION OUTLINE

This dissertation is organized into seven chapters. The first chapter reviews the development made in computer vision field and their application in fields of food process engineering. Chapter 2 presents the kinetic study of chemical marker M-2 formation in mashed potato. Identification of the limiting factors for chemical marker formation and a prediction of kinetic parameters including, order of reaction, reaction rate constant and energy of activation, were determined in this study. Chapter 3 explores identification and development of a novel approach to determine the heating patterns in microwave sterilization processes. In Chapter 4 sensitivities of the computer vision heating patterns were tested with different levels of salt contents and tray sizes. In Chapter 5 upgrading of the developed computer vision method was done by inserting the mashed potato scale samples to facilitate the comparison of the heating patterns for multiple trays in repeated experiments. Chapter 6 investigates the principle and application of the chemical marker M-2 based computer vision method to emulate the heating patterns in real food systems. Chapter 7 provides a protocol to identify and validate the location of cold spot in real foods. A brief conclusion from each chapter along with future recommendation is provided in the last section of this dissertation.

A list of the published chapters and chapters accepted for publication as of Nov.15, 2006.

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Chapter 3
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CHAPTER 1

A COMPUTER VISION METHOD TO DETERMINE COLD SPOT LOCATION IN FOODS STERILIZED IN 915 MHZ MICROWAVE STERILIZATION SYSTEM

1. Introduction

Microwave sterilization method developed in our laboratory is a thermal method that has promise to produce high quality and shelf stable foods. Sterilization of foods in 915 MHz microwave system reduces process time and improve product quality (Pathak, Liu, & Tang, 2003; Guan, Plotka, Clark, & Tang, 2002; Guan et al., 2003). The sterilization process involves a physical phenomenon dependent on the dielectric properties of foods and configuration of microwave cavities. Contrary to conventional heating methods, microwave heating provides much faster rate of heating. The cold or hot spots locations depend on product geometry (Campañone, & Zaritky, 2005; Yang, & Gunasekaran, 2004). Cold spots are regions in foods which receives the least thermal energy during sterilization processes. In order to develop an advanced thermal process, it is necessary to determine the cold spots in food to ensure commercial sterilization. To meet the stringent requirement of food regulatory bodies we decided to measure the time-temperature history of the determined cold spot when developing the microwave sterilization processes.

Determination of cold spot locations in foods during microwave sterilization is a major challenge for researchers in developing processes to ensure that the

processed foods are safe to consumers. Computer simulation models can help in understanding the sterilization process (Pathak, Liu, & Tang, 2003; Zhang, & Datta, 2000). But simulation models, requires validation and may not always be reliable due to complexity of the coupling of heat transfer and dielectric heating in complex microwave sterilization cavities (Ayappa, Davis, Davis, & Gordon, 1991; Pandit, & Prasad, 2003; Romano, Marra, & Tammaro, 2005). For any geometrically complex system used to produce safe foods for consumers, an approach of double validation for process development was emphasized by US food regulatory organizations (Food and Drug Administration, 2005).

Multi-point online monitoring of time temperature profile in industrial scale microwave sterilization system is impractical. Metallic thermocouples can not be placed in a microwave field while fiber optics probes are expensive and inconvenient to monitor multiple points in a tray. It was impractical to identify the cold spots in packaged foods during microwave sterilization processes by point temperature measurement methods. In order to meet the stringent requirements of food regulation bodies for sterilization processes of food products much effort in both industry and academic communities, has been made for designing a method to determine the location of cold and hot spots (Oliveira, & Franca, 2002; Fernandez, Castillero, & Aguilera, 2005, Ghani et al., 2002; Sale, 1976). Chemical marker methods were studied as indirect means to evaluate relative heating absorptions in selected food systems (Lau, et al., 2003; Wang, Wig, Tang, & Hallber, 2003; Pandit et al, 2006). Quantification of chemical marker M-1 and M-2 formed through Maillard reaction between amino acids and reducing sugar such as ribose and glucose required intensive laboratory analyses using High Performance Liquid

Chromatography (HPLC). For example, to analyze a 3-D heating pattern in processed mashed potato containing ribose in 10 oz trays with HPLC, two persons were needed for 2.5 days to quantify M-2 yield at 40 evenly distributed points in one tray. In process development, repeated tests were necessary with multiple trays. Analyzing M-2 yield in those many trays using HPLC became impractical.

It was, therefore, desirable to develop a rapid and reliable method to determine the cold spots locations in sterilized foods. To meet this goal, the major area of emphasis for this research program was to develop a novel computer vision method based on the yield of chemical marker (M-2) to determine the cold spot location in microwave sterilization process. In this chapter, introductory information is provided about concept of microwave sterilization, formation of chemical marker (M-2), and principle of computer vision. In the preceding chapters, kinetics of chemical marker M-2 formation and other studies need to develop a novel method will be discussed.

2. Concept of microwave sterilization

Microwave occupies the portion of the electromagnetic spectrum between 300 MHz and 30 GHz. Focusing or internal concentration is one of the most significant features of microwave sterilization as compared with conventional heating. The electrical properties of materials known as dielectric properties are of critical importance in understanding the interaction between microwave electromagnetic energy and foods. The dielectric properties of a material are described by the complex relative permittivity (ε^* relative to that of free space) in the following relationship (Tang, 2005):

$$\varepsilon^* = \varepsilon' - j\,\varepsilon'' \qquad (1)$$

The real part ε' is the dielectric constant that reflects the ability of the material to store energy in an electromagnetic field; the imaginary part ε" is the dielectric loss factor that influences the conversion of electromagnetic energy into thermal energy. The amount of thermal energy converted in food is proportional to the value of the loss factor (ε"). The power absorbed per unit volume, Q (W/m^3) in the dielectric can be calculated from (Tang, 2005):

$$Q = 5.56 \times 10^{-11} f E^2 \varepsilon'' \quad (2)$$

where E (V/m^{-1}) is electric field intensity, f (Hz) is frequency .These properties along with thermal and other physical properties (specific heat, thermal conductivity), and the characteristic of the microwave electromagnetic fields determine the absorption of microwave energy and consequent heating behavior of food materials in microwave sterilization. Heat conduction inside the parallelepiped shaped foods trays during microwave sterilization in rectangular co-ordinate system can be given as:

$$\rho C_p \frac{\partial T}{\partial t} = \frac{\partial}{\partial x}(k_{xx} \frac{\partial T}{\partial x}) + \frac{\partial}{\partial t}(k_{yy} \frac{\partial T}{\partial y}) + \frac{\partial}{\partial z}(k_{zz} \frac{\partial T}{\partial z}) + \phi \quad (3)$$

. In Equation (1) ρ is density (kg/m^3), C_p is specific heat (kJ/kg ^{o}C) and k is thermal conductivity of foods. In case of homogeneous isotropic foods $k_{xx} = k_{yy} = k_{zz} = k$ Microwave power source term (ϕ, w/m^3) gives the microwave power absorbed density at any location of the foods. When electric field passes through the dielectric medium it attenuates exponentially in the direction of propagation. The attenuation of power at distance x from the surface of incidence can be estimated using Lambert's law as:

$$\phi_x = \phi_o \exp(-2 \alpha x) \quad (4)$$

where ϕ_o is the net power incident on the surface of the food, ϕ_x is power incident at distance x from surface, α is the attenuation factor which depends on the dielectric constant and loss factor:

$$\alpha = \frac{2\pi}{\lambda_o}\left[\frac{1}{2}\varepsilon'\left(\sqrt{1+\left(\frac{\varepsilon''}{\varepsilon'}\right)} - 1\right)\right]^{1/2} \quad (5)$$

where λ_0 is the free space wavelength. Dielectric properties of the food change with salt content among other composition. Penetration depth (D_p) is defined as the distance at which the power drops to 37 % of its value at the surface. Penetration of microwave power inside food depends on both dielectric loss, dielectric constant of the foods and operating frequency as (Tang, 2001):

$$D_p = \frac{c}{2\pi f\sqrt{2\varepsilon'}}\left[\sqrt{1+\left(\frac{\varepsilon''}{\varepsilon'}\right)^2} - 1\right]^{-1/2} \quad (6)$$

where c is velocity of light (m/s), f is operating frequency (915 MHz).

One of the major problems in air circulated microwave heating was the high intensity of electromagnetic fields at the edges and corners of the foods. To overcome this problem the newly designed 915 MHz system at Washington State University was facilitated to flow hot water at 125 ^oC across the direction of food package to match the dielectric properties of foods with surrounding. Hot water not only helps in matching the dielectric properties between foods and the surrounding but it also absorbs the energy from hot region when temperature goes beyond 125 ^oC. Another advantage of hot water flow at 125 ^oC inside the cavity was to transfer the energy to locations where temperature is below 125 ^oC.

The novel WSU 915 MHz single-mode microwave sterilization system was tuned to generate a single mode. The excitation of wave through waveguide was of TE_{10} mode. Microwave energy at single modes with zero degree phase shift feeds energy in the middle of the foods while hot water deliver energy to the edges. Combination of hot water and zero degree phase shift single-mode microwave heating shorten a complete sterilization process to 12 minutes. Because of the constraints with existing direct methods including fiber optics probe, metallic thermocouple, infrared sensor and spectrophotometer, a chemical marker M-2 yield based method was developed to determine the cold spot in the microwave sterilization process.

3. Chemical marker (M-2) yield as indirect means to evaluate sterilization process

Chemical marker offers an alternative as a time-temperature integrator to determine heating patterns. A chemical marker method was developed at the United States Army Natick Research Center (Kim & Taub, 1993) to determine heating patterns in food system for various thermal processes. Three markers 2, 3-dihydro-3, 5-dihydrixy-6-methyl-(4H)-pyran-4-one (referred to as M-1), 4-hydroxy-5-methyl-3(2H)-furanone (M-2) and 5-hydroxymethylfurfural (M-3) have been identified by scientists in Natick US Army laboratory. Kim et al.(1996b) and Ramaswamy,et al., (1996) have used the M-1 yield as a temperature-time integrator to study Ohmic heating and aseptic processing.

Chemical marker kinetics for M-1 and M-2 has been studied with whey protein gel (Lau, Tang, Taub, Yang, 2003, Wang, Wig, Tang, & Hallber, 2003). In a whey protein gel, first order reaction leading to M-2 formation was fast and ultimately gave a shorter time to reach the saturation point (Wang et al., 2003). M-1 cannot be used for

high temperature short time processes. Hence, the Microwave Heating Group at Washington State University (Pullman, WA) selected mashed potato as a model food to locate cold and hot spots for regulatory approval of the microwave sterilization unit.

Chemical marker M-2 (*4-hydroxy-5-methyl-3(2H))-furanone*) is formed by rearranging Amadori compound product (Fig.1) through the reaction of D-ribose and amino acids in the presence of weak acidic (PH>5) environment (Prakash, Kim, & Taub, 1997). 1, 2 enolozation is favored in acidic media (pH< 4) and leads to the formation of 2-furaldehyde from ribose.

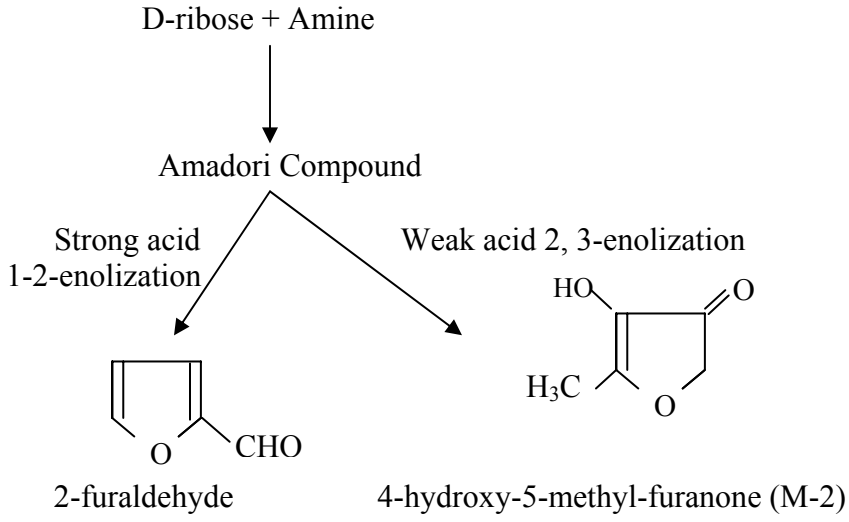

Fig. 1. Reaction pathways leading to the chemical markers formation.

Application of 4-hydroxy-5-methyl-3(2H)-furanone to mapping the lethality distribution within foods for high temperature short time process has been demonstrated by Kim et al., (1996a). Kinetics study of M-2 formation in whey protein gels were reported by Lau et al., 2003. However, a lack of kinetics information for M-2 in other food systems prohibited researchers from quantitatively relating the chemical marker yields to time-temperature effect in microwave sterilization process. In general, a given chemical

marker concentration can be arrived at through many different time-temperature histories. While reviewing the literature on this subject, we observed there was not enough research work regarding application of chemical marker as time-temperature integrator to evaluate the high temperature short time processing. To fill this gap, an extensive study was necessary to determine reaction order, rate constant and activation energy for M-2 formation in a models food (mashed potato) at range of sterilization temperature. Knowledge of kinetics parameters was only a preliminary step in order to determine the cold spot locations based on concentration of chemical marker M-2 formed during sterilization operation. After collecting the kinetic information an emphasis was given to develop a chemical marker M-2 assisted method to determine the heating patterns. In our preliminary study, we found that computer vision would be a possible option to locate the cold spot in microwave sterilization process.

4. Computer vision method to determine cold spot location

Computer vision is the science that develops the theoretical and algorithmic basis by which useful information about an object or scene can be automatically extracted and analyzed from an observed image, image set or image sequence (Haralick and Miller, 1972). Computer vision is a relatively young discipline with its origin traced back to the 1960s (Baxes, 1994). The basic principle of computer vision is described in Fig.2. Applications of this technique have now expanded to various areas such as medical diagnostic, automatic manufacturing, surveillance, remote sensing, technical diagnostics, autonomous vehicle and robot guidance (Sonka, Hlavac, & Boyle, 1999).

Computer vision system is a new technology in food industry for inspection and evaluation purpose as they provide suitable rapid, economic, consistent and objective assessment. So far, this technology has been applied to a wide variety of foods for quality evaluation, including apples (Lu, 2004), oranges (Kondo, Ahmad, Monta, & Murase, 2000), potatoes (Tao, Heinemann, Varghese, Morrow, & Sommer, 1995);

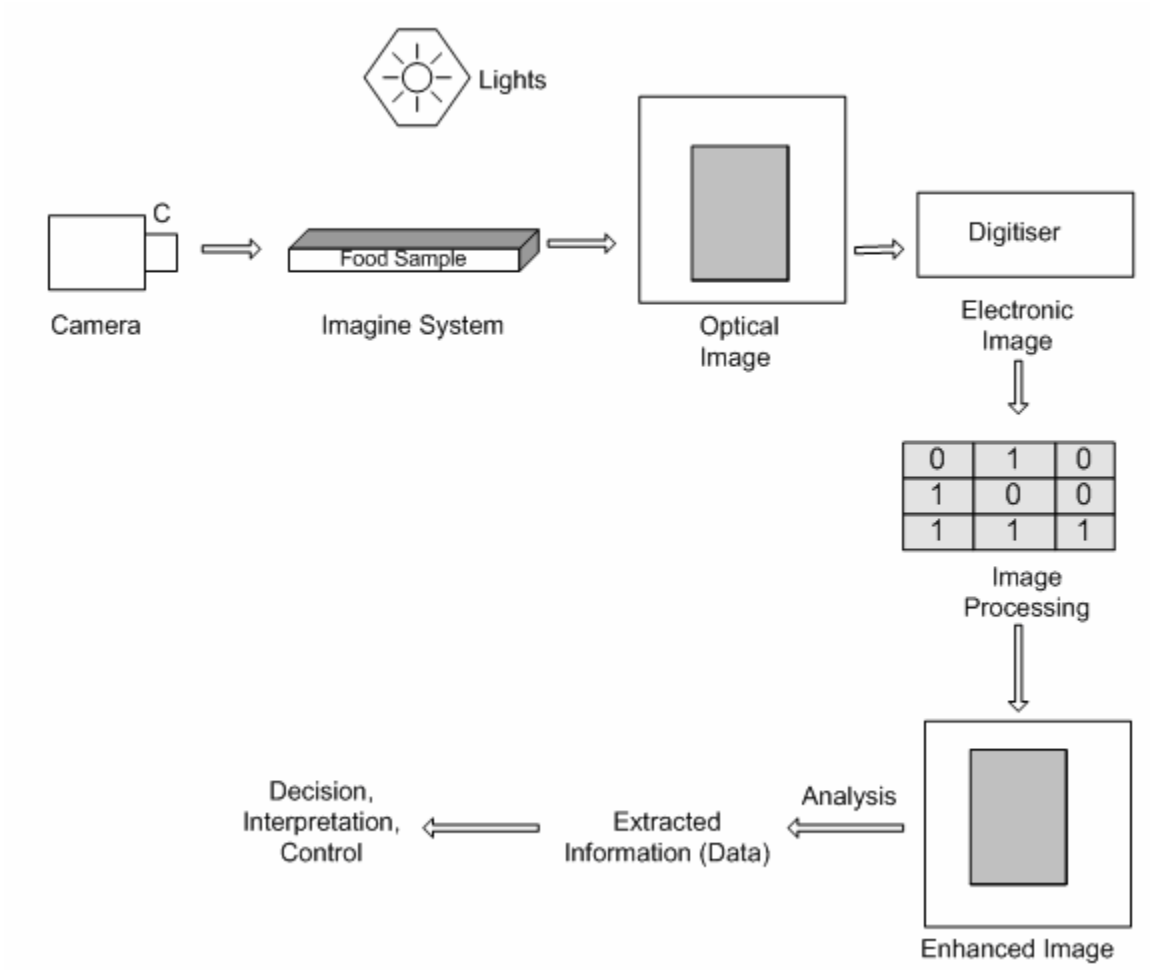

Fig. 2.Principle of computer vision system

carrots (Andersen, Henriksen, Laursen, & Nielsen, 1999), beef (Yoshikawa et al., 2000) and pork (Vestergaards, Risum, & AdlerNissen, 2004), etc. Functions of computer vision are wide-ranging including analysis of chemical properties (Bertram, Whittaker, Shorthose, Andersen, & Karlsson, 2003), grading and discriminating of foods (Du & Sun,

2004); and processing (Wang & Sun, 2003). In most of the computer vision systems, information or data extracted from the image taken from the original samples is obtained from pixel of the images. Pixels contain two main types of information, their position and brightness value, which is also known as color of the images. The color information is represented by three color components (red, green, blue). Thus the color information can be easily extracted from the system by simply re-analyzing the stored image.

A color based computer vision investigation has been conducted for different products such as pizza topping (Du & Sun, 2005; Munkevik, Hall, & Duckett 2005), chocolate (Briones & Aguilera, 2004), noodle (Hatcher, Symons, & Manivannan, 2003) as well as meat quality evaluation (Carpenter, Cornforth, & Whittier, 2001). Due to several benefits, food industry continues to be among the fastest growing segments for computer vision applications. In fact food industry now ranks among the top ten industries using computer vision technology (Gunasekaran, 1996).

In the past, color of a food product has been correlated to sensory score (Lu, Tan, Shatadal, & Gerrard, 2000), pH value (Abriel et al., 2001), moisture (Chaoxin, Sun, & Zheng, 2006) and so on. But, there was no clear relationship established between color value and cumulative thermal lethality (F_o) for *C. botulinum*. For microwave process development, our research group had an urgent need to develop a method which can speed up the cold spot determination process in a batch or semi-continuous treatments system involving multiple trays. A method which would be easy to use, document the performance of microwave sterilization system and provide illustrative heating patterns would be advantageous for seeking regulatory approval. Because of these necessities and benefits a computer vision method was developed in my research program to determine

the cold spot location. Outcomes of this research work will overcome the limitation of the single point limited sensors for monitoring the sterilization operations.

5. Major studies in order to develop a novel method

The main objective of this research work was to develop a rapid and reliable computer vision method for determining the cold spots in packaged foods sterilized in the 915 MHz microwave system. This objective was accomplished in several steps and each separate study had specific objectives to meet the goal. Following six major chapters were considered to discuss the related issues involved in designing a novel method to determine cold spot:

1. Kinetic studies of chemical marker M-2 formation with model food mashed potatoes. Under this study a suitable chemical marker was identified for microwave sterilization process. Limiting factors for chemical marker formation, kinetic parameters for predicting the chemical marker yield and order of reaction were determined in this study.
2. Development of a novel approach to determine the heating patterns using computer vision and chemical marker (M-2) yield. An IMAQ Visio Builder script was developed through interacting programming to determine the cold spot location in sterilized foods.
3. Sensitivity analysis and validation of computer vision heating patterns for microwave sterilization processes. This capter tests the affect of salt content and tray sizes on the heating patterns. Computer vision heating patterns were validated using microbial study and fiber optics temperature measurement sensor.

4. Upgrading the developed computer vision method to facilitate the comparative study of heating patterns. The developed computer vision was modified to compare heating patterns for multiple trays. A kinetics study was also performed to establish the relationship among thermal lethality (F_o) for C. *botulinum*, chemical marker yield and color value. New image system was also designed as part of this study.
5. Principle and application of chemical marker (M-2) based computer vision method to locate the cold spots in real food systems. This chapter investigate the principle for applying model food to simulate the heating patterns in other foods namely salmon in Alfredo sauce.
6. Application of the developed computer vision method to determine cold spot in salmon with Alfredo sauce and its validation. A complete protocol to simulate the heating pattern in salmon with Alfredo sauce using whey protein gel is provided in this study. An inoculated pack study was also performed to confirm the identified cold spot.

Preceding chapters of this thesis justify the need of a specific study performed and objective set to address the related issues. Information available in these studies comprises a novel method to study the microwave sterilization processes.

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CHAPTER 2

KINETICS OF CHEMICAL MARKER M-2 FORMATION IN MASHED POTATO-A TOOL TO LOCATE COLD SPOTS DURING MICROWAVE STERILIZATION

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Abstract

Chemical marker M-2 (4-hydroxy-5-methyl-3(2H)-furanone) can be used as a tool to evaluate heating patterns of foods under microwave sterilization. This research studied the kinetics of the M-2 formation in mashed potato as influenced by temperature and salt content. Mashed potato (83.12 % moisture content) with 1.5 % D-ribose was heated in the capillary tubes at four temperatures. Chemical marker M-2 yield was determined using high performance liquid chromatography. Formation of M-2 in plain mashed potato was a first-order reaction. The rate constant changed with temperature following an Arrehenius relationship. For kinetic parameters estimation, one-step non-linear regression was the best followed by modified two-step regression. The amino acid substrate was the limiting element in the formation M-2 in mashed potato. The salt content of zero to one percent had no influence on the chemical marker yield. Addition of L-lysine more than 1 % resulted in dark color.

Keywords: Chemical marker M-2; M-2 kinetics; D-ribose; L-lysine; Mashed potato; Microwave sterilization; Cold and hot spots.

1. Introduction

Microwaves have been used widely in food processing operations, including drying, pasteurization and sterilization of foods (Decareau, 1985). Because of the direct interaction between microwaves and food products, microwave volumetric heating can overcome the slow heat transfer between heating media and packaged foods during conventional heating (Ohlsson, 1992). A fast and reliable method to monitor and predict microwave-heating pattern in foods during sterilization is needed for successful development of commercial microwave sterilization processes. In order to design an effective thermal process to ensure adequate sterility for shelf-stable foods, it is essential to determine the location of cold and hot- spots in packaged foods.

Microwave heating is different from conventional heating in which the heating patterns is usually dependent upon the direct interaction between microwave energy and food and are difficult to predict (Decareau, 1985). Thus, assessment of temperature distribution within packaged foods during microwave sterilization is essential but it can not be determined with single point each or even various points' temperature measurements (Ohlsson, 1972). Similar challenges were experienced in the development of a pilot scale microwave heating system at Washington State University, USA. Issues that need to be addressed before obtaining regulatory and industrial acceptances include: determination of the locations of cold and hot spots and the nature of their mobility and repeatability, a reliable monitoring procedure to ensure a safe level of microwave sterilization (Guan, Plotka, Clark, & Tang, 2002). Kinetics of chemical marker M-2 formation in mashed potato has been studied (Kim, Taub, Choi, & Prakash, 1996a) to develop a method, which can lead to the detection of cold and hot spot locations.

Direct measurement of time-temperature history for all points in food packages is not possible in microwave sterilization. Chemical marker offers an alternative as a time-temperature integrator to determine heating patterns. A chemical marker method was developed at the United States Army Natick Research Center (Kim & Taub, 1993) to determine heating patterns in food system for various thermal processes. Three markers 2, 3-dihydro-3, 5-dihydrixy-6-methyl-(4H)-pyran-4-one (referred to as M-1), 4-hydroxy-5-methyl-3(2H)-furanone (M-2) and 5-hydroxymethylfurfural (M-3) have been identified by scientists in Natick US Army laboratory. Kim et al.(1996b) and Ramaswamy, Awuah, Kim and Choi (1996) have used the M-1 yield as a temperature-time integrator to study ohmic heating and aseptic processing.

Chemical marker kinetics for M-1 and M-2 has been studied with whey protein gel (Lau, Tang, Taub, Yang, 2003, Wang, Lau, Tang, Mao, 2004). But that information can not be used to determine the location of cold spot for newly developed microwave sterilization system. In whey protein gel, reaction leading to M-2 formation was fast and ultimately giving a shorter time to reach the saturation point. M-1 could not be used for high temperature short time processes. The Microwave Heating Group at Washington State University (Pullman, WA) selected mashed potato as a model food to locate cold and hot spots for approval of the sterilization unit by regulatory bodies. Hence, kinetics information for M-2 with mashed potato was needed to monitor the microwave sterilization process qualitatively

Understanding kinetics of the chemical marker M-2 formation in mashed potato as well as information about order of reaction, correlation of M-2 yield with cumulative lethality (F_o), M-2 yield with degree of cooking (Cook value), limiting factor of the

reaction, effect of D-ribose and amino acids on the chemical marker M-2 yield will guide us to develop a reliable method for determining heating patterns.

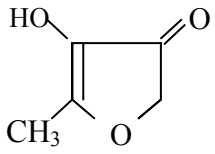
The objectives of this study were: 1) to determine the reaction order, rate constant and energy of activation for the M-2 formation in mashed potato at four temperature; 2) to find the limiting factor and the influence of different sources of mashed potato on the M-2 yield; 3) to study the M-2 formation over the range of dielectric properties of model food by changing the salt content of mashed potato.

This kinetics study will help in establishing a process to develop a reliable method for determining the location of the cold and hot spots in 915 MHz microwave sterilization system.

2. Materials and methods

Chemical marker M-2 (*4-hydroxy-5-methyl-3(2H))-furanone*) is formed by rearranging Amadori compound product (Fig.1) through the reaction of D-ribose and amino acids in the presence of weak acidic (PH>5) environment (Prakash, Kim, & Taub, 1997). Early consumption of any component either D-ribose or amino acids will limit the yield of the chemical marker M-2 during sterilization process. Amino acids especially lysine, arginine, histidine and methoinine are of prime importance during formation of chemical marker M-2 in presence of D-ribose. A yield point after which there will be no significant effect of heating on chemical marker yield is called marker yield at saturation

D-ribose + Amine ⟶ Amadori Compound ⟶



4-hydroxy-5-methyl- 3(2H)-furanone
(M-2)

Fig. 1. Formation of chemical compound 4-hydroxy-5-methyl- 3(2H)-furanone (M-2) in presence of D-ribose with mashed potato.

(C_∞). Chromatographic detection showed that chemical marker M-2 has a UV absorption maximum at 285 nm with a retention time of 5.8 min (Kim & Taub, 1993). Mashed potato used in this study contained added 1.5 % D-ribose (Sigma, St. Lous, MO). Concentrations of amino acids in the sample were as follows: methionine 1.41 μM/g, lysine 1.7 μM/g, histidine 1.33 μM/g, arginine 3.70 μM/g. Concentration of amino acids in the model food was much lower than that of D-ribose due to the chemical composition of potato. Equation for studying the kinetics of chemical marker (M-2) formation with mashed potato can be given as (Lau et al., 2003):

$$\frac{dC}{dt} = k (C_\infty - C)^n \quad (1)$$

The above equation conveys that the rate of formation $\frac{dC}{dt}$ is proportional to n^{th} power of difference between concentration of marker yield at saturation, C_∞ gm /gm of sample) and marker yield at any time, (C gm /gm of sample), while n is order of the reaction and k is reaction constant.

2.1. M-2 yield determination

Mashed potato was selected as a model food in study because of homogeneity and availability. During sample preparation, 1.5 g of D-ribose was dissolved first in 83.12 g of distilled water at room temperature and was then mixed with 15.38 g dry mashed potato flakes (Washington Potato Company, Warden, WA). About 2 ml of the dispersed paste was carefully injected into the glass capillary tubes (inner diameter=1.75 mm;

length=15 cm). Both ends of the tubes were sealed with a hot flame. During tube sealing, precaution was taken to avoid heating of mashed potato. The transition temperature for mashed potato (MP) in 0.7 M phosphate buffer at pH =5.92 was taken 68 and 83 ^{o}C (Palumbo, 1992). Experiments were carried using an oil bath set at 116, 121, 126 and 131 ^{o}C at several time intervals in order to cover a range of likely temperature-time combinations in the microwave sterilization processes. At each temperature level experiment was conducted in two replicates and both replicates were considered for estimation of kinetics parameters and order of reaction. The come-up time for the capillary tube filled with mashed potato to reach set temperature level was between 12-14 sec. Tubes were heated in the oil bath at each temperature to 60 minutes. After predetermined heating times, the tubes were removed from the oil bath and immediately placed in a basin containing crushed ice. Cooled tubes were opened; the potato was removed and immediately weighed. A sample weighing between 0.16-0.17 g was placed in a mortar and carefully ground in 2 ml extraction buffer (10 mM sulphuric acid and 5 mM citric acid) according to the modified extraction procedure described by Lau et. al., (2003). Extracts were collected and sealed tubes were stored overnight in a -20ºC freezer. Upon thawing at room temperature, extracts were mixed thoroughly and centrifuged for 10 min at 14,000 rpm (Eppendorf centrifuge, Brinkman Instruments, Westbury, NY). Supernatants were transferred into fresh tubes and were centrifuged again at the same conditions (10 min at 14,000 rpm). The supernatants were filtered using a PTFE syringe filter with a 0.45 nm pore size and placed into a high performance liquid chromatography sample holder for analysis to obtain M-2 yield.

The Agilent 1100 HPLC system (Agilent Technology, USA) was equipped with a diode array detector. Twenty five µl aliquots were run through the fast acid analysis column, 100 × 7.8 mm (Bio-Rad Laboratories, Hercules, CA). The mobile phase was 10 mM at a flow rate of 1 ml/min. Absorbance of was determined at 285 nm as per Kim and Taub (1993). A calibration curve (Fig. 2.) was established using a commercial sample of chemical marker M-2 obtained from Givaudan Flavor Corporation (Cincinnati, Ohio).

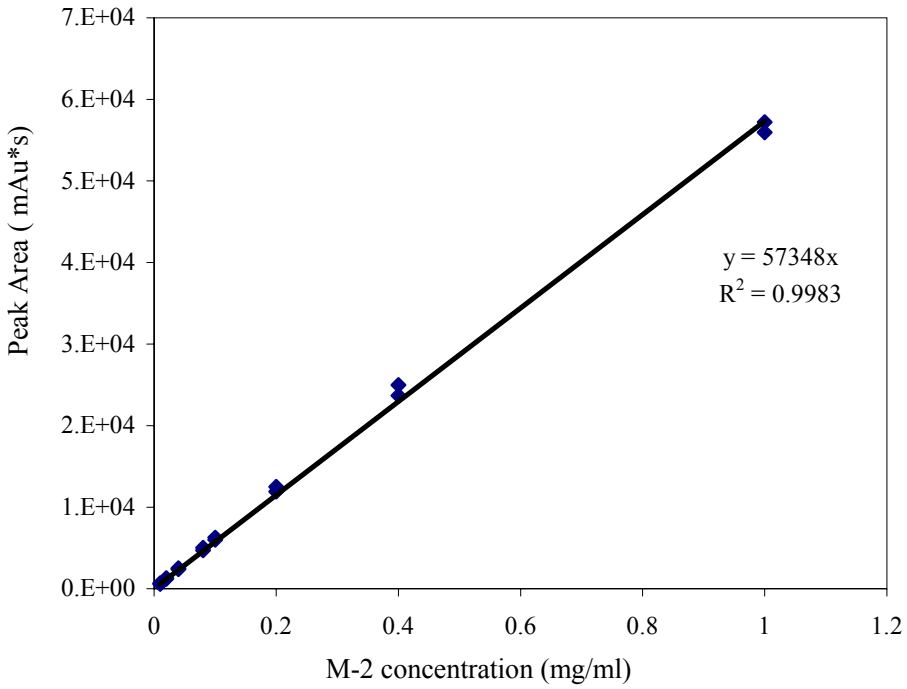


Fig. 2. Calibration curve of chemical marker M-2 (4-hydroxy-5-methyl-3(2H)-furanone) in 10mM sulfuric-5mM citric acid buffer.

Standard dilutions were prepared using the same extraction buffer as for samples of mashed potato in kinetic studies. Calibration curve contributes in expressing yield as mg of marker per gram of sample. The following equation was used in this study to express peak area of the sample as mg of marker per gram of sample:

$$\text{Marker Yield} = \frac{\text{Peak Area (sample)}}{57348} \times \frac{\text{volume of extract}}{\text{weight of sample}} \quad (2)$$

Initial M-2 yield in plain mashed potato was not observed even at high sensitivity of the HPLC, indicating that the marker yield observed is a product of reaction between added D(-) ribose and amino-acids endogenous to potato flakes. The kinetic experiments were carried out in two replicates.

2.2. Limiting factors M-2 formation

Instant mashed-potato flakes from two manufacturing lots: old (2002), new (2003) were acquired from Washington Potatoes Co. (Warden, WA) and were analyzed for amino acid composition. Since potato flakes are high in starch content (~66% according to the Data of National Grain and Feed Association), direct amino acid analysis with the raw material was not possible due to a high glucose concentration that interfered with the spectral detection of amino acids. Instead the potato flakes were pre-extracted prior to the amino acid analysis according to the modified method described by Yang (1997). One gram of flakes was extracted with 25 ml of 80 percent ethanol by shaking in a gyratory shaker (New Brunswick Scientific Precision Inc, M-3, Brunswick, NJ) at room temperature for 15 min and centrifuging at 1200 g at 5 °C for 10 min. The supernatant was filtered through ashless Whatman filter paper of size 40 to remove potato particles. The total volume of extracts was brought up to 25 ml final volume with 80% ethanol. An aliquot was then applied to the column of Beckman 6300 Automatic amino acid analyzer (Beckman Instruments Co., Palo Alto, CA).

The moisture of potato flakes was determined according to the official AOAC method 7.003 (AOAC, 1980). Total protein content of potato flakes was determined using a Leco protein analyzer (Leco Corporation, St. Joseph, MI). All analyses of potato flakes samples were conducted in at least two replicates. Total protein content of mashed potato was 7.3 percent while the content of four major amino acids which participate in chemical marker M-2 formation are: methionine 1.41 μM/g, lysine 1.7 μM/g, histidine 1.33 μM/g, and arginine 3.70 μM/g.

To determine the limiting factor in M-2 formation, mashed potato sample was prepared with 1.5 %, D-ribose. Chemical marker yield of the sample heated in an oil bath for predefined time at 121 ^{o}C temperature was obtained using HPLC. Experiments were repeated for higher levels of D-ribose: 3, 4.5 %. Levels of the D-ribose were selected to investigate the heating time at which amino acid content of the mashed potato become inadequate for M-2 formation. Chemical marker yield obtained at different levels were plotted to analyze the results. At each level five different time intervals were selected to obtain M-2 yield with two replicates.

2.3. Statistical Analysis

Order of reaction and kinetics parameters can be determined by different methods statistically (Hill and Grieger-Block, 1980). Following three methods are commonly used: modified two-step, multi-linear regression and one-step non-linear regression analyses. Those methods were chosen due to their ability to accurately estimate the kinetic parameters of the Arrhenius model (Haralampu, Saguy & Karel, 1985). It was reported that among the above three methods, modified two-step regression gives least

accurate estimation of the Arrhenius parameters because of the need to calculate many intermediate values before estimating the kinetics parameters. (Haralampu, Saguy & Karel, 1985). Multiple linear regressions showed bias with little improvement over modified two-step regression (Ramaswamy, Awuah, Kim, & Choi, 1996). Non-linear regression is probably the most appropriate theoretical method because it does not estimate unnecessary parameters (Arabshahi & Lund, 1984). These three methods were used to estimate the kinetics parameters in this study.

SAS Systems Release 8.1 (SAS Institute Inc., Cary, NC, 2000) was used to perform the statistical analysis. Modified two-step, multi-linear regression and one-step non-linear regression analyses were considered to estimate the kinetic parameters. Integrating equation (1) between C_o to C for a time interval of zero to time interval t we have:

$$\int_{C_o}^{C} \frac{dC}{(C_\infty - C)^n} = \int_0^t k \, dt \tag{3}$$

$$(C_\infty - C)^{1-n} - (C_\infty - C_o)^{1-n} = -(1-n)\, k\, t \tag{4}$$

$$(C_\infty - C) = [(C_\infty - C_o)^{1-n} - (1-n)\, k\, t]^{\frac{1}{1-n}} \tag{5}$$

The above equation does not apply to n=1 because the term $\frac{1}{1-n}$ will become indeterminate. After rearranging the equation (5) we get:

$$C = C_\infty - [(C_\infty - C_o)^{1-n} - (1-n)\, k\, t]^{1/1-n} \quad (n \neq 1) \tag{6}$$

The above model was fitted with the experimental data using procedure NLIN of SAS Systems Release 8.1 (SAS Institute Inc., Cary, NC, 2000). PROC NLIN (nonlinear procedure) fits nonlinear regression models using the least square method. Experimental data supplied were marker yield at various time interval and values estimated from model were order of reaction, marker yield at saturation (C_∞).Values of C_o were considered as zero during estimation.

Coefficient of determination is the best measure to show the degree of fitting between experimental data and prescribed model. However, along with R-square of non-linear regression we also validated the estimation using graphical analysis. In graphical analysis, exponent n in equation (1) was set to zero, half, one, and two to compare the coefficient of determination among zero, half, first, and second order reactions respectively. Results obtained are presented in Table 2.

Fitting of the experimental data to model (6) predict the order of reaction as first. (Table 1). Graphical analysis also supports the predicted order of reaction. Estimation of the kinetic parameters was obtained considering n =1 in equation (1) and integrating between C_o to C:

$$\ln (C_\infty - C) = \ln (C_\infty - C_o) - k\, t \tag{7}$$

Equation (6) in exponential form can be written as:

$$C = C_\infty - (C_\infty - C_o)\ e^{-k\, t} \tag{8}$$

The activated complex theory for chemical reaction rates is the basis for the Arrhenius equation, which relates reaction rate constants to the absolute temperature. The Arrhenius equation is (Holdsworth, 2000):

$$k = A\ e^{-\frac{E_a}{R\,T}} \tag{9}$$

where E_a (kcal/mol) is the activation energy, A is the rate constants and T is absolute temperature (K). R is the universal gas constant (1.987 cal/mol K). Another form of the Arrhenius equation involves the reaction rate constant at a reference temperature. Under this study reference temperature T_o (396.7 K) was taken as average of all four temperature considered. If k_o is the reaction rate constant at T_o then equation (8) can be modified by replacing constant A as:

$$k = k_o \, e^{-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_o}\right)} \tag{10}$$

Equation (9) can be written in linear form by taking logarithmic both sides:

$$\ln k = \ln k_o - \frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_o}\right) \tag{11}$$

Following three statistical methods: modified two-step, multi-linear and one-step non-linear regression methods were considered for estimation of the kinetics parameters.

(i*) Method I:* In *Modified two-step* regression method experimental data were fitted to the model (8) using NLIN procedure of SAS, which is based on the Marquardt algorithm to obtain M_∞ and k at each temperature. Marquardt iterative algorithm regresses the residuals onto the partial derivatives of the model with respect to the parameters until the estimates converge. Reaction rate constants (k) at all temperatures were fitted into the linear model (11) to obtain $\frac{E_a}{R}$ as slope and $\ln(k_o)$ with intercept term. Energy of activation E_a and k_o were calculated from the slop and intercept term, respectively.

(ii) Method II: In *Two-step multi-linear regression,* times were introduced as pseudo-dummy variables t_{Ti} at different temperatures, T_i in equation (7).The dummy time

variable were created by associating the reaction times at a particular temperature ,T_i, with a parameter, k_i , and setting the dummy times associated with the other temperature levels to zero. In this study, m was taken as four because of four temperature levels:

$$-\ln(C_\infty - C) = \sum_{i=1}^{m} k_i t_{Ti} - \ln(C_\infty - C_0) \tag{12}$$

Detailed information on using Eq. (12) is provided in Haralampu, Saguy & Karel, (1985). C_∞ value obtained from non-linear regression method was used in calculating ln (C_∞ - C). ln (C_∞ - C) and pseudo-dummy variables t_{Ti} were fitted with model (12) to obtain reaction rate constant k_i at each temperature level. After all the k values at different reaction temperatures were obtained, they were fitted to model (11) and E_a, k_o were estimated same as in case of modified two-step regression.

(iii) Method III: One-step non-linear regression performs a single regression on all of the data to estimate $\frac{E_a}{R}$ and k_o without calculating the reaction rate at each temperature. An equation without reaction rate constant can be obtained by replacing the k terms in equation (8):

$$C = C_\infty - (C_\infty - C_o) \times exp\left\{ -t\, k_o\, e^{-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_o}\right)} \right\} \tag{13}$$

Substituting k in equation (8) with Eq. (10), we obtain:

$$C = C_\infty - (C_\infty - C_o) \times \exp\left\{ -t\, k_o \exp\left[-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_o}\right) \right] \right\} \tag{14}$$

Transformation of the equation to this form improves the accuracy of the estimation because of involvement of the several parameters (Nelson 1983). The non-linear

regression procedure in SAS Systems was used to fit the marker yield (C) versus time (t) data to model (14), to estimate the E_a and k_o at each temperature level. Reaction rate constant at each temperature was calculated back using equation (11).

2.4. Effect of salt content and additional L-lysine on M-2 yield

Mashed potato with zero and one percent salt content was filled into the capillary glass tubes and experiment was carried out at 121 ^{o}C. Tubes were taken out after 5 min interval from an oil bath and samples were prepared for HPLC analysis as mentioned above. M-2 yield obtained after HPLC analysis at each salt level with two replicates were plotted to determine the effect of salt level on M-2 yield.

Effects of additional L-lysine on chemical marker yield was studied with added L-lysine, ranging from 0.5, 1.0, and 2 .0 %. Mashed potato sample containing L-lysine in specified proportions were filled into tubes and heated at 121 ^{o}C. First tube of two percent L-lysine taken out at 5 min interval was very dark because the marker yield reached the saturation point. Microwave sterilization processing lower than five minutes can not be anticipated on industrial scale unit. Hence, we discarded any experiment of additional L-lysine higher than two percent. Chemical marker yield of different products with different chemical composition: Potato Buds (8.7 % protein), Russet potato (5.2 % protein) and Idahoan Real potato (8.69 % protein) and samples from 2002 and 2003 lots were compared.

3. Results and discussion

Increases in marker yield (M-2) as a function of time at four different temperatures are shown in Fig.3. Those data were used to determine kinetics information for formation of M-2 in mashed potato. Chemical marker yield (M-2) in the unheated mashed potato sample was considered as zero, because no peak was detected during HPLC analysis. An analysis of variance (ANOVA) shows that the marker yields at saturation level for different temperatures were not different on 95 % confidence interval. Estimated marker yield at the saturation level matched with the marker yield obtained by HPLC analysis.

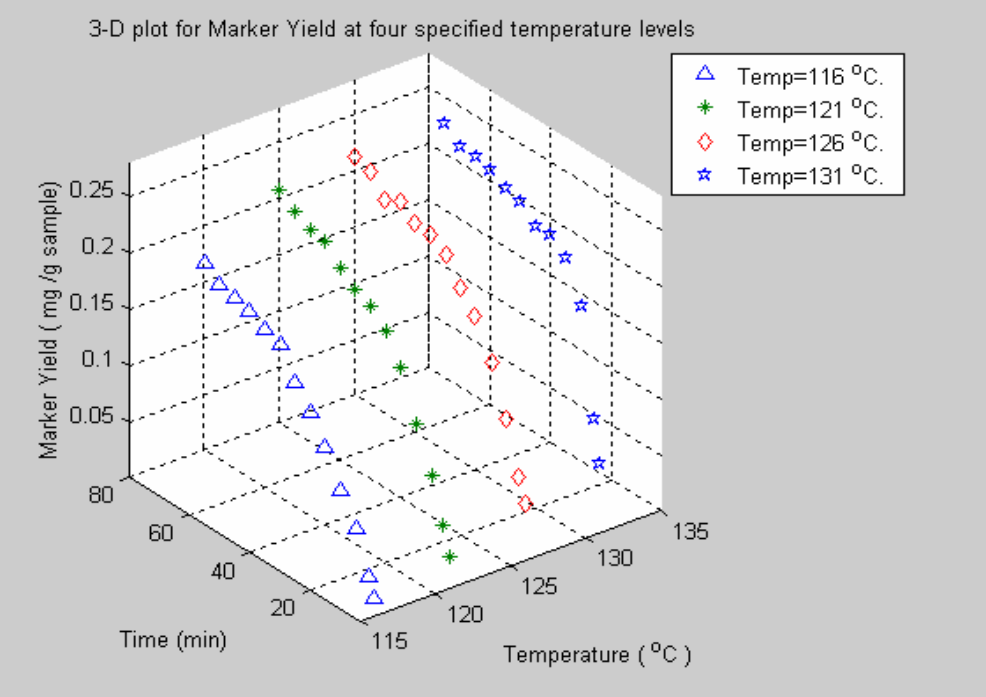


Fig. 3. Chemical marker yield (M-2) for different temperature levels obtained during experimental work, scattered data represent means of two replicates.

3.1. Estimated chemical reaction parameters

The chemical marker formation in mashed potato followed a first order reaction. Graphical analysis also confirmed the first order of reaction. Lau et al., (2003) & Wang, Lau, Tang, Mao (2004) also obtained a first order reaction for M-2 and M-1 formation in whey protein gels. Table 1 summarizes the order of reaction and chemical marker yield at saturation. Results obtained by the graphical analysis for reaction order are presented in Table 2.

Table 1. Estimated order of reaction (n), marker yield at saturation (C_∞) values by non-linear regression based on two replicates of experimental data.

T (^oC)	n	C_∞	R- square
116	1.050 ± 0.44	0.267 ± 0.04	0.989
121	1.0271 ± 0.23	0.284 ± 0.018	0.990
126	1.062 ± 0.18	0.273 ± 0.012	0.991
131	1.066 ± 0.40	0.268 ± 0.015	0.981

Table 2. Estimation of the order of chemical marker (M-2) formation by examining r^2 from plot of zero, half and second order reactions based on two replicates of experimental data.

Temperature (^oC)	Zero order (C_∞ - C) versus time	Half order (C_∞ - C) $^{0.5}$ versus time	First order ln(C_∞ - C) versus time	Second order 1/(C_∞ - C) versus time
116	0.855	0.921	0.987	0.715
121	0.866	0.942	0.973	0.765
126	0.829	0.917	0.978	0.776
131	0.672	0.672	0.959	0.416

First order kinetics (n = 1) for M-2 formation was used to calculate the reaction constant and activation energy. Kinetic parameters (k, E_a) for M-2 formation were obtained by modified-two-step, two-step multi-linear and one-step non-linear regression methods. Results obtained using these statistical methods are given in Table 3. In case of one-step non-linear regression standard error in reaction rate constant was calculated using:

$$\Delta k = k\left[\left|\frac{\Delta k_o}{k_o}\right| + \left|\frac{\Delta E_a}{R}\left(\frac{1}{T} - \frac{1}{T_o}\right)\right|\right] \qquad (15)$$

Coefficient of determination (r^2) and standard error for k, and E_a estimated are provided in Table 3. One-step non-linear method is the best among considered methods followed

by the modified two-step method based on the values of r^2 and the standard error. One step non-linear method has advantage of using whole data set, as well as estimating fewer parameters during analysis. The multi-linear regression gives the smallest r^2 and the largest standard error for estimation of activation energy.

Table 3. Rate constant (min^{-1}), and activation energy, E_a (kcal/mol), for M-2 formation in mashed potato at four temperatures levels based on two replicates of experimental data.

Reaction rate, activation energy	Modified Two-step regression	Two step multi-linear regression	One step non-linear regression	M-2 formation with whey protein gel (Lau, et al., 2003).
k_{116}	0.031 ± 0.014	0.029 ± 0.004	0.030 ± 0.001	0.110
k_{121}	0.040 ± 0.007	0.0451 ± 0.004	0.044 ± 0.002	0.152
k_{126}	0.056 ± 0.007	0.053 ± 0.005	0.063 ± 0.003	0.207
k_{131}	0.096 ± 0.024	0.072 ± 0.006	0.089 ± 0.005	0.281
k_o	0.050 ± 0.048	0.048 ± 0.066	0.052 ± 0.002	0.178
E_a (kcal mol^{-1} K^{-1})	22.96 ± 2.73	19.45 ± 3.79	22.23 ± 1.54	19.48
	r^2= 0.978	r^2= 0.914	r^2= 0.983	

Activation energy estimated for M-2 formation (22.23 ± 1.54 kcal/mol) is within the range cited in the literature for non-enzymatic browining on model food systems (ranging from 16 to 30 kcal/mol) (Labuza and Baisier, 1992). Kinetic parameters estimated in this study were compared with the kinetic parameters for M-2 formation with whey protein gel (Lau et. al., 2003) in Table 3. Reaction rate constant (k) increases with temperature for both cases. Mashed potato has lower amino acid content than whey protein gel, which resulted in lower k and higher E_a values.

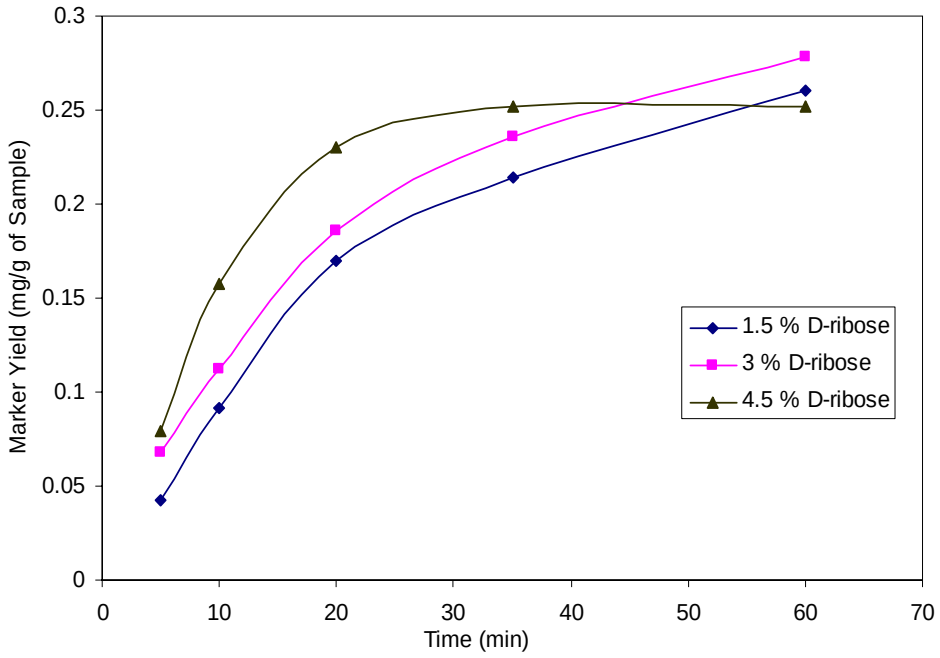


Fig. 4. Chemical marker (M-2) yield with different percentage of D- ribose at 121 ^{o} C, each point represent mean of two replicates.

3.2. Estimation of limiting factor

Chemical marker yield increased with time until 60 minutes for 1.5 and 3 percent D-ribose while yield reached to the saturation point around 20 min with 4.5 percent D-ribose as shown in Fig.4. The amino acid content of mashed potato was adequate for 1.5 ,

3 % D-ribose until 60 minutes, while amino acid was consumed completely around 20 minutes in case of 4.5 % D-ribose (Fig.4). Analytical study showed that total amino acid content in 15 g of mashed potato is much less compare to 1.5 g D-ribose. Hence, amino acid content was observed as the limiting factor during chemical marker M-2 formation.

3.3. Estimation of effect of additional Lysine

L-lysine content higher than 2 % was discarded due to fast reaction and shorter time of saturation. L-lysine above 0.5 percent produced dark color and reduced the saturation time. Mashed potato with 0.5 percent L-lysine had higher yield than plain mashed potato. This study showed that quick appearance of saturation especially at hot spots would be misleading. A longer linear range was observed with plain mashed potato in comparison of 0.5 % L-lysine (Fig.5). Moreover, L-lysine is costly so that determination of hot and cold spots for microwave sterilized foods was recommended without L-lysine.

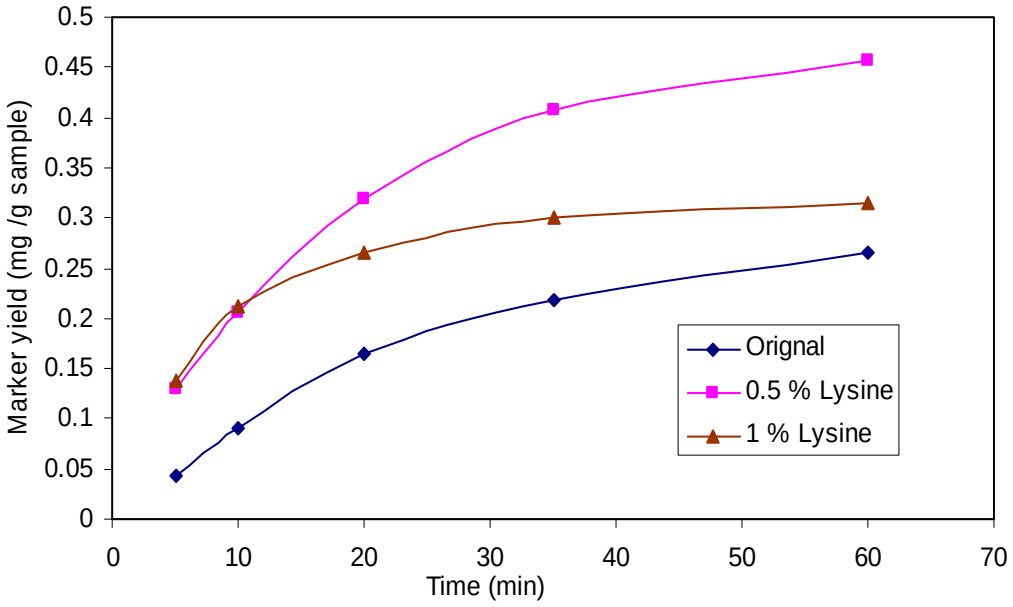


Fig. 5. Effect of additional L-lysine on marker yield in plain mashed potato at 121 ^{o}C, each point represents the mean of two replicates.

3.4. Variability among different mashed potato sources

Three locally available brands of potato flakes: Potato Buds with (8.7 % protein), Russet potato with (5.2 % protein) and Idahoan Real potato with (8.69 % protein) along with old and new batches of mashed potato flakes (8.3 % and 7.3% protein, respectively) were considered for estimating the variability of mashed potatoes sources on chemical marker yield. M-2 yield obtained (Fig. 6) were statistically analyzed using SAS software. M-2 yields from different sources of potatoes were not significantly different (P-value = 0.989).

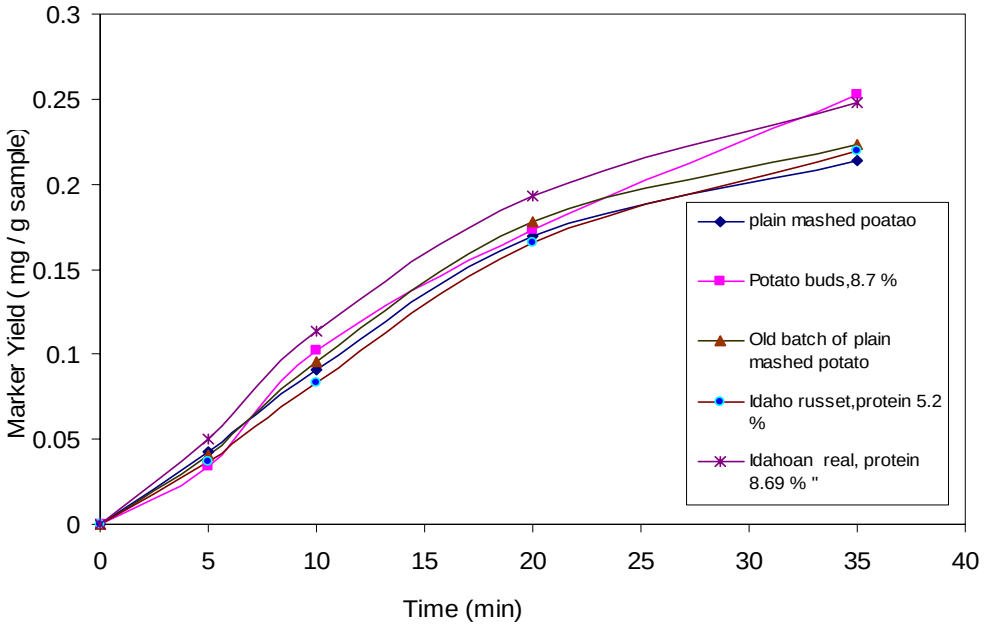


Fig. 6. Chemical marker (M-2) yield for various sources of mashed potato at 121 ^{o}C, each point represents the mean of two replicates.

3.5. Effect of salt on marker yield

Two replicates for each level of salt were evaluated and statistical analysis of chemical marker yield was performed using SAS software. Analysis (P>0.99) showed that chemical marker yield was independent of salt content (Fig.7).

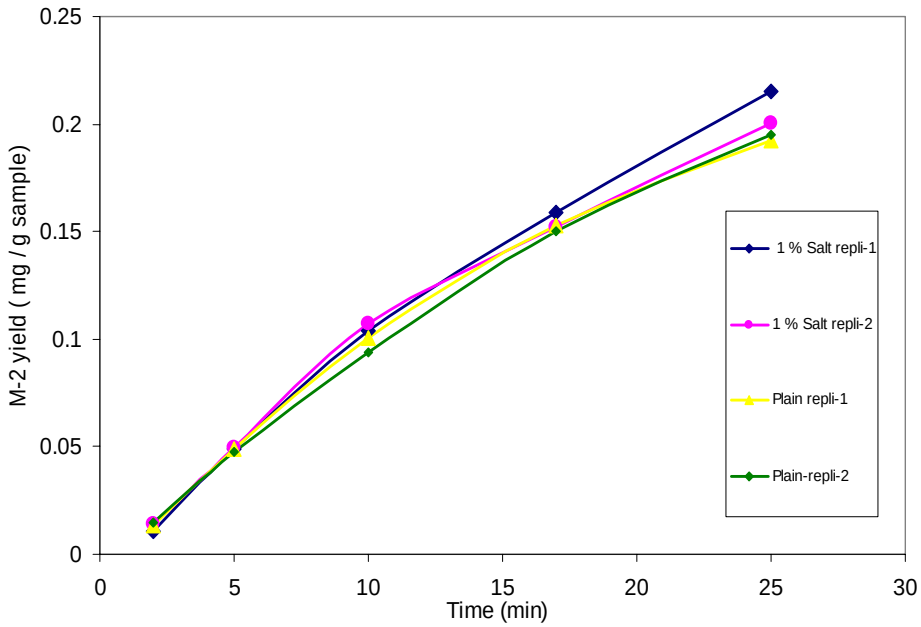


Fig. 7. Effect of salt content on chemical marker (M-2) yield at 121 ^{o}C, each level has two replicates.

4. Conclusion

Chemical marker (M-2) formation in mashed potato with 1.5 percent D-ribose was predicted as a first order reaction. Kinetic parameters were predicted much accurately by one-step non-linear regression method followed by modified two step regression. One-step non-linear regression was a more appropriate method since it does not estimate unnecessary parameters and gives both unbiased and precise estimation of

the parameters. Modified two-step regression was a second option with higher standard error in estimation of the activation energy. Multiple linear regression method gives broader confidence interval in the estimation of activation energy along with the lowest overall coefficient of determination (r^2). Amino acid content was the limiting factor in formation of M-2 from D-ribose and mashed potato. Chemical marker yields of the considered sources of potatoes were not significantly different. Marker yield was found to be independent of the level of salt. The kinetic parameters obtained in this study can be used for determining the hot and cold spots locations during high temperature short time microwave sterilization processes**.**

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Nomenclature

C	Chemical marker (M-2) concentration at any time (mg / g of sample)
C_∞	Chemical marker (M-2) concentration at saturation (mg / g of sample)
E_a	Activation energy for chemical reaction (kcal mol^{-1})
F_o	Cumulative thermal lethality (min)

k	Chemical reaction rate constant (min^{-1})
k_o	Rate constant at reference temperature (min^{-1})
n	Order of the chemical reaction
R_o	Universal gas constant at reference temperature
t	Time (min)
T	Absolute temperature (K)

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CHAPTER 3

DEVELOPMENT OF A NOVEL APPROACH TO DETERMINE HEATING PATTERN USING COMPUTER VISION AND CHEMICAL MARKER (M-2) YIELD

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Abstract

In this study, a novel approach to determine heating patterns using chemical marker (M-2) yield and computer vision was developed for packaged foods after microwave sterilization. Due to various constraints of temperature measurement devices such as fiber optic temperature sensors, thermocouples, and infrared sensors, there is a need to develop an accurate and rapid method to determine heating patterns in packaged food trays after microwave sterilization. Yield of a heat sensitive chemical marker (M-2) was used as a coloring agent and digital images of the processed trays were analyzed using a computer vision system. A script in IMAQ Vision Builder software was written to obtain a 3-D heating pattern for the sterilized trays. Relationship between chemical marker (M-2) yield and cumulative thermal lethality (F_o) was also studied. Validation of the locations of cold and hot spots determined by computer vision were performed by fiber-optics temperature measurement sensor. Results show that computer vision in combination with chemical marker M-2 and other accessories can be used as a rapid, accurate and cost efficient tool to specify the location of cold and hot spots after microwave sterilization.

Keywords: IMAQ vision builder; Chemical marker M-2; Computer vision; Machine vision; Digital image processing; Cold and hot spots; Microwave heating; Sterilization

1. Introduction

Computer vision is a technology for acquiring and analyzing a digital image to obtain information or to control processes. Computer vision can be a successful tool for online measurement of several food products with applications ranging from routine inspection to complex vision monitoring (Gunasekaran, 1996). Du and Sun (2004) presented a review about recent developments in the application of image processing techniques for food quality evaluation and pointed out many opportunities for image processing in the food industry. Computer vision includes capturing, processing and analyzing images to asses the visual quality characteristic in food products (Gonzalez & Wintz, 1991). It is the construction of an explicit and meaningful description of physical objects from images (Ballard & Brown, 1982). Computer vision is a branch of science that develops the theoretical and algorithmic basis by which useful information about an object or scene can be automatically extracted and analyzed from an observed image, image set or images sequence (Haralick & Shapiro, 1992). The technology aims to duplicate and augment human vision by electronically perceiving and understanding an image (Sonka, Hlavac, & Boyle, 1999). Recent advances in hardware and software have expanded this technology by providing low cost powerful solutions, leading to more studies on the development of computer vision systems in the food industry (Locht, Thomsen, & Mikkelsen, 1997; Sun, 2000).

The increased awareness and sophistication of consumers has created the expectation for improving quality in food products. This in turn has increased the need for enhanced quality monitoring. Microwave sterilization is a thermal method that has promise to produce high quality and shelf stable foods (Zhang, Datta, Taub, & Doona, 2001; Guan et al., 2003). To design new thermal processes that ensure adequate sterility for shelf stable foods, it is necessary to determine the locations of cold and hot spots during microwave sterilization. The cold spot is the region which receives the lowest thermal energy and the hot spot is the region of highest thermal energy reception. In order to meets the stringent requirement of food regulatory bodies, there is a need to develop reliable and rapid methods to determine the heating pattern, especially the location of cold spots.

A chemical marker method was developed at the United States Army Natick Research Center (Kim & Taub, 1993) to correlate heating intensity with development of brown color through the Maillard reaction. The chemical marker M-2 (4-hydroxy-5-methyl-3(2H) furanone) is formed by reaction between D-ribose and amines through non-enzymatic browning reaction after enolization under low acid condition (pH>5). The yield of M-2 can be used as means to detect the degree of thermal treatment at any location of processed homogeneous foods.

High performance liquid chromatography (HPLC) can be used to determine the chemical marker yield (M-2 yield) at different locations of microwave-sterilized food (Pandit, Tang, Mikhaylenko, & Liu, 2006). It was observed that HPLC analysis is a costly and time consuming method to determine the heating pattern. A rapid analysis of the heating pattern for different layers of processed foods was not possible by HPLC

method. Automated visual inspection may be the best possible option because of its cost effectiveness, consistency, superior speed and accuracy.

This study deals with the application of Image Acquisition (IMAQ) Vision Builder software, digital imagine systems, and chemical marker M-2 yield as a tool to map heating patterns of thermally treated homogeneous food products. Specific objectives of this study were as follows: 1) to establish a relationship between thermal cumulative lethality (F_o) and chemical marker (M-2) yield 2) to develop a novel method using computer vision systems and chemical marker (M-2) yield to locate the cold/hot spots. 3) to verify the specified cold/hot spots locations.

This information will help in evaluating the heating uniformity as well developing a monitoring procedure to ensure safe level of microwave sterilization.

2. Materials and methods

2.1. M-2 marker yield as a coloring agent

Instant mashed-potato flakes acquired from Oregon/Washington Potatoes Co. (Boardman, OR) was selected as the model food and the accumulation of M-2 marker yield as a coloring agent to quantify the amount of thermal energy at a point. The mashed potato sample was prepared with 1.5 % D-ribose and a moisture content of 83.12 % (wb). Polymeric trays 14 × 9.5 × 2.67 cm (7 oz) were filled with 200 g of mashed potato and vacuum-sealed at 18 inch of Hg vacuum. Each tray was fitted with a thermowell located in the middle to monitor temperature at the single point using a fiber-optic temperature sensor. The single-mode 915 MHz microwave sterilization system developed at Washington State University, Pullman, WA was used a source of energy and trays were

kept in stationary at the center of pressurized microwave cavity. Trays were heated to various F_o values ranging from 3.7 to 18.

F_o is a quantitative measurement of the degree of cumulative thermal lethality which is explained as (Holdsworth, 1997):

$$F_o = \int_0^t 10^{\frac{T - T_{ref}}{z}} dt \qquad (1)$$

where z is defined as change in temperature to increase the rate of inactivation by a factor of ten (Holdsworth, 1997). T is a temperature in ^oC at any time t during microwave heating. For thermal processes to produce shelf-stable low acid foods, C. b*otulinum* type A and B are the targeted bacteria, z for this bacterium is about 10 ^oC and T_{ref} is considered as 121.1 ^oC (Prescott, Harley, & Klein, 2002). Typically, in commercial canning practices, F_o varies between 3 to 12 min depending upon raw material and storage conditions for the processed foods.

The temperature history of the monitored point was saved at two second intervals in each experiment. A sample weight between 0.20 to 0.21 g was taken out precisely from the region around the sensor tip for HPLC analysis. M-2 marker yield for each F_o at any given power level was calculated from M-2 peak area obtained after HPLC analysis. Chemical marker (M-2) yield was expressed as mg/g of sample using slope of the calibration curve established for a commercial chemical marker sample by Givaudan Flavor Corporation (Cincinnati, Ohio). Mathematically yield of chemical marker (M-2) during heating process is expressed as:

$$C(t) = C_\infty - (C_\infty - C_o) \times \exp\{\int_0^t -k_o \exp(-\frac{E_a}{R}[\frac{1}{T(t)} - \frac{1}{T_o}])\, dt\} \qquad (2)$$

where C (t) is marker yield at any time, C_∞ marker yield at saturation, E_a is energy of activation, R molar gas constant, T(t) is recorded temperature-time history at the measured point, T_o is reference temperature. Initial marker yield before heating, C_o, was determined as zero for mashed potato sample with 1.5 % D-ribose. Kinetic parameters were calculated using statistical analysis (Lau et al., 2003; Wang, Lau, Tang, & Mao, 2004; Pandit, Tang, Mikhaylenko, & Liu, 2006) software SAS System Release 8.1 (SAS Institutes, CARY, NC, 2000).

Experiments were also conducted to correlate the intensity of color measured using IMAQ vision builder software and degree of thermal lethality (F_o) during microwave sterilization. Mashed potato with 1.5 % D-ribose in 7 oz trays with fiber optic temperature sensors fitted in the center of trays were sterilized for various level of F_o. IMAQ vision builder software was used to determine the color value at the tip of the fiber optic probes for each level of F_o.

2.2. Microwave as a source of energy

Ten ounce trays (14 × 9.5 × 3.3 cm) were selected for studying the heating pattern of the sterilization process in the pilot-scale scale 915 MHz microwave sterilization system. Mashed potato samples with 1.5 % D-ribose were prepared with three different salt content levels: 0, 0.5 and 1 %. Salt concentration was changed to alter the dielectric loss of the mashed potato so that the study can be applied to a broad range of homogenous foods with different dielectric properties. Trays were heated at 2.67 kW power levels with a repeatable heating pattern. Fiber optic sensors were fitted at the center of each tray and the trays were heated to a temperature of 121 ± 2 ^oC. After

heating up to the set temperature, trays were rapidly cooled down to room temperature and taken out from the microwave cavities. To stop further chemical marker formation as well as to harden the processed mashed potatoes, the microwave processed trays were cooled in the deep-freezer at -35 ^{o}C for 30-45 minutes. Hardening of the processed trays in the deep-freezer made it easy to cut the mashed potatoes into layers. In this study, the mashed potatoes were cut into vertical and middle layers and pictures of each layer were taken to analyze the results.

2.3. Image processing system configuration

Computer vision systems generally consist of five basic components: a digital camera, an image capturing box, illumination, computer hardware and software as shown in Fig. 1. A digital camera (Olympus C -750 Ultra Zoom) was set on top of a wooden box (40 × 30 × 24 cm) and an illuminating round light was mounted inside the box. The resolution of digital camera was 2288 ×1712 pixel. Even illumination is an important prerequisite in image acquisition for food quality evaluation. A wide variety of light sources and lighting arrangements are available (Tao, Chance, & Liu, 1995). The quality of the captured image can be greatly affected by the lighting condition and a well-designed illumination system can help to improve the success of the image analysis by enhancing the image contrast (Novini, 1995). The camera was mounted perpendicular to the surface of the tray. The lighting system was arranged to provide the contrast necessary between the object under inspection and the background. The used camera was providing enough resolution to meet the minimum requirements of the software. National

Instrument software Image Acquisition Vision Builder version 6.1 was installed on 80 GB Pentium IV of RAM 1GB dell desktop system.

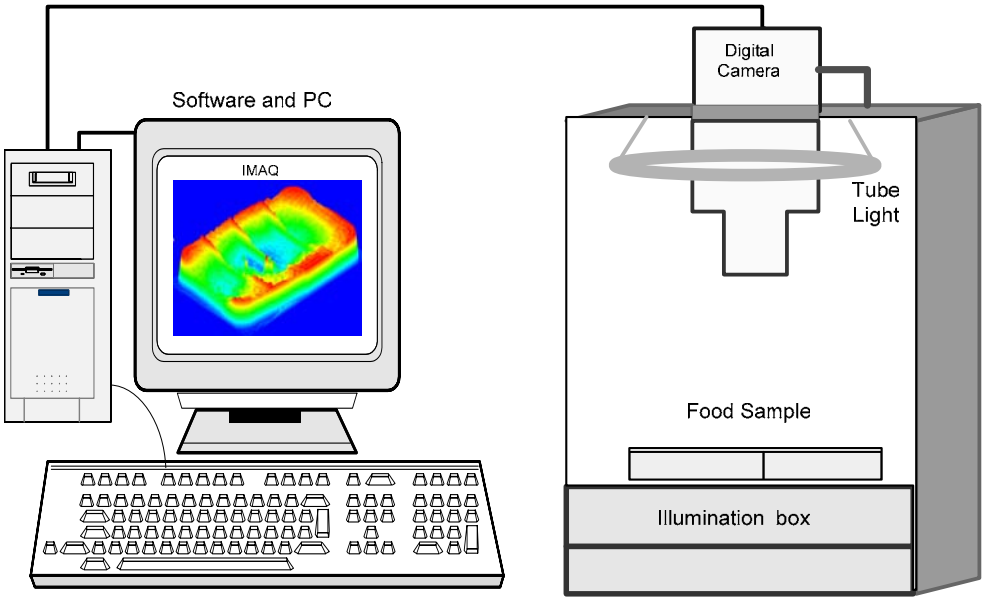


Fig. 1. Components of a computer vision system.

2.4. IMAQ vision builder to locate cold and hot spots

Image processing analysis with the above-mentioned system consisted of six steps (Fig. 2): 1) sample preparation, 2) image acquisition, 3) system calibration, 4) noise filtering, 5) script development, and 6) result analysis.

Image acquisition, that is the capturing of an image in digital form, is the first step in any image processing system. IMAQ Vision is a library of LabVIEW (National Instrument product, Austin, TX) that can be used to develop a computer vision work. Through interactive programming on sample trays, the following batch script was developed:

i) *Simple calibration* - When camera axis is perpendicular to the image plane and lens distortion is negligible, a simple calibration is used to calibrate the image setup. In the

sample calibration, a pixel coordinate is transformed to a real-world coordinates through scaling in the x and y directions. To express measurements in real-world units; a coordinate system was defined by specifying origin, angle, and axis direction.

ii) Extract color planes: HSL - saturation breaks down a color image into various set of primary components such as HSL (Hue, Saturation, and Luminance). Each component becomes an 8-bit image that can be processed as gray scale image. Two principal factors- the coupling of the intensity component from the color information and close relationship between chromaticity and human perception of color makes the HSL space ideal for developing machine vision applications.

iii) Image mask-from ROI - Region of interest (ROI) is an area of an image in which we want to focus our image analysis. ROI can be defined interactively, programmatically, or with an image mask; and an area of the image that is graphically selected from a window displaying the image.

iv) *Look up table (LUT) Equalize*- to highlight image details in an area containing significant information at the expense of the other areas. A LUT transformation converts input grayscale values in the source image into other grayscale values in the transformed image. IMAQ Vision provides four VI's that directly or indirectly apply lookup tables to images. IMAQ Equalize distributes the grayscale values evenly within a given grayscale range. This is used to increase the contrast in image.

v) Gray morphology-Dilate - Grayscale morphology helps in removing or enhancing isolated features. Grayscale morphological transformations compare a pixel to those pixels surrounding it. The transformation keeps the smallest pixel values when performing erosion or keeps the largest pixel values when performing dilation. Dilation

increases the brightness of pixels surrounded by neighbors with a higher intensity. They mainly are used to delineate objects and prepare them for quantitative inspection analysis.

vi) *FFT filters-truncate low pass*-Fast Fourier Transform (FFT) is used to convert an image into its frequency domain. An image can have extraneous noise, such as periodic stripes, introduced during the digitization process. In the frequency domain, the periodic pattern is reduced to a limited set of high spatial frequencies.

A low pass frequency filter attenuates or removes high frequencies present in the FFT (Fast Fourier's Transforms) plane. This filter suppresses information related to rapid variation of light intensities in the spatial image *i.e.*, frequency components above the ideal cut-off frequency are removed, and the frequency component below it remains unaltered. This generally helps in smoothing the sharp edges.

vii) Advance morphology-Remove small objects- Morphological transformations extract and alter the structure of objects in an image. We can use these transformations to prepare objects for quantitative analysis, observe the geometry of regions, and extract the simplest forms for modeling and identification purposes. The advanced morphology functions are conditional combinations of fundamental transformations such as the binary erosion and dilation. This function eliminates tiny holes isolated in objects and expands the contour of the objects based on the structuring element.

viii) Image-3D View- This function gives a pictorial color base three-dimensional heating pattern. IMAQ Vision has several color scales to depict the heating pattern. Under this study, the program was set so that the red color of the spectrum represents the hot area having higher microwave thermal treatment and this region was shown as ridge region.

Similarly the deep blue color represents the cold area having less thermal treatment and was shown as depressed region.

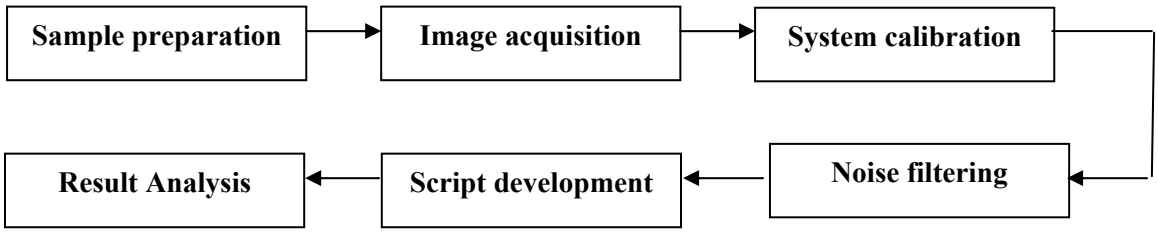


Fig. 2. Major steps involved in heating pattern analysis using computer vision.

ix) Quantify- The Grayscale quantify tool provide a numeral value for the color intensity. Interpretations of the numerical value depend on the selection of color palette. For *Rainbow* color palette the number zero is assigned to deep blue and 255 to dark red. These dimensions less numbers relatively compares the degree of color intensity varying from deep blue to dark red.

System calibration and noise filtering were done to improve the accuracy and visibility of the trays. After running the script for each layer results were analyzed to locate cold and hot spots.

3. Results and discussion

For a 2.67 kW power level, experiments were conducted for various values of F_o. At each F_o the M-2 marker yield was obtained using HPLC method. A linear correlation (R^2 = 0.97) was obtained between the accumulated marker yield and F_o (Fig. 3). The linear relationship between F_o and the marker yield suggests that darker regions of the tray had higher marker yield and higher degrees of thermal treatment. Similarly, lighter

regions had lower marker yield and lower degrees of thermal treatment. Increases in marker yields for selected points were best fitted with linear relationship.

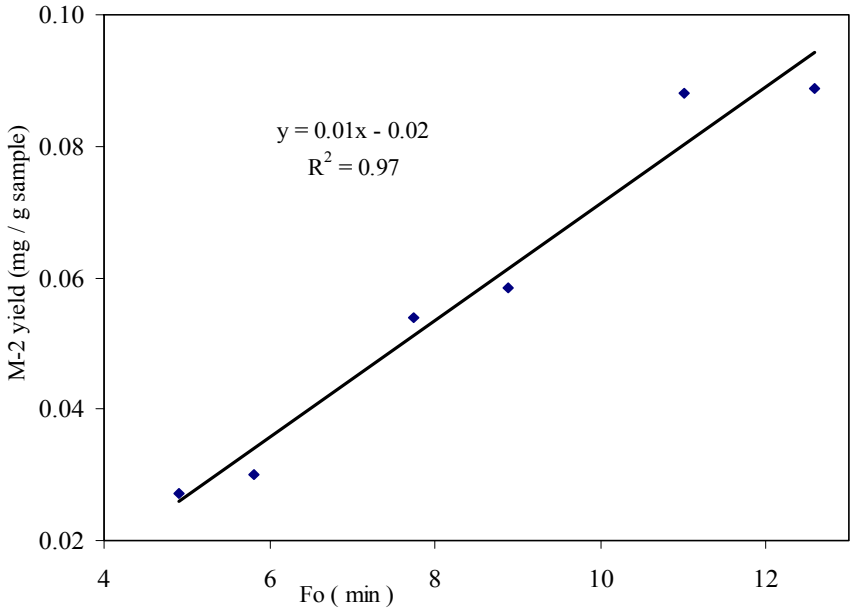


Fig. 3. Relationships between M-2 yield and F_o accumulation in mashed potato during microwave sterilization, each point represents the mean of two replicates.

A linear relationship ($R^2 = 0.98$) was also observed between color value at the tip of fiber optics probes (Fig. 4) and F_o. The deep red color has the highest color value and the deep blue has the lowest color value. This result suggests that the degree of the color intensity increases with level of the thermal treatment.

The deep blue color shows cold spot location and dark red color shows the hot spot location (Fig. 5). It was observed that center of the middle layer in the packaged food was less processed than the edges in each tray (Fig. 5). Cold spot was specified at middle of middle layer and hot spot at the location close to right farther corner in ten-ounce tray. Locations of cold and hot spots were independent to the levels of salt content.

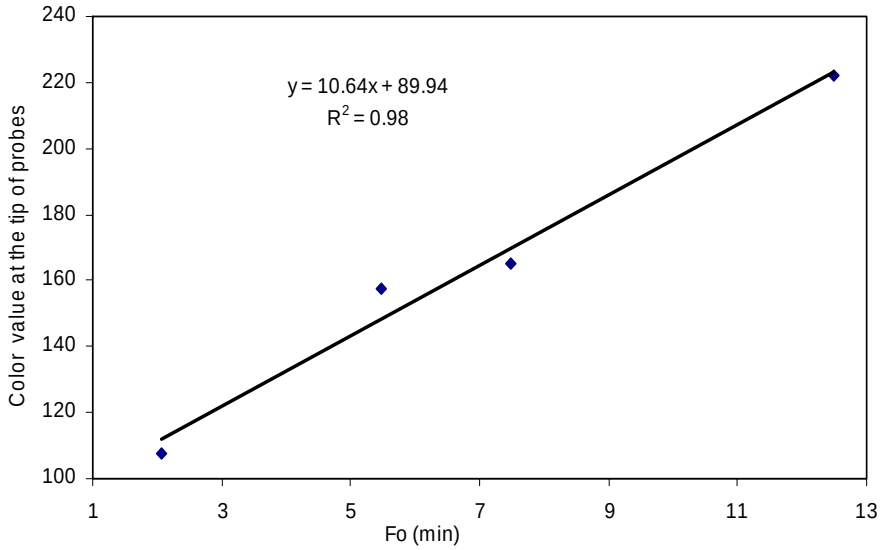


Fig. 4. Relationships between color values obtained by IMAQ vision builder and F_o for processed sample, each point represents the mean of two replicates.

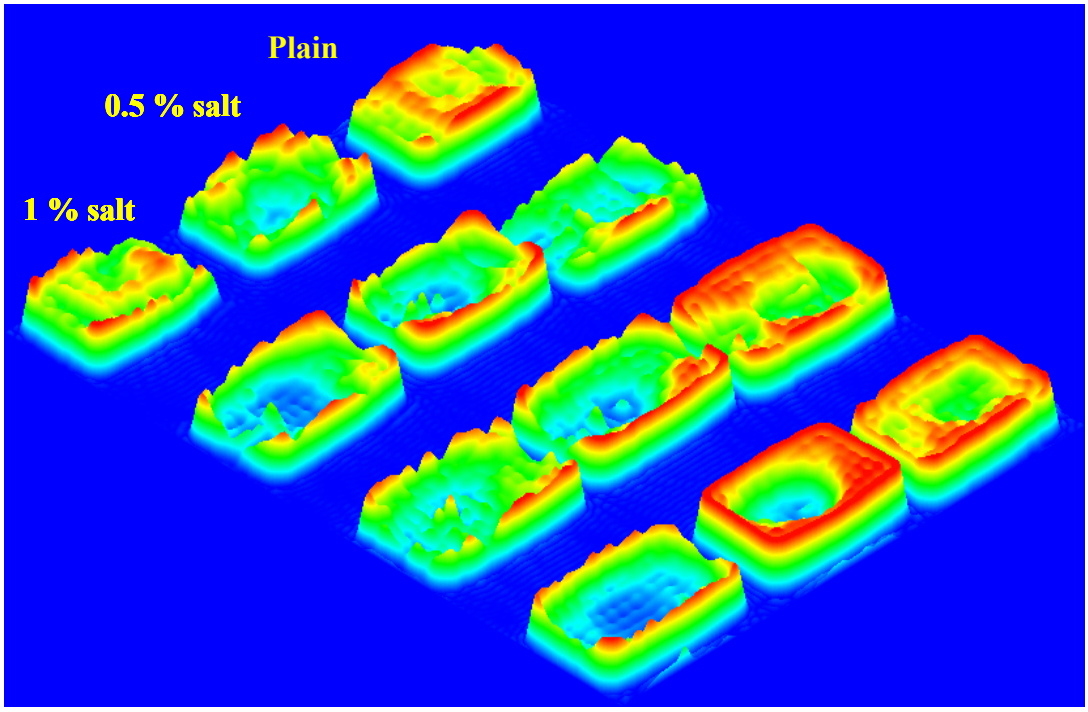


Fig. 5. Comparison of heating patterns of 10 oz trays with three levels of salt content after microwave sterilization, tested in replicates.

4. Validation of locations specified by computer vision

In order to evaluate the accuracy of the cold and hot spot locations determined by computer vision, experiments were conducted using microwave as a source of energy. Experiments were carried in two replicates to test the repeatability of heating profile and temperature difference between cold and hot spots at 2.67 kW power levels. Fiber optic temperature sensors were inserted at those two selected specified locations and experiments were carried at 2.67 kW power level.

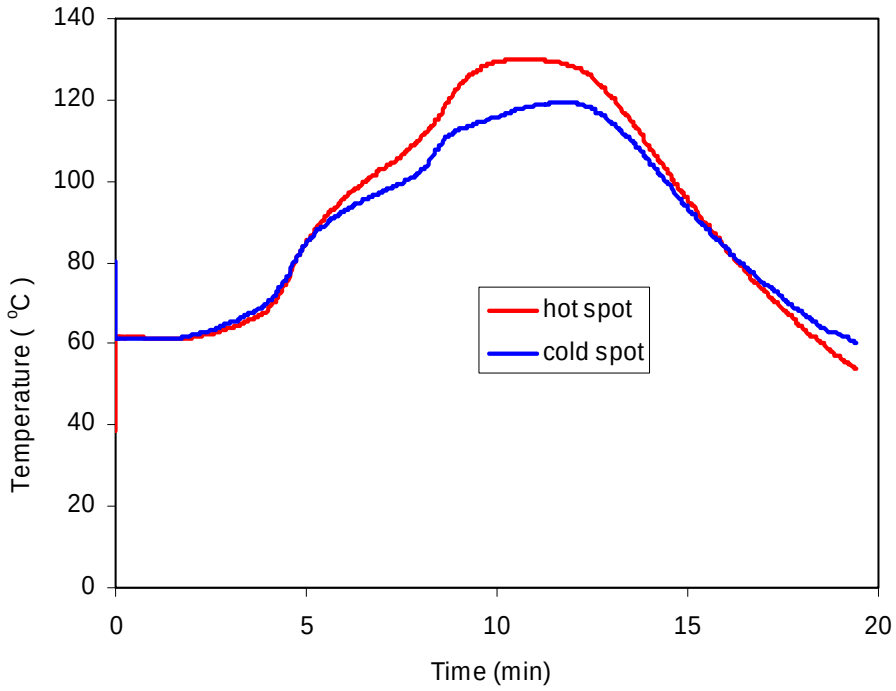
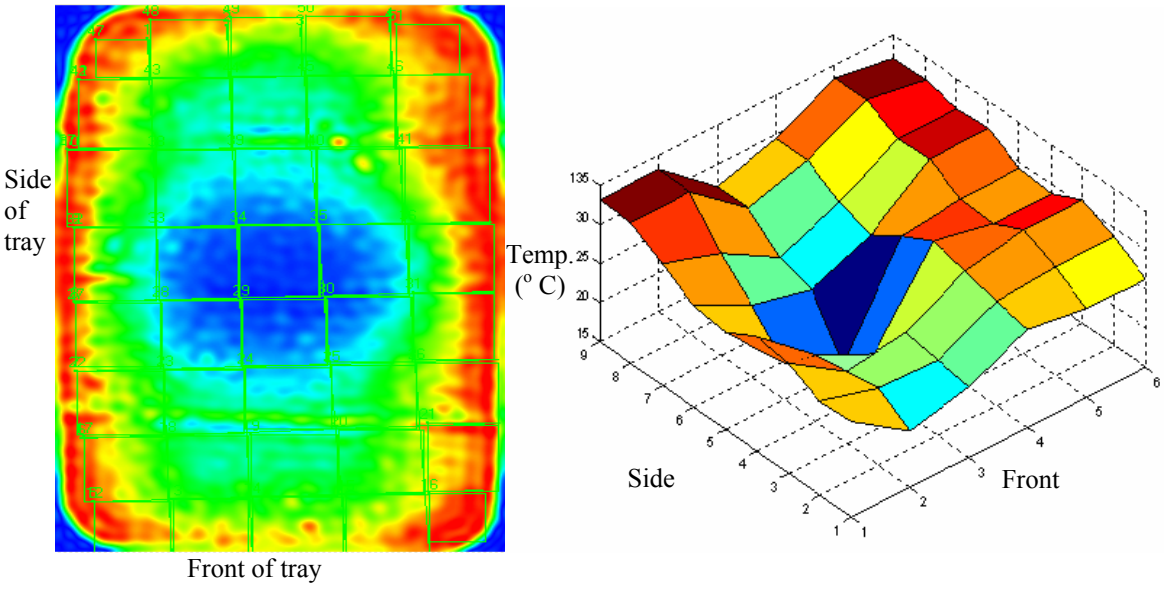


Fig. 6. Heating profile of hot and cold spots locations in 10 oz trays during microwave sterilization at 2.67 kW, tested in two replicates

The measured temperature confirmed the hot and cold spots for each replicate (Fig. 6). To further confirm the location of the cold spot in relationship with other parts of the tray additional 13 tests were conducted. In each case of the test four fiber optic sensors were placed in the sample tray during sterilization process. One of the sensors was always

inserted at the cold spot identified by the computer vision method while the other three were placed in the three different locations. Compiling the temperature measurement for 13 tests provided temperature of 40 points (8 × 5) evenly distributed in the middle layer of the tray. Computer vision heating patterns and temperature mapping obtained using fiber optic probes are compared in Fig. 7. Results showed that heating pattern and cold spot location obtained by both methods are in good agreement. This indicates that the novel method can indeed be used to study general heating patterns in homogeneous foods after microwave sterilization processes.



a). IMAQ heating pattern b). Actual temperature profile

Fig. 7. Comparison of computer vision heating patterns and actual temperature mapping for 10 oz trays.

5. Conclusions

In this paper, a novel approach has been developed to determine the heating pattern using the chemical marker M-2, digital imaging, and computer vision software (IMAQ) vision builder. A linear correlation was obtained between the M-2 marker yield and the degree of thermal treatment (F_o), which suggests that darker color corresponds to higher thermal lethality. Relationship between color at the tip of fiber optics probe and F_o was observed as linear. Locations of cold and hot spots obtained after image processing were also verified using fiber-optic temperature sensors. Locations of the cold and hot spots specified by computer vision matched well with temperature measurements using fiber optics probes. The experiments prove that computer vision (IMAQ Vision Builder) with the chemical marker M-2 and other accessories can be used as an effective tool to identify the location of cold and hot spots in microwave processed foods. Due to its cost effectiveness, consistency, fast speed and accuracy in comparison to HPLC, this method can be considered as the best option to determine the heating pattern.

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Nomenclature

C	marker yield (gm/ ml of sample)
C_o	initial marker yield (gm/ ml of sample)
C_∞	marker yield at saturation (gm/ ml of sample)
E_a	activation energy (kcal mol^{-1})
F_o	cumulative thermal lethality (min)
k_o	reaction constant at reference
LUT	look up table
R	universal gas constant at reference temperature (cal/mol K)
t	time (min)
T_o	reference temperature
T	temperature (K)

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CHAPTER 4

SENSITIVITY ANALYSIS AND VALIDATION OF COMPUTER VISION HEATING PATTERNS FOR MICROWAVE STERILIZATION PROCESSES

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Abstract

To develop a computer vision method for evaluating the heating characteristic of microwave sterilization system, it is necessary to identify and validate the locations of the cold and hot spots for different sizes of tray for food products of differing composition, most importantly, salts levels. A computer vision method based on the yield of chemical marker M-2 was used to determine the location of cold and hot spots in microwave sterilized trays. Locations of the identified cold and the hot spots were tested by changing the dielectric properties of mashed potato. Addition of salt changed the dielectric loss significantly but the heating patterns were not affected by the salt level. An inoculated pack study was performed to detect the number of survivors at the cold spot location specified by computer vision method. A finite difference time domain software QuickWave-3D was used to match the computer vision heating patterns for stationary states. The time-temperature trends of the cold and hot spots were compared during microwave sterilization process. Microbiological validation and real temperature measurement confirmed the identified cold spot locations. This study provided

convincing results to indicate that computer vision method can be used to predict the cold spot location for microwave sterilized foods.

Keywords: Automated machine vision; Computer or machine vision; Chemical marker M-2 yield; Cold and hot spots; Microwave heating pattern; Sterilization; FDTD method; M-2 Kinetics; Image analysis

1. Introduction

Microwave sterilization is a thermal method that has promise to produce high quality and shelf stable foods (Guan et al., 2004 & 2003) Pathak, Liu, & Tang, 2003; Zhang & Datta, 2000; Datta & Anantheswaran, 2001). The sterilization process is a complex physical phenomenon dependent on dielectric properties of foods and configuration of microwave cavities. (Lin, Anantheswar, & Puri, 1995; Zhou, Puri, Anantheswarn, & Yeh, 1995; Pandit, & Prasad 2003). Contrary to conventional heating methods, microwave heating delivers much faster rate of heating. However, the corollary of the volumetric heating method is that if uneven heating occurs, the location of cold or hot spots may vary and depend on product geometry (Campañone, & Zaritky, 2004; Yang, & Gunasekaran, 2004). Being a fast method of sterilization if a food process does not get sufficient time to equilibrate by the end of heating operation, may results into development of cold or hot spots (Ma, Paul, & Pothecary, 1995). Microbes can survive at cold spot while food quality degradation may occur at hot spot (Ghani, Farid, & Chen, 2002; Sale, 1976). Sterilization as a method to guarantee lethality temperatures (121 ^{o}C)

for microorganism, overheating occurring in hot spots zones can cause quality losses (Ayappa, Davis, Crapisite, Davis, & Gordon, 1991; Dinčov, Parrott, & Pericleous, 2004).

Multi-point online monitoring of time temperature profile in industrial scale microwave sterilization system was meticulous task. Metallic thermocouples can not be placed in microwave field while fiber optics probes are expensive and inconvenient to monitor multiple points in a tray. High performance liquid chromatography (HPLC) can be used as an indirect means to determine chemical marker (M-2) yield in process trays. But using HPLC method was time and resource consuming (Pandit, Tang, Mikhaylenko, & Liu, 2006). In order to meet the stringent requirements of food regulation bodies for sterilization processes of food products much effort at both industry and academic level has been made to design a method to determine the location of cold and hot spots (John, Maria, Ruth, & Andrew, 1999; Oliveira, & Franca, 2002; Munkevik, Hall, & Duckett, 2005; Yam, & Papadakis, 2004; Fernandez, Castillero, & Aguilera, 2005). Microwave Heating Group at Washington State University (Pullman, WA) has developed a computer vision method based on chemical marker M-2 yield to locate the cold spot (Pandit, Tang, Liu, & Pitts, 2007).

Previous studies involved kinetics of chemical marker M-2 formation with mashed potato followed by development of a novel approach to determine the heating patterns using computer vision method based on chemical marker (M-2) yield (Pandit, Tang, Mikhaylenko, & Liu, 2006 ; Pandit, Tang, Liu, & Pitts, 2007). The specific objectives of this study were 1) to determine the locations of cold and hot spots with different sizes of trays using computer vision method; 2) to investigate the effect of salt

levels on cold/hot spots locations, and 3) to validate the specified cold spots locations using simulation, microbial study and fiber optics temperature measurement sensors.

This study can be used as a protocol to evaluate the heating patterns of sterilization system with combinations of tray size, salt levels, and MW power levels.

2. Materials and methods

Investing the nature and locations of cold spots for different size of trays are needed to ensure safe level of microwave sterilization process. Variation of heating patterns with different sizes of trays as affected by salt contents was considered in this study to test the sensitivity of heating patterns to dielectric properties changes.

2.1. Effect of salt content on chemical marker M-2 yield

Amino acids (Lysine, Arginine, Histidine and Methionine) and D-ribose are principal reactants in chemical marker M-2 formation (Kim, & Taub, 1993). Instant mashed-potato flakes acquired from Oregon/Washington Potatoes Co. (Boardman, OR) was selected as a model food and accumulated M-2 yield as a coloring agent to quantify the amount of thermal energy at a point (Lau et al, 2003). Mashed potato (15.38 %) sample was prepared with 1.5 % D-ribose and with a moisture content of 83.12 % (w.b). Chemical marker yield for the reaction was also studied in this research in presence of salt. Capillary tubes filled with mashed potato sample for 0.0, 1.0 % levels of salt were heated in oil bath at 121 ^{o}C and were taken out from the oil bath at intervals of 5 minutes. Chemical marker M-2 yield of the extracted mashed potato sample was determined using

HPLC method (Agilent Technology, USA) (Pandit, Tang, Mikhaylenko, & Liu, 2006). Each test was duplicated.

2.2. Dielectric properties measurement

Dielectric properties of mashed potato supplies were determined over range of temperatures from 20 to 121 ºC at 1 to 1800 MHz using a Hewlett-Packard 85070B open ended coaxial probe connected to an Agilent 4291B Impedance Analyzer (Agilent Technologies, Inc., Palo Alto, CA). Mashed potato sample with 0.5 %, 1 % salt added and 1.5 % D-ribose composition was prepared for measuring dielectric properties at 915 MHz in two replicates (Guan, Cheng, Wang, &Tang, 2004). Detailed description of the calibration and measurement procedure is provided in Wang, Wig, Tang, Hallberg (2003).

2.3.Microwave sterilization of mashed potato samples

Seven ounce (14 × 9.5 × 2.67 cm), ten ounce (14 × 9.5 × 3.3 cm), thirteen ounce (14 × 9.5 × 4.2 cm) and twenty ounce (19.5 × 14.4 × 3.2 cm) plastic trays were filled with 200 g, 300 g, 400 g and 600 g of mashed potato sample respectively. Trays were vacuum-sealed at 18 inch of Hg vacuum twice with time setting of 3 and 1 second. To measure the sample temperature using optical fiber sensors (Fiso Technologies, Inc. Quebec, Canada)., a polyimide tubing of outer diameter 0.075 inch (Cole-Parmer, IL, USA) was sealed at one end using silicone sealant (Dow Corning, Midland, MI, USA). The tube was inserted through a hole in the side of the tray so that the temperature of the located point can be monitored. Twenty ounce trays had two fiber-optic probes, one

fitted in middle and another close in one end to left side of the tray. Experiments were conducted in a 915 MHz single mode water circulated microwave heating system at 2.67 kW power level with 35 liter per minute flow rate of water at 125 ^{o}C temperatures. To investigate the heating patterns of 180 and zero degree phase shift, trays were situated at the center of the cavities during microwave heating. In order to study the combined effect of phase shift the tray support was moved from 180 degree to zero degree phase shift cavity. Initial temperature of sample was 22 ± 1 ^{o} C. Speed of the tray support was set to 0.23 inch per second so that temperature at middle point in holding section could reach to 121 ^{o} C. The tray support was made of the Tempalux material (Ultem Polyetherimide Resin, Lenni, PA, USA). Ultum tray support held four trays across length of size 7, 10, 13 oz trays or two trays along length for 20 oz trays. After reaching the sterilization temperature (121 ^{o}C) processed trays were cooled fast by passing the tap water at 16 ^{o}C through the cavity. Fast cooling minimizes formation of chemical marker M-2. Processed trays were stored in the deep-freezer at -35 ^{o}C for 60 minutes to harden the mashed potato before cutting into the layers. Trays were cut into vertical, middle and bottom layers and images of each layer were taken using digital camera (Olympus C -750 Ultra Zoom) mounted with computer vision system.

2.4.Computer vision heating patterns based on chemical marker (M-2) yield

Location of cold spots was determined by analyzing heating patterns of middle and bottom layers using IMAQ vision builder software (National Instrument product, Austin, TX). Vertical layers heating patterns were used to determine microwave power penetration, middle and bottom layer were considered to specify the location of cold and

hot spots for each size of tray. A script developed in IMAQ vision builder software was used to determine the 3-D heating patterns of processed sample (Pandit, Tang, Liu, & Pitts, 2007). Through interactive programming the script was developed by selecting following functions tools of the IMAQ software: color plane extraction, look up table equalize-dilate and erosion, gray morphology, filters smoothing and high-lights details, fast Fourier's Transform low pass filters, and grayscale quantization. Fourier's transform is one of the most important tools which have been extensively used not only for understanding the nature of an image and its formation but also for processing the image. The two-dimensional Fourier's transform of a continuous function *f (x, y)* is given as (Acharya, & Ray, 2005):

$$F(\omega, \psi) = \int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty} f(x,y)\exp[-j\,2\pi(\omega x+\psi y)]dx\,dy) \quad (1)$$

Using Euler's formula the exponential function can be decomposed into:

$$\exp[-j\,2\pi(\omega x+\psi y)] = \cos(2\pi(\omega x+\psi y)) - j\sin(2\pi(\omega x+\psi y)) \quad (2)$$

which implies that the function *f(x, y)* is essentially multiplied by the terms $\cos(2\pi\omega x)$ $\cos(2\pi\psi y)$, $\sin(2\pi\omega x)$ $\sin(2\pi\psi y)$, $\sin(2\pi\omega x)$ $\cos(2\pi\psi y)$, and $\cos(2\pi\omega x)$ $\sin(2\pi\psi y)$. If the function is doubly symmetric function along both the X and Y directions, then Fourier transform of *f(x, y)* involves only the multiplication of $\cos(2\pi\omega x)$ $\cos(2\pi\psi y)$ term. During this study the *f(x, y)* multiplication involved all four terms. Thus integrand $F(\omega, \psi)$ gives the limit summation of an infinite number of sine and cosine terms. The variable ω in Eq.1 denotes number of waves per unit length in X direction, and ψ indicates the number of waves along the Y direction. For a certain pair of

frequency components (ω, ψ) the integrand gives amplitude of the chosen component. The amplitude spectrum of two dimensional function is:

$$|F(\omega,\psi)| = \sqrt{R^2(\omega,\psi) + I^2(\omega,\psi)} \tag{3}$$

where R (ω, ψ) is real part and I(ω, ψ) is an imaginary part of the F (ω, ψ). Low pass filtration of the image was done by taking the weighted average of the neighborhood pixels. The output image in that case would be expressed as:

$$g(m, n) = \sum\sum a(k,1)\ f(m-k, n-1), \qquad (k,1) \in W \tag{4}$$

where *f(m, n)* and *g(m, n)* are the input and output images respectively, W is neighborhood around the pixel at location *(m, n)*, and *a(k, 1)* are the filters weights. All weights were assigned equal values in this study and in that case equation (4) reduced to:

$$g(m, n) = \frac{1}{N}\sum\sum\ f(m-k, n-1), \qquad (k,1) \in W \tag{5}$$

where N is the number of pixels in the neighborhood W. The spatial averaging operation on an image was used to smooth the noise. If an observed image is given as:

$$g(m, n) = f(m, n) + \eta(m, n) \tag{6}$$

then the spatial average will be calculated as:

$$g(m, n) = \frac{1}{N}\sum\sum\ f(m-k, n-1) + \bar{\eta}(m, n), \qquad (k,1) \in W \tag{7}$$

where $\bar{\eta}(m, n)$ is the spatial average of the noise component *η (m, n)*.

The dark red color of the rainbow color palette was set in the pattern to depict hot spot region and deep blue as a cold spot region. For each size of tray two replicates at each salt level were analyzed to determine the cold spot. The middle layer of each tray was divided into rectangular grids of 8 × 5 (rows × column) and color value equivalent to

grayscale value of each grid was obtained using IMAQ vision software. A grid common in each tray with lowest color value for each level of salt was considered as the cold spot. Similarly, the hot spot was detected as rectangular grid common in each tray with highest color value. Distances of the middle point of the region identified as cold spot and hot spot were measured in Cartesian co-ordinate system using IMAQ vision builder software.

2.5. FDTD simulation using QW-3D

Heating patterns based on computer vision analysis were compared with the absorbed power distribution patterns using QuickWave-3D program. QuickWave-3D solves the Maxwell equation using the finite difference time domain method (FDTD). It makes use of finite difference approximation to electric and magnetic fields components, which are staggered both in time and space domain. (Yee, 1996; Peterson, Ray, & Mitra, 1997). Detail of the mathematical modeling and experimental validation is provided elsewhere in Chen et al., (in press).

2.6. Microbial validation

Locations of cold spots detected by chemical markers were validated using inoculated pack study. *Clostridium sporogenes* spores were inoculated in mashed potato to make a final concentration of 7.5×10^6 CFU/10 oz trays. The mashed potato and spores were mixed thoroughly and filled into the trays. The trays were microwave processed at different F_o values based on the thermal characteristics of the spores and the temperature history of the potential cold spot in the mashed potato. After microwave processing, the trays were divided into 40 small cubes (1.75 x 1.9 cm) and numbered from 1 to 40 from

rear side of the trays. For trays processed at low F_o values (< 3.0), the cubes were diluted and plated on agar medium, and survivors were enumerated after incubation. Cubes from trays with F_o values higher than 3.0 were incubated in enrichment media for 48 hours at 32˚C and presence or absence of growth was determined.

3. Results and discussion

3.1. Dielectric properties modeling

Statistical analysis of the chemical marker yield with 0.0 and 1.0 % salt confirmed (P > 0.9988) that there was no influence of salt content on chemical marker yield (Pandit et al., 2006). Dielectric loss of mashed potato was significantly changed by addition of salt from 0 to 1 % (Table 1). Dielectric loss of mashed potato with 1 % salt content was 3.5 times higher than plain mashed potato at 121 ^oC (Table 1). The range of variation in dielectric loss may cover a wide range of homogeneous foods namely mashed potato, whey protein gel etc (Guan et al., 2004). Effect of food temperature (T ^oC) and percentage of salt content (S) on dielectric constant (ε') and dielectric loss (ε") of mashed potato were modeled. Based on coefficient of determination ($R^2_{adj.}$ = 0.999), using statistical analysis software SAS (SAS Institute Inc., Cary, NC, 2000), the fitted models are:

$$\varepsilon' = -0.148.\,T + 1.45.\,S + 68.176 \qquad R^2 = 0.999 \qquad (8)$$

$$\varepsilon'' = 0.00108.\,T^2 + 0.535.\,T.\,S + 8.6171.\,S + 15.3216 \qquad R^2 = 0.999 \qquad (9)$$

where penetration depth (D_p) is the distance at which the power drops to 37 % of its original value at the surface. Penetration depth is affected by dielectric constant, dielectric loss factor, and operating frequency as (Tang et. al., 2002):

$$D_p = \frac{c}{2\pi f \sqrt{2\varepsilon'}}\left[\sqrt{1+\left(\frac{\varepsilon''}{\varepsilon'}\right)^2}-1\right]^{-1/2} \qquad (10)$$

where c is velocity of light (m/s), f is operating frequency (915 MHz). Table 1 summarizes the penetration depth with different level of salt and 1.5 % D-ribose at 915 MHz. Increase in dielectric loss (ε") decreases the depth of penetration (Fig. 1). Because of increase in tray thickness microwave penetration became limiting factor in transmitting the microwave energy to middle layer of trays.

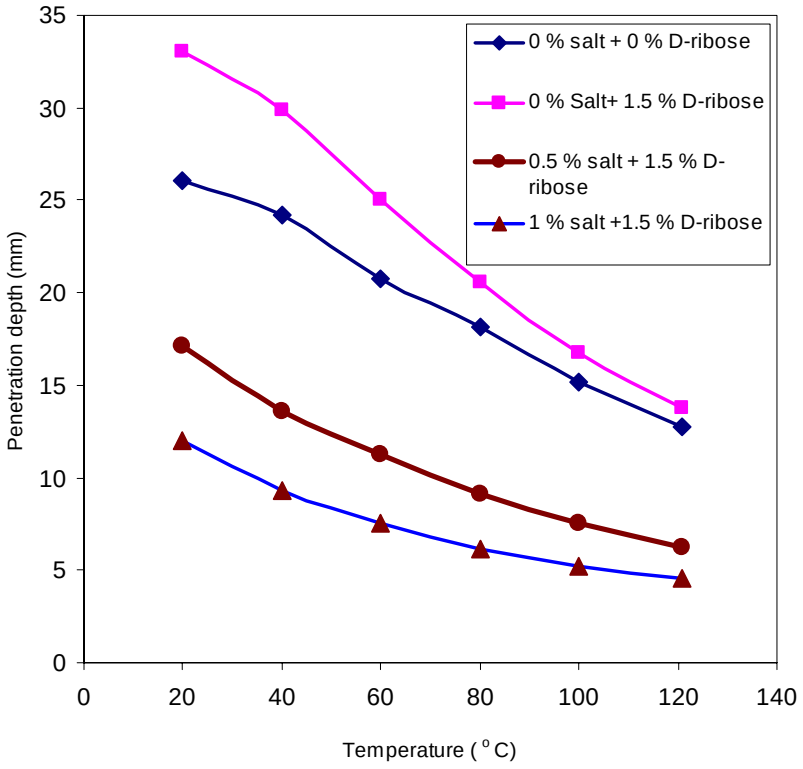


Fig. 1. Penetration depth versus temperature of mashed potato for different levels of salt, each data point represents the mean of two replicates.

Table 1. Mean ± standard deviation (two replicates) of dielectric constant, dielectric loss and penetration depth with mashed potato (83.12 % wb) at different levels of salt (maximum 1 %) and 1.5 % D-ribose as a function of temperature at 915 MHz.

Sample	T (^{o}C)	ε’	ε’’	Pd (mm)
Mashed potato + 0 % salt	20.00	70.17 ± 2.05	16.88 ± 2.95	26.05
	40.00	68.44 ± 1.12	17.951 ± 3.10	24.23
	60.00	63.26 ±2.43	20.218 ± 2.85	20.77
	80.00	59.86 ± 2.67	22.6015 ± 1.79	18.16
	100.00	56.01 ± 1.21	26.3215 ± 0.65	15.21
	121.00	52.105 ± 0.36	30.695 ± 0.71	12.75
Mashed potato + 0 % salt + 1.5 % D-ribose	20.00	66.865 ± 0.60	12.9785 ± 0.85	33.01
	40.00	62.925 ± 0.36	13.943 ± 0.83	29.85
	60.00	59.18 ± 0.34	16.161 ± 0.85	25.05
	80.00	56.26 ± 0.40	19.261 ± 0.94	20.59
	100.00	53.745 ± 0.73	23.3 ± 1.80	16.77
	121.00	50.85 ± 0.014	27.88 ± 2.24	13.80
Mashed potato + 0.5 % salt + 1.5 % D-ribose	20.00	62.05 ± 7.78	24.36 ± 1.05	17.17
	40.00	61.925 ± 5.92	31.035 ± 0.80	13.61
	60.00	62.38 ± 0	38.08 ± 0.39	11.27
	80.00	54.55 ± 0.57	45.27 ± 1.97	9.12
	100.00	52.79 ± 0.46	55.49 ± 1.28	7.56
	121.00	51.19 ± 0.48	69.18 ± 1.85	6.24
Mashed potato + 1 % salt + 1.5 % D-ribose	20.00	67.12 ± 2.10	36.81 ± 1.87	12.01
	40.00	64.795 ± 0.29	47.49 ± 2.73	9.35
	60.00	61.885± 0.66	59.78 ± 2.35	7.50
	80.00	58.91 ± 1.053	73.83 ± 1.82	6.18
	100.00	54.605 ± 0.62	88.61 ± 1.05	5.24
	121.00	51.21 ± 0.64	105.26 ± 0.53	4.54

Salt is strong electrolyte, which increases the electrical conductivity or rate of dissipation (dielectric loss) of power. Increasing temperature also decreases the relaxation time of the water molecules, which resulted into higher dielectric loss. Hence, at high salt content (1%) in higher temperature range (>121 ^{o}C) depth of penetration was observed to be minimum with mashed potato (Fig. 1).

3.2. Cold spots location in each size of tray

Microwave penetration was sufficient to reach the energy to middle layers in mashed potato sample and heating patterns were similar for all levels of salts in 10 oz trays. For cold spot determination, distances were measured considering the left bottom corner of the middle layer of the mashed potato sample as the reference point i.e., (x = 0, y=0). Cold spots for seven-ounce tray was located at x =1.5 cm, y = 4.45 cm and hot spot at x = 1.35 cm, y =13.05 cm. For 10 and 13 oz trays heating patterns were similar to conventional heating i.e., cold spot was observed in middle of the tray (Fig. 2). Due to limitation on microwave power penetration middle layer of thirteen ounce tray received lesser energy. Combined affects of both hot water and 180 degree phase shift were creating dominant edge heating in thirteen ounce tray until middle layer reached to 121 ^{o}C. Location of cold spot and hot spot were obtained in middle layers at x = 6.82 cm, y = 4.75 cm and x = 1.35 cm, y = 13.05 cm for both 14 × 9.5 × 3.3 cm and 14 × 9.5 × 4.2 cm trays. Cold spot area was much wider and appearing with increasing level of salt. Thirteen-ounce tray with 1% salt level had lowest power penetration and significant edge heating. Cold spot was observed at x =12 cm, y = 12 cm and hot spot at x = 11 cm, y = 1.35 cm with 20 oz tray. Heating patterns were repeatable for each level of salt (Fig. 3).

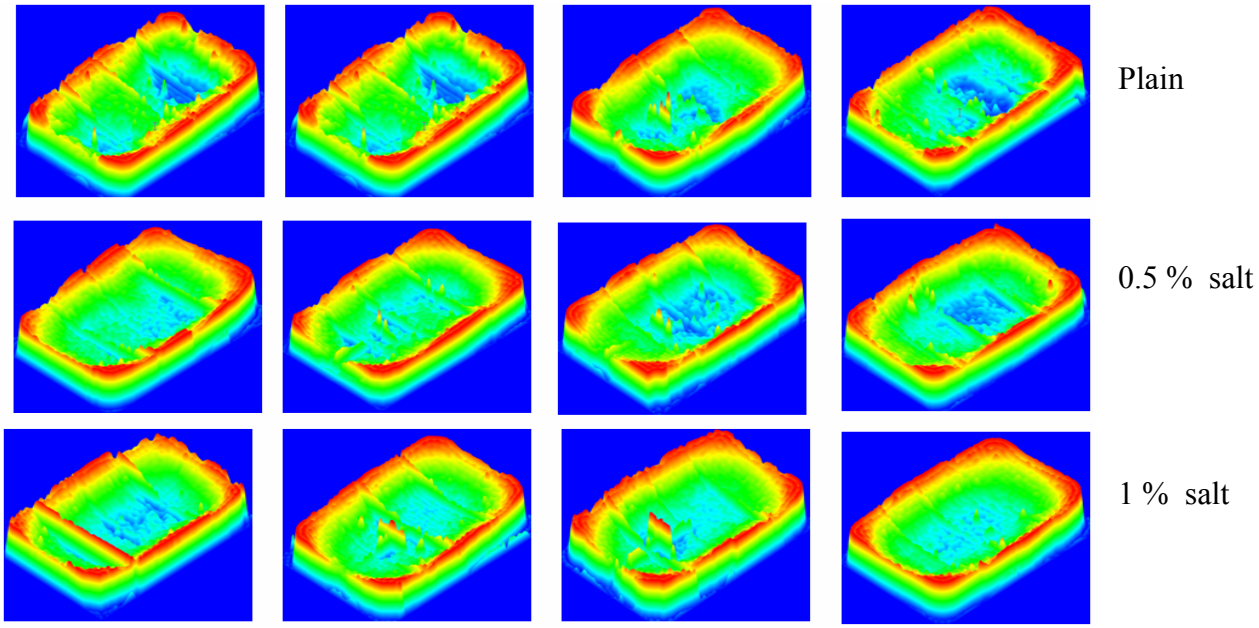


Fig. 2. Computer vision heating patterns of middle layers for three different levels of salt with 14 × 9.5 × 4.2 cm tray at 2.67 kW microwave power level, tested in two replicates.

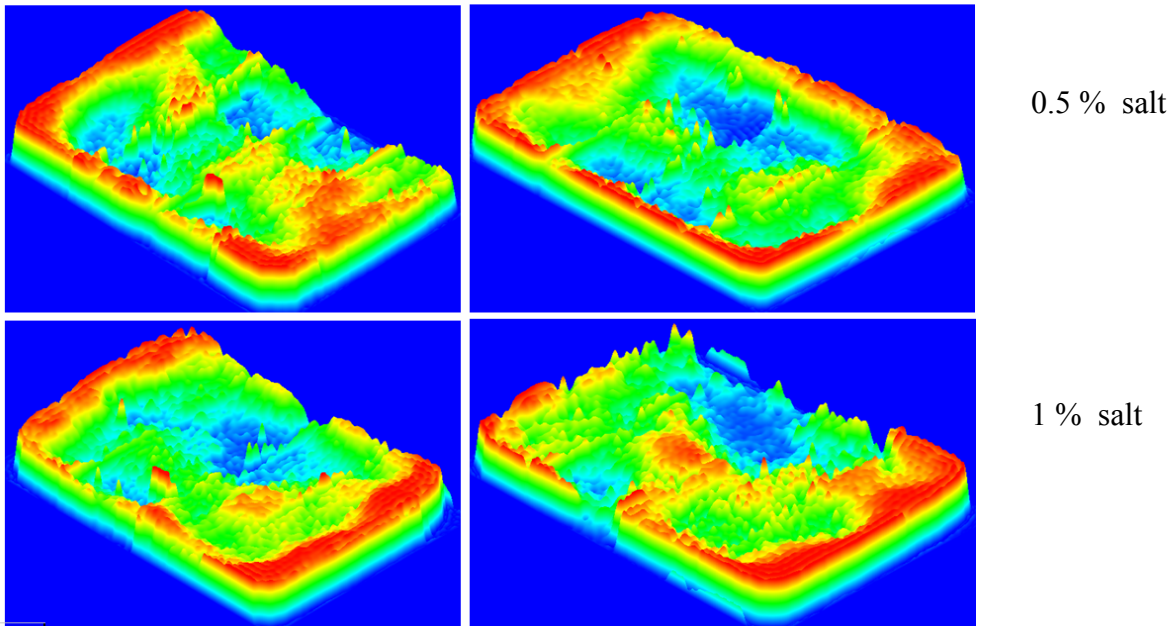


Fig. 3. Computer vision heating patterns of middle layers with two different levels of salt with 19.5 × 14.4 × 3.2 cm tray at 2.67 kW microwave power level, tested in two replicates.

3.3. Effect of tray size and system configuration on heating patterns

Locations of cold spots were dependent on the size of food packages, orientation of the tray and configuration of the microwave sterilization cavity. Boundary regions were receiving higher amount of energy for each size of food package. Hot water (125 ^{o}C) and 180 degree phase shift both had combined affect on edge heating. Hot spots were located at right farthest corner for each size of package. Considerably, edge heating can be minimized by lowering the hot water temperature or by changing of degree of phase shift. Application of zero degree phase shift for both the cavities would be the best option to improve the degree of uniformity.

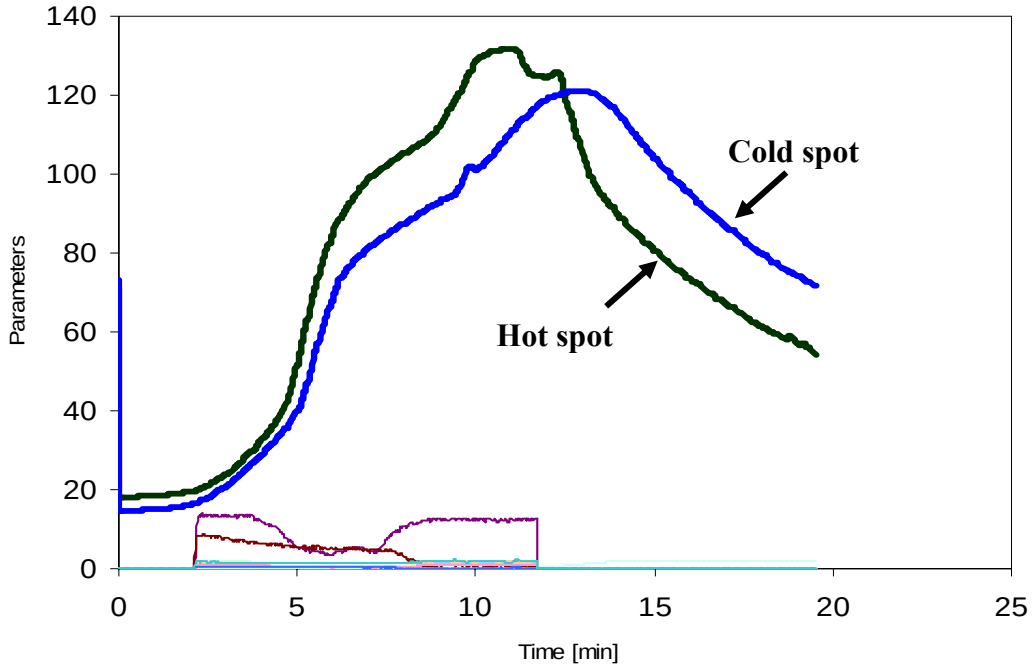
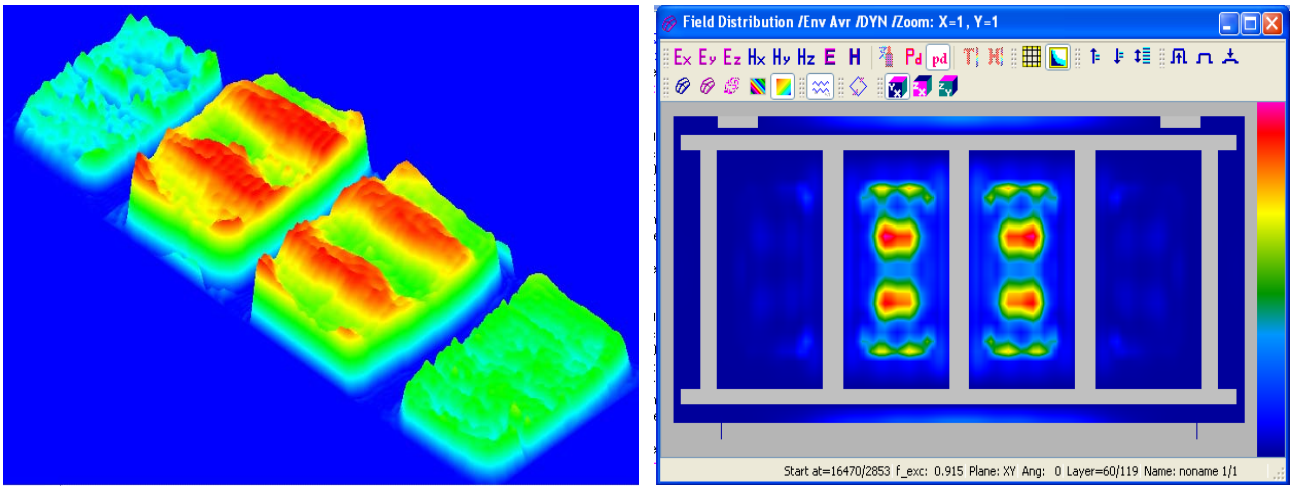


Fig. 4. Temperature measured by fiber optics probes at cold spot x =12 cm, y = 12 cm and hot spot x = 11 cm, y = 1.35 cm in 20 oz tray specified by computer vision method in the middle of trays during microwave sterilization, tested in two replicates.

3.4. *Heating patterns validation*

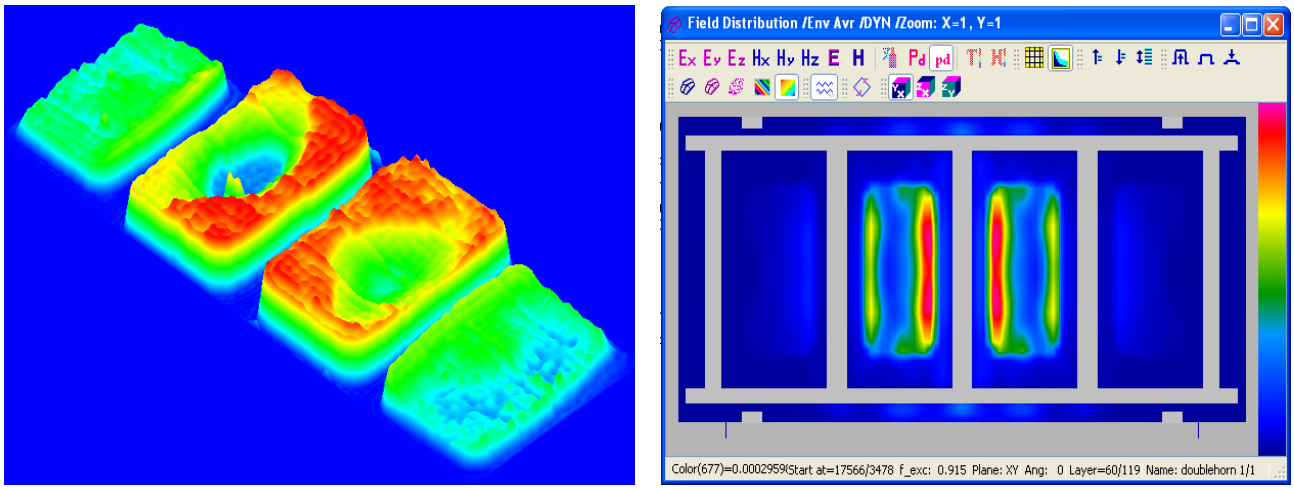
The temperature measured by fiber optics probe confirmed the hot and cold spots for each replicate. Computer vision heating patterns and temperature mapping obtained using fiber optics are compared in Fig. 6 for middle layers. Computer vision heating patterns and cold spot location obtained by fiber optics probes are in good agreement.



a) Computer vision heating pattern

b) Microwave power absorption using QW-3D

Fig. 5. Matching of computer vision heating patterns and QW-3D power absorption patterns for middle layer of 14 × 9.5 × 3.3 cm tray inside the zero degree phase shift cavity in stationary state.



a) Computer vision heating pattern b) Microwave power absorption using QW-3D

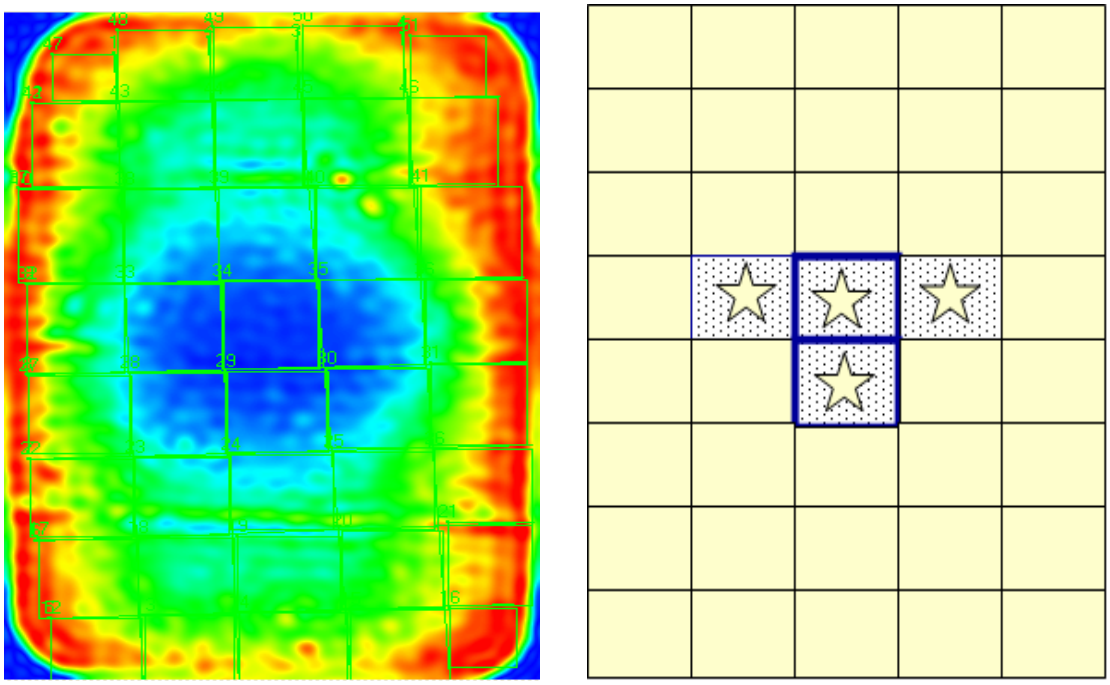
Fig. 6. Matching of computer vision heating patterns and QW-3D power absorption patterns for middle layer of 14 × 9.5 × 3.3 tray inside the 180 degree phase shift cavity in stationary state.

Fig. 5 and Fig. 6 shows matches between heating patterns of developed chemical marker method and simulation for zero and 180 degree phase shift cavities in stationary state. Heating patters comparisons were made only for middle layer of the mashed potato sample. Matching of the QW-3D absorbed power distribution patterns with computer vision heating patterns shows this method can indeed be used as an optional means to determine the heating patterns of foods rapidly and accurately.

3.5. Results of microbial validation

D-value and z value of the spore in mashed potato were found to be 1.02 min at 121 ^{o}C and 10.79 ^{o}C respectively. Based on the D_{121}-value of the spore in mashed potato, for 7.5 × 10^{6} CFU microbial concentration of target F_o was set to 7.7 min. Number of

survivors was counted at each cube during enumeration. For the trays processed higher than F_o 3 min, no survivors were detected. Survivors were detected at cold spot for trays processed at low F_o (< 3 min). The location of cubes showing survivals or growth was compared to that determined by heating patterns using chemical marker yield and computer vision system. The microbiological validation also supported the cold spots locations specified by the computer vision method (Fig. 7).



a) dark blue region showing cold spot in computer vision heating patters

b) Cold spot location observed in microbial validation

Fig. 7. A cold spot location identified by computer vision was observed as cold spot in microbial validation study (☆).

Table 2. Positive growth of spores was shouted in microbial validation at cold spot location identified by computer vision method in case of under process sample during microwave sterilization.

Inoculated levels of spores/300g	Process levels	Designed sterilization value (SV)	Number of potato cubes	Number of positive growth
7.5×10^6	Under target	3.21	40	4
7.5×10^6	Target	7.78	40	0
7.5×10^6	Target	8.64	40	0
7.5×10^6	Over target	12.66	40	0

4. Conclusions

A computer vision method based on yield of chemical marker M-2 was used to locate cold and hot spots with different sizes of tray in microwave sterilization system. Locations of cold and hot spots were found to be independent to the salt contents for each case. Hot water at 125 ^{o}C and 180 degree phase shift combined created edge heating, which caused hot spot close to the edge in each tray. Heating patterns were found to be sensitive to the cavity configuration and tray support structure. Temperature measured at specified locations by fiber optics probes confirmed the hot and cold spots for each replicate. Power distribution pattern using QW-3D and heating pattern by computer vision were matching well for stationary state. Confirmation of the locations specified by computer vision using QW-3D software, fiber optics probes and microbial validations

proved this method as potential means to identify cold spots in microwave sterilization processes.

Acknowledgements

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Nomenclature

ε'	dielectric constant
ε''	dielectric loss
ω	number of waves per unit length in X direction,
ψ	number of waves along the Y direction.
^{o}C	degree centigrade
c	speed of light (m /s)
D_p	depth of penetration (cm)
F (ω, ψ)	Fourier's transforms of the function *f (x, y)*
F_o	cumulative lethality (min)
f	operating frequency
P_o	microwave power absorption density (w / m^3)
S	salt content (%)
t	time (min)
T	temperature (^{o} C)

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CHAPTER 5

DEVELOPING A COMPUTER VISION METHOD BASED ON CHEMICAL MARKER M-2 YIELD TO LOCATE COLD-SPOT IN MICROWAVE STERILIZATION PROCESSES

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Abstract

A major challenge in developing advanced thermal process based on electromagnetic heating is to determine the location of cold spots in foods, when developing a thermal process to ensure commercial sterilization. A rapid and reliable method was developed with the aim to effectively locate the cold spot in model food sterilized in microwave systems. The developed method involved application of chemical marker M-2 yield to a model food, mashed potatoes, using computer vision system and IMAQ Vision Builder program. A systematic study was conducted to establish relationships among M-2 yields, color values from captured images of cut food samples, and thermal lethality (F_o). Several factors including consistency of imaging background and positions of lights over the diffuser box were considered to standardize the method. To facilitate the comparative study of heating characteristic for different combinations of power levels and F_o, a mapping scale using unheated and saturated mashed potato samples was developed by fixing the lowest and upper most gray-scale values. Color values equivalent to gray-level values were positively correlated to F_o and M-2 yield. The specified cold spot location determined by computer vision method was validated in the

915 MHz single-mode microwave sterilization system. The results showed that the computer vision method can potentially be used as an effective tool in microwave sterilization process development for regulatory approval and industrial applications.

Keywords: Color values; computer vision; image processing; chemical marker; heating patterns; microwave sterilization; process validation; IMAQ vision builder; cold spot

1. Introduction

Microwave sterilization holds promise to reduce process time and improve product quality (Guan, Plotka, Clark, & Tang, 2002; Guan et al., 2003). Determination of cold spot locations in foods during microwave sterilization, however, is a major challenge for researchers when developing processes to ensure that the processed foods will be safe for consumers. Computer simulation models can help in understanding the sterilization process (Pathak, Liu, & Tang, 2003; Zhang, & Datta, 2000). Simulation models, however, require validation and may not always be reliable due to complexity of the coupling of heat transfer and dielectric heating in complex microwave sterilization cavities (Ayappa, Davis, Davis, & Gordon, 1991; Pandit, & Prasad, 2003; Romano, Marra, & Tammaro, 2005). For any geometrically complex system used to produce safe foods for consumers, an approach of double validation for process development was emphasized by US food regulatory organizations (Food and Drug Administration).

It is impossible to identify the cold spots in packaged foods during microwave sterilization processes by point temperature measurement methods. Chemical marker methods were studied as indirect means to evaluate relative heating absorptions in

selected food systems (Lau, et al., 2003; Wang, Lau, Tang, & Mao, 2003; Pandit, Tang, Mikhaylenko, & Liu, 2006). Quantification of chemical marker M-1 and M-2 formed through Maillard reaction between amino acids and reducing sugar such as ribose and glucose required intensive laboratory analysis using High Performance Liquid Chromatography (HPLC). For example, to analyze 3-D heating pattern in processed mashed potato containing ribose in 10 oz trays with HPLC, two persons were needed for 2.5 days to quantify M-2 yield at 40 evenly distributed points in one tray. In process development, repeated tests would be necessary with multiple trays and analyzing M-2 yield in those many trays using HPLC became impractical. It was, therefore, desirable to develop a rapid and reliable method to capture the color intensities of chemical markers (M-2) formation that reflect 3-D heating patterns.

A novel approach based on combination of chemical marker (M-2) yield and computer vision has been proposed as an option for evaluating the heating patterns of microwave-sterilized foods (Pandit, Tang, Liu, & Pitts, 2007). More research was needed to standardize the method and to establish correlation between color intensity and process cumulative lethality. In recent years, computer vision has reached wide-spread applications for quality inspection, classification, evaluation of product and process in the agri-food industry (Abdullah, Guan, Lim, & Karim, 2004; Wang & Sun, 2002; Yam & Papadakis, 2004; Hwang, Park, Nguyen, & Chen, 1997). Computer vision method has been approved as an optional technology for acquiring and analyzing an image to obtain information reflecting important product attributes (Brosnan & Sun, 2004; Gunasekaran, 1996; Cheng & Sun, 2004, Tan, Shatadal & Gerrard, 2000; Zion, Shklyar, & Karplus, 1999). A similar approach may be applied in thermal processing applications.

The specific objectives of this study were to: 1) establish a standard method that is not influenced by artifacts; 2) study the correlations among color values, chemical markers (M-2) yield and lethality (F_o); 3) validate this method in identifying the cold spot location in packaged foods processed with the microwave sterilization system by direct temperature measurement with fiber optics probe

The ultimate goal of this study was to develop an effective and reliable method for cold spot detection in support of an FDA approval process for microwave sterilization system and for future process development in industrial applications.

2. Materials and Methods

2.1. Sample Preparation

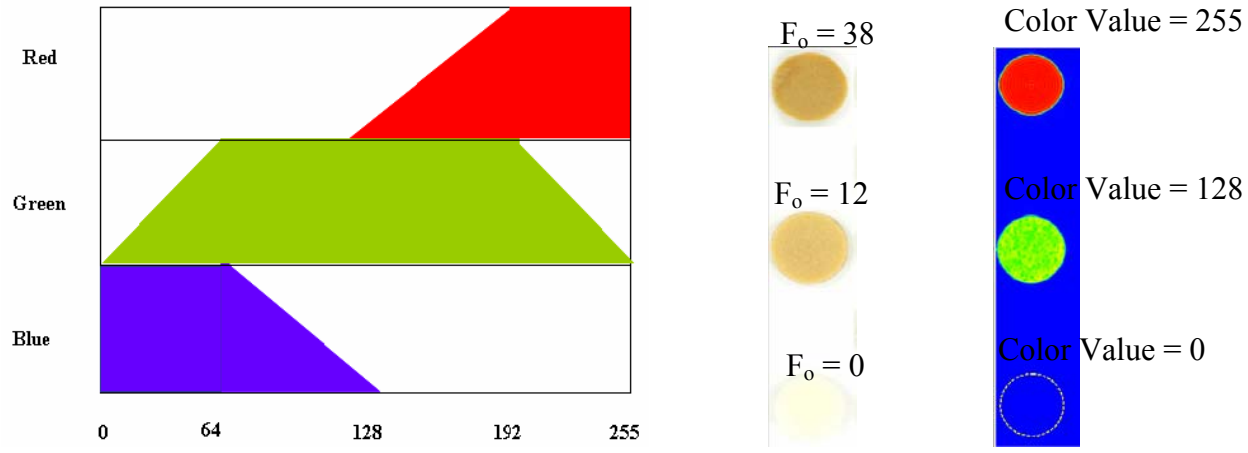
Mashed potato samples with 83.12% moisture content and 1.5% D-ribose were prepared similar as Pandit, Tang, Mikhaylenko, & Liu, 2006. Eight grams of mashed potato sample were placed and sealed into custom-built aluminum containers (diameter 3.5 cm × height 1.4 cm) with an air-tight lid for heating to elevated temperatures in oil baths. A type-T thermocouple was fitted into the lid of the box. The tip of the thermocouple was set to monitor sample temperature at the geometric center of the aluminum container during heating.

2.2. Color palette and development of a new scale

A set of RGB (red, green, blue) values defines the rainbow palette of the IMAQ (Image Acquisition) program (National Instrument, Austin, TX, USA) in which varying degree of red, green, and blue colors are mathematically combined to produce a color in

gray-level range. National Instrument IMAQ Vision Builder 6.1 (National Instrument, Austin, TX, USA) assigned color value equivalent to gray-level value 255 to the darkest pixels of the image while color value to a lightest pixel was not fixed.

Since the full scale for color values varies depending upon the range of color intensity distribution in an image, for comparative study it was necessary to fix the gray-level values to certain pixel intensity. This was done by selecting saturated and unheated mashed potato as upper and lower limits to fix the full scale. Saturated samples were obtained when limiting factor amino acids in mashed potato were consumed during heating process and the formation of chemical marker would reach a saturation point. Beyond this point further heating would not yield to significant chemical marker formation and color changes. The unheated mashed potato sample (marker M-2 yield = 0 mg / g sample) was used as the lowest point of the scale by setting color value to zero, and saturated mashed potato sample (F_o = 38 min; marker yield = 0.268 mg / g sample) was used as the upper most point of the scale by setting color value to 255. A third point in the middle with color value 127 ± 5 (F_o = 12 min) was set to improve the color resolution (Fig. 1).



a). Rainbow palette b). New developed scale

Fig. 1. Concept of converting the gray-level values to color values using pseudo color palette of IMAQ vision builder program and a method to fix the scale using mashed potato sample processed at different F_o.

To produce samples with different levels of marker formation, mashed potato samples were heated in air-tight aluminum containers with oil baths at 121°C to F_o = 12 min or until the chemical marker reached saturation. The *Look- up table* function of the IMAQ Vision Builder software version 6.1 was used to maintain the brightness of the scale-sample to minimize the variation in the color value equivalent to gray scale value during color analysis. The developed scale was also tested for several levels of cumulative lethality (F_o) to obtain the distinctive color value for each level of F_o. The gray-level value of the sample was transformed to a one dimensional color value using the color scheme of Fig. 1. F_o was calculated using the following formula:

$$F_o = \int_0^t 10^{\frac{T-121.1}{z}} dt \quad (1)$$

where, T is the sample temperature in °C at any time t during heating, z was taken as 10 °C for bacterial inactivation (Holdsworth, 1997). Value of z for chemical marker (M-2) formation in mashed potato was calculated at 32 °C using kinetic data (Pandit, Tang, Mikhaylenko, & Liu, 2006).

2.3. Computer vision system

The computer vision system consists of a light pod; helical compact fluorescent bulb; a digital camera with right-angle viewing attachment; automatic image acquisition software and computer vision software installed in a 1.6 GHz RAM Dell station (Fig. 2).

A Nikon D 70 (Nikon Instrument, Melville, NY, USA) digital camera with 18-70 mm DX Nikkor lens was fitted on top of the Paterson Light Pod (Paterson Photographic Inc., Douglassville, GA, USA). The CCD (Charge Coupled Device) camera could move vertically on the stand to adjust the magnification and its distance from the sample. Nikon Capture 4 Editor version 4.3.0 (Nikon Instrument, Melville, NY, USA) software was used to acquire and download the images to a Dell Workstation.

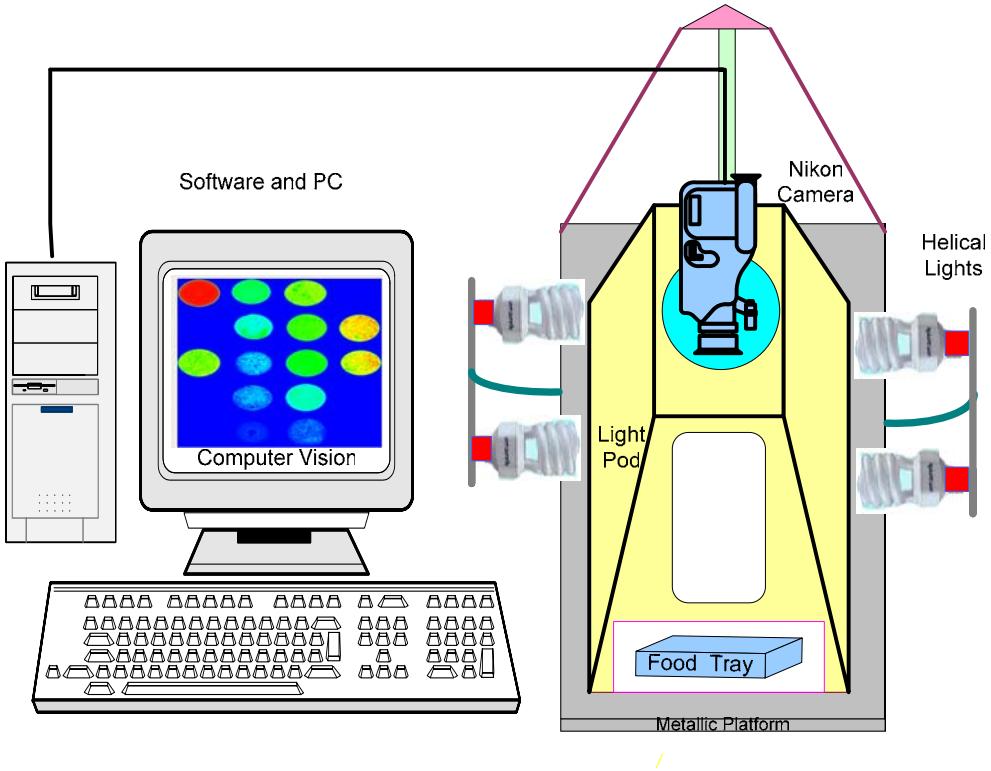


Fig. 1. Computer vision system designed in this study.

2.4. Effect of lights positions on diffuser box

In the computer vision system, lights were mounted outside the light pod to maintain an even luminosity inside the box**.** Four helical 26W (120VAC, 60Hz) bulbs (GE, Schenectady, NY, USA) were mounted on a stand at an angle of 45^{o} around a

Paterson light pod "Cocoon" style medium diffusion shooting tent (43 × 50 ×70 cm). The diffusion light pod was used to prevent the incident monochromatic light source to the object. The incident light intensity inside the diffuser box was measured using a Sekonic exposure meter, FLASHMATE L-308BII (Sekonic, Elmsford, NY, USA). High quality images were captured by matching the exposure meter readings, F (Aperture value = 30) and f/s (number of frame per second = 11), to Nikon D 70 digital camera through manual setting.

Computer vision analysis was performed to test the consistency in background for each image. To evaluate the effect of light positions on heating patterns, lights were mounted at top, middle and bottom positions of the light pod. Computer vision patterns for five samples heated to 110, 116, 121, 126 and 131 ^{o}C temperatures were compared at each position of lights to investigate the affect of light position. Images of the heated samples taken at each position were analyzed using IMAQ Vision Builder software to determine the RGB value equivalent to a gray-scale value.

2.5. Color value, M-2 yield and F_o relationship

2.5.1. Sample preparation and HPLC analysis

The activation energy of chemical marker formation in mashed potato E_a = 22.23 ± 1.54 kcal /mol is different from that of bacterial inactivation (E_a = 80 ± 10 kcal /mol). It was anticipated that time-temperature history for mashed potato samples may affect the chemical marker yield even for the same final F_o. Due to these variables two different pathways: 1) direct heating to a set temperature and 2) holding at 121 ^{o}C for different F_o were considered in this study. In the first set of tests, samples sealed in aluminum

containers were heated to 110, 116, 121, 126, and 131 ^{o}C temperatures (T) in an oil-bath to reach different levels of F_o. In another set of tests, oil-bath temperature was set at 121 ^{o}C and samples were held for a different period of time to cumulative lethality (F_o) of 1.5, 3, 6, 9, 12, 15 and 18 min. The samples were then rapidly cooled by immersing the aluminum containers into ice cubes. The purpose of fast cooling was to minimize additional cumulative lethality (F_o) after reaching the desired F_o. Each experiment condition was repeated twice. Temperature and F_o of the heated samples were monitored using data logging software MS Visual Basic 6 with measurement-computing software Active X, Omega IDRX thermocouple (Omega Engineering Inc., Stamford, CT, USA) isolator controls, and hardware with serial output mounted on a personal computer. Software was logging data at an interval of six seconds.

Chemical marker M-2 yield for both sets of samples were determined using the Agilent 1100 HPLC system (Agilent Technology, Palo Alto, CA , USA). Before the analyses, samples weighing between 0.20 g and 0.21 g were ground in 2 ml extraction buffer (10 mM sulphuric acid and 5 mM citric acid). Sample extraction and HPLC analysis procedures were the same as described in Pandit, Tang, Mikhaylenko, & Liu (2006). Additionally, chemical marker (M-2) yield during heating process was predicted from measured temperature using the mathematical equation (Lau, Tang, Taub, & Yang, 2003):

$$C(t) = C_\infty - (C_\infty - C_o) \times \exp\{\int_0^t -k_o \exp(-\frac{E_a}{R_o}[\frac{1}{T(t)} - \frac{1}{T_o}])\, dt\} \qquad (2)$$

where C (t) is marker yield at any time, C_∞ marker yield at saturation (0.268 mg/g sample), E_a is energy of activation (22.23 kcal mol^{-1} K^{-1}), R_o is molar gas constant (1.988 cal mol^{-1} K^{-1}), T(t) is recorded time-temperature history at the measured point, and T_o is

reference temperature (396.7 K). Initial marker yield before heating, $C_{o,}$ was considered to be zero for mashed potato samples with 1.5 % D-ribose. An experiment was also conducted to compare the M-2 yield of a sample taken from the middle point of the container and a sample taken by mixing the whole container (3.5 × 1.4 cm) for HPLC analysis.

2.5.2. Image Acquisition and Image editing using Adobe Photoshop

Nikon's Capture camera control tool was used for automatically acquiring and downloading the image. Sizes of images taken were 3008 × 2000 with 24 bit per pixel, and were saved in Joint Photographic Experts Group (JPEG) format.

Adobe Photoshop CS version 8.0 (Adobe Systems, San Jose, CA, USA) was used to insert scale images and images to be analyzed into one package. A 20 × 25 cm automatic picture package was divided into 5 × 4 layouts (Thomas, 2003). The first column of the layouts was reserved for the scale samples and other columns were used for images to be analyzed for heating patterns. Resolution of images in picture package was set to 500 pixels per inch.

2.5.3. Functions in computer vision script

A computer vision script was developed through interactive programming to determine the color patterns of heated samples. Developed script in IMAQ Vision Builder contains functions as shown in Fig. 3. Selection of those function tools were made to meet the desired output, as a result of the sequential mathematical computation over original pixels of an image. Main functions are described in details in the following:

(i) *Look-up Table*- Look-up Tables (LuT) was used to set the brightness of the scale samples. An image (I) in rectangular matrix was defined as (Thomas, 2003):

$$I = f[\, s_{in}\,(x, y)] \tag{3}$$

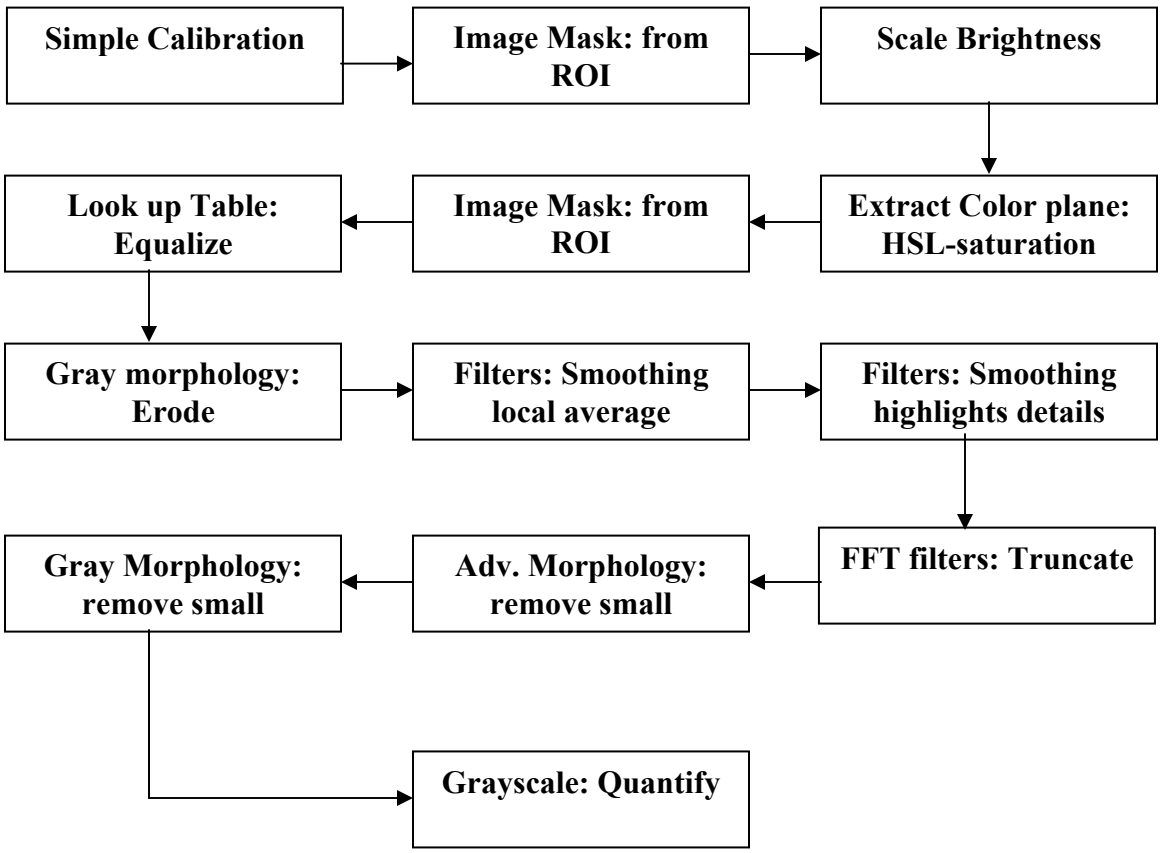


Fig. 3. Functions of the developed IMAQ Vision Builder script for heating pattern analysis.

where x is row index and y is column index. The original gray-scale values $s_{in}\,(x, y)$ can be assigned any value out of the gray-scale set g = {0, 1,……., 255} for an 8-bit image. Resulting set of output values $s_{out}\,(x, y)$ using LuT would be:

$$s_{out}\,(x, y) = f[\, s_{in}\,(x, y)] \tag{4}$$

For each value of g, function f (g) will have 256 possible values in a look-up table, therefore:

$$\text{LuT (g)} = f\text{(g)} \tag{5}$$

In that case, the computed output of the LuT function becomes:

$$s_{out}(x, y) = LuT\,(\,s_{in}(x, y)) \tag{6}$$

(ii) *Extract Color Planes: HSL*- IMAQ Vision Builder provides a pre-defined unique feature based on this concept to represent the gray-level value of a pixel into corresponding one dimensional color value comprised of varying amounts of red, green, and blue (Fig. 1)**.** Color plane HSL (Hue, Saturation, and Luminance) saturation was extracted from each image to adjust the lowest gray-level value to the lightest pixel and the highest gray-level value to the darkest pixel of an image. The coordinate system for HSL color space is cylindrical. The hue (H) value runs from 0 to 360^{o}, the saturation (S) ranges from 0 to 1, and luminance (L) also ranges from 0 to 1, where 0 is black and 1 is white. Following equations describe the nonlinear transformation that maps the RGB color space to the HSL color space:

$$\text{L} = 0.3 \times \text{R} + 0.59 \times \text{G} + 0.11 \times \text{B} \tag{7}$$

$$\text{V2} = \sqrt{3} \times (\text{G} - \text{B}) \tag{8}$$

$$\text{V1} = 2 \times \text{R} - \text{G} - \text{B} \tag{9}$$

$$\text{H} = 256 \times tan^{-1}\left(\frac{V2}{V1}\right)/(2\times\Pi) \tag{10}$$

$$\text{S} = 255 \times \left(\ 1 - \frac{3\times min(R,G,B)}{(R+G+B)}\ \right) \tag{11}$$

(iii) *Gray Morphology: Erosion and Dilation*- These two functions are fundamentals for almost all morphological operations. Dilation increases the brightness of pixels surrounded by proximate pixels with a higher intensity, while erosion is a function that basically reduces brightness of each pixel that is surrounded by proximate pixels with a lower intensity. In erosion, the value of output pixels is set to the minimum of coefficients $s_{in}(x, y)$ as (Davies, 1997; Thomas, 2003):

$$s_{out}(x, y) = min.\ (s_{in}(x, y)) \tag{12}$$

while in dilations, output of the pixels is set to the maximum value of coefficients $s_{in}(x, y)$ as:

$$s_{out}(x, y) = max.\ (s_{in}(x, y)) \tag{13}$$

(iv) *Filters: Smoothing Local Average*- Averaging of the brightness intensity of a pixel was performed by taking the weighted average of the proximate pixels. The output image in that case would be expressed as (Acharya & Ray, 2005; Ritter & Wilson, 2000):

$$g(m, n) = \sum\sum a(k,1)\ f(m-k, n-1), \qquad (k,1) \in W \tag{14}$$

where $f(m, n)$ and $g(m, n)$ are the input and output images respectively, W is the neighborhood of the pixel at location (m, n), and $a(k, 1)$ are the filter weights assigned. All weights were assigned equal values in this study; and therefore equation (14) was reduced to:

$$g(m, n) = \frac{1}{N}\sum\sum\ f(m-k, n-1), \qquad (k,1) \in W \tag{15}$$

where N is the number of pixels in the proximate of W. The purpose of spatial averaging operation on an image was to smooth the noise. In case of an observed image, defined as:

$$g(m, n) = f(m, n) + \eta(m, n) \tag{16}$$

the spatial average of the image was calculated as:

$$g(m, n) = \frac{1}{N}\sum\sum f(m-k, n-1) + \bar{\eta}(m, n), \qquad (k, 1) \in W \tag{17}$$

where $\bar{\eta}(m, n)$ was an average of the noise component $\eta\ (m, n)$ in the spatial domain of the image.

(v) *Filters: Smoothing Highlight Details*- Filtering improved the quality of the image by calculating the new pixel value by using the original pixel value and those of its proximities. Mathematical computation on each pixel was performed by using the equation (Gerhard & Joseph, 1996; Thomas, 2003):

$$s_{out}(x, y) = \frac{1}{m^2}\sum_{u=0}^{m-1}\sum_{v=0}^{m-1} s_{in}(x + k - u,\ y + k - v)\ .\ f(u, v) \tag{18}$$

The output pixel values $s_{out}(x, y)$ depends on the size of kernel matrix ($m \times m$). IMAQ has three predefined kernel matrices of size m = 3, 5 and 7. Under this study, most of the calculations were done with m = 3 and parameter k was defined as:

$$k = \frac{(m-1)}{2} \tag{19}$$

Indices u, and v depend on x and y with k in terms of filter kernel function (Gerhard and Joseph 1996). In case of kernel size 3 × 3, all nine neighboring pixels were represented as:

$$\mathbf{F} = (f(u, v)) = \begin{pmatrix} f(0,0) & f(0,1) & f(0,2) \\ f(1,0) & f(1,1) & f(1,2) \\ f(2,0) & f(2,1) & f(2,2) \end{pmatrix} \tag{20}$$

Using indices x and y equation (20) can be elaborated as:

$$\mathbf{F} = \begin{pmatrix} f(x-1, y-1) & f(x, y-1) & f(x+1, y-1) \\ f(x-1, y) & f(x, y) & f(x+1, y) \\ f(x-1, y+1) & f(x, y+1) & f(x+1, y+1) \end{pmatrix} \tag{21}$$

this includes a pixel (x, y) with its eight proximities pixels.

(vi) *Fast Fourier Transforms (FFT: Low pass truncation)* - The 2D *Fourier Transforms* transforms a spatial function *f(x, y)* of an image into frequency domain *F(u, v), w*hich in continuous domain, was defined as [Thomas, 2003; Shapiro, & Stockman, 2001):

$$F(u, v) = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} f(x,y)e^{-j2\pi(xu+yv)}dxdy \tag{22}$$

The exponential function was expressed using Euler's identity as:

$$\exp(-j2\pi(xu + yv)) = \cos(2\pi(xu + yv)) - j\ \sin(2\pi(xu + yv)) \tag{23}$$

where *f(x, y)* was the light intensity of the points *(x, y),* and *u, v* were the horizontal and vertical spatial frequencies. Equation (22) implies that the function *f(x, y)* is essentially multiplied by the terms $\cos(2\pi ux)$ cos $(2\pi vy)$, $\sin(2\pi ux)$ $\sin(2\pi vy)$, $\sin(2\pi ux)$ $\cos(2\pi vy)$, and $\cos(2\pi ux)$ $\sin(2\pi vy)$. In case of a symmetric function, along both the X and Y axes, Fourier transform of *f(x, y)* involves the multiplication of cos $(2\pi ux)$ cos $(2\pi vy)$ term only. But in this study, the *f(x, y)* multiplication involved all four terms due to asymmetric images. Inversely *Fast Fourier Transformed F (u, v)* can be transformed back into a spatial image *f(x, y)* of resolution N × M:

$$f(x, y) = \sum_{u=0}^{N-1}\sum_{v=0}^{M-1} F(u,v)\ e^{\,j2\pi(\frac{ux}{N}-\frac{vy}{M})} \tag{24}$$

where *F (u, v)* consists of an infinite sum of sine and cosine terms, which are determined by the corresponding frequency. For the given set of *u* and *v* all values of *f(x, y)* contribute to *F (u, v)*. The FFT computation on the image before quantifying the RGB values took between 2-10 minutes depending upon the size of the image to be analyzed for cold spot detection.

2.6. Computer vision heating patterns for food samples using IMAQ Vision Builder

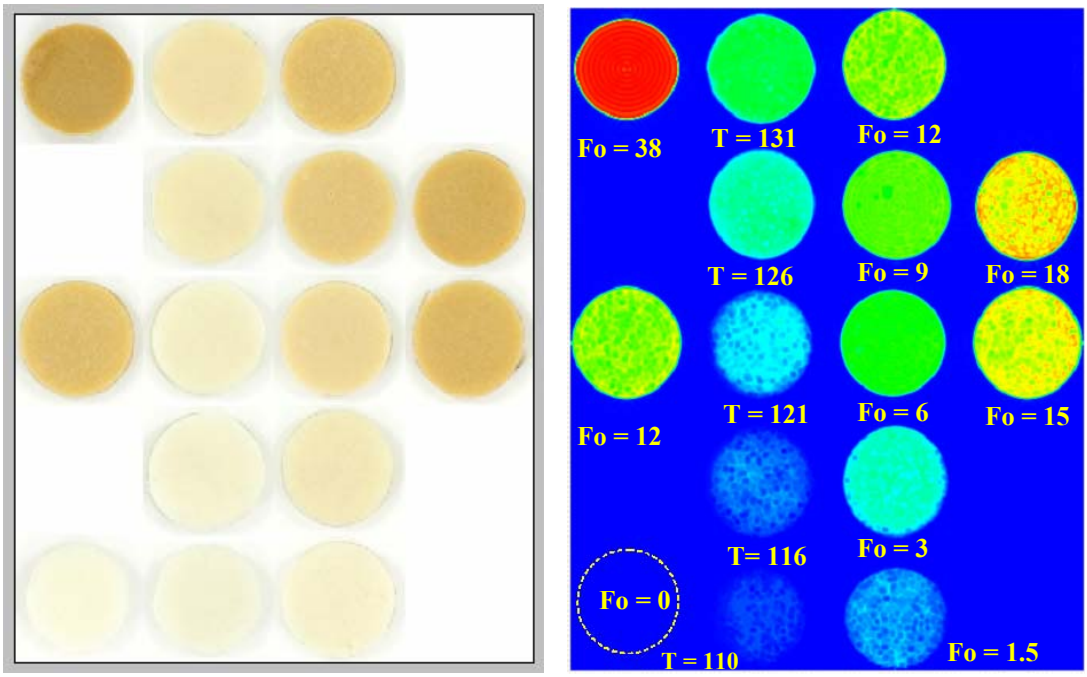
A picture package including images of mashed potato samples in Adobe Photoshop was analyzed using IMAQ Vision Builder program. Brightness of the scale samples were fixed using look-up table, and regions of interest (ROI) were selected using image mask. A developed function script (Fig. 3) was run to determine the heating patterns. Forty (8 × 5) rectangular grids were generated on the heating patterns of each tray. The color values of the grids for each tray were directly extracted to Microsoft Office program Excel (MS Office-2003, USA). Similar steps were followed to collect color values from other images of the package. Using MS Excel, a grid with lowest color value was selected among all of the grids and detected as the cold spot region of the microwave sterilization process.

3. Results and Discussion

3.1. Computer vision color patterns

Colors of heated samples were analyzed by referencing the developed scale in each picture package using Adobe Photoshop. Computer vision showed different color as a result of different M-2 yield at each level of F_o (Fig. 4). HPLC analysis revealed that the sample held at 121 ^oC for F_o = 6 min had much higher chemical marker yield (0.089 mg/g) than a sample directly heated to 126 ^oC (0.029 mg/g) for a similar level of F_o (Table 1). Due to a short heating duration and higher temperature than 121 ^oC, directing heating to 126, 131 ^oC temperatures leads to higher F_o in the tested samples, while chemical marker yields were all comparatively lower. This is because of the earlier stated

difference between the activation energy for M-2 formation and that for thermal inactivation of *C. botulinum* spores used in F_o calculation.

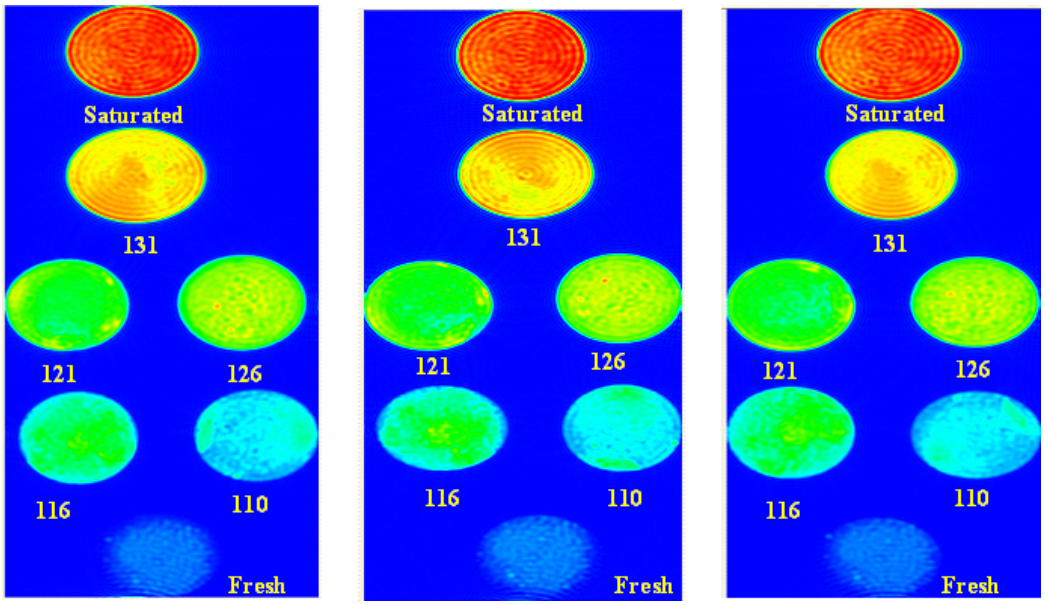


a). Original sample b). Computer vision patterns

Fig. 4. Computer vision patterns for the mashed potato samples heated to a set temperature (T) or held to 121 ^oC for different F_o, results were tested in two replicates.

It is clear that correlation between M-2 formation and F_o are dependent of temperature pathway which will be discussed in details.

Our test results also showed that positions of lights around the diffuser box had no effect on the color images captured by the computer vision system (Fig. 5). A separate study was also conducted to compare the color values of the sample analyzed right after heating and samples stored maintaining a protocol (storage protocol: 1 hour at -35^oC, 12 hours at 5 ^oC, and 1 hour again -35^oC). This study showed no significant difference (P-value> 0.98) in color value for both set of samples.



a). Bottom b). Middle c). Top

Fig. 5. Comparison of computer vision color patterns with mashed potato samples heated to different temperature levels for three positions (bottom, middle and top) of lights. Number denotes the set temperature to which sample was heated.

3.2. Color value equivalent to gray-level value and M-2 yield

Gray-scale quantification tool was used to obtain the color value equivalent to a gray-level value for each sample. A representative color value along with the standard deviation of selected ROI (Region of Interest) was obtained using IMAQ Vision Builder. Our tests showed that chemical marker yield of the sub-sample taken at center of the sample and that of the mixed whole sample in the same container was not significantly different (P-value = 0.985) (SAS, Institutes Inc., Cary, NC, USA). To expedite the extraction procedure, a sub-sample from the middle section of the treated sample was taken at each level of F_o for determination of the M-2 yield using HPLC. M-2 yield of analyzed samples were positively correlated with the cumulative thermal lethality F_o (Fig.

6). M-2 yield of the samples were also correlated with imagine parameter, color value, to establish a relationship (Table -1). Results showed a unique positive correlation between M-2 yield and color values (Fig. 7) regardless of heating pathways. This indicates that computer visions based on the color value equivalent to gray scale can uniquely reflect M-2 yields in thermal processes.

3.3. Color value equivalent to gray-level value and F_o

The color values measured for each sample using IMAQ Vision Builder were plotted against F_o values. Two different positively correlated trends, one for samples heated by holding at 121 ^oC and another for samples directly heated to a set temperature (ramp up) were obtained (Fig. 8). For each different heating pathway, plotted result showed that each level of F_o will lead to unique M-2 yield and color value (Fig. 6, Fig. 8). These relationships between M-2 yield vs. F_o, and color value vs. F_o are unique for a given condition of heating. Based on these relationships, by referring a scale, color value can be used as a representative for the thermal lethality (F_o) and chemical marker (M-2) yield.

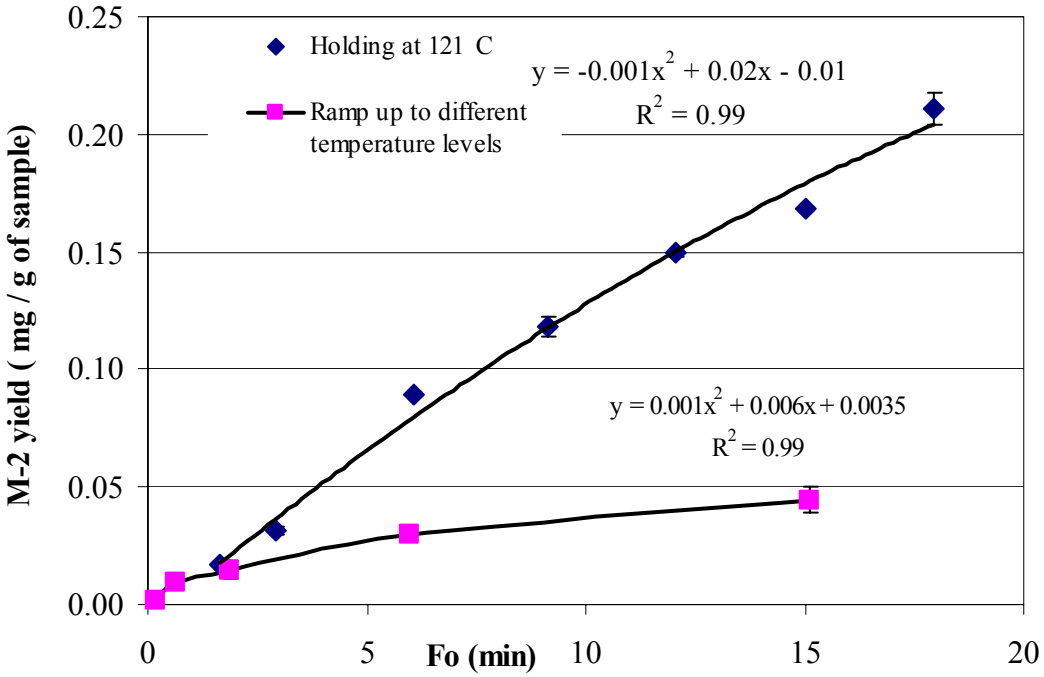


Fig. 6. M-2 yield and F_o correlation with mashed potato samples heated to different set temperature (ramp up) levels or held at 121 ^oC for different F_o, data points are based on two replicates.

Table 1. Color values equivalent to gray-scale values and chemical marker M-2 yield for two different heating conditions, each point represents mean of two replicates.

Data Collected for	Temperature (^oC) or target F_o (min)	F_o (min)	M-2 yield (mg / g of sample)	Color value equivalent to Gray-level
Ramp up to temperature levels	110.00	0.15 ± 0.00	0.005 ± 0.004	10.17 ± 2.48
	116.00	0.53 ± 0.15	0.013 ± 0.005	29.70 ± 8.65
	121.00	1.85 ± 0.03	0.021 ± 0.008	47.78 ± 7.71
	126.00	6.17 ± 0.28	0.029 ± 0.001	84.69 ± 2.16
	131.00	17.81 ± 3.83	0.053 ± 0.011	108.78 ± 3.13
Holding at 121 ^oC for F_o	1.50	1.57 ± 0.09	0.016 ± 0.001	29.08 ± 9.55
	3.00	2.98 ± 0.10	0.034 ± 0.004	78.70 ± 3.26
	6.00	6.08 ± 0.01	0.089 ± 0.001	125.84 ± 2.28
	9.00	9.05 ± 0.08	0.124 ± 0.008	143.54 ± 5.25
	12.00	12.02 ± 0.04	0.152 ± 0.003	158.01 ± 0.04
	15.00	15.05 ± 0.04	0.167 ± 0.002	184.68 ± 3.57
	18.00	17.98 ± 0.04	0.201 ± 0.014	196.05 ± 2.28

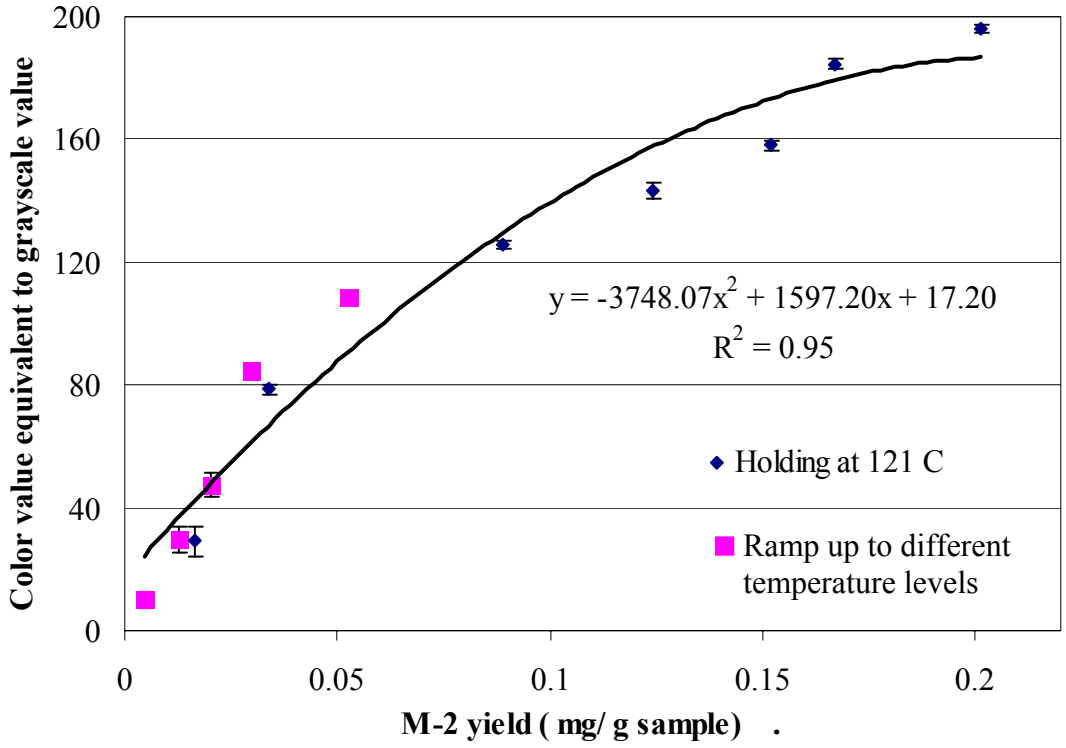


Fig. 7. Color value and M-2 yield relationship with mashed potato samples heated to different set temperatures (ramp up) levels or held at 121 ^{o}C for different F_o, data points are based on two replicates.

4. Validation of locations specified by computer vision

In order to validate the accuracy of the cold and the hot spot locations determined by the computer vision method, experiments were conducted in a 915 MHz single-mode pilot-scale microwave sterilization system in two replicates. Thermo wells that separate sealed sample from fiber-optic sensors were fitted into the tray at the measured distance of cold and hot spots locations. Each tray was filled with 200g of mashed potato sample mixed with 1.5% D-ribose and then vacuum-sealed at 18 in. of Hg vacuum. Trays were set on an Ultum support and a proper speed-time combination was chosen to move the tray support from the loading section to the holding section through two single-mode

microwave cavities. Water at 125 °C was circulated across the tray support inside the pressurized microwave cavities at a flow rate of 35 liter per minute. Fiber optic temperature sensors inserted into the thermo wells measured temperature at the specified cold and hot spot

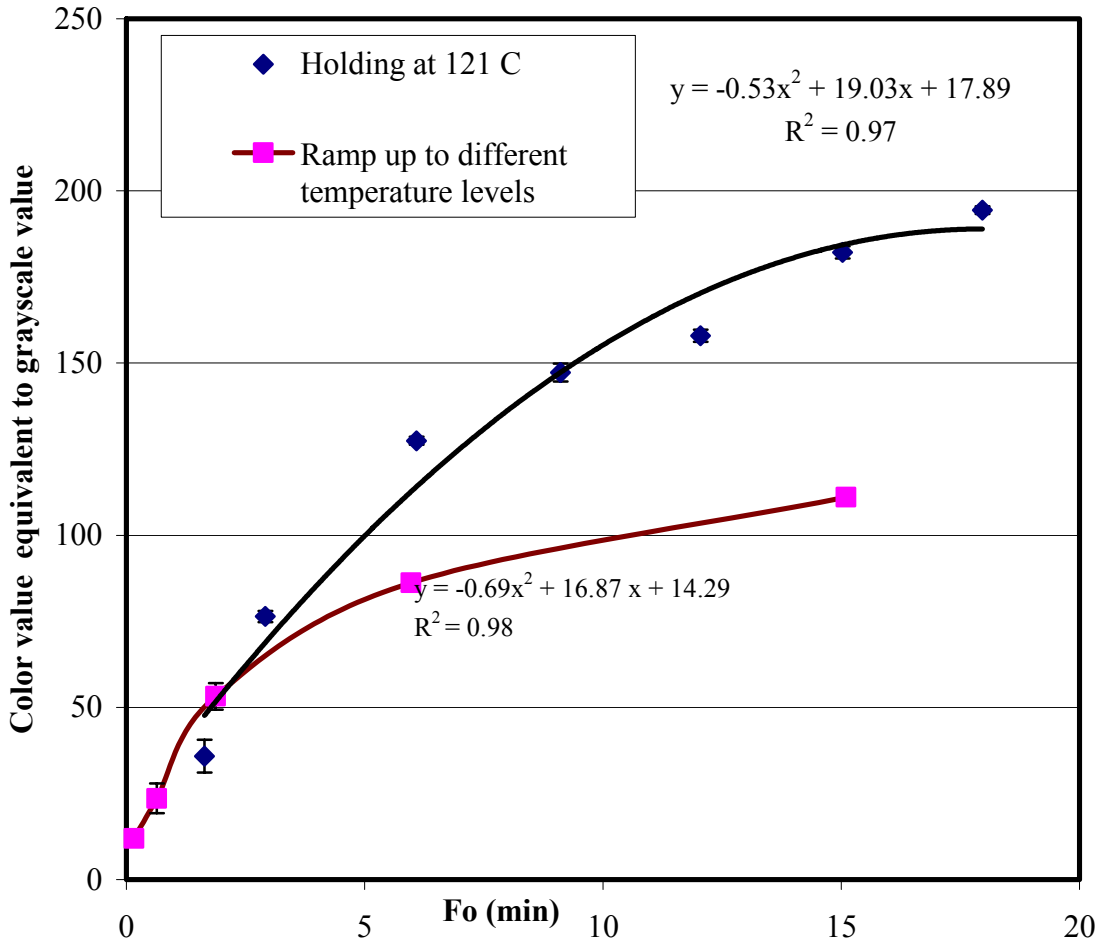


Fig. 8. Color value and F_o relationship with mashed potato samples heated to different set temperatures (ramp up) levels or held at 121 °C for different F_o, data points are based on two replicates.

locations identified by computer vision method. The experiments were conducted at a 2.67 kW microwave power level. The measured temperature confirmed that the

temperature measured at the perceived hot spot was indeed always higher than the cold spot for all tests, as shown by a representative curve in Fig. 9.

To further confirm the locations of the cold spots relative to other parts of the tray, additional 13 tests were conducted. In each of the tests, four optic sensors were

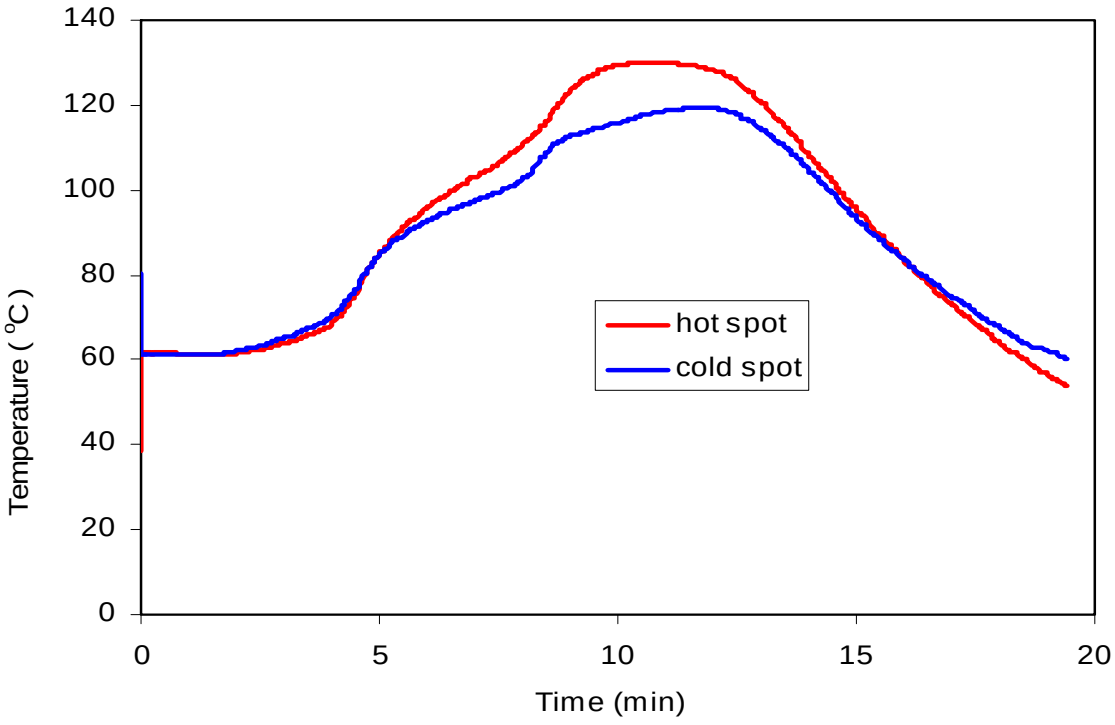
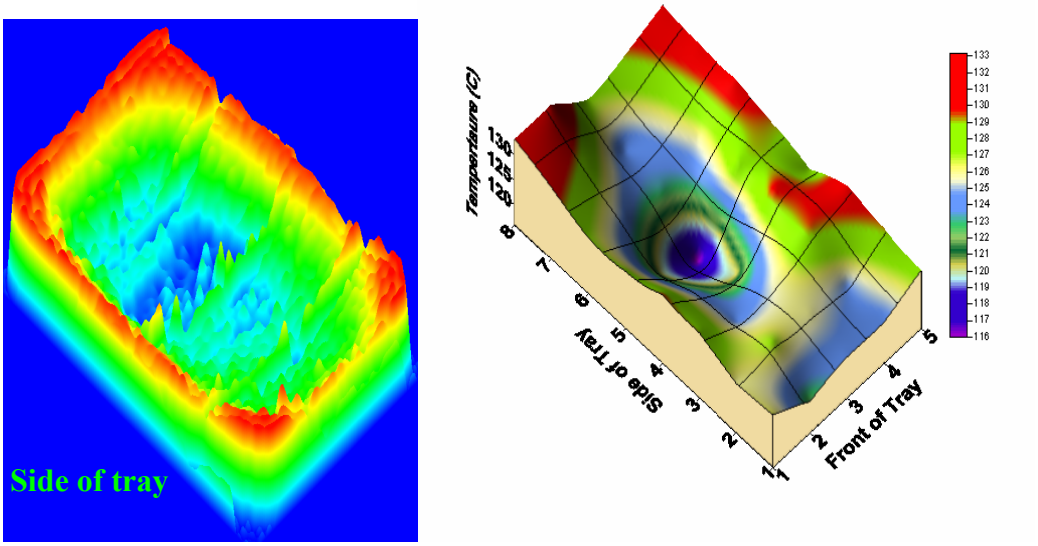


Fig. 9. Validation of cold and hot spots locations specified by computer vision method in 10 oz trays during microwave sterilization at 2.67 kW power level, typical temperature profile from repeated tests in the middle layer of the tray.

placed in a sample tray during the microwave sterilization process. One of the sensors was always inserted at the cold spot identified by the computer vision method while the others placed in three different locations. Compiling all the measured temperatures from the 13 tests, provided temperature profile for a total of 40 different points (8 × 5) evenly distributed in the middle layer of a tray. Computer vision patterns and temperature

mapping, for middle layers, obtained using fiber optics are compared in Fig. 10. It shows that heating pattern and cold spot location obtained by both the methods were concordant. This indicates that the novel computer vision method indeed reliably reveal the cold spots in foods and can be used to study general heating patterns in foods after microwave sterilization processes.



(a) Heating pattern by developed computer by vision method.

(b) Temperature distribution measured by fiber optic probes.

Fig. 10. Matching of the experimental and developed method heating patterns for the middle layer of a 10 oz tray with mashed potato processed at 2.67 kW microwave power level.

5. Conclusions

The designed computer vision system provided consistent background for the images. The developed scale can be used to compare the heating patterns of microwave-sterilized foods for combinations of power levels and F_o. Shooting tent worked well as an

effective diffuser, and positions of lights for a fixed setting of exposure intensity had no influence on the heating patterns.

Color value equivalent to gray scale value was positively correlated with chemical marker yield and cumulative thermal lethality (F_o). For a given F_o, chemical marker (M-2) yield of the samples heated directly to 126, 131 ^oC temperatures were lower than holding the sample at 121^oC. Separate pathway provides different correlation between M-2 yield and F_o. But correlation between color values and M-2 yield are independent of heating pathway. Based on these relationships, a computer vision method was developed to identify the cold and the hot spot regions of a processed food sample. Validation tests confirmed that the computer vision method based on chemical marker M-2 yield can accurately determine the location of cold spots. The developed knowledge base will support application of this method for evaluation of microwave sterilization processes.

Currently this method is being used to locate the cold spots in pink salmon fillets in an Alfredo sauce to develop filing documents for FDA approval of the 915 MHz microwave sterilization system.

Acknowledgments

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Nomenclature

a(k, l)	filters weight
B	blue color value
C	marker yield (mg/ g of sample)
C_o	initial chemical marker yield (mg/ g of sample)
C_∞	chemical marker yield at saturation (mg/ g of sample)
E_a	activation energy (kcal mol^{-1})
F_o	cumulative thermal lethality (min)
F	aperture value
f(x, y)	light intensity of the points (x, y)
F(u, v)	frequency domain of an image
FFT	Fast Fourier's Transforms
g	gray level values
G	green color value
H	hue
L	luminance
k_o	reaction constant at reference temperature
M-2	chemical marker M-2
m	size of matrix array
N	number of pixels
R_o	universal gas constant at reference temperature (cal/mol K)
R	red color value
RGB	red, green, and blue color value

S	saturation
$s_{in}(x, y)$	original gray-scale values
$s_{out}(x, y)$	output pixel values
t	time (min)
T	temperature (^{o}C)
T(t)	temperature (K)
T_o	reference temperature
u	horizontal spatial frequency
v	vertical spatial frequency
W	neighborhood around the pixel

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CHAPTER 6

PRINCIPLE AND APPLICATION OF CHEMICAL MARKER (M-2) BASED COMPUTER VISION METHOD TO LOCATE THE COLD SPOTS IN REAL FOOD SYSTEMS

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Abstract

This paper investigates and develops a protocol for determining the location of cold spots for microwave sterilization process for pink salmon fillets with Alfredo sauce (2:1) using computer vision method to assist the development of novel microwave sterilization processes. Whey protein gel was selected as the model food to emulate the heating patterns in a real food system, salmon fillet. Dielectric property of the whey protein gel was adjusted by adding salt to match that of pink salmon fillet. Kinetic studies was performed to establish relationship among cumulative lethality (F_o), chemical marker (M-2) concentration, and color value in terms of gray-scale value in whey protein gels containing ribose as a pre-cursor for M-2 formation through Mallard reaction during thermal processing. Heating patterns of slab shaped whey protein gel processed in a pilot-scale 915 MHz microwave (MW) sterilization system were analyzed using a computer imaging system. The region with lowest color value was identified as the cold spot location. Two other locations having color value closer to the cold spot were selected for validation using fiber optic temperature sensors in the pilot scale microwave sterilization system. Time-temperature profiles for the three selected points, including the cold spot,

in whey protein gel slabs processed with added sauce were found repeatable during microwave sterilization. Time-temperature profiles for the points selected in the whey protein gel also matched with that in salmon fillet processed in the same sauce. Results showed that the cold spot location identified in whey protein gels was indeed the cold spot in the salmon fillet. This study demonstrated the potential of using computer vision for chemical marker M-2 formed in specially formulated whey protein gels to locate the cold spots in real food systems in microwave sterilization processes.

Keywords: Computer vision method; microwave heating; salmon fillet; dielectric properties; cold spot; whey protein gel

1. Introduction

Computer vision is increasingly used by the food industry for various application, including food quality inspection, process monitoring and classification system because it provides an alternative as automatic and cost-effective technique (Steenhoek, Misra, Hurburgh, & Ben, 2001; Li, Wang & Gu, 2002; Du & Sun, 2004; Tan, 2004; Kumar, Ganjyal, Jones & Hanna, 2006). For similar benefits, a computer vision method based on the yield of chemical marker M-2 was developed in our laboratory to locate the cold spots in microwave sterilization processes. After successful development of a computer vision method to locate the cold spot in homogeneous model food (Pandit, Tang, Liu & Pitts, 2007), our next step was to develop a methodology to determine the cold spot in real food systems. In order to develop microwave sterilization processes for industrial application, determining the cold spots in heterogeneous food system was a major challenge (Wang, Lau, Tang, & Mao, 2004; Yam & Papadakis, 2004; Dworkin & Nye,

2006). To apply this method to real food systems, we decided to provide a complete protocol to determine the cold spot locations.

Several physical and chemical changes occur in food during thermal processing. Due to the chemical reactions, color of the heterogeneous food components changes in sterilization operations (Godereaux, Goullieux, & Kint, 2003; Kim & Ball, 1994; Ross, 1993). Size shrinkage and syneresis can also be noticed with several food systems during sterilization process. The deep pink fillets of salmon became white with shrinkage in shape after processing. Because of these constraints we decided to use a generic model food system with predictable thermal sensitivity color change characteristic to assist identification of the cold spots in real foods. It is desirable that the extent of color development in the model food system is positively correlated to the thermal lethality received in the heated food systems when exposed to the same heat treatment (Kim & Taub, 1993; Wnorowski & Yaylayan, 2001).

A model food system which can be shaped to simulate real food geometry and yield chemical marker proportional to the degree of thermal treatment would be the best choice for this study. Two model food system mashed potato and whey protein gel with the addition of D-ribose (Sigma-Aldrich), have been used in our laboratory for computer vision analysis of heating patterns (Lau et al, 2003; Wang et al, 2004 and Pandit, Tang, Mikhaylenko, & Liu, 2006). Salmon in Alfredo sauce was selected as example of real food system to document the microwave processing for FDA approval. Whey protein gel was chosen as a model food for salmon since there is no diffusion of whey-sauce phase as would took place with the mashed potato. The next step for finding the heating pattern of the product was to find the composition of whey protein that would resemble dielectric

behavior of salmon fillet. Adjustment of dielectric properties of whey protein gel was accomplished by additional salt. A proper composition of salt was found by matching the dielectric constant and dielectric loss of the whey protein gel and salmon.

Whey protein concentrate (Alacen 878, Fonterra, New Zealand) was used to form whey protein gels. It contains about 73% protein on dry basis. Other constitutes of the whey protein concentrate were as follows: moisture 5.5 %, carbohydrates 12.89%, ash 5%, fat 3.72 % and Sodium 1546mg/100g. Due to high amount of amino acids, several levels of D-ribose (Sigma, St. Louis, MO) were tested to find suitable proportions to cover the time temperature range of microwave sterilization. Chemical marker formation and bacterial inactivation have different z and D-values (Prescott, Harley, & Klein, 2002). These refers that temperature changes have different influence on the marker formation and bacterial inactivation. Because of these reasons, we tested the influence of path of heating on the relationship of chemical marker formation and bacterial inactivation. Before applying this method to real food system, we studied how formation of chemical marker (M-2), bacterial inactivation parameter (F_o) and color value equivalent to gray-scale value are correlated.

After matching the dielectric properties of the whey protein gel with salmon fillet it was desirable to compare the time-temperature profiles of the cold spot locations in both food systems during microwave sterilization. In order to accomplish this study to provide sufficient support for predicting heating patterns in the real foods, following specific objectives were considered: 1) Matching the dielectric properties of the model food and real food, 2) Establishing relationships among M-2 formation, cumulative lethality (F_o)

and color value equivalent gray scale value and 3) Comparing the time-temperature profiles for model and real food systems during microwave sterilization.

This study will provide basis for application of the developed computer vision method to emulate the heating patterns and the cold spot with real food systems.

2. Principle and application of chemical marker (M-2) to determine the heating patterns

Chemical marker M-2 (*4-hydroxy-5-methyl-3(2H)-furanone*) was developed at the United States Army Natick Research Center (Kim & Taub, 1993; Prakash, Kim, & Taub, 1997). It is the product of non-enzymatic browning reaction (Maillard reaction) between D-ribose and amines. Rate of chemical marker M-2 formation during heating was found suitable for monitoring high temperature short time processes (Lau et al, 2003, Pandit, et al., 2006). The concept of using chemical marker for monitoring sterilization process is based on the fact that measured concentration of the thermally generated brown color (marker M-2) serves as a time-temperature integrator. The concentration of the brown color formed in food during sterilization operation followed a 1st order reaction (Lau, et al, 2003) is good indicator of the absorbed thermal energy and sterility to *Clostridium botulinum* (Wnorowski & Yaylayan, 2001).

Pandit, Tang, Mikhaylenko & Liu (2006) and Lau et al., (2003) established a first order reaction for chemical maker M-2 formation in mashed potato and whey protein gel. The concentration of M-2 yield during a thermal process is related to processing time as:

$$C(t) = C_\infty - (C_\infty - C_o) \times \exp\{\int_0^t -k_o \exp(-\frac{E_a}{R}[\frac{1}{T(t)} - \frac{1}{T_o}])\, dt\} \qquad (1)$$

where C (t) is M-2 marker yield at any time, C_∞ marker yield at saturation, E_a is activation energy, R molar gas constant, T (t) is recorded temperature-time history at the measured point, and k_o is the reaction rate constant at reference temperature (T_o). Using the kinetics parameters obtained in Pandit, Tang, Mikhaylenko & Liu (2006) for mashed potato, the predicted yield of chemical marker (M-2) was compared with measured yield in mashed potatoes processed in aluminum container after heating in oil bath during ramp up and holding processes. Comparison showed (Fig. 1) that equation (1) can be used to predict the chemical marker yield during microwave sterilization process. But direct compare of Predictions of chemical marker yield for multiple points were impossible because it requires the time-temperature history of all the points.

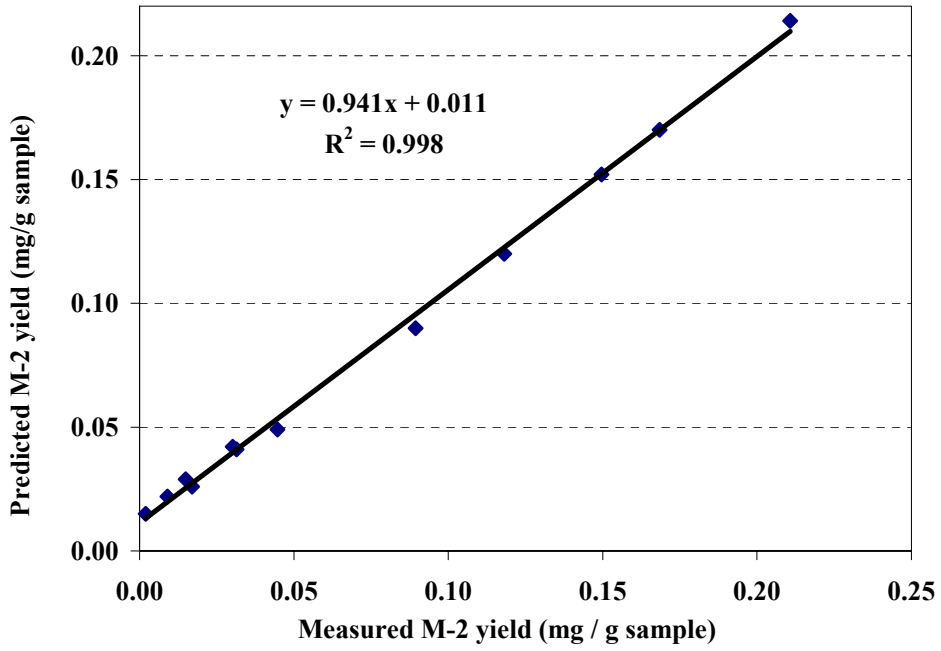

Fig. 1. Comparison of measured and predicted chemical marker yield based upon the kinetics parameters.

Direct measurement, using HPLC and spectrophotometer, of chemical marker M-2 yields C*(t)* at adequate points in microwave sterilized model foods to allow accurate identification of the cold spot is time-consuming and impractical. In addition, reliable determination requires multiple experiments. Hence, there was a need for rapid and accurate method to evaluate the sterilization operation. A computer vision method was developed to capture the color images of processed model foods. The acquired images were in three–dimensional RGB color space, which requires a large storage memory (18.04 Mb) for multiple trays with high resolution. In 3D-RGB color space, each pixel (commonly known as *bits per pixel*) is usually apportioned with 8 bits each for red, green and blue, giving a range of 256 possible values, or intensities for each hue. With this (non-optimal) system, 16,777,216 (256^3 or 2^{24}) discrete combinations of hue and intensity can be specified. This in practice is much larger than gamut of color that can be reproduced. It is claimed that the human eye can distinguish as many as 10 million discrete hues (Cowlishaw, 1985). This number varies from person to person depending upon the condition of the eye and the age of the individual. However, at the resolution of latest computer screens (1280 × 800 pixels) and at a standard viewing distance people cannot distinguish more than a few hundred hues (Cowlishaw, 1985). For these reasons, it was chosen to use grayscale to represent the brown color distribution. Equation (2) was used in our study to convert RGB image into a grayscale image on a pixel- by- pixel basis (IMAQ Vision Builder, 2000):

$$grayscale\ value = 0.30\,R + 0.59\,G + 0.11\,B \qquad (2)$$

This above equation is used in the National Television System Committee (NTSC) standard for luminance. An alternative conversion from RGB to grayscale is a simple average:

$$grayscale\ value = \frac{(R+G+B)}{3} \tag{3}$$

In mathematical computation on pixel, a grayscale digital image is an image in which the value of each pixel is a single sample. Grayscale images intended for visual display are typically stored with 8 bits per sampled pixel (2^8 = 256 colors), which allows 256 intensities (i.e., shades of gray) to be recorded. The accuracy provided by this format is sufficient to avoid visible banding artifacts, and very convenient for programming. To maintain a better appearance on the relatively low color depth, 8-bit color, the stored value was indexed into a color map or palette. The colors available in the palette itself may be fixed to the pseudo-color derived from a grayscale image by mapping each pixel value to a color according to a table or function (Fig. 2).

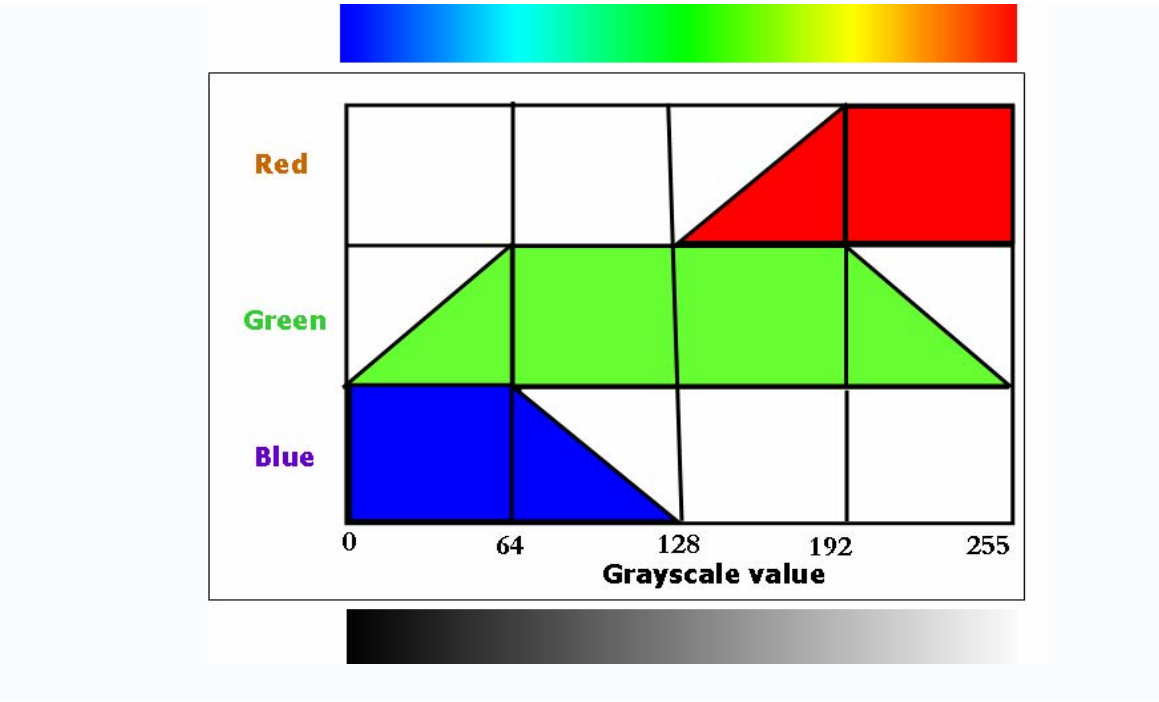

Fig. 2 .A scheme to convert the grayscale image into pseudo color image (IMAQ Vision Builder, 2000).

Although pseudo-coloring does not increase the information contents of the original image, it can make details more visible, by increasing the distance in color space between successive gray levels. This convention of representing the color value in the range 0 to 255 is widespread makes it convenient to store each color value in one 8-bit byte(Cowlishaw, 1985). In our method development, the fresh sample was assigned to grayscale value 0 and fully saturated sample to grayscale value 255 (Pandit, Tang, Liu & Pitts, 2007). This implies that a region with lowest color value corresponds to the least amount of the brown color formed during thermal treatment of the food samples. Based on these concepts we developed a computer vision method to determine the location of the cold spot (Pandit, Tang, Liu & Pitts, 2007).

3. Materials and methods

3.1. Selection of the model food system

Whey protein was selected in this study because it has specific amino acids (methionine, lysine, histidine, and arginine) needed for chemical marker (M-2) formation (Lau et al, 2003; Wang et al, 2004 and Pandit, Tang, Mikhaylenko, & Liu, 2006). Whey protein can easily disperse in water and form firm gel. Whey protein dispersion forms gels after cooking at 80 ^{o}C for 40 minutes. Those gels can then be cut into a desired geometry to simulate different solid foods. Because of these advantages, whey protein gel was selected as model food in this study.

Several formulations of whey protein with the marker precursor D-ribose and different levels of salt were tested to find best possible match for the product of interest. Throughout the heating process, a phase comes after which there would be no significant chemical marker formation is called saturation point (Lau et. al., 2003). Heating patterns analysis after reaching the saturation point is impossible because no significant marker formation would be observed after that. Whey protein preparation with 1.5% D-ribose had saturation point at F_o = 12 min and a shorter linear time-span (8-10 min) at 121 ^{o}C. Preliminary study showed that whey protein sample with 1% D-ribose reached a saturation point when F_o = 18 min at 121 ^{o}C temperature (Wang, Wig, Tang & Hallberg, 2003; Wang, Lau, Tang & Mao, 2004). Experiments suggested that for 2.7- 4 kW microwave power settings the addition of 1% ribose to basic whey protein gel formulation is effective to visualize the heating patterns. The time span equivalent to 1% D-ribose was more suitable for evaluation of sterilization process.

3.2. Dielectric properties measurement

Dielectric properties of salmon were determined over range of temperatures from 20 to 120 ºC at 1 to 1800 MHz using a Hewlett-Packard 85070B open ended coaxial probe connected to an Agilent 4291B Impedance Analyzer (Agilent Technologies, Inc., Palo Alto, CA) (Guan, Cheng, Wang, & Tang, 2004; Wang, Wig, Tang, Hallberg, 2003). Five randomly selected fish fillet (*Oncorhyncus gorbuscha*). The fish was frozen in Kodiak, AK and filleted, deep-skinned, shipped, stored frozen and thawed in Pullman. These were female fish recovered from a terminal fishery. Two replications of Ocean Beauty Seafood shipments were used for this analysis. The salmon sample (15 g) after being broken into small pieces was placed into the test cell. The dielectric probe was sealed and kept in close contact with samples through pressure from a stainless steel spring and piston. Detailed description of the calibration and measurement procedure is provided in Wang, Wig, Tang, Hallberg (2003).

To match the dielectric properties, addition of salt in whey protein precursor with 1% D-ribose was considered a preferred method. Several levels of salt was tried to match the dielectric properties with salmon. Additional 0.3% salt in whey protein formulation matched well with the dielectric properties of salmon fillet.

3.3. Sample preparation

Additional salt was mixed in whey protein concentrate (Alacen 878, Fonterra, New Zealand) to match the dielectric loss of the whey protein gels to that of the salmon fillet. Added 0.3% salt and 1% D-ribose (Sigma, St. Louis, MO) were first dissolved in deionized water at 20 ^{o}C to form whey protein gel of 78.07 % moisture content (w.b).

Then, small amount of water with dissolved salt and D-ribose was added to the whey protein concentrate and mixed with spatula. Osterizer blender (Oster Appliances, Boca Raton, Florida, USA) was used to achieve homogeneity of mixture. The left over water was used to rinse beaker and the blender. This preparation allowed for the uniform distribution of whey protein throughout the solution.

A suitable temperature-time combination (80^{o}C for 40 minutes) was used to set 200g of the whey mix into gel of 78.07 % et basis in a 7 oz (14 × 9.5 × 2.67 cm) tray for heating patterns analysis (Lau et. al., 2003; Wang, Lau, Tang & Mao, 2004)

For kinetics study, 7 g of whey protein samples was cooked in several custom-built aluminum containers (inner diameters 3.5 cm × height 1.4 cm; Fig. 3).

3.4. Kinetics of chemical marker (M-2) formation with whey protein gel

Due to difference in activation energy for chemical marker formation (E_a = 19.48 ± 0.76 kcal /mol) and bacterial inactivation (E_a = 80 ± 10 kcal /mol) it was anticipated that the path of heating may influence the chemical marker yield and consequently the heating patterns (Toledo, 2000). Because of these variables two different pathways: 1) direct heating to a set temperature; and 2) holding at 121 ^{o}C to different F_o. In the first set of tests, aluminum containers were heated to 110, 116, 121, 126, and 131 ^{o}C temperatures (T) in an oil-bath to different levels of F_o. In another set, oil-bath temperature was set at 121 ^{o}C and samples were held for a different period of time to cumulative lethality (F_o) of 1.5, 3, 6, 9, 12, and 15 min. The samples were then rapidly cooled by immersing the aluminum containers into the ice. The purpose of fast cooling was to minimize additional cumulative lethality (F_o) after reaching the desired F_o. Each experimental treatment was

repeated twice. Temperature of the center of the sample was measured using pre-calibrated thermocouple (Fig. 3). Cumulative lethality F_o was calculated using the following formula (Holdsworth 1997):

$$F_o = \int_0^t 10^{\frac{T-121}{z}} dt \tag{4}$$

where, T is the sample temperature in ^oC at any time t during heating, z was taken as 10 ^oC for inactivation of *Clostridium botulinum* (Prescott et. al., 2002).

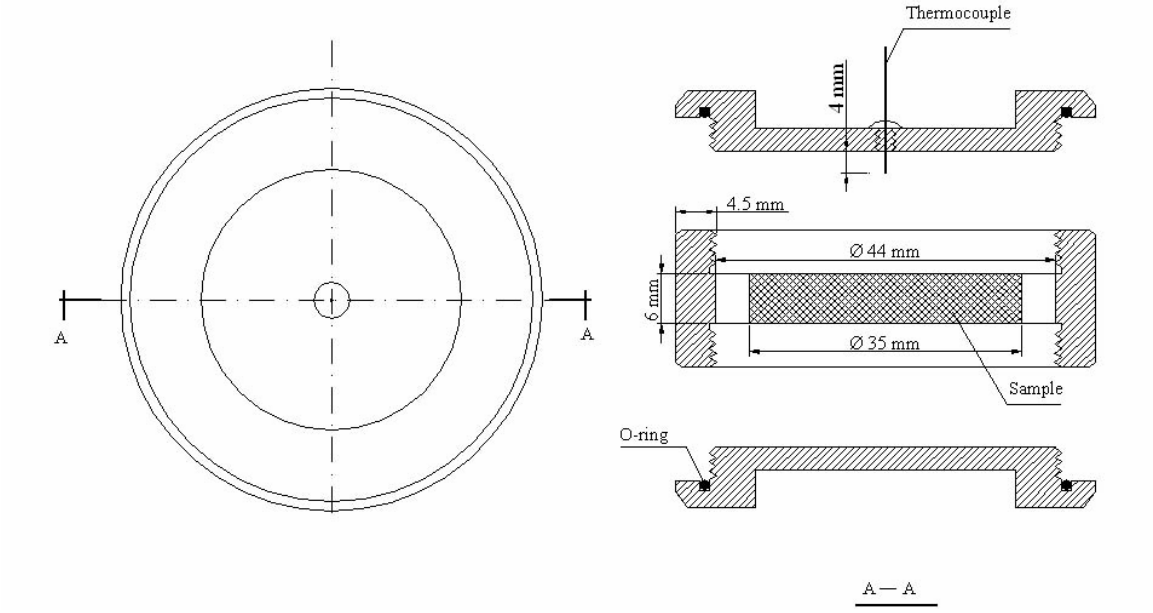

Fig. 3. Diagram of the aluminum cell used to collect the kinetic data with whey protein gel.

Chemical marker M-2 yield for both sets of samples were determined using the Agilent 1100 HPLC system (Agilent Technology, Palo Alto, CA, USA). The samples weighing between 0.20 g and 0.21 g were ground in 2 ml extraction buffer (10 mM sulphuric acid and 5 mM citric acid) for analyses. Sample extraction and HPLC analysis procedures are described in Pandit, Tang, Mikhaylenko & Liu (2006).

Images of the samples after heating in aluminum container were taken (3008 × 2000 with 24 bit per pixel), adjusted and inserted in a picture package using Adobe Photoshop CS version 8.0 (Adobe Systems, San Jose, CA, USA) for color value determination. Color value in terms of gray-scale value for each processed sample was collected after executing a computer vision script developed in IMAQ Vision Builder software (National Instrument, Austin, TX, USA). Other, details of the computer vision analysis was similar as provided elsewhere in Pandit, Tang, Liu & Pitts (2007).

3.5. *Computer vision method to specify cold spot*

Whey protein gel of dimensions 14.5 × 9.5 × 2.67 cm, formed in 7 oz tray was cut into a slab shaped gel (12 × 8 × 1.4 cm) to emulate the salmon fillet. Each tray was filled with 140g of slab shaped gel and 70g sauce and then vacuum-sealed at 18 in of Hg. Alfredo sauce made with fresh cream and aged parmesan cheese with composition: fat 15%, saturated fat 25%, carbohydrate 1%, cholesterol 11% and sodium 17% was used in this study (Bertolli, Englewood Cliff, NJ). Five vacuum sealed polymeric trays moving on conveyor belt were sterilized in the microwave system in each experimental run. Other experimental conditions of microwave processing were same as mentioned elsewhere in Pandit, Tang, Liu & Pitts (2007).

Slabs of the whey protein gels were taken out from trays after microwave sterilization process. Each tray was cut exactly in middle layer using the cutting frame by knife. Image of each layer of the microwave sterilized gel was automatically acquired and downloaded using computer vision system developed in our lab. Sizes of images taken were 3008 × 2000 with 24 bit per pixel, and were saved in Joint Photographic Experts Group (JPEG) format.

Adobe Photoshop CS version 8.0 (Adobe Systems, San Jose, CA, USA) was used to insert images to be analyzed into one package. Fresh and saturated whey protein samples images were also inserted in each picture package as scale images. A 20 × 25 cm automatic picture package was divided into 5 × 4 layouts. The first column of the layouts was reserved for the scale samples and other columns were used for images to be analyzed for heating patterns. Resolution of images in picture package was set to 500 pixels per inch.

A script developed in IMAQ Vision Builder program was used to determine the heating patterns and then cartesian co-ordinates of the cold spots locations. Other details related to developed computer vision method are provided elsewhere in Pandit, Tang, Liu & Pitts (2007).

3.6. Time-temperature profiles during microwave sterilization

In order to gather time-temperature profiles for considered points, experiments were conducted in a 915 MHz single-mode pilot-scale microwave sterilization system with whey protein gel in Alfredo sauce. Thermowell were secured at the specified locations to insert fiber optics probes during microwave sterilization. Each tray was filled with 140g slab shaped whey protein gel cut with 70g sauce and then vacuum-sealed at 18 in of Hg. Five trays were set on the conveyor belt in the microwave sterilization system and a proper speed-time (0.81 inch per second) combination was chosen to move the belt from the loading section to the holding section through two single-mode microwave heating sections. Water at 125 ^{o}C was circulated across the movement of conveyor belt inside the pressurized microwave cavities at a flow rate of 40 lpm. The experiments were

conducted at a 2.67 kW microwave power level. Initial temperatures of the samples were maintained at 7 ^{o}C. The cold spot location was preheated to 60 ^{o}C before trays movement started from loading to holding section until temperature of the cold spot reached to 121 ^{o}C. The desired F_o (6 min) was achieved by holding trays at 121 ^{o}C in the holding section before cooling was started. F_o during microwave heating was calculated using Equation (1) and C-value (Cook Value) was calculated as:

$$\text{C-value} = \int_0^t 10^{\frac{T-T_{Ref}}{z_c}} dt \qquad (5)$$

where, T is the sample temperature at any time t during microwave sterilization and z_c value for cooking was obtained by using the kinetics parameters provided in Lau et. al, 2003. T_{Ref} is normally taken as boiling point of water (100 ^{o}C) at atmospheric pressure and z_c value for cooking whey protein was gel was found to be 32.59 ^{o}C.

In another set of experiments, salmon was processed with Alfredo sauce. Similar experimental conditions were maintained to measure the time-temperature profiles in salmon with Alfredo sauce. Data was collected in replicates to compare the time-temperature trends for considered points.

4. Results and discussion

4.1. Dielectric properties matching

Trend of the dielectric loss and dielectric constant with temperature for salmon and adjusted whey protein gel formulation are presented in Figs. 4 and 5. The formulation of whey protein gel with 20% whey protein concentrate + 1% D-ribose + 0.3% salt was shown to be close to the measured dielectric properties of salmon fillets.

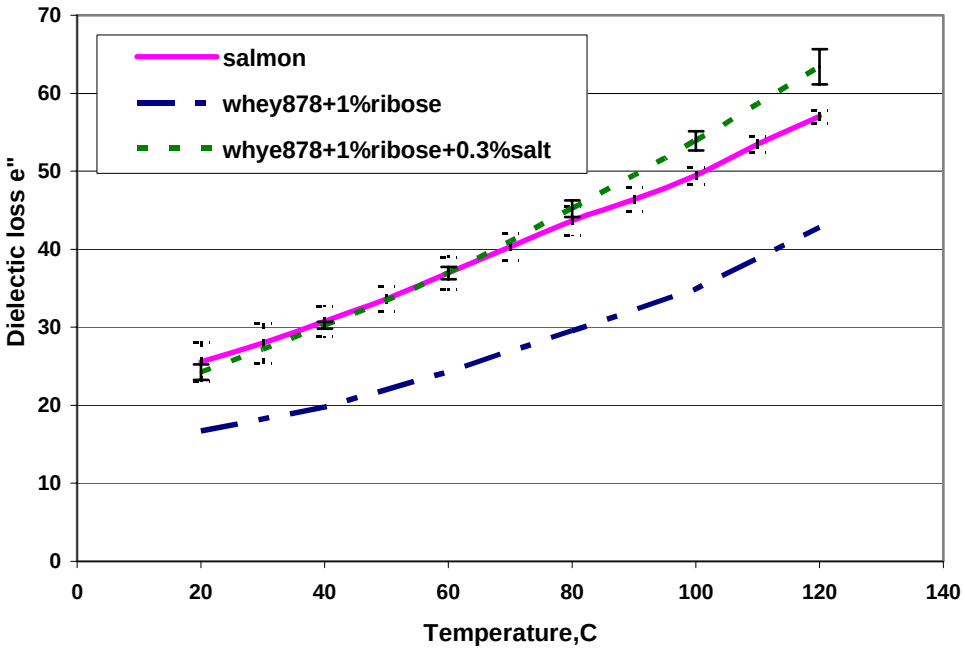

Fig. 4. Matching of the dielectric loss of salmon fillet and whey protein gel formulation by adding 0.3 % salt.

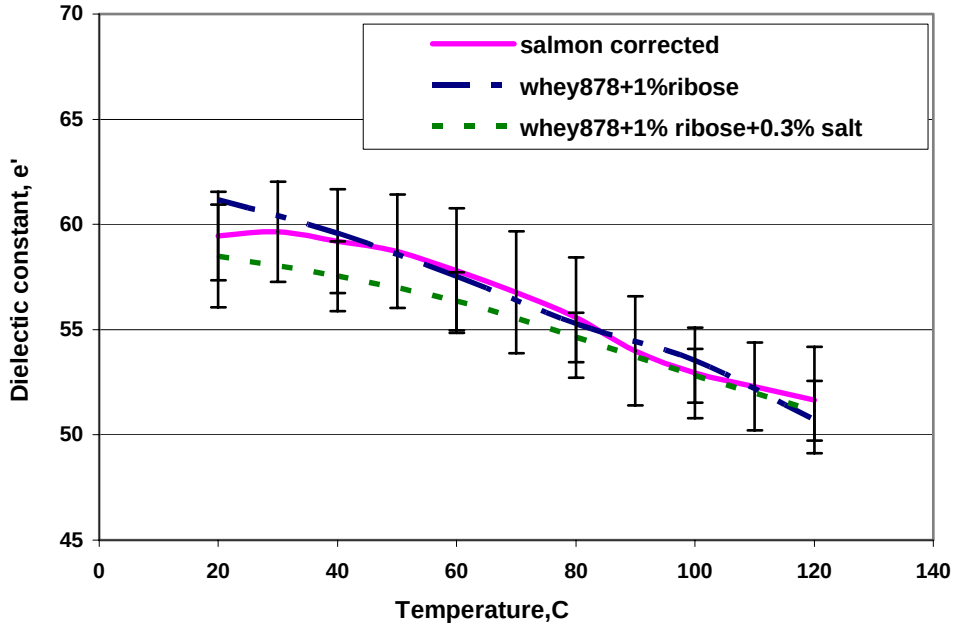

Fig. 5. Matching of the dielectric constant of salmon fillet and whey protein gel formulation by adding 0.3 % salt.

4.2. Relationship among color value, M-2 yield and F_o

Chemical marker yield during holding processes was found higher than ramp up heating (Fig. 6). This confirms that the rate of chemical marker formation is less sensitive to temperature change than bacterial inactivation as indicated by kinetics parameter in (Table 1).

Table 1. Comparison of the kinetic parameters of marker formation and bacterial inactivation. Parameters were calculated from kinetics data available in Pandit et. al., 2006.

Parameters	Chemical Marker formation	Bacterial inactivation (*C. botulinum*)
z-value (^oC)	32.57	10
D-value (min) @ 121 ^oC	0.0198	3.77
k (min^{-1}) @ 121 ^oC	116.31	0.61

Chemical marker yields of points corresponding to F_o = 9 min were compared for both ramp up and holding processes (Fig. 7). The longer cooking during holding (C-value = 47 min) at 121 ^oC for F_o = 9 min compared to ramp up heating to 128 for F_o = 11.15 min (C-value = 9.17 min) resulted into higher chemical marker yield (Fig. 6). Differences in the F_o, C-value and M-2 yield for both methods of heating showed the path dependent nature of these parameters. Fig. 7 supports the statement that microbial inactivation occurred faster than chemical marker formation at high temperature. Which is the main attraction of the high temperature short time (HTST) sterilization processes.

Color value equivalent to gray scale value is another variable which was obtained using computer vision method to represent the concentration of chemical marker formed during heating (Fig. 8).

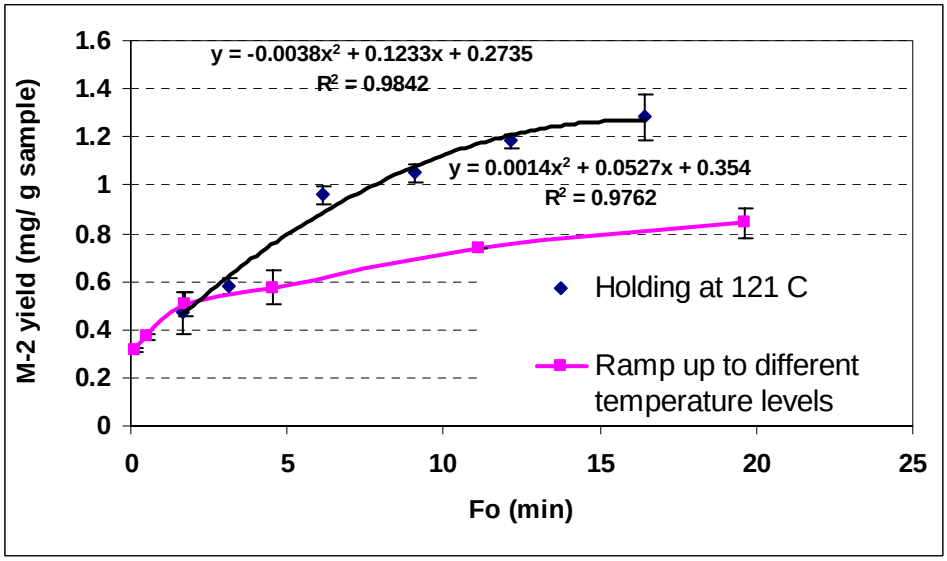

Fig. 6. M-2 yield and F_o relationship for whey protein samples heated to different set temperatures (ramp up) levels or held at 121 ^oC, data points are based on two replicates.

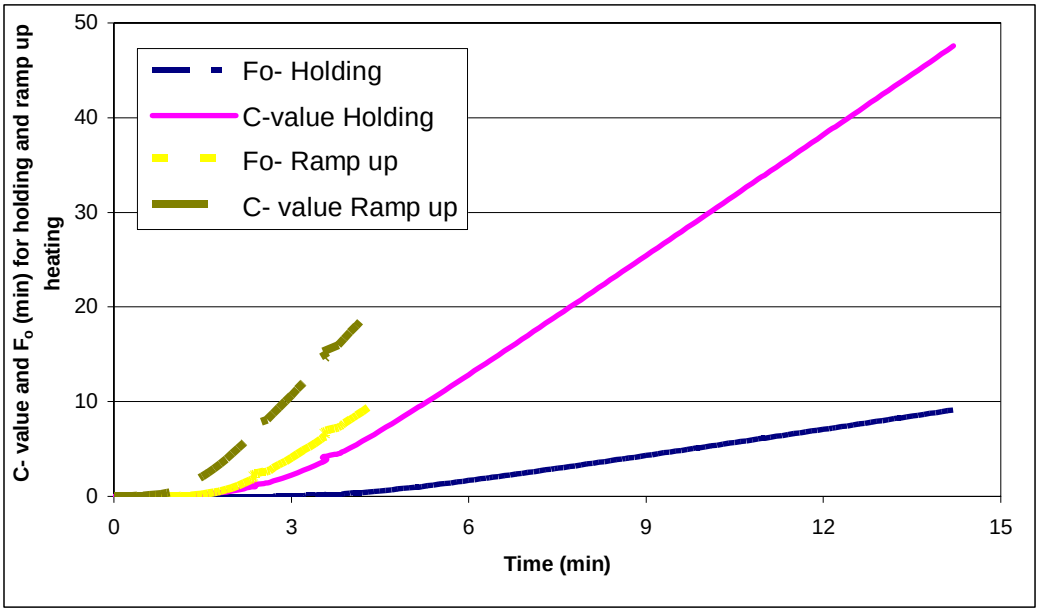

Fig. 7. Comparisons of cook values during holding and ramp up heating at same cumulative lethality (F_o = 9 min)

Color values collected for each sample heated during ramp up and holding processes were correlated with F_o and M-2 yield. Relationship between color value equivalent to gray scale value and F_o showed the similar trends for holding and ramp up heating (Fig. 9). These relationships confirmed that F_o can not uniquely represent the chemical marker concentrations.

A unique positive correlation was observed between color value equivalent to gray-scale value and M-2 yield for whey protein samples (Fig. 10).

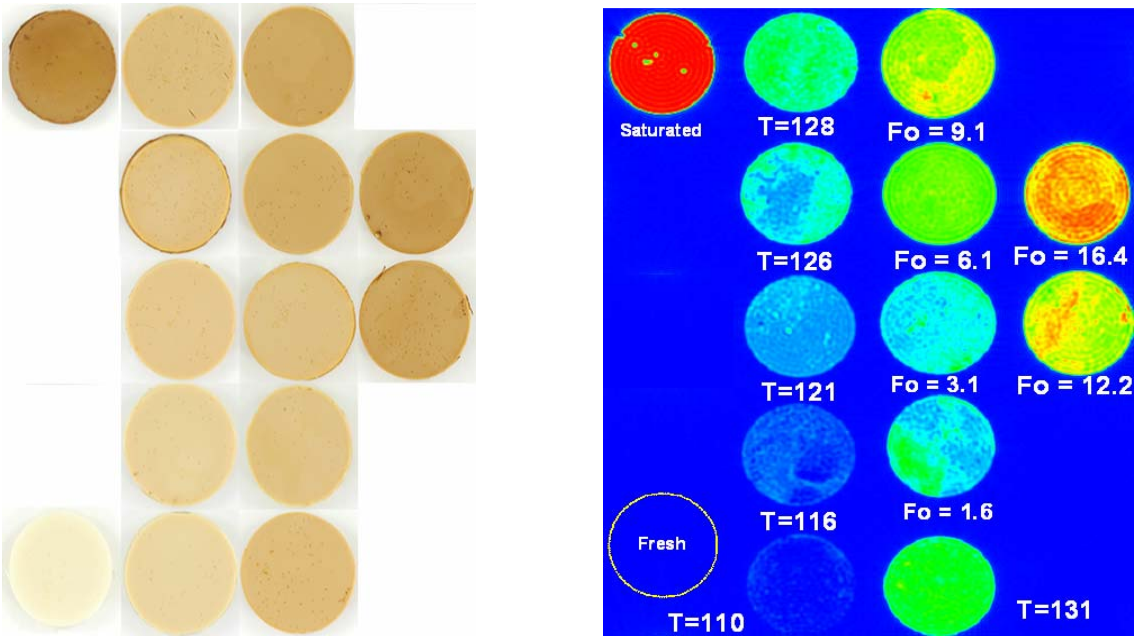

Fig. 8. Computer vision color patterns of the whey protein gel samples processed at different temperature level and F_o in oil bath. Experiments were tested in replicates.

The color value equivalent to gray-scale value increases linearly in this case with chemical marker M-2 yield. This relationship explains that color value is a true indicator of concentration of chemical marker formed during heating process.

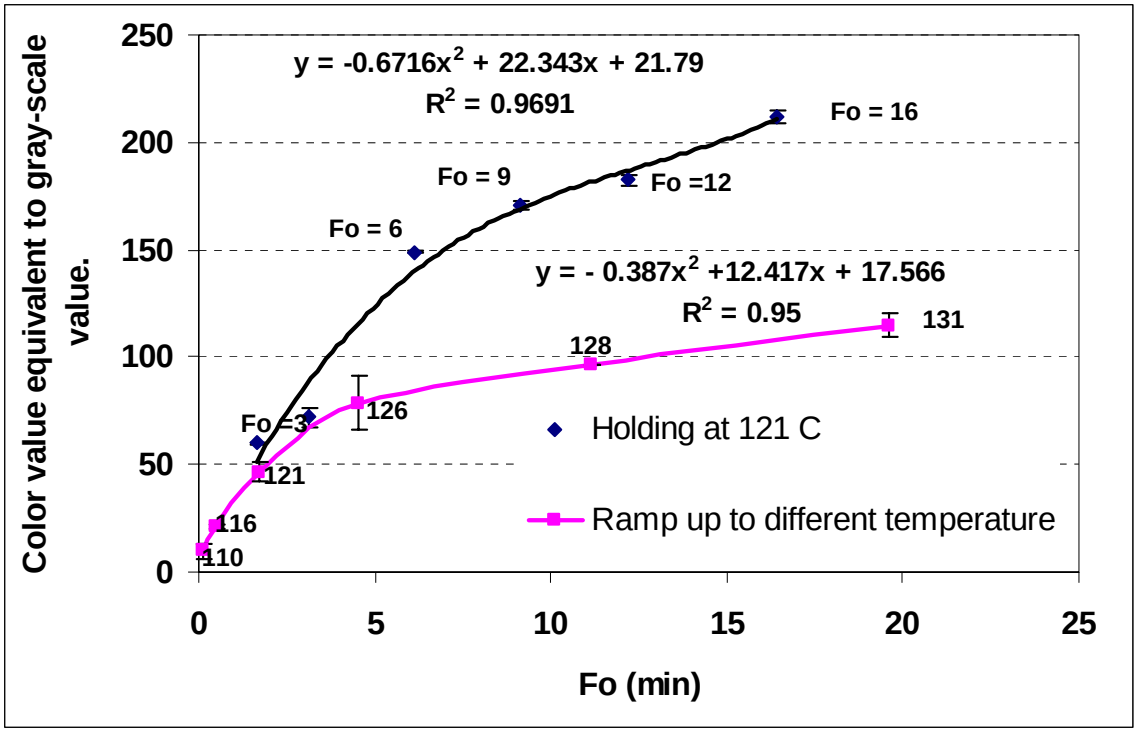

Fig. 9. Color value and F_o relationship for whey protein samples heated to different set temperatures (ramp up) levels or held at 121 ^oC, data points are based on two replicates.

4.3. Locations identified for comparisons

The IMAQ Vision Builder script was executed to identify the coldest layers. Middle layer was observed as coldest layer during microwave processing. A repeatable computer vision heating patterns was observed for each layer. In middle layer the second tray (Tray-2) from left was observed as coldest tray. Tray-2 was divided into 40 grids (8 × 5) to collect the color values equivalent to gray-scale value. Based upon the color values three spots were identified for confirmation in microwave sterilization system (Fig. 11). A region with lowest color value was identified as cold spot while a

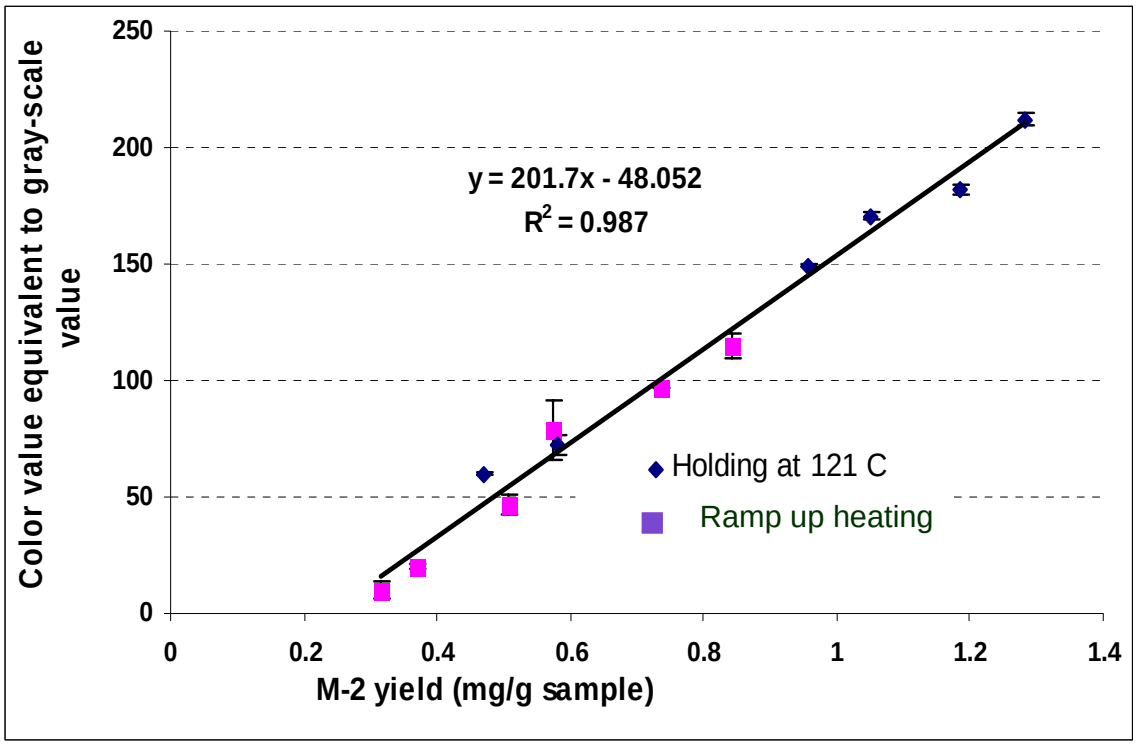

Fig. 10. Color value and M-2 yield relationship for whey protein samples heated to different set temperatures (ramp up) levels or held at 121 ^{o}C, data points are based on two replicates.

Table 2. Three spots based upon color values were specified in the slab shaped whey protein gel (12 × 8 × 1.4 cm) for comparisons.

Tray	Location of spots based on color value	Color values	Distance in X direction from lower right corner of slab (cm)	Distance in Y direction from lower right corner of slab (cm)
Tray-2	Cold	2.05	3.79	0.72
Tray-2	Hot	12.86	4.15	5.66
Tray-2	Warm	8.55	5.33	10.7

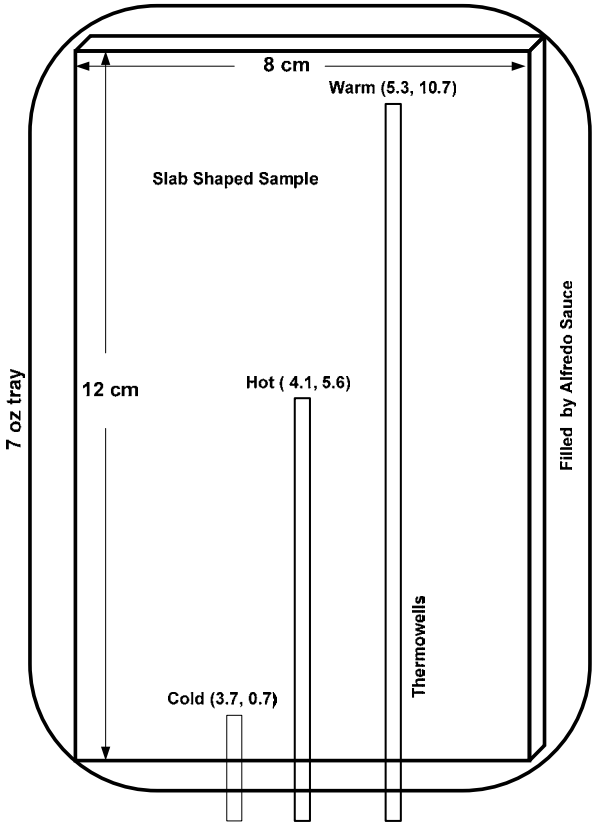

Fig. 1. Locations of fiber optics probe inserted in salmon fillet and whey protein gel to compare the time-temperature profile during microwave sterilization process.

region with highest color value as hot spot. Locations of the selected points, their color values and positions are presented in Table 2, and Fig.11.

4.4. Validation and matching of time-temperature profiles

Time-temperature profiles of the three points specified by computer vision method were compared to test the repeatability of the result with whey protein gel. Result showed a repeatable heating pattern (Fig. 12). Region with lowest color values

has lowest temperature, F_o, and C-value (Cook Value). Location 2, which was geometric center, of the tray was found to be hot spot with highest temperature, F_o and C-value.

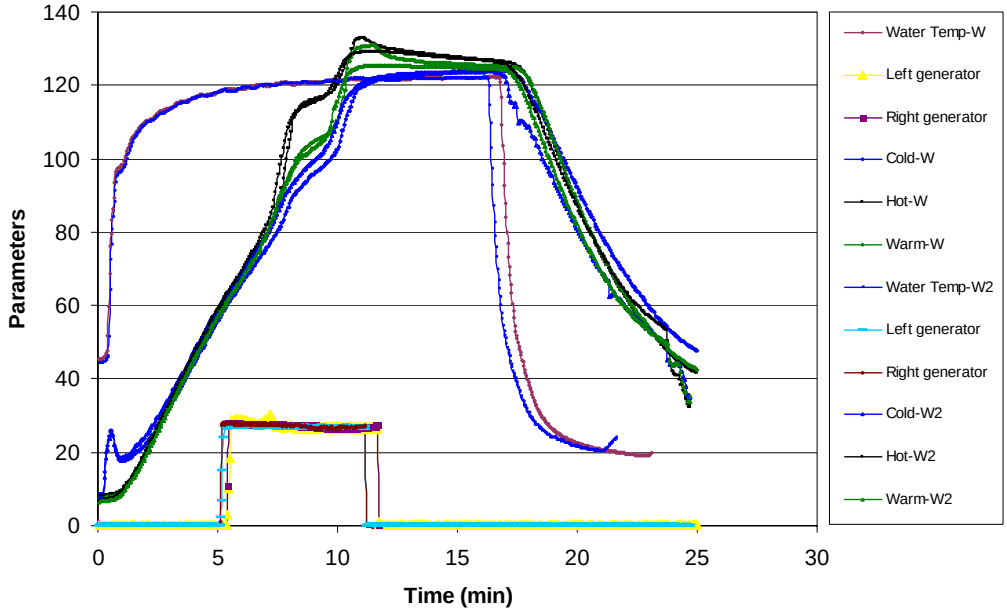

Fig. 12. Time-Temperature profiles for three specified points located in whey protein slab showing the repeatability of the experimental runs.

Time-temperature profiles obtained in whey protein gel were also compared with salmon (Fig. 13). We observed that cold spots in both food systems were at same locations. Trends of temperature variations at considered locations were noticed similar. Table 3 provides the initial and end point data collected at cold, warmer and hot spots locations for whey protein gel and salmon processed under microwave system. Preheating temperatures were maintained same for both foods and cold spot were heated until temperature reached to 121 ^{o}C. Microwave heating time for whey protein gel and salmon with sauce were observed to be very close. Cumulative lethality and cook values

were found in accordance at cold, warm and hot spots location with both the food systems.

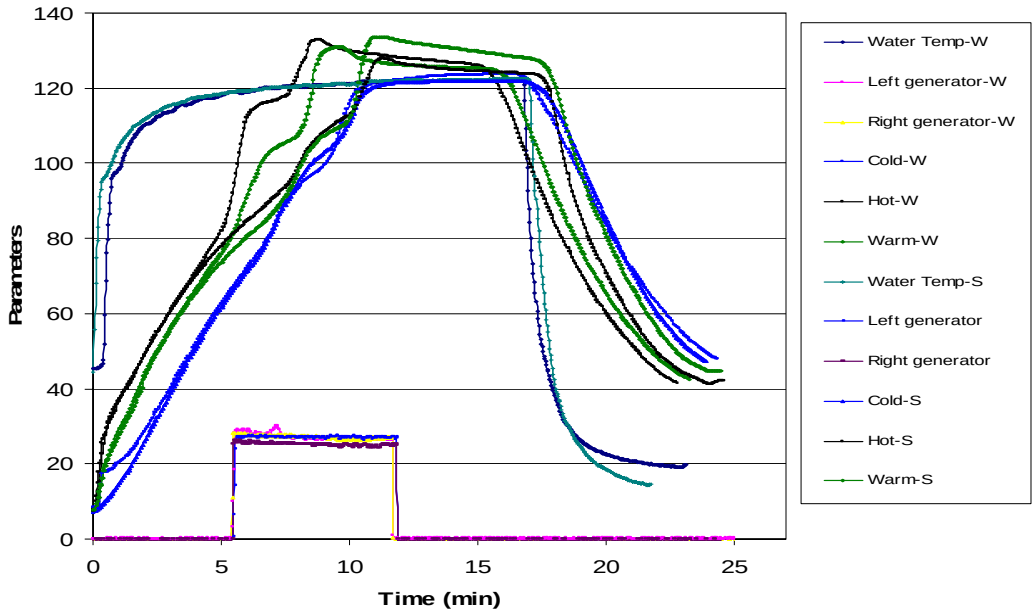

Fig. 13. Comparisons of the time-temperature profiles (S = salmon, & W = whey) at three specified locations in whey protein gel and salmon with Alfredo sauce during microwave sterilization.

Matching of the time-temperature profiles for both whey protein gel and salmon confirmed that whey protein gel can be used as model food to emulate the heating patterns of real food systems. This resemblance of time-temperature profile also suggests that dielectric properties of model food need to match close enough with the food to predict the cold spot.

Table 3. Comparisons of the microwave heating parameters for cold, warm and hot spots locations in salmon and whey protein gel processed with Alfredo sauce, data points are based on two replicates (mean ± SD).

Parameters	Salmon fillet			Whey protein gel		
	Cold	Warm	Hot	Cold	Warm	Hot
Preheating Temperature (C)	61.06 ± 0.01	72.38 ± 7.10	81.84 ± 0.42	61.58 ± 0.32	61.45 ± 3.63	62.1 ± 1.75
Final Temperature (C)	120.87 ± 0.99	133.86 ± 1.50	130.81 ± 5.98	121.32 ± 0.19	127.85 ± 3.57	129.89 ± 1.17
Fo (min)	8.1 ± 0.028	26.325 ± 11.22	53.07 ± 9.57	10.53 ± 0.18	24.04 ± 9.24	40.53 ± 7.74
Cook Value (min)	36.55 ± 5.15	47.82 ± 2.30	60.66 ± 2.39	41.27 ± 1.38	53.005 ± 5.89	64.99 ± 2.93
MW Heating Time (min)	5.96 ± 0.63			6.16 ± 0.01		

The methodology developed in this study can be used to predict the cold spot and heating patterns in other food system.

5. Conclusions

Dielectric properties of the whey protein gel can be adjusted to match the real foods. Due to difference in kinetics parameters, chemical marker formation and bacterial inactivation were found to be dependent on the path of heating. Color value equivalent to gray-scale value can be used to indicate the concentration of chemical marker M-2 formed during microwave sterilization process. A region of lowest color value can be identified as cold region of the microwave sterilization process. During microwave sterilization, time-temperature profiles of the points selected by computer vision method were found repeatable. Cold spots identified in model food systems matched those for a

real food consisting of pink salmon in Alfredo sauce during microwave sterilization. Time and temperature trend at the warmer and hot points in whey protein gel also matches well with salmon during microwave sterilization. This study confirmed that whey protein gel can be used to emulate the heating pattern in real food system. Protocol developed in this study can be applied for other food system to predict the cold spot of microwave processing.

Acknowledgments

This research work was supported by the US Army Natick Soldier Center, Natick, MA and the Washington State University Agricultural Research Center, Pullman WA.

Nomenclature

C	cook value (min)
D	decimal reduction time (min)
C_o	initial marker yield (gm/ ml of sample)
C_∞	marker yield at saturation (gm/ ml of sample)
E_a	activation energy (kcal mol^{-1})
F_o	cumulative thermal lethality (min)
t	time (min)
T_{Ref}	reference temperature
T	temperature (K)
z_c	z value (^oC)

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CHAPTER 7

A COMPUTER VISION METHOD TO LOCATE THE COLD SPOTS IN AN ENTRÉE: SALMON WITH ALFREDO SAUCE, DURING A MICROWAVE STERILIZATION PROCESS

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Abstract

Salmon fish with sauce was selected as a real food to study the heating characteristics of 915 MHz single-mode microwave sterilization system. This paper presents a methodology to locate the cold spots in an entrée style product consisting of a pink salmon fillet in an Alfredo sauce using computer vision method. Due to color change of salmon after microwave processing it was not possible to locate the cold spots directly in salmon fillet using computer vision method. Therefore, whey protein gels containing 1% D-ribose cut into rectangular slabs, to simulate fish fillet, were used to locate the cold spots. Dielectric properties of whey protein gels were matched with those of salmon by adding 0.3 % salt to the basic whey protein gel formulation. The rectangular slabs of whey protein gels (140 g) were sterilized in microwave system with sauce (70 g). Alfredo Sauce made with fresh cream and aged parmesan cheese with composition: fat 15%, saturated fat 25%, carbohydrate 1% and sodium 17% was used in this study. Color intensity of chemical marker (M-2) yield in whey protein gels after microwave processing was captured and analyzed using computer vision system to obtain the heating patterns. The cold spots corresponded to lightest regions in the gel. The cold spot

locations determined in whey protein gels were validated using salmon in sauce. Direct temperature measurement using fiber optics probes at different locations in salmon fillet during microwave sterilization process confirmed the identified cold spot locations. Microbiological validation was also conducted to support the study. Results showed that adjusted dielectric properties of whey protein gel in combination with a computer vision method can predict the cold spot in real food system. The developed computer vision procedures will be used to validate and provide documentation for the system effectiveness as needed in order to receive FDA approval for a microwave sterilization scheduled process for this food.

*Keywords***:** Computer vision; chemical marker M-2 yield; heating patterns; microwave sterilization; image processing; IMAQ vision builder; cold spot; salmon fillet.

1. Introduction

Computer vision method has been proven as a promising tool to locate the cold spots in microwave sterilization processes (Pandit, Tang, Liu, & Pitts, 2007). Due to intricacy in the designed microwave sterilization system, our research group was in need to develop a rapid and efficient tool to determine cold spot and monitor the machinery system as well other related unit operations (Pathak, Liu, & Tang, 2003). In our research, we have developed a computer vision system and method to determine the heating patterns of the microwave sterilized foods (Pandit, Tang, Liu, & Pitts, 2007).

A successful development and validation of a computer vision method with model food, mashed potato, to identify cold spot of microwave sterilization processes

was accomplished. It was always a concern regarding application of the developed method to real food systems. Interacting with FDA, Microwave Heating Group of Washington State University, Pullman, USA, selected salmon with Alfredo sauce the real food system to study the efficacy of the sterilization system before FDA approval. The computer vision method to locate cold spot is based on the development of brown-reddish color corresponding to the accumulation of chemical marker (M-2) yield in mashed potato or whey protein gel (Pandit, Tang, Mikhaylenko, & Liu, 2006) during sterilization process. Chemical marker (M-2) is a product of the reaction between amino acids and D-ribose (Lau et. al., 2003). Due to the color of the salmon fillet, the cold spot detection method based on chemical marker yield can't be applied directly to sterilize salmon.

In our previous studies, we developed a protocol for analyzing the heating patterns using mashed potato as a model food system. But mashed potato used in previous studies was impractical to identify the cold spot in fish with sauce since it would 'fall apart' during the microwave treatment. Whey protein would be better choice compared to mashed potato to simulate the heating patterns of fish because it can be shaped to simulate salmon fillet to yield chemical marker during thermal treatment. An exhaustive study including kinetics of whey protein gel was performed to establish whey protein gel as model to emulate the heating patterns of a salmon fillet. Comparison of time and temperature profiles between whey protein gel and salmon products were performed for microwave sterilization process. Our preliminary study showed that whey protein gel can emulate the heating patterns for this particular food system.

In light of the above mentioned facts, this study presents a method to identify and validate the cold spots locations in salmon with sauce after microwave sterilization. In order to develop the complete protocol, following specific objectives were set: 1) identify the cold spot locations in whey protein gel, shaped to simulate salmon fillet, with Alfredo sauce; 2) validations of the identified cold spot locations in salmon with Alfredo sauce.

Identification and validation of the cold spots with fish and sauce in this study were accomplished to prepare documentation for FDA filing of a scheduled process developed for 915 MHz single-mode microwave sterilization system.

2. Materials and methods

2.1. Selection *of the model food system*

Whey protein has specific amino acids (methionine, lysine, histidine, and arginine) needed for chemical marker (M-2) formation. In addition to amino acids availability, whey protein can also be easily dispersed in water and form firm gel. Whey protein dispersion forms gels after cooking at 80 ^{o}C for 40 minutes, they can then be cut into a rectangular slab to simulate the salmon fillet (Lau et. al., 2003; Wang, Lau, Tang, & Mao, 2004.). Because of these advantages, whey protein was selected as model food.

During a heating process, a phase after which there would be no significant chemical marker formation is referred to as at the saturation point (Lau et. al., 2003, Wang, Lau, Tang, & Mao, 2004). Several levels of D-ribose (0.75%, 1% and 1.5 %) were tried to find the suitable combination of ribose and potato flakes levels. It was necessary to find the appropriate combination of D-ribose to facilitate the suitable time, and F_o range for microwave sterilization study. Whey protein preparation with 1.5% D-ribose had

saturation point at F_o = 12 min and a shorter linear time-span (8-10 min) at 121 ^oC. Whey protein with 0.75 % ribose has not sufficient browning for duration of microwave heating. Our preliminary studies indicated (data not shown) that whey protein sample with 1% D-ribose has saturation point of F_o = 18 min at 121 ^oC temperature. This time span was more suitable for evaluation of sterilization process.

2.2. Sample preparation for heating pattern analysis

An appropriate amount of salt (0.3 %) was added to whey protein concentrate (Alacen 878, Fonterra, New Zealand) in preparing a whey protein gels to match its dielectric property with that of salmon. Several formulations of whey protein with the marker precursor D-ribose and different levels of salt were tested to find best possible match for the product of interest. A considerable matching of dielectric properties was obtained for 0.3 % salt (Pandit, Tang, Mikhaylenko, Liu & Tang, 2007a). The formulation of whey protein 20 % + 1 % ribose + 0.3 % salt was chosen to be close to the determined dielectric properties of salmon filets. The added 0.3% salt and 1% D-ribose (Sigma, St. Louis, MO) were first dissolved in 78.07 % (wb) of water at 20 ^oC. Then, small amount of water with dissolved salt and D-ribose was added to the whey protein concentrate and mixed with spatula. Osterizer blender (Oster Appliances, Boca Raton, Florida, USA) was used to achieve homogeneity of mixture. The left-over water was used to rinse beaker and the blender. This preparation allowed for the uniform distribution of whey protein throughout the solution.

2.3. Chemical marker formation versus bacterial inactivation kinetics

Activation energy for chemical marker formation (E_a = 19.48 ± 0.76 kcal /mol) is lower than bacterial inactivation (E_a = 80 ± 10 kcal /mol) (Prescott et al, 2002). Due to difference in z-values, bacterial inactivation (z-value = 10 ^oC) seems to be much more sensitive to temperature variation than chemical marker formation (z-value = 32.59 ^oC). Another kinetics parameters such as, D-value and reaction constant (k) for both chemical marker formation and bacterial (C. *botulinum*) inactivation, are noticeable different (Table 1). In general, a given chemical marker concentration can be reached through many different time-temperature histories (Wnorowski and Yaylayan, 2001; Kim and Ball, 1994; Kim and Choi, 1998). Because of these variables two different pathways namely, 1) direct heating to a set temperature; and 2) holding at 121 ^oC to different F_o, were considered in to test the validity of these facts. Kinetics parameters for chemical marker formation and bacterial destruction are presented in Table-1. Presented data is based upon the kinetics information available in Pandit et al., 2006.

Table 1. Comparison of the kinetic parameters of marker formation and bacterial inactivation. Parameters were calculated from kinetics data available in Pandit et. al., 2006.

Parameters	**Chemical Marker formation**	**Bacterial inactivation (*C. botulinum*)**
z-value (^oC)	32.57	10
D-value (min) @ 121 ^oC	0.0198	3.77
k (min^{-1}) @ 121 ^oC	116.31	0.61

In experiments, seven gm of whey protein samples were cooked in custom-built aluminum containers, (inner diameters 3.5 cm × height 1.4 cm), and were heated to 110, 116, 121, 126, and 131 ^{o}C temperatures (T) in an oil-bath. The cumulative lethality (F_o) during ramp up to each temperature level was also stored. In another set, oil-bath temperature was set at 121 ^{o}C and samples were held for a different period of time to cumulative lethality (F_o) of 1.5, 3, 6, 9, 12, and 15 min. In both sets of the experiments, samples were rapidly cooled by immersing the containers into the ice. Fast cooling minimized the additional cumulative lethality (F_o) after the heating operation. Each experimental treatment was repeated twice. F_o was calculated using the following formula:

$$F_o = \int_0^t 10^{\frac{T-121.1}{z}} dt \qquad (1)$$

where, T is the sample temperature in ^{o}C at any time t during heating, z was taken as 10 ^{o}C for inactivation of *Clostridium botulinum* (Holdworth, 1997).

Study of M-2 Kinetics **Development of a method to locate the cold spot** **Designing a computer vision system**

Relationship between color values F_o, and M-2 Yield **Position of cold spots with several food systems** **Temperature and microbial validation**

Fig. 1. Major steps involved in computer vision study to determine the location of cold spots in microwave sterilization process.

Chemical marker M-2 yield for both sets of samples were determined using the Agilent 1100 HPLC system (Agilent Technology, Palo Alto, CA , USA). The samples weighing between 0.20 g and 0.21 g were ground in 2 ml extraction buffer (10 mM sulphuric acid and 5 mM citric acid) for analyses. Sample extraction and HPLC analysis procedures are described in Pandit et al. (2006).

Images (3008 × 2000) of the samples heated in aluminum container were taken using the automatic image acquisition system with 24 bit per pixel. Images resolution and sizes were adjusted before inserting each image into a picture package. Adobe Photoshop CS version 8.0 (Adobe Systems, San Jose, CA, USA) was used to insert all images in one package for comparing the heating intensity. Experiments were done in replicate and each package was analyzed in IMAQ Vision Builder software (National Instrument, Austin, TX, USA) for color determination. Color value equivalent to gray-scale value for each processed sample was collected after executing a computer vision script developed in IMAQ Vision Builder.

2.4 Computer vision heating patterns

Computer vision method developed in this research work to simulate the heating patterns in salmon with Alfredo sauce involved several major steps. These steps in order are presented in Fig. 1. Details of the principle and application of the computer vision method to determine the cold spot in real food system is provided elsewhere (Pandit, Tang, Mikhaylenko, Liu, & Tang, 2007a).

2.4.1. Color palette and development of a new scale

On grayscale the color distribution in an image is represented by positive integer between 0 and 255. The lowest number in heating pattern analysis corresponded to the lightest intensity of brown color of the processed tray. Similarly, the darkest color corresponded to highest grayscale value in each analysis. Color intensity of the processed trays varies upon the level of the thermal treatment (F_o). Due to these variations it was impossible to compare heating patterns of two separate experiments, or even two trays in same experiment. For comparative study, it was necessary to fix the gray-level values to certain pixel intensity in each analysis. This was done by inserting fully saturated and unheated (Fresh) whey protein gels samples in each analysis. The saturated whey protein gel sample (F_o = 18 min; marker yield =1.14 ± 0.09 mg/g sample) was always fixed to the upper most point of the scale by setting color value to 255. The unheated whey protein gel sample (marker M-2 yield = 0.12 ± 0.07 mg/g sample) was fixed to the lowest point of the scale by setting color value to zero. For these purpose, samples processed in Aluminum container were inserted in each picture package of the Adobe Photoshop 8.0 to fix the scale and to facilitate the comparative heating patterns analysis.

2.4.2. Image Acquisition and Image editing using Adobe Photoshop

Nikon's Capture camera control program was used for automatically acquiring and downloading the image on Dell Workstation. Sizes of images taken were 3008 × 2000 with 24 bit per pixel, and were saved in Joint Photographic Experts Group (JPEG) format.

Adobe Photoshop CS version 8.0 (Adobe Systems, San Jose, CA, USA) was used to insert scale images and images to be analyzed into one package. A 20 × 25 cm automatic picture package was divided into 5 × 4 layouts (Thomas, 2003). The first column of the layouts was reserved for the scale samples and other columns were used for images to be analyzed for heating patterns (Fig. 2). Resolution of images in picture package was set to 500 pixels per inch.

2.4.3. Functions in computer vision script

A computer vision script in IMAQ Vision Builder was developed through interactive programming to determine the heating patterns of microwave processed samples. Several additional functions were added into the script to generate the grid and extract the color values. The extracted color values were compared to locate cold spot in the tray. A flow chart and functions of IMAQ Vision Builder script is provided in the Fig. 3. Details of the functions are described elsewhere in Pandit (2007b).

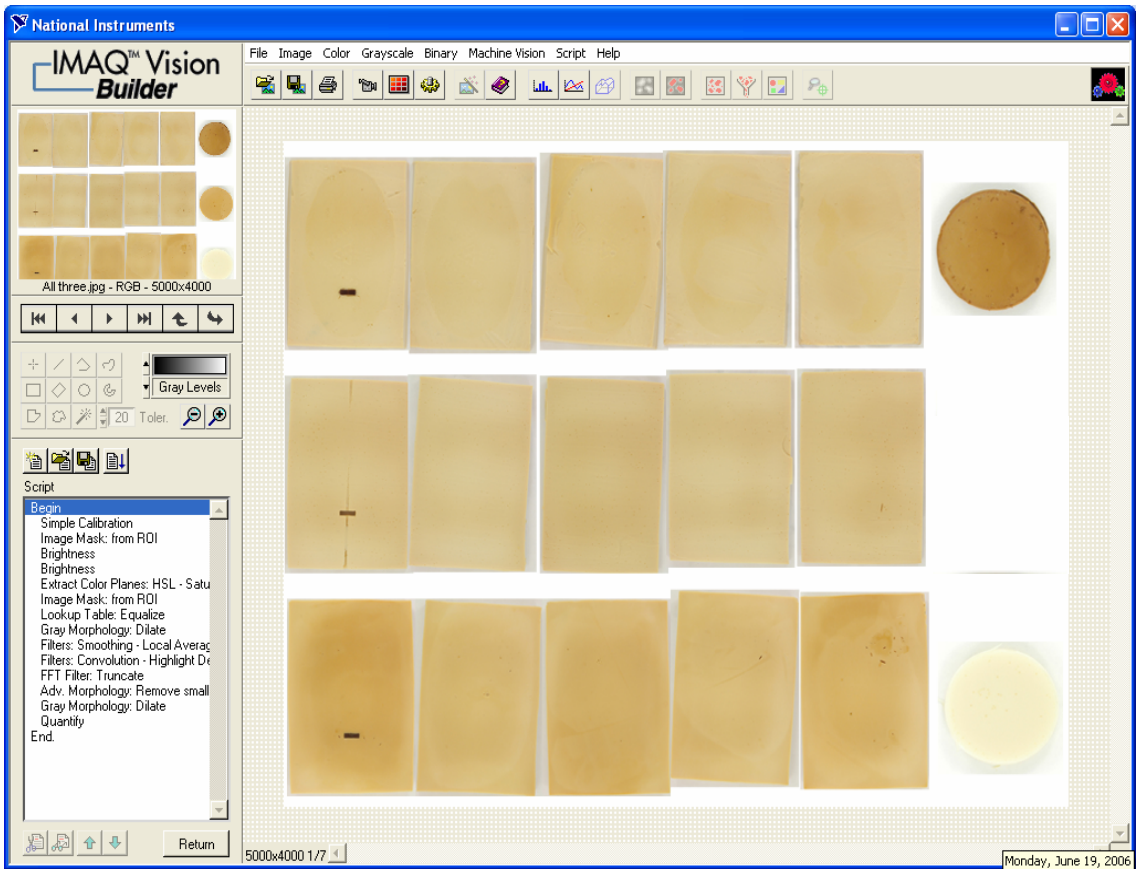


Fig. 2. Images of top, middle, and bottom layers along with the scale sample inserted in one picture package of Adobe Photoshop before analyzing the heating patterns.

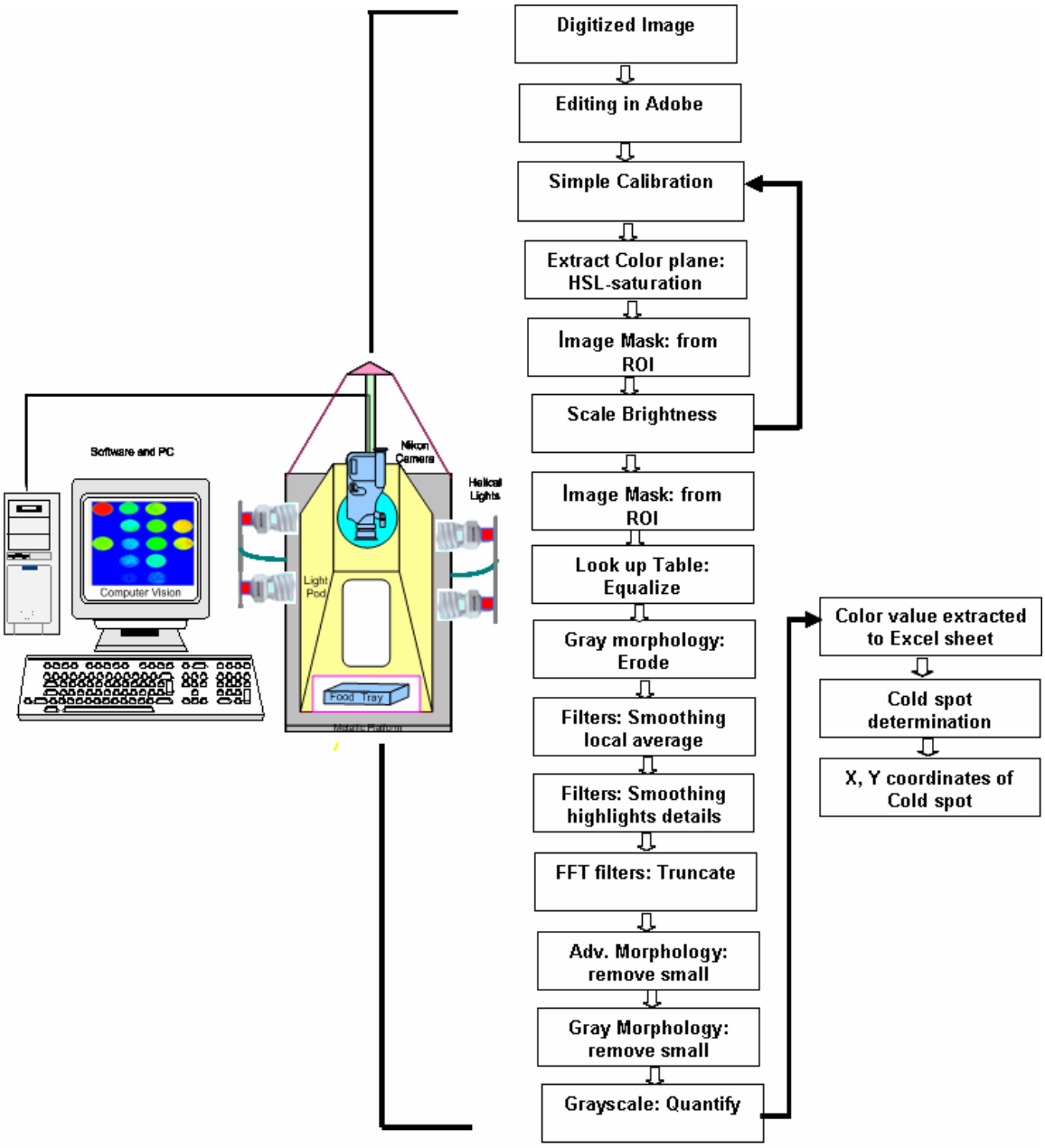


Fig. 3. Flow chart of major steps developed in computer vision method to determine the cold spot location.

Several additional functions with the option of selecting either dilation or erosion were added into the scripts. These grayscale morphological operators filter or smooth the pixel intensities that results into noise filtering, background correction and gray-level feature extraction. Selection of anyone of these operators which influences the brightness of the neighborhood pixel may lead to much clear heating patterns. The neighborhood is defined by structuring elements.

Sternberg's formulae for computing the gray value dilation and erosion are the most straightforward. This formula is based on the concept of umbra. Let $\boldsymbol{X} \subset R^{\boldsymbol{n}}$ and $f: X \to R$ be a function. Then the umbra of f, denoted by $\mathcal{U}(f)$ *is* the set $\mathcal{U}(f) \subset R^{\boldsymbol{n+1}}$, defined by (Ritter & Wilson, 2000):

$$\mathcal{U}(f) = \left\{ (x_1, x_2, x_3, \ldots\ldots\ldots\ldots x_n, x_{n+1}) \in R^{n+1} : (x_1, x_2, x_3, \ldots\ldots\ldots\ldots x_n) \in X \text{ and } x_{n+1} \leq f(x_1, x_2, x_3, \ldots\ldots\ldots\ldots x_n) \right\} \quad (2)$$

Since $\mathcal{U}(f) \subset R^{\boldsymbol{n+1}}$, we can dilate $\mathcal{U}(f)$ by any other subset of $R^{\boldsymbol{n+1}}$**.** This observation provides the clue for dilation of gray-valued images. In general dilation of a function $f : R^n \to R$ by a function g: $X \to R$, where $\boldsymbol{X} \subset R^{\boldsymbol{n}}$ is defined through the dilation of their umbra $\mathcal{U}(f) \times \mathcal{U}(g)$ as follows:

Let, $$\mathbf{x} = (\ x_1, x_2, x_3, \ldots\ldots\ldots\ldots x_n\ ) \in R^n \quad (3)$$

and a function $d: R^n \to R$ which can be define as*:*

$$d(\mathbf{x}) = max\ \{ z \in R : (\mathbf{x}, z) \in \mathcal{U}(f) \times \mathcal{U}(g) \} \quad (4)$$

then we define dilation as:

$$f \times g \equiv d. \quad (5)$$

and similarly erosion of f by g is defined as the function:

$$f / g \equiv e \tag{7}$$

where e = -[(-*f*) × $\hat{g}$] and $\hat{g}$ ***(x)*** **=** *g* ***(-x)***.

For two-dimensional calculation, the new functions $d = f \times g$ and $e = f / g$ for dilation and erosion respectively reduces (Acharya & Ray, 2005):

$$d(\mathbf{y}) = \max_{x \in X} \{ f(y + x) + g(x) \} \tag{8}$$

for the dilation, and

$$e(\mathbf{y}) = \min_{x \in X} \left\{ f(x + y) - g^*(x) \right\} \tag{9}$$

for the erosion, where $g^* : X^* \to R$ is defined by:

$$g^*(x) = g(-x) \forall x \in X^* \tag{10}$$

In practice the function *f* represents the image and *g* represents the structuring element. IMAQ Vision Builder operates with structural element of dimension 3×3, 5×5 and 7×7. In our study a structuring element of size 3 × 3 was selected for most of cases.

The color intensity meter function of the script measures the grayscale image statistics in an image or regions in an image. IMAQ Vision Builder contains the following densitometer parameters: minimum gray value, maximum gray value, mean gray value and standard deviation. An area with minimum standard deviation of the color value was selected as grid size to extract the color values. If *G* represents the set of grayscale *G* = {0, 1, 2, ……..255} values of the selected region of interest (ROI) then mean ($\bar{G}$) is commonly referred as average:

$$\bar{G} = \frac{1}{N} \sum_{i=1}^{N} G_i \tag{11}$$

The standard deviation ($\bar{V}$) of the color value was calculated as:

$$\bar{V} = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(G_i - \bar{G})^2} \quad (12)$$

The last step in this application was measurement of the distances in real word units. The dimensional measurements obtained from the image must be compared with values that are specified in real units. For this purpose, we converted the measurement from the image into real-word units using the calibration tools (IMAQ Manual, 2004).

2.4.4. Heating patterns analysis with whey protein sample

Whey protein gel of dimensions 14.5 × 9.5 × 1.4 cm was first formed in 7 oz tray at 80 ^{o}C, then cut into a slab shaped gel (12 × 8 × 1.4 cm) to emulate the salmon fillet. Alfredo sauce made with fresh cream and aged parmesan cheese with composition: fat 15%, saturated fat 25%, carbohydrate 1%, cholesterol 11% and sodium 17% was used in this study (Bertolli, Englewood Cliff, NJ). Seventy grams of sauce was added to the tray, with half of the sauce on the bottom and half on top of the whey protein gel's slab prior to sealing and microwave heating. Five vacuum sealed polymeric trays (18 inch of Hg vacuums) moving on a conveyor belt were sterilized in the microwave system in each experimental run. Other experimental conditions of microwave processing were same as mentioned elsewhere in Chapter 3 (Pandit et al., 2007). In the first step, the coldest layer in microwave sterilization was obtained by comparing top, middle and bottom layers of the trays. In the next step, the coldest tray was identified in the coldest layer. Total 40 rectangular grids (5 × 8) were generated on the coldest tray to collect the grayscale values equivalent to color value. Color values were directly extracted into the Excel spreadsheet to obtain the lowest color value. A rectangular grid with lowest color value was declared as the cold spot of the microwave sterilization process. A script developed in IMAQ

vision builder program was used to determine the Cartesian co-ordinates of the cold spots.

For microbiological validation and real temperature measurement we identified three spots corresponding two three lowest color values or appearing as blue in computer vision heating patterns. The specified locations were validated using salmon in Alfredo sauce.

3. Validation of Computer Vision Heating Patterns

3.1. Validation of the cold spot using microwave system

In order to validate the accuracy of the cold spots determined by the computer vision method, experiments were conducted in a 915 MHz single-mode pilot-scale microwave sterilization system with salmon and sauce. Thermo wells that separate sealed sample from fiber-optic sensors were fitted into the tray at the measured co-ordinates of cold spots locations. Each tray was filled with 140g of salmon fillet and 70g sauce and then vacuum-sealed at 18 in of Hg. A mesh belt with several small pins was used to hold and move the belt from the loading section to the holding section through two single-mode microwave heating section during the microwave processing. Water at 125 ^{o}C was circulated across the movement of conveyor belt inside the pressurized microwave cavities at a flow rate of 40 liter per minute. The experiments were conducted at a 2.67 kW microwave power level.

3.2. Validation of the cold spot using inoculated pack studies

Clostridium sporogenes PA 3679 spores (batch 308, NFPA strain) were used to validate the cold spot. The inoculums level in this study was set to 10^6 CFU. The heat resistance of these spores was evaluated previously in phosphate buffer (D_{121}=0.8 min). The D-values of the spores in salmon and Alfredo sauce were 1.03 and 1.05 min at 121°C, respectively.

The schedules for automatic processing of the salmon fillets were established for different F_o levels in microwave sterilization system. Preliminary inoculation pack studies were carried out to verify the correctness of selected auto schedules for different F_o levels. Large scale inoculation pack studies were carried out to verify the effectiveness of sterilization process in Washington State University microwave sterilization system. The cold spots determined by the heating pattern of whey protein gels were validated using the real product temperature profile.

Spores were injected into salmon fillet in several locations of potential cold spots identified by computer vision method of microwave-processed whey protein gel that simulated salmon fillet. Three levels of F_o, 3.0, 6.0, and 12.0 (target F_o = 6.0 min) based on information used to define scheduled process for processing were considered in inoculated pack test. After processing, the salmon trays were subjected to incubation 32ºC for a week and trays with positive growth were sought (Guan et al., 2003).

4. Results and discussion

A unique positive correlation was established between color value equivalent to gray-scale value and M-2 yield for whey protein samples heated during ramp up and holding method (Pandit, 2007a). This relationship confirmed that color value equivalent

to gray-scale value increases uniquely with increase in chemical marker M-2 yield (Fig.4).

Heating patterns of the top, bottom and middle layers of each experimental replicate were compared to find out the coldest layer (Fig. 5). Due to limitations of the microwave power penetration and heat conduction, the middle layer of each tray was determined as the coldest layer. Comparisons of the middle layers heating patterns for the five trays are shown in Fig. 6 Second tray from left (Tray-2) was observed as the coldest tray during sterilization process. In Tray-2 three minimum color values regions: 2.05, 8.55 and 12.86 were identified for experimental validation (Table 2). Considering bottom left corner of the Tray-2 as the reference point, location of first point was measured as x = 3.79 cm, y = 0.72 cm, second point as x =5.66 cm, y = 4.12 cm and third point as x = 5.33 cm, y =10.7cm.

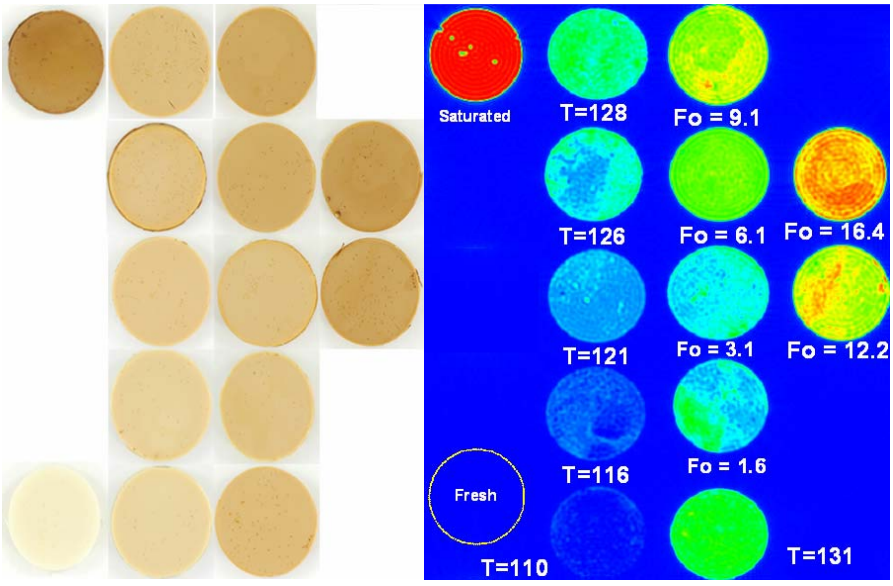
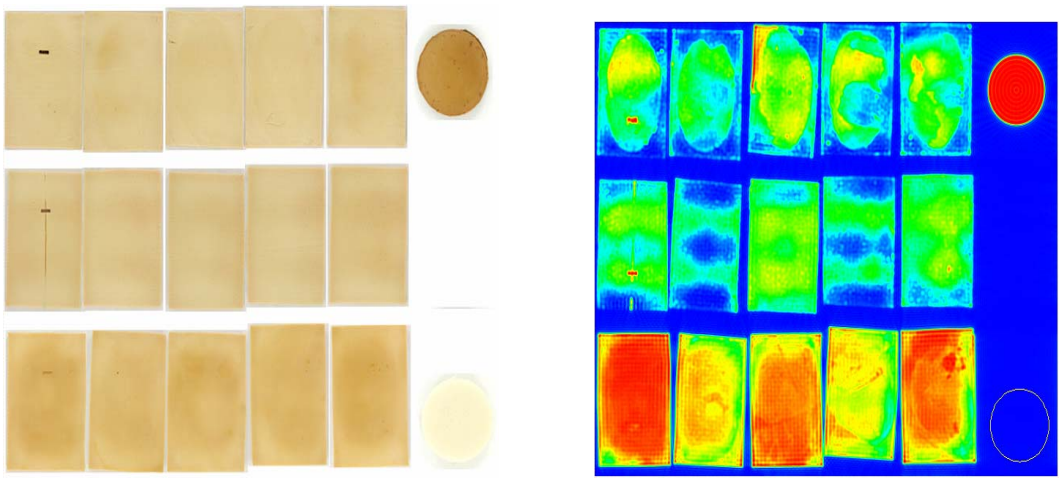


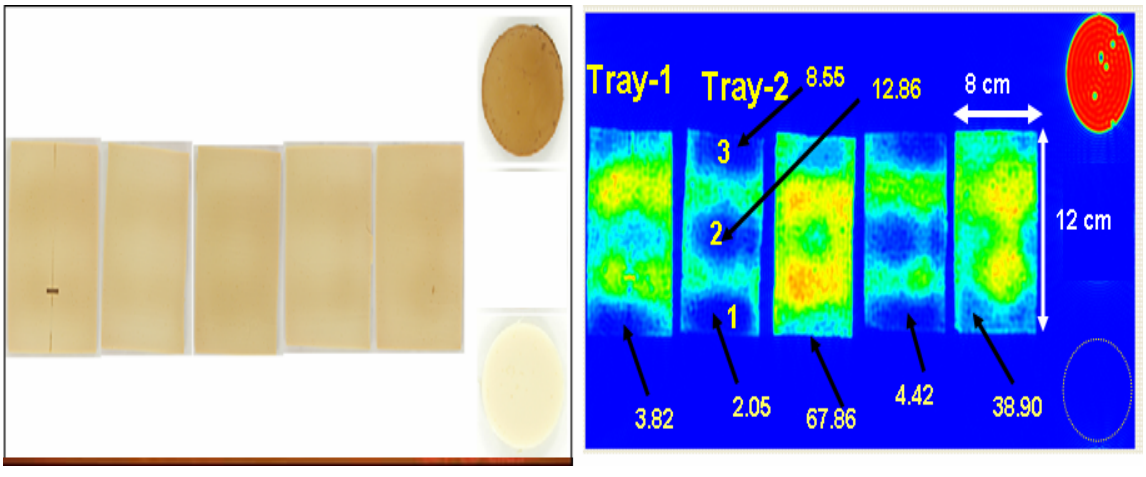
Fig. 4. Computer vision color patterns of the whey protein gel samples processed at different temperature level and F_o in oil bath. Experiments were tested in replicates.



(a) (b)

Fig. 5. Comparison of computer vision heating patterns for top, middle and bottom layers to find the coldest layer. Cold spot was identified in middle layer. (a) MW processed trays (b) Computer vision heating patterns.

Fig. 7 shows the temperatures measured at three different locations using fiber optics probes during microwave sterilization. Temperature measured at first location, with color value 2.05, was lowest (121 ^{o}C) and temperature at third location, with color value 12.86, was highest (126.26 ^{o}C) during experimental validation. This validation confirmed that a region with lowest color value indeed was the coldest region during temperature measurement using fiber optics probes.



(a) MW processed tray (b) Computer vision heating pattern

Fig. 6. Heating patterns in middle layers of the five microwave sterilized trays, number indicate the color values at those locations, (a) Original rectangular shaped whey protein sample. (b) Computer vision heating pattern.

The results of microbial validation studies using inoculated packs of salmon in Alfredo sauce showed that microwave processing delivered designed lethality to the selected heat resistant PA 3679 spores at each processing levels. At lower F_o, 8 packages

Table 2.Three spots based upon color values were specified in the slab shaped whey protein gel (12 × 8 × 1.4 cm) for comparisons.

Tray	Location of spots based on color value	Color values	Distance in X direction from lower right corner of slab (cm)	Distance in Y direction from lower right corner of slab (cm)
Tray-2	Point 1	2.05	3.79	0.72
Tray-2	Point 2	12.86	4.15	5.66
Tray-2	Point 3	8.55	5.33	10.7

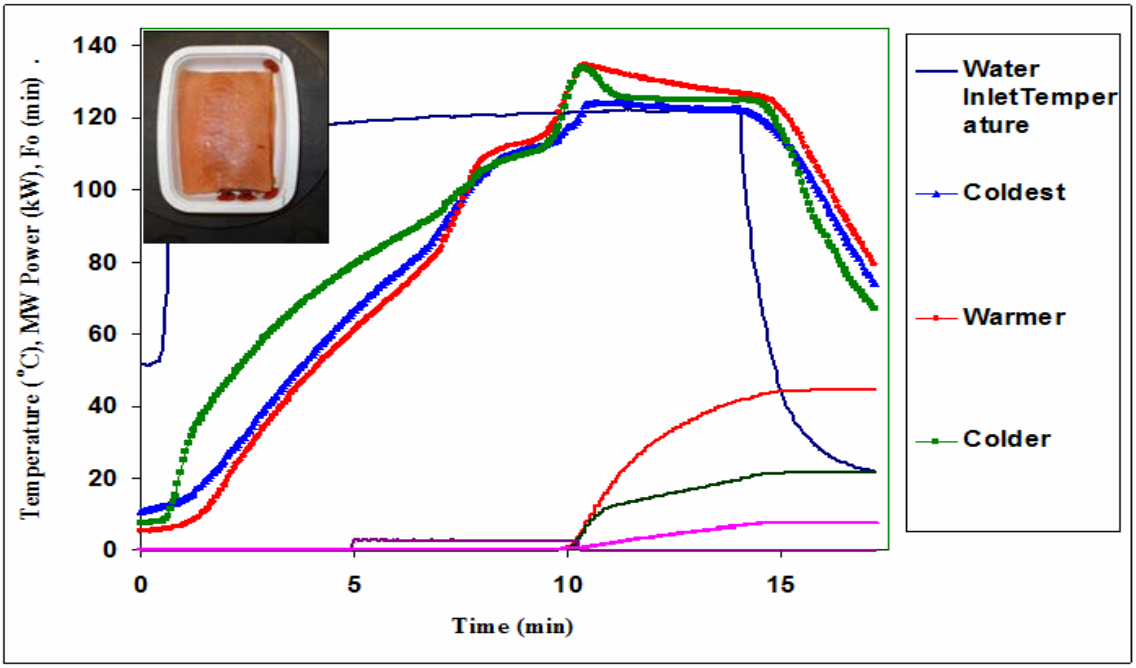


Fig. 7. Time-temperature history at three different locations of color values 2.05, 8.55 and 12.86 specified by computer vision method in 7 oz trays during microwave sterilization of salmon with sauce at 2.67 kW power level, typical temperature profile from repeated tests.

Table 3. Results of the microbiological validation studies using inoculated packs (PA 3679 spores) of salmon in Alfredo sauce to validate the identified cold spot using computer vision method.

Inoculation level of spores/tray	Process level	F_0 (min)	Number of processed trays	Number of positive trays*
1.0×10^6	Control Samples	N/A	6	6
1.0×10^6	Under Target	3.0	12	8
1.0×10^6	Target	6.0	12	0
1.0×10^6	Over Target	12.0	12	0

*: Indicated by gas production and characteristic odor (storage period: 7 days at 32 °C)

of processed foods showed survival of PA 3679 spores. In case of target (F_o = 6 min) and over target microwave sterilization processes no survivor was detected (Table 3).

5. Conclusions

A positive correlation between chemical marker (M-2) yield and color value equivalent to gray-scale value was observed during this study. Temperature measured at three different location identified by computer vision method signifies that color value can be used to represent the level of thermal treatments. The results of inoculation pack study supported the specified cold spots. Whey protein gel after adjusting the dielectric properties can be used to emulate the heating patterns for real food during microwave sterilization system. The computer vision method developed in this study, based on chemical marker (M-2) yield, accurately determined the cold spots location in salmon with sauce. Protocol developed in study to emulate the cold spot and heating patterns can be applied for other food systems as well. Microwave sterilization system delivered sufficient sterility at target $F_{o.}$ The heating patterns validation tests suggest that newly designed microwave sterilization system can be scaled up for the commercial application.

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Nomenclature

D	decimal reduction time (min)
E_a	activation energy (kcal mol^{-1})
$f: X \to R$	f is a function from X into R
$f \times g$	*Minskowski addition* of f and g
f / g	*Minskowski subtraction* of f and g
F_o	cumulative thermal lethality (min)
G	gray value
k	reaction constant
R	set of real numbers
t	time (min)
T	temperature (^oC)
x	elements of an point set
X	represent point sets
z	z-value (^oC)

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CONCLUSIONS AND RECOMMENDATIONS

A chemical marker (M-2) based computer vision method was developed to determine the locations of the cold spot in 915 MHz single mode microwave sterilization process. Kinetics study of the chemical marker M-2 formation was accomplished with mashed potatoes. Formation of chemical marker was observed as first order reaction. One step nonlinear regression modeling matched well with the experimental data of marker yield calculated at four temperatures. Reaction rate constant, energy of activation confirmed that chemical marker formation is slower than bacterial inactivation. 1.5 % D-ribose was most suitable with mashed potatoes for heating pattern analysis.

An IMAQ Vision Builder script was successfully developed to identify the cold spot locations in processed foods. A new image system was developed to minimize the interference by image system environment on heating patterns. Locations of the cold and hot spots specified by computer vision matched well with temperature measurements using fiber optics probes. The experiments results confirm that computer vision method (IMAQ Vision Builder) with the chemical marker M-2 and other accessories can be used as an effective tool to identify the location of cold and hot spots in microwave processed foods.

A sensitivity analysis was also accomplished to test the variability of salt content on the heating patterns. Addition of 1% salt into the mashed potato formulation changed the dielectric loss by three and half times but heating patterns were not affected by salt composition. Mathematically simulated heating patterns using finite difference time domain method (QW-3D) and finite element analysis supported the computer vision heating patterns. During microbiological validation, no survival of surrogates (PA 3679)

was detected in microwave processed tray after monitoring the identified cold spot using computer vision method.

Due to difference in energy of activation and z-value of chemical marker formation and bacterial inactivation, cumulative lethality (F_o) and chemical marker (M-2) formation were observed to be dependent on the path of heating. A unique relationship was observed among color value equivalent to grayscale value and chemical marker yield. Fresh mashed potatoes and saturated mashed potatoes sample were used as lowest and upper most point of the scale to facilitate the comparative heating patterns analysis for the trays processed at different microwave processing levels.

A comprehensive theoretical explanation with support of the experimental data was provided for application of this method to salmon. Salmon with Alfredo sauce was selected as a foods system to evaluate in preparation of a validated process and requisite the documentation for FDA filing of a scheduled process. Because of color change during microwave processing it was impractical to use salmon directly to determine the cold spot locations. Whey protein with adjusted dielectric properties was used to emulate the heating patterns in this particular food. A kinetic study to correlate the chemical marker yield and cumulative lethality with color value demonstrated the similar trend as mashed potatoes. Whey protein concentrate with 1% D-ribose was found more suitable to cover the time-temperature range of the microwave processing. Matching between the time-temperature profiles of adjusted whey protein gel and salmon confirmed that whey protein gel can be used to emulate the heating patterns.

The computer vision method developed in this study, based on chemical marker (M-2) yield, accurately determined the cold spots location in salmon with sauce. Protocol

developed in study to emulate the cold spot and heating patterns can be applied for other food systems as well. No microbial survivor was detected in sterilized foods at target F_o. Microbial validation showed that microwave sterilization system delivered sufficient sterility at target F_o. The heating patterns validation tests suggest that newly designed microwave sterilization system can be scaled up for the commercial application. Due to its cost effectiveness, consistency, fast speed and accuracy the computer vision method developed in this study can applied for industrial application and regulatory approval of the 915 MHz microwave sterilization system.

Microwave sterilization system developed at WSU has potential for approval as advanced thermal process for industrial application. The developed computer vision method based on chemical marker M-2 yield accurately determined the cold spot location in processed foods. Depending upon the requirement of industrial application and regulatory approval the future research work area may be focused in the following directions:

1. Identification of the critical factors for microwave process development.
2. Sensitivity of the computer vision heating patterns with critical factors.
3. Application of computer vision method to identify the cold spots in selected heterogeneous food systems e.g., beef with gravy.
4. Development of a procedure/method to evaluate the level of sterility given to each tray in continuous sterilization operation. Determining an index to test the uniformity of microwave sterilization process.
5. Testing/identification of the portable data tracer and equipments.

6. Facilitate the microwave system with video camera or tracking device to monitor or visualize the position of the tray during microwave processing.
7. Design/selection of the suitable packaging materials and machine.