Biological Validation of Thermal Processing in Bi-axially Rotating Cans Using Alginate Formulated Food Particles Suspended in Non-Newtonian Fluids

By

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ABSTRACT

Using thermocouples that are positioned within the test container, time temperature profiles can be easily obtained for establishing selected thermal processes. In bi-axial agitation processing of liquid particulate canned food products, modeling and biological validation are used as alternative methods to validate such processes. This involves processing of cans filled with alginate based simulated particles, with known counts of spores of heat resistant microorganisms uniformly distributed in their matrices, and computing the accumulated process lethality from their count reduction following processing. For this purpose, the heat resistance of two non-pathogenic surrogates microorganisms, *Geobacillus stearothermophilus* and *Clostridium sporogenes,* in two food matrices, carrot and meat alginate purees, was investigated and modeled using the conventional log-linear as well as Weibull models. D-values for *G. stearothermophilus* ranged between 1.9 and 40.8 min with a z-value of 11.7^{o}C and D-values for *C. sporogenes* ranged between 0.9 and 28.7 min with a z-value of 10.1^{o}C. For carrot alginates, D-values for *G. stearothermophilus* ranged between 1.9 and 42.6 min with a z-value of 11.5^{o}C and D-values for *C. sporogenes* ranged from 1.1 and 31.0 min with a z-value was 10.2^{o}C. The results were also fitted to Weibull model, but the model did not result in any better fit than the conventional first-order model.Alginate fabricated food particles need to have the appropriate textural rigidity to withstand the processing conditions and to have similar thermophysical properties to those of real food particles. Using a response surface methodology, optimum conditions of meat and carrot alginate fabricated particles giving the desirable hardness, adhesiveness and similar thermophysical properties to the real food particles were determined. Carrot alginate particles fabricated using these optimum conditions showed no changes in their hardness when subjected to thermal processing, unlike fresh carrot particles. Modeling of the associated heat transfer process required data on overall heat transfer coefficient (U) and fluid-to-particle heat transfer coefficient (h_{fp}) in the cans during the thermal process. Effect of process variables on U and h_{fp} in canned particulates suspended in non-Newtonian fluid undergoing bi-axial rotation was then evaluated in a pilot-scale rotary autoclave using spherical Nylon particles (d = 1.9 cm) and optimum heat transfer

conditions were determined. Retort temperature, rotational speed and carboxymethyl cellulose concentration were found to have a significant effect on U and h_{fp}. The heating rate of Nylon particles employed in the above studies was nearly matched with the experimental carrot and meat alginate fabricated particles (by varying the diameter of these particles) in order to select the alginate particles having similar heating behavior to the Nylon particles. Cans containing the simulated spore loaded alginate particles were processed in the rotary retort involving biaxial rotation of cans for pre-selected times to achieve approximate process lethality values (F_o) of 3 and 15 min. Using initial and final spore counts in the fabricated particles, the number of log reductions achieved and experimental F_o were computed. F_o values were also computed from the time-temperature data obtained from the heat transfer model based on h_{fp} and U values. The experimental and model predicted F_o values were statistically not different ($p > 0.05$) providing strength to the biological validation process.

RESUME

Les profils de température en fonction du. temps peuvent être facilement obtenus avec l'utilisation de thermocouples placés dans la boite d'essai pour l'analyze optimale des procédés thermiques. Dans le procédé d'agitation bi-axiale de boites de conserve contenant des particules, la modélisation et la validation biologique sont utilisées comme méthodes alternatives pour valider tels procédés. Cela implique la transformation de boîtes remplies avec des particules simulées à base d'alginate contenant un nombre connu de spores de microorganismes résistants à la chaleur, uniformément répartis dans leurs matrices, et le calcul de la létalité accumulée du processus par le calcul de la réduction du nombre des spores suivant le processus. A cet effet, la résistance thermique de deux micro-organismes non-pathogènes substituts, *Geobacillus stearothermophilus* et *Clostridium sporogenes*, dans deux matrices alimentaires, soit les purées de carotte et de viande avec alginate, a été étudiée et modélisée en utilisant le modèle classique log-linéaire, et le modèle de Weibull. Les particules d'alginate doivent avoir la rigidité appropriée pour résister aux conditions de transformation thermique et avoir des propriétés thermophysiques similaires à celles de différentes particules d'aliments. Grâce à une méthodologie de surface de réponse, des conditions optimales pour fabriquer les particules de viande et de carotte à base d'alginate, ayant la dureté et l'adhérence souhaitables ainsi que les propriétés thermophysiques des différentes particules d'aliments, ont été déterminées. La modélisation du processus de transfert de chaleur nécessite des données sur le coefficient général du transfert de la chaleur (U) et le coefficient du transfert thermique entre le fluide et la particule (h_{fp}) dans les boîtes de conserve au cours du processus thermique. L'effet des variables du procédé sur U et h_{fp} dans des boîtes de conserve avec des particules en suspension dans un liquide non-Newtonien subissant une rotation bi-axialle a ensuite été évaluée dans un autoclave rotatif à échelle pilote à l'aide des particules sphériques de Nylon (d = 1,9 cm) et les conditions optimales de transfert de chaleur ont été déterminées. Le taux de chauffage des particules de Nylon utilisées a été presque équivalent à celui des particules de carotte et de viande fabriquées à base d'alginate (en faisant varier le diamètre de ces particules) afin de sélectionner les

particules d'alginate ayant un comportement thermique similaire à celui des particules de Nylon. Les boîtes contenant des particules simulées d'alginate chargées de spores ont été traitées dans l'autoclave rotatif en mode de rotation biaxiale pour des durées prédéterminées pour atteindre des valeurs approximatives de mortalité (F_o) de 3 et de 15 min. Les nombres initial et final de spores dans les particules fabriquées ont permis de déterminer les réductions en log et la mortalité expérimentale. F_o a également été calculé à partir des données du temps vs. température obtenues à partir du modèle de transfert de chaleur basé sur des valeurs de U et h_{fp}. Les valeurs expérimentales et prédites du modèle n'ont pas été statistiquement différentes ($p > 0.05$) supportant la validité du processus de validation biologique.

CONTRIBUTIONS OF AUTHORS

Several presentations have been made based on the thesis research and some manuscripts have been prepared for publication. Two authors have been involved in the thesis and their contributions to the various articles are as follows:

Hussein Hassan is the PhD candidate who planned and conducted all the experiments, in consultation with his supervisor, gathered and analyzed the results, and drafted all the manuscripts for scientific publications.

Dr. H. S. Ramaswamy is the thesis supervisor, under whose guidance the research was carried out, and who assisted the candidate in planning and conducting the research as well as in correcting, editing, reviewing and processing the manuscripts for publications.

LIST OF PUBLICATIONS AND PRESENTATIONS

Part of this thesis has been prepared as manuscripts for publications in refereed scientific journals:

Hassan, H. and Ramaswamy, H. S., 2011. Heat resistance of *G. stearothermophilus* and *C. sporogenes* in carrot and meat alginate purees. Journal of Food Processing and Preservation (accepted).

Hassan, H. and Ramaswamy, H. S., 2011. Optimization of texture of carrot and meat alginate simulated particles for use in thermal processing biological validation studies. International Journal of Food Properties (accepted).

Hassan, H. and Ramaswamy, H. S., 2011. Evaluation and optimization of thermophysical properties of carrot and meat alginate simulated food particles. Journal of Food Engineering (accepted).

Hassan, H. and Ramaswamy, H. S. Effect of Process Variables on Heat Transfer Rates to Canned Particulate non-Newtonian Fluids during Free Bi-axial Rotary Processing (in preparation).

Hassan, H. and Ramaswamy, H. S. Biological validation of bi-axial rotation processing using carrot and meat alginate fabricated particles (in preparation).

Part of this thesis has been presented in scientific conferences:

Hassan, H. and Ramaswamy, H. S. Simulated particles for microbiological validation in rotary autoclaves. Journée scientifique et technique au Centre de Recherche de L'Agriculture (CRDA), St-Hyacinthe, Canada, March 2009.

Hassan, H. and Ramaswamy, H. S. Textural properties of meat alginate particles fabricated for process evaluation studies. Canadian Meat Science Association (CMSA) Annual Meeting, Montreal, Canada, May 2009.

Hassan, H. and Ramaswamy, H. S. Heat resistance of *Geobacillus stearothermophilus* and *Clostridium sporogenes* in carrot and meat alginate dispersions. Institute of Food Technologists (IFT) Annual Meeting, Anaheim, USA, July 2009.

Hassan, H. and Ramaswamy, H. S. Optimization of textural and thermophysical characteristics of carrot and meat alginate reconstituted particles used in the biological validation of thermal processing. World Congress of the International Commission of Agricultural and Biosystems Engineering, Quebec City, Canada, June 2010.

Hassan H. and Ramaswamy H. Effect of process variables on heat rates to canned particulate non-Newtonian fluids during free bi-axial rotary processing. Institute of Food Technologists (IFT) Annual Meeting, Chicago, USA, July 2010.

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… To my Lebanon and my Baalbeck.

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NOMENCLATURE

A	Surface area, m^3
BBI	Biological indicator unit
c	Can
CCRD	Central composite rotatable design
CMC	Carboxymethyl cellulose
CoV	Coefficient of variation
C_p	Heat capacity, Kj/Kg.^oC
CV	Coefficient of variation, %
D	Decimal reduction time, min
d_p	Diameter of the particle, m
DSC	Differential scanning calorimetry
EOE	End over end
f_h	Heating rate index, min
f_c	Cooling rate index, min
F	Heating time at temperature T, min
F_o	Heating time at 120^oC, min
F_s	Integrated lethality, min
FrAR	Free axial rotation
FxAR	Fixed axial rotation
H_{ap}	Apparent heat transfer coefficient between retort and particle, W/m^2.^oC
h_{fp}	Fluid to particle heat transfer coefficient, W/m^2.^oC
J_{ch}	Heating lag factor
J_{cc}	Cooling lag factor
l	Liquid
N_o	Initial number of microorganisms, CFU/ml
N	Final number of microorganisms, CFU/ml
n	Number of log reductions
p	Particle
Q	Heat flux, W/m

r	Retort
RS	Rotation speed, rpm
RSM	Response surface methodology
R^2	Coefficient of determination
T	Temperature, ^oC
t	Time, s
TDT	Thermal death time, min
TPA	Texture profile analysis
TTI	Time temperature integrator
U	Overall heat transfer coefficient, $W/m^2.^oC$
U_a	Apparent overall heat transfer coefficient, $W/m^2.^oC$
z	Temperature sensitivity, ^oC

Greek symbols

α	Thermal diffusivity, m^2/s
β	Shape parameter in Weibull model
ρ	Density, kg/m^3
κ	Thermal conductivity, W/m.K

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CHAPTER 1
INTRODUCTION

Thermal processing or canning is one of the most effective methods to preserve foods from harmful microorganisms by applying heat, in combination with pH, vacuum, water activity or chemical modifications, for long enough time at a high enough temperatures (Ramaswamy and Marcotte, 2005). Conventional canning involves filling of the food product in metal cans, glass jars, retortable semi-rigid plastic containers or pouches, double seaming or heat sealing, followed by heating and cooling in a pressurized batch or continuous retorts, resulting in commercially sterile and shelf stable products. In low-acid foods, the target microorganism is *C. botulinum*, which, if not destroyed by heat, will produce the *botulinum* toxin. With consumers becoming more educated and health conscious, the demand for convenient and high quality foods has increased over time. Canning usually involves excessive thermal treatment of the product, resulting in degradation of color, flavor, texture and nutritional value (David et al., 1996). The quality of canned food can be improved by applying high temperature-short time (HTST) processing, as quality factors are more resistant to heat than the microorganisms, and their temperature sensitivity is less severe than those of microbial spores. HTST processing techniques include aseptic processing, agitation processing and thin profile packaging. Aseptic processing consists of heating the food without any package to a high temperature, holding it for a short time, cooling down and packaging it into a sterilized container in a sterile chamber (Lund, 1987). Thin profile packaging is getting popular. In this type of packaging, retort pouches and semi-rigid containers are used, where the heat transfer is faster because containers have larger surface area as compared to the conventional cans (Ramaswamy and Marcotte, 2005). In agitation processing, mixing is enhanced inside the cans placed in rotary retorts. A key factor in mixing the container's content is the headspace bubble of the cans. The modes of rotation include end-over-end, fixed axial and free bi-axial rotation. In the industry, employing bi-axial continuous agitation processing using FMC Turbo cookers results in a faster heat transfer in liquid-particulate canned food products compared to conventional batch processing; thus, shorter processing time, better quality retention and lower energy consumption will be achieved.

In order to successfully design a thermal process for a canned food, heat penetration data are required. Mathematical models can be used to predict the transient heat penetration of canned foodstuffs; therefore, they can reduce the cost and number of experiments that are necessary to achieve product safety and quality (Teixeira et al., 1969). For liquid particulate canned foods, both overall heat transfer coefficient from the retort medium to the canned liquid, U, and the fluid to particle heat transfer coefficient, h_{fp}, are needed in order to predict heat transfer rates to the particle at the coldest point inside the can. Since it is practically difficult to monitor the transient temperature history of the particle moving inside an agitating can, h_{fp} is one of the important gaps in our knowledge of heat transfer (Maesmans et al., 1992). The effect of different product and process parameters, including rotation speed, retort temperature, headspace volume, liquid viscosity, particle size and concentration on the associated heat transfer coefficients in agitation processing was found to be significant (Anantheswaran and Rao, 1985; Lekwauwa and Hayakawa, 1986; Britt, 1993; Sablani and Ramaswamy, 1995, 1996, 1997; Meng and Ramaswamy, 2007a,b; Dwivedi, 2008). Very few studies have been conducted on the determination of heat transfer coefficients in cans subjected to free axial rotation (Lenz and Lund, 1987; Deniston et al., 1987; Fernandez et al., 1988; Hassan, 1984; Stofors and Reid, 1992; Dwivedi and Ramaswamy, 2008); therefore, there is still a good opportunity for more research in this area.

In free bi-axial rotation, there are two levels of rotation, one at the can level and another one at the cage level when the cans move in a rotary fashion along with the cage. In case of liquid particulate canned food products subjected to free axial rotation, temperature data collection is challenging due to difficulties involved in attaching the temperature measuring devices to the liquid and particles. Due to the difficulty of collecting time temperature history at the particles centers in both systems, biological validation may be necessary (Chandarana, 1992). Nowadays, immobilizing indicators in a matrix of alginate food puree forming a particle and then calculating process lethality using initial and final counts is the most commonly used technique in biological validation (Brown et al., 1984). Alginate exists naturally in the cell wall of brown algae and commercially in the form of sodium salt. Sodium alginate is water soluble, producing viscous solutions and is used as a thickening and stabilizing agent in the food industry.

In the presence of calcium ions, sodium alginate has the ability of forming gels (Phillips and Williams, 2000). Reconstituted alginate food particles possess enough mechanical strength to withstand high processing temperatures and have similar physical and thermal properties to the real food particles. In addition, in contrast to real food particles, simulated particles normally have uniform size and spore concentration (Marcotte et al., 2000). No previous studies were carried out on the optimization of fabricating meat and carrot alginate simulated particles.

Few studies on sterilization processes have used marker microorganisms instead of *C. botulinum* spores because not only of the hazards associated with their handling, but also of the very low number of surviving spores that would result from a commercial process because of their small D-value. In biological validation, when using *C. botulinum*, the marker organism can be spores of *Geobacillus stearothermophilus* (D = 5-6 min) or *Bacillus sporogenes* (D = 1-2 min), which have close z-value (10^{o}C) to the target microorganism. Chemical environment, including the pH, NaCl concentration, sugar and fat concentration, has a significant effect on the heat resistance of the bacterial cell or spore. Hence, the food composition would have a dominating effect on the destruction kinetics of microbial spores intended for biological validations and no such data are available in the literature for meat and carrot alginate infused preparations (Stumbo, 1973).

Based on the available knowledge and recognizing the need for biological validation studies on free axial agitation processing involving canned food alginate fabricated particles suspended in non-Newtonian fluids, the following general objectives were formulated for this study:

1. Studying the heat resistance of *Geobacillus stearothermophilus* and *Clostridium sporogenes* in carrot and meat alginate slurries.
2. Evaluating the relationship between levels of alginate addition, concentration of calcium chloride solution and holding time in the calcium chloride solution on the textural and thermophysical properties of formulated carrot and meat alginate particles in order to obtain particles of desired structural integrity and stability.

3. Optimizing the heat transfer to non-Newtonian liquid particulate cans subjected to fixed and bi-axial rotation.
4. Biologically validating free axial agitation processing by comparing calculated and predicted process lethality values in carrot and meat alginate formulated spores-inoculated particles.

No such detailed studies exist with processing of liquid particulate cans subjected to thermal processing under bi-axial rotation. This includes all steps from preparation of spore impregnated particles filled in cans and subjected to bi-axial rotation processing under conditions which have been characterized and optimized to permit computation and comparison of process lethality both by experimental approach (through spore count-recount from simulated particles) and modelling (using validated thermophysical and heat transfer property values).

CHAPTER 2

LITERATURE REVIEW

2.1 Thermal Processing

2.1.1 General overview

Thermal processing is a traditional, yet among the most effective methods to preserve foods. In 1973, Stumbo defined thermal processing as "the application of heat for the purpose of shelf-life extension and promotion of safety of food". Thermal processing involves heating foods for a specific time and temperature in order to destroy pathogenic and spoilage causing microorganisms. When referring to thermal process, the term "sterile" is often used; however, it is not completely accurate since sterility implies the full destruction of all microorganisms. That is why the term "commercially sterile" is more appropriate to use as the thermal process is designed to kill the microorganisms of public health concern and those causing spoilage (Ramaswamy and Marcotte, 2005).

In 1998, twenty four billion food cans were produced and more than eleven billion cans were used to ship fruits, vegetables and juices. Thermal processing in cans/glass jars was discovered by Apert in 1809 and since then, food canning industry has witnessed remarkable changes in terms of processing methodology, equipments, energy efficiency and product quality and safety (Judge, 2001).

In 1864, the scientific foundation for thermal processing was established when Louis Pasteur discovered that the microorganisms and enzymes can be destroyed by heat. The first method to calculate minimum process conditions for sterilization was given by Bigelow in 1920 and was called "General method". In 1923, Ball presented a "Formula method", which is a mathematical method in order to determine sterilization process. In 1950s, Stumbo revised the "formula method" and made the process calculation more accurate. In 1957, Ball and Olson published an important book on thermal processing. Since then, the mathematics of heat process determination concepts and application are being refined (Dwivedi, 2008).

2.1.2 Principles of thermal processing

2.1.2.1 Pasteurization and Sterilization

In order to successfully establish a thermal process for a canned food, understanding the heat transfer and knowing microbiology are the essential basics. During thermal processing, most spoilage-causing and pathogenic microorganisms are destroyed in a container, where an environment is created in order to inhibit the growth of remaining pathogens and/or their spores. For instance, low oxygen level in the hermetically sealed cans creates a non-supporting environment of the aerobic microorganisms; thus, the target should be the anaerobic microorganisms, which are pH dependent.

From a thermal processing point of view, foods are divided into three groups: high-acid, medium-acid and low-acid foods. Examples of each group are presented in Table 2.1.

Table 2.1 Classification of foods based on pH (Ramaswamy and Abdelrahim, 1991)

pH class	Typical Foods
High Acid pH < 3.7	Apple, apple juice, apple cider, apple sauce, berries, cherry, cranberry juice, cranberry sauce, fruit jellies, grapefruit juice, grapefruit pulp, lemon juice, orange juice, pineapple juice, sour pickles, vinegar
Acid 3.7 < pH < 4.4	Fruit jams, fruit cocktails, grapes, tomato, tomato juice, peaches, pineapple slices, potato salad, prune juice, vegetable juice
Low Acid pH > 4.5	All meats, fish, vegetables, mixed entrees (beans and pork, chicken with noodles, etc), most soups

In low-acid foods, the target problem microorganism is *C. botulinum*, which, in case not destroyed, can produce the deadly *botulinum* toxin. *C. botulinum* can neither grow nor produce *botulinum* when the food pH is lower than 4.5; therefore, it has been generally recognized that the dividing pH between the low and high acid groups is 4.5 as

when the pH is less than 4.5, it's not necessary to worry about *C. botulinum*. Other more heat resistant microorganisms may include *B. stearothermophilus*, *B. thermoacidurans* and *C. thermosaccolyaticum*, but these are of little concern if the storage temperature of the processed cans is less than 30^{o}C as these are thermophilic bacteria (optimal growth temperature: 50-55^{o}C). Pasteurization can be enough for the low-acid foods (pH > 4.5), but in this case, only vegetative microorganisms are destroyed and the spores remain active; thus, products can be stored for short-term under refrigerated conditions only. In medium-acid and acid (pH < 4.5) foods, *C. botulinum* cannot grow and the target microorganism is usually the heat resistant, spoilage type vegetative bacteria or enzymes, which can easily be destroyed by pasteurization (Ramaswamy and Abdelrahim, 1991).

Determining the proper sterilization process time and temperature for a can is a complex procedure as there are numerous factors involved. These factors include the type and resistance of the target microorganism, pH of the food, storage conditions after the process, heating conditions, thermophysical properties of the food and container shape and size (Sablani, 1996).

In order to produce a commercially sterile canned food, authorities require establishing a process schedule that includes thermal process parameters, namely product initial temperature, process temperature, process time and any critical factors that might influence the achievement of commercial sterility (Sablani, 1996).

2.1.2.2 Microbial Destruction Kinetics

In order to establish an appropriate thermal processing schedule at a specified temperature, it is essential to know beforehand the thermal destruction rate of the test microorganism or enzyme under the existing conditions in the can. Earlier evidence support that the inactivation of microorganisms follows the first order reaction kinetics:

$$\ln (N / N_o) = -k\text{t} \qquad (2.1)$$

which can be also expressed as:

$$\log_{10} (N / N_o) = -k\text{t} / 2.303 \qquad (2.1a)$$

or

$$N / N_o = e^{-kt} \qquad (2.1b)$$

where N_o is the number of microorganisms at time zero and N at time t.

Survivor curve, decimal reduction time and thermal death time

The survivor curve is a straight line plot of the logarithm of the number of surviving microorganisms against the heating time at a particular temperature (Figure 2.1)

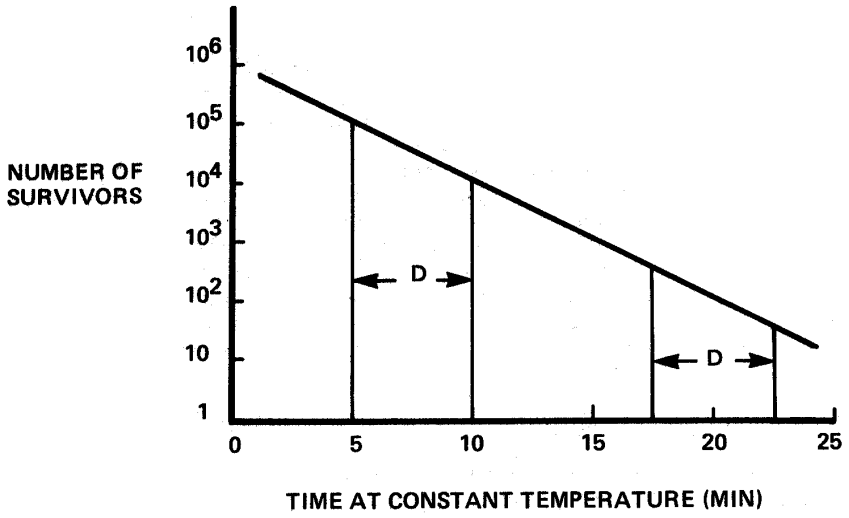


Figure 2.1 A typical survivor curve

At any given temperature, the microbial destruction rate can be defined as decimal reduction time, D, which is the heating time needed to decrease the number of microorganisms by one log cycle i.e. by one decimal reduction (Equation 2.3)

$$D = \frac{t2 - t1}{\log \frac{N1}{N2}} \qquad (2.2)$$

Where:

N_1: number of survivors after heating treatment for time t_1 (min)

N_2: number of survivors after heating treatment for time t_2 (min)

The complete destruction of any bacterial population is not possible due to the logarithmic nature of the survivor curve. In other words, after an infinite number of D-values, a decimal fraction of the population will remain. Thermal death time (TDT) (Equation 2.3) is a term used in food microbiology pointing out to the heating time required to cause a complete destruction of microorganisms, which can be demonstrated by the failure of a given population to grow in culture media after the heat treatment.

$$TDT = nD \tag{2.3}$$

where n is the number of decimal reductions.

For example, if TDT represents time to reduce the population from 10^{10} to 10^{0}, TDT would be a measure of 10 D-values.

Temperature dependence and z-value

The temperature sensitivity of D-values is expressed in form of z-value, which is the temperature range resulting in one log reduction in D-values (Equation 2.4). Graphically, z-value is the temperature range through which D-value passes though one log cycle (Fig. 2.2).

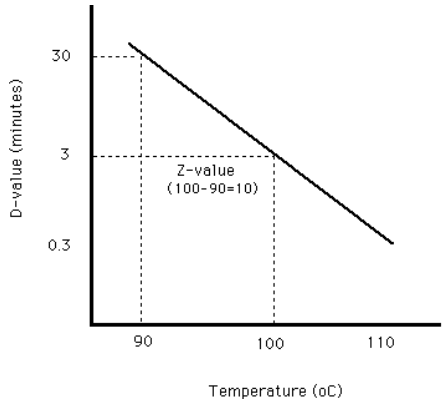


Figure 2.2 A typical thermal resistance curve

Mathematically:

$$z = \frac{T2 - T1}{\log \frac{D1}{D2}} \quad (2.4)$$

where:

D_1: D value at temperature T_1

D_2: D value at temperature T_2

Process Lethality

In a real process, the food passes through a time-temperature profile and the lethal effect of temperatures is integrated over the heating time in order to give process lethality, F_o. The sterilization process is measured using F_o value. A unit of lethality is equivalent to heating 1 min at a reference temperature T_o – 121°C / 250 F. At T_o, the integrated process lethality can be obtained using Equation 2.5:

$$F_o = F * 10^{(T\text{-}To)//z} \quad (2.5)$$

where:

T_o: reference temperature

F: heating time at temperature T

Therefore, at a z-value of 10°C, an F value of 10 min at 115°C is equivalent to an F_o value of 2.78 min; whereas, an F value of 10 min at 125°C is equivalent to a F_o value of 27.8 min.

The minimal F_o must be based on two microbiological considerations. First consideration is decline in the number of spoilage causing bacteria. Second one is destruction of pathogens of public health concern. In low-acid foods, the microorganism of concern is *C. botulinum* and it has been randomly established that the minimum process should reduce the *C. botulinum* contamination by 12D. A D-value of 0.21 min at 121°C is assumed for *C. botulinum*; thus 12D equals to 2.52 min, which is the minimum process lethality (F_o) required. However, for low acid foods, it is more common to have

an F_o of 5 min so that more heat resistant microorganisms, which are not of public health concern, will be destroyed and an F_o of 5 min would achieve 5 decimal reductions with reference to those spoilage microorganisms. That's why, the quality of raw materials must be controlled so that the initial count of these microorganisms remain below 100 in each container in order to keep the spoilage rate below one can in a thousand (Meng, 2006).

2.1.2.3 Characterization of heat penetration data

In order to establish a thermal process schedule, heat penetration data must be obtained through the use of copper-constantan thermocouples that are inserted into the product center through the can wall. An example of product time temperature profile is shown in Figure 2.3.

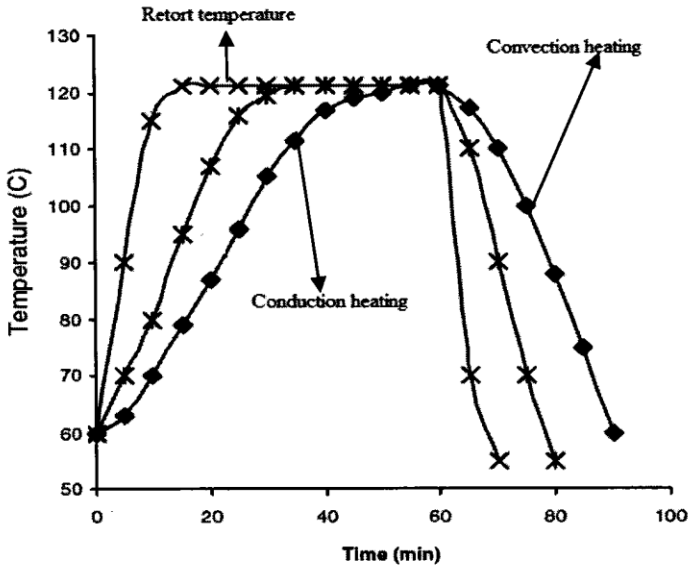


Figure 2.3 Example of time temperature profile in the retort, liquid and particle centers

The accuracy of the time temperature profile depends on the thermophysical properties of the food product, size and shape of the container and the operating conditions of the retort; therefore, it is essential to have cans that are as close as possible to the commercial product. Process time can be calculated by determining heating and cooling rate indices (f_h and f_c) and lag factors (j_{ch} and j_{cc}) from the heat penetration data. It was proved that on a linear scale, when the logarithm of the retort-product center

temperature difference (T_r-T), known as temperature deficit, is plotted against time, a straight line is created after an initial lag. By extending this straight line to the Y axis (T_r-T), intercept, T_{pih}, is obtained and j_{ch} can thus be calculated, as follows:

$$jch = \frac{Tr - Tpih}{Tr - Tih} \tag{2.6}$$

where j_{ch}, called heating lag factor, is a measure of the delay of the beginning of uniform heating in the product. j_{cc} is called cooling lag factor and is the corresponding value for j_{ch} during cooling.

$$jcc = \frac{Tw - Tpic}{Tw - Tic} \tag{2.7}$$

Part of the lag is due to the slow come-up time of the retort and this is accounted for by determining the new zero time for the process. Ball and Olson (1957) used 58% of the come-up time as useful input to the process and it is widely accepted (Holdsworth, 1997). In other words, 42% of the come up time should be added to the process time at retort temperature.

Heating rate index, f_h, is the negative reciprocal of the slope of the line in Figure 2.4. It represents time required for the curve to pass one log cycle and it is an indicator of the heating rate. Higher f_h implies longer time for the log to traverse one log cycle; therefore, a lower heat penetration rate into the product.

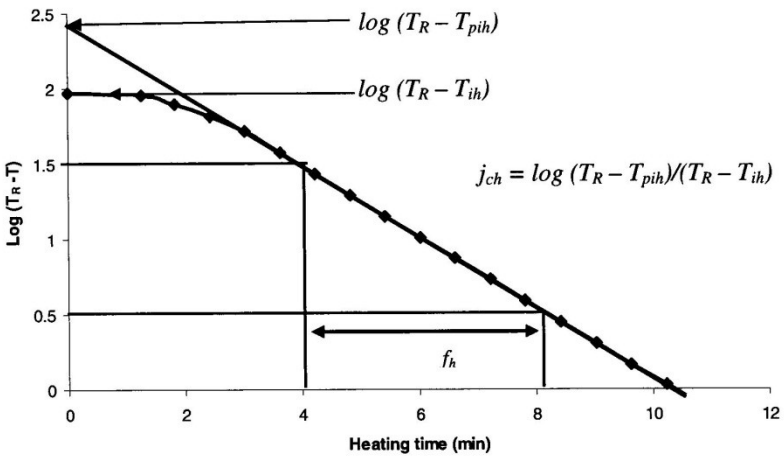


Figure 2.4 Heating curve

The corresponding value for the cooling period is f_c, the cooling rate index (Figure 2.5)

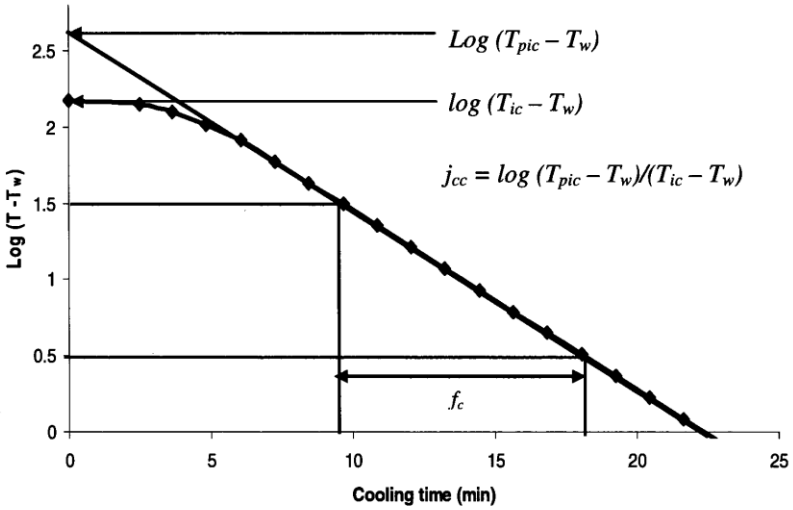


Figure 2.5 Cooling curve

2.1.2.4 Thermal process calculation

In order to understand the thermal process, heat penetration data of a specific food product and thermal destruction kinetics of target microorganisms are used (Figure 2.6).

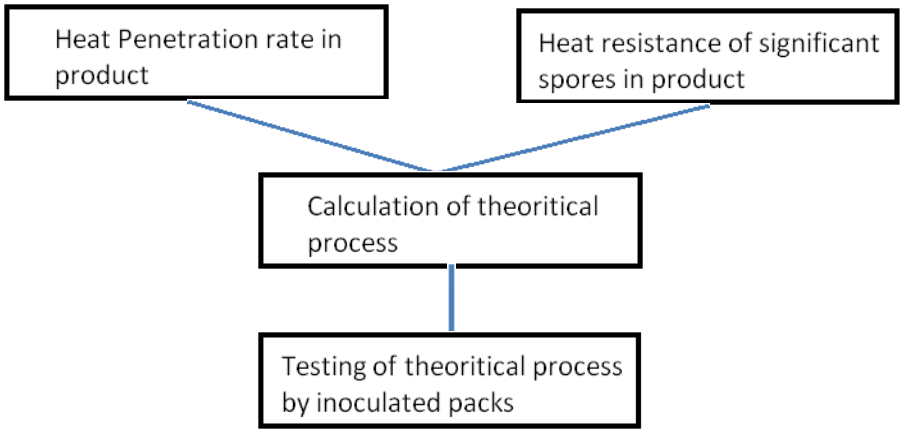


Figure 2.6 Establishment of adequate thermal process (Lopez, 1987)

The objective of the thermal process calculations is to establish the required process time under a given set of heating conditions, resulting in required process

lethality, or to approximate the lethality achieved by a process. In a processing situation, the product goes through a temperature ramp and the process calculation methods integrate the lethal effects of the transient temperature profile. In general, the desired degree of lethality F_o is pre-established and the processes are designed to bring the minimum of this value to the thermal center. The process calculations are divided into two groups: General methods and Formula methods. In the General methods, the lethal effects are integrated by either a graphical or numerical integration procedure, based on time-temperature data obtained from test containers under real commercial processing conditions. On the other hand, in the Formula methods, the parameters obtained from time-temperature data along with many mathematical procedures are used in order to integrate the lethal effects (Ramaswamy et al., 1992).

2.1.3 Food quality

The main objective of the thermal processing is to destroy the pathogenic and spoilage causing microorganisms; however, there has been an increasing concern among consumers regarding the lower quality of thermally processed canned products. This concern has resulted in numerous studies on minimizing this quality degradation in canned foods (Sablani, 1996).

Exploiting the higher sensitivity of microorganisms than quality factors, such as color, texture, flavor and nutrients, to the elevated temperatures, high temperature short time (HTST) and ultra high temperatures (UHT) processes have been developed in order to minimize quality degradation of canned food products while ensuring safety (Reuter, 1993). HTST and UHT concepts have been successfully applied in liquid foods and not in liquid particulate canned food products because of the relatively slow rate of heat transfer and the existence of large temperature gradients between the surface and center of the container (Teixeira et al., 1975).

Aseptic processing consists of heating the food to a high temperature, holding for a short time, cooling down and packaging into a sterilized container in a sterile chamber. Due to the fact that foods are sterilized without any package in aseptic processing, measures can be taken to improve the heat transfer. These methods include high

efficiency heat exchangers, such as plate heat exchangers, scraped surface heat exchangers and tubular heat exchangers. The application of this type of HTST processing is currently limited to liquid and not for liquid particulate canned food products due to remaining problems, such as heat transfer coefficients of the particles and residence time distribution (Ramaswamy et al., 1997). In order to solve these problems, other technologies, such us microwave heating and ohmic heating, have been tested. Aseptic processing has been used successfully in convective heating of liquid food products and not in conductive heating of solid ones as the heat transfer in the latter is relatively slower and large temperature differences exist between the surface and center of the container. Alternatives include thin profile packaging and agitation processing (Willhoft, 1993).

Thin profile packaging is gaining popularity. In this type of packaging, retort pouches and semi-rigid containers are used, where the heat transfer is faster because containers have larger surface area compared to the ones used in conventional processing and the distance for the heat to get the coldest point is shorter. Despite its disadvantages of slow line, labor-demanding operations and fragile packages, thin profile packaging is considered a potential alternative to aseptic processing (Ramaswamy and Marcotte, 2005). During the thermal process, internal pressures of these containers might exceed the saturation pressure of the steam due to the residual air. Also, during the cooling phase, the internal pressure might be higher than the external one resulting in sudden pressure drop in the retort; thus, deformation of containers, loss of seal integrity and chance of explosion of containers will take place, in case the internal pressure is not counterbalanced with the external one. The counterbalancing is achieved through the compressed air (Sablani, 1996).

In agitation processing, rotary retorts can improve the heat transfer in liquid-particulate canned food products, resulting in shorter processing time, better quality retention and lower energy consumption. This type of processing is another alternative to aseptic processing for liquid particulate canned food products.

2.1.4 Type of retorts

2.1.4.1 Still retorts

A still retort is non-agitating, batch-type, vertical or horizontal pressure vessel used to process hermetically sealed containers. In general, containers are put in racks or trays in order to load or unload the retort. In order to get the high temperature needed for commercial sterilization, steam or super heated water under pressure is used.

2.1.4.2 Agitating/rotary retorts:

In order to increase the heat transfer to the product, mechanical agitation is used. Types of agitation include end-over-end (EOE), fixed axial, free axial and Shaka system.

End-Over-End rotation

Clifcorn et al. (1950) suggested using EOE rotation in order to increase the heat transfer to canned food products. EOE involves rotating sealed cans vertically around a circle (Figure 2.7a). As the can rotates, the headspace bubble moves along the length of the can resulting in the agitation of the can content.

Free axial rotation

Continuous types of retorts are constructed with at least two cylindrical shells, where processing and cooling take place and where cans are subjected to axial rotation. The Sterilmatic (JBT Corp. / FMC Corp., San Jose, ZA) is a widely used continuous agitation retort where an entering can is carried along by a revolving reel. The Steritort is a pilot scale simulator of the Sterilmatic series. There are three phases of motion in a Steritort: fixed reel, transitional and free reel motion across the retort bottom (Figure 2.7b). The fixed reel motion takes places over 220^{o} of the cycle, the free rotation over the bottom 100^{o} and the transitional phase on either side of the free rotation. Advantages of continuous retorts over batch retorts include higher production rate, less floor space, less consumption of steam and water and reduced labor. Continuous retorts consist of a system where can enter the retort, get indexed into a revolving reel and is moved

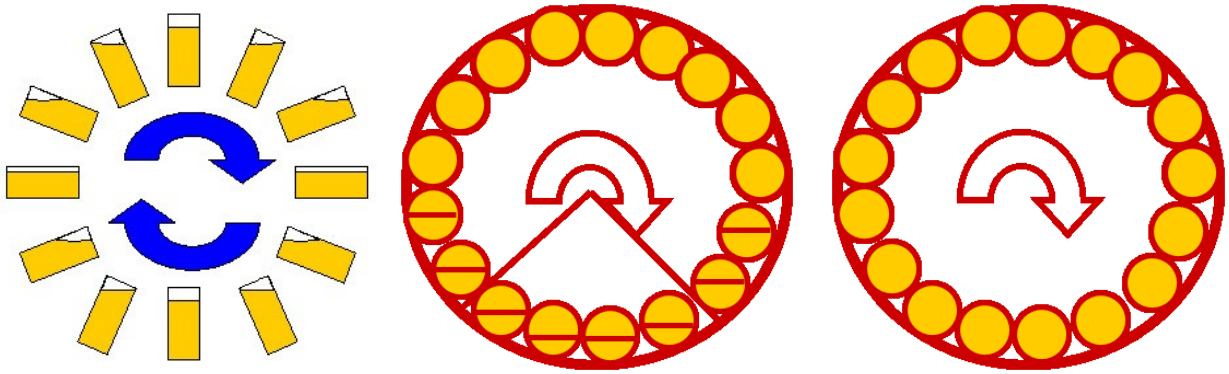
throughout the retort in a spiral pattern. In this system, agitation is provided by allowing the cans to roll freely across the bottom of the retort (Dwivedi, 2008).

Fixed axial rotation

In fixed axial rotation, the sealed can is rotated horizontally around a circle in a single direction (Figure 2.7c). As the can rotates, the headspace bubble moves along the length of the can resulting in the agitation of the can content.

Shaka system

Using Shaka system, sealed cans are rapidly agitated inside the retort. It consists of filling the retort with sealed cans and then shaking vigorously in a back and forth motion, resulting in thorough mixing and rapid heat transfer. In comparison with rotary agitation, using Shaka system results in better heat transfer rates presumably due to greater turbulence (Dwivedi, 2008).



(a) **(b)** **(c)**

Figure 2.7 Different modes of agitation inside STOCK retort

2.1.5 Heat Transfer Coefficients U and h_{fp}

There are three forms of heat energy transfer: conduction, convection and radiation. In the retorts used for canned food products, conduction and convection forms are involved. From the heat transfer point of view, there are three categories of foods:

solid foods, such as fruits, vegetables and meats which are heated by conduction, liquid foods, such as milk and soups which are heated by convection and liquid particulate foods, such as fruits in syrup and vegetables in brine which are heated by convection and conduction.

In liquid particulate foods, heat is first transferred from the can wall to the liquid inside the can by convection. Overall heat transfer coefficient, *U*, is used to describe the heat transfer between the heating or cooling medium in the retort and the liquid in the can. Then, heat is transferred from the liquid to the particle surface by convection and from the particle surface to the particle center by conduction. h_{fp} is used to express the liquid to particle heat transfer coefficient (Meng, 2006).

In order to establish the process schedule, it is required to have both the kinetics of thermal destruction of microorganisms and the time-temperature profile at the coldest point of the product. Time-temperature data can be measured using devices that monitor the temperature changes in the food product. Many studies have been conducted in order to understand the time-temperature relationships for pure conduction or convection products, but few have been done on liquid particulate products. The data obtained from these studies cannot be generalized as they are specific for the type and formulation of food, the containers size and the kind of retort used. In addition, mathematical models can be used to get the time-temperature data at the coldest point of the product. These models can be important in designing, optimizing and validating the thermal food processing. Also, they are essential in order to reduce the number and costs of experiments needed to achieve product safety and quality (Clark, 1978).

The overall heat transfer coefficient (*U*) between the heating medium and liquid inside the can is calculated by using the following equation: (Equation 2.8)

$$U A_c (T_R - T_l) = m_l c_{pl} \frac{dT_l}{dt} + m_p c_{pp} \frac{d\langle T_p \rangle}{dt} \qquad (2.8)$$

where:

T_l: bulk mean temperature of the liquid in the container

T_R: temperature of the heating medium

T_p: temperature of the particle

A_c: can surface area

m_l: mass of the liquid in the can

m_p: mass of the particle in the can

C_{pl}: specific heat of the liquid in the can

C_{pp}: specific heat of the particle in the can

The fluid to particle heat transfer coefficient h_{fp} can be defined with the following equation (Equation 2.9):

$$m_p C_{pp} \frac{d<T_p>}{dt} = h_{fp} A_p (T_l - T_{ps}) \quad (2.9)$$

Heat transfer mode inside the particle is conduction and the temperature of the particle is given by (Equation 2.10)

$$\frac{\partial T}{\partial t} = \alpha_p \nabla^2 T \quad (2.10)$$

With initial and boundary conditions

$$T = T_i \qquad \text{at } t = 0 \quad (2.11)$$

$$-k_p \frac{dT}{dt} = h_{fp} (T - T_l) \quad (2.12)$$

2.1.5.1 Determination of U and h_{fp}

Based on how well *U* and h_{fp} are calculated under simulated process conditions then the prediction of particle center temperature through mathematical models would be accurate. Usually, *U* and h_{fp} are determined from measuring the temperature responses of the particle and liquid under well characterized initial and boundary conditions (Maesmans et. al, 1992). In general, particle and liquid temperatures are measured using

thermocouples. When the particle is attached to rigid thermocouple, it will not simulate the motion in rotary retorts; thus, there will be deviations in the measured U and h_{fp}. In order to determine the particle temperature while moving inside the can, many attempts have been made. In addition to the difficulties in measuring moving particle temperature in rotary retorts, there is a complexity in solving the governing equation of the energy balance in the can containing liquid and particles because of the time variant temperature of the can liquid (Sablani, 1996).

h_{fp} is determined using an inverse heat transfer approach where the boundary condition is determined using measured transient temperatures. The governing partial differential equations, which describe the conduction heat flow inside the particle with appropriate initial and boundary conditions, have to be solved. Experimental data needed for this analysis are: the liquid and particle transient temperatures and the particle thermophysical properties. The liquid bulk temperatures are measured using a needle-type copper constantan thermocouple whose tip is placed at the can center; whereas, the particle transient temperatures are measured using thermocouple embedded in the particle center (Sablani, 1996).

2.1.5.2 Temperature measurement

Several studies have used a fixed particle at the geometric center of the can due to difficulties in measuring the temperature history of the particle without affecting its movement (Lenz and Lund, 1978; Lekwauwa and Hayakawa, 1986; Deniston et al., 1987; Fernandez et al., 1988). Recently, many technologies have been developed so that temperature history of the particle can be monitored without affecting its movement.

From liquid temperature only

In cans subjected to axial rotation, Stoforos and Merson (1990) used a mathematical method that requires the liquid temperature only in order to determine U and h_{fp}. They solved an overall heat balance equation (Equation 2.8) for a can and the differential equation for a spherical particle with appropriate initial and boundary conditions (Equations 2.8 - 2.12). Since the can liquid temperature depends on U and h_{fp},

they estimated the heat transfer coefficients by varying these coefficients and minimizing the errors between experimental and predicted liquid temperatures.

Liquid crystal

In order to determine U and h_{fp}, Stoforos and Merson (1991) used a liquid crystal whose color changes with temperature in order to monitor particle surface temperature. Their method involves coating the particle surface with an aqueous solution of liquid crystals, videotaping the color changes of the particle surface as a function of temperature and comparing them with standard color chart after calibration. Experiments were carried out in a temperature range of 20 to 50^{o}C. Using this method, there were no restrictions on particle motion.

Time Temperature Integrators (TTIs)

In order to determine convective heat transfer coefficient U, combining time temperature integrator (TTI) and a mathematical model has been proposed. TTIs can be in the form of microorganisms, chemicals or enzymes. In 1992, Weng et al. used TTI in the form of immobilized peroxidase in order to determine U and h_{fp} in cans at pasteurization temperatures. A polyacetal sphere loaded with the indicator at the center was attached to a thermocouple and placed at the can geometric center. They calculated the time-temperature history and associated lethality from the equation of heat conduction, with assumed h_{fp} and known thermophysical properties. Then, h_{fp} was modified and lethality was re-calculated until the difference between predicted and calculated lethalities fell within a tolerance limit. They called this approach as Least Absolute Lethality Difference (LALD). Furthermore, during heating and cooling phases in the same experiments, they collected transient temperature data for liquid and particle and estimated h_{fp} by minimizing the sum of least squared temperature difference (LSTD) between measured and predicted particle center temperatures using the mathematical model. Haentjens et al. (1998) and Guiavarac'h et al. (2002) used α-amylase enzyme at reduced water content as TTI.

2.1.5.3 Factors affecting U and h_{fp}

In rotary retorts, faster heat transfer to both liquid and particles and better quality retention compared to still retorts are due to mechanical agitation of cans. Few studies have determined *U* and h_{fp} in thermally processed liquid particulate canned food products. Many papers have been published regarding liquid foods and the effect of system and product parameters on *U* (Quast and Siozawa, 1974; Duquenoy, 1980; Naveh and Kopelman, 1980; Soule and Merson, 1985; Anantheswaran and Rao, 1985, Rao et al., 1985, Sablani and Ramaswamy, 1995, 1996, 1997, Meng and Ramaswamy 2005, 2007a, 2007b, Dwivedi, 2008) and a review paper has been published by Rao and Anantheswaran (1988). On the other hand, the studies done on convective heat transfer in the presence of particles have focused only on the liquid portion of the can by determining the system and product parameters on *U* only (Berry et al., 1979; Berry and Bradshaw, 1980; Berry and Dickerson, 1981).

Rotational speed

In early literature, the effect of rotational speed on heat transfer rate of liquid and liquid-particulate canned food products has been well documented (Conley et al., 1951). Lenz and Lund (1978) studied the effect of rotation speed on heat transfer coefficients in cans subjected to axial rotation at 121^{o}C. As the speed increased, both *U* and h_{fp} increased in all processing conditions. On average, there was an increase of 33% in *U* and 44 % in h_{fp} upon increasing the rotation speed from 3.5 to 8 rpm. The effect of rotational speed was more evident at lower particle concentration. Hassan (1984) measured U with Teflon, aluminum and potato spheres in a can subjected to axial rotation and found out that increasing the rotational speed from 9.3 to 101 rpm had more effect on *U* than on h_{fp}. h_{fp} was found to be highest at 9.3 rpm, intermediate at 101 rpm and lowest at 55.5 rpm in the case of 34.9 mm diameter potato spheres with 30% particle concentration and 25.4 mm diameter Teflon spheres with 20% particle concentration (Maesmans et al., 1992).

Upon increasing the rotational speed from 9.3 to 101 rpm, Deniston et al. (1987) found out that *U* increases 1.2-2.0 times and the effect was greater with larger size particles, but the h_{fp} values measured were insensitive to the rotational speed changes.

This was attributed to small relative particle to liquid velocity because of (1) restricted movement of the particle attached to rigid thermocouple, (2) particle settling as liquid and particle densities were close; thus, the gravity was minimal and (3) small centrifugal force acting on the particle as it was located in the center.

Stoforos and Merson (1992) found out that upon increasing the rotational speed from 15.5 to 100 rpm in axial rotation, U values increased by 50%, but h_{fp} decreased by 80% when the rotational speed increased from 54.5 to 100 rpm and they attributed this decline to lower relative particle to liquid velocity at high rotational speed. Their particle motion study revealed that due to larger centrifugal forces, the high density Teflon particles moved as a solid body at increased rotation speed.

Sablani (1996) observed that for Nylon particles, h_{fp} increased by 56% in oil bath and by 53% in distilled water when the rotational speed increased from 10 to 20 rpm in EOE rotation. His results indicated that the rotational speed effects on U values were more pronounced in viscous liquids. Same observation on the effect of rotation speed on the heat transferred coefficients was reported by Meng (2006) and Dwivedi (2008).

Liquid viscosity

The higher the liquid viscosity is, the lower the turbulence and liquid to particle velocity are; thus, the heat transfer will be slowed down. Lenz and Lund (1978) found out that the heat transfer coefficients decreased with increasing liquid viscosity. According to their study, when processed in 60% sucrose solution, the heat transfer coefficients were lower by around 30% for particles, compared to those processed in water. Hassan (1984) used Teflon particles and found out that as the liquid viscosity increased, the convective heat transfer coefficient decreased. Same thing was noticed in aluminum particles, except that for 3.17 cm diameter particles, higher h_{fp} was found in the case of 350 centistokes (cst) silicone oil compared to 50 cst. Stoforos and Merson (1992) noticed that the more viscous the liquid was, the lower the U value, but opposite trends were found with h_{fp} due to increased particle to liquid velocity at increased liquid velocity, as seen in the particle motion study. Sablani (1996) observed that the heat transfer coefficients were higher with water than with oil.

Particle concentration

U and h_{fp} might be influenced by the presence of particles in cans as this influences the flow pattern and level of mixing; thus, secondary agitation takes place, but higher particle concentration can affect the velocity gradient surrounding each particle. Lenz and Lund (1978) noticed that *U* and h_{fp} in water and 60% sucrose solution decreased when real food particles have been added. Hassan (1984) found out that h_{fp} increased with increasing the particle concentration from 20 to 31%. Deniston et al. (1987) observed that increasing the particle concentration up to a certain level resulted in increasing *U*. Same trend was found for h_{fp} but at lower magnitude. Stoforos and Merson (1992) observed that the particle motion contributed to homogeneous temperature distribution, especially in high viscous canned food products. Sablani (1996) found out that the effect of particle concentration was more pronounced on h_{fp} than *U* as when the particle concentration increased from a single particle to 20%, *U* increased by 20% for oil and 5% for water; however, h_{fp} increased 3 times for oil and 3.4 times for water. Upon increasing the particle concentration to 40%, *U* decreased by 31% for oil and 27% for water and h_{fp} decreased by 12% for oil and 7% for water.

Particle Size

Although the particle size was found to have a significant effect on heat transfer coefficients, no clear relationship has been established. Lenz and Lund (1978) observed that increasing the particle size resulted in increasing *U* in 60% sucrose solution. Same trend was noticed with h_{fp} between water and lead particles, but *U* was lowest when having medium size particles. Hassan (1984) observed that h_{fp} increased with decreasing particle size. Deniston at al. (1987) found no clear relationship between particle size and heat transfer coefficients, noticing however a small decrease in h_{fp} with increasing particle size. Sablani (1996) observed that upon increasing the Nylon particle diameter from 19 to 25 mm, *U* values decreased by 9% for oil and 6% for water and h_{fp} decreased by 9% for water and 24% for oil. On the other hand, upon increasing the Nylon particle diameter from 19.05 to 22.25 mm, h_{fp} values decreased by 13% for oil.

Particle Shape

Sablani (1996) observed a significant effect of Nylon particle shape (sphere, cylinder and cube) on U values when having multiple particles in the can. For oil, U values for cube shaped particles were 3% lower than those of the cylinder; this in turn was 6% lower than those of the sphere. For water, U values for cube shaped particles were 6% lower than those of the cylinder; this in turn was 6% lower than those of the sphere. The particle shape effect was attributed to the void spaces created which resulted in differences in liquid mixing. On the other hand, for oil, h_{fp} values for cube shaped particles were 6% higher than those of the cylinder; this in turn was 20% lower than those of the sphere. The particle shape influence was more predominant in water. The authors explained that the particle shape effect was more predominant due to disturbance in the flow field near the surface of a cylinder and a cube. As for the multiple particles in the can, the trend of h_{fp} reversed with particle shape as the highest values were obtained in the case of sphere and the lowest ones in the case of cube. This was explained by the extent of void spaces between different shapes and the packability of the particles in the can.

Particle Density

Particle density can influence the heat transfer in the can by affecting the particle-fluid motion pattern. Sablani (1996) observed a significant effect of particle density on h_{fp} when using single particle. Also, he noticed that the higher density particle can have a higher settling velocity in the liquid; thus, a higher particle to liquid relative velocity and a higher h_{fp} value. Besides, he found out that particles having the same density (Nylon and acrylic) had similar h_{fp} values in both oil and water; thus, particle thermal properties did not influence h_{fp}. On the other hand, Stoforos and Merson (1992) reported that teflon particles had higher h_{fp} values compared to aluminum particles of the same size and besides the particle density, the thermal properties of the particle matter were presumed to be the explanation.

Can Headspace

Anantheswaran and Rao (1985) noticed that can headspace had no significant influence on U for particles suspended in 60% sucrose solution and subjected to EOE rotation. On the other hand, Sablani (1996) observed a significant effect of can headspace on both heat transfer coefficients. An increase in the headspace from 6.4 to 10 mm increased the U values by 16% and the h_{fp} values by 75%.

2.2 Biological Validation

2.2.1 Overview

As the number and variety of processed food products increase, food industries are faced with the challenge of proving that their products are safely pasteurized or sterilized, which can sometimes be difficult to achieve if conventional temperature probe systems cannot be used and other more complex approaches need to be implemented. The main product categories having these complexities include products cooked in continuous ovens or fryers (e.g. poultry joints, chicken nuggets, burgers, bread) and products with discrete pieces cooked in steam-jacketed agitated vessels (e.g. ready meals, soups, cook-in sauces, fruit preparations) or in heat exchangers (e.g. cook-in-sauces, preserves, dressings). Biological validation can be used as an alternative to the temperature probes in order to verify the microbiological process safety. The traditional practice to biological validation is to inoculate several dozens of cans for many months so that each can and its contents become both culture container and culture medium. Monitoring of spore survival will be possible through gas production and can swelling (Stumbo, 1965).

In 1989, the Food and Drug Administration (FDA) identified the aspects of the manufacturers' responsibility to approve aseptic processing. Of these, the requirement of biological validation of the heat treatment applied to the product has shown to be the most difficult (Dignan et al. 1989). Consequently, the National Food Processors Association (NFPA) has been concentrating its energy on the development of protocols for the validation of aseptic processes for particulate foods (Chandarana and Unverferth 1996).

2.2.2 Methods of biological validation

In order to monitor the effectiveness commercial thermal processes in many fields (food industry, medical and pharmaceutical fields), bacterial spores are used. Biological validation can be categorized based on whether or not bio-indicators are in contact with food (Dignan et al., 1989).

2.2.2.1 Non-contact methods

Non-contact methods include Biological Indicator Units (BIUs), which are leak proof, small diameter plastic rods containing spore solutions (Pflug et al., 1980), spores in glass spheres (Hersom and Shore, 1981), in stainless steel differential scanning calorimetry - DSC - pans or aluminum DSC pans. Non-contact methods have many advantages, including known spore location, complete spore recovery and elimination of the influence of environmental factors (food pH and ionic strength) on spore thermal response.

2.2.2.2 Contact or direct methods

Contact methods include inoculating food with a bacterial spore suspension and producing simulated food particles. This method has many disadvantages including possible spore leaching, but it offers many advantages including uniform inoculum and controlled particle size (Berry et. al, 1985; Sastry, 1988)

2.2.3 Marker organisms

Few studies on sterilization processes used marker microorganisms instead of *C. botulinum* spores because not only of the hazards associated with their handling, but also of the very low number of surviving spores that would result from a commercial process because of their small D-value (D = 0.21 min). In biological validation, incubating and testing full production runs is required; however, in case of using *C. botulinum*, there will be a little chance of finding the surviving spores due to the low D-value; therefore, the process F-value cannot be calculated and doubts would be raised as to where or when the total pathogenic kill took place. In other terms, D-value should allow sufficient log reductions to be measured in order for the process to be correctly calculated. On the other

hand, it is critical that the z-value of the marker organism is close to that of the target microbial species; otherwise, significant errors in the calculated process values can be introduced. In a sterilization process where the target is *C. botulinum* spores, the marker organism can be spores of *Geobacillus stearothermophilus* (D = 5-6 min) or *Bacillus sporogenes* (D = 1 - 2 min), which have close z-value (10^oC) to the target microorganism. An alternative is to use a gas-producing organism and estimate the severity of the process by counting the number of blown cans. When bacterial spores are used in contact with the food, many environmental factors, including type and ionic concentration and medium pH, are known to affect heat resistance of the spores (Cameron et al., 1980).

2.2.4 Biological validation calculations

The thermal process has to be validated in order to ensure that the target process value has been achieved. Commercial sterility for a thermally processed product is the target condition, which depends on the types and numbers of organisms present before and after the process, and on the intended storage conditions. The integrated lethal value of heat received by any particle is calculated using Equation 2.13 given by Stumbo (1973):

$$F_s = D (\log N_o - \log N) \qquad (2.13)$$

where F_s: integrated lethal value

D: decimal reduction time

N_o and N are initial and final number of spores, respectively

Both F_s and F_o are expressed as the equivalent number of minutes at 121^oC (250^oF). By definition, F_o is the equivalent time (min) at 121^oC at the coldest point of the container; whereas, F_s represents the average lethality received within the spore container. Convection F_s values are close to F_o values because product heating in convection is isothermal; however, in conduction, F_s values tend to be much higher than F_o.

2.2.5 Reconstituted food-alginate particles

2.2.5.1 Alginate – chemistry and functional properties

Alginates were first described by the British chemist Stanford in 1881. They are found in the cell walls and intercellular spaces of brown seaweed and provide the plant with both flexibility and strength, which are necessary for its growth in the sea. Alginates are also synthesized by some bacteria, such as some *Azotobacter* and *Pseudomonas* species. The industrial production of alginates consists of more than twenty stages. The final one consists of precipitation in order to recover the product as insoluble alginic acid (Phillips and Williams, 2000).

Alginic acid has limited stability. In order to produce water-soluble, functional and stable alginate products, alginic acid is transformed into different salt forms to make commercial alginate products. The most commonly used alginates in food and food related applications are sodium alginates. In addition to food uses, the other two major outlets are pharmaceutical applications and technical uses as print paste in the textile industry. Alginate is a polysaccharide, like cellulose, composed of several (1000–3000) building units linked together in a partly stiff and partly flexible chain; however, it is not composed of glucose molecules, but of two sugars - both urinates - the salts of mannuronic and guluronic acid. The chemical composition of alginate is to a certain extent variable. It varies according to seaweed species, even within different parts of the same plant and it is subject to seasonal changes. Alginate is used as additives in order to improve, modify and stabilize the texture of certain foods. Usages of alginate in food industry are listed in Table 2.2 (Phillips and Williams, 2000).

Table 2.2 Usages of alginate in the food industry

Used as	Used in
Thickening Agent	Beverages
Gel-Forming Agent	Bakery, Custard, Jams and Marmalades
Film-Forming Agent	Cakes and Frozen Fish and Meat
Stabilizer	Ice Cream

2.2.5.2 Alginate in inoculation studies

In the presence of calcium ions, alginate has the ability of forming gels. This fact was exploited in order to immobilize marker microorganisms in a matrix of food alginate puree and immerse it in a calcium chloride solution, resulting in firm and thermally stable particles. Using food alginate simulated particles is the most commonly used technique for biological validation (Brown et al., 1984).

In general, reconstituted food particle must be able to imitate a food particle as closely as possible. Also, it must have sufficient mechanical strength to withstand pumping as well as the scraper blades beating without disintegration (Abdelrahim, 1994). The particles must also have similar thermophysical properties, especially the density. Moreover, the particle size and thermal conductivity were found to have significant effects (Lee and Singh, 1990). These requirements are found in the food alginate formulated particles (Marcotte et al., 2000). Alginate was also found to provide moist heating conditions similar to those in food particles (Dallyn et al., 1977). Selected inoculation studies using alginate that are reported in the literature are presented in Table 2.3

Table 2.3 Inoculation studies using alginate

Author	Bacterial Spores Used	Inoculated Particles
Dallyn et al. (1977)	*G. stearothermophilus*	Alginate Beads
Russell (1982)	*Propionibacterium shermunii*	Alginate Beads
Brown et al. (1984)	*G. stearothermophilus* and *C. sporogenes*	Potato, Pea and Meat Alginate
Cacaci et al. (1994)	Yeast cells	Potato Alginate
Ocio et al. (1996)	*G. stearothermophilus*	Mushroom Alginate

2.3 Viscous fluids

Viscous fluids tend to deform continuously under the effect of applied stress and can be categorized as either Newtonian or non-Newtonian fluids.

Newton's law of viscosity is given according to Equation 2.14:

$$\tau_{yz} = -\mu . \gamma_{yz} \qquad \text{(Eq. 2.14)}$$

Where:

τ_{yz} = shear stress (N/m^2), stress which is applied parallel or tangential to a face of a material

μ = viscosity (Pa.s), the resistance of a fluid to flow,

γ_{yz} = shear rate (1/s), the rate at which a deformation is applied

2.3.1. Newtonian fluids

Fluids that follow Newton's law of viscosity (2.14) are called Newtonian fluids. The slope of the shear stress versus shear rate graph, which is viscosity, is constant and independent of shear rate in Newtonian fluids. Gases, oils, water and most liquids that contain more than 90% water, such as tea, coffee, beer, carbonated beverages, fruit juices and milk show Newtonian behavior (Sahin and Sumnu, 2006).

2.3.2. Non-Newtonian fluids

Fluids that do not follow Newton's law of viscosity are known as non-Newtonian fluids. The slope of the shear stress versus shear rate graph is not constant in these fluids (Figure 2.8). For different shear rates, different viscosities are observed; therefore, apparent viscosity or a consistency term is used for non-Newtonian fluids. The variation of apparent viscosities with shear rates for different types of non-Newtonian fluids is presented is Figure 2.8.

2.3.2.1.Shear-thinning (Pseudoplastic fluids)

In these types of fluids, as shear rate increases, friction between layers decreases. Typical examples of shear thinning fluids are paint and ink in a ballpoint pen.

2.3.2.2.Shear-Thickening

In these types of foods, as shear rate increases, the internal friction and apparent viscosity increases. If the increase in the viscosity is accompanied with an increase in the volume, shear thickening fluids are called dilatant fluids.

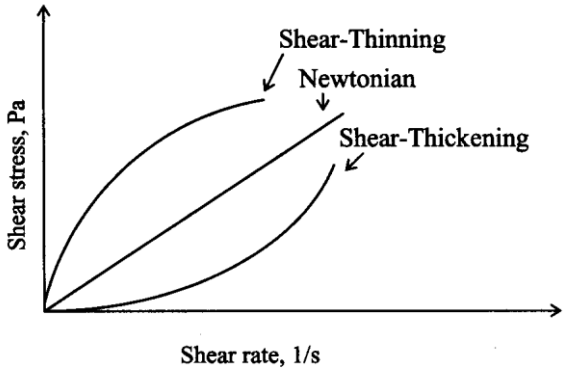


Figure 2.8 Non-Newtonian fluids (Sahin and Sumnu, 2006)

2.4 Carboxymethyl cellulose

Carboxymethyl cellulose (CMC) or cellulose gum is a cellulose derivative with carboxymethyl groups bound to some of the hydroxyl groups of the glucopyranose monomers that make up the cellulose backbone. It is often used as its sodium salt, sodium carboxymethyl cellulose. CMC is used in the food industry as a viscosity modifier or thickener, and to stabilize emulsions in various products including ice cream. It is used primarily because it has high viscosity, is non-toxic, and is non-allergenic (Sahin and Sumnu, 2006). The effect of CMC concentration on the heat transfer to liquid particulate cans subjected to EOE rotation was previously studied by Meng (2006). He found that increasing CMC concentration resulted in a significant decrease in U values due to increased viscosity.

2.5 Textural and thermophysical properties

Texture is one of the most important quality characteristics of foods. Food texture can be evaluated by sensory or instrumental methods. Sensory methods need a taste panel

containing trained panelists and it is hard to repeat the results. On the other hand, instrumental methods are less expensive and less time consuming as compared to sensory methods (Sahin and Sumnu, 2006).

Thermophysical properties (heat capacity, thermal conductivity, thermal diffusivity and density) of food are essential parameters in describing various thermal processes and in optimizing the heating system design (Karunakar et al., 1998). Also, these properties are essential for modeling and evaluating processing operations involving heat transfer in the food industry, where energy costs and food quality and safety are the main considerations. The properties of all water-rich foods are highly temperature dependent (Heldman, 1982).

2.5.1 Texture profile analysis

Single compression or penetration tests are the mostly used instrumental methods to measure the food texture attributes (Gupta et al., 2007). Instrumental texture profile analysis (TPA) was developed by Szczesniak, (1975) and its use has become widespread with the appearance of multitalented computer assisted texturometers, such as TA-XT2 (Stable Micro systems Ltd., Surrey, England). In this type of equipments, it is possible to obtain all the TPA parameters directly by means of its software. Bite-size pieces of food are compressed in the instrument and from this simulated mastication, the force-time curves are obtained to give textural parameters (Friedman et al., 1963; Szczesniak, 1963; Szczesniak 1975). TPA simulates the exercise of two bites of product by the appropriate probe in the texture analyzer. The texture analyzer measures force, distance, and time, thus providing a 3D product analysis (Gupta et al., 2007).

Many textural properties can be quantified using the TPA graph, including hardness, (maximum force required to compress the sample), adhesiveness (work necessary to pull the compression anvil away from the sample), springiness (how well a product physically springs back after it has been deformed during the first compression), among others (Gupta et al., 2007).

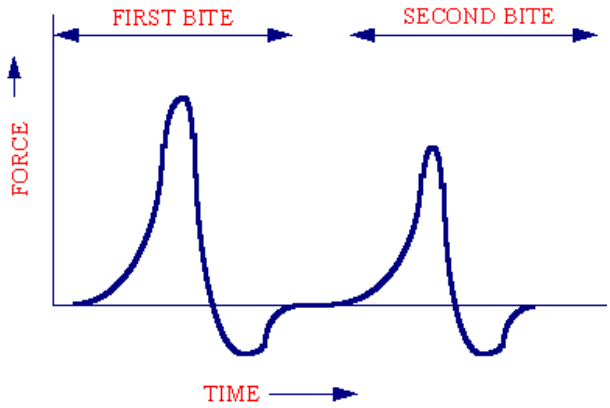


Figure 2.9 A typical Texture Profile Analysis (TPA) of a foodstuff

2.5.2 Thermophysical properties

In the case of biological validation studies involving food alginate fabricated particles inoculated with microbial spores, these particles should have identical thermophysical properties to the real food particles (Marcotte et al., 2000).

2.5.2.1 Heat capacity

Heat capacity ($kJ.kg^{-1}.^{o}C^{-1}$) is the amount of heat required to increase the temperature of 1 g of the substance by $1^{o}C$. Heat capacity of foodstuffs is highly dependent on their composition. Knowing the heat capacity of each component of a mixture is usually enough to predict the heat capacity of the mixture (Sweat, 1995). Heat capacity data for different food materials were given in Rahman (1995) and Singh (1995). Heat capacity of high-moisture foods is largely dominated by water content (Sahin and Sumnu, 2006). Siebel (1892) was the first one to propose an equation to predict heat capacity for aqueous solutions, such as fruit juices or pastes. Many other prediction models were developed later (Heldman, 1982; Choi and Okos, 1986).

There are many methods available to measure heat capacity in foodstuffs, including method of mixture, method of guarded plate, method of comparison calorimeter, adiabatic agricultural calorimeter and differential scanning calorimeter. The latter is extensively used since it reports heat flow, which is directly proportional to the heat capacity of the sample, as a function of temperature. Using this technique, the

difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment (Sahin and Sumnu, 2006).

2.5.2.2 Thermal conductivity

Thermal conductivity ($W.m^{-1}.K^{-1}$) of a material is defined as a measure of its ability to conduct heat. In porous solids, like foods, thermal conductivity depends mostly on the composition, but also on many factors that affect the heat flow paths through the material, such as shape, size and homogeneity. Thermal conductivity in foods having fibrous structures, such as meat, cannot be the same in different direction since heat flow paths through the material change with respect to fiber direction (Sweat, 1995).

Thermal conductivity increases with moisture content. Dry porous solids are very poor heat conductors because of the air in the pores (Sahin and Sumnu, 2006). Thermal conductivity data for more than 100 food materials in the recent literature were classified and analyzed by Krokida et al. (2001). Also, the thermal conductivity of various food materials was given by Rahman (1995) and Sweat (1995).

Many methods can be used for determining thermal conductivity of food stuffs. These can be categorized into steady state and unsteady state methods. Steady state methods include longitudinal heat flow method, radial heat flow method, heat of vaporization method, heat flux method and differential scanning calorimetry method. On the other hand, unsteady state methods include transient hot wire method, modified Fitch method, point heat source model, comparative method and probe method. The latter is widely used for its accuracy and ease of use (Sahin and Sumnu, 2006). Probe method consists of a heater wire insulated over its length and a chromel-constantan thermocouple (type E) at the center of this length (Figure 2.10). The probe is connected to a power supply and the thermocouple to a data acquisition system was connected to a computer. The probe is placed at the core of the sample and the power is turned on in order to pass the 200mA current for 2 min of data acquisition before stopping it. The thermal conductivity can be calculated by plotting temperature vs. ln (time) and calculating the

slope of this graph. Using Equations 2.14 and 2.15, thermal conductivity is calculated (Sahin and Sumnu, 2006):

$$Q = RI^2 \tag{2.15}$$

$$k = \frac{Q}{4\pi m} \tag{2.16}$$

where: Q is the heat flux ($W.m^{-1}$), R the probe resistance ($\Omega.m^{-1}$), k the thermal conductivity ($W.m^{-1}.K^{-1}$), I the current (mA) and m the slope of the linear part of the temperature vs. ln (time) graph.

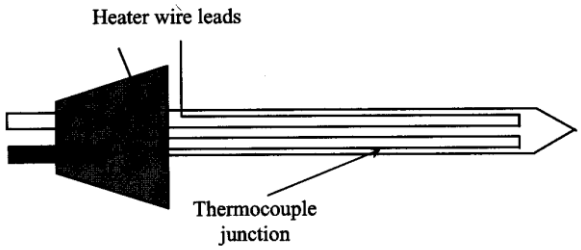


Figure 2.10 Cross section of the thermal conductivity probe (Sastry and Cornelius, 2002)

2.5.2.3 Thermal diffusivity

Thermal diffusivity ($m^2.s^{-1}$) is a physical property associated with transient heat flow. It is a derived property that measures the ability of a material to conduct thermal energy relative to its ability to store thermal energy. Materials with high thermal diffusivity respond quickly to changes in their thermal environment while materials of small thermal diffusivity respond more slowly, taking longer time to reach a new equilibrium condition (Sahin and Sumnu, 2006).

Thermal diffusivity can be determined using indirect or direct methods. Indirect method involves back calculating thermal diffusivity from measured thermal conductivity, density and heat capacity, using Equation 2.17:

$$\alpha = \frac{k}{\rho Cp} \tag{2.17}$$

On the other hand, direct methods include temperature history method, thermal conductivity probe and Dickerson method (Sahin and Sumnu, 2006).

2.6 Response surface methodology

Response surface methodology (RSM) was developed in 1951 by Box and Wilson. It is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes. In addition, it has important applications in the design, development and formulation of new products and in the improvement of existing product designs. RSM is mostly used in the industry, mainly in situations where several input variables (independent variables) influence potentially the quality characteristics (response variables) of the product or process. The main idea of RSM is to use a sequence of designed experiments to obtain an optimal response. In order to do this, Box and Wilson (1951) suggested using a second-degree polynomial model and acknowledged that this model is only an approximation; however, this model was easy to estimate and apply, even when little was known about the process (Myers et al., 2009). Central composite rotatable design (CCRD) is an experimental design used in RSM and consists of three distinct sets of experimental runs:

- A factorial design in the factors studied, each having two levels,
- A set of centre points, experimental runs whose values of each factor are the medians of the values used in the factorial portion. This point is often replicated in order to improve the precision of the experiment,
- A set of axial points, experimental runs identical to the centre points except for one factor, which will take on values both below and above the median of the two factorial levels, and typically both outside their range. All factors are varied in this way (Myers et al., 2009).

PREFACE TO CHAPTER 3

For the thermal process establishment, gathering time temperature data from test cans under real processing conditions is required. In many situations like aseptic processing and continuous flow bi-axial rotary processing, temperature measurements are often difficult. Biological validation using food alginate simulated particles that are inoculated with spores is generally considered useful in such cases to validate the processes as well as computer simulations.

Stumbo (1973) reported that the chemical environment, including pH, salt and sugar concentrations and fat content, has a significant effect on the heat resistance of the bacterial cell or spore. Hence, the food composition would have a dominating effect on the destruction kinetics of microbial spores intended for biological validations. That is why, prior to embarking on biological validation studies in continuous flow systems, the first task is to evaluate the thermal destruction behavior of target spores of *Geobacillus stearothermophilus* and *Clostridium sporogenes* in meat and carrot alginate purees using the first-order semi-log model and the non log-linear Weibull model.

Part of this research was presented in 2009 in the Journée scientifique et technique, CRDA, St-Hyacinthe, Canada. Another part was presented in 2009 in the IFT Annual Meeting, Anaheim, USA. One manuscript has been accepted in the Journal of Food Processing and Preservation.

The experimental work and data analysis were carried out by the candidate under the supervision of Dr. H. S. Ramaswamy.

CHAPTER 3

HEAT RESISTANCE OF *G. STEAROTHERMOPHILUS* AND *C. SPOROGENES* IN CARROT AND MEAT ALGINATE PUREES

3.1 Abstract

The main objective of this study was to investigate the heat resistance of non-pathogenic, heat-resistant *Geobacillus stearothermophilus* and *Clostridium sporogenes* spores in meat and carrot alginate purees. Alginate is used as a viscosity modifier in liquid foods and has been used to fabricate thermally stable and firm simulated particles for biological validation studies of thermal processing, when conventional temperature gathering is not possible. Pureed carrot and meat were mixed with sodium alginate and inoculated with high concentrations of spores of *Geobacillus stearothermophilus* or *Clostridium sporogenes* and filled into capillary tubes, flame sealed and heat treated in an oil bath for different times at selected temperatures. D values were computed from the slopes of survivor curves - log (N/N_o) vs. time - and z values from the slopes of thermal resistance curves - log (D) vs. temperature. Survivor and thermal resistance curves were well described by the first-order linear regression model with the regression coefficients (R^2) above 0.9. For meat alginates, D-values for *G. stearothermophilus* ranged between 1.9 and 40.8 min with a z-value of 11.7^{o}C and D-values for *C. sporogenes* ranged between 0.9 and 28.7 min with a z-value of 10.1^{o}C. For carrot alginates, D-values for *G. stearothermophilus* ranged between 1.9 and 42.6 min with a z-value of 11.5^{o}C and D-values for *C. sporogenes* ranged from 1.1 and 31.0 min with a z-value was 10.2^{o}C. The results were also fitted to Weibull model, but the model did not result in any better fit than the conventional first-order model.

3.2 Introduction

Thermal processing is a traditional, yet among the most effective methods to preserve foods. Stumbo (1973) defined thermal processing as "the application of heat for the purpose of shelf-life extension and promotion of safety of food". Destruction of spoilage and pathogenic microorganisms in canned foods; thus achieving safety and shelf-stability, is the main objective of thermal processing (Ramaswamy and Marcotte, 2005). The main pathogen of public concern in low-acid canned foods is the spore forming, anaerobic, rod-shaped, heat resistant and toxin producing bacteria, *Clostridium botulinum.* Destroying this pathogen, and thereby preventing the formation of *botulin* toxin, is the major goal for the low-acid food canning industry (Lopez, 1987).

Consumers constantly demand products that are safe, shelf-stable and of high-quality. The fact that quality factors are more heat stable than the microorganisms has resulted in the development of high temperature – short time (HTST) processing, including aseptic processing, thin profile packaging and agitation processing (end-over-end, fixed axial and free axial rotation) (Reuter, 1993). Aseptic processing consists of heating the food without any package to a high temperature, holding it for a short time, cooling down and packaging it into a sterilized container in a sterile chamber. In the case of particulate liquid foods, the particles move during the process posing difficulties in attaching thermocouples and thus in gathering temperature profiles of the particles (Lund, 1987). In free axial rotation, there are two levels of rotation, one along the helical reel (upper $2/3^{rd}$ section of the helical path) and other one at the can level when the cans rotate on their own axis along with the retort shell (lower $1/3^{rd}$ section). In case of liquid particulate canned food products subjected to free axial rotation, temperature data collection is challenging because of the difficulties involved in attaching the temperature measuring devices to the liquid and particles. Usually, the temperature should be measured without affecting the normal motion of the fluid mixture in the can, which becomes impossible.

Many technologies have been developed so that temperature history of the particle can be monitored when temperature probe systems cannot be used. Stoforos and Merson (1991) used a liquid crystal whose color changes with temperature in order to

monitor particle surface temperature. Their method involves coating the particle surface with an aqueous solution of liquid crystals, videotaping the color changes of the particle surface as a function of temperature and comparing them with standard color chart after calibration; however, experiments were limited to a temperature range of 20 to 50^{o}C. Time temperature integrators (TTI) have also been used in several studies for estimating the degree of severity of thermal processes. TTIs can be in the form of microorganisms, chemicals or enzymes. Weng et al. (1992) used TTI in the form of immobilized peroxidase. Ramaswamy et al. (1996) used a chemical marker for process lethality measurements under continuous tube flow conditions.

Alginate is a polysaccharide used as a food additive in the food industry. Properties of alginate include gel-forming, film-forming, stabilizing and thickening abilities. Gel-forming property of alginate has been invested in order to fabricate particles that simulate the heating behavior of real foods (Marcotte et al., 2000; Phillips and Williams, 2000). These alginate fabricated particles can be used in order to biologically validate thermal processing, where temperature probe systems cannot be used. Biological validation consists of embedding spores of a heat resistant and non-pathogenic marker organism, mainly *Geobacillus stearothermophilus* or *Clostridium sporogenes*. These heat resistant spores have been considered to follow similar thermal destruction profile (different D values with nearly same z-value, both detailed later) to the target pathogen, *C. botulinum*. Using initial and final counts, integrated process lethality (F_o) can be calculated (Dallyn et al., 1977; Brown et al., 1984; Ocio et al., 1996).

Stumbo (1973) reported that the chemical environment, including the pH, salt and sugar concentrations, and fat content, has a significant effect on the heat resistance of the bacterial cell or spore. Hence, the food composition would have a dominating effect on the destruction kinetics of microbial spores intended for use in biological validations. Hence, prior to embarking on biological validation studies in continuous flow systems, it is necessary to evaluate the thermal destruction behavior of target spores in meat and carrot alginate media and to successfully fabricate typical particles that can be filled into cans.

The objectives of this study were (1) to evaluate the heat resistance of *Geobacillus stearothermophilus* and *Clostridium sporogenes* spores in carrot and meat alginate formulations (pH 6.0) and compare their destruction behavior with available published information and (2) to compare D and z-values calculated from both first-order semi-log model and non log-linear Weibull model. This was done to establish a baseline database on their destruction kinetics for use in biological validation studies.

3.3 Methodology

3.3.1 *Clostridium sporogenes* Spores Preparation

Freeze-dried cultures of *C. sporogenes* (ATCC-7955) were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and stored at -40^{o}C. Spores preparation was done according to the method used by Shao (2008). The culture was hydrated in 10 ml Reinforced Clostridial Medium (RCM) broth (Oxoid, Basingstoke, Hampshire, UK) at 37^{o}C for 24 h under anaerobic conditions and stored at 4^{o}C. To prepare the inoculation culture, 0.1 ml of the broth was transferred to 50 ml of freshly prepared RCM and incubated at 37^{o}C for 24 h under anaerobic conditions. Two similar transfers were made in order to obtain a culture with viable count of approximately 10^{6} CFU/ml. A volume of 0.2 ml of this culture was then transferred and spread on Campdem Sporulating Agar plate (CSA) and incubated at 37^{o}C for 7 days under anaerobic conditions in order to grow and form bacterial spores. The CSA medium contained 2.5 g tryptone (Oxoid, Basingstoke, Hampshire, UK), 2.5 g bacterial peptone (BD, Co., Spark, MD), 0.5 g Lab Lemco meat extract (Oxoid, Basingstoke, Hampshire, UK), 1 g yeast extract (BD, Co., Spark, MD), 0.028 g calcium chloride (BDH, Inc., Toronto, ON), 0.031 g $MnSO_4.H_2O$ (Fisher Scientific, Fair Lawn, NJ), 0.5 g glucose (Fisher Scientific, Fair Lawn, NJ) and 7.5 bacterial agar (Fisher Scientific, Fair Lawn, NJ) in 500 ml of distilled water. Spores were collected by flooding the agar plate surface while scrapping the colonies via sterile glass spreader. After harvest, the spores were washed three times by centrifuging at 4000 x g for 15 min each at 4^{o}C and suspended in sterile distilled water to give approximately 10^{6} CFU/ml. The spores' solution was stored at 4^{o}C until use (Shao, 2008).

3.3.2 *Geobacillus stearothermophilus* Spore Preparation

Freeze-dried cultures of *G. stearothermophilus* (ATCC-10149) were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and stored at -40^{o}C. Spores preparation was done according to the method used by Kim and Naylor (1966). The culture was hydrated in 10 ml TYG broth at 55^{o}C for 24 h under aerobic conditions in a shaking water bath (SW22, Julabo, Labortechnik GMBH, Germany). Two similar transfers were made in order to obtain a culture with viable count of approximately 10^8 CFU/ml. Then, the solution was stored at 4^{o}C. The TYG medium consisted of 5 g tryptone (BD, Co., Spark, MD), 2.5 g yeast extract (BD, Co., Spark, MD) and 1 g K_2HPO_4 (Acros Organics, NJ, USA) in 500 ml distilled water and the pH was adjusted to 7.2. Inoculation culture was prepared by transferring 1 ml of the cultured broth to 50 ml freshly prepared TYG broth and incubating at 55^{o}C for 24 h under aerobic conditions. A volume of 0.2 ml of this culture was then transferred and spread on a sporulation agar plate and incubated at 55^{o}C for 7 days under aerobic conditions in order to grow and form bacterial spores. The sporulation medium contained 4 g nutrient broth (BD, Co., Spark, MD), 2 g yeast extract (BD, Co., Spark, MD), 0.05 g $MnCl_2.4H_2O$ (Acros Organics, NJ, USA) and 10 g bacterial agar (Fisher Scientific, Fair Lawn, NJ) in 500 ml of distilled water. Spores were collected by flooding the agar plate surface while scrapping the colonies via sterile glass spreader. After harvest, the spores were washed three times by centrifuging at 4000 x g and suspended in sterile distilled water to give approximately 10^8 CFU/ml. The spores' solution was stored at 4^{o}C until use.

3.3.3 Sample Preparation

Sodium alginate was obtained from ICC Corporation (New York, NY). Baby carrots and ground minced beef meat (19% fat) were purchased from a local supermarket. Carrot juice was prepared from the carrots using a juice extractor and sodium alginate (2%) was added and mixed thoroughly. Mixture was autoclaved for 15 minutes. On the other hand, 100 g of meat was autoclaved for 20 min for sterilization and softening purposes and then mashed using a laboratory blender. Meat was transferred to a bag and 100 ml of water was added and mixed thoroughly using a stomacher for 10 min. Sodium alginate (2%) was added to the puree and mixed again using the stomacher. Both spores' solutions were heated at 80^{o}C for 10 min in order to kill any vegetative bacteria. 1 ml of

each spore solution was added to 10 g each of carrot and meat alginate purees in a centrifuge tube and mixed thoroughly for 20 min using a vortex mixer. 0.05 g of the inoculated carrot or meat alginate puree were syringed into capillary tubes (75 x 1.2 mm) which were then flame sealed and stored at 4^{o}C until use. Figure 3.1 shows the flame sealed capillary tubes containing the spore loaded meat and carrot alginate puree and the spore inoculated simulated meat and carrot alginate particles to be used in biological validation studies.

Fig. 3.1 Flame sealed capillary tubes containing the spore loaded meat and carrot alginate puree and the simulated meat and carrot alginate particles

3.3.4 Thermal Treatment

A temperature controlled oil bath (SL26, Julabo, Labortechnik GMBH, Germany) was used o conduct the thermal treatment. The heating medium was pure glycerine G33-20 (Fisher Scientific Corp., Whiteby, ON). The come up time in capillary tube was measured and found to be 15 s. Based on literature data on thermal destruction kinetics, for the capillary tubes containing meat or carrot alginate inoculated with *G. stearothermophilus*, four temperatures (110, 115, 120 and 125^{o}C) and six holding times (5, 10, 15, 20, 25 and 30 min, excluding the come-up time) were used. On the other hand, for the capillary tubes containing meat or carrot alginate inoculated with *C. sporogenes*, four temperatures (105, 110, 115 and 120^{o}C) and five holding times (2, 4, 6, 8 and 10 min, excluding the come-up time) were used. Five capillary tubes were treated at each time-temperature combination and taken out by the time interval. Experiments were done

in duplicate. After the heat treatment, capillary tubes were immediately cooled and held in ice water until enumeration within 30 min.

3.3.5 Enumeration of Surviving Spores

The capillary tubes were dipped in ethanol and aseptically opened. Then, their contents were poured into dilution tubes. Serial dilutions were made with 0.1% peptone water and enumeration was done using the pour plate technique. Enumeration was done according to the method used by Shao (2008). Spores of *C. sporogenes* were enumerated in modified PA3679 agar (Ocio et al., 1996). This agar contains 5 g tryptone (Oxoid, Basingstoke, England, UK), 1 g yeast extract (BD, Co., Spark, MD), 1 g K_2HPO_4 (Acros Organics, NJ, USA), 1 g soluble starch (Sigma, St Louis, MO) and 7.5 g bacterial agar (Fisher Scientific, Fair Lawn, NJ) in 500 ml distilled water. The plates were incubated at 37^{o}C under anaerobic conditions for 5 days before counting. Spores of *G. stearothermophilus* were enumerated in Tryptic soy agar (TSA) (BD, Co., Spark, MD) plates, which were incubated at 55^{o}C under aerobic conditions for 5 days before counting.

3.3.6 Data analysis

As has been mostly done in literature, a first-order thermal destruction model was used to evaluate kinetic parameters. Mathematically, the model is represented by the following equation:

$$\ln N = \ln No - kt \qquad (3.1)$$

Where N_o the initial number of microorganisms, N is the number of survivors following a heat treatment of *t* and *k* is the first order rate constant. This equation can be arranged to:

$$\log \frac{N}{No} = -\frac{t}{D} \qquad (3.2)$$

Where D is the decimal reduction time: time needed to decrease N/N_o by one log cycle. In other words, it is the time required to destroy 90% of the microorganisms. The reaction rate constant *k* is 2.303/D. The semi-logarithmic curve resulting when log N/N_o is plotted

versus time is called the survivor curve. D-value is the negative reciprocal of the slope of the survivor curve.

Log D-value is plotted against temperature. The resulting graph is called the heat resistance curve. The thermal sensitivity parameter of D value is defined as a z-value which is the temperature range through which D-value is reduced by one log cycle. This can be obtained from D values obtained at two temperatures using Eq. 3.3 or it can be obtained as the negative reciprocal of the slope of the heat resistance curve.

$$\log \frac{D1}{D2} = \frac{T2 - T1}{z} \tag{3.3}$$

Recently survivor curves have been recognized not to necessarily obey first-order kinetics and to generally show shoulders, tails or downward and upward concavity (Shull et al., 1963; Juneja and Marks, 2005; Bialka et al., 2008). The Weibull model (Martinus and Van Boekel, 2002) has been reported to be a better fit for such non-linear survivor curves. Weibull model is basically a statistical model of distribution of inactivation times. It takes biological variation, with respect to thermal inactivation into account. This model uses two parameters, the scale parameter, α (time) (same as rate constant k in Eq. 3.1) and the dimensionless shape parameter, β. The classical first-order approach is a special case of the Weibull model, where β = 1 (Martinus and Van Boekel, 2002).

Weibull model is shown below:

$$S(t) = \exp\left(-(\frac{t}{\alpha})^{\beta} \right) \tag{3.4}$$

and

$$\log S(t) = -\frac{1}{2.303}\left(\frac{t}{\alpha}\right)^{\beta} \tag{3.5}$$

Since $S(t) = N/N_o$, solving this equation comes to:

$$\ln\left(-2.303 \log\left(\frac{N}{No}\right)\right) = \beta \ln t + \beta \ln \alpha \tag{3.6}$$

Plotting vs. $\ln t$ gives a linear curve with a slope=β and an intercept= $\beta \ln \alpha$.

Once α parameter is calculated, D value can be obtained, as follows:

$$D = \frac{1}{\alpha} 2.303 \qquad (3.7)$$

3.3.7 Statistical Analysis

T-test function of the Microsoft Excel Software was used in order to compare D-values of meat and carrot alginates calculated from both first-order and Weibull models.

3.4 Results and discussions

3.4.1 First Order Model

Figures 3.2 (a, b) show the survivor curves [logarithm of the survival fraction (N/N_o) against heating time (min)] for *C. sporogenes* 7955 in carrot and meat alginate, respectively, under thermal treatment ranging from 105 to 120^{o}C and holding time (excluding come up) ranging from 2 to 10 min. The linear regression lines fitting the experimental data points (excluding the zero point) are also shown on the curve as the trend curve from the Excel program (same as the regression model) from which the line slopes and R^2 values were obtained. D values were then computed as the negative reciprocal of the slope of the regression line. Figures 3.2 (c, d) show similar survivor curves for *G. stearothermophilus* ATCC-10149 in carrot and meat alginate, respectively, under thermal treatment ranging from 110 to 125^{o}C and holding time ranging from 5 to 30 min.

In general, the survivor curves were well described by the first-order linear regression model with the regression coefficients (R^2) above 0.98. As expected, results also indicated that higher treatment temperatures resulted in steeper curves and higher rates of destruction of spores resulting in lower D values. Table 3.1 summarizes the associated D-values of *G. stearothermophilus* ATCC-10149 and *C. sporogenes* 7955 spores in carrot and meat alginate purees, respectively, calculated using the Bigelow first-order model. For meat alginate puree, D-values for *G. stearothermophilus* ranged between 1.9 and 40.8 min and D-values for *C. sporogenes* ranged between 0.9 and 28.7

min. On the other hand, for carrot alginate puree, D-values for *G. stearothermophilus* ranged between 1.9 and 42.6 min and D-values for *C. sporogenes* ranged from 1.1 and 31.0 min.

Figures 3.3 (a, b) show the heat resistance curves for *C. sporogenes* 7955 in carrot and meat alginate purees, respectively. Figures 3.3 (c, d) show the heat resistance curves for *G. stearothermophilus* ATCC-10149 in carrot and meat alginate purees, respectively. These heat resistance curves were obtained by plotting the regression line of the logarithm of the D-value against the heating temperature and the z-values were computed as the inverse negative of the slope of the regression line.

The z-values were found to be 11.6 and 11.5^{o}C in meat and carrot alginate purees, respectively for *G. stearothermophilus* ATCC-10149 and 10.1 and 10.2^{o}C in meat and carrot alginate purees, respectively for *C. sporogenes* 7955 (Table 3.1).

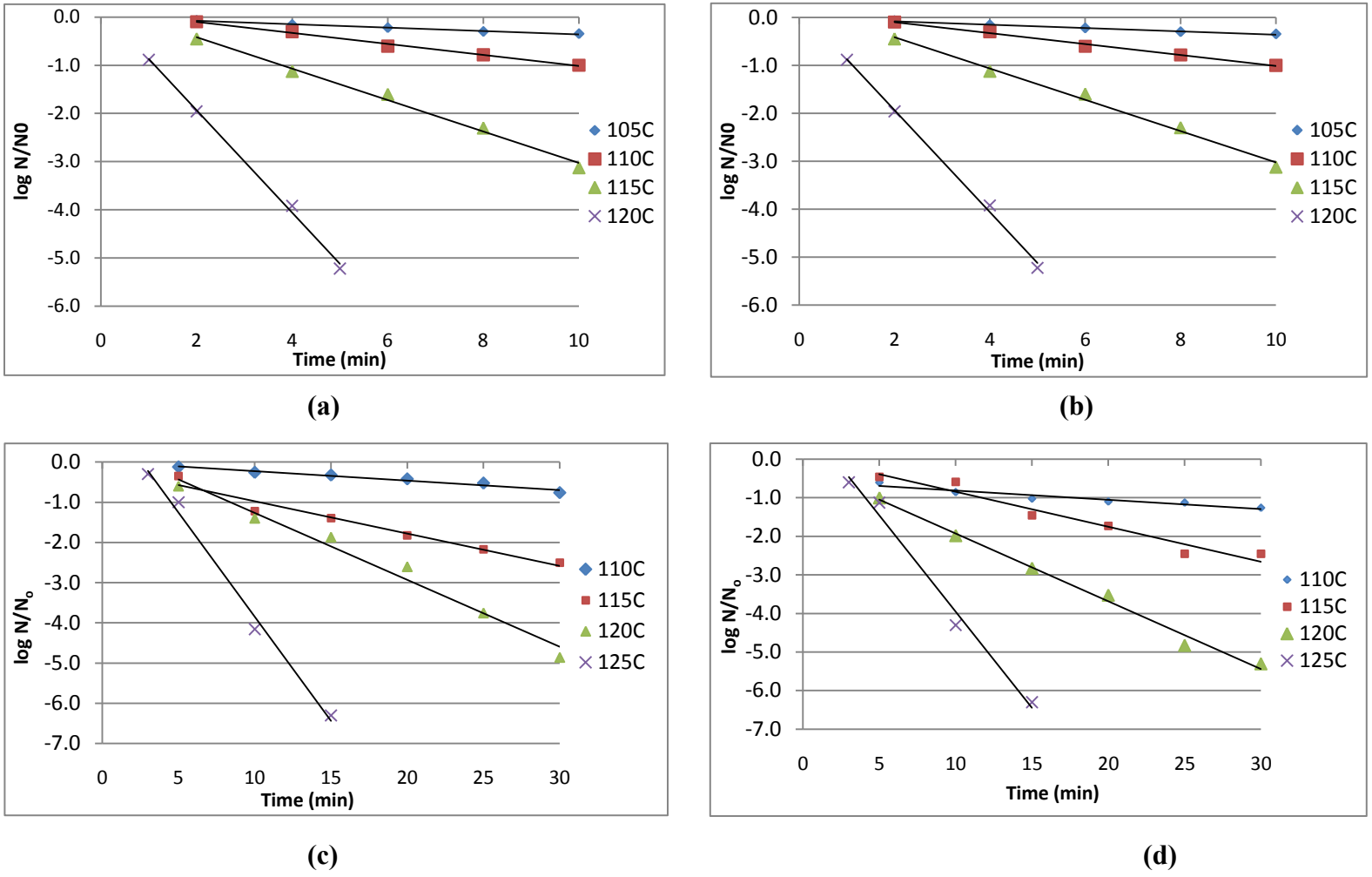


Figure 3.2 Survivor curves of *C. sporogenes* (a, b) and *G. stearothermophilus* (c, d) in carrot and meat alginate purees, respectively, at different temperatures, using first-order Bigelow model

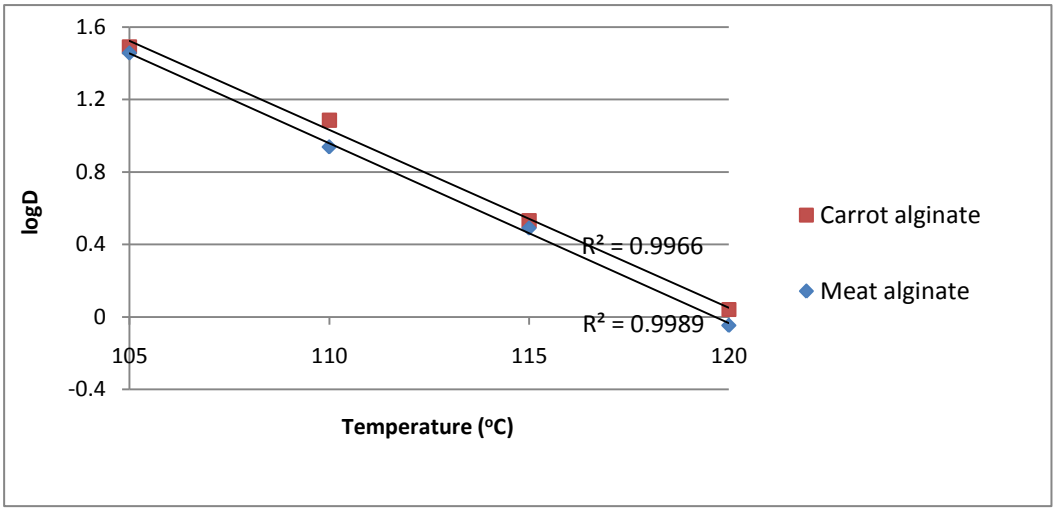
Table 3.1 Decimal reduction times (D) (Mean ± SD) and z-values of (a) *Geobacillus stearothermophilus* ATCC-10149 and *Clostridium sporogenes* 7955 spores in carrot and meat alginate purees using Bigelow model

(a) *Geobacillus stearothermophilus*

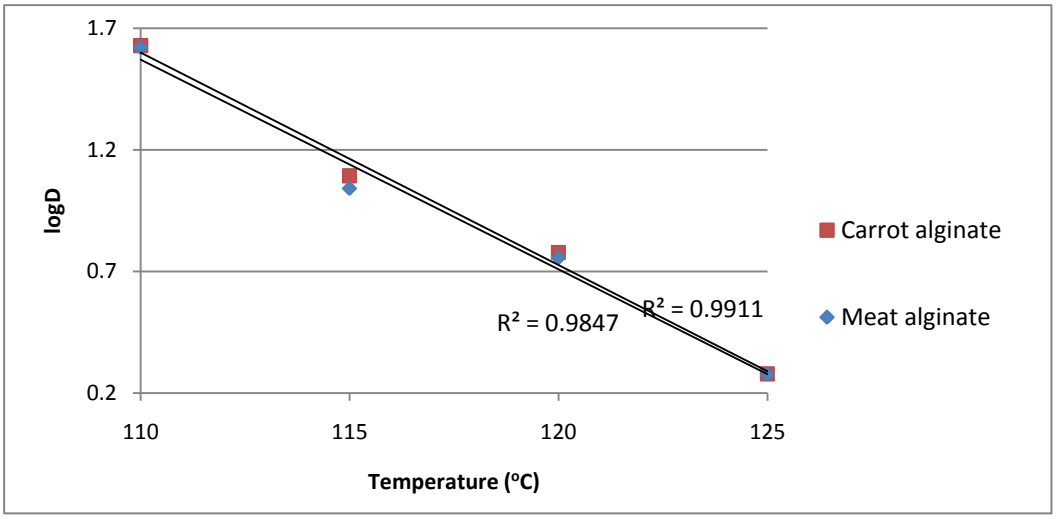
Temperature (^oC)	Carrot		Meat	
	D (min)	R^2	D (min)	R^2
110	42.6 ± 1.21	0.981	40.8 ± 1.47	0.994
115	12.4 ± 0.81	0.987	11.0 ± 0.73	0.997
120	6.0 ± 0.20	0.998	5.7 ± 0.24	0.991
125	1.9 ± 0.08	0.988	1.9 ± 0.08	0.983
z-value (^oC)	11.5		11.6	
$SE_{y/x}$	0.07		0.09	

(b) *Clostridium sporogenes*

Temperature (^oC)	Carrot		Meat	
	D (min)	R^2	D (min)	R^2
105	31.0 ± 0.26	0.994	28.7 ± 0.20	0.991
110	12.2 ± 0.14	0.997	8.7 ± 0.14	0.994
115	3.4 ± 0.08	0.996	3.1 ± 0.02	0.992
120	1.1 ± 0.07	0.999	0.9 ± 0.06	0.997
z-value (^oC)	10.2		10.1	
$SE_{y/x}$	0.05		0.03	



(a)



(b)

Figure 3.3 Heat resistance curves (log D vs. Temperature) of *C. sporogenes* (a) and *G. stearothermophilus* (b) in meat and carrot alginate purees

There are no specific reports on the thermal destruction kinetics of *G. stearothermophilus* or *C. botulinum* spores in carrot and meat alginate purees at pH 6.0 used in this study. However, there are some published values for their destruction kinetics in other food bases. Stumbo (1973) reported a general range of D-values of 0.1 to 1.5 min and z-value of 7.8 to 10°C for *C. sporogenes* PA3679. Cameron et al. (1980) reported D_{110}, $D_{112.8}$, $D_{115.6}$, $D_{118.3}$ and D_{121} for *C. sporogenes* in pea puree (pH = 6) to be 24.5, 10.7, 6.5, 3.8 and 2.0 min, respectively. Brown et al. (1984) reported that D values of *G. stearothermophilus* in potato, meat and pea alginates were 3.5, 2.8 and 2.5 min, respectively and z-values were found to be 11.4, 11.7 and 11.8°C, respectively. Ocio et al. (1996) found out that D_{118}, D_{121} and D_{125} of *G. stearothermophilus* in mushroom alginate were 1.01, 2.80 and 6.61 min, respectively with a z-value of 8°C. Periago et al. (1998) reported that D_{121} for *G. stearothermophilus* in mushroom extract (pH = 6.6) was 4.3 min. Rajan et al. (2006) stated that D_{120} values of *G. stearothermophilus* in deionised water and egg patties were 6.95 and 8.50 min, respectively. Shao (2008) studied the resistance of *G. stearothermophilus* 10149 in milk (pH = 6.5) (no alginate base) and found out that the D_{110}, D_{115} and D_{120} were 49.4, 16.1, 6.3 min, respectively with a z-value of 11.2°C. D_{110}, D_{115}, D_{120} and D_{125} of *G. stearothermophilus* in our study were 42.6, 12.4, 6.0, 1.9 min, respectively in carrot alginate puree and 40.8, 11.0, 5.7 and 1.9 min, respectively in meat alginate puree. Calculated D and z values in our study fall within the overall range of values reported in the literature; however, it is not possible to specifically classify the kinetic values to pH or food groups.

Few studies on sterilization processes have used *C. botulinum* spores because not only of the hazards associated with their handling, but also of the very low number of surviving spores that would result from the thermal process due of their small D-value (0.21 min); therefore, process lethality, F_o, cannot be calculated (Perkins, 1969). Spores of surrogate microorganisms *G. stearothermophilus* or *C. sporogenes*, which have close z-value to the target microorganism, *C. botulinum*, are used instead (Koutchma et al., 2005). The z-value for *C. botulinum*, which is used in process calculations, is equal to 10°C (Stumbo, 1973). Joy and Brown (1988) reported a z-value of 11°C for *C. botulinum*. The z-values found in our study were close to the z-value of *C. botulinum*, indicating the

appropriateness of using spores of *G. stearothermophilus* or *C. sporogenes* as marker microorganisms.

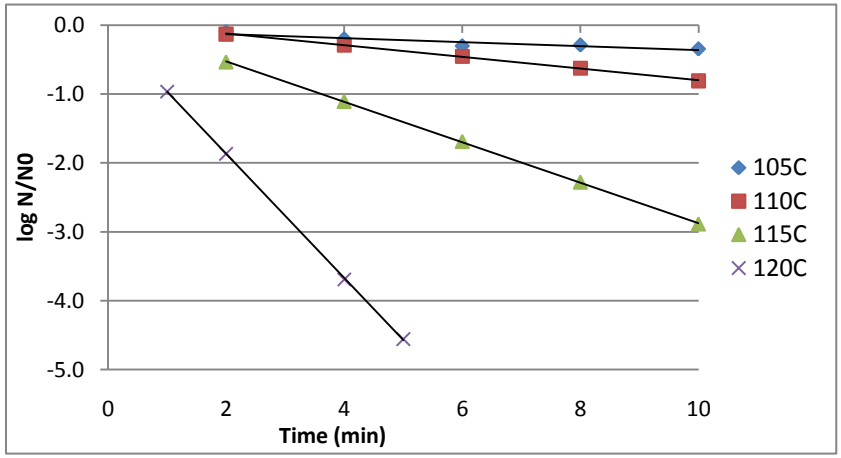
Stumbo (1973) reported that pH of food matrix has a direct influence on D-values. In other words, the hydrogen ion concentration has a profound effect on the heat resistance of microorganisms. For most spore-bearing bacteria, maximum resistance generally occurs in the region of neutrality. Increasing the hydrogen ion concentration causes a corresponding decrease in heat resistance. Sognefest et al. (1948) stated that the lower the pH in the range of 4.5 to 9.0 is, the lower the required heating will be. Meat and carrot alginate purees used in this study had similar pH values (5.9-6.0); however, for the same temperature, D-values for *G. stearothermophilus* and *C. sporogenes* in carrot alginate puree were slightly but significantly ($p < 0.05$) higher than D-values in meat alginate puree, as shown in Table 3.1. Meat alginate purees were distinctly different from carrot alginates with respect to fat content which were 10% and 0%, respectively. A number of investigators have found that microorganisms suspended in oily media are more difficult to destroy by heat than when suspended in aqueous media (Lang, 1935; Jensen, 1945). Lang (1935) suggested that this may be accounted for by the poor conductivity of fat. The results from this study however cannot be explained based on the fat content because D-values in meat alginate purees were in fact slightly lower than those in the carrot alginate purees. Moisture content also has a considerable effect as fat on the heat resistance of the microorganism. Generally, when the moisture content is relatively very low which can restrict the water activity, the associated thermal resistance has been recognized to increase. Interestingly, Bullock and Lightbown (1947) reported that increasing water content of the medium results in a higher resistance, and therefore, in higher D-values. This is perhaps true in the high water activity zone; something that has not been noticed in other studies. The moisture content of the alginate purees used in this study was evaluated to be 92% (±1.1) and 74% (±1.9) in carrot alginate and meat alginate purees, respectively. The confounding effects of fat and moisture content of carrot and meat alginate purees might have moderated the thermal resistance of the bacterial spores in these purees with slightly higher D-values in carrot alginate puree compared to D-values in meat alginate puree.

3.4.2 Weibull Models

Figures 3.4 (a, b) show the survivor curves for *C. sporogenes* 7955 in carrot and meat alginate, respectively, under thermal treatment ranging from 105 to 120^{o}C and holding time (excluding come up) ranging from 2 to 10 min. Figures 3.4 (c, d) show similar survivor curves for *G. stearothermophilus* ATCC-10149 in carrot and meat alginate, respectively, under thermal treatment ranging from 110 to 125^{o}C and holding time ranging from 5 to 30 min.

Table 3.2 shows α, β, R^2 and calculated D-values from the Weibull model. The β values ranged from 0.8 to 1.3. The average β values were 0.99 and 1.11, for *C. sporogenes* in carrot and meat alginates and 1.0 and 1.02 *G. stearothermophilus* in carrot and meat alginates.

Except for the *C. sporogenes* in meat alginates, the β values were nearly 1.0 indicating that the survivor curves for *C. sporogenes* and *G. stearothermophilus* in carrot and meat alginate purees were log-linear. There was no significant ($p > 0.05$) difference between D-values calculated from both Weibull and Bigelow models (Tables 3.1 and 3.2). Figure 3.5 shows that D-values of *C. sporogenes* and *G. stearothermophilus* in carrot and meat alginate purees calculated from Weibull model were in good agreement with the ones calculated using Bigelow model ($R^2 > 0.97$).



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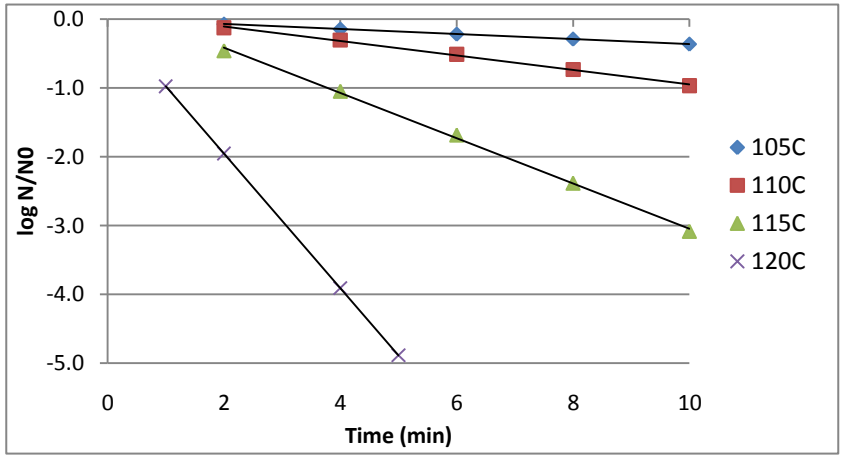

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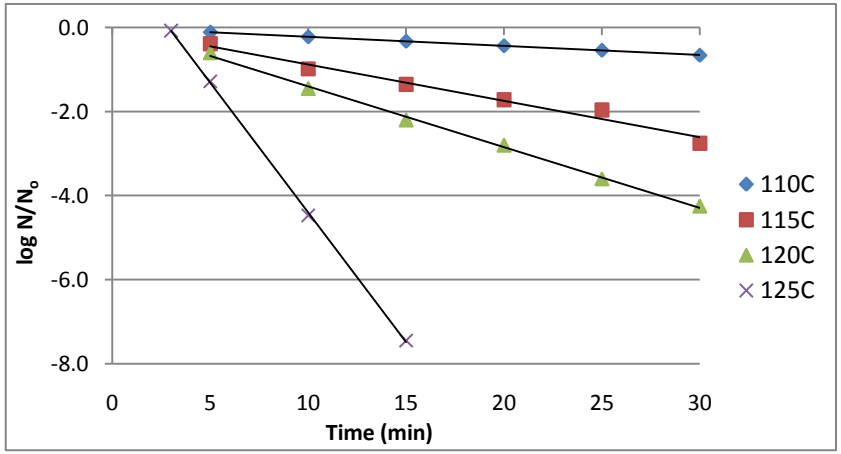

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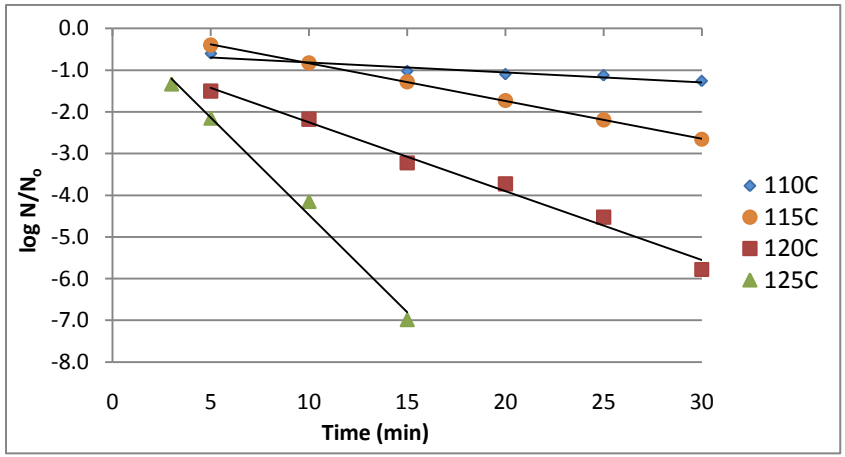


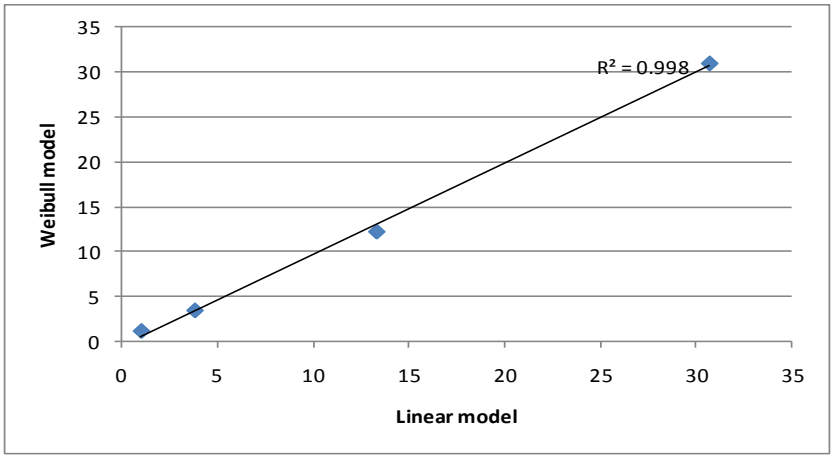
(d)

Figure 3.4 Survivor curves of *C. sporogenes* (a, b) and *G. stearothermophilus* (c, d) in carrot and meat alginate purees, respectively, at different temperatures, using Weibull model

Table 3.2 α (Mean ± SD), β (Mean ± SD), R^2 and D values calculated from Weibull models for *C. sporogenes* and *G. stearothermophilus* in carrot and meat alginate purees

Microorganism	Puree	Temperature	α (min)	β	R^2	D (min)
C. sporogenes	Carrot	105	7.51E-02 ± 0.001	0.81 ± 0.02	0.993	30.7
		110	0.173 ± 0.001	1.13 ± 0.01	0.995	13.3
		115	0.607 ± 0.01	1.05 ± 0.03	0.997	3.8
		120	2.267 ± 0.04	0.97 ± 0.02	1.000	1.0
	Meat	105	8.42E-02 ± 0.001	1.00 ± 0.01	0.994	27.4
		110	0.189 ± 0.002	1.27 ± 0.04	0.989	12.2
		115	0.526 ± 0.01	1.18 ± 0.03	0.995	4.4
		120	2.251 ± 0.03	1.00 ± 0.03	1.000	1.0
G. stearothermophilus	Carrot	110	5.00E-02 ± 0.001	0.98 ± 0.01	0.977	46.1
		115	0.173 ± 0.01	0.83 ± 0.01	0.980	13.3
		120	0.337 ± 0.01	1.22 ± 0.02	0.980	6.8
		125	1.08 ± 0.01	0.98 ± 0.01	0.975	2.1
	Meat	110	5.40E-02 ± 0.001	0.85 ± 0.02	0.985	42.6
		115	0.184 ± 0.01	1.06 ± 0.01	0.994	12.5
		120	0.376 ± 0.02	1.24 ± 0.03	0.996	6.1
		125	1.102 ± 0.02	0.94 ± 0.02	0.974	2.1

There is no complete agreement in the literature on whether Weibull model or the classical first-order model is better to use for describing the inactivation of microorganisms. The classical first order model developed by Bigelow, Ball and Stumbo is widely accepted and practiced (Stumbo, 1973). Its usefulness is clear from a proven record in the canning industry (Martinus and Van Boekel, 2002). This method assumes first-order kinetics as a model to describe inactivation of microorganisms. A mechanistic explanation for this is that death is caused by inactivation of some critical enzyme and it is commonly known that enzyme inactivation is governed by first-order kinetics. Hence, microbial inactivation is also considered to obey first-order kinetics (Martinus and Van Boekel, 2002). Even though the use of D and z values is widely practiced, it has been noted that many survival curves do not really seem to obey first-order kinetics. It has already been noted since the introduction of the D and z concept that many exceptions occur, in the form of shoulders or tails in the survival curves (Cerf, 1977) and Weibull model was able to model these exceptions (Martinus and Van Boekel, 2002).



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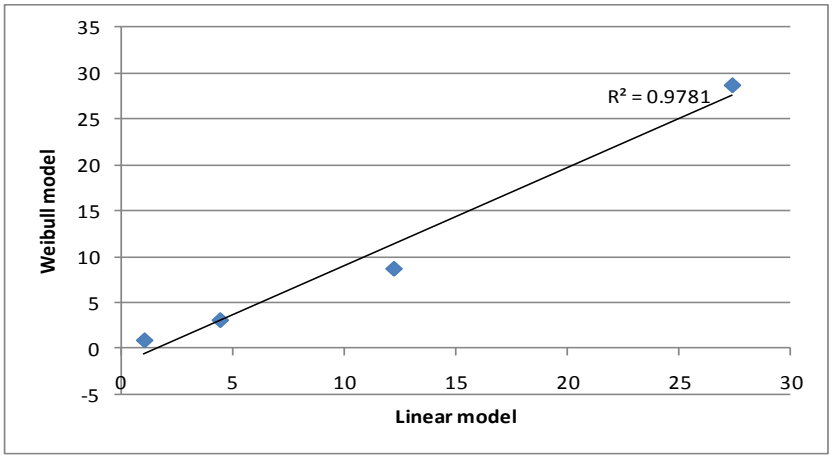

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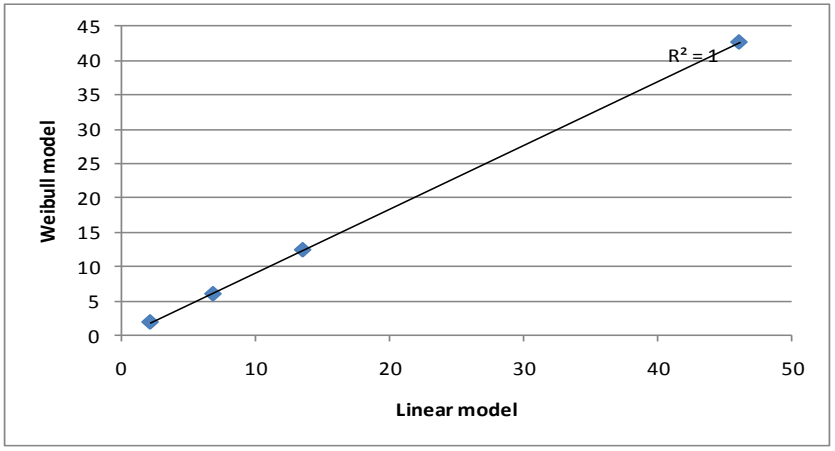

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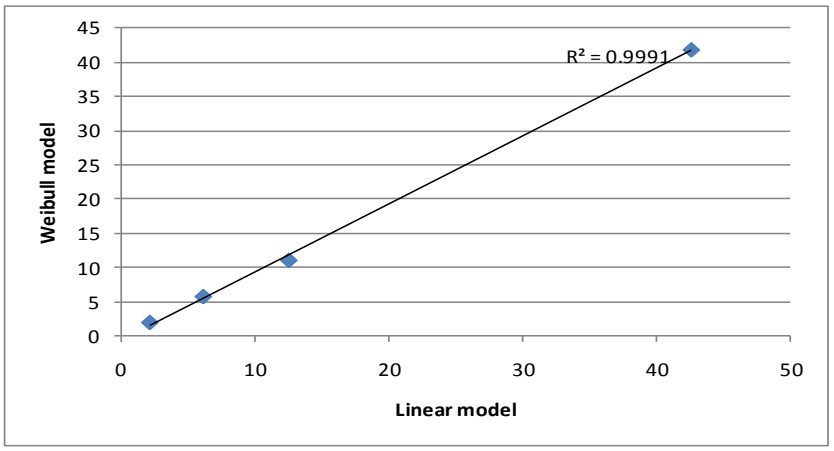


(d)

Fig. 3.5 Comparison between D-values calculated from Weibull and linear models for *C. sporogenes* (a, b) and *G. stearothermophilus* (c, d) in carrot and meat alginate puree, respectively

Martinus and Van Boekel (2002) retrieved data from 55 heat resistance papers and analyzed them. They found out that in only seven cases, the shape parameter (β) was significantly dependent on temperature. Another conclusion from their results is that, in most cases, the shape parameter (β) was higher than 1. In other words, their study indicated that the classical first-order kinetics approach is the exception rather than the rule. Chen and Hoover (2004) and Panagou et al. (2006) found out that the Weibull model produced a better fit than the linear model for the inactivation of *L. monocytogenes* and *P. damnosus*. The Weibull model, in this study, did not produce any better fit than the linear model. Also, the single parameter Bigelow model is easier to use due to the fact that less calculations were involved in it.

3.5 Conclusions

In aseptic processing and continuous rotary autoclaving of liquid particulate food products, temperature probe systems cannot be used. The alternative is to use the biological validation, which is gaining interest, especially due to the fact that in 1989, the Food and Drug Administration (FDA) mandated the biological validation of the aseptic processing of liquid particulate products (Dignan et al. 1989). Inoculating a matrix of firm and thermally stable food alginate simulated particles with spores of *G. stearothermophilus* or *C. sporogenes* can be used in biological validation studies. Using initial and final counts, the integrated process lethality, F_o, can be calculated to ensure the safety of the thermal process. Z-values obtained in this study for *G. stearothermophilus* and *C. sporogenes* were close to the reported z-value of the target microorganism, *C. botulinum*, indicating the appropriateness to use spores of both microorganisms as surrogates. Weibull model was used to model the destruction kinetics and was found to adequately describe the destruction patterns; however, it did not demonstrate any superiority over the Bigelow first-order model and the D value computed from the both models correlated well.

PREFACE TO CHAPTER 4

In the food canning industry, temperature probes are positioned within the test container in order to obtain time temperature profiles and then, process lethality can be calculated. In thermal processes involving free axial rotation processing, it is difficult to attach thermocouples to the particles and the alternative available to evaluate the lethality at the particle center is biological validation.

Alginate based simulated particles with spores of heat resistant microorganisms distributed in them are used in biological validation studies and the process lethality is usually calculated using initial and final counts. Such particles need to have appropriate textural rigidity to withstand the processing conditions. Many process variables, including sodium alginate concentration, calcium chloride concentration and immersion time in the calcium chloride solution, have reported to affect the textural properties of the food alginate fabricated particles. This chapter evaluates the effect of process variables in order to arrive at optimum fabricating conditions using response surface methodology.

Part of this research has been presented in the CMSA annual meeting in 2009 in Montreal, Canada. Another part has been presented in the CIGR conference in 2010 in Quebec City, Canada. One manuscript has been accepted in the International Journal of Food Properties.

The experimental work and data analysis were carried out by the candidate under the supervision of Dr. H. S. Ramaswamy.

CHAPTER 4

OPTIMIZATION OF TEXTURE OF CARROT AND MEAT ALGINATE SIMULATED PARTICLES FOR USE IN THERMAL PROCESSING BIOLOGICAL VALIDATION STUDIES

4.1 Abstract

Alginate based simulated particles with spores of heat resistant microorganisms distributed in them are used in the biological validation studies and process lethality determination using initial and final counts. Such particles should be hard enough to maintain their integrity till the end of the thermal process. Also, they should not adhere to each other inside the can and to the can inner wall. In this study, a response surface methodology was used to determine the optimum conditions that give maximum hardness and minimum adhesiveness of meat and carrot alginate fabricated particles. Sodium alginate concentration (1.5-6.5%), calcium chloride concentration (1.0-3.0%) and immersion time in the calcium chloride solution (4-44 hours) were employed as factors at five levels and the textural properties were assessed using texture profile analysis. For each response (hardness and adhesiveness), a second-order polynomial model was developed using multiple linear regression analysis. Hardness of the fabricated particles was found to significantly increase ($p < 0.05$) with increasing sodium alginate concentration and immersion time in the calcium chloride solution, while calcium chloride concentration had no significant effect. On the other hand, adhesiveness (in absolute values) of the reconstituted particles increased significantly ($p < 0.05$) with increasing sodium alginate while the other two parameters had no significant effect. Applying desirability function method, optimum conditions were found to be sodium alginate concentration of 4.7%, calcium chloride concentration of 2.6% and immersion time of 32.0 and 30.8 hours for carrot alginate and meat alginate fabricated particles, respectively. Fabricated particles using the optimum conditions did not show any variability in hardness values, unlike real food particles, when subjected to thermal processing. This investigation could help food industries using continuous agitation

processing to fabricate firm and thermally stable reconstituted particles to be used in biological validation studies.

4.2 Introduction

Thermal processing is a traditional, yet among the most effective methods to preserve foods. According to Stumbo, thermal processing is the application of heat in order to extend the shelf-life and to promote the safety of food products (Stumbo, 1965). Destroying spoilage and pathogenic microorganisms in canned foods and thus, achieving safety and shelf-stability is the main objective of thermal processing (Ramaswamy and Marcotte, 2005). Lately, there has been an increasing concern among consumers regarding the lower quality of thermally processed canned products. This concern has resulted in numerous studies on minimizing the quality degradation in canned foods. Exploiting the higher sensitivity of microorganisms than quality factors, such as color, texture, flavor and nutrients, to the elevated temperatures, high temperature short time processing (HTST), including aseptic processing, thin profile packaging and agitation processing, has been developed in order to minimize quality degradation of canned food products (Reuter, 1993).

In the food canning industry, temperature probes are positioned within the test container in order to obtain time temperature profiles and then, process lethality can be calculated. In thermal processes involving container agitation (end-over-end and free axial), it is more difficult to attach thermocouples to the particles. In the free axial agitation rotation (as is common in continuous turbo cookers), there are two levels of rotation, one at the can level and other one at the cage level when the cans move in a rotary fashion along with the cage. In these situations, temperature data collection becomes challenging because of the difficulties involved in attaching the temperature measuring devices to the liquid and particles. In the absence of any physical means for temperature measurement at the particle center in free axial rotation processing, the alternative available to evaluate the lethality at the particle center is the biological validation. In 1989, the Food and Drug Administration (FDA) identified the aspects of

the manufacturers' responsibility to approve aseptic processing. Of these, the requirement of biological validation of the heat treatment applied to the product has shown to be the most difficult (Dignan et al., 1989). Consequently, the National Food Processors Association (NFPA) has been concentrating its energy on the development of protocols for the validation of aseptic processing for liquid particulate foods (Chandarana and Unverferth, 1996).

In biological validation, bacterial spores are used (Perkins, 1969). Biological validation can be categorized based on whether or not bacterial spores are in contact with the food (Dignan et al., 1989). Non-contact methods include spores placed in biological indicator units, which are leak proof and small diameter plastic rods (Pflug et al., 1980), spores in glass spheres (Hersom and Shore, 1981), spores in stainless steel (Knap, 1994) or spores in aluminum differential scanning calorimetry pans (Ramaswamy and Ghazala, 1990), positioned at the center of the particle. Non-contact methods have many advantages, including known spore location, complete spore recovery and elimination of the influence of environmental factors (food pH and ionic strength) on spore thermal response. On the other hand, contact methods include inoculating food with a bacterial spore suspension and producing simulated food particles (Berry et al., 1985; Sastry, 1988). This method has many disadvantages including possible spore leaching, but it offers many advantages including uniform inoculum and controlled particle size. Initially, Perspex (poly-methylacrylate) beads containing *Bacillus anthraxis* spores were used to monitor processing of particles in continuous flow systems (Hunter, 1972). Later attempts involved spores immobilized in calcium alginate gel beads (Dallyn et al., 1977) and large food alginate cubes containing spores of *Clostridium sporogenes* or *Bacillus stearothermophilus* (Brown et al., 1984).

Alginate exists naturally in the cell wall of brown algae and commercially in the form of sodium alginate. Sodium alginate is water soluble, producing viscous solutions, and is used as a thickening and stabilizing agent in the food industry. Also, in the presence of calcium ions, alginate has the ability of forming gels (Phillips and Williams, 2000). Nowadays, immobilizing indicators in a matrix of food alginate puree and

immersing it in a calcium chloride solution, resulting in firm and thermally stable particles is the most commonly used technique for biological validation. Inoculated food alginate fabricated particles can be used to determine the process lethality using initial and final counts (Brown et al., 1984; Abdelrahim, 1994; Walsh et al., 1996; Marcotte et al., 2000; Naim et al., 2008). Textural properties of the fabricated particles are essential as these should be hard enough to maintain their integrity till the end of the thermal process in order to minimize the leaching of the spores to the liquid surrounding the particle. Also, the fabricated particles should not adhere to each other inside the can and to the can inner wall (Marcotte et al., 2000).

In order to optimize the process of fabricating the reconstituted particles, Response Surface Methodology (RSM) can be used. RSM is a collection of statistical and mathematical techniques useful for optimizing a variety of food processes (Myers et al., 2009). The principles and foundations of RSM were first introduced by Box and Wilson (1951). The main advantage of RSM is the reduced number of experimental runs that provide sufficient information for statistically valid results. RSM is simpler and more informative than full factorial designs (Koocheki et al., 2009).

The objectives of the study were: (1) to evaluate the effect of sodium alginate concentration, calcium chloride concentration and immersion time in the calcium chloride solution on the hardness and adhesiveness of the carrot alginate and meat alginate fabricated particles and (2) to optimize the process of alginate gel formation by employing Response Surface Methodology and to verify the stability of simulated particles under typical thermal processing conditions.

4.3 Methodology

4.3.1. Sample preparation

Carrot alginate and meat alginate reconstituted particles were fabricated according to the method used by Brown et al. (1984) and Marcotte et al. (2000). Extra lean raw beef and baby carrots were purchased from a local supermarket. Beef paste was made by mincing the beef in a blender with 20% (w/w) distilled water. Carrot paste was made by

boiling 300 g peeled carrots in boiling water for 20 minutes for softening purpose and blending them in a blender with 100 ml of distilled water. A quantity of 0.075 g of tri-sodium citrate was mixed with sodium alginate and added to 100 g of puree in a high speed mixer for 5 min to assure dissolution. Sodium citrate is a food approved sequestrate that was added to control the reaction by competing with the alginate for the calcium ions. Besides, it acts as a buffer in acid systems and can sequester calcium in slightly acid or natural systems. It can also act as a reversible sequestrate when going from near natural condition to acid pH (King, 1983). A quantity of 0.3 g of calcium sulphate suspended in 10 ml distilled water was added to the mixture and mixed thoroughly. Mixture was then transferred to a mould made of one shaped cylinder (45 mm in diameter and 9 mm in height). Molded discs were subsequently immersed into calcium chloride solution for a certain period of time for firming. Then, using a perforator, discs were cut into smaller discs (9 mm in diameter and 9 mm in height) for measuring the textural properties. Figure 4.1 shows the steps involved in the fabrication of the food alginate reconstituted particles.

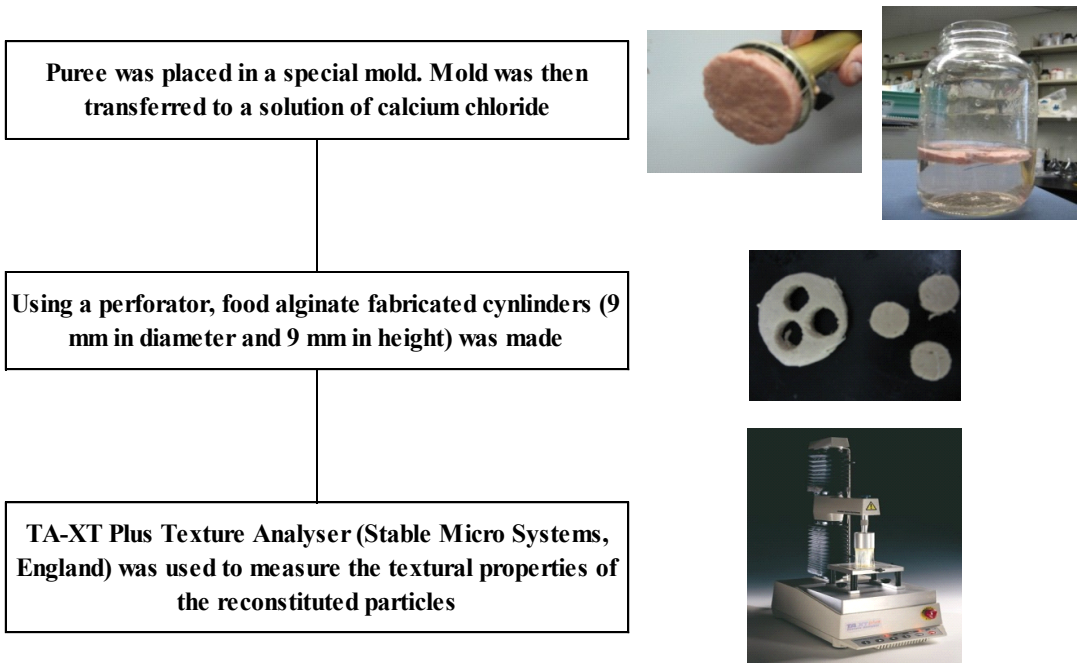


Figure 4.1 Summary of the steps involved in the fabrication of meat and carrot alginate reconstituted particles

4.3.2. Experimental design and statistical analysis

RSM was used to estimate the effect of independent variables (sodium alginate concentration (referred to as X_1); calcium chloride concentration (X_2) and immersion time in the calcium chloride concentration (X_3) on the hardness (N) and adhesiveness (N.s) of the meat alginate and carrot alginate fabricated particles. A Central Composite Rotatable Design (CCRD) was employed for designing the experimental data. The RSM was applied to the experimental data using a commercial statistical package, Design-Expert version 6.01 (Statease Inc., Minneapolis, USA). Experiments were randomized in order to minimize the effects of unexplained variability in the observed responses due to extraneous factors. The experimental design included six centre points in order to calculate the repeatability of the method (Montgomery, 2001). Five levels of sodium alginate concentration (1.5, 2.5, 4.0, 5.5 and 6.5% w/w), five levels of calcium chloride concentration (1.0, 1.4, 2.0, 2.6 and 3.0% w/v) and five immersion times in calcium chloride solution (4, 12, 24, 36 and 44 hours) were used. Range of independent variables was chosen based on preliminary results. The response functions (Y) were hardness and adhesiveness. These values were related to the coded variables (xi, i = 1, 2 and 3) by a second order polynomial using Eq. 4.1:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X^2 + b_{23}X_1X_3 + b_{31}X_2X_3 \quad \text{(Eq. 4.1)}$$

Numerical optimization technique of the Design-Expert software was used for simultaneous optimization of the multiple responses. The desired goals for each variable were chosen within the experimental range. Desired goals for hardness and adhesiveness were maximization and minimization, respectively.

4.3.3. Textural Profile Analysis (TPA)

A TA-XT Plus Texture Analyser (Texture Technologies Corp., Scarsdale, NY / Stable Micro Systems, Godalming, Surrey, UK) with a 2 kg load cell and a circular aluminum probe (d = 5.0 cm) was used in this study. Pre-test, test and post-test speeds were 1.0, 1.5 and 1.5 mm/s, respectively and trigger force was 0.02 N. Samples were

compressed twice to 40% of their original height and texture profile analysis (TPA) was obtained for each disc. The following parameters were quantified: hardness (N), which is maximum force required to compress the sample and adhesiveness (N.s), which is work necessary to pull the compression anvil away from the sample, in other words, it is the negative force that is expressed as the magnitude of the energy below the base level as positive number, with higher number indicating higher adhesiveness. Experiments were carried at room temperature (20^{o}C).

4.4 Results and discussions

A typical TPA is shown in Fig. 4.2. Plots for all carrot alginate and meat alginate fabricated particles were similar, except the fact that the rupture points were either higher or lower indicating different hardness values and the areas under the abscissa after the first compression were larger or smaller indicating different adhesiveness values.

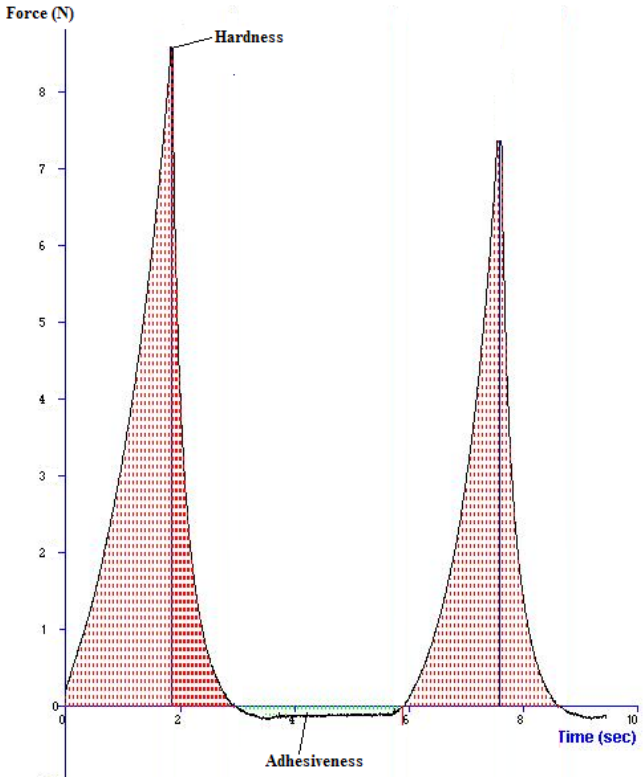


Figure 4.2. A typical Texture Profile Analysis (TPA) of simulated carrot alginate fabricated particle

Mean values (n=5) of hardness (N) and absolute values of adhesiveness (N.s) of reconstituted carrot alginate and meat alginate particles along with their coefficients of variation as function of sodium alginate concentration, calcium chloride concentration and immersion time in the calcium chloride solution are shown in Table 4.1.

Table 4.1 Experimental design used in the study

Assay no	Variables						Response Variables			
	Coded			Actual						
Sl. No	X_1	X_2	X_3	Sodium Alginate (%)	$CaCl_2$ Concentration	Immersion Time (hrs)	Y_1 (Hardness) (N)		Y_2 (Adhesiveness) (N.s)	
							Meat	Carrot	Meat	Carrot
1	-1	-1	-1	2.5	1.4	12	6.5 ± 3.54	6.7 ± 1.04	0.45 ± 2.22	0.43 ± 2.33
2	1	-1	-1	5.5	1.4	12	17.7 ± 0.51	18.0 ± 1.39	2.31 ± 0.87	2.24 ± 3.57
3	-1	1	-1	2.5	2.6	12	7.7 ± 2.21	7.8 ± 0.51	0.83 ± 2.41	0.78 ± 7.69
4	1	1	-1	5.5	2.6	12	19.3 ± 3.11	18.4 ± 0.33	2.06 ± 2.91	2.10 ± 2.86
5	-1	-1	1	2.5	1.4	36	6.8 ± 4.41	6.7 ± 1.64	0.62 ± 3.23	0.64 ± 3.13
6	1	-1	1	5.5	1.4	36	18.3 ± 5.63	18.7 ± 1.50	2.13 ± 1.41	2.17 ± 0.46
7	-1	1	1	2.5	2.6	36	8.8 ± 5.91	8.7 ± 0.11	0.63 ± 6.35	0.62 ± 6.45
8	1	1	1	5.5	2.6	36	19.5 ± 2.77	19.5 ± 1.79	2.10 ± 2.86	2.10 ± 0.48
9	-1.68	0	0	1.5	2.0	24	3.3 ± 0.91	4.1 ± 4.88	0.17 ± 5.88	0.16 ± 6.25
10	1.68	0	0	6.5	2.0	24	18.8 ± 8.72	18.5 ± 3.08	2.87 ± 1.05	2.84 ± 2.82
11	0	-1.68	0	4.0	1.0	24	16.3 ± 0.55	15.6 ± 1.79	0.88 ± 1.14	0.92 ± 9.78
12	0	1.68	0	4.0	3.0	24	18.7 ± 0.80	18.8 ± 4.95	0.82 ± 1.22	0.83 ± 7.23
13	0	0	-1.68	4.0	2.0	4	8.0 ± 0.75	8.1 ± 0.49	0.72 ± 2.78	0.71 ± 9.86
14	0	0	1.68	4.0	2.0	44	16.3 ±0.80	16.6 ± 1.63	1.11 ± 9.91	1.15 ± 1.74
15	0	0	0	4.0	2.0	24	16.0 ± 0.75	16.1 ± 6.27	1.02 ± 7.84	0.99 ± 4.04
16	0	0	0	4.0	2.0	24	14.1 ± 0.71	13.7 ± 1.24	1.41 ± 5.67	1.34 ± 5.97
17	0	0	0	4.0	2.0	24	13.2 ± 2.65	13.7 ± 1.02	0.76 ± 7.89	0.73 ± 8.22
18	0	0	0	4.0	2.0	24	16.0 ± 3.56	16.2 ± 3.02	1.02 ± 6.86	0.97 ± 6.19
19	0	0	0	4.0	2.0	24	14.1 ± 1.49	14.2 ± 1.69	1.41 ± 2.84	1.36 ± 6.62
20	0	0	0	4.0	2.0	24	13.2 ± 0.98	13.7 ± 1.61	0.76 ± 7.89	0.80 ± 5.00

Mean ± COV (SD/Mean x 100) (n = 5)

X_1: Sodium alginate concentration, X_2: calcium chloride concentration and X_3: immersion time in calcium chloride solution

Hardness values ranged from 3.3 to 19.5 N and from 4.1 to 19.5 N for meat alginate and carrot alginate fabricated particles, respectively. Adhesiveness values for meat alginate and carrot alginate fabricated particles ranged from 0.17 to 2.87 N.s and from 0.16 to 2.84 N.s, (in absolute values), respectively. The results indicate that the texture parameters are quite sensitive to the process variables justifying the need for the study and for evaluating the optimal conditions.

4.4.1. Model fitting

The second-order polynomial response surface model (Eq. 4.1) was fitted to each of the two response variables (Y). For judging the fitting of the explanatory models and the variation of the hardness and adhesiveness values, the sum of squares of the sequential model was analyzed. These analyses indicated that adding up the quadratic terms significantly improved the model for the hardness and the adhesiveness. Regression analysis and ANOVA were used to fit the model and to examine the statistical significance of the terms. The estimated regression coefficients of the models for the response variables along with the corresponding coefficients of determination (R^2) are given in Table 4.2.

The lack of fit is an indication of the failure for a model representing the experimental data at which points were not included in the regression or variations in the models cannot be accounted for random error (Montgomery, 2001). If there is a significant lack of fit which could be indicated by a low probability value, the response predictor is discarded. The lack of fit, as illustrated in Table 4.2, did not result in a significant *p*-value for the models, meaning that these models were significant for predicting the relevant responses.

Table 4.2 ANOVA and regression coefficients of the second-order polynomial model for the response variables (actual values)

Source	DF	Hardness						Adhesiveness					
		Meat			Carrot			Meat			Carrot		
		Coefficient	Sum of squares	*p*-value	Coefficient	Sum of squares	*p*-value	Coefficient	Sum of squares	*p*-value	Coefficient	Sum of squares	*p*-value
Model	9	-6.50	449.77	<0.0001	-7.7	423.56	<0.0001	-0.55	9.06	0.0003	-0.38	9.150	0.0001
Linear													
b_1	1	8.26	369.82	<0.0001	8.26	347.79	<0.0001	-0.07	8.24	< 0.0001	-0.10	8.330	<0.0001
b_2	1	-9.73	7.38	0.138	-7.36	6.86	0.163	0.58	6.05E-06	0.993	0.38	3.19E-05	0.983
b_3	1	0.40	19.12	0.027	0.33	21.15	0.025	8.01E-03	0.02	0.656	0.01	0.037	0.482
Quadratic													
b_{11}	1	-0.57	23.94	0.016	-0.56	23.28	0.020	0.10	0.72	0.014	0.10	0.73	0.009
b_{22}	1	2.75	14.17	0.049	2.26	9.57	0.106	-3.69E-02	2.56E-03	0.864	9.73E-03	2.22E-04	0.956
b_{33}	1	-6.25E-03	11.67	0.070	-6.25E-03	11.67	0.078	6.71E-05	1.35E-03	0.901	1.59E-04	7.60E-03	0.748
Interaction													
b_{12}	1	-0.06	0.02	0.935	-0.26	0.45	0.708	-0.09	0.06	0.428	-0.08	0.039	0.471
b_{13}	1	-4.17E-03	0.05	0.902	6.25E-03	0.1	0.859	-7.63E-04	1.51E-03	0.895	8.33E-04	2.25E-03	0.861
b_{23}	1	6.94E-03	0.02	0.935	0.02	0.21	0.797	-2.60E-03	2.81E-03	0.857	-5.21E-03	0.011	0.701
Residual	10		28.33			30.32			0.82			0.69	
Lack of fit	5		20.16	0.172		22.92	0.120		0.39	0.537		0.34	0.517
Pure error	5		8.17			7.4			0.43			0.35	
Total	19		478.1			453.9			9.88			9.85	
R^2		0.94			0.93			0.92			0.93		

Coefficient of determination, R^2, is the proportion of variation in the response attributed to the model rather than to random error and was suggested by Little and Hills (1978) and Mendenhall (1975) that for a model to have a good fit, the R^2 should not be less than 0.8. When R^2 approaches unity, it signifies excellent suitability of fitting empirical model to the actual data. The lower value of R^2 shows the inappropriateness of the model to explain the relation between variables. For hardness, R^2 values were found to be 0.93 and 0.94, for carrot alginate and meat alginate fabricated particles, respectively. On the other hand, for adhesiveness, R^2 values were found to be 0.93 and 0.92, for carrot alginate and meat alginate fabricated particles, respectively. R^2 values for both response variables were higher than 0.80, indicating that the regression models were suitable to correlate the behavior.

Coefficient of variation (CV) describes the extent to which the data were dispersed. As a general rule, the coefficient of variation should not be greater than 10%. Daniel (1991) reported that a high CV indicates that variation in the mean value is high and does not satisfactorily develop an adequate response model. For hardness values, the

coefficients of variation (s.d/mean x 100) ranged from 0.51 to 8.72% in meat alginate and from 0.11 to 6.27% in carrot alginate particles. On the other hand, for adhesiveness, the coefficients of variation ranged from 0.87 to 9.91% in meat alginate and from 0.46 to 9.86% for carrot alginate fabricated particles. Our results showed that the coefficients of variation were less than 10% for both responses (Table 4.2), representing an acceptable precision and reliability of the conducted experiments**.**

4.4.2. Hardness

The *p*-values were used to check the significance of each coefficient. This value is necessary to understand the pattern of mutual interactions between the test variables. The smaller the magnitude of the *p*-value, the more significant is the corresponding coefficient. Values of *p* less than 0.05 indicate that the model terms are significant (Montgomery et al., 2001).

From the model of hardness, linear and quadratic effects of sodium alginate concentration and linear effect of immersion time were significant ($p < 0.05$) (Table 4.2). Based on the sum of squares, the importance of the independent variables on hardness of carrot alginate and meat alginate reconstituted particles could be ranked in the following order: sodium alginate concentration > immersion time in calcium chloride > calcium chloride concentration. It can be seen that among the interaction terms, none were significant ($p > 0.05$). The results also showed that variables with the largest effect were the linear and quadratic terms of sodium alginate concentration followed by the linear effect of immersion time.

The relationship between independent and dependent variables is illustrated in the form of three-dimensional response surface plots as generated by the model. The response surfaces were based on the coefficients presented in Table 4.2. The data were generated through keeping one variable at its respective zero level (centre value of the testing ranges) and varying the other two within the experimental range.

The variation of hardness with sodium alginate concentration and immersion time at constant calcium chloride concentration (2%) is presented in Figures 4.3b and 4.3d for

carrot alginate and meat alginate fabricated particles, respectively. On the other hand, the variation of hardness with sodium alginate concentration and calcium chloride concentration at constant immersion time (24 h) is presented in Figures 4.3a and 4.3c for carrot alginate and meat alginate fabricated particles, respectively. As shown in these figures, hardness of the carrot alginate and meat alginate fabricated particles increased exponentially with sodium alginate concentration and immersion time.

Marcotte et al. (2000), Manjunatha and Das Gupta (2006) and Roopa and Bhattacharya (2008) also reported that increasing sodium alginate concentration resulted in an increase in the hardness of food alginate gel, as increasing sodium alginate concentration increases the gel formation and therefore the hardness of the reconstituted particles. King (1983) found out that increasing immersion time in the calcium chloride solution resulted in an increase in hardness as well, as alginate gels are produced by interaction with calcium ions and the gel strength will usually increase with time. On the other hand, Manjunatha and Das Gupta (2006) and Roopa and Bhattacharya (2008) reported a significant effect of the calcium salt concentration on the hardness of alginate gels.

On the other hand, the carrot alginate reconstituted particles had significantly ($p < 0.05$) higher moisture content than the meat alginate reconstituted particles; however, there was no significant ($p > 0.05$) difference in the hardness values between both types of particles fabricated from the same levels of sodium alginate concentration, calcium chloride concentration and immersion time.

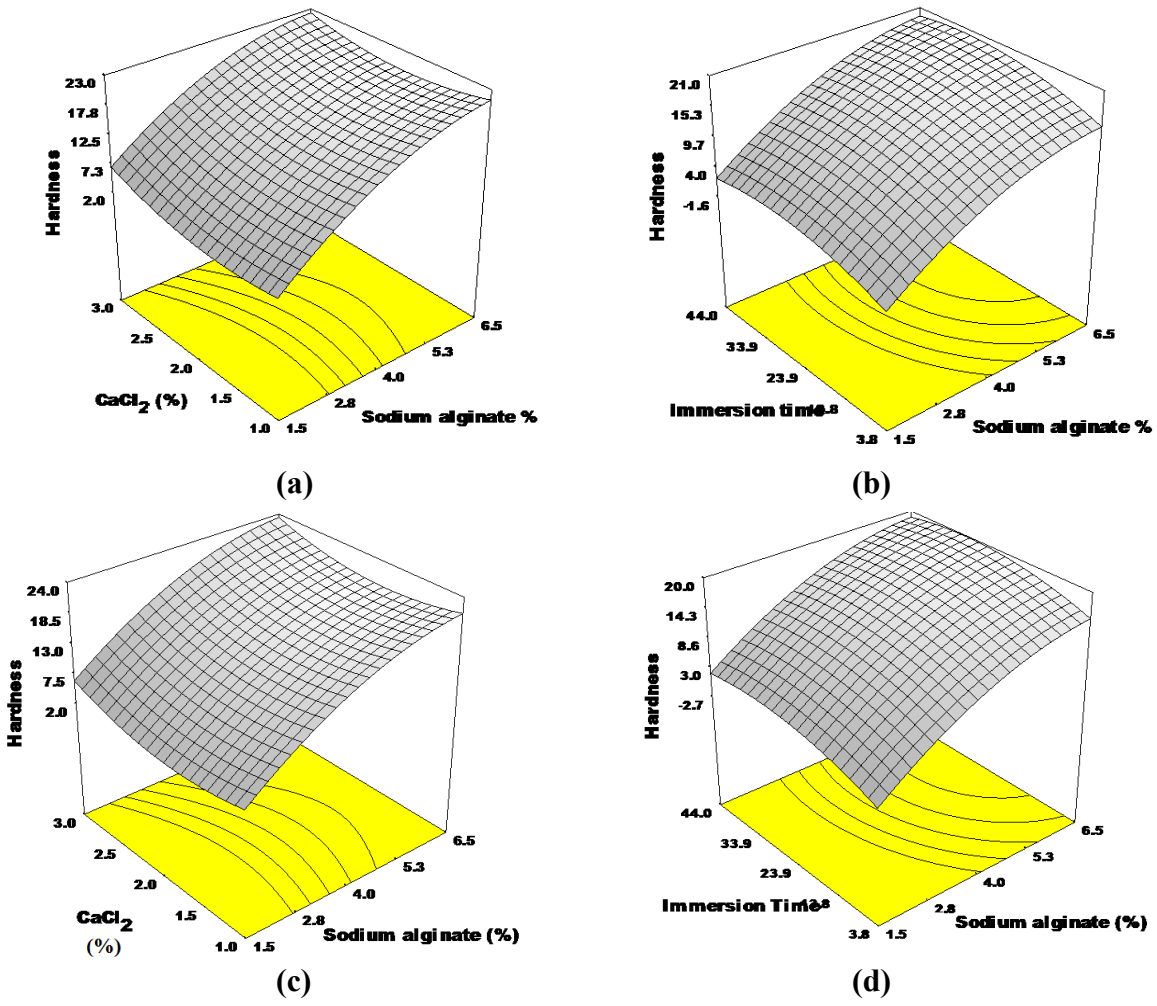


Fig 4.3. Response surface for the effect of sodium alginate concentration and calcium chloride concentration (immersion time = 24 hours), sodium alginate concentration and immersion time (calcium chloride concentration = 2% on the hardness of the carrot/alginate (a, b) and meat/alginate (c, d) fabricated particles

Fat content of the meat alginate reconstituted particles (~10%) probably counterbalanced the effect of the lower moisture content as increasing fat content results in a decrease in the hardness values, caused by a less dense food matrix. This observation was in agreement with other studies (Bryant et al., 1995; Rudan et al., 1999; Tunick et al., 1993; Kahyaoglu et al., 2005).

As can be seen in Table 4.1, hardness values of meat alginate and carrot alginate fabricated particles ranged from 3.3 to 19.5 N. Gelled whole eggs, egg yolk and egg white particles inoculated with microorganisms can be used instead of the food alginate fabricated particles in biological validation studies (Raikos et al., 2006; Dev et al., 2009). Raikos et al. (2006) measured the hardness values of egg yolk and egg white gels at different pH, salt and sugar concentrations and reported hardness values ranging from 1.96 to 14.2 N. This clearly indicates that the food alginate reconstituted particles in our study were harder than the egg white and egg yolk gels and thus, they may better withstand the high temperatures of the thermal processing.

4.4.3. Adhesiveness

The results in Table 4.2 for adhesiveness indicated that the linear and quadratic effects of sodium alginate concentration were significant ($p < 0.05$), while the effects of calcium chloride concentration and immersion time in the calcium chloride solution were not ($p > 0.05$). The mutual interaction between sodium alginate concentration, calcium chloride concentration and immersion time in the calcium chloride concentration were found to be non-significant ($p > 0.05$).

The variation of adhesiveness with sodium alginate concentration and immersion time at constant calcium chloride concentration (2%) is presented in Figures 4.4b and 4.4d for carrot alginate and meat alginate fabricated particles, respectively. On the other hand, the variation of adhesiveness with sodium alginate concentration and calcium chloride concentration at constant immersion time (24 hours) is presented in Figures 4.4a and 4.4c for carrot alginate and meat alginate fabricated particles, respectively. As it can

be shown in the figure, the adhesiveness, in absolute values, increased with increasing sodium alginate concentration.

Increasing the sodium alginate concentration from 1.5 to 6.5%, therefore decreasing the moisture content, resulted in a significant ($p < 0.05$) increase in the adhesiveness (absolute values) from 0.16 to 2.84 N.s for carrot alginate and from 0.17 to 2.87 N.s for the meat alginate fabricated particles. Increasing the moisture content makes the fabricated particles adhere more to the compression anvil and work necessary to pull the compression anvil away from the sample will be higher. This observation has been reported by Rahman and Al-Farsi (2005) who found out that increasing moisture content of dates from 17.1 to 58.2% resulted in a decrease in their adhesiveness values from 4.60 to 0.55 N.s (in absolute values). On the other hand, despite the significant ($p < 0.05$) difference in the moisture content between carrot alginate and meat alginate reconstituted particles fabricated using the same concentration of sodium alginate and calcium chloride and immersion time in calcium chloride solution, there was no significant ($p > 0.05$) difference in the adhesiveness values. This can be due to the counterbalance effect of the fat content of the meat alginate reconstituted particles (~10%). Presence of fat makes the food matrix loose and open; therefore, adhesiveness value will increase (Bryant et al. 1995).

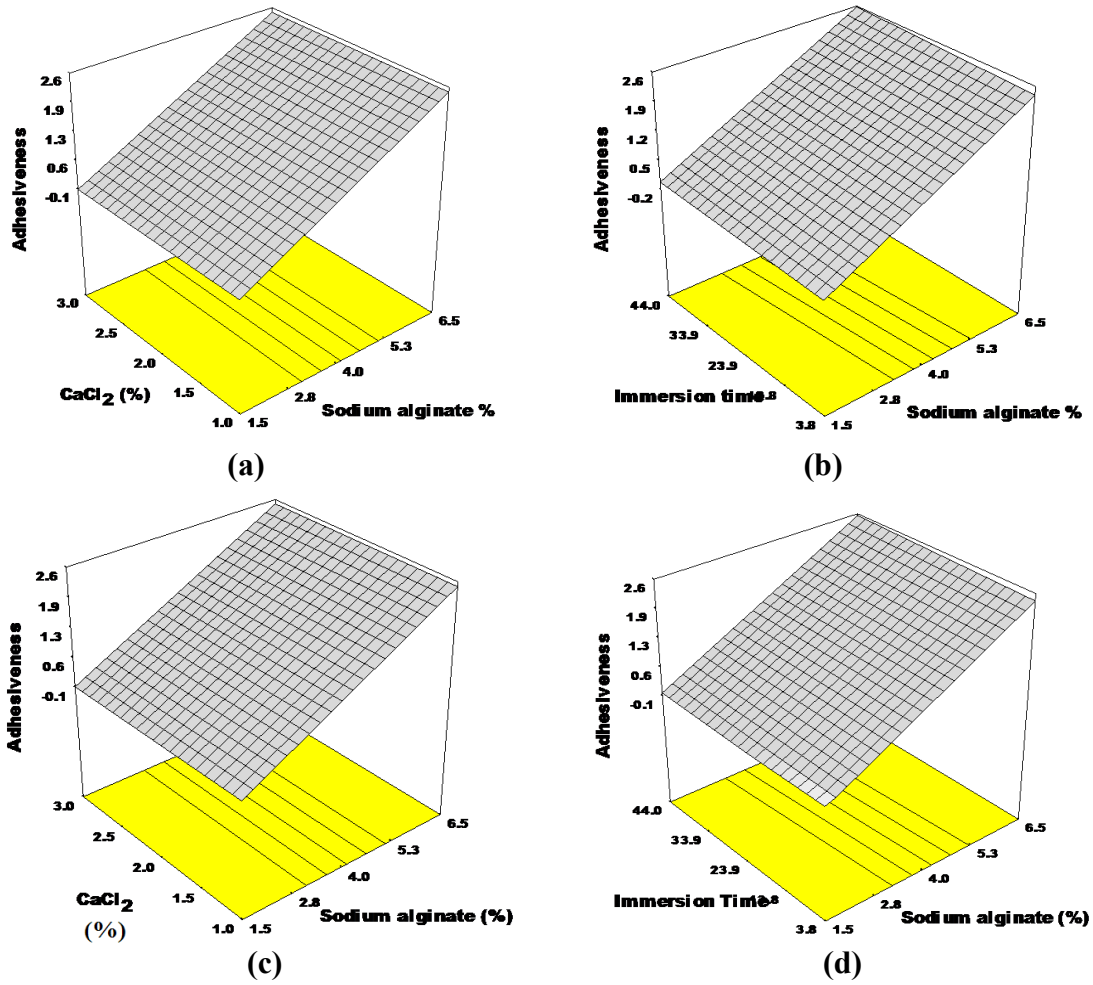
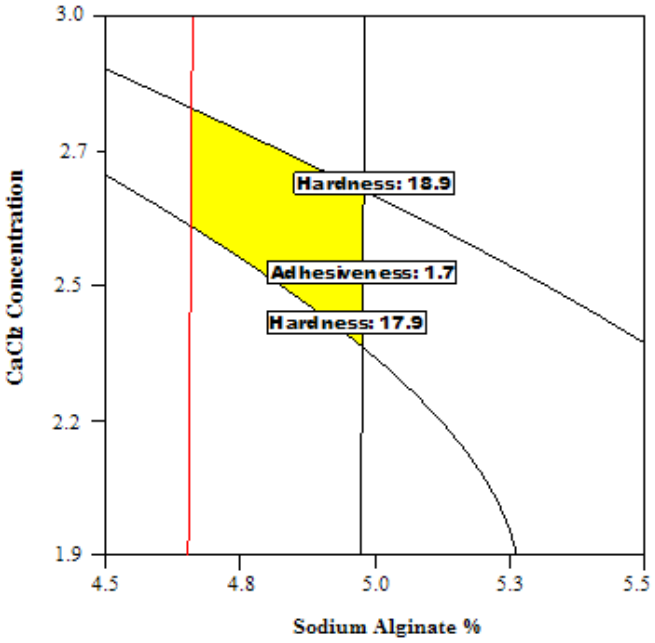


Fig 4.4. Response surface for the effect of sodium alginate concentration and calcium chloride concentration (immersion time = 24 hours), sodium alginate concentration and immersion time (calcium chloride concentration = 2%) on the adhesiveness of the carrot/alginate (a, b) and meat/alginate (c, d) fabricated particles

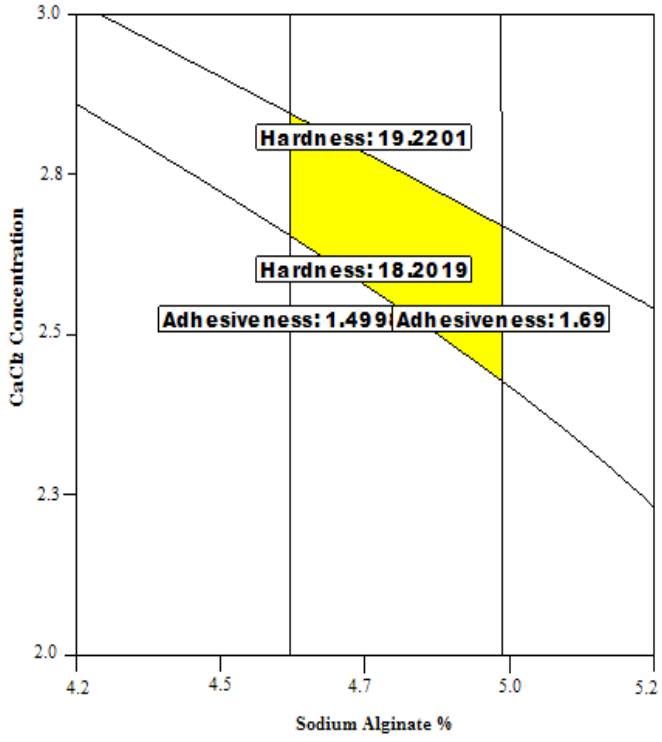
4.4.4. Optimization

Optimum conditions for the fabrication of meat alginate and carrot alginate reconstituted particles were determined to obtain maximum hardness with minimum adhesiveness in the particles. Maximum hardness is essential in order to have firm and stable particles that can withstand the high thermal processing temperatures and therefore avoiding the leaching of the spores out from the matrix to the surrounding liquid. On the other hand, minimum adhesiveness is essential in order to prevent sticking of the particles to each other and to the can inner wall during thermal processing. Optimum fabrication conditions were determined by superimposing the contour plots of all the responses as shown in Fig. 4.5.

The optimum conditions for maximum hardness, for minimum adhesiveness and for both maximum hardness and minimum adhesiveness are tabulated in Table 4.3. For maximum hardness of 20.2 and 19.9 N in meat and carrot alginate reconstituted particles, respectively, optimum conditions were sodium alginate concentration of 5.45 and 5.47%, calcium chloride concentration of 2.59 and 2.56% and immersion time of 30 and 28.3h, for meat alginate and carrot alginate fabricated particles, respectively. For minimum adhesiveness of 0.91 and 0.88 N.s in meat and carrot alginate reconstituted particles, respectively, optimum conditions were sodium alginate concentration of 3.5%, calcium chloride concentration of 1.40 and 2.57% and immersion time of 12 h, for meat alginate and carrot alginate fabricated particles, respectively. For both maximum hardness and minimum adhesiveness, optimum conditions were sodium alginate concentration of 4.74 and 4.71%, calcium chloride concentration of 2.6% and immersion time of 30.8 and 32.1 h, for meat alginate and carrot alginate fabricated particles, respectively.



(a)



(b)

Fig 4.5. Superimposed contour plots of hardness and adhesiveness in carrot (a) and meat (b) alginate reconstituted particles

Table 4.3 Predicted optimum conditions for producing meat and carrot alginate particles with highest hardness, lowest adhesiveness and both highest hardness and lowest adhesiveness properties

Goal	Alginate Particle	[Sodium Alginate]	[$CaCl_2$]	Immersion Time (hrs)	Hardness (N)	Adhesiveness (N.s)	Desirability
Maximum Hardness	Carrot	5.39	2.47	29.43	19.55		1.00
		5.28	2.53	32.07	19.66		1.00
		5.47	**2.56**	**28.31**	**19.91**		1.00
		5.48	2.30	36.00	19.34		0.99
		5.50	1.40	32.19	19.13		0.98
	Meat	5.43	2.57	31.50	20.18		1.00
		5.45	**2.59**	**30.01**	**20.24**		1.00
		5.47	2.50	35.24	19.86		1.00
		5.50	2.37	33.45	19.50		1.00
		5.50	1.40	31.05	18.98		0.97
Minimum Adhesiveness	Carrot	**3.50**	**2.57**	**12.00**		**0.88**	0.73
		3.50	2.25	12.00		0.88	0.73
		3.50	2.46	12.19		0.88	0.73
		3.50	2.22	12.00		0.88	0.73
		3.50	2.20	12.00		0.88	0.73
	Meat	**3.50**	**1.40**	**12.00**		**0.91**	0.73
		3.50	1.52	12.00		0.91	0.73
		3.50	1.56	12.00		0.91	0.73
		3.50	1.40	12.06		0.91	0.73
		3.50	1.75	12.00		0.91	0.73
Maximum hardness and minimum adhesiveness	Carrot	**4.71**	**2.60**	**32.05**	**18.70**	**1.60**	0.77
		4.72	2.60	32.01	18.73	1.60	0.77
		4.80	2.60	33.15	18.93	1.65	0.77
		4.93	1.40	29.97	17.85	1.70	0.72
		4.93	1.40	29.93	17.85	1.70	0.72
	Meat	**4.74**	**2.60**	**30.80**	**18.80**	**1.61**	0.78
		4.71	2.60	31.04	18.71	1.59	0.78
		4.69	2.60	31.09	18.67	1.58	0.78
		4.92	2.60	29.01	19.18	1.69	0.78
		4.53	2.60	30.58	18.24	1.50	0.78

4.4.5. Thermal stability

Fresh carrot and carrot alginate discs (9 mm in diameter and 9 mm in height), fabricated according to the optimum conditions, were placed together inside a can and thermally processed at 100 and 120^{o}C inside water immersion rotational processing conditions (15 rpm) in the pilot scale retort in order to compare the hardness changes due to heat. Fig. 4.6 shows the effect of cooking on the hardness of carrot alginate reconstituted particles. Hardness of fresh and cooked carrots came down from 110 N for

the fresh to about 10 N for the processed particles. On the other hand, hardness of the carrot alginate fabricated particles stayed nearly same at 19 N both before and after processing.

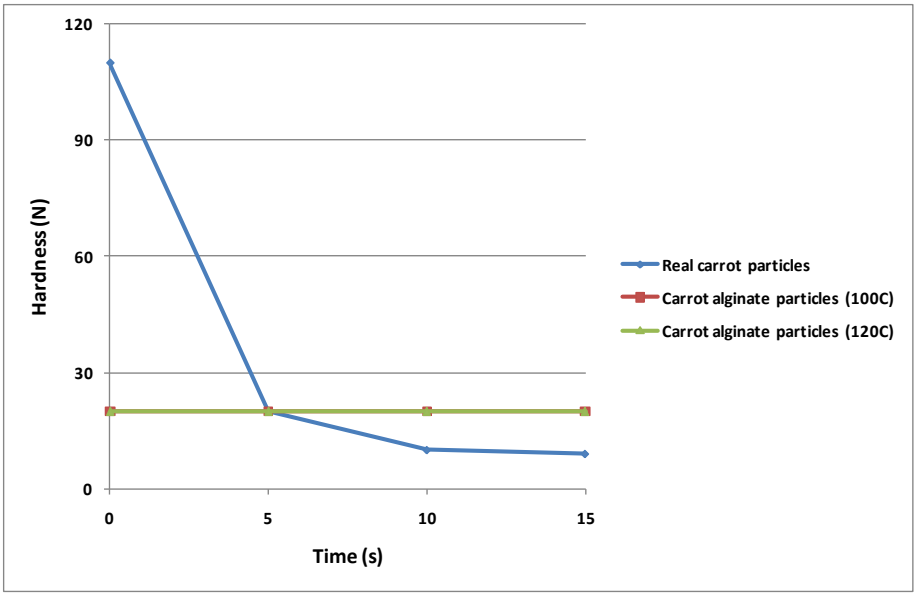


Fig 4.6 Softening curves for carrot cooked at 100°C and for carrot/alginate fabricated particles at 100°C and 120°C

Fresh carrot particles have been reported to follow two mechanisms of softening losing about 75% of their hardness during the first five minutes of cooking after which rate of softening slows down (Huang and Bourne, 1983; Taherian, 1995 and Marcotte et al., 2000). Spiess et al. (1987) reported a hardness of 14 N for cooked carrots at 95°C for 20 min. Besides, Rahman et al. (1971) reported a hardness of 21 N for carrots cooked for 10 min. The hardness values for fresh and cooked carrots were within this range. Softening curve of the fabricated carrot/alginate particles showed a different trend than the carrots, indicating that the reconstituted particles have much higher thermal stability than carrots. Hardness of carrot/alginate fabricated particles was twice higher than the one of the cooked carrots.

4.5 Conclusions

The results of the study showed that the effects of sodium alginate concentration and immersion time in calcium chloride solution were statistically significant ($p < 0.05$) for the hardness of carrot alginate and meat alginate reconstituted particles. On the other hand, sodium alginate concentration had a statistically significant effect ($p < 0.05$) on the adhesiveness of the reconstituted particles. Second-order polynomial models were obtained for predicting hardness and adhesiveness. Increasing the sodium alginate and immersion time resulted in an increase in the hardness values; however, increasing sodium alginate concentration only resulted in an increase in the adhesiveness values. Optimum fabrication condition for maximizing hardness and minimizing adhesiveness comprised the condition in which the sodium alginate concentration was 4.7%, the calcium chloride concentration was 2.6% and the immersion times in calcium chloride solution were 30.8 and 32.1 hours, respectively for meat alginate and carrot alginate fabricated particles, respectively. Fabricated particles using the optimum conditions did not show any variability in hardness values, unlike real food particles, when subjected to thermal processing. This investigation could help food industries using continuous agitation processing to fabricate firm and thermally stable reconstituted particles to be used in biological validation studies.

PREFACE TO CHAPTER 5

In thermal processes involving aseptic processing and free axial rotation processing, there are many difficulties in attaching thermocouples to the particles. The alternative available to evaluate the lethality at the particle center is to conduct biological validation, where bacterial spores are inoculated inside the matrix of food alginate simulated particles and process lethality can be calculated from initial and final counts.

An important pre-requisite for such particles is to have similar thermophysical properties (density, heat capacity, thermal conductivity and thermal diffusivity) to the real food particles. Many variables, including sodium alginate concentration, calcium chloride concentration and immersion time in the calcium chloride solution, are involved in the particles making and may affect the thermophysical properties of the food alginate fabricated particles. This chapter evaluates the effect of different process variables in order to arrive at optimum conditions using response surface methodology.

Part of this research has been presented in the CIGR conference in 2010 in Quebec City, Canada. One manuscript has been accepted in the Journal of Food Engineering.

The experimental work and data analysis were carried out by the candidate under the supervision of Dr. H. S. Ramaswamy.

CHAPTER 5

EVALUATION AND OPTIMIZATION OF THERMOPHYSICAL PROPERTIES OF CARROT AND MEAT ALGINATE SIMULATED FOOD PARTICLES

5.1 Abstract

The objective of the study was to investigate the thermo-physical properties of meat and carrot based alginate particles as influenced by the formulation variables. A response surface methodology (RSM) was used to study the effect of sodium alginate concentration, calcium chloride concentration and dipping time in the calcium chloride solution, at five levels each, on the thermo-physical properties of the fabricated particles (9 mm in diameter and 9 mm in height). Density was similar for all conditions. Increasing sodium alginate concentration resulted in a significant decrease in the heat capacity and thermal conductivity values. These changes were primarily attributed to the lowering of moisture content of the particles resulting from the treatment. Using RSM, optimum conditions for fabricating particles having similar thermo-physical properties to real foods were obtained as 5.3 and 4.9% sodium alginate, 2.2% calcium chloride and 14.2 and 36.0 h immersion in the calcium chloride solution for meat and carrot alginate particles, respectively.

5.2 Introduction

Thermal processing is amongst the most effective methods to preserve foods by applying heating, in combination with pH and vacuum, for sufficient time at a high enough temperature to create commercial sterility (Ramaswamy and Marcotte, 2005). Conventional canning involves filling of the food product in metal cans, glass jars, retortable semi-rigid plastic containers or pouches followed by double seaming or heat sealing and thermal processing in a pressurized batch or continuous retorts, resulting in commercially sterile and shelf stable products (Prahbat, 2006). With consumers becoming more educated and health conscious, the demand for convenient and high quality foods has increased over time. Canning usually involves excessive thermal treatment of the product, resulting in considerable degradation of color, flavor, texture and nutrients (David et al., 1996). The quality of canned food can be improved by applying high temperature-short time (HTST) processing, as most quality factors are less temperature sensitive than the microorganisms. HTST processing includes aseptic processing, agitation processing and thin profile processing. Aseptic processing consists of heating of food prior to packaging to a high temperature, holding it for a short time, cooling it down and then packaging it in pre-sterilized containers in a sterile chamber (Lund, 1987). The extension of aseptic processing to particulate foods has been generally hindered by limitations to demonstrate an adequate thermal treatment as some particles move faster than the others, posing difficulties in gathering temperature profile at the particle center. In continuous flow agitation processing especially involving free axial rotation like in FMC turbo-cookers, there are two levels of rotation, one at the can level and another one at the helix-level when the cans move in a rotary fashion along through the helix. In both aseptic and agitation processing systems, temperature data collection is challenging due to difficulties involved in attaching the temperature measuring devices to the moving particles/cans. Because of these difficulties, biological validation becomes necessary (Chandarana, 1992). FDA has recognized quantitative biological validation of lethality in such processes (Dignan et al. 1989).

Biological validation can be carried out in many ways. It can be categorized based on whether or not bacterial spores are in contact with food (Dignan et al., 1989). Non-

contact methods include incorporating spores in biological indicator units, in small diameter plastic rods (Pflug et al., 1980), in glass spheres (Hersom and Shore, 1981), in stainless steel pans (Knap, 1994) or in aluminum differential scanning calorimetry pans (Ramaswamy and Ghazala, 1990), and then positioning them at the center of the particle. On the other hand, contact methods include inoculating food with a bacterial spore suspension or producing spore loaded simulated food particles (Berry et al., 1985; Sastry, 1988). Immobilizing suitable indicators in a matrix of alginate food puree forming a particle and then calculating process lethality using initial and final concentration of the indicator is the most commonly used technique in process validation when temperature measurements are not possible (Brown et al., 1984).

Alginates exist naturally in the cell wall of brown algae and commercially in the form of sodium salt. Sodium alginate is water soluble, producing viscous solutions and is used as a thickening and stabilizing agent in the food industry. Also, in the presence of calcium ions, sodium alginate has the ability of forming gels (Phillips and Williams, 2000). Simulated alginate food particles possess enough mechanical strength to withstand high processing temperatures and have similar physical and thermal properties to the real food particles. In addition, in contrast to real food particles, simulated particles can have uniform size and spore concentration (Marcotte et al., 2000).

Thermo-physical properties (heat capacity, thermal conductivity and density) of food are essential parameters in modeling various thermal processes and in optimizing the heating system design (Karunakar et al., 1998). Also, these properties are essential for characterizing heat transfer processes in the food industry, where energy costs and food quality and safety are the main considerations. These properties are also temperature dependent (Heldman, 1982). In the case of biological validation studies involving food alginate fabricated particles inoculated with microbial spores, it is desirable that these particles have thermo-physical properties similar to the real food particles (Marcotte et al., 2000).

Response surface methodology (RSM) is a combination of statistical and mathematical techniques useful for optimizing a variety of food processes (Myers et al.,

2009). The principles and foundations of RSM were first introduced by Box and Wilson (1951). The main advantage of RSM is the reduced number of experimental runs that provide sufficient information for statistically valid results. In other words, RSM is faster and more informative than full factorial designs (Koocheki et al., 2009).

The objectives of the study were: (1) to evaluate the effect of different formulations on the thermo-physical properties of meat and carrot based alginate particles and (2) to optimize the process of making these simulated particles so that they have thermo-physical properties similar to those of real food particles.

5.3 Methodology

5.3.1. Samples preparation

Preparation methodology for meat and carrot alginate particles was similar to those used by Brown et al. (1984) and Marcotte et al. (2000). Briefly, extra lean beef (18% fat) and baby carrots were purchased from a local supermarket. Beef puree was made by mincing the beef in a blender with 20% w/w distilled water. Carrot puree was made by placing 300 g peeled carrots in boiling water for 20 minutes for softening purpose and then blending them in a blender with 100 ml of distilled water. Tri-sodium citrate (0.075 g) was mixed with the predetermined amount of sodium alginate and added to 100 g of the puree in a high speed mixer for 5 minutes to assure dissolution. Sodium citrate is a food-approved sequestrate that was added to control the reaction by competing with the alginate for the calcium ions. Also, it acts as a buffer in acid systems and can sequester calcium in slightly acid or natural systems. Besides, it can act as a reversible sequestrate when going from near natural condition to acid pH (King, 1983). A quantity of 0.3 g of calcium sulphate suspended in 10 ml distilled water was added to the mixture and mixed thoroughly. Mixture was then transferred to a mould shaped to make a cylindrical disc (45 mm in diameter and 9 mm in height). Moulded discs were subsequently immersed into calcium chloride solution for a certain period of time for structure formation. Then, using a perforator, discs were cut into smaller ones (9 mm in diameter and 9 mm in height) that were used for measuring the thermo-physical properties.

5.3.2. Experimental design

Response surface methodology (RSM) was used to estimate the effect of independent variables (sodium alginate concentration, x_1; calcium chloride concentration, x_2 and immersion time in the calcium chloride solution, x_3) on the density ($g.cm^{-3}$), heat capacity ($kJ.kg^{-1}.^oC^{-1}$) and thermal conductivity ($W.m^{-1}.K^{-1}$) of the meat alginate and carrot alginate particles. A central composite rotatable design (CCRD) was employed for designing the experimental data. RSM methodology was applied to the experimental data using a commercial statistical package, Design-Expert version 6.01 (Statease Inc., Minneapolis, USA). Experiments were randomized in order to minimize the effects of unexplained variability in the observed responses due to extraneous factors. The experimental design involved 20 experiments with 6 replications of the central point in order to calculate the repeatability of the method (Montgomery, 2001). Experiments were done in triplicates. Five levels of sodium alginate concentration (1.5, 2.5, 4.0, 5.5 and 6.5% w/w), five levels of calcium chloride concentration (1.0, 1.4, 2.0, 2.6 and 3.0% w/v) and five immersion times in calcium chloride solution (4, 12, 24, 36 and 44 h) were used. The response functions (y) were density, heat capacity and thermal conductivity. Values were related to the coded variables (x_i, i = 1, 2 and 3) by a second order polynomial using the equation below:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{23}x_1x_3 + b_{31}x_2x_3$$

(Eq. 5.1)

The coefficients of the polynomial model were represented by b_0 (constant term), b_1, b_2 and b_3 (linear effects), b_{11}, b_{22} and b_{33} (quadratic effects), and b_{12}, b_{13} and b_{23} (interaction effects). Statistical significance of the terms in the regression equations was examined. The significant terms in the model were found by analysis of variance (ANOVA) for each response. The adequacy of model was checked by the R^2 values. Numerical optimization technique of the Design-Expert software was used for simultaneous optimization of the multiple responses. The desired goals for each variable and response were chosen. All the independents variables were kept within range while the responses were the measured values.

The general linear model (GLM) procedure within SAS (1993) was used in order to evaluate the significance of moisture content and temperature on the heat capacity of carrot alginate and meat alginate fabricated particles. T-test in the Microsoft Excel (2007) was used in order to assess the difference between means.

5.3.3. Moisture and fat determination

Moisture content: The oven drying method was used. Carrot alginate and meat alginate simulated particles were homogenized and dried at 100^{o}C for 24 h (Nollet, 2004).

Fat content: AOAC Official Method 991.36 for determination of fat in meat and meat products was used. 2 g of sample was dried, and then, petroleum ether was added and lipids in the sample were extracted using the Soxhlet apparatus. After that, the solvent was evaporated and the fat residue was cooled to room temperature and weighed.

5.3.4. Density measurements

A liquid displacement method was used for the density measurement. The volume of the sample was measured directly by the volume of liquid displaced by the test material. A non-wetting liquid, toluene, was used (Sahin and Sumnu, 2006).

5.3.5. Heat capacity measurements

A differential scanning calorimeter (DSC Q100, TA instruments Inc., New Castle, DE) was used with a nitrogen cooling system. At first, the cell constant of the instrument was evaluated by running an experiment with sapphire (Al_2O_3). The ratio of the experimental heat capacity to the theoretical heat capacity of the sapphire gave a cell constant of 1.774. A 50 g quantity of meat and carrot alginate discs were homogenized in order to get a representative samples. A sample of 10-15 mg was placed in the aluminum pan which was then sealed using an encapsulating press from TA instruments Inc. An empty pan that had been already weight-matched with the sample pan was also sealed for reference. The method consisted of equilibrating the sample at 5^{o}C, and then heating at a rate of 5^{o}C/min from 5^{o}C to 130^{o}C. Nitrogen was used as a purging gas at a flow of 50 ml

min^{-1}. The instrument automatically gives heat capacity curves at temperatures ranging from 5 to 130^{o}C.

5.3.6. Thermal conductivity measurements

Thermal conductivity of various samples were evaluated using the line-heat source probe method developed by Sweat (1974). This method consisted of a heater wire insulated over its length and a chromel-constantan thermocouple (type E) at the center of this length. The probe was connected to a power supply (Hewlett-Packard, 6236B) and the thermocouple was connected to a data acquisition system, which was connected to a computer. The probe had an internal resistance of 226.67 Ω m^{-1} and was calibrated with glycerol. Calibration values were within 10% of the value reported in the literature. The probe was placed at the core of the sample (45 mm in diameter and 9 mm in height) and the data acquisition system was switched on for 8 s to record the initial temperature. Then, the power was turned on in order to pass the 200mA current for 2 min. Thermal conductivity was calculated by plotting temperature vs. ln (time) and calculating the slope of straight line. Using the following equations, thermal conductivity was calculated:

$$Q = RI^2 \qquad \text{(Eq. 5.2)}$$

$$k = \frac{Q}{4\pi m} \qquad \text{(Eq. 5.3)}$$

where:

Q is the heat flux ($W.m^{-1}$), R the probe resistance ($\Omega.m^{-1}$), k the thermal conductivity ($W.m^{-1}.{}^{o}C^{-1}$), I the current (mA) and m the slope of the linear part of the temperature vs. ln (time) graph.

5.4 Results and Discussions

Moisture content, density, heat capacity and thermal conductivity values of the carrot alginate and meat alginate simulated particles, as function of sodium alginate concentration, calcium chloride concentration and immersion time in the calcium chloride solution are summarized in Tables 5.1 to 5.3. Moisture content varied from 91.7 to 96.3% for the carrot alginate and 64.3 to 69.0% for the meat alginate fabricated

particles. Density values were similar for all particles. Heat capacity (C_p) values varied from 3.31 to 3.85 $kJ.kg^{-1}.^oC^{-1}$ for meat alginate and from 3.56 to 4.28 $kJ.kg^{-1}.^oC^{-1}$ for carrot alginate particles, respectively, in the temperature range, 40 to 120°C. Thermal conductivity values ranged from 0.41 to 0.51 $W.m^{-1}.^oC^{-1}$ for meat alginate and from 0.53 to 0.64 $W.m^{-1}.^oC^{-1}$ for carrot alginate particles. The results indicated a fairly wide range of values of responses, except for density, depending on the treatment conditions. In other words, thermo-physical properties were quite sensitive to the process variables justifying the need for the study and for evaluating the optimal conditions for formulations.

Table 5.1. Moisture (%) and density ($g.cm^{-3}$) of reconstituted carrot and meat alginate particles as a function of sodium alginate, calcium chloride concentration and immersion time in calcium chloride solution

	Coded			Actual			Y_1 (Moisture) (%)		Y_2 (Density) ($g.cm^{-3}$)	
Sl.No.	X_1	X_2	X_3	Sodium Alginate (%)	$CaCl_2$ (%)	Dipping Time (hours)	Carrot	Meat	Carrot	Meat
1	-1	-1	-1	2.5	1.4	12	95.0 ± 0.7	68.1 ± 2.9	1.02 ± 0.0	1.04 ± 1.9
2	1	-1	-1	5.5	1.4	12	93.0 ± 0.8	65.3 ± 1.5	1.04 ± 3.8	1.05 ± 3.8
3	-1	1	-1	2.5	2.6	12	94.7 ± 0.6	68.5 ± 0.0	1.06 ± 5.7	1.02 ± 3.9
4	1	1	-1	5.5	2.6	12	92.5 ± 1.3	65.6 ± 4.6	1.02 ± 2.0	1.02 ± 0.0
5	-1	-1	1	2.5	1.4	36	95.2 ± 0.7	68.3 ± 0.6	1.06 ± 1.9	1.04 ± 5.8
6	1	-1	1	5.5	1.4	36	93.0 ± 1.5	65.4 ± 0.8	1.07 ± 0.9	1.05 ± 1.9
7	-1	1	1	2.5	2.6	36	95.1 ± 3.7	68.3 ± 1.5	1.03 ± 0.0	1.02 ± 0.0
8	1	1	1	5.5	2.6	36	93.0 ± 0.0	65.7 ± 1.5	1.07 ± 1.9	1.02 ± 0.0
9	-1.682	0	0	1.5	2.0	24	96.3 ± 1.3	69.0 ± 0.7	1.04 ± 3.8	1.04 ± 1.9
10	1.682	0	0	6.5	2.0	24	91.7 ± 5.5	64.3 ± 0.8	1.04 ± 3.8	1.04 ± 1.0
11	0	-1.682	0	4.0	1.0	24	93.9 ± 2.2	66.9 ± 1.5	1.07 ± 3.7	1.04 ± 1.9
12	0	1.682	0	4.0	3.0	24	94.4 ± 0.7	66.4 ± 0.6	1.02 ± 0.0	1.05 ± 5.7
13	0	0	-1.682	4.0	2.0	4	94.1 ± 2.2	66.3 ± 0.9	1.05 ± 5.7	1.02 ± 5.9
14	0	0	1.682	4.0	2.0	44	93.4 ± 1.5	66.8 ± 1.5	1.03 ± 3.9	1.02 ± 0.0
15	0	0	0	4.0	2.0	24	93.8 ± 1.8	66.4 ± 1.1	1.02 ± 5.9	1.04 ± 3.8
16	0	0	0	4.0	2.0	24	94.0 ± 0.7	66.6 ± 1.1	1.02 ± 2.0	1.04 ± 1.9
17	0	0	0	4.0	2.0	24	93.9 ± 0.6	66.6 ± 0.3	1.02 ± 2.0	1.04 ± 0.0
18	0	0	0	4.0	2.0	24	93.9 ± 1.5	66.5 ± 0.9	1.03 ± 7.8	1.05 ± 1.9
19	0	0	0	4.0	2.0	24	93.8 ± 1.3	66.4 ± 0.0	1.03 ± 0.0	1.02 ± 7.8
20	0	0	0	4.0	2.0	24	93.8 ± 1.3	66.5 ± 0.0	1.04 ± 0.0	1.04 ± 5.8

Mean ± CoV (n = 3)

Table 5.2. Heat capacity ($kJ.kg^{-1}.^oC^{-1}$) of carrot and meat alginate reconstituted particles at different temperatures as a function of sodium alginate, calcium chloride concentration and dipping time in calcium chloride solution

Alginate Particle	Sodium Alginate (%)	$CaCl_2$ (%)	Dipping Time (hours)	Cp ($kJ.Kg^{-1}.^oC^{-1}$)				
				40°C	60°C	80°C	100°C	120°C
Meat	2.5	1.4	12	3.36 ± 3.27	3.49 ± 3.61	3.73 ± 3.22	3.78 ± 3.33	3.86 ± 3.11
	5.5	1.4	12	3.35 ± 2.99	3.41 ± 3.23	3.65 ± 3.45	3.61 ± 3.32	3.78 ± 3.17
	2.5	2.6	12	3.41 ± 3.52	3.57 ± 2.80	3.65 ± 3.01	3.69 ± 3.41	3.85 ± 3.27
	5.5	2.6	12	3.34 ± 3.89	3.50 ± 3.43	3.62 ± 2.76	3.77 ± 2.92	3.83 ± 3.29
	2.5	1.4	36	3.44 ± 2.62	3.60 ± 3.61	3.74 ± 3.21	3.78 ± 2.65	3.80 ± 2.89
	5.5	1.4	36	3.31 ± 3.14	3.49 ± 2.58	3.59 ± 3.62	3.73 ± 3.22	3.77 ± 2.65
	2.5	2.6	36	3.44 ± 3.66	3.51 ± 2.96	3.70 ± 2.43	3.71 ± 3.50	3.77 ± 3.18
	5.5	2.6	36	3.41 ± 3.72	3.47 ± 3.63	3.65 ± 2.47	3.69 ± 2.44	3.75 ± 3.47
	1.5	2.0	24	3.50 ± 2.86	3.62 ± 3.51	3.75 ± 3.36	3.79 ± 2.74	3.85 ± 2.33
	6.5	2.0	24	3.31 ± 3.63	3.42 ± 2.92	3.57 ± 3.25	3.66 ± 3.64	3.70 ± 2.81
	4.0	1.0	24	3.43 ± 3.79	3.62 ± 3.31	3.69 ± 2.71	3.73 ± 3.40	3.74 ± 3.37
	4.0	3.0	24	3.45 ± 2.61	3.48 ± 3.74	3.62 ± 3.31	3.71 ± 2.70	3.76 ± 3.38
	4.0	2.0	4	3.43 ± 3.03	3.55 ± 2.54	3.62 ± 3.59	3.75 ± 3.20	3.74 ± 2.67
	4.0	2.0	44	3.44 ± 3.37	3.52 ± 2.95	3.62 ± 2.49	3.76 ± 3.46	3.80 ± 3.16
	4.0	2.0	24	3.38 ± 3.73	3.59 ± 3.23	3.61 ± 2.88	3.74 ± 2.41	3.80 ± 3.42
	4.0	2.0	24	3.40 ± 3.53	3.49 ± 3.23	3.61 ± 3.49	3.75 ± 2.77	3.79 ± 2.37
	4.0	2.0	24	3.43 ± 3.79	3.53 ± 3.40	3.66 ± 3.44	3.73 ± 2.41	3.79 ± 2.74
	4.0	2.0	24	3.40 ± 2.65	3.44 ± 3.78	3.67 ± 3.00	3.71 ± 2.43	3.74 ± 2.40
	4.0	2.0	24	3.44 ± 2.62	3.47 ± 2.59	3.67 ± 3.27	3.81 ± 3.15	3.80 ± 3.18
	4.0	2.0	24	3.45 ± 2.61	3.55 ± 2.54	3.68 ± 2.45	3.65 ± 3.56	3.81 ± 3.15
Carrot	2.5	1.4	12	3.66 ± 3.31	3.80 ± 5.57	3.91 ± 3.04	3.94 ± 3.27	4.28 ± 3.27
	5.5	1.4	12	3.55 ± 3.63	3.77 ± 4.77	3.87 ± 3.33	4.06 ± 3.45	4.08 ± 3.28
	2.5	2.6	12	3.64 ± 5.77	3.77 ± 3.21	3.99 ± 3.51	4.00 ± 3.35	4.26 ± 3.03
	5.5	2.6	12	3.56 ± 3.40	3.69 ± 3.79	3.90 ± 3.44	3.93 ± 3.03	4.11 ± 3.14
	2.5	1.4	36	3.68 ± 3.80	3.84 ± 3.15	3.97 ± 3.00	4.08 ± 5.15	4.26 ± 3.03
	5.5	1.4	36	3.69 ± 3.22	3.83 ± 3.16	3.85 ± 3.48	3.90 ± 4.62	4.19 ± 3.20
	2.5	2.6	36	3.66 ± 3.31	3.81 ± 3.52	3.97 ± 5.29	4.02 ± 3.33	4.24 ± 3.09
	5.5	2.6	36	3.62 ± 3.56	3.68 ± 3.29	3.84 ± 5.31	3.87 ± 4.86	4.16 ± 5.05
	1.5	2.0	24	3.71 ± 3.48	3.84 ± 3.15	3.98 ± 3.29	4.03 ± 4.47	4.28 ± 4.77
	6.5	2.0	24	3.56 ± 3.40	3.71 ± 5.96	3.85 ± 5.45	3.91 ± 5.78	4.16 ± 4.90
	4.0	1.0	24	3.64 ± 3.27	3.69 ± 3.28	3.92 ± 3.34	3.92 ± 5.77	4.27 ± 3.14
	4.0	3.0	24	3.70 ± 5.08	3.65 ± 6.05	3.96 ± 4.74	3.87 ± 3.46	4.26 ± 5.19
	4.0	2.0	4	3.69 ± 3.28	3.83 ± 3.16	3.86 ± 3.47	4.01 ± 5.64	4.20 ± 5.26
	4.0	2.0	44	3.59 ± 5.24	3.76 ± 3.72	3.92 ± 5.87	4.05 ± 3.31	4.18 ± 5.50
	4.0	2.0	24	3.61 ± 4.99	3.75 ± 3.22	3.92 ± 3.57	3.83 ± 5.91	4.27 ± 3.14
	4.0	2.0	24	3.64 ± 3.32	3.81 ± 3.18	3.85 ± 5.97	3.86 ± 3.63	4.20 ± 5.50
	4.0	2.0	24	3.68 ± 5.54	3.85 ± 5.45	3.94 ± 3.32	4.12 ± 2.89	4.25 ± 3.29
	4.0	2.0	24	3.66 ± 5.57	3.81 ± 3.18	3.90 ± 5.89	3.93 ± 5.75	4.22 ± 2.82
	4.0	2.0	24	3.55 ± 3.41	3.89 ± 3.11	3.90 ± 3.36	4.13 ± 2.88	4.23 ± 4.26
	4.0	2.0	24	3.67 ± 5.72	3.78 ± 5.85	3.94 ± 4.57	4.05 ± 5.58	4.10 ± 4.59

Mean ± CoV (n = 3)

Table 5.3. Thermal conductivity ($W.m^{-1}.^{o}C^{-1}$) of carrot and meat alginate reconstituted particles as a function of sodium alginate concentration, calcium chloride concentration and immersion time in calcium chloride solution

Sl.No.	Coded			Actual			Y_1 (Thermal Conductivity) $x10^{-6}(W.m^{-1}.K^{-1})$	
	X_1	X_2	X_3	Sodium Alginate (%)	$CaCl_2$ (%)	Dipping Time (h)	Carrot	Meat
1	-1	-1	-1	2.5	1.4	12	0.61 ± 8.20	0.49 ± 0.00
2	1	-1	-1	5.5	1.4	12	0.56 ± 7.14	0.43 ± 6.98
3	-1	1	-1	2.5	2.6	12	0.60 ± 8.33	0.49 ± 8.16
4	1	1	-1	5.5	2.6	12	0.55 ± 7.27	0.43 ± 6.98
5	-1	-1	1	2.5	1.4	36	0.60 ± 6.67	0.49 ± 4.08
6	1	-1	1	5.5	1.4	36	0.55 ± 3.64	0.44 ± 4.55
7	-1	1	1	2.5	2.6	36	0.59 ± 6.78	0.48 ± 8.33
8	1	1	1	5.5	2.6	36	0.56 ± 7.14	0.42 ± 7.14
9	-1.682	0	0	1.5	2.0	24	0.64 ± 3.13	0.51 ± 5.88
10	1.682	0	0	6.5	2.0	24	0.53 ± 3.77	0.41 ± 7.32
11	0	-1.682	0	4.0	1.0	24	0.56 ± 1.79	0.46 ± 4.35
12	0	1.682	0	4.0	3.0	24	0.58 ± 5.17	0.46 ± 4.35
13	0	0	-1.682	4.0	2.0	4	0.56 ± 1.79	0.46 ± 2.17
14	0	0	1.682	4.0	2.0	44	0.59 ± 5.08	0.47 ± 4.26
15	0	0	0	4.0	2.0	24	0.58 ± 0.00	0.46 ± 0.04
16	0	0	0	4.0	2.0	24	0.58 ± 0.00	0.47 ± 0.00
17	0	0	0	4.0	2.0	24	0.58 ± 3.45	0.46 ± 8.70
18	0	0	0	4.0	2.0	24	0.57 ± 7.02	0.46 ± 8.70
19	0	0	0	4.0	2.0	24	0.58 ± 0.02	0.46 ± 0.00
20	0	0	0	4.0	2.0	24	0.58 ± 3.45	0.46 ± 6.52

Mean ± CoV (n = 3)

5.4.1. Model fitting

The second-order polynomial surface model (Eq. 5.1) was fitted to each of the response variables (y). The sum of squares of the sequential model was analyzed for the corresponding fitting of the explanatory models and the variation of the moisture content, heat capacity and thermal conductivity. Regression analysis and ANOVA were used for developing and evaluating the statistical significance of the terms (Table 5.4). The estimated regression coefficients of the models for the response variables, along with the corresponding coefficients of determination (R^2) are given in Table 5.4.

Montgomery (2001) defined the lack of fit as an indication of the failure for a model to represent the experimental data at which points were not included in the regression or variations in the models cannot be accounted for random error. If there is a significant lack of fit, which could be indicated by a low probability value, the response predictor is discarded. The lack of fit in our study, as indicated in Table 5.4, was not significant (p>0.05), meaning that the model was sufficiently accurate for predicting the relevant responses.

According to Mendenhall (1975) and Little and Hills (1978), the coefficient of determination R^2, is the proportion of variation in the response attributed to the model rather than to random error. It was suggested that R^2 should not be less than 0.8 in a model with a good fit. Lower values of R^2 indicate that the model is not appropriate to explain the relation between variables. The R^2 values for moisture, heat capacity and thermal conductivity were found to be 0.94, 0.82 and 0.86, respectively, for carrot alginate particles and 0.96, 0.83 and 0.97, respectively, for meat alginate particles. In other words, the results showed that the R^2 values for the response variables were higher than 0.80, meaning that the regression models were suitable to correlate the experimental variations.

Coefficient of variation (CV) can be used to describe the extent to which the data were dispersed. In general, the coefficient of variation (CV) should not be greater than 10%. Daniel (1991) reported that a high CV indicates that variation in the mean value is high and does not satisfactorily develop an adequate response model. Our results (Tables

5.1 to 5.3) show that the coefficients of variation were less than 10% for all the responses.

5.4.2. Moisture and fat content

As shown in Figure 5.1, the moisture content of the alginate fabricated particles decreased significantly ($p < 0.05$) as the sodium alginate concentration was increased from 1.5 to 6.5%; however, calcium chloride concentration and immersion time in the calcium chloride concentration had no significant ($p > 0.05$) effect. Sodium alginate was directly added to the formulation and hence higher amounts sodium alginate progressively replaced the moisture content. Values of the moisture content of carrot alginate simulated particles were comparable to the ones reported by Marcotte et al. (2000). The fat content in the meat alginate fabricated particles were not significantly different and averaged 10.5 ± 0.42 % for all samples while the raw meat had 17.0 ± 0.50 % fat.

5.4.3. Density

Density values were also similar for all samples and were not significantly different ($p>0.05$). The gel making process did not result any swelling during the immersion in the calcium chloride solution step and therefore, did not result in changes in the density values. Observed values were comparable to the density of raw carrots (1.04 $g.cm^{-3}$) and raw beef meat (1.02 $g.cm^{-3}$) reported by Sweat (1974). Marcotte et al. (2000) also found no effect of the formulation on the density values of carrot alginate simulated particles.

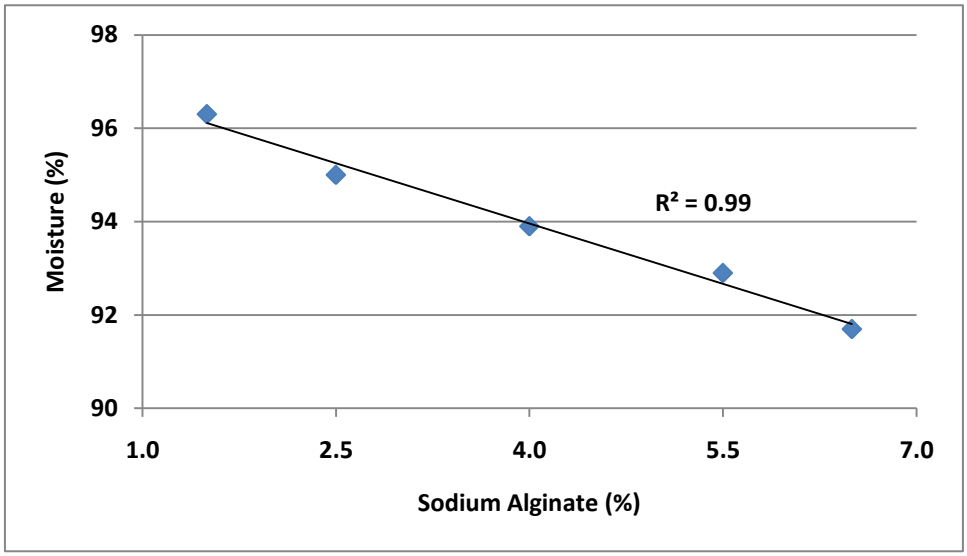
5.4.4. Heat capacity

The heat capacity values of carrot and meat alginate particles increased significantly ($p < 0.05$) with a decrease in sodium alginate concentration; however, calcium chloride concentration and immersion time in the calcium chloride solution did not have any significant ($p > 0.05$) influence on the associated heat capacity values. The response surface plots of heat capacity as a function of the principal variable sodium alginate concentration and the minor variable calcium chloride concentration are

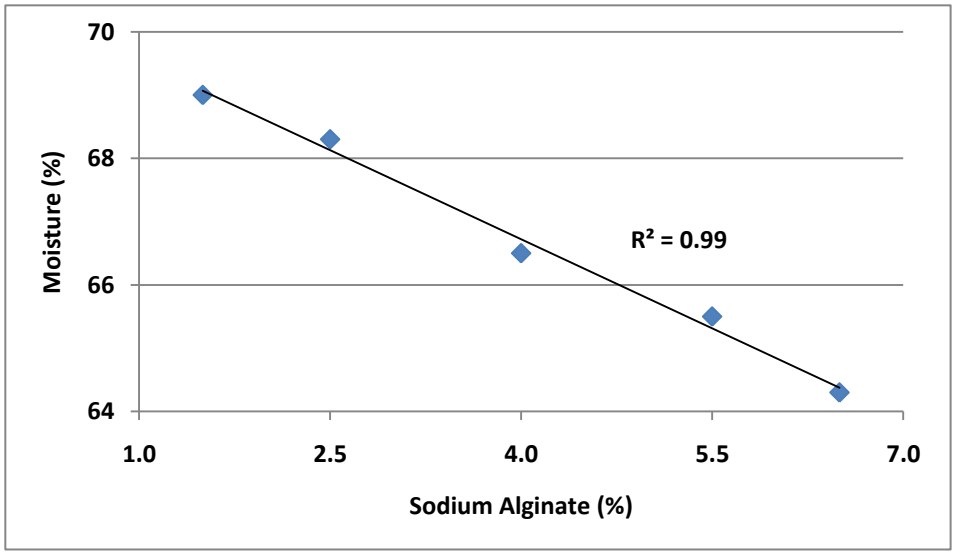
illustrated in Figures 5.2 and 5.3, for carrot alginate and meat alginate fabricated particles, respectively.

Table 5.4. ANOVA and regression coefficients of the second-order polynomial model for the response variables (actual values)

	Source	DF	Moisture (%)			DF	C_p (kJ.Kg^{-1}.°C^{-1})		
			Coefficient	Sum of squares	*p*-Value		Coefficient	Sum of squares	*p*-Value
	Model	3	97.121	19.3	< 0.0001	9	3.830	0.035	0.0005
Linear	b_1	1	-0.793	19.3	< 0.0001	1	1.24E-03	0.026	0.0002
	b_2	1	-7.21E-03	2.56E-04	0.955	1	-1.38E+02	2.05E-03	0.138
	b_3	1	-4.71E-04	4.37E-04	0.941	1	1.00E-02	2.72E-04	0.571
Quadratic	b_{11}					1	7.75E-04	4.38E-05	0.819
	b_{22}					1	2.94E-02	1.61E-03	0.183
	b_{33}					1	-4.93E-05	7.25E-04	0.361
Interaction	b_{12}					1	-8.33E-03	4.50E-04	0.468
	b_{13}					1	-8.33E-04	1.80E-03	0.162
	b_{23}					1	-2.08E-03	1.80E-03	0.162
	Residual	16		1.23		10		7.90E-03	
	Lack of fit	11		1.20E-05	0.320	5		2.22E-03	0.838
	R^2		0.94				0.82		
			Thermal Conductivity (W.m^{-1}.K^{-1})						
	Model	3	0.641	9.84E-03	< 0.0001				
Linear	b_1	1	-0.02	9.76E-03	< 0.0001				
	b_2	1	1.66E-03	1.36E-05	0.712				
	b_3	1	1.86E-04	6.79E-05	0.413				
	Residual	16		1.54E-03					
	Lack of fit	11		3.30E-04	0.265				
	R^2		0.86						
			Moisture (%)				C_p (kJ.Kg^{-1}.°C^{-1})		
	Model	3	70.234	26.81	< 0.0001	9	4.134	0.04	0.0009
Linear	b_1	1	-0.933	26.72	< 0.0001	1	-0.085	0.027	0.0001
	b_2	1	1.94E-02	1.85E-03	0.866	1	-0.233	3.16E-03	0.073
	b_3	1	6.35E-03	7.90E-02	0.277	1	1.89E-04	6.59E-05	0.778
Quadratic	b_{11}					1	3.59E-03	9.42E-04	0.300
	b_{22}					1	1.75E-02	5.75E-04	0.413
	b_{33}					1	-4.21E-05	5.29E-04	0.431
Interaction	b_{12}					1	2.08E-02	2.81E-03	0.088
	b_{13}					1	-6.25E-04	1.01E-03	0.283
	b_{23}					1	2.26E-03	2.11E-03	0.132
	Residual	16		1.00		10		7.87E-03	
	Lack of fit	11		0.96	0.81	5		2.87E-03	0.722
	R^2		0.96				0.83		
			Thermal Conductivity (W.m^{-1}.K^{-1})						
	Model	3	0.545	1.20E-02	< 0.0001				
Linear	b_1	1	-0.019	1.20E-02	< 0.0001				
	b_2	1	-3.66E-03	6.59E-05	0.131				
	b_3	1	4.16E-05	3.40E-06	0.722				
	Residual	16		4.16E-04					
	Lack of fit	11		3.33E-04	0.265				
	R^2		0.97						



(a)



(b)

Fig 5.1. Relationship between sodium alginate concentration and moisture content of carrot alginate (a) and meat alginate (b) fabricated particles

As the sodium alginate concentration decreased from 6.5 to 1.5%, the moisture content of carrot alginate particles increased ($p < 0.05$) from 91.7 to 96.3% and that of meat alginate particles significantly ($p < 0.05$) increased from 64.3 to 69.0%. Heat capacity of foods depends strongly on their moisture content and increases with an increase in the moisture content (Shmalko, 1996). For moist foods, water content affects the heat capacity much more than any other food components and the lower the moisture content, the lower is the heat capacity (Unklesbay et al., 1999). Between the carrot and meat alginate particles, the meat particles had lower moisture content than carrot alginates and hence their heat capacities were considerably lower. Heat capacity comparison between meat and carrot alginate particles cannot be made only on the basis of moisture content since the meat alginate particles have other components like fat and protein which are mostly non-existent in carrot particles.

Based on the sum of squares, the importance of the independent variables on heat capacity of carrot alginate and meat alginate simulated particles could be ranked in the following order: sodium alginate concentration > calcium chloride concentration > immersion time in calcium chloride solution. It can be seen that among the interaction terms, none were significant ($p > 0.05$).

In Figures 5.4a and 5.4c, the experimentally measured heat capacity values were compared with the model predicted values for carrot alginate and meat alginate fabricated particles, respectively. The regression model was in a good agreement ($R^2 > 0.80$) with the experimental results.

Temperature had a highly significant ($p < 0.05$) influence on heat capacity values of meat and carrot alginate simulated particles. The relationship between heat capacity and temperature is shown in Figure 5.5. Heat capacity increased with temperature between 40 and 120^{o}C. Heat capacity has been generally recognized to increase with temperature (Karunakar et al., 1998; Marcotte et al., 2008).

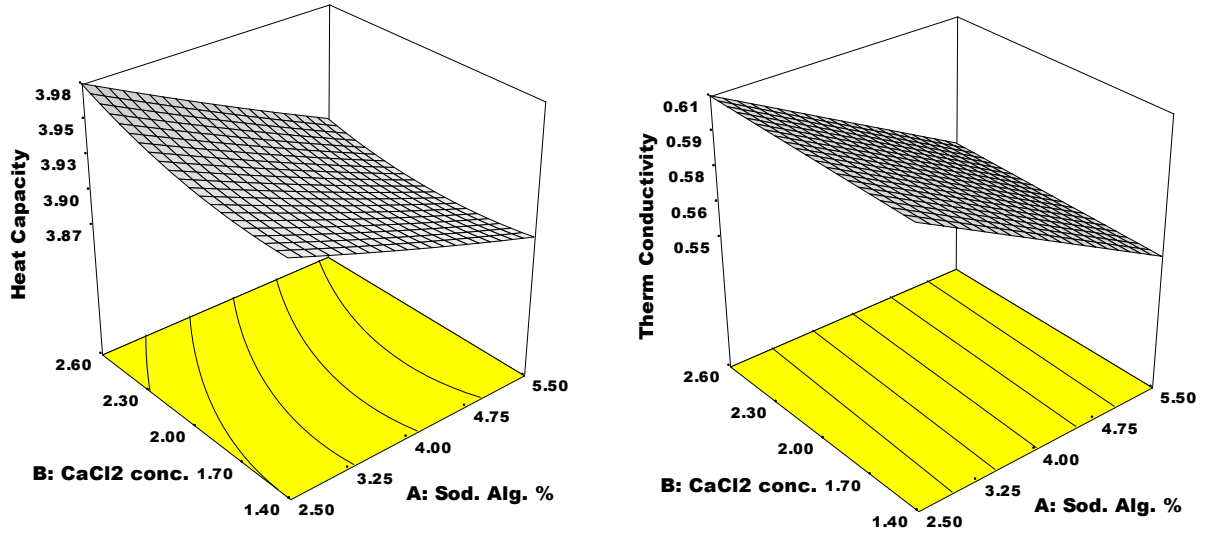


Fig 5.2. Effect of sodium alginate concentration and calcium chloride concentration on the heat capacity ($kJ.kg^{-1}.^{o}C^{-1}$) at $80^{o}C$ and thermal conductvity ($W.m^{-1}.^{o}C^{-1}$of the carrot alginate fabricated particles

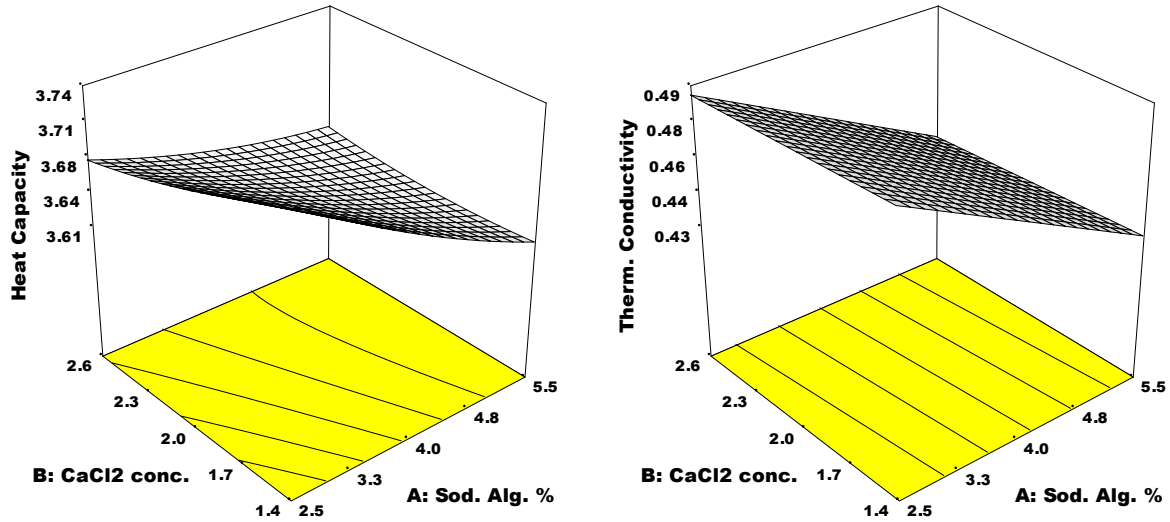


Fig 5.3. Effect of sodium alginate concentration and calcium chloride concentration on the heat capacity ($kJ.kg^{-1}.^{o}C^{-1}$) at $80^{o}C$ and thermal conductvity ($W.m^{-1}.K^{-1}$) of the meat alginate fabricated particles

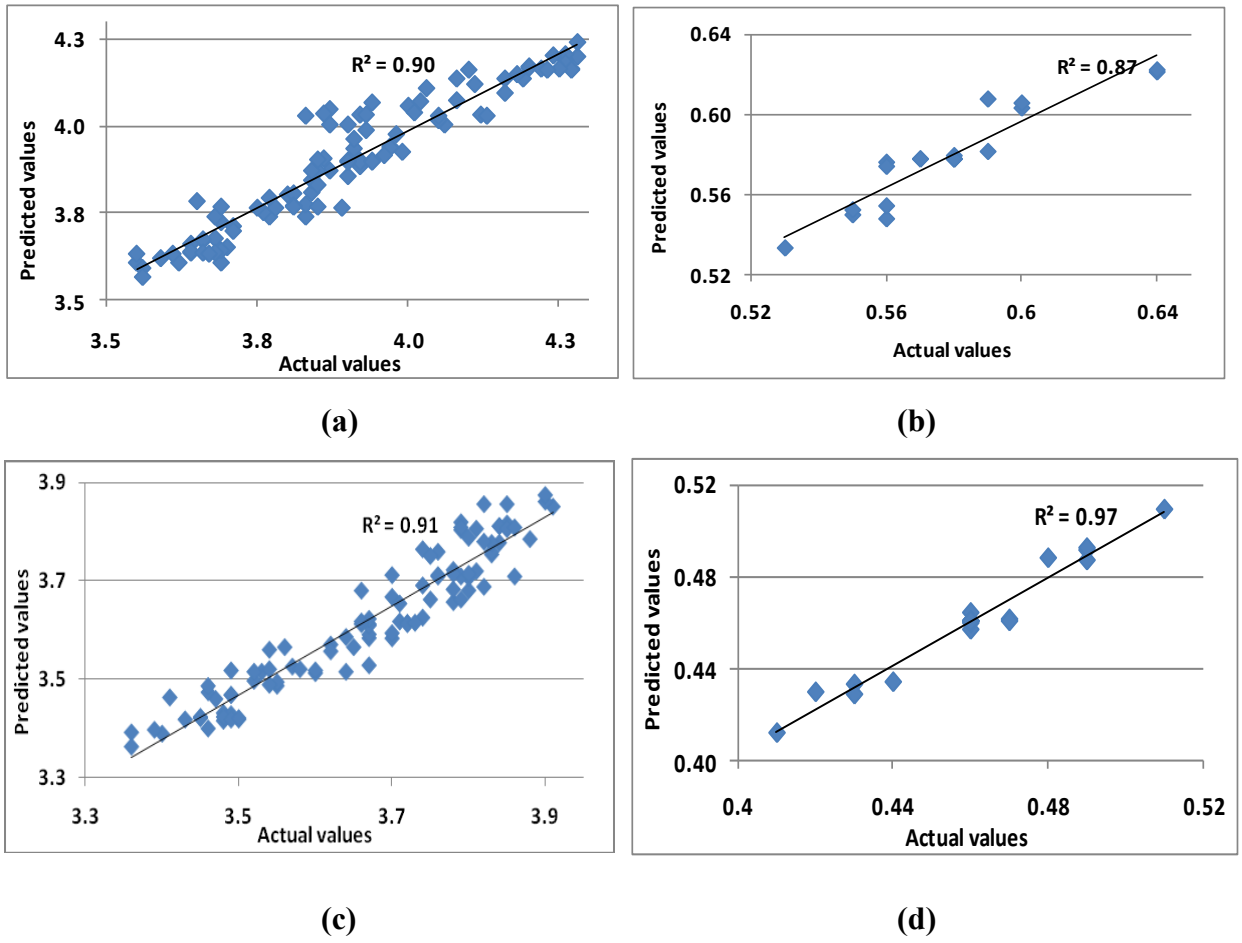


Fig. 5.4. Comparison between predicted and actual values of (a, c) heat capacity (kJ.kg^{-1}.^{o}C^{-1}) at 80^{o}C and (b, d) thermal conductvity (W.m^{-1}.K^{-1}) of carrot alginate and meat alginate fabricated particles, respectively

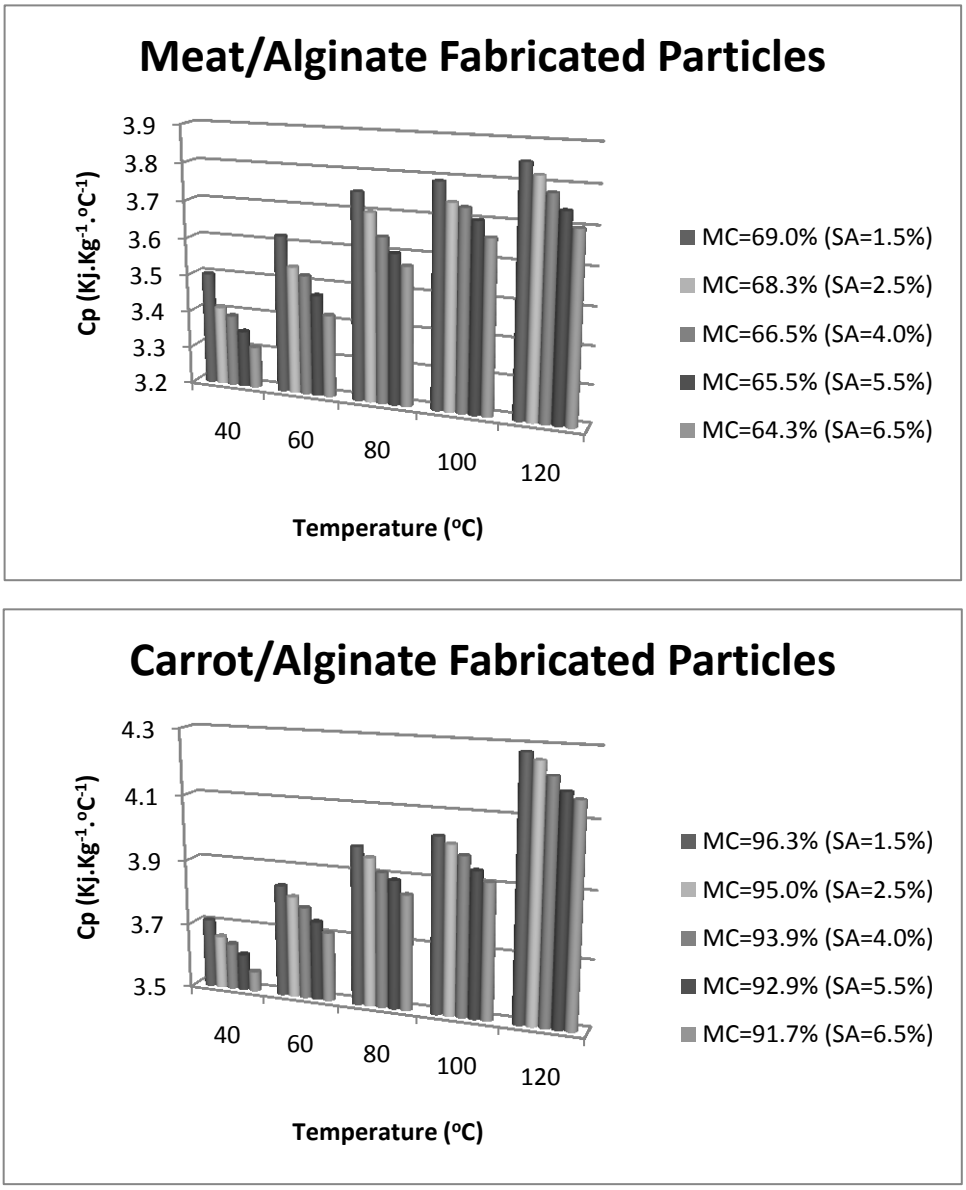


Fig. 5.5. Effect of temperature and sodium alginate concentration (SA) / moisture content (MC) on heat capacity values of meat and carrot alginate reconstituted particles

5.4.5. Thermal conductivity

Thermal conductivity of meat alginate and carrot alginate particles also increased significantly ($p < 0.05$) as the sodium alginate concentration decreased. Again, calcium chloride concentration and immersion time in the calcium chloride solution had no significant ($p > 0.05$) effect on the thermal conductivity values as shown in Figures 5.2 and 5.3 for carrot alginate and meat alginate fabricated particles, respectively. Other studies have also demonstrated that thermal conductivity is highly moisture dependent (Shmalko et al., 1996; Marcotte et al., 2008).

In Figures 5.4b and 5.4d, the observed thermal conductivity values for carrot alginate and meat alginate fabricated particles, respectively, were compared with the predicted values calculated from the polynomial regression models. Both figures show that the models were in good agreement ($R^2 > 0.8$) with the experimental results.

Values of thermo-physical properties predicted from the various published models were computed and compared with the experimental results (data not included as per editorial suggestions). These predicted values were quite different from the experimentally determined values for the simulated particles. These particles were specially fabricated through a gelling process to be structurally more stable and to have thermal properties in the range appropriate for food products, but compositionally they are different from the real food particles, and hence the conventional models did not work in this case. This makes the results of the study even more meaningful.

5.4.6. Optimization

In this study, optimization was applied within the experimental ranges of sodium alginate and calcium chloride concentrations, and immersion time in calcium chloride solution in order prepare particle with nearly same thermo-physical properties of real carrot and meat particles as determined experimentally in this study (Table 5.5). Polley et al. (1980) and Sweat (1995) reported that heat capacity of carrot was 3.77 and 3.90 kJ/kg.^{o}C, respectively. Sweat (1995) found out that the heat capacity of lean beef was 3.52 kJ/kg.^{o}C. Backstrom and Emblick (1965) reported that the thermal conductivity of

lean beef was $4.6x10^{-7}$ $W.m^{-1}.^oC^{-1}$. Our results for real food particles were in general agreement with values reported in the literature.

Table 5.5. Density, heat capacity and thermal conductivity of real carrot and meat particles

Food	Density ($g.cm^{-3}$)	C_p ($kJ.Kg^{-1}.^oC^{-1}$)	Thermal Conductivity ($W.m^{-1}.K^{-1}$)
Carrot	1.03 ± 0.01	3.86 ± 0.04	0.56 ± 0.02
Meat	1.04 ± 0.02	3.60 ± 0.04	0.44 ± 0.02

Mean ± SD (n = 3)

Using the numerical optimization technique of the Design-Expert software, optimum conditions for fabricating the particles were determined by superimposing the contour plots of all the responses. The resulting conditions are presented in Table 5.6. For meat alginate fabricated particles, optimum conditions were 5.2-5.4% of sodium alginate, 2.2-2.5% of calcium chloride concentration and 35.3-36.0 h of immersion time in the calcium chloride solution. On the other hand, for carrot alginate fabricated particles, optimum conditions were 4.9-5.0% of sodium alginate, 2.4-2.6% of calcium chloride concentration and 13.1-24.5 h of immersion time in the calcium chloride solution.

Table 5.6. Predicted optimum conditions for producing meat and carrot alginate particles having identical Thermophysical properties to the real carrot and meat

Alginate Fabricated Particle	Sod. Alg. %	CaCl2 conc.	Immersion Time (hrs)	Density ($g.cm^{-3}$)	C_p ($kJ.Kg^{-1}.^oC^{-1}$)	k ($W.m^{-1}.K^{-1}$)	α ($x10^{-6}$) ($m^2.s^{-1}$)	Desirability
Carrot	4.9	2.4	36.0	1.04	3.87	0.56	0.14	1
	5.0	2.4	35.3	1.04	3.87	0.56	0.14	1
	5.0	2.6	35.8	1.04	3.87	0.56	0.14	1
	5.0	2.5	35.3	1.04	3.87	0.56	0.14	1
Meat	5.4	2.5	13.1	1.04	3.61	0.43	0.12	1
	5.2	2.2	13.1	1.04	3.62	0.44	0.12	1
	5.3	2.4	24.5	1.04	3.62	0.43	0.12	1
	5.3	2.2	16.4	1.04	3.62	0.43	0.12	1

For meat alginate fabricated particles, optimum conditions were 5.2-5.4% of sodium alginate, 2.2-2.5% of calcium chloride concentration and 13.1-24.5 hours of immersion time in the calcium chloride solution. On the other hand, for carrot alginate fabricated particles, optimum conditions were 4.9-5.0% of sodium alginate, 2.4-2.6% of calcium chloride concentration and 35.3-36.0 hours of immersion time in the calcium chloride solution.

The desirability function approach is one of the most widely used methods in industry for the optimization of multiple response processes. Desirability concept for multi-criteria optimization in industrial quality management was introduced by Harrington (1965). It is based on the idea that the "quality" of a product or process that has multiple quality characteristics is unacceptable when one of them stays outside of some "desired" range. The method finds operating conditions that provide the "most desirable" response values. The desirability function model has the potential to compare responses with different scales, transforming easily the responses to one measurement in order to be applied for both qualitative and quantitative responses (Shi et al., 2008). The desirability values reported in our study were 1, making the suggested values for sodium alginate concentration, calcium chloride concentration and immersion time in calcium chloride solution highly accurate.

5.5 Conclusions

Biological validation of aseptic processing and continuous agitation processing is gaining interest due to the difficulties in using temperature sensors in these systems. Biological validation can be done through the fabrication of meat and carrot alginate simulated particles that are thermally stable and having similar thermo-physical properties to the real food particles. Increasing sodium alginate level resulted in significant ($p < 0.05$) decrease in moisture content, which caused a significant decrease in the heat capacity and thermal conductivity values of the fabricated particles, as the latter properties are known to be influenced by the water content of foods. On the other hand, calcium chloride concentration and immersion time in calcium chloride solution had no significant ($p > 0.05$) effect on the thermo-physical properties of the fabricated particles.

Density values were similar for all the fabricated particles. Optimum conditions for fabricating particles having thermo-physical properties that are similar to the real food particles were obtained. This investigation could help aseptic processing and continuous agitation processing food industries in ensuring process safety by employing biological validation using food fabricated alginate particles.

PREFACE TO CHAPTER 6

Previous studies have showed that in rotational retorts, rates of heat transfer can be affected by several process and product parameters, mainly retort temperature, rotational speed and liquid viscosity. The knowledge of the effect of these factors on the heat transfer rates is essential to establish the thermal processing procedure in rotary retorts.

Therefore, in this chapter, the study was focused on effectively using the previously established methodology for bi-axially rotating cans with particles suspended in Newtonian fluids (Dwivedi, 2008) for quantifying the effects of rotation speed, retort temperature and liquid viscosity on U and h_{fp} associated with non-Newtonian fluids and arrive at optimum conditions.

The experimental work and data analysis were carried out by the candidate under the supervision of Dr. H. S. Ramaswamy.

CHAPTER 6

EFFECT OF PROCESS VARIABLES ON HEAT TRANSFER RATES TO CANNED PARTICULATE NON-NEWTONIAN FLUIDS DURING FREE BI-AXIAL ROTARY PROCESSING

6.1 Abstract

Heat transfer to canned particulates in non-Newtonian fluids (Nylon particles suspended in aqueous carboxymethyl cellulose - CMC - solution) during fixed and free bi-axial rotation was studied in a pilot-scale, full water-immersion single-cage rotary retort. A response surface methodology was used in order to study the effect of different process parameters, including rotational speed (7-23 rpm), CMC concentration (0.0-1.0%) and retort temperature (110-130^{o}C), at five levels of each, on overall heat transfer coefficient (U) and fluid to particle heat transfer coefficient (h_{fp}) in fixed and free axial rotation modes. The analysis of variance showed that the rotational speed, CMC concentration and retort temperature were significant ($p < 0.05$) factors for h_{fp} in the following order: rotation speed > CMC concentration > retort temperature; however, only rotational speed and CMC concentration were significant ($p < 0.05$) factors for U. With an increase in rotational speed and retort temperature, there was an increase in the associated U and h_{fp} values; however, increasing the CMC concentration resulted in the opposite. Using the numerical optimization of the Design Expert software, optimum heat transfer was found at a rotational speed of 20 rpm, CMC concentration of 0.6% and retort temperature of 126^{o}C. T-test revealed that both U and h_{fp} were significantly ($p < 0.05$) higher in the free bi-axial mode as compared to the fixed axial mode of rotation.

6.2 Introduction

With consumers becoming more educated and health conscious, the demand for convenient and high quality foods has increased over time and food industry has started to shift away from the conventional thermal processing towards high-temperature, short-time (HTST) processing, including aseptic processing, thin profile packaging and agitation processing (David et al., 1996). Due to the fact that quality factors are more heat stable than microorganisms, HTST processing offers the potential to provide the same level of sterility with better quality retention (Holdsworth, 1997). In order to effectively use the HTST processing, rapid heating systems are a necessity. Agitation processing is one such system by inducing forced convection in liquid particulate canned products instead of conduction heating in static processing; therefore, better quality retention can be achieved due to product mixing. There are currently two methods for inducing agitation in cans, namely, end-over-end rotation (EOE) and axial rotation. EOE rotation is common in batch processing systems and involves rotating vertically placed sealed cans secured in a cage around a central axis. On the other hand, axial rotation is encountered with continuous systems such as reel and spiral cookers. It involves rotating sealed cans around the circle in a horizontal plane (Dwivedi, 2008).

In thermal processing, heat penetration parameters are commonly used in combination with the process calculation methods, such as Ball and Stumbo, for the establishment and optimization of thermal processes. Theoretical models can be useful tools for designing, optimizing and validating such food systems; however, the usefulness of these models depends upon the accuracy of the input physical parameters. Data on overall heat-transfer coefficient (U) and the fluid-to-particle heat-transfer coefficient (h_{fp}) are necessary parameters, in addition to the thermophysical properties of the product, for the modeling programs (Sablani and Ramaswamy 1996).

The early studies on rotational processing (Clifcorn et al., 1950; Berry et al., 1979; Berry and Bradshaw, 1980; Berry et al., 1985) have dealt with the effect of agitation on the heat penetration parameters of the product. Rao and Anantheswaran (1988) provided an overview on U values for canned liquids in rotary retorts. The more recent studies have focused on U and h_{fp}, both of which are essential parameters that

influence the heating rate of the liquid particulate mixture. A number of studies have evaluated the different process variables that influence the heat transfer coefficients and it has been found that various factors, including rotational speed, retort temperature, headspace volume, system geometry, liquid viscosity, rotation radius, particle size and particle density are key influencing factors in EOE and axial agitation processing (Anantheswaran and Rao, 1985; Lekwauwa and Hayakawa, 1986; Britt, 1993; Sablani and Ramaswamy, 1995, 1996, 1997; Meng and Ramaswamy, 2007a,b; Dwivedi, 2008). Other studies evaluated the effect of EOE agitation on nutrient, texture and color retention of the food products (Abbatemarco and Ramaswamy 1994, 1995). Some studies have also been done on free axially rotating cans by evaluating the effect of process variables on U and h_{fp} (Lenz and Lund, 1978; Deniston et al., 1987; Fernandez et al., 1988; Hassan, 1984; Stoforos and Reid, 1992). In these studies, particles were fixed inside the can; therefore, this did not simulate the real free motion of the particles during axial agitation processing. Stoforos and Merson (1992) used liquid crystal coated particles for allowing a free rotation of the particles inside the can; however, the study was carried out at a low temperature range of 26 to 50°C. Also, contrary to the bi-axial motion of the particles across the bottom of the retort found in continuous systems, the can set used by Hassan (1984), Deniston et al. (1987) and Stoforos and Merson (1992) had only a circular, one directional motion, further compromising their results. Some studies (Weng et al., 1992; Haentjens et al., 1998; Guiavarc'h et al., 2002) used time-temperature integrators in the form of microorganisms, chemicals or enzymes in combination with a mathematical model in order to determine the convective heat transfer coefficient without affecting the motion of particles in real processing conditions.

The major reason for the shortage in studies on U and h_{fp} in free axially rotating cans is the fact that time temperature profiles of liquid and particle are difficult to measure under commercial processing conditions. Traditional computation of U and h_{fp} requires data on both particle and liquid temperatures. While some techniques could be used to measure the liquid temperature, gathering time temperature heating profile at the particle center is not feasible. Dwivedi (2008) established a unique methodology for evaluating U and h_{fp} in bi-axially rotating cans under commercial processing conditions.

This method involved developing correlations between U and h_{fp} using actual time temperature data gathered through the usage of thermocouples from sealed cans in the fixed axial mode of rotation, then coupling these values with experimentally evaluated U from liquid temperature gathered using wireless sensors and finally back computing h_{fp} for bi-axially rotating cans from the correlations. This method was based on the assumption that within a can, factors that influence U will also influence h_{fp}; therefore; U and h_{fp} are generally interrelated. Dwivedi (2008) tested this method under different processing conditions for Newtonian fluids and found out that it was adequately responsive to process variables. No such data exist for biaxial rotation processing of particulate cans containing non-Newtonian fluids.

Carboxymethyl cellulose (CMC) is a derivative of cellulose, an insoluble homopolymer of repeating β-D-glucopyranosyl units joined by 1-4 glycosidic linkages. Rheological properties of CMC are based on long rigid molecules that are anionic due to many ionized carboxyl groups. Due to electrostatic repulsion, these molecules tend to be extended in solutions and adjacent chains repel each other; therefore, CMC solutions tend to be highly viscous and stable (BeMiller and Whistler, 1996). As a typical hydrocolloid, CMC has many applications in the food industry, product development and processing due to its specific rheological properties (Meng, 2006). Meng (2006) reported that the temperature distribution in the canned fluid was found to be large as CMC solution concentration increased beyond 0.6%, making the conventional calculations of U and h_{fp} impractical; therefore, he proposed the concept of apparent heat transfer coefficient, h_{ap}, between the retort and the particle.

Many process parameters affect the heat transfer in free axially agitating cans, and quantifying their influence on coupled heat transfer rates to the product (U and h_{fp}) is important for establishing thermal processing procedure in rotary retorts. The objective of this study was to use the previously established methodology by Dwivedi (2008) for quantifying the effects of rotation speed, retort temperature and liquid viscosity on U and h_{fp} associated with particulates suspended in a non-Newtonian fluid in order to arrive at optimum conditions.

6.3 Methodology

6.3.1. Sample Preparation

A CMC solution was the non-Newtonian fluid used in the study. An aqueous solution of CMC was prepared by dissolving the required weight of commercial grade CMC (Sigma, St. Louis, MO) in appropriate quantity of distilled water under constant stirring and leaving it for 24 hours for complete dispersion of the lumps and elimination of dissolved air (Awuah et al., 1993). Nylon spheres of 19 mm diameter (Small Parts Inc., Miami, FL) were used as food simulating particulates. Only spherical shaped particles were used in order to avoid non-uniformity associated with other geometries and so that heat transfer to the particle center is uniform from all directions. Table 6.1 summarizes the thermophysical properties of the CMC and Nylon particles as reported in the literature (Sablani and Ramaswamy, 1997; Meng and Ramaswamy, 2007a).

Table 6.1. Thermophysical properties of test materials (25^{o}C) (Meng, 2006)

Material	Density ($Kg.m^{-3}$)	Heat capacity ($J.\ kg^{-1}.C^{-1}$)	Thermal conductivity ($W.m^{-1}.C^{-1}$)	Thermal diffusivity ($m^2.s^{-1}$)
Nylon	1128	2073	0.369	1.52E-07
CMC solution 0.0% 0.2% 0.5% 0.8% 1.0%	1000	4180	0.599	1.43E-07

Because of the small concentrations of CMC aqueous solutions, heat capacity was assumed to be the same as the one of water (Anantheswaran and Rao, 1985). Cans of size 307×409 (Home Canning Co., Montreal, QC) were filled with the CMC solution along with 30% of Nylon particles to a headspace of 12 mm and were closed by a manual closing machine (Home Canning Co., Montreal, QC).

6.3.2. Retort cage modifications for free axial rotation

A pilot-scale rotary single cage retort (Stock Rotomat PR 900; Herman Stock Maschinenfabrick, Neumünster, Germany) was modified, according to the method used by Dwivedi (2008), in order to simulate the rotational motion in Steritort (FMC Corp., San Jose, CA) continuous cookers. In summary, the retort basket was retrofitted with a stainless steel enclosure in which a test sealed can was held in an axial direction. On this enclosure, the end plate facing the retort shell has been removed so that the can slides down and rolls along the retort shell during a part of the rotation cycle. Sealed can rolled on its own axis (in a direction counter to that of retort cage) when inclined downward (approximately one-third of the rotation). In the top two-third of the rotation, sealed can was restrained in the enclosure only rotating with the cage. This process resulted in a bi-axial rotation of the can in the free axial mode versus only a single clockwise direction in the fixed axial mode. On the other hand, sealed cans subjected to fixed axial rotation were restrained inside the cage and rotated around the cage in a horizontal way. Further details of the retort cage modification are provided in Dwivedi (2008).

6.3.3. Thermocouple fitting

Time temperature profiles of the liquid part of the cans subjected to fixed axial rotation were obtained using CNS copper constantan needle-type thermocouples (locking connector, C-10, Ecklund Harrison Technologies, Inc. Cape Coral, FL) with tips located at the geometric centers. On the other hand, time temperature profiles of the particles were obtained using CNS copper constantan wire thermocouples (d = 0.0762 mm, Omega Engineering Corp., Stamford, CT) introduced into the center through a fine hole and fixed by a small amount of epoxy glue. Thermocouple leads from the retort were connected to a slip ring assembly at the end of the rotating shaft. The thermocouple outputs were recorded using a data acquisition system (HP34970A, Hewlett Packard, Loveland, CO) at 1 s intervals. In the free axial mode, the liquid temperature was measured using Track Sense Pro (Ellab Inc., Centennial CO), a FDA-compliant wireless multi-channel data-logging system.

6.3.4. Calculation of U and h_{fp} in fixed axial mode

The governing differential equation for the liquid particulate systems during agitation processing was derived from thermal energy balances by assuming that the fluid temperature was uniform, the initial fluid and particle temperatures were uniform, the heat transfer coefficients were constant, the resistance to heat transfer at the particle surface was finite, the diameter of the spherical particles was constant and the thermophysical properties of the fluid and particle were isotropic. The overall heat transfer coefficient, *U*, can be determined by the equation below, provided that the heat transferred across the wall of the can is equal to the heat absorbed by the particle and by the fluid (Sablani and Ramaswamy, 1996).

$$UA_c \int_0^{t_{pt}} (T_R - T_l)dt = m_l.c_{pl} \int_{t=0}^{t_{pt}} dT_l + h_{fp}.A_p \int_{t=0}^{t_{pt}} (T_l - T_{ps})dt \qquad \text{(Eq. 6.1)}$$

In this equation, the integrating time, t_{pt}, is the time required to achieve a lethality of 10 min at the particle centre. This could be obtained from determining h_{fp}.

The differential equation describing the heat transfer from continuous phase to the particle center is based on Fourier's equation:

$$\frac{\partial T}{\partial t} = \alpha \nabla^2 T \qquad \text{(Eq. 6.2)}$$

$\alpha = k/\rho C_p$, where ρ is the density, C_p, the heat capacity, k, the thermal conductivity, α, the thermal diffusivity and , Laplace operator given by:

$$\nabla^2 = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2} \qquad \text{(Eq. 6.3)}$$

It is also assumed that the particles receive heat only from the liquid and not from the can wall. In other words, heat is first transferred from the can wall to the liquid and from the liquid to the particle. Heat flow in a spherical particle immersed in liquid can be described by the partial differential equation, as follows:

$$\frac{\partial T}{\partial t} = \alpha_p \left(\frac{\partial^2 T}{\partial r^2} + \frac{2}{r} \frac{\partial T}{\partial r} \right) \qquad \text{(Eq. 6.4)}$$

The initial and boundary conditions are:

$$T(r, 0) = T_i \text{ at } t=0 \qquad \text{(Eq. 6.5)}$$

$$k_p \frac{\partial T}{\partial r} = h_{fp} \left(T_l - T_{ps} \right) \text{ at } r = a \qquad \text{(Eq. 6.6)}$$

Process lethality, F_o, indicated its severity with respect to microbial destruction and is the key to establish process time. F_o can be calculated for each run by numerical integration of temperature as follows:

$$F_o = \int 10^{(T-121.1)/z} \qquad \text{(Eq. 6.7)}$$

If the particle and liquid transient temperatures are available, h_{fp} can be determined by solving Eq. 6.4 (Sablani and Ramaswamy, 1996). First, a value of h_{fp} was assumed, the temperature history at the particle center was predicted and the resulting F_o predicted at the particle center was obtained using Eq. 6.7 based on the measured liquid temperature data. The predicted F_o was then compared with the actual F_o at the particle center based on the measured temperature history at the particle center. The objective function, which was the difference between predicted and experimental F_o values, was minimized through iteration (Sablani and Ramaswamy, 1996); however, this can also be based on predicted and experimental temperatures at particle center. By sequentially changing h_{fp} value, the objective function was minimized, which nearly matched the predicted and measured F_o values at the particle center to a preset value of 10 min, and the corresponding h_{fp} was considered to represent the specific processing conditions. U was then computed by solving the energy balance equation (Eq. 6.1) by carrying out the integration up to the same process time. Analytical solution for Eq. 6.2 with a conductive boundary condition is complex due to varying liquid temperatures over time. Numerical solutions based on finite differences are simpler and were used in this study as well as in

the literature (Teixeira et al., 1969; Sablani and Ramaswamy, 1996; Meng and Ramaswamy, 2005; Dwivedi, 2008).

6.3.5. Calculation of U and h_{fp} in free axial mode

The computational technique for evaluating U and h_{fp} from liquid and particle temperature data that is used during the fixed axial mode could not be used for the free axial mode as it was not possible to gather temperature profile at the particle center; therefore, in order to get estimates of U and h_{fp} in the free axial mode, the empirical approach developed by Dwivedi (2008) was used. After the determination of U and h_{fp} in the fixed axial mode, the h_{fp}/U ratio was obtained. It has been shown that there is a good correlation between U and h_{fp} for any given processing condition; thus, it was assumed that a similar relationship would exist in free axial rotation as well. On the other hand, in order to measure U in free axial rotation, U and h_{fp} are needed. First, a value of U was assumed for the free axial mode and multiplied by the value of the ratio in order to get an estimate of h_{fp}. Then, Eq. 6.1 and Eq. 6.6 were solved in order to compute the liquid temperature in the free axial mode. This was then compared with the liquid temperature in free axial rotation and the difference was minimized. U value that has met the minimization was taken in order to represent the free axial rotation and the corresponding h_{fp} was then easily computed.

6.3.6. Statistical analysis

Response surface methodology (RSM) was used to estimate the effect of independent variables (retort temperature, x_1, CMC concentration, x_2 and rotation speed, x_3) on the overall heat transfer coefficient, U and fluid to particle heat transfer coefficient, h_{fp}. A central composite rotatable design (CCRD) was employed for designing the experimental data. RSM was applied to the experimental data using a commercial statistical package, Design-Expert version 6.01 (Statease Inc., Minneapolis, USA). Experiments were randomized in order to minimize the effects of unexplained variability in the observed responses due to extraneous factors. The experimental design involved 20 experiments with 6 replications of the central point in order to calculate the repeatability

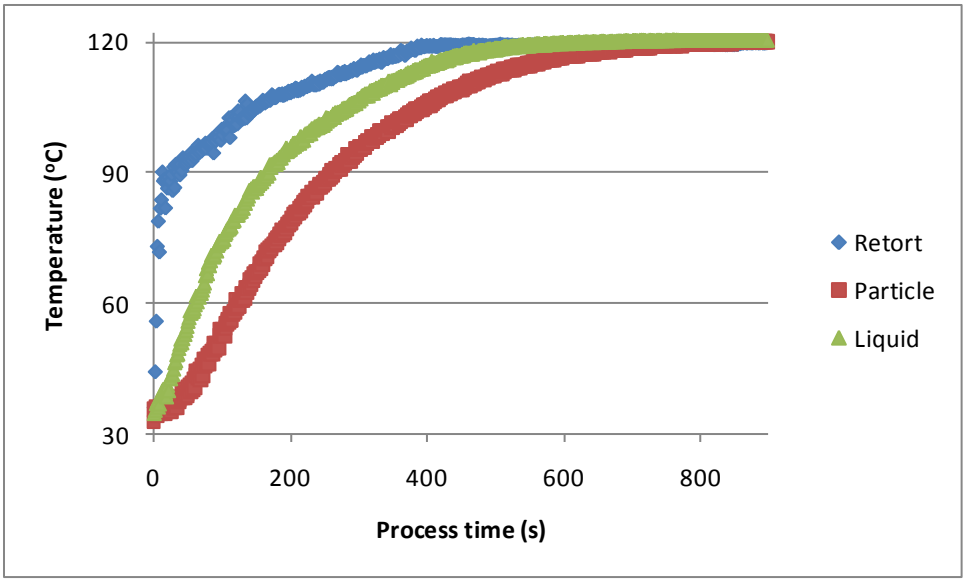
of the method (Montgomery, 2001). Five levels of retort temperature (110, 114, 120, 126, 130^{o}C), five levels of CMC concentration (0.0, 0.2, 0.5, 0.8 and 1.0%) and five levels of rotation speed (7, 10, 15, 20 and 23 rpm) were used. The response functions (y) were U and h_{fp}. Values were related to the coded variables (x_i, i = 1, 2 and 3) by a second order polynomial using the equation below:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{23}x_1x_3 + b_{31}x_2x_3 \quad \text{(Eq. 6.8)}$$

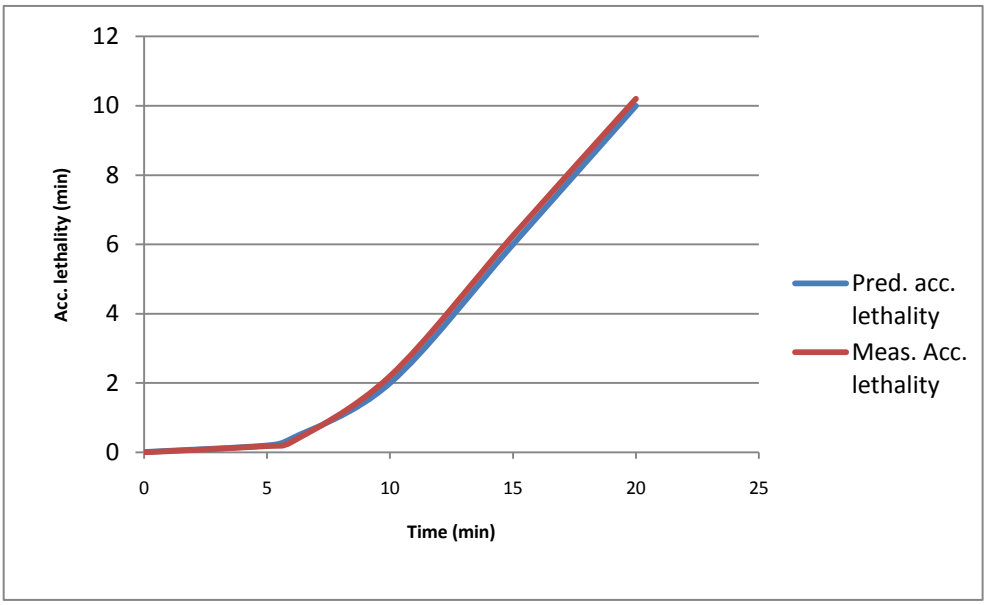
The coefficients of the polynomial model were represented by b_o (constant term), b_1, b_2 and b_3 (linear effects), b_{11}, b_{22} and b_{33} (quadratic effects), and b_{12}, b_{13} and b_{23} (interaction effects). Statistical significance of the terms in the regression equations was examined. The significant terms in the model were found by analysis of variance (ANOVA) for each response. The adequacy of model was checked by looking at the R^2 and adjusted-R^2 values. Numerical optimization technique of the Design-Expert software was used for simultaneous optimization of the multiple responses. The desired goals for each variable and response were chosen. All the independent variables were kept within range while the responses were the maximum values.

6.4 Results and discussion

Typical temperature profiles of the retort medium, the liquid and the particle during a test run involving fixed axial rotation is shown in fig. 6.1a. As can be seen, the retort reached the set point temperature much faster than the liquid and followed by the particle. Experiments were run for an accumulated lethality value of 10 min at the particle center. Fig. 6.1b shows a typical lethality plot that is used for the prediction of h_{fp}, demonstrating an excellent fit for the predicted and experimental lethality values when the objective function was minimized.



(a)



(b)

Fig. 6.1. Typical temperature profiles of the retort medium, liquid and particle (a) and comparison between measured and predicted accumulated lethality at the particle center when the objective function is minimized (b)

Table 6.2 shows U and h_{fp} values associated with selected conditions in fixed and free axial rotation.

Table 6.2. CCRD experimental design for evaluating U and h_{fp} in fixed and free axial modes of rotation

Temperature (^oC)	CMC conc. (%)	Rotation speed (rpm)	U Fixed Axial ($W/m^2.C$)	h_{fp} Fixed Axial ($W/m^2.C$)	h_{fp}/U (fixed axial)	U Free Axial ($W/m^2.C$)	h_{fp} Free Axial ($W/m^2.C$)
126(1)	0.8(1)	20(1)	390	515	1.3	610	810
126(1)	0.2(-1)	20(1)	440	630	1.4	705	1010
114(-1)	0.8(1)	20(1)	360	460	1.3	570	745
114(-1)	0.2(-1)	20(1)	445	585	1.3	705	930
126(1)	0.8(1)	10(-1)	280	385	1.4	440	605
126(1)	0.2(-1)	10(-1)	370	520	1.4	590	840
114(-1)	0.8(1)	10(-1)	290	335	1.2	445	525
114(-1)	0.2(-1)	10(-1)	380	475	1.3	595	750
120(0)	0.5(0)	23(1.68)	455	620	1.4	720	1005
120(0)	0.5(0)	7(-1.68)	305	425	1.4	490	695
130(1.68)	0.5(0)	15(0)	365	510	1.4	565	825
110(-1.68)	0.5(0)	15(0)	360	420	1.2	575	670
120(0)	1.0(1.68)	15(0)	265	415	1.6	430	675
120(0)	0.0(-1.68)	15(0)	415	585	1.4	650	940
120(0)	0.5(0)	15(0)	350	500	1.4	560	810
120(0)	0.5(0)	15(0)	350	500	1.4	550	810
120(0)	0.5(0)	15(0)	350	490	1.4	550	805
120(0)	0.5(0)	15(0)	350	460	1.3	540	780
120(0)	0.5(0)	15(0)	340	480	1.4	530	790
120(0)	0.5(0)	15(0)	345	500	1.4	560	780

In the fixed axial mode of rotation, values of U ranged between 265 and 455 $W/m^2.C$ and those of h_{fp} ranged between 335 and 630 $W/m^2.C$. In the free axial mode of rotation, values of U ranged between 430 and 720 $W/m^2.C$ and those of h_{fp} ranged between 605 and 1010 $W/m^2.C$. The results indicate that the heat transfer coefficients are quite sensitive to the process variables justifying the need for the study and for evaluating the optimal conditions.

6.4.1. Model fitting

The second-order polynomial response surface model (Eq. 6.8) was fitted to each of the response variables (Y). For the corresponding fitting of the explanatory models and the variation of U and h_{fp} values, the sum of squares of the sequential model was analyzed. These analyses indicated that adding terms up to the quadratic significantly

improved the model for the heat transfer coefficients in both fixed and free axial modes of rotation. Regression analysis and ANOVA were used to fit the model and to examine the statistical significance of the terms. The estimated regression coefficients of the models for the response variables, along with the corresponding coefficients of determination (R^2) are given in Table 6.3 for the fixed axial rotation and in Table 6.4 for the free axial rotation. Coefficient of variation (CV) and adjusted-R^2 were also calculated to check the model adequacy.

Table 6.3. ANOVA and regression coefficients of the second-order polynomial model for the U and h_{fp} in fixed axial rotation mode (actual values)

Source	DF	$U_{fixed\ axial}$			$h_{fp\ fixed\ axial}$		
		Coefficient	Sum of squares	p-value	Coefficient	Sum of squares	p-value
Model	9	3.67E+03	5.09E+04	< 0.0001	-3.87E+03	1.07E+05	< 0.0001
Linear							
b_1	1	-50.02	12.29	0.6773	69.93	8.77E+03	< 0.0001
b_2	1	-479.61	2.36E+04	< 0.0001	-393.03	4.70E+04	< 0.0001
b_3	1	-31.55	2.36E+04	< 0.0001	-4.59	4.72E+04	< 0.0001
Quadratic							
b_{11}	1	0.19	691.37	0.0093	-0.28	1.44E+03	0.0268
b_{22}	1	-10.33	12.28	0.6773	28.27	91.97	0.5264
b_{33}	1	0.52		0.0001	0.41	1.54E+03	0.0230
Interaction							
b_{12}	1	2.46	157.13	0.1563	1.04	28.13	0.7242
b_{13}	1	0.19	248.04	0.0830	0.02	3.13	0.9061
b_{23}	1	3.71	248.04	0.0830	2.92	153.13	0.4169
Residual	10		668.85			2134.90	
Lack of fit	5		531.35	0.0821		851.56	0.6682
Pure error	5		137.5			1.28E+03	
Total	19		5.16E+04			1.09E+05	
R^2		0.987			0.980		
Adj-R^2		0.975			0.963		
CV		2.27			2.98		

As noted earlier, the lack of fit is an indication of the failure for a model representing the experimental data at which points were not included in the regression or variations in the models cannot be accounted for random error (Montgomery, 2001). The

lack of fit, illustrated in Tables 6.3 and 6.4, did not result in a significant *p*-value for selected variables, meaning that these models were sufficiently accurate for predicting the relevant responses.

Table 6.4. ANOVA and regression coefficients of the second-order polynomial model for the U and h_{fp} in free axial rotation mode (actual values)

Source	DF	$U_{free\ axial}$			$h_{fp\ free\ axial}$		
		Coefficient	Sum of squares	*p*- value	Coefficient	Sum of squares	*p*- value
Model	9	5.07E+03	1.29E+05	< 0.0001	-9.27E+03	2.81E+05	< 0.0001
Linear							
b_1	1	-68.45	12.72	0.7647	159.07	2.43E+04	< 0.0001
b_2	1	-631.97	5.93E+04	< 0.0001	-190.53	1.22E+05	< 0.0001
b_3	1	-41.02	6.02E+04	< 0.0001	11.80	1.23E+05	< 0.0001
Quadratic							
b_{11}	1	0.27	1.33E+03	0.0104	-0.62	7.26E+03	0.0049
b_{22}	1	-8.94	9.20	0.7990	-10.52	12.74	0.8833
b_{33}	1	0.88	6.97E+03	< 0.0001	0.55	2.74E+03	0.0516
Interaction							
b_{12}	1	2.78	200.00	0.2507	-1.74	78.13	0.7170
b_{13}	1	0.21	312.50	0.1584	-0.10	78.13	0.7170
b_{23}	1	5.83	612.50	0.0586	6.25	703.13	0.2895
Residual	10		1.35E+03			5.62E+03	
Lack of fit	5		661.84	0.5136		4.60E+03	0.0621
Pure error	5		683.33			1.02E+03	
Total	19		1.30E+05			2.87E+05	
R^2		0.990			0.980		
Adj-R^2		0.980			0.963		
CV		2.04			3		

As noted earlier, coefficient of determination, R^2, is the proportion of variation in the response attributed to the model rather than to random error. The R^2 values for U and h_{fp} in the present study were found to be 0.99 and 0.98 (Tables 6.3, 6.4), respectively, in fixed and free axial rotation, indicating that the regression models were suitable to explain the behaviour. The adj-R^2 values were found to be higher than 0.96 for both responses in fixed and free axial rotation. Higher adj-R^2 indicated that non-significant terms have not been included in the model.

As noted earlier, coefficient of variation (CV) should not be greater than 10%. Our results showed that the coefficients of variation for U and h_{fp} were 2.27 and 2.28%, respectively, in fixed axial rotation (Table 6.3) and 2.04 and 3.00%, respectively, in free axial rotation (Table 6.4), representing a better precision and reliability of the conducted experiments.

The following models were developed for response surface plot for U and h_{fp} in fixed and free axial modes of rotation as influenced by the process variables (retort temperature, T, rotational speed, R, and CMC concentration, C):

$$U_{\text{fixed axial}} = 3668.08 - 50.02*T - 479.61*C - 31.55*R + 0.19*T^2 - 10.33*C^2 + 0.52*R^2 + 2.46*T*C + 0.19*T*R + 3.71*C*R \quad \text{(Eq. 6.9)}$$

$$h_{fp\ \text{fixed axial}} = -3867.40 + 69.93*T - 393.03*C - 4.59*R - 0.28*T^2 + 28.27*C^2 + 0.41*R^2 + 1.04*T*C + 0.02*T*R + 2.92*C*R \quad \text{(Eq. 6.10)}$$

$$U_{\text{free axial}} = 5066.24 - 68.45*T - 631.97*C - 41.02*R + 0.27*T^2 - 8.94*C^2 + 0.88*R^2 + 2.78*T*C + 0.21*T*R + 5.83*C*R \quad \text{(Eq. 6.11)}$$

$$h_{fp\ \text{free axial}} = -9273.54 + 159.07*T - 190.53*C + 11.80*R - 0.62*T^2 - 10.52*C^2 + 0.55*R^2 - 1.74*T*C - 0.10*T*R + 6.25*C*R \quad \text{(Eq. 6.12)}$$

Fig. 6.2 shows that the polynomial regression model was in good agreement with the experimental results. In this figure, each of the observed values is compared to the predicted value calculated from the model. Our results ($R^2 > 0.9$) suggest that the models used in this study were able to identify heat transfer coefficients in free and fixed axial modes of rotation.

6.4.2. Effect of process parameters on U and hfp

Analysis of variance presented in Table 6.3 and Table 6.4 was used to evaluate the effect of rotation speed, CMC concentration and temperature on the associated heat transfer coefficients in fixed and free axial rotation. Linear and quadratic effects of rotation speed, linear effect of CMC concentration and quadratic effect of temperature on

U were significant ($p < 0.05$), while the linear effect of temperature and quadratic effect of CMC concentration were not ($p > 0.05$). The mutual interaction between CMC concentration, temperature and rotation speed were found to be non-significant ($p > 0.05$) on U. Based on the sum of squares, the importance of the independent variables on U in fixed and free axial rotation could be ranked in the following order: rotation speed > CMC concentration > temperature. Dwivedi and Ramaswamy (2008) used a Newtonian fluid (glycerin) and reported the same order. The effects of retort temperature, rotational speed and CMC concentration were imperative to heat transfer coefficients. Fig. 6.3 and 6.4 show the effect of the three parameters on U and h_{fp} in fixed and axial modes of rotation.

a. CMC concentration

Heat transfer coefficients, U and h_{fp}, decreased significantly ($p < 0.05$) with increasing CMC concentration in fixed and free axial modes of rotation. For the same levels of temperature and rotational speed, increasing CMC concentration from 0.2 to 0.8% resulted in an average decrease of ~22% in the corresponding heat transfer coefficients. Our results have shown an increase in the resistance to heat transfer with increasing CMC concentration and this could be due to dramatic increase in the shear resistance (increased apparent viscosity or consistency) at higher CMC concentrations, as reported by Abdelrahim (1994). At higher CMC concentrations, fluid viscosity increased and thickness of boundary layer around the can wall and particle surface became larger, resulting in slower the heat transfer.

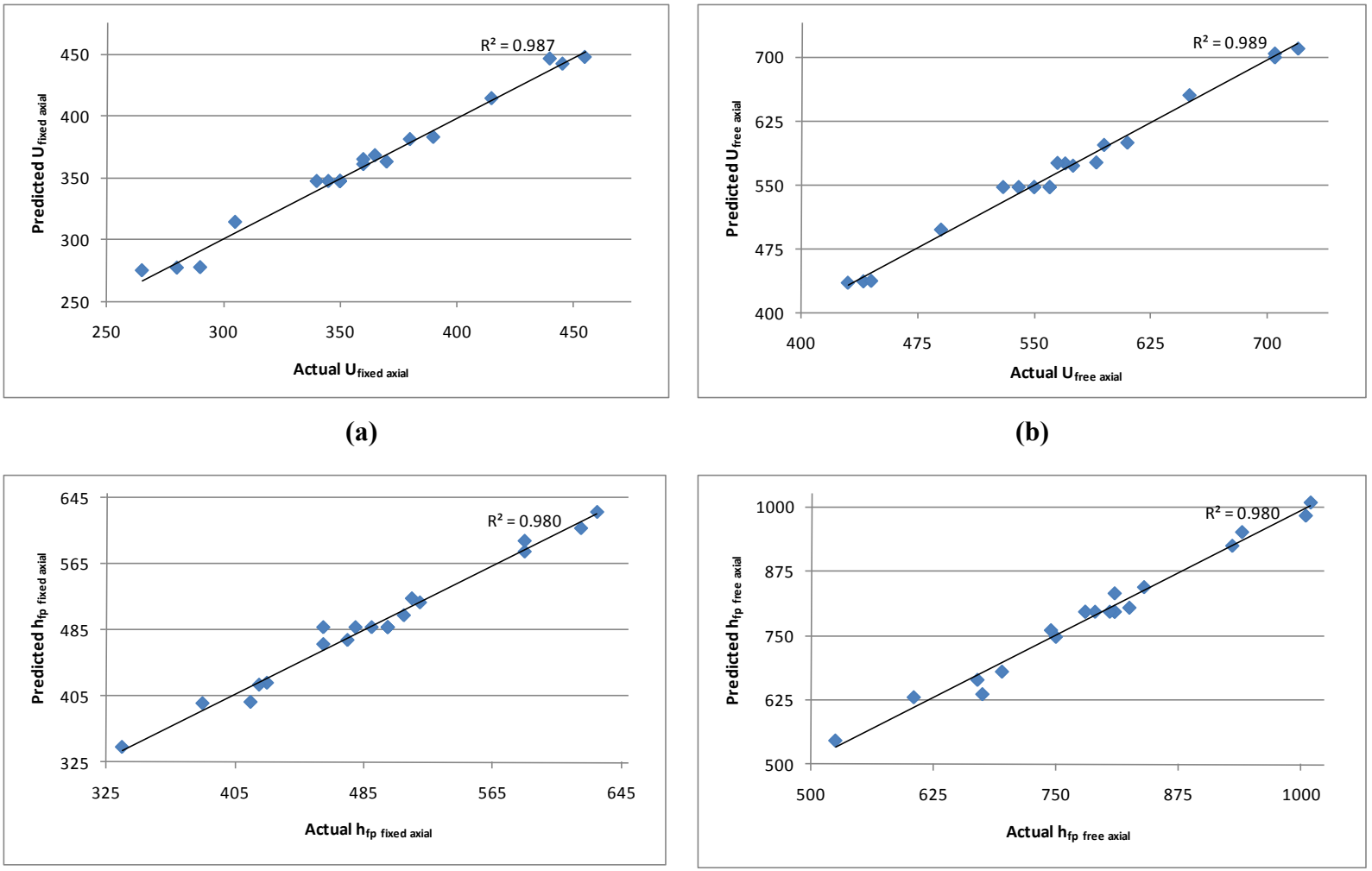
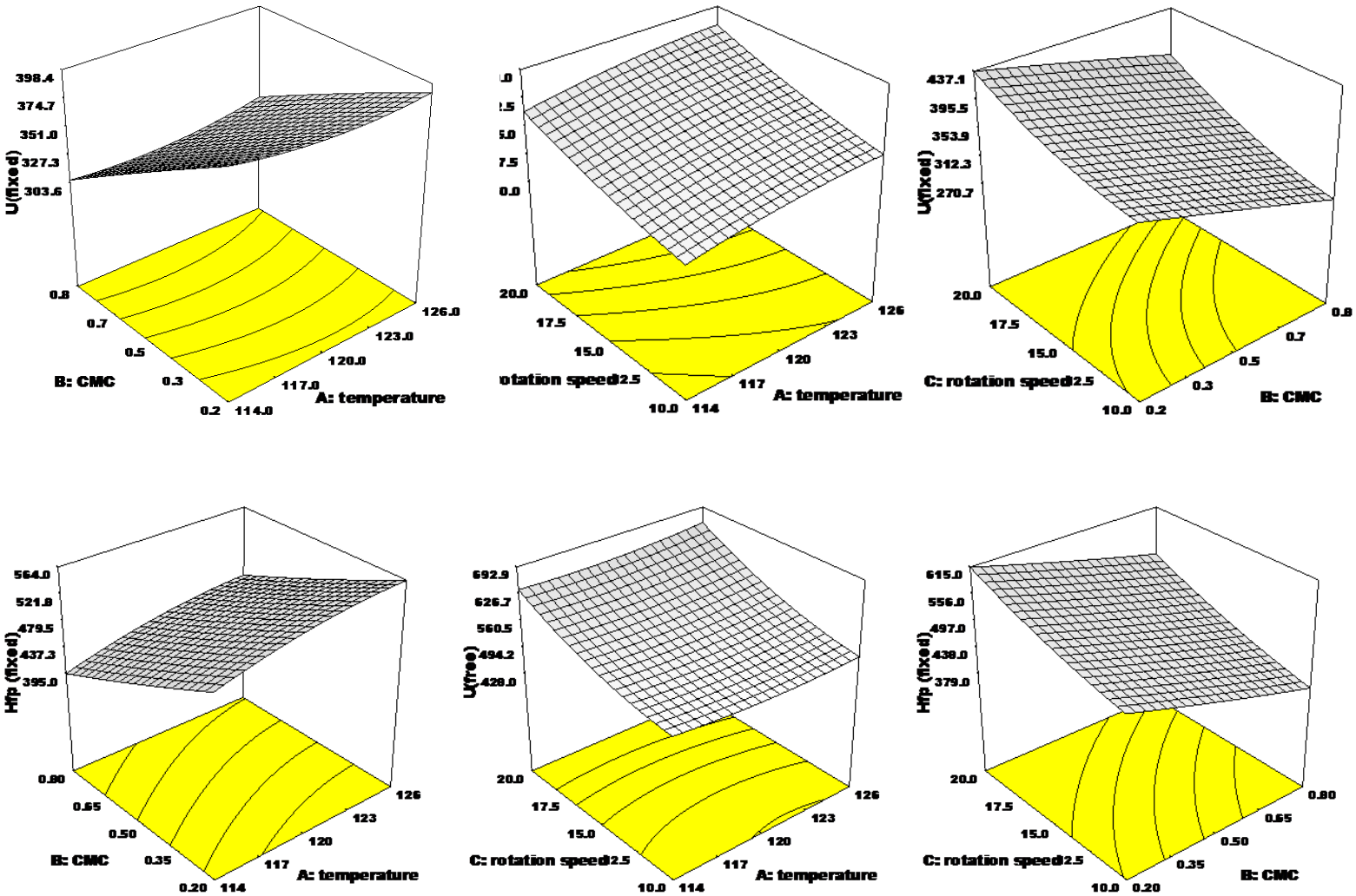


Fig. 6.2. Comparison between predicted and actual values of *U* (a, b) and h_{fp} (c, d) in fixed and free axial modes of rotation, respectively



(D)

Fig. 6.3. Effect of rotational speed, temperature and CMC concentration on U (a) and h_{fp} (b) in fixed axial mode of rotation

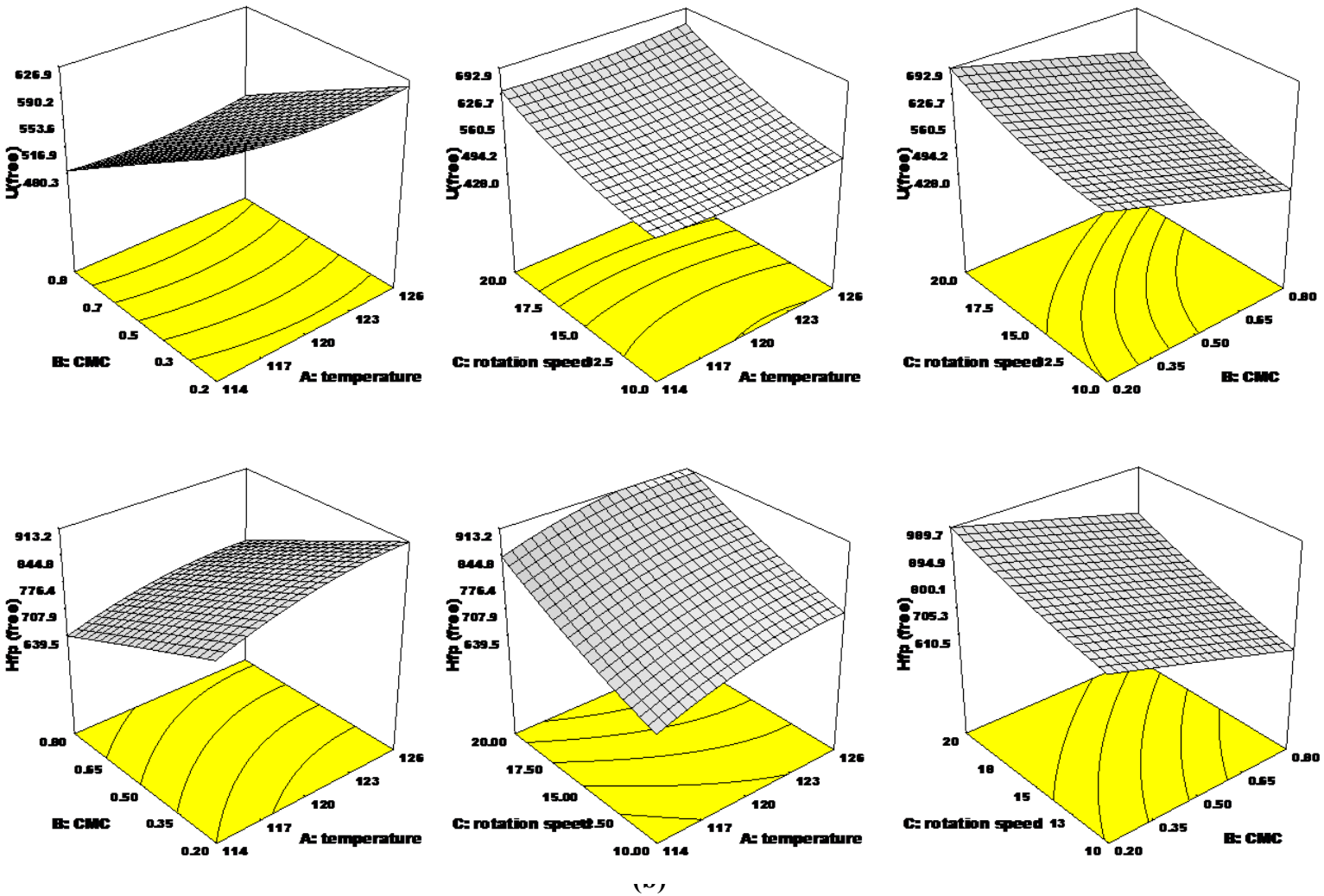


Fig. 6.4. Effect of rotational speed, temperature and CMC concentration on U (a) and h_{fp} (b) in free axial mode of rotation

Meng (2006) found that U and h_{ap} values decreased with an increase in CMC concentration. Hassan (1984) reported a decrease in overall heat transfer coefficient with increasing fluid viscosity. Lenz and Lund (1978) found that heat transfer coefficients were higher in water than in 60% aqueous sucrose solution. Sablani (1996) studied the heat transfer to Nylon particles in oil and water and found out that U and h_{fp} values were higher in water than oil. On the other hand, Stoforos and Merson (1992) studied fluid to particle heat transfer coefficient with teflon spheres in Newtonian fluids subjected to axial rotation and reported that h_{fp} values increased with increasing fluid viscosity; however, they explained this observation with their particle motion study, which showed that particle to fluid relative velocity increased in high viscous liquids. Our findings on the effect of CMC concentration on the heat transfer were consistent, in general, with the results from most previous studies.

b. Rotational speed

Heat transfer coefficients, U and h_{fp}, increased significantly ($p < 0.05$) with increasing rotational speed in fixed and free axial modes of rotation. For the same levels of CMC concentration and temperature, increasing rotational speed from 10 to 20 rpm increased the corresponding heat transfer coefficients by ~20%. This can be explained by the improved mixing due to higher degree of turbulence, resulting in increased relative particle to fluid velocity (Anantheswaran and Rao, 1985; Sablani, 1996; Sablani and Ramaswamy, 1997). A definite advantage of agitation processing over still processing at 0 rpm is evident at all rotation speeds. Early literature has already documented the effect of rotational speed on heat transfer rates and the resulting processing time (Conley et al., 1951). Lenz and Lund (1978) found that increasing the reel speed from 3.5 to 8.0 rpm significantly increased the heat transfer. Hassan (1984) reported that increasing the rotational speed from 9.3 to 101 rpm had more effect on U than on h_{fp}, but he could not explain his findings. Stoforos (1988) found that increasing rotational speed from 9.3 to 101 rpm resulted in increasing U by 1.2-2.0 times. Sablani (1996) observed that increasing rotational speed from 10 to 20 rpm resulted in h_{fp} increase of 56% for oil and 53% for water and in U increase of 24% for oil and 13% for water. Meng (2006) and Dwivedi (2008)

reported a significant effect of the rotational speed on the associated heat transfer coefficients. These observations in the literature conform to the findings of the present study.

c. Temperature

Retort temperature had a positive, although the least effect among the process parameters, on improving the heat transfer in fixed and free axial modes of rotation. For the same levels of CMC concentration and rotational speed, increasing retort temperature from 114 to 126^{o}C increased the corresponding heat transfer coefficients by ~5%. The effect of retort temperature could be due to the reduction in CMC viscosity and better liquid convection at higher temperature. This trend was also reported by Sablani and Ramaswamy (1996), Meng and Ramaswamy (1996) and Dwivedi (2008).

6.4.3. Modes of rotation

Most common modes used in the canning industry are end-over-end mode in batch retorts and free axial mode in continuous retorts. Fixed axial rotation is not widely used in the canning industry. *T*-test showed a significant ($p < 0.05$) difference in U and h_{fp} values between fixed and free axial modes and heat transfer coefficients in free axial mode were on average 35-40% higher than in fixed axial mode. This improvement could be explained by the enhanced mixing of the can contents provided by the bi-axial agitation of the freely rotating cans. The unidirectional can agitation in fixed axial rotation resulted in lower values of heat transfer coefficients.

Meng (2006) reported U values ranging from 298 to 532 W/m^2.C and h_{fp} values ranging from 369 to 987 W/m^2.C for Nylon spheres placed in 0.0-0.4% CMC and subjected to end-over-end rotation at a temperature of 120^{o}C and rotation speed of 10 rpm. For similar conditions in fixed axial mode of rotation, U values in our study ranged from 305 to 415 W/m^2.C and h_{fp} ranged from 425 to 585 W/m^2.C. On the other hand, for similar conditions in free axial mode of rotation, U values in our study ranged from 490 to 650 W/m^2.C and h_{fp} ranged from 695 to 940 W/m^2.C, indicating that heat transfer in canned non-Newtonian fluid particulates is highest in free axial rotation, followed by end-over-end and fixed axial rotation. This conclusion conforms

to the observation of Naveh and Kopelman (1980) who reported that heat transfer coefficients in end-over-end rotation were 2-3 times higher than in fixed axial rotation. Also, Dwivedi (2008) found that heat transfer in Newtonian fluid particulates can was most rapid in free axial rotation, followed by end-over-end rotation and then by fixed axial rotation.

6.4.4. Optimization

Using the numerical optimization technique of the Design Expert software, optimum condition for heat transfer in non-Newtonian fluid particulates subjected to free and fixed axial rotation was determined in order to obtain maximum fluid to particle heat transfer coefficient, h_{fp}, and overall heat transfer coefficient, U. Our objective was to select a range of CMC solution viscosity that encompasses the viscosity values of different commercial soups. Viscosity of CMC solutions at different concentrations vs. some types of commercial soups at 30^{o}C are presented in Table 6.5.

Table 6.5. Viscosity of CMC solution at different concentrations and some commercial soups at 30^{o}C

Author	Solution	Consistency coefficient (Pa s^{n})	Flow behaviour index
Meng (2006)	CMC		
	0.2%	0.087	0.74
	0.4%	0.344	0.64
	0.6%	1.068	0.56
	0.8%	2.188	0.51
	1.0%	4.145	0.46
Sopade et al. (1993)	Bean soup	1.77	
	Melon soup	1.42	
Ibanoglu (1992)	Yogurt soup		0.43
	Lentil soup		0.32
	Lentil and tomato soup		0.32
Harper (1965)	Tomato soup		0.40

CMC concentration (0.6-1.0%), retort temperature (110-130^{o}C) and rotation speed (7-23 rpm) were selected as objective goals for the independent variables. Optimum conditions of process parameters are tabulated in Table 6.6, providing the highest rate of heat transfer. These conditions were: retort temperature of 126^{o}C, CMC concentration of 0.6% and rotational speed of 20 rpm.

Table 6.6 Optimal conditions of heat transfer to non-Newtonian fluid particulates cans subjected to free and fixed axial modes of rotation

Rotation mode	Temperature (^{o}C)	CMC concentration (%)	Rotational Speed (rpm)
Fixed axial	126.0	0.60	20.0
	125.5	0.60	20.0
	126.0	0.62	20.0
	121.9	0.60	20.0
Free axial	126.0	0.60	20.0
	125.4	0.60	20.0
	122.4	0.60	20.0
	116.0	0.60	20.0

6.5 Conclusions

The present study investigated the effects of different process parameters on the overall heat transfer coefficient, U and fluid to particle heat transfer coefficient, h_{fp} for Nylon particles suspended in non-Newtonian fluid (CMC) and subjected to fixed and free axial modes of rotation. The values of U and h_{fp} in free axial mode were significantly ($p < 0.05$) higher than in fixed axial mode of rotation. Rotational speed, CMC concentration and retort temperature had a significant ($p < 0.05$) effect in the following order: rotation speed > CMC concentration > temperature. With the increase in rotational speed and retort temperature, heat transfer coefficients increased while with further increase in CMC concentration, heat transfer coefficients decreased. Optimum conditions for maximum values of U and h_{fp} were: retort temperature of 126^{o}C, CMC concentration of 0.6% and rotational speed of 20 rpm.

PREFACE TO CHAPTER 7

In order to establish any thermal process, time temperature data from test cans must be collected; however, in continuous flow biaxial rotary processing, temperature measurements are often difficult. Biological validation using food alginate simulated particles that are inoculated with spores is generally considered a useful alternative.

Since temperature probe systems cannot be attached to the particle center in the case of bi-axial rotary retorts, time temperature profile can be predicted using fluid to particle heat transfer coefficient, h_{fp}, liquid temperature and thermophysical properties of the particle. Then, using the General Method, process time can be calculated. Using initial and final spores' count in the food alginate simulated particles, process lethality can be measured.

A validated process model is useful in optimization approaches. The objective is to verify that the process lethality values determined by a validation methodology match those predicted under practical conditions.

The experimental work and data analysis were carried out by the candidate under the supervision of Dr. H. S. Ramaswamy.

CHAPTER 7

BIOLOGICAL VALIDATION OF BI-AXIAL ROTATION PROCESSING USING CARROT AND MEAT ALGINATE FABRICATED PARTICLES

7.1 Abstract

Biological validation in bi-axial rotation processing of canned food products is necessary due to the difficulty of using temperature measuring devices to collect time temperature history at the particles centers and therefore, to calculate process lethality. In this study, carrot and meat alginate fabricated particles inoculated with spores of *C. sporogenes* and *G. stearothermophilus*, respectively, were filled into cans along with a non-Newtonian liquid (carboxy-methyl cellulose) and processed for predetermined time at three temperatures (110-125^{o}C) and two rotation speeds (5-25 rpm). Process times ranged from 19.5 to 36.4 min for carrot alginate and from 19.3 to 40.1 min for meat alginate fabricated particles. Heating behavior of these particles were previously matched with those involving Nylon spheres (d=1.9 cm) used in earlier heat transfer studies for process optimization. Using initial and final spores counts of the particles, number of log reductions, n, and the process lethality, F_o, was determined. These were then compared with the model predicted values which were obtained through simulated time temperature profiles of the alginate fabricated particles. There were no significant differences ($p > 0.05$) in the computed F_o values between those obtained from biological validation and numerical simulation under the experimental conditions. This investigation could thus help food industry employing bi-axial agitation processing to use the biological validation approach for assuring the safety of their products.

7.2 Introduction

With today's consumers becoming more educated and health aware, the demand for convenient foods with high quality has boosted over time. Canning, in general, involves excessive thermal treatment of the food product, resulting in degradation of quality attributes (David et al., 1996). High temperature-short time (HTST) processing can be applied in order to improve the quality of the canned food products as quality factors are significantly more heat stable than the microorganisms. HTST processing includes aseptic processing, agitation processing and thin profile packaging. Aseptic processing consists of heating the food without any package to a high temperature, holding it for a short time, cooling down and packaging it into a sterilized container in a sterile chamber (Lund, 1987). Thin profile packaging involves using retort pouches and semi-rigid containers, where the heat transfer is faster due to larger surface area compared to conventional cans (Ramaswamy and Marcotte, 2005). In agitation processing, mixing is enhanced inside the cans placed in rotary retorts, resulting in shorter processing time and better quality retention. The modes of rotation include end-over-end, fixed axial and free axial rotation (Dwivedi, 2008).

In thermal processing, theoretical models can be useful tools for designing, optimizing and validating food systems; however, the usefulness of these models depends upon the accuracy of the input physical parameters, such as overall heat-transfer coefficient (U) and the fluid-to-particle heat-transfer coefficient (h_{fp}) (Sablani and Ramaswamy 1996). Various process parameters, including rotational speed, retort temperature, headspace volume, system geometry, liquid viscosity, rotation radius, particle size and particle density were reported to significantly influence U and h_{fp} (Anantheswaran and Rao, 1985; Lekwauwa and Hayakawa, 1986; Britt, 1993; Sablani and Ramaswamy, 1995, 1996, 1997; Meng and Ramaswamy, 2007a, b; Dwivedi, 2008). In the liquid particulates heat transfer studies, it is necessary to have well-defined thermophysical properties for particles in order to obtain consistent heat transfer coefficients; that is why Nylon spheres were in general used instead of real food as there is variability in the thermophysical properties of the latter due to differences in the structure and chemical composition (Dwivedi, 2008).

In free axial rotation, there are two levels of rotation, one at the can level and another one at the cage level when the cans move in a rotary fashion along with the cage. In case of liquid particulate canned food products subjected to free axial rotation, temperature data collection is challenging due to difficulties involved in attaching the temperature measuring devices to the liquid and particles; therefore, biological validation is necessary to assure the safety of the thermal process (Chandarana, 1992). No work has been reported in the literature about using food alginate simulated particles in the biological validation of bi-axial rotation processing.

Biological validation can be categorized based on whether or not bio-indicators are in contact with food (Dignan et al., 1989). Non-contact methods include Biological Indicator Units (BIUs), which are leak proof, small diameter plastic rods containing spore solutions (Pflug et al., 1980), spores in glass spheres (Hersom and Shore, 1981) or in stainless steel differential scanning calorimetry - DSC - pans or aluminum DSC pans. However, nowadays, contact method, which consists of immobilizing spores of heat resistant microorganisms in a matrix of alginate food puree forming a particle and then calculating process lethality, F_o, is the most commonly used technique in biological validation (Brown et al., 1984). Inoculated food alginate fabricated particles can be used to determine the process lethality using initial and final spores' counts of the inoculated particles (Brown et al., 1984; Abdelrahim, 1994; Walsh, 1996; Marcotte et al., 2000; Naim et al., 2008). In low-acid canning industry, target microorganism is the *C. botulinum*, which, in case not destroyed by heat, *botulinum* toxin will be produced. Few biological validation studies have used *C. botulinum* spores because not only of the hazards associated with their handling, but also of the very low number of surviving spores that would result from the thermal process due of their small D-value (0.21 min); therefore, process lethality cannot be calculated (Perkins, 1969). Spores of heat-resistant surrogate microorganisms, such as *G. stearothermophilus* or *C. sporogenes*, which have close z-value to the target microorganism, *C. botulinum*, can be used instead (Koutchma et al., 2005).

The objectives of the study were: (1) to compare the heat behavior of carrot and meat alginate fabricated particles at different diameters to the one of Nylon spheres usually used in heat transfer studies (2) to predict time temperature profile at the alginate fabricated particle in order to calculate the process times using the Improved General Method (3) using initial and

final spores count for carrot and meat fabricated particles inoculated with spores of *C. sporogenes* and *G. stearothermophilus*, respectively, to determine the number of log reductions, n, in order to calculate process lethality, F_o, and to compare the latter with the model predicted values, which were obtained through simulated time temperature profiles of the alginate fabricated particles.

7.3 Materials and Methods

7.3.1 Spores preparation

C. sporogenes and *G. steaorthermophilus* spores preparation was done according to the methods used by Shao (2010) and Kim and Naylor (1966), respectively, as exaplained earlier in Chapter 3.

7.3.2 Alginate particle making

Carrot alginate and meat alginate fabricated particles were fabricated according to the method used by Brown et al. (1984) and Marcotte et al. (2000). Extra lean beef and baby carrots were purchased from a local supermarket. Beef paste was made by autoclaving 100 g of lean beef for 15 min for softening and sterilization purposes and then mincing it in a blender with 20 ml of *G. steaorthermophilus* spores solution. Carrot paste was made by autoclaving 100 g of peeled carrots and blending them in a blender with 35 ml of *C. sporogenes* spores solution. A quantity of 0.075 g of tri-sodium citrate was mixed with sodium alginate and added to the puree in a high speed mixer for 5 min to assure dissolution. A quantity of 0.3 g of calcium sulphate suspended in 10 ml distilled water was added to the mixture and mixed thoroughly. Mixture was then molded manually into spheres under aseptic conditions. Molded spheres were subsequently immersed into calcium chloride solution for a certain period of time to allow the gel to harden. In biological validation studies, food alginate fabricated particles should be hard enough to save their integrity till the end of the thermal process. Also, they should have identical thermophysical properties to real food particles (Marcotte et al. 2000). As noted earlier in Chapters 4 and 5, optimization of the process of fabricating carrot and meat alginate simulated particles has been carried out. It was found that the optimum conditions were sodium alginate concentration of

4.7%, calcium chloride concentration of 2.6% and immersion time of 32.0 and 30.8 hours for carrot alginate and meat alginate fabricated particles, respectively.

7.3.3 Spores recovery and enumeration

Recovery of spores from the meat and carrot alginate particles was done according to the method used by Dallyn et al. (1977) and Brown et al. (1984). Particle was aseptically chopped up and the resulting small pieces were dissolved in 35 ml 5% tri-sodium citrate in a 50 ml centrifuge tube with glass beads for approximately 20 min on a vortex mixer in order to assure a complete breakdown of the particles. Serial dilutions were made with 0.1% peptone water and enumeration was done using the pour plate technique. Enumeration of *C. sporogenes* in carrot alginate fabricated particles was done in a modified PA3679 agar (Ocio et al., 1994) and the plates were incubated at 37^{o}C under anaerobic conditions for 5 days before counting. Spores of *G. stearothermophilus* in meat alginate fabricated particles were enumerated in Tryptic soy agar (BD, Co., Spark, MD) plates, which were incubated at 55^{o}C under aerobic conditions for 5 days before counting. Initial count (N_o) in carrot alginate and meat alginate fabricated particles was found to be 10^5 and 10^7 CFU/ml, respectively.

7.3.4 Water bath experiments

In previous heat transfer studies conducted to investigate the effect of process and product parameters on the rate of heat transfer to liquid particulate cans, Nylon spheres were used and assumed to be food simulating particles. In order to validate this assumption, meat and carrot alginate spheres with diameters ranging from 1.6 to 1.9 cm were fabricated and thermocouple wires (d = 0.0762 mm, Omega Engineering Corp., Stamford, CT) were introduced into the center of these particles as shown in Fig. 7.1. On the other hand, thermocouple wires were placed at the center of a Nylon sphere (d = 1.9 cm) through a fine hole drilled using a horizontal lathe and were fixed using epoxy glue. Thermocouples were connected to a data acquisition system (HP34970A, Hewlett, Packard, CO) and temperatures were computer recorded. Then, particles were dipped in a water bath (Thermo Electron Corporation, HAAKE C10, MA) at three temperatures (60, 80, 100^{o}C) and time temperature profiles at the particle center were plotted.

Heating behavior of Nylon versus meat and carrot alginate fabricated particles was evaluated by comparing the heating rate index, f_h, of each particle diameter at each water bath temperature. Heating rate index is the time required for the straight line portion of the heating curve, temperature deficit (T_r-T) vs. heating time, to pass through one log cycle. In other words, it is the negative reciprocal slope of the heating curve.

7.3.5 Can preparation and processing

A pilot-scale rotary single cage retort (Stock Rotomat PR 900; Herman Stock Maschinenfabrick, Neumünster, Germany) was modified, according to the method used by Dwivedi (2008), in order to simulate the rotational motion in continuous cookers. Cans of size 307×409 (Home Canning Co., Montreal, QC) were filled with 0.6% caboxymethyl cellulose (Fisher Scientific Ltd., Montreal, QC) solution and 30% of meat or carrot alginate fabricated particles to a headspace of 12 mm and were closed by a manual closing machine (Home Canning Co., Montreal, QC). Cans were then placed in the retort and subjected to thermal processing at 115 and 120^{o}C for cans of carrot alginate fabricated particles inoculated with *C. sporogenes* spores and at 120 and 125^{o}C for cans of meat alginate fabricated particles inoculated with *G. stearothermophilus* spores, using a rotation speed of 5 and 25 rpm. Experiments were carried in duplicate. Further details of the retort cage modification and can preparation are provided in Dwivedi (2008).

7.3.6 Predicting time temperature profiles

In free axial rotation processing, measuring the time temperature profile at the fabricated alginate particle center is challenging since attaching the temperature measuring devices fail as the can touches the bottom of the retort (Dwivedi, 2008). Therefore, time temperature profile at the particle center in this case was predicted using fluid to particle heat transfer coefficient, h_{fp} (determined in Chapter 6), liquid time temperature profile and particle thermophysical properties, according to the method used by Dwivedi (2008). In order to check the validity of this method, time temperature profile at the particle center in fixed axial rotation was predicted and compared to the actual one as shown in Fig. 7.2. There was no significant ($p > 0.05$) difference

between predicted and actual temperature history at the particle center in fixed axial rotation, showing the validity of predicting the temperature history at particle center in free axial rotation.

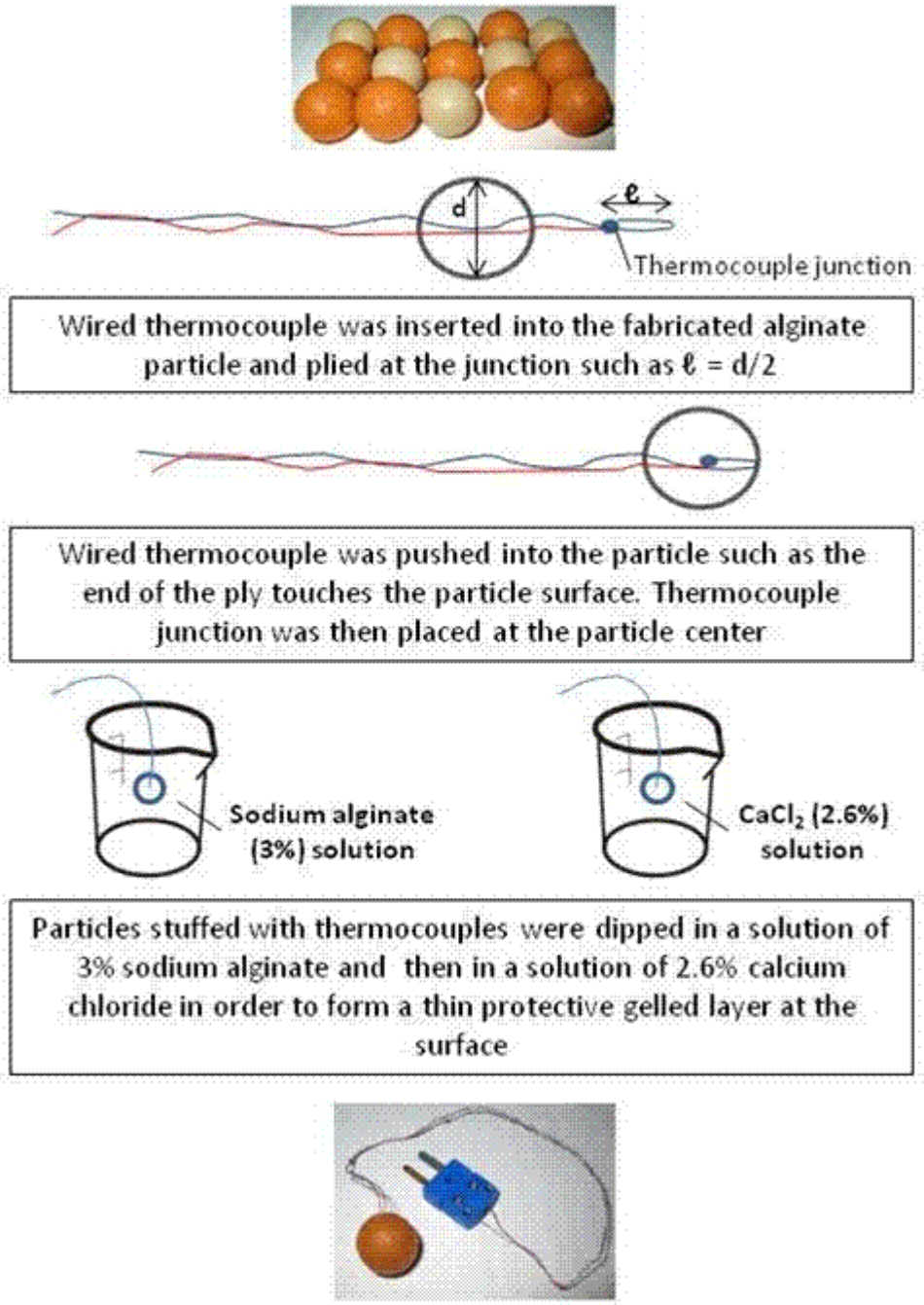


Fig. 7.1 Steps of making the meat and carrot alginate fabricated particles used in water bath experiments

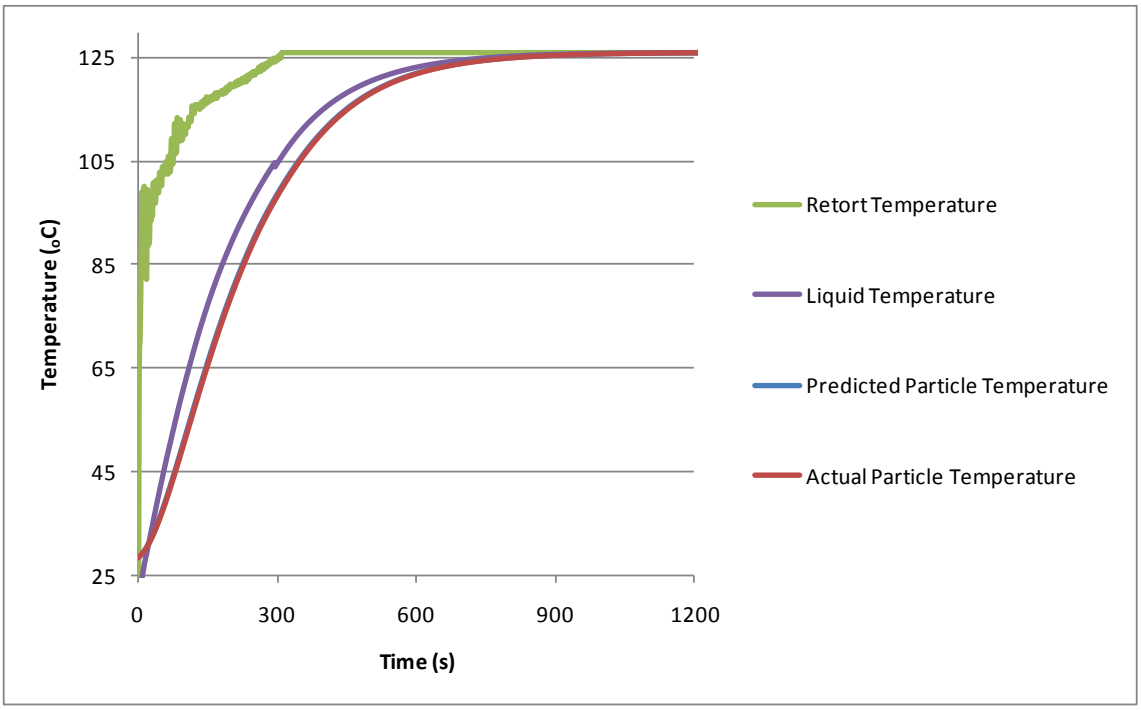


Fig. 7.2 Time temperature profile of retort (set T = 126°C), CMC solution (0.8%), predicted and actual Nylon particle center (d = 1.9 cm) in a can under fixed axial rotation at 20 rpm

7.3.7 Process time calculation

Process lethality, F, is a measure of the heat treatment or sterilization. In order to compare the relative sterilizing capacities of heat processes, a unit of lethality needs to be established. For convenience, this is defined as equivalent heating of 1 minute at a reference temperature, 121°C. Thermal processes are generally designed to deliver a minimum of this preset value, F, at 121°C, labeled F_o at the particle center (Ramaswamy and Marcotte, 2005).

$$F = F_o \times 10^{[(121-T)/z]} \qquad \text{(Eq. 7.1)}$$

Where F is the process lethality at temperature T, F_o the process lethality at reference temperature and z is temperature range at which there is one log reduction in the decimal reduction time, D.

The Improved General Method was developed by Ball (1923) and is one of the methods used to calculate the thermal process. It integrates the lethal effects by a numerical procedure based on time temperature data. The lethal rate (L) at any temperature can be obtained as:

$$L = 10^{[(T-121)/z]} \qquad \text{(Eq. 7.2)}$$

Process lethality, F_o, is computed then by multiplying the lethal rate, L, by the time interval and summing up the F_o values.

$$F_o = \int L.dt \qquad \text{(Eq. 7.3)}$$

Process time (P_t) is the time at which there was an accumulated lethality, F_o, of 3 min for particles inoculated with *C. sporogenes* spores and 15 min for meat alginate fabricated particles inoculated with *G. stearothermophilus* spores. Retorts do not reach design process temperature immediately once the steam is on; however, it requires a finite heating time, called come-up-time (CUT) in order to achieve the operating temperature. Ball (1923) suggested a CUT effectiveness of 42% and this has since been used in most thermal process calculations. Ramaswamy (1993) reported that the effectiveness of CUT in thin profile packaging was 82%. A CUT effectiveness of 70% was chosen as a compromise between Ball and Ramaswamy's values in the present study and the CUT was measured to be 4.8 min. The corrected process time (B) is calculated as:

$$B = P_t + 0.7.CUT \qquad \text{(Eq. 7.4)}$$

On the other hand, the integrated lethal value of heat received by any point of the particle is calculated using the equation given by Stumbo (1973):

$$F_s = D_r \log n \qquad \text{(Eq. 7.5)}$$

Where F_s is the integrated lethal value, D_r the decimal reduction time, n the number of log reductions ($n = \log N_o/N$; where N_o and N are the initial and final count numbers of spores, respectively).

7.3.8 Statistical Analysis

T-test function of the Microsoft Excel Software was used in order to compare the computed F_o values between those obtained from biological validation and numerical simulation under the experimental conditions

7.4 Results and discussion

7.4.1 Heating rate index, f_h, of particles

Heating rate index, f_h, values (Table 7.1) ranged from 89 to 144 seconds and from 111 and 152 seconds for carrot alginate and meat alginate fabricated particles, respectively, at diameters ranging from 1.6 to 1.9 cm. On the other hand, f_h for Nylon particle was found to be ~139 s.

T-test revealed that there was no significant ($p > 0.05$) difference between f_h values at different temperatures using the same particle diameter as shown in Fig. 7.3. This was not in agreement with the findings of Amr and Yaseen (1994) and Taiwo et al. (1997) who reported an increase in f_h values with decreasing sterilization temperatures. This can be due to the fact that the temperatures of the water bath in the present study were low ($\leq 100^oC$) compared to the sterilization ones. On the other hand, particle diameter had a significant ($p < 0.05$) effect on f_h. As the carrot alginate fabricated particle diameter increased from 1.6 to 1.9 cm, f_h increased by ~35%; whereas, there was a ~21% increase as the meat alginate fabricated particle diameter increased from 1.7 to 1.9 cm. This finding conforms to Heldman and Hartel (1997) who reported that for conduction heating products, the magnitude of f_h increased with increasing particle size and this is due to faster heat penetration at lower diameter particles.

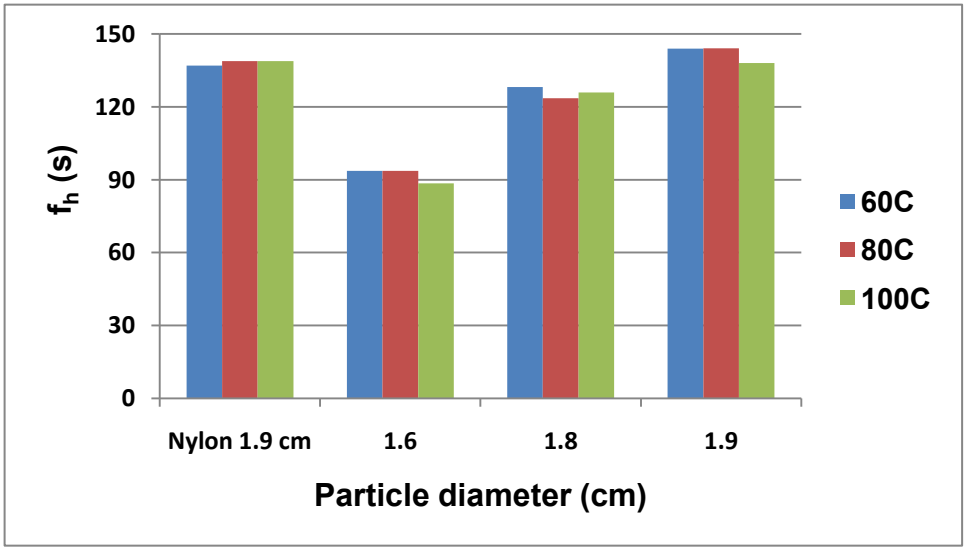
Carrot alginate and meat alginate fabricated particles with a diameter of 1.9 and 1.8 cm, respectively, were found to have nearly similar heating rate index, f_h, to the one of the 1.9 cm Nylon sphere as shown in Fig. 7.3. Thermophysical properties of carrot and meat alginate particles and Nylon sphere were measured in a previous work and are shown in Table 7.2.

Heldman and Hartel (1997) reported that f_h depends on the values of the thermophysical properties. Thermal diffusivity, α, in carrot alginate was higher than in meat alginate fabricated

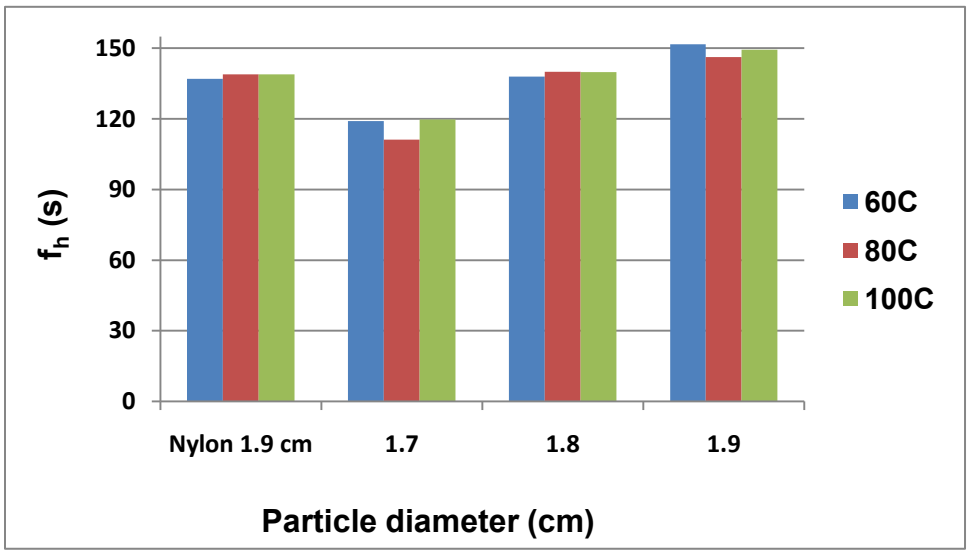
particles due to higher moisture content in the carrot alginate fabricated particles. This explains the lower f_h values in carrot alginate compared to meat alginate fabricated particle for the same diameter. Both meat and carrot alginate fabricated particles have higher specific heat, C_p, and thermal conductivity, *k*, than Nylon sphere; however, Nylon sphere has a higher density value than carrot and meat alginate fabricated particles. On the other hand, thermal diffusivity, α, which was back calculated using C_p, *k* and density, was found to be nearly similar for both alginate fabricated particles and Nylon sphere, explaining the reason for obtaining close f_h values for the alginate fabricated particles and Nylon sphere of the same diameter.

7.4.2 Process times

In the low-acid food canning industry, adequate calculation of any corrected process time must be based on two microbiological considerations: (1) destruction of *C. botulinum* spores and (2) reduction in the number of spoilage causing bacteria. It has been randomly established that the minimum B should result in reducing the *C. botulinum* population by 12 decimal reductions (D = 0.21 min at 121^{o}C; F_o = 2.5) (Stumbo, 1973). This explains the reason for choosing the accumulated lethality of 3 min for *C. sporogenes* inoculated carrot alginate particles. Since D values associated with *G. stearothermophilus* is nearly equal five times the D value associated with *C. sporogenes*, accumulated lethality value of 15 min for the *G. stearothermophilus* inoculated meat alginate particles was chosen. Corrected process times (B) were calculated according to the Improved General Method and are presented in Table 7.3.



(a)



(b)

Fig. 7.3 Heating rate index, f_h, of carrot alginate (a) and meat alginate (b) fabricated particles at different diameters vs. Nylon sphere (d = 1.9 cm)

Table 7.1. f_h (s) of meat and carrot alginate fabricated particles vs. Nylon sphere at different temperatures

T (°C) \ Diameter (cm)	Nylon	Carrot Alginate			Meat Alginate		
	1.9	1.6	1.8	1.9	1.7	1.8	1.9
60	137.0 ± 2.7	93.6 ± 3.7	128.2 ± 0.0	144.0 ± 6.6	119.1 ± 2.1	138.0 ± 1.3	151.7 ± 6.5
80	138.9 ± 2.7	93.6 ± 3.7	123.6 ± 4.3	144.1 ± 7.4	111.2 ± 3.5	140.0 ± 4.2	146.2 ± 7.6
100	138.9 ± 2.7	88.5 ± 0.0	126.0 ± 5.6	138.0 ± 1.3	119.8 ± 1.1	139.9 ± 1.3	149.3 ± 8.7

Table 7.2. Thermophysical properties of meat and carrot alginate fabricated particles vs. Nylon sphere

Particle	Density ($kg.m^{-3}$)	C_p ($j.kg^{-1}.^oC^{-1}$)	k ($W.m^{-1}.^oC^{-1}$)	α ($m^2.s^{-1}$)
Carrot alginate	1040	3870	0.56	$1.40x10^{-7}$
Meat alginate	1040	3610	0.43	$1.20x10^{-7}$
Nylon sphere	1128	2073	0.37	$1.57x10^{-7}$

Table 7.3. Process times, B (min) with an accumulated F_o of 15 min for meat alginate (a) and 3 min for carrot alginate (b) fabricated particles

Run #	Temperature (°C)	Rotation speed (rpm)	B (min)
1	120	5	40.1
2	120	20	38.0
3	125	5	21.2
4	125	20	19.3

(a)

Run #	Temperature (°C)	Rotation speed (rpm)	B (min)
1	115	5	36.4
2	115	20	35.1
3	120	5	21.6
4	120	20	19.5

(b)

For carrot alginate fabricated particles, B values ranged between 19.5 and 36.4 min at 120 and 150°C. On the other hand, for meat alginate, B values ranged between 19.3 and 40.1 min at 125 and 120°C. Increasing the temperature resulted in shorter process time. This could be due to the reduction in CMC viscosity and better liquid convection at higher temperatures. This trend was also reported by Sablani and Ramaswamy (1996), Meng and Ramaswamy (1996) and Dwivedi (2008). On the other hand, increasing rotation speed from 5 to 25 rpm resulted in decreasing the corrected process times for both carrot and meat alginate fabricated particles. Early literature has already documented the effect of rotational speed on heat transfer rates and the resulting processing time (Conley et al., 1951). This can be explained by the improved mixing due to higher degree of turbulence, resulting in increased relative particle to fluid velocity (Anantheswaran and Rao, 1985; Sablani, 1996; Sablani and Ramaswamy, 1997; Meng and Ramaswamy, 2006; Dwivedi, 2008).

Enujiugha and Akanbi (2010) used the General Method in order to calculate the corrected process times, B, for Nigerian ugba, inoculated with *G. stearothermophilus* spores and placed in

tomato sauce, groundnut oil and brine cans, respectively. B values were found to be 39.3, 41.6 and 42.7 min for brine, oil and tomato sauce, respectively. Our B values for meat alginate fabricated particles inoculated with *G. stearothermophilus* were ~ 39 min, which is close to their values.

7.4.3 Number of log reductions and process lethality

Carrot and meat alginate particles inoculated with *C. sporogenes* and *G. stearothermophilus*, respectively, were subjected to the different corrected process times, B, calculated using the Improved General Method as per Table 7.3. Spore count in the particles, N (CFU/ml) after the thermal process was measured in order to calculate the number of log reductions, n ($\log N_o/N$), using the initial spores' count in the particles, N_o (CFU/ml). Process lethality was then calculated using Eq. 7.5. All computed parameters, log N (CFU/ml), number of log reductions, n, decimal reduction time at 121°C, D (min), calculated process lethality, F_o (min) for carrot and meat alginate fabricated particles are presented in Table 7.4.

For carrot alginate fabricated particles inoculated with *C. sporogenes* spores, *n* ranged from 2.5 to 2.9 and for meat alginate fabricated particles inoculated with *G. stearothermophilus* spores, *n* ranged from 2.2 to 2.7. On the other hand, calculated process lethality, F_o, ranged from 2.8 to 3.2 min for carrot alginate fabricated particles inoculated with *C. sporogenes* spores and from 12.5 to 15.4 min for meat alginate fabricated particles inoculated with *G. stearothermophilus* spores.

Calculated F_o values were in general slightly lower than predicted ones (3 min for *C. sporogenes* and 15 min for *G. stearothermophilus*). Decimal reduction time, D_o, used in Eq. 7.5 to calculate F_o was determined using capillary tubes where the come-up time was found to be 15 seconds. Come-up time in carrot alginate (diameter = 1.9 cm) and meat alginate (diameter = 1.8 cm) fabricated particles was found to be ~ 400 seconds. The larger the particle is, the longer the come-up time will be (Newman and Steele, 1978) and the longer the process time required. This means that the large particle cannot be considered as an instantaneously heated system and in practice this means that the spores near the surface of the carrot or meat alginate fabricated particle received a more severe process than those in the centre.

Table 7.4. log N (CFU/ml), number of log reductions, n, decimal reduction time at 121°C, D (min), calculated process lethality, F_o (min) for carrot and meat alginate fabricated particles

Alginate particle	Temperature (°C)	Rotation speed (rpm)	log N (CFU/ml)	Number of log reductions (n = log N_o/N)	Decimal reduction time at 121°C (D_o)	Calculated F_o (n.D_o)
Carrot with *C. sporogenes*	115	5	2.8 ± 0.13	2.6	1.1	2.9
	115	20	2.9 ± 0.09	2.5		2.8
	120	5	2.7 ± 0.14	2.7		3.0
	120	20	2.5 ± 0.10	2.9		3.2
Meat + *G. stearothermophilus*	120	5	4.6 ± 0.12	2.4	5.7	13.7
	120	20	4.8 ± 0.15	2.2		12.5
	125	5	4.3 ± 0.23	2.7		15.4
	125	20	4.4 ± 0.18	2.6		14.8

The higher the processing temperature, the greater this difference becomes (Brown et al., 1984). This is why the spore beads used by Bean et al. (1979) and Dallyn et al. (1977) were of sufficiently small size to not require come-up time corrections. On the other hand, T-test revealed that the difference between calculated and predicted F_o values was not significant ($p > 0.05$). This can be due to the fact that the bi-axial agitation results in an intensive mixing of the can content; thus, alginate fabricated particles witnessed a uniform heating.

7.5 Conclusions

In this study, carrot and meat alginate fabricated particles inoculated with spores of *C. sporogenes* and *G. stearothermophilus*, respectively, were used to biologically validate bi-axial agitation processing of liquid particulates, where temperature monitoring devices cannot be used in order to obtain the time temperature profile at the particle center and therefore to calculate process times. Using predicted time temperature profiles, process times were calculated by employing the Improved General Method and process lethality, F_o, was determined using initial and final spores' counts. This latter was compared to model predicted values which were obtained through simulated time temperature profiles of the alginate fabricated particles. The difference observed between calculated and predicted F_o values was not significant ($p > 0.05$) and the results were sufficiently encouraging to suggest that biological validation using food alginate fabricated particles inoculated with spores of surrogate microorganisms can be used for assuring the process and product safety.

CHAPTER 8

GENERAL CONCLUSIONS, CONTRIBUTION TO THE KNOWLEDGE AND RECOMMENDATIONS

GENERAL CONCLUSIONS

1. Food composition has a dominating effect on the destruction kinetics of microbial spores intended for use in biological validation studies. Hence prior to embarking on such studies, it was necessary to evaluate the thermal destruction behaviour of target spores in meat and carrot alginate media. D values were computed from the slopes of log (N/N_o) vs. time, and z values from log (D) vs. temperature curves. The results were also fitted to Weibull model, but the model did not result in any better fit than the conventional Bigelow first order model.

2. Response surface methodology was used to determine the optimum conditions that give maximum hardness and minimum adhesiveness of meat and carrot alginate fabricated particles since fabricated particles used in biological validation studies should be hard enough to save their integrity till the end of the thermal process and should not adhere to each other inside the can and to the can inner wall. These optimum conditions were found to be sodium alginate concentration of 4.7%, calcium chloride concentration of 2.6% and immersion time of 32.0 and 30.8 h for carrot alginate and meat alginate fabricated particles, respectively. Fabricated particles using the optimum conditions did not show any variability in hardness values, unlike real food particles, when subjected to thermal processing.

3. Another requirement for biological validation studies is that food alginate simulated particles should have identical thermophysical properties to the real food particles. Thermophysical properties of meat and carrot alginate fabricated

particles as influenced by process variables were investigated. Density values were similar for all conditions. Increasing sodium alginate concentration resulted in a significant ($p < 0.05$) decrease in the heat capacity, thermal conductivity and thermal diffusivity values, while the other two factors had no significant effect. Optimum conditions were found to be 5.3 and 4.9% sodium alginate, 2.2% calcium chloride and 14.2 and 36.0 h immersion time in the calcium chloride solution for meat alginate and carrot alginate reconstituted particles, respectively.

4. Various product and process parameters are key influencing factors in the heat transfer coefficients of agitation processing. The effect of rotational speed, carboxy-methyl cellulose (CMC) concentration and retort temperature on overall heat transfer coefficient (U) and fluid to particle heat transfer coefficient (h_{fp}) in fixed and bi-axial rotation modes using non-Newtonian fluid (CMC) was investigated. Optimum conditions were a rotational speed of 20 rpm, CMC concentration of 0.6% and retort temperature of 126^{o}C. T-test revealed that both U and h_{fp} were significantly ($p < 0.05$) higher in the free bi-axial mode as compared to the fixed axial mode of rotation.

5. Heating behaviour index (f_h) of the carrot and meat alginate fabricated particles of different diameters was evaluated in order to select the diameter at which f_h values matches the ones for Nylon sphere used in earlier heat transfer studies. Using predicted time temperature profiles at the meat and carrot alginate fabricated particles centers, at the selected diameter, time temperature profiles were simulated and process times were calculated at three temperatures (115-125^{o}C) and two rotation speeds (5-25 rpm) by employing the Improved General Method to achieve an accumulated central lethality, F_o, of 3 min for use with *C. sporogenes* (D_o value is close to 1.0 min and hence this should result in a measureable 3-log reduction in the *C. sporogenes* spore population) and 15 min for *G. stearothermophilus* (Do ~ 5 min and hence this would result in similar 3-log spore count reductions) inoculated particles. Using initial and final spores'

counts of the particles, number of log reductions, and hence the resulting F_o was determined and compared to the F_o predicted from simulations. The experimental values were slightly lower than the ones from simulations; however, the difference was not significant ($p > 0.05$).

CONTRIBUTIONS TO KNOWLEDGE

1. Previous literature reported that the chemical environment, including the pH, salt and sugar concentrations and fat content, has a significant effect on the heat resistance of the bacterial cell or spore. No heat resistance study was done on carrot or meat alginate media. In this study, D and z-values in meat and carrot alginate media were determined.

2. There is no complete agreement in the literature on whether Weibull model or the classical first-order model is better to use for describing the inactivation of microorganisms. Our study showed that Weibull model did not result in any better fit than the conventional first-order one.

3. Few studies have been done on the textural properties of carrot and meat alginate fabricated particles, especially using texture profile analysis (TPA). No work on the thermophysical properties of carrot and meat alginate fabricated particles has been reported. It can be said now that optimum conditions to fabricate carrot and meat alginate particles can be determined and applied in biological validation studies.

4. No work has been done before to compare the rate of heat transfer between fixed and bi-axial mode of rotation using non-Newtonian fluids. Our study showed that

heat transfer coefficients are 35 - 40% higher in bi-axial compared to fixed mode of rotation.

5. Although Improved General method is widely used in the industry in order to calculate process time, no work has been reported in the literature to actually measure the number of log reductions caused by subjecting an inoculated food matrix to this calculated process time, in the context of bi-axially rotating cans with particles suspended in a non-Newtonian fluid. It can be said now that actual number of log reductions is not significantly ($p > 0.05$) different from the predicted one using numerical integration technique (Improved General method).

RECOMMENDATIONS FOR FUTURE RESEARCH

This research has demonstrated several important findings. Meanwhile, it also showed some ideas of interest for future research and development, which could be summarized as follows:

1. Investigating the heat resistance of other types of surrogate microorganisms (such as *B. subtilis*) and employing them to inoculate meat and carrot alginate fabricated particles.

2. Using egg patties as a matrix to immobilize bacterial spores instead of food alginate.

3. Investigating the effect of the type of alginate (high in guluronic acid and low in guluronic acid) and water addition on the textural properties of the fabricated particles.

4. Extending this study to quantify heat transfer coefficients in other shear-thickening non-Newtonian (such as starch) fluids in free axially rotating cans.

5. Quantifying the influence of can size, can material, particle shape, head space volume and heating medium on heat transfer coefficients in free axially rotating cans.

6. Comparing process times calculated using Improved General Method vs. Original General Method, Ball, Stumbo and other process calculation methods.

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