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Development of Thermal Process for Gaeng Phed Gai in Retort Pouches

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Development of Thermal Process for Gaeng Phed Gai in Retort Pouches

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Abstract

Gaeng Phed Gai - a traditional Thai dish containing chicken in coconut milk with red chili and striped bamboo shoot - packed in retort pouches was processed to commercial sterility in a still retort. Process lethality was determined by temperature measurements and use of bioindicators. The quality of the product was evaluated by microbiological tests, analysis of nutritional contents and sensory assessment. The product was commercially sterile after the heat treatment, but the sensory quality was insufficient. For taste, juiciness and overall acceptability, heat-treated product received significantly lower scores than the untreated product. Calculations of cook value and further reinforced the impression that the product was overheated due to the slowness of the retort used.

Searches in scientific databases revealed reports from several Asian studies of similar products and processes. These show that it is possible to produce food consisting of large pieces in liquid medium, retaining an acceptable quality for extended periods of storage at ambient temperatures.

The conclusion is that it should be possible to produce shelf-stable Gaeng Phed Gai with a good quality, if further tests are carried out with a faster retort. It is recommended that pouches with Gaeng Phed Gai are heat-treated to a process lethality (F_0) of 10 minutes, as this would guarantee consumer safety and low levels of economical spoilage. An F_0 as low as 6 minutes can be used if the initial levels of heat resistant spores in the raw materials are proven to be low by practical tests.

Keywords: Thai food, thermal process, retort pouch

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1 Introduction

1.1 Background

In 2011, Herman Drotz and Kanitta Arnljots, students at the Swedish University of Agricultural Sciences, carried out literature studies and market tests to investigate the possibilities of producing Thai food in retort pouches. The results indicated that it would be possible to produce Thai food, containing sauce as well as pieces of meat and vegetables, with a long shelf-life at ambient temperatures. The Swedish food company Santa Maria was interested in a cooperation with the aim to investigate this subject further including practical tests. Santa Maria has an assortment of Asian spices and plain sauces, but no products containing pieces of meat and vegetables. Their assortment would be widened if we could approve that it is possible to produce Thai food with acceptable quality in retort pouches.

Searches in scientific databases revealed reports from several similar studies, e.g. of Kerala style fish curry, (Gopal *et al.*, 2001), Seer fish curry (Ravishankar *et al.*, 2002), prawn kuruma (Mohan *et al.*, 2008), Chettinad style goat meat curry (Rajkumar *et al.*, 2010), and Chettinad chicken (Rajan *et al.*, 2011), which were all processed in retort pouches.

1.2 Objectives

The objective of this project was to investigate a method for thermal processing of Gaeng Phed Gai - a traditional Thai dish containing chicken in coconut milk with red chili and striped bamboo shoot - packed in pouches and processed in a retort. This method would give the product a shelf-life of one year or more. The task was to test the thermal process in a small scale and evaluate the product in regard to microbiological status, nutritional values and sensory quality.

Santa Maria does not have any retort processing operations in Sweden and therefore lacks knowledge of thermal processing of retort pouch products. For this reason, it was also important to include an extensive theoretical background of retort processing and retort pouch packaging.

1.3 Delineation

The aim with this report is to include both theoretical studies as well as a presentation of the practical tests. This report includes an extensive part of theory required for the development of retort pouch processes, as well as a presentation of the small-scale test of Gaeng Phed Gai production.

A traditional Thai recipe for Gaeng Phed Gai was used in the practical tests. Alternative formulations or use of food additives were not tested. Because of the limited time available for the project, experiments were mainly confined to direct effects of the thermal process. The only storage tests conducted were microbiological tests and pH measurement after a short time of storage. However, some theory about shelf-life testing and expected effects of storage is included as a foundation for further studies.

Ideally, the initial levels of heat resistant spores in Gaeng Phed Gai should be evaluated by extensive practical tests. However, such tests are very time-consuming and expensive. Hence, general figures known to be adequate for most food products have been used in combination with information found in literature about the raw materials. The use of such general data can lead to overestimation of spore levels and heat resistance, but, on the other hand, gives a large margin of safety.

Parallel to this study of the thermal process, another SLU student, Kanitta Arnljots, conducted investigations about economical and marketing aspects of the same product. For further reading about these aspects, contact kanitta_arnljots@yahoo.com to receive her report “Thai Food in Retort Pouch Packaging”.

2 Background theory

2.1 Introduction to thermal processing

Thermal processing normally refers to in-container sterilization of food (Awuah *et al.,* 2007). Thermal processing is the most widely used method for preserving food and extending the shelf-life. The first calculation of the minimum treatment for achieving safe products was made in 1920. The processes have developed a lot since then but the fundamental concept remains the same; apply high temperature for a sufficiently long time to destroy microorganisms that can be a danger to public health or cause product spoilage. The air-tight and microbiologically tight container prevents recontamination and maintains an environment that reduces the possibilities for microorganisms with high heat-resistance to grow.

According to the theoretical models of destruction of microorganisms it is not possible to reach complete sterility (Awuah *et al.,* 2007). Trying to achieve complete sterility would require conditions leading to a terrible product. For practical purposes the term commercial sterility has been developed. According to the Codex Alimentarius Commission (1993, 2), "commercial sterility of thermally processed food means the condition achieved by application of heat, sufficient, alone or in combination with other appropriate treatments, to render the food free from microorganisms capable of growing in the food at normal non-refrigerated conditions at which the food is likely to be held during distribution and storage."

Designing a good thermal process is far from easy. It requires a lot of knowledge of processing methods, how the components of the food product reacts to heat treatment and how the microorganisms are affected (Awuah *et al.,* 2007). The outcome of the thermal process depends on physical properties of the product, the size and shape of the packaging, the type of microorganisms present and their heat resistance. In addition, knowledge of the product's pH, water activity and salt con-

tent is needed as these properties affect the survival of microorganisms. Low pH, low water activity and high salt content are factors inhibiting microbial growth.

Nowadays there is a lot more to consider in heat-treatment than just food safety and shelf stability (Awuah *et al.,* 2007). Factors such as quality, added values and user convenience are of high importance as well. The traditional processing of canned foods has severe effects on sensory properties and nutritional values. Hence, research has been directed at improving the process. Advancements have been made through optimizing temperatures and reducing heating time. The retortable pouch is one of the advancements enabling rapid heating.

2.2 Retort processing

Retorting, also known as pressure cooking or autoclave processing, is a common method for in-container sterilization of foods. In the traditional retorting method, the food containers are placed on racks where they are kept still while heated by water, steam or a mixture of steam and air (Awuah *et al.,* 2007). Retort pouches are relatively vulnerable, which makes overpressure necessary to avoid damages with resulting loss of seal integrity. The pressure needed in the retort is approximately 10 psi above atmospheric pressure for sterilization at 116 °C, 15 psi at 121 °C and 20 psi at 127 °C (Potter & Hotchkiss, 1998). To provide the overpressure, compressed air or additional steam is introduced during the processing cycle.

The development of retorts has been towards use of a steam-air mixture for heating, instead of water or saturated steam (Toledo, 2007). This system is said to be the best for fragile containers, such as retort pouches. The reason is that no exhaustion is needed, removing the danger of sudden pressure changes breaking the packages.

Still retorting is rather slow as it takes a long time for the entire content to reach the right temperature. In a still retort the maximum temperature used is 121 °C. If a higher temperature is used, the food is cooked against the wall (Potter & Hotchkiss, 1999). Because there is little movement within the package it takes a long time for the cold point to reach the sterilization temperature. In conduction heated food products which are not agitated there will be a temperature gradient from the surface of the package to the centre of the product (Ryley & Kajda, 1994). The outer part will get a severe treatment, leading to quality loss.

The processing time can be reduced significantly by agitating the containers, creating circulation within them and increasing the rate of heat transfer (Potter & Hotchkiss, 1998). The containers can either be turned end over end or spinned on their long axis. One of the methods might be more effective than the other, depending on the specific physical properties of the product. Mixing reduces the risk of cooking the food against the wall, thereby allowing a higher temperature to be used. As will be described later, a higher temperature and shorter time is generally beneficial for product quality. Agitation is most effective for liquid or semi-liquid foods, which can be easily mixed through movement of the packages. Some free head space within the packages is needed for optimum product turnover.

Common alternatives to in-container sterilization are HTST (high temperature short time) and UHT (ultra high temperature) treatment where the food passes through a heat exchanger (Awuah *et al.,* 2007). It is kept in the cell until the right temperature and time has been reached. The food is then packed in sterilized packaging materials. So far this has mostly been used for liquid foods. Authorities have approved use of this method for food with small particles, but it has not yet been approved for food with large particles. The reason is difficulties of proving lethality for microorganisms in the large particles when moving through the heat exchanger.

2.3 Conduction and convection

The heat transfer mechanisms important for retort products are conduction and convection (Potter & Hotchkiss, 1998). In conduction, heat moves from one particle to another in more or less straight lines. This is the case in solid foods and other situations when the food does not move. Convection, on the other hand, involves movement of the food. Natural convection occurs when the heated parts of the food rises due to decreasing density, causing circulation within the product. The circulation speeds up the heat transfer throughout the entire product. Forced convection occurs when circulation is created mechanically, e.g. by agitation.

The coconut milk in Gaeng Phed Gai set in convection heating motion due to the heat received through the package wall. The solid pieces of chicken meat and bamboo shoots, on the other hand, will only be heated by conduction of heat received from the surroundings. Convection heating is much more rapid, so the liquid is heated more quickly than the pieces.

2.4 Slowest heating zone

When heat is applied from the outside, as it is done in retorts, the food closest to the surface reaches the final sterilization temperature first (Potter & Hotchkiss, 1998). The point in the food last to reach the sterilization temperature is called the cold point or slowest heating point. To ensure commercial sterility, the slowest heating point must reach the sterilization temperature and remain there for the time required to destroy the most resistant bacterial spores.

The location of the cold point depends on product characteristics and the geometry of the container (Richardson, 2004). In rigid cans the cold point is always somewhere along the vertical axis. In products heated by conduction only, the cold point will be in the geometrical centre. In convection heated foods, the cold point will be shifted downwards to a point which depends on the amount of movement. The temperature in packages of complex geometries, such as plastic trays or pouches, will have a less predictable cold point. The same applies to foods which are mixes of liquids and solids. Temperature measurements at many points in the containers may be needed to determine the cold point.

Ghani *et al.* (2002) studied the temperature distribution in beef-vegetable soup in retort pouches lying flat in a stationary retort using saturated steam for heating. They showed through computer simulation that the slowest heating zone covers the whole cross-section of the pouch in the early stages of heating and then migrates to locate itself at a distance of 30 % from the bottom of the lying pouch. For broccoli-cheddar soup the slowest heating zone (SHZ) settles at 30 % from the bottom and 35-40 % of the length from the widest end (Ghani *et al.*, 2006). For carrot-orange soup the corresponding figures was shown to be 30 % from the bottom and 20-30 % from the widest end.

One of the authors of the book “Sterilization of Food in Retort Pouches” was asked if he had any ideas of where the SHZ should be in a flat (i.e. pillow-shaped) pouch containing chicken and bamboo shoots in coconut milk. The answer he gave was (M. Farid, personal communication, March 12, 2012): “Since you have solid and liquid it is difficult to know where the slowest heat region SHZ will be. If the chicken pieces are small then the SHZ region will be pushed down but if the pieces are big conduction will dominate and the SHZ will lie in the centre of the geometry.” The product used in this study contains a large amount of pieces, many of them large. Hence, the SHZ in Gaeng Phed Gai should be in or close to the geo-

metrical centre. The cold point will be in a chicken piece, as these are the largest pieces and heated by conduction only.

The above applies mostly to products processed in still retorts. In products heated in agitating retorts, temperature distribution is fairly uniform throughout the product (Holdsworth & Simpson, 2007). Therefore, the point of temperature measurement is less critical.

It is important to not only determine the cold point inside the containers, but also to be aware of the cold point of the retort (Richardson, 2004). In a properly vented steam retort, the temperature at different points in the retort will vary by 0.5 °C at worst during the hold phase of the process. During the come-up and cooling phases, on the other hand, great variations can occur. Steam/air and water retorts can have even less consistent temperature. Just as for pouches, the cold point in the retort should be determined experimentally.

2.5 Temperature measurements

The temperature in the cold point must be monitored during the development of a heating process. This can be done using thermocouples or thermistors. The data loggers should be set at the smallest possible time interval, to get as precise measurements of the heat treatment as possible (Richardson, 2004). For most processes, the interval should be no longer than 30 seconds.

Holdsworth & Simpson (2007, 150) states the following about thermocouple placement, which is highly relevant for the product in this study:

> "For mixed products, which heat by both convection and conduction simultaneously, e.g. potatoes in brine, it is necessary to ensure that the centre of the potatoes receive a satisfactory process and the thermocouples should therefore be located in the center of the potato with the largest dimensions."

To consider the worst case, containers in which temperature measurements are conducted can be overfilled, resulting in a slower heat transfer (Richardson, 2004). A 10 % overfill is usually adequate.

2.6 Effects of heating on product quality

In addition to the effects on microbial survival, effects on sensory properties, nutritional values and other quality factors must also be considered. The high temperatures and times needed to achieve a shelf-stable product will cause reactions affecting the quality. The effects are both of a qualitative and quantitative nature. Changes in appearance, taste and texture are examples of qualitative losses (Awuah *et al.,* 2007). Nutritional degradation, on the other hand, is an example of a quantifiable loss that will occur.

An acceptable thermal process must fulfill the microbial inactivation objectives at the same time as the accompanying unwanted chemical reactions are minimized (Toledo, 2007). Data on the effects of elevated temperatures on chemical reactions are valuable for determining the quality changes resulting from a thermal process. Food scientists use a parameter called z-value for describing the temperature dependence of chemical reactions (Brennan, 2006). It is defined as the temperature change that results in a tenfold change in the decimal reduction time, which is the time required for a 90 % reduction of the substance under study. The lower the z-value, the more temperature sensitive is the reaction. The z-value of most chemical reactions associated with quality losses in food products is at least two times higher than the z-values for microbial inactivation, which generally have z-values around 10 °C (Toledo, 2007). The z-values of some quality degrading chemical reactions are shown in Table 1. As can be understood from these figures, properties such as flavor, color, texture and nutritional values are generally more sensitive to long times than to high temperatures. For this reason, it is common practice in the food industry to increase the temperature to decrease the time, thereby minimizing the quality degrading reactions without lowering the microbial inactivation (Toledo, 2007).

Table 1. z-values for quality degrading chemical reactions. From Toledo (2007, 298) with minor alterations

Texture	Z (°C)	*Overall sensory quality*	Z (°C)
Apples	21-28	Beets	19
Black beans	35	Broccoli	44
Navy beans	37	Carrots	17
Soy beans	42	Corn kernels	32
Beef	4	Green beans	29
Beets	40	Peas	32
Brussel sprouts	21	Potatoes	26
Carrots	18	Squash	26
Potatoes	11		
Shrimp	23	*Color loss*	
		Green pigment	30
		Red pigment	31
Nutrients		Browning	32
Carotene	25		
Thiamine	27	*Enzymes*	
Pyroxidine	29	Peroxidase	28
Folic acid	20	Catalase	8
Ascorbic acid	27	Lipozgenase	9
		Polyphenol oxidase	8
		Pectin esterase	16

Vitamins are the most sensitive food components, and these should be the main focus in nutrient evaluation of processed food (Awuah *et al.,* 2007). Loss of vitamins occurs due to degradation into inactive substances, creation of compounds of lower potency, or irreversible binding to other food components (Ryley & Kajda, 1994). These changes are accelerated by oxygen, light and transition metals. Temperature, duration of heat treatment, pH and moisture are other factors affecting vitamin loss (Lešková *et al.*, 2006). The extent of vitamin loss depends on cooking conditions and type of food. The heat sensitive vitamins are (Awuah *et al.,* 2007):

- The fat soluble vitamins A (in presence of oxygen), D, E and β-carotene.
- The water soluble vitamins B1 (thiamine), B2 (riboflavin) in acid environment, C (ascorbic acid), nicotinic acid, pantothenic acid and biotin C.

Prediction of the effects on vitamins is difficult due to lack of kinetic data, which is mainly available for thiamine and ascorbate (Ryley & Kajda, 1994). Reactions

between food components, causing product-specific nutrient changes, also make it difficult to make correct predictions. The success of mathematical predictions of vitamin retention is dependent on correct predictions of time-temperature profiles at many points within the container. Accurate time-temperature estimations requires an adequate heat transfer model, based on knowledge of the heat transfer coefficient from the heating medium to the package and the thermal characteristics of the food product. These extensive requirements in combination with lack of kinetic data for some vitamins make this approach difficult to use.

Proteins will also be affected during heat treatment. Changes in primary protein structure are of greatest concern; as such changes can reduce the digestibility or even make the protein biologically unavailable (Awuah *et al.,* 2007). Other structural changes rather make the protein easier to digest. Protein damages due to Maillard reactions can be reduced through lowering pH and temperature or removing the sugar components active in the reaction.

Lipids, carbohydrates and minerals are virtually unaffected by heat treatment (Awuah *et al.,* 2007).

The pigments responsible for the color of the food are also affected by heat treatment. Fortunately, when using processing methods with high temperature and short processing time, these changes are usually quite small (Awuah *et al.,* 2007).

In this context it is interesting to notice that effects on nutrients from thermal processes are not as severe as one might think. Studies by Rickman *et al.* (2007, 1 & 2) showed that the nutritional values of fresh, frozen and canned foods are similar by the time they are consumed. The loss of nutrients in fresh food during storage and cooking is significant; up to half the initial content. Canned food loses some of its nutrient content during heat treatment but, on the other hand, the nutrient stability during storage is much better thanks to the low oxygen levels in the package. The oxygen level in frozen foods is higher, resulting in more oxidation of nutrients, but less is lost during the initial heat treatment because of shorter time. Fatty acids, carbohydrates and proteins are not significantly affected by canning.
Mineral and fiber content is also similar in canned, fresh and frozen products. As vitamins are most sensitive, some differences can occur here. However, all the three product types mentioned have their positive and negative sides and it is hard to say that one is better or worse than the others even for vitamins.

2.7 Determinants of shelf-life

Catauro & Perchonok (2012) showed that the major determinants of shelf-life of retort processed foods are development of off-flavor and off-color resulting from Maillard and oxidation reactions. For meat products, texture change due to moisture migration can also be one of the main mechanisms of quality loss over time. The nutritional value of most products is maintained throughout the shelf-life, except for vitamin B and C which are lost to a great extent. The shelf-life of retort processed foods range from 1 to 8 years, with some meat and vegetable products showing the longest shelf-life.

To maintain the quality of shelf-stable food, the product should be protected from oxygen and light (Ryley & Kajda, 1994). Storage temperature is also a major factor for quality retention, as temperature is a major determinant of chemical reaction rates.

2.8 Accelerated shelf-life testing

When there is a need to know the shelf-life of a product quickly, but no time for waiting several years for the product to deteriorate, accelerated shelf-life testing (ASLT) is very useful. It builds upon the fact that deterioration of food depends on extrinsic factors such as temperature, humidity, gas atmosphere and light (Robertson, 1999). The deterioration can be accelerated by storing the food in an environment where one or several of these factors have been increased. The effects of the intrinsic factors on the acceleration can be quantified by applying principles of chemical kinetics, and then used to calculate the relation between the shelf-life at the accelerated condition and the shelf-life under normal storage conditions. The most commonly used acceleration factor is temperature.

Before deciding which temperatures and times to use in ASLT, one must know what effect a given temperature change has on the shelf-life. For this purpose it is common to use the concept of Q10, which is the ratio of shelf-life between two temperatures which are 10 °C apart (e.g. if an increase in temperature of 10 °C leads to a halving of the shelf-life, then the Q10 is 2). It can also be described as change in reaction rate when the temperature is changed by 10 °C.

As different kinds of reactions have different rates, one type of food has many Q10s. The lipid oxidation may have one Q10 while the Maillard browning has another, for example (Perchonok, 2002). There is also the difficulty that the prop-

erties of a specific food product affect the reaction rates. Hence, there is no definitive Q10 for a specific type of food. To know the exact figure, practical test must be conducted. However, an approximation can be made by studying literature. According to Robertson (1999), typical Q10 values for canned foods are 1.1 to 4.0. Toledo (2007) states that the Q10 for microbial growth, and the reactions behind non-enzymatic browning, color changes and flavor changes is usually around 2.

According to Catauro & Pertchonok (2012), the correlation between the shelf-life at two different temperatures is:

$$t_s = t_0 e^{-aT}$$

Where
t_s = shelf-life at temperature T_s
t_0 = shelf-life at temperature T_0
a = lnQ10/10
$T = T_s - T_0$

For shelf-life tests of packed foods normally stored in room temperature, Robertson (1999) recommends using at least two of the following temperatures: 5 °C, 23 °C (room temperature), 30 °C, 35 °C and 40 °C. If a too high or too low temperature is used there is a risk of inducing reactions resulting in erroneous results.

2.9 Retort pouch packaging

Packaging has a large influence on the shelf-life of food products (Rodríguez *et al.*, 2002). Rigid metal containers are still the most common packaging type for thermally sterilized food, but there are other packaging types with many advantages. The retort pouch is a packaging type that allows faster heat transfer than the traditional metal or glass containers, owing to their slimmer profile, or more specifically, the higher surface area to volume ratio (Awuah *et al.*, 2007; Rodríguez *et al.*, 2002). The same microbial lethality can be achieved with a 30-50 % shorter processing time, compared to retorting of metal cans. The difference in processing time is largest for foods in which there is no natural convection (Snyder & Henderson, 2007). It should be pointed out that the comparison between pouches and cans is only true as long as the same type of process is used. Comparing agitated cans with still-cooked pouches can give a different result. For food products consisting of particles in a liquid medium, for example, agitated cans require a shorter processing time than still-cooked pouches.

Thanks to the shorter processing time, retort pouches generally give a better product quality. One example of this is the study by Mohan *et al.* (2008), which showed that prawn kuruma packed in retort pouches were superior to canned products with regard to color, firmness, hardness, chewiness and overall acceptability.

Other commonly mentioned advantages of retort pouches are low weight, space efficient at storage, ease of opening and preparation, and packaging economy (Mohan *et al.* 2008). In addition, they save tremendous amounts of energy compared to cans thanks to the shorter processing times, and are also more easily disposed (Holdsworth & Simpson, 2007).

Material structure

Most retort pouches are constructed as four-ply laminates of different packaging films that can withstand high process temperature and pressure (Jun *et al.*, 2006). The typical pouch has an outer polyester (polyethylene terephtalate) layer for heat resistance and printability, an aluminum foil layer as a barrier for oxygen and light, biaxial oriented nylon for resilience, and an inner layer of cast polypropylene for pack sealing (Holdsworth & Simpson, 2007). An adhesive between each layer bind the materials. The pouch structure is illustrated in Figure 1.

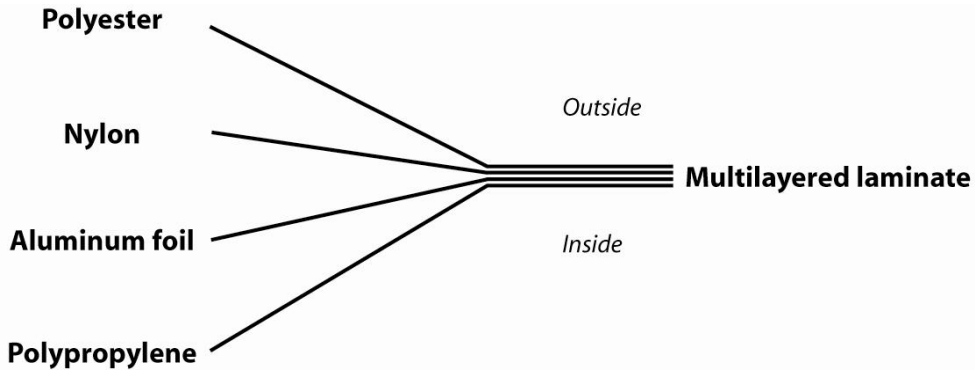

Figure 1. Cross-section sketch of a retort pouch.

Instead of using aluminum foil, some pouch materials contain silicium oxide (Holdsworth & Simpson, 2007), polyvinylidene chloride (Jun *et al.*, 2006), ethylene vinyl alcohol or nylon. The reason is that they are transparent and allow vi-

sion of the content. Another reason is that it makes the pouch microwaveable. These plastic materials are good barriers to oxygen molecules but they are not complete barriers. Hence, severely reduced shelf-life of non-foil containers is a direct implication.

Removal of residual gas

Before sealing the packages, the amount of air inside should be minimized to avoid negative effects on sensory properties and nutrient levels (Rodríguez *et al.*, 2002). The risk of adverse effects from oxidation during processing and storage can be minimized by removing oxygen by vacuum (non-liquid products) or steam flushing. Removal of air has been shown to increase the shelf-life of retort products (Bindu *et al.*, 2007). In addition to reducing oxidation effects, e.g. on color and nutrients, it also reduces bacterial growth compared to aerobic packaging. What is more, minimizing the residual gas increases the heating rate of the product substantially and reduces the risk of pouch damage from gas expansion during heating (Rodríguez *et al.*, 2002).

Pouch quality

It is important that the pouches used have a sufficient quality, to avoid puncture, breakage, oxygen entrance, migration of chemical compounds etc. The seal integrity can be tested by conducting bursting tests by injecting gas under pressure, measuring seal thickness and testing seal strength. The suitability of the pouches can also be ascertained by measuring properties such as overall migration residue, sterility, process resistance and tensile strength at break (Bindu *et al.*, 2007).

The key bottlenecks that have been identified for retort pouches are entrapment of product at the seal interface and possible leaking channels allowing microbial invasion (Awuah *et al.*, 2007). A contamination-free seal is crucial for the shelf-life (Holdsworth & Simpson, 2007).

2.10 Spoilage with health consequences

In thermal processing of foods there are two types of spoilage risks that must be considered; spoilage by pathogenic bacteria of danger to public health and spoilage of non-pathogenic bacteria causing commercial damage (Stoforos, 1995). Common causes of spoilage are pre-process spoilage, under-processing, inadequate cooling and contamination resulting from package leakage (Ghani *et al.*,

2002). Spoilage of danger to public health is described in this section, and economical spoilage is described in the next.

Relatively few of the microorganisms known today are pathogenic, i.e. disease causing, and few diseases can be transmitted through food (FPA & FPI, 2009). For food processors bacteria are the most problematic microorganisms. Bacteria can be divided into groups depending on different characteristics; e.g. ability to form spores (spore-forming or not spore-forming), oxygen sensitivity (aerobic or anaerobic), temperature requirements for growth (psychrotrophic, mesophilic or thermophilic), metabolic characteristics (e.g. putrefactive/proteolytic or non-proteolytic/saccharolytic), etc.

Some bacteria have the ability to produce spores, which is a dormant stage in the normal life cycle (FPA & FPI, 2009). Spores have the ability to survive long periods of environmental stress, e.g. periods of high temperature, and then germinate and start growing when the conditions are favorable again. Bacteria which are not in a spore state are much less heat resistant. Therefore, spore-forming bacteria are the concern in heat treatment of foods. The major spore-forming bacteria belong to the genera *Clostridium* or *Bacillus*. Typically the most problematic microorganism is *Clostridium botulinum*, as it can thrive in the anaerobic conditions inside food containers and produce the very dangerous botulinum toxin (Awuah *et al.*, 2007). For thermal processing of food with a pH above 4.6, the generally accepted limit below which *C. botulinum* cannot grow, special attention is given to this bacteria.

The botulinum toxin is an extremely potent neurotoxin that results in botulism, which is a severe neuroparalytic disease (Novak *et al.*, 2005). *C. botulinum* can grow in many food products and produce the toxin. Consumption of as little as 30 ng of toxin causes illness or even death. Consumption of as little as 0.1 g of food in which neurotoxin producing species has grown is enough to cause botulism. Because botulism is so severe, any incidence of the disease is considered unacceptable (Anderson *et al.*, 2011). In any food production, the level of risk for botulism resulting from ingesting the product must approach zero. However, it is difficult to define this level exactly. The reason is that none of the outbreaks of food borne botulism from commercially canned foods have been associated with properly processed products, i.e. there is no experience that can be used for determining where the limit between sufficient and insufficient treatment is.

The botulinum toxin is actually produced by six distinct gram-positive obligately anaerobic spore-forming bacteria; four groups of *C. botulinum* and certain strains *C. baratii* and *C. butyricum* (Novak *et al.*, 2005). These six groups are shown in Table 2. Proteolytic (group I) and non-proteolytic (group II) *C. botulinum* are responsible for most cases of food borne botulism.

Proteolytic *C. botulinum* are mesophilic (mesophiles grow well between 20 °C and 45 °C and have an optimum temperature between 30 °C and 40 °C) and produces spores of high heat resistance. A severe heating process is required to inactivate them, such as the 121 °C for 3 min used as a standard for low-acid canned foods (EFSA, 2005).

Non-proteolytic *C. botulinum* are psychrophic (psychrotrophs grow well at or below 7 °C and have an optimum temperature between 20 °C and 30 °C) and produces spores of moderate heat resistance. Spores of non-proteolytic anaerobes will generally not survive minimal retort processing (Downes & Ito, 2001). Non-proteolytic *C. botulinum* spores are inactivated by heating at 90 °C for 10 min, or an equivalent process (EFSA, 2005).

Table 2. Organisms producing the botulinum toxin, according to Novak et al. (2005, 384)

Neurotoxigenic organism	Neurotoxins formed	Non-neurotoxigenic equivalent organism
C. botulinum group I (proteolytic/putrefactive)	A, B, F	*C. sporogenes*
C. botulinum group II (non-proteolytic/saccharolytic)	B, E, F	No name given
C. botulinum group III	C, D	*C. novyi*
C. botulinum group IV (*C. argentinense*)	G	*C. subterminale*
C. baratii	F	All typical strains
C. butyricum	E	All typical strains

Clostridium perfringens is another anaerobic spore-forming bacteria commonly involved with food-borne illness (EFSA, 2005). It is more common than *C. botulinum*, but the intoxication due to *C. perfringens* is usually brief and self-limiting. The illness lasts only 12-24 hours and is usually not serious enough for consulting a physician. Deaths of elderly due to *C. perfringens* are reported occasionally. The heating processes that eliminate proteolytic *C. botulinum* spores eliminate *C. perfringens* as well.

2.11 Spoilage with economical consequences

Food processors must not only consider threats to food safety. There is also a risk of product spoilage with economical consequences. If the product contains bacteria with higher heat resistance than the target pathogen, then a more stringent process than that needed to inactivate pathogens is required. Because of the higher heat resistance of many bacteria with the ability to cause economical spoilage, processes applied to obtain shelf-stable foods are commonly two to three times more severe than those necessary to inactivate *C. botulinum* spores.

The most heat resistant food spoilers are spores of thermophilic bacteria (thermophiles grow well at temperatures above 45 °C and have optimum temperatures between 55 °C and 65 °C). Most thermophilic bacteria of importance for foods belong to the genera *Clostridium*, *Bacillus*, *Geobacillus*, *Paenibacillus*, *Alicyclobacillus* or *Thermoanaerobacter* (Jay *et al.*, 2005). According to Ghani *et al.* (2002), low acid foods (pH ≥4.6) are commonly spoiled by bacteria from the thermophilic flat-sour group (*G. stearothermophilus, B. coagulans*), sulfide spoilers (*C. nigrificans, C. bifermentans*) and gaseous spoilers (Thermoanaerobacterium thermosaccharolyticum). Thermophilic facultative aerobic spore-forming bacteria are the ones typically responsible for flat-sour spoilage, i.e. spoilage through acid production with little or no gas generation, of low-acid canned foods.

The most common economical spoiler is *G. stearothermophilus*, which is also the most heat resistant of all microorganisms encountered in foods (Ghani *et al.*, 2002). These spores are up to 20 times more heat resistant than *C. botulinum*, and commonly have D-values in the range 4.0-5.0 min and z-values of 7.8-12.2 °C (the concepts of D- and z-values will be described in the next section). For this reason it is often used in inactivation studies. If *G. stearothermophilus* is inactivated, all other microorganisms will be inactivated as well. As a comparison, a high level of economical spoilage is expected if the process is based only on achieving a 12 $\log_{10}$ reduction of *C. botulinum*.

It should be pointed out though that flat-sour product spoilage does not develop as long as the temperature is kept below 43 °C. The reason is that thermophilic bacteria do not germinate below that limit (Downes & Ito, 2001). Thermal processes for products which are to be stored at lower temperatures are often tested using *Clostridium sporogenes* instead, which is a mesophilic putrefactive anaerobe of higher heat resistance than *C. botulinum* (Anderson *et al.*, 2011).

Flat-sour spoilage of thermally processed low acid food can also be caused by growth of mesophilic aerobic spore formers (Downes & Ito, 2001). Spores of these bacteria have a relatively low heat resistance as long as the product is fully hydrated, but if they are trapped in unhydrated particles they may survive. Mesophilic aerobic sporeformers of importance in food spoilage belong to the genera *Bacillus* and *Sporolactobacillus*. *Bacillus* is by far the most important of these, as *Sporobacillus* occur only in low numbers in food and have low heat resistance. Bacillus species are either aerobic or facultative anaerobic.

2.12 Describing heat resistance and inactivation of microorganisms

The temperature and time needed for inactivation of bacteria varies depending on species, the amount of bacteria to be killed and properties of the food product, e.g. pH, water activity and preservatives (FPA & FPI, 2009). The thermal resistance of a microorganism is usually expressed by a "D-value" and a "z-value".

The decimal reduction time, D, is the time at a specific set of conditions required to reduce the number of survivors of a certain microorganism by 90 %, i.e. one $1\log_{10}$ (FPA & FPI, 2009). The D-value can be determined by exposing high levels of spores to sterilization conditions over increasing durations of clock time, and then determining the number of surviving spores at several points in time (SGM Biotech, 2010). By plotting the logarithm of the number of surviving spores against time, the D-value can be determined as the slope of the curve.

The z-value is the number of temperature degrees between a 10-fold (one $\log_{10}$) change in the microbial survival (FPA & FPI, 2009). The z-value can be derived from the thermal death time curve, which is the straight line observed when the logarithm of the D-value is plotted against time (Holdsworth & Simpson, 2007). The slope of thermal death time curve is -1/z.

The thermal death time curve shows that when the temperature increases, the time required for achieving the same microbial destruction decreases logarithmically (Heldman & Hartel, 1997). In other words, the rate of microbial destruction increases as the temperature increases. From this it can be concluded that the required processing time will be shorter if a higher temperature is used. Any time-temperature combination causing microbial destruction can be used (Potter & Hotchkiss, 1998). As an example, 0.78 minutes at 127 °C is equally destructive to *C. botulinum* in low acid foods as 2.78 min at 121 °C or 10 min at 116 °C. It is important to remember that it is not only the holding time at the maximum temperature that is of importance. Microorganisms are exposed to lethal temperatures dur-

ing heating up to the target processing temperature and during cooling, and it is the cumulative effect of the whole processing cycle that is of importance.

The rate of inactivation of a microorganism is known as the lethal rate (Holdsworth & Simpson, 2007). The lethal rate (L) at any temperature can be calculated by the formula:

$$L = D_{ref}/D = 10^{(T-Tref)/z}$$

As shown by the formula, the lethal rate is the ratio of the D-value of the microorganism at a certain temperature, T, to the D-value of that same organism at the reference temperature, T_{ref}.

Lethal rates are additive which can be used to calculate the total lethality of a thermal process (also called "process lethality" or "process equivalent time") known as the F-value (Holdsworth & Simpson, 2007). The F-value is determined by plotting the lethal rate value against process time and then calculating the area under the curve. Two common methods for calculating the area under the curve are the trapezoidal rule and Simpson's rule, which are both used to determine the area of irregular geometrical figures. Using the trapezoidal rule, the total lethality of a thermal process can be calculated by the following procedure (SGM Biotech, 2010):

First the lethal rate at each time point at which a temperature measurement has been made is calculated by the formula:

$$L = t_A x10^{((T_A-T_{ref})/Z_A)}$$

Where
t_A = process clock time interval, e.g. 1 min
T_{ref} = Process reference temperature
T_A = Temperature at the point in time
Z_A = Actual Z-value for the spores under study

Then the values for all measuring points are summarized to the total process lethality:

$$F_{(Tref,ZA)} = \Sigma F_{T1, ZA} + F_{T2, ZA} + F_{T3, ZA} + \ldots + F_{Ti, ZA}$$

The above formulas are used for calculating the lethality of a specific process with a specific reference temperature and an organism with a specific z-value. In calculations of F_0, which is a standard measure, the reference temperature is set to 121 °C and the z-value to 10 °C.

To elaborate on the concept of the F-value of a process, it is the number of minutes at a specific temperature required to achieve a certain destruction of microorganisms, e.g. a 12D reduction of *C. botulinum* (Potter & Hotchkiss, 1998). As mentioned above, F_0 is the standard where a temperature of 121 °C and a z-value of 10 °C are used. To give an example, an F_0-value of 9.50 means that the studied retort process is equivalent to a heat treatment of 121°C for 9.50 minutes against an organism with a z-value of 10 °C. The practice of converting the lethality of the process to a time at a constant reference temperature allows comparison of the lethality of different thermal processes (Ryley & Kajda, 1994).

2.13 General targets for microbial reduction

Stumbo *et al.* (1975) state that a high degree of safety is achieved if only one viable *C. botulinum* spore, with a $D_{121°C}$ -value of 0.20 min and a z-value of 10 °C, is remaining in every 10^{12} processed containers. Anderson *et al.* (2011) recommend using a $D_{121°C}$–value of 0.25 minutes instead, as this is the highest know D-value of proteolytic *C. botulinum* spores.

In a later study by Pflug (1987), a less strict target was recommended; one viable *C. botulinum* spore in every 10^9 processed containers, i.e. a 10^{-9} probability of spore outgrowth. This was based on a conservative assumption that the initial value in low-acid canned foods is 10^3 spores, to which a process resulting in a 12 $\log_{10}$ inactivation is applied.

From an economical point of view, the process can be considered adequate when only one viable spore of a high heat resistant mesophilic organism ($D_{121°C}$ = 1.5 min; z = 8 °C) remains in every 10^4 containers processed (Stumbo *et al.*, 1975). This level is the standard used by European food industry (Anderson *et al.*, 2011).

2.14 Regulatory demands

The European Union is working to harmonize the food control systems of the member countries (Anderson *et al.*, 2011). The European Parliament and the Council of the European Union regulation on the hygiene of foodstuffs lists re-

quirements for heat-treated food products in hermitically sealed containers. They state that the process used should conform to an internationally recognized standard, such as pasteurization, ultra high temperature or sterilization (European Commission, 2004). The Scientific Panel on Biological Hazards of the European Food Safety Authority refers to the application of the botulinum cook as a way of controlling *C. botulinum* (EFSA, 2005; 27): "The application of the 'botulinum cook' is defined as equivalent to 3 minutes heating at 121 °C. This value is also the F_0 value or the process value. This heating regime is used for low acid canned food products and results in a 12 $\log_{10}$ units reduction in numbers of spores." The required F_0-value of 3 minutes is derived from the highest known D-value of proteolytic C. botulinum spores, which is 0.25 minutes at 121 °C (Anderson *et al.*, 2011). Hence, the required F_0 is 12 x 0.25 min = 3 min. These values are also the practice in the US (Holdsworth & Simpson, 2007).

The minimum reduction of 12 $\log_{10}$ for *C. botulinum* in a commercially sterile product has an adequate safety factor incorporated (Stoforos, 1995). The 12 $\log_{10}$ criterion has been used for more than 90 years, and decades of experience of using this reduction value in commercial sterilization show that it is effective (Anderson *et al.*, 2011).

2.15 Food properties protecting microorganisms

Sugar in high concentration, starch and proteins protect microorganisms against heat (Potter & Hotchkiss, 1998). Fats and oils protect microorganisms to a large extent by making it more difficult to the wet heat to reach and enter the cells. Dry heat is less lethal than wet heat, because it is more difficult for dry heat to penetrate into the cells. Fat is also a poorer heat conductor than water, which further complicates the sterilization of food containing a lot of fat. More heating time is needed for destruction of organisms in the fat/oil phase. Sterilization of meat packed in oil, for example, is very difficult. The high fat content of the coconut milk in Gaeng Phed Gai might interfere with the sterilization to some extent.

The consistency of the food can also have protecting effects (Potter & Hotchkiss, 1998). A higher viscosity gives less convection heating. Addition of starch or other thickeners will slow down the heat penetration. Special thickeners which thicken after retorting instead of when heated has been developed to avoid this problem. No thickeners were added to Gaeng Phed Gai in this study.

2.16 Controlling bacteria with pH, a_w, salt or nitrite

Growth and heat resistance of bacteria is influenced by the composition of the product (EFSA, 2005). The main parameters are pH and water activity (a_w).

Lower pH reduces the heat resistance of bacterial spores (Anderson *et al.*, 2011). Lower pH also reduces the growth of many bacteria (FPA & FPI, 2009; EFSA, 2005). Hence, the growth of bacteria can be controlled by reducing the pH to levels where they cannot grow. The definition of a low-acid food product is a product which has a pH below 4.6; the limit below which proteolytic *C. botulinum* cannot grow. The corresponding figure for non-proteolytic C. botulinum is pH 5.0. Spores will not germinate in acid environment, which is the reason why high-acid products can be processed at lower temperatures. Only the vegetative cells must be inactivated, and bacteria in the vegetative state are killed much more easily than bacterial spores.

Water activity is the vapor pressure of a product divided by the vapor pressure of pure water. It shows how much of the water that is bound to non-aqueous constituents and solids. A higher water activity can support more microorganisms. Proteolytic *C. botulinum* cannot grow at a_w <0.94 (EFSA, 2005). The corresponding figure for non-proteolytic *C. botulinum* is a_w <0.97.

Water activity also impacts the heat resistance of bacterial spores (Anderson *et al.*, 2011). In general, the heat resistance of spores is higher at low a_w and lower as a_w approaches 1.0, which is the highest possible value.

According to Anderson *et al.* (2011), salt, i.e. NaCl, and other preservatives, such as nitrite, can inhibit growth of bacteria in heat-treated foods. The underlying mechanism is an inhibition of recovery of heat damaged spores. However, salt is likely to play a rather small role, as higher concentrations of NaCl lower the water activity and thereby increases the resistance of spores during heating.

2.17 Inoculation tests

The heat treatment process cannot be decided from theoretical calculations only (Toledo, 2007; Potter & Hotchkiss, 1998). To be reasonably sure of the safety of the thermal process, theoretical results must be checked by inoculated pack studies. In such studies, packages are inoculated with an organism with a known heat resistance, higher than that of the background microflora in the product. The in-

oculation is set to a level which makes it easy to evaluate the spoilage. After sterilization, retorted packages are incubated at a temperature which favors microbial growth and are then checked for signs of growth and spoilage, e.g. by visual analysis and bacteriological tests.

An alternative to adding bacterial spores directly into the product is using ampoules containing spores. Any surviving spores in the ampoules are shown as a color change when the ampoules are incubated at an optimal temperature for a certain time (SGM Biotech, 2010).

2.18 Bioburden

Raw materials and ingredients are the primary sources of microorganisms in food products (FPA & FPI, 2009). When using a risk-based approach to food processing it is necessary to quantify the initial amount of microorganisms, i.e. bioburden, of the raw materials as this is the basis for the implementation of control measures and food safety objectives (Anderson *et al.*, 2011). Ideally, the quantification should be based on tests of large number of raw material samples. However, it can be very expensive to conduct new studies and very difficult make accurate quantifications of organisms occurring in low numbers. Making conservative estimations including a margin of safety, based on knowledge of occurrence of a broader group of microorganisms, can be the only realistic path to take. An example of such a broader group is the mesophilic putrefactive anaerobic spore formers (PA), of which *C. botulinum* is a member.

Below follows a description of the information found about spore concentrations in the raw materials of Gaeng Phed Gai.

Poultry

Meat and poultry are commonly contaminated during slaughter and processing (FPA & FPI, 2009). The pathogenic bacteria of primary concern for meat and poultry are *Salmonella*, *Campylobacter*, *Listeria monocytogenes*, *Clostridium perfringens* and *Bacillus cereus* (and *Yersinia enterocolita* for pork). *Clostridium botulinum* is rare in these products and when it occurs it is usually in very low numbers; 0.1-7 spores per kg. This is in line with the findings of Anderson *et al.*

(2011), who state that studies tend to report *C. botulinum* spore levels of 0.1-10 per kg of raw meat and poultry. They also state that the incidence of PA spores in general is 2000-20000 times greater than the incidence of *C. botulinum* spores. As an example, Greenberg *et al.* (1966) tested 1078 samples of chicken meat from the United States and Canada and found an average of 2.5 PA spores per gram. Only one of the 8093 PA spores found was a *C. botulinum* spore.

Vegetables

High numbers of bacterial spores are likely to be found in root crops (e.g. onions, garlic, beets, carrots and potatoes) mushrooms, some spices and honey (FPA & FPI, 2009). Vegetables are easily contaminated by *C. botulinum*, because they are often in contact with soil (Anderson *et al.*, 2011). Vegetables also support the germination, outgrowth and toxin production of some strains. Vegetables are also likely to contain other spore-forming bacteria, both mesophilic and thermophilic, which might cause product spoilage if the product is stored at high temperatures. For this reason canned vegetables are often processed much more than what is required to inactivate *C. botulinum*.

Spices

Spices and herbs are commonly contaminated with microorganisms originating from soil, plants or animals (McKee, 1995). The microorganisms can survive harvest, primary processing and drying processes. Bacterial spores are able to survive for long periods in dehydrated spices (FPA & FPI, 2009).

The predominant bacteria (50-95 %) in spices are mesophilic aerobic spore-formers of the genus *Bacillus* (Sperber & Doyle, 2009). These bacteria will not spoil the spices as they cannot grow in the dry environment, but when the spices are added to other food products they can start growing and cause spoilage. Even though spices contain bacterial spores, incorporation of spices in low acid foods in hermetically sealed containers is usually not a big issue. The reason is that spores of mesophilic aerobic bacteria have a relatively low heat resistance as long as the product is fully hydrated (Downes & Ito, 2001). If trapped in unhydrated particles, on the other hand, they may survive and cause flat-sour spoilage.

Thermophilic spores, which are more heat resistant, also occur in spices but in much lower numbers. Spoilage by thermophilic flat-sour spore-formers and thermophilic anaerobes is prevented if the product is cooled properly after thermal processing and then stored at temperatures below 43 °C (Downes & Ito, 2001).

Most thermophiles require storage temperatures of 45-55 °C to cause spoilage (Teixeira, 2007).

Black pepper has often been reported as the spice containing the highest number of bacteria, but all spices are susceptible to contamination (McKee, 1995). Grecz *et al.* (1986) state that commercial spices are generally contaminated by 10^5 to 10^8 microorganisms per gram. They found the number of heat resistant bacterial spores per gram of spices to be $1x10^7$ for black pepper, $2x10^6$ for thyme, $7x10^4$ for anise, $4x10^5$ for curry powder, $8x10^4$ for poultry seasoning and $1.5\text{-}3x10^4$ for pickling spice, cardamom and cumin.

The occurrence of thermophilic organisms in a number of food ingredients was investigated in a Canadian study by Castell (1944). Among the 20 spices investigated Portuguese paprika, white pepper and Batavia cassia where those containing the highest numbers of anaerobic spores; $1x10^6$ cfu/g. The cane sugar investigated contained 0-10 anaerobic spores per g.

McKee (1995) mentions two other studies of interest. The first is a US study by Hall (1951) which showed that the mesophilic spore counts ranged from 0 for cinnamon to $7.6x10^4$ for paprika. The number of thermophilic spores ranged from 0 for cinnamon to $2.4x\ 10^4$ per gram for red pepper in the same study. The second is a study by Singh *et al.* (1988) who investigated the microbial loads of chili, black pepper and turmeric and found the spore counts to be in the range 10^2 to 10^5 cfu/g.

Fujisawa *et al.* (2004) studied the occurrence of *C. botulinum* in 100 commercially available samples of spices. Chili powder was one of them. Clostridia were isolated from 47 % of the samples but no *C. botulinum* was found. *C. perfringens* was found in 27 % of the samples, but none of them had the enterotoxin gene. Carlin *et al.* (2004) made a similar study. They tested 65 samples of spices, herbs and dehydrated mushroom for *C. botulinum* type A, B E and F. They did not find any such bacteria in their samples and concluded that the most probable number of *C. botulinum* in these products is less than 0.6 per kg.

Fish sauce

Fish sauce production is dependent on microorganisms, predominantly halophilic aerobic spore-formers, as these are used in the fermentation of fish (Lopetcharat *et al.*, 2001). Crisan & Sands (1975), investigated the microbial flora in four fermented fish sauces and found that species of the genus *Bacillus* dominates the mi-

croflora after fermentation, as their spores are able to survive the fermentation. They also showed that obligate anaerobes were absent in fish sauce.

Germination and growth of microorganisms in fish sauce is inhibited by the high salt content (Lopetcharat *et al.* 2001). The product is boiled before packaging which reduces the levels of microorganisms.

Carlin *et al.* (2004) tested 62 samples of aroma, sauce and gravy for *C. botulinum* type A, B E and F. One of the samples was positive. They concluded that the most probable number of *C. botulinum* in these products is 0.3-0.6 per kg. They also tested 10^2 samples of fish and shellfish and found *C. botulinum* in 26 of them, which led them to the conclusion that the most probable number in these products is 2-3 per kg.

Rapeseed oil

No indications of high occurrence of *C. botulinum* or other spores in pure edible oils were found in literature. Several outbreaks of botulism have occurred from vegetable tubers or roots (e.g. garlic, onions and potatoes) in oil, but it is likely that these spores originated from the vegetables and roots (Morse *et al.*, 1990).

Tap water

In drinking water, microbial levels are low. Conventional methods of water treatment have been shown to be effective at removing *C. botulinum* spores from drinking water (Long & Taucher, 2006). The quality of drinking water in Sweden is well monitored. *Clostridium perfringens* is used as the indicator for resistant microorganisms and is tested regularly, both in "raw water" before water treatment and during water treatment (National Food Administration, 2006). The levels are generally low and a remark is given if the species is found.

Coconut milk and bamboo shoots

Spore counts in the coconut milk and bamboo shoots used in this project are very close to zero, because these ingredients have been retorted once before.

2.19 Maximum spore concentrations in Gaeng Phed Gai

From the information presented above, we can conclude that the ingredients in Gaeng Phed Gai with a high probability of contamination with *C. botulinum* are spices and chicken meat. To include a margin of safety and estimate the worst case

level of contamination, a statement by Stumbo *et al.* (1975, 1316), who are authorities in the field, can be used. They state that the prior processing population of *C. botulinum* spores of approximate maximum heat resistance ($D_{121°C}$ = 0.20 min, z = 10 °C) in low-acid foods should seldom, if ever, be greater than one per gram of food. This level of contamination has been used in practice by Anderson *et al.* (2011), for example, in the bioburden estimation of a pesto sauce, which includes many ingredients that are easily contaminated of spores of *C. botulinum*; sweet basil, garlic and pine nuts. The reported contamination levels for the ingredients of Gaeng Phed Gai are low in relation to those used in pesto sauce. By combining all this knowledge, it can be concluded that calculations with a contamination level of one spore per gram of Gaeng Phed Gai includes a considerable margin of safety. This level has been used in this study for a high level of conservatism.

For spores of higher heat resistance than *C. botulinum*, Stumbo *et al.* (1975, 1316) state that the prior processing population of spores of mesophilic bacteria of greater heat resistance than *C. botulinum* ($D_{121°C}$ = 1.0-1.5 min, z = 8 °C), in low-acid foods should seldom be greater than one per gram of food.

2.20 Target process lethality for Gaeng Phed Gai

As described above, it is appropriate to use one spore per gram of product as the maximum initial level of spores, both for *C. botulinum* and for mesophilic spores with higher heat resistance than *C. botulinum*. For a unit size of 200 grams, this means 200 spores per unit, i.e. 2.3 log CFU per unit.

The target value for the probability of *C. botulinum* survival is set to 10^{-9} per unit in this study, as recommended by Pflug (1987). The heat resistance values for *C. botulinum* used by Anderson *et al.* (2011), $D_{121°C}$ = 0.25 min and z = 10 °C, is used in the calculations. For economical spoilage, the target recommended by Stumbo *et al.* (1975) is used: one viable spore of a high heat resistant mesophilic organism ($D_{121°C}$ = 1.0-1.5 min; z = 8 °C) remaining in every 10^4 containers processed (Stumbo *et al.*, 1975).

The process lethality, F_0, required to achieve a given reduction of a target organism can be calculated by the formula (Toledo, 2007):

$F_0 = \log(N_0/N)*D_0$

Where
N_0 and N is the spore load before and after heat treatment respectively
D_0 is the D-value of the organism at the reference temperature 121.1 °C

For *C. botulinum*, the target F_0 becomes: $\log(200/10^{-9})*0.25 = 2.83$

For mesophilic organisms with higher heat resistance than *C. botulinum*, with a D-value in the order of 1.00-1.50, the target F_0 becomes 6.30-9.45 min.

It is interesting to note that the probability of spoilage from a given organism can be calculated by the formula $N = N_0*10^{-(F0/D0)}$ (Toledo, 2007). Using the values above for *C. botulinum* ($N_0 = 200$, $D_0 = 0.25$) shows that the probability of spoilage from *C. botulinum* after processing to an F_0 of 9.45 min is as low as:

$N = 200*10^{\wedge -(9.45/0.25)} = 3*10^{-36}$.

3 Methods

3.1 Food preparation

The product was prepared by hand by Thai chefs using ingredients according to a traditional Thai recipe. The ingredients are shown in Table 3. Chicken breast filets were cut into 0.5-1.0 cm thick slices (maximum slice dimension: 8x3x1cm) and fried in rapeseed oil and chili spices for 15 minutes, using a liquid pressure gas burner. The other ingredients were added, and the whole mix was cooked for another 15 minutes.

Table 3. Ingredient composition of Gaeng Phed Gai at preparation

Ingredient	Weight %
Coconut milk	40.7
Chicken meat	20.4
Water	20.4
Bamboo shoots	10.2
Fish sauce	2.9
Rapeseed oil	2.6
Chili spices	1.6
Sugar	1.2

3.2 Packaging

Retortable pouches (Flair Flexible Packaging Corporation) with a size of 255 x 180 mm were used. The pouches consisted of four layers: an inner layer of 80 µm cast polypropylene, a 9 µm aluminum layer, a 15 µm nylon layer, and an outer layer of 12 µm polyethylene terepthalate. Detailed pouch characteristics, provided by the manufacturer, are given in Table 4.

Table 4. Pouch characteristics, according to Flair Flexible Packaging Corporation

Parameter	Typical Value	Test Method
Total thickness	116 µm	FTM
Thickness of aluminum foil	9 µm	
Thickness of cast polypropylene	80 µm	
Thickness of polyethylene layer	12 µm	
Thickness of nylon	15 µm	
Tensile strength, kg/15mm		ASTM D882
machine direction	9.50	
transverse direction	9.50	
Elongation at break, %		ASTM D882
machine direction	130.00	
transverse direction	120.00	
Heat seal strength, kg/15mm	5.00 at 200 °C	FTM
	6.00 at 210 °C	
	6.80 at 220 °C	
Bursting strength, kgf/cm^2	9.5	ISO 2759
Oxygen transmission, at 23 °C, dry	0.00 cc/m^2	ASTM D3985
Water vapor transmission at 38 °C, 90 % RH	0.00 cc/m^2	ASTM F1249

The pouches were filled with chicken pieces to a weight close to 100 g without dividing any chicken pieces. The pouches were then filled up with sauce, containing bamboo shoots, to a total weight of 200 ± 2 g. After filling, the pouches were put in a water bath to heat the product to 90 °C before sealing. This step was included to reduce the risk of pouch breakage due to product expansion during heating. It is a step that would not have been necessary in a retort allowing manual adjustment of retort pressure during cooling. This issue will be discussed in more detail later.

The residual gas was removed by a vacuum pump (N026.3 AN.18, Neuberger) before sealing the packages with an impulse heat sealer (Fermant 400, JOKE Technology GmbH).

3.3 Retort processing

The retort processing was conducted in Linköping, Sweden, in facilities owned by the dairy company Arla Foods. The packages were laid flat on trays in a still overpressure retort of laboratory model (PS/QCS/EV150 Top Loading Priorclave, Pri-

orclave Ltd), which uses a steam/air mixture for heating. The target temperature of the retort was set to 121 ºC, which is the maximum temperature that should be used in a still retort.

The retort used does not have a vacuum pump assisting with air removal. Instead a period of free steaming is incorporated in the process. Free steaming introduces a stage during heating up to process temperature when a solenoid valve at the rear of the autoclave is opened for a pre-set time. The valve opens at a factory set temperature just above 100 ºC and is held open for five minutes. During this time steam is being generated in the chamber in large volumes, which creates turbulence as it passes through the load before escaping through the valve. This turbulence assists with air removal and reduces temperature lag between the load and the retort, reducing process time.

An F_0 of 10 min was targeted for full assurance that the probability of product spoilage from high heat resistant mesophilic organisms would be lower than 10^{-4}. A large number of test batches were run to identify the time at 121 °C necessary to achieve this level of lethality. First, the cold point in the retort was identified by measurements in all three baskets in the retort in which pouches could be processed. Then, several batches were run with only a single pouch, in which a temperature probe and bioindicators were put for measurement of the process lethality. Regarding the cold point inside the pouches, it was assumed to be in the geometrical centre because of the large number and size of pieces in the pouches making conduction the dominating mechanism of heat transfer.

When the time required to achieve an F_0 of 10 minutes had been identified (to 9 minutes at 121 °C) – a larger scale test was carried out, processing three batches containing 12 pouches each. In addition to a batch with the target F_0 of 10 minutes, one batch with three minutes longer time and one batch with 6 minutes longer time were included. The reason for the inclusion of these more severely heated batches was a desire of full assurance that at least one batch would be commercially sterile, and also a wish to create a material that allowed for comparison of the effects of different levels of heat treatment.

The retort used does not have any system for active cooling under pressure. Hence, when each process cycle was finished, the retort had to be left to cool down passively to 100 °C. At this point the pressure in the retort is back at atmospheric level. The retort was then opened and the pouches put in a water bath hold-

ing a temperature of 8 °C for 10 minutes. There were two reasons for this procedure of cooling: First, the product should be cooled as quickly as possible after sterilization to prevent unnecessary heat damages to the food (Potter & Hotchkiss, 1998). Second, it is necessary to make sure that the product temperature is taken down below 43 °C, to avoid germination of spores causing flat-sour product spoilage (Ghani *et al.*, 2002).

3.4 Procedure for temperature measurements

Temperatures were measured by use of two temperature probes (10K NTC Termistors in PB-5002 probes, Intab Interface-Teknik AB) – one probe measuring the temperature in the retort chamber and one probe measuring the temperature at the geometrical centre of a pouch at the cold point in the retort. Temperature loggers (Tinytag TGP-4020, Intab Interface-Teknik AB) were used to record the temperature every 30 seconds.

For measurement of product temperature, one pouch was fixed with a packaging gland (home-made from a fuel system nipple filled with silicone, imitating the function of the packaging glands from Ellab Co. Denmark used by e.g. Bindu *et al.* 2007) through which the temperature probe was inserted. The part of the probe which was not in the geometrical centre was isolated with silicone. The pouch in which temperature measurements was conducted was filled with 10 % more product than the other pouches, as per the recommendations by Richardson (2004), to a total product weight of 220 g.

3.5 Calculation of process characteristics

When the thermal processing had been finished, several process characteristics were calculated from the time-temperature data collected. The process lethality (F_0) was calculated as described in the Theoretical background. To get a measure of the heat treatment with respect to the nutrient degradation and textural changes occurring during the heat treatment, the cook value (C_0) was determined. This was done using the reference temperature 100 °C and the z-value 33 °C, which is the level where thiamine is denaturized, as per Bindu *et al.* (2007). The cook value is defined similarly to the F_0-value (Holdsworth & Simpson, 2007):

$$C_o = \int_0^t 10^{\frac{T-100}{z_c}} dt$$

The calculation of the C_0 was done using the trapezoidal rule, just as for the calculation of F_0.

3.6 Practical tests of microbial inactivation

Ampoules containing bacterial spores were used to compare the practically achieved process lethality to the value calculated from temperature data. Two packages in each of the three batches were prepared with bioindicator ampoules (SterilAmp II, SGM Biotech) containing $3x10^4$ spores of *Geobacillus stearothermophilus* 7953 with a D-value of 1.7 min and a z-value of 9.0 °C. In total, 15 ampoules were used in each batch; whereof 10 ampoules were put inside chicken pieces and 5 ampoules were put among the bamboo shoots. These pouches were filled by 10 % more product than the other pouches, i.e. by 220 g product.

The Certificate of Analysis for the ampoules, provided by the manufacturer, shows that 0 of 20 ampoules are negative after a thermal treatment with an F_0-value of 7.0 or less, and 20 of 20 ampoules are negative after a thermal treatment with an F_0-value of 11.0 or more.

After the thermal treatment, the ampoules were incubated at 55 ºC for 48 hours and visually analyzed for colour shifts due to bacterial growth.

In addition to the use of bioindicators, microbiological analysis was conducted by the accredited laboratory Eurofins. Two packages from the shortest heat treatment were sent to the laboratory for analysis of aerobic bacteria (3M Petrifilm), anaerobic bacteria (in-house method), yeast (NMKL 98, 4. Ed., 2005) and mould (NMKL 98, 4. Ed., 2005), after 10 days of storage at 30 ± 0.5 °C. This storage temperature was chosen for two reasons:

- Downes & Ito (2001) state that an incubation temperature of 30-35 °C is favorable for culturing of mesophilic sporeformers important to food microbiologists.
- NACMCF (2010) states that the storage temperature should be representative of the expected temperature that the product will be exposed to during commercial distribution and storage. Typical temperatures for shelf-stable foods are 24-35 °C.

3.7 pH and water activity

The pH of the product was measured using a pH meter with a built-in thermometer (Orion 320, Thermo Scientific) available at the laboratory of Arla Foods in Linköping. Measurements were conducted before heat treatment, right after heat treatment and after 30 days of storage at 21 ± 1°C. For the measurements, the guidelines given in Codex Alimentarius Commission (1993) were followed: The instrument was rinsed and calibrated using standard buffers with pH 4.0 and 7.0 respectively. The accuracy was checked using a buffer with pH 5.0. The product was mixed to a uniform paste, whereupon the temperature was adjusted to 25 °C before determining the equilibrated pH.

Samples of the product before heat treatment and after the shortest heat treatment were sent to the accredited laboratory Eurofins for analysis of water activity (a_w). The samples were analyzed using the method NMKL No. 168.

3.8 Nutritional values

Two samples of the product after heat treatment (9 min at 121 °C) were sent to the accredited laboratory Eurofins for analysis of water content, protein content, fat content and ashes. Calculations of carbohydrate and energy content were also conducted by the laboratory. These analyses are part of an analysis package, "Nutritional Values Group 1", attended by the laboratory for a discounted price.

3.9 Sensory evaluation

Sensory analysis was performed to compare the acceptability of the product before and after (9 min at 121 °C) heat treatment. Samples of approximately 30 grams were assigned three digits random codes, heated to 60 ºC and then served to the sensory panel one at a time in a balanced order. The panel was asked to rate appearance, colour, flavour, juiciness, texture and overall acceptability, using a hedonic scale ranging from 9 (like extremely) to 1 (dislike extremely), as per Rajkumar *et al.* (2010). The full sensory scale is shown in Table 5. The evaluations were conducted in an existing sensory laboratory at Arla Foods in Linköping.

Table 5. The hedonic scale used in sensory analysis

Number	Word description
9	Like extremely
8	Like very much
7	Like moderately
6	Like slightly
5	Neither like nor dislike
4	Dislike slightly
3	Dislike moderately
2	Dislike very much
1	Dislike extremely

The sensory panel consisted of ten semi-trained assessors who were introduced to the attributes (appearance, colour, flavour, juiciness, texture and overall acceptability) in the beginning of the session.

According Srilakshmi (2003, 283), semi-trained panels "are constituted of technical people and their families, who are normally familiar with the qualities of different types of food. They are capable, with few preliminary test runs, of following instructions for tests given, discriminating differences and communicating their reactions". The panelists used in this project are all members in a panel for assessing milk products and, therefore, are only semi-trained on Thai food assessment. It is well known that good sensory analysis requires trained assessors, but using fully trained assessors can be very costly (Ruiz-Capillas & Moral, 2007). Using semi-trained panelists is usually cheaper. It also has the advantage that semi-trained assessors' judgment is closer to that of an average consumer, at the same time as they produce results which are largely correlated to that of trained assessors.

The data were evaluated using a one-tailed paired t-test to determine the statistical significance of effects of retort processing on quality traits. Significance was established at $p < 0.05$.

4 Results and Discussion

4.1 Process lethality

Measured retort and product temperatures, and the calculated F_0-value for the batch with the shortest processing time, are shown in Figure 2. For the other two batches, the same process was used, with the only difference that the time at 121 °C was extended from 9 minutes to 12 and 15 minutes respectively (i.e. adding an extra 3 and 6 minutes to the F_0-value). The figure shows that an F_0 of 12 was accomplished. However, the bioindicator ampoules indicate that the temperature measurements overestimated the process lethality slightly. 1 of the 15 ampoules used in the shortest heat treatment was positive after heat treatment, indicating that the actual F_0 was lower than 11, which is the limit where all ampoules are negative according to the Certificate of Analysis provided by the ampoule manufacturer. There are at least two possible reasons for the temperature sensor's overestimation of F_0:

1. The low filling degree of the pouches resulted in an asymmetrical geometry, making the location of the cold point less predictable. The cold point might not have been located in the geometrical centre where temperature measurements were made.
2. The temperature probe is designed to measure the temperature at its tip, but some heat might be conducted from points with higher temperature through the sensor stick itself which has a metal coating.

The degree of filling would be easily solved by filling the pouches with more food, which would be possible when using a retort able to retain full overpressure during cooling.

A situation with an unknown cold-point can be handled by using multiple temperature probes in the same package and then use the result showing the lowest temperature, as done by e.g. Rajkumar *et al.*, (2010). Unfortunately, this could not be done in this study, as the equipment available did not allow more than two probes to be inserted in the retort.

An attempt to avoid the problem of heat conducting probe sticks was made by isolating the stick with a silicone tube, but the effectiveness of this solution was not investigated. In any case, other researchers should have faced the same problem, as similar equipment is used. The temperature probe used by Bindu *et al.* (2007), for example, is also a stick with a metal coating, with the difference that it contains a thermocouple instead of a thermistor. For the sake of conformity with other researchers, the author would recommend using equipment from Ellab Co. Denmark in further studies, as done by e.g. Bindu *et al.* (2007), Mohan et al. (2008) and Rajkumar *et al.* (2010).

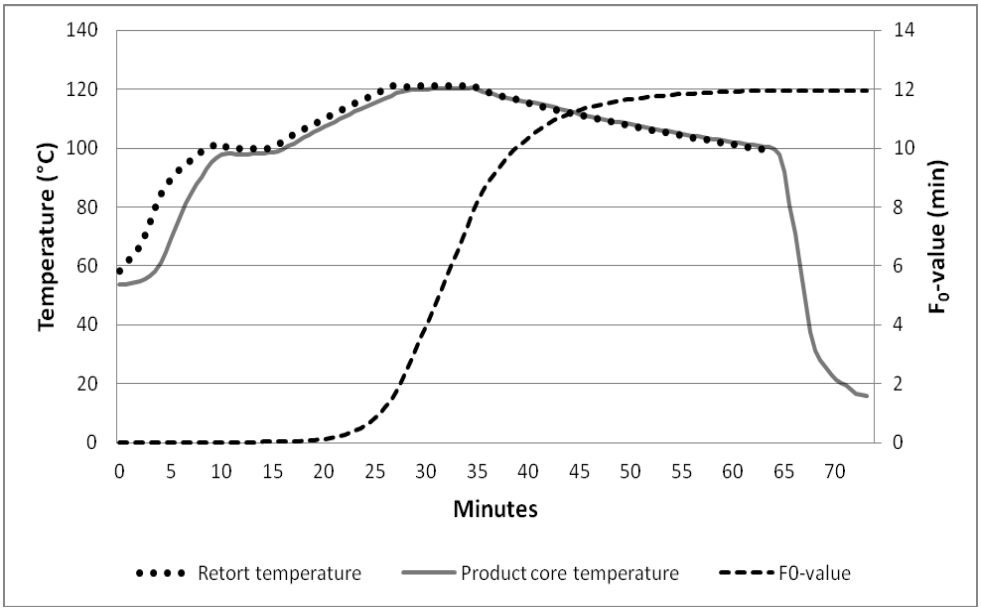

Figure 2. Heat penetration characteristics and F_0-value of Gaeng Phed Gai in retort pouches.

The process lethality adopted was in agreement with recommendations and findings of other authors. Mohan *et al.*, (2008) recommends a minimum F_0 value is 6 minutes. Frott & Lewis (1994) recommended an F_0 value between 8 and 20 min for retort processed meat products. Ranganna (2000) reported F_0 values between 8 and 12 for meat products. Gopal *et al.* (2001) processed Kerala style fish curry at 121.1 °C to F_0 values of 6.56 min and 8.43 min. Ravishankar *et al.* (2002) proc-

essed Seer fish curry in retort pouches to an F_0 value of 11.5 min, obtaining a product that remained in good condition for 24 months at room temperature. Prince Devadason *et al.* (2010) processed buffalo meat block in retort pouches to an F_0 value of 6.52, which corresponded to a 5D reduction of Clostridium sporogenes PA 6379. Rajkumar *et al.* (2010) processed Chettinad goat meat curry in retort pouches to an F_0 value of 12.10. Rajan *et al.* (2011) found that Chettinad chicken processed in retort pouches at 121.1 °C and a corresponding F_0 value of 5.2 was sufficient to be able to store the product at 35±2 °C for 180 days.

Microbiological analysis of the food confirmed an adequate level of sterility. Two pouches from the shortest heat treatment were tested for total count of aerobic bacteria, anaerobic bacteria, yeast and mould. None of these microorganisms could be detected after 10 days of storage at 30 °C, as shown in Table 6.

Table 6. Analysis results for microorganism in the product heat-treated for 9 minutes at 121 °C

Analysis	Results pouch 1 (log cfu/g)	Results pouch 2 (log cfu/g)
Total count aerobic bacteria	<3.0	<3.0
Total count anaerobic bacteria	<2.0	<2.0
Yeast	<2.0	<2.0
Mould	<2.0	<2.0

The results are in agreement with the findings of other researchers. Rajkumar *et al.* (2010) determined total viable, anaerobic, coliform, staphylococcal, streptococcal, clostridial and yeast and mould counts of Chettinad goat meat curry retorted to an F_0 value of 12.1 minutes and showed that the product was commercially sterile. Rajan *et al.* (2011) could not detect any *E. coli*, *Salmonella spp.*, *Staphylococci spp.*, yeast or mold during their 180 day storage at 35±2 °C of Chettinad chicken, which was processed in retort pouches to an F_0 value of 5.2.

It should be remembered that the microbiological test described only proves the microbial quality after a short time of storage. Before commercialization of any food product, longer storage studies should be conducted. Challenge studies should be conducted for at least the intended shelf-life of the product (NACMCF, 2010). Even longer times could be needed for an additional margin of safety, and to account for slow recovery of heat-injured cells and for users who might consume the product beyond the declared shelf-life.

4.2 Cook value

Figure 3 shows that the thermal process resulted in a cook value of 123 minutes. This value is higher than those reported by other researchers working with similar processes. Rajkumar *et al.* (2010) reported a cook value of 75 min for goat meat curry processed to an F_0 of 12.1 min, Ravishankar *et al.* (2002) reported 95 min for Seer fish curry processed to an F_0 of 11.5 min, Bindu *et al.* (2007) reported a cook value of 99 min for black clams processed to an F_0 of 9 min, and Bindu *et al.* (2010) reported a cook value of 66 min for Fish Peera processed to an F_0 of 7 min. The high cook value indicates more severe nutritional degradation and textural changes.

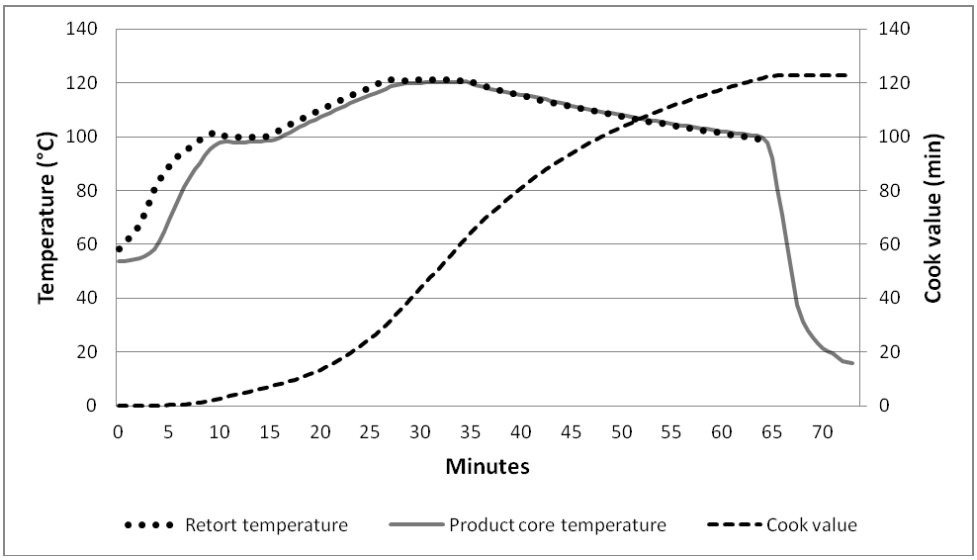

Figure 3. Heat penetration characteristics and cook value of Gaeng Phed Gai in retort pouches.

The reason for the high cook value is the slow heating and cooling of the retort used, which lead to a long processing time. The come-up time of the retort (i.e. the time from initiation of heating until the target retort temperature was reached) was 26.5 minutes. The reason for the slow heating is that the retort creates steam by heating water during processing, which takes a long time. Come-up time is significantly lower when using a process in which pre-made steam is used. Ravishankar *et al.* (2002), for example, had a come-up time of 3 minutes in their processing of Seer fish curry.

The method for cooling is also of great importance. The retort used did not allow any active cooling under pressure. It had to be left to cool passively to 100 °C before it could be opened and the pouches cooled in water, making 33 minutes

pass during cooling from 121 °C to a temperature below 43 °C. Ravishankar *et al.* (2002) used cooling by water spray inside the retort, giving an almost instant drop in retort temperature and a product cooling time of about 10 minutes.

4.3 Choice of retort

The retort found for this project was a lab-scale still retort. As described above, this model was too slow during both heating and cooling to give a satisfying result. In addition, it adjusted the pressure automatically and did not allow manual controlling of pressure. This resulted in a too low overpressure during cooling, causing many of the fragile pouches to break. Ideally, a retort for processing of retort pouches should allow retention of a high overpressure during the whole processing cycle, including the cooling phase.

For the best result, a retort allowing agitation of the pouches should be used instead of a still retort, as this reduces processing time. Snyder & Henderson (2007) showed that agitation is an important factor for processing time for products consisting of particulates in liquid medium. In addition, agitation makes the use of a higher processing temperature possible, further reducing processing time.

4.4 The suitability of the pouch used

The size of the pouches was far from optimal. The initial plan was to fill them with 400 g of product. The fact that the retort pressure could not be set manually led to a too low pressure inside the retort during cooling, leading to expansion of the pouch contents. Hence, the pouches had to be filled with as little as 200 g of product instead, to avoid rupture of all pouches during cooling. The lower degree of filling probably lead to a slightly faster heat transfer than would otherwise have been the case, and also increased difficulties of determining the cold point due to a more irregular shape of the container.

The pouch material used is similar to or better compared to those used in similar studies. For example it has a water transmission of 0.00 cc/m^2. This can be compared with e.g. the 0.18 g/m^2 for the pouches used by Rajkumar *et al.* (2010) and 0.21 g/m^2 for the pouches used by Mohan *et al.* (2008). According to Rajan *et al.* (2011), a low water vapour transmission is an indicator of suitability of the pouches for retort processing. It is also positive for the shelf-life of the product.

The strength of the pouch and seal is important for avoiding bursting during processing or handling. The seal strength is also an indicator of shelf-life (Rajan *et al.*, 2011). The heat seal strength of the pouch used is 6.80 kg/15mm if a sealing temperature of 220 °C is used. This can be compared with the pouches used by Rajkumar *et al.* (2010), for example, which showed a heat seal strength of 5.9-6.5 kg/15mm.

4.5 pH and water activity

The results from measurements of pH and water activity (a_w) are shown in Table 7. As expected, these parameters have values allowing growth of proteolytic *C. botulinum* (pH >4.6 and a_w >0.94), both before and after heat treatment. The results support the use of a high temperature treatment for reduction of bacterial spores.

Table 7. Analysis results for pH and water activity

Sample	pH	Water activity
Before heat treatment	6.10, 6.10	0.96, 0.96
9 min at 121 °C	6.11	0.96, 0.97
12 min at 121 °C	6.07 (6.08*)	-
15 min at 121 °C	6.07 (6.06*)	-

*Measured after 30 days of storage. The other measurements were made right after processing.

The results indicate that pH did not change much due to retort processing. This was a bit surprising; a small increase was expected. Mittal and Blaisdell (1984) reported an increase of 0.16-0.30 pH units during cooking. Vasanthi *et al.* (2007) reported increased pH as temperature and time of cooking was increased. Rajkumar *et al.* (2010) reported an increase in 0.4 pH units during retorting of Chettinad goat meat curry. An increased pH is a result from splitting of hydrogen bonds during heating, releasing positive charges (Rajkumar *et al.*, 2010). On the other hand, heating also leads to formulation of new hydrogen bonds. Equilibrium between splitting and formulation of hydrogen bonds might be the reason for the lack of change in Gaeng Phed Gai.

The pH in Gaeng Phed Gai did not change during the first 30 days of storage, though it is likely that it would decrease during a longer time of storage. Such a development was observed by Bindu *et al.* (2010) for Fish Peera, by Prince Devadson *et al.* (2010) for buffalo meat block, by Rajkumar *et al.* 2010 for Chetti-

nad goat meat curry, and by Rajan *et al.* (2011) for Chettinad chicken. The commonly observed decrease in pH might be due to degradation of proteins and liberation of free amino acids (Rajan *et al.*, 2011).

4.6 Nutritional values

The analytical results for nutritional values of the heat treated product are presented in Table 8. These figures should be interpreted as indications rather than absolute values, because the procedure of pouch filling resulted in some variation in proportions of the different ingredients, and the number of analyzed samples was low.

Table 8. Average nutritional values (± STD) for the product heat-treated for9 min at 121 °C

	Value per 100 g	Analytical method
Calculated energy content (kJ)	416 ± 39	Calc. acc. to SLV FS 1993:21
Calculated energy content (kcal)	99 ± 9	Calc. acc. to SLV FS 1993:21
Protein (g)	15 ± 3	Mod NMKL nr 6, Kjeltec
Carbohydrates (g)	2.0 ± 1.6	Calc. acc. to SLV FS 1993:21
Fat (g)	3.5 ± 0.4	NMKL 131, Lidfett0A.10
Ash (g)	2.2 ± 0.1	NMKL 173 2nd ed
Water content (g)	78 ± 2	NMKL 23, 1991

As expected, the protein content is relatively high and the contents of fat and carbohydrates low, due to a high proportion of chicken in the product.

An inexplicably large standard deviation was observed for carbohydrates. Coconut milk is the clearly dominating source of carbohydrates, but a difference in coconut milk content should be reflected in fat content as well, for which it dominates even more.

4.7 Sensory analysis

The sensory scores of the untreated product and the heat-treated product are shown in Table 9. The average responses for all sensory attributes were within the range "neither like nor dislike" to "like very much". The averages of the two samples differed by 0-1.4 units on the 9-point scale, depending on the attribute being evaluated. Some statistically significant differences were identified. The product which had not undergone any heat treatment received significantly better results

for taste, juiciness and overall acceptability. A common comment was that the chicken was a bit dry in the heat-treated product. However, the crispiness of the bamboo shoots was well preserved. Some of the panellists identified an unfresh smell of the heat-treated sample. The comments also indicated that some of the flavour of spices was lost during heat treatment, especially in the chicken pieces. The latter could be compensated by more seasoning before heat treatment.

Table 9. Sensory scores of Gaeng Phed Gai

	Appearance	Color	Taste	Juiciness	Texture	Overall acceptability
Avg. score of untreated product	6.6	6.4	7.6	6.8	6.9	7.2
Avg. score of heat-treated product	6.3	6.4	6.3	5.4	6.1	5.8
Avg. difference of the two samples	0.3	0	1.3	1.4	0.8	1.4
Std of the difference of the two samples	1.16	0.82	1.49	1.26	1.62	1.56
t-value	0.82	0	2.75*	3.50*	1.56	2.81*

*Statistically significant difference. The t-value exceeds the upper α-critical value of the t-distribution with n-1 degrees of freedom; $t_{0.05,\,9} = 1.833$.

The findings from the sensory analysis are in line with what was shown by the cook value described earlier. The significant sensory difference is probably a reflection of the severity of the heat treatment, which is worse than what would be the case if a faster retort had been used. In other words, the slow heating and cooling of the retort is clearly reflected in the sensory properties. It is impossible to tell what the result had been with a faster retort, but it would probably have been better.

Researchers using better retorts for processing similar products have shown better results. Gopal *et al.* (2001) evaluated Kerala style fish curry and showed an overall acceptance of 8.0 on a 10-point scale right after heat treatment, which decreased to 7.5 after 12 months of storage. Rajkumar *et al.* (2010) evaluated appearance, colour, flavour, juiciness, texture and overall acceptability for Chettinad goat meat curry, showing scores of 8.0-8.4 on a 9-point hedonic scale after heat treatment. Rajan *et al.* (2011) evaluated appearance flavour, juiciness, texture, saltiness, mouth coating and overall acceptability of Chettinad chicken, and showed results of 7.5-7.8 right on an 8-point hedonic scale right after heat treatment.

The method shows that differences exist in some characteristics of the heat-treated and untreated products, but one should be careful not to draw the conclusions too

far. The results can be used as an indication of consumer opinions, but one cannot be entirely sure that the results are in line with the opinions of consumers in general. The semi-trained panel used in this study consisted of 10 assessors, and no selection on Thai food usage had been made. Consumer tests generally involve 100-500 target consumers from three or four cities (Meilgaard *et al.*, 2007). To be confident of the consumer acceptance of Thai food in retort pouches, such large consumer tests should be conducted later in the development process.

The development process can also include other sensory tests to map the characteristics more specifically and identify improvement potentials. Descriptive profiling by a panel trained on Thai food is one example of a test that can become helpful in optimizations of product formulations and process details.

5 Conclusions and recommendations

Results from temperature measurements and microbiological tests show that the product is commercially sterile after the heat treatment. Unfortunately, the sensory quality of the foods is insufficient. The calculation of cook value supports the conclusion that the product received a too drawn-out heat treatment. However, results from the literature studies shows that it is possible to produce food consisting of large pieces in liquid medium in retort pouches, retaining an acceptable quality for extended periods of storage at ambient temperatures. The conclusion is that it should be possible to produce shelf-stable Gaeng Phed Gai in retort pouches with a good quality.

The main issue of this study was the retort. It was too slow in heating, did not allow manual setting of the overpressure and did not have any cooling water system. Further tests must be performed in a retort allowing very fast heating, using steam, and very fast cooling, using cooling water. The retort must allow retention of high pressure during the whole process, including the cooling phase, to avoid rupture of the containers. It is recommended that further tests and future large-scale production should be done with a retort that allows agitation of the containers. Agitation results in a faster heat transfer, shortening the processing time and resulting in a better product quality.

It is recommended that any thermal process of Gaeng Phed Gai has the target process lethality (F_0) 10 minutes. Such a heat treatment gives a probability of *C. botulinum* survival of $<10^{-9}$, with a significant margin of safety, and a probability of economical spoilage of $<10^{-4}$. If future practical tests of spore levels in the raw materials show low levels of contamination, or a low heat resistance of the existent spores, a process lethality of as low as 6 minutes can be used to achieve a product with better sensory and nutritional qualities.

Many of the results from the practical tests in this study should be seen as indicative values, because a relatively low number of samples were analysed in this first round of tests. When a process leading to a better sensory quality has been established, more extensive sensory tests with large numbers of consumers should be conducted for a thorough evaluation of consumer acceptance. More exact evaluations of nutritional values should also be performed by analysis of a larger number of pouches. Shelf-life studies will be required to determine how long the product retains an acceptable sensory quality. Microbial status should be evaluated throughout the whole shelf-life, because recovery of heat damaged spores can be slow. If the company's assortment of retort pouch products will consist of several Thai dishes, tests are required for every dish, as processing results depend on food composition.

For a commercialized product, consumers should be aware of the fact that a cool storage is advantageous for the product. Use of a statement such as "store away from direct sunlight" is appropriate. The reasons are that temperature is a major determinant of the rate of quality degrading reactions, and that storage at temperatures above 43 °C can cause germination and growth of extremely heat-resistant thermophilic bacteria which may have survived the process.

To sum up, the author believes in the concept and is almost certain that it will be possible to produce Thai food with a good quality in retort pouches. It is likely that ready-meals in retort pouches will soon be a part of the Swedish food market, as it is a concept with many advantages.

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