NUTRITIONAL AND QUALITY CHANGES IN A SOUS VIDE PRODUCT PASTEURIZED AT VARIOUS TEMPERATURES

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NUTRITIONAL AND QUALITY CHANGES IN A *SOUS VIDE* PRODUCT PASTEURIZED AT VARIOUS TEMPERATURES

by

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ABSTRACT

A test product using harp seal (*Phoca groenlandica)* meat (Seal Meat Shepherd's Pie) was developed and processed by the *sous vide* method. Changes in nutrient content were tested after the product was pasteurized at five different time/temperature schedules (65°, 70°, 75°, 80° and 85°C for 105, 60, 43, 35 and 30 min respectively) and were also tested after the product was prepared by the conventional method of cooking. A non-pasteurized sample acted as control. The nutrients tested were thiamin, fatty acids, protein and amino acids. Percent fat and moisture content and pH and water activity were recorded for all the variations studied. Results showed small variations in the amounts of nutrients as a result of some of the treatments when compared statistically with the non-pasteurized sample. Microbial changes in the product processed at the 85°C for 30 min pasteurizing schedule, non-pasteurized product and conventionally prepared samples were monitored. Changes in the texture of the meat were tested after the heat treatments and in the non-pasteurized samples. Optimum pasteurization schedules were determined for some of the factors as follows: for Ω-3 fatty acids (65°C for 105 min), for amino acids (85°C for 30 min) and for texture (70° to 80°C for 60 to 35 min). The pasteurized product did not show any increase in the growth of aerobic and anaerobic bacteria when stored at both 2.2°C and 8°C for 21 days.

For

Kevin

Geoff, Jill and Tim

and Mom

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TABLE OF CONTENTS

PAGE

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS AND SYMBOLS

ANOVA	analysis of varience
a_w	water activity
AOAC	Association of Official Analytical Chemists
C	quality degradation rate
CFU	colony forming unit
CV	conventionally prepared product
EFA	essential fatty acid
D	decimal reduction time
EST	estimated number of microorganisms
F	thermal destruction rate of microorganisms
NP	non-pasteurized product
MUFA	monounsaturated fatty acids
PUFA	polyunsaturated fatty acids
SFA	saturated fatty acids
SMSP	seal meat shepherd's pie

1. INTRODUCTION

1.1. *Sous vide* technology

In response to consumer demands for high quality, easy to prepare and fresh tasting convenience foods, food scientists have been applying their knowledge to provide an increasing array of new, partially processed, minimally preserved, nonsterile, high quality extended shelf-life foods (Rhodes, 1991). *Sous vide*, meaning "under vacuum", has been a method of controlled atmosphere food processing in use since approximately 1978. The process is also known as cuisine en papillote *sous vide*, cuisson *sous vide* and *sous vide* cook-chill (SVCC) (Mason *et al.*, 1990; Church and Parsons, 1993).

1.1.1. *Sous vide* methodology

For the *sous vide* method, a raw or partially cooked portion of food is placed in a pouch or rigid tray. After removal of the air, the package is sealed. Using steam or hot water, the packaged food is then subjected to a pasteurization temperature range of 65° to 90°C (with corresponding time periods), followed by immediate chilling to 3°C or lower. When it is processed under highly controlled conditions of preparation, packaging, time and temperature, the food will remain fresh-tasting and safe for consumption for up to 28 days if stored below 3°C. Tests on the sensory quality of cook-chill and *sous vide* products have been extensively reviewed by Mason *et al.* (1990) and results indicate the superior quality of *sous vide* processed foods in comparison to foods prepared by other cook-chill

non-vacuum methods. Mason *et al.* (1990) concluded that, "Conventional cook-chill can be enhanced in terms of both product quality and storage life by the application of vacuum techniques". The complete technique of food preservation using the *sous vide* method has been described in detail by Schafheitle (1990).

Only high quality, food grade, oxygen impermeable, flexible pouches or bags, or thin rectangular trays should be used for vacuum cooking and storage (see Figure 1.1. which shows an example of the semi-rigid tray system). The thin profile geometry of the containers allows rapid heating and cooling of the product, a process which cannot be realized with cylindrical metal cans (Ghazala *et al.*, 1995). The permeability of the plastics and films used is affected by the composition, temperature, relative humidity and integrity of the films and plastic containers used (Beauchemin, 1989). The *sous vide* process involves heat treatment in a water saturated atmosphere, followed by a relatively long storage period at refrigerated temperatures and this, plus the chemistry of the food, determines the selection of the ideal plastic. The plastics used are multi layered, by co-extrusion or with adhesives, to maintain barriers after thermal processing. The integrity of the heat seal is just as important as the choice of plastic materials for barrier purposes (Campbell, 1993).

1.1.1.1. Advantages and disadvantages of *sous vide* technology Eighteen years is a relatively short length of time for a new food technology to become established and, as well as the accolades it has received, it has also had some setbacks. Since its introduction in France by Georges Pralus, it has continued to remain popular at many food establishments and food chains in Europe. Lioutas (1988) has considered possible reasons

Figure 1.1. Picture of a sous vide product in a single portion semi-rigid tray.


Seal Shepherd's Pie

why controlled atmosphere packaging has been applied so successfully in Europe but is still in its infancy in the U.S., listing the advantages of geographically smaller countries/distribution systems, frequency of consumer shopping and higher awareness/acceptance of refrigerated foods in Europe versus non acceptance in the U.S. of the higher price associated with *sous vide*, plus the absence of large food chains dedicated to high quality refrigerated foods as in Europe.

Advantages: *Sous vide* food processing has many advantages over traditional food processing methods: 1) vacuum packaging removes oxygen and thus retards growth of aerobic bacteria and reduces the effect of deterioration due to oxidation of fats and vitamins, 2) because the foods are fully or partially cooked after sealing in the pouch or bag, juices and volatile flavours are retained, as are water soluble nutrients, 3) foods prepared by the *sous vide* method usually do not have any preservatives, a positive aspect for many consumers, and 4) when prepared at controlled central locations, *sous vide* has the potential to consistently deliver superior cuisine on a large scale to the food industry, or on a smaller scale, to the individual consumer (Rhodehamel, 1992).

Disadvantages: The main concern with *sous vide* technology is with the potential health hazard of improperly prepared foods. The psychrotropic food-borne pathogens, especially *Clostridium botulinum*, are of particular concern because the methods of preparing, distributing and storing provide prime areas for contamination. Rhodehamel (1992) lists four concerns related to the technique. These concerns are: 1) *sous vide* products are generally formulated with very little or no preservatives and even salt may be omitted, 2) the products receive minimal thermal processing, i.e. are only pasteurized, 3)

the anaerobic environment created by the removal of oxygen encourages the growth of anaerobic foodborne pathogens if the product is not stored at correct refrigeration temperatures, and 4) it is difficult to monitor temperature control of the product once it leaves the processing plant, and temperature abuse may occur.

The advantages of the *sous vide* method can only be realized if precautions are taken at each step of the process to prevent or minimize microbial contamination. An important step to include in *sous vide* production is the calculation and use of the *D* (decimal reduction time) value, i.e. the time (min) at a specified temperature needed to reduce a microbial population by 90%.

1.1.2. Microbiological considerations

The most important determining factors to ensure safety during the shelf life of *sous vide* products are: the initial concentration of bacteria in the raw materials, and refrigeration that is properly controlled. In addition, factors that will increase the safety of the food are: a high thermal destruction rate of microorganisms (*F* value) during the heat treatment; pH; water activity (a_w); relative humidity; and expert packaging (Beauchemin, 1990). For food products that cannot tolerate a high processing temperature, control of *C. botulinum* can be improved by using a lower storage temperature combined with a food pH of 5.0 or lower, an a_w of 0.97 or lower and/or a salt concentration of 3.5% or higher (Betts and Gaze, 1995).

The *F* value is affected by a number of factors. These factors include the initial concentration of bacteria before heat treatment, the severity of the heat treatment in

relation to time and temperature, the thermal diffusivity into the food, the geometry of the package and the presence of entrapped gas in the package, the type of heat treatment and the thermal resistance of the bacteria (Beauchemin, 1990). The cooking process has to be planned to utilize the maximum microbiological destruction rate with no adverse effects on the quality, by considering the value of F and the value of the quality degradation rate (C). The temperatures achieved during the heating period are monitored using a probe or thermocouple in the product.

1.1.2.1. Shelf life studies Shelf life studies measure the physical, chemical, microbiological and sensory changes during controlled storage conditions. The time when the quality or sensory attributes of the food product become unacceptable is the maximum shelf life of the food product (Burn and Prentice, 1994).

The most important factors affecting the shelf life of food are the content of microorganisms and the metabolites they produce during their growth. The metabolites, such as organic acids, can cause chemical and sensory changes or physical changes (eg. swelling of packed foods from carbon dioxide production). Each food product, either raw or manufactured, has a different microbiological quality standard. It is generally accepted that meat spoilage occurs when the number of colony forming units (CFU) of bacteria reach 10^7/g (Stiles, 1991; Bailey *et al*, 1993; Burn and Prentice, 1994). Therefore, a shelf life study on products containing meat should continue until this value is reached.

1.1.3. Nutritional aspects

Apart from the better organoleptic quality obtainable through retention of flavour and juiciness in the sealed *sous vide* package, a positive effect on retention of nutrients would be expected, since this method offers the possibility of retarding both the oxidation and the leaching of water soluble vitamins (Petersen, 1993). However, according to Creed (1995) and Church and Parsons (1993), there is little objective evidence to support the theory that high quality taste and texture means an automatic high nutrient retention. Nutritional labelling of processed foods has become mandatory in many countries and of interest to many consumers. Therefore it is important to know which nutrients are affected by the *sous vide* method. Various experimental techniques have been used to analyse vitamin retention in meal components. Only a few analyses have been made of prepared dishes, including those prepared by the *sous* vide method (Creed, 1995). When research on combination foods has been reported, the temperatures used for processing have usually been >100°C. Pasteurizing temperatures used in the *sous vide* technique do not reach this level.

While a certain amount of work has been done on vitamin retention during *sous vide* processing, little or no attention has been paid to retention of fatty acids and amino acids (Bognar *et al.*, 1990) in *sous vide* products. No change in the content of protein, fat, carbohydrate and minerals is expected (Creed, 1995). Smith and Alvarez (1988) reported that amino nitrogen, non-protein nitrogen and pH did not change during storage of vacuum packaged turkey breast rolls. In chilled and pasteurized chilled meals, it has been reported by Bognar (1990) that no significant changes were found in the content of

protein, fat, carbohydrates and minerals after 10 and 28 days storage at 2°C, followed by reheating. Fatty acids did not change and only sulphur containing amino acids showed slight change. There is need for more research in the area of fatty acids and amino acids in *sous vide* products.

1.2. Harp seal (*Phoca groenlandica*) meat

Harp seal (*Phoca groenlandica*) is the most abundant species of seal in the Atlantic region of Canada, particularly off the coast of Newfoundland and Labrador, where its population has been estimated at 3 million during the winter months (Stenson, 1995). Seal meat is a nutritious meat which is underutilized as a human food source. Seal meat constitutes a large portion of the native diet in Northern Canada (Kuhnlein and Soueida, 1992; Wein, 1994). However, seal meat consumption by non-native Canadians is extremely limited.

1.2.1. Fat composition of harp seal meat

The fat content of harp seal meat ranges from 1.8 to 3.7% (g/g, wet muscle mass) depending on the sex of the animal and time of year (Beck *et al.*, 1993; Shahidi and Synowiecki, 1993; Gales *et al.*, 1994). Furthermore, the fat is composed of a higher percent of polyunsaturated fatty acids (PUFA) than meat from land animals. Pork and beef meat contain, respectively, 10.4 and 6.2% PUFA (% of total fatty acids) compared to 24.6% PUFA for seal meat. Saturated fatty acids in seal meat are approximately 17% of

total fatty acids, compared to 37% and 47% in pork and beef respectively. Monounsaturated fatty acid (MUFA) levels are similar in seal meat, pork and beef (Shahidi and Synowiecki, 1993). The omega-3 (Ω-3) fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are present in seal meat but not in meat from land animals. The skeletal muscle fat of the adult female harp seal contains from 2.6 - 7.4% EPA and from 2.5 - 8.0% DHA on a total fatty acid basis (Engelhardt and Walker, 1974). Much attention has been focused on these long chain fatty acids as having a possible therapeutic use in preventing certain heart conditions (Holub, 1990; Kinsella, 1986; Turley and Strain, 1993).

Oxidation of PUFAs proceeds at a faster rate than does that of saturated fatty acids (SFAs) (Nawar, 1985), so that intramuscular fat in raw seal meat would be expected to deteriorate more quickly than the saturated fat from beef or pork. The primary products of fat oxidation, hydroperoxides, decompose to produce a range of secondary products such as aldehydes, ketones and alcohols which give undesirable flavours to the fat and hence, the meat. Much of the strong flavour and odour attributed to seal meat is the result of buildup of these byproducts in the fat. Properly handled fresh or fresh frozen raw and cooked seal meat should benefit from the vacuum packaging involved with *sous vide* processing.

1.2.2. Protein and mineral composition of seal meat

Table 1.1. lists the protein and minerals found in 100g of various cooked high biological value protein foods. Seal meat values for iron from two species of seal indicate a very high content of this mineral in comparison with land animal values.

1.2.3. Use of seal meat for the test product

Numerous combination dishes can be prepared using the meat of harp seal. Use of the *sous vide* technique to produce these food products has not been investigated. It is suspected that this processing form, which considerably reduces the amount of oxygen present, would be a highly acceptable method for seafood preparation. In addition, combination of the seal meat with other ingredients would reduce the contribution of any strong flavour from the meat. If the meat dishes were prepared in a controlled setting, using high quality ingredients, convenience mixed dishes containing seal meat could provide a way to utilize this nutrient dense meat. A traditional mixed dish containing meat was chosen for this research on quality and nutritional changes. Seal Meat Shepherd's Pie, (SMSP) with ground seal meat, gravy and vegetables, contains 24% seal meat. It is one of many dishes that can be used as a vehicle for this meat.

Table 1.1. Protein and minerals found in 100 g of various cooked high biological value protein foods.

Meat or Fish (100 g)	Protein (g)	Iron (mg)	Copper (mg)	Magnesium (mg)	Calcium (mg)	Manganese (mg)
Seal, harp*	23.2	64.6	0.10	34	591***	<0.01
Seal, ringed **	34.1	27.1	0.21	32	14	0.02
Beef (flank)	28.1	3.5	0.12	24	6	0.02
Pork (loin,lean)	28.5	1.1	0.08	21	6	0.02
Chicken (dark,w/o skin)	23.3	1.3	0.07	20	11	0.02
Salmon (pink, canned)	19.7	0.8	0.11	34	213***	-
Oysters (canned)	7.1	6.7	4.46	54	45	-

Source: Pennington, J.A.T. (1994)

*Harp seal. Source: Shahidi, F. and Synoweicki, J. (1993)

**Ringed seal. Source: Kuhnlein, H.V. and Soueida, R. (1992)

***calcium high due to ground/softened bone throughout flesh

1.3. Fats and fatty acids

1.3.1. Composition of fats and fatty acids

Most fats and oils can be classified as mixed glycerides. A food fat or oil is a combination of three fatty acids on a glycerol molecule and is called a triacylglycerol. Many of the properties of food fats are a reflection of their fatty acid components, the arrangement of the fatty acids on the glycerol and interactions between the fatty acids.

The most common and important fatty acids contain between 12 and 22 carbons and are found in many different plant and animal fats. In general, the fatty acids have an even number of carbon atoms. The naturally occurring fatty acids can be grouped on the basis of the number of double bonds in the fatty acid chain. Fatty acids containing a single double bond between two of the carbon atoms in a fatty acid chain are referred to as monounsaturated fatty acids (MUFA). If a fatty acid contains two or more double bonds it is referred to as a polyunsaturated fatty acid (PUFA) (deMan, 1992). In PUFAs, the double bonds are arranged in the *cis* configuration in most naturally occurring fats. The *trans* configuration of double bonds occurs mainly in fats that have undergone the hydrogenation process.

Long chain fatty acids having the first double bond occur at carbon 3 or carbon 6 from the methyl end of the fatty acid chain are referred to as Ω-3 or Ω-6 fatty acids. Fatty acids belonging to the Ω-3 and Ω-6 families of PUFAs cannot be synthesized *de novo* by animals. Because they are important components of cell membranes and serve as precursors for a number of biologically active compounds, some of these fatty acids are

considered to be essential fatty acids (EFA). However, because all but linoleic acid (C18:2 n-6) and linolenic (C18:3 n-3) can be provided by conversion of other fatty acids, these two are the only true EFAs.

1.3.2. Dietary requirements for fat and fatty acids

Dietary lipids or fats are necessary to the body for a number of reasons. They serve as concentrated sources of energy and a source of essential fatty acids. They also act as carriers of the fat soluble vitamins. Keen interest in fat in the diet has been ongoing for the past two to three decades. The average intake of fat (as a percentage of total energy) in Canada is 38% (Health and Welfare Canada, 1990) and in Britain is 42% (British Nutrition Foundation, 1992). The recommendations from the National Research Council (NRC) (1989) of the USA, as cited by the British Nutrition Foundation (1992), and from Health and Welfare Canada (Nutrition recommendations. Report of the Scientific Review Committee, 1990) are for 30% of energy from fat compared to 33% recommended by the British Committee on Medical Aspects of Food Policy (British Nutrition Foundation, 1992).

Not only has an interest developed in the total fat content of foods, but the type of fat has importance. Recommendations for the type of fatty acids to be included in the diet have also been made. A balance of unsaturated fatty acids:saturated fatty acids, in a ratio of 2:1, is recommended (Health and Welfare Canada, 1990). Recent investigations have shown that dietary *trans* fatty acids may adversely affect plasma cholesterol risk factors for heart disease (Mensink and Katan, 1990; Mensink *et al.*, 1992; Troisi *et al.*, 1992;

Zock and Katan, 1992; Wood *et al.*, 1993; Judd *et al.*, 1994). Although foods containing *cis* double bonds are recommended over foods that are high in *trans* double bonds, no specific ratio of *cis* to *trans* fatty acids in the diet has been recommended (Health and Welfare Canada, 1990). The governments of many nations have made recommendations to the public regarding the issue of dietary fat reduction and fat and fatty acid intakes, with improved health benefits being the driving force behind these directives (British Nutrition Foundation, 1992).

Evidence supporting a beneficial role for long chain, highly unsaturated, Ω-3 fatty acids in the diet continues to accumulate (Kinsella, 1986; Nelson and Ackman, 1988; Ackman, 1990; Holub, 1990; Nettleton, 1991). Epidemiological studies have suggested that the consumption of fish and/or oils containing EPA and DHA can reduce the risk of arterial thrombosis and thus offer protection against cardiovascular disease (Turley and Strain, 1993). It has been suggested that the position of the Ω-3 fatty acids, i.e. sn1, sn2 or sn3, on the triacylglycerol molecule is important and that marine mammals have a more favourable arrangement than do fish (Ackman, as cited in Kyle, 1991). Nutrition recommendations from Health and Welfare Canada (1990) suggest that Ω-6 fatty acids should be present in the diet providing at least 3% of energy, and that Ω-3 fatty acids should be included in the diet at 0.5% of total energy content. Except for infants, the health risk of underconsuming adequate amounts of fatty acids is far less than the health risk of overconsuming them.

1.3.3. Fat deterioration

In addition to chemical analysis of foods and food fats for fat and fatty acid content, research is ongoing into the chemical and physical changes to fatty acids that occur when foods are processed and formulated. In spite of the recommendations for fat reduction, foods continue to be manufactured with high percentages of fat. The question arises as to whether the fats and fatty acids that may be declared on the label are actually present in the form and amount listed. Changes that can occur to fats during preparation and storage include lipolysis, oxidation and thermal decomposition (Nawar, 1985). Of these, the main concern is with oxidation which can proceed during both food production and distribution whether or not the food is heated or unheated during preparation. Lipid oxidation is one of the major causes of food spoilage. It leads to the development of off-flavours and off-odours (rancidity) and these changes in the fat can lead to a decrease in the nutritional quality of the food. Fats that are high in unsaturated fatty acids are particularly susceptible to oxidation which, once it is initiated, proceeds rapidly.

1.3.4. Oxidation

The reaction with oxygen is referred to as autoxidation (Nawar, 1985; Cosgrove *et al.*, 1987) and can be precipitated by any number of catalysts. The production of free radicals from oxygen and the fatty acid is thermodynamically difficult, requiring an activation energy of about 35 kcal/mol (Nawar, 1985). Extending the induction period, which is prior to activation and during which time oxidation has not yet begun, is important for the overall control of oxidation. The first few radicals necessary to start the propagation

reaction are produced through some catalytic process. Metal catalysis and/or exposure to light are most often implicated in the reaction, although more recently, it has been suggested that singlet oxygen is the active species involved, with plant and animal pigments such as chlorophyll or myoglobin acting as the sensitizers. Once there are sufficient free radicals to begin the chain reaction, hydrogen ions are removed from the unsaturated fatty acid chains at the allylic position. Oxygen is added at this location with the result that peroxy radicals form and these in turn remove hydrogen from the α-methylenic groups of other molecules to yield hydroperoxide and free radical groups. The free radicals react with oxygen to continue the sequence described. There is usually a shift in the position of double bonds during the reaction, resulting in isomeric hydroperoxides. Hydroperoxides are unstable and enter into numerous and complex breakdown reactions resulting in a variety of products, some of which are responsible for flavour and odour changes in the food (Nawar, 1985).

Delaying the activation or initiation step prolongs the shelf life of the product. Anything that can be considered to catalyze the reaction should be controlled. Elimination of as much oxygen as possible removes a main factor in the equation.

1.3.5. Factors which initiate oxidation of fatty acids

Factors that would affect oxidation in a food product include moisture content, metal catalysts and temperature.

1.3.5.1. Water activity (a_w) It has been shown that oxidation rates depend strongly on the water activity of foods. An a_w of 0.1 to 0.3 induces rapid oxidation, but at activities

from 0.3 to 0.5, the oxidation slows down. Above 0.5, oxidation proceeds at a more normal rate (Nawar, 1985).

1.3.5.2. Metals The transition metals, such as cobalt, copper, iron, manganese and nickel, are major pro-oxidants. If present in amounts even as low as 0.1 ppm, they can decrease the length of the induction period and increase the rate of oxidation (Nawar, 1985).

Iron is present in relatively high amounts in seal meat. As reported by Rhee (1989), non-heme iron, released from meat that has been cooked, appears to accelerate lipid oxidation, but it is not known whether some fatty acids are more susceptible to this than others. Rhee and Ziprin (1987) found that the higher total pigment and myoglobin concentration in beef compared to chicken and pork correlated with increased lipid oxidation. Ahn *et al.* (1993) found that a combination of antioxidant, in particular metal chelators, plus vacuum packaging of hot cooked turkey patties provided better protection from oxidation than cold packaging. Ringed seal meat (*Phoca hispida)* contains approximately 6 to 10 times more iron than does beef (Kuhnlein and Soudeida, 1992; Pennington, 1994) (see Table 1.1.) and the iron could be expected to have an effect on lipid oxidation. Iron content of harp seal (*P. groenlandica*) has been recorded at even higher levels than ringed seal meat (Shahidi and Snyoweicki, 1993).

1.3.5.3. Temperature

The rate of oxidation increases as the temperature is increased. Temperature is also important in terms of the effect of oxygen partial pressure on the rate of oxidation. As the temperature increases, the increase in rate with increasing oxygen concentration becomes

less evident, since oxygen becomes less soluble as the temperature is raised (Nawar, 1985).

1.4. Thiamin

Thiamin (Vitamin B1) was officially discovered in 1897 by Christian Eijkman (Gubler, 1984). Eijkman, observing poultry fed polished rice and noticing the appearance of symptoms similar to those of beriberi in humans, wondered if there was a connection between refining of cereal products and the symptoms of weakness and polyneuritis in humans. He and his associate, Gerrit Grijns, fed kitchen scraps, and later rice bran or polishings, to the birds and found that they improved dramatically. From these findings Eijkman and Grijns were able to establish that a substance in the bran and germ prevented the symptoms from occurring. In 1911, Casimir Funk isolated what was known as the antiberiberi principle and, because it was determined that it contained an amine, referred to it as a "vitamine", the name that was eventually adopted in general to designate the whole class of trace nutritional factors. After isolation and crystallization of the active substance from rice bran by B.C.P. Jansen and W. Donath in 1926, Robert R. Williams and his group, under the auspices of Capt. E.B. Vedder, succeeded in presenting a chemical formula in 1936. As well, they proposed the name of thiamine, also accepted with the spelling, thiamin.

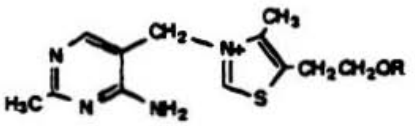
Thiamin is present in practically all plant and animal tissues, however its content in many foods is small and food preparation may result in considerable losses. Thiamin is essentially absent in fats and oils and in any highly refined foods. Considerable variation in recorded values is evident when comparison is made of thiamin values from different information sources.

Health and Welfare Canada (1990) recommends 0.4 mg thiamin/1000 kcal (0.48 mg/5000 kJ) energy per day. Recommendations are based particularly on energy from carbohydrate sources.

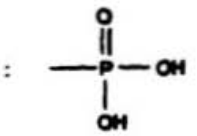
1.4.1. Chemical structure and forms of thiamin

The chemical structure of thiamin is shown in Figure 1.2. Williams and his group studied the molecular structure of thiamin and found that thiamin can be cleaved at the methylene bridge into a pyrimidine derivative (2-methyl-4-amino-5-hydroxy methylpyrimidine) and a substituted thiazole ring (4-methyl-5-hydroxyethylthiazole) (Gubler, 1984; Lambert and deLeenheer, 1992). About 80-90% of the total thiamin, i.e. thiamin plus thiamin phosphate ester content in cells, is thiamin pyrophosphate (TPP), the coenzyme form of thiamin. In some animal tissues, thiamin triphosphate (TTP) is present in amounts of 70-80% total thiamin, (Kawasaki, 1992). In plant products, thiamin occurs predominantly in the nonphosphorylated, or free, form (Gubler, 1984).

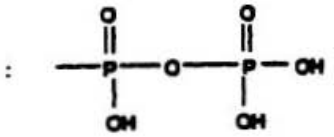
The double salt form of thiamin with hydrochloric acid ($C_{12}H_{18}Cl_2N_4OS$; molecular weight 337.28) is soluble in water, slightly less soluble in methanol and glycerol, nearly insoluble in ethanol and insoluble in ether and benzene (Merck Index, 1983). This



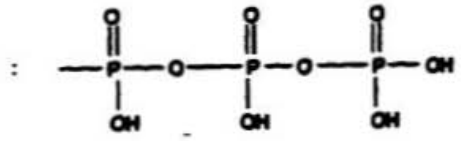
R: H — Thiamin



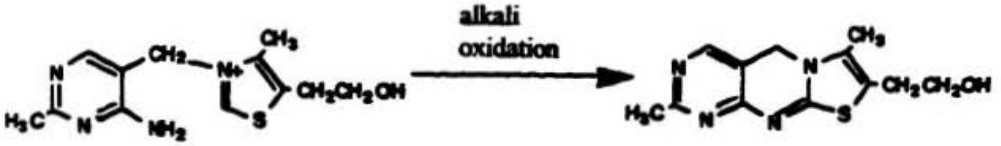
Thiamin monophosphate



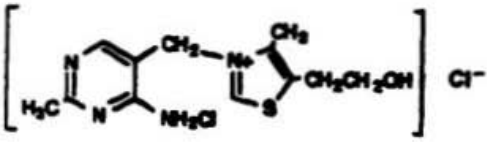
Thiamin diphosphate (pyrophosphate)



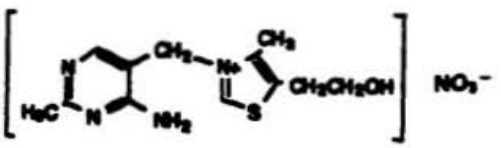
Thiamin triphosphate



Thiamin — Thiochrome



Thiamin hydrochloride



Thiamin mononitrate

Figure 1.2. Chemical structures of thiamin and its conversion to thiochrome.

form of thiamin is used most often in experimental studies. The more stable form, thiamin mononitrate ($C_{12}H_{17}N_5O_4S$: molecular weight 327.36), is most often used in fortification of foods, especially enrichment of flour mixes.

1.4.2. Physiological functions of thiamin

The physiological functions of thiamin were first demonstrated in 1936 by R.H.S. Thompson and R.E. Johnson who showed a correlation between a high blood pyruvate level and Vitamin B1 deficiency. Soon after, R.A. Peters and coworkers demonstrated that thiamin was essential for carbohydrate metabolism (Gubler, 1984).

Thiamin must be converted into thiamin phosphates before exerting its physiological function as a coenzyme in cells. Thiamin pyrophosphate functions as a coenzyme for several important enzymes in carbohydrate and amino acid metabolism (Ottaway, 1993). Many of these reactions are important for the generation of an adequate supply of ATP and so a deficiency of the active form of thiamin will affect those tissues that have a high energy requirement, such as nerves, muscles and heart. During a deficiency of thiamin, the main pathology occurs primarily in these areas. Thiamin deficiency is known to affect nerve function, especially in peripheral nerves. The disease state, known commonly as beriberi, has been associated with those countries of the world which rely heavily on refined grain carbohydrates as the chief food energy source. In industrialized countries, deficiency is usually associated with alcoholism, due to the poor dietary intake of thiamin and impaired absorption, metabolism and storage. Thiamin

deficiency, with central nervous system involvement, results in Wernicke Korsakov syndrome.

1.4.3. Determination of thiamin

Thiochrome is quantitatively formed from thiamin by alkaline (pH >8) oxidation with cyanogen bromide or potassium ferricyanide (Figure 1.2.). Thiochrome is a highly fluorescent blue compound with an excitation maximum of about 365 nm and an emission maximum close to 435 nm.

1.4.4. Factors affecting availability of thiamin

Temperature, pH and time of heating, processing and storage, are the most important factors contributing to the loss of thiamin in food products (Dwivedi and Arnold, 1973). Investigations into the effects of sulphur, iron, copper and phenols on thiamin stability have been done and have shown the difficulty of working with real and complex food systems. In addition, water activity, light and oxygen interactions are factors to consider in the degradation and analysis of thiamin.

1.4.4.1. pH Destruction of thiamin is more rapid in alkaline than in acid conditions (Dwivedi and Arnold, 1973). When thiamin solutions at pH 7.0 and above were heated, hydrogen sulfide was a major product. Heating of thiamin solutions at pH 6.0 or below resulted in cleavage of thiamin at the methylene bridge between the thiazole and pyrimidine moieties, producing 4-methyl-5-(ß-hydroxyethyl) thiazole as the principle sulfur containing product (Dwivedi and Arnold, 1973). Alazamora *et al.* (1992)

suggested that significant improvement in thiamin stability can be achieved with slight lowering of pH of canned low acid foods, such as meats and vegetables, from a pH of 5.9 to 6.4 down to ~pH 5. A dramatic retention of thiamin after processing was observed in another experiment with canned low acid foods. Briozzo *et al.* (1987) lowered the pH of pea and corn purees from 6.9 and 6.5 (respectively) to 5.6 and achieved a 95% and 63% (respectively) increase in thiamin retention. Model systems have also been used to demonstrate the beneficial effect of low pH on thiamin (Fox *et al.*, 1982).

1.4.4.2. Sulphites Bisulphites and other inorganic bases are known to degrade thiamin by rupturing the methylene bridge and splitting the pyrimidine and thiazole rings apart (Gubler, 1984). Thiosulphites are found in onion and garlic (Clydesdale *et al.*, 1991; Carson, 1987), but the proportion of these ingredients in the SMSP product (1.0% of onion and 0.1% of garlic) is not expected to have an influence on thiamin retention.

1.4.4.3. Proteins and carbohydrates Thiaminases, which are present in small concentrations in vegetable and animal food products also degrade thiamin. During cooking, the enzymes will be denatured and therefore should not have any effect on thiamin. Naturally occuring proteins and amino acids are known to have a protective effect on thiamin (Dwivedi and Arnold, 1973), but once these proteins have been denatured by the heating of the product, this protective effect would not be significant.

A review of research, which involved the reactions of thiamin with various carbohydrates, indicated that few reactions of any significance occured. Only when carbohydrates which participate in the Maillard type reaction were involved were there noticeable results. Tests using thiamin hydrochloride with glucose solution (Dwivedi and

Arnold, 1973) and thiamin hydrochloride with xylose, maltose and glucose (Doyon and Smyrl, 1983) indicated a destruction of thiamin. In the research of Doyon and Smyrl the loss of thiamin was 37%. If these reducing sugars do not figure prominently in the food product under investigation and the moisture content of the product is not low enough to promote the Maillard reaction within the time of the food preparation, it can be assumed this reaction would not be significant.

1.4.4.4. Minerals Copper is known to complex with thiamin causing decomposition of the vitamin (Dwivedi and Arnold, 1973). Iron, within the thermostable hemin products of animal tissues, binds thiamin, making it unavailable. Porzio *et al.* (1973) refer to thiamin modifying factors containing hemoglobin and myoglobin in tuna, beef and pork which have antithiamin activity. Seal meat used in our test product is extremely high in iron and seal meat is also a significant source of copper. To date, studies have not been done on the possible influences of these minerals on the stability of thiamin in cooked food products containing seal meat.

1.4.4.5. Phenols Interaction of thiamin with phenols has been noted. Initial studies were conflicting, but investigation by Yang and Pratt (1984) showed that two cinnamic acid derivatives, caffeic acid and chlorogenic acid, degraded thiamin with the major product being thiamin disulphide. Tannins have also been implicated in thiamin breakdown (Rungruangsak *et al.*, 1977). On the other hand, plant antioxidants such as quercetin, found in onion in amounts five to ten times higher than many other vegetables (Hertog *et al.*, 1992), might have a protective effect on this vitamin (Benterud, 1977). Further

investigation into the amounts needed for a beneficial effect and the result of processing on the antioxidant are needed.

1.4.4.6. Temperature and time The importance of thiamin retention versus temperature variation has been examined at length. It has been shown that thermal degradation of thiamin follows the Arrhenius equation (Feliciotti and Esselen, 1957). This also applies to thermal destruction during processing of foods. There have been many studies on the thermal destruction kinetics of thiamin (Feliciotti and Esselen, 1957; Mulley *et al.*, 1975b; Skjöldebrand *et al*, 1983). Increases in temperature lead to increased destruction of thiamin. A correlation between the destruction of thiamin and the destruction of target microorganisms has shown that the destruction rate of the vitamin can be safely used to assure the destruction rate of the microorganisms (Feliciotti and Esselen, 1957). The degradation of the vitamin has long been used as an indication that temperatures reached in thermal processing were sufficient to destroy target microorganisms (Teixeira *et al.*, 1969; Fox *et al.*, 1982).

Thiamin, bound to protein, as in tissues, is more stable to thermal destruction than is free thiamin. In the *sous vide* method, essentially waterless cooking takes place where the nutrients remain sealed with the product. Research has shown that this method of preservation should be very beneficial to retention of this vitamin. Recommendations for minimal loss of thiamin during processing suggest deaeration or inert gas treatment where possible (Benterud, 1977). Most studies of the effect of heat on thiamin have used temperatures >100°C (Dwivedi and Arnold, 1972; Mulley *et al.*, 1975a; Augustin *et al.*, 1980; Skjöldebrand *et al.*, 1983; Briozzo *et al.*, 1987; Ramaswamy *et al.*, 1990;

Alazamoro *et al.*, 1992; Banga *et al.*, 1992; Steet and Tong, 1994). Some studies using lower temperatures have been performed with model systems (Fox *et al.*, 1982).

1.4.4.7. Water activity (a_w) Water activity in model systems has been studied (Arabshadi and Lund, 1988) with the conclusion that intermediate to high water activity was beneficial to thiamin retention. When both a_w and pH observations were combined the a_w was deemed to have little effect (Fox *et al.*, 1982).

1.4.5. Choice of thiamin as nutrient index

As part of our overall study of the effects of various pasteurization time-temperatures on the quality of a *sous vide* product, thiamin was chosen as a suitable index because of its sensitivity to heat. Single pure foods have been tested for thiamin stability versus microbial level using low temperature (70° to 100°C) cooking methods (Awonorin and Ayoade, 1993). Some studies using model systems of the *sous vide* method have been explored. However, very little research into thiamin stability has been carried out using food components processed by the *sous vide* method.

1.5. Protein and amino acids

Proteins are complex, multifaceted, and multireactive structures composed of linear chains of amino acids that are joined by peptide bonds. They play an important role in the structure and function of cells. Numerous proteins have been isolated, purified and

sequenced. It is the ratio and sequence of the amino acids that are specific for each protein which give the protein its characteristics.

For optimal growth and repair of the body, humans require sufficient amounts of nine of the main twenty amino acids found in foods. These nine amino acids; histidine, methionine, lysine, phenylalanine, leucine, isoleucine, tryptophan, threonine and valine (Henley and Kuster, 1994), are considered to be essential to the body and a food protein containing all of them in adequate amounts is referred to as a complete protein. Food proteins containing lesser amounts of one or more of the essential amino acids are referred to as incomplete proteins. Complete proteins are found mainly in animal products.

Energy from protein should comprise 10 to 13% of total energy depending on age, sex and level of activity (Health and Welfare Canada, 1990).

1.5.1. Reactions of proteins during food processing

The properties of proteins give them superb characteristics for functioning in biological systems, but these characteristics also make many proteins susceptible to environmental stresses. Food processing, which includes the many steps a food undergoes between harvest and consumption, can modify proteins and change the functionality, nutritional quality or safety of the food.

Heat treatment of food proteins brings about changes in texture and flavour of food products. The physical and chemical conditions a protein may encounter during processing can result in changes, which range from slight changes in the hydration of the protein or its relation with other molecules in the system, to moderate chemical changes in

the amino acid side chains, and to actual thermal destruction at very high temperatures. When optimally processed, these changes can contribute to improvement in the characteristics of the food.

1.5.2. Protein denaturation

Heat is the most common physical agent capable of changing proteins through denaturation. Protein denaturation is any modification in conformation (secondary, tertiary or quaternary) not accompanied by the rupture of the peptide bonds involved in the primary structure. The ultimate step in denaturation may be a totally unfolded polypeptide structure. The rate of denaturation by heat depends on many factors, such as the nature of the protein, protein concentration, water activity, pH, ionic strength and the kind of ions present.

1.5.3. Food processing effects on amino acids

Heat can cause the chemical alteration of amino acid residues, especially in alkaline environments. Arginine, cystine, threonine, serine and cysteine are destroyed. The amide (acid) type amino acids, glutamine and asparagine, are deaminated under alkaline conditions. In acid environments, tryptophan is destroyed at high temperatures, and hydroxyl containing amino acids, serine and threonine, are slowly destroyed. Ultraviolet light destroys the aromatic amino acids tryptophan, phenylalanine and tyrosine (Finley, 1985). Thermal treatments carried out in the presence of oxygen lead to the partial destruction of the tryptophan residues in proteins (Finley, 1985). All amino acids are

sensitive to dry heat. Lysine is particularly sensitive to the Maillard reaction involving a reducing sugar, an amino acid and alkaline treatment and/or dry heat (Cheftel *et al.*, 1985).

Isopeptides are formed during heating of proteins. As noted by Otterburn (1989), the ease of formation of isopeptide crosslinks in any specific protein is dependent on, among other things, the concentration of glutamic acid and aspartic acid in relation to lysine. Due to the competitive nature of reducing sugars and lipids, their presence together in a food can reduce the formation of the isopeptides.

Fay *et al.* (1991) conclude from literature research that significant isomerization only occurs under excessive conditions of temperature, alkaline pH, and/or treatment time. Temperature and pH prevailing under normal food processing conditions produce negligible isomerization.

The sulphur-containing amino acids, methionine and cystine, are damaged by reaction products from lipid oxidation. Methionine has been studied as an amino acid that reacts with fat in protein containing foods. Finley (1985) notes that lysine losses appeared to occur during the initial oxidation induction period whereas tryptophan losses occurred predominantly through the peroxidation period of lipid breakdown. Examination of free amino acids before and after pasteurizing might indicate any changes that may have occurred due to heating.

1.5.4. Protein and amino acid considerations in Seal Meat Shepherd's Pie

One must consider that different proteins and food systems have very different susceptibilities to any damage resulting from processing. In the present study, the effect of additional heat on already heat denatured protein is of interest. According to Finley (1985), in the literature there is no agreement on the effects of food processing on the nutritional quality of food proteins. Food processing factors expected to influence the product would include heat, heat in the presence of oxygen and reaction with lipid oxidation products. It is not expected that the Maillard reaction would be a factor in this research because of the very moist conditions and the acidic environment. Hamm (1977) reports on several research papers which conclude that normal cooking does not have much effect on essential amino acids, except the sulphur containing amino acids. However, meat dishes containing added carbohydrates did show a greater loss of amino acids (Hamm, 1977).

1.6. Texture

Texture, along with taste, odour and appearance, is one of the sensory properties of foods. Food texture is related to the microstructure of the food. Food processing, which generally makes use of heating, freezing, emulsification, etc. can have important influences on the microstructure and physical properties, and hence the texture, of a food. The texture of protein based muscle foods is significantly affected by cooking. Connective

tissue denaturation by heat can result in softening, while thermal denaturation of contractile proteins (myosin and actin) can lead to toughening. Fiber breaking, resulting from grinding of the muscle tissue will also affect the texture of the meat. Breaking the fibers will increase the tenderness of the meat (Stanley, 1987).

1.6.1. Influence of heat on texture

Meat tenderness is one of the most important quality criteria when evaluating results of cooking conditions. Thermal treatment of tough meat would have a beneficial effect on the tenderness if the meat were cooked for sufficient time. Connective tissue, which is predominantly collagen and found in higher amounts in the tougher cuts of meats, will begin to soften or break down to gelatin at 50° to 60°C in a moist environment. Muscle fiber proteins in beef will begin to denature, shrink and toughen at approximately 60° to 70°C (Bennion, 1980). This was confirmed by Bertola *et al.* (1994) in testing time/temperature variations on cylinders of beef muscle heated in water baths from 60° to 81°C. They observed that denaturation of actin began at 66°C. After 30 min at 68°C the protein was completely denatured. Protein denaturation was followed by Differential Scanning Calorimetry (DSC) for actin, myosin, sarcoplasmic proteins and collagen. The *sous vide* technique should create a favourable climate for tenderization of meats because of the lower temperatures used. However, the temperature range for denaturation of proteins is species specific and therefore the muscle protein of each animal species would require investigation to determine if an ideal time/temperature range for optimal texture can be found.

1.6.2. Measurement of texture

The measurement of texture may be accomplished directly by the use of sense of touch, which is subjective. However, because the stimulus of texture perception is mainly mechanical in nature, it can be measured by physical or objective methods. Instrumental tests for texture rely on deformation and flow characteristics (rheology) of the food material. Precision of objective measurements is potentially superior to subjective measurement, but testing equipment and procedures must be carefully controlled.

In most uniaxial compressive tests, a food specimen is deformed at a constant deformation rate (Peleg, 1987). The force that develops is recorded continuously and the relationship between force and time is shown by an upward curving line. The shape and size of the test sample must be specified and maintained. The larger the number of units in the food speciman to be tested, the more precise and reliable the results will be.

1.7. Objectives

The overall objectives of the present study are:

1. To develop a value-added food product using an underutilized marine species.
2. To prepare the product using the *sous vide* method with optimization of time/temperature schedules.
3. To compare the product with conventionally prepared and non-pasteurized sample products for nutritional and quality changes.

2. MATERIALS AND METHODS

2.1. Product formulation

2.1.1. Preparation of product

Figure 2.1. shows a summary flow chart of the preparation of seal meat shepherd's pie (SMSP). Harp seal meat was obtained from animals populating the waters surrounding Newfoundland and Labrador in the winters of 1993 and 1995. Other ingredients were obtained at local supermarkets. Seal meat that had been stored at -40°C was allowed to thaw in a refrigerator. Seal meat from the flank and rump sections (see Figure 2.2.) was cut into about 4 cm cubes, mixed together, ground and reground using an Omas TS8 food grinder fitted with a 4 mm die (Omcan, Mississauga, ON). The meat, 600 g, was cooked in 28 g corn oil for 10 min at which time the red colour of the meat disappeared. Chopped onion, 35 g, plus 3 g minced garlic was added to the fried meat and cooked for 3 min. All purpose flour, 16 g, was stirred into the mixture of meat. One beef bouillon cube (6 g), dissolved in 250 mL boiling water, was blended with the mixture. In a separate container, 135 g peeled diced carrots were cooked in simmering water for 5 min, and then drained and added to the meat along with the following: 484 g drained canned peas, 50 g tomato paste, 3 g dried parsley, 1 g dried thyme, 6 g salt and 1 g black pepper. This mixture was further cooked for 8 min and then homogenized in a food processor for 5 min.

FLOW CHART FOR PREPARATION OF SEAL MEAT SHEPHERD'S PIE

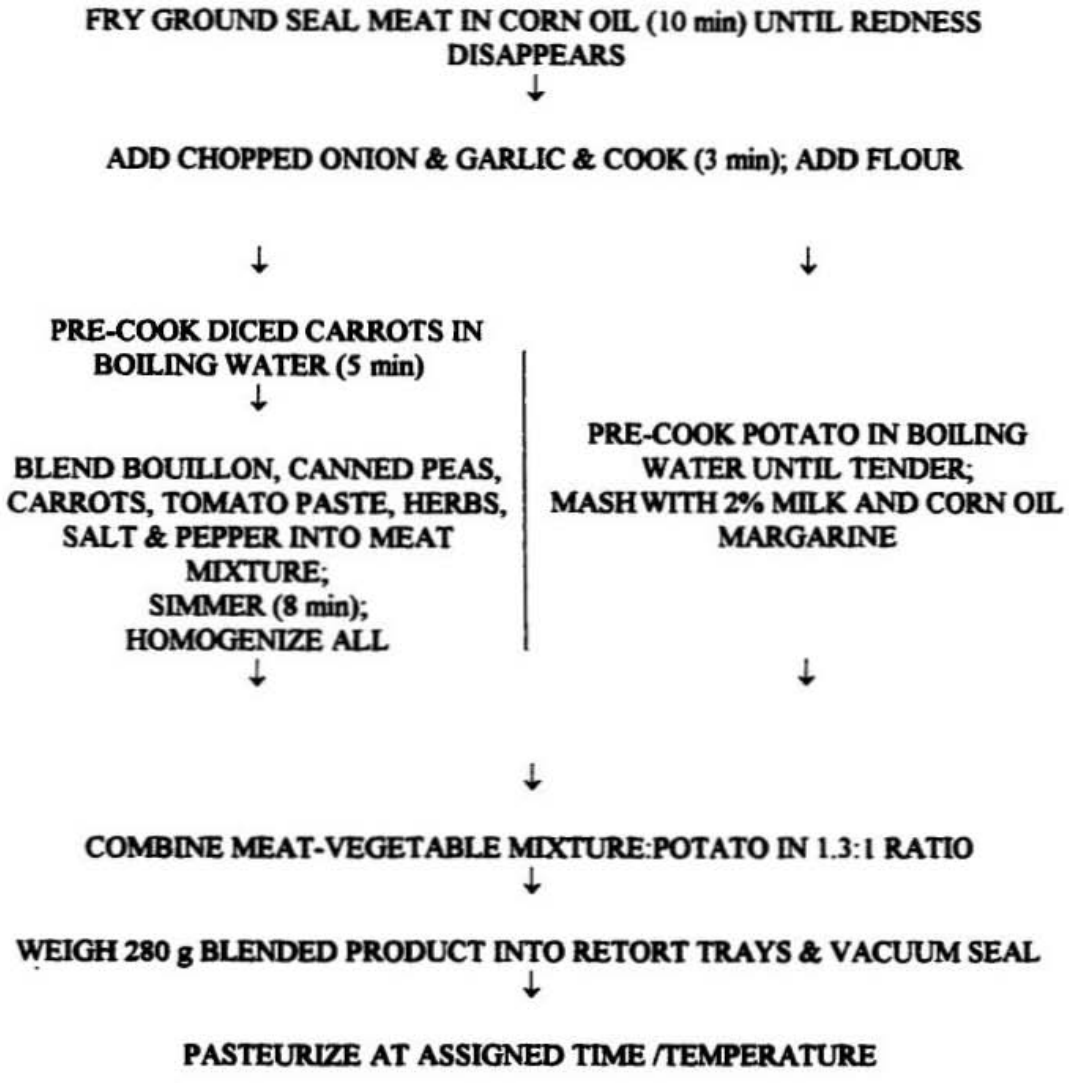


Figure 2.1. Flow chart for preparation of seal meat shepherd's pie (SMSP).

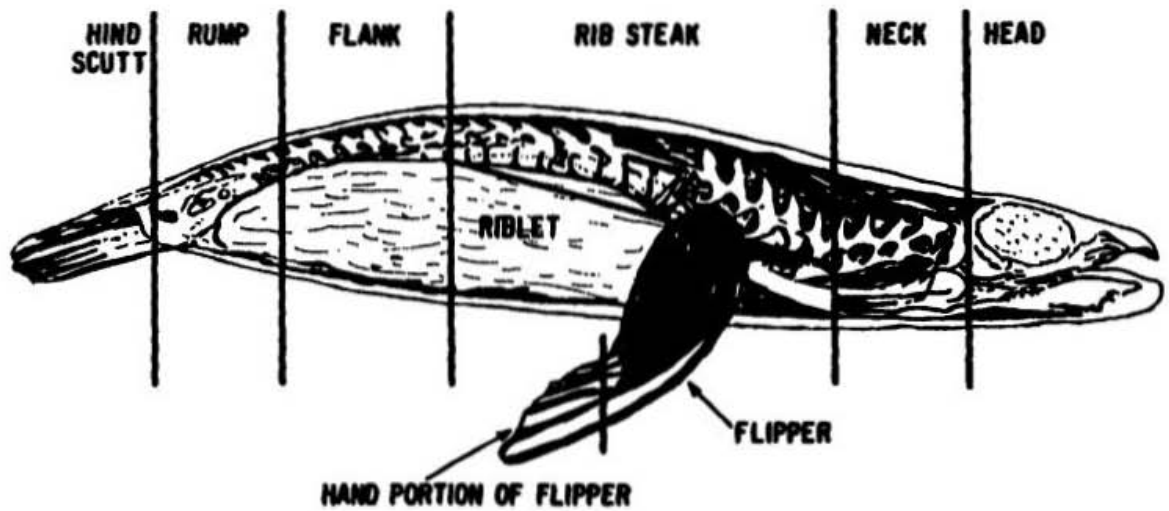


Figure 2.2. Diagram of harp seal (*P. groenlandica*) indicating typical cuts of the carcass.

One kilogram of peeled white potatoes was cooked separately in boiling water until fork tender, drained and then mashed with 250 mL 2% M.F. homogenized milk plus 120 g corn oil margarine. The amount of mashed potato produced by this formula was in excess of that needed for the correct ratio of meat-vegetable mixture:potato and the excess potato was discarded. Prior to processing and pasteurization, mixtures of the meat-vegetable and potato were blended together in a 1.3:1 (w/w) meat-vegetable:potato ratio. The final product contained 41% (w/w) of the meat-vegetable mixture as seal meat. The procedure that was followed gave a yield of seven individual trays, each containing 280 g of product. Approximately 65 to 70% of the original weight of the ingredients was used. Moisture losses and discard loss from the mashed potato formula account for a difference in raw weight and finished total weight.

While this preparation technique involved homogenization to ensure adequate blending for sample analysis, a product for the market would not include this procedure.

2.1.2. *Sous vide* processing and pasteurization

Two hundred and eighty grams of the product were placed in a Dynopak PE-HE (Dyno CP 505) 145 x 105 x 34 mm rectangular vacuum packaging tray supplied by Stock Steri-Technic Canada Inc. (Ste. Genevieve, PQ). The tray was covered with a Dynoseal ST 1580 Top Web film (Stock Steri-Technic Canada, Inc.). The product was vacuum sealed (950 mbar) using the Stock Dyno Sealing Machine Model 462 VGA (Stock Steri-Technic Canada, Inc.). Following sealing of the trays of samples, a predetermined number of trays

were removed, cooled to refrigerator temperature (2° to 5°C) and stored at -40°C for use as non-pasteurized samples.

Trays filled with the product were pasteurized at 65°, 70°, 75°, 80° or 85°C for 105, 60, 43, 35, or 30 min, respectively, using a Julabo 8VC water bath (Julabo Labortechnik, Schwarzwald, Germany) with 5 L capacity and ±0.2°C accuracy. After removal from the water bath, the trays were immediately chilled to about 5°C in ice water for 15 min and then stored for 30 days at -40°C in a walk-in type freezer. Freezing was necessary to preserve the product until analysis could be performed within 30 days.

2.1.3. Conventional treatment

For the conventional treatment, the meat-vegetable mixture was cooked on a stovetop at 85°C to 90°C for a further 25 min before being homogenized and blended with the potato in the 1.3:1 ratio. The product was placed in an aluminum tray and covered with plastic wrap plus aluminum foil, allowed to cool overnight to refrigerator temperature (2° - 5°C) and then stored at -40°C. The formula yield was seven 280 g trays.

2.1.4. Variations of product

The product was prepared in quantities sufficient to complete the particular study being undertaken. For each study the product was prepared on three separate occasions and on each occasion some containers were processed using the *sous vide* protocol, some were prepared by the conventional protocol and some were left as non-pasteurized. An attempt was made to use the same types and brands of ingredients for each set to reduce variability

between sets. The type of 100% corn oil margarine used in the preparation of Set 1 was the hard, block form of margarine. The type of margarine used for Sets 2 and 3 was the soft, tub form due to non-availability of the hard type during the preparation of these latter two sets.

Procedure for the preparation of the product for fatty acid analysis followed the steps outlined above. Procedure for the preparation of the product for protein and amino acid analyses followed the steps outlined above and, in addition, samples of the product prepared at each temperature variation plus the conventional and non-pasteurized variations were freeze dried prior to analysis. Procedures for preparation of the product for microbiological, thiamin and texture tests were adjusted to meet the needs of those tests.

2.1.4.1. Microbiological variation One hundred and ninety trays of the SMSP were prepared. Fifty-six trays of the product were pasteurized at 85°C for 30 min using a Stock Pilot-Rotor 900 model retort (Stock America, Inc. Milwaukee, WI). This heat treatment, one of the five time/temperature research pasteurization variations giving a 12 *D* in the target bacteria, is equivalent to the water bath method which also gives a 12 *D* reduction. The retort, which can pasteurize 60 trays together, was used because this experiment required many more samples. Half of the trays of pasteurized samples, plus half of the non-pasteurized and conventional sample trays, were stored in an upright refrigerator maintained at 8°C (representing an abusive refrigerator temperature) and the remaining half were stored in a walk-in refrigerator at 2.2°C (representing the recommended refrigerator temperature).

2.1.4.2. Thiamin variation The product was spiked with thiamin hydrochloride. The total weight of the meat mixture obtained from a preparation of a complete set (65°C to 85°C plus conventional treatment and non-pasteurized treatment trays) was determined. This weight was used to calculate the amount of thiamin hydrochloride to add based on 0.35 mg/g of meat mixture. The thiamin hydrochloride was dissolved in 50 mL of distilled water, added to the meat mixture and dispersed throughout the mixture using a food mixer. The prepared mashed potato was added to the meat mixture in a ratio of 1.3:1 (w/w) of meat-vegetable:potato. Addition of the potato gave a final content of 0.2 mg thiamin hydrochloride per gram of SMSP product. To avoid the excessive moisture loss that would occur during the conventional preparation method of the SMSP for thiamin analysis, a covered aluminum pan was used and the product was baked at 180°C for 1 h.

2.1.4.3. Texture variation In this variation, following the preparation of pasteurized and non-pasteurized and conventional samples, the meat mixture was not put through the food processor. Mashed potato was not used with this test. Two hundred and seventy grams of the meat mixture were weighed into retort trays for pasteurization treatment and for the non-pasteurized treatment. This amount of meat mixture allowed for effective and correct sealing of the trays. Portions of the meat were cooked by the conventional method and stored in plastic containers until measurement. All of the meat product samples were stored at 2.2°C until analysis.

2.2. Microbiological method

2.2.1. Microbiological analysis

Figure 2.3. shows a summary flow chart of the microbiological testing procedures. All of the five time/temperature pasteurization combinations result in a 12 *D* reduction of microorganisms, making it unnecessary and impractical, from a procedural perspective, to use all of the pasteurization schedules. The 85°C for 30 minutes pasteurization schedule was chosen to represent the *sous vide* process. Both storage temperature variations of non-pasteurized, pasteurized (85°C) and conventionally prepared SMSP product were analyzed for aerobic and anaerobic bacteria after day 0 and at 2 day intervals for the first week. Following this, these variations of product and storage temperature were analyzed once each week until 21 days, the suggested minimal shelf life for a similar *sous vide* processed product, had elapsed.

Sterilization of all equipment and the work area was performed routinely. On each sampling day, 50 g duplicate samples (weighed to the nearest 1/1000th) of each treatment; pasteurized, non-pasteurized and conventionally prepared, each stored at the two temperatures of 2.2°C and 8°C, were each weighed into a separate blender jar. (This level or accuracy was also used for the methods for fat, fatty acid, thiamin, protein and amino acid determinations.) Each sample was blended with 450 mL 0.1% sterile peptone water (Difco, Detroit, MI) in a Waring commercial blender (Dynamic Co. of America, New Hartford, CO) for two minutes. The pH of this dilution (10^{-1}) was measured using the Orion pH meter model 720 (Orion Research Inc., Chicago. IL) and serial dilutions of 10^{-2}

FLOW CHART FOR MICROBIOLOGICAL PROCEDURES

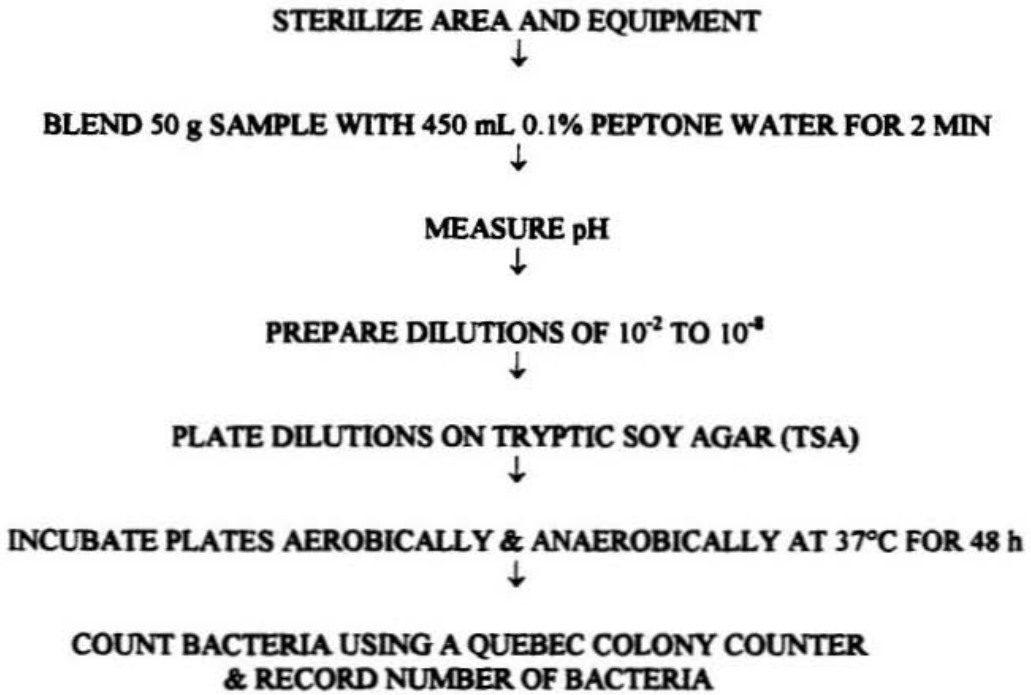


Figure 2.3. Flow chart of microbiological procedures.

to 10^{-8} were prepared. One millilitre of blended sample was pipetted into 9 mL of the peptone water in a test tube and vortexed. One millilitre of this dilution was pipetted into another 9 mL of peptone water and vortexed. This procedure was continued until a 10^{-8} dilution was obtained. Test tubes were vortexed between pipetting and the mouth of the test tube was sterilized before and after each dilution. Sterile cotton balls were placed in the openings of the test tubes.

Total bacterial counts were determined by plating appropriate amounts of each dilution on Tryptic soy agar(TSA) plates (Difco, Detroit, MI). To obtain total anaerobic and acid bacteria counts, the plates were incubated anaerobically using a Nalgene transparent polycarbonate desiccator (Fisher Scientific, Fair Lawn, NJ) fitted with the BBL Gas Pak system and gas pak catalyst (Becton Dickinson Microbiology Systems, MD). To ensure the efficiency of the anaerobic system, the initial air was removed from the desiccator using the GAST vacuum pump motor (GAST Manufacturing. Co., Benton Harbour, MI).

All plates were incubated for 48 h in a low temperature incubator (Model 307, Fischer Scientific, Fair Lawn, NJ) maintained at 37°C.

2.2.2. Additional tests

Any physical change, i.e. formation of gas in the trays, was noted on each sampling day. Chemical changes, i.e. pH and total acidity, were also measured on each sampling day. pH was measured as in Section 2.2.1. Total acidity of the samples was measured by the method of Simpson *et al.* (1994) with the following modifications. One hundred milliliters

of deionized water was added to 10 g of the sample and mixed thoroughly using a Fisher magnetic stirrer (Fisher Scientific, Fair Lawn, NJ). The solution was filtered using Whatman filter paper No.4 (Whatman Int. Ltd. UK) and the filtrate was titrated with 0.1M NaOH using five drops 1% phenolphthalein (1 g phenolphthalein dissolved in 100 mL ethyl alcohol) as indicator. The total acidity was calculated from the following equation from Simpson *et al.* (1994) who used a similar meat based product:

g lactic acid/100 g sample = 0.009 x V (mL) of 0.1M NaOH

This is recorded as lactic acid as it is the acid found in the highest percent in meat based products.

2.3. Fat and fatty acids method

2.3.1. Fat extraction

Figure 2.4. shows a summary flow chart of procedures used for fat extraction and transmethylation of fatty acids. The Bligh & Dyer (1959) procedure for extraction of fat was followed. Ten grams of the food product were weighed into a 400 mL beaker. As recommended in the procedure, two mL of distilled water were added to bring the moisture content to the required level. Ten milliliters of chloroform and 20 mL of methanol were added and the mixture was blended with an Ultra-Turpax T25 (polytron) blender (IKA Labortechnik, Cincinnati, OH) for exactly 2 min. Thirty milligrams of the internal standard (I.S.), (triheptadecanoin, C17:0) (Sigma, St.Louis, USA) were added so

FLOW CHART FOR FAT EXTRACTION AND TRANSMETHYLATION

WEIGH 10g FOOD & 2mL WATER INTO BEAKER

↓

BLEND WITH 10mL $CHCl_3$ & 20mL MeOH FOR 2min

↓

ADD I.S. & 10mL $CHCl_3$ & BLEND 30s

↓

ADD 10mL DISTILLED WATER & BLEND 30s

↓

FILTER WITH VACUUM & TRANSFER FILTRATE TO SEPARATING FUNNEL

↓

BLEND FILTER RESIDUE WITH 10mL $CHCl_3$ FOR 15s, SCRAPE, & BLEND 15s

↓

TRANSFER 2ND AMOUNT FILTRATE TO THE SEPARATING FUNNEL

↓

ALLOW TO SEPARATE FOR 1h

↓

COLLECT BOTTOM $CHCl_3$/FAT LAYER IN WEIGHED ROUND BOTTOM FLASK

↓

REMOVE SOLVENT USING ROTARY EVAPORATOR

↓

OBTAIN WEIGHT OF FAT IN FLASK

↓

STORE FAT OR PROCEED WITH TRANSMETHYLATION

↓

Figure 2.4. Flow chart of procedures used for fat extraction and transmethylation (to be cont'd).

↓

WEIGH 0.05g FAT INTO CONICAL REACTION VESSEL WITH MeOH/HCl(94:6)

↓

INCUBATE FOR 16h AT 70°C

↓

COOL AND POUR INTO TEST TUBE

↓

EXTRACT FATTY ACID METHYL ESTERS WITH HEXANE THREE TIMES

↓

CLEAN HEXANE EXTRACT WITH DISTILLED WATER TWICE

↓

CHILL FOR 3h, THEN EVAPORATE HEXANE WITH NITROGEN

↓

TRANSFER RESIDUE TO AUTOSAMPLER USING CS_2

↓

STORE IN FREEZER (-20°C) UNTIL TESTED ON GC

Figure 2.4. Flow chart of procedures used for fat extraction and transmethylation (cont'd).

that it represented approximately 1/20th the weight of the fat in the product and 10 mL more of chloroform were added and the mixture was blended for 30 s. Distilled water (10 mL) was added and all was blended a further 30 s. The homogenate was then filtered through a Whatman #1 filter paper (Cat.No. 1001 090, Whatman International Ltd., Maidstone, Eng.), set in a Buchner funnel over a 500 mL suction flask attached to a GAST vacuum pump (GAST Manufacturing. Co., Benton Harbour, MI). When the residue appeared dry, it was pressed with the bottom of a beaker to express as much of the liquid as possible. The filtrate was poured into a separating funnel. The filter paper and residue were transfered to a Waring blender (200 mL jar size) along with 10 mL chloroform and blended for 15 s. The sides of the blender were scraped down and the mixture blended a further 15 s. This mixture was then filtered through a #1 filter paper in the Buchner funnel as before. The blender jar was swished with 5 mL chloroform to clean sides and this was also filtered. The filtrate from this was added to that already in the separatory funnel and then was allowed to separate for 1 h. The funnel was protected from light by a covering of aluminum foil.

The bottom chloroform/fat layer was drawn into a weighed round bottom flask and the chloroform was evaporated using a Caframo VV 2001 Rotary Evaporator (Heidolph-Elektro GmbH and Co KG, Kelheim, Germany) set at 90 rpm and Caframo WB 2001 water bath (Heidolph-Elektro) set at 55°C. The gas lines were flushed with nitrogen before evaporation proceeded. After all chloroform had been removed, the round bottom flask was allowed to cool and it was then weighed to obtain the weight of fat in the sample. Percent of fat in the sample was then calculated.

As much of the fat as possible was suctioned from the flask into a 5 mL amber vial using a Pasteur pipette. A small amount of hydroquinone (~0.05 g) was added and the vial flushed with nitrogen. The vial was stored at -15°C.

2.3.2. Transmethylation of fatty acids.

Transmethylation followed the method of Keough and Kariel (1987) with some modification. The fat was allowed to come up to room temperature and 0.05g was placed in a 3 mL conical reaction vessel (Supelco, Supelco Canada Ltd. Mississauga, ON). Two milliliters of methanol:hydrochloric acid (94:6) reactant was added along with a small amount (~0.01g) of hydroquinone. The vessel was tightly capped with a fresh screw cap and placed in a metal tube. The tubes of fat were heated for 16 h in a convection oven set at 70°C.

After cooling, the contents of the conical reaction vessel were poured into a small test tube, 1.5 mL hexane were added and the tube vortexed. The top layer (avoiding any bottom layer) was removed to a large test tube. One and one half milliliters of hexane were added to the original test tube and again vortexed. The top layer was again removed to the large test tube. This step was repeated once again after which 1.5 mL distilled water were added to the hexane layers in the larger test tube, the tube was vortexed and the top layer removed to a second large test tube. One and one half milliliters of water were added to this tube, vortexed and the top layer removed to a second small test tube.

The test tube was placed in a freezer at -15°C for 3 to 4 h after which the contents were immediately poured into a third small test tube where all of the hexane was evaporated off under a fume hood using a stream of nitrogen. To the residue left in the

test tube, 1 mL carbon disulphide was added and this was pipetted into a 2 mL autosampler vial and capped. If the sample did not reach the neck of the vial, a few extra drops of carbon disulphide were added. The vials were stored at -20°C until analysis on the gas chromatograph which was performed within 5 days.

2.3.3. Gas Chromatograph (GC) analysis

The transmethylated fatty acids were analyzed with a Hewlett Packard 5890 Series 2 Gas Chromatograph (Hewlett Packard, Atlanta, GA) equipped with 30 m x 0.53 mm I.D. and 1 mm film thickness Supelcowax 10 (Supelco Canada Ltd. Mississauga, ON) fused silica capillary column and a flame ionization detector. The oven temperature was set at 220°C and the injection port and detector were maintained at 230°C. Identification of fatty acids was based on two standards, a mixture of PUFA-1 (Supelco Cat.No. 4-7033) and lipid standards (181-1 and 181-15, Sigma). Eight replicates at each temperature variation were tested for Set 1 and three replicates were tested at each temperature variation for Sets 2 and 3. A sample printout of the fatty acid gas chromatogram is shown in Figure A.1. Appendix A.

2.4. Thiamin analysis method

Figure 2.5. shows a summary flow chart of the procedures for thiamin extraction and quantification. A modification of the official method of analysis published by the

Association of Official Analytical Chemists (AOAC) (1990) was used for determination of thiamin in the spiked product. The food was digested with acid, hydrolyzed with enzymes to convert thiamin di- and tri- phosphate to thiamin, the hydrolysate filtered and then thiamin was converted to the highly fluorescent thiochrome for reading fluorimetrically. The procedure used in thiamin determination was identical to the AOAC procedure except for replacement of the purification step in the AOAC method with a filtration step using a C18 Sep Pak.

2.4.1. Extraction method

Each test day, two product samples were tested along with a standard. Each sample was analyzed in triplicate and the standard was analyzed in duplicate. A two gram portion of the SMSP was weighed into a 100 mL volumetric flask. Twenty-five millilitres of 0.1N HCl were added and the mixture shaken to disperse. Approximately 25 mL more of the 0.1N HCl were added and the mixture was shaken gently. After covering the flask with aluminum foil, it was autoclaved at 121°C for 30 minutes, during which time acid hydrolysis of the product occurred. After cooling for fifteen minutes, the flask was brought to volume with 0.1N HCl, the mixture was shaken to disperse contents and a 5 mL aliquot was transfered to a 125 mL conical flask, to which approximately 62 mL of 0.1N HCl was added. The pH was adjusted to between 4.0 and 4.5 with 2N NaOAc using the Orion SA720 pH meter (Orion Research Inc., Chicago, IL). Five milliliters of the enzyme mixture (α-amylase and papain) was added. The flask was sealed with parafilm and aluminum foil, shaken gently and allowed to incubate, with occasional shaking, for

FLOW CHART FOR THIAMIN EXTRACTION AND QUANTIFICATION

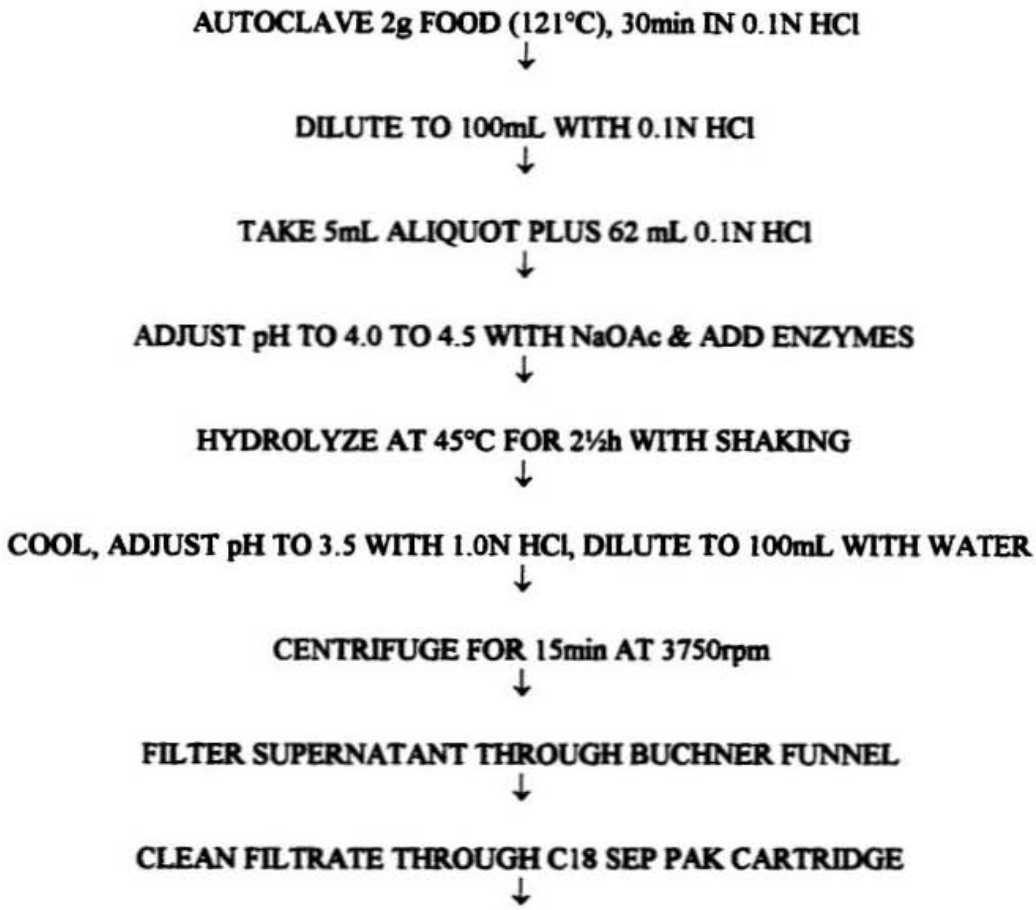


Figure 2.5. Flow chart of procedures used for thiamin extraction and quantification (to be cont'd).

↓

ADD 5mL FILTRATE TO 1.5mL NaCl IN CENTRIFUGE TUBE

↓

SWIRL, ADD 3mL OXIDIZING AGENT & 13mL ISOBUTANOL, SHAKE 15s

↓

REPEAT PREVIOUS FOUR STEPS; SIMILARLY PREPARE 2 BLANKS & SHAKE ALL FOR 2min

↓

CENTRIFUGE 10min AT 1850rpm

↓

PIPETTE OFF ABOUT 10mL ISOBUTANOL LAYER

↓

TEST ISOBUTANOL LAYER AT 365nm EXCITATION & 435nm EMISSION

↓

CALCULATE AMOUNT THIAMIN

Figure 2.5. Flow chart of procedures used for thiamin extraction and quantification (cont'd).

2½ h at 45°C in a Julabo 8 VC water bath (Julabo Labortechnik, Schwarzwald, Germany) with a 5 L capacity and ±0.2°C accuracy.

Following enzyme hydrolysis, the pH was adjusted to 3.5 with 1N HCl, the mixture was poured into a 100 mL graduated cylinder and the volume was brought to 100 mL with distilled water. This volume was poured into 2 centrifuge tubes (ca. 50 mL) which were centrifuged for 15 min at 3750 rpm on a IEC Clinical centrifuge (International Equipment Co., Needham Hgts., MA). After removal from the centrifuge, the tubes were allowed to chill in crushed ice for 30 min which allowed for easier removal of the supernatant. The supernatant was filtered through a Whatman #42 ashless filter paper (Whatman International Ltd., Maidstone, Eng.) in a Buchner funnel using a GAST vacuum pump motor (GAST Manufacturing. Co., Benton Harbour, MI). The filtrate was then further cleaned using a C18 Sep Pak Plus cartridge (Cat. No. 20515, Millipore Waters Chromatography, Marlborough, MA) that had been previously conditioned with 5 mL methanol and 15 mL distilled water. A 30 mL aliquot of the filtrate from the Buchner funnel was drawn into a 50 mL syringe, 10 mL was pushed through the sep pak and discarded. The remaining 20 mL of clarified filtrate was pushed through the sep pak into a small holding flask. This filtrate was the Assay Sample Solution.

A stock standard solution was prepared by weighing 50 mg thiamin hydrochloride (that had been dried to a constant weight overnight in a convection oven) and dissolving it in 20% acidified alcohol to a volume of 500 mL. The intermediate standard solution was prepared by diluting 50 mL of stock solution with 20% acidified alcohol to a volume of 500 mL. These standards were stored at 4°C and used within one month. To prepare the

Assay Standard Solution, 10 mL of intermediate standard solution was pipetted into a 100 mL volumetric flask with approximately 50 mL of 0.1N HCl, mixed and autoclaved at 121°C for 30 min. This was brought to volume with 0.1N HCl. This was designated the Working Standard Solution (containing 1µg/mL thiamin HCl). Twenty milliliters of the working standard solution was pipetted into a 125 mL conical flask with 50 mL 0.1N HCl, the pH was adjusted to 4.0 to 4.5 and the working solution was clarified as described above for the sample solution. This filtrate was designated as the Assay Standard Solution.

2.4.2. Conversion of thiamin to thiochrome

Five milliliters of the Assay Sample Solution or the Assay Standard Solution was pipetted into each of four numbered 50 mL centrifuge tubes containing 1.5 g NaCl each. Using a 5 mL pipet, 3 mL of oxidizing solution (1% potassium ferricyanide in 15% sodium hydroxide) was added to tube number 1 immediately after the tube had been gently swirled on the vortex mixer. Isobutanol (13 mL) was immediately added using an automatic pipetter and the tube shaken manually for 15 s. Tube 2 received the identical treatment and both tubes were labelled isobutanol extract from assay sample or standard solution. Tubes 3 and 4 were treated with 3 mL of NaOH instead of the oxidizing reagent and labelled isobutanol extract from sample or standard blank. After the four tubes received the initial treatment they were all shaken manually for 2 min before centrifuging at 1850 rpm for 10 min. Approximately 10 mL of the upper isobutanol layer were pipetted into a small test tube. The tube was wrapped and capped with aluminum foil. In addition to testing the

assay sample solution and the assay standard solution using the thiochrome method, two dilutions of the stock solution, prepared daily, plus a blank or zero standard (without thiamin), were analyzed. These results were used to prepare a standard curve.

2.4.3. Fluorescence measurements

The reading on the fluorimeter-spectrometer (Perkin-Elmer LS5, Perkin-Elmer, Montreal, P.Q.) were performed within 1 h of sample preparation. The fluorimeter reading was taken at 365 nm excitation and 435 nm emission after the machine was zeroed with the blank or zero standard.

2.5. Protein and free amino acid method

2.5.1. Kjeldahl method for determination of crude protein

Figure 2.6. shows a summary flow chart of the crude protein determination. The Kjeldahl procedure for determination of crude protein content was followed. Prior to initiation of this procedure, the product samples were freeze dried. Product variations were thawed at refrigerator temperature (2-3°C) and duplicate samples from each tray were freeze dried using an Edwards Model 12K Supermodulyo Freeze Dryer (Edwards High Vacuum (Canada) Ltd., Burlington, ON). The average wet weight of the samples was 14.2 g. The average weight of the freeze dried samples was 3.7 g. Following the recommended procedure of Kjeldahl, 400 mg of the freeze dried product was weighed onto nitrogen free

FLOW CHART FOR CRUDE PROTEIN DETERMINATION (KJELDAHL METHOD)

WEIGH 0.4 g FREEZE DRIED SAMPLE INTO KJELDAHL TUBE

↓

ADD KJELTABS AND 20 mL SULPHURIC ACID

↓

DIGEST IN BUCHI DIGESTION UNIT FOR 60 TO 70 min

↓

COOL, ADD 60 mL DISTILLED WATER & PLACE ON BUCHI DISTILLATION UNIT

↓

ADD NaOH & START STEAM DISTILLATION

↓

DISTIL UNTIL 150 mL DISTILLATE COLLECTED

↓

TITRATE DISTILLATE WITH O.5N H_2SO_4

↓

WHEN INDICATOR RETURNS TO PINK, RECORD mL ACID

↓

CALCULATE % CRUDE PROTEIN

Figure 2.6. Flow chart of procedures used for crude protein determination (Kjeldahl method).

paper and the weight recorded. The product was placed in a Kjeldahl tube along with 2 Kjeltabs (potassium sulphate and selenium) and 20 mL sulphuric acid. Five tubes and a blank were put through each run. Duplicate samples of non-pasteurized, pasteurized and conventional treatments were tested.

The SMSP was digested using the Buchi 426 Digestion Unit (Brinkmann Instruments (Canada) Ltd. Rexdale, ON), first at setting #3 for 20 min and then at setting #9 for 30 to 40 min until the digest was clear (to slightly opaque) and slightly yellow. The digester was then set at #10 for 10 min. The tubes were removed from the machine and allowed to cool for 15 to 30 min.

Distillation was done on a Buchi 315 Distillation Unit (Brinkmann Instruments (Canada) Ltd. Rexdale, ON). Sixty milliliters of distilled water were added to the digestion tube and the tube was placed in the distillation unit. The conical titration flask, containing 50 mL indicator was put in place, 100 mL NaOH was added to the digestion tube and distillation started. When the 200 mL level was reached in the titration flask, indicating that 150 mL of distilate had been collected (after 7 to 9 min), distillation was stopped. The digestion tube was removed and the contents discarded.

Titration of the distillate was carried out immediately after the distillation. The digital buret (Brinkmann Instruments (Canada) Ltd. Rexdale, ON) was zeroed by bringing the 0.5N sulphuric acid to the tip of the dispenser, removing the drop and then zeroing. The acid was added slowly to the distillate until the color changed back to the original pink of the titrator (as compared with the blank). The number of milliliters of acid used was recorded for the final calculation of percent crude protein.

2.5.2. Amino acid analysis for physiological free amino acids

Figure 2.7. shows a summary flow chart of the procedure used for amino acid analysis. The weight of a 15 mL centrifuge tube was recorded using a Sartorius MCI balance (Sartorius Canada Inc., Mississauga, ON) and 0.5 g freeze dried sample was placed in it. Five milliliters of 14% sulphosalicylic acid ($C_7H_6O_6S.2H_2O$) (Sigma Chemical Co.) made with lithium citrate buffer (96.0% water, 1.0% lithium citrate, 1.0% thioglycol, 0.7% hydrogen chloride, 0.5% benzoic acid) (Beckman Instruments Inc.) and 0.01% EDTA, pH of 2.2 (Sigma Chemical Co.) at a was added to the tube and all was vortexed well for 15 s. The sample was placed in the refrigerator for 30 min after which it was vortexed and poured into 3 Eppendorf tubes (ca. 1.5 mL). The tubes were centrifuged on an Eppendorf 5412 centrifuge (Brinkmann Instruments (Canada) Ltd. Rexdale, ON) for 6 min and the supernatants were transfered to 2 Eppendorf tubes as the supernatant was still not clear. Extraction was carried out in triplicate or quadruplicate on the freeze dried samples.

Supernatants from each tube were combined and the volume recorded. One milliliter of the supernatant was placed in a small labelled test tube and 2 mL of lithium citrate buffer containing internal standard (IS) ((S)-2-aminoethyl-L-cysteine. Hydrochloride) (Sigma Chemical Co.) was added, the tube covered with parafilm and stored at 4°C until analysis.

Analysis of the amino acids was carried out using a Beckman amino acid analyzer, Model 121-M in the Amino Acid Analysis Facility at Memorial University of Newfoundland. (NOTE - this method did not allow for detection of hydroxyproline.)

FLOW CHART FOR AMINO ACID ANALYSIS

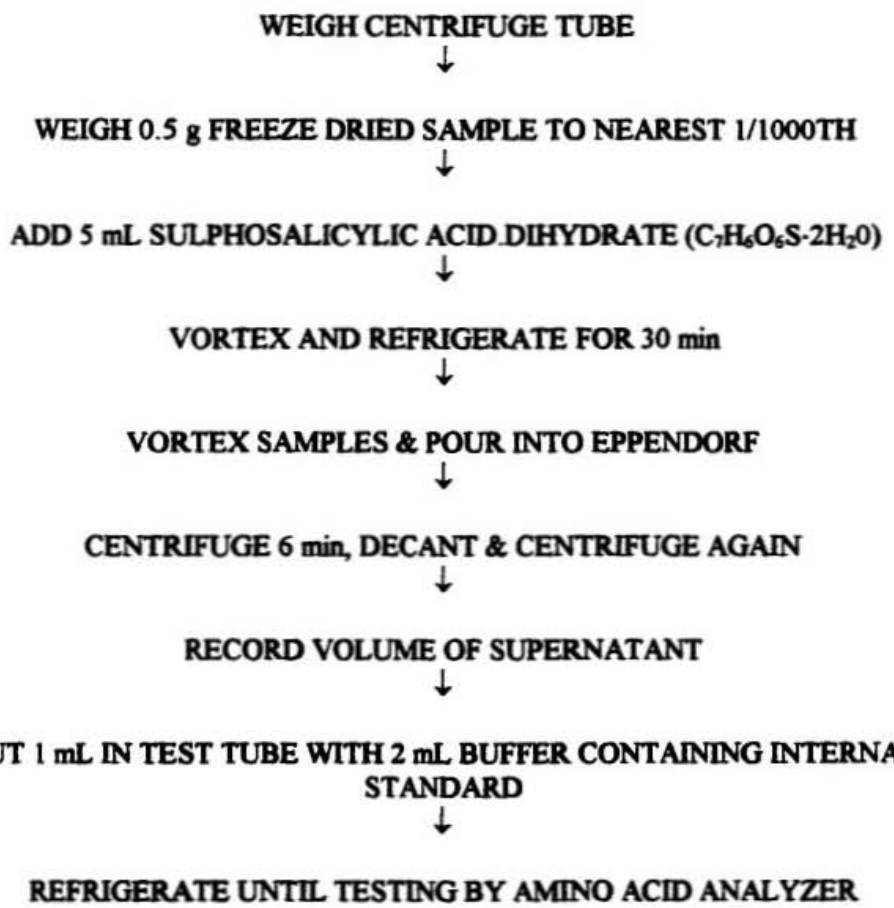


Figure 2.7. Flow chart of procedures used for amino acid analysis.

2.6. Texture method

2.6.1. Preparation of sample

The SMSP for texture analysis was prepared as per Section 2.1.4.3. Only cooked, ground seal meat pieces were tested for texture changes. The *sous vide* trays containing SMSP were brought to room temperature and then the contents were emptied into a sieve. The product was washed briefly with water at 18° - 20°C to separate the meat from the gravy and vegetables. Representative pieces of cooked ground seal meat ranging from 10 mm to 12 mm in cross section and between 8 mm and 15 mm in depth were chosen for testing and given a gentle second washing.

2.6.2. Measurement of texture

Six to 8 pieces of meat were compression/penetration tested on the Lloyd Texturemeter (Omnitronix Ltd., Missassauga, ON) using a 5 newton load and fitted with a 1.6 mm diameter probe (plunger). Crosshead speed was set at 3 mm/min and the probe penetrated 6 mm into the meat before being automatically returned to zero. Figure A.2. Appendix A shows a printout of the machine settings. Data were recorded on the Tatung TCS-7000 computer and stored in a Lotus file format for further analysis.

2.7. Statistical methods

Results of testing for fatty acids and amino acids were analyzed statistically at the Statistical Consulting Centre, Memorial University of Newfoundland using SAS's General Linear Model (GLM) for an analysis of variance. When significance was shown between values at the different temperature variations, a multiple comparison test was performed. For pre-planned comparisons, a decision was made to compare the non-pasteurized sample values with the pasteurized and conventionally prepared sample values and only probabilities of $p \leq 0.05$ and $p \leq 0.01$ associated with these were discussed.

Results of testing for thiamin, protein and texture were analyzed statistically using analysis of variance, followed by the Bonferroni t-test if significance was evident.

3. RESULTS

The estimated nutrient composition of the ingredients used in SMSP and the estimated percent fatty acids in the fats used in SMSP have been compiled and presented in Tables B.1. and B.2. (Appendix B). The mean percent moisture content, the pH and a_w of the product variations are given in Table B.3. (Appendix B). All calculations of the means for all tests include the standard deviations.

3.1. Microbiological analysis

The results of studies on pasteurized (85°C), non-pasteurized and conventionally treated SMSP stored at both 2.2°C and 8°C and tested for the presence of aerobic and anaerobic bacteria, changes in pH and the presence of acid are presented in Tables B.4. and B.5. (Appendix B). A decrease in pH corresponds to an increase in % acid.

Data on the bacterial counts are plotted in Figures 3.1. and 3.2. Aerobic and anaerobic bacterial counts in pasteurized SMSP were < log 3 CFU/g throughout the 21 day storage period that was followed. Counts in non-pasteurized (NP) and conventionally treated (CV) SMSP stored at the higher (8°C, abuse) temperature rose steadily from 3.4 and 3.69 to 9.49 and 9.88, respectively, for aerobic bacteria and 3.4 and 3.75 to 9.42 and 9.68 for anaerobic bacteria. Bacterial counts in samples stored at 2.2°C (recommended refrigerator temperature) remained fairly stable for 6 days (3.49 to 5.24 and 3.63 to 5.17)

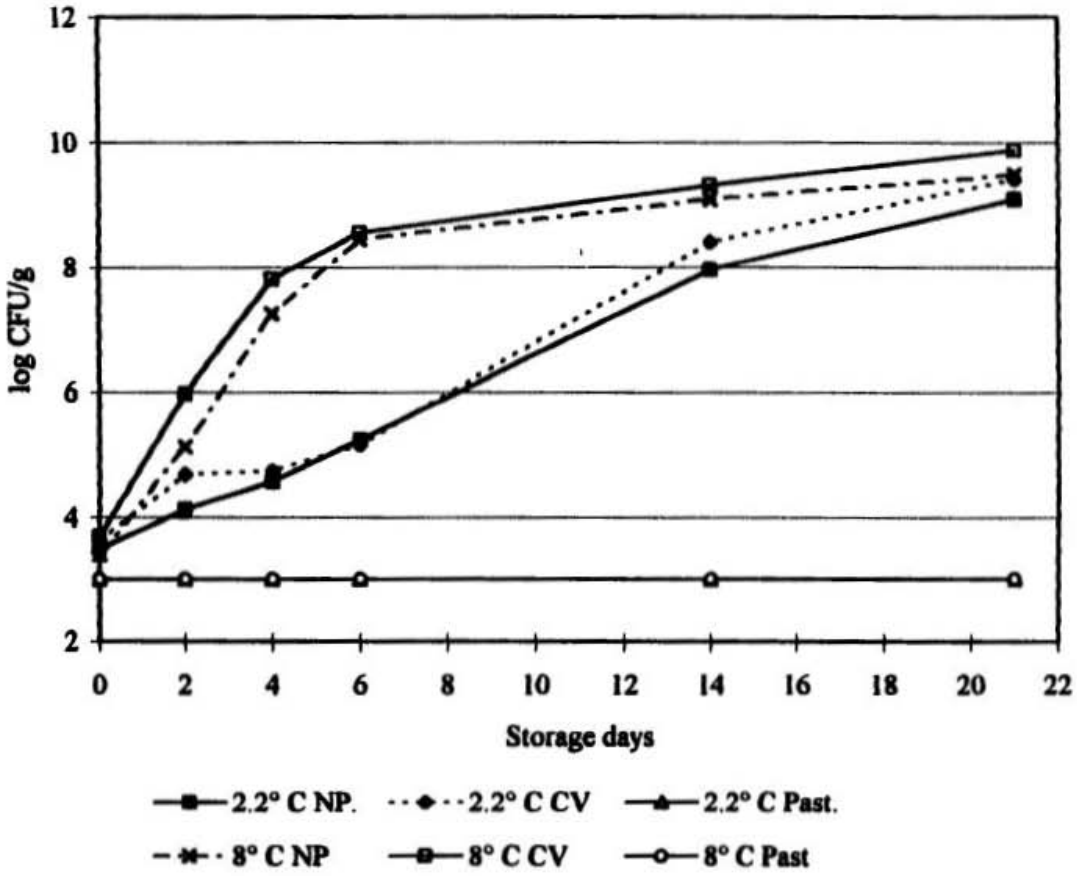


Figure 3.1 Aerobic bacterial counts in non-pasteurized (NP), conventional (CV) and pasteurized (P) SMSP stored at 2.2°C and 8°C.

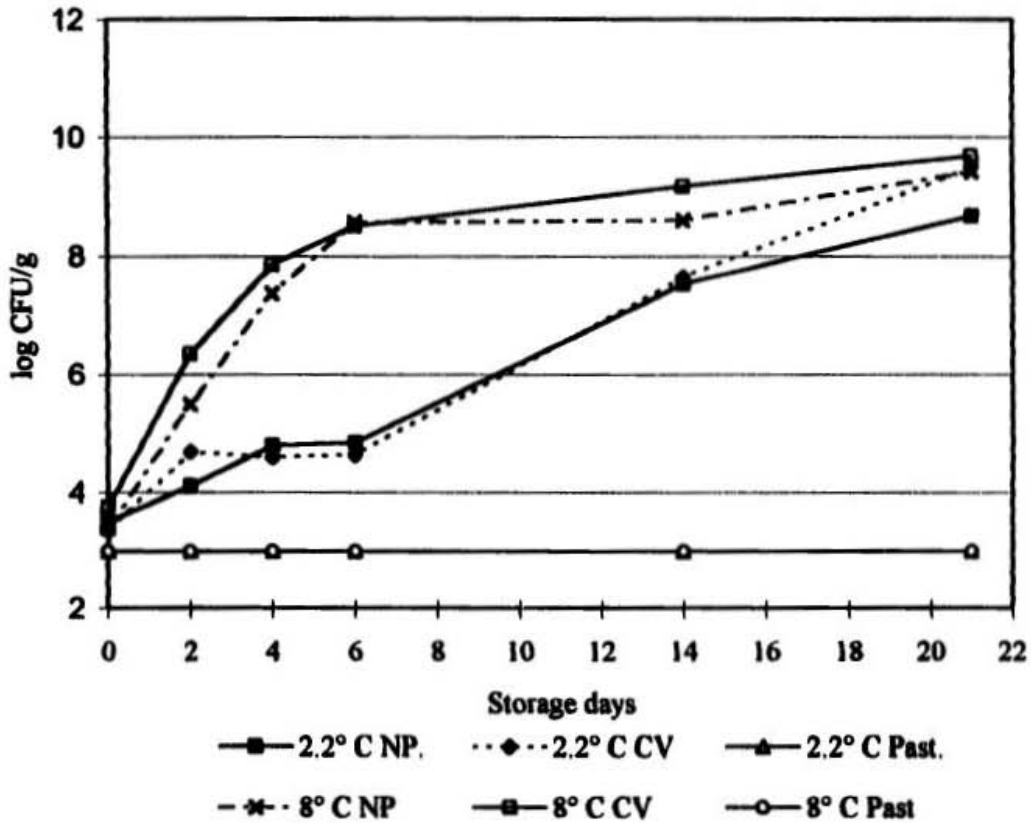


Figure 3.2 Anaerobic bacterial counts in non-pasteurized (NP), conventional (CV) and pasteurized (P) SMSP stored at 2.2°C and 8°C.

in both non-pasteurized and conventional samples respectively but then reached levels similar to the 8°C samples by day 21.

Figure 3.3. shows changes in acidity of variations stored at both temperatures over 21 days. Acid levels in non-pasteurized samples reached 0.047% and 0.05% (w/w) in samples stored at 2.2° and 8°C, respectively. The acidity in conventional samples stored at 2.2° and 8°C reached 0.04% and 0.039%, respectively, while that in pasteurized increased by only 0.005% from 0.028% acidity at either storage temperature.

Physical changes were noted in the non-pasteurized samples and the conventionally treated samples stored at 8°C. These included gas production and swelling of the containers after 14 days. There was no gas production in trays stored at 2.2°C during the 21 day trial period.

3.2. Fat and fatty acid analysis

The mean % fat in SMSP prepared at each pasteurization time-temperature plus the conventional temperature and the non-pasteurized sample is represented graphically in Fig. 3.4: Twelve different fatty acids were analysed to study the effects of heat on the fatty acid composition of SMSP. Data were collected from three separate sets of SMSP prepared using the five pasteurization schedule treatments, the conventional treatment and non-pasteurized treatment. Table B.6. (Appendix B) lists the mg fatty acid/g fat of each set of data plus the mean of the three sets. These values indicate some variability between sets for some of the fatty acids. This variability in values is not consistent across one set.

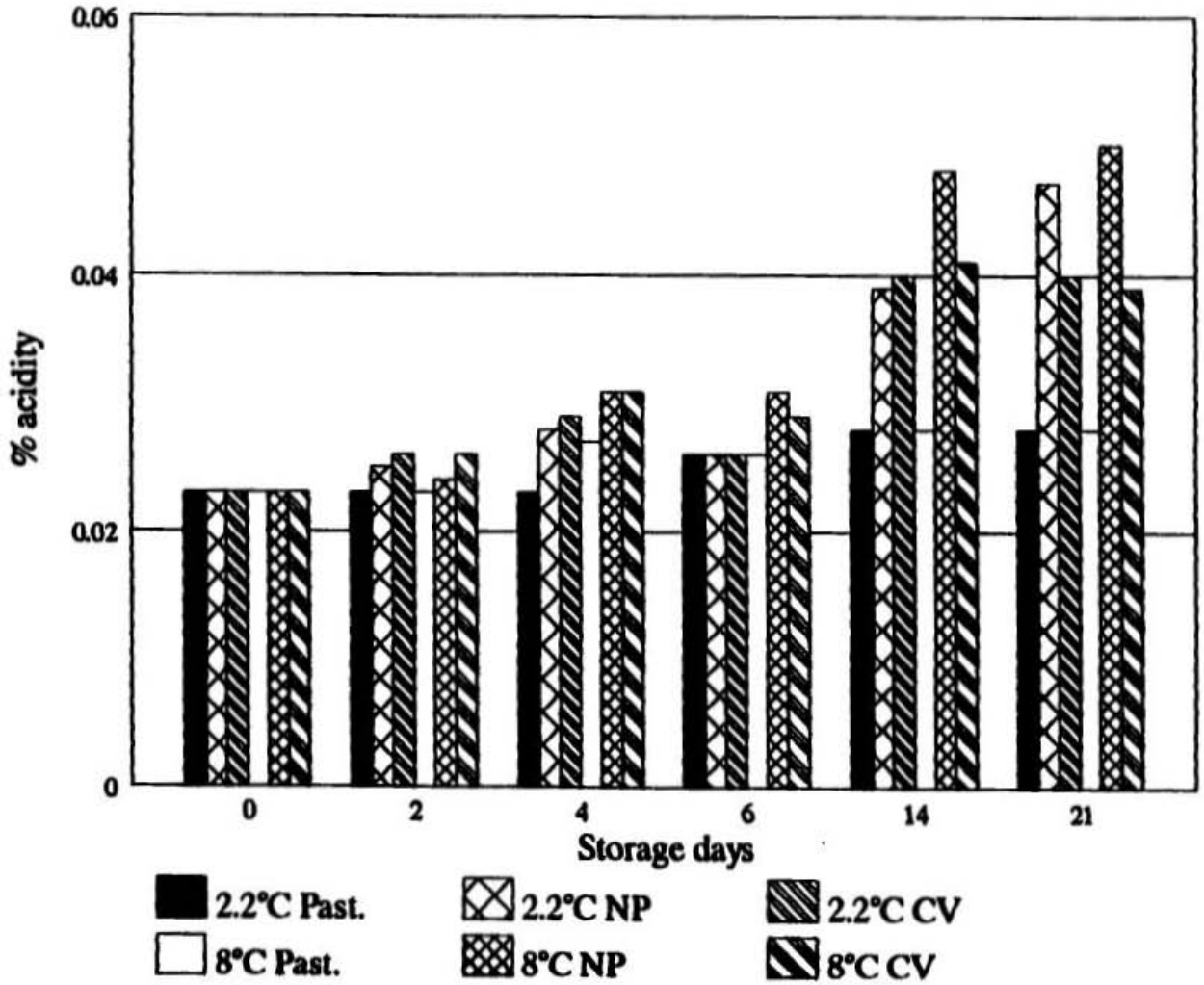


Figure 3.3. Acidity in non-pasteurized (NP), conventional (CV) and pasteurized (P) SMSP stored 21 days at 2.2°C and 8°C.

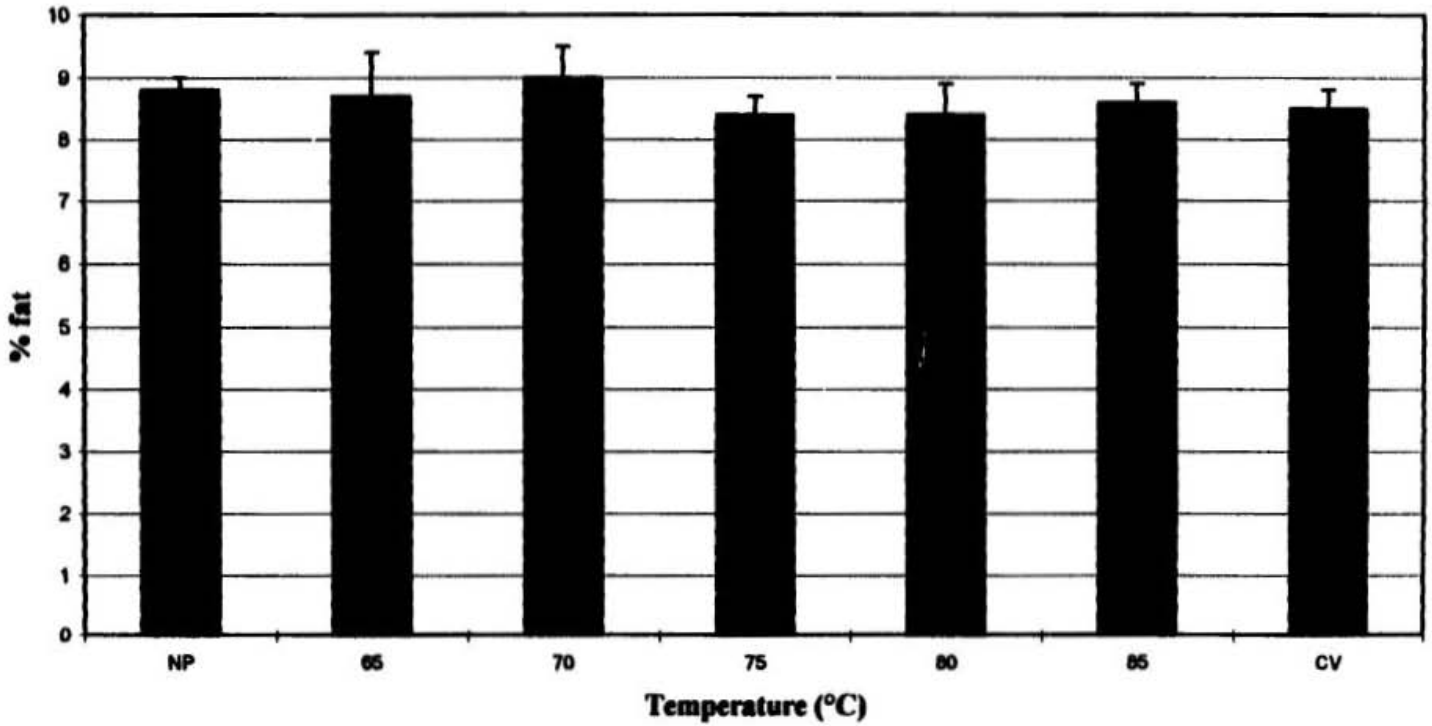


Figure 3.4. Mean (± standard deviation) percent fat from all sets' means in SMSP tested at 5 pasteurization time-temperatures plus non-pasteurized (NP) and conventional (CV).

For example, from Table B.6., values for Set 1 for C18:1 are higher than Sets 2 and 3, but values for C16:0 are more consistent across three sets.

Table B.7. (Appendix B) gives a summary of the p-values for the repeated measures analysis of variance (ANOVA) test. Comparison between the three sets (lots), between the temperatures and between lot*temperature shows significant differences occurring in all factors studied but not with all fatty acids. The effect of lot or set is evident at $p<0.01$ in the amounts of fatty acids C18:1, C18:2, C18:3, C20:4, C22:5 and C22:6 and at the $p<0.05$ level in C16:1 and C18:4 fatty acids. Temperature effects are significant at $p<0.01$ in the amounts of fatty acids C14:0, C18:1, C18:4, C20:5, C22:5 and C22:6 and at the $p<0.05$ level in the amount of fatty acid C16:1. The effect of lot*temperature values is significant at the $p<0.01$ level for the amounts of fatty acids C14:0, C16:1, C18:1, C18:4, C20:5, C22:5 and C22:6 and at the $p<0.05$ level in the amount of fatty acid C16:0.

In order for the results from the ANOVA to be viewed in a meaningful way, it was decided to compare the non-pasteurized sample results with the pasteurization temperature sample results and the conventional treatment sample results. A summary of the p-values, at significance levels of $p<0.01$ and $p<0.05$, of the multiple comparison test between the mean value of each of the 12 fatty acids at each of the five pasteurization time-temperatures plus the conventional temperature and the mean of the non-pasteurized sample is given in Table B.8. (Appendix B). Only those fatty acids which showed statistical significance between temperature treatments are displayed. The value for which there is a significant difference, when compared with the non-pasteurized treatment value,

is highlighted with ↑ or ↓ indicating if the value is higher or lower than the non-pasteurized sample value.

Figure 3.5. shows a comparison of the saturated fatty acid values in the SMSP. From Figure 3.5. and Table B.6., it can be seen that the level of the saturated fatty acid, C14:0, is higher at the pasteurization temperature of 65°C than the non-pasteurized sample (6.115 versus 5.208 mg/g fat). This is significant at the $p<0.05$ level (Table B.8.). From Figure 3.6. and Table B.6. and Table B.8. the level of the MUFA C16:1 is higher in the SMSP pasteurized 65°C than in the non-pasteurized sample (8.915 versus 7.717 mg/g fat) at a $p<0.05$ level and the level of the MUFA C18:1 is lower in the SMSP prepared by the conventional treatment (169.098 versus 203.168 mg/g fat) at $p<0.01$ when comparison with the non-pasteurized sample is made. The levels of the PUFAs studied show variation in response to the different temperatures. From Figure 3.7. and Table B.6. and Table B.8. the levels of the fatty acids C18:4, C20:5, C22:5 and C22:6 are significantly ($p<0.01$) higher (0.594, 6.093, 2.419 and 6.384 mg/g fat, respectively) in the SMSP pasteurized at 65°C than in the non-pasteurized sample (0.280, 4.189, 1.859 and 4.402 mg/g fat, respectively). However the level of the fatty acid C18:4 is significantly ($p<0.05$) lower in the SMSP prepared at the conventional temperature than in the non-pasteurized sample (0.056 versus 0.280 mg/g fat). The omega-3 fatty acids, including C20:5, C22:5 and C22:6, which had significantly different values, are viewed again on a larger, more clear scale in Figure 3.8.

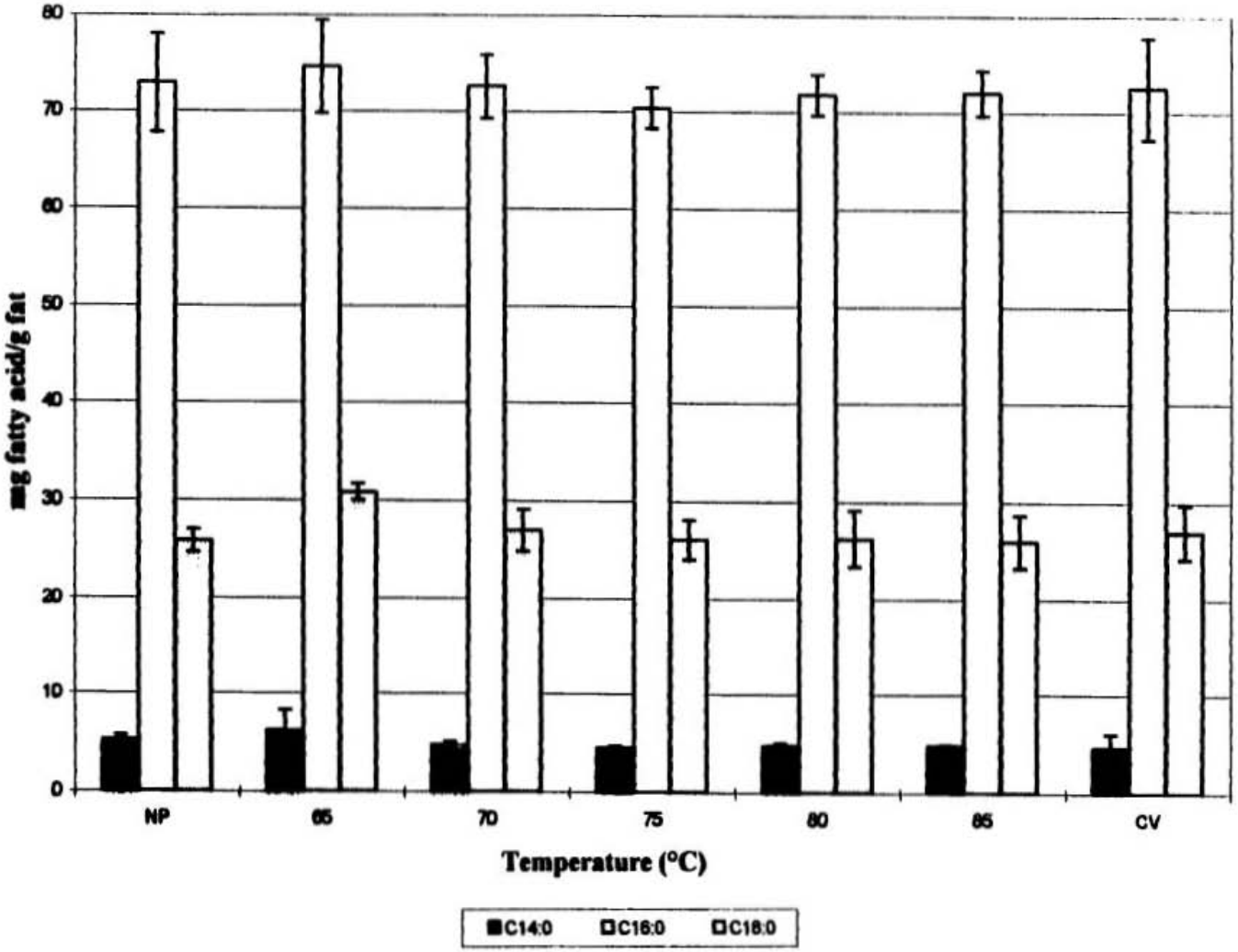


Figure 3.5. Mean (± standard deviation) mg saturated fatty acids/g fat in all sets' means for SMSP tested at 5 pasteurization time-temperatures plus non-pasteurized (NP) and conventional (CV).

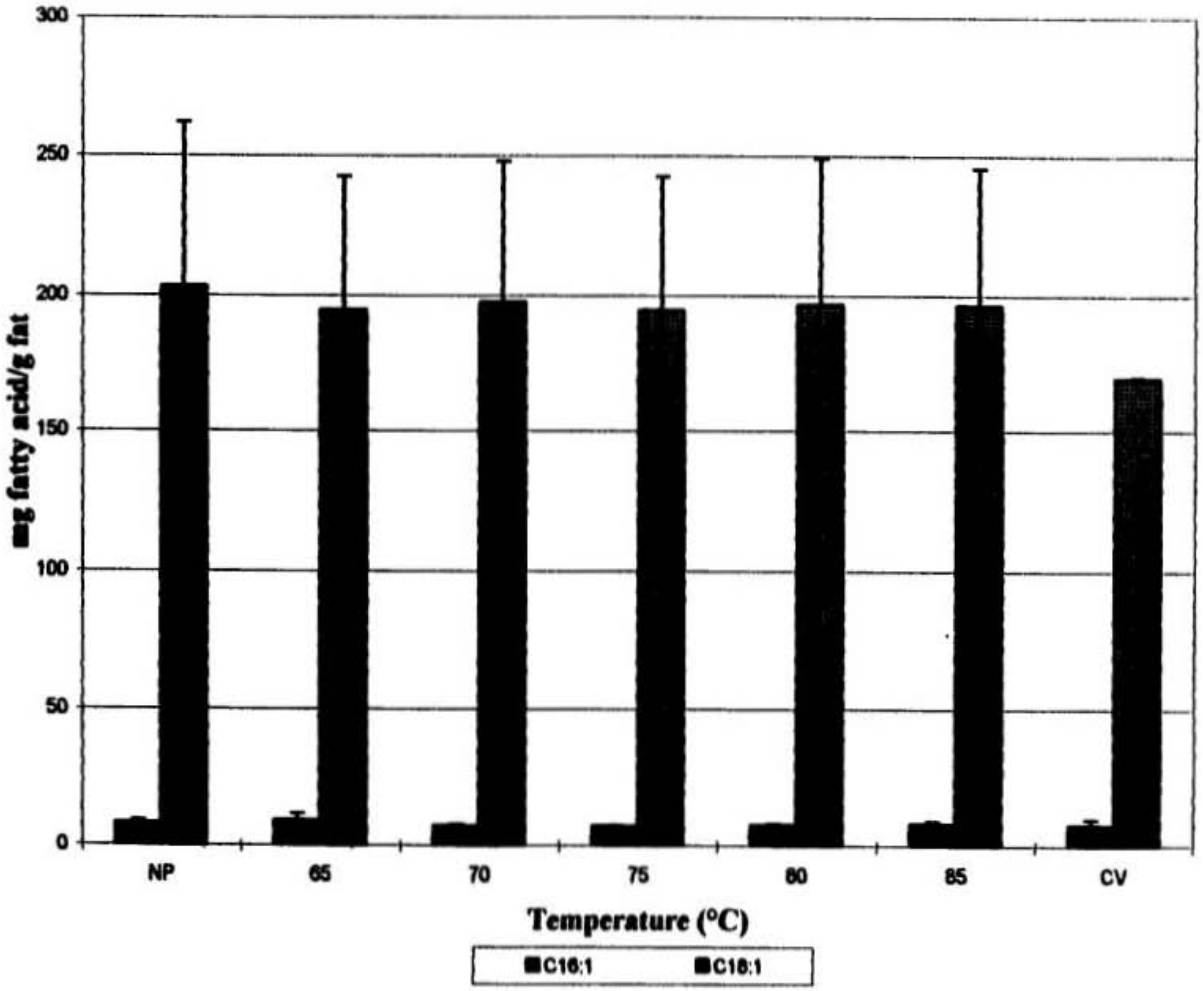


Figure 3.6. Mean (± standard deviation) mg monounsaturated fatty acids/g fat in all sets' means for SMSP tested at 5 pasteurization time-temperatures plus non-pasteurized (NP) and conventional (CV).

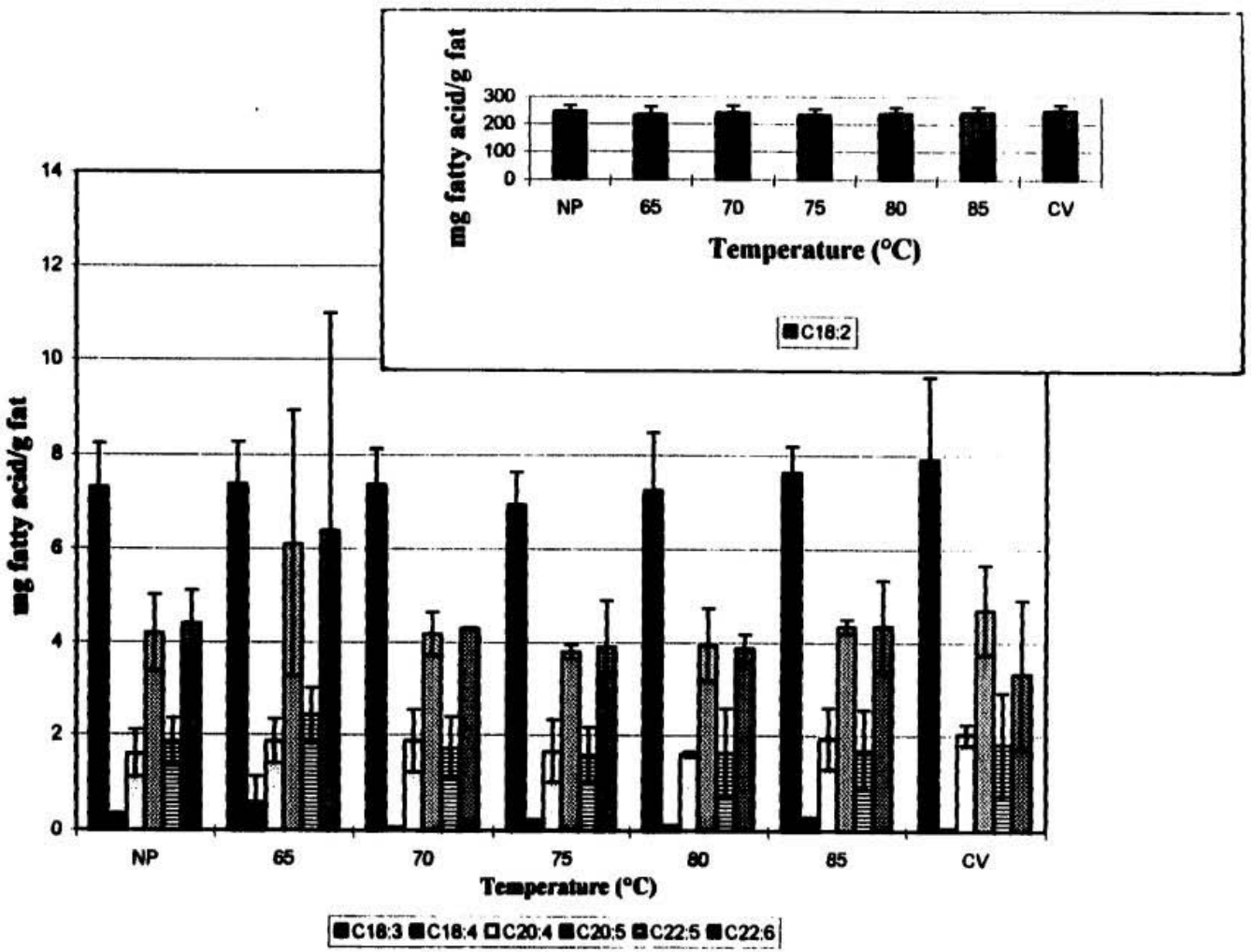


Figure 3.7. Mean (± standard deviation) mg polyunsaturated fatty acids/g fat in all sets' means for SMSP tested at 5 pasteurization time-temperatures plus non-pasteurized (NP) and conventional (CV).

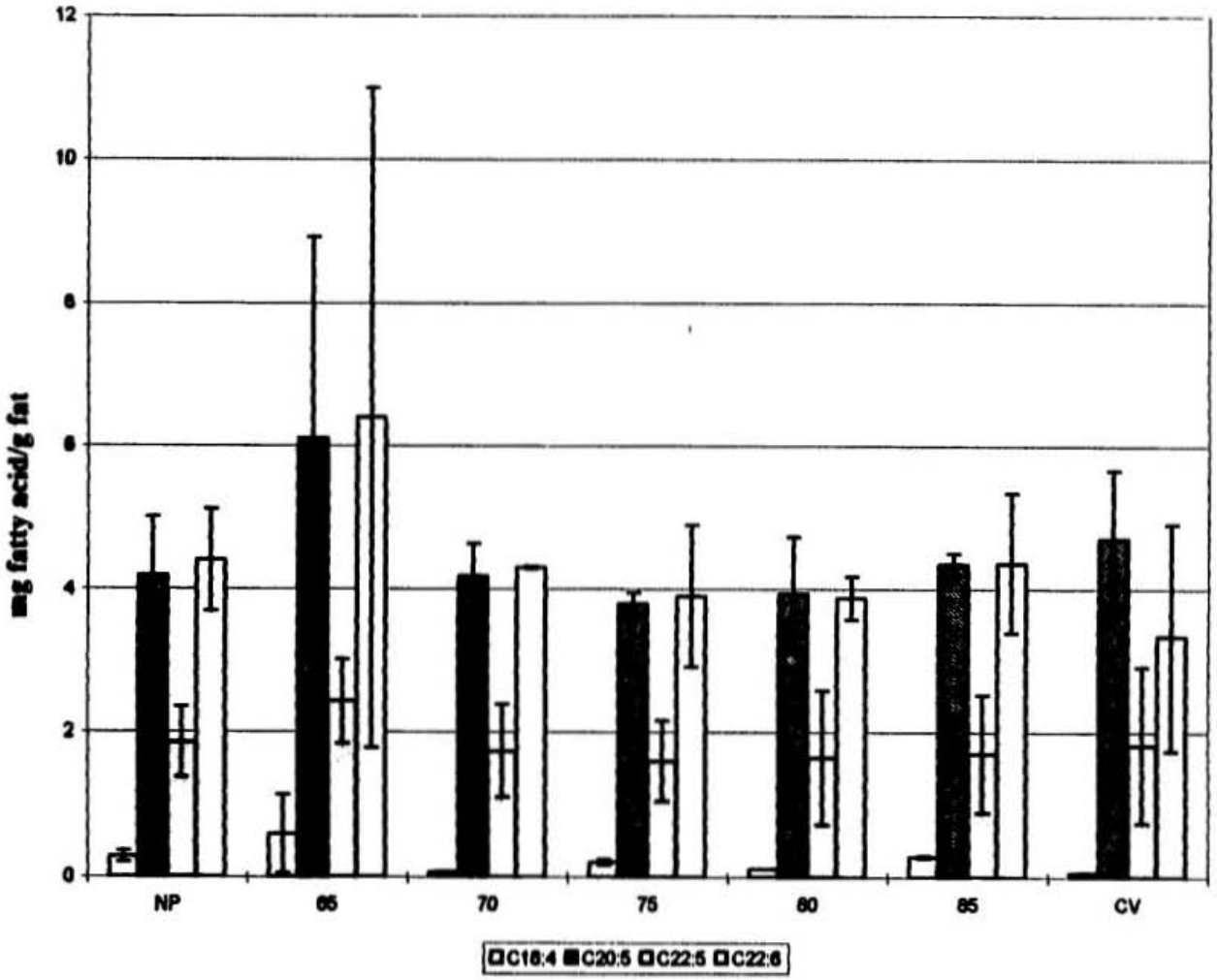


Figure 3.8. Mean (± standard deviation) mg omega-3 fatty acids/g fat in all sets' means for SMSP tested at 5 pasteurization time-temperatures plus non-pasteurized (NP) and conventional (CV).

3.3. Thiamin analysis

Table B.9. (Appendix B) lists the thiamin data collected from three sets of SMSP. The mean thiamin values across the three sets ranged from 0.217 mg thiamin hydrochloride/g non-pasteurized product to 0.191 mg/g of the conventional product. Values of 0.202 to 0.192 mg thiamin hydrochloride/g of product were found for 65° to 85°C respectively. This is represented graphically in Figure 3.9. A simple ANOVA performed on the data collected from three sets of SMSP indicated no significant differences in the levels of thiamin between the pasteurizing temperatures, non-pasteurized and conventional treatments.

3.4. Protein analysis

The results of crude protein analysis are presented in Table B.10. (Appendix B) listing mean percent of each set and mean percent of the three sets. This is illustrated graphically in Figure 3.10. A simple ANOVA performed on the data indicates that there is a significant difference between the values of the set means (see Table B. 11., Appendix B). The uncorrected p-value for all values for the SMSP pasteurized at 5 time/temperatures and the non-pasteurized SMSP when compared with the SMSP prepared by the conventional method indicates significance at p-value <0.05 and <0.01. There was no significant difference between the pasteurized sample values and the non-pasteurized

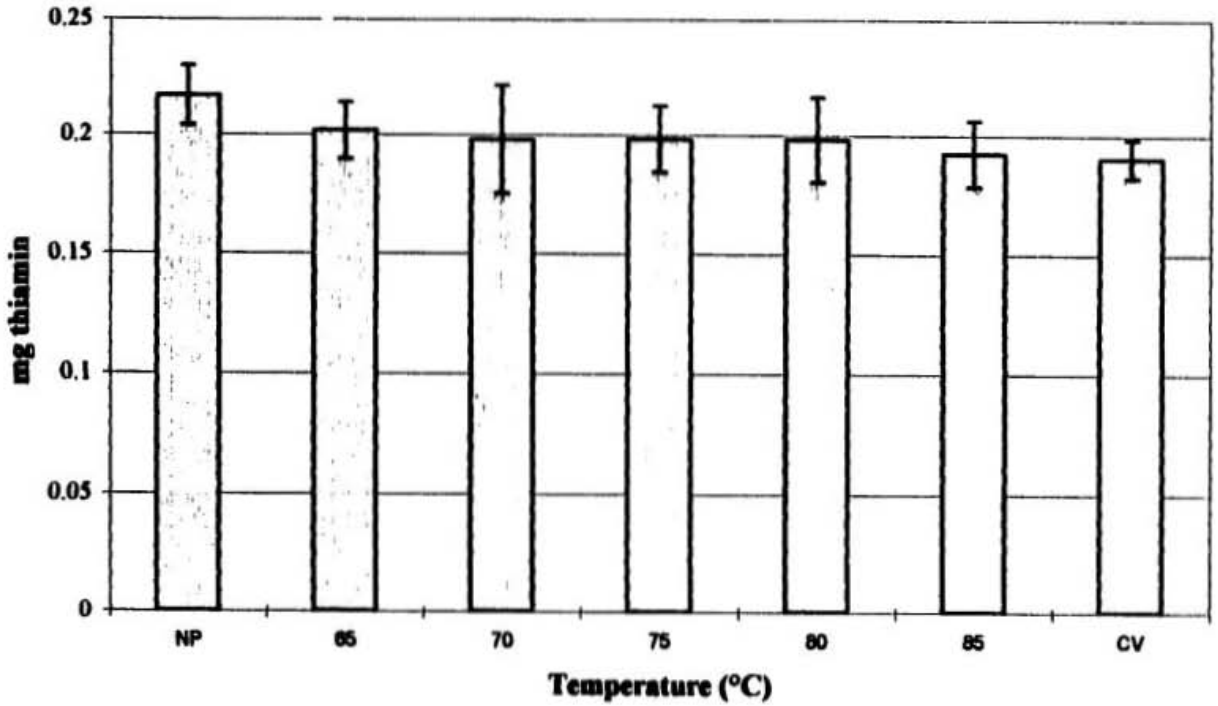


Figure 3.9. Mean of all sets (± standard deviation) mg thiamin/g food in SMSP tested at 5 pasteurization time-temperatures plus non-pasteurized (NP) and conventional (CV).

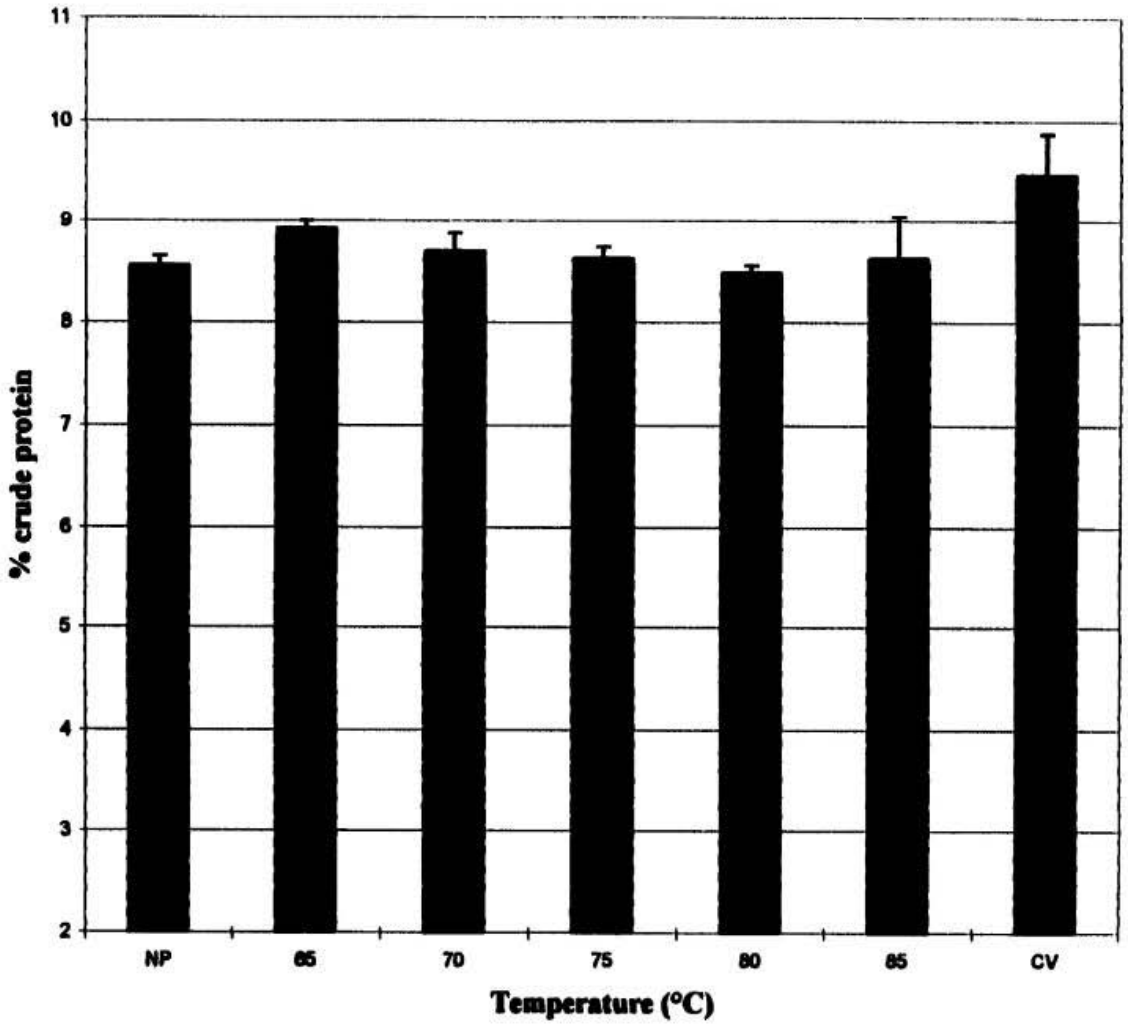


Figure 3.10. Mean (± standard deviation) percent crude protein (wet weight) of all sets' means for SMSP tested at 5 pasteurization time-temperatures plus non-pasteurized (NP) and conventional (CV).

sample value. Mean amount of crude protein for all samples at all temperatures is 8.77%. Seal meat contributes 73.7% protein to the total protein and as such might be expected to confer the most influence on any protein changes.

3.5. Free amino acid analysis

Twenty different amino acids (including the nine essential amino acids) were quantified to study the effects of heat on the free amino acid composition of SMSP. Data from all three sets of SMSP were collected. Set 1 includes values from duplicate analyses of two samples from each of the 5 pasteurization time-temperature treatments plus the conventionally prepared treatment and the non-pasteurized treatment. Sets 2 and 3 include values from triplicate analyses of one sample of each treatment. Table B.12. (Appendix B) lists the mean milligram free amino acid per gram crude protein (dry weight) of each set of data plus the standard deviation of the mean of each set. The total amount of all free amino acids at each temperature is included. Figure 3.11. shows the total of all mean values of all 20 free amino acids at each temperature.

Table B.13. (Appendix B) gives a summary of the p-values from the repeated measures analysis of variance test. Comparison between the sets (lots), between the temperatures and between lot*temperature shows a significant difference for almost all factors studied. Effect of lot or set is significant at $p<0.01$ for all but serine, lysine and arginine and lysine is significant at $p<0.05$. From this table, temperature has a significant

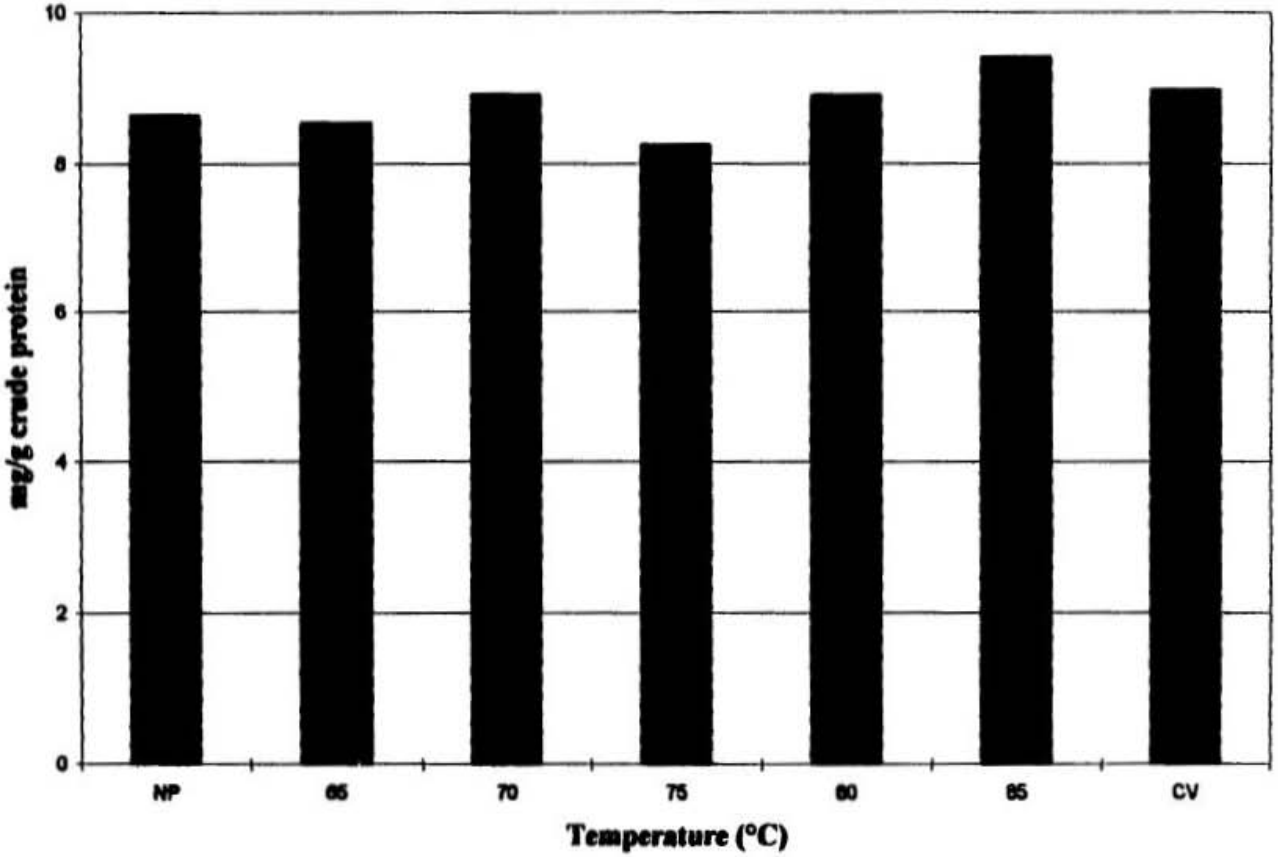


Figure 3.11. Mean total (mg/g crude protein) of all sets' means of all amino acids in SMSP tested at 5 pasteurization time-temperatures plus non-pasteurized (NP) and conventional (CV).

effect on the values for the amino acids aspartic acid, asparagine, glutamic acid, proline, glycine, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan and arginine at $p<0.01$ and for the amino acid serine at the $p<0.05$ level. The effect of lot*temperature was significant at the $p<0.01$ level in all amino acids except aspartic acid, threonine and valine and at the $p<0.05$ level for aspartic acid and threonine.

In order for the results from the ANOVA to be viewed in a meaningful way, it was decided to compare the non-pasteurized sample with the sample pasteurized at different temperatures and with the conventional treatment sample. A summary of p-values, at significance levels of $p<0.01$ and $p<0.05$, of the multiple comparison test between the mean value of each of the 20 free amino acids at each of the five pasteurization time-temperatures and the conventionally prepared sample and the mean of the non-pasteurized sample is given in Tables B.14. - B.20. (Appendix B). Only those free amino acids which showed statistical significance between temperature treatments are displayed in these tables. The amino acids are separated into aliphatic, hydroxyl-containing, acidic, basic, sulphur-containing, aromatic and the imino acid proline, in the tables B.14. - B.20, respectively. The p-value at which there is a significant difference when compared with the non-pasteurized treatment p-value is highlighted with ↑ or ↓ indicating if the mg free amino acid/g crude protein is higher or lower than the free amino acid in the non-pasteurized treatment.

Pasteurization at 85°C for 30 min resulted in retention of significantly (at either $p<0.05$ or $p<0.01$ levels) higher levels of nine of the twenty free amino acids in the SMSP.

Four of the nine essential amino acid values are significantly (at either $p<0.05$ or $p<0.01$ levels) higher in the 85°C sample than in the non-pasteurized sample.

The aliphatic amino acids, valine and isoleucine show an increase over the non-pasteurized control at 85°C at a $p<0.01$ level and leucine at the $p<0.05$ level. In comparison to the non-pasteurized sample glycine and leucine decrease during conventional cooking while valine increases. Serine, of the hydroxyl-containing amino acids, is significantly ($p<0.01$) increased at 85°C. Of the acidic amino acids and their amides, aspartic acid and asparagine values significantly ($p<0.05$) increase at conventional temperatures, glutamic acid increases ($p<0.01$) at 85°C and asparatic acid values decrease significantly ($p<0.01$) at both 65° and 75°C. The basic amino acid arginine is increased ($p<0.01$) at 85°C. Amounts of the sulphur-containing amino acids are increased ($p<0.01$) with the temperatures of 80° and 85°C for both amino acids and amounts of methionine are increased significantly ($p<0.05$) at 70°C. Amounts of cystine are increased ($p<0.01$) with the conventional treatment. In the aromatic group, tryptophan is decreased significantly ($p<0.05$) at 70° to 80°C and at $p<0.01$ for 65°C and tyrosine decreases ($p<0.05$) at 75°C. Tyrosine increases ($p<0.05$) at 85°C and at the $p<0.01$ level for the conventional treatment. The imino acid proline shows a significant ($p<0.01$) decrease with the conventional treatment.

3.6. Texture analysis

From the values of force recorded by the texturemeter at points along the graph (see representative graph, Figure A.3. Appendix A), the value at 3 mm of depth of probe was chosen as the marker value for each testing. A 3 mm penetration of the probe is approximately 75% of the maximum height of the peak representing force. The mean and standard deviation of force (N, newton) for each temperature variation for 3 sets is presented in Table B.21.(Appendix B). Values for force ranged from 1.03471N for non-pasteurized to 0.87551N for 70°C. This is represented graphically in Figure 3.12..

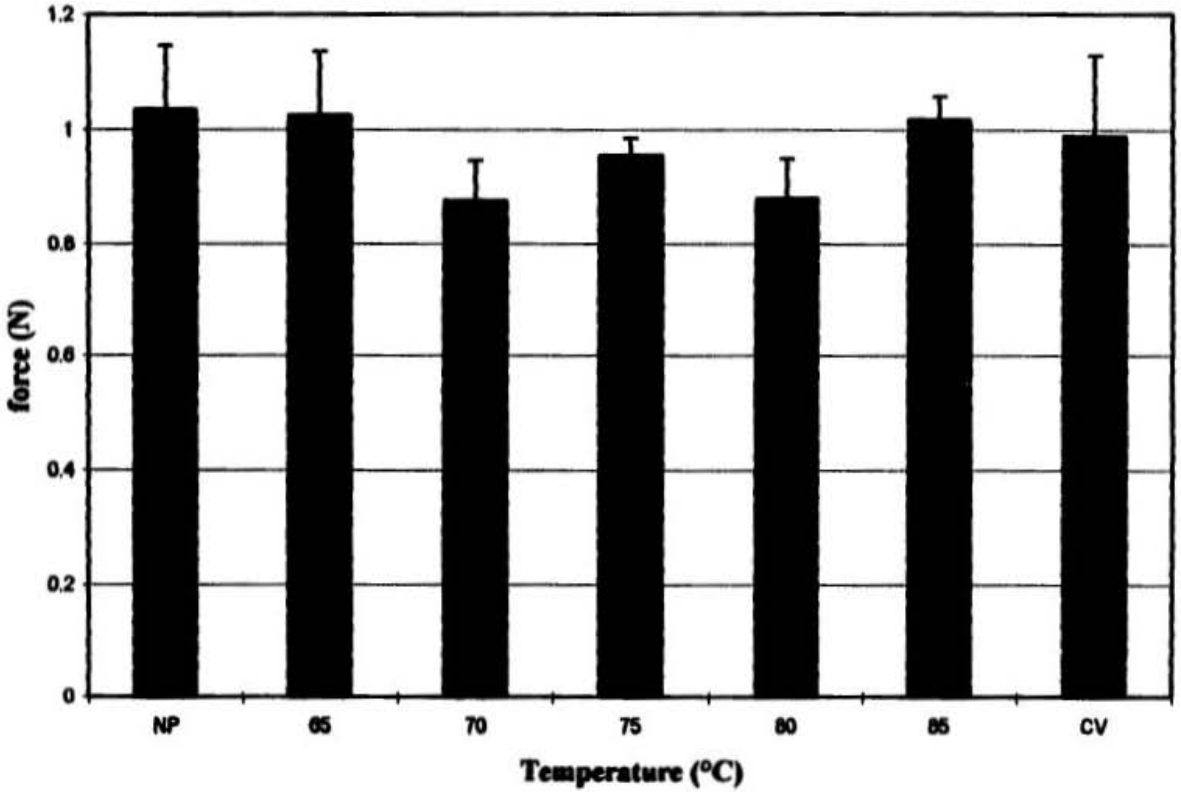


Figure 3.12. Mean of all sets (± standard deviation) force (newtons) required at 3 mm for SMSP meat tested at 5 pasteurization time-temperatures plus non-pasteurized (NP) and conventional (CV).

4. DISCUSSION

4.1. Microbiological analysis

The results of microbiological testing were as predicted with regard to the effects of different storage temperatures. In the non-pasteurized and conventional samples stored at 8°C, there was a steady increase in both aerobic and anaerobic bacteria over the 21 day trial period, whereas at the lower temperature of 2.2°C bacterial growth was retarded, at least until day 6. The results of Simpson *et al.* (1994), testing *sous vide* products pasteurized at 65° and 75°C and stored at 5° and 15°C over a 35 day period, showed a steady rise in bacterial levels at the higher storage temperature, but a delay in bacterial growth at the lower temperature. Our product was pasteurized at a higher temperature (85°C) and had a *D* value of 12 compared to 5*D* and 13*D* used in the Simpson trial, but similarly shaped curves were observed at both the high and low storage temperatures in both tests.

It is interesting to note that aciduric bacterial growth occurred to a greater extent at the higher temperature as indicated by both a small increase in % acid and a slightly lower pH in both non-pasteurized and conventional products at 8°C. Final bacterial counts were similar for both treatments and a study of the microbiological population would probably show a high level of lactic acid bacteria predominating at 8°C. After sufficient time or temperature abuse, the pasteurized sample might show evidence of psychrotropic microaerophilic lactic acid bacteria growth. Presence of the acid produced

by these bacteria has been noted to have an inhibiting effect on *C. botulinum* growth (Doyle, 1991). This could be viewed as a positive attribute of vacuum packaging.

As expected, the pasteurized product did not show bacterial growth within the 21 day trial period, confirming the suitability of the *sous vide* process as a preservation method.

4.2. Fat and fatty acid analysis

There is a scarcity of information on the effect of heat treatment at <100°C on food fats and fatty acids. Most researchers have investigated the changes occurring with temperatures used in deep fat frying (>180°C) and usually only the fat in pure form is studied. As with any nutrient, the effects of other components in the foodstuff must be taken into consideration as having a possible beneficial or detrimental effect on the nutrient in question. The SMSP product is very complex, containing moderately large quantities of protein and carbohydrate as well as iron and other minerals.

Examination of the results of fatty acid analysis of eight readings from each treatment for Set 1 and triplicate readings of Sets 2 and 3 of the SMSP product show differences between the various time/temperature combinations. The twelve fatty acids can be examined together, and they can also be analyzed in separate groups of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and the Ω-3 fatty acids. The amounts of the SFAs, palmitic (C16:0) and stearic (C18:0) acids in the pasteurized and conventionally treated samples did not show a

significant difference when compared with the amounts in the non-pasteurized control, as indicated in Table B. 8. (Appendix B). The amounts of myristic acid (C14:0) in SMSP pasteurized at a temperature of 65°C were significantly higher than in the non-pasteurized sample. These results indicate a certain stability within this group of fatty acids. Armstrong and Bergan (1992) cite temperatures of >150°C as being necessary in order to see an effect of heat on saturated fatty acids.

The two MUFAs, palmitoleic (16:1) and oleic (C18:1) acids, do not show a trend in relation to each other and the heat treatments. The conventional treatment appears to have had a detrimental effect on the levels of C18:1 in the SMSP but it did not significantly affect the levels of C16:1. The temperature of 65°C is marginally beneficial in retention of C16:1. Of the two MUFAs, C18:1 is the more sensitive in our experiment. This sensitivity might be explained by the different form of the margarine used for set 1 and for sets 2 and 3 (as previously noted in Chapter 2). Oleic acid, which is present in high amounts in the corn oil margarine, exists as *cis* and *trans* isomers. Most block or hard margarines have somewhat higher levels of saturated and/or *trans* unsaturated fatty acids than the tub or soft margarines (Slover *et al.*, 1985). In set 1, containing block or hard margarine, a higher percentage of *trans* would be present, whereas the tub or soft margarine used in sets 2 and 3 normally would contain more of the *cis* isomer. The *trans* isomer is more thermodynamically stable and less susceptible to oxidative damage than the *cis* configuration (Craig-Schmidt, 1992, Jandacek, 1992) and the levels of C18:1 in all of the set 1 SMSP preparations, except for that prepared by the conventional treatment, are higher (see Table B.6. Appendix B). In addition, levels of oleic and linoleic acids are

generally adjusted in the hard and soft margarines, so that in set 1, the levels of C18:1 are higher than the levels of C18:2, while in sets 2 and 3, C18:2 is present in the higher amounts. The statistically significant difference noted for C18:1 between lots ($p = 0.0001$) (Table B. 7., Appendix B) is between lots 1 and 2 and lots 1 and 3. However, the two lower values contributed by sets 2 and 3 for C18:1, would have skewed the mean of the 3 sets to the lower value. Therefore, we cannot see an upward trend in values for the two MUFAs. The lower value seen with the conventional treatment could be explained by the greater exposure of the conventional product to air causing some oxidation of C18:1. Although oleic acid is present in seal meat fat in high amounts, some of it would have been destroyed during frying. The values obtained reflect more the SMSP margarine content which masks the seal meat content and makes it difficult to distinguish changes, which might be due to heat, in the two main fat sources. However, changes seen in the values for this fatty acid would be attributed to the margarine type since the seal meat levels of the fatty acid would presumably remain constant throughout the three sets.

Seven fatty acids are included in the category of PUFAs: C18:2, C18:3, C18:4, C20:4, C20:5, C22:5 and C22:6.

From preliminary literature searches for our research of the effect of heat on fatty acids it was expected that the level of linoleic acid (C18:2) would show a significant variability with increasing processing temperature because of the length of the carbon chain and presence of two double bonds. Our results show that this has not been the case. In a study by Cosgrove *et al.* (1987) it was shown that, when using linoleate and mono-, di- and tri-acylglycerols in a homogeneous chlorobenzene solution at fixed temperature

and pressure, linoleic acid did not follow the classic autoxidation kinetics of the other PUFAs investigated and it was recommended that more study be made of the mechanisms of its autoxidation.

The levels of th Ω-3 fatty acid, linolenic acid, C18:3, were statistically as stable as the dienoic linoleic acid. The levels of the Ω-3 fatty acid, C18:4, although present in only small amounts, was also not significantly reduced at any of the pasteurization time-temperatures. Generally, the higher number of double bonds is thought to lead to more instability. Eicosapentaenoic acid (C20:5), docospentaenoic acid (C22:5) and docosahexaenoic acid (C22:6), longer chain Ω-3 fatty acids, are examined in Figure 3.8. There is a noticable difference in the effects of heat treatment at 65°C when compared with the other treatments. The levels of these fatty acids are in higher amounts at 65°C than in the non-pasteurized sample. The lower temperature and low oxygen environment may have contributed to this improved retention. Values did not vary significantly between the remaining temperatures compared with the control NP. Docosahexaenoic acid appears to be more sensitive than eicosapentaenoic acid to the conventional treatment (Figure 3.8.), but this was not statistically significant.

A review of the literature of research on Ω-3 fatty acids in foods and in model systems suggests that these fatty acids are more stable than would be expected in a long chain, highly unsaturated fatty acid. Ratnayake *et al.* (1989) concluded, from an experiment using DHA and EPA heated at temperatures from 100° to 240°C, that EPA in fish oils is quite resistant to thermal abuse. They observed that the fatty acids with an

uneven number of double bonds, such as EPA, were more stable than those with an even number of double bonds. Fujita *et al.* (1994) tested the oxidative stability of sardine lipids (high in EPA and DHA) through heat treatment and found that phospholipids and non-polar lipids produced a heat induced stabilization in PUFAs. The complex nature of the SMSP product may provide some protection for these normally unstable fatty acids. Bruna *et al.* (1989) also observed lower oxidation rates for EPA and DHA when seven long chain PUFAs in an aqueous medium were photoirradiated. They suggested that the number of double bonds, rather than chain length, was the stability factor, and also noted the greater stability of C18:3 when compared with the other PUFAs, but not when compared with the Ω-3 fatty acids. Miyashita *et al.* (1993) conducted a similar experiment and also found the Ω-3 fatty acids to be more stable, with DHA the least affected. Nawar *et al.* (1990) carried out an experiment to observe losses/deterioration in Ω-3 fatty acids in fish cooked by various methods and demonstrated a possible sensitivity to oxidation once the oil is removed from its natural source indicating that within a food product these fatty acids are stable. Gall *et al.* (1983) found basically no change in fatty acid composition of four fish species after cooking when compared with the raw value.

4.3. Thiamin analysis

Little or no change in thiamin levels occurred with the additional heat treatment, i.e. pasteurizing of the product, as is indicated by comparison of values from pasteurized product versus non-pasteurized product. Figure 3.9. demonstrates this graphically. Any of the time/temperature combinations used for pasteurization should retain added thiamin based on this experiment.

Retention of thiamin, i.e. the amount of the vitamin remaining after heat treatment, within the time/temperature range of this experiment was higher than expected when reference to the literature on this heat senstive vitamin is considered. However, most research work on thiamin degradation in food systems has been done at >100°C or, if at lower temperatures, the work has been done on model systems. Although it is difficult to make direct comparisons between my work and the results from other investigations due to the variability of cooking conditions, a reference to some research will be made.

Benterud (1977) writes, during a description of an experiment on thiamin mononitrate levels in sugar solutions at temperatures from 110° to 130°C for 15 min., that "In food processing, the vitamin stability situation is more complicated than this (model system) because other factors are involved in addition to temperature and time." Teixeira *et al.* (1969) used a digital computer technique for the determination of bacterial lethality versus nutrient retention in conduction heated foods to predict thiamin retention in canned foods and found that, contrary to the popular theory that a short treatment time at high temperature favoured higher retention, the optimum point was at 248°F (120°C) for 90

min over a treatment range of 236° to 293°F (113° to 145°C) for 160 to 40 min. Mulley *et al.* (1975a) compared the rate of destruction of thiamin hydrochloride in pea puree, beef puree and peas in brine puree with that of thiamin hydrochloride in a phosphate buffer at temperatures of 250°F(121°C), 260°F(127°C), 270°F(132°C) and 280°F(138°C), and found that thermal stability of thiamin decreased with rising temperature, as expected, and that thiamin was more stable to heat in these food systems than in the model buffer system.

Bertelsen *et al.* (1988) did not find any trends in thiamin retention over the cooking range 20 to 55 min at 190°C used for roasted chicken breast and drumstick. They commented on the need for simplification and improvement in existing extraction procedures. Awonorin and Ayoade (1993) conducted an experiment with four species of ground meat at 70° to 90°C for 15 min to 5 h and found the rate of decrease in thiamin content was greater at the higher temperature. They also noted that losses were greater in meats which had more moisture loss. Moisture is retained in the vacuum packaged *sous vide* SMSP.

In a review of vitamin losses in food processing, Ryley and Kajda (1994) note that apparent increases may occur in foods due to the release of chemically bound forms, or to improvements in digestibility and extraction when the matrix of the food has been damaged. Feliciotti and Esselen (1957) reported that thiamin in foods is more resistant to thermal breakdown than is the pure vitamin in aqueous or buffered solutions, and that pH did not appear to be as significant an influence in foods. They attributed this to the relative proportions of free and combined thiamin. The papers reviewed all indicate the

sensitivity of this vitamin, and the difficulty in obtaining consistent results from one experiment to another, especially when working with a food system..

During the chilling step in the production of the SMSP, it is not expected that any thiamin loss will occur. Bognar (1990) reported only a 0.02 - 0.97% per day loss in "pasteurized chilled meals" stored at 2°C. And no significant loss was observed in thiamin in various foods (gravy and mashed potato) (as reported in Bognar *et al.*, 1990) when cooled from a pasteurization temperature of 80°C to 15°C over a 5 h chilling time.

It is possible that the iron content of the SMSP had an influence on thiamin retention, but within the scope of this experiment, this cannot be determined. The pH of the product, ~5.9, is slightly higher than that recommended (5.0 to 5.6), but is still not considered to be in the detrimental range for optimum thiamin retention. And since precautions were taken to avoid light exposure during the extraction and detection section of the experiment, the only effect ultraviolet light might have had would be during the preparation of the product when natural thiamin could have been destroyed.

4.4. Protein analysis

The conventional cooking treatment resulted in values for crude protein that were significantly higher than those for every other temperature when judged by ANOVA. Denaturation of the meat and vegetable protein would have begun and been almost complete during initial pre-pasteurization steps. Further heat treatment was insufficient to cause any major change in protein content. Percent moisture content and a_w of the

conventional product were slightly less than that of all other samples. This could have caused a small but higher concentration of protein in the conventional product. The protein level, 9.47% [CV] versus 8.65% average of other temperatures, represents less than 1% (2 - 3 g) when viewed in relation to overall nutrient values. No correlation was seen between protein content and free amino acid content. As reported in Chapter 1 of this thesis, little, if any, change was expected to be seen due to the effect of the mild heat treatments on protein.

4.5. Free amino acid analysis

It appears that the set (lot) or batch of product had a significant effect on the levels of all amino acids except serine and arginine (see B.13., Appendix B). This indicates that a larger number of sets would be necessary to eliminate the variability in batch production and amino acid hydrolysis. This would be an important point if this test were to be repeated.

Threonine, glutamine, alanine, lysine and histidine remained stable within the temperature parameters of this study. Histidine is not known to be sensitive to heat treatments, whereas threonine is mainly affected by extremes of pH (Finley, 1985) combined with heat. The slightly acidic nature of the SMSP product does not appear to have had an influence here. Lysine destruction in fresh albacore tuna was studied at temperatures of 140° to 180°C and it was concluded that these standard thermal processing temperatures did not significantly affect the nutritional parameters of lysine and

protein digestibility (Banga *et al.*, 1992). This basic amino acid was also the most stable during various time temperature treatments of faba beans in water. Cooking temperatures between 100° and 125°C for 1 to 12 h resulted in an increase in the amount of free lysine for both hard-to-cook and easy-to-cook beans (Ziena *et al.*, 1991). Lysine is more reactive in amino-carbonyl reactions in dry heat environments (Feeney *et al.*, 1985). In the moist heat of the *sous vide* treatment notable changes in lysine would not be expected.

Values for methionine and cystine indicate an increase in amounts of these amino acids at several temperature variations. Studies have shown that combinations of meat and vegetables rather than meat alone result in more destruction of these amino acids which readily participate in the Maillard reaction. Methionine, arginine and histidine have also been noted for sensitivity to reducing sugars, glucose and ribose, in meat (Baardseth, 1977) but these amino acids did not show any significant losses in our product. The high moisture content (75%) of the SMSP may have provided protection. The sulphur containing amino acid, methionine, has been studied in meat for its heat sensitivity (Hamm, 1977), and losses of 10% were noted in normal cooking procedures. However, no significant losses of this amino acid were seen at any of the pasteurizing temperatures tested.

The heat sensitivity of tryptophan is well known. This amino acid has been used in mutagenicity studies because it is released more readily than other amino acids. It binds with mutagens that are released by heat creating tryptophan derivatives of the mutagen. Since these mutagenic tryptophan derivatives have been observed in foods that have been cooked at low temperatures for extended cooking times (Commoner *et al.*, 1978), it

would seem to indicate a sensitivity of this amino acid to low temperature/long time heat treatments. Table B.19. (Appendix B) indicates a statistically significant reduction in tryptophan at the lower pasteurization temperatures of 65° to 80°C and not at the higher temperatures of 85°C and the conventionally treated sample. It is possible that the shorter cooking times used with these higher pasteurization time-temperatures actually had a positive effect on retention of tryptophan. The longer processing times of 105, 60, 43 and 35 minutes used with the lower temperatures in the SMSP pasteurization could have led to increased denaturation of tryptophan. Although traditionally there has been a tendency to prefer low temperature/long time heat treatment for *sous vide* processed products, preliminary trials, as reported by Sheard and Rodger (1995), have indicated that relatively high temperature/short time treatments may be preferable for some dishes prepared by *sous vide* processing.

The amounts of leucine, glycine and proline in SMSP were significantly lowered by conventional cooking. These amino acids have not been studied extensively in food products. It is possible that the more simple configuration of glycine resulted in its destruction during the uneven heating occurring during the conventional treatment.

Attention is routinely focused on the changes in essential amino acids during cooking. The four essential amino acids with significantly higher values at 85°C, valine, isoleucine, leucine, methionine, may have been released due to proteolysis at this higher pasteurization time-temperature schedule. The shorter cooking time associated with this temperature resulted in retention, rather than further breakdown, of these amino acids.

It was expected that the conventional treatment would show a detrimental effect on more of the free amino acids because of the slightly more intense heat treatment during the stove top cooking. Instead, it appears that this more intense cooking method, i.e. frying temperatures plus fluctuating stove top temperatures, for approximately the same time as the 85°C (30 min), has enhanced the free amounts of five of the amino acids studied. This reflects the probability that these amino acids were released from proteins during the more intense treatment and contributed to the free amino acid pool.

Overall, statistical analysis of the other time/temperature combinations used in this study did not show the same trend as seen with this highest temperature/shortest time pasteurization schedule. This temperature (85°C) is not considered to be in the destructive range (i.e. >100°C) and combined with the short cooking time, is probably sufficient to release some bound amino acids.

4.6. Texture analysis

The results indicated a tenderization of the seal meat in the mid range of the time/temperature combinations. The non-pasteurized sample did not have sufficient heating time and collagen breakdown was incomplete. Long time/low temperature heat treatments have been recommended for the tenderization of meats with high connective tissue contents. The collagen (connective tissue) content of harp seal meat ranges between 0.48 to 0.59% for manually separated meat from beaters (immature seals) and bedlamers (more mature seals) (Shahidi *et al.*, 1990). Seal meat is relatively high in

connective tissue and it was expected that the 65°C pasteurization schedule would produce the most tender meat. However, results for meat pasteurized at 65°C/105 min indicated the second highest reading, i.e. less tender. As this temperature is beyond the range for the start of collagen breakdown (50° to 60°C), we would expect to see noticeable tenderization. It is possible that the temperature was not actually high enough or the time was not really long enough for solubilization of the amount of collagen in this particular species. Pasteurization at 70° to 80°C, with the accompanying processing times, produced the most tender pieces of meat.

The higher temperatures reached in the conventional treatment appear to have resulted in a balance between the toughening of the myofibrillar protein and a breakdown of the collagen so as to result in only a moderate amount of tenderization. In addition, the slightly lower moisture content of the conventional treatment might have contributed to higher stress values, i.e. toughness.

McCrae and Paul (1974) in their study of beef semitendinosus muscle found that the slowest heat penetration rates were between the 60° to 70°C range, over the range of 10° to 70°C tested. They felt that the rate of heat penetration affected the breakdown of collagenous tissue. It is possible that, even if the temperature is maintained at 65°C for 105 min, it is not sufficient to degrade the seal collagen to gelatin and this might explain the higher stress (force) values at the 65°C temperature variation. The faster heat penetration rates of the 70° to 80°C variations may have allowed some solubilization to occur within those ranges.

Paul *et al.* (1973) found that increasing coagulation of the contractile (myofibrillar) proteins was a more important factor than the breakdown of the collagenous tissue in controlling tenderness changes in beef semitendinosus and biceps femoris muscles when seen as a decrease in pentrometer (penetration) readings with increase in temperature from 58° to 82°C. They also found considerable animal to animal variation and suggested that this could be reduced by use of animals of controlled management. Results from the above studies on beef can only be viewed with interest in comparison to this study on ground seal meat.

5. CONCLUSIONS

The *sous vide* method of preservation of Seal Meat Shepherd's Pie (SMSP) has favourable effects on some of the nutritional and quality factors studied. Microbial studies confirmed the effectiveness of the *sous vide* pasteurization technique in reducing bacterial spoilage and thus lengthening the shelf life of the product. The pasteurization schedule of 65°C for 105 min resulted in significantly higher values for the Ω-3 fatty acids, C20:5, C22:5 and C22:6. Thiamin was not significantly affected by any of the heat treatments, but results pointed to a reduced thiamin content with use of the conventional treatment. Further studies on this vitamin may show, more conclusively, the benefits of the milder pasteurization treatments. Values for the twenty free amino acids studied indicate that the pasteurization schedule of 85°C for 30 minutes gives significantly higher values for nine of the amino acids. Texture of the seal meat improved in the pasteurization schedule range of 70° to 80°C.

Because SMSP involved many different food ingredients, such as the seal meat, vegetables, herbs, etc, it is not possible to definitely conclude that a nutritional change is due to one particular pasteurization process. Experimentation using *sous vide* processing techniques on seal meat alone might answer some of the questions regarding the influence of the high mineral content and/or the direct effects of the processing method on the Ω-3 fatty acids in the seal meat.

This study has confirmed the beneficial effects of *sous vide* processing of foods with regards to minimal destruction of those nutrients studied and has indicated the necessity of examining various pasteurization schedules for each new food product.

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APPENDICES

Appendix A

Figure A.1. Sample of gas chromatogram of fatty acid analysis of SMSP (C18:4 not found).

Test Parameters	Lloyd Instruments Ltd			F1 Help
Test Type	Compression	Sample Width	20.00 mm	
No of Stages	1	Sample breadth	20.00 mm	
No of Samples	1	Sample thickness	8.000 mm	
Y Axis	2.500 N	Return mode	Automatic	
X Axis	8.000 mm	Zero mode	Automatic	
Extensometer	Internal	Extension Range	1000.0 mm	

	Speed	Limit	Hold Time	Measure	Variable
Stage 1	3.000 mm/min	6.000 mm	0.0000 min	NONE	STAGE_1
Stage 2	10.00 mm/min	8.000 mm	0.0000 min	NONE	STAGE_2
Stage 3	10.00 mm/min	8.000 mm	0.0000 min	NONE	STAGE_3
Stage 4	10.00 mm/min	8.000 mm	0.0000 min	NONE	STAGE_4
Stage 5	10.00 mm/min	8.000 mm	0.0000 min	NONE	STAGE_5
Stage 6	10.00 mm/min	8.000 mm	0.0000 min	NONE	STAGE_6
Stage 7	10.00 mm/min	8.000 mm	0.0000 min	NONE	STAGE_7
Stage 8	10.00 mm/min	8.000 mm	0.0000 min	NONE	STAGE_8
Stage 9	10.00 mm/min	8.000 mm	0.0000 min	NONE	STAGE_9
Stage 10	10.00 mm/min	8.000 mm	0.0000 min	NONE	STAGE_10
Repeat until	CYCLE = 1				

Figure A.2. Printout of Lloyd Texturemeter machine settings.

Appendix A (cont..)

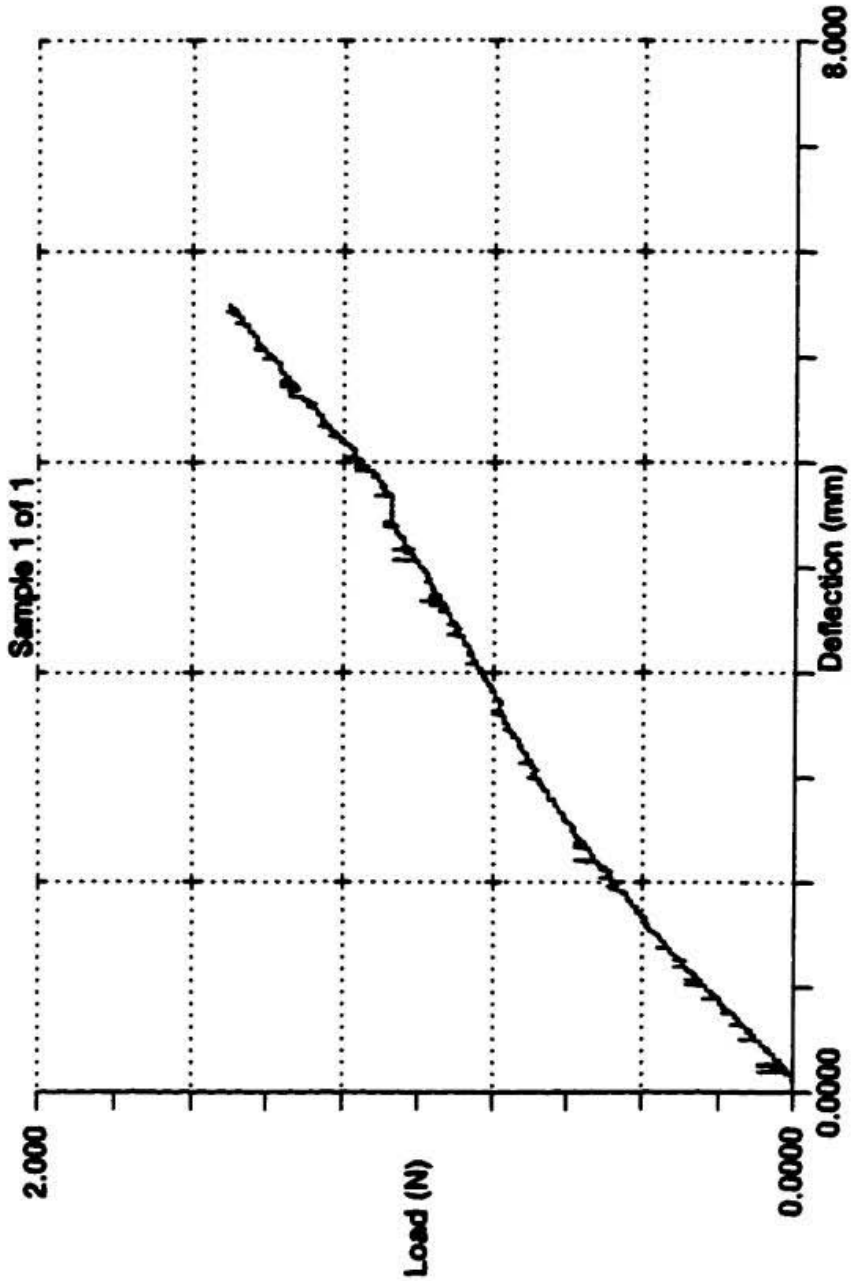

Figure A.3. Sample of a texturemeter graph for a conventionally prepared seal meat sample.

Table B.1. Estimated nutrient composition of ingredients used in Seal Meat Shepherd's Pie (SMSP).

Food	Wgt. g	Moist. %	Calories kCal	Protein g	Fat g	Carbohydrate g	PUFA g	SFA g	Thiamin mg	Iron mg	Copper mg	Vit.C mg
Seal, raw	600	*70	680	142.0	7.8	0.0	**5.8	**1.7	0.9	*115.0	0.71	2.1
Oil, corn	28	0	246	0.0	28.0	0.0	*16.0	*4.0	0.0	0.0	0.00	0.0
Onions, raw	35	*132	13	0.4	0.0	3.0	0.0	0.0	0.0	0.1	#0.0	0.4
Garlic, raw	3	40	4	0.2	0.0	1.0	0.0	0.0	0.0	0.1	-	1.0
Flour, all purpose	16	12	58	1.0	0.0	12.0	0.0	0.0	0.1	0.6	0.00	0.0
Beef bouillon cube	6	*3	10	1.0	0.0	1.4	0.0	0.0	0.0	0.1	-	0.0
Carrots, raw	135	*87	58	1.4	0.3	13.7	0.1	0.0	0.1	0.6	#0.1	2.5
Peas, canned	484	*102	333	21.4	1.7	60.9	0.8	0.0	0.5	4.6	0.40	46.5
Tomato paste	50	*87	42	1.9	0.5	9.4	0.0	0.0	0.1	0.5	-	0.3
Parsley, dried	3	10	8	0.9	0.0	1.3	0.0	0.0	0.0	1.7	0.02	0.0
Thyme, dried	1	10	4	0.1	0.0	0.9	0.0	0.0	0.0	1.7	0.02	0.0
Salt	6	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0
Pepper	1	10	3	0.0	0.0	0.7	0.0	0.0	0.0	0.3	0.02	0.0
Potatoes, raw	1000	77	790	20.7	1.0	179.0	0.0	0.0	0.7	7.2	2.46	132.0
Margarine, corn oil	120	16	816	0.0	91.2	0.0	36.0	36.0	0.0	0.0	0.00	0.0
Milk, 2%	258	*89	128	8.6	5.0	12.4	0.2	3.0	0.1	0.1	0.02	1.6
Water	250	100	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0

Yield: 7 - 280 g cooked servings

Source : Elizabeth Warwick (1993)

* Health and Welfare Canada. Nutrient Values of Some Common Foods (1987)

Pennington (1994)

** Shahidi & Synowiecki (1991)

Appendix B (cont..)

Table B.2. Estimated percent fatty acid of total fatty acids in the fats used in Seal Meat Shepherd's Pie (SMSP)

	Fat Sources in SMSP			
Fatty Acid	Seal meat (raw)	Corn oil	Corn oil margarine	Milk(2%)
C14:0	3.4	1.0	~0.5	12.0
C16:0	13.3	14.0	~10.7	26.0
C16:1	9.3	tr	tr	3.0
C18:0	4.3	2.0	5.9t - 6.9b	11.0
C18:1	25.5	30.0	37.5t - 50.3b	28.0
C18:2	2.8	50.0	43.7t - 30.1b	2.0
C18:3	0.3	2.0	1.0t - 0.7b	1.0
C18:4	0.5	0.0	0.0	0.0
C20:4	2.6	0.0	0.0	0.0
C20:5	4.6	0.0	0.0	0.0
C22.5	3.0	0.0	0.0	0.0
C22.6	5.3	0.0	0.0	0.0
Other	22.6	0.0	0.0	16.0
t tub margarine (soft)				
b block margarine (hard)				
Reference	1	2	3	2

1 Engelhart and Walker (1974)
2 British Nutrition Foundation (1992)
3 Slover (1985)

Table B.3. Mean (± standard deviation) percent moisture content plus pH and water activity of SMSP tested at 5 temperatures plus non-pasteurized (NP) and conventional (CV) treatments.

Temperature °C	Moisture	pH	a_w
NP	75.4(±0.408)	5.90(±0.028)	0.955(±0.005)
65	75.8(±0.386)	5.91(±0.016)	0.954(±0.003)
70	75.3(±0.109)	5.93(±0.015)	0.958(±0.009)
75	75.6(±0.428)	5.93(±0.013)	0.960(±0.006)
80	75.4(±0.321)	5.90(±0.040)	0.954(±0.007)
85	75.5(±0.439)	5.90(±0.034)	0.957(±0.005)
CV	73.2(±0.225)	5.92(±0.017)	0.952(±0.003)

Appendix B (cont..)

Table B. 4. Aerobic and anaerobic bacterial counts/gram of sample, pH & % acid for SMSP stored at 2.2°C.

Pasteurized

Storage Days	aerobic	anaerobic	pH	% Acid
0	<3 Est*	<3 Est	5.88	0.023
2	<3 Est	<3 Est	5.95	0.023
4	<3 Est	<3 Est	5.89	0.023
6	<3 Est	<3 Est	5.79	0.026
14	<3 Est	<3 Est	5.77	0.028
21	<3 Est	<3 Est	5.86	0.028

Non-pasteurized

Storage Days	aerobic	anaerobic	pH	% Acid
0	3.49	3.5	5.84	0.023
2	4.12	4.11	5.85	0.025
4	4.57	4.79	6.01	0.028
6	5.24	4.84	5.9	0.026
14	7.96	7.54	4.65	0.039
21	9.1	8.68	5.06	0.047

Conventional

Storage Days	aerobic	anaerobic	pH	% Acid
0	3.63	3.36	5.84	0.023
2	4.68	4.69	5.91	0.026
4	4.74	4.6	5.94	0.029
6	5.17	4.64	5.9	0.026
14	8.42	7.66	5.15	0.04
21	9.52	9.49	5.37	0.04

Values for the aerobic and anaerobic bacteria are logarithms of the number of colony forming units (CFUs)

*<3Est = <1000 CFU/g (Post, 1988)

Appendix B (cont..)

Table B. 5. Aerobic and anaerobic bacterial counts/gram of sample, pH & % acid for SMSP stored at 8°C.

Pasteurized

Storage Days	aerobic	anaerobic	pH	% Acid
0	<3 Est*	<3 Est	5.84	0.023
2	<3 Est	<3 Est	5.92	0.023
4	<3 Est	<3 Est	5.9	0.027
6	<3 Est	<3 Est	5.9	0.026
14	<3 Est	<3 Est	5.75	0.028
21	<3 Est	<3 Est	5.85	0.028

Non-pasteurized

Storage Days	aerobic	anaerobic	pH	% Acid
0	3.4	3.4	5.67	0.023
2	5.13	5.5	5.84	0.024
4	7.25	7.38	5.95	0.031
6	8.46	8.57	4.99	0.031
14	9.1	8.61	4.43	0.048
21	9.49	9.42	4.57	0.05

Conventional

Storage Days	aerobic	anaerobic	pH	% Acid
0	3.69	3.75	5.79	0.023
2	5.97	6.35	5.89	0.026
4	7.81	7.86	5.91	0.031
6	8.57	8.51	5.61	0.029
14	9.32	9.17	4.45	0.041
21	9.88	9.68	4.86	0.039

Values for the aerobic and anaerobic bacteria are logarithms of the number of colony forming units (CFUs)
*<3Est = <1000 CFU/g (Post, 1988)

Table B.6. Mean (± standard deviation) mg/g fat of 12 fatty acids in SMSP tested at 5 pasteurization temperatures plus non-pasteurized (NP) and conventional (CV).

	NP			65°C			70°C			75°C			80
Fatty Acid	**Sets**			**Sets**			**Sets**			**Sets**			S
	1	2	3	1	2	3	1	2	3	1	2	3	1	
C14:0	5.712	4.939	4.973	8.411	4.693	4.973	4.458	5.096	4.558	4.368	4.693	4.648	4.603	5
Mean±S.D. 3 sets		**5.208**	**±0.4368**		**6.115**	**±2.0707**		**4.704**	**±0.3432**		**4.570**	**±0.176**		4.
C16:0	78.814	69.910	69.843	80.158	71.523	72.050	75.006	73.864	68.802	72.654	68.634	69.664	73.371	72
		72.856	**±5.16**		**74.581**	**±4.8407**		**72.554**	**±3.3023**		**70.314**	**±2.0885**		71.
C16:1	9.038	7.414	6.709	11.906	7.504	7.336	6.698	7.784	6.619	7.123	7.056	6.754	7.672	7.6
		7.717	**±1.1946**		**8.915**	**±2.5911**		**7.034**	**±0.651**		**6.978**	**±0.1969**		-
C18:0	25.099	27.194	25.278	26.891	28.538	27.832	24.886	29.154	26.958	23.722	27.216	27.272	23.016	28
		25.8608	**±1.1609**		**27.7536**	**±0.826**		**26.992**	**±2.1339**		**26.0736**	**±2.8339**		26.
C18:1	270.850	165.715	172.962	249.693	172.962	160.619	255.651	172.715	163.968	250.186	166.040	167.541	257.242	168
		203.168	**±58.719**		**194.421**	**±48.26**		**197.445**	**±50.598**		**194.589**	**±48.154**		19
C18:2	219.666	251.922	261.800	199.651	250.757	251.026	212.094	261.117	249.458	206.931	243.768	247.722	215.522	256
		244.462	**±22.635**		**233.811**	**±29.584**		**240.89**	**±25.61**		**232.803**	**±22.496**		23
C18:3	6.250	7.750	7.918	6.418	7.515	8.187	6.507	7.930	7.638	6.238	7.000	7.605	5.835	8.
		7.302	**±0.9188**		**7.370**	**±0.8933**		**7.358**	**±0.7514**		**6.944**	**±0.6847**		-
C18:4	0.291	0.358	0.202	1.198	0.414	0.157	0.000	0.213	0.000	0.000	0.202	0.202	0.000	0.
		0.280	**±0.0787**		**0.594**	**±0.5425**		**0.067**	**±0.009**		**0.202**	**±0.0346**		
C20:4	2.139	1.523	1.176	2.341	1.445	1.814	2.621	1.624	1.400	2.408	1.210	1.400	1.635	1.
		1.613	**±0.4878**		**1.870**	**±0.4583**		**1.882**	**±0.6499**		**1.669**	**±0.644**		
C20:5	4.98	4.24	3.34	9.34	4.18	4.76	4.21	4.61	3.70	3.96	3.67	3.75	3.06	4
		4.189	**±0.8246**		**6.093**	**±2.8279**		**4.178**	**±0.4683**		**3.797**	**±0.1507**		
C22:5	1.333	2.274	1.960	3.091	2.106	2.072	1.042	2.285	1.882	0.963	1.971	1.870	0.616	2.
		1.859	**±0.479**		**2.419**	**±0.579**		**1.736**	**±0.6343**		**1.602**	**±0.5552**		
C22:6	6.317	3.875	3.013	11.704	3.696	3.774	5.398	4.122	3.394	5.051	3.371	3.282	3.976	4
		4.402	**±0.7137**		**6.384**	**±4.601**		**4.301**	**±0.0148**		**3.898**	**±0.9968**		

cids in SMSP tested at
ized (NP)

Appendix B (cont..)

70°C			75°C			80°C			85°C			CV		
	Sets			Sets			Sets			Sets				
1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
4.458	5.096	4.558	4.368	4.693	4.648	4.603	5.051	4.648	4.726	4.906	4.838	3.125	5.499	5.466
	4.704	**±0.3432**		**4.570**	**±0.176**		**4.771**	**±0.2467**		**4.827**	**±0.0985**		**4.693**	**±1.2835**
75.006	73.864	68.802	72.654	68.634	69.664	73.371	72.531	69.350	74.626	70.134	71.198	66.494	76.530	74.458
	72.554	**±3.3023**		**70.314**	**±2.8885**		**71.747**	**±2.1209**		**71.982**	**±2.3469**		**72.498**	**±5.298**
6.698	7.784	6.619	7.123	7.056	6.754	7.672	7.694	6.787	8.781	7.381	7.090	4.827	8.960	8.075
	7.034	**±0.651**		**6.978**	**±0.1969**		**7.381**	**±0.5174**		**7.750**	**±0.9042**		**7.291**	**±2.1761**
24.886	29.154	26.958	23.722	27.216	27.272	23.016	28.549	27.138	22.859	27.440	27.597	24.170	29.725	27.026
	26.992	**±2.1339**		**26.0736**	**±2.8339**		**26.2304**	**±2.8749**		**25.9616**	**±2.6911**		**26.9696**	**±2.778**
255.651	172.715	163.968	250.186	166.040	167.541	257.242	168.078	164.707	253.288	164.002	171.629	168.851	169.512	168.918
	197.445	**±50.598**		**194.589**	**±48.154**		**196.672**	**±52.479**		**196.302**	**±49.495**		**169.098**	**±0.3637**
212.094	261.117	249.458	206.931	243.768	247.722	215.522	256.502	245.862	216.630	252.370	254.475	223.115	254.845	266.325
	240.89	**±25.61**		**232.803**	**±22.496**		**239.288**	**±21.265**		**241.158**	**±21.268**		**248.091**	**±22.382**
6.507	7.930	7.638	6.238	7.000	7.605	5.835	8.042	7.851	7.034	7.717	8.109	5.958	8.781	9.005
	7.358	**±0.7514**		**6.944**	**±0.6847**		**7.246**	**±1.2226**		**7.627**	**±0.5441**		**7.918**	**±1.6979**
0.000	0.213	0.000	0.000	0.392	0.202	0.000	0.030	0.000	0.000	0.437	0.414	0.000	0.157	0.000
	0.067	**±0.009**		**0.202**	**±0.6346**		**0.112**	**±0.0014**		**0.280**	**±0.0158**		**0.056**	**±0.007**
2.621	1.624	1.400	2.408	1.210	1.400	1.635	1.669	1.568	2.677	1.523	1.635	2.195	2.083	1.792
	1.882	**±0.6499**		**1.669**	**±0.644**		**1.624**	**±0.0513**		**1.938**	**±0.6362**		**2.027**	**±0.2081**
4.21	4.61	3.70	3.96	3.67	3.75	3.06	4.59	4.17	4.52	4.24	4.27	3.64	5.48	5.00
	4.178	**±0.4603**		**3.797**	**±0.1587**		**3.942**	**±0.7921**		**4.346**	**±0.1556**		**4.704**	**±0.9524**
1.042	2.285	1.882	0.963	1.971	1.870	0.616	2.386	1.938	0.784	2.274	2.061	0.616	2.643	2.229
	1.736	**±0.6343**		**1.602**	**±0.5552**		**1.646**	**±0.92**		**1.702**	**±0.8056**		**1.826**	**±1.071**
5.398	4.122	3.394	5.051	3.371	3.282	3.976	4.122	3.539	5.477	3.920	3.662	1.557	4.614	3.797
	4.301	**±0.0148**		**3.898**	**±0.9968**		**3.875**	**±0.3031**		**4.357**	**±0.9817**		**3.326**	**±1.582**

Table B. 7. Summary of p-values from ANOVA with repeated measures test for 12 fatty acids tested in SMSP at 5 pasteurization temperatures plus non-pasteurized (NP) and conventional (CV).

Source	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C18:4	C20:4	C20:5	C22:5	C22:6
Lot	0.6929	0.0896	0.0342*	0.8401	0.0001**	0.0001**	0.0001**	0.0296*	0.0001**	0.1816	0.0001**	0.0001**
Temperature	0.0012**	0.6457	0.0321*	0.5804	0.0001**	0.0881	0.0553	0.0001**	0.3509	0.0042**	0.0020**	0.0011**
Lot*Temperature	0.0001**	0.0361*	0.0001**	0.3883	0.0001**	0.9401	0.0730	0.0001**	0.2614	0.0001**	0.0001**	0.0001**

* : $p < 0.05$
** : $p < 0.01$

Table B.8. Summary of p-values for fatty acids (tested in SMSP) from a pre-planned multiple comparison test between the mean of 6 temperatures (5 pasteurization temperatures and a conventional (CV) temperature) and the mean of the non-pasteurized (NP) sample.

Temperature (°C)	65	70	75	80	85	CV
Fatty Acid						
C14:0	*0.0160 ↑	0.2709	0.0839	0.2486	0.3320	0.1533
C16:1	*0.0418 ↑	0.3346	0.1984	0.5773	0.9696	0.4440
C18:1	0.1825	0.4605	0.1912	0.3643	0.3137	**0.0001 ↓
C18:4	**0.0008 ↑	0.0668	0.4900	0.0752	0.8900	**0.0147 ↓
C20:5	**0.0017 ↑	0.9924	0.5388	0.6858	0.7740	0.2000
C22:5	**0.0038 ↑	0.5773	0.2374	0.3935	0.5172	0.8890
C22:6	**0.0031 ↑	0.9118	0.4530	0.4320	0.9369	0.1480

* = $p<0.05$

** = $p<0.01$

↑ = value is significantly higher than non-pasteurized value

↓ = value is significantly lower than non-pasteurized value

Table B. 9. Mean (± standard deviation) mg thiamin/g food in SMSP tested at 5 pasteurization temperatures plus non-pasteurized (NP) and conventional CV).

Temperature(°C)		NP	65	70	75	80	85	CV
Set 1	Mean	0.22856	0.202707	0.215084	0.191358	0.197612	0.188304	0.183742
	±S.D.	±0.0030	±0.0034	±0.0084	±0.0162	±0.0139	±0.0109	±0.0090
Set 2	Mean	0.21793	0.208657	0.205508	0.204705	0.208922	0.208368	0.194638
	±S.D.	-*	±0.0013	±0.0065	±0.0038	±0.0102	±0.0027	±0.0036
Set 3	Mean	0.20338	0.183912	0.167671	0.183731	0.176812	0.182142	0.193014
	±S.D.	±0.0014	±0.0037	±0.0039	±0.0015	±0.0073	±0.0017	±0.0028
Mean all Sets		0.216623	0.201803	0.19819	0.198393	0.198318	0.192455	0.190465
S.D. between Sets		±0.0126	±0.0108	±0.0181	±0.0116	±0.0133	±0.0097	±0.0048

* data from only 1 group of tests available

Table B. 10. Mean (± standard deviation) percent crude protein (wet weight) in SMSP tested at 5 pasteurization temperatures plus non-pasteurized (NP) and conventional (CV).

Temperature (°C)		NP	65	70	75	80	85	CV
Set 1	Mean	8.695	8.972	8.785	8.470	8.543	9.096	9.421
	S.D.	±0.146	±0.150	±0.146	±0.234	±0.078	±0.152	±0.221
Set 2	Mean	8.465	8.799	8.867	8.661	8.399	8.693	9.007
	S.D.	±0.083	±0.141	±0.294	±0.071	±0.080	±0.029	±0.084
Set 3	Mean	8.496	8.977	8.455	8.753	8.539	8.090	9.992
	S.D.	±0.060	±0.167	±0.062	±0.162	±0.120	±0.144	±0.083
Mean all Sets		8.552	8.916	8.703	8.628	8.494	8.626	9.473
S.D. between Sets		±0.102	±0.083	±0.178	±0.118	±0.067	±0.413	±0.404

Table B.11. p-values (uncorrected) for crude protein in SMSP at 5 pasteurization temperatures plus non-pasteurized (NP) and conventional (CV).

Temperature (°C)	NP	65	70	75	80	85	CV
NP	-	0.1362	0.484	0.7186	0.7798	0.7238	**0.0092 †
65		-	0.3368	0.2145	0.0969	0.2125	*0.0462 †
70			-	0.7208	0.3451	0.7156	*0.0169 †
75				-	0.5305	>0.800	**0.0124 †
80					-	0.5348	**0.0074 †
85						-	**0.0123 †
CV							-

* $p<0.05$

** $p<0.01$

† conventional is significantly higher than pasteurization temperatures and non-pasteurized.

Table B.12. Mean (± standard deviation) mg amino acid/g crude protein in SMSP tested at 5 pasteurization temperatures plus non-pasteurized (NP) and conventional (CV).

Temperature (°C)		NP			65			70			75			
Amino Acid		Set 1	Set 2	Set 3	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3	Set
Aspartic acid	Mean each set *	0.43	0.65	0.69	0.33	0.51	0.64	0.46	0.57	0.62	0.37	0.59	0.63	0.4
	Mean±S.D.		**0.59 ±0.12**			**0.48 ±0.19**			**0.55 ±0.07**			**0.53 ±0.12**		
Threonine		0.25	0.30	0.33	0.29	0.22	0.32	0.33	0.26	0.32	0.25	0.29	0.32	0.2
			0.29 ±0.03			**0.28 ±0.04**			**0.31 ±0.02**			**0.28 ±0.03**		
Serine		0.16	0.18	0.20	0.20	0.16	0.19	0.22	0.17	0.19	0.21	0.17	0.19	0.1
			0.18 ±0.02			**0.18 ±0.02**			**0.19 ±0.02**			**0.19 ±0.01**		
Asparagine		1.81	2.48	3.02	2.01	2.27	2.70	2.21	2.45	2.70	1.71	2.42	2.71	2.0
			2.44 ±0.50			**2.33 ±0.29**			**2.45 ±0.20**			**2.28 ±0.42**		
Glutamic acid		1.07	0.97	0.98	1.37	0.82	0.93	1.46	0.90	0.92	1.14	0.91	0.91	1.3
			1.01 ±0.05			**1.04 ±0.24**			**1.09 ±0.26**			**0.99 ±0.11**		
Glutamine		0.86	0.86	0.93	0.99	0.80	0.95	1.07	0.86	0.80	0.75	0.86	0.97	0.9
			0.88 ±0.03			**0.91 ±0.08**			**0.91 ±0.12**			**0.86 ±0.09**		
Proline		0.24	0.10	0.12	0.27	0.09	0.12	0.28	0.09	0.11	0.23	0.09	0.11	0.2
			0.16 ±0.06			**0.16 ±0.08**			**0.16 ±0.08**			**0.14 ±0.06**		
Glycine		0.08	0.06	0.07	0.09	0.05	0.07	0.11	0.05	0.06	0.08	0.06	0.06	0.1
			0.07 ±0.01			**0.07 ±0.02**			**0.07 ±0.02**			**0.07 ±0.01**		
Alanine		0.47	0.42	0.48	0.59	0.36	0.45	0.63	0.40	0.44	0.49	0.40	0.44	0.5
			0.46 ±0.03			**0.47 ±0.10**			**0.49 ±0.10**			**0.44 ±0.04**		
Valine		0.24	0.25	0.30	0.28	0.23	0.28	0.31	0.25	0.29	0.24	0.24	0.27	0.2
			0.26 ±0.03			**0.27 ±0.02**			**0.28 ±0.03**			**0.25 ±0.01**		
Cystine		0.07	0.08	0.08	0.10	0.07	0.08	0.09	0.08	0.08	0.07	0.08	0.08	0.0
			0.08 ±0.00			**0.08 ±0.01**			**0.08 ±0.00**			**0.08 ±0.01**		
Methionine		0.11	0.03	0.03	0.12	0.03	0.03	0.14	0.03	0.04	0.10	0.03	0.03	0.1
			0.05 ±0.04			**0.06 ±0.04**			**0.07 ±0.05**			**0.06 ±0.03**		
Isoleucine		0.12	0.12	0.14	0.15	0.11	0.13	0.16	0.11	0.13	0.13	0.11	0.13	0.1
			0.13 ±0.01			**0.13 ±0.02**			**0.14 ±0.02**			**0.12 ±0.01**		
Leucine		0.22	0.12	0.14	0.27	0.11	0.14	0.26	0.11	0.14	0.22	0.11	0.14	0.2
			0.16 ±0.04			**0.17 ±0.07**			**0.17 ±0.06**			**0.16 ±0.05**		
Tyrosine		0.13	0.25	0.28	0.16	0.23	0.27	0.17	0.24	0.27	0.13	0.24	0.27	0.1
			0.22 ±0.06			**0.22 ±0.05**			**0.22 ±0.04**			**0.21 ±0.06**		
Phenylalanine		0.21	0.16	0.19	0.25	0.16	0.19	0.26	0.16	0.18	0.20	0.15	0.18	0.2
			0.18 ±0.02			**0.20 ±0.04**			**0.20 ±0.04**			**0.18 ±0.02**		
Tryptophan		0.05	0.11	0.11	0.04	0.09	0.13	0.12	0.10	0.11	0.04	0.11	0.12	0.0
			0.09 ±0.03			**0.09 ±0.03**			**0.11 ±0.01**			**0.09 ±0.04**		
Lysine		0.23	0.26	0.28	0.27	0.23	0.29	0.24	0.24	0.27	0.22	0.24	0.28	0.2
			0.26 ±0.02			**0.26 ±0.02**			**0.25 ±0.01**			**0.25 ±0.03**		
Histidine		0.10	0.12	0.13	0.12	0.11	0.13	0.13	0.11	0.13	0.10	0.11	0.13	0.1
			0.12 ±0.01			**0.12 ±0.01**			**0.12 ±0.01**			**0.12 ±0.01**		
Arginine		0.90	0.99	1.02	1.06	0.87	0.98	1.14	0.93	0.95	0.88	0.93	0.96	1.0
			0.97 ±0.05			**0.97 ±0.08**			**1.01 ±0.10**			**0.92 ±0.03**		
Total			8.59			8.48			8.88			8.21		

*Set 1, mean of 4 tests. Sets 2 and 3, mean of 3 tests.

Appendix B (cont..)

z crude protein in SMSP tested
asteurized (NP)

65			70			75			80			85			CV		
Set 2	Set 3	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3
0.64		0.46	0.57	0.62	0.37	0.59	0.63	0.41	0.68	0.64	0.49	0.66	0.68	0.54	0.69	0.70
1.48 ±0.19			0.55 ±0.07			0.53 ±0.12			0.57 ±0.12			0.61 ±0.09			0.64 ±0.07	
0.32		0.33	0.26	0.32	0.25	0.29	0.32	0.28	0.29	0.32	0.32	0.29	0.34	0.27	0.28	0.33
1.28 ±0.04			0.31 ±0.02			0.28 ±0.03			0.30 ±0.02			0.32 ±0.02			0.29 ±0.03	
0.19		0.22	0.17	0.19	0.21	0.17	0.19	0.19	0.20	0.19	0.22	0.19	0.20	0.17	0.20	0.20
1.18 ±0.02			0.19 ±0.02			0.19 ±0.01			0.19 ±0.00			0.20 ±0.01			0.19 ±0.02	
2.70		2.21	2.45	2.70	1.71	2.42	2.71	2.01	2.53	2.76	2.32	2.51	2.94	2.58	2.49	2.91
2.33 ±0.29			2.45 ±0.20			2.28 ±0.42			2.43 ±0.31			2.59 ±0.26			2.66 ±0.18	
0.93		1.46	0.90	0.92	1.14	0.91	0.91	1.35	1.07	0.91	1.52	1.04	0.96	0.92	1.02	0.96
1.04 ±0.24			1.09 ±0.26			0.99 ±0.11			1.11 ±0.18			1.17 ±0.24			0.97 ±0.04	
0.95		1.07	0.86	0.80	0.75	0.86	0.97	0.91	0.83	0.79	1.02	0.79	0.85	1.21	0.79	0.88
0.91 ±0.08			0.91 ±0.12			0.86 ±0.09			0.84 ±0.05			0.89 ±0.10			0.96 ±0.18	
0.12		0.28	0.09	0.11	0.23	0.09	0.11	0.27	0.07	0.11	0.30	0.10	0.13	0.13	0.10	0.09
0.16 ±0.08			0.16 ±0.08			0.14 ±0.06			0.15 ±0.08			0.18 ±0.09			0.11 ±0.02	
0.07		0.11	0.05	0.06	0.08	0.06	0.06	0.10	0.07	0.06	0.11	0.07	0.07	0.06	0.06	0.07
0.07 ±0.02			0.07 ±0.02			0.07 ±0.01			0.08 ±0.01			0.08 ±0.02			0.06 ±0.00	
0.45		0.63	0.40	0.44	0.49	0.40	0.44	0.55	0.45	0.46	0.61	0.44	0.42	0.39	0.48	0.51
0.47 ±0.10			0.49 ±0.10			0.44 ±0.04			0.49 ±0.05			0.49 ±0.08			0.46 ±0.05	
0.28		0.31	0.25	0.29	0.24	0.24	0.27	0.28	0.29	0.30	0.31	0.29	0.31	0.29	0.27	0.31
0.27 ±0.02			0.28 ±0.03			0.25 ±0.01			0.29 ±0.01			0.30 ±0.01			0.29 ±0.02	
0.08		0.09	0.08	0.08	0.07	0.08	0.08	0.08	0.19	0.08	0.09	0.18	0.08	0.07	0.19	0.08
0.08 ±0.01			0.08 ±0.00			0.08 ±0.01			0.12 ±0.05			0.12 ±0.05			0.11 ±0.05	
0.03		0.14	0.03	0.04	0.10	0.03	0.03	0.12	0.06	0.03	0.14	0.05	0.03	0.09	0.06	0.03
0.06 ±0.04			0.07 ±0.05			0.06 ±0.03			0.07 ±0.04			0.07 ±0.05			0.06 ±0.03	
0.13		0.16	0.11	0.13	0.13	0.11	0.13	0.15	0.13	0.13	0.16	0.13	0.14	0.13	0.13	0.14
0.13 ±0.02			0.14 ±0.02			0.12 ±0.01			0.14 ±0.01			0.15 ±0.01			0.13 ±0.00	
0.14		0.26	0.11	0.14	0.22	0.11	0.14	0.26	0.13	0.14	0.29	0.13	0.14	0.13	0.12	0.14
0.17 ±0.07			0.17 ±0.06			0.16 ±0.05			0.18 ±0.06			0.19 ±0.07			0.13 ±0.01	
0.27		0.17	0.24	0.27	0.13	0.24	0.27	0.15	0.27	0.27	0.17	0.26	0.28	0.23	0.25	0.27
0.22 ±0.05			0.22 ±0.04			0.21 ±0.06			0.23 ±0.06			0.24 ±0.05			0.25 ±0.02	
0.19		0.26	0.16	0.18	0.20	0.15	0.18	0.23	0.19	0.18	0.27	0.18	0.19	0.16	0.17	0.18
0.20 ±0.04			0.20 ±0.04			0.18 ±0.02			0.20 ±0.02			0.21 ±0.04			0.17 ±0.01	
0.13		0.12	0.10	0.11	0.04	0.11	0.12	0.04	0.10	0.13	0.05	0.10	0.15	0.08	0.09	0.13
0.09 ±0.03			0.11 ±0.01			0.09 ±0.04			0.09 ±0.04			0.10 ±0.04			0.10 ±0.02	
0.29		0.24	0.24	0.27	0.22	0.24	0.28	0.26	0.28	0.25	0.29	0.19	0.27	0.27	0.26	0.27
0.26 ±0.02			0.25 ±0.01			0.25 ±0.03			0.26 ±0.01			0.25 ±0.04			0.27 ±0.00	
0.13		0.13	0.11	0.13	0.10	0.11	0.13	0.11	0.13	0.11	0.13	0.13	0.12	0.12	0.13	0.12
0.12 ±0.01			0.12 ±0.01			0.12 ±0.01			0.12 ±0.01			0.13 ±0.00			0.12 ±0.00	
0.98		1.14	0.93	0.95	0.88	0.93	0.96	1.04	1.06	0.94	1.20	1.01	0.99	0.90	0.94	0.98
0.97 ±0.08			1.01 ±0.10			0.92 ±0.03			1.01 ±0.05			1.07 ±0.09			0.94	0.03
			8.88			8.21			8.87			9.35			8.92		

Table B.13. Summary of p-values from ANOVA with repeated measures test for 20 amino acids tested in SMSP at 5 pasteurization temperatures plus non-pasteurized (NP) and conventional (CV).

Source	Asp	Thr	Ser	Asn	Glu	Gln	Pro	Gly	Ala	Val
Lot	0.001**	0.0001**	0.2477	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.0003**
Temperature	0.001**	0.1159	0.0235*	0.0001**	0.0022**	0.0683	0.0001**	0.0095**	0.1944	0.0003**
Lot*Temp	0.0214*	0.0238*	0.0003**	0.0037**	0.0001**	0.0001**	0.0002**	0.0004**	0.0001**	0.0781

Source	Cys	Met	Ile	Leu	Tyr	Phe	Trp	Lys	His	Arg
Lot	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.0139*	0.0015**	0.0964
Temperature	0.0001**	0.0027**	0.0066**	0.0038**	0.0001**	0.0013**	0.0048**	0.0802	0.244	0.0031**
Lot*Temp	0.0001**	0.0001**	0.0079**	0.002**	0.0001**	0.0001**	0.0001**	0.0008**	0.0023**	0.0037**

* $p<0.05$

** $p<0.01$

Appendix B (cont..)

Table B.14. Summary of p-values for aliphatic free amino acids (tested in SMSP) from a pre-planned multiple comparison test between the mean of 6 temperatures (5 pasteurization temperatures and a conventional (CV) temperature) and the mean of the non-pasteurized (NP)sample.

Temperature (°C)	65	70	75	80	85	CV
Amino Acid						
Glycine	0.2432	0.4681	0.0875	0.9109	0.2432	**0.0095↓
Valine	0.9806	0.1628	0.2987	0,0694	**0.0021↓	*0.0172↓
Isoleucine	0.2680	0.0748	0.6427	0.0556	**0.0011↓	0.1087
Leucine	0.6437	0.4016	0.9162	0.1566	*0.0376↓	*0.0359↓

Table B.15. Summary of p-values for hydroxyl-containing free amino acids (tested in SMSP) from a pre-planned multiple comparison test between the mean of 6 temperatures (5 pasteurization temperatures and a conventional (CV) temperature) and the mean of the non-pasteurized (NP)sample.

Temperature (°C)	65	70	75	80	85	CV
Amino Acid						
Serine	0.9171	0.4065	0.8081	0.0758	**0.0042↓	0.1911

Table B.16. Summary of p-values for acidic free amino acids (tested in SMSP) from a pre-planned multiple comparison test between the mean of 6 temperatures (5 pasteurization temperatures and a conventional (CV) temperature) and the mean of the non-pasteurized (NP)sample.

Temperature (°C)	65	70	75	80	85	CV
Amino Acid						
Aspartic Acid	**0.001↓	0.0845	**0.0101↓	0.5195	0.3386	*0.0116↓
Glutamic Acid	0.5924	0.1.56	0.7183	0.0585	**0.0026↓	0.4366
amide						
Asparagine	0.2189	0.8410	0.0902	0.9771	0.0797	*0.0127↓

* = p<0.05
** = p<0.01
↑ = value is significantly higher than non-pasteurized value
↓ = value is significantly lower than non-pasteurized value

Table B.17. Summary of p-values for basic free amino acids (tested in SMSP) from a pre-planned multiple comparison test between the mean of 6 temperatures (5 pasteurization temperatures and a conventional (CV) temperature) and the mean of the non-pasteurized (NP)sample.

Temperature (°C)	65	70	75	80	85	CV
Amino Acid						
Arginine	0.9508	0.3675	0.2582	0.2668	*0.0197↑	0.4644

Table B.18. Summary of p-values for sulphur-containing free amino acids (tested in SMSP) from a pre-planned multiple comparison test between the mean of 6 temperatures (5 pasteurization temperatures and a conventional (CV) temperature) and the mean of the non-pasteurized (NP)sample.

Temperature (°C)	65	70	75	80	85	CV
Amino Acid						
Cystine	0.8360	0.8766	0.7173	**0.0001↑	**0.0001↑	**0.0001↑
Methionine	0.1310	*0.0142↑	0.8331	**0.0047↑	**0.0005↑	0.2715

Table B.19. Summary of p-values for aromatic free amino acids (tested in SMSP) from a pre-planned multiple comparison test between the mean of 6 temperatures (5 pasteurization temperatures and a conventional (CV) temperature) and the mean of the non-pasteurized (NP)sample.

Temperature (°C)	65	70	75	80	85	CV
Amino Acid						
Tyrosine	0.5969	0.9351	*0.0503↓	0.4645	*0.0503↑	**0.0002↑
Phenylalanine	0.3344	0.0742	0.4355	0.0958	0.0609	0.0609
Tryptophan	**0.0050↓	*0.0444↓	*0.0158↓	*0.0135↓	0.3530	0.6636

Table B.20. Summary of p-values for proline (tested in SMSP) from a pre-planned multiple comparison test between the mean of 6 temperatures (5 pasteurization temperatures and a conventional (CV) temperature) and the mean of the non-pasteurized (NP)sample.

Temperature (°C)	65	70	75	80	85	CV
Amino Acid						
Proline	0.9128	0.8437	0.2075	0.5695	0.1005	**0.004↓

* = $p<0.05$

** = $p<0.01$

↑ = value is significantly higher than non-pasteurized value

↓ = value is significantly lower than non-pasteurized value

Table B.21. Mean (± standard deviation) of all sets' means of force (newtons) required at 3mm for SMSP tested at 5 pasteurization temperatures plus non-pasteurized (NP) and conventional (CV).

Temperature(°C)	NP	65	70	75	80	85	CV
Mean force(N)	1.0347 ±0.1138	1.0264 ±0.1090	0.8755 ±0.0666	0.9559 ±0.0278	0.8891 ±0.0688	1.0181 ±0.0398	0.9895 ±0.1396