STABILISATION AND CHARACTERISATION OF A PEANUT BASED READY-TO-USE SUPPLEMENTARY FOOD (RUSF)

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BY

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DECLARATION

This is to certify that this thesis is the result of research undertaken by Agartha Adwoa Krah towards the award of the MPhil Food Science in the Department of Nutrition and Food Science, University of Ghana, Legon.

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ABSTRACT

The prevalence of under nutrition in many communities requires the need for the availability of nutrient dense affordable food supplements such as Ready-to-use supplementary foods. An energy dense, drinkable ready to use supplementary food produced by enzyme hydrolysis of cooked mixture of peanuts, cowpeas and rice with added vitamin mineral mix was both acceptable to consumers and showed promise of efficacy in improving the nutritional status of women of child bearing age through nutritional intervention studies. However the product was physically, chemically and microbiologically unstable, and did not store for long at ambient temperatures. This work was done to improve the physical and microbiological stability of the drinkable peanut based ready-to-use supplementary food.

Roasted peanut, cooked cowpeas and rice were mixed in a ratio of 3:3:4 (dmb) and slurried in a colloid mill, hydrolysed with alpha-amylase and bromelain enzymes at 50˚C and 43˚C respectively to obtain a liquefied product of about 20% solids. Calculated amounts of vitamin and mineral mix were added to meet the requirements of the target population for the supplement. The product was thermally processed by either pasteurizing (10, 20 and 30 minutes) at 95˚C or retorting at 119˚C for 10 minutes. The effectiveness of the thermal processes on the microbial and chemical stability of the product was monitored at 25, 35 and 45˚C for five weeks. The flow behaviour of the product and viscosity were characterised using the Brookfield digital viscometer. The effects of particle size on product separation rate were determined, and the application of stabilizers (CMC, carrageenan and Xanthan gum) to modify product viscosity examined. The shelf life of the product was calculated based on chemical deterioration rate by modelling free fatty acids data on Arrhenius kinetics.

The finer particles in the product (≤106µ) had faster settling rates than the larger ones (≥300 and 500µ). The apparent viscosity of the product was 440cP, and generally increased by the use of stabilizers. Addition of Xanthan gum at 1% level gave the greatest increase in apparent viscosity (1111.0 cP), and its subsequent application in the product formulation substantially reduced the settling rate of particles, and provided some measure of physical stability to the product.

Optimal starch hydrolysis was achieved after 1 hour incubation with alpha amylase at 50˚C-55˚C. Protein hydrolysis by bromelain was highest at 43˚C, and SDS-PAGE zymograms showed extensive hydrolysis of all proteins within I hour of incubation with bromelain. Prolonged incubation times led to reformation of peptides as was noticeable on the zymograms of 3 hour hydrolysates, suggesting that plastein-like reactions probably occurred under the conditions of incubation with bromelain.

Microbiological stabilization of the product by thermal processing proved inadequate as was evidenced by high microbial counts in all treatments. Heat penetration data from both pasteurization and sterilization processes showed that thermal processing was inadequate to assure microbial stability of the product. Accelerated shelf life studies (based on lipid oxidation data) showed the product to be chemically stable at room temperature (25˚C) for 22 days. Practically however, the product shelf stability did not go beyond three days at ambient conditions due to microbial spoilage.

While it was possible to improve the physical stability of the product, microbiological stability was not attainable using physical treatments, and other options need to be explored. It is essential to improve the shelf life of the product at ambient temperature storage, in order to enhance its distribution and usage among the rural poor and undernourished who need it the most.

DEDICATION

I dedicate this work to the Almighty God; my banner. To the two wonderful ladies in my life; my mother Agnes and my sister Christiana for believing in me, nurturing my dreams and sacrificing so much for me to come this far.

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TABLE OF CONTENTS

Content **Page**

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Under nutrition is a prevalent condition in many African communities and a pre-disposing factor to various forms of diseases. This to a large extent has been a result of poverty which makes it very difficult for vulnerable groups to afford nutritionally adequate food. As a result they are heavily dependent on high carbohydrate foods with little or inadequate supplies of proteins and essential vitamins. In poor and deprived communities, children, the aged, pregnant women and the sick are particularly vulnerable to under nutrition (Van Der Kam *et al*., 2012; Stratton *et al*., 2003). For the short term it is frequently an emergency that requires immediate nutritional intervention with foods that are nutritionally potent enough to either prevent or correct the conditions of under nutrition. Such foods should also be able to provide enough energy to meet or supplement the daily requirements of the consumer.

Ready to use therapeutic foods (RUTF) is a generic term referring to different types of foods such as spreads or compressed products useful for feeding children and adults who are seriously malnourished or chronically sick (Manary, 2006). Due to the high energy density of RUTFs (about 5.5kcal/g), severely malnourished and chronically sick individuals can consume just a few spoonfuls several times a day and obtain sufficient nutrients for complete restoration of health. RUTFs are formulated and processed such that they need no further processing before eating. They also have a reasonably long shelf life and are very palatable and acceptable to the consumer (Annan, 2014). Formulation of RUTFs to provide the calories, and nutritional profile needed to meet the requirements of specific vulnerable groups can be challenging depending upon the

recommended daily allowances (RDA) of the target group. In the case of meeting the requirements of adult vulnerable groups, rather than provide RUTF, it is a lot easier to supplement their food with better sources of nu

3trition, or formulate a product that can be used as ready to use supplementary food (UNICEF, 2008). Asante (2009) described the development of a ready to use supplementary food by formulating cowpeas, peanuts and rice using constrained mixture designs (Cornell, 1995). The three commodities were chosen in part because they are easily available locally and also because when mixed in the right proportions they provided the needed nutritional energy necessary for a supplementary food. A pre-hydrolyzed mixture of roasted peanuts (0.35-0.45), cooked cowpea (0.2-0.25) and rice (0.35-0.4) was drum dried and evaluated for its energy and protein content among other properties. The product had nutritional properties which met the recommended dietary allowances of energy, macro- and micronutrients for vulnerable groups (children, pregnant and lactating mothers). The formulation was further modified into protease and amylase pre-hydrolyzed liquefied drinkable product to contain 15% solids, such that it would have the following attributes:

1. Sufficiently liquid enough to be swallowed by consumers who may be so malnourished or severely ill that consuming solid food might be difficult.
2. Highly digestible.
3. Pleasant nutty flavour with some sweetness deriving from the process of amylase hydrolysis of the starches and the addition of sugar.
4. Nutrient dense with high levels of protein to promote recovery. Vitamins and minerals were added at pre-determined amounts.
5. No detectable levels of aflatoxins.

Product quality and shelf life are critical to its success and acceptability by consumers, no matter its nutritional quality (NZFSA, 2005). For a liquid product, some quality characteristics may include physical attributes (i.e. colour, appearance, flow properties, structural degradation), microbiological stability, chemical characteristics (including degradation of some vitamins, and lipid oxidation) as well as sensory acceptability.

1.2 Rationale

The peanut based ready to use supplementary food (RUSF) formulation under consideration is a drinkable beverage that was designed to be readily digestible when ingested and to release needed energy to the consumer as quickly as possible. Initial tests have shown the product to be quite acceptable to consumers (Anim-Fofie, 2011). Its efficacy was tested in a nutritional intervention study among women of childbearing age (Agbemefle, 2013; under review) in a community from which previous baseline studies showed the cohort group to be grossly undernourished (Anim-Fofie, 2011). However, the product was microbiologically and physically unstable as it easily separated (i.e. sedimentation of particles) upon standing, and did not store longer than one week even after pasteurization. To extend the shelf life, the product was usually kept in refrigerated storage after pasteurization. The target consumers may not have access to house hold refrigeration facilities, or even afford electrical energy to keep them refrigerated. The physical separation of the product also needs to be addressed, by reducing the mean particle size or varying the medium viscosity in order to minimize the rate of particle sedimentation. A reduced mean particle size will improve product mouth feel and probably increase consumer acceptance.

1.3 Objectives

The objective of the study was to improve the physical and microbiological stability of the peanut based drinkable RUSF.

The specific objectives were to:

- Investigate the effects of product particle size distribution on separation rate during storage.
- Investigate the effects of stabilizers in minimizing product separation and characterize the flow behaviour of the drinkable RUSF.
- Determine the degree of hydrolysis of the macromolecules (proteins and starches).
- Conduct shelf life studies and microbial stability under different temperature storage conditions.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Malnutrition

Malnutrition or abnormal nutrition occurs in human beings as a result of nutritional imbalance and could mean an excess or a deficit of nutrients depending on the physiological requirements of the body. Based on the requirements of normal nutrition, the body mass index (BMI) is used as an indicator of the risk of an individual being over or under-nourished (Stratton *et al.*, 2003). Though malnutrition can imply inappropriate intake of any of the essential nutrients, protein-energy malnutrition (PEM) which is due to an inappropriate intake of the macro-nutrients especially protein and fats is the commonest form of malnutrition (Stratton *et al.*, 2003).

In developing countries where living conditions of majority of the people are characterised by poverty, lack of resources and skills with its accompanying food insecurity, protein-energy under-nutrition is very common and children under the age of five (5) years are the most vulnerable (Wardlaw, 1993). It is reported (Defourny *et al*., 2009) that about 146 million children under five in developing countries are under weight whiles in sub-Saharan Africa about 38.6% of the children are stunted, 28.4% underweight, and 7.2% wasted (Olwedo *et al*., 2008) with 3% suffering from severe acute malnutrition (Briend and Collins, 2010) at any point in time. According to Huybregts *et al.* (2012), from analysis based on 139 countries, about 10.2% of deaths were attributed to wasting, which was mainly as a result of malnutrition. Black *et al*., (2010) asserted that wasted children are three times more likely to die than their well nourished counterparts. Additionally malnutrition has a profound effect on the health and cognitive performance of these children as child nutrition is known to be vital in brain development (Pollitt

and Brown, 1996). Under-nutrition has also been implicated in child mortality, morbidity (Gera, 2010) and disability-adjusted life years together with intrauterine growth restriction (PLoS Medicine Editors, 2008).

Furthermore, under nutrition affects immune function leading to compromised immune defences and increased susceptibility to all forms of diseases (Heikens et al, 2008). It is also known to cause mortality through its potentiating effect on diseases (Pelletier, 1995) such as malaria (Van Der Kam *et al*., 2012) and HIV/AIDS (Ndekha, 2009) as well as aging etc. which could be a result of inefficient absorption of nutrients (Olwedo *et al*., 2009). Under-nutrition is also known to have a synergistic effect with these co-morbidities as well as parental characteristics such as poor health and nutritional status (Grantham-McGregor, 1995). The long term effect of this is a reduction in the human resource base as a result of deaths or a diminishing of intellectual ability of victims thus perpetuating the poverty cycle (Huybregts *et al.*, 2012). There is therefore the need for an urgent remedy if the cycle should be broken (Pelletier, 1995).

2.2 Ready-to-use Therapeutic Foods (RUTFs)

Malnutrition in children with its accompanying effects such as wasting, stunting, morbidity and mortality has for a long time been a public health issue worldwide (Van der Kam *et al.*, 2012). Food security is one of the leading causes of malnutrition and hence makes children and other vulnerable groups targets of interest (Amsel and Hirsch, 2009).

Interventions in the form of food aid programs have normally been centred on the provision of cereal legume blends distributed in areas facing food security problems but these have been reported to be too low in energy density for the problems of malnutrition (Karakochuks *et al*., 2012; Bahwere, Sadler and Collins, 2009) as compared to a more energy dense formulation

intended for feeding of severe acute malnourished (SAM) patients (Manary, 2006). Ready-to-use therapeutic foods (RUTFs) belong to the group of lipid rich nutrition supplements (LNS). They are energy-dense, protein-rich fortified with micronutrient and formulated to have similar nutrients composition with UNICEP's F-100 formula. RUTFs have been used at the rehabilitation stage of treating SAM and have been proven successful in the implementation of community based therapeutic care (CTC) (Briend and Collins, 2010; Ndekha *et al.*, 2009). This concept was developed as an alternative to the in-patient therapeutic care system which has been relatively ineffective in curtailing the effect of SAM (Defourny *et al.*, 2009), due to cost (Defourny *et al.*, 2009), the availability of facilities (Beesabathuni and Natchu, 2010) and their effect on the number of people reached (Karakochuks *et al*., 2012) by these interventions.

The first RUFT plumpy nut^{R} by Nutriset is basically made up of peanuts, sugar, oil and milk but is quite expensive because it has to be imported. Also in the case of local production, milk powder which is imported is used as protein source, not making it any less expensive. Researchers have therefore been concentrating on the use of locally available raw material for a formulation that will provide nutrient composition very similar to the proven plumpy nut^{R} (Karakochuks *et al*., 2012; Briend and Collins, 2010).

2.3 The Concept of Food Supplementation

Food supplementation or dietary supplementation is a practice in which people are provided with a product to complement their daily dietary intake with the intention to improve the nutritional value of their diet (http://www.fda.gov/AboutFDA/Transparency/Basics/ucm195635.htm).

Food supplementation is normally done in the form of food aids in areas of food shortages where nutritious foods such as cereal soy blend (CSB) are distributed to people (Nackers *et al.*,

2010). In children with SAM this may come as RUTF whereas in those with moderate acute malnutrition (MAM) it has been described as ready to use supplementary food (RUSF) (Defourny et al, 2009). There have been a number of studies on the efficacy of RUSF in enhancing the rate of recovery from MAM and SAM or even preventing them. Defourny *et al*. (2009) found that adding child-targeted RUSF supplementation to a general food distribution resulted in increased haemoglobin status and linear growth. In a similar study Karakochuks *et al*. (2012), realized that the treatment of moderate acute malnutrition with RUSF resulted in higher recovery rates in children, despite the higher amount and energy content cereal soya blend (CSB). RUTF supplementation has been found to improve the rate of weight gain and improvement of appetite, a shorter period of convalescence as well as a higher rate of recovery from cough in children after an episode of acute uncomplicated Plasmodium falciparum malaria (Van der Kam *et al.*, 2012). In addition RUSF in a research to determine the efficacy of two different food supplements on body mass index (BMI) in wasted Malawian adults with HIV, supplementary feeding with fortified spread was proved (Ndekha *et al*., 2009) to result in a greater increase in BMI and lean body mass than feeding with corn-soy blend yet proving the fact that food supplementation can be employed to enhance the health status of sick adults.

2.4 Recommended Dietary Allowances (RDAs) for Vulnerable Groups

For normal growth and healthy life people need a well balanced diet having the nutrient composition that is needed to fully satisfy the metabolic requirements of the body. There are different requirement of nutrients for different groups of people with different characteristics age, gender, health status etc (Naicker, 2004). Child nutrition is vital for proper growth and development (Campos *et al*., 2010) and as the sick and aged they are considered vulnerable

because they have special nutritional requirement (RDAs) to achieve the required rate of growth for a period (Hermoso *et al*., 2010). When these needs are not met these people are exposed to malnutrition and its accompanying complications (Brown and Pollitt, 1996). Naicker, (2004) stated the RDA for nutrient intake of children between 0-12months and 12-24months (Table 2.1) and indicated based on a 24 hour dietary recall, that children from 0-24months in Umbumbulu (South Africa) met only about 70% and 75% of their protein and energy RDA respectively. Marin (2004) stated that there could be up to 33% increase in energy requirements hence frequent under nutrition cases in patients with cystic fibrosis. WHO has recommended that apart from proteins and vitamins and minerals the daily energy intake of HIV and AIDS patients should be increased by half the requirements of an active HIV negative adult of 2430 Kcal for male and 2170 kcal for female (Bahwere, Sadler and Collins, 2012). Among institutionalized old women in India, Reddy and Syeda (2011) found that the women's carbohydrate and fat was not significantly different from RDAs whereas protein, fibre and micro nutrients were lower than their respective RDAs.

Table 2.1: RDAs of Children within the Age Range of 0-12 and 12-24 Months

Nutrient	**RDA**	
	0-12months	**12-24months**
Energy	2869	5440
Proteins	13.5	14
Vitamin C	30	40
Vitamin E	3	6
Riboflavin	0.4	0.8
Niacin	5	9
Vitamin B6	0.3	1
Vitamin B12	0.3	0.7
Phosphorus	300	800
Magnesium	40	80
Selenium	10	20

Source: Naicker, 2004

2.5 The Application of Enzymes in Food Processing

Enzymes are often referred to as biological catalysts which enhance the rates of reactions in biological systems. They are predominantly made up of proteins and are very specific in terms of the reaction they catalyze, the conditions required for optimum activity (temperature, pH and other chemical) etc. Enzymes are naturally occurring in biological organisms and are involved in different activities; for example they perform different functions in food crops from cultivation to post harvest periods in the supply chain of food (Tawil *et al.*, 2012). In the food processing industry, various endogenous enzymes have been employed to enhance the quality of food, bioavailability of nutrients, increase energy density foods etc. (Songré-Ouattara, 2009).

One way in which enzyme hydrolysis has been utilized in the food processing industry is in alcoholic beverage production. In beer production, endogenous cereal enzymes (amylases and proteases) developed during germination breakdown storage starches and proteins into soluble forms to be fermented by yeasts into alcohol (Sim and Berry 1996). In addition to malt enzymes,

various works have been done on isolating enzymes from micro-organisms. Amylases for instance have been isolated from various micro-organisms such as Bacillus licheniformis ATCC 9945a (Nataˇsa Boˇzi *et al*.,2011 Sodhi et al., (2005) lactic acid bacteria (Songré-Ouattara, 2009), Bacillus amyloliquefacien (Yook and Robyt, 2002) etc. Amylases have also been isolated from animals; an example of which is the porcine pancreas (Yook and Robyt, 2002).

The primary concern of most researches using enzymes has been on the efficiency in yielding the desired end product. To validate the enzyme activity, appropriate substrates have been used to explore the conditions of optimum hydrolysis. Usually the conditions of interest for enzyme activity have frequently been the temperature, pH, moisture content, the state of the substrate.

2.5.1 Amylases in Food Processing

Amylases are hydrolases that act on long chain polysaccharides such as starches to break them down into oligosaccharides and monosaccharides such as maltose and glucose by hydrolysing the α-1,4 and α-1,6 glycosidic linkages of the polysaccharides (Aiyer, 2005). Amylases can be obtained from animal, micro-organism and plant sources.

Amylases are classified into endo-amylases or exo-amylases based on how the glycosidic bond is attacked. α-amylase is an endo-amylase attacking the randomly breaking the inner α-1, 4 glycosidic linkages of the starch structure (Konsula and Liakopoulou-Kyriakides, 2004) while beta-amylase and gluco-amylase are examples of exo-enzymes which hydrolyse from the non-reducing end of the chain producing short chain product (Gupta *et al*., 2003). Glucoamylases are debranching enzymes thus able to hydrolyse the α-1, 6 glycosidic linkages leading to complete hydrolysis of starch together with alpha and/beta amylases (Aiyer, 2005). These amylases have

been used in the production of dextrose, syrups, dextrins and their products also used as sources of sweetness in various food products (Aiyer, 2005).

Alpha and beta amylases have been isolated from organisms of varying sources including plant and microbial and have been used in foods for various outcomes (Matsuo *et al*., 2012; Bozic *et al*., 2011; Sanni *et al*., 2002). Alpha and beta amylases have also been used to produce hydrolysates of starchy foods in order to improve upon their digestibility, nutritional quality and enhance their taste (L´opez, 2005; Traore, 2004). These enzymes have been used extensively in the brewing industry especially in the malting process in which they are used to produce fermentable sugars (Sim and Berry, 1996).

The effects of processing conditions on enzyme hydrolysis have been investigated (Mousia *et al*., 2004; Eneje, 2001; Warparla and Pandiella, 2000). Gebremariam *et al*. (2013) found that alpha and beta amylases were deactivated at temperatures higher than 50°C whilst Sim and Berry (1996) obtained 65°C for alpha amylase hydrolysis. Using different concentrations of α- amylase at different temperatures, Yook and Robyt (2002) also found that the rate constant of α-amylase hydrolysis increased with temperature from 40 to 70°C with 50^0C giving the optimum degree of hydrolysis. In a similar study, Sodhi *et al*., (2005) indicated that alpha amylase from *Bacillus* sp. PS-7 showed maximum activity at 60^oC though activity was observed within a range of 40-80^oC. The rate of enzyme activity depended on nature of the solid substrate, the moistening agent, level of moisture content, incubation temperature, presence or absence of surfactant, carbon, nitrogen, mineral, amino acid and vitamin supplements.

2.5.2 Cysteine Proteases

Cysteine proteases also known as thiol proteases refer to a class of proteases in which the nucleophilic cysteine residue of the protease forms a covalent bond with the carbonyl group of the scissile peptide bond in substrates (Bromme, 2000). They can be derived from a wide range of organisms including plants, and even micro-organisms. The papain family proteases such as papain, bromelain and ficin are the most predominant cysteine proteases of plant sources and have various applications in the textile, pharmaceutical and food industries (González-Rábade *et al*., 2011). Papain has a popular use in the tenderisation of meat (Minh *et al*., 2012) and protein coagulation (Zhong, *et al*., 2005) whereas ficin is known (González-Rábade *et al*., 2011) to have casein hydrolysing properties thus ability to function as a milk clotting protease. These proteases also used for the treatment and reduction of many illnesses including tumours, diarrhoea, digestion enhancers among others (González-Rábade *et al*., 2011).

Bromelain is a cysteine protease from the family of bromeliaceae obtained from pineapple plant mainly from the stem or fruit extract of pineapple (Crude bromelain). The crude extract is constituted by a complex mixture of different thiol-endopeptidases (González-Rábade *et al*., 2011). Bromelain can be obtained from cooled pineapple pulp or juice as well as the stem though the stem bromelain for economic reason is preferred for commercial purposes since it is not consumed (Leite *et al*., 2012). Bromelain has been found to have numerous important health benefits apart from acting as a digestive enzyme; a property that is employed in food processing (Bharttacharyya, 2008). Bromelain has also been used extensively in foods for meat tenderization (Calkins and Sallivans, 2007), milk clotting, brewing and functional protein pre-digestion (Corzo *et al*., 2012). It is believed to have a wide range of activities, and acts on

specific bonds in the subtrates and also has specific conditions under which it acts (González-Rábade *et al*., 2011).

Researches on the activity of bromelain indicated that the conditions required for optimum activity depends on the source of the enzyme and the conditions of incubation (Gautam *et al*., 2010; Jutamongkon and Charoenrein, 2010). Tochi *et al*., (2008) stated that bromelain is stable over a wide range of pH range from 2-9. Work done by Corzo, (2012) showed that fruit bromelain showed optimum activity at 55 °C azoalbumin for azocasein, 59 °C for sodium caseinate and 37°C for haemoglobin all at different pHs. For stem bromelain, Gautam *et al*., (2010) indicated that the optimum temperature for activity was within 50°C-60°C. Yet Jutamongkon and Charoenrein, (2010) established from their work on the thermal stability of fruit bromelain that the enzyme is stable at 40°C, above which 40°C, loss of activity begins, and the rate of activity loss increasing with temperature.

2.5.3 Plastein Reactions

When a protein hydrolysate is incubated with a protease, the plastein reaction may occur in which small molecular polypeptides may undergo rearrangements, into new protein-like substance. In the plastein reaction, peptide fragments of a protein hydrolysate are enzymatically joined through peptide bonds (Belitz, *et al*., 2009). Madzlan *et al*., (2006) reported that electrophoresis analysis of plastein synthesised by papain showed the presence of one major band with molecular weight of 2.8 KDalton while, synthesis with bromelain showed the presence of two bands with a molecular weight of less than 6.5 KDalton and 3.5 KDalton. The mechanisms of the reaction have been suggested to involve condensation (Yamashita, *et al*., 1976a), transpeptidation (Combes and Lozano, 1994) and physical forces (Andrews and

Alichanidis, 1990). These may occur separately or simultaneously (Stevenson *et al*., 1998). The plastein reaction has been applied in debittering protein hydrolysates (Stevenson *et al.,* 1998), fortifying food proteins deficient in some essential amino acids (e.g., methionine), reducing the content of some undesirable amino acids (e.g., phenylalanine) for special dietetic applications (Yamashita *et al*., 1976b) or improving functional properties of food proteins (Andrews and Alichanidis, 1990).

2.6 Rheological Behaviour of Fluids

Rheologically fluids are categorized into (a) Newtonian flow in which shear rate (γ) is directly proportional to shear stress (σ) and (b) and non-newtonian which requires additional parameters in describing their flow behaviour because the relationship between γ and σ is inadequate in describing the relationship (Steffe, 1996). Non- Newtonian fluids could be shear thinning (pseudoplastic), shear thickening (dilatants), time dependent and time independent (Steffe,1996). A lot of fluid foods have been found to be non-Newtonian, pseudoplastic and the power law model has been used to describe their rheological behaviour. Examples of these include fruit juices (Dak *et al*., 2008), molasses (Kaur, 2008), etc.

The Power Law Model

$\sigma = K (\gamma)^{n}$

where;

σ is the shear stress which is the force applied on the fluid for example the revolutions per minutes (RPM) applied for the determination of viscosity.

γ is the shear strain; the effect of the shear stress on the viscosity of the fluid.

K is the consistency index which is a measure of the apparent viscosity

n is the power law index; an indicator of the flow properties of the fluid.

The consistency co-efficient of power law fluids has been found to increase with increase in the concentration of solids contents of fluid foods (Shamsudin *et al*., 2005; Zainal *et al*., 2000). In the same way gums such as dextran (Kaur, 2008) xanthan gum (Ahmed and Ramaswamy, 2004), carboxymethyl cellulose (CMC), carrageenan (Steffe, 1996) etc when they are used in food systems cause viscosity increments with increase in concentration which forms the basis for their use as thickeners and stabilizers (Kaur, 2009).

2.7 Emulsifiers and Stabilizers

Apart from safety and quality considerations in food processing, the structure and appearance of a food product is important for consumer acceptability. The structure and texture of food well depends on its composition which in turn has an effect on sensorial characteristics of the food (Dalgleish, 2006). Emulsions are a group of foods in which various components which otherwise are immiscible are made by dispersing one component in the other. To facilitate the dispersion process the surface tension is reduced through the use of surfactants (as emulsifiers) and/or by increasing the interfacial viscosity (Charcosset, 1998). Examples of food emulsions include dairy products, salad dressing, etc.

Emulsifiers are small surface active molecules which have both hydrophilic and hydrophobic sites, and they include compounds such as mono and diglycerides, polysorbates, phospholipids etc. Proteins such as those derived from milk and eggs are also frequently used as emulsifying agents in a number of food products.

The properties of a protein stabilized food emulsions such as dairy product and beverages depend both on the surfactant used and the nature of the continuous layer (Charcosset, 1998) meanwhile, the nature and composition of the interfacial layer is dependent on the interactions between the proteins and the surfactants (Dickinson, 1992). For these reasons surface properties such as viscosity, surface adsorbed layer composition and interfacial tension which are responsible for emulsion stability could be changed as a result of an alteration of the protein-surfactant composition (Xu *et al*., 2005). This means that the composition of the emulsion should be taken into consideration when deciding the type of stabilizing agent used in any food system. Jayasundera *et al*., (2009), concluded that small amounts of surfactants do not enhance the stability of a protein stabilized emulsion.

The choice of an emulsifying agent therefore depends greatly on the food system and the behaviour and mechanism of particular stabilizers and emulsifiers as well as the type of food, its components and the structure desired (Thakur *et al*., 2006). The stability of an emulsion is influenced by the size of the emulsion droplets; the smaller the droplets the smaller the interfacial tension and hence the higher the stability (Chung *et al*., 2013). Surfactants are generally known for reducing the interfacial tension and enhancing the formation of smaller droplets. The type and concentration of the surfactants used in emulsions therefore have a very significant effect on the droplet formation and stability.

Stabilizers are hydrocolloids which are large molecular polymers predominantly polysaccharides that act in food products normally as gelling agents, thickening agents, stabilizing agents etc. They can also act in emulsions as stabilizing agents or to a lesser extent emulsifying agents (Dolz *et al*., 2008).

The ability of hydrocolloids to modify the properties of an emulsion depends on its molecular characteristics (molar mass, conformation, charge etc.) as well as their impact on the physico-chemical properties of the food system (Philips and Williams, 2009). For example gum arabic is a highly compact, branched hydrocolloid with a protein covalently bound to it and is normally used as a stabilizer. However its effectiveness remains an issue since so much of it is needed and the effectiveness of an emulsifying agent is related to the amount of it that is required to create and stabilize an emulsion.

The ability of food colloids to thicken aqueous solution is utilized in emulsions to prevent creaming and hence enhance stability when fat level is low and hence close packing of fat globules is impossible (Chung *et al*., 2013). The stabilizing action of hydrocolloids such as xanthan, carboxymethycellose and carrageenan in emulsions is normally attributed to the structuring, thickening and gelation of the aqueous continuous phase depending on their properties and concentration. The addition of hydrocolloids to emulsions can disturb stability through phase separation due to thermodynamic incompatibility with the emulsion droplets (Dalgleish, 2006).

Emulsifiers and stabilizers have been used in various researchers for the attainment of emulsion stability. For instance, using a mixed system of Tween 80 and locust bean gum, Chung et al., (2013) found that beyond a certain concentration, the particle size of droplets increased with increased concentration of locust bean gum as the samples increased in consistency and pseudoplasticity. It has also been hypothesized (Aryanti, Hou and Williams, 2009) that the viscosity of the continuous phase (aqueous phase) in oil in water emulsions affect the droplets size and stability. In their work (Hellerbach *et al*., 2013) proved that at a given concentration of emulsifier, an increase in the concentration of xanthan gum increases the viscosity of the

continuous phase. However there was an observed increase in the droplets size which was suspected to be a result of the shear thinning nature of xanthan. William and Philips (2009) explained that the high viscosity at low concentration helps prevent sedimentation and the shear thinning enhance easy flow from bottle upon shaking.

2.8 Causes of Food Spoilage.

Food is obtained from biological organisms within which various biochemical reactions occur. The termination or continuation of these processes after the conversion of these living organisms into food could be a cause of spoilage. Processing techniques that are applied to food may enhance its quality and/or induce spoilage by changing some of their properties. Furthermore with storage depending on the nature of the food it gets exposed to other organisms and environmental conditions which could lead to their deterioration. Based on the factors involved food spoilage and/or deterioration could be classified as physical, chemical or biological.

2.8.1 Physical

The physical quality of food is related to attributes such as texture, colour, appearance, mouthfeel, consistency and therefore the deterioration of physical quality is the alteration or deviation of the characteristics of food from the consumers' expectation. The effect of the heat treatment on sensory and nutritional attributes as a result of some chemical reactions during food processing can lead to changes in the physical properties of some foods. (Miri *et al*., 2008). Enzymatic and non enzymatic browning can lead to changes in colour due to the changes in natural pigments for example in foods containing green vegetables during thermal processing (Shin and Bhowmik, 1995). Change in the texture (softening) of foods such as fruits and

vegetables (Sun, Tang and Powers, 2007), change in viscosity of fluids such as milk products (Mussa and Ramaswamy, 1997) and fruit juices, alteration of the colour of milk, meat and fruit juices (Durance 1997), changes in organoleptic properties of foods as a result of the destruction of some nutrients (Awuah, Ramaswamy and Economides, 2007) may also be undesirable changes that can occur in foods. In food products in which structure and texture are vital such as in emulsions, the physical quality is paramount as small deviations in the form of flocculation, creaming, phase separation and ultimately serum separation are completely unacceptable (Philips and Williams, 2009).

2.8.2 Chemical

A number of chemical reactions occur in food during processing and storage which can lead to changes in physical characteristics and loss of important nutrients (Lindley, 1998). In thermally processed foods, thermal degradation reactions, such as maillard reactions, lipid oxidation etc., occur to a varying extent depending on the type of food and the prevailing conditions. Maillard reactions for instance between carbohydrates, free amino acids and proteins can lead to discolouration or browning of food (non-enzymatic browning). Enzymatic browning in fruits has been linked to the presence of oxygen, a reason for which they are de-aerated before pasteurization (Awuah *et al*., 2007). Also vitamin degradation of heat treated food is a very significant phenomenon in thermal processing and has been used extensively in optimization of heat processes as an indicator of quality retention (Leskova, 2006; Abakarov and Nunez, 2013). Loss of vitamins is dependent on a number of factors such as oxygen, heat and processing conditions as well as on the constituents and formulation (Awuah *et al*., 2007). Additionally, exposure of proteins to heat leads to the destruction of most of the tertiary and quaternary

structure. This leads to changes in the protein conformation which in most cases leads to an increased digestibility as a result of the exposure of peptide bonds which may be the active sites of digestive enzymes (Thomas *et al.*, 2007).

Lipid oxidation is a very common cause of food deterioration and contributes to the development of undesirable flavours in foods that are rich in fats or oils. The rancidity development in high lipid foods is due to the formation of numerous aliphatic aldehydes, and alcohols, which are expressed as off-flavours (Farhoosh *et al.*, 2009). For example, peanuts contain approximately 50–55% oil with 30% of the oil being linoleic acid, which becomes susceptible to development of rancid and off-flavours through lipid oxidation (Grosso *et al.*, 2008). The rate of oxidation is affected by factors such as water activity, storage conditions; temperature, light, and mineral contaminant etc.

2.8.3 Biological

Biological substances like enzymes and micro-organisms are very important in food deterioration and spoilage. This is because most enzymes are present in food in their natural form and micro-organisms are present in natural bodies such as soil, water, air etc, with which food stuffs are in constant contact. Apart from microbial spoilage of fresh and high moisture foods, food crops undergo changes induced by respiration, transpiration and other metabolic activities, mediated by enzymes (Paull and Chen, 1997). Also storage related parameters such as water activity, temperature, oxygen content, light etc can also have adverse effect on the keeping quality of some foods (Ahvenainen, 1996).

Microbial spoilage and pathogenicity have been the major factors in food processing considerations and have been the goal of most food processing techniques. Most enzymes in

foods are destroyed at temperatures lower than those needed for microbial sterility (Negi and. Roy, 2000). Enzymes such as polymethylesterase, polyphenol oxidase, galacturonase and peroxidase are normally present in fruits and are inactivated using pasteurization temperatures which are generally lower than 100^{o}C (Awuah *et al*., 2007).

Lipolytic micro-organisms can also cause lipid hydrolysis by the release of extracellular lipolytic enzymes that can be active even after the destruction of the cells that produced them. These microbial lipases degrade lipids in foods resulting in the production of undesirable characteristics such as excessive foaming during churning in butter making and the development of rancidity in the finished product and the production off-flavours and rancidity in pasteurized milk (Ledenbach and Marshall, 2009). In addition some psychrotropic bacteria can produce proteinases which have been found to cause off-flavours, for example bitter flavours resulting from the production of bitter peptides (Ledenbach and Marshall, 2009).

2.9 Thermal Processing

The use of heat in treating food is a very old and common practice often intended to enhance the quality of food by destroying micro-organisms, reducing or removing anti-nutritional substances, enhance the digestibility, availability of nutrients, the flavour of foods (Wandsnider, 1997; De Corcuera *et al*, 2003). Food preservation is the main aim of thermal processing and is mostly based on the control of micro-organisms which are destroyed by heat based on their nature, environmental conditions and consequently their response to the heat treatment. On this basis there are different techniques in thermal processing including blanching, pasteurization and sterilization that can be applied based on the purpose of the thermal treatment.

2.9.1 Microbial Destruction Kinetics

Attaining an optimum thermal process requires that the heat resistance of the micro-organism of interest at a given temperature for a given time is known (Miri *et al.*, 2008). Microbial destruction by lethal agents such as heat has been reported to be a first order reaction and can be obtained from the survivors curve expressed as:

$\text{Log}_e (N/N_O) = -kt$ where

N = number of surviving microorganisms after a treatment with lethal agent for time t (min),

N_O = initial number of microorganisms

k = reaction rate constant in min^{-1} (Mussa and Ramaswamy, 1997).

The treatment time (constant lethal agent) that will destroy 90% of the existing microbial population is normally expressed in thermal processing as the decimal reduction time (D value) and can be obtained as the negative reciprocal slope of the $\log_{10} (N_O/N)$ *vs*. time curve and is therefore reciprocally related to k as; D = 2.303/k. The z value which measures the sensitivity of the organism to change in temperature is defined as the temperature required to cause a change in D-value by a factor of 10 and can be obtained from a plot of the log of *D*-value against temperature (Mussa and Ramaswamy, 1997). Depending on the target micro-organism and food formulation there are different D and z values for different products. Heat labile micro-organisms generally have lower D and z values than heat resistant micro-organisms. For this reason the thermal process of any food system is set using the most heat resistant micro-organisms present (Silva and Gibbs, 2010).

2.9.2 Blanching

Blanching is a form of heat treatment normally applied to fruits and vegetables with the intention of deactivating enzymes, modifying texture, flavour (Kidmose and Martens, 1999) colour (Genovese *et al*.,1997) and in some cases removing trapped air (Reyes De Corcuera, *et al.*, 2004). Blanching can also be used in destroying surface microorganisms or reducing microbial load in food (Forsythe, 2011).

In spite of the need for utilisation of legumes such as soya beans and cowpeas as sources of protein, they contain anti-nutritional component such as tannins, trypsin inhibitors and Phytates that have to be removed to enhance the nutritional quality of the proteins (Wang *et al*., 1997). Because these anti-nutritional factors are heat labile, they can be removed by traditional preparation procedures such as cooking and blanching that also allows for the absorption of water and heat to enhance digestibility of the legumes (Gowen *et al*., 2007). Blanching of legumes also stops the action of enzymes as well as leads to a reduction in the microbial load of the food (Gowen *et al*., 2007).

Being a thermal process, blanching can have negative effects on the desirable characteristics of blanched foods. For instance blanching is said (Prestamo, Fuster and Risueno, 1998) to have an effect on the texture of treated carrots intended for frozen storage, which they attributed to changes in pectic substances when exposed to heat. Blanching of some fruits and vegetables may also lead to loss of nutritional substances such as vitamins (Kidmose and Martens, 1999) colour and (Negi and. Roy, 2000). Similarly blanching of legumes can lead to the loss of important nutrients such as vitamins, sugars etc (song *et al*., 2003).

However some methods of blanching lead to a better retention of this quality attributes. Microwave blanching has been proved to result in better texture of carrots as a result of a lower

extent of cell destruction and the removal of water leading to an increase in mechanical strength and also that steam blanching and microwave blanching are better in terms of nutrients retention since there is no tendency of nutrients leaching into the water as in the case of water blanching (Kidmose and Martens, 1999). Peroxidase inactivation which is said to occur faster at higher temperature is often used to assess the efficiency of blanching (Negi and. Roy, 2000) which is normally done at temperature from 90^{o}c to 110^{o}c (Forsythe, 2011) although the optimum time and temperature appropriate for a particular food item depends on the quality expectation of the end product (Reyes De Corcuera, Cavalieri and Powers, 2004). The negative effects of blanching can be reduced by optimising the thermal process (Shi *et al*., 2004) or by using other alternative methods of blanching such as steam blanching instead of water blanching (Wang *et al*., 1997).

2.9.3 Pasteurisation

Pasteurisation is a food preservation method aimed at reducing food micro-organisms to levels that will make the food safe to the consumer in the state in which it is presented, for a specified time within which it should be consumed (Kalchayanand *et al*., 1998). Traditionally pasteurisation is a thermal process that is based on the elimination of pathogenic organisms and reducing spoilage organisms to acceptable levels. During pasteurisation temperatures are controlled such that the effect on microbial destruction is obtained and yet the loss of desirable properties of the food for instance the colour and flavour of milk is minimised (Franco *et al*., 2008).

The process of pasteurisation is widely applied to milk and milk products and the effectiveness of the pasteurisation is normally determined by the inactivation of the enzyme alkaline phosphatase or peroxidases (Mussa and Ramaswamy, 1997) which can be destroyed by

temperatures within 62°C and 80°C depending on the time used. Meat and meat and meat products are also pasteurised at similar temperature to destroy surface micro-organisms. In fruits juices such as orange juice the target of pasteurisation is the destruction of the enzyme pectin methylesterase which require higher heat of about 95-98°C (Vervoort *et al*., 2011).

Generally due to the low temperatures involved in pasteurisation (65-95°C), products obtained after pasteurisation are not stable other means of preservation such as refrigeration, modified atmosphere packaging or storage, vacuum packaging, addition of antimicrobial additives etc must be employed to enhance shelf stability (Silva and Gibbs, 2010).

Traditionally, pasteurisation can be classified either as low temperature-long time (63.5°C for 30min) or high temperature-short time (71.7 for 15s) pasteurisation (Van Brandt *et al*., 2011). Apart from these however, ultra high temperature pasteurization (UHT) is an improvement upon the high temperature short time (HTST) treatment, where a more sever temperature is used at an extremely short time. A temperature range of 135-150°C (for 1-10s) has been used in milk processing to yield products with extended shelf life (Heilig *et al.,* 2008). Additionally, temperatures around 145°C for as low as 25milli seconds have been used in surface pasteurisation of meat and poultry to reduce pathogenic microbes on the surfaces (Morgan et al., 1996; Cygnarowicz-Provost et al., 1994). UHT pasteurisation (though occurs at a higher temperature) has been considered a relatively milder heat treatment intended for microbial sterility whilst minimising the effects of heat on the properties of foods that determine their quality (Newstead *et al.,* 2006).

2.9.4 Sterilisation

The primary aim of thermal sterilisation has been the achievement commercial sterility: a state in which most of the microorganisms are destroyed and yet nutritive and organoleptic properties are as much as possible maintained (Awuah *et al*., 2007). This is because an attempt to render food completely free of micro-organisms will render food unsuitable for consumption because it will not meet the consumers' expectation of quality (Simpson *et al*., 2008). A number of food processing techniques intended to preserve the more natural colour and flavour, and yet assure safer, more convenient, more nutritious foods to the consumer are available. These include high-pressure processing (Mussa and Ramaswamy,1997), pulsed electric field (Zhang, Barbosa-Canovas and Swanson, 1995), ohmic heating, radio frequency, microwave (Sun, Tang and Powers, 2007), pulsed light , ionizing radiation, ultra violet light, ozone treatment (Ozen and Floros, 2001), soft electron processor (Baba *et al*., 2004).

Like all the other processing techniques the success of a thermal process depends on a number of factors. Different food systems require different temperatures and times at which they attain sterility which depend on factors such as the composition of the food, the processing technique used, the type and size of container (Kannan and Sandaka, 2008; Abdul Ghani et al, 2001). For instance solid foods are heated by conduction whilst liquids heat by natural convection and a mixture of solids and liquids mix by both mechanisms (Abdul Ghani and Farid, 2006), yet some processing techniques are more suited to liquid foods of low viscosity whilst others will be best for highly viscous liquids (Awuah *et al*., 2007). In addition, foods that contain heat labile nutrients or other quality parameters are less thermally processed than those that are rather enhanced or less affected by the application of heat (Choi *et al.*, 2006). This is also because the

heat resistance of micro-organisms changes in different environment due to different factors including pH, water activity, chemical composition etc (Miri *et al*., 2008).

Retorting has been used to refer to thermal sterilization and has gained a lot of research attention in enhancing the safety and quality of sterilized food. The type of retort used has been implicated in the efficiency of sterilization due to its effects on heat penetration, lethality and the loss of food quality (Berry and Bradshaw, 1982). Agitated retorts have been found to be better in terms of effectiveness and efficiency of sterilization as the time and temperature of microbial destruction is concerned product quality retention for that matter (Ansar Ali *et al*., 2006).

Many researchers have worked on thermal sterilisation and various process parameters have been established based on the properties of the food and especially the organisms relevant to food safety and public health concern. For low acid canned foods the sterilization value is based on the destruction of the spores of *Clostridium botulinum*. The spores of *Clostridium botulinum* which is an anaerobic heat resistant organism of public health concern have been found not to germinate in a pH below 4.8 based on which a pH of 4.6 has been set as the standard for acidity in foods (Awuah *et al*., 2007). Generally a D-value of 0.21 min. at 121^{o}c has been established based on the 12D reduction required for minimum sterility, which has become a basis for the calculation of the integrated lethality value F_o. The F_o value for minimum sterility has been calculated to be 2.52min based on the spores of clostridium (Forsythe, 2011).

2.9.5 Quality Deterioration of Food during Thermal Processing

Quality concerns during thermal processing are mostly on the change in physico-chemical properties of foods. The application of heat has deteriorative effects on food quality due to the potential loss of nutrients, change in colour, flavour, texture and consistency (Sun *et al*., 2007).

Just as in microbial destruction, the sensitivity of these desirable characteristics to heat follows the first order reaction kinetics (Durance, 1997). For instance Shin and Bhowmik (1995) used the thermal reduction of the colour in green pea puree as the indicator of the extent of heat damage to quality during thermal processing obtained 31.10 min and 42.87 as D and z values respectively based on (-La/b) for their product. Simpson *et al*., (2008), indicated that temperature uniformity is very vital to quality retention in thermal processing and hence efforts should be made to integrate temperature over the volume of container and time. Although both microbial destruction kinetics and quality deterioration kinetics of food are first order reactions, the increase of the rate of microbial destruction with increase in temperature is higher than that of loss of quality; a reason for the usefulness of high temperature short time operations in quality retention (Durance, 1997). This has been confirmed in the degradation rates of nutrients, colour and texture of asparagus which were found (Sun *et al*., 2007) to increase at a lower rate than microbial spores at 121^{o}C with time. Based on that, the Optimum temperature of sterilization with minimum quality loss has been found to be a function of the z of the quality attribute and the specific location of the quality loss within the container (Durance, 1997).

2.10 Food Spoilage Due to Lipid Hydrolysis and Oxidation

Oily foods are normally unstable mainly due to the presence of triacylglycerides which are hydrolysed in the presence of water to form free fatty acids resulting in the hydrolytic rancidity of the fatty foods. The free fatty acids if unsaturated are prone to oxidation, leading to the production of short chain acids and carbonyl compounds that cause the undesirable flavours characteristic of oxidative rancidity.

2.10.1 Hydrolytic Rancidity

Hydrolytic rancidity is a fat degradation reaction in which the fatty acids of the triglyceride bonds are cleaved to release the free fatty acids with their characteristic odours. This occurs either through the action of lipase enzymes, or very high temperatures. Foods containing high levels of polyunsaturated fatty acids such as linoleic acid and linolenic acid have been found (Sewald and DeVries, 2012) to be much more susceptible to rancidity than moderately saturated compounds such as oleic acid because they can form many different off-flavour molecules (Cesa *et al*., 2012). Apart from those naturally present in food some lipolytic micro-organisms are known (Ledenbach and Marshall, 2009) to produce microbial lipases that cause rancidity in food during storage. Because the presence of free fatty acids (FFA) in oily products exposure to high storage temperatures can cause off-flavours and it is important to reduce the rate of deterioration of oil due to rancidity (Chotimarkorn and Silalai, 2008) by maintaining low levels of FFAs throughout storage.

2.10.2 Oxidative Rancidity

The enzymes peroxidase and lipoxygenase act on the double bonds of free fatty acids and begin a chain of reactions with various undesirable products reducing the quality of food and is one of the routes of oxidation in fats. Another route which the oxidation reaction could take is the free radical route which requires the presence of a catalyst such as light, high energy radiation or the presence of metal ions which is propagated by the formation of free radicals (Sewald and DeVries, 2012). The photo-oxidation route is the third one and is driven by the absorption of light and oxygen. The oxidation process brings about intermediate products such as hydroperoxides which are further broken down to form low molecular weight products such as

alcohols, aldehydes, and ketones, ultimately leading to oxidative rancidity (Farhoosh *et al*., 2009).

Oxidation reactions take place in the presence of light, heat, energy etc. Beltran *et al*., (2003) found that, oxidation of minced chicken depended on the amount of pressure treatment. Also working on Jameed, a milk product, Al-Isma *et al*., (2007), found that both oxidation and hydrolytic rancidity increased with air drying and also with storage at room temperature. Similar results were obtained by Nieto et al. (2011) on their work on cooked lamb as they realized that volatiles from lipid oxidation increased with increase storage time and the exposure for retail.

2.11 Shelf-Life Testing

The shelf life of processed foods is very vital for quality retention and economic considerations of the processed foods. This is because the aim of food processor is to deliver safe foods that retain their quality over a stipulated period of time which is termed as the shelf-life of the food (Fu and Labuza 1991). The shelf-life of a particular food product depends on factors such as product formulation, processing technique used, storage and distribution (Kilcast and Subramaniam, 2000). The shelf-life of a product may be determined by the growth of micro-organisms with time based on safety concerns whereas in other cases it may be the loss of quality which have no harmful effects but indicates that the food no longer conforms to its consumers' acceptability standards (NZFSA, 2005). Shelf-life studies require knowledge of the quality reduction parameters which could be microbial, chemical or physical and shelf life studies could be indirect or direct.

2.11.1 Indirect Methods

Indirect methods of shelf-life estimation can be done by the use of accelerated shelf-life studies (ASLS) involving the use of elevated temperatures to increase the rate of deterioration of food with the intention of hastening spoilage. The rate of spoilage at actual storage temperature can be deduced by extrapolating (NZFSA, 2005). To do this the principles of predictive modelling using appropriate models such as the Arrhenius theorem, Q_{10} concept or (FSAI, 2011) may be used.

2.11.1.1 Predictive Modelling

Predictive modelling is a means of estimation of the shelf-life of a product based on changes in the various factors affecting spoilage and their interaction with each other (Kilcast and Subramaniam, 2000). The deterioration can be due to the effects environmental conditions such as temperature, water activity, presence or absence of oxygen and their resultant changes in biochemical reactions and physiological activities (Ahvenainen, 1996). Predictive modelling is used widely to study microbial behaviour in specific environment, their control in particular food systems and the estimation of shelf life of foods (Podolak *et al*., 2010). The use of microbial models in the prediction of shelf life is common in the fish and fish product (Taoukis *et al*., 1999), minimally processed fruits and vegetables (Corbo *et al*., 2006), milk and milk products (Duyvesteyn *et al*., 2001) where microbial spoilage is the main mode of deterioration. In the use of microbial growth models in the prediction of shelf life, the specific spoilage organisms which are normally the most important organisms to the spoilage of the food are used. This is done by isolating the most predominant of the micro flora present in natural storage conditions of the product and studying its growth pattern in model substrates through which the shelf life is predicted (Dalgaard, 1995). The obtained shelf life is normally validated by the use of real data from normal storage conditions or information derived from sensory data (Dalgaard *et al*., 1997).

Shelf life estimation based on Predictive models can be done by evaluating the effects of the mechanism of spoilage such as temperature on spoilage using such as the Arrhenius equation, Q_{10} concept and other kinetic models depending on the mechanism(Taoukis *et al*., 1999; Dalgaard, 1995).

2.11.1.2 Application of Q_{10} Concept in Predicting Shelf Life

The Q_{10} concept of shelf life prediction is based on the increase in the rate of reaction corresponding to a 10° increase in the temperature (Berges *et al*., 2002). Normally the shelf life estimation based on a criteria which could be sensory, lipid stability, microbial degradation etc (Dalgaard and Jørgensen, 2002). Based on any such criteria Q_{10} can be calculated as the shelf life at T°C/shelf life at T+10°C and the figure is used to predict the shelf life of the product (Al-Kadamany *et al*., 2002). The Q_{10} model has been applied in modelling microbial behaviour (Davidson *et al*., 2006), respiration of rate in plants (Gifford, 2003) and other biological and biochemical phenomena. In food processing, the Q_{10} concept can be used in modelling the temperature sensitivity of food spoilage reactions to the mechanisms of spoilage such as temperature and water activity. For instance Evranuz, (1993) calculated the Q_{10} value of unblanched salted and roasted peanuts based on the rate of peroxidation. The shelf life of Hass avocado was also predicted at 7 and 25°C based on Q_{10} of data obtained at 10 and 20°C (Perez *et al*., 2004). Additionally based on the effect of temperature, the Q_{10} of guava fruits was calculated as a measure of the rate of change in respiration and ripening behaviour to help in optimising storage and retail conditions and reduce quality losses (Bron *et al*., 2005). The Q10 concept can be related to the Arrhenius principle by the equation,

$$Q_{10} = \exp [10\ Ea/(R\ T\ (T+10)]$$

where the Q_{10} depends on the Ea and the absolute temperature (Fu and Labuza, 1997).

2.11.1.3 Application of the Arrhenius Theorem in Predicting Product Shelf Life

The Arrhenius concept is used to model the temperature dependence of deterioration of zero order and first order reaction such as microbial activity, lipid oxidation, etc (Fu and Labuza, 1997). The Arrhenius equation is given as:

$K = K_0 e^{-Ea/RT}$ or

$\ln (K) = -Ea/R \cdot 1/T + \ln (Ea)$

Where:

K: Rate of reaction constant

K_0: is the pre-exponential factor

Ea: the activation energy in cal/mol

R: the gas constant in cal/mol

T: the temperature in ^oK

A plot of shelf-life (lnK) against temperature (-Ea/R · 1/T) gives a straight line; the slope indicating temperature sensitivity of the reactions involved. The steeper the slope the smaller the change in temperature needed to cause significant changes in deterioration (Fu and labuza, 1997). Based on these the predictions shelf life of the product at different temperatures can be made (Petrou *et al*., 2002).

2.12 Conclusion

Malnutrition remains a problem in Africa including Ghana. Food supplementation has been identified as one of the effective ways of reducing malnutrition and can be a tool for improving food security especially for vulnerable groups. Food supplements such as ready to use supplementary foods (RUSF) provide (macro and micro-) nutrients calculated to meet minimum

requirements of the target group's daily requirements. The formulation and processing of such products require an understanding of the functionality of the ingredients, their degradation kinetics during processing and storage, as well as the nutritional requirements of the target group for which the food is intended. In the processing of such supplementary foods the calorie content, bioavailability and absorption rates of the nutrients are usually of great consideration. Consequently the application of enzymes such as amylases and proteases has been used to enhance the nutritional quality of various supplementary foods.

The acceptability of supplementary foods by the target consumers is vital to the achievement of the purpose for which they are intended. In fluid supplementary foods the flow properties as well as emulsion stability are also important quality criteria, and are modified by the application of appropriate stabilizers and emulsifiers. Apart from consumer acceptability of the food, the keeping quality of the food is equally important, especially for its application in wide variations of space and time. It is important therefore, to determine the period within which the food can retain its quality (shelf life) taking into consideration the factors that can contribute to the loss of quality.

CHAPTER THREE

3.0 MATERIAL AND METHODS

3.1 Raw Materials

Groundnuts (*Arachis hypogea*) a cultivar locally known as Chinese, a locally available long grain rice (Uncle Sam) (*Oryza sativa L*.) and cowpeas (*Vigna unguilata*) were purchased from a local market (Madina market). Sugar used as sweetener was also purchased from a shop. Bromelain was obtained from herbal extract plus, (USA) and amylase was obtained from DSM (South Africa).

3.2 Sample Preparation

Groundnuts and cowpeas were well sorted to remove broken, pecks and discoloured grains, before passing through the various units of operations of the process.

3.2.1 Roasting

Peanuts were roasted at 150^{o} C for 45minutes in an electric oven (to about L50 value in colour) to enhance flavour and to inactivate the enzymes.

3.2.2 Soaking

Cowpeas were decorticated using the method described by Sefa-Dedeh, (1978) and then soaked in 1% sodium bicarbonate ($NaHCO_3$) for 16 hours. This was done in order to soften the

ingredients and also reduce the beany flavour and yet prevent putrefaction from occurring in the process.

3.2.3 Cooking

Cowpeas and rice where cooked by boiling. Rice was boiled for 30minutes while it took 180minutes to boil decorticated cowpeas.

3.2.4 Homogenisation

The roasted peanuts, cooked cowpeas and rice were mixed in the ratio of 3:3:4 (on dry matter bases) and co-milled in a colloid mill (Machine No. 84492, Premier colloid mills limited, Walton-on Thame, Surrey, England) with sufficient added water to obtain a homogenized mixture containing 20% solids. The product was warmed to about 40°C and amylase enzyme added. The product was stood for about 1 hour at 40°C to allow time for the maximum hydrolysis of the starches by amylase enzymes. Afterwards bromelain enzymes (proteases) were also added to the product and left to stand for 3 hours to enable hydrolysis of the proteins in the product.

3.2.5 Thermal Processing

The product was heated in an open jacketed kettle and maintained at 95°C for 15 minutes to inactivate the enzymes. It was hot-filled into 330mL bottles, capped and pasteurized by immersing in boiling water for 10minute, 20minute or 30minute. Some of the bottled product was sterilized by retorting at 119^{o}C for 10minutes, and then left to cool at room temperature.

3.3 Process Flow Chart for RUSF Production

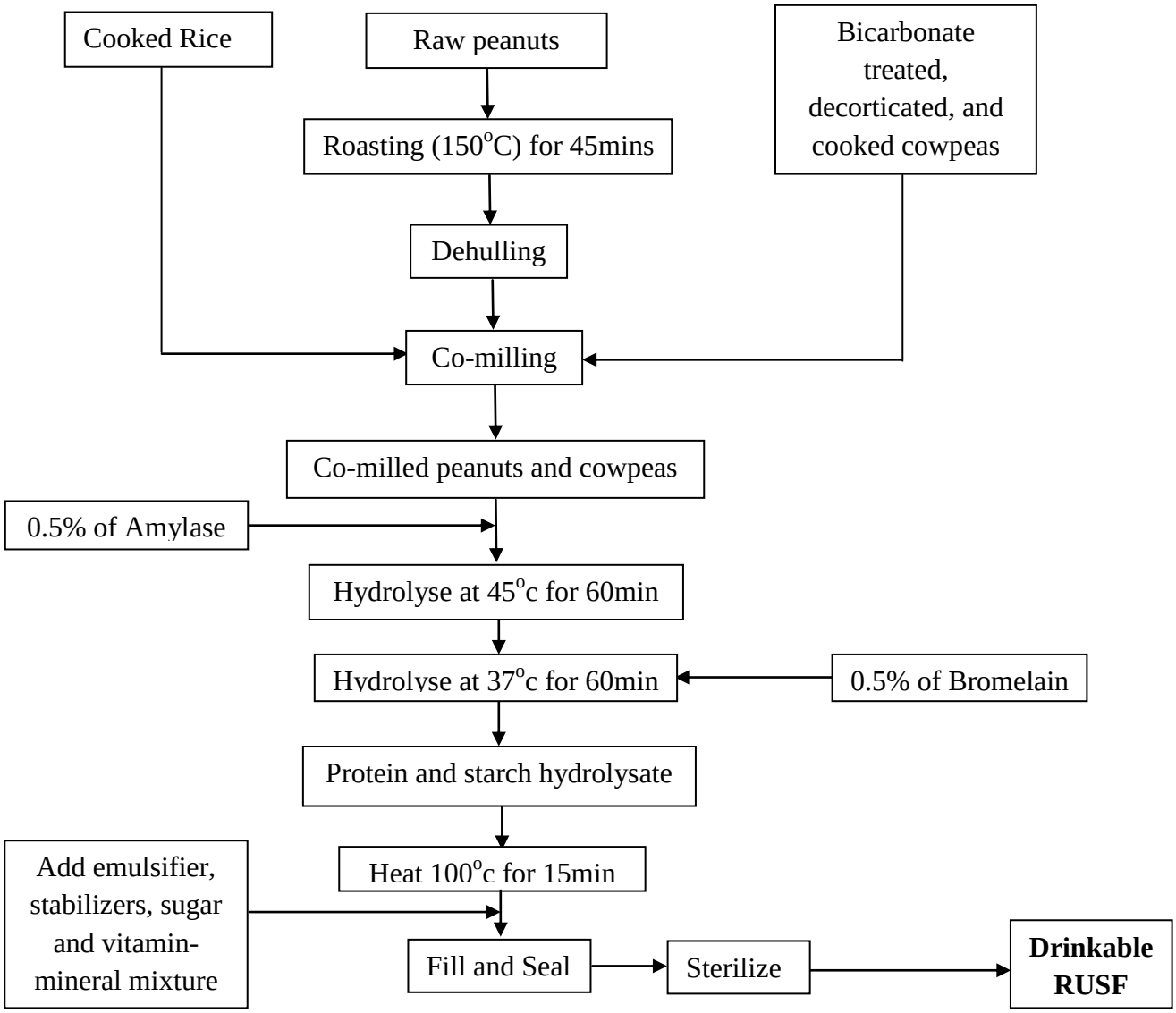

Figure 3.1: Process Flow Chart for RUSF Production

3.4 Chemical Determinations

3.4.1 Proximate Analysis

Total solids and Ash were analyzed on all the samples using AOAC methods 925.09 and 923.03 respectively. Crude fibre was also determined using standard procedures method 962.09 (AOAC, 1990). To determine the moisture content of the liquid sample, the samples were kept in the oven at a temperature of 50^{o}C for about 10hours and then increased to 105^{o}C for two hours according to method 925, AOAC (2005) with modifications. Carbohydrate was determined by difference. Protein was analysed using a conversion factor of 6.25 using kjeldahl method; 955.04, AOAC (2005). Fat was determined using the Werner-Schmid process in which the sample was heated with 6M HCL in a ratio 1:1 to dissolve the protein and the fat settled as a layer on the top of the acid. The fat was extracted by shaking the mixture with diethyl ether which was evaporated and the fat calculated as % fat based on the weight taken (about 5g in this work) (Pearson, 1976).

3.4.2 Mineral Analysis

3.4.2.1 Determination of Na and K

Na and K were determined using Spectra AA 220FS flame photometer (Varian Co., Mulgrave, Australia) with an acetylene flame following the manufacturer's procedure in the manual. Na and K standards were prepared from a stock standard of 1000µg/ml using deionised water which was also used as blank.

3.4.2.2 Determination of Phosphorus

P was determined spectrophotometrically as stated by Bray and Kurtz (1945). One (1) ml of filtered sample was added to 30ml of deionised water. A drop of P-nitrophenol and Ammonium solution was added until solution turned light yellow. A blue colour was developed by adding 8ml of L-ascorbic acid. The solution in the 50ml volumetric flask was made to the mark using deionised water and absorbance was read at 712nm on a Shimadzu UV-120-02 spectrophotometer. Percentage Phosphorus is calculated as:

$$\% P = \text{Spectrophotometer reading} \times \frac{\text{vol. of extract}}{\text{Aliquot}} \times \frac{100}{10^6}$$

3.4.2.3 Determination of Ca, Cr, Cu, Fe, Mg, Mn, Zn, Ni and Pb

Atomic absorption spectroscopy (AAS) was used to determine the concentrations of Ca, Cr, Cu, Fe, Mg, Mn, Zn, Ni and Pb. The AAS (Model: PERKIN ELMER Analyst 400) was equipped with the winlab 32 software to select a wavelength, slit, current and the mineral of interest. The air-acetylene (10:2.5) flame at a temperature of about 2300^0C was used for igniting the aspirated sample standards were prepared from commercial stock standards of 1000µg/ml of each mineral using deionised water. The blank (deionised water) and standard solutions were aspirated by the machine to create a calibration curve. Concentration of the each mineral was given as ppm and converted to mg/100ml.

3.5 Determination of the Degree of Hydrolysis of the Macromolecule by Enzymes

3.5.1 Starch

The degree of starch hydrolysis was determined by monitoring reducing sugars levels. Luff schoorl method of determining reducing sugars (Egan *et al*. 1981). Twenty five (25) ml of copper reagent (Copper II pentahydrate, Citric acid and Sodium carbonate) was measured into 25ml of the sample and refluxed for 10minutes. It was cooled and titrated with 0.1N Sodium thiosulphate after adding 3.0 g of potassium iodide and 20 ml of 6N HCL. 1.0 ml starch indicator was added, and titration continued until the blue colour changed to give a white precipitate of cuprous iodide. The reducing sugars were estimated by calculating from a standard based on the amount of thiosulphate used (titre value).

3.5.2 Protein Hydrolysis by (Bromelain) Protease Enzymes

3.5.2.1 Preparation of Samples

Fifty (50) ml of the homogenized mixture of rice peanuts and cowpeas were taken and 0.5%, 1% and 1.5% commercial bromelain was added to them and then incubated for 1hr, 2hr and 3hrs each at 37^{o}c, 40^{o}c and 43^{o}c. The samples were then evaluated for the degree of hydrolysis by monitoring the production of $-NH_2$ using 2, 4, 6-trinitrobenzenesulphonic acid (TNBSA). The process was assessed qualitatively using sodium dodecyl sulphate-poly acrylamide gel electrophoresis (SDS-PAGE).

3.5.2.2 Trinitrobenzene Sulphonic Acid (TNBSA) Method

The samples were reacted with TNBSA to produce a chromogenic derivative that was measured quantitatively at 340nm. The standard curve was generated using L-leucine concentration between 0.5 and 1.

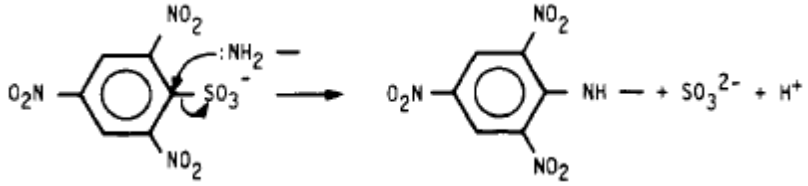

Figure 3.2 Reaction of TNBSA with amino groups

3.5.2.3 SDS-PAGE

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was done according to the method described by Laemmli, (1970) on the bromelain hydrolyzed and non hydrolyzed products. Proteins were extracted from the sample using ammonium sulphate precipitation. In the process the samples were centrifuged at14500 rev/min and the filtrate that contains the proteins was collected to which ammonium sulphate was added to 40% saturation. The precipitated proteins were collected after centrifugation at 10000 rev/min. More ammonium sulphate was added to the supernatant to make 70% saturated to obtain more proteins. The proteins collected at both stages were pooled and dissolved together with a solution containing 50mM pyridine, 10 mM thiourea, 1% SDS and 5mM phenylmethanesulphonylflouride. The protein solution was then diluted in a ratio 1:2 respectively with the sample buffer tris buffer (pH 6.8), 10% SDS, glycerol, dithiotrietol and bromophenol blue. 15µl of this mixture sample was loaded into the wells and 10µl of the molecular weight marker was also loaded as a standard (low molecular

weight standard). The samples were run with the Bio Rad^{R} mini protean II. The running or electrode buffer was diluted ten-fold and used to fill the space in between the plates in the tank and the samples were run at 200 volts until the blue marker dye reached the bottom of the gel.

3.6 Effect of Particle Size Distribution on Product Separation.

The mixture of roasted peanuts, cooked rice and cowpeas was passed through the colloid mill (Machine No. 84492, Premier colloid mills limited, Walton-on Thame, Surrey, England). The milled mixture had particle size range between 500µ-75µ. Different particle size products were obtained by passing the mixture through the sieves (500µ, 300µ and 106µ) thus yielding products with particle sizes ≤ 500µ, 300µ and 106µ respectively. The filtrates were poured into a well graduated 100ml measuring cylinder and their sedimentation rates monitored at the intervals of 3, 6, 12, 24, 48, 72 and 96 hours each. The rate of sedimentation was estimated as the difference between the previous level of clear solution and the level at the time of inspection.

3.7 Effects of Stabilizers on Physical Stability and Flow Behaviour of Product.

Three commercially available gums: carboxymethylcellulose (CMC), Xanthan gum and carrageenan were each incorporated into the product and passed through the colloid mill. The flow behaviour (i.e. shear rate shear stress relationships) of the products were characterized at room temperature (25°C). The rate of product separation was determined with time by monitoring the volume of the clear solution on top of the product. Three separate levels of each stabilizer (0.05%, 0.075% and 0.1%) were used. As was done for the particle size, the readings were taken at time intervals of 3, 6, 12, 24, 48, 72 and 96 hours each and again the rate of sedimentation was used as an indicator of the efficacy of the stabilizer for the product.

3.7.1 Apparent Viscosity

Viscosity measurement of the different samples was done using a Brookfield digital viscometer (model DV-1; Brookfield engineering Labs Inc., Middleboro, U.S.A) with a LV spindle number 2. The viscosity of all the samples with different stabilizers was taken at different spindle speeds. Viscosity readings for each sample were taken at six (6) different shear rates in (cp).

3.8 Storage Stability and Shelf-life Studies

Accelerated shelf-life studies were done on the product over a period of five weeks. The shelf life of the product was determined by applying the Arrhenius model which uses the effect of temperature on product deterioration with time. Microbial growth, hydrolytic rancidity and oxidative rancidity were used to assess the rate of deterioration of the product. However, only the data for hydrolytic rancidity was used for the estimation of product shelf life.

3.8.1 Microbial Analysis

Microbial examinations were done to detect the rate of growth of micro-organisms whose presence in food products at certain levels may be used to assess the quality and\or safety. Enumeration tests including total plate count, E. coli, yeast and moulds were done as indicators of the rate of the build-up of microbial load. The entire tests were done over a period of 5weeks at different temperatures (25°C, 35°C and 45°C).

Plate count agar, Eosin methylene blue and potato dextrose agar were used to culture for total plate count, E. coli, yeast and moulds respectively. The media were prepared using methods specified on the labels and peptone water was used as the diluents in all analysis. Two dilutions were used and two replicates were done for each. The drop plate method as described by

Herigstad *et al*., (2001) with a few modifications was used for all the microbial analysis. Each plate was divided into four quadrants and 0.025ml of the sample was used to obtain about 4-6 drops on each quadrant. The samples were incubated in ovens set to temperatures of 37^{o}C, 44.5^{o}C, 25^{o}C total plate count, E. coli, yeast and moulds respectively. Microbial colonies were counted with the aid of a colony counter and then multiplied by (4*10) to give the count per ml.

3.8.2 pH and Titratable Acidity

pH was determined according to the method described by AOAC (1990), method 981.12.

Titratable acidity was determined according to the AOAC (2005) method 970.21 and expressed as the percentage of lactic acid by titrating samples with 0.1M NaOH and the values reported as moles of sodium hydroxide per 25ml of sample.

3.8.3 Free fatty Acids and Peroxide Value Test

Fat extractions were done using a solvent extraction method. About 200ml of 40-60°C petroleum ether was added to 50ml of RUSF samples and shaken for about 2hours to obtain the fats in the petroleum ether which was decanted and evaporated on a water bath to obtain the fat.

For lipid stability, free fatty acids (FFA) content and peroxide value (PV) of the extracted oil were determined over the storage period of the product as an indicator of lipid hydrolysis and oxidation. The FFA content was determined according to the AOAC general method 965.33, whilst the PV was determined using AOAC general method 940.28.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 Nutrient Composition of Drinkable RUSF

The drinkable RUSF was formulated as an intervention food. It was deliberately formulated to have high protein and energy content, using locally available raw materials that had high protein, high carbohydrate and fat. Table 4.1 shows that even though the product had a very high moisture content, and consequently fluid, it also had a very high energy content of 564.52 ± 2.76 Kcal/100g.

Being a supplementary food the nutrients available in the drinkable RUSF, should provide a percentage of the recommended daily allowances of nutrients for the target consumers which were women of reproductive age. Table 4.1 shows that a (330mL) bottle of RUSF contained 316.13 Kcal of energy, and could meet about 25% RDA of carbohydrate, and 43% of the fat requirement of adult women thus providing them with a lot of energy needed for their daily activity which will be supplemented by other foods they may eat. It also provided 28% needed proteins. The Table also shows that while a good fraction (at least 25%) of macromolecules were met by the formulation, the requirements for micronutrients were marginally met.

Table 4.1: Nutrient Composition of RUSF and the Percentage of RDAs of WRA to be Met

Macronutrients	**g/100g**	**g/bottle DMB**	**RDA of WRA (g/d)**	**% RDA met for WRA**
Moisture content	83.03 ± 0.02	-	-	-
Energy (Kcal)	564.52 ± 2.76	316.13	-	-
Carbohydrate (DMB)	59.15 ± 0.76	33.12	130[1]	25.48
Protein	22.93 ± 0.77	12.84	46[1]	27.91
Fat	15.26 ± 0.04	8.55	20[2]	42.75
Fibre	12.70 ± 0.50	7.11	-	-
Ash	2.66 ± 0.20	1.49	-	-
Micronutrients	**mg/100g DMB**	**mg/bottle DMB**	**RDA of WRA (mg/d)**	**% RDA met for WRA**
Calcium	50.50 ± 2.61	28.28	1000[1]	2.83
Iron	2.18 ± 0.01	0.37	18[1]	2.06
Magnesium	6.84 ± 0.07	1.16	310[1]	0.35
Manganese	0.88 ± 0.00	0.15	1.8[3]	8.33
Zinc	1.47 ± 0.01	0.25	8[1]	3.13
Phosphorus	58.75 ± 1.63	9.97	700[1]	1.42
Potassium	489.69 ± 0.36	83.10	4700[3]	1.77
Sodium	712.02 ± 0.65	120.83	1500[3]	8.06

DMB= Dry matter basis; WRA= Women of reproductive age; [1]recommended dietary allowances (RDAs) [dietary reference intakes (DRI, 2002/2005)].

4.2 Degree of Hydrolysis of Macromolecules

To enhance the digestibility of the product and increase the energy density, the mixture of rice cowpeas and peanuts was treated with starch digestive enzymes (amylases) and proteases (bromelain) to hydrolyse the starches and proteins. This was done at varying conditions to determine the optimum conditions of hydrolysis of the polymers by the enzymes in the substrate (RUSF).

4.2.1 Hydrolysis of Starches in the Product Formulation

Reducing sugars analysis was done to determine the degree of hydrolysis of starches. The overall average amount of reducing sugars obtained from the samples was 31.24±1.34 mg/ml. The concentration of amylase enzyme did not show any significant effect on the hydrolysis. Figure 4.1 shows that as the temperature of hydrolysis of starch by the amylase enzyme increased the reducing sugars content increased to a maximum at about 50°C and remained constant between 50° and 55°. This implies that optimum hydrolysis occurred at 50°C.

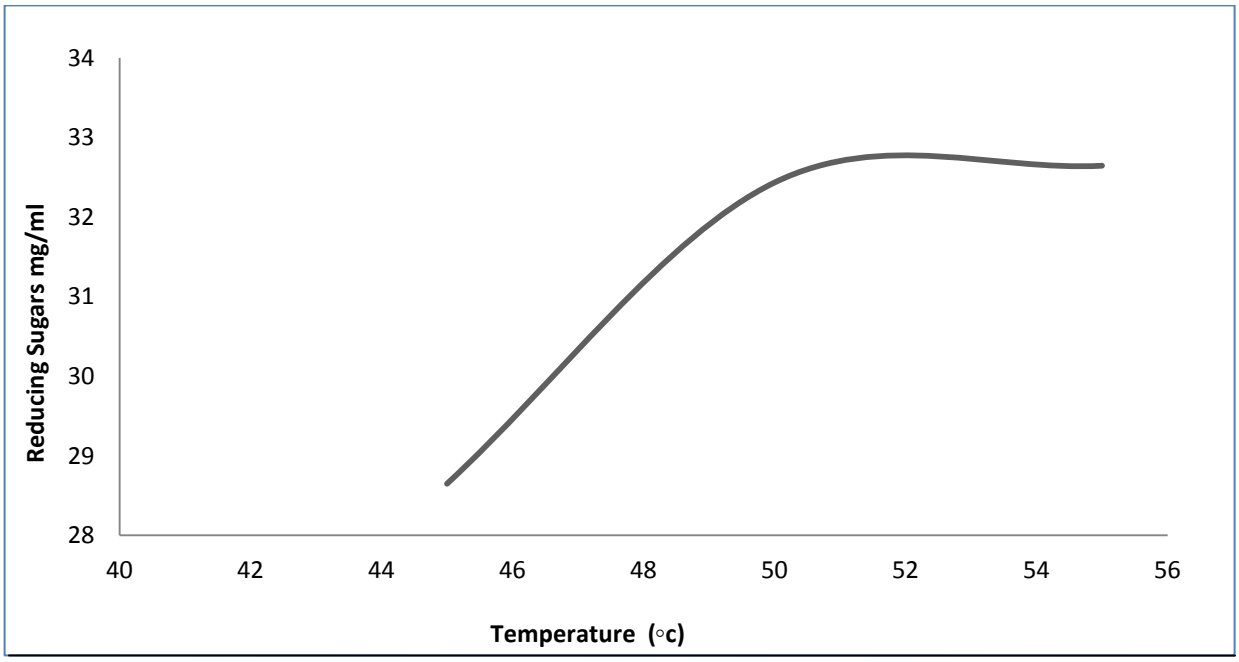

Figure 4.1: Effects of temperature on the degree of hydrolysis of starches of the drinkable RUSF

This result agreed with the findings of other researches on the activity of amylases. The activity of alpha amylases has been shown to be between 40-75°C (Sodhi et al., 2005; Yook and Robyt 2002) and the optimum activity was obtained at 50°C (Yook and Robyt, 2002).

However Sodhi *et al*., (2005) had optimum amylase activity at higher temperature of 65°C as was also documented by Sim and Berry (1996). Concerning the time of hydrolysis, starch hydrolysis increased almost linearly with a higher rate as the time increased from 45 to 60 minutes (Figure 4.2). The effect of hydrolysis time on reducing sugars content was significant at all incubation temperatures. Also at the same incubation time an increase in temperature led to a higher amount of reducing sugars (Figure 4.3). The highest degree of starch hydrolysis was obtained at the temperature range of 50-55°C and for the highest time of one hour.

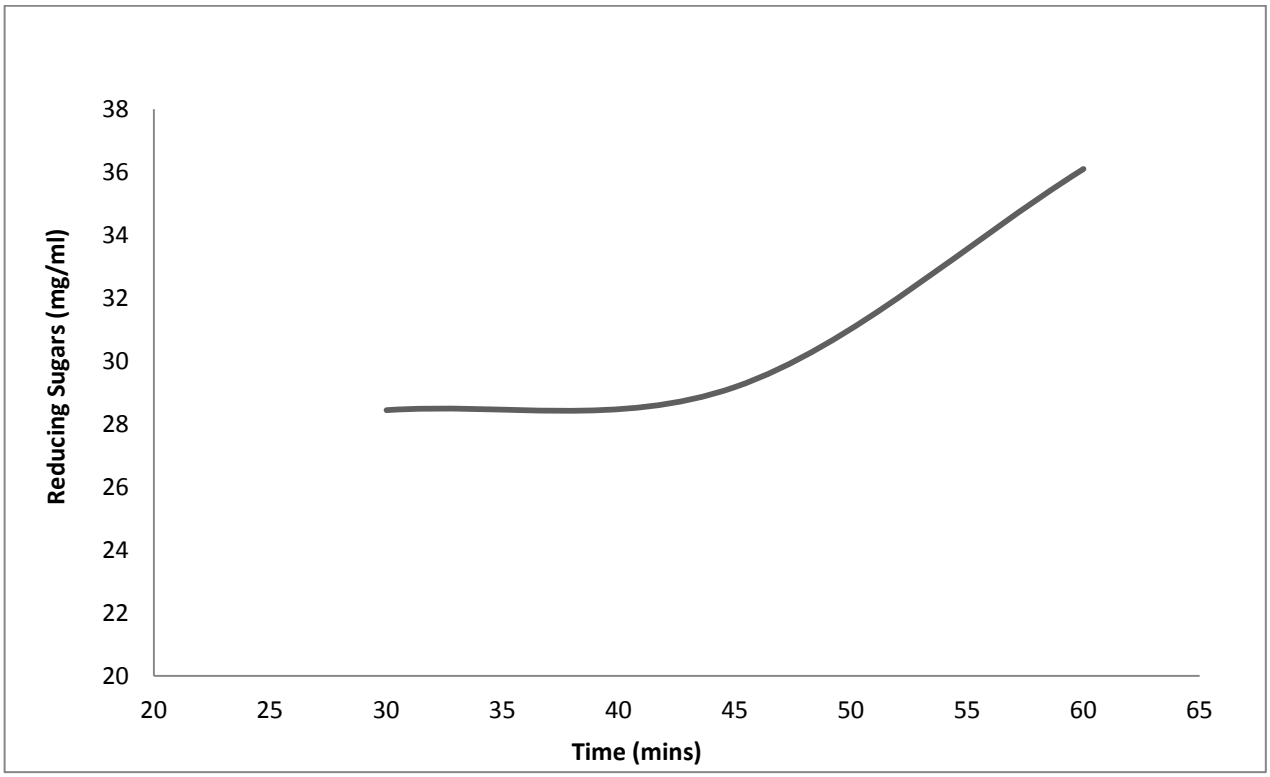

Figure 4.2: Effects of time on the degree of hydrolysis of the starches of the drinkable RUSF

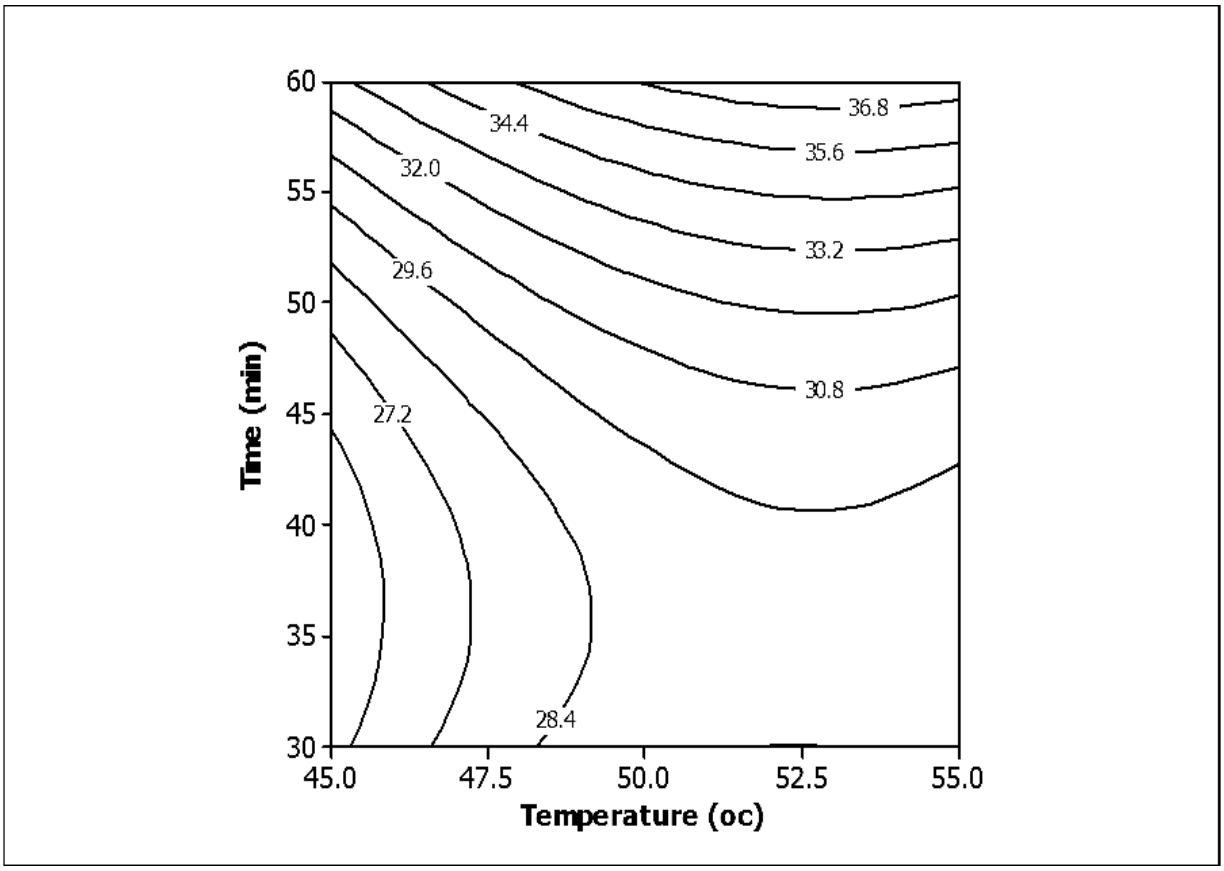

Figure 4.3: Combined effects of temperature and time on the degree of hydrolysis of the starches of the drinkable RUSF

4.2.2 Trinitrobenzene Sulphonic Acid Determination of $-NH_2$ groups (as a Measure of Protein Hydrolysis)

There was a linear relationship between incubation temperature and protein hydrolysis. As incubation temperature increased for bromelain hydrolysis of the proteins, there was an increase in TNBSA bound $-NH_2$ groups (Figure 4.4). This implies that the activity of the enzyme (commercial bromelain) was well within the incubation temperature range used (37-43°C). Bromelain is a complex mix of natural enzymes that is known to work within a wide range of temperature depending on the substrate. Corzo *et al*., (2012), indicated 55°C, 59°C and 37°C as optimum temperature of activity on three different subtrates. The linear

relationship (Figure 4.4) indicates the probability of an increase in bromelain activity with further increases in temperature. The observation may be due to the fact that optimum temperature for bromelain activity may not have been met in this work contradicting the observation made by Jutamongkon and Charoenrein, (2010) on fruit bromelain that there was loss of activity at temperatures beyond 40°C. This however may be agreeing with the optimum range (50°C-60°C) obtained by Gautam *et al*., (2010) for their stem bromelain.

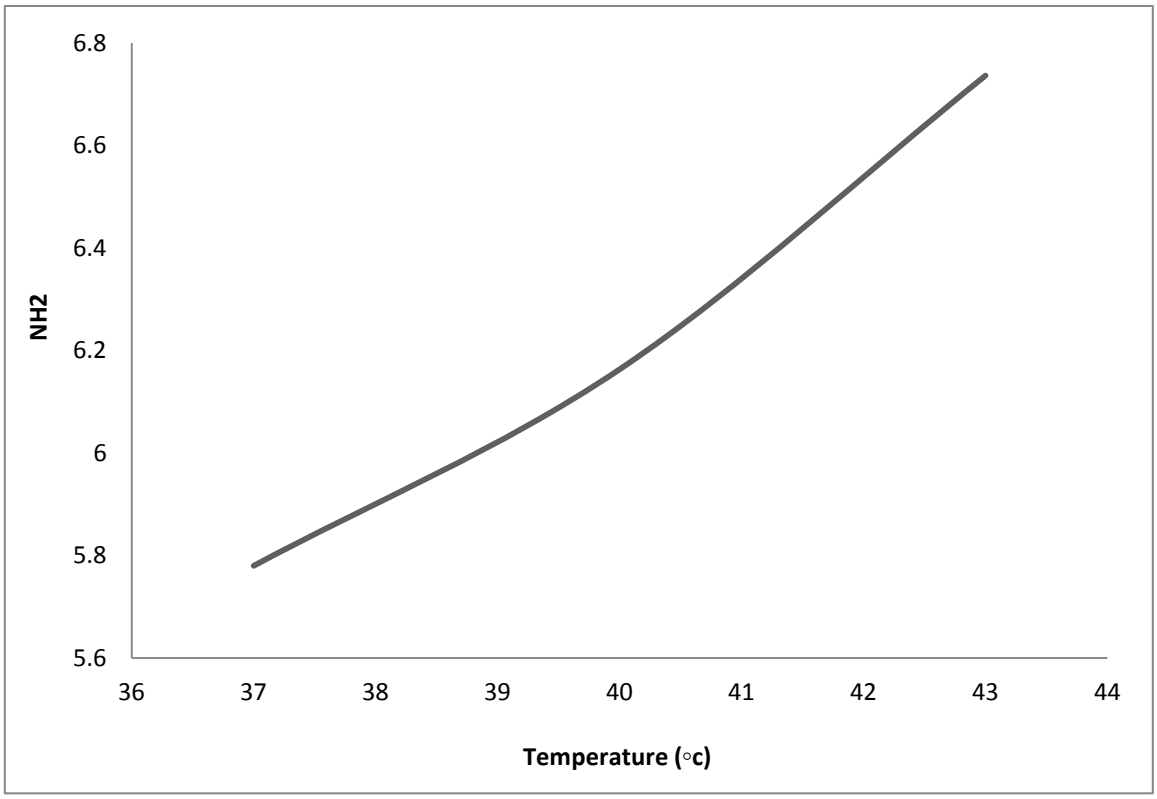

Figure 4.4: Effects of temperature on the degree of hydrolysis of the RUSF proteins

Similar to temperature, increasing the time of hydrolysis also increased the degree of hydrolysis however there was a peak after 120minutes beyond began to drop (figure 4.5). This implies that after 120 minutes free -NH_2 groups were no longer available to react with TNBSA probably because they recombined after some time in plastein reactions. Plastein

reaction involves the enzymatic hydrolysis of proteins into small molecular polypeptides and the rearrangement of these peptides, into new protein-like substances. Electrophoresis analysis of plastein synthesised by papain showed the presence of one major band with molecular weight of 2.8 KDalton, while synthesis with bromelain showed the presence of two bands with a molecular weight of less than 6.5 KDalton and 3.5 KDalton (Madzlan *et al*, 2006). Beyond 2hours, there was an apparent reduction of hydrolysis probably because of condensation of free amino groups in the plastein reaction.

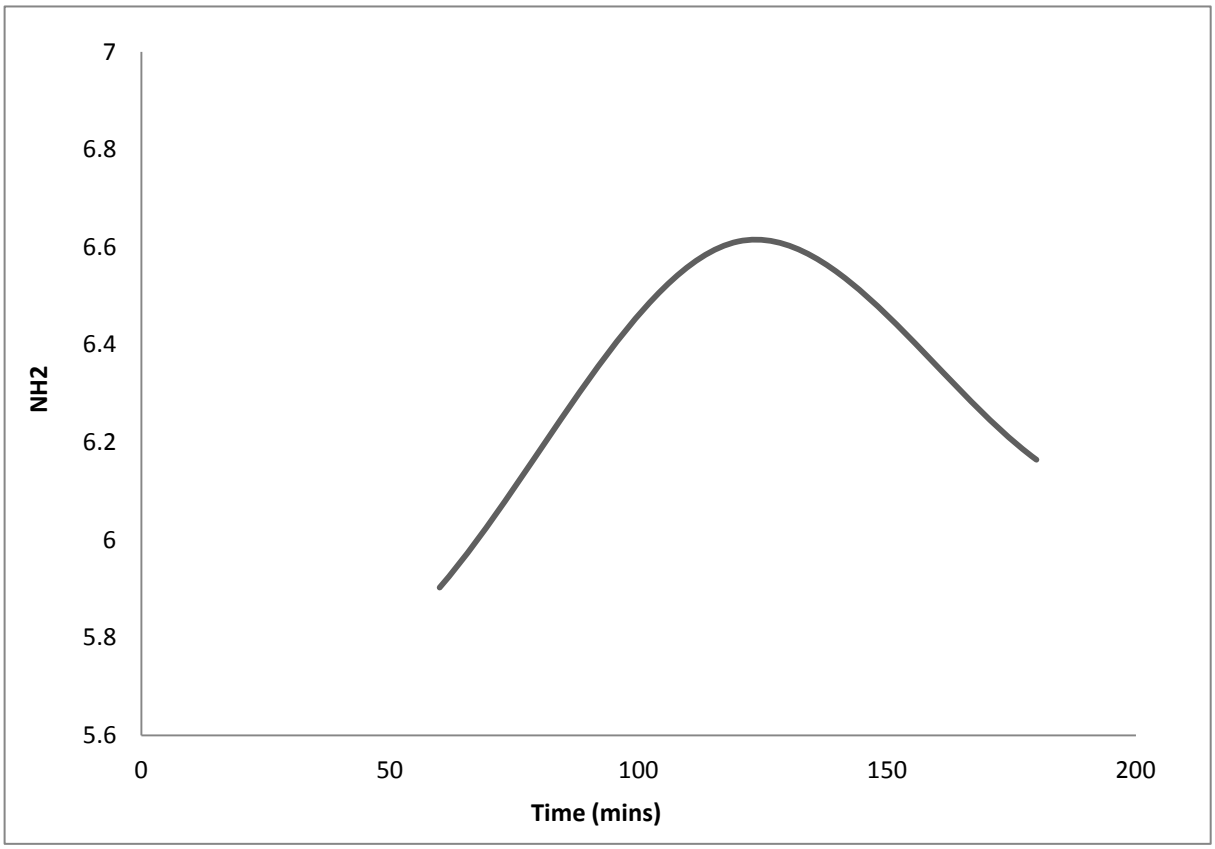

Figure 4.5: Effects of time on the degree of hydrolysis of the drinkable RUSF proteins

The combined effects of incubation temperature and time are shown in Figure 4.6. At lower temperatures (i.e. below 39°C), increasing hydrolysis time increased the degree of

hydrolysis. The maximum protein hydrolysis by bromelain was attained at 2 hours incubation above 42°C. Beyond 2 hours of incubation at low temperatures (i.e. below 39°C), there was no further change in hydrolysis. However at higher temperatures (i.e. 42°C), there was a decrease in amino groups after incubation for longer periods than 2 hours (Figure 4.6). Madzlan *et al.*, (2006) reported the formation of plastein reaction products at 37°C after 9-24 hours of incubation.

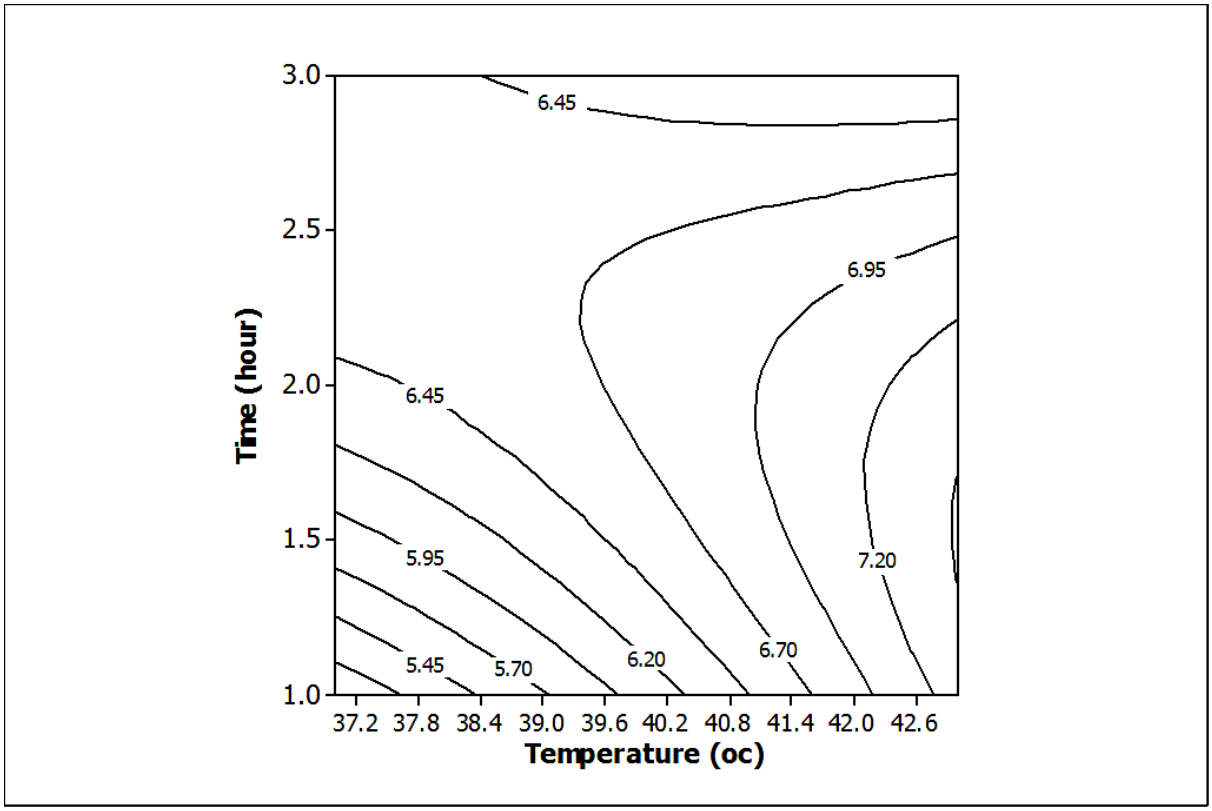

Figure 4.6: Combined effects of temperature and incubation time on the degree of hydrolysis of the drinkable RUSF proteins**.**

In this work the degree of hydrolysis as determined by -NH_2 yield was optimum at the highest temperature (Figure 4.6) and though the decline in the amount of -NH_2 occurred after 120 minutes of incubation, it is clear that within the time range used in this experiment,

60minutes of incubation could also give the same effect at 42°C. Consequently, although time had a significant effect on the degree of hydrolysis, its effect was not as high as that of incubation temperature. The highest amount of $–NH_2$ (7.2) obtained was due to the effect the highest temperature used (43°C). The amount of $–NH_2$ obtained at the highest time 6.45 was much lower (figure 4.6).

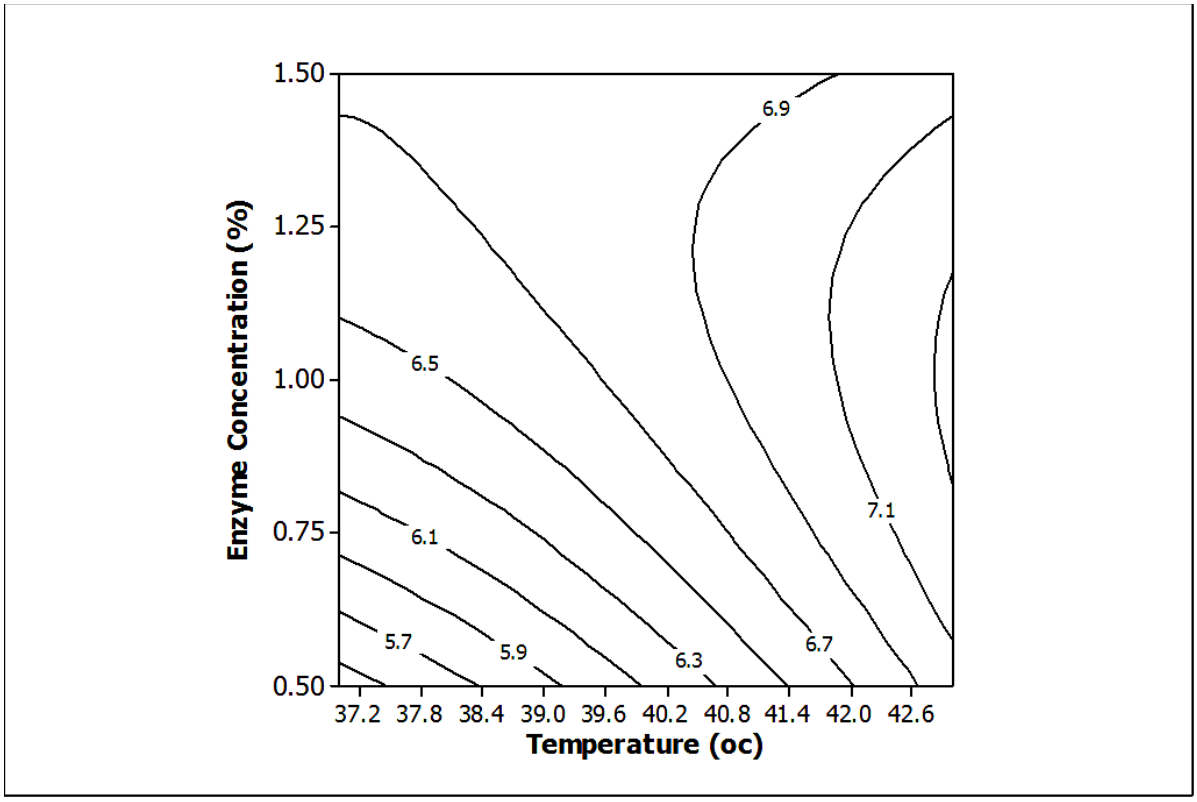

Figure 4.7: Combined effects of temperature and concentration of the enzyme on the degree of hydrolysis of the drinkable RUSF proteins

Figure 4.7 shows that although the enzyme concentration had an effect on the bromelain hydrolysis, it was observed that its effect was more profound as the temperature increased. It was realised that the rate of increase was reducing with increased concentration, still confirming the higher effect of temperature on hydrolysis.

4.2.3 Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of Protein Hydrolysates.

The effectiveness of bromelain hydrolysis of the food proteins was monitored using sodium dodecyl sulphate polyacrylamide gel electrophoresis, and the gels are presented as plate 4.1. Molecular weight markers with bands ranging from 97.4Kda to 14.4Kda were used. The two zymograms in plate 1 represent (a) protein hydrolysates obtained after 1 hour incubation with bromelain and (b) protein hydrolysates after 3 hours incubation with bromelain. For the non hydrolyzed sample, there were clear protein bands with weights above 97.4 Kda. The absence of those bands in the bromelain treated samples (for both the 1 hour and 3 hour hydrolyzed samples) implies that those proteins were successfully hydrolysed by the protease (bromelain).

However, in the samples that were hydrolysed for three hours (Plate 1) there appeared low molecular weight bands (below 21.5 and above 14.4Kda), that were neither present in the non hydrolyzed samples nor the 1 hour hydrolyzed samples. This is probably further proof of the possibility of recombination of amino groups to form new peptides in a plastein reaction. The formation of two electrophoretic bands after bromelain induced plastein reaction has been reported (Madzlan *et al*., 2006). The authors noted that electrophoresis analysis of plastein synthesised by papain showed the presence of one major band with molecular weight of 2.8 KDalton while, synthesis with bromelain showed the presence of two bands with a molecular weight of less than 6.5 KDalton and 3.5 KDalton. In the current work the electrophoretic analysis of the protein hydrolysates with bromelain showed the bands, which however were much bigger than those reported by Madzlan *et al*., (2006).

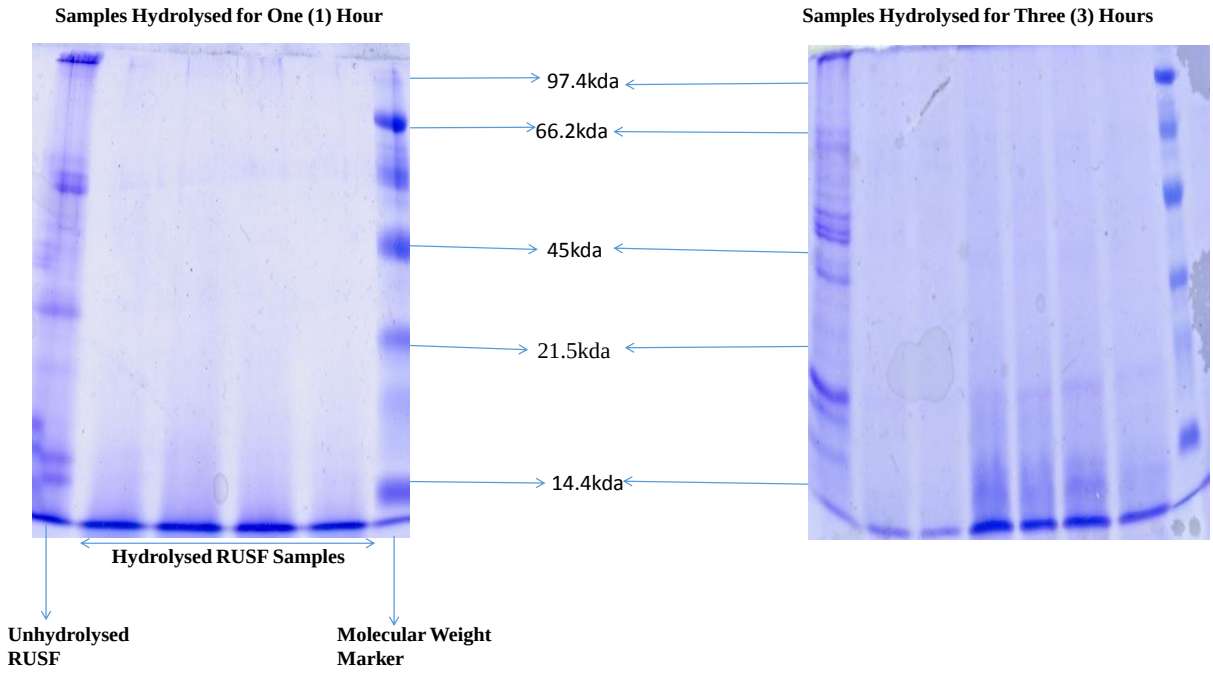

Plate 4.1: SDS PAGE zymogram showing the effect of bromelain hydrolysis on RUSF proteins

4.3 Physical Stability of the Product (RUSF).

The physical stability experiments were done to determine the most significant cause(s) of product separation and hence to ascertain the factors to be put in place to render the product physically stable. Different particle sized products were obtained by passing different batches of the colloid milled product mixture through ASTM sieve sizes 500µ, 300µ and 106µ respectively. This yielded different RUSF products with particle size distribution ranges within ≤ 500µ, 300µ and 106µ respectively and these were used for the settling rate analysis. Additionally three stabilizers applied to the product to assess the effect of stabilizers on the product's physical stability.

4.3.1 The Effect of Particle Size on the Rate of Separation

Figure 4.8 and Table 4.2 show that the samples with the finest particle sizes (≤ 106µ) gave the steepest slope (i.e. fastest settling rate) indicating the rate of decay was higher than that of the samples with larger sizes (≤ 500µ, and ≤ 300µ).

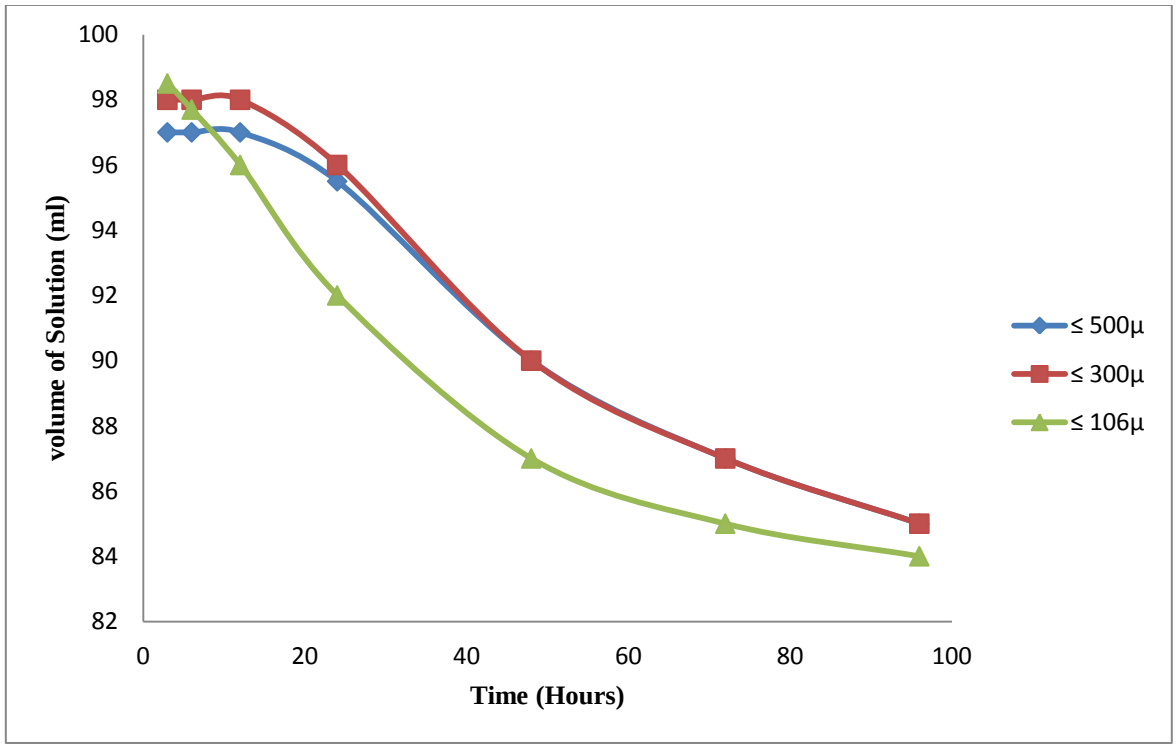

Figure 4.8: Effects of product particle size on the settling rate of RUSF samples with different particle sizes.

*Volume of solution refers to the volume of RUSF suspension without supernatant.

Table 4.2: Degradation Rate Values Showing the Rate of Sedimentation of RUSF Samples with Different Particle Sizes

Product Particle Size	**Rate**	**R^2**
106	-0.09[a]	0.8347
300	-0.074[b]	0.6635
500	-0.068[c]	0.6778

Stoke's law states that the settling or sedimentation velocity (V_m) of a moving particle in a medium is directly proportional to the square of its size (or diameter, D), the differential in the densities of the particle and medium) and inversely proportional to the viscosity of the fluid:

$V_m = D^2 g (\rho_p - \rho_f)/18\mu$..... Equation 4.1

Where

V_m = terminal or settling velocity

D = Diameter of particle (assuming it is a sphere)

ρ_p and ρ_f = density of the particle and the fluid respectively

μ = viscosity of the fluid.

Based on Stoke's law the observations made in Figure 4.8 and Table 4.2, indicate that the particle sizes of the samples used did not influence settling rate significantly since the samples with the least sizes were settling faster. The density differential between the particles and the fluid which also has a direct influence on the rate of sedimentation was an important factor in this experiment since during milling the lightest fractions such as the cellulose, and pectin that make up the fibre component of the food are the most resistant to

size reduction and generally remain as the larger particles. On the other hand, components such as starches and proteins with higher densities are far more friable and hence form the finer fractions of the milled product (Hemery *et al*., 2009; Glitsø, and Bach Knudsen, 1999). Consequently the smaller or finer particles obtained after milling, had high densities which outweighed the effect of the diameter of the particles thus causing a higher sedimentation rate in the finer than the coarser components. However since the ≤ 500µ samples contained in addition to the coarse, the denser particles which were contained in the ≤ 106µ samples, the observation could not solely be due to the densities of the particles. This notwithstanding the terminal velocity of a moving particle is affected inversely by viscosity of the medium. Therefore since the samples used in this work were not significantly affected by particle size, the effect of density was similar for all samples and yet there was a very glaring difference between the fine samples and the others which was attributed to a high difference in viscosity of the fine samples than the others. A lower fluid viscosity of the finer samples which had less particles will markedly increase their velocity since there will be less resistance to movement.

4.3.2 Effects of Stabilizers on the Flow Behaviour of the drinkable RUSF.

From the Stokes Law (Equation 4.1) the viscosity of the fluid had a strong influence on the particle separation (or phase separation) of the product. The less viscous the fluid medium, the greater the sedimentation rate. Consequently the effects of hydrocolloids (as stabilizers and viscosity modifiers) on the flow behaviour of the product as well as particles sedimentation rates were examined. Shear stress and shear strain data obtained from apparent viscosity determinations of the product (using the Haake rotational viscometer)

were fitted to the power law model (Steffe, 1996), from which the flow behaviour parameters were estimated. Table 4.3 gives the means of the consistency coefficients (k) and the flow behaviour indices (n) for the three stabilizers (carboxy methyl cellulose, carrageenan and xantham gum) used in the product to modify its flow behaviour. All the stabilizers increased the apparent viscosity of the fluid 441.457±4.234. The highest concentrations of all stabilizers gave products with the highest consistency (apparent viscosity)

Table 4.3: Effects of Stabilizers on some Rheological Characteristics of the Drinkable RUSF

Stabilizer	**Concentration (%)**	**k**	**n**
CMC	0.05	447.60±3.74^{c}	0.30±0.01^{c}
CMC	0.075	853.46±45.98^{b}	0.43±0.24^{b}
CMC	0.1	1013.43±61.36^{a}	0.45±0.01^{a}
Carrageenan	0.05	700.27±24.39^{c}	0.41±0.01^{c}
Carrageenan	0.075	1049.16±83.54^{b}	0.51±0.01^{b}
Carrageenan	0.1	1090.56±54.49^{a}	0.49±0.01^{a}
Xanthan Gum	0.05	802.39±13.25^{c}	0.40±0.01^{a}
Xanthan Gum	0.075	894.13±10.40^{b}	0.45±0.01^{b}
Xanthan Gum	0.1	1111.31±4.99^{a}	0.50±0.00^{c}

* (Figures with the same superscript are not significantly different)

The flow behaviour parameters k and n in Table 4.3 show that the stabilizers modified the viscosity of the products differently. For each stabilizer increasing levels of incorporation from 0.05% to 0.1% significantly ($p \leq 0.05$) increased the apparent viscosity, k. Among the three stabilizers, xanthan gum Produced the most profound increase in the apparent viscosity of the product while carboxy methyl cellulose (CMC) had the least effect. They have been found to increase the consistency of food products by acting as thickeners, the efficiency of that depending on the molar mass and hydrodynamic size of the polymer which is also

affected by the molecular structure as well as the charge of the polymer (Philips and Williams, 2009). Though all three gums used here have similar structure xanthan gum is known to have a higher viscosity at low shear rate which is drastically reduced with increase in shear indicating high psuedoplasticity. This behaviour according to Philips and Williams (2009) results from the ability of the xanthan molecule to form intermolecular aggregates through hydrogen bonding and polymer entanglements in solution leading to a highly ordered network of entangled stiff molecules making it a preferred choice when stabilizing emulsions and suspensions and when good flow properties are required. This was also evident in this work since xanthan gum gave the lowest rate of separation (Table 4.4) as illustrated in Figure 4.9 using the highest concentrations of the gums where xanthan gum is showing a more gradual rate than carrageenan and CMC.

In all cases however, the flow behaviour index (n) was less than unity (1) suggesting that no matter the stabilizer used the flow behaviour was always non-Newtonian, and psuedoplastic (or shear thinning). It has been suggested that most cereal fluid products are shear thinning and can be described by the power law model. As an example, Wang et al. (1999) observed that the cooked slurries of rice were shear-thinning, with no obvious yield stress at experimental levels of concentration and temperature. The shear-thinning nature of the fluid implies that with a little force applied by shaking the product will readily flow which is a desirable flow property for the drinkable RUSF since it has to be readily available to the consumer.

4.3.2 Effects of Stabilizer on Sedimentation Rates of Particles in the drinkable RUSF

Table 4.4 shows the sedimentation rates and the R-squared values of the fitted plots. As discussed in section 4.3.1 in particulate fluid foods particles tend to sediment upon standing of the product. The rate of sedimentation is influenced by the particle size, the differential densities between the particles and the fluid and inversely dependent on the viscosity of the fluid. The various types of hydrocolloids influence the viscosity of the fluid foods differently based on their hydrodynamic mass, volume and stokes radius. Figure 4.9 shows the influence of three stabilizers (CMC, Carageenan and xanthan gum) on the settling rate of the drinkable RUSF. The settling rates of the various are summarised in table 4.4. The influence of the hydrocolloids on the settling rates is reflected in the viscosities of the product when the products containing hydrocolloids (section 4.3.2). CMC having the lowest viscosity also had the fastest settling rate. Consequently in order to reduce to the settling rate of the product it may be desirable to use a hydrocolloid that will increase the viscosity as in the case of xanthan gum.

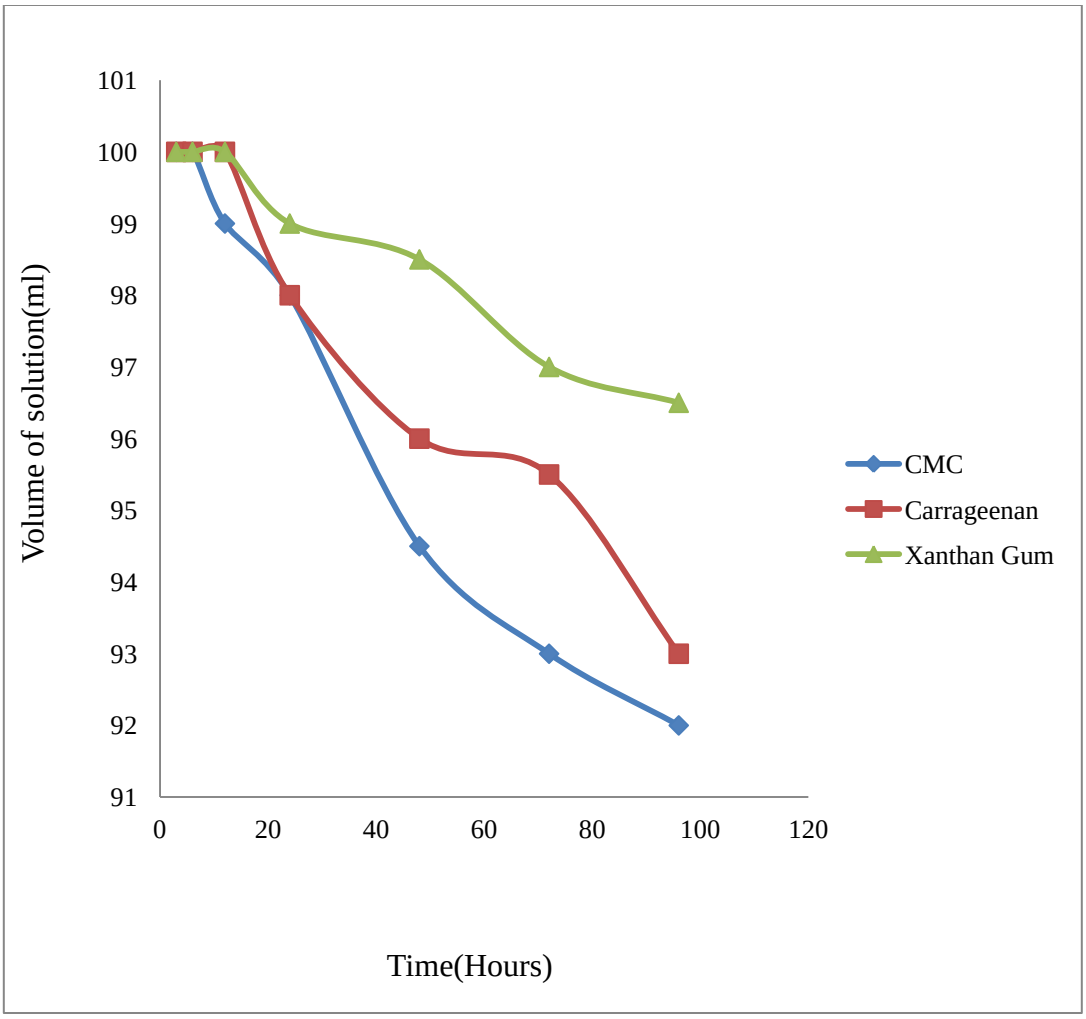

Figure 4.9: Effects of three different stabilizers on the sedimentation of RUSF

Table 4.4: Degradation Rate Values Showing the Effects of Stabilizers on the Rate of Sedimentation of the drinkable RUSF

Stabilizer	Rate	R^2
CMC		
0.05	-0.059[a]	0.7535
0.075	-0.049[b]	0.7906
0.1	-0.045[c]	0.7599
Carrageenan		
0.05	-0.048[a]	0.6796
0.075	-0.035[b]	0.6726
0.1	-0.034[c]	0.7988
Xanthan Gum		
0.05	-0.047[a]	0.6835
0.075	-0.039[b]	0.7411
0.1	-0.018[c]	0.6621

4.4 The Effect of Pasteurisation Time, Storage Temperature and Storage Time on Microbial Growth, and Physico-Chemical Properties of the RUSF

Critical parameters evaluated for the microbial stability were the process severity (pasteurisation and sterilisation) using pasteurisation time, storage temperature and storage time. The pasteurisation schedule was such that it took about forty three (43) minutes to get the samples to the temperature of pasteurisation (95°c) (figure 4.10) where it stayed for 10, 20, or 30 minutes. On the other hand for the retorted (sterilized) samples (figure 4.11) it took 25 minutes for it to come up to 119°c where it was maintained for 10minutes.The importance of the rate of heat penetration is based on its lethal effect on the micro-organisms of and the quality deterioration of the food product.

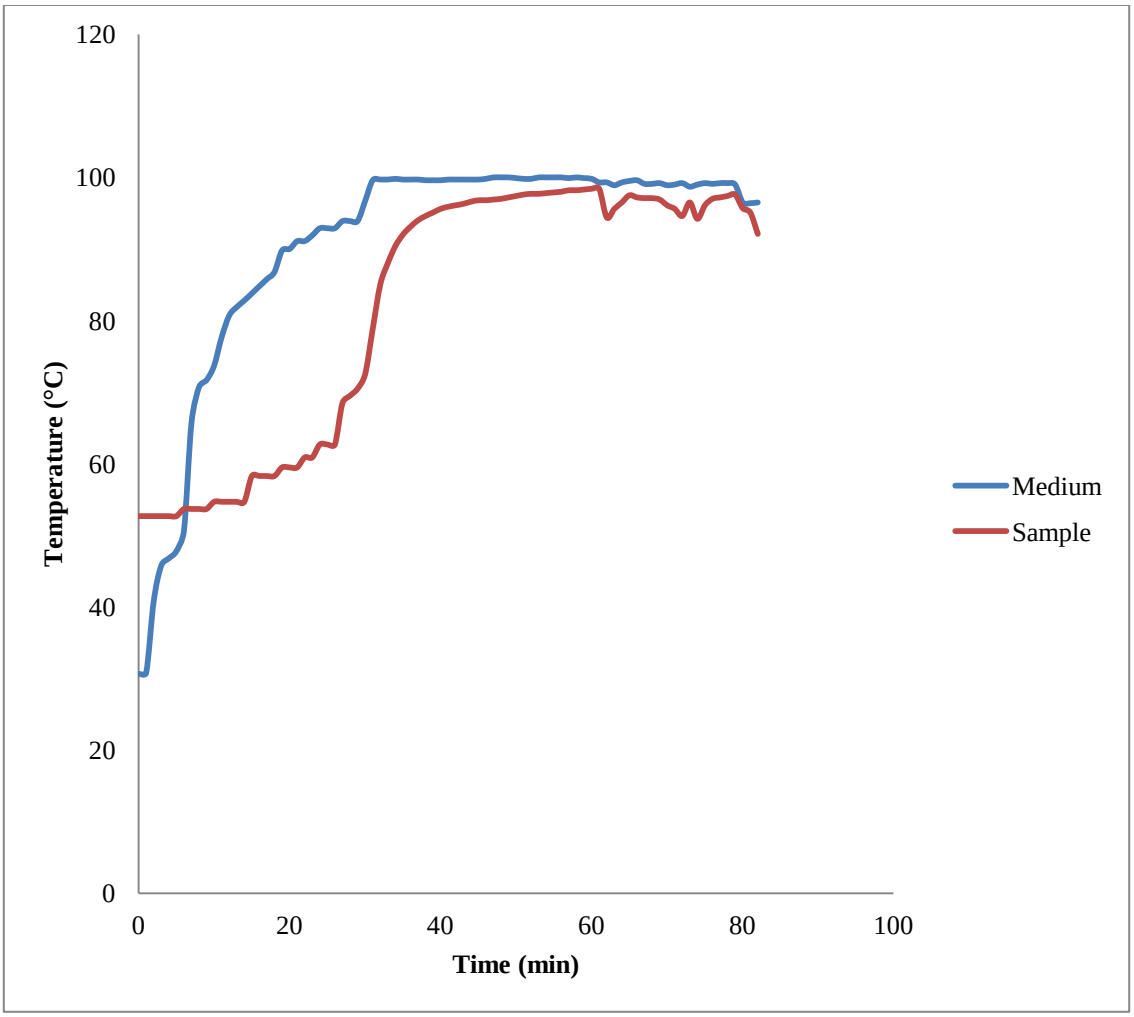

Figure 4.10: Heat penetration curve of pasteurised RUSF samples (at 95°C for 10,20 &30min)

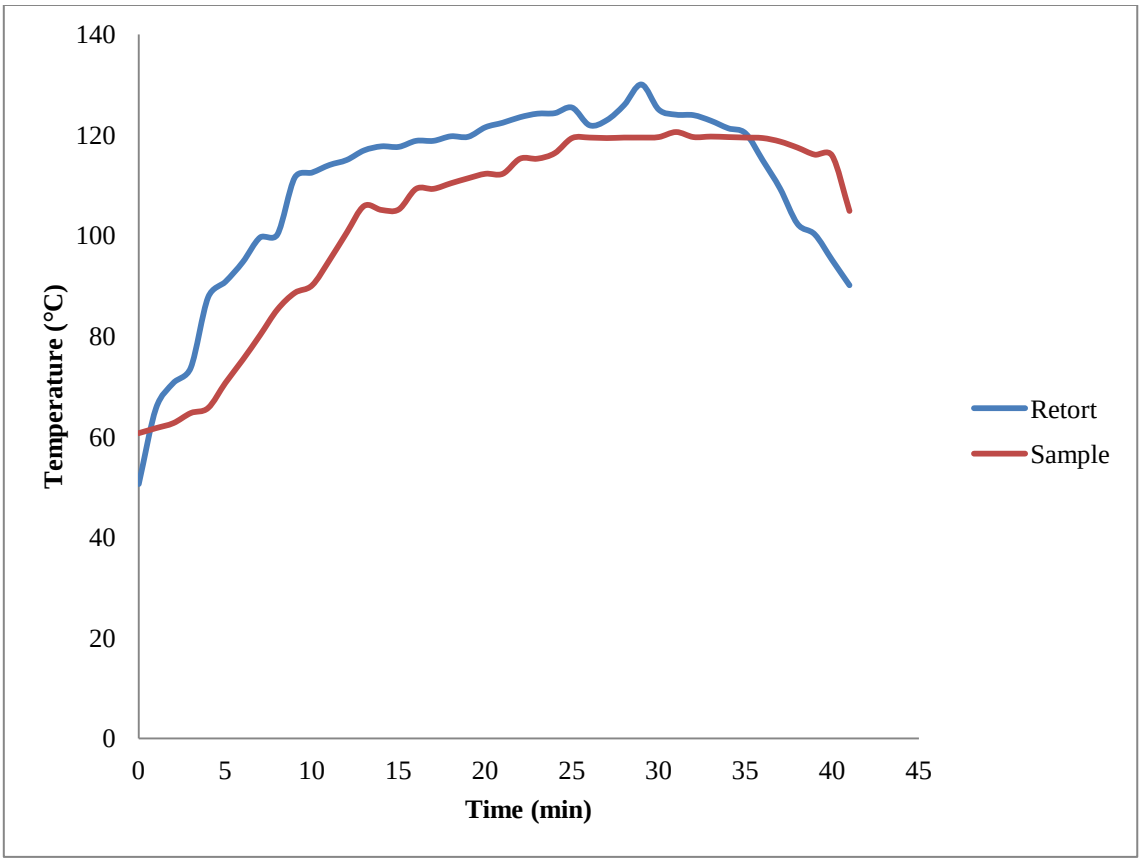

Figure 4.11: Heat penetration curve of retorted RUSF samples (sterilised at 119°C for 10min)

The effects of these processing schedules on the microbial stability of the product under different conditions of storage (time and temperature) were monitored using microbial counts. This approach is supported by Awuah *et al*., Ramaswamy and Economides, (2007) who observed that in thermal processing the primary objective is to eliminate spoilage micro-organisms and pathogenic micro-organisms or reduce them to levels that will make the food product safe for consumption and this can be used as means of assessing the efficiency of a thermal process.

Table 4.5 shows the ANOVA summary table of the effects of processing and storage variables on microbial counts of the product. All the main effects had significant influence on microbial growth. There were also significant interactions between the variables such that the microbial counts due to storage temperature and storage time depended on the severity of the heat treatment regime. The effects of Storage time on microbial growth on the other hand also depended on the storage temperature.

Similar observations were made for the effects of the pasteurisation time, storage temperature and storage time on the FFA and PV of the products (Table 4.6). The effects of storage time and temperature on FFA and PV depended on the pasteurisation regimes. Furthermore the effects of the storage time depended on the storage temperature.

Table 4.5: Anova Summay (P-Values) Showing the Effects of Different Processing and Storage Variables on Microbial Parameters of RUSF Samples

Variable	P-value		
	TVC	E.coli	Yeasts and Moulds
Pasteurization Time (A)	<.0001	0.0006	0.0013
Storage Temperature (B)	<.0001	<.0001	<.0001
Storage Time (C)	<.0001	<.0001	<.0001
A*B	<.0001	<.0001	0.0012
A*C	<.0001	<.0001	<.0001
B*C	<.0001	<.0001	<.0001
A*B*C	<.0001	<.0001	<.0001

Table 4.6: Anova Summay (P-Values) Showing the Effects of Different Processing and Storage Variables on Physico-Chemical Parameters of RUSF Samples

Variable	P-value			
	Free Fatty Acids	Peroxide Value	pH	Titratable Acidity
Pasteurization Time (A)	<.0001	<.0001	0.4722	<.0001
Storage Temperature (B)	<.0001	<.0001	<.0001	<.0001
Storage Time (C)	<.0001	<.0001	0.8656	<.0001
A*B	<.0001	<.0001	0.0468	<.0001
A*C	<.0001	<.0001	0.0233	<.0001
B*C	<.0001	<.0001	0.5397	<.0001
A*B*C	<.0001	<.0001	0.0949	<.0001

4.4.1 Comparison between Retorted RUSF (119°C) and Pasteurised (95°C) RUSF Samples

It was observed that the TVC for the retorted samples was significantly higher than that of the samples pasteurised for 20 and 30 minutes but was equivalent with 10minutes pasteurised samples. This suggests that pasteurising the samples at 95°C for 30minutes was far more efficient in reducing the microbial load than retorting for 10minites at 119°C (Figure 4.12). The effects of retorting at 119°C for 10minutes and pasteurising at 95°C for 10minutes on microbial loads were not significantly different (figure 4.12). Sterilising particulate fluids in a still retort may not be as efficient as using agitated retorts due to slow rate of heat transfer. Consequently 10 minutes of retort time was probably too short for adequate heat transfer to cause microbial destruction (Ansar Ali *et al*., 2006).

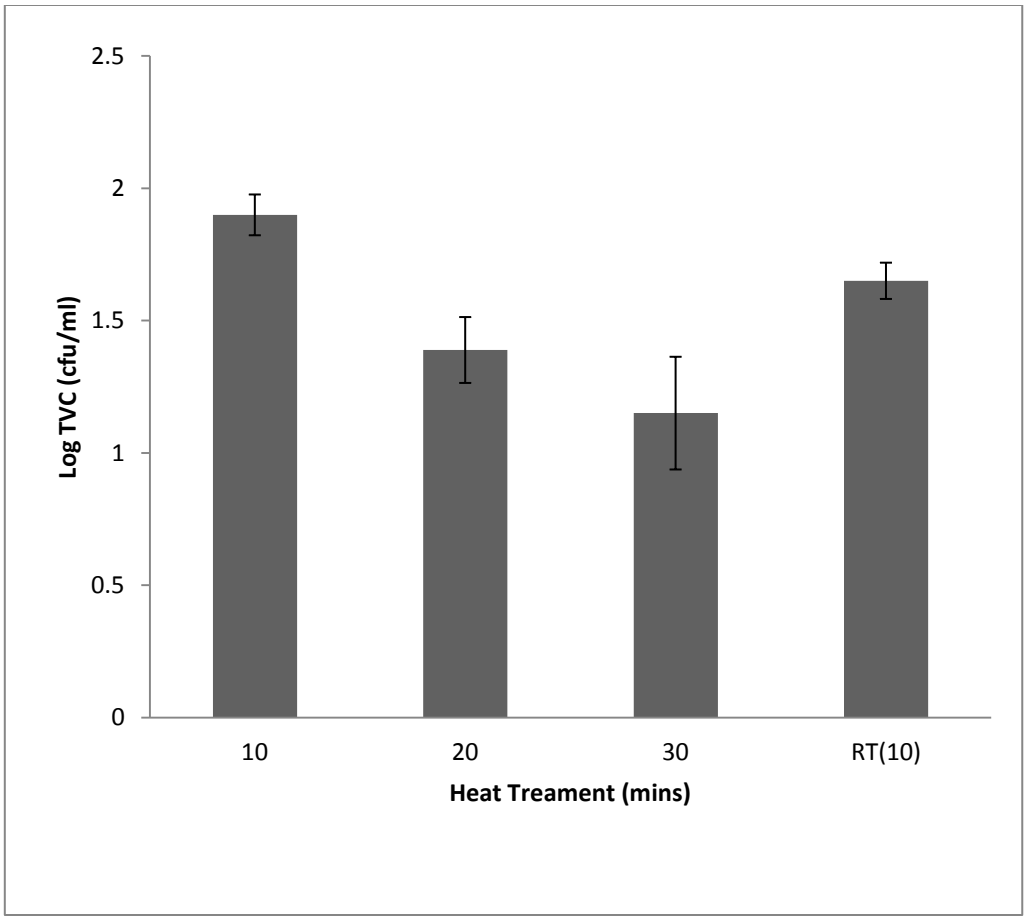

Figure 4.12: Effects of heat treatment on microbial loads (TVC) of RUSF samples

The observations made in this experiment are confirmed by this principle as the time taken to destroy the same number of micro-organisms (D-value) is higher at lower temperature than at higher temperatures and explains why the microbial count reduces with an increase in time (from 10minutes to 30minutes) (Stoforos, 1995).

The FFA and PV of the samples differed significantly with processing schedules (Figure 4.13 and 4.14). Samples that were pasteurised for 10minutes showed relatively lower FFA and PV. As the pasteurisation time increased to 30minutes, FFA and PV also increased, suggesting that increasing the severity of heat treatment increased the deterioration rate of

the fats. The FFA and PV of the samples that were sterilised by retorting for 10minutes were not significantly different from 20minutes pasteurised samples. This trend suggests that the severity of the heat treatment of the retorted samples was probably not different from the 20minutes pasteurised samples confirming that still retorts are not efficient for sterilising particulate fluids.

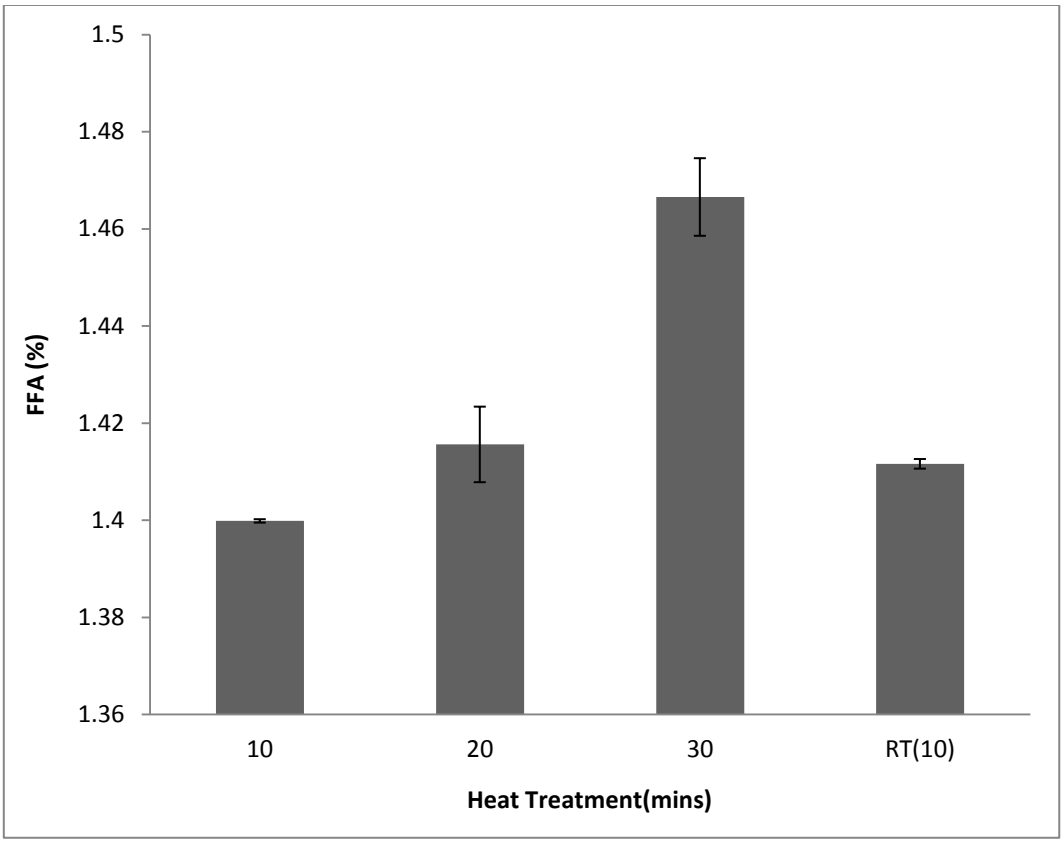

Figure 4.13: Effects of heat treatment on free fatty acids of RUSF samples

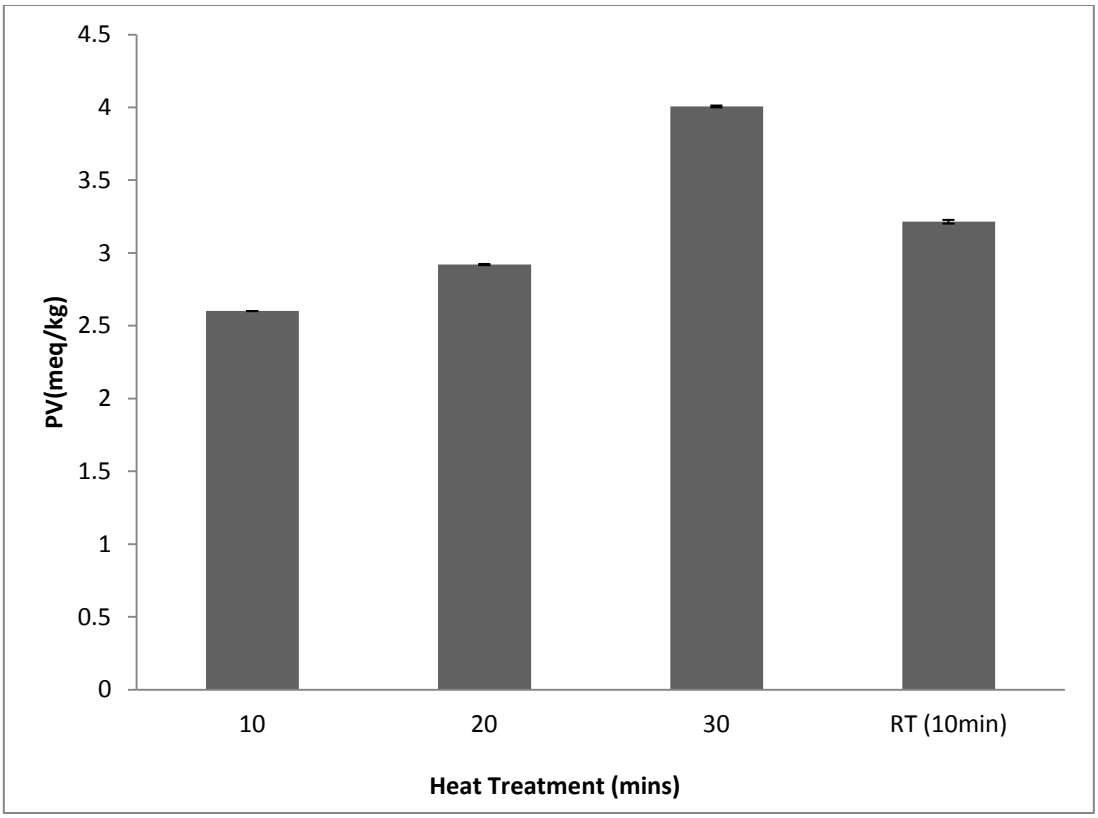

Figure 4.14: Effects of heat treatment on peroxide value of RUSF Samples

4.5 Product Stability and Shelf-life Estimation

The stability of a product is a function of time during which it may deteriorate chemically or undergo microbial spoilage. The rate at which these occur influences the rate of spoilage and consequently how long it may store under given conditions before it becomes unacceptable for human consumption (i.e. shelf life). The samples obtained after pasteurisation were stored at three different temperatures (25, 35 and 45°C) for five weeks. Samples were obtained from each of the storage temperatures and the microbial load, FFA and PV monitored on weekly basis. Figure 4.15 shows the trends of microbial load (log cfu) with storage time at the three different storage temperatures. The rates of microbial growth were different for each of the storage temperatures. For each storage temperature there were

increases in load until week three. Sample stored at 45°C showed a faster growth whilst those stored at 35°C had slower rate of growth throughout the study. There was no obvious peak for the one stored at 35°C whilst the one stored 45˚C peaked between third and fourth week. On the other hand even though there was a rapid growth of micro-organism in sample stored at 25˚C it peaked between weeks two and three and then started to decline.

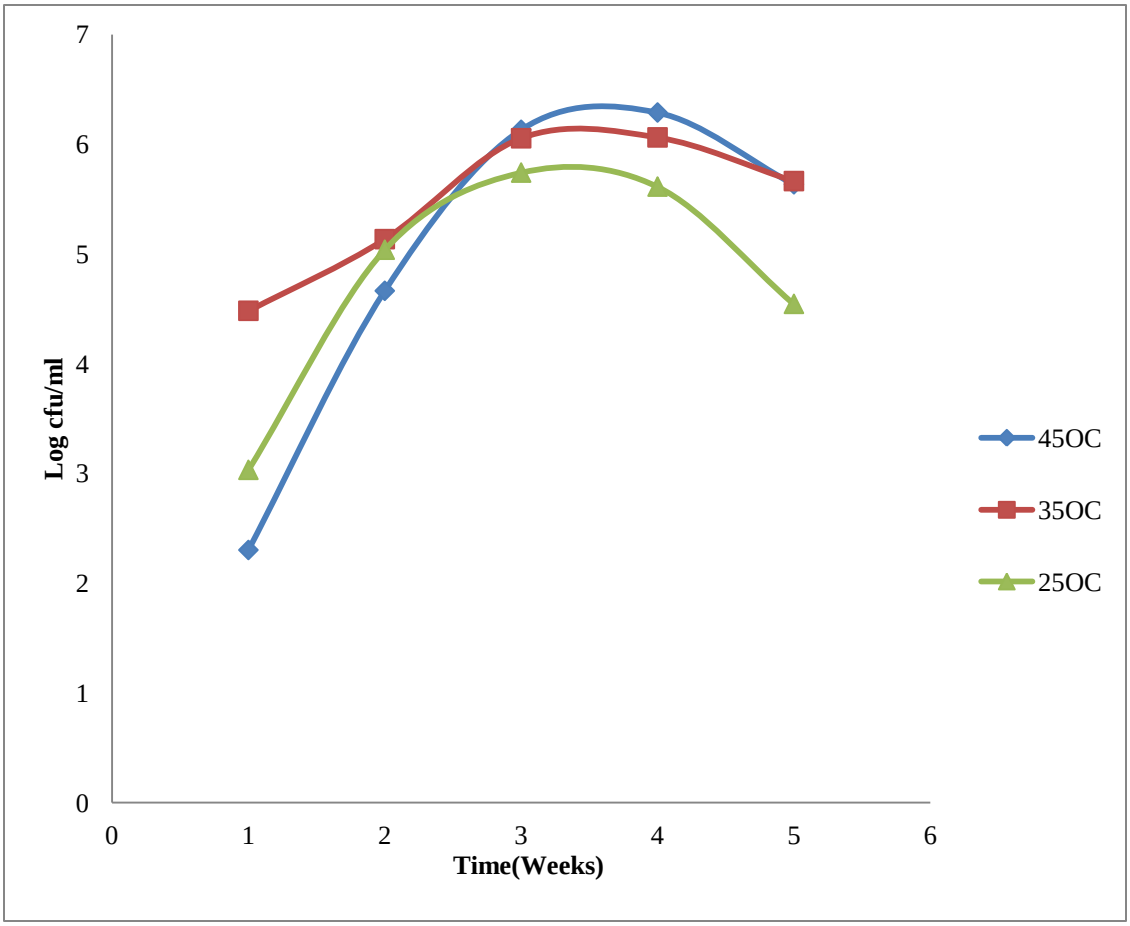

Figure 4.15: Bacteria growth (TVC) in RUSF samples during 5 weeks of storage at 25°C, 35°C and 45°C

4.5.1 Microbial Growth as Indices for Product Stability

The growth of micro-organisms depends on a number of factors of which the medium and environmental factors such as temperature and pH are very vital. In a given medium, different micro-organisms have different temperatures at which the rate of growth is maximum (Prescott *et al*., 2002). In this work the effect of temperature changes on the quality indicators of food (RUSF) were investigated (figure 4.15). Total plate count was high for all the storage temperatures. This was because the growth of most spoilage bacteria (mesophiles) has been found to be within the temperature range of 20°c to 45°c (Hogg, 2005). It was also observed that, though the counts at 25°c were lower than 35°c right from week one (1), they were higher than 45°c for the first two (2) weeks after which the value for 45°c shot up beyond that of 35°c.

In relation to time it was found that the growth of micro-organisms slowed down by the fourth (4^{th}) week. A decline in the growth may be the result of a change in their environmental conditions that requires a re-organisation of their metabolic activities (Prescott *et al*., 2002). With time in a microbial population, the metabolic activities of rapidly growing micro-organisms result in the production of waste which may have toxic effect on the micro-organisms. In addition as micro-organisms continue to multiply and increase in number the competition for nutrients and oxygen increases. In this work the depletion of oxygen may have had the most remarkable effect on microbial growth since the product was bottled and there was a limited source of oxygen to the organisms.

4.5.2 pH and Titratable Acidity

One way in which micro-organisms alter their environmental conditions is to change the pH by the production of organic acids as metabolic wastes. The production of acids was very typical of microbial growth in this work since the pH of the product dropped from 6.7±0.3 to 4.27±0.22. The changes in pH seemed to correlate with the rate of microbial growth at the different storage temperatures. The pH drop was higher for product stored at 35 and 45 storage condition than at 25˚C (figure 4.16).

The trends in pH and titratable acidity were probably influenced by the hydrolysis of the proteins. This is because the hydrolysis produced peptides, carboxylic and amino groups. On the other hand bacteria growth and fermentation of sugars in the product also produced organic acids. The increasing amino groups tend to create a buffer system against changes in pH and consequently there seemed not to be a discerning trend in the pH and titratable acidity with storage time (Figures 4.16 and 4.17).

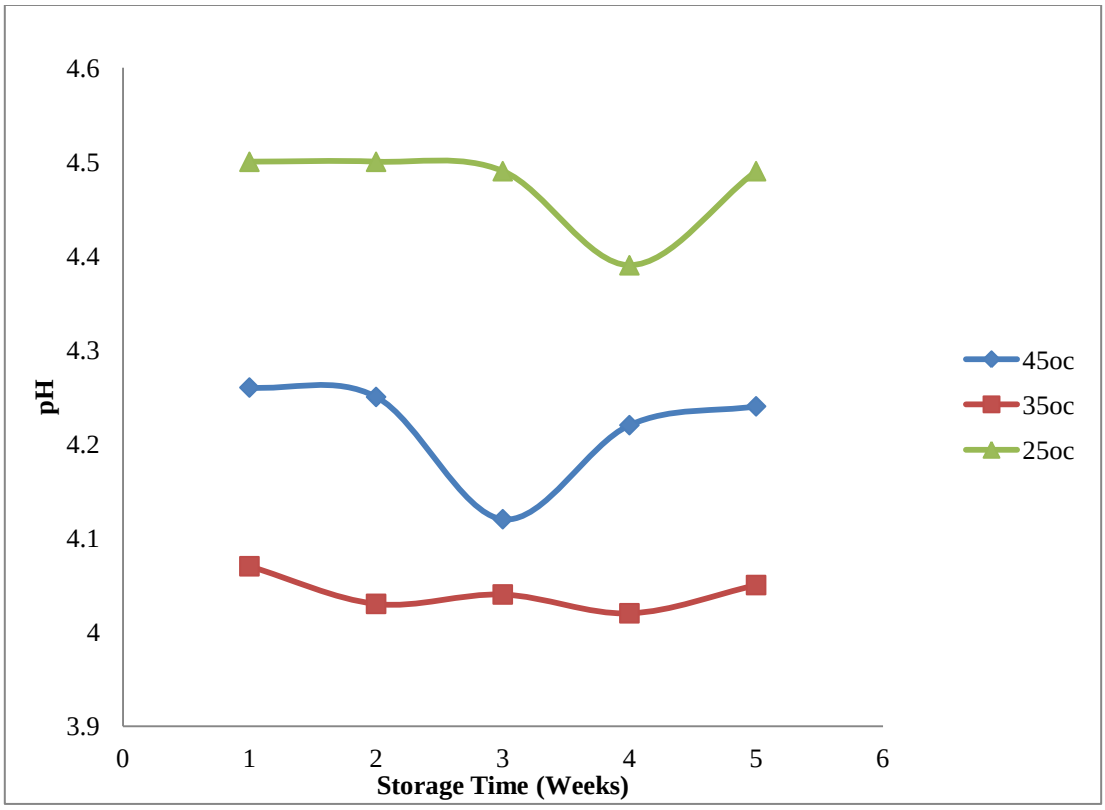

Figure 4.16: Changes in pH of RUSF samples during 5 weeks of storage at 25°C, 35°C and 45°C

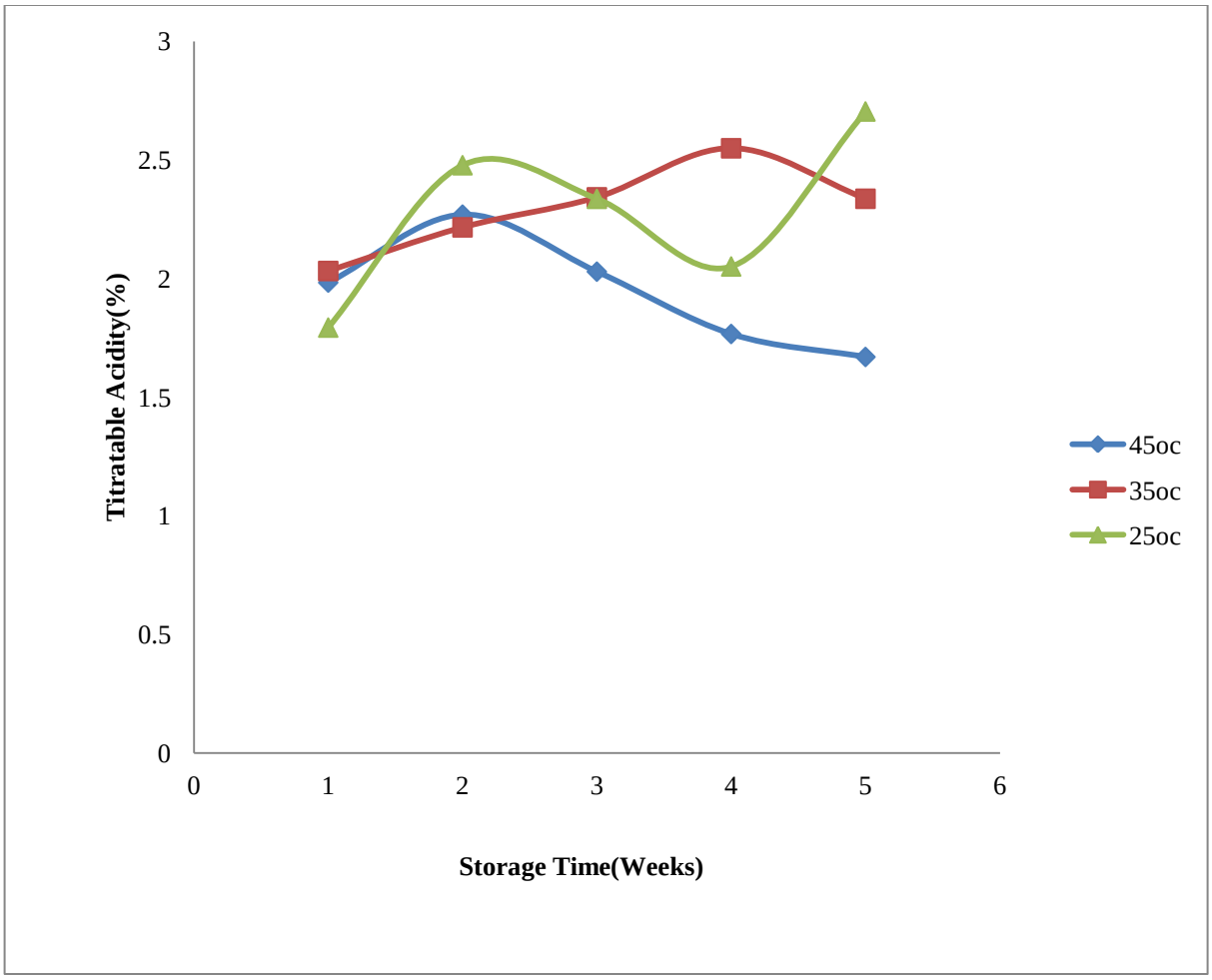

Figure 4.17: Changes in titratable acidity of RUSF samples during 5 weeks of storage at 25°C, 35°C and 45°C

Figure 4.18 shows that there was a general increase in the growth of yeasts and moulds throughout the storage period. This was contrary to what was observed for the bacteria growth in figure 4.15. Yeasts and moulds are known to be more tolerant to acid environment because they are better able to make use of acids (Prescott *et al*., 2002).

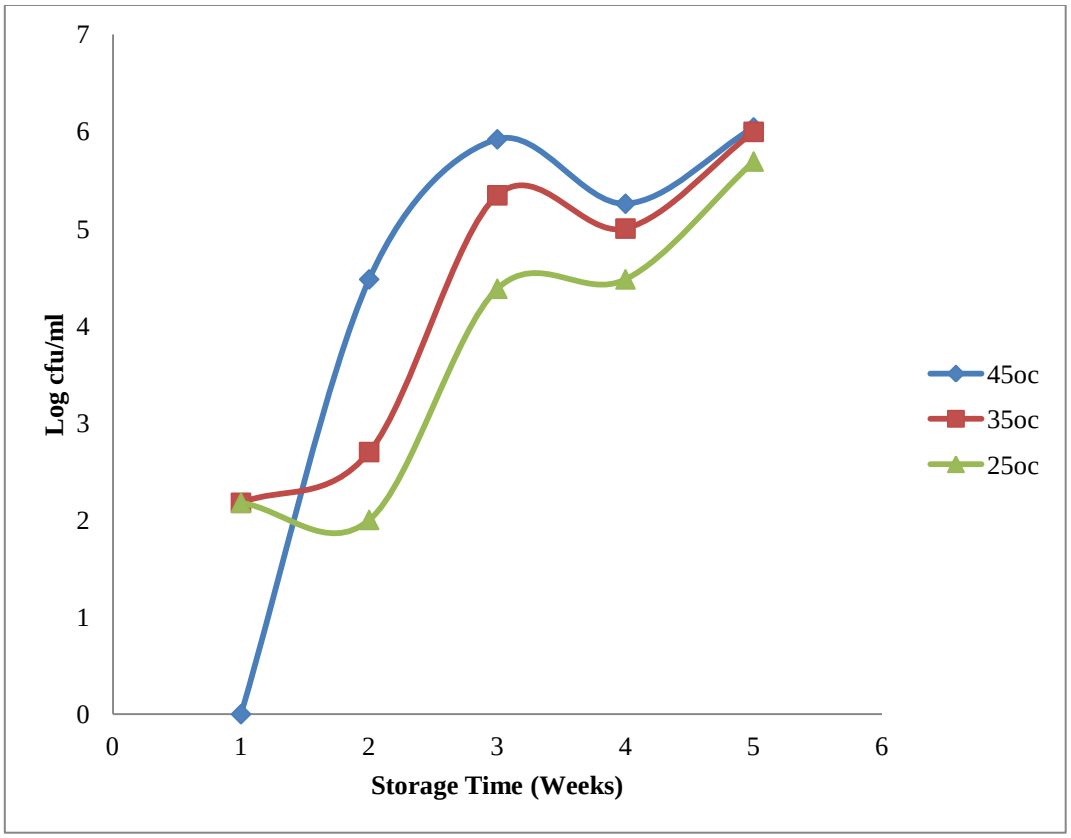

Figure 4.18: Changes in the growth of Yeasts and Moulds Growth in RUSF samples during 5 weeks of storage at 25°C, 35°C and 45°C.

4.5.3 Free Fatty Acids and Peroxide Value as Indices of Product Stability

FFA and PV are frequently monitored as indices of lipid degradation or otherwise. For an oily or fatty product, it is desirable that both indices should remain low to indicate product quality stability under the storage conditions. Figure 4.19 shows a gradual increase in FFA for the first three weeks and a rapid increase thereafter. Even though they followed the same trends, samples stored at 25°C had lower FFA values whilst samples stored at 45°C generally had higher FFA values throughout the storage period. FFAs are a result of triacylglycerols (TAG) hydrolysis especially under aqueous conditions. TAG hydrolysis to FFA was

enhanced by the increasing storage temperature and hence the higher values at 45˚C than at 25°C.

Figure 4.20 shows the trends in peroxide values of the products that were stored at different temperatures. At all three temperatures there was an induction period of three weeks during which there was a gradual increase of PV. The PV values of the samples stored at 45˚C were higher than those stored at 25˚C even during the induction period. Autoxidation of lipids proceed with heat it is therefore the reason why PV increased with increased storage temperature (from 25°C to 45°C). Many other researchers obtained similar results on the exposure fatty products to heat with time. Nieto et al., (2011) for instance observed an increase in oxidation product in their cooked lamb with the storage time and exposure to environmental temperatures. Al-Ismail *et al*., (2007) also observed an increase in rancidity of milk product with storage time and air drying.

For the products stored at 25°C there was increase in the PV up to week 4 after which it levelled off. On the other hand, there were no obvious trends in PV for product stored at 45°C after week three. Hydroperoxides formation in lipids are primary oxidation products that are known to be weak and transient and easily breakdown to secondary oxidation products that are more stable (Sewald and DeVries, 2012). Consequently a decrease in hydroperoxides does not necessarily means a discontinuation of oxidation but rather that the oxidation process is near completion.

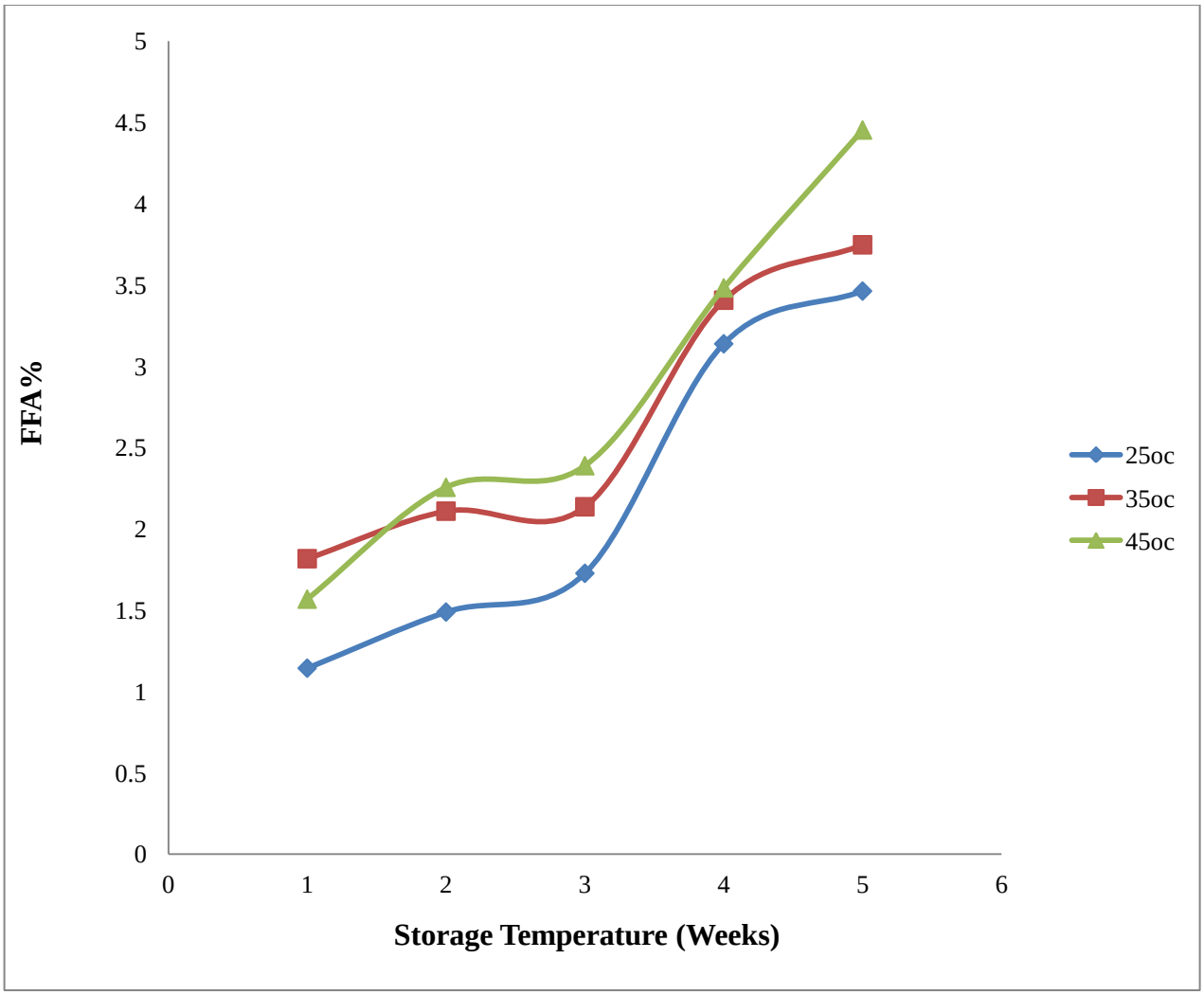

Figure 4.19: Changes in free fatty acids values of RUSF samples during 5 weeks of storage at 25°C, 35°C and 45°C

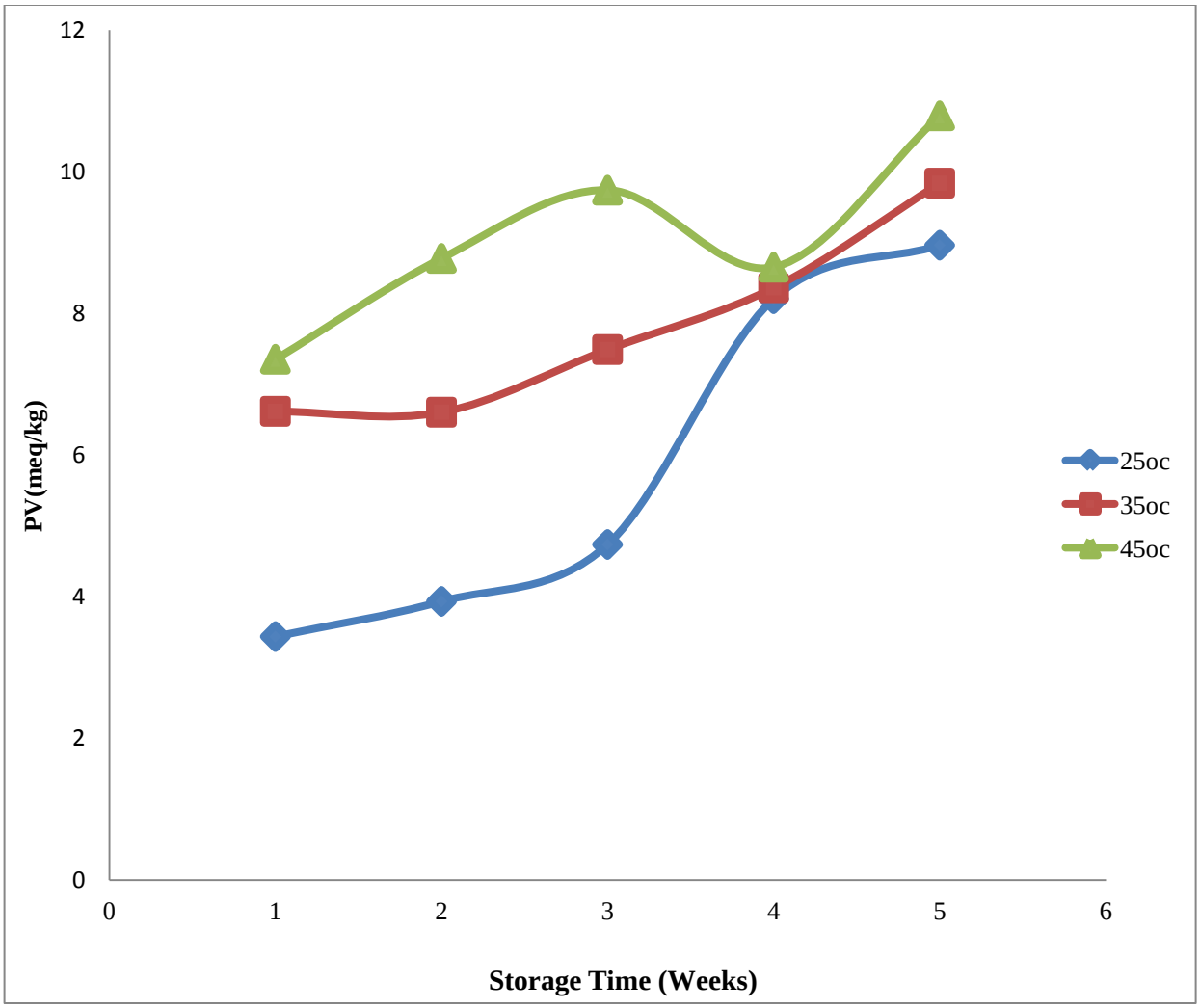

Figure 4.20: Changes in peroxide values of RUSF samples during 5 weeks of storage at 25°C, 35°C and 45°C

4.6 Estimation of Product Shelf-life

Processed foods being biological materials undergo biological and chemical deterioration upon storage. The rate of deterioration influences how long the product maintains its keeping quality under given holding conditions before it becomes unacceptable for human consumption (i.e. shelf life). In order to estimate the shelf life of a product critical quality parameters that indicate product acceptability are monitored under specified conditions. The

monitoring process could be direct or indirect. In the indirect method usually done using accelerated shelf life studies the product is held at elevated temperatures and the product quality parameters (microbial load and lipid oxidation indices, water activity, etc) are monitored over a reasonable period of time. The basis of this method is that product spoilage (whether chemical or biological) are based on three basic mechanisms (chemical reaction kinetics, Q_{10} factor and Arrhenius model) (Fu and Labuza, 1997). Furthermore to estimate the shelf life of a product the product must be well understood and the possible modes of deterioration well explored to establish the most suitable means of spoilage, and determine the mechanism (NZFSA, 2005). Table 4.7 shows the design of shelf life studies done in this work using the indirect method.

Table 4.7 Experimental Design for Shelf Life Studies on the Drinkable RUSF

Method	Indirect(Accelerated)
Desired Shelf Life	6 months
Actual Test Time	5 weeks
Temperatures	25°C , 35°C, 45°C
Microbial	Total Plate count, yeast and moulds, E. coli
Chemical tests	FFA, PV

The modes of deterioration of the products as established in section 4.5.0 were microbial degradation, lipid hydrolysis and oxidation. However as observed in section 4.4.1 the problem of microbial growth was more due to inadequate heat process schedule which could easily be solved by the use of better processing equipment. On the other hand product deterioration due to lipid hydrolysis and oxidation is a more intrinsic problem that is not easily solvable by mere change of equipment. Consequently the shelf life estimation was

done based on lipid hydrolysis (FFA) as a measure of chemical deterioration and the onset of rancidity. The FFA values were therefore used as the most suitable mode of quality deterioration for the estimation of the shelf life of the product. Based on the observation made in section 4.5.3 that high lipid degradation reactions occurred at elevated temperatures (35°C and 45°C), the effect of temperature on lipid hydrolysis (FFA production) was used as the mechanism of deterioration. The shelf life estimation of the product was therefore based on the time before the surge in FFA values. The Arrhenius theory was used in the predictive modelling of shelf life.

The Arrhenius equation is given as follows

$K = K_0 e^{-Ea/RT}$ where,

K: Rate of reaction constant

Ea: the activation energy in cal/mol

R: the gas constant in cal/mol

T: the temperature in °K (°C +273)

The Arrhenius equation can be linearised into a log form as follows

$Ln(K) = -Ea/R \cdot 1/T + ln(Ea)$

The FFA data obtained from the samples stored at the three different temperatures were fitted into the linearised Arrhenius model as presented in figure 4.21. The slope of the curves in the figure shows that the degradation rate i.e. FFA hydrolysis was steeper in samples stored at 45°C and least for samples stored at 25°C. Based on figure 4.19 the FFA values rapidly increased after the third week. Consequently three weeks was used as the maximum acceptable storage period under accelerated storage conditions.

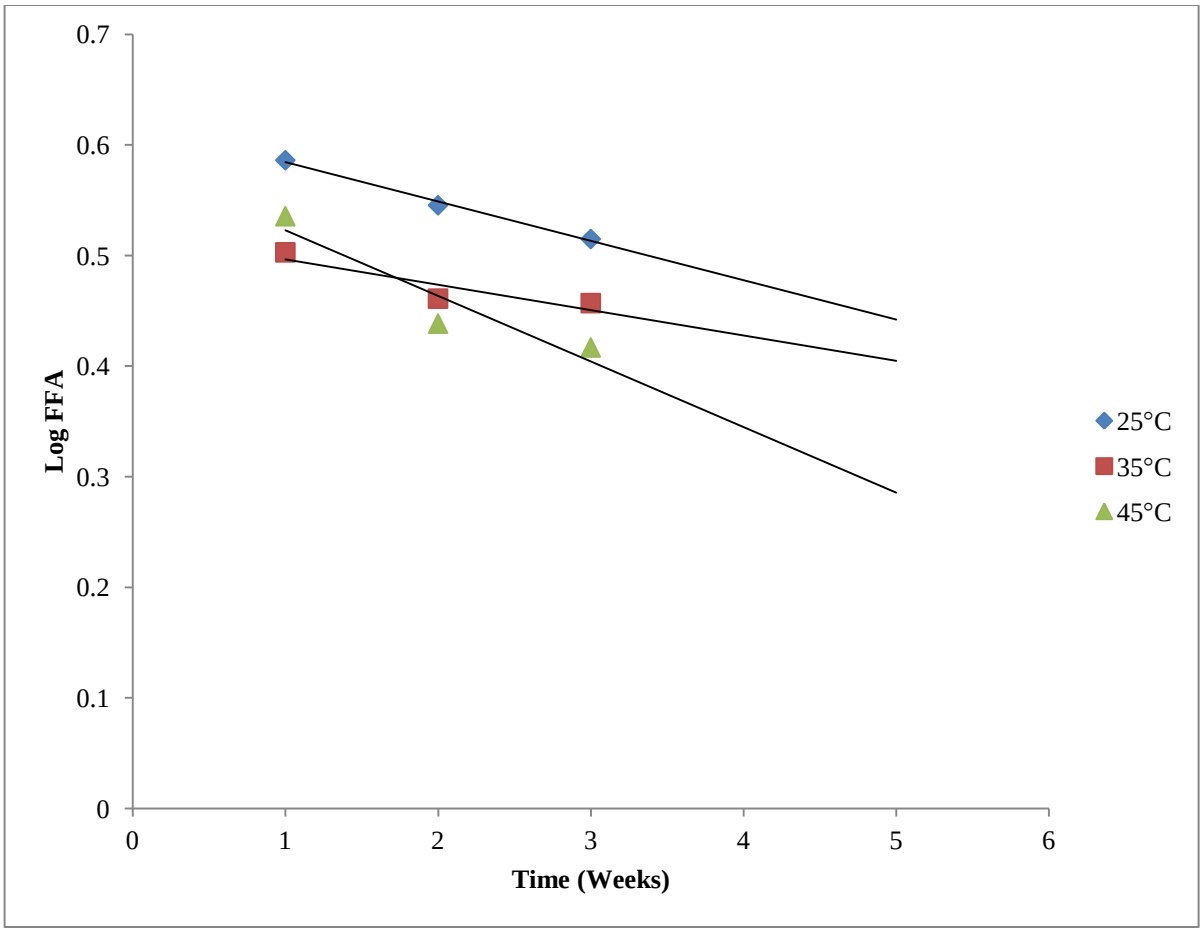

Figure 4.21: Effect of storage time on the rate of deterioration of fat (log FFA).

Table 4.8 Degradation Rate Models for RUSF at Different Storage Temperatures

Storage Temperature(°C)	Degradation Rate Equation	R^2
25	Y= -0.0356x+0.6201	0.881
35	Y=-0.023x+0.5195	0.8158
45	Y=-0.0593x+0.5822	0.9932

Where Y is the log of FFA and X is time in weeks

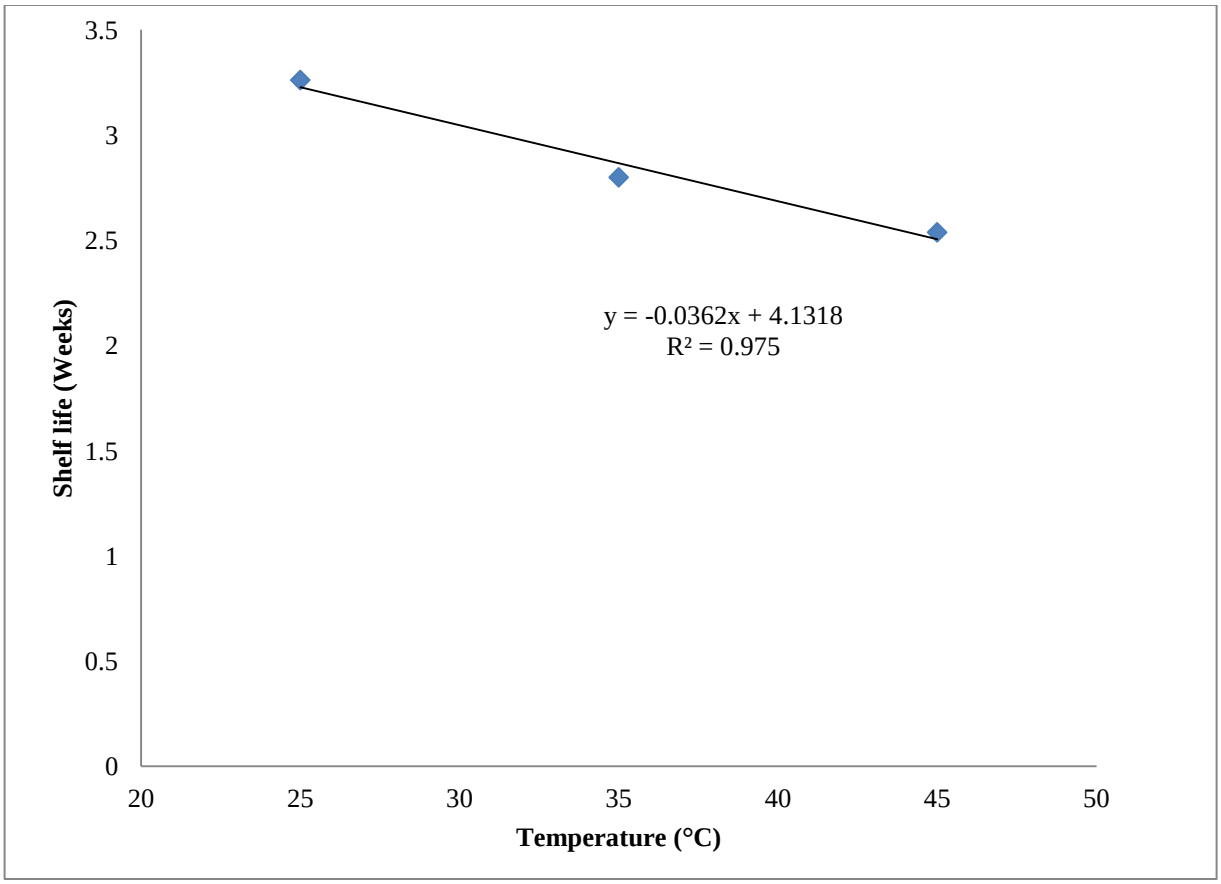

Figure 4.22: Predictive shelf life of RUSF as a function of storage temperature based on the Arrhenius model

Table 4.9: Predicted Shelf Life of RUSF as a Function of Temperature

Storage Temperature (°C)	Shelf Life (days)
25	22
35	20
45	18

Based on the model derived from Figure 4.22 the shelf-life of the product can be estimated for each temperature as presented in Table 4.9. The product stored at lower temperature (25°C) had a higher shelf life (22days) while those stored at higher temperature had a lower

shelf life (18 days). For the product to store longer than three weeks therefore refrigeration storage conditions need to be explored.

CHAPTER FIVE

5. 0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The RUSF samples made up of small particle sizes (≤106µ) had faster settling rate as compared to larger particle sizes (≤ 300µ and ≤500µ) indicating that the effect of particle sizes on product separation was based on the density differential of the particles and the fluid.

The viscosity of the fluid had a significant effect on product separation rate and the increase in the viscosity using different stabilizers reduced the settling rate of the drinkable RUSF. Of the three different stabilizers used, xanthan gum proved to be the best stabilizer and its application in the product greatly slowed down the separation rate and provided some measure of physical stability to the product.

Enzyme hydrolysis of the macromolecules (starch and proteins) was extensive as monitored by the increased amounts of reducing sugars, and amino groups (reaction with TNBSA).

Maximal hydrolysis of starches by alpha amylase occurred after 1 hour at 50°C while that of proteins by bromelain peaked after 2hours at 43°C. Beyond 2 hours, plastein-like reactions occurred to reduce the concentration of free amino ends. Reformation of peptides as was observable on the SDS-PAGE zymogram of 3hours hydrolysates gave credence to the possibility of Plastein reactions under the incubation conditions.

Stabilisation of the product by thermal processing using pasteurisation or sterilisation techniques proved inadequate as determined by microbial count (TVC). Pasteurisation at 95°C for 30minutes or retorting at 119°C for 10minutes still showed microbial count. Heat

penetration data from both pasteurization and sterilization processes showed that thermal processing was inadequate to assure microbial stability of the product.

Accelerated shelf life studies using lipid hydrolysis indicator of FFA showed the product to be stable at room temperature (25°C) for 22days. Practically however, the product shelf stability did not go beyond three days at ambient conditions due to microbial spoilage. Heat penetration data from both pasteurization and sterilization processes confirmed that thermal processing was inadequate to assure microbial stability of the product.

5.2 Recommendations

- Options, such as the use of chemical preservatives and other physical methods to improve the microbiological stability of the product need to be explored.
- Alternative means of packaging may be considered if thermal processing of the product will be continued in order to explore more severe thermal process schedules.
- Alternative means of processing e.g. UHT processing, extrusion into cakes or biscuits could be considered in order to change the state of the product thus eliminating the effects of water activity in enhancing microbial growth.
- Higher temperatures for bromelain hydrolysis and longer times for amylase hydrolysis could be considered to maximize the activity of the enzymes in further research.
- Further work on plastein reactions and the reformation of peptide bonds during incubation of protein hydrolyzates with bromelain need to be carried out to take advantage of this phenomenon to improve the functional and nutritional quality of high protein foods.

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APPENDICES

Appendix 1: Analysis of Variance (ANOVA) Table for Degree of Starch Hydrolysis

Source	DF	SS	Mean Square	F -Value	Pr > F
Temp	2	181.9863111	90.9931556	50.33	<.0001
Time	2	643.1525333	321.5762667	177.87	<.0001
Concentration	2	7.3381333	3.6690667	2.03	0.1510
Temp*Time	4	82.3246222	20.5811556	11.38	<.0001
Temp*Concentration	4	90.8035556	22.7008889	12.56	<.0001
Time*Concentration	4	2.7050667	0.6762667	0.37	0.8250
Temp*Time*Concentration	8	89.2039111	11.1504889	6.17	0.0002

Appendix 2: ANOVA Table for Degree of Protein Hydrolysis

Source	DF	SS	Mean Square	F Value	Pr > F
Temp	2	8.35318412	4.17659206	30.95	<.0001
Time	2	4.61869927	2.30934964	17.11	<.0001
Conc	2	5.12500198	2.56250099	18.99	<.0001
Temp*Time	4	17.46599747	4.36649937	32.36	<.0001
Temp*Conc	4	21.66291865	5.41572966	40.13	<.0001
Time*Conc	4	14.95476322	3.73869081	27.70	<.0001
Temp*Time*Conc	8	13.90152361	1.73769045	12.88	<.0001

Appendix 3: ANOVA Table for Particle size

Source	DF	SS	Mean Square	F Value	Pr > F
Particle size	2	30.175658	15.087829	143.33	<.0001
Holding Time	6	1044.322511	174.053752	1653.51	<.0001
Particle size* Holding Time	11	18.476190	1.679654	15.96	<.0001

Appendix 4: Viscosities of Samples Using Different Stabilizers

Stabilizer	RPM					
	100	60	50	20	10	5
No Stabilizer	97.1	104.4	114.1333	205.5	216.1	228.5667
0.05%CMC	115.22	130.92	137.09	210.7	207.8	293.33
0.075%CMC	131.37	155.56	163.92	256.86	363.72	489.58
0.1%CMC	131.37	155.56	163.92	256.86	363.72	489.58
0.05%Carrageenan	109.76	140.5	130.5	187.88	266.98	387.17
0.075%Carrageenan	108.49	135.76	131.27	208.8	353.75	465.05
0.1%Carrageenan	117.18	143.14	152.69	244	352.1	500.27
0.05%Xanthan gum	122.33	141.16	144.69	227.62	330.13	438.45
0.075%Xanthan gum	120.02	144.62	150.44	241.13	351.31	519.1
0.1%Xanthan gum	141.45	148.41	154.78	240	313.15	440.42

Appendix 5: ANOVA Table for Stabilizer Treated Samples

Source	DF	SS	Mean Square	F Value	Pr > F
Stabilizer (A)	2	45.107143	22.553571	51.90	<.0001
Concentration (B)	2	95.583333	47.791667	109.99	<.0001
Holding Time (C)	6	1115.686508	185.947751	427.93	<.0001
A*B	4	5.166667	1.291667	2.97	0.0259
A*C	12	25.503968	2.125331	4.89	<.0001
B*C	12	38.111111	3.175926	7.31	<.0001
A*B*C	24	17.055556	0.710648	1.64	0.0618

Appendix 6: Values for Microbial Growth, pH and Titratable Acidity at Different Pasteurisation Time, Storage temperature and Storage Time.

Pasteurisation Time (min)	Storage Temperature (°C)	Storage Time (weeks)	Replicate	pH	Titratable Acidity	TVC1	E. coli	Yeast and Molds
10	25	1	1	4.44	1.90609	100	0	0
10	25	1	2	4.48	2.07544	100	0	0
10	25	2	1	4.5	1.44128	340	0	0
10	25	2	2	4.42	1.51424	240	0	0
10	25	3	1	4.34	1.98987	160	0	16
10	25	3	2	4.42	1.58901	164	0	36
10	25	4	1	4.8	1.16744	84	0	20
10	25	4	2	4.86	1.37012	108	0	4
10	25	5	1	4.7	4.1653	0	0	92
10	25	5	2	4.76	3.92388	0	0	140
10	35	1	1	4.23	3.24288	416	0	12
10	35	1	2	4.43	2.82491	360	0	0
10	35	2	1	4.1	2.9267	20	4	0
10	35	2	2	3.94	3.24288	16	0	0
10	35	3	1	4.05	1.16744	92	0	16
10	35	3	2	4.13	1.16744	96	0	8
10	35	4	1	4.1	2.07544	100	12	0
10	35	4	2	4.2	2.252	148	4	4
10	35	5	1	4.14	2.53035	92	20	128
10	35	5	2	4.22	2.43576	60	16	160
10	45	1	1	4.2	1.44128	0	0	0
10	45	1	2	4.02	1.74395	0	0	0
10	45	2	1	4.05	1.90609	480	0	56
10	45	2	2	4.13	1.82412	560	0	48
10	45	3	1	4.03	1.98987	100	16	116
10	45	3	2	4.03	1.90609	184	4	80
10	45	4	1	4	1.58901	300	0	32
10	45	4	2	4.1	1.82412	308	0	24
10	45	5	1	4.3	1.44128	108	48	72
10	45	5	2	4.32	1.51424	84	32	92
20	25	1	1	4.3	1.10348	0	0	0
20	25	1	2	4.5	0.98097	0	0	0

[1] These are raw values. They are multiplied by 10 to arrive at the colony forming units of TVC, E. *coli*, yeast and mould per ml.

20	25	2	1	4.35	2.9267	28	0	0
20	25	2	2	4.65	3.13568	32	0	0
20	25	3	1	4.5	2.82491	8	0	4
20	25	3	2	4.5	2.72492	48	0	0
20	25	4	1	4.3	2.62673	16	0	0
20	25	4	2	4.46	2.9267	12	0	0
20	25	5	1	4.33	2.43576	0	0	0
20	25	5	2	4.39	2.07544	0	0	4
20	35	1	1	4.05	1.66558	292	0	0
20	35	1	2	3.91	1.82412	336	0	0
20	35	2	1	4.2	2.07544	8	12	0
20	35	2	2	3.86	1.82412	0	4	0
20	35	3	1	4.05	2.53035	92	0	12
20	35	3	2	4.01	2.43576	140	0	36
20	35	4	1	4.03	2.34298	56	0	4
20	35	4	2	4.09	1.98987	120	0	0
20	35	5	1	4	1.66558	12	0	72
20	35	5	2	4.08	1.82412	8	0	68
20	45	1	1	4.8	2.07544	0	0	0
20	45	1	2	4.84	1.82412	0	0	0
20	45	2	1	4.2	1.37012	200	0	28
20	45	2	2	4.3	1.30076	208	0	52
20	45	3	1	3.95	2.53035	156	4	172
20	45	3	2	4.05	2.53035	188	0	152
20	45	4	1	4.15	1.37012	172	0	20
20	45	4	2	4.35	1.30076	164	0	12
20	45	5	1	4.31	1.98987	36	0	92
20	45	5	2	4.33	1.74395	8	0	52
30	25	1	1	4.4	1.51424	172	0	0
30	25	1	2	4.46	1.51424	128	0	0
30	25	2	1	4.4	3.57528	32	0	0
30	25	2	2	4.52	3.24288	48	0	0
30	25	3	1	4.5	2.252	20	0	4
30	25	3	2	4.56	2.53035	40	0	12
30	25	4	1	4	1.58901	24	0	0
30	25	4	2	3.88	1.66558	20	0	0
30	25	5	1	4.4	2.07544	4	0	4
30	25	5	2	4.6	1.98987	0	0	16

30	35	1	1	4	1.37012	8	0	0
30	35	1	2	3.84	1.51424	8	0	0
30	35	2	1	4.01	1.10348	12	8	0
30	35	2	2	3.97	1.10348	8	12	0
30	35	3	1	4.53	2.53035	112	8	4
30	35	3	2	4.59	2.53035	24	0	0
30	35	4	1	3.92	2.07544	124	0	0
30	35	4	2	3.98	2.16282	100	0	0
30	35	5	1	4.06	1.98987	20	20	116
30	35	5	2	4.02	1.74395	12	40	128
30	45	1	1	4	1.51424	0	0	0
30	45	1	2	4.36	1.51424	4	0	0
30	45	2	1	4.24	3.57528	160	0	40
30	45	2	2	4.28	2.9267	220	0	20
30	45	3	1	4.1	1.30076	192	0	28
30	45	3	2	4.18	1.37012	200	0	36
30	45	4	1	4.15	2.252	176	0	0
30	45	4	2	4.05	2.53035	200	0	0
30	45	5	1	4.03	1.66558	28	8	112
30	45	5	2	4.13	1.58901	20	0	180
40	25	1	1	4.56	2.62673	200	0	8
40	25	1	2	4.5	2.62673	160	0	4
40	25	2	1	4.52	1.82412	108	0	0
40	25	2	2	4.64	2.16282	44	0	0
40	25	3	1	5.52	2.34298	0	0	68
40	25	3	2	3.6	2.43576	0	0	52
40	25	4	1	4.41	2.43576	44	0	0
40	25	4	2	4.43	2.62673	24	0	0
40	25	5	1	4.2	2.62673	8	0	92
40	25	5	2	4.6	2.34298	16	0	48
40	35	1	1	4.02	1.82412	508	0	0
40	35	1	2	4.1	1.98987	492	0	0
40	35	2	1	4.03	2.62673	464	0	0
40	35	2	2	4.11	2.82491	560	0	4
40	35	3	1	4.06	3.13568	192	0	100
40	35	3	2	4.12	3.24288	160	0	0
40	35	4	1	4	3.80588	112	24	48
40	35	4	2	3.9	3.68968	164	4	28

40	35	5	1	3.9	2.9267	100	40	60
40	35	5	2	4	3.57528	64	76	60
40	45	1	1	4.01	2.82491	12	0	0
40	45	1	2	3.89	2.9267	0	0	0
40	45	2	1	4.4	2.62673	224	0	0
40	45	2	2	4.46	2.62673	240	0	0
40	45	3	1	4.3	2.53035	48	12	28
40	45	3	2	4.32	2.07544	12	4	52
40	45	4	1	4.44	1.44128	112	0	24
40	45	4	2	4.52	1.82412	120	0	32
40	45	5	1	4.2	1.58901	36	4	128
40	45	5	2	4.26	1.82412	28	12	152

Appendix 7: ANOVA Table for pH

Source	DF	SS	Mean Square	F Value	Pr > F
Pasteurisation Time (A)	3	0.21121000	0.07040333	1.79	0.1586
Storage Temperature (B)	2	3.61410667	1.80705333	45.96	<.0001
Storage Time (C)	4	0.06312000	0.01578000	0.40	0.8069
A*B	6	0.45560000	0.07593333	1.93	0.0903
A*C	12	0.76304000	0.06358667	1.62	0.1110
B*C	8	0.12446000	0.01555750	0.40	0.9187
A*B*C	24	1.32690000	0.05528750	1.41	0.1439

Appendix 8: ANOVA Table for Titratable Acidity

Source	DF	SS	Mean Square	F Value	Pr > F
Pasteurisation Time (A)	3	5.32005308	1.77335103	62.33	<.0001
Storage Temperature (B)	2	3.09896179	1.54948090	54.46	<.0001
Storage Time (C)	4	2.12654363	0.53163591	18.69	<.0001
A*B	6	4.51656171	0.75276028	26.46	<.0001
A*C	12	9.29712548	0.77476046	27.23	<.0001
B*C	8	4.88889057	0.61111132	21.48	<.0001
A*B*C	24	25.63676168	1.06819840	37.54	<.0001

Appendix 9: ANOVA Table for TVC

Source	DF	SS	Mean Square	F Value	Pr > F
Pasteurisation Time (A)	3	183775.4667	61258.4889	90.76	<.0001
Storage Temperature (B)	2	148824.2667	74412.1333	110.25	<.0001
Storage Time (C)	4	278139.4667	69534.8667	103.02	<.0001
A*B	6	285690.9333	47615.1556	70.55	<.0001
A*C	12	196127.2000	16343.9333	24.22	<.0001
B*C	8	498995.7333	62374.4667	92.42	<.0001
A*B*C	24	394254.4000	16427.2667	24.34	<.0001

Appendix 10: ANOVA Table for E. Coli

Source	DF	SS	Mean Square	F Value	Pr > F
Pasteurisation Time (A)	3	491.733333	163.911111	6.68	0.0006
Storage Temperature (B)	2	1156.266667	578.133333	23.57	<.0001
Storage Time (C)	4	2731.466667	682.866667	27.83	<.0001
A*B	6	977.066667	162.844444	6.64	<.0001
A*C	12	1429.600000	119.133333	4.86	<.0001
B*C	8	2059.733333	257.466667	10.49	<.0001
A*B*C	24	3241.600000	135.066667	5.51	<.0001

Appendix 11: ANOVA Table for Yeast and Mould

Source	DF	SS	Mean Square	F Value	Pr > F
Pasteurisation Time (A)	3	4251.2000	1417.0667	5.94	0.0013
Storage Temperature (B)	2	22120.8000	11060.4000	46.39	<.0001
Storage Time (C)	4	118356.5333	29589.1333	124.12	<.0001
A*B	6	6090.4000	1015.0667	4.26	0.0012
A*C	12	19755.4667	1646.2889	6.91	<.0001
B*C	8	19505.8667	2438.2333	10.23	<.0001
A*B*C	24	40762.9333	1698.4556	7.12	<.0001

Appendix 12: Values of FFA PV at Different Pasteurisation Time, Storage temperature and Storage Time.

Pasteurisation Temperature (°C)	Storage Temperature (°C)	Storage time (weeks)	Replicate	FFA	PV
10	25	1	1	1.4651	2.9567
10	25	1	2	1.4588	2.9876
10	25	2	1	1.7468	6.1889
10	25	2	2	1.7484	6.1969
10	25	3	1	2.6905	7.5901
10	25	3	2	2.6891	7.5932
10	25	4	1	3.3419	8.1252
10	25	4	2	3.3833	8.1894
10	25	5	1	2.4255	9.605
10	25	5	2	2.4261	9.8456
10	35	1	1	1.4575	5.3654
10	35	1	2	1.4598	5.3391
10	35	2	1	2.0268	5.653
10	35	2	2	2.0249	5.5855
10	35	3	1	2.7519	7.1552
10	35	3	2	2.78043	7.1894
10	35	4	1	3.4373	8.321
10	35	4	2	3.4356	8.34
10	35	5	1	2.4255	9.1245
10	35	5	2	2.4261	9.2071
10	45	1	1	1.7361	5.3654
10	45	1	2	1.7484	5.3391
10	45	2	1	2.143	7.2057
10	45	2	2	2.1419	7.182
10	45	3	1	2.1112	7.1562
10	45	3	2	2.158	7.1479
10	45	4	1	3.1392	6.9562
10	45	4	2	3.1574	6.9479
10	45	5	1	2.3986	10.4997
10	45	5	2	2.4005	10.3855
20	25	1	1	1.564	3.18
20	25	1	2	1.5726	3.173
20	25	2	1	1.7765	6.2875
20	25	2	2	1.7632	6.2894
20	25	3	1	2.7437	7.8511

20	25	3	2	2.7423	7.8266
20	25	4	1	3.5274	8.7614
20	25	4	2	3.5532	8.7423
20	25	5	1	2.4663	9.454
20	25	5	2	2.44789	9.3967
20	35	1	1	1.7935	5.578
20	35	1	2	1.6769	5.9878
20	35	2	1	2.2182	6.1882
20	35	2	2	2.2158	6.1845
20	35	3	1	2.4856	7.4875
20	35	3	2	2.4315	7.6755
20	35	4	1	3.485	8.7475
20	35	4	2	3.4825	8.7155
20	35	5	1	2.3892	10.0123
20	35	5	2	2.389	10.1987
20	45	1	1	1.5058	6.9493
20	45	1	2	1.5108	6.9721
20	45	2	1	2.2268	7.1835
20	45	2	2	2.233	7.1917
20	45	3	1	2.2474	7.2235
20	45	3	2	2.2445	7.3517
20	45	4	1	3.1474	7.5255
20	45	4	2	3.1405	7.5517
20	45	5	1	2.5226	10.9671
20	45	5	2	2.5161	10.9813
30	25	1	1	1.5172	4.5954
30	25	1	2	1.5163	4.59
30	25	2	1	1.7956	6.4694
30	25	2	2	1.7996	6.4514
30	25	3	1	2.858	8.0767
30	25	3	2	2.8532	7.9984
30	25	4	1	3.6612	9.7406
30	25	4	2	3.651	9.7619
30	25	5	1	2.5823	9.5566
30	25	5	2	2.5939	9.6666
30	35	1	1	1.6769	5.9878
30	35	1	2	1.6742	5.9075
30	35	2	1	2.2922	6.6876

30	35	2	2	2.3213	6.6354
30	35	3	1	2.5897	7.6936
30	35	3	2	2.5888	7.7242
30	35	4	1	3.4825	8.7155
30	35	4	2	3.5397	9.1926
30	35	5	1	2.399	10.5676
30	35	5	2	2.3895	10.6889
30	45	1	1	1.621	7.3493
30	45	1	2	1.627	7.3602
30	45	2	1	2.2922	6.6876
30	45	2	2	2.3213	6.6354
30	45	3	1	2.2444	7.7062
30	45	3	2	2.2568	7.6962
30	45	4	1	3.2334	8.7562
30	45	4	2	3.2378	8.7562
30	45	5	1	2.5926	11.7965
30	45	5	2	2.5879	11.7929
40	25	1	1	1.409	3.5978
40	25	1	2	1.4332	3.596
40	25	2	1	1.691	6.6275
40	25	2	2	1.695	6.6421
40	25	3	1	2.501	6.596
40	25	3	2	2.494	6.5993
40	25	4	1	3.2937	7.7899
40	25	4	2	3.2992	7.7542
40	25	5	1	2.3645	7.7001
40	25	5	2	2.3624	7.5878
40	35	1	1	1.6748	6.9492
40	35	1	2	1.6861	6.9993
40	35	2	1	2.0598	5.1772
40	35	2	2	2.0671	5.186
40	35	3	1	2.2515	7.4993
40	35	3	2	2.2504	7.4842
40	35	4	1	3.2465	7.3793
40	35	4	2	3.2414	7.3742
40	35	5	1	2.3201	8.9001
40	35	5	2	2.294	8.8822
40	45	1	1	1.6814	7.9302

40	45	1	2	1.6841	7.942
40	45	2	1	1.9644	9.0785
40	45	2	2	1.9671	9.0184
40	45	3	1	2.0431	9.073
40	45	3	2	2.0427	9.1855
40	45	4	1	3.0175	9.573
40	45	4	2	3.0341	9.5855
40	45	5	1	2.2976	9.9404
40	45	5	2	2.3089	9.9483

Appendix 13: ANOVA Table for FFA

Source	DF	SS	Mean Square	F Value	Pr > F
Pasteurisation Time (A)	3	0.96425795	0.32141932	652.47	<.0001
Storage Temperature (B)	2	6.97562002	3.48781001	7080.10	<.0001
Storage Time (C)	4	92.85365675	23.21341419	47122.2	<.0001
A*B	6	0.05529211	0.00921535	18.71	<.0001
A*C	12	0.27277348	0.02273112	46.14	<.0001
B*C	8	2.92311819	0.36538977	741.73	<.0001
A*B*C	24	0.44862985	0.01869291	37.95	<.0001

Appendix 14: ANOVA Table for PV

Source	DF	SS	Mean Square	F Value	Pr > F
Pasteurisation Time (A)	3	16.1171339	5.3723780	2823.01	<.0001
Storage Temperature (B)	2	208.5032246	104.2516123	54780.9	<.0001
Storage Time (C)	4	250.3641765	62.5910441	32889.6	<.0001
A*B	6	3.1207495	0.5201249	273.31	<.0001
A*C	12	11.5664477	0.9638706	506.48	<.0001
B*C	8	69.6502363	8.7062795	4574.87	<.0001
A*B*C	24	17.6058904	0.7335788	385.47	<.0001

Appendix 15: L-leucine Standards for TNBSA

Concentration	Absorbance
0.25	0.84
0.25	0.868
0.5	1.314
0.5	1.189
0.75	1.454
0.75	1.506
1	1.485
1	1.518