KEEPING QUALITY OF YOGHURT IN STORAGE AS INFLUENCED BY INCLUSION OF BLACK CUMIN (*Nigella sativa)* SEED OIL EXTRACTS

BY

HUSSAIN, Yunus Rahmah
MTech/SAAT/2017/7210

DEPARTMENT OF ANIMAL PRODUCTION
SCHOOL OF AGRICULTURE AND AGRICULTURAL TECHNOLOGY
FEDERAL UNIVERSITY OF TECHNOLOGY
MINNA, NIGER STATE.

OCTOBER, 2021

ABSTRACT

As a result of negative and potential life-threatening side effects of chemical preservatives used in dairy products, it is worthwhile to seek alternative toxic free preservatives from natural sources for diary food products. As such, phytochemical constituents of *Nigella sativa* seed oil extract (*NSOE*) were determined before being utilized in the preservation of yoghurt produced at concentrations of (T2)2, (T3)4, (T4)6, (T5)8, and (T6)10 % and (T1) 0.1 % of Sodium benzoate which served as control in their respective treated groups. The yoghurt samples were refrigerated for 42 days and their physical, chemical and microbial and sensory properties were evaluated at 7 days' interval. The *Nigella sativa* oil extract revealed the presence of phenols, flavonoids, tannins, terpenoids, saponins, alkaloids, reducing sugar, anthraquinones, cardiac glycosides and steroids while protein was absent. Quantitatively, tannins (17.05 ± 0.24 mg/g) were relatively highest phytoconstituents while alkaloids (45.77 ± 0.56 µg/g) and saponins (29.37 ± 5.06 µg/g) were in minor quantities. A significant ($p< 0.01$) difference was observed in the mean pH of the yoghurt samples with T5 and T6 (8 and 10 % v/v NSOE infused) having the lowest mean pH value (3.55) and the highest pH value(4.61) was observed for yoghurt included with 6 % v/v NSOE. The mean of the titratable acidity of the yoghurt is significantly ($p< 0.01$) affected. A significant ($p< 0.01$) difference was observed in the mean of non-fat solid values of the yoghurt samples with T1 (0.1% Benzoic acid), having the lowest value of 29.35, the highest non-fat solid values were observed for yoghurt infused with 10% v/v *NSOE* (33.22). Also There is no significant ($p>0.01$) difference in the total solid mean values of (NSOE) infused yoghurt no significant ($p>0.01$) difference in the viscosity mean of the yoghurt samples. The moisture content mean value of T1 (0.1% Benzoic acid infused) have the highest value of 68.28 as compared to T6 (63.02). The mean fat content value of the yoghurt samples with T6 having the highest value (3.68), the lowest was observed in T1 (2.12). there was a significant difference ($P< 0.01$) for the values of crude protein highest was observed in T6 (7.76). Significant ($p< 0.01$) differences were observed in the mean value of the ash content of the all the yoghurt samples (T1 - T6). The mean value of bacteria count for the control (0.1% Benzoic acid) is 27.14 cfu/mL which is the highest as compared to T6 having the lowest value 13.09 cfu/mL. Finally, no significant ($p> 0.01$) difference in the mean values of colour, consistency, flavour and overall acceptability during the cold storage for all the treatment. Conclusively, the inclusion of *Nigella sativa* seed oil in yoghurt could lead to production of a better keeping quality yoghurt. And as acceptable alternative to chemical preservation.

TABLE OF CONTENTS

CHAPTER THREE

LIST OF TABLES

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

Yoghurt is a Turkish name for a fermented milk product. It is originated by early nomadic herdsmen especially in Asia, Eastern and Southern Europe. Yoghurt consumption has steadily increased over the years. The per-capita consumption of yoghurt is expected to show considerable growth in the future (Choi *et al*., 2016; Rafaat and Ramadan, 2018). Yoghurt is a coagulated product of milk, obtained by the addition of acid forming bacteria into milk that is homogenized, pasteurized and fermented.

The microorganisms employed in the making of yoghurt is referred to as "starter culture", they include *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Ramchandran, 2009). During fermentation, protein in milk undergoes hydrolysis, viscosity is increased, pH decreases and bacteria metabolites are produced which contributes to the taste and possibly, to the health promoting attributes of yoghurt. Consequently lactose (sugar) in milk is converted to lactic acid which causes the formation into the characteristics curd (Ani *et al*., 2018).

Yoghurt plays an important role in human nutrition, health maintenance, therapeutic and dietetic function (Yabaya and Idris, 2012). It has been shown to increase digestion, fight off infections, boost immunity, assist people with lactose intolerance; also, it is an ideal vehicle for fortification with nutraceuticals which are substances isolated, extracted and purified from plants that confers health benefits (Rastogi *et al*., 2013). Yoghurt like fresh milk is highly vulnerable to spoilage by microorganisms and susceptible to contamination (Pal *et al*., 2015).

Fungi mostly are the cause of spoilage in addition to bacteria as low pH level produces selective media for their growth. Low quality milk, unfavourable storage temperature, unclean utensils; unsuitable starter culture affects quality of yoghurt. In order to prevent the growth of spoilage organisms and to improve the overall quality of yoghurt, conventional synthetic or artificial additives have been added to yoghurt during its manufacturing. Moreover, their rich bio-active components with health benefits constitute a potential source of minerals and vitamins in addition to their antimicrobial properties which could consequently and positively affect the shelf-life of yoghurt.

Nigella sativa L belong to the family *Ranunculacae.* It is a spice that has been used for decades for culinary and medicinal purposes. It is also used as natural remedy for asthma, hypertension, diabetes, inflammation, cough, bronchitis and fever (Singh *et al*., 2014). Fixed oil from black cumin is rich in linoleic, oleic acids, bioactive phytosterols and tocopherols (Ramadan *et al*., 2014). The major compound found in *Nigella Sativa* is thymoquine (30 -40 %) (Naz, 2011; Aftab *et al*., 2013; Abbasnezhaad *et al.*, 2015). The seeds are the most commonly utilized part of the plants; it has a pungent and bitter taste with considerable amount of oil inside its follicles which mostly are utilized as a food preservative and spice and, the oil used for therapeutic purpose. Black seed contains 26 % proteins, 25 % carbohydrates, 84 % crude fibres and 4.8 % ash; it contains a good level of carotene, and minerals which includes Copper, Phosphorus, Zinc and Iron (Ramadan *et al*., 2015).

The use of natural additives could be of more benefits to the consumers, addition of herbs and spices could be an effective approach or strategy to improve the functionality, quality and bio preservation of yoghurt (Aswal *et al*., 2012).

1.2 Statement of the research problem

i. Yoghurt which is the most sought-after dairy product, despite its therapeutic benefits, has a number of draw backs as regards spoilage and contamination.

ii. Synthetic anti microbial such as butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA) and tert-butylated hydroquinone (TBHQ) were the conventional widespread preservatives employed in the manufacture of yoghurt.

iii. Additives that are meant to minimi ze deterioration, maintain nutritional value or quality of yoghurt are unfortunately synthetic which have been proven not to be completely safe and are linked to a number of food sensitivities such as allergies, gastro intestinal diseases and cancers; there is also resistance on the part of microbes to these synthetic additives making them thrive in the medium especially spore formers and methicilin resistant *Staphylococcus aureus,*and psychotrophic bacteria that recontaminate finished products. (Salman *et al*., 2008).

1.3 Justification for the study

i. There are evidences that plant extracts are increasingly gaining precedence over synthetic alternatives by virtue of their fewer side effects and greater affordability (Debjani, 2019).

ii. Black cumin extracts have been reported to extend the shelf-life of milk products (Merai *et al.,* 2003)

iii. Despite all concerted efforts to preserve and protect the quality of yoghurt using natural additives, there are no previous investigations that approached the incorporation of an oil extracts of *Nigella sativa* as a preservative or improver of the keeping quality of yoghurt as well as extending its shelf–life. This lack of information on these areas is a justification for the current study. Therefore, this study is directed towards evaluating the keeping quality of yoghurt incorporated

with oil extracts of *Nigella sativa* varying inclusion levels during refrigerated storage at 5^0C for 42 days.

1.4 Aims and objectives of the study

The aim of this study is to evaluate the keeping quality of yoghurt during cold storage as influenced by inclusion of black cumin (*Nigella sativa)* seed oil extract

The Objectives are to;

i. determine the quantitative and qualitative phytochemical profiles of oil extract of *Nigella sativa* seeds,
ii. determine physico-chemical and proximate composition of yoghurt preserved with oil extracts of *Nigella sativa* seeds during cold storage at 5^0C for 42days
iii. evaluate microbiological properties of yoghurt during cold storage following treatment with oil extracts of *Nigella sativa* seeds.
iv. Determine sensory properties of the *Nigella sativa* oil infused yoghurt during cold storage.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description of food

Food is any substance that provides nutritional benefit to the body via the supply of adequate quantity of vitamins, fats, proteins, carbohydrates, or minerals, originating from plants or animals. The main function of food is to provide energy, stimulate growth, make the body work, and maintain life (Joardder and Masud, 2019). However, any food which provides additional benefits to the body of an organism or human other than the primary function could be regarded as functional foods. These include provision of benefits such as antioxidant, antimicrobial, antilipidemic, and antidiabetic benefits. Production of this type of food could be through additional processing such as fermentation.

2.1.1 Shelf life of food

Food items can be classified under shelf life as perishable, semi-perishable, and non-perishable. Perishable foods have shelf life ranging from several days to about three weeks (0-21days). Milk and dairy products, meats, poultry, eggs, and seafood are the examples of perishable food items. Semi-perishables are different food items that can be preserved for long time (about six months) under proper storage conditions. Examples of such foods include vegetables, fruits, cheeses, and potatoes. Non-perishables are natural and processed foods that have indefinite shelf life. These foods can be stored for several years or longer. Dry beans, nuts, flour, sugar, canned fruits, mayonnaise, and peanut butter are few examples of non-perishable foods (Doyle, 2009).

2.2 The yoghurt

2.2.1 Production, preservation, nutritive and health benefits

Yoghurt is one of the most popular dairy milk-based products with recognition as a healthy food consume by different class of people across the world. When consume, it supplies wide-array of nutrients with balanced ratio of energy and fat content which makes it a nutrient-dense and safe food consumed for a healthy living. It is a rich source of minerals because it supplies the body with significant amounts of calcium in biologically useful forms for normal body functions and well-being. Furthermore, yoghurt can be regarded as a functional food because it has several health benefits beyond the basic nutrition for provision of protein. This functional usefulness includes improved lactose tolerance, body weight changes and fat loss, and a variety of health attributes associated with probiotic bacteria. (Ndambi *et al*, 2008).

The production of yoghurt is diverse depending on available resources and the know-how; although, the general preparation of yoghurt follows pasteurization of the milk either in a steel pot or any other suitable container at temperature of about 85^0C for some minutes, then rapid cooling to a lower temperature of about 43^0C then incubation of the cooled milk with relevant *Lactobacilli* bacteria (Ndambi *et al*, 2008).

Yoghurt is the most popular fermented milk product in the world, and its consumption has increased due to consumer concern about healthier foods (Trujillo *et al*., 2002). Although yoghurt is an alternative for milk preservation, its shelf life is still short when compared to other dairy products, such as some types of cheese, and its nutritional value also makes the product suitable for the development of some spoilage microorganisms (Ledenbach and Marshall, 2009). In this context, dehydration appears as a process for yoghurt preservation, and freeze drying and spray drying are the most recommended drying methods, mainly due to the maintenance of the sensory and nutritional characteristics of the food (Trachoo, 2002).

Yoghurt is a product of the lactic acid fermentation of milk by addition of a starter culture containing beneficial bacteria including *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus*. In some countries, the production of yoghurt involves the use of less traditional microorganisms, such as *Lactobacillus helveticus* and *Lactobacillus delbrueckii ssp. lactis*, which are sometimes mixed with the starter culture (Michelle, 2005). Yoghurt is an excellent source of protein, calcium, phosphorus, riboflavin (vitamin B2), thiamin (vitamin B1) and vitamin B12, and a valuable source of folate, niacin, magnesium and zinc. The protein it provides is of high biological value by containing all the amino acids essential for a heathy living, as well as containing the vitamins and minerals found in milk and other dairy foods in appreciable bio available quantities (Hjartåker *et al*., 2002).

2.2.2 Physico-chemical properties of yoghurt

Physico-chemical properties are properties defined, measured and expressed in physical and physico-chemical ways. However, there is no clear dividing line between these two types of properties. Paulus (1989) classified physical properties as: mechanical properties, thermal properties, transport properties, and other properties (electrical and optical). It is considered misleading to use transport as a subclass of physical properties, since many mechanical, thermal, and electrical properties are considered transport properties, e.g electrical conductivity and thermal conductivity. Moreover, among thermal properties, specific heat is a constitutive property, whereas thermal conductivity and diffusivity are transport properties. The classification proposed here is similar to the classification of physical properties in Jowitt (1974), but firstly two new subclasses, thermodynamic properties and mass transfer properties, replace Jowitt's subclass of diffusional and related properties; most of the properties included in Jowitt's subclass are in fact thermodynamic ones. The new mass transfer properties subclass now proposed includes both mass

transfer by diffusion and mass transfer by other mechanisms, and thus is more general. Secondly, a new subclass of physico-chemical constants has been added.

2.2.3 The pH of yogurt

Acidification of milk leads to the disruption of the internal structural properties of casein micelles due to the solubilization of colloided calcium phosphate (Dalgleish and Law, 1989). As caseins approach their isoelectric point (pH 4.6), the net negative charge on casein is reduced, which decreases electrostatic repulsion between charged groups, including the phosphoserine residues that are exposed when the CCP is solubilized. Electrostatic attraction increases and protein-protein attraction also increases through enhanced hydrophobic interactions (Lucey, 2004). Physico-chemical mechanisms for the formation of acid milk gels can be discussed for three pH regions (Lucey, 2004). Moreover, acidification values increase as pH of a medium decreases.

When the pH of milk decreases from 6.6 to 6.0, the net negative charge on the casein micelles decreases, which results in a decrease in electrostatic repulsion. Since only a small amount of CCP is solubilized at pH >6.0, the size of the casein micelles is largely unchanged.

As the pH of milk decreases further from pH 6.0 to 5.0, the net negative charge on casein micelles greatly decreases and the charged “hairs” of κ-casein may shrink (or curl up). This results in a decrease in electrostatic repulsion and steric stabilization, which are both responsible from the stability of casein micelles in the original milk. At pH ≤6.0 the rate of solubilization of CCP increases, which weakens the internal structure of casein micelles and increases electrostatic repulsion between the exposed phosphoserine residues. In milk, CCP is completely solubilized in casein micelles by pH ~5.0. However, in rennet-coagulated cheese, a significant amount of CCP is not solubilized at this pH,

probably because of a protective role on CCP solubility from the higher solids content of curd compared with milk. Dalgleish and Law (1988) reported that the amounts and proportions of caseins dissociated from the micelles were both temperature- and pH-dependent. More caseins are dissociated from micelles into the serum as temperature decreases from 30 to 4°C. The pH at which maximum dissociation occurs is between pH 5.6 and ~5.1 (Dalgleish and Law, 1988), which may be attributed to a partial loosening of bonds within and between caseins due to loss of CCP (Lucey, 2004). At low temperatures, hydrophobic interactions involved in casein association are very weak.

When the pH of milk becomes close to the isoelectric point of casein (pH 4.6), there is a decrease in the net negative charge on casein, which leads to a decrease in electrostatic repulsion between casein molecules. On the other hand, casein-casein attractions increase due to increased hydrophobic and plus-minus (electrostatic) charge interactions (Horne, 1998). The acidification process results in the formation of three-dimensional network consisting of clusters and chains of caseins (Mulvihill and Grufferty, 1995). Nevertheless, the pH yoghurt is within the range of 4.15 to 4.50 according to the literature (Kavas *et al.,* 2003; Matos *et al*., 2013).

2.2.4 The titratable acidity

The titratable acidity might be influenced by the discrepancy due to the action of the starter cultures that hydrolysed lactose into lactic acid during the fermentation process. Acidification rate of yogurts (fortified and nonfortified) can also be influenced by many factors, including growth characteristics, specific growth rate, fermentation time, sugar or amino acid consumption, and product yields (Marafon *et al.,* 2011; Wang *et al.,* 2012).

2.2.5 The viscosity of yogurt

There have been many studies on the viscosity and flow properties of stirred yogurts (Haque *et al.,* 2001; Lee and Lucey, 2006). In most studies, stirred yogurts were tested

on a viscometer or rheometer to determine the flow properties after the original set gels were empirically agitated using a spoon or a high-speed mixer (Van Marle *et al.*, 1999). During the mixing or loading steps there are structural changes in yogurt, which affect the flow properties. For stirred yogurt products it should be recognized that steps, such as, mixing result in a reduction in viscosity that is only partially restored after shearing is stopped. Recovery of structure is called "rebodying" and is a time-dependent phenomenon. Structural recovery also affects the apparent viscosity of yogurts. Arshad *et al.,* (1993) reported that glucono-δ- lactone (GDL)-induced gels had only 30% recovery of the original value of the dynamic moduli even after allowing 20 h for recovery after shearing.

2.2.6 Total solid content

The total solid content is the total amount of solid particles in the medium. As for the total solid content of yoghurt, the change in pH or titratable acidity might influenced the increased total solid content, which might be as a result in a modification of the pH–acidity relationship due to the buffering capacity of compounds, including proteins, phosphates, citrates, and lactates, during storage (Wang *et al.*, 2012).

2.2.7 Sensory properties

A sensory property can be defined as the human physiological-psychological perception of a number of physical and other properties of food and their interactions. The physiological apparatus (fingers, mouth, eyes, taste and aroma receptors, ears) examines the food and reacts to the food's properties. Signals are sent to the brain which interprets the signals and comes to a decision about the food's sensory quality; this is the psychological bit. Sensory properties are measured subjectively by trained or untrained sensory panels or individuals. Sensory properties can be subdivided into: tactile

properties, textural properties, colour and appearance, taste, odour, and sound (Shafiur and McCarthy, 1999).

The main difference between texture and other sensory attributes is that texture is perceived mainly by biting and mastication, i.e. by the mouth. Many of the sensory properties are related to physical and physico-chemical properties as measured objectively with instruments. However, this does not mean that instrumentally measured characteristics are sensory properties. The following discussion will help to highlight the difference. The rheological nature of a food, and the food's texture, are two different things. Rheological properties are measured objectively using suitable instruments that allow controlled deformation of the food. Texture, however, has to be measured subjectively. It will depend partly, of course, on the food's rheological properties, but also, potentially, on a number of other properties (e.g shape, size, porosity, thermal properties) and on the expectations and prior experience of the person (s) assessing the texture. In many cases, texture can be correlated quite well with an instrumentally measured rheological property (often an empirical or imitative one), but texture as such can be measured only by subjective means (Shafiur and McCarthy,1999).

2.3 Food spoilage

Food spoilage can be defined as food shelf-life deterioration as a result of chemical, physical or microbial, actions which lead to devaluation of colour, nutrition, texture and edibility of food (Amit *et al.*, 2017). There are many types of food spoilage and they are treated below.

2.3.1 Physical spoilage

Food spoilage due to physical changes or instability is defined as physical spoilage. Moisture loss or gain, moisture migration between different components, and physical separation of components or ingredients are the examples of physical spoilage. The key

factors affecting physical spoilage are moisture content, temperature, glass transient temperature, crystal growth, and crystallization (Amit *et al.*, 2017).

2.3.2 Microbial spoilage

Microbial spoilage is a common source of food spoilage which occurs due to the action of microorganisms. It is also the most common cause of food borne diseases. Perishable foods are often attacked by different microorganisms. Microorganisms involved in food spoilage can be divided into three major categories: e moulds, yeasts, and bacteria. The growth of most microorganisms can be prevented or lingered by adjusting storage temperature, reducing water activity, lowering pH, using preservatives, and using proper packaging (Tianli *et al*., 2014).

There are intrinsic and extrinsic factors that can affect microbial spoilage in foods. The intrinsic properties of foods determine the expected shelf life or perishability of foods and also affect the type and rate of microbial spoilage. Endogenous enzymes, substrates, sensitivity to light, and oxygen are the primary intrinsic properties associated with food spoilage (Amit *et al.*, 2017). To control food quality and safety, these properties can be controlled during food product formulation (Doyle, 2009). Intrinsic factors of food spoilage also include pH, water activity, nutrient content, andoxidation–reduction potential (Steel, 2004; Doyle, 2009). Extrinsic factors of food spoilage include relative humidity, temperature, presence, and activities of other microbes (Amit *et al.*, 2017).

2.3.3 Chemical spoilage

Chemical and biochemical reactions occur naturally in foods and lead to unpleasant sensory results in food products. Fresh foods may undergo elementary quality changes caused by: (a) microbial growth and metabolism which results in pH changes, (b) toxic

compounds, and/or (c) the oxidation of lipids and pigments in fat which results in undesirable flavours and discoloration, Chemical spoilage is interrelated with microbial actions. However, oxidation phenomena are purely chemical innature and also dependent on temperature variations (Van-Boekel, 2008)

2.4 Oxidation

In the presence of oxygen, amino acids convert into organic acid and ammonia. This is the elementary spoilage reaction in refrigerated fresh meat and fish (Jay, 2000).The term 'rancidification' is used to denominate lipids oxidation through which unsaturated fats (lipids) undergo reaction with oxygen (Amit *et al.*, 2017). The consequences in food items are colour alteration, off-flavour, and toxic substances formation. Rancidification can be catalysed by the presence of metal oxides and exposure to light increases the reaction rate. After this reaction, carbonyl compounds are responsible for rancid taste of foods are produced (Amit *et al.*, 2017).

Hydrolytic rancidity causes lipid degradation by the action of lipolytic enzymes. In this reaction, free fatty acids are cleaved off triglyceride molecules in the presence of water. These free fatty acids have rancid flavours or odour (Steele, 2004). The released volatile fatty acids have a stiffmal odour and taste; therefore, hydrolytic rancidity is extremely noticeable in fats, such as butter (Amit *et al.*, 2017)

2.5 Proteolysis

Proteolysis is an ubiquitous and irreversible post translational modification, involves limited and highly specific hydrolysis of peptide and iso-peptide bonds of a protein. The entire phenomena require the presence of miscellaneous protease enzymes (Rogers and overall, 2013). Different specialized proteases play key roles in various regulatory processes. Moreover, highly specific proteolytic events are associated with normal and pathological conditions (Igarashi *et al.*, 2007). Foods containing nitrogen compounds

frequently incur this reaction. Proteins, after being incurred through proteolysis, eventually get converted into small-sized amino acids. The following reaction presents proteolysis mechanism (Igarashi *et al*., 2007).

2.6 Putrefaction

Putrefaction refers to the series of anaerobic reactions through which amino acids detour to a mixture of amines, organic acids, and stiff-smelling sulphur compounds, such as mercaptans and hydrogen sulphide. This is a biochemical phenomenon as the presence of bacteria is exigent all through the process. Along with amino acids, indole, phenols and ammonia are also formed due to protein putrefaction Most of these chemicals have displeasing odour. Putrefaction is quite common in meats and other protein-rich foods at temperatures greater than 15^0C. This elevated temperature facilitates microbial activities (Igarashi *et al*., 2007).

2.7 Maillard reaction

Non-enzymatic browning, which is also known also as Maillard reaction, is another primary cause of food spoilage. This reaction occurs in the amino group of proteins, or the amino acids present in foods. Colour darkening, reducing proteins solubility, developing bitter flavours, and reducing nutritional availability of certain amino acids are the common outcomes of Maillard reaction. This reaction occurs during the storing of dry milk, dry whole eggs, and breakfast cereals (Desrosier and Singh, 2014)

2.8 Pectin hydrolysis

Pectins are complex mixtures of polysaccharides that make up almost one-third of the cell wall of dicotyledonous and some monocotyledonous plants. Indigenous pectinases are synthesized or activated during ripening of fruits and cause pectin hydrolysis which softens the structure of food. Damages of fruits and vegetables by mechanical means may

also activate pectinases and initiate microbial attack (Desrosier and Singh, 2014). Pectin substances may also be de-esterified by the action of pectin methyl esterase. This esterification process is initiated in situ on damaged tissues, firm fruits, and vegetables by strengthening the cell walls and enhancing intercellular cohesion via a mechanism involving calcium. Metal ions catalyze the decomposition of heat-labile fruit pigments, which consist of pectin ingredients. This process causes the colour change in fruit jams or jellies (Amit *et al.*, 2017). Therefore, jams and jellies are preserved in glass containers rather than metallic jars.

2.9 Food preservation

Food preservation refers to the process or technique undertaken in order to avoid spoilage and to increase shelf life of food (Rodriguez-Gonzalez *et al.*, 2015). Different preservation and processing techniques such as chemical, biological and physical methods have been widely applied globally. However, biological methods of preservation known as fermentation is broadly applied to most dairy products (Amit *et al.*, 2017).

2.9.1 Chemical processes and preservatives

Food preservation using chemical reagents is one of the ancient and traditional methods,Effectiveness of this method depends on the concentration and selectivity of the chemical reagents, spoilage-causing organisms, and the physical and chemical characteristics of food items (Amit *et al.*, 2017). The global consumption and application of food additives and preservatives are extending. Using chemical reagents as food additives and preservatives is a sensitive issue because of health concerns (Mursalat *et al.*, 2013). In different countries, the applications of chemical preservatives and food additives are monitored and regulated by different acts, rules, and government authorities (Islam *et al.*, 2015)

Preservatives are defined as the substances capable of inhibiting, retarding, or arresting the growth of microorganisms or any other deterioration resulting from their presence Food preservatives extend the shelf life of certain food products. Preservatives retard degradation caused by microorganisms and therefore maintain the colour, texture, and flavour of the food item (Adam and Moses, 2008). Examples of chemical preservative include; lactic acid, benzoic acid, sulphur dioxide, sulphur sulphate, ascobyl palmitate, sodium nitrate, gallate among others.

2.9.2 Biological processes.

Fermentation method uses microorganisms to preserve food. This method involves decomposition of carbohydrates with the action of microorganisms and/or enzymes bacteria, yeasts, and moulds are the most common groups of microorganisms involved in fermentation of a wide range of food items, such as dairy products, cereal-based foods, and meat products. Fermentation enhances nutritional value, healthfulness, and digestibility of foods. This is a healthy alternative to many toxic chemical preservatives (Adam and Moses, 2008).

It promotes involvement of microorganisms in the conversion of food nutrients including carbohydrates into alcohol or organic acids (Vuppala and Murthy, 2015). It improves raw foods to become more stable, safe, acceptable and nutritive for human consumption. It is economically viable because it demands less cost and simple in nature. In addition to this, longer shelf life of the fermented food is obvious compared with unfermented foods (Steinkraus, 1995).

Fermentation increases foods shelf life up to months in addition to their established perishable periods. For example, fermentation has been reported to increase shelf-life of vegetable to about a year; while fermentation of wheat bread was also identified to intensified shelf-life extensively (Dal, 2007). The extension of shelf life due to

fermentation mainly depends on the proliferation in the food by favourable microorganisms majorly the *Lactobacilli*. Meanwhile, the effectiveness of fermentation depends on the quality of water, presence of air, and temperature of the system. Therefore, inappropriate fermentation temperature and poor water quality, and other related issues could lead to relatively shorter shelf life which has been observed in fermented foods (Joardder and Masud, 2019).

Fermentation is the food processing type of choice whenever drying of food is impossible, and fermentation can lead to production of quality foods that show fabulous safety record even in the developing countries where fermentation is been carried out by people with little or no formal education in chemistry and or microbiology. Fermentation is also contributing to sustainable health growth because it can be an avenue of lessening the spike of nutritional diseases and enhancement of superior resistance to intestinal and other diseases in infants by sufficient intake of fermented foods for the promotion of an overall beneficial effects of high-quality fermented foods. However, fermentation despite being an effective means of improving food quality and shelf life, is faces challenges serving as impediment of deriving the expected benefits from fermented foods and these include insufficient oxygen supply, temperature fluctuation, and presence of obnoxious minerals in water as well as the selection of the appropriate facilities for the implementation of the process

2.10 Food additives

The key objectives to use food additives are to improve and maintain nutritional value, to enhance quality, to reduce wastage, to enhance customer acceptability, to make food more readily available, and to facilitate processing food items (Adam and Moses, 2008). Food additives can be either natural or synthetic chemical substances that are used intentionally during processing, packaging, or storage of foods to bring desired changes in food

characteristics. Food additives can be divided into two major groups intentional and incidental. Among these two, intentional additives are strictly controlled by government authority (de Man, 2018). Additives are used to disguise faulty process, to hide spoilage, damage, or other inferiority, and apparently to deceive consumer. Moreover, if additives cause substantial reduction in nutrition, then their uses are also unaffiliated (de Man, 2018).

2.10.1 Classification of fermentation process

Fermentation process can be classified as alcoholic, acetic acid and lactic acid fermentation.

2.10.2 Alcohol fermentation

This occurs as a result of yeast action on the simple sugar called 'hexose' converting it into alcohol and carbon dioxide. The quality of fermented products depends on the presence of alcohol. In this process, air is excluded from the product to avoid the action of aerobic microorganisms, such as acetobacter. This process ensures the longer shelf life of the products. The following equation illustrates alcohol fermentation by conversion of hexose (Amit *et al.*, 2017)

$C_6H_{12}O_6 + O_2 = C_2H_5OH + CO_2$

Hexose + Oxygen= Ethanol + Carbon dioxide

2.10.3 Vinegar (acetic acid) fermentation

This type of fermentation takes place after alcohol fermentation. Acetobacter converts alcohol to acetic acid in the presence of excess oxygen (Kartz, 2001). Under this method, food products are preserved as pickles, relishes among others (Amit *et al.*, 2017). Vinegar fermentation results in acetic acid and water by oxidation of alcohol

$C_2H_5OH + O_2 = CH_3\,COOH + H_2\,O$

Ethanol + Oxygen = Acetic Acid + Water.

2.10.4 Lactic acid fermentation

This takes place due to the presence of two types of bacteria: homo-fermenters and hetero-fermenters. Homo-fermenters produce mainly lactic acid via the glycolytic (Embden–Meyerhof) pathway. Hetero-fermenters produce lactic acid plus appreciable amounts of ethanol, acetate, and carbon dioxide, via the 6-phosphogluconate/phosphoketolase pathway. Homolactic fermentation involve 1 mol of glucose to yields two moles of lactic acid (Kartz, 2001).

$C_6H_{12}O_6$(Glucose) - $2CH_3CHOHCOOH$ (Lactic Acid).

Heterolactic fermentation involve 1 mol of glucose to produce 1 mol each of lactic acid, ethanol, and carbon dioxide (Kartz, 2001).

$C_6H_{12}O_6 = CH_3CHOHCOOH + C_2H_5OH + CO_2$

Glucose = Lactic Acid + Ethanol + Carbon dioxide.

2.10.5 Possible health risk of food preservatives and additives

Chemical food additives and preservatives are mostly considered safe, but several of them have negative and potentially life-threatening side effects. For example, nitrates upon ingestion, are converted to nitrites that can react with haemoglobin to produce met-haemoglobin; a substance that can cause loss of consciousness and death, especially in infants. Different artificial food colourings, such as tartrazine, Allura red, ponceau, and benzoate preservatives, have adverse effects on the behaviour of infants; these additives are credited as the cause of the hyperactive behaviours of infants (Amit *et al.*, 2017). Preservatives also have intolerances among people who have asthma. Sulphites (including sodium bisulfite, sodiummeta-bisulfite, and potassium bisulfite) found in wine, beer, and dried fruits are known to trigger as thematic syndromes and cause migraines in people who are sensitive to them. Sodium nitrate and sodium nitrite are also classified as ‘probable carcinogenic elements’ to humans by International Agency for Research of

Cancer (IARC) (Nogrady, 2013). Nitrites and benzoates may have adverse effects on pregnant women. Sodium nitrite intake lowers haemoglobin and hematocrit values of pregnant women. Both benzoate and nitrite induce decrease in serum bilirubin and increase in serum urea. Nitrites after ingestion, get converted into nitrosamines, which could be harmful to a foetus (Amit *et al.*, 2017).

2.10.6 Use of herbs and spices for food preservation

Substances that are naturally occurring, and directly derived from biological systems without alteration or modification in a laboratory setting are recognized as natural antimicrobials. These can be obtained from different sources including plants, animals, bacteria, algae and fungi. Plant extracts have the advantage of having been consumed by humans for thousands of years, and besides antimicrobial ability, several plants are being used in different areas of human health such as traditional medicine, functional foods, dietary supplements and recombinant protein manufacturing. Their role as antimicrobials derives from their capacity to control natural spoilage processes (food preservation) and to prevent/control growth of microorganisms, including pathogenic micro- organisms (Tajkarimi *et al*., 2010). The potential antimicrobial properties of plants have been related to their ability to synthesize, through secondary metabolism, several chemical compounds of relatively complex structures with antimicrobial activity. These antimicrobial components are naturally produced to boost the defense system of a plant during adverse conditions (Makwana *et al*., 2015).

2.10.7 Benefits of herbs and spices in food preservation

A wide variety of plant materials are categorized as herbs or spices. Herbs and spices and derived extracts have been used since ancient times to improve sensory characteristics of food, as preservatives, for their nutritional and healthy properties and also for their antimicrobial effect (Gyawali and Ibrahim, 2014). The main advantage is that these are

residue free and generally recognized as safe and are therefore potential alternatives to chemical additives (Sanchez and Aznar, 2015). The terms ‘herbs’ and ‘spices’ have more than one definition in common languages but the most commonly used are those which consider herbs to be obtained from the green parts of a plant, such as the stem and leaves. They differ from other plants used in foods in that they are used in small amounts to import flavour rather than larger quantity or using it as food itself.

Herbs and are dietary sources of polyphenols and contribute to the total polyphenol intake. Pérez-Jiménez *et al.* (2010) identified the 100 richest dietary sources of polyphenols showing spices and herbs with the highest concentrations. Spices such as cloves, black seed, star anise contain high amounts of phenolic flavours such as eugenol (cloves) or anethole (star anise). Dried herbs contain high amounts of flavonones such as eriocitrin (peppermint) or pinocembrin (Mexican oregano) and/or high amounts of hydroxycinnamic acids such as rosmarinic acid in herbs from the *Laminaceae* family (pepper- mint, sage rosemary, spearmint, thyme). Most of the consumed polyphenols are metabolized by intestinal microbiota, in some cases resulting in metabolites with greater biological activity than their predecessors (Valdes *et al*., 2015). Out of the herbs and spice reported to have been used for the preservation of foods, *Nigella sativa* has been widely reported by various researchers based on its health benefit. (Halamova *et al.,* 2010).

2.11 *Nigella sativa*

2.11.1 Morphology of the plant

Nigella sativa is a bushy, self-branching plant of about 50 to 60 cm in height. Leaves are divided into linear segment 2 to 3 cm long; they are apposite in pairs on either side of the stem. Its lower leaves are small, and petiolate and upper leaves are long. The plant has finely divided foliage and pale bluish or white flowers. The flowers grow terminally on its branches. *Nigella sativa* reproduces with itself and forms a fruit capsule which consist

of many white trigonal seeds, once the fruit capsule has matured, it opens up and the seeds contained within are exposed to the air becoming black in colour (black seeds), seeds are triangular in shape, black in colour and possess a severe pungent smell, contains considerable amount of oil (Chevallier, 1996).

2.11.2 Cultivation and collection of *Nigella sativa*

The plant is widely grown in different part of the world and is an annual herb cultivated in India and Pakistan. *Nigella sativa* is cultivated during winter season in much the same way as wheat. The areas where maize, green gram or black grams are grown can be used after harvesting these crops. Before sowing the seeds, 2 to 3 times ploughing is enough for good crops and weed control. Heavy soils need more ploughing than light soils. The seeds are sown 30 cm apart. The seeds should not be sown deep because the germination is delayed. About 12 to 15 kg seeds per hectare are sown. Three to five irrigations are required that is, presowing, seeding stage, flowering stage, and fruit formation stage and seeds development stage. Crop matures during April and May. It should be harvested early in the morning. The crop is harvested when the fruit/capsule turn yellowish. The late harvesting may result in shattering the seeds. After harvesting and proper drying, it can be threshed by trampling the crop with tractor or proper thresher. After threshing, the seeds should be properly stored in bags or containers (Ahmad *et al.,* 2004).

2.11.3 Chemical constituents of *Nigella sativa*

In view of its wide range of medicinal uses, the plant has under gone extensive phytochemical studies. *Nigella sativa* seeds contain 28 % to 36 % fixed oil, proteins, alkaloid, saponin and 0.4 to 2.5% essential oil. The fixed oil is mainly composed of unsaturated fatty acid that includes arachidonic, eicosadienoic, linoleic and linolenic acids. The saturated fatty acid present in the oil are palmitic, stearic and myristic acid (Hajhashemi *et al.*, 2004).

The crystalline active principle, nigellone is the only constituent of the carbonyl fraction of the oil. The other constituents of the volatile oil of the seed are p-cymene carvacrol, t-anethole, 4-terpineol and longifoline. Four alkaloids have been reported as constituent of *Nigella sativa* seeds. Recently, a triterpene saponin alfa here in was isolated from the seeds of *Nigella sativa.* α-hered in is known to have antitumor activity (Kumara and Haut, 2001).

The ethanolic extracts of the seeds was found to contain three flavonoids namely quercetin and kaempferol 3- glucosyl (1-2) galactosyl (1-2) glucoside and quercitin –3-(6-ferulolyl glucosyl) (1-2) galactosyl (1-2) glucoside (Merfort *et al.,* 1997). Other than those triglycoside quercetin 3-glucosi de, kaempferol 3-glucoside and rutin were also isolated from the seeds of *Nigella sativa.*

Nigella sativa seeds contain other ingredient including nutritional components such as carbohydrates, fats, vitamins, mineral elements and proteins including eight or nine essential amino acid. Fractionation of whole *Nigella sativa* seeds using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) shows bands ranging from 94 to 100 KDa molecular mass (Haq *et al.,* 1999). Monosaccharide in the form of glucose rhamnose, xylose and arabinose are also found. The seeds also contain carotene, which is converted by liver to vitamin A, the *Nigella sativa* seeds are also a source of calcium, irons and potassium (Salem and Hossain, 2000).

2.11.4 Pharmacological properties of *Nigella sativa* seeds

Many studies have been conducted particularly during the last two decades on the effect of *Nigella sativa* seeds extracts or its active compounds on the various body systems in vivo or in vitro. The following is the selection of some of these studies.

2.11.5 Antioxidant activity

Generation of free radicals may be at least partially the basis of many human diseases and conditions. Therefore, the antioxidant action of *Nigella sativa* may explain its claimed usefulness in folk medicine. The essential oil of *Nigella sativa* was tested for a possible antioxidant activity. The essential oil, thymoquinone and other components like carvacrol, anethole and 4-terpineol demonstrated respectable radical scavenging property. The free radical scavenging effect of thymol, thymoquinone and dithymoquinone were studied on the reactions generating reactive oxygen species such as superoxide anion radical, hydroxyl radical and singlet oxygen using the chemiluminescence and spectrophotometer methods (Kruk *et al.,* 2000). Thymoquinone and fixed oil of *Nigella sativa* were also reported to inhibit non-enzymatic pero-xidation in ox brain phospholipid liposomes (Houghton *et al.,* 1995). The antioxidant effect of thymoquinone (TQ) and a synthetic structurally related ter-butyl thymoquinone (TBHQ) were examined in vitro. Interestingly, both TQ and TBHQ efficiently inhibited iron dependent microsomal lipid peroxidation in a concentration dependent manner (Badary *et al.,* 2003).

2.11.6 Hepatoprotective activity

Hepatotoxicity is associated with alteration in the levels and activities of certain enzymes such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), oxidant scavenger enzymes system including glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT). The protective action of thymoquinone against the hepatotoxin: terbutylhyderoperoxide has been demonstrated using isolated rat hepatocytes (Daba *et al.,* 1998). In this study, the hepatoprotective activity of thymoquinone (TQ) was compared with that of silybin a known hepatoprotective agent. The mechanism of hepatoprotection of TQ is not certain but may be related to the preservation of intracellular gluathione (GSH), the depletion of which by oxidative stress is known to increase the susceptibility of cells to irreversible injury. It has also been

shown that pretreatment of rats with *Nigella sativa* oil for 4 weeks was effective in protection against CCl4 and D-galactosamine induced hepatic damage. No ill effects on liver function were observed when the oil was given orally at a dose of 100 mg/kg/day for 4 weeks. In mice thymoquinone, 8 mg/kg/day for 5 days before and 1 day after CCl4 treatment was found to protect against the biochemical and histological markers of liver damage (Nagi *et al.,* 1999). Recently, it is also found to show protective effects against ischemia reperfusion injury on liver (Fahrettin *et al.,* 2008).

2.11.7 Anti nephrotoxic activity

Administration of seed extract with cysteine, vitamin E and *Crocus sativa* before administrating the nephrotoxic drug cisplatin was effective in ameliorating the biochemical and physiological indices of nephrotoxicity (El-Dally, 1996). This conform with the result of (Tembhurne *et al.,* 2008; Ali, 2004) who reported the Nephroprotective activity of *Nigella sativa* seed oil in nephrotoxicity induced by Cisplatin and Gentamycin. The reason for the protective action is not certain but may be related to the antioxidant action of the drug and the fact that the neprotoxic drug may induce its effects via generation of free radicals (El-Dally, 1996). Fanconi syndrone (FS) induced by ifosfamide is characterized by wasting off glucose, electrolyte and organic acids along with elevated serum creatinine and urea as well as decreased creatinine clearance rate. Administration of thymoquinone with the drinking water before and during ifosfamide treatment ameliorated the severity of ifosfamide induced renal damage and improved most of the alteration of biochemical parameters (Badary, 1996)

2.11.8 Anti-cancer activity

Salomi *et al.* (1992) have shown that the crude methanolic extract of the seeds of this plant exhibited a strong cytotoxic action on Elrich ascites carinoma, Dalton's ascites lymphoma and sarcoma 180 while exerting minimal cytotoxicity to the normal

lymphocytes. In another study, the aqueous and alcoholic extract of *N. sativa* alone or in combination with H_2O_2 as an oxidative stressor were found to be effective in in vitro inactivating MCF-7 breast cancer cells (Farah and Begum, 2003).

The antitumor effect of thymoquinone and β-elemene were investigated both in vivo and in vitro in male albino rats on fibro sarcoma induced by 20-methyl cholanthrene and it was found to inhibit tumor incidence and tumor burden significantly. The possible modes of action were discussed as its antioxidant activity and interference with DNA synthesis coupled with enhancement of detoxification process (Badary and Gamal-el-Din, 2001; Zhou *et al.*, 2003; Gali-Muhtasib *et al.*,2006; Amr, 2009). A fraction of the ethanolic extract of *N. sativa* seeds was studied in mice against intraperitoneally implanted murine P388 leukemia and subcutaneously implanted Lewis lung carcinoma cells. The life span of treated mice increased by 153% as compared to directly sulphoxide treated control mice. α- Hederin, a triterpene isolated from this fraction, produced significant tumor inhibition rates while the underline mechanism(s) of antitumor activity of hederin remained to be established. Topical application of *N. sativa* and *C. sativa* extracts inhibited two-stage skin carcinogenesis in mice induced by dimethyl benzanthracene and croton oil. The in vivo and in vitro inhibitory effect of thymoquinone against benzo (a) pyrene induced stomach carcinogineses are also reported in mice (Salomi *et al.*, 1991). Worthen *et al.* (1998) have tested in vitro a crude gum, a fixed oil and two purified components of the seeds thymoquinone (TQ) and dithymoquinone (DTM) for their cytotoxicity to several parental and multi-drug resistant tumor cell lines.

The gum and the oil were devoid of cytotoxicity while both TQ and DTM were cytotoxic to all of the cell lines. Both the parental cell lines and their corresponding MDR variant (that were resistant to several) standard antineoplastic drugs were equally sensitive to TQ and DTM. The study was also conducted on the structural activity relationship of 27

different analogy of TQ. Among these compounds, TQ-2G, TQ-4A1 and TQ-5A1 were found to be more potent than TQ in terms of inhibition of cell growth, induction of apoptosis and modulation of transcription factor-NF-κB. The novel analogs were also able to sensitize gemcitabine and oxaliplatin-induced apoptosis in MiaPaCa-2 (gemcitabine resistant) PC cells, which was associated with down-regulation of Bcl-2, Bcl- xL, survivin, XIAP, COX-2 and the associated prostaglandin E2 (Banerjee *et al.*, 2010).

2.11.9 Antidiabetic activity

Al-Awadi and Gumma (1987) have reported the use of a plant mixture containing *N. sativa,* myrrh, gum, asafetida and aloe by diabetics in Kuwait. They studied the effect of these drugs for their glucose lowering effect in rats and found it to be effective. Further studies on the plant mixture containing N. sativa revealed that the blood glucose lowering effect was due to the inhibition of hepatic gluconeogenesis and the plant extracts mixture may prove to be useful therapeutic agent in the treatment of non-insulin dependent diabetes mellitus (Al-Awadi *et al.,* 1991; Mohamed *et al.,* 2009). The volatile oil of *Nigella sativa* alone also produced a significant hypoglycemic effect on normal and alloxan induced diabetic rabbits without changes in insulin levels (Al-Hader *et al 1993)* *Nigella sativa* seed extract when given orally decreased the elevated glucose levels in alloxan induced diabetic rabbits after two months of treatment. Another study was designed to investigate the possible insulinotropic properties of *Nigella. sativa* oil in streptozotocin plus nicotinamide induced diabetes mellitus in hamsters. After four weeks of treatment with *Nigella sativa* oil significant decrease in blood glucose level together with significant increase in serum albumin level were observed (Farah *et al.*, 2002). The study was also confirmed for it protective effects in diabetes for crude extracts and n-Hexane extract of *Nigella sativa* seed (Matira and Zesmin, 2008). The clinical study of

Nigella sativa on 60 diabetic patients demonstrates significant improvement with reference to total cholesterol, low density lipoprotein cholesterol (LDL- C), and fasting blood glucose indicating effective as an add-on therapy in patients of insulin resistance syndrome (Najmi *et al.,* 2008).

In another study, Nadia and Taha (2009) evaluated the effect of *Nigella sativa* seed oil and thymoquinone on oxidative stress and neuropathy in Streptozotocin induced diabetic rats. The results indicated to marked increase in norepinephrine and dopamine concentrations and a marked decrease in serotonin concentration compared to the control group. These findings were partly reversed by oral administration of either *Nigella sativa* oil or TQ.

2.11.10 Antimicrobial activity

The antibacterial effect of the phenolic fraction of *Nigella sativa* oil was first reported by Topozada *et al*. (1965). The extract and the oil have been reported to have a broad spectrum of activity against a number of microbes. In vitro antibacterial effects of the essential oil showed pronounced activity even in 1:1000 dilutions against several organisms that include *Staphylococcus aureus, E. coli, Salmonella typhi, Vibrio cholera.* The oil was more effective against gram positive than gram negative organism. El-Kamali *et al.* (1998) using the plate diffusion method confirmed the report and showed that essential oil was effective against gram positive (*Bacillus substilis* and *Staphylococcus aureus*) and gram-negative bacteria (E. coli and *Pseudomonas aeruginosa*) the antibacterial effect was maximal when *Bacillus substilis* was used. The oil was found to have excellent antifungal activity particularly against Aspergillus species. In a study using murine cytomegalovirus as a model intraperitoneal administration of oil substantially decreased the viral load in liver and spleen (Salem and Hossain, 2000).

2.11.11Antiparasitic activity

Nigella sativa oil has been shown to possess anticestodal and antinematodal properties. In a recent study. *Nigella sativa* oil was shown to be effective in reducing the number of *Schistosoma mansoni* worms in the liver and decreased the total number of ova deposited in both the liver and the intestine (Mahmoud *et al.,* 2002; ElShenawy *et al.,* 2008).

Nigella sativa has also recently been shown to be effective against other helminths such as *Hymenolepsis nana* (Ayaz *et al.,* 2007), It performs this function by augmenting host immunity. Similar protective effects were seen against other worms such as *Trichinella spiralis* and *Aspiculuris* (AbuElEzz, 2005).

2.11.12 Antimalarial

Various extracts of *Nigella sativa* found to show anti-plasmodial activity against both in vivo and in vitro plasmodia infections. It shows 100 % inhibition of the parasite growth (*Plasmodium falciparum*) at concentration 50 ug/ml. *Nigella sativa* shows dose dependent activity against parasite (Abdulela and Zainal-Abidin,2007; El-Hadi *et al.,* 2010).

2.11.13 Analgesic and anti-inflammatory activity

Houghton *et al.* (1995) reported that crude fixed oil of *Nigella sativa* and an active principle thymoquinone (TQ) inhibits cycloxygenase and 5-lipooxygenase pathway of arachi-donate metabolism in rat peritoneal leukocytes. The effect was demonstrated via the dose dependent inhibition of the formation of thromboxane B2 and leukotrienes B4. This effect was later confirmed in experimental animal studies conducted using aqueous suspension of *Nigella sativa* crushed seed by Al-Ghamdi (2001). In this study, formation of edema in rat hind paw was inhibited and these effects were comparable with aspirin used as a standard antiflammatory drug. Khanna *et al*. (1993) using three antinociceptive tests in rats and mice (hotplates test, tail pinched test, acetic acid induced writhing) conclude that the fixed oil of the seeds is endowed with strong antinociceptive actions

and these actions were due to an opioid principle in the oil as they were antagonized by naloxone. The mechanism of anti-inflammatory and analgesic effect seems to be related to the inhibition of eicosanoid synthesis as suggested by the study of Houghton *et al*. (1995).

2.11.14 Antinociceptive effects

Study showed that the oral administration of *Nigella sativa oil* extracted from Egyptian *Nigella sativa* seeds produces a suppressive effect on nociceptive responses caused by thermal, mechanical and chemical nociceptive stimuli in mice, and that the antinociceptive effect of *Nigella sativa* oil is partly attributable to its component, thymoquinone. It also revealed that at least the supraspinal opioid systems are involved in the antinociceptive effect of thymoquinone (Al-Shebani and Al-Tahan, 2009).

2.11.15 Anti-ulcer activity

The aqueous extracts of *N. sativa* seeds was effective in reducing the ulcer index induced by aspirin by about 36% (Rajkapoor *et al.,* 1996). In other study oil of seed of *Nigella sativa* found to show protective effects on the formation of stress gastritis in hypothyroidal rats (Abdel-sater *et al.,* 2009). A clinical study also supported the eradication of *Helicobacter pylori* in patient with non-ulcer dyspepsia (Salem *et al.,* 2010).

2.11.16 Anti-histaminic action

The antihistaminic effect was first investigated by El- Dakhakhany (1982) who reported the protective action of thymoquinone and carbonyl fraction of *N. sativa* against histamine-induced bronchospasm in guinea pigs. Furthermore, an in vitro study demonstrated that nigellone, isolated from *Nigella sativa*, effectively inhibited the release of histamine from mast cells, possibly through decrease in intracellular calcium and inhibition of protein kinase C (Chakarvarti, 1993). These effects together with analgesic

and anti-inflammatory actions perhaps can be correlated with the use of *Nigella sativa* in eczema and asthma, for scorpion and spider stings and for the bites of cat, dog and snake, recommended in the folk medicine (Al-Jishi and Hoziafa 2003).

2.11.17 Antihyperlipidemic effects

Seeds of *Nigella sativa* were evaluated in several animals' models for lipid lowering activity in which orally administered extracts of the seed showed promising activity. It reduces the serum cholesterol and lipoprotein level significantly (Le *et al.,* 2004; El DakhaKhani *et al.,* 2000; Muhammad & Muhammad 2007; Khadiga *et al.,* 2009; Bahram *et al.,* 2009; Ghanya *et al.,* 2011). The study was also conducted on human being by administering the powder of seeds of *Nigella sativa* before breakfast for two months and was found to reduce the total cholesterol, triglycerides, LDL-cholesterol to a highly significant extent (Inayat *et al.,* 2009; Datau *et al.,* 2010).

2.12 Effect *Nigella sativa* on cardiovascular system

Nigella sativa alone or in combination with honey or garlic are promoted for the treatment of hypertension which drew the attention of El-Tahir *et al.* (1993) to investigate the action of the volatile oil of *Nigella sativa* and its active constituent thymoquinone on the arterial blood pressure and heart of anaesthetized rats. Both agents produce a dose dependent decrease in the arterial blood processor and heart rates. These effects were significantly antagonized by atropine, cyproheptadiene and hexamethonium. This suggests that these effects were centrally antagonized mainly via the involvement of 5-hydroxy tryptaminergic and muscarinic mechanism. Oral dose of 0.6 ml/kg/day of *Nigella sativa* extract produced a significant hypotensive effect in spontaneously hypertensive rats.

These findings were significantly comparable with the standard anti-hypertensive drug nifedipine (Zaoui *et al.,* 2002). The effect of the drug was concluded to be partially due to its diuretic effect which was comparable to 0.5 mg/kg/day furosemide. In one study,

two-month dietary supplementation with *Nigella sativa* extract to normal rats has shown a homogenous cardiac hypertrophy and enhanced cardiac contractility at baseline conditions. The hearts of Nigella-treated rats developed a moderate but significant hypertrophy that was evident by an increase in the heart weight to body weight ratio. The observed Nigella-induced cardiac hypertrophy was associated with an increase in the baseline cardiac inotropic properties (Yar *et al.,* 2008).

In Unani medicine *Nigella sativa* is used for stomachache and as a digestive, carminative, laxative and anti-jaundice (Chopra *et al.,* 1956). Oral *Nigella sativa* powder was reported to relieve flatulence. While Nigellone, an active principle of *Nigella sativa* was found to antagonize histamine induced contractions of guinea pig intestine. In addition, to this a choleretic effect of *N. sativa* oil and its active principles (thymoquinone, thymohydroquinone and dithymoquin-one) reported, respectively (Mahfouz and El-Dakhakhany, 1960). El-Dakhakhani *et al*. (1965, 2000) investigated the effect of *Nigella sativa* oil on gastric secretion and ethanol-induced ulcer in rats. Reported to significant increase in mucin content, glutathione level as well as a significant decrease in mucosal histamine content and ulcer formation, with a protection ratio of 53.56%, was found in the *Nigella sativa* oil pretreated group. More recently, the crude extract of *Nigella sativa* was shown to cause a dose dependent (0.1 to 3.0 mg/ml) relaxation of spontaneous contractions of rabbit jejunum as well as inhibition of K +- induced contractions in a similar dose range, suggestive of calcium channel blockade (Gilani *et al.,* 2001). Abdel-Sater (2009) investigated the protective effects of *Nigella sativa* on hypothyroidism induced development of acute cold restraint stress gastritis in rats.

2.12.1 Effect of *Nigella sativa* on respiratory system

El-Tahir *et al*. (1993) reported that volatile oil of *Nigella sativa* seeds produces dose dependent increases in the respiratory rate and intratracheal pressure of guinea pig. When

the study was conducted only using thymoquinone, in the active principle of volatile, it was found that it only increased the intratracheal pressure without having a significant effect on the respiratory rate, thus the author suggest that volatile oil could be used as potential respiratory stimulant if thymoquinone is removed from the oil. Thus, the oil then can be used in asthma. Gilani *et al*. (2001) studied the effect of a crude extract of *Nigella sativa* seed on isolated rabbit jejunum and guinea pig tracheal preparation. The extract was found a dose dependent relaxation of spontaneous contraction in the rabbit jejunum and inhibition of KCl induced contractions. These actions were similar to those produced by verapamil, a Ca ++ - channel antagonist. The above pharmacological activities of the petroleum ether fraction of the extracts were about 10 times higher than those of the crude extract. In an in vitro experiment carried out by Chakravarti (1993) it is suggesting that nigellone, a carbonyl polymer of thymoquinone isolated from seeds of *Nigella sativa* was found to inhibit effectively the histamine release from the mast cells thus showing the basis for its traditional use in asthma. The results of clinical study of *Nigella sativa* conducted in children showed to manage the wheeze associated with lower respiratory tract illness (Jameel *et al.,* 2009). In another clinical study on forty (40) chemical war victims, Mohammad and Javad (2008) investigated the effect of *Nigella sativa* on respiratory symptoms. They were recorded symptoms score in three different visits and found significant improvement in all respiratory symptoms score and wheezing in second and third visits compared to first visits.

2.12.2 Effect of *Nigella sativa* on nervous system

Nigella sativa seeds revealed promising narcotic analgesic activity mediated possibly through opioid receptors (Khanna *et al.,* 1993). The oil from the seeds exhibited central nervous system (CNS) depressant and potential analgesic effect. It was also found to potentiate pentobarbitone induced sleeping time. The study conducted on cultured

cortical neurons and influence of neurotransmitters release showed to indicate increased secretion of neurotransmitters. It also modulates amino acid release in cultured neurons. There was increased in Gamma Amino Butyric Acid (GABA) activity while secretion of glutamate, aspartate and glycine was found to decrease. All the results represented the sedative and depressive effects of *Nigella sativa* seed extract (Tarek *et al.,* 2010). Repeated administration of *Nigella sativa* was also found to decrease the turnover of 5HT and produces anxiolytics activity (Perveen *et al.,* 2009).

Thymoquinone is the major constituent of *Nigella sativa* seeds. In one of the study conducted in mice, thymoquinone reported to show the anticonvulsant activity (Hosseinzadeh *et al.,* 2004; Hosseinzadeh and Paravardeh, 2005).

2.12.3 Effect of *Nigella sativa* on immune system

As a natural remedy, people take *Nigella sativa* seeds or oil is a promoter of good health and for the prophylaxis of common cold and asthama. In view of that, El-Kadi and Kandil, (1986) investigated the effect of *Nigella sativa* on immune system and found that the drug has immuno potentiating properties in human T-cells in vitro. This was confirmed by Haq *et al.* (1995) who showed that *Nigella sativa* seeds activate T-lymphocyte to secrete the interleukin, IL-3 andIL-1B production. In further experiment, they purified the proteins in the whole *Nigella sativa* seeds and it should be noted that some proteins have suppressive and other shave stimulatory properties in lymphocyte culture (Haq *et al.,* 1999).

2.12.4 Effect of *Nigella sativa* on *Genitourinary System*

The study showed that the volatile oil of *Nigella Sativa* inhibited spontaneous contraction of rats and guinea pig uterine smooth muscle induced by oxytocin (Aqel and Shaheen 1996). It was also reported that *Nigella sativa* crude oil induced uterine contractions both in vivo in pregnant rabbits and in vitro of non-pregnant rat uteri (El-Naggar and El-Deib,

1992). Similarly, it was found that the hexane extract of *Nigella sativa* exhibited mild uterotropic activity and prevented pregnancy in rats when given on day 1 to 10 post-coitum (Keshri *et al.,* 1995).

2.12.5 Effect of *Nigella sativa* on reproductive system

Sixty days study of *Nigella sativa* seeds shows an increase in the weight of reproductive organs, sperm motility and count in cauda epidydimides and testicular ducts. Spermatogenesis was found to increase at primary and secondary spermatocyte. While in fertility, there was increase in number of female pregnant rats (Mukhallad *et al.,* 2009; Al-Sa'aidi *et al.,* 2009).

2.13 Effect of *Nigella sativa* on blood

In view of that the petroleum ether extract of *Nigella sativa* was studied for its action on blood coagulation and was reported to shorten the whole blood clotting time, plasma clot time and kaolin-cephalin clotting time of male rabbits when compared to control. In addition, a significant shortening of bleeding time in rats was also observed. However, there were no significant effects on the thrombin time or prothrombin time but the partial thromboplastin time was shortened while euglobulin time was prolonged (Ghoneim *et al.,* 1982).

The seed extracts and its constituent appear to have a low level of toxicity. The toxicity of fixed oil (10 ml/kg for 12 weeks) of *Nigella sativa* seeds in mice and rats were investigated through the determination, of LD50 values and examination of possible biochemical, hematological and histopathological changes. The low toxicity of *Nigella sativa* fixed oil was evidenced by high LD50 values (11.915 ml/kg), key hepatic enzyme stability and organ integrity values. This suggests a wide margin of safety for therapeutic doses of fixed oil and *Nigella sativa* seeds. The LD50 value of thymoquinone was found to be 2.4 g/kg.

Inclusion of thymoquinone in the drinking water of mice at concentration of 0.03% for 90 days resulted in no signs of toxicity except for significant decrease in fasting plasma glucose concentration (Zaoui *et al.,* 2002).

In a recent study of diazinon induced organ toxicity, with *Nigella sativa* seeds extracts given orally for three and six weeks, the study observed attenuated extensive changes of hematological and biochemical parameters in diazinon- treated rats. Based upon these results, they suggested *Nigella sativa* seeds can be considered as a promising therapeutic agent against hematoxicity, immunotoxicity, hepatoxicity, nephrotxicity and cardiotoxicity induced by diazinon and may be against other chemical pollutants, environmental contaminants and pathogenic factors (Atef *et al.,* 2010). Some other studies also demonstrate that treatment with *Nigella Sativa* resulted in significant decrease of haematological disorders induced by aflatoxin (Abdel-Wahhab and Aly, 2005) and cadmium (Demir *et al.,* 2006). No remarkable pathological changes were recorded in bone marrow of animals treated with suspension of *Nigella Sativa* in carbon tetrachloride induced bone marrow toxicity (Abou gabal e*t al.,* 2007).

2.14 The use of *Nigella sativa* seed as preservative for yoghurt and other food products

Nigella sativa is a confluence of functional bioactive compounds and phytochemicals. Apart from the chemical components of the seed, oil extract from the seed is another rich source of beneficial bioactive compounds. The seed contains fixed oil which ranges between 28 to 36 % and the oil is majorly composed of unsaturated fatty acids including arachidonic, eicosadienoic, linoleic and linolenic, saturated fatty acids including palmitic, stearic, and myristic (Hajhashemi *et al*., 2004). The oil and seed constituents of *Nigella sativa* are reported to have different immunomodulatory and immunotherapeutic potentials and possess reproducible antioxidant effects through enhancing the oxidant

scavenger system; the oil has also been reported to possess a hepatoprotective effect in some models of liver toxicity (Salem, 2005).

Although, most studies investigated pharmaco-medicinal benefits of *Nigella sativa* seed and its oil, emerging scientific evidences are supporting the beneficial effects of the black seed as preservative for milk and other food products because of its bioactive component. In advancing of the possibility of exploiting the antioxidant properties of the black seed on yoghurt, Okur (2021) reported that the augmentation of yoghurt with black seed increased the total phenolic characteristic and antioxidant activity of yoghurt and the outcomes significantly indicate that yoghurt with black cumin might become a popular dairy product in the future. Similarly, preservation of cheese with *Nigella sativa* oil was reported to increase the shelf-life, nutritional qualities, and elimination of pathogenic microorganisms from the cheese (Alnaemi and Alsawaf, 2011).

In a contrast study where extract of the black cumin was used in meat preservation, black cumin-treated meat samples had lowered thiobarbituric acid reacting substances, free fatty acids, peroxide, formation of protein carbonyls, and off-odour or rancid odour development were lower than the control and values that were treated with synthetic antioxidant butylated hydroxyl tuolene (BHT) which is a conventional meat preservative (Pranav *et al*., 2018).

Microencapsulation of *Nigella sativa* seeds oil for producing functional yogurt led to the recommendation that the black seed could be used for elongating the shelf-life of yoghurt, improved its chemical composition, and sensory properties as reported by Abedi *et al*. (2016). Also, *Nigella sativa* was reported to exert antimicrobial cum antioxidant effect when used as a nano composite film based for preservation of fish (Sani *et al*., 2021). The addition of *Nigella sativa* oil was also reported as a safe natural antimicrobial and immunostimulant additive in cheese because it exerts lethal effect on *L. monocytogenes*

at refrigeration temperature, hence improving the keeping quality of cheese (Fadel and Ismail, 2016).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location of the experiment

The research was carried out at the Animal Production Laboratory in Federal University of Technology, Gidan Kwano Campus, Minna, Niger State. Minna is located between Latitude 9°37' North and and 11^0 30' North and Latitude 3030' North and 70 20' East. The temperature rande of Minna is between 23^0C and 34^0C with humidity between 90%-98% and atmospheric pressure of about 1012 – 1015mb. (Accuweather, 2021).

3.2 Source of raw materials and identification

The *Nigella sativa* seeds were purchased from a reputable herbal shop at the Kure Ultra-modern market in Minna, Niger State, Nigeria. The seeds specimen was identified and authenticated by a plant taxonomist at the Department of Biological Sciences, Faculty of Life Sciences, Federal University of Technology, Minna. The raw milk for yoghurt preparation was obtained from a Maizube farm, Niger State. The milk was aseptically collected over ice packs and immediately transported to the Animal Production Laboratory. Starter culture was purchased from a reputable store in the market.

3.3 Source of chemical and reagents

The solvents, gallic acids, quercetin, tannic acid, media, methanol were obtained from Sigma Aldrich U.S were of analytical grade.

3.4 Preparation of *Nigella sativa* seeds

Seed materials were decontaminated by removing extraneous substances including soil dust, particles and other residues that may influence analytical result, This involved briskly brushing the seeds, washing with water and rinsing with distilled water, followed by drying in the oven at 30°C to stabilize the seeds samples as described by Imtiaz *et al.* (2013).

3.5 Extraction of *Nigella sativa* oil from the seeds

Oil extract of *Nigella sativa* was obtained using n-hexane solvent with as light modification of the procedure of Erkan *et al*. (2008). One, (1kilogram) of *Nigella sativa* seed was subjected to decoction with distilled water. The strong decoction was collected exhaustively with n-hexane and evaporated to remove the n hexane fraction. This was labeled as *Nigella sativa* oil extracts (NSOE) and stored in refrigerator at 4^{o}C until when required for use.

3.6 Determination of phytochemical constituents of *Nigella sativa* oil

The qualitative and quantitative tests were carried out on the powdered samples of the dried and roasted seeds using standard procedures described as follow:

3.6.1 Qualitative phytochemical screening of seed

3.6.2 Test for Flavonoids

The method of Harbone (1973) and sofowora (1993) was used to test for Flavonoids in the seeds. A portion of the powdered plant in each case was heated with 10 ml of ethyl acetate in a test tube over a steam bath for 3 minutes. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. Yellow colouration was observed thus indicating the presence of flavonoids.

3.6.3 Test for tannins

The methods of Harbone (1973) and Sofowora (1993) were used to test for tannins using the seeds. Briefly, A portions of (0.5g) of the dried powdered seed sample was boiled in 20ml distilled water in a test tube and filtrate. 0.1% ferric chloride ($FeCl_3$) solution was added to the filtrate. The appearance of brownish green or a blue-black colouration indicate the presence of tannins in the test sample.

3.6.4 Test for saponins

The method of Harbone (1973) and Sofowora (1993) was used to test for saponins in the seeds. 0.2g of the powdered sample of *Nigella sativa* was boiled in 20ml of distilled water

in a test tube in boiling water bath and filtered. Ten (10) ml of the filtrate was mixed with 5 ml of distilled water and was shaken vigorously to the formation of a stable persistent froth. The frothing was mixed with drop of olive oil and shaken vigorously with the formation of emulsion thus a characteristic of saponins (Harborne, 1973)

3.6.5 Test for alkaloid

The method of Harbone (1973) and Sofowora (1993) was used to test for alkaloid in the seeds. Exacts 0.5g of *Nigella sativa* extract was stirred with $5cm^3$ of 1% aqueous HCI on a steam bath, few drops of picric acid solution was added to $2cm^3$ of the extract. The formation of a reddish-brown precipitate was taken as a preliminary evidence for the presence of alkaloids (Trease and Evans, 1978).

3.6.6 Test for steroids

The method of Harbone (1973) and Sofowora (1993) was used to test for steroids in the seeds. Exacts 0.5g of *Nigella sative* extract was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The colour changed from violet to blue or green in some samples indicated the presence of steroids

3.6.7 Test for cardiac glycosides (Keller-Killani test)

The method of Harbone (1973) and Sofowora (1993) was used to test for flaronoids in the seeds. Exact (5mL) of *Nigella sativa* extracts was mixed with 2ml of glacial acetic acid containing one drop of ferric chloride (Fecl3) solution, followed by the addition of 1ml concentrated sulphuric acid. Brown ring was formed at the interface indicates deoxysugar characteristics of cardenloides. A violet ring was also observed beneath the brown ring, while in the acetic acid layer; a greenish ring was formed gradually throughout the thin layer (Harborne, 1973).

3.6.8 Test for anthraquinones (Borntrager's test)

Exact 0.5g of plant extract was shaken with 5ml chloroform, the chloroform layer was filtered and $0.5cm^3$ of 10% ammonia was added to the filtrate. The mixture was shaken

thoroughly, the formation of a pink/violet or red, yellow colour in the ammonical phase indicates the presence of anthraquinones (Harborne, 1973).

3.6.9 Test for reducing sugar (Benedict test)

A quality exact 0.5g of *Nigella sativa* extract was mixed thoroughly with 3cm^{3}distill water and filtered, 3 drops of the filtrate were added to 3cm^3 Benedict reagents and placed in a boiling water bath for 5minutes. The formation of a brick red precipitate indicates reducing sugar (Harborne, 1973).

3.7 Data collection on quantitative phytochemical screening of *Nigella sativa seed o*il

3.7.1 Determination of total phenol

The method of Harbone (1973) and Sofowora (1993) was used to test for phenol in the seeds. The sample were defatted with 100ml of diethyl other using a soxhlet apparatus for 2hr. the fat free sample was boiled with 50ml of petroleum ether for the extraction of the phenolic component for 15min. 5ml of the *Nigella sativa* extract was pipetted into a 50ml flask, then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30min for colour development. This was measured at 505nm. Tannic acid was used to establish the calibration curve (Edeoga *et al*., 2005)

3.7.2 Determination of total flavonoid

Total flavonoid was determined using aluminium chloride colorimetric method (Chang *et al*., 2002). Quercetin was used to establish the calibration curve. Exactly 0.5ml of the diluted sample was added into test tube containing 1.5ml of methanol. 0.1ml of 10% $AlCl_3$ solution and 0.1ml sodium acetate ($NaCH_3COO^-$) were added, followed by 2.8ml of distilled water. After incubation at room temperature for 30min, the absorbance of the reaction mixture was measured at 415nm. The amount of 10% $AlCl_3$ was substituted by the same amount of distilled water in blank.

3.7.3 Determination of total akaloids

0.5g of the sample was dissolved in 96% ethanol -20% H_2SO_4 (1:1). 1ml of the filtrate was added to 5ml of 60% tetraoxosulphate (VI) and allowed to stand for 5min. Then; 5ml of 0.5% formaldehyde was added and allowed to stand for 3h. The reading was taken at absorbance of 565nm. The extinction coefficient (E296, ethanol {ETOH} = 15136$M^{-1}cm^{-1}$) of vincristine was used as reference alkaloid (McDonald *et al.*, 2001)

3.7.4 Determination of saponins

0.5g of the sample was added to 20ml of 1NHCl and was boiled for 4h. After cooling it was filtered and 50ml of petroleum ether was added to the filtrate for ether layer and evaporated to dryness. 5ml of acetone ethanol was added to the residue. 0.4mls of each was taken into 3 different test tubes. 6ml of ferrous sulphate reagent was added into them followed by 2ml of concentrated H_2SO_4. It was thoroughly mixed after 10min and the absorbance was taken at 490nm. Standard saponin was used to establish the calibration curve (Oloyede, 2005).

3.7.5 Determination of tannin

0.2g of sample was measured into a 50ml beaker. 20ml of 50% methanol was added and covered with para film and placed in a water bath at 77-80^{O}C for 1hr. it was shaken thoroughly to ensure a uniform mixture. The extract was quantatively filtered using a double layered whatman No.41 filter paper into a 100ml volumetric flask, 20ml water added, 2.5ml Folin-Denis reagent and 10ml of Na_2CO_3 were added and mixed properly. The mixture was made up to mark with water mixed well and allowed to stand for 20min for the development of a bluish-green colour. The absorbances of the tannic acid standard solutions as well as samples were read after colour development on a UV-spectrophotometer model 752 at a wavelength of 760nm (AOAC, 2000).

3.8 Production of yoghurt

Stirred yoghurt was prepared with slight modification to the procedure described by Ihemeje *et al*., (2015). The basic ingredient for the stirred yoghurts includes 100 % whole milk, skimmed milk, water, 4 % starter culture, and sugar. The whole milk (raw milk) composition was adjusted to raise its total solid content to 14-16 g/100 g by dissolving non-fat dry milk (Arla Dano skimmed milk) into the whole milk in a plastic bowl and it was then homogenized properly. The milk solution obtained was pasteurized at 65 °C for 20 minutes while stirring frequently. Cooling of the pasteurized milk to about 47 °C was achieved by immersing into a basin of cold water. On cooling,500g of sugar was added to the milk solution followed by inoculation of the milk with commercially bought starter cultures containing culture mix of *Lactobacillus spp* and *Streptococcus thermophilus*. This was then properly homogenized. The inoculated milk was incubated at 42 °C for 12-24 hours. After incubation, the yoghurt produced (with pH of about 4.3-4.5) was cooled in an ice bath and stirred before refrigerating. This was followed by addition of various inclusion levels of the extracts of *Nigella sativa* before refrigeration.

3.9 *Nigella sativa oil* inclusion levels

Yoghurt produced was separated into six (6) aseptic flasks of 100 mL each and categorized as treatment1 without the addition of *Nigella sativa* oil extract but with conventional preservative (Sodium benzoate) at 0.1% v/v and was tagged $NSOE_0$. Treatment 2 (2 %v/v $NSOE_2$), Treatment 3, 4, 5 and 6, contained 4, 6, 8 and 10 % v/v *Nigella sativa* oil extracts and tagged as $NSOE_3$, $NSOE_4$, $NSOE_5$ and $NSOE_6$, respectively.

3.10 Experimental design

Completely randomized design was used for the experiment. There were six treatments each with three replicates. A total number of 105 samples were established and analyzed,

with each treatment having 15 samples. The sample analyses were carried out at 7 days interval (0, 7, 14, 21, 28, 35 and 42), under cold storage.

3.11 Data collection on analysis of yoghurt samples

The analytical units (yoghurt samples) were homogenized and kept under refrigeration for further analysis. Prior to refrigeration, the initial pH, total bacteria count and proximate analysis of the samples were carried out. The sample units were stored in the refrigerator at 4^0C for 42 days. They were assessed or analyzed at 7 days interval for 42days.

3.12 Proximate composition of the yoghurt samples

The moisture and ash contents of the yoghurt samples were determined using the indirect method of employing oven drying and furnace incineration. Soxhlet's method was used to determine the fat contents of the samples on wet weight basis. Crude protein and total ash in the samples were determined by the procedures of AOAC (2002).

3.13 Determination of physical properties

The following physical property: colour was determined according to Adriana *et al.,* (2018).

3.14 Physicochemical analysis

3.14.1 Total titratable acidity (TTA):

The titratable acidity was determined using the AOAC (2012) method. Ten (10) ml of the samples were measured into conical flasks and two drops of phenolphthalein indicator (2 ml) was added to each yoghurt sample and titrated with 0.I M NaOH to the first permanent pink colour. The acidity was reported as the percentage lactic acid by weight.

$$\text{Titrable acidity (\%)} = \frac{\text{Quantity of NaOH (ml)} \times 0.009 \times 100}{\text{Quantity of sample}}$$

3.14.2 Determination of pH

The pH was determined according to AOAC (2012), using a pocket pH meter. The pH meter was calibrated using a 7.0 buffer solution and rinsed with water; it was then put into 25 ml of sample and reading was recorded after stabilization.

3.14.3 Determination of viscosity

The viscosity was measured using a viscometer (Model 800, OFITE; OFI Testing Equipment, Inc., Houston, Texas, U.S.A). Samples were put in a stainless measuring cylinder and viscosity readings taken on the viscometer at 600 rev/min

3.14.4 Determination of total solids

The total solid was determined using the method described by AOAC (2012). Three (3)grams of the sample was weighed into a dry petri dish of a known weight. The total portion was pre-dried for 25 minutes on a steam bath and then dried for 3 hours at 100°C in a forced draft air oven. The total solid of the sample was estimated as the weight of the dried sample residue and was calculated as:

$$\%\ \text{Total solid} = \frac{\text{W2} - \text{W1}}{W1 - W} \times 100$$

Where: W = weight of the petric dish W1 = weight of the petric dish and sample test portion, and W2

= weight of petric dish and dry sample. Total solids (dry matter) was checked by subtracting % moisture content from the mass (100)

3.14.5 Determination of total solids-non-fat:

The total solids-not-fat was determined as described by AOAC (2012). It was obtained by taking the difference between % total solids and % fat content.

That is: % Solids-Non-Fat = % Total Solids - % Fat content

3.14.6 Microbiological analysis of samples

The apparatus used for the bacteriological analysis were washed and sterilized using autoclave. Each treatment sample was serially diluted by aseptically introducing 1ml of inoculated yoghurt sample into a test-tube containing 9 mls of sterile distilled water and homogenized by shaking to make 9:10 dilutions, followed by further dilution up to 10:10cfu/ml concentrations; 10^6cfu/ml concentrations was chosen for inoculating freshly prepared agars. A1ml quantity of 10^6cfu/ml concentration (appropriately diluted sample) was used to inoculate freshly prepared media. The plates were allowed to solidify and then incubated in the incubator for 24hours at 37°C.The organisms in the cultured plates were sub-cultured on different selective media such as *Bacilluscereus* agar (BCA), Nutrient agar (NA), Mac-conkey agar (MCA), Mannitol salt agar (MSA), *Salmonella-shigella* agar (SSA) and *Brucella* agar (BrA), for the enumeration of *Bacilluscereus*, total bacteria counts, *coliform*, *Staphylococcus* and *Salmonella*/*Shigella* counts.

The media was aseptically prepared and sterilized by autoclaving at I21°C for 15minutes and allowed to solidify before inoculation. Inoculated plates of the various agars used were inoculated at 37 °C for 48 hours. The counts were expressed in colony forming units per millilitre of samples (cfu/ml). The isolates were subculture severally on a fresh media to obtain pure cultures using streak plate count method. These pure cultures were maintained on agar slants for further characterization and identification of bacteria isolates. The colonies that appeared were counted using colony counting chamber and the counts recorded.

3.15 Enumeration of microbial population:

Total plate counts for the nutrient and Mac-Conkey agar were done by counting colonies at the reverse side of the culture plates. Total colony count was expressed in colony

forming units per millilitre (cfu/ mL) (Harrigan and McCance, 1990). Plate counts were done using colony counter for the yeasts and hand lens for moulds.

3.16 Sensory evaluation

The product samples were evaluated using a 9-point hedonic scale for sensory characteristics and overall acceptability by a twenty untrained panellist selected randomly. They were served coded samples of yoghurt, which were scored based on taste, texture, appearance and overall acceptability. These 9 hedonic rating scales ranged from like extremely (9) to dislike extremely (1) as described by (Ihemeje *et al.*, 2015).

3.17 Storage study

The yoghurt samples were stored at 4°C for 42 days. Samples were taken and analyzed at 7 days intervals for total solid non-fat, total titratable acidity, total solids, viscosity, pH, microbial quality and sensory attributes.

3.18 Data analysis

Data obtained was subjected to analysis of variance, for a completely randomized design using S.P.S.S statistical package version 9.2.Where differences occurred, the means were separated using the Duncan Multiple Range Test. The model used for the experiment was

$$Y_{ij} = \mu + a_i + E_{ij}$$

Where Y = Treatment observation

μ = population mean

a_i = treatment effect of the i^{th} treatment where i = (6), i.e *Nigella sativa* inclusion levels (0, 2 ,4, 6 ,8 and 10 % v/v)

E_{ij}= random error

CHAPTER FOUR

4.0 RESULTS

4.1 Qualitative and quantitative phytochemical constituents of *Nigella sativa* oil extracts

Table 4.1 shows the qualitative and quantitative phytochemical constituents of *Nigella sativa* seed oil extracts. Phenol, flavonoids, tannins, terpenoids, saponins, alkaloids, reducing sugar, anthraquinone, cardiac glycosides, steroids, protein, anthocyanins were all present in the oil sample while protein was absent. The quantitative phytochemical constituents of *Nigella sativa* seed oil. Tannins (17.05 mg/g) was found to be the highest phyto constituents of the oil sample, followed by total phenol (11.79 mg/g), terpenoids (5.47 mg/g), flavonoids (2.12mg/g), alkaloids (45.77 µg/g) and), saponins (29.37 µg/g) respectively.

4.2 Physico-chemical parameters of *Nigella sativa* oil extract infused yoghurt during cold storage at 5ºC for 42 days

4.2.1 Changes in pH of *Nigella sativa* oil extract (*NSOE)* infused yoghurt during the cold storage at 5ºC for 42 days

The result of changes in pH of the various samples during the cold storage is presented in Table 4.2.1 The result showed changes in pH of the various samples during the cold storage indicated a significant ($p < 0.01$) for day 0, 7, 28, 35, and 42. A significant ($p < 0.01$) difference in the mean pH of the yoghurt samples with T4 (6 % v/v NSOE infused) having the highest pH value (4.61) and T5 and T6 (8 and 10 % v/v NSOE) which were statically similar having the lowest pH value (3.55). Day 14 and 21 were not significantly ($p>0.01$) affected.

Table 4.1 Qualitative and quantitative phytochemical constituents of *Nigella sativa* oil extracts

Phytochemicals	Qualitative inferences	Quantitative
Phenols	+	11.79 ± 0.03 (mg/g)
Flavonoids	+	2.12 ± 0.12(mg/g)
Tannins	+	17.05 ± 0.24 (mg/g)
Terpenoids	+	5.47 ± 0.26 (mg/g)
Saponins	+	29.37 ± 5.06(µg/g)
Alkaloids	+	45.77 ± 0.56 (µg/g)
Reducing Sugar	+	_
Anthraquinones	+	_
Cardiac glycosides	+	_
Steroids	+	_
Protein	-	_
Anthocyanins	+	_

+ = Present, - = Absent

Table 4.2:1 Changes in pH of *Nigella sativa* oil extracts (*NSOE)* infused yoghurt samples (Mean ± SEM) during the cold storage at 5^0C for 42days

	T1	T2	T3	T4	T5	T6	SEM	p-value
Day 0	4.17^b	4.13^b	4.12^b	4.14^b	4.55^a	4.52^a	0.04	0.001
Day 7	4.41^a	4.45^a	4.38^a	4.42^a	4.05^b	4.14^b	0.03	0.001
Day 14	3.78	3.94	3.83	3.94	3.93	4.12	0.04	0.080
Day 21	3.91	3.70	6.13	5.63	3.51	4.83	0.25	0.800
Day 28	3.54^b	3.67^a	3.68^a	3.46^c	3.43^c	3.53^b	0.05	0.001
Day 35	3.54^a	3.66^a	3.68^a	3.46^b	3.43^b	3.53^a	0.04	0.001
Day 42	4.44^a	4.28^a	4.38^a	4.24^a	4.20^a	4.06^b	0.03	0.001
Mean	4.27^b	4.31^b	3.92^b	4.61^a	3.55^c	3.55^c	0.07	0.001

Means with different superscripts across the same row are significantly different ($p < 0.01$).

T1 = yoghurt + 0.1 % V/V of Sodium Benzoate

T2 = yoghurt + 2 % V/V of *NSOE*

T3 = yoghurt + 4 % V/V of *NSOE*

T4 = yoghurt + 6 % V/V of *NSOE*

T5 = yoghurt + 8 % V/V of *NSOE*

T6 = yoghurt + 10 % V/V of *NSOE*

4.2.2 Changes in total titratable acid values of *Nigella sativa* oil extracts (*NSOE)* infused yoghurt during the cold storage 5^0 for the period of 24days

The result of the total titratable of yoghurt samples during cold storage is presented in Table 4.2.2. The result indicated that significant difference ($p<0.01$) existed in the total titrable acid across all the samples except for day 42 that was not significantly ($p>0.001$) affected. At day 0, 7, 14, 21, 28 and 35 of storage, the values ranges between 0.11 – 0.63 %. T4 recorded the highest significant total titretable acid value (0.63 %) at day 28 of storage and the highest means of (0.38 %).

4.2.3 Changes non-fat solid values of *NIGELLA sativa* oil extracts (*NSOE)* treated yoghurt during the cold storage

The result of the non – fat solid value of yoghurt samples during cold storage is presented in (Table 4.2.3). The result showed that there was significant ($p<0.05$) difference across all the storage days measured. At day (0) of the storage the results ranges between 26.63 % and 35.43% the highest value was recorded by T6 35.43% while the least was recorded by T1 (26.63%). At day (21) of the storage, the results ranges between 28.97 % and 32.40% the highest value was recorded by T2 (32.40%) while the least was recorded by T1 (28.97%). At day (28) of the storage, the results showed that T5 and T6 had similar values while T2, T3 and T4 also had similar values.

However, the highest non solid values was recorded by T5 (32.92 %). At day (35) of the storage, the results showed that T2, T4, T5 and T6 had similar values while T1, and T3 also had similar values. However, the highest non solid values was recorded by T2 (33.85 %). At day (42) of the storage, the results showed that T2, T3, T4 and T5 had similar values while T1, and T6 also had similar values. However, the highest non solid values was recorded by T5 (35.68 %). The mean values of the non solid showed that T6 perform better in terms of the non - fat solid 33.22 %.

Table 4.2:2 Changes in total titratable acid values of *Nigella sativa* oil extracts (*NSOE)* infused yoghurt samples during the cold storage at 5^0C for 42days

	T1	T2	T3	T4	T5	T6	SEM	p-value
Day 0	0.17^{c}	0.13^{c}	0.24^{b}	0.24^{b}	0.14^{a}	0.15^{a}	0.01	0.001
Day 7	0.11^{c}	0.13^{c}	0.12^{c}	0.16^{c}	0.28^{b}	0.34^{a}	0.02	0.001
Day 14	0.38^{ab}	0.45^{a}	0.37^{ab}	0.44^{a}	0.28^{c}	0.33^{ab}	0.01	0.100
Day 21	0.34^{c}	0.29^{c}	0.51^{a}	0.55^{a}	0.30^{c}	0.39^{b}	0.02	0.001
Day 28	0.35^{bc}	0.33^{c}	0.55^{a}	0.63^{a}	0.31^{c}	0.44^{b}	0.03	0.001
Day 35	0.42^{d}	0.44^{a}	0.32^{b}	0.29^{c}	0.28^{c}	0.26^{c}	0.02	0.001
Day 42	0.45	0.41	0.37	0.40	0.34	0.34	0.03	0.320
Mean	0.31^{a}	0.311^{a}	0.35^{a}	0.38^{a}	0.27^{b}	0.32^{a}	0.02	0.05

Means with different superscripts across the same row are significantly different ($p < 0.01$).

T1 = yoghurt + 0.1 % V/V of Sodium Benzoate

T2 = yoghurt + 2 % V/V of *NSOE*

T3 = yoghurt + 4 % V/V of *NSOE*

T4 = yoghurt + 6 % V/V of *NSOE*

T5 = yoghurt + 8 % V/V of *NSOE*

T6 = yoghurt + 10 % V/V of *NSOE*

The result for total solids shows that there is a significant ($p > 0.01$) difference amongst the yoghurt samples for day 0,14,21,35 and 42, however no significant ($p > 0.05$) difference was observed in the mean values of the yoghurt samples Table 4.7.

Table 4.2.3: Changes in total solid (TS) of *Nigella sativa* oil extract (*NSOE)* Treated yoghurt during the cold storage at 5^0C for the period of 42 days

	T1	T2	T3	T4	T5	T6	SEM	p-value
Day 0	28.96a	32.17ab	33.08b	34.02b	37.78c	38.03c	0.87	0.001
Day 7	32.12a	32.95a	31.70a	34.27a	35.17a	38.75b	0.67	0.004
Day 14	30.71a	31.61ab	32.88b	34.30cd	35.70cd	34.42cd	0.44	0.001
Day 21	31.14a	33.04b	34.95b	34.81b	34.63b	36.29c	0.45	0.001
Day 28	30.54a	33.04a	33.34ab	33.46ab	36.92c	36.26b	0.61	0.005
Day 35	32.70a	36.00b	32.81a	36.29b	36.04b	37.30b	0.47	0.001
Day 42	34.07a	36.78b	36.71b	37.74bc	39.12c	37.40bc	0.41	0.001
Mean	31.46a	33.65b	33.64b	34.98b	36.48c	36.92c	0.56	0.050

Means with different superscript across the same row are significantly different ($p < 0.05$).

T1 = yoghurt + 0.1 % V/V of Sodium Benzoate

T2 = yoghurt + 2 % V/V of *NSOE*

T3 = yoghurt + 4 % V/V of *NSOE*

T4 = yoghurt + 6 % V/V of *NSOE*

T5 = yoghurt + 8 % V/V of *NSOE*

T6 = yoghurt + 10 % V/V of *NSOE*

Table 4.2:4 Changes in non-fat solid values of *nigella sativa* oil extracts (*NSOE)* infused yoghurt samples during the cold storage at 5^0C for 42days

	T1	T2	T3	T4	T5	T6	SEM	p-value
Day 0	26.63^{c}	29.67^{c}	30.42^{b}	31.36^{b}	35.28^{a}	35.43^{a}	0.85	0.001
Day 21	28.97^{b}	30.38^{a}	32.40^{a}	31.81^{a}	30.79^{a}	32.19^{a}	0.35	0.001
Day 28	28.54^{c}	30.83^{b}	30.81^{b}	30.86^{b}	32.92^{a}	31.80^{a}	0.44	0.01
Day 35	30.71^{b}	33.85^{a}	30.47^{b}	33.29^{a}	32.38^{a}	33.35^{a}	0.38	0.01
Day 42	32.07^{b}	34.79^{a}	34.63^{a}	34.78^{a}	35.68^{a}	33.48^{b}	0.34	0.01
Mean	29.35^{c}	31.25^{b}	31.15^{b}	32.04^{a}	32.96^{a}	33.22^{a}	0.47	0.05

Means with different superscripts across the same row are significantly different ($p < 0.01$).

T1 = yoghurt + 0.1 % V/V of Sodium Benzoate

T2 = yoghurt + 2 % V/V of *NSOE*

T3 = yoghurt + 4 % V/V of *NSOE*

T4 = yoghurt + 6 % V/V of *NSOE*

T5 = yoghurt + 8 % V/V of *NSOE*

T6 = yoghurt + 10 % V/V of *NSOE*

4.2.4 Changes in viscosity (mm/s^2) of *Nigella sativa* oil extracts (*NSOE)* infused yoghurt during the cold storage at 5^{0}C for 42 days

The result of the viscosity of yoghurt samples during cold storage are presented in (Table 4.2.4). The results showed that there were significant ($p<0.05$) difference across all the storage days measured. At day (0) - day (42) of storage, the result of the 7 types of storage duration ranged between 750.33 **mm/s^2** to 885.33 **mm/s^2**. T1 at day 42 recorded the highest significant total physical observable viscosity (885.33 **mm/s^2**) while the least physical observable viscosity value (750.33 **mm/s^2**) was recorded by T1 at day 7 of storage and the highest means of 783.52mm/s2.

4.3 Proximate composition of *Nigella sativa* oil extracts (*NSOE)* infused yoghurt during cold storage at 5^{0}C for 42 days

4.3.1 Changes in crude protein of *Nigella sativa* oil extracts (*NSOE)* infused yoghurt during the cold storage at 5^{0}C for 42 days

The result of changes in crude protein of the various samples during the cold storage is presented in Table 4.3.1 The result showed that there was significantly ($p<0.01$) difference across all the storage days measured from day 0, 7, 14, 21, 28, 35 and 42. A significant ($p<0.01$) difference in the mean crude protein of the yoghurt samples showed that T6 (10 % v/v NSOE) showed the highest crude protein value (7.76) while T1 (0.1 % v/v sodium benzoate) had the lowest crude protein value (5.09). However, the mean value showed that T5 and T6 were statistically similar.

4.3.2. Changes in moisture content of *Nigella sativa* oil extracts (*NSOE)* infused yoghurt during the cold storage at 5^{0}C for 42 days

The result of changes in moisture content of the various samples during the cold storage is presented in Table 4.3.2. The result showed that there was significantly ($p<0.01$) difference across the storage days measured from day 0, 7, 21, 28, 35 and 42. However day 14 was not significantly ($p>0.01$) affected. A significant ($p < 0.01$) difference in the

Table 4.2.5 Viscosity (mm/s^2) of *Nigella sativa* oil extracts (*NSOE)* infused yoghurt samples during the cold storage at 5^{0}C for 42days

	T1	T2	T3	T4	T5	T6	SEM	p-value
Day 0	751.33^{c}	751.00^{c}	755.33^{ab}	753.00^{ab}	761.00^{b}	761.66^{a}	0.66	0.001
Day 7	750.33^{c}	751.33^{c}	757.33^{b}	756.00^{b}	759.33^{a}	762.33^{a}	1.03	0.004
Day 14	751.66^{c}	752.00^{c}	756.66^{a}	757.00^{a}	757.00^{a}	762.00^{a}	0.90	0.001
Day 21	789.00^{a}	766.00^{b}	760.00^{b}	758.33^{ab}	756.66^{c}	763.66^{b}	2.66	0.001
Day 28	753.66^{b}	753.00^{b}	755.66^{ab}	757.33^{ab}	756.33^{ab}	763.00^{a}	0.86	0.01
Day 35	803.33^{a}	772.33^{b}	771.33^{b}	758.33^{d}	756.33^{d}	764.00^{c}	3.82	0.001
Day 42	885.33^{a}	849.33^{b}	821.33^{c}	769.66^{d}	756.33^{e}	767.33^{d}	11.61	0.001
Mean	783.52^{a}	770.71^{b}	768.23^{c}	758.52^{d}	757.571^{d}	763.42^{b}	3.08	0.05

Means with different superscripts across the same row are significantly different ($p < 0.01$).

T1 = yoghurt + 0.1 % V/V of Sodium Benzoate

T2 = yoghurt + 2 % V/V of *NSOE*

T3 = yoghurt + 4 % V/V of *NSOE*

T4 = yoghurt + 6 % V/V of *NSOE*

T5 = yoghurt + 8 % V/V of *NSOE*

T6 = yoghurt + 10 % V/V of *NSOE*

Table 4.3.1: Changes in crude protein of *nigella sativa* oil extracts (*NSOE)* infused yoghurt samples during the cold storage at 5^0C for 42days

	T1	T2	T3	T4	T5	T6	SEM	p-value
Day 0	4.49^{e}	5.08^{d}	5.74^{c}	7.00^{b}	7.76^{a}	7.52^{a}	0.30	0.001
Day 7	4.61^{d}	5.39^{c}	6.14^{b}	6.23^{b}	7.53^{a}	7.65^{a}	0.26	0.001
Day 14	5.08^{d}	5.83^{c}	5.92^{b}	6.24^{b}	7.52^{a}	7.82^{a}	0.24	0.001
Day 21	5.54^{d}	5.97^{c}	6.13^{c}	7.51^{c}	7.42^{b}	7.69^{a}	0.20	0.001
Day 28	5.60^{c}	5.66^{c}	6.49^{c}	7.46^{a}	7.93^{a}	7.92^{a}	0.24	0.001
Day 35	5.02^{d}	4.75^{d}	5.76^{c}	6.93^{b}	7.88^{a}	7.79^{a}	0.30	0.001
Day 42	4.97^{e}	5.02^{e}	5.13^{d}	6.07^{c}	7.19^{b}	7.75^{a}	0.26	0.001
Mean	5.09^{d}	5.39^{d}	5.98^{c}	6.78^{b}	7.69^{a}	7.76^{a}	0.26	0.001

Means with different superscripts across the same row are significantly different ($p < 0.01$).

T1 = yoghurt + 0.1 % V/V of Sodium Benzoate

T2 = yoghurt + 2 % V/V of *NSOE*

T3 = yoghurt + 4 % V/V of *NSOE*

T4 = yoghurt + 6 % V/V of *NSOE*

T5 = yoghurt + 8 % V/V of *NSOE*

T6 = yoghurt + 10 % V/V of *NSOE*

mean moisture content of the yoghurt samples showed that T1 (0.1 % v/v sodium benzoate) have the highest moisture content value (68.28) while T6 (10 % v/v NSOE) had the lowest moisture content value (63.02). However, the mean value showed that T2 T3 and T4 were statistically similar, while T5 and T6 were also similar.

4.3.3 Changes in fat content of *Nigella sativa* oil extracts (*NSOE)* infused yoghurt during the cold storage at 5ºC for 42 days

The result of changes in fat content of the various samples during the cold storage is presented in Table 4.3.3 The results showed that there were significantly ($p<0.01$) differences across the storage days measured from day 7, 14, 21, 28, 35 and 42. However day 0 was not significantly ($p>0.01$) affected. A significant ($p<0.01$) difference in the mean fat content of the yoghurt samples showed that T6 (10 % v/v NSOE) have the highest fat content value (3.68) while T1 (0.1 % v/v sodium benzoate) had the lowest fat content value (2.12). However, the mean value showed that T1, T2 and T3 were statistically similar, while T5 and T6 were also similar.

4.3.4. Changes in ash contents of *Nigella sativa* oil extracts (*NSOE)* infused yoghurt during the cold storage at 5ºC for 42 days

The result of changes in ash contents of the various samples during the cold storage is presented in Table 4.3.4 The result showed that there were significantly ($p<0.01$) difference across the storage days measured from day 7, 21, 35 and 42. There were no significant ($p>0.01$) differences observed at 0, 14, and 28. A significant ($p<0.01$) difference in the ash contents of the yoghurt samples showed that T4 at day 7 (6 % v/v NSOE) had the highest ash contents value (3.48) while T6 at day 21 (10% v/v NSOE)) had the lowest fat content value (2.20). However, the mean value was not significantly affected

Table 4.3:2 Changes in moisture content of *Nigella sativa* oil extracts (*NSOE)* treatments on the yoghurt sample (mean ± sem) quality parameters during the cold storage at 5^0C for 42days

	T1	T2	T3	T4	T5	T6	SEM	p-value
Day 0	70.97^{a}	67.82^{b}	66.91^{b}	65.98^{b}	62.22^{c}	61.96^{c}	0.86	0.001
Day 7	66.21^{a}	67.04^{a}	68.29^{a}	65.75^{a}	64.83^{a}	61.24^{b}	0.66	0.01
Day 14	69.28	58.39	67.12	65.70	64.30	65.58	1.62	0.564
Day 21	69.45^{a}	68.13^{ab}	66.66^{b}	66.54^{b}	63.08^{c}	63.73^{c}	0.62	0.001
Day 28	68.85^{a}	66.95^{b}	65.05^{bc}	65.19^{ab}	65.37^{bc}	63.37^{d}	0.46	0.001
Day 35	67.29^{a}	63.86^{b}	65.55^{ab}	63.71^{c}	63.92^{b}	62.69^{c}	0.40	0.001
Day 42	65.89^{a}	63.18^{ab}	63.28^{ab}	62.25^{b}	60.87^{cd}	62.59^{ab}	0.41	0.001
Mean	68.28^{a}	65.05^{b}	66.12^{b}	65.01^{b}	63.51^{c}	63.02^{c}	0.72	0.050

Means with different superscripts across the same row are significantly different ($p < 0.01$).

T1 = yoghurt + 0.1 % V/V of Sodium Benzoate

T2 = yoghurt + 2 % V/V of *NSOE*

T3 = yoghurt + 4 % V/V of *NSOE*

T4 = yoghurt + 6 % V/V of *NSOE*

T5 = yoghurt + 8 % V/V of *NSOE*

T6 = yoghurt + 10 % V/V of *NSOE*

Table 4.3:3 Changes in fat content of *Nigella sativa* oil extract (*NSOE)* infused yoghurt sample during the cold storage at 5^0C for 42days

	T1	T2	T3	T4	T5	T6	SEM	p-value
Day 0	2.33	2.50	2.67	2.66	2.50	2.61	0.08	0.10
Day 7	2.25^{b}	2.30^{b}	2.20^{b}	2.31^{b}	3.16^{a}	2.85^{ab}	0.11	0.001
Day 14	2.00^{c}	3.00^{b}	3.00^{b}	3.67^{a}	3.93^{a}	3.83^{a}	0.17	0.001
Day 21	2.17^{c}	2.21^{c}	2.53^{bc}	2.57^{bc}	3.99^{b}	4.46^{a}	0.22	0.001
Day 28	2.17^{d}	2.66^{c}	2.57^{bc}	3.16^{b}	3.83^{a}	4.10^{a}	0.17	0.001
Day 35	1.98^{c}	2.15^{bc}	2.34^{ab}	3.00^{a}	3.66^{a}	3.94^{a}	0.18	0.001
Day 42	2.00^{d}	1.98^{d}	2.09^{d}	2.94^{c}	3.44^{b}	3.92^{a}	0.18	0.001
Mean	2.12^{c}	2.40^{c}	2.48^{c}	2.97^{b}	3.51^{a}	3.68^{a}	0.16	0.001

Means with different superscripts across the same row are significantly different ($p < 0.01$).

T1 = yoghurt + 0.1 % V/V of Sodium Benzoate

T2 = yoghurt + 2 % V/V of *NSOE*

T3 = yoghurt + 4 % V/V of *NSOE*

T4 = yoghurt + 6 % V/V of *NSOE*

T5 = yoghurt + 8 % V/V of *NSOE*

T6 = yoghurt + 10 % V/V of *NSOE*

Table 4.3:4 Changes in ash contents of *Nigella sativa* oil extracts (*Nsoe)* infused yoghurt sample during the cold storage at 5^0c for 42days

	T1	T2	T3	T4	T5	T6	SEM	p-value
Day 0	3.21	3.41	2.61	3.08	2.44	2.76	0.16	0.56
Day 7	2.64^{bc}	3.03^{abc}	3.00^{abc}	3.48^{a}	3.33^{ab}	2.29^{c}	0.12	0.02
Day 14	3.76	2.95	3.21	4.08	3.77	2.90	0.18	0.30
Day 21	4.43^{a}	2.74^{a}	3.22^{bc}	2.68^{ab}	2.30^{ab}	2.20^{ab}	0.20	0.001
Day 28	3.31	3.66	3.22	3.33	3.44	3.60	0.10	0.72
Day 35	2.84^{c}	3.46^{a}	3.02^{bc}	3.35^{ab}	3.28^{ab}	3.37^{ab}	0.16	0.36
Day 42	2.32^{c}	3.39^{a}	3.02^{bc}	3.07^{bc}	3.23^{ab}	3.23^{ab}	0.08	0.001
Mean	3.21	3.23	3.01	3.49	3.22	3.09	0.14	0.28

Means with different superscripts across the same row are significantly different ($p < 0.01$).

T1 = yoghurt + 0.1 % V/V of Sodium Benzoate

T2 = yoghurt + 2 % V/V of *NSOE*

T3 = yoghurt + 4 % V/V of *NSOE*

T4 = yoghurt + 6 % V/V of *NSOE*

T5 = yoghurt + 8 % V/V of *NSOE*

T6 = yoghurt + 10 % V/V of *NSOE*

4. 4 Microbiological parameter of *Nigella sativa* oil extracts (*NSOE)* Infused yoghurt during cold storage at 5^0C for 42 days

4.4.1 Changes in bacterial count (CFU/mL) of *Nigella sativa* oil extracts (*NSOE)* infused yoghurt during the cold storage

The results of changes in bacterial count of the various samples during the cold storage are presented in Table 4.4.1. The results showed that there were significantly ($p<0.01$) difference across the storage days measured from day 0, 7, 14, 21, 28, 35 and 42.

A significant ($p<0.01$) difference in the mean bacterial count of the yoghurt samples showed that T1 (0.1 % v/v sodium benzoate) had the highest bacterial count value (27.14) while T6 (10 % v/v NSOE) had the lowest bacterial count value (13.09). However, the mean value showed that T1, T2, T3 and T4 were statistically similar.

4.5 Sensory properties of *Nigella sativa* oil extracts (*NSOE)* infused yoghurt during cold storage at 5^0C for 42 days

4.5.1. Changes in colour of *Nigella sativa* oil extracts (*NSOE)* infused yoghurt during the cold storage

The result of the change in colour is presented in Table 4.5.1. The result showed that there was no significant differences across all the days (0-42) of cold storage and the means was also not significantly affected.

4.5.2. Changes in consistency level of *Nigella sativa* oil extracts (*NSOE)* infused yoghurt during the cold storage

The result of the change in consistency level is presented in Table 4.12. The result showed that there were no significant differences across all the days (0-42) of cold storage and the mean was also not significantly affected.

Table 4.4:1 Changes in microbial counts (CFU/ml) of *Nigella sativa* oil extracts (*NSOE)* infused yoghurt sample during the cold storage at 5^0C for 42ddays.

	T1	T2	T3	T4	T5	T6	SEM	p-value
Day 0	27.00^{b}	29.66^{a}	26.66^{b}	30.33^{a}	14.00^{c}	16.33^{c}	1.60	0.001
Day 7	31.33^{b}	31.66^{b}	28.00^{c}	34.66^{a}	28.66^{c}	18.66^{d}	1.28	0.001
Day 14	32.00^{a}	27.00^{b}	21.66^{c}	24.33^{c}	20.33^{c}	10.66^{d}	1.90	0.010
Day 21	27.33^{a}	27.00^{a}	22.00^{b}	23.33^{b}	15.66^{c}	12.00^{c}	1.50	0.001
Day 28	30.00^{a}	27.66^{b}	23.33^{b}	17.33^{c}	12.33^{c}	9.66^{c}	1.94	0.001
Day 35	23.33^{a}	19.66^{b}	23.66^{a}	20.66^{b}	23.33^{a}	15.00^{c}	1.17	0.001
Day 42	19.00^{a}	15.00^{a}	17.66^{a}	19.00^{a}	17.00^{a}	9.33^{b}	0.92	0.002
Mean	27.14^{a}	25.37^{a}	23.28^{a}	24.23^{a}	18.75^{b}	13.09^{c}	1.47	0.001

Means with different superscripts across the same row are significantly different ($p < 0.05$).

T1 = yoghurt + 0.1 % V/V of Sodium Benzoate

T2 = yoghurt + 2 % V/V of *NSOE*

T3 = yoghurt + 4 % V/V of *NSOE*

T4 = yoghurt + 6 % V/V of *NSOE*

T5 = yoghurt + 8 % V/V of *NSOE*

T6 = yoghurt + 10 % V/V of *NSOE*

Table 4.5:1 Changes in colour of *Nigella sativa* oil extracts (*NSOE)* infused yoghurt sample during the cold storage at 5^0C for 42days

	T1	T2	T3	T4	T5	T6	SEM	p-value
Day 0	7.69	7.58	7.68	7.69	7.05	7.02	0.07	0.50
Day 7	7.64	7.57	7.64	7.50	6.86	6.79	0.06	0.10
Day 14	7.67	7.58	7.58	7.58	6.92	6.83	0.07	0.11
Day 21	7.75	7.60	7.82	8.00	7.36	7.45	0.08	0.33
Day 28	6.27	6.36	6.55	8.00	7.36	7.45	0.07	0.50
Day 35	5.92	6.01	6.20	7.65	7.01	7.10	0.28	0.50
Day 42	6.65	6.66	6.85	7.88	7.25	7.34	0.14	0.50
Mean	7.08	7.05	7.19	7.76	7.12	7.14	0.11	0.36

Means with different superscript across the same row are significantly different ($p < 0.01$).

T1 = yoghurt + 0.1 % V/V of Sodium Benzoate

T2 = yoghurt + 2 % V/V of *NSOE*

T3 = yoghurt + 4 % V/V of *NSOE*

T4 = yoghurt + 6 % V/V of *NSOE*

T5 = yoghurt + 8 % V/V of *NSOE*

T6 = yoghurt + 10 % V/V of *NSOE*

Table 4.5:2 Changes in consistency level of *Nigella sativa* oil extracts (*NSOE)* infused yoghurt sample during the cold storage at 5^0C for 42days

	T1	T2	T3	T4	T5	T6	SEM	p-value
Day 0	7.64	8.00	7.71	7.64	7.50	7.64	0.05	0.10
Day 7	7.64	7.57	7.64	7.50	6.86	6.79	0.06	0.10
Day 14	7.67	7.58	7.58	7.58	6.92	6.83	0.07	0.10
Day 21	7.75	7.60	7.82	8.00	7.36	7.45	0.08	0.33
Day 28	6.27	6.36	6.55	8.00	7.36	7.45	0.07	0.50
Day 35	7.67	8.00	7.75	7.75	7.58	7.58	0.05	0.21
Day 42	6.83	7.10	7.64	7.45	7.82	7.73	0.12	0.18
Mean	7.35	7.46	7.53	7.70	7.34	7.35	0.07	0.12

Means with different superscripts across the same row are significantly different ($p < 0.01$).

T1 = yoghurt + 0.1 % V/V of Sodium Benzoate

T2 = yoghurt + 2 % V/V of *NSOE*

T3 = yoghurt + 4 % V/V of *NSOE*

T4 = yoghurt + 6 % V/V of *NSOE*

T5 = yoghurt + 8 % V/V of *NSOE*

T6 = yoghurt + 10 % V/V of *NSOE*

4.5.3 Changes in flavour of *Nigella sativa* oil extracts (*NSOE)* infused yoghurt during the cold storage

The result of the change in flavour is presented in Table 4.5.2. The result showed that there was no significant differences across all the days (0-42) of cold storage and the means was also not significantly affected.

4.5.4. Changes in overall acceptability of *Nigella sativa* oil extracts (*NSOE)* infused yoghurt during the cold storage

The result of the change in overall acceptability is presented in Table 4.5.4. The result showed that there was no significant differences across all the days (0-42) of cold storage measured and the same trend was also observed for all the mean values

Table 4.5:3 Changes of flavour of *Nigella sativa* oil extracts (*NSOE)* infused yoghurt sample during the cold storage at 5^0C for 42days

	T1	T2	T3	T4	T5	T6	SEM	p-value
Day 0	7.64	8.00	7.71	7.64	7.50	7.64	0.05	0.10
Day 7	7.79	7.64	7.50	6.86	6.29	6.36	0.08	0.10
Day 14	7.75	7.67	7.50	6.92	6.33	6.33	0.09	0.10
Day 21	6.83	6.60	7.36	6.82	6.55	6.64	0.15	0.71
Day 28	6.27	6.36	6.55	8.00	7.36	7.45	0.07	0.50
Day 35	7.67	8.00	7.75	7.75	7.58	7.58	0.05	0.21
Day 42	6.83	7.10	7.64	7.45	7.82	7.73	0.12	0.13
Mean	7.25	7.34	7.43	7.35	7.06	7.10	0.09	0.26

Means with different superscripts across the same row are significantly different ($p < 0.01$).

T1 = yoghurt + 0.1 % V/V of Sodium Benzoate

T2 = yoghurt + 2 % V/V of *NSOE*

T3 = yoghurt + 4 % V/V of *NSOE*

T4 = yoghurt + 6 % V/V of *NSOE*

T5 = yoghurt + 8 % V/V of *NSOE*

T6 = yoghurt + 10 % V/V of *NSOE*

Table 4.5.4: Changes in overall acceptability of *Nigella sativa* oil extracts (*NSOE)* infused yoghurt sample during the cold storage at 5^0C for 42days

	T1	T2	T3	T4	T5	T6	SEM	p-value
Day 0	7.57	7.29	7.36	7.14	7.00	7.00	0.06	0.20
Day 7	7.79	7.64	7.50	6.86	6.29	6.36	0.08	0.10
Day 14	7.58	7.33	7.42	7.17	7.00	6.92	0.06	0.30
Day 21	6.50	6.40	7.27	6.82	6.73	6.27	0.14	0.37
Day 28	6.20	6.27	6.36	6.82	6.73	6.27	0.07	0.50
Day 35	7.67	8.00	7.75	7.75	7.58	7.58	0.05	0.21
Day 42	6.83	7.10	7.64	7.45	7.82	7.73	0.12	0.13
Mean	7.16	7.15	7.33	7.14	7.02	6.88	0.08	0.23

Means with different superscripts across the same row are significantly different ($p < 0.01$).

T1 = yoghurt + 0.1 % V/V of Sodium Benzoate

T2 = yoghurt + 2 % V/V of *NSOE*

T3 = yoghurt + 4 % V/V of *NSOE*

T4 = yoghurt + 6 % V/V of *NSOE*

T5 = yoghurt + 8 % V/V of *NSOE*

T6 = yoghurt + 10 % V/V of *NSOE*

CHAPTER FIVE

5.0 DISCUSSION

The qualitative and quantitative parameters of the oil revealed the presence of vital phytochemical constituents such as phenolic compounds (total phenol, flavonoids and tannins) which are very important in antioxidant effects (Reddy *et al.*, 2018; Busari *et al.,* 2021). Polyphenols, are considered as powerful active compounds expressing strong antioxidant activities (Busari *et al.,* 2021).The antimicrobial activities demonstrated by *Nigella sativa* seed oil as observed is not amazing as it is mainly due to phenolic compound redox potential, which can play an important role in adsorbing and neutralizing free radicals, quenching reactive oxygen species which disrupt the cell membrane and destroy the DNA of microbes **(**Thasniya *et al.,* 2021).Therefore, having observed a reduction in the bacteria load due to the inclusion of the black seed oil in this study, it can be confidently suggested as a suitable agent for promoting functional consumption of yoghurt.

The improvement of the pH as observed in this study could be because of the rich phytochemical components of the black seed. In agreement with this, black seed oil extract was reported to keep yoghurt pH and acidity values during 14 days storage at 4°C while also reducing the growth of mycotoxins which led to suggestion that, the use of the oil extracts from black seed could have implications on both the safety and preservation of yoghurt (Ahmed-Noah *et al.,* 2019). Similarly, in a short communication, it was reported by Kwon *et al.,* (2019) that basically the enhancement of physicochemical and antioxidant properties of yoghurt under cold storage was possible due to the inclusion of black seed oil and or its water extracts on the pH regulation and maintenance, (Kwon *et al*., 2019). The influence of the black seed oil extract on the pH could also be linked to its

high level of phenolic compounds which makes its an antioxidant agent and the lactic acid bacteria activities of utilizing fermentable sugar and the production of organic acid, which subsequently decreases the pH. This same effect was also seen in black carrot extracts with its high level of phenolic compounds such as anthocyanin was observed to influence pH of yoghurt under cold storage (Baria *et al.,* 2020). The maintenance of the yoghurt pH at acidic level during the cold storage is beneficial because it could be responsible for the elimination of non-desirable microbes and increased population of the probiotics which could have a beneficial nutritional implication on consumers (Tsevdou *et al*., 2020).

There were significant difference in the percentages of the total titrable acid percentage of the yoghurt samples with the black cumin oil in this present study which is in agreement with the outcome of a study which indicated a significant difference in the total titrable acid percentage when using black seed and honey concentrates as additive to yoghurt under cold storage. The range of values by Bakr *et al*. (2017) is within the same range as the total titrable acids percentage reported in this study. However, the authors reported that the difference in level of significance between the control and treatment could be attributed to the inclusion of honey with the black seed cumin and the shorter duration of the storage. Meanwhile, since the values obtained in this current study were within the same range, despite the longer duration of the storage, as well as an observed increase in the total titrable acids percentage in the treated yoghurt which was observed between days 14 and 28, it could be inferred that the inclusion of black cumin seed oil extracts enhanced the yoghurt quality.

The increase of the non-fat solid of the treated samples of yoghurt indicate that the inclusion of the black seed cumin oil extract improved the yoghurt quality despite duration of storage. This could be attributed to the antioxidant component of the oil which

prevented the lipid peroxidation and breakdown of the proteins in the milk. Although, this has scarcely been reported for black seed, the antioxidant effect of the phenolic compounds in the black seed can be linked with the activities of (transglutaminase) and (oxidoreductase) enzymes which are reported to increase non-fat solid contents of yoghurt under storage as reported in separate reports of Yüksel and Erdem (2010) and Martin *et al*. (2010). These reports both linked inhibition of lipid peroxidation and protein degradation as means of increasing non-fat solid in yoghurt. Although, there was no significant difference in the quantity of non-fat solid in the yoghurt samples of the control and the treatments on 2, 4, 6 and 10 % v/v NSOE, the higher concentration of non-fat solid in these treatment groups could be linked with better texture and appearance of the yoghurt samples. This is in agreement with a report that, storage time and increasing non-fat solid could improve colorimetric, syneresis and water holding capacity of yoghurts compared to yoghurt with lower non-fat yoghurt constituents when stored at low temperature (Nouri *et al*., 2013).

The increased viscosity of the treated yoghurt could be associated with a suspected level of better protein because nutritive value of proteins in yoghurt is related to their amino-acid composition as well as to the availability of these amino-acids which are the main protein end products in milk and its derivatives such as yoghurt (Đurđević-Denin *et al*., 2002). The increased viscosity of the untreated yoghurt could be because the samples were of lower quality because increasing viscosity could be attributed to the changes in the microstructure of the yogurt curd as well as polysaccharides since the higher the viscosity, the higher the quantity of polysaccharides production in the yoghurt samples (Bouzar *et al*., 1997; Jumah *et al*., 2001).

Addition of black seed oil in the yoghurt led to the production of more viscous yoghurt compared with the control. The implication of this observation is that, the treated yoghurt

samples were of better quality compared with the untreated yoghurt samples. This improvement could be linked with the antioxidant richness of the black seeds leading to an improved consolidation of the gel structure, tighter interacting water, and less susceptibility of the yoghurt samples to shearing and temperature changes of the treated samples compared with the control. In agreement with this, a report on fortification of yoghurt with tea which is also an antioxidant-rich material accounted that the viscosity of yoghurt samples were improved which made the samples better than the untreated ones which were more viscous (Najgebauer-Lejko *et al*., 2020).

In agreement with observations recorded in this present study, increase in protein with corresponding reduction in moisture can be linked with the antioxidant properties of the black seed because as an antioxidant agent, it is capable of preventing protein carbonylation which is a major protein-related degradation process with significant implications on milk nutrients composition with emphasis on protein (de Oliveira *et al*., 2016; Nevin and Rajamohan, 2004; Kaseke *et al*., 2021).

Although, there are scarcity of report on the effect of *Nigella sativa* inclusion on yoghurt nurtitive values, this present study however demonstrated that the inclusion of the black seed improved the nutritive values for instance the increase in crude protein values of the yoghurt which increased untill 42 days storage of the yoghurt. Similar inclusion of Sesame seed in yoghurt was also reported to have significantly affected the nutritive values of protein content of yoghurt and in a similar observation with this present study, despite the increase in protein content, the inclusion of the sessame seeds was reported not to have significant impact on the overall acceptability of yoghurt (Mohammed and Soba, 2018).

The inclusion of *Nigella sativa* oil exracts as preservative additive to the yoghurt in this study also demonstrated that it improves the proximate composition of the milk under cold storage. It was observed that the inclusion of the black seed oil improved the fat and ash composition of the yoghurt while moisture was reduced. The reduction in moisture could be linked with the reduction in the microbial load, stable appearance, less viscosity and firmness observed in the yoghurt (Gharibzahedi *et al*., 2018). Likewise, the improvement in ash and fats content might be as a result of antioxidant activity of the extract which prevent oxidation of fat and carbohydrate of the yoghurt samples.

The use of *Nigella sativa* oil extract as preservative for prevention of microbial damage of the yoghurt samples was achieved in this study because the result obtained from the microbial studies of the samples indicated that increasing the oil extract in the milk corresponded with a reducing microbial load of bacteria in the milk as the duration of the storage increases (Ahmed *et al*., 2021). Yoghurt could be susceptible to microbial damages during storage because these damages occur mainly due to microbial, chemical, and enzymatic reactions and physical factors which make yoghurt susceptible to attack, growth, and reproduction of microorganisms (Rawat, 2015). However, the use of plant materials such as *Nigella sativa* oil because of its antimicrobial attributes, is capable of eliminating these microbes and as such promote the storage-ability and shelf-life of the yoghurt; this ability as being due to the active principles in the black seed which help to eliminate the microbes (Tavakkoli *et al*., 2017).

Yoghurt as a fermented food which has been subjected to the action of microorganisms or enzymes leading to desirable biochemical changes, can still pose as a source of risk for humans because of possibility of undesirable microorganisms which could grow in the milk (Giri and Mangaraj, 2012). This justifies the need for the inclusion of plant materials such as black seed which has been reported to have antimicrobial activities as one of its

health beneficial features. The antimicrobial properties of black seed oil in the reduction of bacteria growth on yoghurt in this study can be possibly linked with the antibiotic potential of the seed which was demonstrated in *in-vitro* studies by El-Sayed and Youssef (2019). They reported the seed extract antibiotic activities against a wide range of different bacterial isolates which comprised both gram negative and gram-positive representatives.

While black seed was reported as an antimicrobial agent, specific capacity of the seed against bacteria was reported for its aqueous and oil extracts according to Hanafy and Hatem (1991). According to the researchers, the specific biological activities and physiological importance of *Nigella sativa* is linked to its ability to eliminate bacteria within a biological system either its extracts from water or oil. Similarly, Morsi (2000) also reported that black seed has multiple anti-bacteria activities either in its crude or purified extracts. Although, there are scarce reports on the exact effect of black seed oil extract on microbial count of yoghurt with specific focus on bacteria load, the outcome of this study showed that the coliform unit of the bacteria per milliliter of the yoghurt was lower in the treated yoghurt compared with the control. Previous reports (Yimer *et al*., 2019). on the antimicrobial properties of the seed could suggest why there was reduce bacteria in the milk which could also be the reason why the yoghurt could be stored for a long time. The submission above agrees with the findings of Hassanien *et al*. (2014) after assessment of soft cheese supplemented with black cumin oil for food borne pathogens and quality during storage. Their reports indicated that the supplemented cheese had reduced population of food borne pathogens compared with the un-supplemented ones. Similarly, the black seed and its derivatives such as the oil used in the present study were suggested as novel additives that could be used to produce functional foods due to its anti-bacteria properties (Hassanien *et al*., 2015). Also, due to the high value components of

the black seed along with its traditional medicinal and biological principles, as well as its oil, the seed was suggested as an additive that can be used for the production of functional foods and nutraceutical for management of varied human diseases (Yimer *et al*., 2019).

Similarly, in agreement with accounts of the present study that *Nigella sativa* oil as a preservative was reported to have potential use as an antioxidant, and antimicrobial; the seed was reported to eliminate microorganisms especially the population of food spoilage microbes with a decrease of 2.6-3.7 log cycles (Bahtiti, 2015). The inhibitory effect of black seed on microbes could also be linked with the change in pH leading to increased acidic condition, thereby extending the shelf-life of the yoghurt which led to its recommendation as a flavouring additive of common foods such as breads and pickles considering its low level of toxicity (Mishra *et al*., 2020). Also, as a natural therapeutic, the inclusion of *Nigella sativa* seed oil in foods such as yoghurt can lead to production of medicinal-foods that can be useful for the management of some human ailments. This was attested to in a report whereby the natural therapeutic features of the oil were investigated which led to recommendation of the oil as a therapeutic agent with potential for the treatment of *sinusitis* due to its antimicrobial and analgesic effects (Mahboubi, 2018). In cold storage, the use of black seed oil can be of significant usefulness as demonstrated in the preservation of mutton which was reported to be very effective – an attribute which was linked to its antimicrobial activities (Mahros *et al*., 2021).

The non-changes in colour, flavour, consistency and general acceptability of yoghurt with some fruits and herbs groups similar to black seed was reported at low temperature (Ayar and Gurlin, 2014)..As such, the general attributes of *Nigella sativa* oil and its derivatives as it was observed in the present study is that the oil can be used in food control systems with specific emphasis on yoghurt and other fermentable milk products whereby the seed and or its oil can eliminate common food borne infectious agents such as *Listeria*

monocytogenes which is a common food poisionous agent found in milk and other foods. This was also reported in a study where by black seed oil was reported to have exhibited a strong antibacterial activity against all the strains of *L. monocytogenes*, yielding a significantly larger inhibition zone compared with a commercial antimicrobial agent known as Gentamicin (Nair *et al*., 2005).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In conclusion, the inclusion of *Nigella sativa* oil extracts (NSOE) in yoghurt exhibited the following;

i. Possesses phytochemicals both quantitatively and qualitatively, with tannins having the highest value of (17.05), alkaloids and saponins having the lowest. And are safe to be used as preservatives in food models.

ii. Improved the physico-chemical characteristics of yoghurt under cold storage as it improved titratable acids, viscosity, pH among others which might contribute to the yoghurt extended shelf life.

iii. Proximate composition of infused yoghurt with black seed oil was improved, reduction in moisture content of the yoghurt under cold storage hence prevent the exposure to spoilage and subsequently extension of shelf life

iv. *Nigella sativa* oil extracts *(*NSOE) phytochemical constituents was able to confer antimicrobial properties against bacterial in yoghurt under cold storage.

v. *Nigella sativa* oil extracts *(*NSOE) inclusion did not affect the yoghurts sensory attributes. Thus, serves as a novel herbal preservative

vi. *Nigella sativa* oil extracts *(*NSOE) with inclusion level of 10% v/v (*Nigella sativa* oil extracts) i.e. treatment six (T6) has a better keeping quality, good flavour, and overall acceptable values including longer viability without whey separation.

6.2 Recommendations

ii. *Nigella sativa* Oil Extract (NSOE) at 2-10% v/v is therefore, recommended for commercial production of yoghurt with a good keeping quality.

iii. However, future studies may focus an increasing the *Nigella sativa* Oil Extract (NSOE) inclusion level, above 10% v/v and economic assessment for the identification of viable strategies for the commercialization of this technology before it can be used for food preservation (this includes technologies to enhance availability, efficiency and stability of novel dairy product especially the likes of the one produced in this research).

REFERENCES

Abbasnezhad, A.A., Hayaldanoudin, I., Niazmand, S. & Mahmoud, A.M. (2015). Effect of hydroalcoholic extract of *Nigella sativa* seeds on oxidation stress in hippocampus of STZ-induced diabetic rats. Avicennia. *Journal of Phytomedicine,* 5(4). 33-340.

Abdel-Sater, K.A., (2009). Gastroprotective effects of *Nigella sativa* oil on the formation of stress gastritis in hypothyroidal rats. *International journal of physiology, pathophysiology and pharmacology*, *1*(2), p.143.

Abdulelah, H.A.A. & Zainal-Abidin, B.A.H. (2007). In vivo anti-malarial tests of *Nigella sativa* (black seed) different extracts. *American Journal of Pharmacology and Toxicology*, *2*(2), 46-50.

Abedi, A. S., Rismanchi, M., Shahdoostkhany, M., Mohammadi, A. & Hosseini, H. (2016). Microencapsulation of *Nigella sativa* seeds oil containing thymoquinone by spray-drying for functional yogurt production. *International Journal of Food Science and Technology*, *51*(10), 2280-2289.

Abou-Gabal, A. A., Essawy, A. E., Abdel-Moneim, A. M., Hamed, S. S. & Elzergy, A. A. (2007). The protective effect of black seed (*Nigella sativa*) against carbon tetrachloride-induced chromosomal aberrations and ultrastructural changes of bone marrow cells. *Arab Journal of Biotechnology*, 10(2):275-288.

Abu El Ezz, N. M. (2005). Effect of *Nigella sativa* and Allium cepa oils on Trichinella spiralis in experimentally infected rats. *Journal of the Egyptian society of parasitology*, *35*(2), 511-523.

Accuweather, (2021). http:/www.accuweather.com/en/minna/254122/cureent-weather/254122.

Adams, M. R. & Moses, M. O. (2008). Food microbiology. 3rd ed. Cambridge: *The Royal Society of Chemistry*; p. 98–99.

Aftab, A., Asifi, H., Mohd, M., Shah, A.K., Abdul, K.N., Nasir, A.S., Zohcir. A.D. & Firoz, A. (2013). A review on therapeutic potential of *Nigella sativa*; A miracle herb. *Asian Pacific Journal of Tropical Biomedicine*, 3(5), 337-352.

Ahmad, Z., Gafoor, A., Aslam, M. (2004). *Nigella sativa* – A potential commodity in crop diversification traditionally used in health care. Project on Introduction of Medicinal herb and species as crop. Ministry of food, agriculture and livestock, Pakistan.

Ahmed, L. I., Ibrahim, N., Abdel-Salam, A. B. & Fahim, K. M. (2021). Potential Application of Ginger, Clove and Thyme Essential Oils to Improve Soft Cheese Microbial Safety and Sensory Characteristics. *Food Bioscience*, 101177.

Ahmed-Noah, B., Marwa M. E., Tamer, M. E. & Adel, G. A., (2019). Non-traditional Oils Encapsulation as Novel Food Additive Enhanced Yogurt Safety Against Aflatoxins. *Pakistan Journal of Biological Sciences, 22: 51-58.*

Al-Awadi, F. M., Fatania, H. & Shamte, U. (1991). The effect of a plant mixture extract on liver gluconeogenesis in streptozotocin-induced diabetic rats. *Diabetes Research (Edinburgh, Scotland)*, *18*(4), 163-168

Al-Awadi, F. M., & Gumaa, K. A. (1987). Studies on the activity of individual plants of an antidiabetic plant mixture. *Acta diabetologialatina, 24*(1): 37-41.

Al-Ghamdi, M. S. (2001). Anti-inflammatory, analgesic and anti-pyretic activity of *Nigella sativa*. *Journal Ethnopharmacology,* 76:45-48.

Al-Hader, A., Aqel, M. & Hasan, Z. (1993). Hypoglycemic effect of volatile oil of *Nigella sativa* seeds. *International Journal of Pharmacology*, 31(2): 96-100.

Ali, B. H. (2004). The effect of *Nigella sativa* in gentamicin nephrotoxicity in rats. *American Journal Chinese Medicine,* 32 (1): 49-55.

AlJishi, S. A. & Hozaifa, B. (2003). Effects of *Nigella sativa* on Blood Homeostatic Function in Rats. *Journal of Ethnology Pharmacology,* 85 (1) : 7-14.

Alnaemi, S. H., & Alsawaf, D. S. (2011). Effect of *Nigella sativa* (seed and oil) on the bacteriological quality of soft white cheese. *Iraqi Journal of Veterinary Sciences,* 25(1): 21-27.

Al-Sa'aidi, J. A. A., Al-Khuzai, A.L.D. & Al-Zobaydi, N. F. H. (2009). Effect of alcoholic extract of *Nigella sativa* on fertility in male rats. *Iraqi Journal of veterinary science,23: 123-128.*

Al-Shebani, W. H. & Al-Tahan, F. J. (2009). Antinociceptive effect of watery suspension of *Nigella sativa* Linn. seeds in mice. *Iraqi Journal Veterinary Science*, 23:245-248.

Amit, S. K., Uddin, M. M., Rahman, R. M., Islam, S. R. & Khan, M. S. (2017). A review on mechanisms and commercial aspects of food preservation and processing. *Agricultural and Food Security, 6:51.*

Amr, E. E. (2009). Anti-Cancer Properties of *Nigella sativa* spp. Essential Oils and their Major Constituents, Thymoquinone and β-Elemene. Cur. Clin. (current clinical) *Pharmacology,* 4: 43-46.

Ani. E, Julius. A, Bibilana. I. (2018). Physicochemical, microbiological, sensory properties and storage stability of plant based yoghurt produced from Bambaranut, soybean and *Moringa oleifera* seed milks. *American journal of Food and Nutrition, 6(4): 115-125.*

Ansari, A. A., Hassan, S., Kenne, L., Atta-ur-Rehman, S. & Wehler, T. (1988). Structural studies on a saponin isolated from *Nigella sativa. Jourrnal of phytochemistry,* 27(12):3977-3979.

AOAC. (2000). Official methods of analysis. 17th ed. Official Methods of Analysis of AOAC International. Gaithersburg (MD): CAB publisher.

AOAC. (2002). Official methods of analysis. 17th ed. Official Methods of Analysis of AOAC International. Gaithersburg (MD): CAB publisher

AOAC. (2012). Official Methods of Analysis. 20th edition. Association of Official Analytic Chemists. Washinton D.C.

Aqel, M. & Shaheen, R. (1996). Effects of the Volatile Oil of *Nigella sativa* Seeds on the uterus Smooth Muscle of Rat & Guinea pg. *Journal of Ethnopharmocology*, 52 (1): 23-26.

Arshad, H., Paulsson, M. & Dejmek, P. (1993). Rheology of buildup, breakdown, and rebodying of acid casein gels. *Journal of Dairy Science,* 76:3310-3316.

Aswal, P., Shukla, A. &Priyadarshi, S. (2012). Yogurt preparation, characteristics and recent advancement. *Cibtech Journal of Bio-Protocols,* 12(1): 34-44.

Atef, M., Al-Attar, W. & Al-Taisan, A. (2010). Preventive Effects of Black Seed (Nigella sativa) extract on Sprague Dawley Rats Exposed to Diazinon. *Australia Journal Basic Apply Science,* 4(5): 957-968.

Ayar, A. & Gurlin, E. (2014). Production and sensory, textural, physicochemical properties of flavored spreadable yogurt. *Life Science Journal*, *11*(4): 58-65.

Ayaz, E., Yilmez, H., Ozbek, H., Tas, Z. & Orunc, O. (2007). The effect of *Nigella sativa* against *Aspiculuristetraptera* and *Hymenolepsis nana* in naturally infected mice. *Saudi Medical Journal*, 28:1654-1655.

Badary, A., Taha, R. A, Ayman, M., El-Din, G. & Abdel-Wahab, M. H. (2003). Thymoquinone is a potent suprroxide anion scavenger. *Drug Chemist Toxicology,* 26(2): 87-98.

Badary, O. A. (1996). Thymoquinone attenuates afosfamide-induced Fanconi syndrome in rats and enhances its antitumor activity in mice. *Journal Ethnopharmacology*, 67:135-142.

Badary, O. A. & Gamal-el-Din, A. M. (2001). Inhibitory effect of thymoquinone against 20-methylcholanthrene–induced fibrosarcoma tumorigenesis. *European Journal of Cancer Detectection and Prevention*, 25:362-368.

Bahram, P. G., Vahide, E. A., Maryam, R. & Abolfazl, G. (2009). Effect of dietary supplementation with *Nigella sativa* L. on serum lipid profile, lipid peroxidation and antioxidant defense system in hyperlipidemic rabbits. *Journal Medicinal Plants Research,* 3(10): 815-821.

Bahtiti, N. H. A. (2015). Chemical investigation and preservative effect of Jordanian *Nigella sativa* L. seed oil on date paste. *International Journal of Research Studies in Biosciences (IJRSB)*, 3: 1-5.

Bakr, I. A., Mohamed, T. H., Tammam, A. A. & El-Gazzar, F. E. (2017). Characteristics of yoghurt fortified with black cumin honey. *Assiut (University of asuit, Egypt) Journal of Agricultural Sciences*, 48(1): 67-79.

Banerjee, S., Azmi, A. S., Padhye, S., Singh, M. W., Baruah, J. B., Philip, P. A., Sarkar, F. H. & Mohammad, R. M. (2010). Structure-Activity Studies on Therapeutic Potential of Thymoquinone Analogs in Pancreatic Cancer. *Pharmacology Reserve,* 27*(*6): 1146-1158.

Baria, B., Singh, A. K., Panjagari, N. R., Arora, S., & Minz, P. S. (2020). Colouring properties and stability of black carrot anthocyanins in yoghurt. *Journal of Food Science and Technology*, 1-10.

Bouzar, F., Cerning, J., & Desmazeaud, M. (1997). Exopolysaccharide production and texture-promoting abilities of mixed-strain starter cultures in yogurt production. *Journal of Dairy Science,* 80(10): 2310-2317.

Burits, M, & Bucar F. (2000). Antioxidant activity of *Nigella sativa* essential oil. *Phytotherapy research,* 14: 8-23.

Busari, L. O., Ojurongbe, O., Adeleke, A. M. A., Olabanti, A. S. & Akindele, A. A. (2021). Biting behaviour & Infectivity of Simulium Damnosum Complex with Onchocera Parasite in Alabameta, Osun State, Southwest, Nigeria. PLOS ONE 16 (6): *doi: 10. 1371 Journal.*

Chakarvarti, N. (1993). Inhibition of histamine release from mast cells by nigellone. *Ann.(annals) Journal of Allergy*, 70(3): 237-242.

Chang, C. C., Yang, M. H., Wen, H. M. & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis,* 10: 178-182.

Chevallier, A. (1996). Encyclopedia of medicinal plants: A practical reference guide to more than 500 key medicinal plants and their uses. New York(NY): Dorling Kindersley (DK) Publishing .237.

Choi, Y. J., Jin, H. Y., Yang, H. S., Lee, S. C & Huh, C. K. (2016). Quality and storage characteristics of yoghurt containing *Lactobacillus sakei* ALI033 and cinnamon ethanolic extract. *Journal of Animal Science2and Technology,* 58:16- 20.

Chopra, R. N., Nayyer, S. L. & Chopra, I. C. (1956). Glossary of Indian medicinal plants. India: *New Delhi Council of scientific and industrial research (CSIR),* 176-188.

Daba, M. H. & Abdel-Rehman, M. S. (1998). Hepatoprotective activity of thymoquinone in isolated rat hepatocytes. *Toxicology. Lett. (letter),* 95: 23-29.

Dal B.F. (2007). Improvement of the quality and shelf life of wheat bread by fermentation with the antifungal strain Lactobacillus plantarum FST 1.7. *Journal of Cereal Sci*ence, 45(3): 309–318.

Dalgleish, D. G. & Law, A. J. R. (1998).pH-induced dissociation of bovine casein micelles. II. Mineral solubilization and its relation to casein release. *Journal of Dairy Research*, 56:727-735.

Datau, E., Eko, A., Surachmanto, E., Pandelaki, K. & Langi, F. A. (2010). Efficacy of *Nigella sativa* on Serum Free Testosterone and Metabolic Disturbances in Central Obese Male. *Acta. Med. Indones. (The Indonesian Journal of Internal Medicine),* 2(3):130-34

De man, J. M., Finley, J. W., Hurst, W. J. & Lee, C. Y. (2018). *Principles of food chemistry*. Springer.http://link.springer.com>book.

De Oliveira, F. C., Coimbra, J. S. D. R., de Oliveira, E. B., Zuñiga, A. D. G. & Rojas, E. E. G. (2016). Food protein-polysaccharide conjugates obtained via the Maillard reaction: A review. *Critical Reviews in Food Science and Nutrition*, *56* (7):1108-1125.

Debjani, S. (2019). A review of the seeds comprising *panchphoron*, a Spice used in Indian Cuisine Department of Zoology, Acharya Prafulla Chandra Roy Government College, Darjeeling, West Bengal, INDIA *International Journal of Pharmaceutical Investigation*, 9(2): 36- 42.

Demir, H. K., Coskun O, Uz, Y. H., Koe, A. & Yildiz, A. (2006). Effect of black cumin (*Nigella sativa*) on heart rate, some hematological value, and pancreatic beta-cell damage in cadmium-treated rats. *Biological Trace Element Res*earch, 1(10): 151-162.

Desrosier, N. W. & Singh, R. P. (2014). Food preservation. Encyclopaedia Britannica Inc., https://www.britannica.com/topic/food-preservation.

Doyle, M. P. (2009). Compendium of the microbiological spoilage of foods and beverages. Food microbiology and food safety. New York: Springer.

Đurđević-Denin, J. D., Maćej, O. D. & Jovanović, S. T. (2002). Viscosity of set-style yogurt as influenced by heat treatment of milk and added demineralized whey powder. *Journal of Agricultural Sciences, Belgrade*, *47*(1): 45-56.

Edeoga, H. O., Okwu, D. E., & Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African journal of biotechnology*, *4*(7): 685-688.

El-Dakhakhani, M. (1965). Studies on the Egyptian *Nigella sativa* L: Some pharmacological properties of its seed's active principle in comparison to its dihydro-compound and its polymer. ArzneimForsch. Drug Reseach,15:1227-1229.

El-Dakhakhani, M., El-Halim, M. A. & Aly, S. M. (2000). Effect of *Nigella sativa* oil on gastric secretion and ethanol-induced ulcer in rats. *Journal Ethnopharmacology*, 72(1-2): 299-304.

El-Dakhakhany, M. (1982). Some pharmacological properties of some constituents of *Nigella sativa* L seeds: The carbonyl fraction of essential oil. Proceeding of the 2nd *International conference on Islamic Medicine*, *Kuwait*, 426-431.

El-Daly, E. S. (1996). Protective effect of cysteine, Vitamin E, *Crocus sativus* and *Nigella sativa* extract on cisplatin induced toxicity in rats. *Journal Islamic Academy Science*s, 9(4):47-55.

El-Hadi, M., Bakri, A., Yousif, Y., Mohammed, G. & Hassan,S. K. (2010). Antiplasmodial Activity of Some Medicinal Plants Used in Sudanese Folk-medicine. *Environmental Health Insights*, 4:1-6.

El-Kadi, A. & Kandil, K. O. (1986). Effect of *Nigella sativa* (the black seed) on immunity. Proceeding of the 4th *International Conference on Islamic Medicine, Kuwait*. Bull. Islamic Medicine, 4: 344-348.

El-Kamali, H. H., Ahmad, A. H., Mohammad, A. S. & Yahia, A. A. M. (1998). Antibacterial properties of essentials oils from *Nigella sativa*. *Fitoterapia (Journal of Physiotherapy)*, 69: 77-78.

El-Naggar, A. R. M. & El-Deib, A. E. M. (1992). A study of some biological activities of *Nigella sativa* (black seeds) "Habbat El-Barka". *Journal Egypt Sociology Pharmacology*, 11(2): 781-800.

El-Sayed, S. M. & Youssef, A. M. (2019). Potential application of herbs and spices and their effects in functional dairy products. *Heliyon*, *5*(6).

ElShenawy, N.S., Soliman, M.F. & Reyad, S. I. (2008). The effect of antioxidant properties of aqueous garlic extract and *Nigella sativa* as anti-schistosomiasis agents in Mice. *Institute for Tropical medicine,* 50:29-36.

El-Tahir, K. E., Ashour, M. & Al-Harbi, M. M. (1993). The respiratory effects of the volatile oil of black seed (*Nigella sativa*) in guinea pigs: elucidation of the mechanism(s) of action. *General Pharmacology*, 24(5): 1115-1122.

Erkan, N., Ayranci, G. & Erol, A. (2008). Antioxidant activities of rosemary*(Rosmarinus officinalis* L.) extract, blackseed(*Nigella sativa* L.) essential oil,carnosis,rosmarinic Acid and sesamol. *Journal of food chemistry*, 110: 6-88.

Fadel, H. M. & Ismail, J. (2016). Detection of listeria monocytogenes in raw milk and ready to eat dairy products with evaluating the antimicrobial effect of cold-pressed *Nigella sativa* oil. *Assiut Veterinary Medical Journal:* 149:13-18.

Fahrettin, Y., Sacit, C., Alpaslan, T., Mustafa, A., Nurten, A., Hale, C., Ali, R.O. & Muharrem, B. (2008). *Nigella sativa* relieves the deleterious effects of ischemia reperfusion injury on liver. *World Journal Gastroenterology*, 14 (33):5204-5209.

Farah, I. O. & Begum, R. A. (2003). Effect of Nigella sativa and oxidative stress on the survival pattern of MCF-7 breast cancer cells. *Biomedical Science Instrumental*, 39:359-364.

Farah, K. M., Atoji, Y., Shimizu, Y. & Takewaki, T. (2002). Insulinotropic properties of *Nigella sativa* oil in streptozotocin plus nicotinamide diabetic hamsters. *Reserved Veternary Science*, 73: 279-282.

Gad, A. M., El-Dakhakhany, M. & Hassan, M. M. (1963). Studies on the chemical constitution of Egyptian *Nigella sativa* L oil. *Planta Med. (Medicinal plants Journal)*, 11(2):134-138.

Gali-Muhtasib, H., Roessner, A. & Schneider-Stock, R. (2006). Thymoquinone: a promising anti-cancer drug from natural sources. *International Journal Biochemistry*, 38(8):1249-1253.

Ghanya Al-Naqeep, A. S., Al-Zubairi, M. I., Zulkhairi, H. A. & Norhaizan, M .E . (2010). Antiatherogenic Potential of *Nigella sativa* Seeds and Oil in Diet-Induced Hypercholesterolemia in Rabbits. *Evidence Based Complementary Alternative Medicine (eCAM)*, 8 (1): 1-8.

Gharibzahedi, S. M. T., Koubaa, M., Barba, F. J., Greiner, R., George, S. & Roohinejad, S. (2018). Recent advances in the application of microbial transglutaminase cross linking in cheese and ice cream products: A review. *International Journal of biological macromolecules*, 107: 2364-2374.

Ghoneim, M. T., El-Gindy, A. R., El-Alami, R., Shoukry, E. & Yaseen, S. (1982). Possible effects of some extracts of *Nigella sativa* L seeds on blood coagulation system and fibrinolytic activity. *Proceeding of 2nd International Conference on Islamic Medicine 12th Apr, Kuwait*, 528-535.

Gilani, A. H., Aziz, N., Khurram, I. M., Chaudhary, K. S. & Iqbal, A. (2001). Bronchodilator, spasmolytic and calcium antagonistic activities of *Nigella sativa* seed (Kalonji): a traditional herbal product with multiple medicinal uses. *Journal Pakistan Medical Association*, 51(3):115-120.

Giri, S. K. & Mangaraj, S. (2012). Processing influences on composition and quality attributes of soymilk and its powder. *Food Engineering Reviews*, *4*(3): 149-164.

Gyawali, R. & Ibrahim, S. A. (2014). Natural products as antimicrobial agents. *Food control*, *46*, 412-429.

Hajhashemi, V., Ghannadi A. & Jafarabadi, H. (2004). Black cumin seed essential oil, as a potent analgesic and antiinflammatory drug. *Phytotherapy Reserch*,18(3): 195-199.

Halamova, K., Ladislav, K., Taroslav, F., Olga, S., Blanka, S. & Petr, M. (2010). In vitro antifungal effect of black cumin seed quinones against dairy foliage yeast at different acidity levels. *Journal of Food Protection*, 73(12): 2291-2295.

Hanafy, M. S. & Hatem, M. E. (1991) Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin). *Journal of Ethnopharmacology*, 34(2-3): 275–278

Haq, A., Abdullatif, M., Lobo, P. I., Khabar, K. S., Sheth, K.V. & Al-Sedairy, S. T . (1995). *Nigella sativa*: Effect on human lympocytes and polymorphonuclear leucocyte phagocytic activity. *Immuno pharmacology*, 30(2):147-150.

Haq, A., Lobo, I., Al-Tufail, M., Rama, N. R. & Sedairy, S. T. (1999). Immunomodulatory effect of *Nigella sativa* proteins fractionated by ion exchange chromatography. *International Journal Immuno pharmacology.* 21:283-285.

Haque, A., Richardson, R. K. & Morris, E. R. (2001). Effect of fermentation temperature on the rheology of set and stirred yogurt. *Food Hydrocolloids,* 15:593-602.

Harborne, J. B. (1973). Phenolic compounds. In *Phytochemical methods Springer, Dordrecht.http://doi.org/10.1009781-940-009-5921-7-2.*

Harrigan, W. F. & McCance, M. E. (1990). Labouratory Methods in Food and Diary Microbiology. Academic Press, London, pp: 210

Hassanien, M. F. R., Mahgoub, S. A. & El-Zahar, K. M. (2014). Soft cheese supplemented with black cumin oil: Impact on food borne pathogens and quality during storage. *Saudi Journal of Biological Sciences*, *21*(3): 280-288.

Hassanien, M. F., Assiri, A. M., Alzohairy, A. M. & Oraby, H. F. (2015). Health-promoting value and food applications of black cumin essential oil: an overview. *Journal of Food Science and Technology*, *52*(10): 6136-6142.

Hjartåker, A., Lagiou, A., Slimani, N., Lund, E., Chirlaque, M. D., Vasilopoulou, E., Zavitsanos, X., Berrino, F., Sacerdote, C., Ocké, M .C., Peeters, P .H .M., Engeset D., Skeie, G., Aller, A., Amiano, P., Berglund, G., Nilsson, S., McTaggart, A., Spencer, E .A., Overad, K., Tjønneland, A., Clavel-Chapelon, F., Linseisen, J., Schulz, M., Hemon, B. & Riboli, E .(2002). Consumption of dairy products in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort: data from 35 955 24-hour dietary recalls in 10 European countries. *Public Health Nutrition,* 5: 1259–1271.

Horne, D. S. (1998). Casein interactions: Casting light on the Black Boxes, the structure in dairy products. *International Dairy Journal*, 8:171-177.

Hosseinzadeh, H. & Parvardeh, S. (2005). "Anticonvulsant effects of thymoquinone, the major constituent of *Nigella sativa* seeds, in mice." *Phytomedicine*, 11(1): 56-64.

Hosseinzadeh, H., Parvardeh, S., Nassiri-Asl, M. & Mansour, M.T. (2004). "Intracerebroventricular administration of thymoquinone, the major constituent of *Nigella sativa* seeds, suppresses epileptic seizures in rats. *Medical Science Monitor,* 11(4):106-110.

Houghton, P. J., Zarka, R., Heras, B. & Hoult, J. R. (1995). Fixed oil of Nigella sativa and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Med. (Journal of Medicinal plants),* 6: 33-36.

Igarashi, Y., Eroshkin, A., Gramatikova, S., Gramatikoff, K., Zhang, Y., Smith, J. W., & Godzik, A. (2007). CutDB: a proteolytic event database. *Nucleic acids research, 35 (1): D546-D549.*

Ihemeje, A., Nwachukwu, C. N. & Ekwe, C. C. (2015). Production and Evaluation of flavoured yoghurt using carrots, pineapple and spiced yoghurt, ginger and pepper fruit. *African Journal of Food Science,* 9(3): 163-169.

Imtiaz, A., Tripthi, J., Sharma. M. & Nayak. A. (2013). Evaluation of Acute Oral Toxicity of *Nigella sativa* LINN seed methanolic extract in mice world. *Journal of Pharmacy and Pharmaceutical Sciences,* 3(4): 973-982.

Inayat, U.B., Fazal, U.R., Muhannad, A. K. & Sarfaraz, K. (2009). Effect of Prophetic medicinal Kalonji (*Nigella sativa* L.) on Lipid Profile of Human Being: An In vivo Approach. *World Applied Science. Journal*, 6(8): 1053-1057.

Islam, M. N., Rahman, A. H. M. S., Mursalat, M., Rony, A. H. & Khan, M. S. (2015). A legislative aspect of artificial fruit ripening in a developing country like Bangladesh. *Chemical Engineering Research Bulletin*, *18*(1): 30-37.

Jameel, A., Rahat, A. K. & Ashraf, M. M. (2009). Study of *Nigella sativa* oil in the management of wheeze associated lower respiratory tract illness in children. *African Journal of Pharmacology,* 3(5): 248-251.

Jay, J. M. (2000). Modern food microbiology. 6th ed. Gaithersburg: Aspen Publishers

Joardder, M. U. H. & Masud M. H. (2019). Food preservation in developing countries: challenges and solutions. *https://doi.org/10.1007/978-3-030-11530-2* .

Jowitt, R. (1974). Classification of Foodstuffs and Physical Properties; *Lebensmittel - Wissenschaft and Technologies (L W T)* 7(6): 378-371.

Jumah, R. Y., Abu-Jdayil, B., & Shaker, R. R. (2001). Effect of type and level of starter culture on the rheological properties of set yogurt during gelation process. *International Journal of food properties*, *4*(3): 531-544.

Kaseke, T., Opara, U. L. & Fawole, O. A. (2021). Novel seeds pretreatment techniques: effect on oil quality and antioxidant properties: a review. *Journal of Food Science and Technology*, 1-14.

Katz, F. (2001). Active cultures add function to yogurt and other foods: Yogurt reinvents itself. *Food Technology (Chicago)*, 55(3): 46-49.

Kavas, G., Uysal, H., Kiliç, S., Akbulut, N. & Kesenkas, H. (2003). Some properties of yoghurts produced from goat milk and cowgoat milk mixtures by different fortification methods. *Pakistan Journal Biology Science*, 6: 1936–1939

Keshri, G., Singh, M. M., Lakshami, V. & Kamboj, V. P. (1995). Post-coital contraceptive effect of the seeds of *Nigella sativa* in rats. *Indian Journal of Physiology and Pharmacology*, 39(1): 59-62.

Khadiga, A., Abdel-Ati, A. E. & Mustafa, H. E. (2009). The effect of dietary *Nigella sativa* seeds on the blood cholesterol and lipoprotein levels of rabbits. *Journal of Animal and Plant Science* 3(3): 227-230.

Khanna, T., Zaidi, F. A. & Dandiya, P. C. (1993). CNS and analgesic studies of *Nigella sativa. Fitoterapia*, 5: 407-410.

Kruk, I., Michalska, T. & Klanda, A .(2000). The effect of thymol and its derivatives on reaction generating reactive oxygen species. *Chemosphere Journal*, 41:1059-1064.

Kumara, S. S. & Huat, B. T. (2001). Extraction, isolation and characterization of anti-tumour principle, alpha-hedrin, from the seeds of *Nigella sativa. Planta Med*. 67: 29-22.

Kwon, H. C., Bae, H., Seo, H. G. & Han, S. G. (2019). Chia seed extract enhances physiochemical and antioxidant properties of yogurt. *Journal of dairy science*, *102*(6): 4870-4876.

Le, P. M., Benhaddou-Andaloussi, A., Settaf, A., Cherrah, Y. & Haddad, P .S . (2004). The petroleum ether extract of *Nigella sativa* exerts lipid lowering action in the rats. *Journal Ethno pharmacology*, 94(2-3):251-259.

Ledenbach, L. & Marshal, R. (2009). Microbiological Spoilage of Dairy Product, Springer Science Business Media. *Doi:10.1007/978-1-4419-0826-12.*

Lee, W. J. & Lucey, J. A. (2006). Impact of gelation conditions and structural breakdown on the physical and sensory properties of stirred yogurts. *Journal of Dairy Science*. 89: 2374-2385.

Lucey, J. A. (2004). Formation, structural properties and rheology of acid-coagulated milk gels. *Doi:10.1016/51874-558×(04)80064-2.*

Mahboubi, M. (2018). Natural therapeutic approach of *Nigella sativa* (Black seed) fixed oil in management of Sinusitis. *Integrative medicine research*, *7*(1): 27-32.

Mahfouz, M. & El-Dakhakhany, M. (1960). Some chemical and pharmacological properties of the new antiasthmatic drug "Nigellone". *Egypt Pharmacology Bulletin,* 42:411-424.

Mahmoud, M. R., El-Abhar, H. S. & Salh, S. (2002). The effect of *Nigella sativa* oil against the liver damage induced by *Schistosoma mansoni* infection in mice. *Journal Ethnopharmacol*ogy, 79(1): 1-11.

Mahros, M. M., Abd-Elghany, S. M., Sayed-Ahmed, M. Z., Alqahtani, S. S. & Sallam, K. I. (2021). Improving the microbiological quality, health benefits, and storage time of cold-stored ground mutton supplemented with black seed. *L W T, 138*:110-673.

Makwana, S., Choudhary, R., Haddock, J. & Kohli, P. (2015). In-vitro antibacterial activity of plant based phenolic compounds for food safety and preservation. *Food Science and Technology, 62*(2), 935-939.

Marafon, A. P., Sumi, A., Granato, D., Alcantara, M. R., Tamime, A.Y. & de Oliveira, M. N. (2011). Effects of partially replacing skimmed milk powder with dairy ingredients on rheology, sensory profiling, and microstructure of probiotic stirred-type yogurt during cold storage. *Journal of Dairy Science*, 94: 5330–5340

Martin, F., Cayot, N., Vergoignan, C., Journaux, L., Gervais, P. & Cachon, R. (2010). Impact of oxidoreduction potential and of gas bubbling on rheological properties of non-fat yoghurt. *Food Research International, 43*(1): 218-223.

Matira, K. & Zesmin, F. D. (2008). Effects of the crude and the n-hexane extract of *Nigella sativa* Linn. (kalajira) upon diabetic rats. *Bangladesh Journal Pharmacol*ogy, 4:17-20.

Matos, S., Pinto, A., Castilho, C., Correia, P. R. & Monteiro, A. C. (2013). Mix goat and sheep yogurt: development and product characterization. *International Journal Biology and Veterinary Agric Food Engineering*, 7:301–304.

McDonald, S., Prenzler, P. D., Antolovich, M. & Robards, K. (2001). Phenolic content and antioxidant activity of olive extracts. *Food Chemistry*, 73:73-84.

Merai, M., Boghra, V. R. & Sharma, R. S. (2003). Extraction of anti toxigenic principles from tulsi leaves and their effects on oxidative stability of ghee (Cheese). *Journal of Food Science and Technology*, 40(1), 52-57.

Merfort, I., Wary, V., Barakat, H., Hussain, A. & Nawwar, A. M. (1997). Flavonoltriglycosides from seeds of *Nigella sativa. Phytochemical*, 46(2): 359-363.

Michelle, M. C. (2005). The nutrition and health benefits of yoghurt. *International Journal of Dairy Technology*, 58, 1-12.

Mishra, A. P., Devkota, H. P., Nigam, M., Adetunji, C. O., Srivastava, N., Saklani, S. & Khaneghah, A. M. (2020). Combination of essential oils in dairy products: A review of their functions and potential benefits. *Lwt, 133*, 110116.

Mohamed, A. M., EL-Sharkawy, F. Z., Ahmed, S. A. A., Aziz, W. M. & Badary, O.A. (2009). Glycemic Control and Therapeutic Effect of *Nigella sativa* and *Curcuma longa* on Rats with streptozotocin-induced Diabetic Hepatopathy. *Journal Pharmacol. Toxicology, 4* (2):45-57.

Mohammad, H. & Boskabady, J. F. (2008). The Possible Prophylactic Effect of Nigella sativa Seed Aqueous Extract on Respiratory Symptoms and Pulmonary Function Tests on Chemical War Victims: A randomised, Double-blind, placebo-controlled trial. *Journal of Alternative and Complementary medicine*, 14 (9): 1137-1144.

Mohammed, J. A. (2008). Microemulsions as Potential Drug Delivery System. PDA *Journal of Pharmaceutical Science and Technology*, 62 (1): 66-79.

Mohammed, A. H. A. & Soba, R. M. A. (2018). *Evaluation of Nutritional Composition of Yoghurt Supplemented With Sesame Paste (Tahini)* (Doctoral dissertation, Sudan university of Science and Technology).

Morsi, N. M. (2000) Antimicrobial effect of crude extracts of *Nigella sativa* on multiple antibiotics-resistant bacteria. *Acta Microbiol Pol (Polish Journal of Microbiology),* 49(1): 63–74.

Muhammad, A. B. & Muhammad, T. (2007). Effect of *Nigella sativa* on Lipid Profile in Albino rats. *Gomal Journal of Medical. Science*, 5(1):28-31.

Mukhallad , A. M., Mohamad, M. J., Mohamad, P. & Hatham, D. (2009). Effects of Black Seeds (*Nigella sativa*) on Spermatogenesis and Fertility of Male Albino Rats. Research *Journal of Medical Science.* 4(2): 386-390.

Mulvihill, D. M. & M. B. Grufferty. (1995). Effect of thermal processing on the coagulability of milk by acid. In Heatinduced Changes in Milk. Special Issue No. 9501 (Ed. P. F. Fox). *International Dairy Federation, Brussels*. 188-205.

Mursalat, M., Rony, A. H., Rahman, A. H. M. S., Islam, M. N. & Khan, M. S. (2013). A critical analysis of artificial fruit ripening: Scientific, legislative and socio-economic aspects. *ChE (Chemical engineering and science magazine), 4*(1): 6-12

Nadia, M. H. & Taha R. A. (2009). Effects of *Nigella sativa* oil and thymoquinone on oxidative stress and neuropathy in streptozotocin-induced diabetic rats. *Pharmacology*, 84: 127-134.

Nagi, M. N., Alam, K., Badary, O. A., Al-Shabanah, O. A., Al-Sawaf, H. A. & AL- Bekairy, A. M. (1999). Thymoquinone protects against carbon tetracholide hepatotoxicity in mice via an antioxidant mechanism. *Biochemistry Molecular Biology International,* 47: 153-159.

Nair, M. K. M., Vasudevan, P. & Venkitanarayanan, K. (2005). Antibacterial effect of black seed oil on Listeria monocytogenes. *Food control*, *16*(5): 395-398.

Najgebauer-Lejko, D., Witek, M., Żmudziński, D., & Ptaszek, A. (2020). Changes in the viscosity, textural properties, and water status in yogurt gel upon supplementation with green and Pu-erh teas. *Journal of Dairy Science*, *103*(12): 11039-11049.

Najmi, A., Nasiruddin, M., Khan, R. A. & Haque, S.F .(2008). Effect of *Nigella sativa* oil on various clinical and biochemical parameters of insulin resistance syndrome. *International Journal Diabetes in* Developing Countries, 28: 11-14.

Ndambi, O. A., Kamga, P. B., Imele, H., Mendi, S. D. & Fonteh, F. A. (2008). Effects of milk preservation using the lactoperoxidase system on processed yoghurt and cheese quality. *African Journal of Food, Agriculture, Nutrition and Development*, *8*(3): 358-374.

Nouri, M., Ezzatpanah, H., Abbasi, S. & Behmadi, H. (2013). Investigating the stability of chemical and physical characteristics of non-fat set yoghurt containing textured milk during the storage time. *Iranian Journal of Food Science and Technology* 10(40): 57-66.

Norgady, B. (2013). The hard facts of food additives ABC health and wellbeing. *http;//www.abc.net.au/health/features/stories/2013/02/14/3684208.htm.*

Okur, Ö. D. (2021). Determination of antioxidant activity and total phenolic contents in yogurt added with black cumin (*Nigella sativa* L.) honey. *Ovidius University Annals of Chemistry*, *32*(1): 1-5.

Oloyede, O. I. (2005). Chemical profile of unripe pulp of *Carica papaya*. *Pakistan Journal of Nutrition*, 4(6): 379-381.

Pal, M., Misgana,T., Asmalash, T. & Jergefa, A. (2015). Hygiene and microbial quality of yoghurt. Beverages and Food World, 42: 25-31

Paulus, K. (1989). Nutritional and sensory properties of processed foods. In: *Food Properties and Computer-Aided Engineering of Food Processing Systems.* Singh, R. P., and Medina, A. G. eds. Kluwer Academic Publishers, New York. 177-200.

Pérez-Jiménez, J., Neveu, V., Vos, F. & Scalbert, A. (2010). Identification of the 100 richest dietary sources of polyphenols: an application of the Phenol-Explorer database. *European journal of clinical nutrition*, *64*(3): S112-S120.

Perveen, T., Haider, S., Kanwal, S. & Haleem, D. J. (2009). "Repeated administration of *Nigella sativa* decreases 5-HT turnover and produces anxiolytic effects in rats," *Pakistan Journal Pharmacology Sci*ence, 22(2):139-144.

Pranav, C., Arun K. D., Nanda, P. K., Vishal K. & Yadav, J. P. (2018). Effect of *Nigella sativa* seed extract on lipid and protein oxidation in raw ground pork during refrigerated storage. *Nutrition & Food Science*, 48(1): 2-15.

Rafaat, M. E. & Ramadan, M. F. H. (2018). Changes in the physiochemical and microbiological properties of probiotics fermented low fat yogurt, enriched with barley B-glucan during cold storage. *Mljekartvo*, 68(4): 295- 309.

Rajkapoor, B., Anandan, R. & Jayakar, B. (1996). Anti-ulcer effect of *Nigella sativa* and *Pongamiapannata* in rats. *Fitoterapia*, 67:195-199.

Ramadan, M. F. H., Samra, M. A. & Khahled, M. E. (2014). Soft cheese supplemented with black cumin oil: Impact on food borne pathogens and quality during storage. *Saudi Journal of biological science,* 21(3): 280-288.

Ramadan, M. F. H., Assiri, A. M., Alzohairy, A. M. & Oraby, H. F. (2015) Health promoting value and food application of black cumin essential oil and overview. *Journal of food science and technology,* 52(10): 6136-42.

Ramchandran, L.(2009). Physico-chemical and therapeutic properties 0f low-fat yoghurt as influenced by fat replacers, exopolys sacharides and probiotics. PhD thesis, School of Biomedical & Health Sciences, Faculty of Health Engineering and Sciences Victoria University Australia.

Rastogi, S., Katara, A., Pandey, M. M., Arora, S., Singh, R. B & Ravat. A. K. S.(2013). Physical stability and HPLC analysis of Indian Kudzu (*Pueraria tuberosalinn*.) fortified milk.Evidence based Complementary and Alternative medicine (eCam), 3: 1-6.

Rawat, S. (2015). Food Spoilage: Microorganisms and their prevention. *Asian Journal of Plant Science and Research*, *5*(4): 47-56.

Reddy, H. S., Adhari, S. A. & Afrah, S. A. (2018). Studies on Phytochemical Screening - Gc-Ms Characterization, Antimicrobial and Antioxidant Assay of Black Cumin Seeds (*Nigella Sativa*) and Senna Alexandria (*Cassia Angustifolia*) Solvent Extracts. *International Journal of Pharmaceutical Sciences and Research*, 9(2): 490-97.

Rodriguez-Gonzalez, O., Buckow, R., Koutchma, T. & Balasubramaniam, V. M. (2015). Energy requirements for alternative food processing technologies—principles, assumptions, and evaluation of efficiency. *Comprehensive Reviews in Food Science and Food Safety*, *14*(5): 536-554.

Rogers, L. D. & Overall, C. M. (2013). Proteolytic post-translational modification of proteins: proteomic tools and methodology. *Molecular and Cellular Proteomics*, *12*(12): 3532-3542.

Salem, E. M., Yar, T., Bamosa, A. O., Al-Quorain, A., Yasawy, M. I., Alsulaiman, R. M. & Randhawa, M.A. (2010). Comparative study of *Nigella sativa* and triple therapy in eradication of *Helicobacter Pylori* in patients with non-ulcer dyspepsia. *Saudi Journal Gastroenterology*,16: 207-214.

Salem, M. L. (2005). Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. *International Immuno pharmacology*, 5:1749–1770.

Salem, M.L. & Hossain, M. S. (2000). Protective effect of black seed oil from *Nigella sativa* against murine cytomegalovirus infection. *International Immunopharmacology*, 22:729-740.

Salman, M. T., Khan, R. A. & Shukla. I. (2008). Antimicrobial activity of black cumin seeds (*Nigella sativa*). Against multi-drug resistant strains of coagulase negative *Staphylococcus*, *Hippocratic Journal of Unani Medicine*, 3(1): 107-1 12.

Salomi, M. J., Nair, S. C. & Panikkar, K. R. (1991). Inhibitory effect of *Nigella sativa* and Saffron (Crocus sativus) on chemical carcinogenesis in mice. *Journal of Nutrition and Cancer*,16(1):67-72.

Salomi, N. J., Nair, S. C., Jayawardhanan, K. K., Varghese, C.D. & Panikkar, K. R. (1992). Antitumour principles from *Nigella sativa* seeds. *Cancer Lett*. *(letter)*, 63(1): 41-46.

Sánchez, G. & Aznar, R. (2015). Evaluation of natural compounds of plant origin for inactivation of enteric viruses. *Food and environmental virology*, *7*(2): 183-187.

Sani, I. K., Rasul, N. H., Asdagh, A., Pirsa, S. & Ghazanfarirad, N. (2021). Development of antimicrobial/antioxidant nanocomposite film based on fish skin gelatin and chickpea protein isolated containing Microencapsulated *Nigella sativa* essential oil and copper sulfide nanoparticles for extending minced meat shelf life. *Doi:http://doi.org/10.21203/rs.3.rs-337479/v1*.

Shafiur, M. R. & Owen J. McCarthy (1999). A classification of food properties, International *Journal of Food Properties*, 2(2): 93-99, *Doi: 10.1080/10942919909524593.*

Sofowora, A. (1993). Phytochemical screening of Medicinal Plants and Traditional Medicines in Africa. *2nd edition. Spectrum books ltd; Nigeria*, 150-156.

Steele, R. (2004). *Understanding and measuring the shelf-life of food*. Woodhead Publishing, *ISBN-13:978-1855737327.*

Steinkraus, K. (1995). Handbook of indigenous fermented foods, revised and expanded. CRC Press, Boca Raton, Florida, United States.

Singh, S., Das S. S., Sigh G., Carola S., De laposuna, M. P. and Catalan C. A. (2014). Composition in vitro antioxidant and antimicrobial activities of essential oil and oleoresins obtained from black cumin seeds (*Nigella sativa*). *Biomedical research International*, 1-10.

Tajkarimi, M. M., Ibrahim, S. A. & Cliver, D. O. (2010). Antimicrobial herb and spice compounds in food. *Food control*, *21*(9): 1199-1218.

Tarek, E., Gomez-Serranillos, M. P., Olga, P., Carmen, A. & Carretero, M. E. (2010). *Nigella sativa* L. Seed Extract Modulates the Neurotransmitter Amino Acids Release in Cultured Neurons in Vitro. *Journal of Biomedicine and Biotechnology*, 2010: 398312.

Tavakkoli, A., Mahdian, V., Razavi, B. M. & Hosseinzadeh, H. (2017). Review on clinical trials of black seed (*Nigella sativa*) and its active constituent, thymoquinone. *Journal of Pharmacopuncture*, *20*(3):179.

Tembhurne, S. V, Firoj, S. & Jagtap, A. G (2008), Nephroprotective activity of *Nigella sativa* seeds against cisplatin induced Nephrotoxicity in Mice. Indian J. of Natural Products. 24(1): 13-16.

Thakur, N., Sareen, N., Sharma, B. & Jagota. K. (2013). In-vitro antifungal activity of *Foeniculum vulgare* against spoilage fungi. *Global Journal of Bio- Science and Technology* 2(3): 427-430.

Thasniya, M., Reshma, K., Arunima, S. H. & Gopika, S. K. (2021). *Nigella sativa*: Properties, processing and food applications. *The Pharma Innovation Journal*, 10(4): 173-184.

Tianli, Y., Jiangbo, Z. & Yahong, Y. (2014). Spoilage by alicyclobacillus bacteria injuice and beverage products: chemical, physical, and combined control methods. *Comprehensive Reviews* in *Food Science* and *Food Safety*,13(5): 771–97.

Topozada, H. H., Mazloum, H. & El-Dakhakhany, M. (1965). The anti-bacterial properties of *Nigella sativa* seeds: Active principle with some clinical application. *Journal Egypt Medicine. Association,* 48: 187-202.

Trachoo, N. (2002). Yogurt: The fermented milk. *Songklanakarin Journal of Science and Technology*, *24*(4): 727-738.

Trease G. E, & Evans, W. C. (1978). Pharmacology 11th Ed. Bailliere Tindall Ltd, London, 115- 222.

Trujillo, A. J., Capellas, M., Saldo, J., Gervilla, R. & Guamis, B. (2002). Applications of high-hydrostatic pressure on milk and dairy products: a review. *Innovative Food Science & Emerging Technologies*, *3*(4): 295-307.

Tsevdou, M., Ouli-Rousi, M., Soukoulis, C. & Taoukis, P. (2020). Impact of high-pressure process on probiotics: Viability kinetics and evaluation of the quality characteristics of probiotic yoghurt. *Foods*, 9(3): 360.

Valdés, L., Cuervo, A., Salazar, N., Ruas-Madiedo, P., Gueimonde, M. & González, S. (2015). The relationship between phenolic compounds from diet and microbiota: impact on human health. *Food & function*, 6 (8): 2424-2439.

Van Boekel, M. A. (2008). Kinetic modeling of food quality: a critical review. *Comprehensive Reviews in Food Science and Food Safety*, *7*(1): 144-158.

Van Marle, M. E., Van den Ende, D., de Kruif, C. G. & Mellema, J. (1999). Steady-shear viscosity of stirred yogurts with varying ropiness, *Journal of Rheology*. 43: 1643-1662.

Vuppala, G. & Murthy, R. K. (2015). Fermentation in food processing. *Journal Microbiology Biotechnology*, 4(1): 1–7.

Wang, W., Bao, Y., Hendricks, G. M. & Guo, M.(2012). Consistency, microstructure and probiotic survivability of goat's milk yoghurt using polymerized whey protein as a co-thickening agent. *International Dairy Journal*, 24: 113–119.

Worthen, D. R., Ghosheh, O. A. & Crooks, P. A. (1998). The in vitro anti-tumour activity of some crude and purified components of black-seed, *Nigella sativa* L. *Anticancer Research,* 18(3A):1527-1532.

Yabaya. A. & Idris, A. (2012). Bacteriological quality assessment of some yoghurt brands sold in Kaduna metropolis. *Journal of Research in National Development (JORIND)*, 10(2):35-39.

Yar, T., El-Hariri, M., EL-Bahai, M. N. & Bamosa, A. O. (2008). Effects of *Nigella sativa* supplementation for one month on cardiac reserve in rats. *Indian Journal Physiology Pharmacology.* 52(2): 141-148.

Yimer, E. M., Tuem, K. B., Karim, A., Ur-Rehman, N. & Anwar, F. (2019). *Nigella sativa* L. (black cumin): a promising natural remedy for wide range of illnesses. *Evidence-Based Complementary and Alternative Medicine, 6:1-16*.

Yüksel, Z., & Erdem, Y. K. (2010). The influence of transglutaminase treatment on functional properties of set yoghurt. *International Journal of dairy technology*, *63*(1): 86-97.

Zaoui, A., Cherrah, Y., Aloui, K., Mahassine, N., Amarouch, H. & Hassar, M .(2002). Effect of *Nigella sativa* fixed oil on blood homeostasis in rat. *Journal Ethno pharmacology*, 79(1): 23-26.

Zhou, Y., Shen, K., Hou, S., Qiu, M. & Luo, Z. (2003). Experimental study on apoptosis induced by elemene in glioma cells. Ai Zheng. *Chin. Journal Cancer*, 22(9): 959-963.

APPENDIX

Acute Toxicological Test for the Black cumin seed (Lorke's method, 1983)

Samples	No of Animal Used	Mortality	Doses	Toxicity signs
		PHASE 1		
Nigella sativa oil	3	0	10mg/kgbw	No observable sign of toxicity
	3	0	1000mg/kgbw	No observable sign of toxicity
	3	0	2000mg/kgbw	No observable sign of toxicity
		PHASE 2		
Nigella sativa Oil	3	0	2500mg/kgbw	No observable sign of toxicity
	3	0	3500mg/kgbw	Inactive at 1st 10 minutes but recovered later
	3	0	5000mg /kgbw	Shivering and Inactive at 1st 15 minutes but later recovered

N.B: $\mathbf{LD_{50}=\sqrt{(minimum\ tolerated\ dose)(max.lethal\ dose)}}$

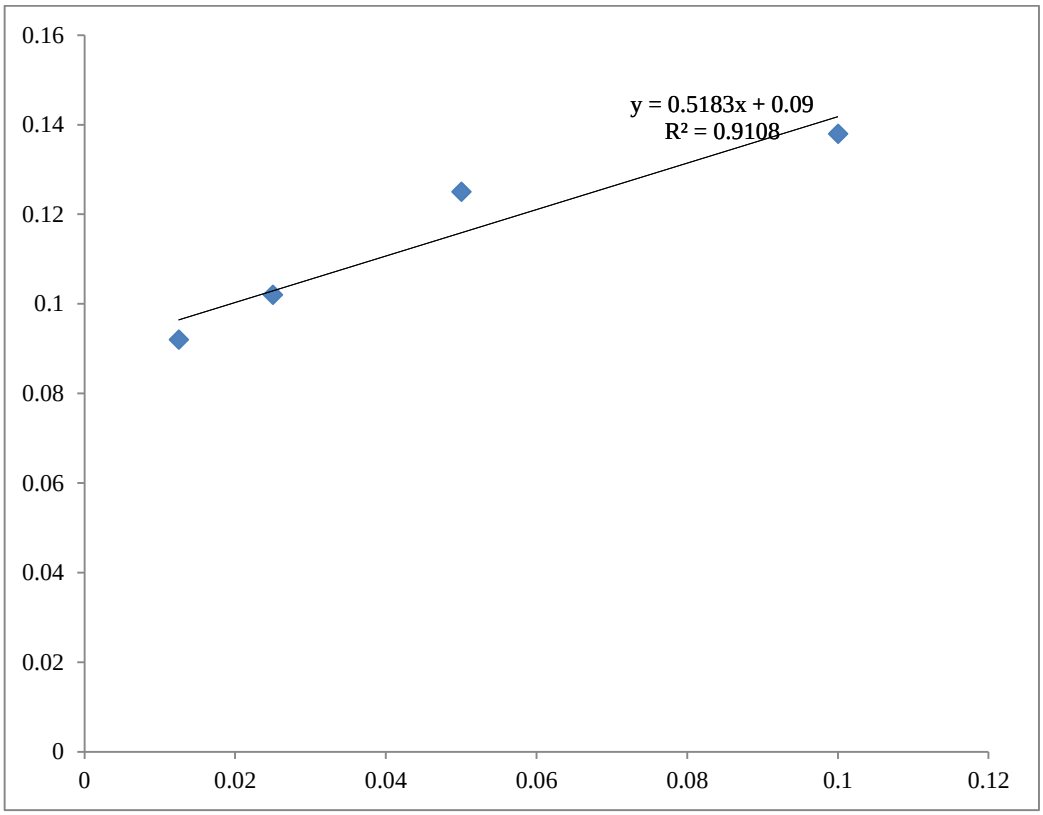
Route of Administration: Oral

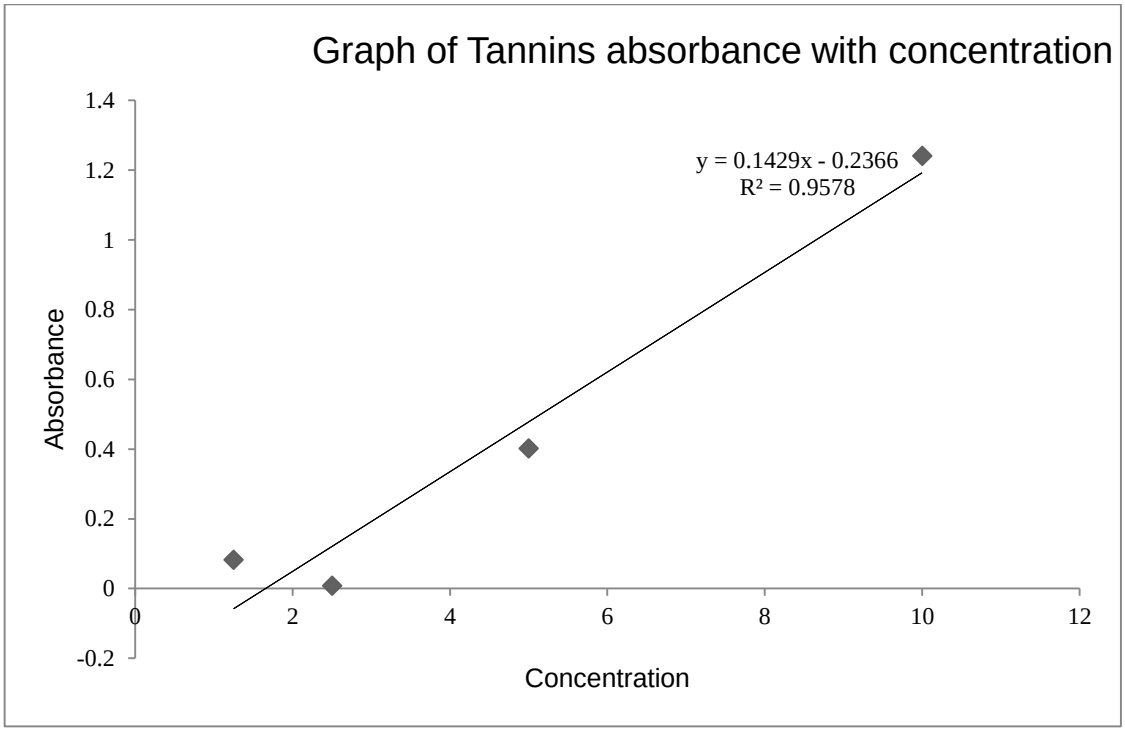
Since no death occurred at the maximum dose (5000mg/kgbw). Therefore, the LD_{50} of *Nigella sativa* oil is greater than 5000mg/kgbw.

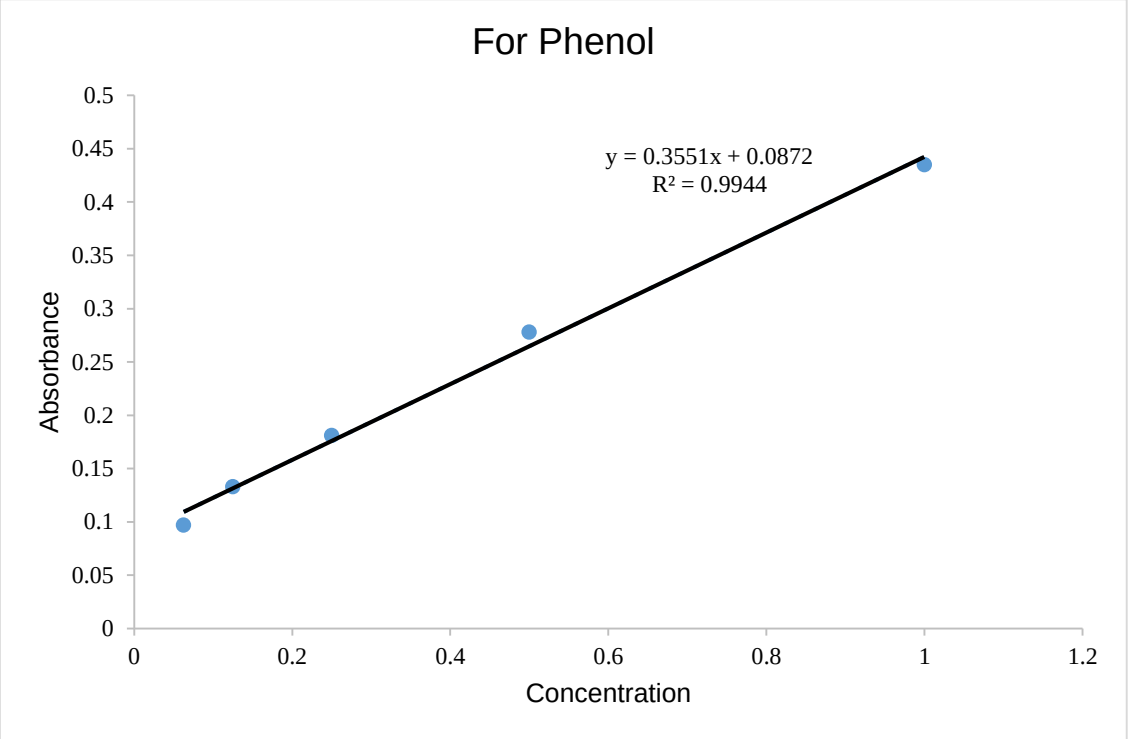
$LD_{50} \geq$ 5000 mg/kg bw.

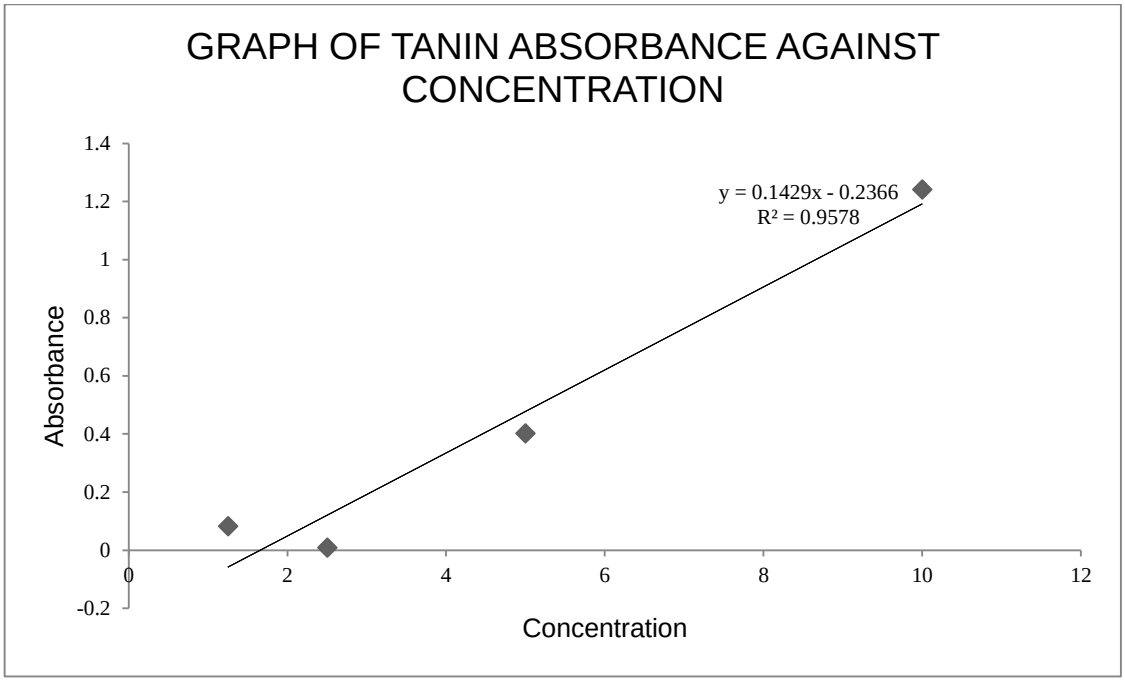
Calibration Curves of Phytochemicals Determination

Flavonoid




Graph of Tannins absorbance with concentration
y = 0.1429x - 0.2366
R² = 0.9578
Absorbance
Concentration
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
2
4
6
8
10
12


For Phenol
y = 0.3551x + 0.0872
R² = 0.9944
Absorbance
Concentration
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
0.2
0.4
0.6
0.8
1
1.2


GRAPH OF TANIN ABSORBANCE AGAINST CONCENTRATION
y = 0.1429x - 0.2366
R² = 0.9578
Absorbance
Concentration
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
2
4
6
8
10
12

Quantitative phytochemical analysis (Raw data)

	T. Phenols (mg/g)	Flavonoids (mg/g)	Tannins (mg/g)	Terpenoids (mg/g)	Saponins (µg/g)	Alkaloids (µg/g)
***Nigella sativa* oil**	11.75, 11.82	2.23, 2.09	17.28, 16.81	5.21, 5.73	24.31, 35.42	45.21, 46.32