ROASTED NIGELLA SATIVA SEED AS A FUNCTIONAL INGREDIENT IN FOODS AND BEVERAGE– EXPLORATORY STUDIES

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by

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LIST OF ABBREVIATIONS

µl	Microliter
µm	Micrometer
^{0}C	Degree Celsius
AAS	Atomic Absorption Spectroscopy
AG	Arabic Gum
DPPH	1, 1-Diphenyl-2-Picrylydrazyl
FRAP	Ferric reducing antioxidant power
g	Gram
GAE	Gallic acid equivalent
GE	Green Tea
GTE	GT Extract
h	Hour
HGTE	Higher level of GTE
RSM	Response Surface Methodology
Kg	Kilogram
L	Liter
M	Molar
mM	Milimolar
MD	Maltodextrin
MDA	Malondialdehyde
N. sativa	*Nigella sativa* L
NSP	*Nigella sativa* powder
NSX	*Nigella sativa* Supercritical CO_2 extraction
rpm	Rate per minute
TFC	Total flavonoid content
TPC	Total Phenolic Content
W/W	Weight/Weight
TBARS	Thiobarbituric Acid Reactive Substances
LGTE	Lower level of GTE

BENIH BIJI NIGELLA SATIVA YANG DIHASILKAN SEBAGAI BAHAN FUNGSI DALAM MAKANAN DAN MINUMAN - KAJIAN EKSPLORAS

ABSTRAK

Nigella Sativa L., juga dikenali sebagai jintan hitam digunakan secara meluas dalam masakan di pelbagai tempat di seluruh dunia. Apabila dipanggang dan dikisar, biji itu bertukar menjadi warna coklat gelap dengan aroma dan juga rasa yang sama seperti biji kopi yang dipanggang. Oleh kerana biji panggang kaya dengan kafein dan sebatian bioaktif, potensi untuk menggunakannya sebagai produk minuman seperti kopi bubuk dapat sangat besar. Selain itu, ekstrak biji dapat dikembangkan menjadi produk nutraseutikal atau digunakan sebagai bahan tambahan fungsional dalam makanan. Fasa pertama kajian ini melibatkan pengoptimuman proses pengeringan semburan ampaian (suspension) biji Nigella Sativa yang dipanggang (roasted-NSS) menggunakan kaedah tindak balas permukaan untuk menghasilkan serbuk Nigella Sativa (NSP) dengan menggunakan dua agen pembawa iaitu maltodekstrina (NSP-MD) dan gam Arab (NSP-AG). Keadaan optimum yang menghasilkan 72.9 % NSP-MD adalah 130 ^{o}C suhu salur masuk pada kadar suapan sebanyak 7 %, manakala keadaan optimum untuk menghasilkan 40.7 % NSP-AG adalah 130 ^{o}C suhu salur masuk pada 12 % kadar suapan. Kandungan kafein dan aktiviti antioksidan bagi roasted-NSS yang digunakan dalam kajian adalah 3.8 %, 72.5 %, 45 mmol/mg dan 27.4%. Selepas pengeringan semburan, kandungan kafein bagi NSP-AG dan NSP-MD masing-masing menurun dengan ketara ($P<0.05$) kepada 1.6 dan 1.9 %. Secara keseluruhan, tiada perbezaan yang signifikan ($P >0.05$) dalam kebolehterimaan roasted-NSS, NSP-MD dan NSP-AG. Kesemua sampel merekodkan nilai yang lebih sedikit daripada 6 yang menunjukkan bahawa semua sampel boleh diterima. Dalam

fasa kedua, kajian kestabilan penyimpanan selama 12 bulan dijalankan untuk menilai keberkesanan NSP-MD dan NSP-AG dalam melindungi kafein, sebatian antioksidan dan ciri-ciri berfungsinya berbanding roasted-NSS. Sebelum fasa penyimpanan, kandungan kafein dalam roasted-NSS, NSP-MD, dan NSP-AG masing-masing adalah 4.3 %, 3.1 % dan 2.6 %, Kandungan kafein menunjukkan penurunan berterusan sepanjang 12 bulan tempoh penyimpanan yang mana telah merekodkan 70% kerugian dalam kedua-dua sampel NSP-MD dan juga NSP-AG manakala 73% bagi sampel roasted-NSS. Berkenaan dengan perlindungan sebatian antioksidan semasa fasa penyimpanan, tiada perbezaan yang ketara ($P>0.05$) antara NSP-MD dan NSP-AG. Oleh itu, MD dianggap calon yang terbaik sebagai ejen penyalutan untuk pengeringan semburan NSP, seperti yang terbukti memberikan perlindungan jangka masa pendek untuk kafein dan penghasilan yang tinggi semasa pengeringan semburan. Fasa ketiga kajian ini melibatkan penyediaan dan penilaian ekstrak biji Nigella Sativa menggunakan pengekstrakan CO_2 super kritis. Dua jenis ekstrak yang disediakan adalah ekstrak biji Nigella Sativa mentah (raw NSX) dan ekstrak Nigella Sativa yang dipanggang (roasted NSX). Ekstrak NSS yang dipanggang, NSS mentah, roasted NSX dan raw NSX dinilai untuk sifat antioksidannya. Nilai yang diperoleh adalah dalam lingkungan 14 hingga 44 mg/g, 12 hingga 30 mg/ml, 33 hingga 50% dan 0.7 hingga 0.9 mmol/mg masing-masing untuk TPC, TFC, DPPH dan FRAP. Susunan kedudukan mengikut aktiviti antioksidan adalah; roasted NSX > raw NSX > roasted NSS > raw NSS. Berdasarkan kedudukan di atas, roasted NSX dan raw NSX dipilih untuk penilaian kapasiti antioksidan radikal oksigen (ORAC) untuk menentukan potensi penggunaan sebagai produk nutraseutikal atau bahan tambahan berfungsi dalam formulasi makanan. Nilai ORAC yang diperoleh untuk raw NSX dan roasted NSX adalah 6370 dan 10561 μmole TE / 100gram. Kesemua nilai ini

adalah lebih tinggi daripada nilai ORAC bagi kebanyakan ekstrak biji benih, serbuk koko, wain dan juga teh hijau seperti yang disenaraikan oleh pangkalan data USDA. Fasa terakhir kajian ini melibatkan penggunaan roasted NSX dan raw NSX sebagai bahan berfungsi dalam patti daging lembu yang mana ekstrak teh hijau (GTE) digunakan sebagai kawalan positif. Analisis pengoksidaan lemak, pengoksidaan protein, komposisi proksimat, hasil memasak, pH, warna, sifat tekstur dan deria telah dijalankan sebelum dan selepas penyimpanan pada suhu -18^{o}C selama 12 minggu. Selepas penyimpanan, berlaku penurunan dalam kelembapan dan kandungan lemak kesemua patti ($P<0.05$), namun kandungan abu dan protein tetap sama ($P>0.05$). Selepas penyimpanan, peringkat pengoksidaan lemak dan pengoksidaan protein adalah; kawalan (4.14 μM / g, lemak, 15.01 ol mol / mg protein)> GTE rendah (3.27 μM / g lemak, 11.06 ol mol / mg protein> GTE tinggi (2.98 μM / g lemak, 9.78 ol mol / mg protein)> NSX mentah rendah (2.16 μM / g lemak, 10 μ mol / mg protein)> NSX panggang rendah (2.09 μM / g lemak, 9.13 ol mol / mg protein)> NSX mentah tinggi (1.88 μM / g lemak 8.4 ol mol / mg protein)> NSX panggang tinggi (1.72 μM / g lemak 7.78 μ mol / mg protein) ($P <0.05$). Selepas penyimpanan, kawalan dan kesemua patti yang digabungkan dengan ekstrak yang tinggi adalah tertakluk kepada penilaian deria. Kebolehterimaan secara keseluruhan berapa pada kedudukan seperti berikut; kawalan < GTE < NSX dipanggang < NSX mentah.

ROASTED *NIGELLA SATIVA* SEED AS A FUNCTIONAL INGREDIENT IN FOODS AND BEVERAGE– EXPLORATORY STUDIES

ABSTRACT

Nigella sativa also known as black cumin or black seed is extensively used for culinary purposes in many parts of the world. Upon roasting and grinding the seed appears dark brown with aroma and taste similar to roasted coffee bean. Since the roasted seed is rich in caffeine and bioactive compounds, the potential for utilizing it as a powdered coffee-like beverage product can be tremendous. In addition, the seed extracts could be developed into a nutraceutical product or used as a functional additive in food. The first phase of this study involved optimization of spray drying process of roasted *Nigella Sativa* seeds (roasted-NSS) suspensions by using response surface methodology to produce *Nigella sativa* powder (NSP) by employing two different carrier agents namely maltodextrin (NSP-MD) and gum Arabic (NSP-AG), The optimum conditions yielding 72.9 % of NSP-MD were 130 ^{o}C inlet temperature at 7 % feed flow rate, whilst the optimum conditions yielding 40.7 % NSP-AG were 130 ^{o}C inlet temperature at 12 % feed flow rate. The caffeine content and DPPH and FRAP of roasted–NSS used in the study was 3.8 %, 72.5 % and 45 mmol/mg respectively. Following spray drying, the caffeine content of NSP-AG and NSP-MD dropped significantly ($P<0.05$) to 1.6 and 1.9 % respectively. All samples scored higher than 6 indicating that all samples were acceptable. In the second phase, a 12-month storage stability study was conducted to evaluate effectiveness of NSP-MD and NSP-AG in protecting caffeine, anti-oxidative compounds and functional properties as compared to Roasted-NSS. Before storage, the caffeine contents of roasted-NSS, NSP-MD and NSP-AG were 4.3 %, 3.1 % and

2.6 %, respectively. The caffeine content continued to show a steady decline during the 12 months of storage where a total loss of 70 % was registered in both NSP-MD and NSP-AG samples and 73% in roasted-NSS. With regard to protection of antioxidant compound during storage, there was no significant difference ($P>0.05$) between NSP- MD and NSP-AG. Therefore, MD was thought as a good candidate as a coating agent for spray drying of NSP as evidenced by better short-term protection of caffeine and a higher yield during spray drying. The third phase of the study involved preparation and evaluation of *Nigella sativa* seed extracts using supercritical CO_2 extraction two types of extracts prepared were raw *Nigella sativa* seeds extract (raw NSX) and roasted *Nigella sativa* extract (roasted NSX). The roasted NSS, raw NSS, roasted NSX and raw NSX extracts were evaluated for antioxidant properties. The antioxidant values obtained were in the ranges of 14 to 44 mg/g, 12 to 30 mg/ml, 33 to 50 % and 0.7 to 0.9 mmol/mg for TPC, TFC, DPPH and FRAP respectively. The ranking in terms of antioxidant activity was; roasted NSX > raw NSX > roasted NSS > raw NSS. Based on the above ranking, roasted NSX and raw NSX were selected for oxygen radical antioxidant capacity (ORAC) evaluation to determine potential uses as nutraceutical product or functional additive in food formulations. The ORAC values obtained for raw NSX and roasted NSX were 6370 and 10561 μmole TE/ 100gram respectively. These values were higher than the ORAC values of most of seed extracts, cocoa powder, wine and green tea as listed by USDA databases. The final phase of the study involved utilisation of roasted NSX and raw NSX as a functional ingredient in beef patty where green tea extracts (GTE) was used as a positive control. Analysis of fat oxidation, protein oxidation, proximate composition, cooking yield, pH, colour, textural and sensory properties were conducted before and after storage at -18^{o}C for 12 weeks. Upon storage, a drop in

moisture and fat content occurred in all patties ($P<0.05$), however, ash and protein content remained the same ($P>0.05$). After storage, the ranking of fat oxidation and protein oxidation was; control (4.14 μM/g, fat, 15.01 μmol/mg protein) > low GTE(3.27 μM/g fat, 11.06 μmol/mg protein > high GTE(2.98 μM/g fat,9.78 μmol/mg protein) > low raw NSX (2.16 μM/g fat, 10 μmol/mg protein) > low roasted NSX (2.09 μM/g fat,9.13 μmol/mg protein) > high raw NSX (1.88 μM/g fat 8.4 μmol/mg protein)> high roasted NSX(1.72 μM/g fat 7.78 μmol/mg protein) ($P<0.05$). Following storage, control and all patties incorporated with high extracts were subjected to sensory properties. The overall acceptability was ranked as; control < GTE < roasted NSX < raw NSX.

CHAPTER ONE

INTRODUCTION

1.1 Background and Rationale

Several medicinal plants and their purified components appear to have valuable therapeutic elements. For instance, *Nigella sativa* seeds (dicotyledon that belongs to Ranunculaceae family) have functioned as food preservative and spices for many years (Cheikh-Rouhou et al., 2007). The seeds are crushed and used to substitute black pepper, at times brewed in hot water and drunk as tea (Goreja, 2003). The roasted seeds flavour is similar to coffee; it is a healthy alternative to coffee. *Nigella sativa* seeds can be sprinkled on salads, become pastries filling, and baked as topping. It has often been used to avert the effects of oxidation (Sultan et al., 2009).

Due to the increasing reports regarding the benefits of *Nigella sativa* seeds, interest to develop *Nigella sativa* seeds into powder form as a competitor against coffee as an instant beverage has risen (Prashanth et al., 2015).Various methods are required to convert *Nigella sativa* from seed to powder; one of them is the spray drying method (Quek et al., 2007).

India, Pakistan, Syria, Turkey, Saudi Arabia, Egypt and Bangladesh are the major *Nigella sativa* seeds producers, however, comparative production data is not available in open-access online sources. Only data for Turkey has been sourced which shows that in 2018 it produced about 3,300 tonnes of *Nigella sativa* seeds with an average yield of 980kg/hectare (FAO & WHO, 2014).

An antioxidant can protect against free radical damage and oxidation by neutralising excess free radicals, which leads to oxidative stress and degenerative diseases (Singh and Sharad, 2004).

Antioxidants can be affected due to storage, handling, and processing, while their combination suggests synergistic or additive impacts. A number of ailments are related to free radicals and oxidative stress, including stroke, cardiovascular disease, atherosclerosis, gastrointestinal dysfunctions, organs ischemia and reperfusion injuries, malignancies, AIDS-related diseases, obstructive sleep apnoea, diabetes mellitus, haemorrhagic shock, hypertension, altered gene expression, osteoporosis, neurodegenerative diseases (Alzheimer and Parkinsonism), cataracts, rheumatoid arthritis, and dementia-related aging. Anti-oxidative vitamins are less effective than dietary biophenols in vitro. Antioxidants are widely used in the food industry (Zhang et al., 2004) and found in many functional foods (e.g. fruits, vegetables, cereals, dry legumes, chocolate, and beverages (tea, coffee or wine) due to phenolic compounds contained in them. Many studies have begun focusing on using antioxidants derived from natural sources (McCarthy et al., 2001). Cloves, cinnamon, *Nigella sativa*, turmeric, ginger, garlic, and onion contain antioxidant properties and are used widely in various food industries, both dried and fresh forms (Ramanathan & Das, 1993; Thorat et al., 2013).

Nigella sativa extracts have exceptional antioxidants that can be embedded into foods to improve shelf-life, retain nutritional values, and maintain quality at storage phase. Preference towards Western diet amongst millennials is a concern. Globalisation brings with it fast foods that has influenced and changed eating habits (Mak et al., 2008). Fourteen phenolic acids which are the predominant compounds and flavonoid compounds were identified in the Tunisian *Nigella sativa* shoots and

roots including chlorogenic acid, ferulic acid, gallic acid, p-dihydroxybenzoic acid, p-coumaric, trans-2-hydroxycinnamic acid, trans-cinnamic acid, epicatechin, (+)-catechin, quercetin, apigenin, amentoflavone, flavone and vanillic acid (Bourgou et al., 2008).

In recent years, various factors affected the extent of meat consumption. Some of these factors are related to the health issues (Hodson & Earle, 2018). As stated earlier, the expectation of food healthiness has recently become an important quality criterion among the consumers choice of a particular food (meat, bread, etc). Likewise, the concept of meat consumption has gradually been ruined by some health related factors. In fact, negative perceptions are mostly related to the consumption of some meat constituents (e.g. fat, saturated fatty acids, cholesterol, sodium, etc.) and the risk of major society chronic disease (e.g. heart disease, cancer, hypertension, and obesity). Several studies reported these relations and the importance of meat and meat products in consumer health (Biesalski, 2005; Demeyer et al., 2008; Ferguson, 2010; Higgs, 2000; Linseisen et al., 2002; Norat et al., 2002; Williamson et al., 2005), nevertheless, the findings of these studies do not seem to always be consistent (McAfee et al., 2010). However, there is a general agreement in recommending moderate meat consumption (smaller portions and less frequent) as part of a balanced diet. In fact, some reports elucidated that reducing meat consumption leads to serious nutritional challenges for some important nutrients (Millward & Garnett, 2010). Growing market competition and changes in consumer demands have provoked enhancement of the quality and image of meat. As a result, market loss due to negative perceptions of meats will be inhibited. Moreover, new market niches through the development of health-beneficial products will be created (Jimenez et al., 2012). Different strategies can be effectively applied to modify

(increase or reduce) the bioactive compounds in meat and meat products and thus develop meat-based functional foods. Most studies into meat based functional foods have been founded on animal production (genetic and nutritional). In addition, technological strategies aimed at affecting the presence of certain constituents with health implications (Arihara, 2004; Jimenez, 2007a; Jimenez et al., 2001).

Endogenous (naturally present in meat) or exogenous (mainly from vegetable and marine sources) bioactive compounds that are used to develop healthier meat and meat products, their implications for health, and the applied strategies to improve their contents, are reviewed by Jimenez et al. (2010). These enhancing strategies include improving fat contents, improving the proteins and amino acid contents, incorporation of probiotics and prebiotics, minerals, vitamins, antioxidants and etc. Incorporation of vitamins and antioxidants such as vitamin E, folic acid, flavonoids and carotenoids are one of the employed strategies to enhance the health beneficial properties of meat products. Flavonoids and simple phenolic compounds are among the most important dietary antioxidants that can be incorporated in meat products and hence, reduce lipid oxidation as well as myoglobin oxidation in meats (Lynch & Kerry, 2000). A variety of plant-derivatives rich in natural antioxidants (flavonoid and phenolic compounds) have widely been utilized to increase the oxidative stability of meat and meat products. Some of these compounds also have anti-inflammatory, anti-cancer and anti-microbial activities (Zhang et al., 2010). In meat they have been incorporated in the animal tissue by feeding (Descalzo & Sancho, 2008; Lynch & Kerry, 2000), while in meat products they have been added as plant extracts or as part of plant ingredients during the industrial production. Antioxidant activity has been widely reported for extracts of fruits such as grape, lycorine root, horsetail, arbutus-berries, etc. (Ganhão et al., 2010; Jongberg et al.,

2011; Nissen et al., 2004; Yildiz-Turp & Serdaroglu, 2010), herbs and spices such as clove, rosemary, cardamom, nutmeg, oregano, green tea, (Banon et al., 2007; Lara et al., 2011; Mohamed and Mansour, 2012; Sanchez-Escalante et al., 2001; Trindade et al., 2010). Some of these antioxidantive compounds can prevent the formation of unhealthy compounds (other than oxidation products) such as heterocyclic amines or N-nitroso compounds (NOC) (Gibis and Weiss, 2010; Jimenez-Colmenero, 2007b). In addition, utilization of dietary components including plant extracts, vit E, and C,pre- and probiotics can control the damage of potent carcinogens (e.g. NOC) caused by consumption of meat products (Demeyer et al., 2008).

Burger patty is a popular meat-based processed food product (Darwish et al., 2011). It is made of ground beef and has high lipid content. Typical ground beef contains 18% lipids, of which 46% are saturated, 51% monounsaturated, and 3% polyunsaturated fatty acids (Demeyer et al., 2008). Meat shelf-life relies on unsaturated fatty acids content, especially those with two double-bonds (Wood et al., 2003). Synthetic and natural antioxidants can also extend meat products shelf-life, apart from inhibiting oxidation process (Estéves and Cava, 2006). Incorporation of *Nigella sativa* extract into beef patty as an example of food system rich in unsaturated fat and protein, may offer a simple yet effective way to enhance its storage stability by minimizing lipid and protein oxidative spoilage.

1.2 Problem Statement

1. *Nigella sativa* is rich in bioactive compounds, which provides health benefits to the body. Roasting the seed appears aroma and taste similar to roasted coffee bean However, furans, ketone, and pyrazines were present only in roasted samples and their concentrations generally increased with roasting time (3-methylbutanal, 2,3-

pentanedione, Furfural, Furans, ketone, and pyrazines). Therefore, differences in the formation of these volatile compounds caused by roasting conditions must play an important role in the flavor characteristic of *Nigella sativa* seeds (Kiralan, 2012). High caffeine content and high in nutritional value it has potential for alternative for coffee beverage like but since limited study has done in processing of seed and considering the ultimate health benefits of *Nigella sativa* seeds they are mostly consumed raw and the problem with this is the sensory issue which would limit its application as beverages. Thus, by processing, we expect to improve sensory appeal in terms of aroma, taste and flavour. (Aroma is defined as an odour, sensed through the nose and retro nasally, and also through the back of the mouth where the nasal and mouth cavities are interlinked. Taste is the sense experienced by the tongue and describes sensations of saltiness, sweetness, sourness, bitterness or umami. Flavour or flavor, however, is a more complex sensation comprising of gustatory, olfactory (smell), optical (visual) and sometimes even auditory perceptions. The commonly known flavours such as chocolate or vanilla have a distinct smell as well as a certain combination of different 'tastes' which is pleasing along with the taste.)

2. There is limited literature on physicochemical and nutritional stability of processed *Nigella sativa* seed under storage. However, presence of caffeine is able to prolong shelf life which is generally advantageous (Nonthakae & Matan, 2015). Caffeine and its catabolic products theobromine and xanthine exhibit both antioxidant and pro-oxidant properties. Therefore, caffeine and its metabolites may also contribute to the overall antioxidant and chemo preventive properties of caffeine-bearing beverages (Azam et al., 2003).

A storage stability study will be carried out to provide more data and make better recommendations for processing methods.

3. *Nigella* seeds are also rich in antioxidants, which could provide a natural alternative to the synthetic/chemical antioxidants widely used in food processing. However, in the raw form, the activity and application of the seeds as antioxidants is limited. Thus, by processing and use of SC-CO_2 extraction method could provide environmentally friendly, recyclable alternative to solvent extraction that we can be able to make use of the antioxidants from *Nigella sativa* seed to retard oxidation in fatty food systems such as meat. Currently, there is no application of *Nigella sativa* seed extracts tested in meat products.

1.3 Objectives

The main objective of this study is to study the potential use of *Nigella sativa* seed as beverage and functional beverage and food.

1. Optimization of spray drying process to produce instant *Nigella sativa* seed;
2. To investigate the effect of process parameter (temperature and feed flowrate) and carrier agents on physicochemical and sensory properties of spray dried *Nigella sativa*;
3. To study the storage stability of spray dried *Nigella sativa*;
4. To evaluate antioxidant activity of *Nigella sativa* seed extracts using super critical CO_2 extraction;
5. To study *Nigella sativa* supercritical CO_2 extract as functional ingredient in beef patties.

1.4 Thesis Outline

In this thesis the potential use of *Nigella sativa* seed as beverage and functional ingredient in foods will be discussed. This thesis consists of 7 main chapters. The first two chapters will provide general information of the study and literature information regarding the study. The following chapters will discuss the processing of *Nigella sativa* seed and stability of seed during storage and also application of this seed as a functional ingredient into the beef patties. Whereas the overall conclusion of this research and several recommendations for further studies and last chapter will be briefly described of each chapter as follows:

CHAPTER 1 Introduction to this study, including the background of the study as well as the current development and challenges. *Nigella sativa* is rich in caffeine and bioactive compounds, the potential for utilizing it as a powdered coffee-like beverage product and substitute to burger can be tremendous. In addition, the seed extracts could be developed into a nutraceutical product or used as a functional additive in food.

CHAPTER 2 Literature review is aimed to provide general information of *Nigella sativa* including its production and distribution, these chapters also provide the information about nutritional value of *Nigella Sativa* and the potential application of functional ingredients in food also, effect of drying methods on the quality of final production.

CHAPTER 3 entitled “effect of conventional roasting on the total phenolic content, total flavonoid content and DPPH radical scavenging activities of *Nigella sativa* seeds” will discuss roasting of Nigella sativa seeds and effect of various time and temperatures on antioxidant activity of *Nigella sativa* seed.

CHAPTER 4 entitled “effect of carrier agents on chemical composition, physical properties and sensory properties of spray-dried roasted *Nigella sativa* seed” will discuss the effect of spray drying process on phyisco-chemical characteristic and sensory properties of *Nigella sativa* seed.

CHAPTER 5 entitled “storage stability of spray dried *Nigella sativa* (black seeds) powder: effect of carrier agents on the physicochemical, phenolic compounds and antioxidant properties” will discuss storage stability of *Nigella sativa* powder and *Nigella sativa* seeds.

CHAPTER 6 entitled “effect of supercritical CO_2 extraction on antioxidant activity of *Nigella sativa* seeds and incorporating the extract in the formulation of beef patties to improve the lipid stability, storage study and sensory attributes” will discuss the effect of supercritical CO_2 extraction on antioxidant activities of raw and roasted seeds and application of *Nigella sativa* extracts tested in meat systems.

CHAPTER 7 will provide the overall conclusion of the finding of this research as well recommendations for further studies regarding to processing of *Nigella sativa* seed.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of *Nigella sativa* seed

Nigella sativa seeds or fennel flower in layman's term refers to an indigenous annual herbaceous dicotyledonous plant (Suresh Kumar., 2011). This plant belongs to Buttercup or Ranunculaceae family (Table 2.1) and it can be found in abundance in the Middle East, Eastern Europe, western Asia, and Mediterranean nations (Venkatachallam et al., 2010). *Nigella sativa* seeds can reach up to 60 cm, has blue flowers with finely-divided foliage (Fig 2.1), and produces black seeds with caraway type. These black seeds are small in size (1–5 mm) with corrugated integuments and are the most useful part of the plant. For thousands of years, these seeds have been added to food as spices and have served as food preservative. Apart from their culinary uses, those from the middle east, Northern Africa, and India have traditionally, for centuries, used the seeds to treat asthma, bronchitis, cough, eczema fever, headache, influenza and rheumatism due to their anti-inflammatory, anti-histamine, and anti-diabetic properties (Gaur et al., 2017). Apart from black cumin, *Nigella sativa* seed has other different names. This seed is known as 'Panacea' in old latin, which means 'cure all', Jintan hitam in Malaysia, while 'Kalonji' in India, and 'Habbatul-Barakah' in Arabic that means 'seeds of blessing' (Padhye et al., 2008; Ramadan, 2007; Sharma et al., 2009). This black cumin is described as the curative black cumin in the holy bible (Salem & Hossain, 2000).

Table 2.1: Scientific classification of *Nigella sativa.*

Scientific classification	
Kingdom	: Plantae
Division	: Magnoliophyta
Order	: Ranunculales
Family	: Ranunculaceae
Genus	: Nigella
Species	: *sativa*

Sources :(http://www.plants.usda.go)

Nigella sativa refers to a flowering plant with tapering green leaves. Its flowers are typically white, yellow, pink, pale purple and pale blue, whereas the fruit is large with an inflated capsule containing dark black tiny seeds when the fruit is ripe (Ahmad et al., 2013; Aljabr et al., 2015).

Herbal products from *Nigella sativa* oil or seed powder are produced in various forms, such as antiseptic cream, ointment, powder, soft gel capsules, tea, vapour rubs, etc., and marketed as treatment for numerous health problems. One such product is Baraka Diabsol soft gel capsules (black seed oil) that reduce blood sugar level among diabetic patients. Apart from the therapeutic products, *Nigella sativa* is used in cosmetics, for example, the Baraka black seed cream, an herbal antiseptic cream. It has been reported that the consumption of a teaspoon of *Nigella sativa* oil thrice a day after meals, along with inhalation of *Nigella sativa* oil vapour, is therapeutic for asthma and cough, while intake of a teaspoon of *Nigella sativa* oil

mixed with yoghurt twice a day prevents diarrhoea-related problems (Ilaiyaraja & Khanum 2010).

Fig 2.1 The plant and flower of *Nigella Sativa* (Racoma, 2013)

2.1.1 Composition

The seeds of *Nigella sativa* seeds are composed of fat (28.5%), protein (26.7%), carbohydrate (24.9%), crude fibre (8.4%), and total ash (4.8%). Minerals, such as phosphorus, potassium, sodium, and iron, as well as other essential elements (e.g. calcium, copper, magnesium, manganese, and zinc) are predominantly found in *Nigella sativa* seeds (Sultan, 2009).in this study focused on oil extraction of *Nigella sativa* seed however, in our study main focused on roasted *Nigella sativa* seed.

Table 2.1 Proximate contents in *Nigella sativa* seeds

Proximate analysis	**Value of analysis (%)**	**References**
Crude protein	22.75	Al-Beitawi and El-Ghousein, 2008
	20-27	Abdel-Aal and Attia, 1993; Salem, 2001; Takruri and Dameh, 1998
	26.7 22.80	Cheikh_Rouhou *et al*, 2007 Sultan, 2009
Moisture	4.40	(Al-Beitawi and El-Ghousein, 2008)
	5.52-7.43	(Abdel-Aal and Attia, 1993; Salem, 2001; Takruri and Dameh, 1998)
	8.65 6.46	(Cheikh_Rouhou *et al*, 2007) (Sultan, 2009
Ash	4.45	(Al-Beitawi and El-Ghousein, 2008)
	3.77-4.92	(Abdel-Aal and Attia, 1993; Salem, 2001; Takruri and Dameh, 1998)
	4.86	(Cheikh_Rouhou *et al*, 2007)
	4.20	(Sultan, 2009)
Crude oil	30-38	(Edris, 2009)
	30-35	(Dandik and Aksoy, 1992)
	34.49-38.72	(Abdel-Aal and Attia, 1993; Salem, 2001; Takruri and Dameh, 1998)
	28.48	(Cheikh_Rouhou *et al*, 2007)
	36.25	(Al-Beitawi and El-Ghousein, 2008)
	31.16	(Sultan, 2009)
Carbohydrate	23.5-33.2	(Abdel-Aal and Attia, 1993; Salem, 2001; Takruri and Dameh, 1998)
	40	(Cheikh_Rouhou et al, 2007)

Nigella sativa seeds contain fixed and essential oils, alkaloid, and saponin. The fixed oil contains unsaturated fatty acid, cycloeucalenol, sterol glucosides, beta-sitosterol, cycloartenol, and sterol esters (Forouzanfar et al., 2014). The alkaloids are made up of two different elements: isoquinoline and pyrazole/indazole. Examples of isoquinoline alkaloids are nigellicimine-N-oxide and nigellicimine, while pyrazole alkaloids are nigellicine and nigellidine. Saponin found in the black seeds is alpha-hederin (Ahmad et al., 2013). *Nigella sativa* seeds are also rich in bioactive components, including thymoquinone (30-48%), thymohydroquinone, dithymoquinone, p-cymene (7-15%), carvacrol (6-12%), 4-terpineol (2-7%), t-anethol (1-4%), sesquiterpene longifolene (1-8%), α-pinene, and thymol (Ahmad et al., 2013). Thymoquinone is predominantly found in essential oil and several investigations have analysed this compound to determine its therapeutic effect (Watkins, 2007).

2.1.2 Significance of *Nigella Sativa* seed

Nigella sativa seeds are peppery in taste with a touch of spiciness and are extensively used as spice and condiment for their carminative and aromatic properties (Gassara et al., 2016).The seeds are normally used in the whole form for cooking curries, baking pastries, and making Mediterranean cheeses; while the ground form is usually sprinkled on salads (Ramadan, 2007). *Nigella sativa* is well-known ever since the ancient Egyptian and Greek civilisations and has been used to improve menstrual flow and increase breastmilk production, apart from treating headache, nasal congestion, toothache, and many other conditions (Ibrahim et al., 2000). It is also a renowned drug during the Islamic civilisation, and in Islamic heritage, it is recognised as a Prophetic medicine because Muslims believe that *Nigella sativa*

seeds are effective in curing all diseases, aside from death, as stated by the Islamic Prophetic statements (Haddith, Sahih Bukhari:5688). Ibn Sina (Avicenna), in his famed manuscript entitled "Al-Kanon fit-tib" or the Canon of Medicine, mentioned that *Nigella sativa* seed was prescribed to energise the body, besides treating fatigue and depression (Hussain & Hussain.2016). *Nigella sativa* seed is used in the form of herb or pressed oil for medicinal purposes (Sharma *et al.*, 2009). In Southeast Asia and the Middle East, the seeds are used to treat various ailments, such as asthma, bronchitis, rheumatism, and inflammatory conditions (Ahmad et al., 2013), including obstinate hiccups, loss of appetite, vomiting, dropsy, and puerperal diseases (Gilani et al., 2004). *Nigella sativa* oil can both treat and prevent skin diseases (Abu-Zinadah, 2009), such as psoriasis and eczema (Several interactomes over-represented oleic acid and lauric acid. The interactomes included genes/transcripts/proteins coded by the gene name. These were FAAH, PMP2, RBP1, APOE, APOB, LDLR, PCSK9, GLTP, ALB, UBC, MTRNR2L2, LTB4R2, GPR68, LY96, TLR4 and FCGRT. In addition, the interactomes also included other chemical substances. Therefore, two of active compounds contained by N. sativa were active substances that had been known having interactions with several proteins Lauric acid, LY96, and TLR4 were over-represented by interactomes that were annotated as LPS detection for the biological process, LPS receptor activity for the molecular function, and LPS receptor complex for the cellular components). The oil, when mixed with beeswax, helps relieve joint pain, treats burns and skin infections, minimises wrinkles, and an excellent moisturiser (Ramadan, 2007).

Several studies have attributed plausible antihyperglycemic characteristics of *Nigella sativa* seed to its antioxidants constituents (Abdelmeguid et al., 2010). Thus, dithymoquinone and thymoquinone are the core antioxidant constituents of *Nigella*

sativa (Mukhtar et al., 2019). Usage of *Nigella sativa* seed, namely injection and ingestion, could increase the antioxidant defence of the body (GaliMuhtasib et al., 2006). Researchers discovered that *Nigella sativa* can decrease lipid peroxidation, but it raises antioxidant enzymes (Abdelmeguid et al., 2010; Houcher et al., 2007; Salama, 2012; Muhammad Tauseef et al., 2014). maintain integrity of pancreatic beta-cells, as well as raise the quantity of islets and their diameters (Alimohammadi et al., 2013). It also decreases resistance of insulin (Bamosa et al., 2010), raises secretion of insulin (Alimohammadi et al., 2013; Mansi, 2005; Rchid et al., 2004), and inhibits advancement of glycation end-product (Loss & Chintalapati, 2011). Furthermore, expansion in glycemic may minimise lipid dysfunction, especially among diabetic patients (Kendall et al., 2006). *Nigella sativa* is also used to treat hypertension, rheumatism, and gastrointestinal disorders such as dyspepsia, flatulence, dysentery, and diarrhoea (Durmuskahya & Ozturk, 2013; Siddiqui et al., 2014). Moreover, *Nigella sativa* in the ointment form is a respite for nasal ulcers, abscesses, swollen joint, eczema, and orchitis. The application of *Nigella sativa* and honey can cure respiratory ailments, namely asthma, bronchospasm, and chest congestion (Nasir et al., 2014). Analyses regarding the impacts of *Nigella sativa* on metabolic syndrome are presented in Table 2.2.

In *Nigella sativa*, the differences were seen in the volatile composition between raw and roasted *Nigella sativa* seeds. Furthermore, different roasting times significantly affect the volatile compounds of *Nigella sativa* seeds like terpenes, which decreased in the roasted samples. Furans, ketone, and pyrazines, on the other hand, were present only in roasted samples and their concentrations usually increased with roasting time. Therefore, differences in the formation of these volatile

compounds brought on by roasting conditions play a crucial role in the flavour characteristic of *Nigella sativa* seeds (Kiralan, 2012).

2.1.3 Antioxidant Properties of *Nigella sativa* seed

Nigella sativa is known for its antioxidant properties and the exploration of its biological activities namely the antioxidative effect of its seed powder, fixed or volatile oil and various kinds of extracts have been studied thoroughly using both in vitro (Burits & Bucar, 2000) and in vivo methods (Khan & Sultana 2005). Several studies have evaluated the antioxidative properties of individual constituents of *Nigella sativa* seeds. In vitro methods that measured the electron transfer and ROS scavenging discovered carvacrol, thymol and thymohydroquinone (2) had a stronger effect than dithymoquinone (1) and thymoquinone (3) (Figure 2.5) (Burits & Bucar 2000; Kruk et al., 2000). Among the methods available for the assessment of antioxidant capacity in vitro, the techniques that measure hydrogen atom transfer are considered relevant to the evaluation of the radical chain-breaking effect of food and biological samples. While the oxygen radical absorbance capacity (ORAC) assay, however, is recommended for measuring hydrogen atom transferability because of its wide-spread validation in a number of laboratories and the availability of comparable data (MacDonald et al., 2006).

Literature has highlighted that *Nigella sativa* seed contains various phytochemicals that are beneficial to human health. (Desai et al.,2015) found *Nigella sativa* seed oil is a rich source of polyphenols (1744 µg/g). Major antioxidants found in *Nigella sativa* essential oil are carvacrol, cymene, thymoquinone, thymol, t-

anethole and 4-terpineol whereas its fixed oil is rich in tocopherols (Sultan et al., 2015). Radical scavenging effects were shown in carvacrol, thymoquinone, t-anethole and 4-terpineol when DPPH assay for non-specific p-hydrogen atom was used. Significant OH radical scavenging effects are also shown in these components when tested with the assay for non-enzymatic lipid peroxidation in liposomes and the deoxyribose degradation assay (Burits & Bucar, 2000).

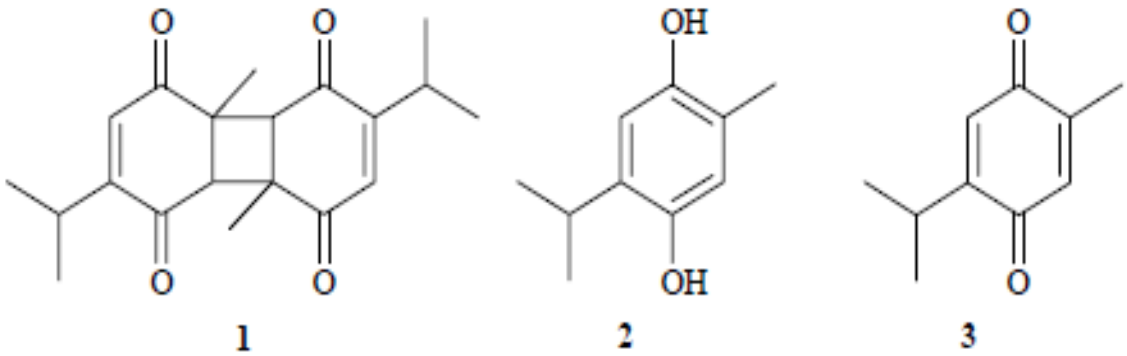

Figure 2.2: Chemical structures of *Nigella sativa* quinones: dithymoquinone (1), thymohydroquinone (2) and thymoquinone (3).

2.2 Antioxidant Properties

Antioxidants defined as "substances in small quantities that can prevent or greatly retard the oxidation of easily oxidisable materials, such as fats". Antioxidants inhibit or delay the production of free radicals (Alenzi et al., 2013). Reactive oxygen species (ROS), which are generated in the human body, are highly reactive pro-oxidants that can cause cell damage (Halliwell, 1991) by attacking membrane lipids, intracellular protein/enzymes, carbohydrates, and nuclear DNA in tissues and cells (Singh & Singh, 2008). Nevertheless, antioxidants may give protection against ROS (Shahidi, 2000). There are numerous approaches to determine antioxidant activities, such as

oxidisation of lipid or lipoprotein substrate, assessment of various assays to ensure inhibitory of oxidation, as well as trapping and measuring the ability of antioxidants to intercept free radicals (Frankel & Meyer, 2000).

Researchers discovered that *Nigella sativa* can decrease lipid peroxidation, but it raises antioxidant enzymes (Abdelmeguid et al., 2010; Houcher et al., 2007; Salama, 2012; Muhammad Tauseef et al., 2014).Reduction in oxidative stress can enhance regeneration of pancreatic beta cells (Kanter, 2008). The direct or indirect decrease in free radical species may affect the metabolism of lipid. The constituents of an antioxidant can safeguard tissues against peroxidation of lipid and advance the function of an enzyme that encompasses metabolism of lipid (Yin & Porter, 2011).

2.2.1 Classification of Antioxidants

Ratnam et al., (2006), divided antioxidants into two classes: enzymatic and non-enzymatic. Those enzymatic antioxidants can be produced endogenously, including low molecular weight molecules, enzyme cofactors, and enzymes (Mirończuk et al., 2018). Meanwhile, non-enzymatic antioxidants can be derived from certain classes of dietary sources, such as polyphenols (the most), flavonoids, and phenolic acids. Vitamins, carotenoids, organosulfur, and minerals are the other classes of dietary antioxidants (Hamid et al., 2010).

Figures 2.2 illustrate the classification and broad scope of antioxidants, respectively. It is noteworthy to highlight the great difference between antiradical and antioxidant activities. Antiradical activities refer to the ability of a compound in reacting with free radicals, whereas antioxidant activities can inhibit oxidation process. As a result, assayed systems that use stable free radicals (DPPH and ABTS)

offer information regarding antioxidant activities or radical scavenging, although unrelated to antioxidant activities. In demonstrating antioxidant activities in regard to lipid/food stabilisation, it is imperative that tests are done on real products despite being seen as a challenge (Schaich, 2016).

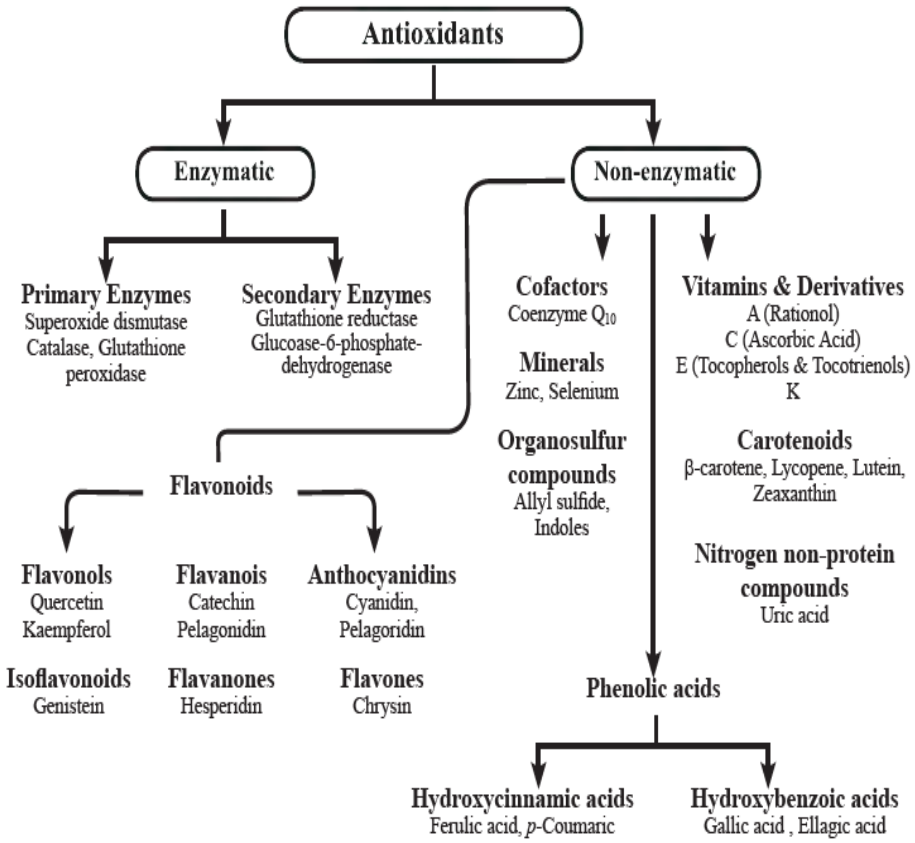

Fig. 2.3: Classification of antioxidants (Carocho & Ferreira, 2013)

2.2.2 Single Electron Transfer (SET) based assays

Simulation of antioxidant activity using SET-based assays can be performed via suitable redox-potential probe Table (2.3), where antioxidants react with fluorescent or coloured probe (oxidising agent), rather than peroxyl radicals (Prior et al., 2005). To measure the antioxidant capacity, spectrophotometric SET-based assays are employed. The assay measures oxidant reduction based on change in colour, whereby colour change degree signifies probe absorbance rate at certain wavelength to indicate antioxidants concentration in samples (Gülcin et al., 2012). Trolox-equivalent antioxidant capacity (TEAC) or 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and 2,2-di (4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) refer to assays of decolourisation (You et al., 2010).Meanwhile, cupric reducing antioxidant capacity (CUPRAC), FC total phenols assay, and ferric reducing antioxidant power (FRAP) display increment in absorbance rate at certain wavelength upon reaction between chromogenic reagent and antioxidants (Berker et al., 2007). For instance, CUPRAC and FRAP form charge transfer complexes in lower valence of iron (Fe (II)) and copper (Cu (I)) after corresponding with ligands, respectively (Moharram et al., 2014).

Table 2.3 In vitro antioxidant capacity (ORAC, TRAP, IOU, TEAC, FRAP and DPPH).

assays involving hydrogen atom transfer reactions ROO• + AH à ROOH + A• ROO• + LH à ROOH + L•	ORAC (oxygen radical absorbance capacity) TRAP (total radical trapping antioxidant parameter) Carbon bleaching assay IOU (inhibited oxygen uptake) Inhibition of linoleic acid oxidation Inhibition of LDL oxidation
Assays by electron-transfer reaction M(n) + e (from AH) à AH• + M (n – 1)	TEAC (Trolox equivalent antioxidant capacity) FRAP (ferric ion reducing antioxidant parameter)
Other assays	DPPH (diphenyl-1-picrythydrazyl) Copper (II) reduction capacity Total phenols assay by Follin-Ciocalteu reagent

Source: Frankel & Finley (2008).

2.2.3 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH)

DPPH refers to a free radical that is stable, natural, and available commercially as organic nitrogen radicals (Pyrzynska & Pękal, 2013). DPPH assay has been widely used in antioxidant studies due to its simple and sensitive method (Kedare, & Singh, 2011). The theory behind DPPH assay lies in the hydrogen donor as the antioxidant to determine radical scavengers (Gülçin et al., 2010). Figure 2.4 portrays both DPPH structure and mechanism, in which hydrogen is accepted. When this occurs, the disappearance of DPPH becomes proportional to impact of antioxidant in samples. The most common technique to monitor DPPH is via UV spectrometer due to its accuracy and simplicity. DPPH shows the effect of antioxidant based on UV

absorption at 517 nm wavelength, where purple changes to yellow when hydrogen is absorbed from antioxidant. A stoichiometric reaction is noted to reflect the amount of absorbed hydrogen atoms.

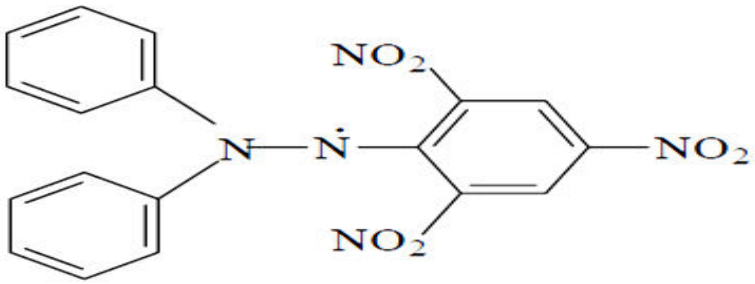

Fig 2.4 The structure of DPPH•

DPPH• +AH ⟶ DPPH-H+A•

The newly formed radical (A•) can follow radical-radical interactions to render stable molecules via radical disproportionation, nevertheless, these secondary reactions are mainly hindered (Juan et al., 2000).

DPPH• + A• ⟶ DPPH-A

A• + A• ⟶ A-A

As a result, the DPPH• disappearance could be an acceptable indication for estimating the degree of free radical scavenging.

2.2.4 Ferric Reducing Antioxidant Power (FRAP)

The method of FRAP looks into decreased complex ferric ion-TPTZ (2,4,6-tri(2-pyridyl)-1,3,5-triazine) due to antioxidant effect. The ligand-Fe^{2+} bond exhibits intense navy blue shade (Apak et al., 2004). The rate of absorbance is determined by reduction in iron that correlates to the amount of antioxidants (Thaipong et al., 2006; Pellegrini et al., 2003; Gil et al., 2002). Ascorbic acid (Gil et al., 2002), and Trolox (Pellegrini et al., 2003) can be applied as the reference.

2.2.5 Total Phenolic Assay by Folin-Ciocalteau

Total phenolic assay by FC colourimetry is extended from Singleton and Rossi's work. A technique of reducing chemical with FC reagent, molybdenum oxides, and tungsten was adopted to analyse proteins, such as tyrosine. Proteins consist of phenolic group and further extension was initiated by Singleton by analysing phenolic in wine (Singleton & Rossi 1965). This technique is not only quantitative and sensitive in nature, but also depends on polymerisation degree of phenols, while correction for nucleic acids, ascorbic acid, and proteins demand certain interference. Reduction in metal oxide exhibits maximum broad light absorption at 765 nm. Due to the dissociated form of many phenolic compounds (conjugate base or phenolate anion) at assay pH (pH~10), they can be oxidised easily using FC reagent (Gupta, 2015).

2.2.6 The ORAC Assay

The technique using Oxygen Radical Absorption Capacity (ORAC) assay (Denev et al., 2010; Thaipong et al., 2006; Ou et al., 2001) determines the scavenging activity