UTILISATION OF SUPERHEATED STEAM AND TORREFACTO ROASTING ON QUALITY ATTRIBUTES OF BLACK SEED (*NIGELLA SATIVA*) FOR BEVERAGE APPLICATION

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by

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LIST OF SYMBOLS & ABBREVIATIONS

ABTS	2,2′-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid)
ANOVA	analysis of variance
AOAC	Association of Official Agricultural Chemists
AV	p-anisidine value
BCA	browned compounds analysis
CIE	Commission Internationale de l'Eclairage
CIELAB	Commission Internationale de l'Eclairage *L**, *a*, b**
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDTA	disodium ethylenediaminetetraacetate acid
FAO	Food and Agriculture Organization
FFA	free fatty acid
FRAP	ferric reducing antioxidant power
GAE	gallic acid equivalent
GC	gas chromatography
HCL	hydrochloric acid
HIV	human immunodeficiency virus
HOSC	hydroxyl radical-scavenging capacity
HPLC	high performance liquid chromatography
IC	inhibition concentration
KI	potassium iodide
MHz	megahertz
NA	not available
NaOH	sodium hydroxide
NRCB	naturally roasted coffee beans
ORAC	oxygen radical absorbance capacity

PV	peroxide value
RSC	radical scavenging capacity
RSM	response surface methodology
SHS	superheated steam
TC	torrefacto coffee
TOTOX	total oxidation value
TPC	total phenolic content
TR	torrefacto roasting
TRBS	torrefacto roasted black seeds
TRBSB	torrefacto roasted black seed brew
TRCB	torrefacto roasted coffee beans
TRH	torrefacto roasted honey
TRHB	torrefacto roasted black brew
TRK	torrefacto roasted kelulut
TRKB	torrefacto roasted kelulut brew
TRS	torrefacto roasted sugar
TRSB	torrefacto roasted brew
TSS	total soluble solids
USA	United States of America
USD	United States Dollar
UV	ultra-violet
WHO	World Health Organization

PENGGUNAAN STIM PANAS LAMPAU DAN PEMANGGANGAN TORREFACTO TERHADAP ATRIBUT KUALITI BIJI HITAM (*NIGELLA SATIVA*) UNTUK APLIKASI MINUMAN

ABSTRAK

Jintan hitam atau *Nigella sativa*, sejenis rempah yang penuh faedah kesihatan, biasanya dipanggang dalam pelbagai aplikasi makanan. Walaupun penting, aplikasi haba memudaratkan kualiti jintan jitam, justeru, teknik inovatif seperti stim panas lampau (SHS) dan torrefacto (TR) mungkin bermanfaat. Objektif penyelidikan ini adalah untuk menghasilkan minuman daripada jintan hitam dipanggang SHS dan torrefacto. Dalam fasa pertama, biji hitam dipanggang dengan SHS dan pemanggangan perolakan, pada 150 ℃, 200 ℃ dan 250 ℃ selama 10, 15, dan 20 minit pada setiap suhu kemudian dibandingkan dari segi pengoksidaan lipid, proksimat, antioksida, dan minyak pati. Keputusan menunjukkan bahawa SHS menghasilkan lebih minyak (SHS, 24.83 %, perolakan, 23.23 %), serta nilai peroksida (dalam meq O_2/kg, nilai tertinggi; 84.00 perolakan, 48.00 SHS), nilai p-anisidine (nilai tertinggi; 28.36 perolakan, SHS, 23.73), kapasiti mengais radikal DPPH (SHS; 92.45 - 69.97 %, perokalan; 92.08 - 65.81 %), dan nilai FRAP (dalam mM $FeSO_4$/g sampel; SHS, 327 - 435, perolakan, 301 - 356) yang lebih baik berbanding perolakan. Keputusan juga menunjukkan pengoksidaan miyak yang melebihi standard yang ditetapkan. Ini menunjukkan cara pengekstrakan pelarut yang tidak sesuai serta kualiti biji yang tidak baik, Profil minyak pati sampel perolakan menunjukkan kehilangan thymoquinone, komponen penting biji hitam. Berdasarkan keputusan ini, biji hitam dipanggang TR dan SHS dalam fasa kedua pada 220℃ selama 10 minit.

Parameter ini ditentukan melalui gerak balas permukaan (RSM) untuk kapasiti antioksidan yang maksimum. Torrefacto (TR) ialah penambahan gula semasa dipanggang untuk mengurangkan kepahitan dan mengekalkan rasa produk. TR turut dijalankan dengan madu dan madu kelulut. Biji hitam torrefacto dengan madu kelulut (TRK) menghasilkan TPC (8.10 mg GAE/g sampel) dan FRAP (719.98 mM $FeSO_4$/g sampel) tertinggi, manakala biji hitam dipanggang madu (TRH) melihatkan DPPH IC_{50} terbaik (5.83 mg/ml). Dari segi oksidasi minyak, TRH mencatatkan FFA terendah (2.78 % asid oleik) manakala biji hitam dipanggang gula (TRS) mencatatkan nilai p-anisidine (12.79) dan TOTOX (180.79) yang terendah. Semua biji hitam dipanggang torrefacto (TRBS) juga mencatatkan kandungan thymoquinone yang jauh lebih tinggi (TRS, 0.48 mg/g; TRK, 0.35 mg/g; TRH 0.43 mg/g) berbanding sampel kawalan. Oleh itu semua TRBS dibru (seperti kopi) untuk analisa deria pada fasa seterusnya. Analisa menunjukkan konsentrasi bru lebih mempengaruhi penerimaan deria berbanding tambahan gula atau madu dalam biji hitam dipanggang torrefacto. Kesimpulannya, pemanggangan SHS berserta TR pada 220 ℃ selama 10 minit boleh menghasilkan biji hitam yang berkualiti serta berpotensi dibru sebagai minuman yang berkhasiat.

UTILISATION OF SUPERHEATED STEAM AND TORREFACTO ROASTING ON QUALITY ATTRIBUTES OF BLACK SEED (*NIGELLA SATIVA*) FOR BEVERAGE APPLICATION

ABSTRACT

Nigella sativa, known as black seed, has many health benefits and is typically roasted prior to consumption. This causes detrimental quality loss, thus, an innovative method such as superheated steam (SHS) and torrefacto roasting may be advantageous. This research aims to develop a beverage from SHS torrefacto (TR) roasted black seeds. In the first phase, SHS was applied to black seeds at 150°C, 200°C and 250°C for 10, 15, and 20 mins at each temperature then compared to convection roasting at the same parameters for lipid oxidation, proximate, antioxidants, and essential oils. Results indicated that SHS roasted seeds yielded more oil (24.83 % vs only 23.23 % oil for convection) and had better peroxide value (highest value for convection, 84.00 versus SHS, 48.00 meq O2/kg of oil), p-anisidine value (highest value; for convection, 28.36 versus SHS, 23.73), FRAP values (SHS; 327 to 435 mM FeSO4/g sample, convection; 301 to 356 mM FeSO4/g sample), and DPPH scavenging capacity (SHS; 92.45 to 69.97 % inhibition, convection; 92.08 to 65.81 % inhibition). Results also indicate unsuitable extraction method and poor seed quality since oil oxidative analysis results were all above standard acceptable levels. Convection roasting also caused loss of thymoquinone, an important characteristic component of black seed essential oil. In the second phase, black seeds were TR and SHS roasted at 220°C for 10 min. This roasting time and temperature was derived via response surface methodology for maximum antioxidant capacity. TR is the process of adding sugar during roasting to reduce bitterness and preserve flavour. Besides sugar TR was also done with honey and kelulut honey. Torrefacto kelulut honey roasted black seeds (TRK) had the highest TPC (8.10 mg GAE/g sample) and FRAP values (719.98 mM FeSO4/g sample) while torrefacto roasted honey black seeds (TRH) had best DPPH IC_{50} value (5.83 mg/ml). Additionally, TRH had the lowest FFA (2.78 % oleic acid) while torrefacto roasted sugar black seeds (TRS) had the lowest p-anisidine (12.79) and TOTOX value (180.79). All torrefacto roasted black seeds (TRBS) also yielded better thymoquinone content than non TRBS (TRS, 0.48 mg/g; TRK, 0.35 mg/g; TRH 0.43 mg/g). In the final phase, all TRBS were brewed into a hot beverage for hedonic sensory analysis. Sensory result revealed that brew concentration had more impact on sensory qualities than addition of sugar or honey. In conclusion, SHS along with TR at 220 ℃ for 10 minutes produced high quality black seed that can be potentially brewed into a nutritious drink.

CHAPTER 1

INTRODUCTION

1.1 Background information

The black seed, scientifically known as *Nigella sativa* from the genus *Nigella* in the family Ranunculaceae, is an indigenous plant native to the Mediterranean region (Ipor & Oyen, 1999; Jansen, 1981; Paarakh, 2010; Ravindran, 2017). Hereafter referred to as just black seeds, these are the titular seeds within this research that possess a world of therapeutic potency among which include its ability to ease many human ailments such as headache, fever, dizziness, and many others (Ali & Blunden, 2003; Ramadan, 2007). Prevalent culinary practice is to heat-treat black seeds before using them, examples include, pickling (boiling) and adding them to bread before baking to give it a distinctive flavour (Aljabre *et al.*, 2005). Black seed oils are also included as a healthy ingredient in pastry goods and it would almost always be roasted, either dry or in oil, before being added to recipes as a flavouring agent (Kiralan, 2012). Roasting the seeds before obtaining its lipid fraction is also a common practice (Atta, 2003). Evidently roasting black seeds is a necessary supplementation for which little study has been done, even as late as 2022 (Suri *et al.*, 2022). All this establish that roasting is an important part of processing black seeds, but roasting comes with both advantages and disadvantages.

Arguably the most significant and marketable benefit of roasting is flavour development which adds to enhance taste of food product (Perren & Escher, 2013). Roasting is also important to accelerate expulsion of water vapour leading to decrease in moisture content of food products (Perren & Escher, 2013). Low moisture environment reduce likelihood for microbes to grow and eliminates toxins which in turn increase shelf life of roasted product (Zambrano *et al.*, 2019). Roasting is also considered important when it can cause structural changes to render products more palatable and flexible, such as when oilseeds are roasted to improve lipid extraction or toasted to make them edible (Bhattacharya, 2015). Roasting also encourage Maillard reaction which elevate antioxidant capacity and lower lipid oxidation in oil rich products (Suri *et al.*, 2022). On the other hand, roasting, especially conventional methods (convection roasting with hot air) leads to loss of important bioactive compounds as a result of thermal degradation (Shan *et al.*, 2016). Roasting also induce lipid oxidation that eventually cause rancidity in oils (Yaacoub *et al.*, 2008). Conventional roasting also cause loss of volatile compounds and end up reducing product marketability (Baggenstoss *et al.*, 2008; Zzaman & Yang, 2014). Particularly in black seeds, roasting causes thymoquinone to degrade (Farag *et al.*, 2017; Kiralan, 2012). Prolonged roasting has also been reported to cause degradation of black seed oxidative indices (Bakhshabadi *et al.*, 2017; Suri *et al.*, 2022). Thus, the use of an innovative heating method to roast black seeds is well worth exploring.

An up and coming method of roasting, superheated steam (SHS) is a dry, clear, colourless gas derived from applying sensible heat to wet steam under normal pressure to higher than 100°C (Head *et al.*, 2010). SHS has been researched as far as three decades ago to study vitamin C preservation in potato (Burg & Fraile, 1995). Recently, it has been reported to be capable in preventing oxidation and can preserve important organic compounds since it has a low/no oxygen environment which is achieved through saturation of the heating chamber with steam (Mujumdar & Law, 2010; Zzaman *et al.,* 2013). SHS has also been proven to reduce microbial load subsequently reducing food spoilage (Park *et al.*, 2021). SHS roasting proved beneficial to process peanuts, cocoa, sesame seeds and even black seeds in terms of lipid oxidation and antioxidant capacity (Idrus *et al.*, 2017; Liang *et al.*, 2018; Ling *et al.*, 2018). As impressive as SHS roasting is, it may still not prevent loss of volatile compounds which happens to be an important constituent of black seeds. Hence a means of preventing loss of black seed volatile components is paramount when subjecting it to roasting. Such a method is torrefacto roasting (TR).

TR is the convenient method of adding sugar to Robusta coffee beans during roasting to conceal its bitter flavour (Rahmad *et al.*, 2017; Caballero *et al.*, 2015). High-quality coffee beans were not only hard to come by but also expensive during the Spanish Civil War, leading to the development of this distinctive roasting procedure to help stretch coffee (Blick, 2016; Lonely Planet Food, 2018). Each coffee bean gets coated with a layer of sugar when roasted that prevents air contact, effectively reducing flavour loss and delays the trigger of oxidative degradation (Sanz *et al.*, 2002). Melanoidins, formed through Maillard reaction from interaction between amino acids and reducing sugars during TR, has enhanced antioxidant qualities and strong antioxidant potential (Pérez-Hernández *et al.*, 2012, Lopez-Galilea *et al.*, 2008b). Torrefacto roasted coffee reportedly contain higher antioxidant content/activity (Pastoriza & Rufián-Henares, 2014). Based on these benefits it is believed that incorporating TR, a simple method of adding sugar during roasting, can prove effective in preserving quality of roasted black seeds. Furthermore, TR has never been attempted with black seeds.

TR, by and large only refers to addition of sugar (Liu *et al.*, 2019) and so, an alternative to sugar is a worthwhile exploration. Sugar has a negative connotation as a primary cause of adverse health effects (Du *et al.*, 2018) and to that affect, honey could be a potential option. Honey, besides containing a wealth of health benefits, has also many local variations such as tualang, kelulut and gelam, all of which are prized commodities (Rajindran *et al.*, 2022). Kelulut honey in particular is fast becoming more accessible and has gained growing interest due to its ease of management and harvest (Nordin *et al.*, 2018). With its characteristic sour taste (Kamal *et al.*, 2021), inclusion of kelulut honey in TR of black seeds can prove effective, not just as a substitute for sugar, but also as an enhancement to both its flavour and as a layer of protection for important components within roasted black seeds.

This study aimed to, (i) compare the effects of SHS roasting to convection roasting on the proximate and antioxidant content of black seeds, and (ii) evaluate the feasibility of torrefacto roasted black seeds from optimum roasting conditions in the production of a black seed beverage.

1.2 Problem Statement

Roasting is a ubiquitous practice of processing black seeds that is not extensively researched in food science thus a research gap exists. Previous work on innovative black seeds roasting methods was relegated to only microwave roasting. Interest into researching roasting of black seeds have gained momentum only within the past decade. There was only one published work on SHS roasting of black seed, with limited roasting parameters, as of the time the current work was undertaken by Liang *et al.,*, which used only 180 °C and roasted black seeds for 10, 15 and 20 minutes (Liang *et al.*, 2018).

Although roasting is seen as a necessary step for reasons ranging from moisture reduction to microbial elimination, traditional roasting media has its fair share of detrimental effects. Even when roasted in innovative methods like microwave oven, roasting happens in an oxygen saturated environment. Traditional roasting media can also cause loss of antioxidant capacity as a result of bioactive compound degradation. Roasting in the presence of oxygen accelerates lipid oxidation of resulting black seed oil and cause inevitable loss of volatile compounds.

Where loss of volatile compounds are concerned, there are no means of avoiding this since there are no barriers that can contain or maintain them during roasting. Since most volatile compounds are contained within the seed coat, common roasting methods accelerate these losses. There is also the potential for loss of antioxidant capacity upon brewing of black seeds from not being able to fully extract bioactive compounds in the beverage. So, it is important that roasting method can maintain antioxidant capacity as much as possible.

Sensory analysis of black seeds or any of its products are very limited. Black seed products are commonly only in the form of whole seeds or oil, so investigation of its organoleptic properties are dismally scarce.

1.3 Justification of study

Despite the prevalent use of heat and roasting to prepare black seeds, the effects of heat and especially variation of roasting temperatures aren’t well studied area of research and has only gained interest within the past decade (Jan *et al.*, 2019; Mazaheri *et al.*, 2019a; Suri *et al.*, 2022) leaving a research gap. Neither preparation methods nor optimal process parameters for these methods have been studied extensively as a food product, leaving the industry to simply add black seed oil or powder into products with no proper prior treatment (Haque *et al.*, 2021). This research could help industry players to pre-treat black seed to optimise its full potential. Additionally, commercial black seed based food products have gained popularity fairly recently with products in the past only being made up of whole seeds and oil (Teshome & Anshiso, 2019). This has left a dearth of commercial food products from a produce with numerous health benefits and to that, this research explored its viability as a beverage.

1.4 Objectives

The main aim of this research is to utilise SHS torrefacto roasted black seeds (*Nigella sativa*) to potentially develop a black seed beverage. More specific objectives are as follows:

1. To compare the effects of different roasting time and temperature between superheated steam and convection roasting on the oil oxidative stability, volatile compounds, proximate content, antioxidant capacity of black seeds and to optimise superior roasting method for maximum antioxidant activity.
2. To assess commercial honey and kelulut honey (stingless bee honey) as potential alternatives to sugar for torrefacto roasting of black seeds in terms of proximate, antioxidant capacity, oil oxidative stability, and essential oil and thymoquinone content.
3. To assess physicochemical, antioxidant activity and sensorial acceptance of beverage brewed from SHS torrefacto roasted black seeds.

1.5 Thesis Outline

Consisting of five chapters, this thesis explores black seed roasting as affected by SHS roasting, torrefacto roasting and acceptance of a brew made from SHS torrefacto roasted black seeds.

Chapter 1 is the customary outline, briefly touching on four aspects of this research namely black seeds, roasting, SHS, and TR. This chapter additionally provides challenges with roasting black seeds, objectives of the research and the thesis outline.

Chapter 2 is a more in depth look into previous literature with regards to the four aspects of this research. Black seeds, the plant, the seeds, its economic significance, and current work with the seeds in food applications are elaborated. Since roasting is a common way of processing black seeds, roasting, its advantages, disadvantages, and innovative roasting methods are also expounded. A specific innovative roasting method and the subsequent aspect of the research i.e., SHS is then detailed including its advantages. Final portion of chapter 2 expands on torrefacto roasting, a method of roasting coffee used to mask bitterness and extend its shelf life.

Chapter 3 is based on the first specific objective of the research through which the effects of multiple roasting temperatures and roasting times on proximate content, antioxidant capacity, oil oxidative stability, volatile component, proximate content, and antioxidant capacity of black seeds with both SHS and convection roasting of black seeds were investigated with both SHS and convection roasting. The superior roasting method, which was incidentally SHS was then optimised for antioxidant assays, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity (RSC) and total phenolic content (TPC) to derive best roasting temperature and time.

Chapter 4 uses the optimum roasting temperature and time to see the effects of TR on black seeds. Sugar is used in TR and thus alternatives to sugar, namely commercial honey and kelulut honey was also incorporated. Proximate content, antioxidant capacity, oil oxidative stability analysis, essential oil yield and thymoquinone quantification was studied.

Chapter 5 evaluates the sensory acceptance of a brew made from torrefacto roasted black seeds in various concentrations. Antioxidant capacity, total soluble solids and pH of the brews were also analysed.

Chapter 6 ties up the findings in a summary and discusses possible new avenue the current research can explore.

CHAPTER 2

LITERATURE REVIEW

2.1 Black seeds (*Nigella sativa*)

Dating as far back as ancient Egypt, the black seeds were even found in the tomb of Tutankhamun as it is customary for pharaohs to prepare resources for the "after-life". Personal healers of the pharaoh often had the seeds within reach as it served as a solution for a variety of conditions among which include its aid for digestion, and as a potent treatment for colds, headaches and even infections (Schleicher & Saleh, 2000). Academy Award winner Helen Mirren starred in "The Hundred - Foot Journey", a movie in which the seeds, known in parts of India as "*kalonji*", makes a presence in a special spice box. This spice box is featured below in Figure 2.1.

Figure 2.1: Screenshot from the film "The Hundred – Foot Journey" at approximately 32 min, 55 sec. Notice the jar containing black seeds labelled "*kalonji*" in the bottom right (Hallström, 2014).

The spice box belonged to the protagonist's late mother and was gifted to him by his father as a family heirloom marking its significance and importance in Indian cuisine since his family represented a quintessential South Asian family rooted in celebrating the wonders of its cuisine (Hallström, 2014).

2.1.1 Plant

From the Genus, *Nigella*, in the Family, *Ranunculaceae*, the black seed, scientifically known as *Nigella sativa*, is native to the Mediterranean, its cultivation ranging several Middle Eastern countries including Northern African countries namely but not limited to, Egypt, Ethiopia, Iran, Israel, Lebanon, Morocco, Somalia, Sudan, Syria, and also spreading east into Asia as far as Afghanistan, Pakistan and India (Ipor & Oyen, 1999; Jansen, 1981; Paarakh, 2010; Ravindran, 2017). The nature of the climate in these countries coupled with sandy soil are very conducive for the germination and cultivation of the black seed plants (Schleicher & Saleh, 2000). The herbaceous plant grows to approximately 70 cm (28 inches) tall and produces oblong, tubular capsules that contain numerous seeds (Ipor & Oyen, 1999). Fruits are gathered when yellow in colour and dried before being threshed to separate seeds from pods (Tembhurne *et al.*, 2014).

2.1.2 Seeds

2.1.2(a) Botanical and structural description

The seeds are perceptibly black in colour, triangular in shape and measuring approximately 1.5 to 3 mm in length with a matte textured surface (Malhotra, 2012). The seeds have flat sides with reticulated ridged patterns which can be seen in Figure

2.2 below. Seed cross section revealed that the endosperm made up of oil globules filled thin walled cells (Paarakh, 2010).

Figure 2.2: *Nigella sativa* seeds observed under a stereo microscope. Picture taken by author.

2.1.2(b) Chemical composition

Even though cultivation climates are similar, the blacks seeds are grown in multiple locations across many different countries causing vast variations in chemical composition arising from disparities in developmental stages, genotype and phenotype (Atta, 2003). This ultimately leads to difference in physiochemical properties from the variables that contribute to these inequalities (Haron *et al.*, 2014). The oil is one of the black seeds most commercially viable and consumer favourite products (Anshiso & Teshome, 2018). It is also considered a highly specialised oil as it has very prominent, characteristic aromas and flavours with very specific uses in culinary (Kamal-Eldin, 2009). A summary of past literature reporting proximate composition of black seeds was presented in Figure 2.3.

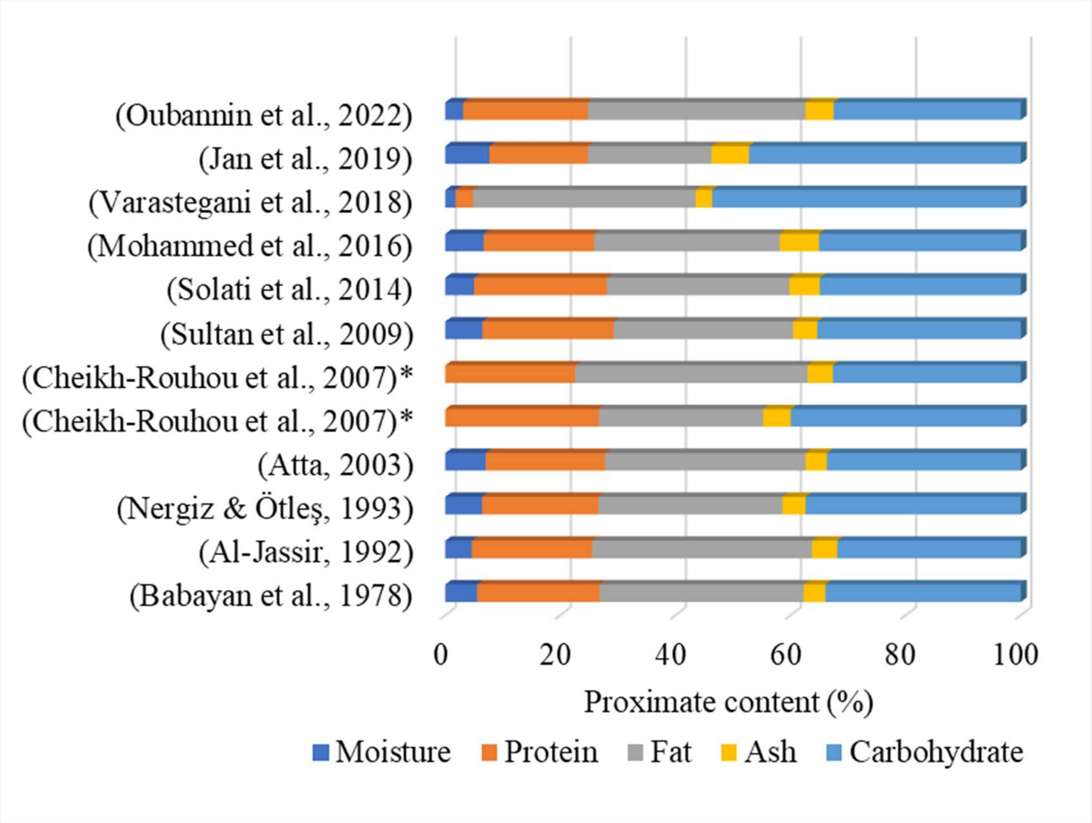

Figure 2.3: Proximate composition of black seed from various sources.
*Expressed as % dry weight and thus excludes moisture content.

From Figure 2.3, it is deducible that the black seeds are a low moisture (ranging 1.8 to 8.14%) and high fat (ranging 21.5 to 38.74%) produce making it a suitable raw material for oil extraction. Black seed oil is constituted of fixed oils and volatile oils. Fatty acids present in the seed oil are comprised mainly of linoleic acid (18:2 n-6), oleic acid (18:1 n-9) and palmitic acid (16:0) (Lutterodt *et al.*, 2010), and also include, albeit in lower proportions; myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0) (Ramadan *et al.*, 2012), palmitoleic acid (16:1), α-linolenic acid (18:3 n-3), arachidic acid (20:0), eicosenoic acid (20:1) (Rudzińska *et al.*, 2016) and, eicosadienoic acid (20:2) (Ramadan *et al.*, 2003). The presence of up to 62.1% linoleic acid makes black seed oil high in polyunsaturated fatty acids and thus contain higher proportion of unsaturated fatty acids (approximately 76.6 to 80.5 %) to saturated fatty acids (approximately 18.2 to 21.7 %) (Edris, 2011).

2.1.2(c) Volatile components

Volatile components is characteristic of the black seeds given that much of its health benefits were ascribed to presence of many bioactive compounds in its volatile oil (Ahmadi *et al.*, 2016). However, it only makes up approximately 1.8 % to 0.15 % of the fixed oil and only 0.4 % to 0.02 % of the entire seeds (Edris, 2010). Compounds present within its volatile component vary vastly based on the origin of the black seeds and thus the exact composition of thymoquinone (Farag *et al.*, 2017). In saying that, black seeds volatile oils are typically made up of carvacrol, p-cymene, α-pinene, 4–terpineol, longifolene, thymol, thymoquinone, and thymohydroquinone (Figure 2.4) among many more (Mazaheri *et al.*, 2019a; Ravindran, 2017).

Thymoquinone is of particular interest since it is unique to the black seeds and has been isolated for use in medical research (Gökce *et al.*, 2016). Thymoquinone on its own has been proven to have anti-inflammatory, anti-microbial, anti-cancer, hepatoprotective, and immunotherapeutic effects among many others (Gholamnezhad *et al.*, 2016). Thymoquinone molecular structure is shown in Figure 2.4 below.

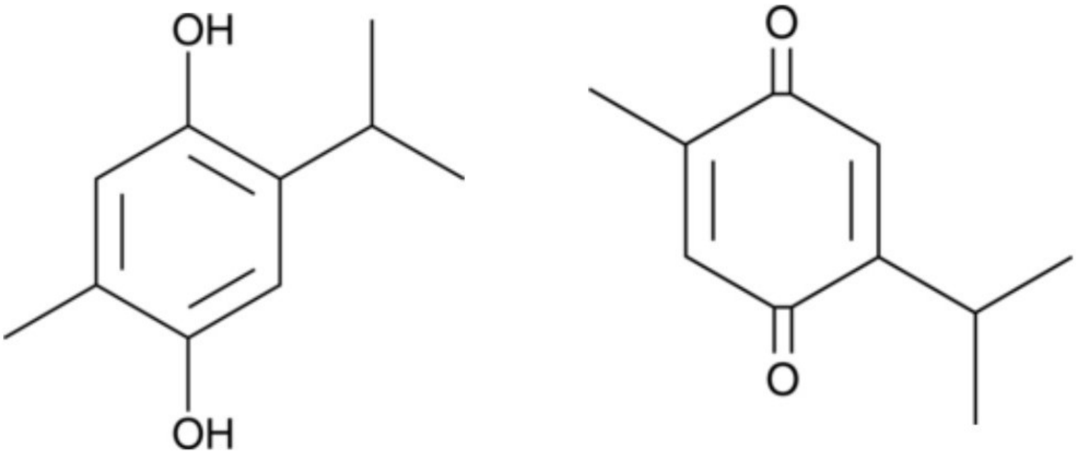

Figure 2.4: Important volatile compounds in black seeds, left, thymohydroquinone, and right, thymoquinone.

As aforementioned, since thymoquinone content is highly dependent on seed origin, the composition of thymoquinone present in black seeds can vary from approximately 31% to 55% within its essential oil (Iranian black seeds) to as little as 1.8% (Indian black seeds) (Farag *et al.*, 2017; Mazaheri *et al.*, 2019). As it is with thymoquinone, so it is with other compounds, for example, while p-cymene is completely undetected in Syrian black seeds, it makes up 59% of Indian black seed within its volatile component (Farag *et al.*, 2017). Another instance would be α-thujene that only consisted 0.2% of Turkish black seed essential oil but made up 10.1% of Iranian black seed essential oil (Mazaheri *et al.*, 2019).

Since volatile components are responsible for the key flavours of black seeds, it would also be important to note their odours and key sensorial characteristics that correspond to the compounds. However, this is an area of research only undertaken over the past decade and only in 2018, Kesen *et al.,* carried out sensory analysis with trained panellist to identify key odour descriptions. Some of these descriptions are enlisted in the table below.

Table 2.1: Descriptions of compounds that contribute to black seed aroma

Compound	Aroma
3-Penten-2-ol	Green
Limonene	Citrusy
Styrene	Balsamic
Acetoin	Buttery
Propanoic acid	Fatty
Isobutanoic acid	Cheesy
Pentanoic acid	Cheesy
Guaiacol	Smoky
Phenethyl alcohol	Honey, floral
Eugenol	Spicy-smoky

2.1.2(d) Antioxidant capacity

Black seeds are well known for having high antioxidant capacity both *in-vitro* and *in-vivo* (Islam *et al.*, 2017). Among *in-vivo* antioxidant analysis, black seeds showed a positive effect when evaluated for ferric reducing ability of plasma (Nili-Ahmadabadi *et al.*, 2018), catalase activity and total antioxidant activity, based on copper ion reduction (Mahmoud *et al.*, 2021), and even a novel total antioxidant capacity using ABTS (2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) assay (Alkis *et al.*, 2021). *In-vitro* antioxidant assays were also extensively studied, and black seeds typically showed prominent antioxidant capacity (Dalli *et al.*, 2022). Black seeds DPPH (2,2-diphenyl-1-picrylhydrazyl) values were reported to be higher compared to various other seeds (Rokosik *et al.*, 2020). Similarly black seed oil total phenolic content (TPC) also recorded significantly higher values when compared to several other vegetable oils (Ornella *et al.*, 2022). Black seeds additionally exhibited considerable FRAP (ferric reducing antioxidant power) and *in-vitro* ABTS values as well (Dalli *et al.*, 2022).

2.1.2(e) Black seed medicinal properties

Medicinal or therapeutic effects on human health of the black seeds have been extensively studied in the past (Dalli *et al.*, 2022). The seeds have known, among others, anti-inflammatory, immunomodulatory, neuroprotective, anti-cancer, anti-dyslipidemic, anti-diabetic, cardioprotective, hepatoprotective, gastroprotective, and nephroprotective properties, based on both in vitro and in vivo analysis (Hannan *et al.*, 2021). The black seeds have an abundance of phytochemical constituents that contribute to its therapeutic effects, which include terpenes, alkaloids, coumarins, saponins, flavonoids, and phenolics (Ahmad *et al.*, 2021). The most studied constituent and incidentally unique to black seeds is thymoquinone, a monoterpene that is present in the black seed essential oil (Dzoyem *et al.*, 2017). Just thymoquinone on its own has reported anti-cancer, neuroprotective, hepatoprotective, anti-inflammatory, and anti-microbial effects among others (Malik *et al.*, 2021). In Hannan *et al.,* comprehensive review of black seeds health benefits published in 2021, black seed potency was researched in either the form of solvent extracts (hydroalcoholic, ethanolic, hexane), aqueous extracts, whole seeds, seed oil, seed oil emulsion, essential oil, or incorporated with honey or into a cream (Hannan *et al.*, 2021). This meant that all these benefits were attributed to either thymoquinone or to what is typically contained within fractions of black seeds vis-à-vis, oleic acid, linoleic acid, eicodadienoic acid, palmitic acid, stearic acid, p-cymene, α-pinene, dithymoquinone, thymohydroquinone, carvacrol, carvone, limonene, 4-terpineol, citronellol, anethol, nigellicimine, kaempferol, quercetin, and vanillic acid, to name a few (Ahmad *et al.*, 2021).

2.1.2(f) Black seed economic value

Public records for black seed production and trade is scarce even up to as recent as 2020, thus prescribing a definitive economic value to the crop is circumscribed at the moment (Rahman *et al.*, 2020). However, being predominantly popular as an oilseed, growing interest among consumers has subsequently initiated burgeoning resurgence in its cultivation for oil production (Anshiso & Teshome, 2018). In USA alone, black seed oil market value stood at 15 million USD as of 2018 and this number is projected to almost double by the mid-2020s (Haque *et al.*, 2021). Ethiopia produced 18000 metric tonnes of black seeds in 2015 and it became its second cash crop trade after ginger (Anshiso & Teshome, 2018). Although readily available information on distinct production numbers are scarce, Turkey, as a main black seed producing country, produced 3300 tonnes from 980 kg/hectare mean yield (Haque *et al.*, 2021). A sizeable South Asian population in Australia is likely the contributing factor to a reported 70.3 tonnes of black seeds imported from India to its local market in 2016 (Rahman *et al.*, 2020).

2.1.2(g) Black seed processing

Black seed processing (Figure 2.5) has two main constituents, the first being processing in domestic practice and processing in industrial level product development which is largely edible oil extraction (Haque *et al.*, 2021).

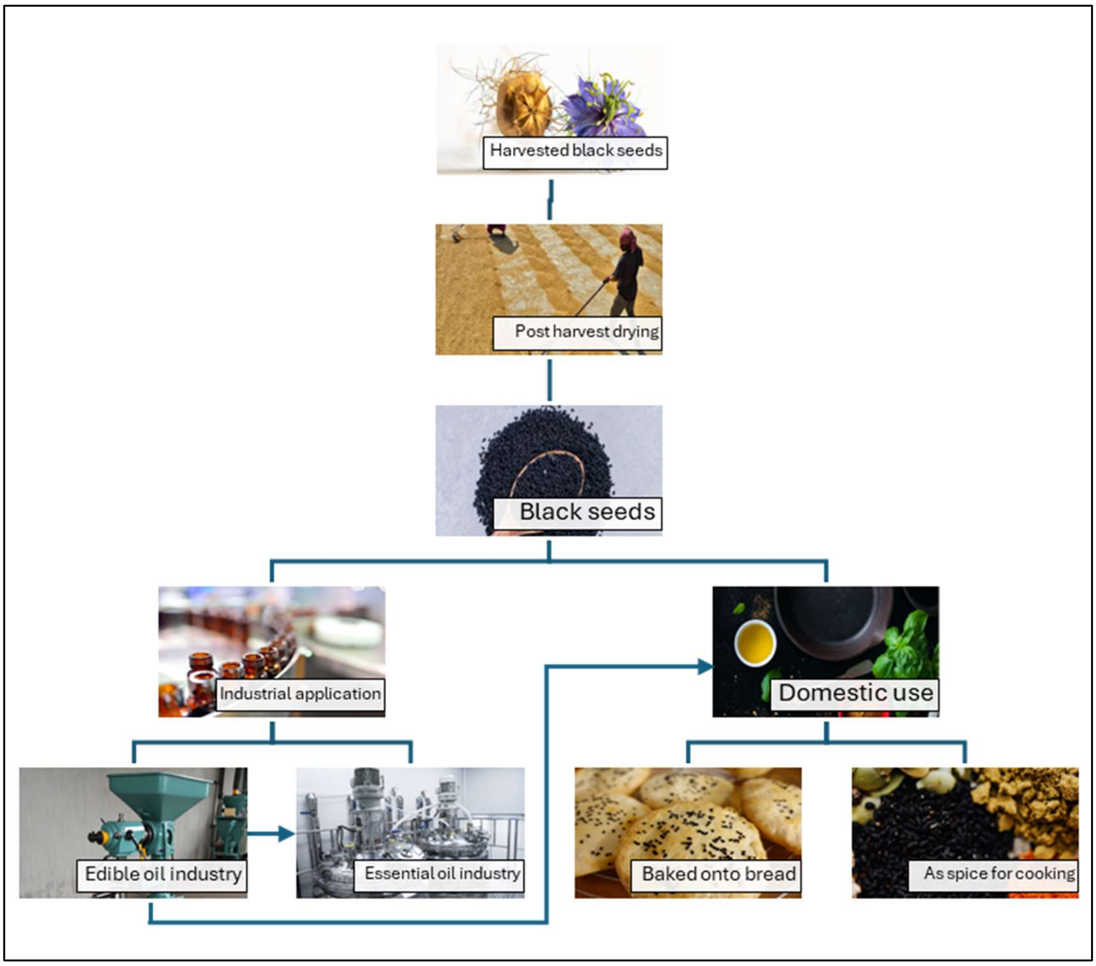

Figure 2.5: General flow of black seed processing

Black seed industrial processing typically involves mechanical extraction of black seeds to produce oil, or, steam or hydro distillation to recover essential oil (Malhotra, 2012). However, pretreatment of black seeds prior to oil recovery is only relegated to drying and threshing, the former aimed at ensuring fruits are completely dry to better release seeds and the latter to remove seeds from fruit (Momin & Jamir, 2021). Screw pressing is among the most commonly used method of oil extraction for black seeds (Sakdasri *et al.*, 2023). All processing methods (screw pressing, steam/hydro distillation, any form of domestic use) after post-harvest handling of black seeds involve heat. Screw press involve at least 50°C (Zzaman *et al.*, 2014) and steam or hydro distillation involve boiling water or steam temperature (Zelelew & Gebremariam, 2018).

Where domestic practice is concerned, black seeds are ubiquitously subjected to some form of heat treatment prior to consumption, for example adding it into pickling liquid involved boiling, while addition onto bread prior to baking to provide a unique taste exposes it to bread baking temperatures which can range from 160°C to 250°C (Aljabre *et al.*, 2005). If not whole seeds, black seed oils are also added into cereal based pastry products to serve as a beneficial component (Farag *et al.*, 2017). Its use in Indian cuisine almost always requires black seeds to be roasted, or in culinary jargon, toasted, either dry or in oil, before being added to dishes as a flavour base (Kiralan, 2012). When not roasted, black seeds make up an important ingredient in *panch phoron* (spelling variations; *panch phoran, paanch phoron, paanch phoran*), a blend of five important spices which is used as a base flavour profile in cooked dishes of Bengali (of West Bengal, state in India, and Bangladesh) cuisine (Ravindran, 2017).

Perhaps the most common black seed product is in the form of its oil which has found a growing interest among consumers (Teshome & Anshiso, 2019). Hence there is a sizeable oil production industry employing small industrial scale apparatus like screw press oil expeller machines (Bakhshabadi *et al.*, 2017). Research into different pressing temperatures, screw press expeller speed, and heat pre-treatment of black seeds to yield optimum amount of oil have been investigated in the past owing to growing consumer interest (Bakhshabadi *et al.*, 2017; Silvia *et al.*, 2012). Bakhsjabadi *et al.,* in 2017 reported 718 W application of microwave roasting prior to screw pressing to be optimal for best black seed oil quality. It is worth mentioning that even in 2023, the screw pressing method of obtaining black seed oil is seen as a non-existent area of research and by that extension, one that has no established guidelines or standards for its working temperature and other extraction parameters or even its final product (Sakdasri *et al.*, 2023).

Roasting the seeds prior acquisition of its lipid fraction isn’t at all a new or novel concept (Atta, 2003). During the early Islamic age, Syrian nomadic healers from Bedouin tribe revered and held the black seeds in high regards, as sacred (Sincich, 2003). These healers described roasting seeds for approximately 5 to 6 minutes as a preparation step for oil extraction; an apparently trivial practice without which seed oil could still be easily obtained but a practice that is still diligently carried out indicating some form of underlying positive effect (Agbaria *et al.*, 2015). Clearly roasting black seeds is an indispensable supplementation for which little research has been done, even as late as 2022 (Suri *et al.*, 2022). Although research exists for the pretreatment of black seeds prior to oil extraction (Bakhshabadi *et al.*, 2018; Mazaheri *et al.*, 2019a; Suri *et al.*, 2022), no information on a widespread industrial level use of those pretreatments exist within literature.

2.2 Roasting

Described as a process involving heat to achieve specific results for specific food products, roasting of oilseed is typically done to achieve two goals, to develop flavour and/or to change structure of product (Perren & Escher, 2013). Within these two goals, oilseed roasting or seed and nut roasting is specified here since roasting of meats like chicken and lamb, vegetables and other forms of roasting each have their own distinctive processes and goals wherein changes caused by heat greatly vary (Vega *et al.*, 2012). Via physicochemical and structural changes within each unique product, roasting and other thermal processing can increase bioavailability of substances while also boosting the digestibility and sensory characteristics of produce (Sruthi *et al.*, 2021). As important as roasting is to food processing, it has both advantages and disadvantages, hence the continuous research into developing new and improved methods to prepared food via heating (Sruthi *et al.*, 2021).

2.2.1 Importance of roasting in food production

Perhaps the most important marketable advantage of roasting is flavour development (Perren & Escher, 2013). A product inescapably intertwined with roasting for the sole purpose of flavour development is coffee. So paramount that roasting techniques and parameters have been extensively studied and meticulously developed to the point of being able to categorically define roasted coffee beans according to doneness vis-à-vis, New England, Italian, French, and Vienna, among others (Sinnott, 2011). The changes that occur to cause browning of coffee beans is a result of heat induced non-enzymatic browning called Maillard reaction in foods containing amino acids and reducing sugars (Pérez-Hernández *et al.*, 2012). Coffee has highly specified roasting apparatus designed to achieved uniformity and exert expert control over roasting parameters such as time, temperature, rotation speed, air speed etc (Hoffmann, 2014).

Cocoa is another example of a confectionary product wherein roasting is necessary for flavour development, driving extensive studies into various heating methods due to its widespread popularity (Gutiérrez, 2017). Roasting, through application of heat, accelerate formation of water vapour leading to decrease in moisture content of food products (Perren & Escher, 2013). Importance of moisture loss lies in the reduced likelihood for microorganisms to grow which in turn increase shelf life of roasted product (Zambrano *et al.*, 2019). Furthermore, just the application of heat alone can assist in reducing or eliminating microbial load and toxins (Sruthi *et al.*, 2021). Next to development of flavour, roasting is deemed necessary when it can induce structural changes leading to roasted product being more edible and malleable for example, oilseed roasting to allow better lipid extraction or peanut roasting that makes it edible (Bhattacharya, 2015). Besides just enhancement of flavours brought about by non-enzymatic browning, presence of browned compounds in foods as a result of Maillard reaction, elevates antioxidant capacity that in turn lowers lipid oxidation in oil rich products (Suri *et al.*, 2022).

2.2.2 Drawback of roasting in food production

The application of heat onto food products, in spite of its necessity, comes with some detrimental effects (Zzaman *et al.*, 2017). Often relegated to conventional methods, roasting often leads to loss of important bioactive compounds that contribute to its antioxidant capacities (Zzaman *et al.*, 2013). This phenomenon is a result of thermal degradation that negatively affect heat susceptible compounds effectively reducing value of roasted products (Shan *et al.*, 2015). The loss of these bioactive compounds also allows for lipid oxidation to occur more freely (Perren & Escher, 2013). Moreover, heat from roasting also induces lipid oxidation causing formation of hydroperoxides which are compounds that can cause rancidity in oils (Yaacoub *et al.*, 2008). Since most food products, even innovative ones like microwave roasting and infrared roasting happens in the presence of oxygen, this further accelerates lipid oxidation (Yodkaew *et al.*, 2017). Hydroperoxides can further devolve into secondary oxidation products such as carbonyls which will eventually cause irrevocable oil spoilage (Suri *et al.*, 2022).

In products revolving around development of flavour as a result of roasting like coffee and cocoa, conventional roasting methods often lead to irreparable loss of volatile compounds reducing marketability of these products (Baggenstoss *et al.*, 2008; Zzaman & Yang, 2014). Affected marketability typically comes from impaired sensorial attributes which, in addition to loss of volatile compounds, can also be a result of prolonged roasting (Nebesny & Budryn, 2006). Negative sensorial attributes usually positively correlated to prolonged roasting and the higher presence of compounds like pyrazines and pyridines (Giacalone *et al.*, 2019).

2.2.2(a) Lipid oxidation

A negative outcome of roasting or heating high fat/lipid products is lipid oxidation which is the degradation of oil quality that cause rancidity, resulting in bad flavour and odour (Loganathan *et al.*, 2022). Besides heat or thermal treatments, other factors such as moisture and light can also cause oil rancidity by triggering processes such as hydrolysis or auto-oxidation (Edelstein, 2019). Oil saturation also contributes to ease of oxidation as highly unsaturated oils are far more susceptible to oxidation than saturated oils (Loganathan *et al.*, 2022).

Lipid oxidation, in addition to causing off flavours, also reduces product shelf life and is capable of causing health complications when consumed and is thus a point of concern when studying high lipid/fat products (Xie *et al.*, 2020). Lipid oxidation (Figure 2.6) is complex process that is initiated by the creation of free radicals, triggered by factors such as heat or light, then quickly reacts with oxygen species to generate peroxyl radicals, which in turn make hydroperoxides, also known as primary oxidation products. Radicals formed in the initiation step target other fatty acids and continuously and exponentially produce additional hydroperoxides and free radicals until it undergoes termination when reacted with other radicals (Edelstein, 2019; Loganathan *et al.*, 2022). Further reaction of these hydroperoxides leads to the formation of secondary oxidation products like aldehydes and alkanes, and subsequent reaction of secondary oxidation products lead to the formation of tertiary oxidation products like ketones (Grebenteuch *et al.*, 2021).

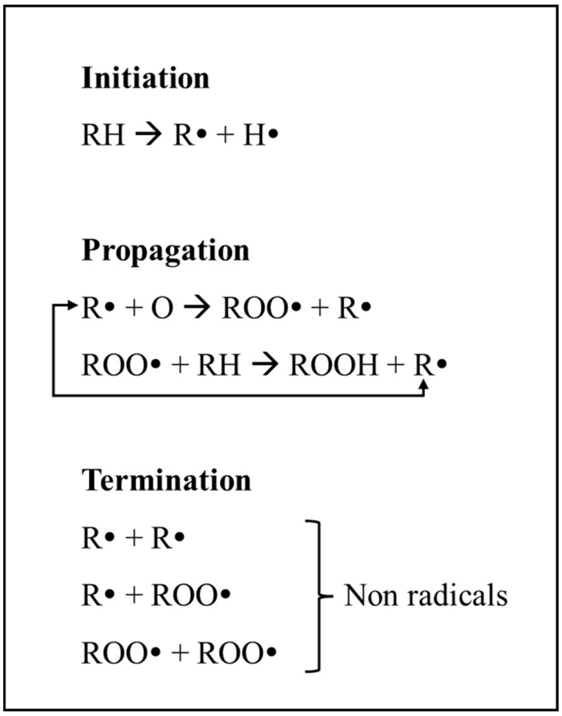

Figure 2.6: Oxidation mechanism, RH (unsaturated fatty acid), R• (fatty acid radical), H• (free hydrogen radical), ROO• (reactive peroxyl radical), ROOH (hydroperoxide).

Lipid oxidation can be measured by several methods the most commonly used include peroxide value, p-anisidine value, iodine value and TOTOX value (Maszewska *et al.*, 2018). Peroxide value is the measure of hydroperoxides in fat samples using the commonly employed method of titration. The method involves hydroperoxides within samples reacting with potassium iodide to form iodine and then titrated with sodium thiosulfate to determine final peroxide value (Zhang *et al.*, 2021). Determining peroxide value can be an indication of the freshness of the lipid sample or its susceptibility to oxidation as a result of treatments (Caballero *et al.*, 2015). Following determination of primary oxidation, anisidine value, the analysis to determine secondary oxidation products within lipid samples follow suit. Anisidine value employs use of the organic compound *para*-anisidine which reacts upon contact with secondary oxidation products, quantified spectrophotometrically (Pike & O'