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DOES *NIGELLA SATIVA* INCREASE THE SHELF LIFE AND CHANGE TEXTURE OF BREAD?

A Thesis

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The Faculty Department of Clinical Nutrition at Life University

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Master of Science in Clinical Nutrition.

By

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Dedications

"And lower to them the wing of humility out of mercy and say, My Lord, have mercy upon them as they brought me up when I was a child." QURAN SURAH AL ISRA (24)

This thesis is dedicated first to my parents who rise, nurture, and supports me.

I also dedicate this work to my beloved husband, who always encourages and supports me in my life.

Table of Contents

Dedications .... **I**

List of Figures .... **IV**

List of Tables .... **VI**

Acknowledgments .... **VIII**

List of Abbreviations .... **IX**

Abstract .... **XII**

Chapter I. Introduction **1**

Objectives of the Study 2

Hypothesis of the Study 2

Primary Scientific Research Questions 3

Chapter II. Review of Literature **4**

Chemical Composition of NS Seeds 4

Traditional Uses of Folk Remedies 8

Added Value of Products from NS seeds 8

Scientific Researches and Pharmacological Potentials 9

In vitro studies 9

Animal studies 11

Human studies 16

Side Effects of *Nigella Sativa* 21

Shelf Life 21

Validation Tools to Assess Shelf Life 22

Texture analyzer *22*

Water activity meter 24

Nigella Sativa and Shelf Life 25

Chapter III. Methods **27**

Study Design and Collection of Raw Material 27

Ingredients and Preparation 27

Tools to Assess Shelf Life 28

Texture analyzer 28

Water activity Meter 28

Physical and Microbial Analysis 29

Statistical Analysis 29
Chapter IV. Results 31
Characteristics of Bread Types 31
Comparison of All Bread Types 31
Control Bread 39
Bread with NSO..44
Bread with NSP 49
Association between Water Activity, Firmness, and Temperature for All Types of Bread……..54
Association for Control Bread 56
Association for Bread with NSO Type..58
Association for Bread with NSP Type 60
Physical and Microbial Activity 61
Chapter V. Discussion 63
Strengths and Limitations 65
Conclusion 65
References...67

List of Figures

Figure Page

1. *Nigella sativa* (NS) flower & seeds …..……………………...……………………….3

2. Chemical structures of active compounds of NS: thymoquinone, thymohydroquinone, carvacrol, carvone, thymol and 4-terpineol. Dithymoquinone ………………...…...…5

3. Means plot for bread firmness according to bread types (control bread, bread with NSO, and bread with NSP)..………………………………………………..……….......33

4. Means plot for water activity (WA) of bread according to bread types (control bread, bread with NSO, and bread with NSP). ……………………………………...…..34

5. Means plot for firmness of bread types overall (control bread, bread with *Nigella sativa* oil [NSO], and bread with *Nigella sativa* powder [NSP]) according to days. ……...…36

6. Means plot for water activity of bread types overall (control bread, bread with *Nigella sativa* oil [NSO], and bread with *Nigella sativa* powder [NSP]) according to days.......36

7. Means plot for firmness of control bread according to days...……………………...…41

8. Means plot for water activity of control bread according to days. ……..…………..…41

9. Means plot for firmness of bread with *Nigella sativa* oil (NSO) over successive days..46

10. Means plot for water activity of bread with *Nigella sativa* powder (NSO) over successive days. ……………………………….…………………………………….46

11. Day by Day changes in Means firmness of bread with *Nigella sativa* powder (NSP)....52

12. Day-by-Day changes in Means water activity of bread with *Nigella sativa* powder (NSP). ……………………………………………………………………………….52

13. . Microbial growth comparison in day 7 between three types of bread: control sample, bread with NSP sample, and bread with NSO sample…………………………………61

14. Microbial growth comparison in day 8 between three types of bread: control sample, bread with NSP sample, and bread with NSO sample…………………………………62

15. Microbial growth comparison in day 10 between three types of bread: control sample, bread with NSP sample, and bread with NSO sample…………………………………62

List of Tables

Table Page

1. Chemical Constituents of *Nigella sativa* (NS) Seeds………………………………………….....6
2. Some Vitamin Contents in *Nigella sativa* (NS) Seeds……………………………......……...7
3. Some Mineral Contents of *Nigella sativa* (NS) Seeds…………………………….……….......7
4. Difference Between Fixed Oil and Volatile Oil………………………………...…....…....8
5. Descriptive Statistics of Bread Firmness and Water Activity ……………………....…….31
6. Test of Homogeneity for Firmness and Water Activity among Bread Types …………....…32
7. Firmness and Water Activity Comparison According to Bread Types ……………….…..32
8. Test of Homogeneity for Firmness and Water Activity among Days for Bread Types Overall……………………………………………………………………………………..35
9. Day-by-Day Means of Firmness and Water Activity for Bread Types Overall …………......35
10. Day-by-Day Multiple Comparisons of Firmness and Water Activity for Bread Types Overall……………………………………………………………………………………..38
11. Test of Homogeneity for Firmness and Water Activity among Days for Control Bread ….....40
12. Day-by-Day Means of Firmness and Water Activity for Control Bread ……….................40
13. Day-by-Day Multiple Comparisons of Firmness and Water Activity for Control Bread …...43
14. Test of Homogeneity for Firmness and Water Activity among Days for Bread with Nigella sativa oil (NSO) ……………………………………………………………………...45
15. Day-to-Day Means of Firmness and Water Activity for Bread with Nigella sativa oil (NSO)……………………………………………………………………………….....45
16. Multiple Day-to-Day Comparisons of Firmness for Bread with Nigella sativa oil (NSO)………………………....…………………………….…………………...48

17. Multiple Day-to-Day Comparisons of Water Activity for Bread with Nigella sativa oil (NSO)………………………………………………………………………………….....49

18. Test of Homogeneity for Day-to-Day Changes in Firmness and Water Activity for Bread with (NSP) ………………………………………………..……………….………...50

19. Means of Firmness and Water Activity for Bread with Nigella sativa powder (NSP) According to Days ……………………..………………………………..……………..51

20. Day-by-Day Multiple Comparisons of Firmness and Water Activity for Bread with (NSP)..53

21. Correlation Matrix between Water Activity, Firmness, and Temperature for All Types of Bread ……………………………………………………………………………………55

22. Linear Regression for Temperature Impact on Water Activity Overall…………………..….55

23. Linear Regression for Temperature Impact on Water Activity Overall Coefficients ……....55

24. Correlation Matrix between Water Activity, Firmness, and Temperature for Control Bread..57

25. Linear Regression for Temperature Impact on Water Activity for Control…….…………57

26. Linear Regression for Temperature Impact on Water Activity for Control Coefficients…...57

27. Correlation Matrix between Water Activity, Firmness, and Temperature for Bread with Nigella sativa oil (NSO)………………………..…………………………………………59

28. Linear Regression for Temperature Impact on Water Activity for Bread with Nigella sativa oil (NSO)…………………………..…………………………………………………….59

29. Linear Regression for Temperature Impact on Water Activity for Bread with Nigella sativa oil (NSO) Coefficients …………………………..……..…………………………………59

30. Correlation Matrix between Water Activity, Firmness, and Temperature for Bread with Nigella sativa powder (NSP)……………………………………………………………60

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List of Abbreviations

(ABR) Auditory brainstem responses

(AGE) Antioxidant and Antischistosomal Activities of Garlic extract

(ALP) Alkaline phosphatase

(Anti-TPO)Anti-thyroid peroxidase

(AR) Allergic rhinitis

(BP) Blood pressure

(CAT) Catalase

(dB) decibels

(FBG) Fasting blood glucose

(FDA) Food and Drug Administration

(GC/MS) gas chromatography/mass spectroscopy

(GCF) Gingival crevicular fluid

(GI) Gingival Index

(GRAS) Generally Recognized as Safe

(GSH) Glutathione

(Hb) Hemoglobin

(HbA1C) Glycated hemoglobin

(HDL-C) High-density lipoprotein cholesterol

X

(I/R) Ischaemia/reperfusion

(L428) Hodgkin's lymphoma

(LC50) Lethal concentration 50

(LDL-C) Low-density lipoprotein cholesterol

(MC38) Mouse colon carcinoma

(MPO) Myeloperoxidase

(MRSA) Methicillin-resistant Staphylococcus aureus

(MWM) Morris water maze test

(NA) Nicotinamide

(NaCl) Sodium chloride

(NF- kB) Nuclear factor kappa B

(NIRS) Near-infrared reflectance spectroscopy

(NS) Nigella sativa

(OSI) Oxidative stress index

(OVA) Ovalbumin

(P) P-value or probability value)

(PCOS) polycystic ovarian syndrome

(PI) Plaque Index

(PPD) Probing Pocket Depth

(RAL)	Relative Attachment Level
(SOD)	Superoxide dismutase
(SRP)	Scaling and root planning
(STZ)	Streptozotocin
(T3)	Triiodothyronine
(TAC)	Total antioxidant capacity
(TBA)	Thiobarbituric acid
(TE)	Telogen effluvium
(TG)	Triglycerides
(TOS)	Total oxidative status
(TQ)	Thymoquinone
(TSH)	Thyroid stimulating hormone
(TVB-N)	Total volatile basic nitrogen
(US)	United States
(VEGF)	Vascular Endothelial Growth Factor
(WA)	Water activity

Abstract

Background: Among medicinal plants, *Nigella sativa* (NS) is emerging as a miracle herb with a rich religious and historical background, since many types of research have shown its broad spectrum of pharmacological potential. The objective of this study was to compare the shelf-life and texture of three types of bread: bread with *Nigella sativa* Oil (NSO), bread with *Nigella sativa* Powder (NSP), and bread without NS.

Methods: An experimental study assessed the texture and shelf-life of three types of bread: bread without NS, bread with NSO, and bread with NSP. The total number of samples was 75, with 25 samples of each type of bread. Texture analyzer TA.XT Express and water activity meter (AquaLab Pre) were used to measure texture and moisture, respectively, and both were applied to measure the shelf life of bread. Physical and microbial activities were monitored during the storage period.

Results: Although there was no statistically significant association found between the all bread types, the bread with NSO had lowest firmness mean ± standard deviation (SD) = 3906.22 ± 3353.99, compared to bread with NSP = 5480.18± 4129.94 and control bread = 6040.78 ± 4234.77. Water activity for the three types of bread had approximately the same mean and SD. NS, particularly the oil form, increased the rate of bread freshness and shelf life. There were statistically significant differences ($P = <0.05$) between firmness and water activity for all bread types. Bread with NSO did not show mold until day 10, compared to control bread and NSP bread that started mold growths at day 7 and day 8, respectively.

Conclusion: There is a relationship between NS and extended shelf-life. NSO has a stronger impact than NSP. Firmness in all types of bread was increased over time, even as the water activity decreased. NSO had the highest effect on growth of mold on bread compared to NSP.

Chapter I: Introduction

Among medicinal plants, *Nigella sativa* (NS) is emerging as a miracle herb with a rich religious and historical background. NS is an annual flowering shrub plant which belongs to the family Ranunculaceae (Beheshti al., 2016). Many types of research have shown its broad spectrum of pharmacological potential (Sahak et al., 2016; Kooti et al., 2016; Kazemi, 2014). NS has the potential to improve the health of people of all ages (Hosseinzadeh et al., 2017).

There are several names for NS in different languages. For example, it is called Habbatussada (or Habat et Baraka) in Arabic; Nigella, love-in-a-mist, fennel flower, black cumin, black caraway, black coriander, and black seed in English; and Neidonkukka in Finnish. NS is an essential drug in traditional Indian systems of medicine, like Unani and Ayurveda, which are forms of alternative medicine. Among Muslims, NS is considered an essential form of curative medicine that is readily available. It was discovered that NS is a remedy for all illnesses except death in one of the Prophetic hadith. It is also recommended for use on a regular basis in Tibb-e-Nabwi (Prophetic Medicine) (Ahmad et al., 2013).

NS is an edible plant commonly identified as black seed. It is native to North Africa, Southern Europe, and Southwest Asia, and it is cultivated in many regions in the world, such as the Middle Eastern and Mediterranean region, South Europe, India, Pakistan, Syria, Turkey, and Saudi Arabia (Dahan et al., 2015). It is grown in a hot climate and sandy soil (Plants for a future, 2012). NS is consumed as seeds, oil, or powder. In countries where it is cultivated, NS is used in the preparation of a traditional sweet dish composed of NS seed paste which is sweetened with syrup or honey, and in the flavoring of foods, especially bakery products and cheeses (Vedavathy, 2004).

NS is a small herb with a height of around 45 cm, with slender, four-centimeter-long, pinnatisect leaves divided into linear segments. It is an herb with pale flowers, blue peduncles, and black trigonous (having three angles or corners) seeds. NS seeds have a pungent, bitter taste and aroma (Srinivasan, 2018). According to many researchers, NS prevents and treats diseases such as hypertension, asthma, diarrhea, diabetes, and skin disorders (Huseini et al., 2013; Boskabady et al., 2008; Duncker et al., 2012; Kanter, et al., 2009; Aljabre et al., 2015). NS is less known in Western Europe and the Americas (Yarnell & Abascal, 2011). Additionally, little research has been published in the United States (U.S.) about the benefits of NS. There have been some studies (Ozpolat & Duman, 2016; Wojtasik-Kalinowska et al., 2017; Taqi et al., 2013) conducted on the ability of NS to increase the shelf life of food.

Objectives of the Study

This descriptive, experimental study was designed to compare the shelf life of three types of bread: bread with *Nigella sativa* Oil (NSO), bread with *Nigella sativa* Powder (NSP), and bread with without NS, and also to compare the texture of the three types of bread at Life University.

Null Hypothesis (Ho) and Alternate Hypothesis (Ha)

Ho of this study is NS has a little effect on increasing shelf life and changing the texture of bread, while the Ha is NS increases shelf life and can change the texture of bread.

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Primary Scientific Research Questions

- Does NSO increase the shelf-life of bread?
- Does NSP increase the shelf-life of bread?
- Do NSO and NSP change the texture of bread?
- Is there a relationship between water activity, firmness, and temperature?

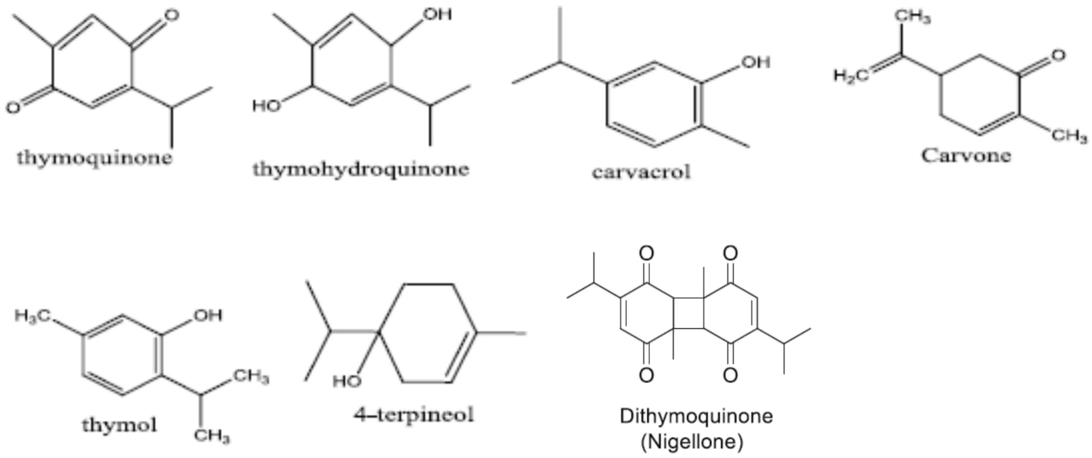
Figure 1. Nigella sativa (NS) flower & seeds (Srinivasan, 2018).

Chapter II: Review of the Literature

Chemical Composition of NS Seeds

NS displays a variety of rich properties in its chemical composition. Carbohydrates, proteins, amino acids, and volatile and fixed oils are contained in the seeds (see table 1). Feroz, (2018) defined gas chromatography/mass spectroscopy (GC-MS) as an analytical technique that combines characteristics of gas-liquid chromatography and mass spectrometry to recognize different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. According to Nickavar et al., (2003), GC-MS determined the chemical composition of the extracted fixed oil (total fatty acid composition) and volatile oil of NS seeds grown in Iran (see table 4). Eight fatty acids (99.5%) and 32 compounds (86.7%) have been recognized in the fixed and volatile oils, respectively. The major fatty acids of the fixed oil are linoleic acid omega-6 (55.6%); oleic acid, a monounsaturated omega-9 (23.4%); and palmitic acid (12.5%). The primary compounds of the volatile oil were p-cymene (14.8%), trans-anethole (38.3%), limonene (4.3%), carvone (4.0%), thymoquinone (TQ), thymohydroquinone, and dithymoquinone (nigellone). NS contains some vitamins and mineral such as B complex vitamins and calcium, phosphorus and iron (see tables 3 & 4) (Zahoor et al 2004).

Figure 2. Chemical structures of active compounds of *Nigella sativa* (NS): thymoquinone, thymohydroquinone, carvacrol, carvone, thymol, 4-terpineol (Nickavar et al., 2003), and Dithymoquinone (Khan & Afzal, 2016).



- Thymoquinone: The most active constituent of the volatile oil in NS seeds (30%–48%). The carbonyl polymer of TQ has medicinal features, decreases blood sugar, and aids in muscle relaxation and antioxidation (Janfaza & Janfaza, 2017).
- Dithymoquinone: Nigellone is the carbonyl dimer of TQ present in NS, and it prevents the release of histamines, giving relief in asthmatic conditions (Khan & Afzal, 2016).
- P-cymene is a monoterpene found in over 100 plant species used for food purposes and medicine. It represents a range of biological activities including anti-inflammatory, antioxidant, antinociceptive, anxiolytic, anticancer, and antimicrobial effects (Marchese et al., 2017).
- Carvacrol is a monoterpenic phenol provided by an abundant number of aromatic plants. It is utilized in low concentrations as a food flavoring ingredient and preservative, as well as a fragrance element in cosmetic formulations. It possesses a variety of biological and pharmacological

characteristics including antibacterial, antioxidant, antifungal, anticancer, anti-inflammatory, and hepatoprotective effects (Suntres, Coccimiglio, & Alipour, 2014).

- 4-terpineol is used as an herbal medicine with several advantages, such as anti-inflammatory, antibacterial, anti-fungal infection, and anticancer effects (Khaw-On & Banjerdpongchai, 2012).
- Trans-anethole, or t-anethole, exerts antimetastatic activity as well as antioxidative, antimicrobial, antiviral, and anti-inflammatory properties. It has also been experimentally shown that anethole has no toxicity at low doses and is considered nongenotoxic and noncarcinogenic and therefore quite safe (Seo et al., 2018).

Table 1*: Chemical Constituents of Nigella sativa* (NS) *Seeds*

Group	**Percentage (%)**
Fixed oil	32–40
Volatile oil	0.4–0.45
Protein	16–19.9
Minerals	1.79–3.74
Carbohydrates	33.9
Fiber	5.5
Water	6

Note. Randhawa & Al-Ghamdi (2002).

Table 2*: Some Vitamin Contents in Nigella sativa* (NS) *Seeds*

Vitamin	**Quantity**
Thiamine	15µg/g
Riboflavin	1 µg/g
Pyridoxine	5 µg/g
Folic acid	610 I.U/g
Niacin	57 µg/g

Note. Zahoor et al. (2004).

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Table 3*: Some Mineral Contents of Nigella sativa* (NS) *Seeds*

Mineral	**Quantity**
Calcium	1.859 mg/g
Phosphorus	5.265 mg/g
Iron	105 µg/g
Copper	18 µg/g
Zinc	60 µg/g

Note. Zahoor et al. (2004).

Table 4*: Difference between Fixed Oil and Volatile Oil*

Volatile Oil	**Fixed Oil**
Also called an essential oil.	Also called natural non-volatile oil.
Volatile oil can evaporate when placed openly under room temperature.	Fixed oils do not evaporate at room temperature.
They can be extracted easily through distillation.	They require some specific techniques for extraction.
Mixtures of cleoptenes & stearoptenes are termed as volatile oils.	Esters of higher fatty acids & glycerin are called as fixed oils.
Possess high refractive index.	Possess low refractive index.
These are optically active.	These are optically inactive.

Note. Guild (n.d.).

Traditional Uses of Folk Remedies

According to Yarnell and Abascal (2011), Avicenna refers to NS seeds in *The Canon of Medicine* as seeds that stimulate the body's energy and help recovery from dispiritedness and fatigue. NS seeds and their oil have a long history of folklore usage in Indian and Arabian civilization as both food and as medicine.

Added Value of Products from NS Seeds

NS seeds have been added as a spice to a variety of Persian foods including yogurt, bread, pickles, sauces, and salads (Hajhashemi et al., 2004). In addition, honey and/or garlic with NS is used to enhance benefits for both elements (Mostafa & Soliman, 2010). The U.S. Pharmacopeial Convention recommended the development of a NS seed monograph for its herbal medicines compendium in May 2013. The Food and Drug Administration (FDA) in the U.S. classifies black cumin (black caraway) NS as Generally Recognized as Safe (GRAS) for use as a spice, natural seasoning, or flavoring (American Botanical Council, 2017).

Scientific Researches and Pharmacological Potentials

***In vitro* studies.**

NS has antibacterial advantages according to Bakathir and Abbas (2011). The antibacterial effect of ground NS seeds was studied through the modified paper-diffusion method. A clear inhibition of the growth of *Staphylococcus aureus* was observed by a concentration of 300 mg/mL with distilled water as a control; this inhibition was confirmed by using the positive control azithromycin, which is an antibiotic used to treat different types of bacterial infections. The inhibition obtained was higher in ground NS seeds from Hadramout compared to seeds from Ethiopia. Positive inhibition can be attributed to two important active components of NS: TQ and melanin (Bakathir & Abbas 2011).

Md (2018) studied different concentrations of NS oils (NSO) for their antibacterial activity against different strains of gram-negative and gram-positive multidrug-resistant bacteria (*Methicillin-resistant Staphylococcus aureus* [MRSA], *Acinetobacter baumannii, Escherichia coli,* and *Pseudomonas aeruginosa*) by applying the diffusion method. For all the strains of *Acinetobacter baumannii* and *E. coli* that were tested against 100% of NSO, there was no recorded zone of inhibition. However, for the different strains of MRSA and *Pseudomonas aeruginosa,* different zones of inhibition were obtained for all the different oil dilutions used. Bacterial growth was inhibited at 100%, 80%, 50%, 40%, 30%, and 20% NSO dilutions.

Anthelminthic drugs are used to treat *Ascaris suum* infestations. They work by destroying the worms on contact, by paralyzing them, or by altering the permeability of their plasma membranes (Simalango & Utami, 2014). An activity of NS against

Ascaris suum is demonstrated in Simalango and Utami (2014), who conducted *in vitro* experimental research to determine the antihelminthic impact and lethal concentration 50 (LC50) of NS ethanoic extract against *Ascaris suum*. Ten *Ascaris suum* were soaked in each ethanoic extract of NS with concentrations of 0.5%, 1%, 2%, 4%, and 8%. Sodium chloride (NaCl) 0.9% solution was applied as a negative control. The amount of dead *Ascaris suum* was measured every 12 hours for 48 hours. The method was tested and replicated three times. The anthelminthic effect was found after 48 hours of observation at the extract concentrations of 0.5%, 1%, and 2%, and after 12 hours at the extract concentrations of 4% and 8%. In conclusion, the NS ethanoic extract had an anthelminthic effect on *Ascaris*.

The traditional preparation process of NSO starts with roasting the seeds. Dahan et al.'s (2015) study aimed to examine the role and boundaries of thermal processing of NS seeds in the preparation of therapeutic extracts and to clarify the underlying mechanism. NS extracts obtained by several seed thermal processing methods were scanned *in vitro* for their antiproliferative effect on mouse colon carcinoma (MC38) cells and for TQ content. The impact of various techniques of thermal processing on the ability of obtained NSO to prevent the nuclear factor kappa B (NF-kB) pathway was investigated in Hodgkin's lymphoma (L428) cells. Several thermal processing protocols yielded three different patterns: heating the NS seeds to 150 °C, 100 °C, or 50 °C produced oil with a high capability to prevent tumor cell growth; heating to 25 °C or no heating had a mild antiproliferative outcome; and heating to 250 °C or 200 °C had no impact. Similar patterns were found for the TQ content of the same oils, which presented a correlation with the antiproliferative data. This suggested that there is an oxidative transition mechanism between quinones after controlled thermal processing of the seeds. While NSO from heated seeds delayed the expression of NF-kB transcription, nonheated seeds resulted in only 50% inhibition. The data showed that controlled thermal processing of NS seeds (at 50 °C–150 °C) produces significantly higher anticancer activity in relation to a higher TQ oil content and prevents the NF-kB signaling pathway.

Animal studies.

NS works as an antischistosomal agent; schistosomiasis is a disease caused by infection from freshwater parasitic worms in certain tropical and subtropical countries. The antioxidant and antischistosomal activities of garlic extract (AGE) and NSO on normal and *Schistosoma mansoni*-infected mice was investigated. The results showed that protection using AGE and NSO prevented most of the hematological and biochemical alterations and increased the antioxidant capacity of schistosomiasis mice compared to infected-untreated ones. These results suggested that AGE and NSO might be promising agents to complement schistosomiasis-specific treatment (Shenawy, Soliman, & Reyad, 2008).

Furthermore, antioxidant, anticancer, anti-inflammatory, and antibacterial activities of methanol extracts from the shoots, roots, and seeds of NS were studied. The three organs exhibited potent antioxidant activity using the oxygen radical absorbance capacity method and a cell-based assay (Khan et al., 2011).

The activity of NS as an antioxidant was evident in Abdel-Wahhab and Aly's (2005) study that aimed to examine the effect of NSO and *Syzygium aromaticum* oils to scavenge free radicals generated during aflatoxicosis. The subjects were 60 male rats which were divided into six treatment groups, including a treatment group and a control group. The groups were experimented on for 30 days, orally (5mg/kg body weight) with *Syzygium aromaticum* oil and orally (5mg/kg body weight) with NSO either with or without aflatoxin. Blood samples were obtained at the end of the experimental period for hematological and biochemical analysis. The findings revealed that exposure to aflatoxins resulted in hematological and biochemical changes typical for aflatoxicosis. Treatment using *Syzygium aromaticum* oil and NSO on rats fed an aflatoxin-contaminated diet produced significant protection against aflatoxicosis. Furthermore, NSO was determined to be more effective than *Syzygium aromaticum* oil in restoring the parameters that were altered by aflatoxin in rats.

Al-Seeni, Rabey, Al-Hamed, and Zamazami (2018) conducted a study aimed at estimating the protective role of NSO against the side effects of tartrazine, a widely used additive in food products, using drugs to improve appearance and taste on male rats. Eighteen albino rats were divided randomly into four groups (n = 6). The first group was the negative control, the second group was a positive control which took 10 mg/kg body weight tartrazine, and the third group received the same dose of tartrazine as the second group and was co-treated with NSO for eight weeks. Tartrazine reduced the total protein, high-density lipoproteins, and antioxidants while increasing liver enzyme, total cholesterol, and kidney function parameters, triglycerides, low-density lipoproteins, and lipid peroxidation in the positive control group. Correspondingly, it caused pathological changes in the tissues of the liver, kidney, testes, and stomach. Treating tartrazine-supplemented rats of the third group with NSO for eight weeks significantly improved all biochemical parameters and restored the tissues of the kidney, stomach, testes, and liver back to normal. The study concluded that NSO succeeded in protecting male rats against the adverse conditions resulting from tartrazine administration.

According to a study conducted by Pari and Sankaranarayanan (2009), the anti-hyperglycemic potential of TQ on the activities of key enzymes of carbohydrate metabolism in streptozotocin-nicotinamide (STZ-NA) induced diabetic rats were evaluated. Oral administration of TQ at 20, 40, and 80 mg/kg body weight for 45 days dose-dependently improved the glycemic status of STZ-NA caused in diabetic rats. The levels of insulin and hemoglobin (Hb) increased, with a significant reduction in glucose and glycated hemoglobin (HbA [1C]) levels. The modified activities of carbohydrate metabolic enzymes were restored to near normal. These results demonstrated that TQ at 80 mg/kg body weight is linked to beneficial changes in hepatic enzyme activities and thereby exerts potential antihyperglycemic effects.

In a study by Duncker et al. (2012), the authors determined the possible alleviating effect of NS and TQ on food allergies. Ovalbumin (OVA) sensitized BALB/c mice were pre-treated with a hexanoic NS seed extract TQ and subsequently challenged intragastrically with OVA. All four treatments significantly decreased clinical scores of OVA-induced diarrhea. NS seed extract TQ decreased intestinal mast cell numbers and plasma mouse mast cell protease-1. It was demonstrated that NS seed extract significantly improved symptoms and immune parameters in murine OVA-induced allergic diarrhea; this impact is at least partially mediated by TQ.

According to El-Abhar, Abdallah, and Saleh (2003), both NS and its constituent TQ were found to possess gastroprotective activity against gastric mucosal injury induced by ischemia/reperfusion in rats. Ischemia/reperfusion (I/R) induced gastric lesions are known to be linked with the free radical formation. Male Wistar rats were subjected to I/R and injected either with NO (2.5 and 5.0 mL/kg) orally or TQ 5, 20, 50, and 100 mg/kg orally. The results showed that I/R elevated the levels of lipid peroxide and lactate dehydrogenase, while it decreased those of reduced glutathione (GSH) and superoxide dismutase (SOD). These biochemical alterations were accompanied by an increase in the formation of gastric lesions, which were decreased by either treatment. This indicates that both NSO and TQ possess gastroprotective impact against gastric lesions, which may be related to the maintenance of the gastric mucosal redox state.

The hepatoprotective activity of NS has been studied. Yildiz et al. (2008) reported that NS (0.2 mL/kg) intraperitoneally relieved harmful impacts of ischemia-reperfusion injury on the liver. Biochemical parameters like the serum aspartate aminotransferase, alanine aminotransferase lactate dehydrogenase levels, total antioxidant capacity (TAC), catalase (CAT), total oxidative status (TOS), oxidative stress index (OSI), and myeloperoxidase (MPO) were defined in hepatic tissue in rats with hepatic ischemia. Results suggested that NS treatment protected the rat liver against hepatic ischemia-reperfusion injury.

The protective effect of NSO on acoustic trauma-induced hearing loss in rats was studied by Culhaoglu, Erbek, Erbek, and Hizal (2017). The study aimed to define the potential preventive impacts of NSO in an animal model of noise-induced hearing loss. Acoustic trauma is a cause of hearing loss. Twenty Sprague Dawley female rats (mean age 12 months; mean weight 250 g) were involved in the study. All of the procedures were under general anesthesia. After otoscope examinations, baseline-hearing thresholds were obtained using auditory brainstem responses (ABR). The rats were separated into two groups: the study group (n = 10) and the control group (n = 10). Two mL/kg/day of NSO were given to the rats in the study group orally. To create an acoustic shock, the mice were then subjected to noise from a white band of 4 kHz with a density level of 107 decibels (dB) in a soundproofed test chamber. On the first day after exposure to acoustic trauma, hearing frequency measurements were repeated. On day four after the acoustic trauma, ABR measurements were repeated. There was no difference between baseline hearing thresholds for rats before acoustic trauma ($P > 0.005$). After the acoustic trauma, hearing thresholds increased, and there were no statistically significant differences between hearing thresholds for study groups and control group ($P = 0.979$). On the fourth day after acoustic trauma, the hearing thresholds of rats in the control group were found to be higher compared to those in the study group ($P = 0.03$). The findings indicate that NSO has a protective influence against acoustic trauma in early periods.

Imam et al. (2016) tested the role of NS and its active constituents in learning and memory. The study was conducted to evaluate the cognitive enhancing effects of NSO in the scopolamine-induced rat model of cognitive impairment. These influences were investigated on the scopolamine-induced dementia model in the Morris water maze test (MWM) and Y-maze test. The hippocampal histoarchitectural responses to scopolamine and NSO were also examined. Scopolamine (1 mg/kg intraperitoneal) was given to cause dementia, followed by oral administration of NSO (1 mL/kg) for 14 consecutive days. MWM and Y-maze paradigms were used to evaluate hippocampal and frontal dependent memory, respectively. Scopolamine resulted in memory impairment, demonstrated by delayed latency in the MWM and decreased percentage alternation in the Y-maze that was coupled with alterations in the cortico-hippocampal neurons. Posttreatment of rats with NSO mitigated scopolamine-induced amnesia by decreasing latency period and increasing percentage alternation and histological changes. The observed anti-amnestic effect of NSO makes it a promising anti-amnestic agent for clinical trials in patients with cognitive impairment.

A laboratory-based, randomized control trial study by Anwar, Hamid, and Butt (2016) aimed to examine the protective impact of NS on cystic follicles in letrozole (a non-steroidal aromatase inhibitor which lowers estrogen production) induced polycystic ovarian syndrome (PCOS) in mice. Forty female BALB/c mice were divided into four groups, each group having 10 animals. Group A was a control group; they were given a normal diet. Group B consumed letrozole at a dose of 1 mg/kg body weight. Group C was treated with NSP at a dose of 10 g/kg body weight once daily, starting at 22 days and continuing up to eight weeks, and with the same dose of letrozole as group B for eight weeks. Group D was treated with NSO at a dose of 4 mL/kg body weight once daily starting at 22 days and continuing up to eight weeks. Animals were divided one day after the last dose. Anwar et al. (2016) observed the shape, size, color, and consistency of the mice's ovaries. Cystic follicles were included and noted. They found that a significant number of cystic follicles were recognized in the ovaries of animals in group B as compared to group A, while their number decreased significantly in groups C and D as compared to group B. The study concluded that NSP and NSO both have a similar protective impact on PCOS in mice by reducing the number of cystic follicles.

Human studies.

Abu Khader (2012) studied the effect of NSO on insulin resistance syndrome. Sixty patients were divided into two groups of 30 patients each to consume statin and metformin tablets for a six-week period. The second group of patients was additionally given 2.5 mL of NSO twice a day during the therapy. The findings exhibited more improvement in serum cholesterol and fasting blood glucose in the patients of the second group compared to the first group, thus illustrating the therapeutic potential of NSO against insulin resistance syndrome.

Anti-inflammatory and analgesic activity of NS in human studies has also been investigated. Allergic rhinitis (AR) is a type of inflammation in the nose which occurs when the immune system overreacts to allergens in the air (Alsamarai et al., 2014). Alsamarai et al. (2014) conducted a study that aimed to estimate the therapeutic efficacy of NS extract as a treatment for allergic rhinitis. Sixty-eight patients with AR were included in the study; 19 patients had mild symptoms, 28 patients had moderate symptoms, and 21 patients had severe symptoms. Each group was divided into active and control groups. A skin test was performed on all patients. Any individual with a negative skin test was excluded. For six weeks, individuals in the active group took NSO, and the control group individuals consumed regular food oil in the form of nasal drops. After six weeks of treatment, out of 100% of patients in the mild active group, 68.7% became symptom free and 25% were improved, while in the severe active group 58.3% became symptom free and 25% were improved. In addition, 92.1% of total patients in the active group confirmed progress in their symptoms or were symptom free, while the corresponding value was 30.1% in the control group (*P* = 0.000). At the end of six weeks of topical treatment, the improvement in tolerability of allergen exposure in the active group became 55.2%, which was significant (*P* = 0.006) as compared with the control group, which accounted for 20% simultaneously. The study concluded that the topical application of NSO was useful in the treatment of allergic rhinitis with minimal side effects.

Ibrahim et al. (2014) tested protective effects of NS on menopausal women with metabolic syndrome. The subjects were 30 menopausal women aged 45 – 60 years old. The participants were randomly selected and divided into two experimental groups. The treatment group was given NSP orally in the form of capsules at a dose of 1g/day after breakfast for two months and compared to a control group who consumed a placebo. Biochemical and anthropometric parameters were measured at the baseline, in the first month, second month, and one month after treatment was completed, to define their body weight, serum lipid profile, and fasting blood glucose (FBG). The findings were that the treatment group presented significant ($P < 0.05$) improvement in total cholesterol, triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and blood glucose ($P < 0.05$). However, a slight decrease with no significant difference in body weight changes of the participants was recorded. The study concluded that treatment using NS has a protective effect of improving lipid profile and blood glucose, which are highly elevated during the menopausal period.

Aljabre et al. (2015) stated the positive effects of using NS on the skin. The study revealed that NS is useful as anti-acne treatment, reducing skin pigmentation, hypersensitivity reactions, and skin cancers. NS seeds significantly decreased skin irritation and improved skin hydration and epidermal barrier function when compared to the placebo group. The authors suggested the potential use of seeds in anti-aging, moisturizing, mitigating, and protective cosmetics due to their antioxidant and anti-inflammatory abilities.

The effects of NS on thyroid function were studied by Farhangi et al. (2016). Hashimoto’s thyroiditis is an autoimmune disease and the most common cause of hypothyroidism. The study aimed to evaluate the effects of NS on thyroid function, serum vascular endothelial growth factor (VEGF) -1, nesfatin-1, and anthropometric features in patients with Hashimoto’s thyroiditis. Forty patients with Hashimoto’s thyroiditis participated in the study. They were randomly divided into treatment and control groups, taking 2 g of NSP or 2 g of placebo daily for eight weeks. The findings were that the NS group significantly decreased body weight and BMI. Serum concentrations of thyroid stimulating hormone (TSH) and anti-thyroid peroxidase (anti-TPO) antibodies were reduced while serum triiodothyronine (T3) concentrations increased after eight weeks. There was a vital decrease in serum VEGF concentrations in the treatment group, and no changes were observed in the placebo group. NS enhanced thyroid status and anthropometric variables in patients with Hashimoto's thyroiditis. Furthermore, NS significantly decreased serum VEGF concentrations in these patients.

Huseini et al. (2013) piloted a study aimed at exploring the influences of NSO on blood pressure (BP). In a double-blind, randomized study, 70 participants aged 34 to 63 years with diastolic BP from 60 to 90 mmHg and systolic BP from 110 to 140 mmHg participated. They were divided into a study group and a placebo group. The study group consumed 2.5 mL NSO twice a day for eight weeks. The systolic and diastolic BPs, BMI, and blood levels of aspartate transaminase, alkaline phosphatase, alanine transaminase, creatinine, and blood urea nitrogen were defined at the baseline and the end of the study. The findings showed that in the NSO-treated group, the systolic and diastolic BPs reduced significantly compared to the placebo group. No significant change in the other parameters in either group was found. The study concluded that the daily oral administration of 5 mL NSO for eight weeks lowers systolic and diastolic BPs without any adverse impacts.

A study by Kapil, Suresh, Bathla, and Arora (2018) aimed to assess the potential advantages of local application of TQ gel as an adjunctive to scaling and root planing (SRP) in patients with chronic periodontitis, which is an inflammation of the tissue around the teeth. Twenty patients were divided into two groups. The first group comprised study patients (TQ in addition to SRP) and the second group consisted of control subjects (only SRP). Clinical parameters such as Gingival Index (GI), Plaque Index (PI), Relative Attachment Level (RAL), and Probing Pocket Depth (PPD) were observed at the baseline and six weeks postoperatively. Alkaline phosphatase (ALP) levels in gingival crevicular fluid (GCF) were assessed at the baseline and six weeks postoperatively using microcapillaries. In addition, the antimicrobial ability of TQ against three bacteria was estimated by applying antimicrobial strains. The findings were that there was a significant decrease in PI, GI, and PPD levels and an increase in RAL and GCF ALP levels in both groups at six weeks from the baseline. In the comparison between the first group and the second group in the microbiological assessment of 0.2% TQ gel, it was observed to be sensitive against *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomita*, and *Prevotella intermedia*. The study concluded that intracrevicular application of 0.2% TQ gel could be a useful adjunct to SRP in treating chronic periodontitis.

Telogen effluvium (TE) is a scalp disease identified by the shedding or thinning of hair resulting from the early entry of hair in the telogen phase. A study by Rossi et al. (2013) aimed to estimate the ability of NSO as a potential treatment for TE. Twenty patients affected by TE were enrolled in the double-blind, placebo-controlled, randomized study. Patients were divided into a treatment group and a placebo group (n = 10 in each group). The treatment group was treated with a scalp lotion containing 0.5% NS for three months. Videodermatoscopic analysis (Trichoscan Dermoscope Fotofinder®) and evaluation by three independent dermatologists were performed before treatment, after three months of treatment, and at the six-month follow-up. The findings presented a significant improvement in 70% of patients treated with NS. The Videodermatoscopic analysis showed a significant increase in hair density and hair thickness in patients treated with NS. NS was also able to decrease the inflammation observed in the majority of patients affected by TE. The study concluded that NS can be considered potentially beneficial for the treatment of TE.

NS has been used as an anticonvulsant agent. Akhondian, Parsa, and Rakhshande (2007) studied the ability of NS to reduce the frequency of seizures in childhood refractory epilepsy in a double-blinded crossover clinical trial. Twenty children with refractory epilepsy were entered in the study, 10 boys and 10 girls aged between 13 months and 13 years old. All patients were taking regular treatment for at least one month before the study. They received an aqueous extract of NS (40 mg/kg/8 h) or placebo. The findings were that the mean frequency of seizures reduced significantly during treatment with extract ($P < 0.05$). The study concluded that the water extract of NS has anti-epileptic effects in children with refractory seizures.

Side Effects of *Nigella Sativa*

There are minimal and rare side effects of NS according to many researchers (Alsamarai et al., 2014; Ali, 2004; Dollah et al., 2013; Khazdair, 2015). NS is considered a safe seed to consume as food or medicine. However, the previous studies applied NS for a specific time, such as eight weeks, and a particular amount, from 1 mg to 10 g of NS.

Shelf-life

A product's "shelf life" is defined as the length of time the customer can expect a product to act and look as expected and to stay safe for use. The period of time differs depending on the type of product, how it is stored, and how it is used. The are some factors that affect shelf life over time; preservatives can break down, allowing fungi and bacteria to grow, and temperature changes or exposure to air, moisture, or sunlight can cause changes in texture and color and may cause the products to smell (U.S. Food and Drug Administration (n.d.).

Bread is a product with a shorter shelf life than most other bakery products. During storage, bread quickly loses its freshness, and this leads to the loss of its organoleptic freshness. Generally, there are two factors that accelerate the rate of freshness loss in bread: first, changes in physical or chemical properties that subsequently result in the decrease of crumb softness during storage time; second, spoilage, which results from a microbial attack (Ho, Aziz, Bhat, & Azahari, 2013). According to Second Harvest Food Bank of Middle Tennessee (n.d.), the shelf life of bread stored at room temperature (70° F) is three to five days.

Validation Tools to Assess Shelf-life

Texture analyzer.

Texture analyzers such as TA.XT Plus are used for research, development, and quality control of food products (Instron, 2013). Nowadays, a texture analyzer is used on other products such as cosmetics, pharmaceuticals, and packaging (Instron, 2013). The texture analyzer is an instrument used to apply a scientific method to determine the textural properties of foods (Food Technology Corporation, n.d.). It uses a double compression test to achieve the results (Texture Technologies, n.d). Samples are compressed twice, mimicking the biting action of the mouth, and the texture analyzer is used to show how the product would behave when chewed (Texture Technologies, n.d.).

The machine is able to analyze multiple textural parameters in one trial (Texture Technologies, n.d.). However, before setting the parameters that are to be tested, one must consider the product (Texture Technologies, n.d.). For example, one would not test the springiness of hard candy, since no useful data can be collected from it. The texture analyzer measures four primary parameters or characteristics (hardness, fracturability, cohesiveness, and springiness) and three secondary

International Journal of Scientific & Engineering Research Volume 10, Issue 7, July-2019
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characteristics (gumminess, chewiness, and resilience) (Food Technology Corporation, n.d.; Instron, 2013; Texture Technologies, n.d.).

Characteristics of the bread measured included firmness, cohesiveness, springiness, and gumminess (Singh, Kaur, & Singh, 2017). Firmness in this case signifies hardness, which measures the maximum initial force on the product, which can reflect the shelf life of the product (Singh et al., 2017). Texture analyzers such as TA.XT Plus are commonly used in different research studies (Xie, Dowell, & Sun, 2003; Asghar et al., 2009; Abu Ghoush et al., 2007).

Dvořáková et al. (2013) studied gluten-free bread along with wheat bread on quality of bread, elasticity, crumb hardness, gumminess, and chewiness. The most significant improvement was found for the mean bread volume (30%). The texture analysis showed a positive impact of rice flour on hardness, chewiness, and gumminess. Hardness decreased while chewiness and gumminess had similar results.

Xie et al. (2003) studied bread firmness as measured by a texture analyzer to study the potential of visible and near-infrared reflectance spectroscopy (NIRS) to identify bread changes according to storage days by comparing NIRS results to a texture analyzer. Twenty-five loaves of wheat white bread from one batch were measured over five days. Texture analyzer and NIRS measurements were made on the same slice at the same time. The analysis was repeated five times using the same type of samples from five different batches. NIRS measures of loaf averages, slices, and daily averages were compared with texture analyzer measures. NIRS measurements correlated with storage time and had smaller standard deviations than the texture analyzer measurements. The findings were that NIRS could follow bread changes during storage time more accurately than the texture analyzer. NIRS measurements are probably based on both chemical and physical changes during bread staling, unlike the texture analyzer method that only measures bread firmness.

Gómez, Talegón, and Hera (2013) studied two different gluten-free bread formulas (80% and 110% hydration), as well as bread quality parameters. Crumb texture was determined by using a TA.XT2 texture analyzer. In less-hydrated bread, no significant differences were found depending on the mixing arm, but mixing time affected the volume of bread, this being higher while mixing time increased. Both mixer arm and mixing speed were found to have a significant impact on bread volume and texture in more-hydrated dough, reaching higher particular volumes and softer bread with the wire whip compared with the flat beater, as well as with lower mixing speeds and longer mixing time. In more-hydrated bread, time improved the bread's particular volume, but in less-hydrated bread the impact was the opposite. This influence was remarked in longer mixing times.

Water activity meter.

AquaLab Pre is one example of a water activity meter, which is a thermodynamic device that measures the energy of water in a product. It is related to the microbial susceptibility of food products and relationships with several reactions that end shelf life in foods. Because it is measured on a scale with a known standard, the water activity meter is particularly well suited to meeting safety and quality specifications. AquaLab Pre is a quick and accurate device for measuring water activity (WA). This instrument gives readings in five minutes or less. Its results and readings are reliable, providing ±0.01 WA accuracy. The instrument is simple to clean, and checking calibration is easy. There is an opposite relationship between water activity and shelf life; if the amount of water in a food product is lower, the shelf life of the product will be longer (Zhang, Sun, & Zhang, 2015). A water activity meter has been used in different research studies to measure shelf life (Sirpatrawan, 2009; Sozer, Bruins, Dietzel, Franke, & Kokini, 2011; Sloan et al., 2016).

Nigella Sativa and Shelf -life

A study by Ozpolat and Duman (2016) aimed to measure the influence of NSO on the shelf-life, sensory quality, and chemical and microbiological properties of fish fillets during storage at 2 ± 1 °C. Acceptability scores for sensory evaluation of all described treatment groups reduced with storage time. Defined limits for mesophilic bacteria and total volatile basic nitrogen (TVB-N) were reached after 21 days for the control group, after 24 days for the 0.2% NSO-treated group, and after 28 days for the 0.4% and 0.6% NSO-treated groups. The findings were that the NSO-treated groups had longer shelf life and higher sensory quality than the untreated control group. Also, no variation was found among groups during storage regarding defined thiobarbituric acid (TBA) values.

Wojtasik-Kalinowska et al. (2017) aimed to determine the impact of adding various levels of NSO to chopped pork on the quality and shelf life of pork patties. The study determined the fatty acids profile, lipid oxidation, color, and total number of aerobic bacteria in raw pork patties stored under refrigeration temperature for eight days. Moreover, sensory evaluation of roasted pork patties was performed. The findings were that the addition of NSO to pork influences the properties of pork patties. The radical activity of NSO was also indicated. The study concluded that there is the potential to use NSO to develop functional pork products with volatile compounds and beneficial fatty acids profile.

Rahman et al. (2017) studied the effect of different levels of NS extract and synthetic antioxidant on fresh and preserved beef meatballs. Treatment groups were control group, 0.1%, 0.2%, and 0.3% NS extract treated. Intervals of the experiment were on days 0, 15, 30, and 60. The findings were that the microbiological assessment was decreased significantly ($p < 0.05$) compared to the control group, while the storage period increased significantly. The study concluded that NS extract was a potential source of antioxidants and antimicrobial activities.

Giri (2012) evaluated for appearance, texture, aroma, taste, and overall acceptability by applied five-point hedonic scale sensory evaluation of NS. Two products, chutney and tea powder, were developed with an incorporation of NSP at various levels. For chutney powder, incorporation of NSP was 40%, 50%, and 60%, and for tea, incorporation of NSP was 0.5, 1, 1.5, and 2 g/2g tea bag in place of regular tea. The results were that chutney powder had the highest acceptance at the 50% level, and the 0.5g/2 g tea bag level was the most accepted for tea powder.

Chapter III: Methods

Study Design and Collection of Raw Material

An experimental study was conducted at Life University. The purpose of the study was to assess the texture and shelf-life of three types of bread (bread without NS, bread with NSO, and bread with NSP). Some of the raw material was purchased from the local market of a Walmart in Marietta, GA, while NSO and NSP were purchased online from the Amazon.com.

Ingredients and Preparation

The recipe that used in this research was following (Mnif et al., 2012), but there was some edits were applied to conduct this study. Each dough was prepared from 7 cups (around 1,000 g) Member's Mark flour, 2 tablespoons (30 mL) Pure Wesson vegetable oil, 3 tablespoons (around 43 g) Domino granulated sugar, 2.25 cups (625 ml) warm water, 3.25 teaspoons (around 19 g) Red Star yeast, 1 teaspoon (5 g) Morton Iodized salt, 3 teaspoons (15 g) Khana Pakana Brand NSP, and 3 teaspoons (around 15 mL) Minas Beauty Brand NSO.

The first dough was made without NS and it was the control sample. The second dough was made with 15 mL of NSO, 10 mL added before baking and 5 mL after baking, and it was called the oil sample. The third was made with 15 g of NSP (before baking only), and it was called the powder sample. For all experiments, the yeast and sugar were added to the warm water for 5 minutes, and then the salt, oil, and flour were added gradually. The doughs were baked at 300 ^{o} F for 15 minutes. The total number of samples was 75; each type of bread had 25 samples. Samples were divided for 5 days, and each day 15 samples of all types of bread (5 oil samples, 5 powder samples, and 5 control samples) were tested to monitor texture and water activity.

Tools to Assess Shelf-life

Texture analyzer.

A TA.XT Plus Texture Analyzer was applied to measure the firmness of bread for 5 days (the first day was the same day of baking). Five samples from each type were randomly selected every day to measure the firmness. The TA.XT Express Texture Analyzer, fitted with a TA-3 1-inch diameter cylinder probe, was selected for this study. Each sample weighed 40 g, and the probe was compressed into the center of the product to a distance of 10mm from the top surface of the product. The parameter settings were as follows: test mode – measure force in compression; pre-test speed – 2.0 mm/s; test speed – 1.0 mm/s; post-test speed – 10.0 mm/s; 10 mm compression distance; force trigger of 5 g. Firmness measures the maximum initial force on the product, which can reflect on the shelf life of the products (Singh et al., 2017). Texture analyzers (such as TA.XT Plus) are used commonly in different research studies (Xie et al., 2003; Gambaro et al., 2004; Abu-ghoush et al., 2008).

Water activity meter.

An AquaLab Pre device was used to measure the shelf-life for 5 days. Five samples from each type were randomly selected to measure the activity of water every day by using AquaLab Pre meter, and the average of the five samples from each type was calculated to estimate the water activity. AquaLab Pre is one example of a water activity meter, which is a thermodynamic device that measures the energy of water in a product. It is related to the microbial susceptibility of food products and relationships with several reactions that end shelf life in foods. There is an opposite relationship between water activity and shelf life; if the amount of water in a food product is lower, the shelf life of the product will be longer (Zhang et al., 2015). Water activity meters have been used in different research studies to measure shelf life (Sirpatrawan, 2009; Sozer et al., 2011; Sloan et al., 2016).

Physical and Microbial Analysis

The samples were separated (control samples, NSO samples, and NSP samples) and saved in three large metal plates covered by a white paper roll. The shelf life of the three types of bread was monitored every day by physically examining and observing the appearance, color, smell, and texture at room temperature storage (25 ^{o}C) for 10 days. Pictures were taken to monitor mold growth during storage period (10 days).

Statistical Analysis

The study used a statistical analysis through the Statistical Package for Social Science (SPSS) program (Version 22.0) to analyze quantitative data collected and recognize the relationship between variables. Data analyses consisted of computing descriptive statistics in the form of frequency, percentage, minimum, maximum, and indicators of central tendencies (means) and variability (standard deviations (SD)). This consisted of using inferential statistics to determine:

- The difference of main variables results according to triple classification using one-way ANOVA test and Tukey test for homogeneous descriptions and Kruskal-Wallis test and Dunnett T3 test for nonhomogeneous.
- The relationships between variables, as initially assessed using bivariate correlational analyses (Pearson).

- The overall fit of the hypothesized model, as tested by using multiple regressions to find the rate of factors' effects on the main variables of study and the direction of that effectiveness.

Chapter IV: Results

Characteristics of Bread Types

Descriptive statistics of bread firmness and water activity for all three types of bread (bread without NS [control], bread with NSO, and bread with NSP) are reported in Table 5. The bread with NSO had the lowest firmness mean ± SD = 3906.22 ± 3353.99, compared to bread with NSP = 5480.18± 4129.94 and control bread = 6040.78 ± 4234.77. Water activity for the three types of bread had approximately the same mean and SD. Control bread mean ± SD = 0.97 ± 0.03, bread with NSO mean ± SD = 0.97 ± 0.02, and bread with NSP mean ± SD = 0.97 ± 0.03. All three types of bread were exposed to the same temperature, mean ± SD = 21.39 ± 0.49.

Table 5*: Descriptive Statistics of Bread Firmness and Water Activity*

Variable	Firmness Min-Max	Firmness Mean ±SD	WA Min-Max	WA Mean ±SD	Temperature Min-Max	Temperature Mean ±SD
Control (n=25)	347.70- 12198.33	6040.78 ±4234.77	0.88- 1.01	0.97±0.03	20.60-22.00	21.39±0.49
Bread with NSO (n=25)	181.68- 10810.94	3906.22 ±3353.99	0.94-1.02	0.97±0.02	20.60-22.00	21.39±0.49
Bread with NSP (n=25)	336.32-12069.72	5480.18 ±4129.94	0.90-1.00	0.97±0.3	20.60-22.00	21.39±0.49
Overall (N=75)	181.68- 12198.33	5142.39 ±3977.93	0.88- 1.02	0.97±0.03	20.60-22.00	21.39 ±0.49

Values are reported as mean±SD. Abbreviations: SD = standard deviation, WA = water activity.

Comparison of All Bread Types

Firmness and water activity had contrasting results among the three types of bread. One-way ANOVA test was used to identify the difference in homogeneous data means among bread types, depicted in Table 6. All results indicated that the test

International Journal of Scientific & Engineering Research Volume 10, Issue 7, July-2019
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of homogeneity of variances was applicable for firmness and water activity among the three types of bread, since (*P* = > 0.05). One-way ANOVA tests of firmness and water activity comparison according to bread type are reported in Table 7. Although the observed results indicated that there was no statistically significant difference between firmness and water activity mean, bread with NSO had the lowest firmness mean ± SD (3906.22 ± 3353.99), and a slightly higher mean ± SD of water activity (0.9724 ± .022) compared to the other bread types (see Figures 3 and 4).

Table 6: *Test of Homogeneity for Firmness and Water Activity among Bread Types*

Variables	Levene Statistic	df1	Sig.
Firmness	1.990	2	NS(0.144)
WA	.102	2	NS(0.903)

Abbreviations: SD = standard deviation, WA = water activity NS = not statistically significant (*P* = > 0.05).

Table 7: *Firmness and Water Activity Comparison According to Bread Types*

Variables		N	Mean	D	95% Confidence Interval for Mean		F	*P*-value
					Lower Bound	Upper Bound		
Firmness	Control	25	6040.7824	±4234.77	4292.75	7788.81	1.986	NS(0.145)
	Bread with NSO	25	3906.2199	±3353.99	2521.76	5290.68		
	Bread with NSP	25	5480.1775	±4129.94	3775.42	7184.93		
	Total	75	5142.3933	±3977.93	4227.16	6057.63		
WA	Control	25	.9720	28	.96	.98	.080	NS(0.923)
	Bread with NSO	25	.9724	22	.96	.98		
	Bread with NSP	25	.9696	.0285	.96	.98		
	Total	75	.9713	26	.97	.98		

Values are reported as mean±SD and analyzed by one-way ANOVA test. Abbreviations: NS = not statistically significant (*P* = > 0.05), SD = standard deviation.

International Journal of Scientific & Engineering Research Volume 10, Issue 7, July-2019
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Figure 3. Means plot for bread firmness according to bread types (control bread, bread with NSO, and bread with NSP). The Y-axis represents the means for firmness of the three types of bread. The X-axis represents the three types of bread.

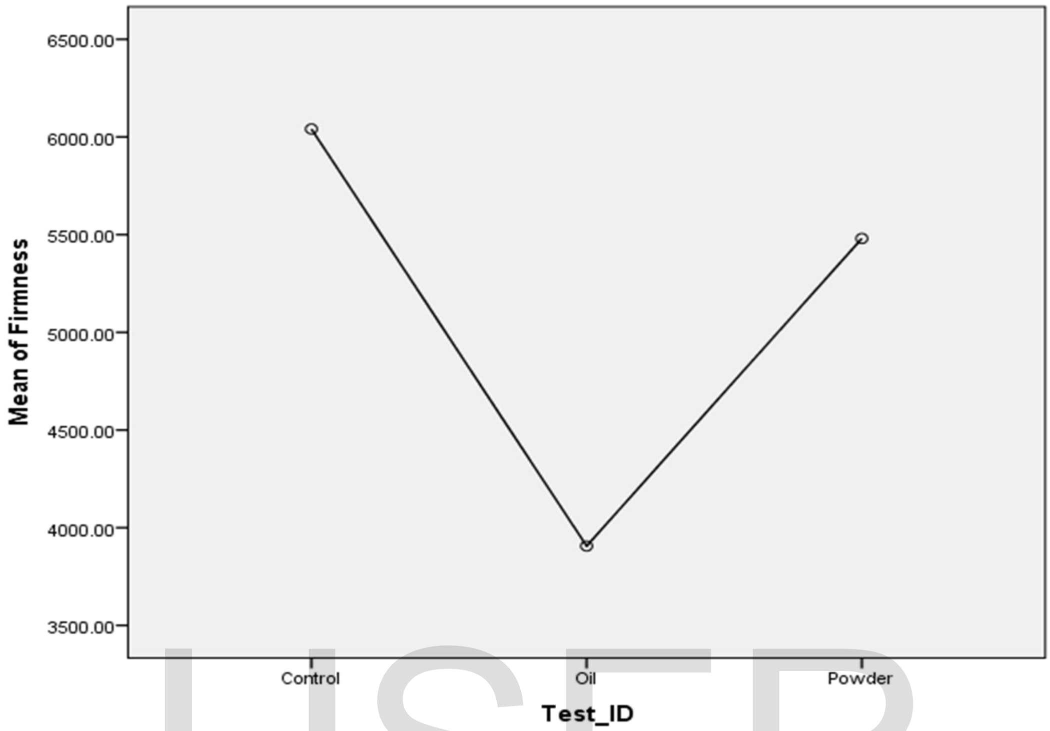
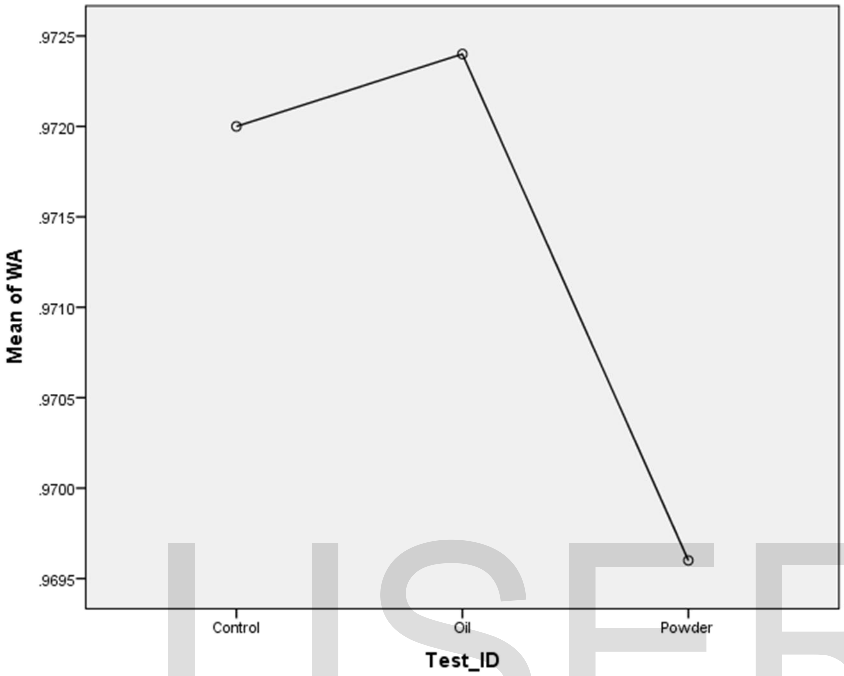


Figure 4. Means plot for water activity (WA) of bread according to bread types (control bread, bread with NSO, and bread with NSP). The Y-axis represents the means for WA of the three types of bread. The X-axis represents the three types of bread.



Firmness and water activity had contrasting findings among five days for the three types of bread overall. Tests of homogeneity for firmness and water activity among days for bread types overall are shown in Table 8. All results indicated that the test of homogeneity for variances was not applicable for firmness and water activity among days for the three types of bread overall since (P = < 0.05). Means of firmness and water activity for bread types overall according to days are illustrated in Table 9. The findings were that the mean of firmness showed statistically significant increases for every successive day (P = 0.00), while the mean of water activity showed fluctuations: the mean was reading at 0.9680 on day one, and then on day five it decreased to (0.9427), and the results were statistically significant (P = 0.00) (see Figures 5 and 6).

Table 8: *Test of Homogeneity for Firmness and Water Activity among Days for Bread Types Overall*

Variables	Levene Statistic	df1	Sig.
Firmness	14.425	4	.000
WA	6.028	4	.000

Table 9: *Day-by-Day Means of Firmness and Water Activity for Bread Types Overall*

Variables		N	Mean	±SD	95% Confidence Interval for Mean		Kruskal-Wallis	
					Lower Bound	Upper Bound	Mean Rank	*P*-value
Firmness	Day 1	15	372.2306	±87.87	323.5674	420.8938	8.00	S(0.00)
	Day 2	15	1902.2817	±799.47	1459.5468	2345.0167	23.13	
	Day 3	15	6258.2319	±2714.85	4754.7966	7761.6671	45.47	
	Day 4	15	8427.9507	±2484.84	7051.8878	9804.0137	56.27	
	Day 5	15	8751.2713	±2639.43	7289.6017	10212.9410	57.13	
	Total	75	5142.3933	±3977.93	4227.1554	6057.6311		
WA	Day 1	15	.9680	±.01	.9610	.9750	30.73	S(0.00)
	Day 2	15	.9980	±.01	.9949	1.0011	65.23	
	Day 3	15	.9707	±.01	.9653	.9760	32.57	
	Day 4	15	.9773	±.03	.9617	.9929	44.40	
	Day 5	15	.9427	±.03	.9260	.9593	17.07	
	Total	75	.9713	±.03	.9653	.9774		

Values are reported as mean±SD and analyzed by Kruskal-Wallis test. Abbreviations: S = statistically significant (*P* = < 0.05). SD = standard deviation.

International Journal of Scientific & Engineering Research Volume 10, Issue 7, July-2019
ISSN 2229-5518

Figure 5. Means plot for firmness of bread types overall (control bread, bread with *Nigella sativa* oil [NSO], and bread with *Nigella sativa* powder [NSP]) according to days. The Y-axis represents the means for firmness of the three types of bread. The X-axis represents the days.

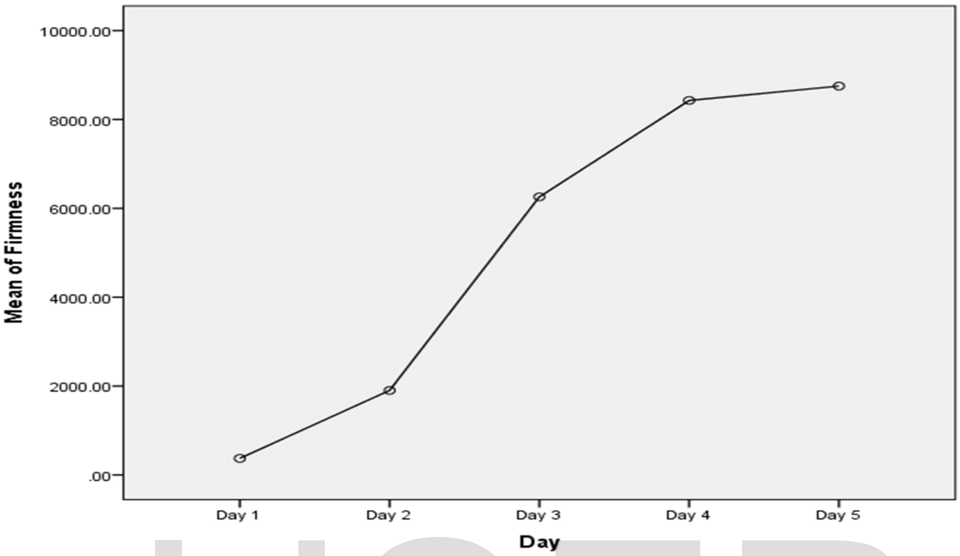
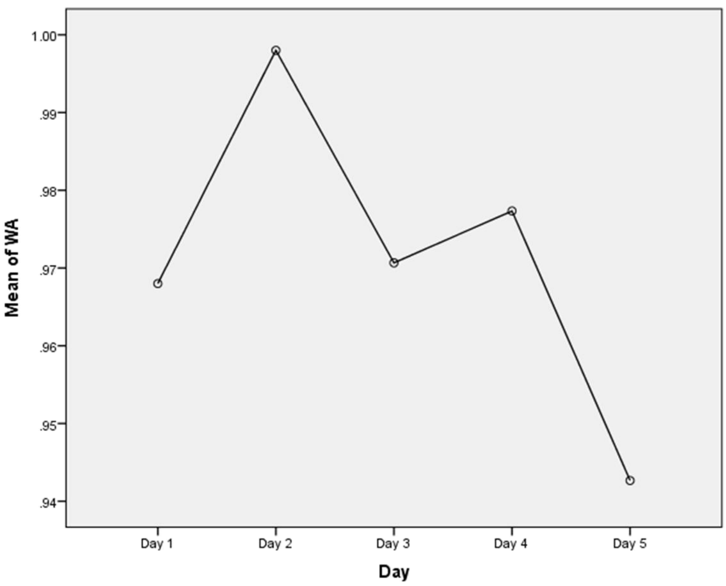


Figure 6. Means plot for water activity of bread types overall (control bread, bread with *Nigella sativa* oil [NSO], and bread with *Nigella sativa* powder [NSP]) according to days. The Y-axis represents the means for water activity of the three types of bread. The X-axis represents the days.



International Journal of Scientific & Engineering Research Volume 10, Issue 7, July-2019
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The differences in firmness and water activity among five days for three types of bread overall are reported in Table 10. The experimental means for WA and firmness were compared with the same control mean with the Dunnett T3 test of multiple comparisons for nonhomogeneous variances. There were statistically significant differences between firmness on day 1 and day 2 ($P = 0.00$), day 1 and day 3 ($P = 0.00$), day 1 and day 4 ($P = 0.00$), and day 1 and day 5 ($P = 0.00$). For day 2, there were statistically significant differences between firmness on day 2 and day 3 ($P = 0.00$), day 2 and day 4 ($P = 0.00$), and day 2 and day 5 ($P = 0.00$). For day 3, 4, and 5, there were statistically significant differences in firmness on day 1 and day 2 ($P = 0.00$). In the case of water activity, the differences in day to day means was statistically significant between day 1 and day 2 ($P = 0.00$), day 2 and day 3 ($P = 0.00$), and day 2 and day 5 ($P = 0.00$). There were statistically significant differences between day 3 and day 5 ($P = 0.030$), and day 4 and day 5 ($P = 0.028$).

Table 10: *Day-by-Day Multiple Comparisons of Firmness and Water Activity for Bread Types Overall*

Dependent Variable	(I) Day	(J) Day	Mean Difference (I-J)	*P* value	95% Confidence Interval	
					Lower Bound	Upper Bound
Firmness	Day 1	Day 2	-1530.05113*	S(0.000)	-2204.6947	-855.4075
		Day 3	-5886.00127*	S(0.000)	-8171.9578	-3600.0447
		Day 4	-8055.72013*	S(0.000)	-10148.0908	-5963.3494
		Day 5	-8379.04073*	S(0.000)	-10601.5193	-6156.5621
	Day 2	Day 1	1530.05113*	S(0.000)	855.4075	2204.6947
		Day 3	-4355.95013*	S(0.000)	-6685.4151	-2026.4852
		Day 4	-6525.66900*	S(0.000)	-8666.5865	-4384.7515
		Day 5	-6848.98960*	S(0.000)	-9116.5103	-4581.4689
	Day 3	Day 1	5886.00127*	S(0.000)	3600.0447	8171.9578
		Day 2	4355.95013*	S(0.000)	2026.4852	6685.4151
		Day 4	-2169.71887	NS(0.247	-5041.4708	702.0331
		Day 5	-2493.03947	NS(0.145	-5445.9945	459.9156
	Day 4	Day 1	8055.72013*	S(0.000)	5963.3494	10148.0908
		Day 2	6525.66900*	S(0.000)	4384.7515	8666.5865
		Day 3	2169.71887	NS(0.247	-702.0331	5041.4708
		Day 5	-323.32060	NS(01.000	-3151.0430	2504.4018
	Day 5	Day 1	8379.04073*	S(0.000)	6156.5621	10601.5193
		Day 2	6848.98960*	S(0.000)	4581.4689	9116.5103
		Day 3	2493.03947	NS(0.145)	-459.9156	5445.9945
		Day 4	323.32060	NS(1.000)	-2504.4018	3151.0430
WA	Day 1	Day 2	-.03000*	S(0.000)	-.0412	-.0188
		Day 3	-.00267	NS(0.999)	-.0151	.0098
		Day 4	-.00933	NS(0.923)	-.0342	.0156
		Day 5	.02533	NS(0.066)	-.0011	.0518
	Day 2	Day 1	.03000*	S(0.000)	.0188	.0412
		Day 3	.02733*	S(0.000)	.0185	.0362
		Day 4	.02067	NS(0.115	-.0032	.0446
		Day 5	.05533*	S(0.000)	.0298	.0809
	Day 3	Day 1	.00267	NS(0.999	-.0098	.0151
		Day 2	-.02733*	S(0.000)	-.0362	-.0185
		Day 4	-.00667	NS(0.988)	-.0310	.0177
		Day 5	.02800*	S(0.03)	.0021	.0539
	Day 4	Day 1	.00933	NS(0.923)	-.0156	.0342
		Day 2	-.02067	NS(0.115)	-.0446	.0032
		Day 3	.00667	NS(0.988)	-.0177	.0310
		Day 5	.03467*	S(0.028)	.0025	.0668
	Day 5	Day 1	-.02533	S(0.066)	-.0518	.0011
		Day 2	-.05533*	S(0.000)	-.0809	-.0298
		Day 3	-.02800*	S(0.030)	-.0539	-.0021
		Day 4	-.03467*	S(0.028)	-.0668	-.0025

Values are reported as mean difference and analyzed by Dunnett T3 test. Abbreviations: NS = not statistically significant (P = > 0.05), S = statistically significant (P = < 0.05).

Control Bread

Firmness and water activity had contrasting findings among days overall for control bread. Tests of homogeneity for firmness and water activity among days for control bread are shown in Table 11. All results indicated that the tests of homogeneity for variances were not applicable for firmness and water activity among days for control bread, since (*P* = < 0.05). Means of firmness and water activity for control bread according to days are illustrated in Table 12. The findings were that the firmness means showed statistically significant increases by every succeeding day (*P* = 0.00), while the water activity mean showed fluctuating results: the mean started on day 1 at (.9720), and then on day 5, the mean decreased to (.9320), and the results were statistically significant (*P* = 0.00) (see Figures 7 and 8).

International Journal of Scientific & Engineering Research Volume 10, Issue 7, July-2019
ISSN 2229-5518

Table 11*: Test of Homogeneity for Firmness and Water Activity among Days for Control Bread*

Variables	Levene Statistic	df1	Sig.
Firmness	4.360	4	S(0.011)
WA	8.969	4	S(0.000)

Abbreviations: S = statistically significant (*P* = < 0.01), S = statistically significant *P* = < 0.05).

Table 12*: Day-by-Day Means of Firmness and Water Activity for Control Bread*

Variables		N	Mean	±SD	95% Confidence Interval for Mean Lower Bound	Upper Bound	Mean Rank	*P*-value
Firmness	Day 1	5	365.0760	±14.63	346.9062	383.2458	3.00	S(0.00)
	Day 2	5	2664.0082	±815.37	1651.5823	3676.4341	8.00	
	Day 3	5	7900.9764	±1942.94	5488.4920	10313.4608	15.60	
	Day 4	5	8638.9312	±2270.55	5819.6685	11458.1939	17.00	
	Day 5	5	10634.9200	±2082.77	8048.8204	13221.0196	21.40	
	Total	25	6040.7824	±4234.77	4292.7546	7788.8101		
WA	Day 1	5	.9720	±.00	.9664	.9776	12.60	S(0.00)
	Day 2	5	1.0000	±.00	1.0000	1.0000	22.00	
	Day 3	5	.9620	±.00	.9564	.9676	8.00	
	Day 4	5	.9940	±.00	.9829	1.0051	19.00	
	Day 5	5	.9320	±.03	.8914	.9726	3.40	
	Total	25	.9720	±.02	.9602	.9838		

Values are reported as mean±SD and analyzed by Kruskal-Wallis test. Abbreviations: S = statistically significant (*P* = < 0.05). SD = standard deviation.

Figure 7. Means plot for firmness of control bread according to days. The Y-axis represents the means for firmness of the three types of bread. The X-axis represents the days.

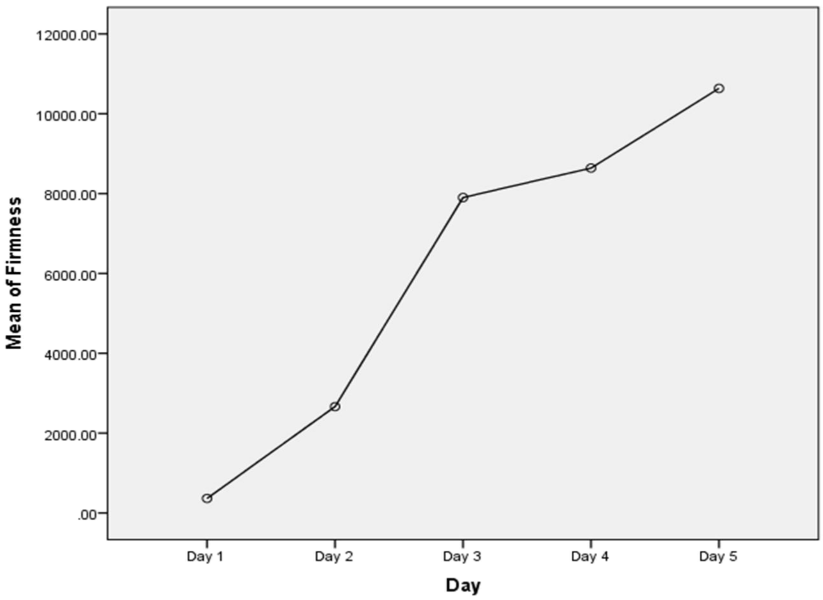
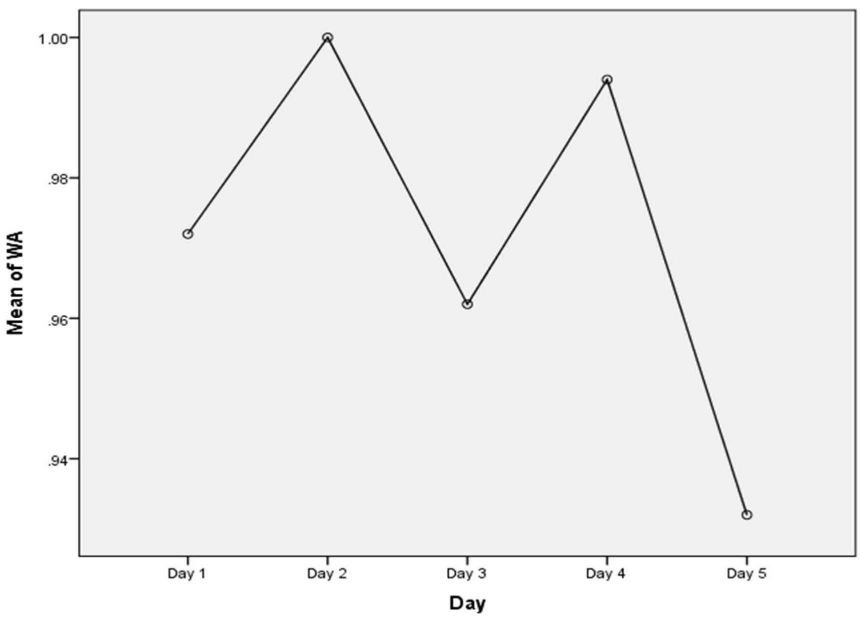


Figure 8. Means plot for water activity of control bread according to days. The Y-axis represents the means for water activity of the three types of bread. The X-axis represents the days.



The differences in firmness and water activity among days for control bread are depicted in Table 13. Dunnett T3 test of multiple comparisons was used for nonhomogeneous variances. As shown in Table 13, there was a statistically significant difference between firmness on day 1 and day 2 (P = 0.019), and statistically significant differences were found between day 1 and day 3 (P = 0.006), day 1 and day 4 (P = 0.008), and day 1 and day 5 (P = 0.002). In the case of day 2, there was a statistically significant difference between firmness on day 2 and day 3 (P = 0.015), and day 2 and day 4 (P = 0.018); between day 2 and day 5, there was a statistically significant difference (P = 0.003). In case of water activity, the means difference between water activity among days were statistically significant between day 1 and day 4 (P = 0.021), and statistically significant differences were found between day 1 and day 2 (P = 0.001), day 2 and day 3 (P = 0.00), and day 3 and day 4 (P = 0.003).

Table 13*: Day-by-Day Multiple Comparisons of Firmness and Water Activity for Control Bread*

Dependent Variable	(I) Day	(J) Day	Mean Difference (I-J)	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Firmness	Day 1	Day 2	-2298.93220*	.019	-4055.0236	-542.8408
		Day 3	-7535.90040*	.006	-11721.1291	-3350.6717
		Day 4	-8273.85520*	.008	-13164.8170	-3382.8934
		Day 5	-10269.84400*	.002	-14756.2843	-5783.4037
	Day 2	Day 1	2298.93220*	.019	542.8408	4055.0236
		Day 3	-5236.96820*	.015	-9179.7071	-1294.2293
		Day 4	-5974.92300*	.018	-10620.7537	-1329.0923
		Day 5	-7970.91180*	.003	-12212.0631	-3729.7605
	Day 3	Day 1	7535.90040*	.006	3350.6717	11721.1291
		Day 2	5236.96820*	.015	1294.2293	9179.7071
		Day 4	-737.95480	.999	-5661.2640	4185.3544
		Day 5	-2733.94360	.387	-7402.3033	1934.4161
	Day 4	Day 1	8273.85520*	.008	3382.8934	13164.8170
		Day 2	5974.92300*	.018	1329.0923	10620.7537
		Day 3	737.95480	.999	-4185.3544	5661.2640
		Day 5	-1995.98880	.776	-7049.4644	3057.4868
	Day 5	Day 1	10269.84400*	.002	5783.4037	14756.2843
		Day 2	7970.91180*	.003	3729.7605	12212.0631
		Day 3	2733.94360	.387	-1934.4161	7402.3033
		Day 4	1995.98880	.776	-3057.4868	7049.4644
WA	Day 1	Day 2	-.02800*	.001	-.0376	-.0184
		Day 3	.01000	.059	-.0004	.0204
		Day 4	-.02200*	.021	-.0401	-.0039
		Day 5	.04000	.266	-.0297	.1097
	Day 2	Day 1	.02800*	.001	.0184	.0376
		Day 3	.03800*	.000	.0284	.0476
		Day 4	.00600	.744	-.0133	.0253
		Day 5	.06800	.056	-.0025	.1385
	Day 3	Day 1	-.01000	.059	-.0204	.0004
		Day 2	-.03800*	.000	-.0476	-.0284
		Day 4	-.03200*	.003	-.0501	-.0139
		Day 5	.03000	.491	-.0397	.0997
	Day 4	Day 1	.02200*	.021	.0039	.0401
		Day 2	-.00600	.744	-.0253	.0133
		Day 3	.03200*	.003	.0139	.0501
		Day 5	.06200	.070	-.0060	.1300
	Day 5	Day 1	-.04000	.266	-.1097	.0297
		Day 2	-.06800	.056	-.1385	.0025
		Day 3	-.03000	.491	-.0997	.0397
		Day 4	-.06200	.070	-.1300	.0060

Values are reported as mean difference and analyzed by Dunnett T3 test. Abbreviations: *= statistically significant (*P* = < 0.05).

Bread with NSO

Tests of homogeneity for firmness and water activity among days for bread with NSO are reported in Table 14. As regards water activity tests showed homogeneity of variances for successive days for NSO bread (P = > 0.05). The means differences measured by one-way ANOVA test are illustrated in Table 15. As regards firmness tests of homogeneity for variances were not applicable for day-to-day bread made with NSO (P = < 0.05), the findings were that the firmness means showed statistically significant increases over successive days (P = 0.00). Thus, the means differences by Kruskal-Wallis test are shown in Table 15. The output for water activity means showed fluctuating results: the mean started on day 1 (.9520), then on day 5, the mean decreased to (.9460), and the findings were statistically significant (P = 0.00) (see Figure 9).

Table 14*: Test of Homogeneity for Firmness and Water Activity among Days for Bread with Nigella sativa oil (NSO)*

Variables	Levene Statistic	df1	Sig.
Firmness	21.595	4	.000
WA	1.950	4	.141

Table 15: *Day-to-Day Means of Firmness and Water Activity for Bread with Nigella sativa oil (NSO)*

				95% Confidence Interval for Mean				*P*-value
		N	Mean ±SD	Lower Bound	Upper Bound	Test	Mean	
Firmness	Day 1	5	346.5046±148.32310	162.3372	530.6720	Anova	346.50	S(0.00)
	Day 2	5	1160.8348±282.20210	810.4346	1511.2350		1160.83	
	Day 3	5	3614.7320±339.46392	3193.2319	4036.2321		3614.73	
	Day 4	5	6360.6146±1680.97334	4273.4105	8447.8187		6360.61	
	Day 5	5	8048.4136±3179.42150	4100.6404	11996.1868		8048.41	
	Total	25	3906.2199±3353.99365	2521.7594	5290.6805			
WA	Day 1	5	.9520±.00447	.9464	.9576	Test	Mean rank	*P*-value
	Day 2	5	.9940±.00894	.9829	1.0051	Kruskal-Wallis	3.00	S(0.00)
	Day 3	5	.9820±.00447	.9764	.9876		8.00	
	Day 4	5	.9880±.01789	.9658	1.0102		13.20	
	Day 5	5	.9460±.01342	.9293	.9627		19.40	
	Total	25	.9724±.02241	.9631	.9817		21.40	

Values are reported as mean±SD and analyzed by one-way ANOVA or Kruskal-Wallis test.

Abbreviations: S = statistically significant (P = < 0.05). SD = standard deviation.

*Figure 9.*Means plot for firmness of bread with *Nigella sativa* oil (NSO) over successive days. The Y-axis represents the means for firmness of the three types of bread. The X-axis represents the days.

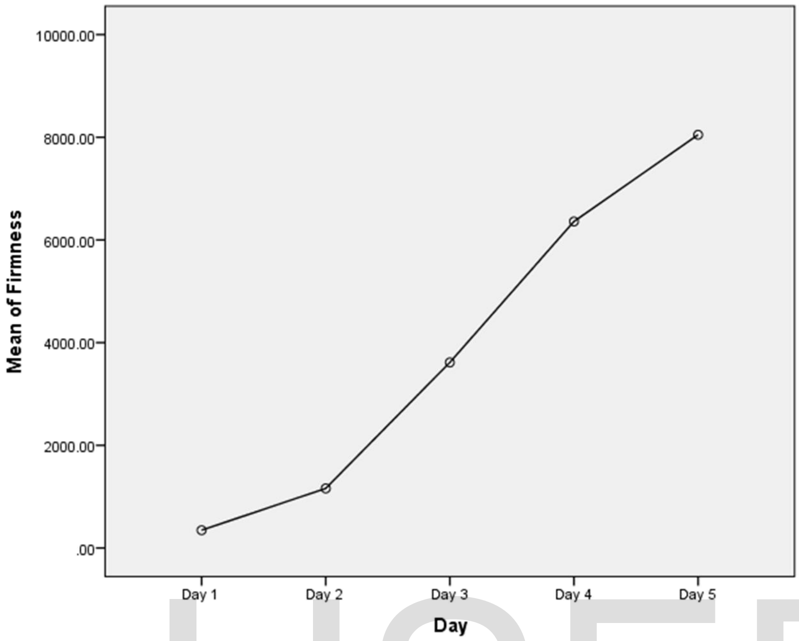
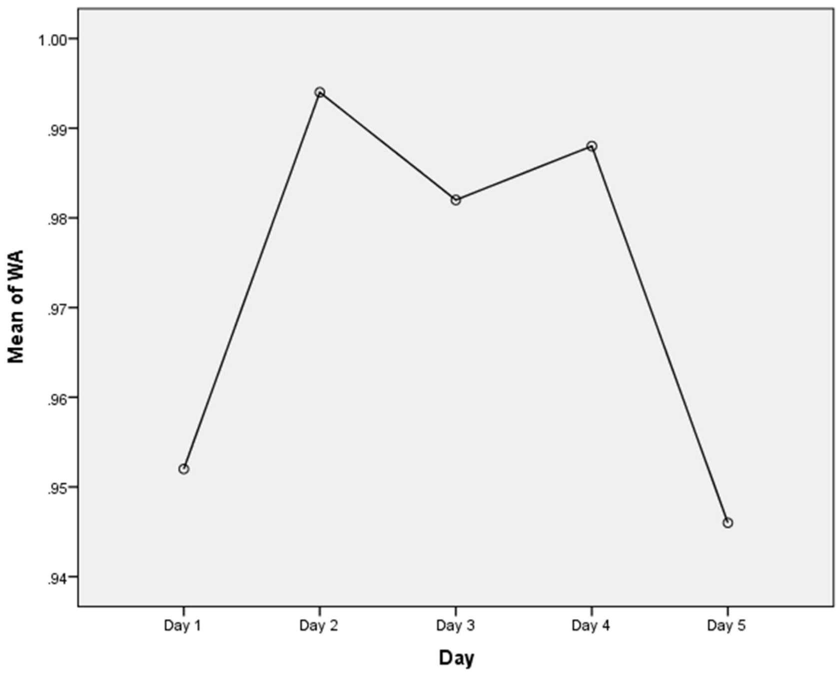


*Figure 10.*Means plot for water activity of bread with *Nigella sativa* powder (NSO) over successive days. The Y-axis represents the means for water activity of the three types of bread. The X-axis represents the days.



International Journal of Scientific & Engineering Research Volume 10, Issue 7, July-2019
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The differences in firmness over days for bread with NSO are described in Table 16. Dunnett T3 test of multiple comparisons of experimental group of bread was used for nonhomogeneous variances with one (the same) control group. As shown in Table 16, there was a statistically significant difference between firmness on day 1 and day 2 (P = 0.009), day 1 and day 3 (P = 0.00), and day 1 and day 4 (P = 0.008); in day 1 and day 5, the result was statistically significant (P = 0.033). In the case of day 2, there was a statistically significant difference between firmness on day 2 and day 3 (P = 0.00). Moreover, there were statistically significant difference found in day 2 and day 4 (P = 0.012), and day 2 and day 5 (P = 0.048).

Multiple day-to-day comparisons of water activity for bread with NSO are reported in Table 17. The means difference between water activity among days was statistically significant between day 1 and day 2 (P = 0.00), day 1 and day 3 (P = 0.003), and day 1 and day 4 (P = 0.00). There was also a statistically significant difference between water activity on day 2 and day 5 (P = 0.03), and a statistically significant difference on day 3 and day 5 (P = 0.00), and day 4 and day 5 (P = 0.00).

Table 16: *Multiple Day-to-Day Comparisons of Firmness for Bread with Nigella sativa oil (NSO)*

Dependent Variable	(I) Day	(J) Day	Mean Difference (I-J)	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Firmness	Day 1	Day 2	-814.33020*	.009	-1383.8796	-244.7808
		Day 3	-3268.22740*	.000	-3955.8860	-2580.5688
		Day 4	-6014.11000*	.008	-9618.3096	-2409.9104
		Day 5	-7701.90900*	.033	-14541.7345	-862.0835
	Day 2	Day 1	814.33020*	.009	244.7808	1383.8796
		Day 3	-2453.89720*	.000	-3183.0415	-1724.7529
		Day 4	-5199.77980*	.012	-8764.1549	-1635.4047
		Day 5	-6887.57880*	.048	-13704.2552	-70.9024
	Day 3	Day 1	3268.22740*	.000	2580.5688	3955.8860
		Day 2	2453.89720*	.000	1724.7529	3183.0415
		Day 4	-2745.88260	.118	-6288.5387	796.7735
		Day 5	-4433.68160	.189	-11236.5375	2369.1743
	Day 4	Day 1	6014.11000*	.008	2409.9104	9618.3096
		Day 2	5199.77980*	.012	1635.4047	8764.1549
		Day 3	2745.88260	.118	-796.7735	6288.5387
		Day 5	-1687.79900	.941	-8104.8886	4729.2906
	Day 5	Day 1	7701.90900*	.033	862.0835	14541.7345
		Day 2	6887.57880*	.048	70.9024	13704.2552
		Day 3	4433.68160	.189	-2369.1743	11236.5375
		Day 4	1687.79900	.941	-4729.2906	8104.8886

Values are reported as mean difference and analyzed by Dunnett T3 test. Abbreviations: * = statistically significant ($P = < 0.05$).

Table 17: *Multiple Day-to-Day Comparisons of Water Activity for Bread with Nigella sativa oil (NSO)*

(I) Day	(J) Day	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Day 1	Day 2	-.04200*	.000	-.0631	-.0209
	Day 3	-.03000*	.003	-.0511	-.0089
	Day 4	-.03600*	.000	-.0571	-.0149
	Day 5	.00600	.911	-.0151	.0271
Day 2	Day 1	.04200*	.000	.0209	.0631
	Day 3	.01200	.454	-.0091	.0331
	Day 4	.00600	.911	-.0151	.0271
	Day 5	.04800*	.000	.0269	.0691
Day 3	Day 1	.03000*	.003	.0089	.0511
	Day 2	-.01200	.454	-.0331	.0091
	Day 4	-.00600	.911	-.0271	.0151
	Day 5	.03600*	.000	.0149	.0571
Day 4	Day 1	.03600*	.000	.0149	.0571
	Day 2	-.00600	.911	-.0271	.0151
	Day 3	.00600	.911	-.0151	.0271
	Day 5	.04200*	.000	.0209	.0631
Day 5	Day 1	-.00600	.911	-.0271	.0151
	Day 2	-.04800*	.000	-.0691	-.0269
	Day 3	-.03600*	.000	-.0571	-.0149
	Day 4	-.04200*	.000	-.0631	-.0209

Values are reported as mean difference and analyzed by Tukey test. Abbreviations: * = statistically significant (P = < 0.05).

Bread with NSP

Tests of homogeneity for firmness and water activity among days for bread with NSP are shown in Table 18. Results of firmness and water activity indicated that tests of homogeneity for variances were not applicable among days for bread with NSP, since (P = < 0.05). Means of firmness and water activity for bread with NSP according to days are illustrated in Table 19. The findings were that the firmness means increased until day 4, then decreased to day 5, and the results are statistically significant (P = 0.00). The water activity mean was high in day 2 (1.00), and then decreased to (.950) in day 5, and the results are statistically significant (*P* = 0.00) (see Figures 11 and 12).

The differences in firmness and water activity among days for bread with NSP are shown in Table 20. Dunnett T3 test of multiple comparisons was used for nonhomogeneous variances. As shown in Table 20, there was a statistically significant difference between firmness on day 1 and day 2 (*P* = 0.001); on day 1 and day 3, the result was a statistically significant difference (*P* = 0.037). Also, there was a statistically significant difference between firmness on day 1 and day 4 (*P* = 0.002), and day 1 and day 5 (*P* = 0.005). For day 2, there was a statistically significant difference between firmness of day 2 and day 4 (*P* = 0.004); on day 2 and day 5, the result was a statistically significant difference (*P* = 0.011). In the case of water activity, the means difference between water activity among days were statistically significant between day 1 and day 3 since (*P* = 0.023); on day 2 and day 3, the results showed a statistically significant difference (*P* = 0.001).

Table 18*: Test of Homogeneity for Day-to-Day Changes in Firmness and Water Activity for Bread with NSP*

Variables	Levene Statistic	df1	Sig.
Firmness	18.399	4	.000
WA	21.347	4	.000

Table 19: *Means of Firmness and Water Activity for Bread with Nigella sativa powder (NSP) According to Days*

		N	Mean ±SD	95% Confidence Interval for Mean		Mean Rank	*P*-value
				Lower Bound	Upper Bound		
Firmness	Day 1	5	405.1112 ±50.69561	342.1643	468.0581	3.00	S(0.00)
	Day 2	5	1882.0022 ±282.14974	1531.6670	2232.3374	8.00	
	Day 3	5	7258.9872±2923.34578	3629.1740	10888.8004	16.00	
	Day 4	5	10284.3064±1980.91027	7824.6814	12743.9314	21.40	
	Day 5	5	7570.4804±1772.27981	5369.9044	9771.0564	16.60	
	Total	25	5480.1775±4129.94240	3775.4210	7184.9339		
WA	Day 1	5	.9800±.00000	.9800	.9800	15.50	S(0.004)
	Day 2	5	1.0000±.00000	1.0000	1.0000	23.00	
	Day 3	5	.9680±.00447	.9624	.9736	8.40	
	Day 4	5	.9500±.03082	.9117	.9883	8.20	
	Day 5	5	.9500±.04123	.8988	1.0012	9.90	
	Total	25	.9696±.02865	.9578	.9814		

Values are reported as mean±SD and analyzed by Kruskal-Wallis test. Abbreviations: S = statistically significant ($P = < 0.05$). SD = standard deviation.

International Journal of Scientific & Engineering Research Volume 10, Issue 7, July-2019
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Figure 11. Day by Day changes in Means firmness of bread with *Nigella sativa* powder (NSP). The Y-axis represents the means for firmness of the three types of bread. The X-axis represents the days.

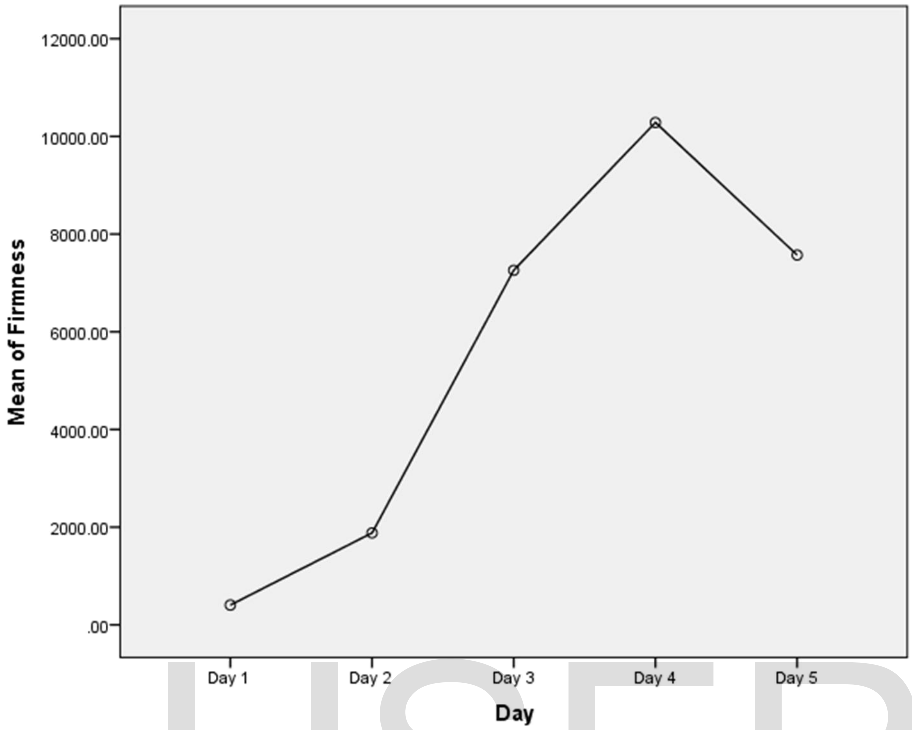
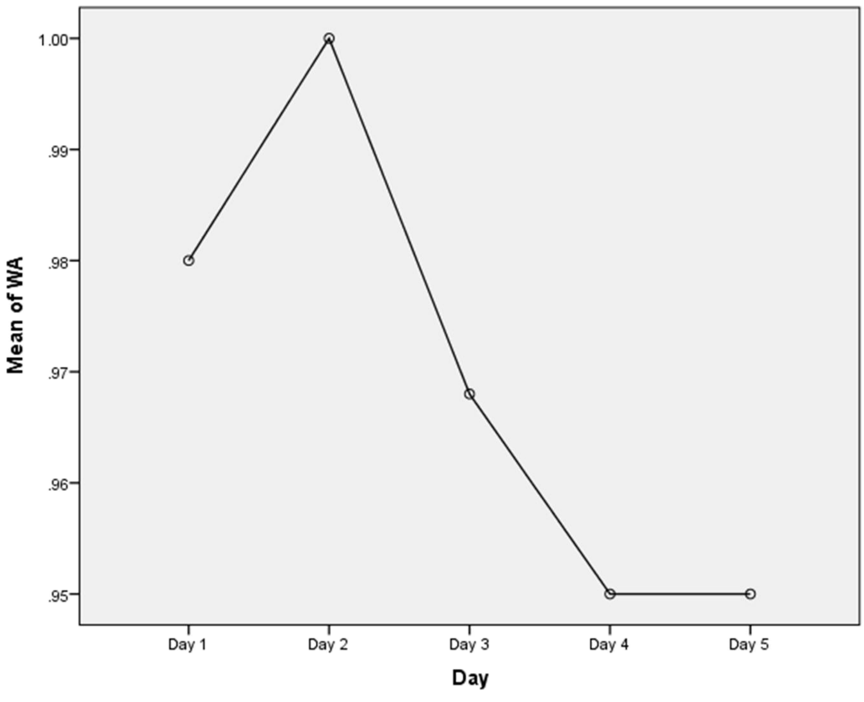


Figure 12. Day-by-Day changes in Means water activity of bread with *Nigella sativa* powder (NSP). The Y-axis represents the means for water activity of the three types of bread. The X-axis represents the days.



International Journal of Scientific & Engineering Research Volume 10, Issue 7, July-2019
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Table 20*: Day-by-Day Multiple Comparisons of Firmness and Water Activity for Bread with* NSP

Dependent Variable	(I) Day	(J) Day	Mean Difference (I-J)	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Firmness	Day 1	Day 2	-1476.89100*	.001	-2073.9430	-879.8390
		Day 3	-6853.87600*	.037	-13150.0085	-557.7435
		Day 4	-9879.19520*	.002	-14144.6386	-5613.7518
		Day 5	-7165.36920*	.005	-10981.1927	-3349.5457
	Day 2	Day 1	1476.89100*	.001	879.8390	2073.9430
		Day 3	-5376.98500	.083	-11639.4094	885.4394
		Day 4	-8402.30420*	.004	-12620.0005	-4184.6079
		Day 5	-5688.47820*	.011	-9451.9296	-1925.0268
	Day 3	Day 1	6853.87600*	.037	557.7435	13150.0085
		Day 2	5376.98500	.083	-885.4394	11639.4094
		Day 4	-3025.31920	.511	-9025.0923	2974.4539
		Day 5	-311.49320	1.000	-6242.0399	5619.0535
	Day 4	Day 1	9879.19520*	.002	5613.7518	14144.6386
		Day 2	8402.30420*	.004	4184.6079	12620.0005
		Day 3	3025.31920	.511	-2974.4539	9025.0923
		Day 5	2713.82600	.327	-1651.4272	7079.0792
	Day 5	Day 1	7165.36920*	.005	3349.5457	10981.1927
		Day 2	5688.47820*	.011	1925.0268	9451.9296
		Day 3	311.49320	1.000	-5619.0535	6242.0399
		Day 4	-2713.82600	.327	-7079.0792	1651.4272
WA	Day 1	Day 2	-.02000	.	-.0200	-.0200
		Day 3	.01200*	.023	.0024	.0216
		Day 4	.03000	.436	-.0364	.0964
		Day 5	.03000	.681	-.0588	.1188
	Day 2	Day 1	.02000	.	.0200	.0200
		Day 3	.03200*	.001	.0224	.0416
		Day 4	.05000	.123	-.0164	.1164
		Day 5	.05000	.271	-.0388	.1388
	Day 3	Day 1	-.01200*	.023	-.0216	-.0024
		Day 2	-.03200*	.001	-.0416	-.0224
		Day 4	.01800	.842	-.0476	.0836
		Day 5	.01800	.953	-.0702	.1062
	Day 4	Day 1	-.03000	.436	-.0964	.0364
		Day 2	-.05000	.123	-.1164	.0164
		Day 3	-.01800	.842	-.0836	.0476
		Day 5	.00000	1.000	-.0861	.0861
	Day 5	Day 1	-.03000	.681	-.1188	.0588
		Day 2	-.05000	.271	-.1388	.0388
		Day 3	-.01800	.953	-.1062	.0702
		Day 4	.00000	1.000	-.0861	.0861

Values are reported as mean difference and analyzed by Dunnett T3 test. Abbreviations: * = statistically significant ($P = < 0.05$).

Association between Water Activity, Firmness, and Temperature for All Types of Bread

The associations between water activity and firmness for all types of bread overall, as well as between water activity and temperature, conducted by Pearson correlation, are reported in Table 21. The results indicated that there was a negative statistically significant relationship between water activity and firmness since ([P = 0.002 < 0.05], r = -0.36). Also, there was a moderate negative statistically significant relationship between water activity and temperature since ([P = 0.00 < 0.05], r = -0.43). Linear regression was used to find the percentage of temperature that impacted on water activity, reported in Tables 22 and 23. The correlation coefficient (R = 0.428) indicated that the independent variable (temperature) had a moderate impact on water activity, and the R^2 or the coefficient of determination 0.183, saying that 18% of the change in WA was accounted for by the temperature variable.

Table 21*: Correlation Matrix between Water Activity, Firmness, and Temperature for All Types of Bread*

		Firmness	WA	Temperature
Firmness	Pearson Correlation	1	-.358*	.008
	Sig. (2-tailed)		.002	.944
	N	75	75	75
WA	Pearson Correlation	-.358*	1	-.428*
	Sig. (2-tailed)	.002		.000
	N	75	75	75
Temperature	Pearson Correlation	.008	-.428*	1
	Sig. (2-tailed)	.944	.000	
	N	75	75	75

Values are reported as Pearson Correlation coefficients and analyzed bivariate correlation test. Abbreviations: * = statistically significant (*P* = < 0.05).

Table 22*: Linear Regression for Temperature Impact on Water Activity Overall*

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.428[a]	.183	.172	.02399

Abbreviation: R = total correlation coefficients.

Table 23: *Linear Regression for Temperature Impact on Water Activity Overall Coefficients*

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.	95.0% Confidence Interval for B Lower Bound	95.0% Confidence Interval for B Upper Bound
1	(Constant)	1.461	.121		12.081	.000	1.220	1.702
	Temperature	-.023	.006	-.428	-4.050	.000	-.034	-.012

Values are reported as linear equation coefficients and analyzed by regression test. Abbreviations: B = beta.

The linear equation model:

Y= B+ a X + SE

Water activity= 1.461-0.023 Temperature +0.006

Water activity= 1.461-0.023 (21.39) +0.006

Water activity= 0.97+0.006

Water activity= 0.98

Association for Control Bread

The associations between water activity and firmness of all types of control bread, as well as between water activity and temperature, conducted by Pearson correlation, are illustrated in Table 24. The results showed that there was statistically no significant relationship between water activity and firmness since ([P = 0.073 > 0.05], r = -0.36), while the relationship between water activity and temperature was a negative statistically significant relationship ([P = 0.001 < 0.05], r = -0.624). Linear regression was used to find the percentage of temperature impact on water activity, reported in Tables 25 and 26. The correlation coefficient (R = 0.624) indicated that the independent variable (temperature) had a moderate impact on water activity for control bread and temperature accounts for 39% of WA (R^2 = 0/389).

Table 24*: Correlation Matrix between Water Activity, Firmness, and Temperature for Control Bread*

		Firmness	WA	Temperature
Firmness	Pearson Correlation	1	-.365	.100
	Sig. (2-tailed)		.073	.634
	N	25	25	25
WA	Pearson Correlation	-.365	1	-.624*
	Sig. (2-tailed)	.073		.001
	N	25	25	25
Temperature	Pearson Correlation	.100	-.624*	1
	Sig. (2-tailed)	.634	.001	
	N	25	25	25

Values are reported as Pearson correlation coefficients and analyzed bivariate correlation test. Abbreviations: *= statistically significant (*P* = < 0.05).

Table 25: *Linear Regression for Temperature Impact on Water Activity for Control*

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.624[a]	.389	.363	.02281
a. Predictors: (Constant), Temperature				

Abbreviation: R = total correlation coefficients.

Table 26*: Linear Regression for Temperature Impact on Water Activity for Control Coefficients*

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	1.736	.200		8.698	.000	1.323	2.149
	Temperature	-.036	.009	-.624	-3.830	.001	-.055	-.016

Values are reported as Linear equation coefficients and analyzed by regression test. Abbreviations: B = beta.

The linear equation model:

Y= B+ a X + SE

Water activity= 1.736 -0.036 Temperature +0.009

Water activity= 1.736 -0.036 (21.39) +0.009

Water activity= 0.97+0.009

Water activity= 0.97

Association for Bread with NSO Type

The associations between water activity and firmness of all types of bread with NSO, as well as between water activity and temperature, conducted by Pearson correlation, are depicted in Table 27. The findings indicated that there was no statistically significant relationship between water activity and firmness since ([P = 0.399 > 0.05], r = -0.176), while the relationship between water activity and temperature was a negative statistically significant relationship since ([P = 0.00 < 0.05], r = -0.685). Linear regression was used to find the percentage of temperature impact on water activity, illustrated in Tables 28 and 29. The correlation coefficient (R = 0.685) indicated that the independent variable (temperature) had a moderate impact on water activity for bread with NSO, and temperature accounts for 47% of WA (R^2 = 0.469).

Table 27*: Correlation Matrix between Water Activity, Firmness, and Temperature for Bread with Nigella sativa oil (NSO)*

		Firmness	WA	Temperature
Firmness	Pearson Correlation	1	-.176	.016
	Sig. (2-tailed)		.399	.939
	N	25	25	25
WA	Pearson Correlation	-.176	1	-.685*
	Sig. (2-tailed)	.399		.000
	N	25	25	25
Temperature	Pearson Correlation	.016	-.685*	1
	Sig. (2-tailed)	.939	.000	
	N	25	25	25

Values are reported as Pearson Correlation coefficients and analyzed bivariate correlation test. Abbreviations: *= statistically significant (P = < 0.05).

Table 28: *Linear Regression for Temperature Impact on Water Activity for Bread with Nigella sativa oil (NSO)*

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.685a	.469	.446	.01668

Abbreviation: R = total correlation coefficients

Table 29*: Linear Regression for Temperature Impact on Water Activity for Bread with Nigella sativa oil (NSO) Coefficients*

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	1.626	.145		11.212	.000	1.326	1.926
	Temperature	-.031	.007	-.685	-4.507	.000	-.045	-.017

Values are reported as linear equation coefficients and analyzed by regression test. Abbreviations: B = beta.

International Journal of Scientific & Engineering Research Volume 10, Issue 7, July-2019
ISSN 2229-5518

The linear equation model:

Y= B+ a X + SE

Water activity= 1.626 -0.031 Temperature +0.007

Water activity=1.626 -0.031 (21.40) +0.007

Water activity= 0.96+0.007

Water activity= 0.979

Association for Bread with NSP Type

The associations between water activity and firmness of all types of bread with NSP, as well as between water activity and temperature, conducted by Pearson correlation, are shown in Table 30. The results indicated that there was a moderate negative statistically significant relationship between water activity and firmness since ([P = 0.015 < 0.05], r = -0.482), while there was no statistically significant relationship between water activity and temperature since ([P = 0.846 > 0.05], r = -0.041).

Table 30*: Correlation Matrix between Water Activity, Firmness, and Temperature for Bread with Nigella sativa powder (NSP)*

		Firmness	WA	Temperature
Firmness	Pearson Correlation	1	-.482*	-.084
	Sig. (2-tailed)		.015	.689
	N	25	25	25
WA	Pearson Correlation	-.482*	1	-.041
	Sig. (2-tailed)	.015		.846
	N	25	25	25
Temperature	Pearson Correlation	-.084	-.041	1
	Sig. (2-tailed)	.689	.846	
	N	25	25	25

Values are reported as Pearson Correlation coefficients and analyzed bivariate correlation test. Abbreviations: *= statistically significant (P = < 0.05).

Physical and Microbial Activity

Day-by-day softness of all bread samples decreased and the firmness increased, while none of the bread samples showed any change of appearance, color, or smell during day 1 to day 10. Mold growth (two colonies) started on day 7 in control samples, compared to bread with NSP samples and bread with NSO samples, which did not show any occurrence of microbial growth (see Figure 13). During day 8, besides the two colonies of mold growth on control samples, one colony of mold growth appeared in bread with NSP sample, compared to bread with NSO samples, which did not appear to be infected with microbial growth (see Figure 14). During day 10, both control and bread with NSP samples appeared with two colonies of growth mold, compared to bread with NSO samples, which appeared with a tiny mold growth (see Figure 15).

Figure 13. Microbial growth comparison in day 7 between three types of bread: control sample, bread with NSP sample, and bread with NSO sample.

Control sample

Bread with NSP sample

Bread with NSO sample

Figure 14. Microbial growth comparison in day 8 between three types of bread: control sample, bread with NSP sample, and bread with NSO sample.

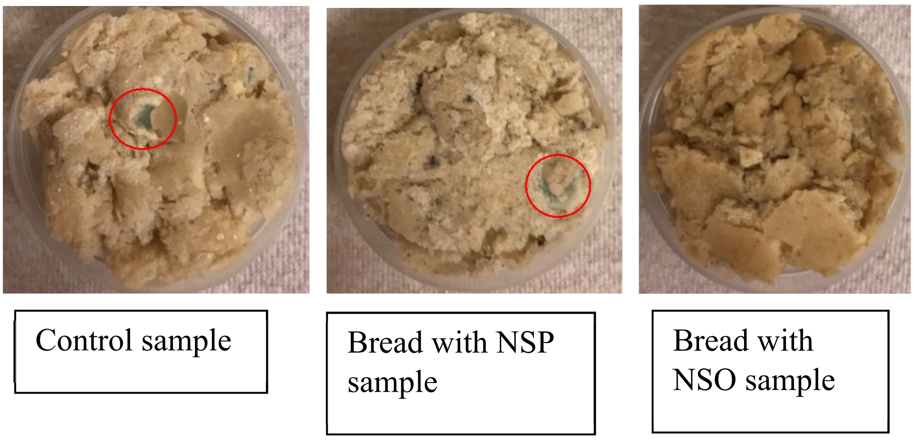
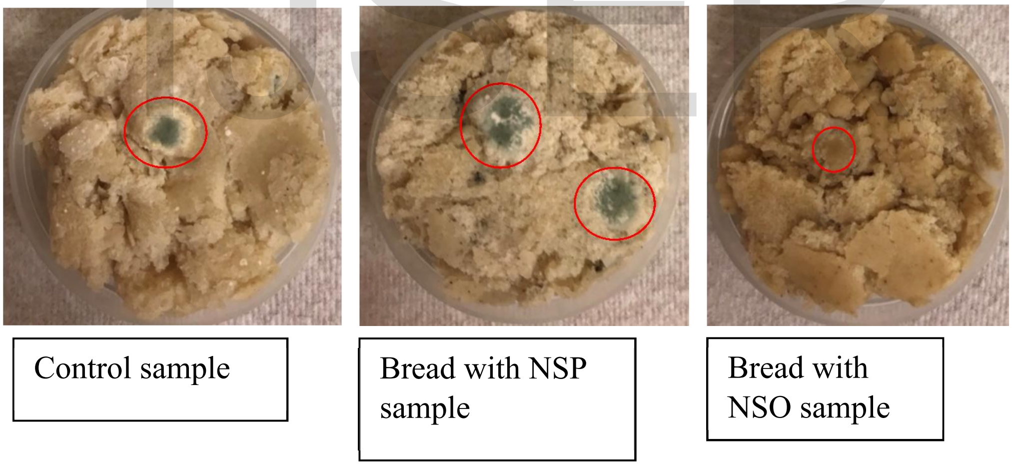


Figure 15. Microbial growth comparison in day 10 between three types of bread: control sample, bread with NSP sample, and bread with NSO sample.



Chapter V: Discussion

The aim of this study was to compare firmness in the three types of bread and to discover whether there is a correlation between firmness and water activity in bread. The study showed the mean differences in the firmness of the three types of bread (bread without NS [control], bread with NSP, and bread with NSO). The findings of the study showed that, although there were no statistically significant differences between all bread types, bread with NSO had the lowest firmness compared to bread with NSP and control bread. The finding indicates that NS, especially bread with NSO, increases the rate of bread freshness and extends the shelf life. Hassanien, et al., (2013) noted reduction in hardness of cheese crude that fortified with black cumin seeds oil. Ozpolat and Duman (2016) measured the influence of NSO on the shelf life of fish fillets during storage at 2 ± 1 °C. Their findings were that the NSO showed longer shelf life and higher sensory quality in fish fillets. The antimicrobial and antioxidants may have the essential roles of decreasing the bread hardness that can increase the shelf life of the bread.

The study compared firmness and water activity means for bread types according to days. The results of the study illustrated that the bread of hardness are significantly increased, and bread becomes harder by increasing days, while water activity significantly decreased by increasing days. Mohamad et al. (2015) provided a justification about the association between time and hardness. Increasing time or days are associated with increased the firmness of bakery products, which is also associated with a decrease in the shelf life of the bakery products. Rosen et al. (2018) conducted a study to investigate the influence of storage time on hardness and water activity in pastry biscuits. The change in hardness and water activity was followed over 14 days. The findings were that the hardness of pastry biscuits increased and water activity decreased with increasing the storage time.

The study investigated associations between water activity, firmness, and temperature for all types of bread. The results of the study indicated disagreement between water activity and temperature in the case of control bread and bread with NSO. Moreover, there were showed disagreement between water activity and firmness of bread with NSP. In the case of all types of bread overall, the results showed disagreement between water activity and firmness, as well as disagreement between water activity and temperature. Mohamad et al. (2015) studied the influence and correlation of different baking parameters on cake qualities during the baking process. The findings showed an association between the firmness of cake with a moisture content that was analyzed at different times and different oven temperatures. The results indicated that there is an association between firmness, moisture content, and temperature that plays an important role in impacting bread quality and shelf life.

The study also compared the physical and microbial activity of the three types of bread. with the major finding of the study is that the NSO samples appeared with a tiny mold growth at day 10, compared to NSP sample that appeared with mold growth at day 8 and control sample that started mold growth early at day7. These results indicated the essential role of NGS as antimicrobial activity, which help to extend the shelf life of bread. Hassanien, et al., (2013) studied the potential of black cumin seed oil as an antibacterial and antioxidant agent during manufacture and storage of cheese prepared with starter cultures including contaminating bacteria as well as artificially inoculated bacterial pathogens. Supplementation of cheese with black cumin seed oil showed antimicrobial impacts growth, and significantly reduced the counts of the inoculated pathogens after 21 days of storage. Ozpolat and Duman (2016) studied the impact of NSO on shelf life and sensory quality on fish fillets. The NSO showed longer shelf life and higher sensory quality in fish fillets. The results indicated that NSO has antimicrobial effects which can naturally increase the shelf life of bread.

Strengths and Limitations

The strength of this study is the study compared the ability of NSO and NSP to increase the shelf life of bread in compared to different studies that assessed the influence of NSO to increase the shelf life on other products such as meat and cheese. Since the bread is popular to consume, there are lack studies that assessed the impact of NS to increase the shelf life of bread. This study has limitations that need to be mentioned. NS is affected by heat, which may damage valuable compounds that have many benefits. The instruments used in the study have some limitations. Measuring moisture content is not a reliable indicator for predicting microbial responses and chemical reactions in foods. Moisture content determination limitation is attributed to differences in the intensity with which water associates with other components in foods. The texture analyzer method is a typical sensory parameter and is extremely hard to assess precisely due to its complexity, multi-factor characteristics, and uncertainty.

Conclusion

The research study provided insight into the effect of NS on texture and shelf life of bread. Although there was no statistically significant difference between all bread types, bread with NSO had the lowest firmness means compared to bread with NSP and control bread. Bread with NSO did not appear with mold growths until day 10, compared to control bread and NSP bread, which started with mold growths at day 7 and day 8, respectively. The means of all types of bread firmness increased and bread became harder by increasing days, while water activity decreased by increasing days. Moreover, all the types of bread showed differences between water activity and firmness, as well as differences between water activity and temperature. The bread in this research is not used for commercial purposes. Further research is needed to assess the acceptability of NS flavor and preservative effect in bread or other products consumed by people in the U.S. If the NS flavor is acceptable to the general American public, one visualizes considerable scope for enhancing the shelf life of bread with NSO in particular. The savings to both the economy and the consuming public would be considerable.

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