Effects of Spice and Herb Extracts on the Thermal and Storage Stability of Mustard Oil

Mohammad Rihan Arefin

Roll No.: 0121/02

Registration No: 999

Session: January-June 2021

A thesis submitted in the partial fulfilment of the requirements for the degree of Master of Science in Food Processing and Engineering

Department of Food Processing and Engineering

Faculty of Food Science & Technology

Chattogram Veterinary and Animal Sciences University

Chattogram-4225, Bangladesh

FEBRUARY 2023

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Mohammad Rihan Arefin

February 2023

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Supervisor	**Co-supervisor**
Dr. Afroza Sultana	**Dr. Shireen Akther**
Associate Professor	Associate Professor and Head
Department of Food Processing and Engineering	Department of Food Processing and Engineering

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Chairman of the Examination Committee

Dr. Shireen Akther

Department of Food Processing and Engineering

Department of Food Processing and Engineering

Faculty of Food Science and Technology

Chattogram Veterinary and Animal Sciences University

Khulshi, Chittagong-4225, Bangladesh

FEBRUARY 2023

PLAGIARISM VERIFICATION

Title of Thesis**:** ***Effects of Spice and Herb Extracts on the Thermal and Storage Stability of Mustard Oil***

Name of the Student: Mohammad Rihan Arefin

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Reg. no: 999

Department: Food Processing and Engineering

Faculty: Food Science and Technology

Supervisor: Dr. Afroza Sultana

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Dr. Afroza Sultana

Associate Professor

Department of Food Processing and Engineering

Faculty of Food Science and Technology

Acknowledgement

Firstly, I would like to express my deepest sense to the “Almighty Allah” whose blessings has enabled me to complete the research work and dissertation successfully in this field and to complete this thesis writing for the degree of **Master of Science (MS)** in **Department of Food Processing & Engineering**.

I would like to show my deepest sense of gratitude, sincere appreciation and profound regards to my respective supervisor, **Dr. Afroza Sultana**, **Associate Professor, Dept. of Food Processing & Engineering** for her scholastic guidance, With immense pleasure, I express my indebtedness to my co-supervisor **Dr. Shireen Akther**, **Associate Professor & Head**, **Dept. of Food Processing & Engineering,** CVASU for his keen inspiration and permission to conduct this study.

I also express my profound appreciation and heartfelt gratitude to **Professor Dr. A.S.M Lutful Ahasan,** Vice Chancellor, Chattogram Veterinary and Animal Sciences University (CVASU), for his tremendous support and **Professor Md. Ashraf Ali Biswas,** Dean, Faculty of Food Science and Technology, CVASU, for providing necessary scope and funding to carry out the work during the whole period.

It is my pleasure to express my sincere gratitude to **Mr. Moinuddin Tutul** (Managing Director, Banga Flavour & Fragrance Pvt. Ltd) for their support and co-operation in performing the research work in their laboratory.

Last but least I express my deepest sense of gratitude, and cordial respect of feelings to my friends & my beloved family members for their immense sacrifice, blessings, and encouragement.

The Author

February 2023

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List of Abbreviation

AOAC	Association of Official Analytical Chemists
CAC	Codex Alimentarius Commission
BHT	Butylated hydroxytoluene
BHA	Butylated hydroxyanisole
TBHQ	tert-Butylhydroquinone
PV	Peroxide Value
AV	Acid Value
CEA Oil	Clove Extract Added Oil
EAA oil	Embanox-2 Amtioxidant Added oil
AEA oil	Asafoetida Extract Added Oil
PPM	Parts Per Million
ANOVA	One way analysis of variance
AOF	Australian Oilseeds Federation

Abstract

The study assesed the impact of oil enrichment with natural antioxidants, a promising method for extending shelf life of edible oil compared to synthetic antioxidants. Clove and asafoetida antioxidants can balance mustard oils and prevent oxidation. Commercial synthetic antioxidants are cheap and effective, so the food industry utilizes them. Embanox-2, a commercial synthetic antioxidant that has displayed similar effectiveness as it was made for that purpose. However, synthetic materials may pose health risks. Thus, this study compared natural and synthetic antioxidants effects in protecting mustard oils from oxidative degradation. Solvent extraction was employed followed by evaporation to obtain bioactive enriched extract from clove and asafetida. The extract were added to enrich the mustard oil and then physiochemical and biochemical test was conducted according to established standard. ANOVA and Tukey's test (applied at a 5% level of significance) were utilized to compare all data obtained. Results showed that physical criteria like specific gravity (0.90 - 0.92) and moisture percentage (≤0.2%) was well under control and the process was also very much successful in diminishing unsaturation by controlling the iodine value from 25.5 g/100g in pure oil to 24.74, 23.8, 23.62 g/100g in enriched oils. Natural antioxidants also impacted mustard oil's thermal stability by controlling acid and peroxide values to safe levels, when compared to pure mustard oil (4.48 mg KOH/g and 6.2 mEq/kg) by both clove extract (3.48 mg KOH/g and 5 mEq/kg), asafoetida extract (2.47 mg KOH/g and 3.2 mEq/kg) & also embanox-2 (2.02 mg KOH/g and 2.8 mEq/kg) enriched oils even during the highest heating time (60 min). Then again, in case of storage stability similar positive outcome was also replicated in treated mustard oil samples throughout the 28 days period. Moreover, the outcome was also positive in case of TPC and TAA, where clove extract added oil (119.125 mg GAE/100 ml and 22.194 mg TE/100 g) and embanox-2 added oil (54.391 mg GAE/100 ml and 28.256 mg TE/100 g) showed better increment with asafoetida extract added oil also demonstrating almost similar value. These results indicate that natural antioxidants can works as replacement to synthetic antioxidants to enrich mustard oil.

Keywords: Natural Antioxidant, Spice & herbs, Embanox-2, Oxidative stability Antioxidant activity, Phenolic compound.

Chapter 01

Introduction

Fats and oils are regarded as significant nutrients in human diets since they serve as a major source of energy. Edible oils have a significant physiological impact because they transport essential fatty acids, which the body cannot generate by itself but must acquire from food in order to keep cell membranes functioning properly. Edible oils are used in cooking as well as in conventional medicine to treat colds, coughs, bronchitis, edema, and burns. Additionally, they are necessary for the production of prostaglandins, which serve a variety of important roles in the body (Ilyas, 2016). In addition to that, it also affects the observable traits of different processed food varieties. The human body uses the fats and oils in the diet for three purposes: as a source of energy, as a building block, and to create potent biological regulators. Oils and fats are crucial for metabolic processes in the human body. Fatty acids, which are found in oils and fats, are vulnerable to attack from a variety of sources, including light and oxygen (Choe and Min, 2007).

Vegetable oils are important in human nutrition as they provide energy, essential fatty acids and facilitate the absorption of fat-soluble vitamins (Grace et al., 2008). These are beneficial and popular due to their cholesterol lowering effect. In contrast to animal fats, which are predominantly saturated and hence do not react readily with other chemicals, especially oxygen, unsaturated vegetable oils are more reactive. Vegetable oils are essential in global nutrition depending on the regional conditions, a variety of oils are produced in different qualities (Tesfaye and Abebaw, 2016). According to a study that was conducted by Jambunathan et al. in 2008, the value of the oil and its ability to maintain its consistency are extremely important for the consumer's preferred use, which is typically as an ingredient in culinary preparation (Bou et al., 2012). Some physicochemical characteristics of oil can be used to determine both its nutritional and physical qualities. These characteristics include iodine value, peroxide value, saponification value, free fatty acid, color appearance, etc. Vegetable oils should be handled carefully when storing them for an extended period of time to preserve oil quality because they are susceptible to oxidative

deterioration, which reduces their shelf life. The hydro peroxides, which are the main oxidation products produced when oxygen reacts with unsaturated fatty acids, are measured by the peroxide value (Tarmizi and Ismail, 2008). Oils in a low oxidation state have peroxide values between 1 and 5 mEq/Kg, whereas those in an average oxidation state have values between 5 and 10 mEq/Kg (O'brien, 2008). The peroxide value is used to determine the level of oil damage. The peroxide value standard for vegetable oils that do not undergo rancidity should be well below 10 mEq/kg. The acid value is often used as an indication of the general condition and the nature of the oil is safe to eat (Pardeshi, 2020).

Mustard oil is one of the most important vegetable oils employed for deep-frying. Mustard oil can be produced using black mustard (Brassica nigra), brown Indian mustard (Brassica juncea), and white mustard (Brassica hirta). Due to presence of ally isothiocyanate it is pungent in nature (Nayak et al., 2016). Mustard oil, extracted from the Brassica nigra plant, is rich in protein (30%), calcium (5%), phytins (5%), phenolics (5%), and natural antioxidants (5%). Due to its high monounsaturated fatty acid content and healthy polyunsaturated fatty acid ratio, mustard oil is beneficial to cardiovascular health. Mustard oil is suitable for those with heart conditions because it contains the fewest saturated fatty acids. Additionally, mustard oil includes glucosinolate, which has antibacterial, antifungal, and anticarcinogenic effects. These qualities are responsible for many of the oil's medical benefits (Yadav and Kumari, 2013). Since there is such a high demand for mustard oil in the Bangladeshi market, it is also very likely to be contaminated. The oil's dark color makes it more susceptible to tampering with any low-quality oil (Khan et al., 2013). The term "rancidity" refers to unappealing flavors and odors in food that is the result of the fat or oil component of the food degrading. There are three possible mechanisms for rancidity. Natural sources of lipids and triacylglycerol are oils and fats. Saturated and unsaturated fatty acids and glycerides are included in their chemical makeup (Pardeshi, 2020).

Mustard oil, which contains erucic acid, is banned in the United States. It appears that erucic acid, when taken in larger quantities, can be poisonous to the heart. While studies on humans have not found any harmful effects from being exposed to erucic acid, studies on animals have allowed researchers to establish tolerance levels for human exposure. Mustard oil has a moderate level of oxidative stability. (Nayak et al., 2016).

The two main factors that determine how long raw or processed foods will last in the storage condition are oxidation and microbial contamination (Yadav et al., 2014). The development of off flavor is caused by oxidation processes, which primarily take place in the unsaturated portion of lipid. Therefore, lipid oxidation lowers the acceptability of food goods among consumers (Falowo et al., 2014). In cooking oils, lipid oxidation is a major degradation process. It has an impact on the organoleptic qualities as well as the shelf life of oil-based foods. Several methods have been implemented to deal with the oxidation chemistry, altering the mechanism, changing the factor that determines the stability of fat & oil by incorporating chemical, antioxidant & by changing the physical state etc. (Chandran et al,. 2017).

To stop lipid oxidation, several antioxidants of synthetic origin are added to food products. The most often used antioxidants are BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), PG (propyl gallate), etc. However, using synthetic antioxidants can have some negative health implications, such as endocrine malfunction; affect organ function, and reproductive disorders. Also, due to their low thermal stability, concern about their long term effects and the increasing demand by consumers for natural products, plant extracts emerged as good alternatives to synthetic antioxidants (Taghvaei and Jafari, 2015). Food processing industry now prefers natural antioxidants, free of any synthetic chemicals, and they provide similar protection on lipid oxidation in different food products (Hygreeva et al., (2014).

Numerous natural antioxidants from various sources have been found and studied for their antioxidative potential in various dietary products. Since ancient times, food has been enriched or fortified and then enhanced with the help of herbs and spices (Shahidi, 2015). Herbs are made from the leaves of a plant whose stem above-ground doesn't turn woody. Generally speaking, a plant's leaf is considered a food herb when it's used in cooking, but any other component of the plant, frequently dried, can be a spice. The term "spice" refers to a variety of plant elements, such as flowers (clove), bulbs (garlic, onion), fruits (cumin, red chilli, black pepper), stems (coriander), bark (cinnamon), roots (ginger), berries (peppercorns), aromatic seeds (cumin), and other parts of the plant (Brewer, 2011).

Depending on their flavor or taste and the portion of the plant they came from, several groupings of spices and herbs can be identified. Essential oils, resins, whole,

oleoresins and aqueous or ethanolic extracts in food are some of the numerous ways that spices and herbs are employed as antioxidants (Peter, 2001).

Considering these facts, a study was done to generate enhanced mustard oil by integrating extracts from spices and herbs then comparing its effectiveness to both pure standard oil (without incorporation of extract) & synthetic antioxidant rich oil in terms of oxidative stability, thermal stability and shelf life.

Aim and objectives

1. To investigate the antioxidant activity of the spice and herb extracts when used in mustard oil.
2. To evaluate and analyze changes in enriched mustard oil's physicochemical properties.
3. To assess the storage stability of enriched mustard oil by monitoring changes in its peroxide value, acidity, and oxidative stability over a period of time.
4. To demonstrate the impact of spice and herb extracts to synthetic antioxidant in enhancing thermal stability of enriched mustard oil under high temperature conditions.

Chapter 02

Review of Literature

2.1 Oils and Fats

Oils and fats of the highest quality are tasteless, odorless, pure, and resistant to oxidation. However, oxidation can happen to oils and fats during preparation, storage, and usage. Some processing steps, like caustic refining and bleaching, may promote oxidation, despite the fact that oil processing is partly intended to remove or destroy oxidized products or factors that may initiate or enhance oxidative reactions. During storage, oxidative reactions can decompose and form compounds responsible for a rancid odor and taste (Chow and Gupta, 1994). There have been many works regarding the prohibition of oxidation in fat & oil. Several approaches have been attempted to increase the shelf life of edible oils, such as modifying their genetic makeup, altering their chemical make-up, and adding synthetic antioxidants (Chandran et al,. 2017). Mustard oil are oil of plant source that contains moderate amount of polyunsaturated (21%), monounsaturated (60%) fatty acid and lower in saturated fat but has a lower amount of antioxidant content that makes both oil prone to oxidative breakdown under optimum condition (Saldaña and Martínez-Monteagudo, 2013). Spices & herbs have higher amount & many varieties of antioxidants present in its constituents. Utilizing the antioxidant extract from these, then utilizing them in mentioned oil can bore many positive changes.

2.2 Synthetic antioxidants

There are many antioxidants of synthetic origin such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) or tertbutylhydroquinone (TBHQ) and various commercial antioxidants are often used to retard lipid oxidation in food systems. It's been proven that these antioxidants cause significant changes to the oxidative stability of oil. There are many other commercial antioxidant available of different name made with combination of various synthetic antioxidant. Embanox-2 is also a commercial synthetic antioxidant which is known for frequent use in various application in the food industry. It is a synergistic mixture of the antioxidants BHA

(Butylated Hydroxy Anisole) and BHT (Butylated Hydroxy Toluene); where vegetable oil is added as a carrier.

Table 2.1: Analytic information's of Embanox-2 (Source: RTC Chemtronics)

Application	Food	Vegetable oil Animal fats Fried Products Baked products
Physical & Chemical Properties	Appearance	Oily liquid
	Odour	Phenolic (Slightly)
	Taste	Not available
	Color	Pale yellowish to golden.
	Boiling Point	264°C (507.2°F)
	Critical Temperature	Not available
	Specific Gravity	0.94 (25 °C)
	Refractive Index	1.47 (25 °C)
Solubility	Water	Insoluble
	Vegetable oils	Unlimited
Doses	20 – 2000 ppm	
Handling Procedure	Normal handling Avoid contact with skin and eyes	
Storage Condition	Normal temperature conditions Not below 0°C - not above 30°C (Optimum 15°C).	

However, using synthetic antioxidants can have some negative health implications, such as endocrine malfunction; affect organ function, and reproductive disorders. The food processing industry now prefers natural antioxidants, free of any synthetic chemicals, and they provide similar protection on lipid oxidation in different food products (Hygreeva et al., (2014).

2.3 Natural antioxidant

Their use in numerous items due to their antioxidant potential has been topic of many studies over the years. Special attention has been focused on the extraction of natural food additives with antioxidant activity from inexpensive or residual sources from the agricultural and food-processing industries. Recently, consumer health consciousness has led to a demand for “natural” alternatives to synthetic food antioxidants. Natural plant based antioxidants, such as phenolics, have received much attention for their antioxidant characteristics.

Over the recent two decades, many studies have looked into ways to improve the healthfulness of various edible oils by adding natural plant extracts with antioxidant activities. The antioxidant and antimicrobial properties of phenolic compounds found in plants have made them a popular topic of study in recent years (Bubonja-Sonje et al., 2011). Various natural product extraction processes may yield a variety of extract compositions. Different extraction techniques can result in significantly different extract product compositions (Pourmortazavi et al., 2004). Therefore, the biochemical properties of extract products obtained from same plant species using different methods may vary. It is stated that garlic prevents thermal degradation of sunflower oil by enhancing the oil's hydrolytic stability, lowering the rate at which double bonds in the oil are conjugated, and decreasing the amount of polyunsaturated fatty acid lost during the process (Iqbal and Bhanger, 2007). It is also further confirmed that the addition of thyme and lavender to sunflower seed oil improved the quality parameters of the oil after it was exposed to frying temperatures (Bensmira et al., 2007). Moreover, adding other Mediterranean aromatic plants like rosemary and sage to olive oil enhanced the oil's thermal resistance and stability. (Ayadi et al., 2011). Studies like these were conducted with the primary objective of decreasing the use of synthetic compounds as antioxidants, which have been linked to a variety of health problems and are in high demand from consumers despite these risks.

2.4 Edible oil and natural extract

It has been found that the utilization of olive plant antioxidants is very effective in the process of stabilizing a variety of edible oils. Olive leaf extract added to edible vegetable oils may boost the oils' radical scavenging activity and oxidative stability. (Salta et al., 2007). Higher concentrations of olive extract led to an improvement in the quality of the sunflower oil. In a study it was concluded that a concentration of

olive extracts of 200 ppm or higher is more impactful than BHT in accelerating both the protection factor and the oxidative stability at ambient temperature and shelf-life. (Abd-Elghany et al., 2010)

Microencapsulated high oleic sunflower oil was shown to benefit from the antioxidant properties of extracts from four different natural plants (rosemary, broccoli sprout, and citrus) (Ahn et al., 2008) Microencapsulated high oleic sunflower oil was found to have significantly reduced lipid oxidation when treated with these natural plant extracts. They found that the lipid oxidation of microencapsulated high oleic sunflower oil was significantly reduced under the accelerated storage conditions when they added a combination of the aforementioned natural extracts rather than a single extract. The antioxidant effects of hazelnut and poppy oils were also studied, and compared to those of butylated hydroxyanisole (BHA) and essential oils of rosemary (Rosmarinus officinalis), clove (Syzygium aromaticum), and cinnamon (Cinnamomum zeylanicum) (Ozcan and Derya, 2011). The oils were infused with essential oils at concentrations of 0.25 and 0.5 percent, with BHA added at a 0.02 percent level for comparison. There were 14 days of storage at 50 degrees Celsius in the dark, with periodic checks on peroxide levels. Compared to the control group, essential oils' antioxidant effect was greater, but BHA was still superior. For preventing lipid oxidation, cinnamon oil, followed by clove and rosemary oils, was the most effective antioxidant of the essential oils studied.

Sesamin and sesamolin combine to form sesamol, a unique and potent antioxidant found in sesame oil. According to research, the unsaponifiable matter found in sesame oil can be used as a substitute for synthetic antioxidants in sunflower oil (Mohamed and Awatif, 1997). After 30 minutes of roasting at 180 °C, sesame seeds were mixed with sunflower oil at varying concentrations of saponification and unsaponifiable matter (0.02%, 0.05%, and 0.1%) to see how they performed compared to a reference (no additives) sample heated to 63 °C. These findings demonstrated that the concentration of natural antioxidants significantly increased the effectiveness of the antioxidant activity. Due to the high concentration of effective antioxidant compounds in roasted sesame oil, these experts recommend using it in food applications as a natural antioxidant. Researchers in a separate study determined whether or not the antioxidant activity of a sesame methanol extract improved the stability of soybean, sunflower, and safflower oils (Suja et al., 2004). Vegetable oils treated with sesame extract at 5, 10, 50, and 100 ppm had their diene value, p-anisidine value, and

peroxide value significantly ($P<0.05$) reduced. They discovered that 200 ppm of sesame extract had a greater antioxidant effect than BHT.

These studies demonstrate that there are a great number of herbal plants that can be utilized for the utilization of natural antioxidants; however, there are two significant factors that should be taken into consideration: a) A natural antioxidant source which is only cultivated in one region of the world is not as desirable as one that can be found everywhere. b) It is important to think about the other applications of these medicinal plants; it may not be the most cost-effective strategy to grow a plant solely for the purpose of harvesting its antioxidants. When trying to extract natural antioxidants, it makes more sense to make use of natural resources that are widely available, well-established, and well-known. (Taghvaei and Jafari, 2013).

2.5 Herb and spices

It was common practice, especially in ancient times, to season cooking oils with a variety of spices and herbs to improve their flavor and aroma. They were able to make products that lasted longer on store shelves and were more well-liked by consumers thanks to an enticing flavor profile of spices and herbs. Spices contain natural antioxidants that can assist in reducing the effects of oxidative stress. Exposure to gamma, ultraviolet, or X-ray radiation; psychological or emotional stress; polluted food; unfavorable environmental conditions; strenuous physical activity; smoking; alcoholism; or drug abuse can all cause high levels of free radicals in cells and tissues. Oxidative stress has been linked to a wide variety of illnesses, including the aging process, cancer, and cardiovascular disease, to name just a few of these conditions. Malondialdehyde and 4-hydroxynonenal are just two of the many byproducts that can react with other biological molecules such as proteins and nucleic acids that are produced when lipids are oxidized. There is a correlation between malondialdehyde and the health problems of mutagenesis and carcinogenesis. It has been demonstrated that malondialdehyde can be formed both enzymatically and non-enzymatically. Herbs and spices are excellent sources of antioxidants due to their high vitamin content. As a consequence of this, spices and herbs have the prospect to be utilized as therapeutic or preventative agents for a wide variety of health problems (Yashin et al., 2017).

Herbs and spices are regarded as the main flavoring agents in a wide variety of cuisines. In addition to giving food its distinctive flavor, spices are a massive origin of

antioxidant phytochemicals (Turek and Stintzing, 2013). Various essential oils or spice & herb extract has been experimented on numerous occasions by adding it to vegetable oils to have the dual effects of adding flavor and acting as a natural antioxidant to increase shelf life & as part of natural treatment it bore positive results most of the times (Sadeghi et al., 2016). The antioxidant activity of herbs and spices is most often due to phenolic acids (gallic, protocatechuic, caffeic, and rosmarinic acids) phenolic diterpenes (carnosol, carnosic acid, rosmanol, and rosmadial), flavonoids (quercetin, catechin, naringenin, kaempferol, epicatechin, gallate, epigallocatechin gallate and rutin), volatile oils (eugenol, carvacrol, thymol, menthol, safrole, 1,8-cineole, α-terpineol, p-cymene, cinnamaldehyde, myristicin and piperine) and phenylpropanoids (thymol, eugenol, carvacrol, p-cymene) (Kapadiya et al., 2016).

2.6 Asafoetida

Asafoetida (*Ferula asafoetida*) which is mainly an oleo-gum-resin that is collected from the roots of F. asafoetida, a medicinal plant native to Iran. All over the world, people use it to add flavor to different foods. Historically, it has been used to treat a wide range of conditions, including asthma, epilepsy, gastrointestinal distress, flatulence, intestinal parasites, poor digestion, and the flu. Asafoetida contains antioxidant, antiviral, antifungal, cancer-preventive, antidiabetic and hypotensive activities, according to recent pharmacological and biological investigations. Asafoetida's significance in antioxidative potential has been a great interest for treatment of dietary products for quite a long time. (Mahendra and Bisht, 2012).

2.7 Clove

It is the dried, aromatic, and unopened floral buds of the clove tree, also known as *Syzygium aromaticum*. Due to its intense flavor, medicinal properties, and antioxidant activity, clove has become a popular flavoring agent in commercial and home kitchens (Kamatou et al., 2012). Additionally, they can be found in betel chew and chewing tobacco, and they lend a pleasant aroma to perfumes. Cloves have the potential to be used in a wide variety of medical settings due to their properties as an anti-inflammatory, an antioxidant, and an antifungal agent. Consumption of synthetic antioxidants is associated with a lower risk of cancer and cardiovascular disease. This association is reciprocal, as it results in an increase in the antioxidant content of fruits

and vegetables. Clove has been shown to effectively neutralize cancer-causing free radicals thanks to the antioxidant properties it possesses. According to the findings of some studies, the antioxidant capacity of various types of clove products varies. Eugenol, the primary component of clove, is responsible for many of the antioxidant properties that the oil possesses (Ogata et al., 2000). The major bioactive compounds besides eugenol is, eugenyl acetate and β-caryophyllene; their different quantitative contribution justifies the unequal aroma and antioxidant properties of clove products (Chow and Gupta, 1994). It is possible that the antioxidant activity is caused by a variety of different mechanisms, such as the scavenging of radicals and the chelation of metal ions. There is evidence in the scientific literature that eugenol participates in photochemical reactions and possesses powerful antioxidant activity (Mihara and Shibamoto, 1982). Thus, cloves become an excellent choice in enhancing both flavor and antioxidant profile of any food materials it is added.

Chapter 03

Materials and Methods

3.1 Purchase of Raw Material

Samples of Mustard oil was collected from local super shops & raw and fresh spices including Cloves (Syzygium aromaticum) and Asafoetida (Ferula asafoetida) were actually bought from a local street market in Chattogram, Bangladesh. Synthetic Antioxidant Embanox-2 was collected from Banga Flavour & Fragrance (Pvt.) Ltd. Ethanol (96%), sodium carbonate, sodium hydroxide, aluminium chloride and Folin-Ciocalteu phenol reagent were bought from Merck (Darmstadt, Germany). The given reagents were acquired from Sigma Aldrich (St Louis, USA): sodium nitrite, and DPPH (2, 2-diphenyl-1-picrylhydrazyl). All the reagents were of the highest purity and analytical grade.

3.2 Extraction from Spice & Herbs

Both the clove and the asafoetida were dried for overnight in an oven set to 55°C. Samples were turned into a powder using an electronic grinder, then put through mesh (40). Solvent extraction was carried out by modifying the method used by Al-Juhaimi and Ghafoor (2011), where diethyl ether was used as the solvent. Both powdered samples (10 g) were extracted in 50 mL of diethyl ether over a 30-minute period with agitation at room temperature (25°C). Following the extraction, the mixture was filtered using Whatman filter paper # 1. The residues were once more extracted and filtered in 50 mL of diethyl ether. A 100 mL extract was created by mixing the filtrates. Then the extracts were taken in round flask to evaporate the solvent in a rotary evaporator at 35°C. Then, until it was required for further investigation, it was kept in a freezer.

3.3 Addition of Extracts to Oil

The extract of asafoetida, clove and solution of embanox-2 was added to mustard oil in a flask. The dosing for addition was 500 ppm. Serial diluted the extracts with some of the cooking oil before adding to the bulk oil. The ingredients were blended or stirred thoroughly in a water bath that was shook vigorously to ensure thorough

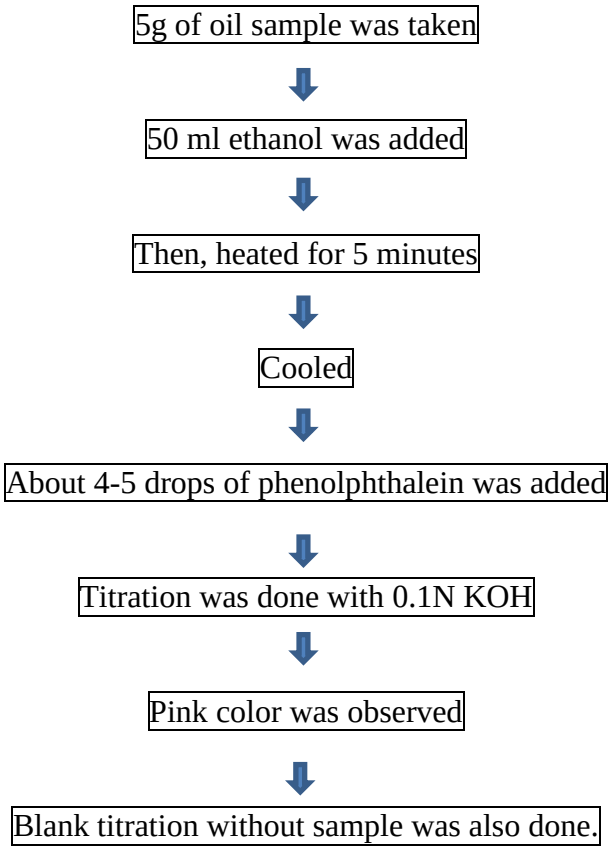
mixing. Following collection, the blended oils were placed in glass bottles and subjected to test of oxidative and thermal stability.

3.4 Determination of Physicochemical Properties of Oils

3.4.1 Acid Value (AV)

The Acid Value is the number of milligrams of potassium hydroxide (KOH) necessary to neutralize the fatty acids in 1 gram of sample. The acid value of oil is determined by titrating a solution of the oil in diethyl ether with an alcoholic solution of sodium or potassium hydroxide (AOCS Cd 3d-63). It is expressed as the amount of KOH (in mg) to neutralize 1 g of oil.

Procedure:



Calculation:

Acid Value = (56.1*(sample-Blank)*Normality)/ (Weight of sample)

3.4.2 Peroxide Value (PV)

It is defined as the amount of peroxide oxygen per 1 kilogram of fat or oil. It gives a measure of the extent to which an oil sample has undergone primary oxidation. The method of determining PV involves a titration of the oil containing potassium iodide in a chloroform-acetic acid mixture. The hydroperoxides oxidise the iodide to iodine, which is determined by titration with sodium thiosulfate. (AOCS Cd 8b–90).

Procedure:

5g of oil sample taken in a stoppered conical flask.

⬇

Addition of 10 ml chloroform was done.

⬇

Addition of 15 ml acetic acid was followed.

⬇

1ml 15% KI was added

⬇

Then, placed in a dark place for 5-10 minutes and then 30 ml distill water was added

⬇

About 1-2 drops slightly boiled starch indicator was added

⬇

Titration with 0.1N $Na_2S_2O_3$ was done

⬇

As a result, dark blue color will disappear

⬇

Blank titration was also done

Standardization of $Na_2S_2O_3$:

10ml 0.01N $K_2Cr_2O_7$ was taken in a stoppered conical flask

⬇

1 ml conc. HCl was added

⬇

Also added 1 ml 15% KI solution

⬇

Then, kept in a dark place for 10 minutes

⬇

Added 20 ml distill water

⬇

Addition of 1-2 drops of starch indicator was done

Titration with 0.1N $Na_2S_2O_3$ was followed.

Calculation:

Peroxide Value = ((Sample-Blank)*N*100)/W

3.4.3 Iodine value (IV)

Iodine value or Iodine number as it is commonly known as is the amount of Iodine that can react with the fat of a common mass (100 grams). This denotes the degree of unsaturation of fats. The protocol for iodine value determination usually comprises a titration procedure (AOCS Cd 1b-87).

Procedure:

First 1-2 ml of sample was collected in a stoppered conical flask

⬇

Addition of 10 ml chloroform was done

⬇

25 ml Henus solution was added

⬇

Then, kept in a dark place for 30 minutes

⬇

After that 10 ml 15% KI was added

⬇

Addition of 100 ml distill water was followed

⬇

Added 1-2 drops of starch indicator

⬇

Titration with 0.1 N $Na_2S_2O_3$ was done.

Preparation of Hanus Solution:

13.2 gm pure resublime iodine was taken in a 500 ml volumetric flask

⬇

Added 100 ml acetic acid

⬇

Addition of 1.5 ml pure Br (sulfur free) was done

⬇

Lastly, Acetic acid was added up to 500 ml

Calculation:

Iodine Value = ((B-S)*Normality*12.69) / (Weight of sample taken).

3.4.4 Specific Gravity

Specific Gravity or relative gravity is a dimensionless quantity that is defined as the ratio of the density of a substance to the density of the water at a specified temperature.

Standardization of Pycnometer:

The pycnometer was filled with a cleaning solution containing chromic acid and was left to sit for a number of hours. Filled the pycnometer with water that has been recently boiled and cooled to about 20 °C, and then it was set in a water bath maintained at a constant 30 °C. After letting the object soak for 30 minutes, took it out, dried it off with a towel, and weighed it.

Procedure:

Removed the cap from the side arm of the pycnometer before using it on the prepared oil sample and filled the instrument without trapping any air bubbles. Placed the sealed container in a water bath preheated to 30 °C and let it sit for 30 minutes. Cleaned the capillary opening thoroughly to remove any oil that may have leaked out. Brought the bottle out of the water, rinsed it out, and dried it completely. When the temperature falls below 30 °C, quickly removed the cap from the side arm and weighed it quickly.

Specific Gravity, C = $\frac{A-B}{C-B}$

Where,

A = weight of SG bottle in g, filled with oil at temperature of 30°C.

B = weight of SG bottle in g, at temperature of 30°C.

C = weight of SG bottle in g, filled with water at temperature of 30°C.

3.4.5 Moisture Content

Moisture content (or water content) refers to the weight of the water contained in a certain object or material. It is usually expressed as a percentage of weight.

Procedure:

Weighed 5–10g of oil or fat that had been well blended by stirring into a dish that had already been dried and tared. Then, the lid of the dish was loosen and heat was applied, in an oven at 105°C for 1 hour. Removed the dish from the oven and the lid was closed. Cooled it in a desiccator containing phosphorus pentoxide or equivalent dessicant and then weighed. Heated in the oven for a further period of 1 hour, cooled and weighed. We repeated this process until change in weight between two successive observations does not exceed 1 mg.

Carry out the determination in duplicate

Moisture and volatile matter = $\frac{W1 \times 100}{w}$

Where,

W_1 = Loss in gm of the material on drying

W = Weight in gm of the material taken for test

3.5 Determination of Thermal Stability of enriched Oil

Every 30 minutes, 10 g of both pure and treated oil samples were taken to test the oil's stability at 170°C for one hour (Perera et al., 2020). The collected samples were then exposed to tests to measure the peroxide value (PV) and acid value (AV) level

3.6 Determination of Oxidative Stability of enriched Oil

To conduct an experiment investigating the possibility of accelerated aging & its effect on oxidation, a total of 16 empty glass bottles were filled with pure and

enriched oil samples & stored at room temperature. Sampling was done every 7 days for four weeks (28 days) (Perera et al., 2020). Then, the peroxide value and the acid value were evaluated.

3.7 Determination of Biochemical Properties of Oils

3.7.1 Total Phenolic Content of Oil

TPC levels in enriched oil were determined using a modified version of the technique described by Singleton et al. (1999). A total of 1 mL of each sample was mixed with 1.5 mL of Folin-phenol Ciocalteu's reagent (10% v/v) and left to react at room temperature for 3 minutes. Following this, sodium carbonate (75 g/L) was added to the reaction mixture at a concentration of 1.5 mL and left for 60 min at room temperature (25 ºC) in the dark and the absorbance of the resulting blue complex was measured at 760 nm in a UV-Visible spectrophotometer. Absorbance versus concentration plots were used to generate a standard calibration curve, which was found to be linear over the tested concentration range.

3.7.2 DPPH Radical Scavenging Activity (Antioxidant Assay)

In order to determine whether or not a compound can scavenge free radicals or donate hydrogen, as well as to measure the antioxidant activity of plant extracts, the DPPH assay is commonly used. Each treated oil sample's DPPH free radical scavenging capacity was assessed using the technique described by Leong and Shui, (2002) with slight changes. First, 2 mL of methanolic DPPH solution were mixed with 1 ml of oil sample and the mixture was left in the dark for half an hour. Utilizing a UV-visible spectrophotometer, an absorbance reading was taken at 517 nm.

3.8 Statistical Analysis

Statistical analyses were carried out using Minitab statistical package (version 21). The outcome of the experiment are displayed as mean ± SD of measurements. One way analysis of variance (ANOVA) was done and their statistical significance ($p<0.05$) was carried out by Tukey’s pairwise comparison analysis.

Chapter 04

Result

4.1 Physicochemical Properties of Oil

It is through analysis of oils' physicochemical properties that their authenticity, quality, and purity can be established. The physicochemical properties of pure and improved mustard oil were measured, and the results are tabulated in Table 4.1. It shows in Table 4.1 that after a week of storage, the PV and AV of pure mustard oil were 1.8 mg KOH/g oil & 2.01 mEq/kg, respectively, and values for the same criteria in enriched mustard oil, which included clove extract added oil, embanox-2 antioxidant added oil, and asafoetida extract added oil were 1.4, 0.8, 1 mg KOH/g oil & 1.67, 1.44, 1.89 mEq/kg respectively. Analysis of the data showed that the p-values for AV and PV were found to be lower than 0.05 ($p < 0.05$). This meant that the AV and PV of enriched mustard oil samples were significantly different from those of pure mustard oil samples.

Table 4.1: Physicochemical properties of all oil samples after a week of storage.

Parameter	Pure oil	CEA oil	EAA oil	AEA oil
Iodine Value	25.51 ± 0.02^{a}	24.74 ± 0.01^{b}	23.8 ± 0.02^{c}	23.62 ± 0.02^{d}
Peroxide Value	1.8 ± 0.15^{a}	1.4 ± 0.15^{b}	0.8 ± 0.11^{c}	1 ± 0.11^{c}
Acid Value	2.01 ± 0.017^{a}	1.67 ± 0.02^{c}	1.44 ± 0.01^{d}	1.89 ± 0.02^{b}
Specific Gravity	0.90 ± 0.01^{a}	$0.91\pm0.01^{a,b}$	$0.92\pm0.01^{a,b}$	0.91 ± 0.01^{b}
Moisture (%)	0.17 ± 0.01^{a}	0.2 ± 0.01^{a}	0.13 ± 0.01^{b}	0.19 ± 0.01^{a}

Results are presented as mean ± SD. *Different superscripted letters (a-c) in each row shows statistically significant differences ($p<0.05$) for all the samples.

Where, **CEA oil** = Clove extract added oil

EAA oil = Embanox-2 antioxidant added oil

AEA oil = Asafoetida extract added oil

Unsaturated fatty acid content is typically measured in iodine numbers when analyzing oils for their nutritional value. Table 4.1 shows the iodine value of both pure mustard oils and those that have been enhanced with clove (CEA oil), embanox-2 (EAA oil) & asafoetida (AEA oil) were 25.51, 24.74, 23.8 and 23.62 g/100g respectively. Figure 4.1 represents the decrease of iodine value over the course of 28 days, where we can see that all the samples had almost similar fall in value. But the oil with lowest value (20.17) was embanox-2 antioxidant added oil. Since the *p* value of all the samples in both scenario was less than 0.05 ($p < 0.05$) and so it turns out that the iodine value of pure and enriched oil samples has significant difference.

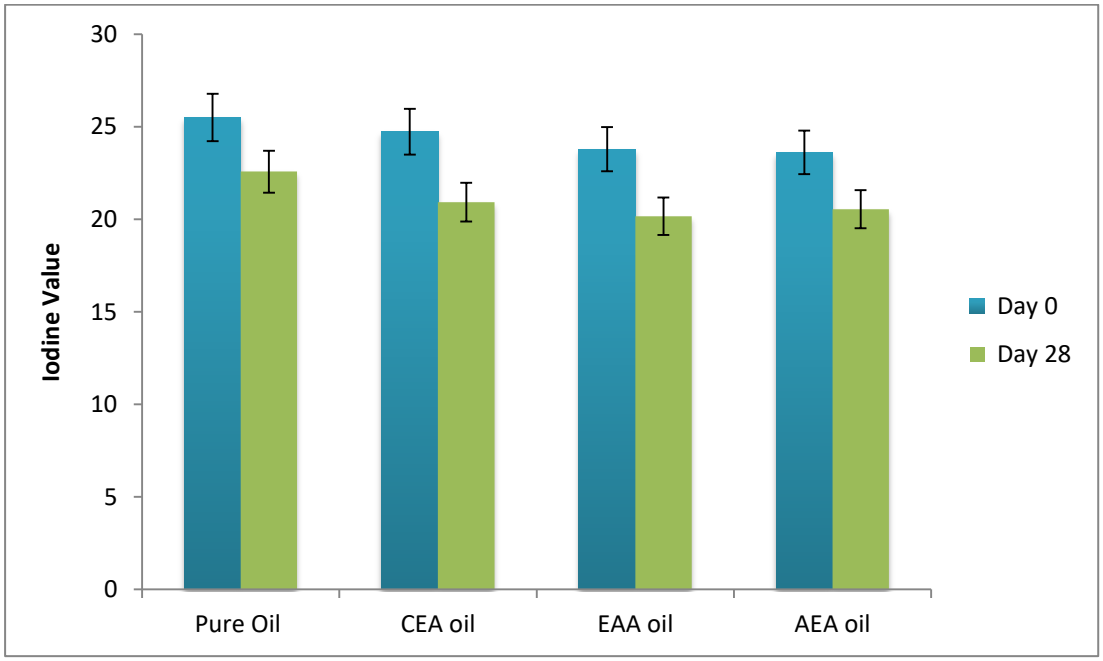


Figure 4.1: IV level changes in 28 days period. Results are presented as mean ± SD. * denotes significant differences at $P < 0.05$ for all the samples.

All of the enriched and pure mustard oil samples used in this analysis had moisture levels ranging from 0.1% to 0.2% (Table 4.1). This research found that after a week of storage, all of the enriched oils except the one with embanox-2 added had higher moisture content than they had initially. The p value for all the samples was less than 0.05 ($p < 0.05$), which means the sample was statistically significant. However, the rise for clove & asafoetida extract added oil (0.2 & 0.19) was not significant because the calculated p value of both samples was higher than 0.05 ($p > 0.05$) when individually compared to pure oil.

The specific gravity of pure and enriched mustard oil samples were 0.92 and 0.91, 0.91 & 0.90 respectively. According to the findings, the specific gravity of the enriched oils were higher than that of the pure oil, and the p value of all of the oil samples was lower than 0.05 ($p < 0.05$). As a consequence of this, there is a significant difference between the specific gravities of the samples of enriched and pure mustard oil.

4.2 Thermal Stability

Nevertheless, heating oils for an extended period of time degrades both their organoleptic and nutritional qualities. As a result of these conditions, the thermal stability of both pure oils and oils that had been enhanced was investigated by heating them to 170 °C and measuring their AV and PV values.

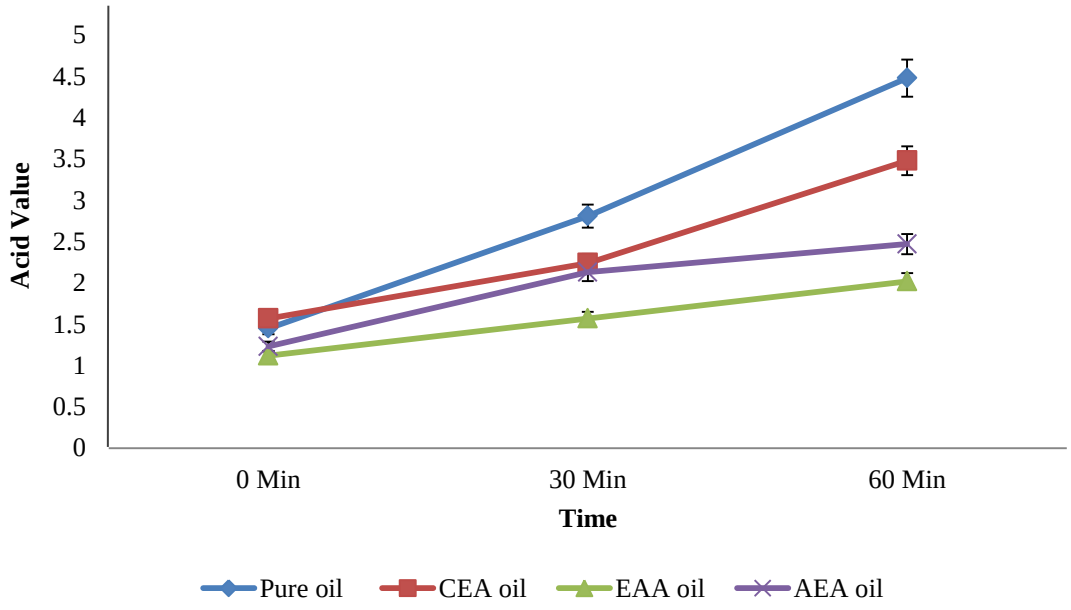


Figure 4.2: AV level changes with heating time. Results are presented as mean ± SD. * denotes significant differences at P < 0.05 for all the samples.

According to the findings presented in Figure 4.2, it seems Acid Value was increasing with each additional increament of heating time for all of the oil treatment investigated in this study. Immediately following the process of oil enrichment, samples of mustard oil with clove extract added exhibited the highest AV as the value stand at 1.45 mg KOH/g oil; On the other hand, when the temperature was maintained at 170 °C for 30 and 60 minutes, it was the pure oil that showed the greatest increase at the end and that is 2.81 & 4.48 mg KOH/g oil respectively. Whereas, for the

enriched mustard oils such as embanox-2 antioxidant added oil showed the lowest rise in AV with value of 1.57 & 2.02 mg KOH/g oil for 30 minute and 60 minute respectively. The other enriched oil has somewhat displayed similar changes under the same conditions. In this experiment, with a calculated p value of less than 0.05 ($p < 0.05$), we can conclude that the AV of enriched and pure mustard oil samples has significant difference across all temperatures.

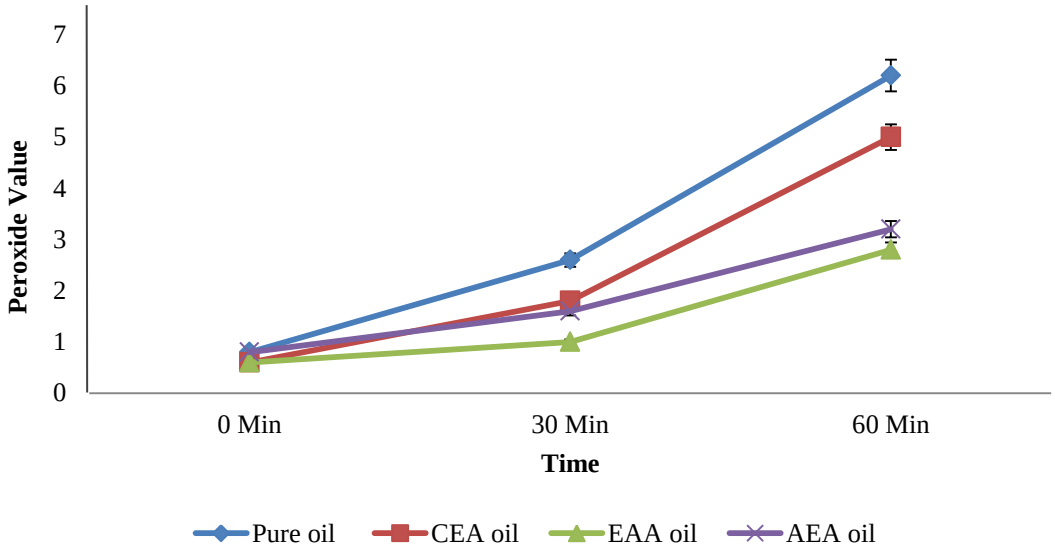


Figure 4.3: PV level changes with heating time. Results are presented as mean ± SD. * denotes significant differences at $P < 0.05$ for all the samples.

The PV of the oils improved as the heating time increased, and pure mustard oil had a higher PV than enriched oil at every heating time (Figure 4.3). However, the PV growth rate of the oil that had been enriched was slower than that of the pure oil. When enriched oil samples was compared among themselves, it is found that synthetic antioxidant cause better effect in controlling the increase of PV with 0.6, 1, 2.8 mEq/kg in respective increase of heating time, but both natural antioxidants can also cause almost identical influence in controlling the PV value (0.6, 1.8, 5 and 0.8, 1.6, 3.2 mEq/kg respectively). Moreover, the statistically analyzed *p* value of all oil samples was less than 0.05 ($p < 0.05$); so there exist a significant difference between the PV of enriched and pure mustard oil samples.

4.3 Oxidative Stability

The oxidative stability of mustard oil which can also be regarded as the storage stability was determined by calculating the PV and AV of samples kept at room temperature for 28 days. Figure 4.4 shows, the preliminary AV level of the mustard oil of pure origin were noticeably greater than the treated samples, with the exception of the clove extract added oil. A gradual and consistent rise in AV has taken place in every one of the samples while they were being kept in storage for a total of 28 days. Throughout the entire storage period, the pure samples displayed the highest AV level, and on the 28th day, that level reached its highest point (4.78 mg KOH/g).

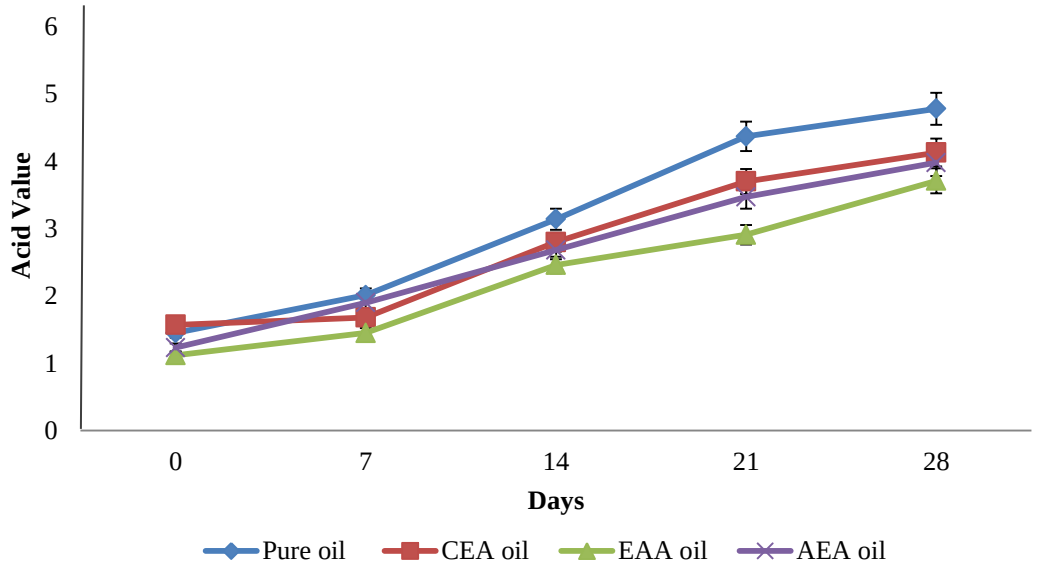


Figure 4.4: Changes of AV level in mustard oil samples during storage. Results are presented as mean ± SD. * denotes significant differences at P < 0.05 for all the samples.

During the initial day, the AV levels of the enriched mustard oils that contained asafoetida, clove, and embanox-2 were as follows, 1.57, 1.12 and 1.23. Whereas the final increment on the 28th day were 4.13, 3.71, 3.98 mg KOH/g respectively. All oil samples increased in AV level with period of storage, but the rate of growth in enriched oil samples was quite lower than the the pure samples (Figure 4.4). Given that both pure and enriched oil samples had p-values below 0.05 ($p < 0.05$), there was significant difference between the AV levels of the enriched and pure oil samples.

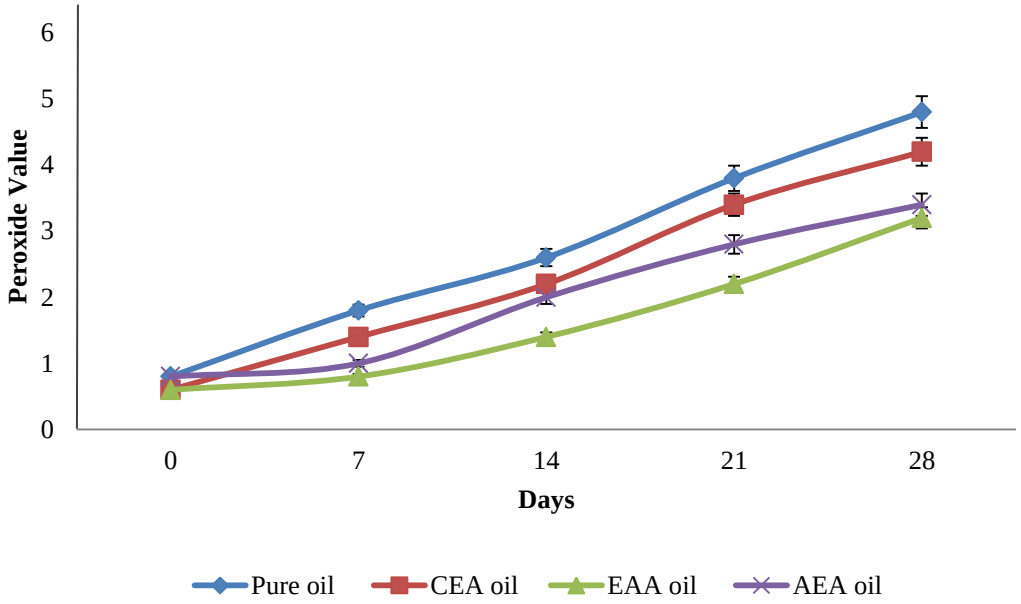


Figure 4.5: Changes of PV level in mustard oil samples during storage. Results are presented as mean ± SD. * denotes significant differences at P < 0.05 for all the samples.

In another case, pure mustard oil displayed the largest PV from the initial days to the final days, as its value was 0.8 mEq/kg in the start and it reached the value of 4.9 mEq/kg in the end. In the case of enriched mustard oil samples, PV were 4.2 and 3.2, 3.4 mEq/kg respectively (at the 28th day period of storage). In addition, the peroxide value increased in all of the oil samples as a function of the length of time they were stored; however, the increase occurred at a shorter rate in the samples of enriched oil than it did in the samples of pure mustard oil (Figure 4.5). Although exerting similar outcome but compared to clove extract, asafoetida extract and embanox-2 antioxidant was slightly effective against controlling PV. Since, the p value of pure and enriched oil samples of this study was less than 0.05 ($p < 0:05$) in all the analysis, there was a significant difference between the PV of all samples.

4.4 Total Phenolic Content

When evaluating the positive effects of a substance for its antioxidant properties, one crucial factor is determining its total phenolic content. This assessment provides an initial glimpse into whether or not the extract addition from spice and herbs is valuable for bettering the oil quality. In Table 4.2, it is clearly visible that TPC of the oil samples ranged from the lowest in pure oil as expected which valued at 50.7 mg GAE/100 ml to highest in clove extract added oil with 119.13 mg GAE/100 ml.

Table 4.2: Biochemical properties of enriched & pure mustard oil samples.

Name	Total Phenolic Content	Total Antioxidant Activity
Pure oil	50.7 ± 0.99^{c}	1.22 ± 0.03^{d}
CEA oil	119.12 ± 0.01^{a}	22.19 ± 0.01^{b}
EAA oil	54.39 ± 1.35^{c}	28.26 ± 0.02^{a}
AEA oil	60 ± 0.98^{b}	12.16 ± 0.01^{c}

Results are presented as mean ± SD. *Different superscripted letters (a-c) in each row shows statistically significant differences ($p<0.05$) for all the samples.

Where, **CEA oil** = Clove extract added oil

EAA oil = Embanox-2 antioxidant added oil

AEA oil = Asafoetida extract added oil

Additionally, concentrations of TPCs also saw a slight increase in case of embanox-2 antioxidant added oil (54.39 mg GAE/100 mL) and asafoetida extract added oil (60 mg GAE/100 mL).

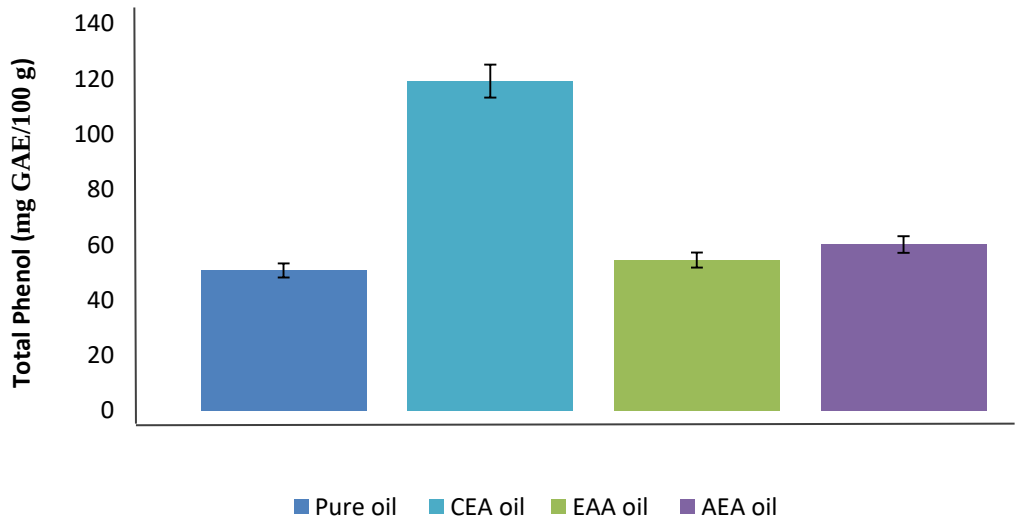


Figure 4.6: Total phenolic content of pure and enriched mustard oils. Results are presented as mean ± SD. * denotes significant differences at $P < 0.05$ for all the samples.

According to the findings that are presented in Figure 4.6, every type of enriched oil had a noticeably greater quantity of phenolic compounds than the pure mustard oil. Additionally, p value for all oil samples was less than 0.05 ($p < 0.05$) and that makes all the oil samples statistically significant.

4.5 Total Antioxidant Activity

The present study also demonstrated that the antioxidant activity of these natural spice & herbs can impart nearly the same effect as commercial synthetic antioxidants.

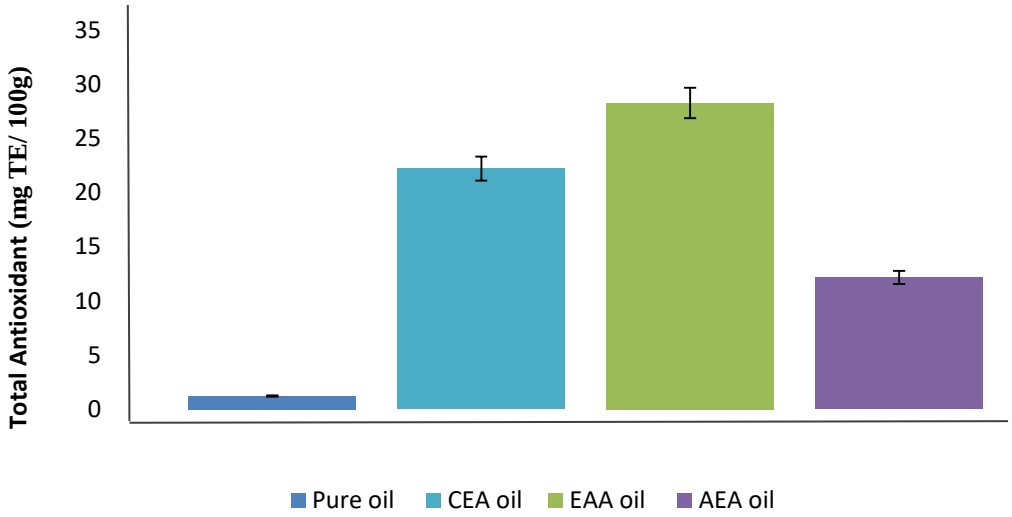


Figure 4.7: Total Antioxidant Activity of pure and enriched mustard oils. Results are presented as mean ± SD. * denotes significant differences at $P < 0.05$ for all the samples.

According to the data presented in Table 4.2, among the natural antioxidants clove extract added oil (22.19 mg TE/100 g) imparted better antioxidant activity than asafoetida extract added oil (12.16 mg TE/100 g). Due to the presence of substances including acetyleugenol, eugenol, and β-caryophyllene, clove has antioxidant properties (Yashin et al., 2017). However, in Figure 4.7 the findings denoted that the experiment with the highest antioxidant activity was 28.26 mg TE/100 g which is when embanox-2 antioxidant was added to mustard oil. As the *p* value of all oil samples was less than 0.05 ($p < 0.05$), differences in antioxidant activity between enriched and pure mustard oil samples are statistically significant.

Chapter 05

Discussion

5.1 Changes in Physicochemical Properties of Oil

Antioxidants derived from natural sources are in high demand because modern consumers are health-conscious and want food that tastes as close as possible to its original and fresh state. The polyphenolic compounds found in spices have a high antioxidant capacity, making them a possible replacement for synthetic antioxidants in food products while also providing additional health benefits (Shahid et al., 2018)

During week long storage, the AV and PV of enriched mustard oils were lower than those of pure mustard oil. Perhaps this is because of the antioxidant effects of the bioactive compounds found in spice and herb extract. We found that these natural compounds have nearly the same antioxidant potential as synthetic antioxidants, suggesting they may be able to inhibit the oxidation of fatty acids in a study of their own (Taghvaei and Jafari, 2015).

When evaluating the health benefits of oils, the percentage of unsaturated fatty acids is typically measured in iodine values. Oils with a high percentage of saturated fatty acids are not as healthy as those with a higher percentage of unsaturated fatty acids. Nonetheless, highly unsaturated fatty acids in oils undergo oxidative degradation due to their double-bond configuration and the presence of free radicals unless sufficient antioxidant is added (Negash et al., 2019). In this study, the result witnessed was fall in IV for all the samples. The natural extract was successful in exhibiting similar impact as their synthetic counterpart. It's possible that this is due to the fact that the extracts contain a biochemical compound whose chemical energy decreases the number of double bonds in mustard oil's polyunsaturated fatty acids (Wardana et al., 2018).

Since the presence of water in oil triggers the hydrolysis of triglycerides, which in turn leads to rancidity. So, low moisture content is necessary for oils to maintain their quality over time (Negash et al., 2019). The Australian Oil seeds Federation Quality Standards of Technical Information & Typical Analysis, specify that edible oils may

contain no more than ≤0.2% moisture (A. O. Federation, 2011) and it was true for the outcome of this study as well. The absence of free water in the diethyl ether base extraction is thought to be the root cause of this phenomenon (Shahrajabian et al., 2019).

The density of a liquid can be determined by its "specific gravity." The addition of bioactive compound of spice and herb, which typically consists of compact and dense pure compounds, was the reason for the increase in specific gravity seen in both naturally enhanced oil samples (Parthasarathy et al., 2008). Then again, in the case of commercial synthetic antioxidants, their chemical formation also involves elements that are heavier and denser, which is why the effect they cause is almost comparable to that of natural antioxidants.

5.2 Thermal Stability of natural and synthetic antioxidant rich oil

Oils are frequently used for frying a wide variety of food products, so the oils themselves must be able to withstand the high temperatures required for frying without breaking down. The hydrolysis, thermal decomposition, oxidation and polymerization are some of the chemical reactions that take place as a result of the thermal degradation of oils that occurs at the temperature at which frying takes place (Perera et al., 2020). During the heating phase of oils, hydrolysis takes place more frequently in oils containing short chain fatty acids than in oils containing long chain saturated fatty acids (Xu et al., 2015). Some studies suggest that, when the temperature of the enriched oils was kept at 170 °C, it was found that the oils displayed improved stability & became less prone to rancidification (Chandran et al., 2017). In this study, similar result was also noticed in case of controlling the AV value to induce quality changes in oil. According to Codex Alimentarius Commission (CAC) standard acid value up to 5 mg KOH/gm of oil is safe for consumption. The acid value in all the treated samples (Figure 4.2) after heating were below 5 mg KOH/gm of oil so it doesn’t deviates from food safety standard.

Then again, in case of peroxide value it's possible that the presence of natural antioxidants in clove and asfoetida, as well as the effect of synthetic antioxidant used, contributes to the lower PV of enriched oil by inhibiting the initiation and propagation of autoxidation reactions (Dapkevicius, 2002). The peroxide value of corn oil

increases dramatically when the temperature rises from 25 to 180 °C. Moreover, when corn oil is infused with thyme extract and subjected to deep-frying, those oil had the lowest peroxide value (Karoui et al., 2011). Pradhananga and Manandhar also carried out an experiment along these segments. Peroxide and acid value were discovered to increase with heating period in sunflower oil, and it was reported that both AV and PV can be reduced by the addition of TBHQ, another commercial synthetic antioxidant. Two separate groups of researchers, Singh et al. and Hou et al., using different oils, also came to the same conclusions.

5.3 Oxidative Stability of natural and synthetic antioxidant rich oil

As the increment in AV of all the oil samples is apparent from the inclining trend line seen in Figure 4.4, there is one thing to notice and that is the influence in controlling the rise of the value is played by both the natural antioxidants & the synthetic antioxidants almost similarly. This study's findings on AV level shifts were consistent with those of a previous one by Iqbal and Bhanger. In order to keep the oils from deteriorating under rapid oxidation conditions, sunflower oil along with garlic extract were combined, but they discovered that the blank treatment led to AV development more quickly than the oil with garlic added. When oils are stored at an elevated temperature while also being exposed to light, the rate at which they deteriorate due to oxidation is significantly quickened as compared to when they are stored under the conditions of in-house conditions (Choe and Min, 2006). In the following study, both natural and synthetic antioxidant treatment of mustard oils, actively participated in controlling the rate of oxidation in unfavorable conditions for longer period of time.

The total amount of peroxide and hydroperoxide that is present in the edible oil system can be determined using the peroxide value parameter. When determining the oxidative stability or storage stability of vegetable oils, the PV is the most commonly used experiment. The presence of peroxides is related to rancidity because their presence indicates the extent of primary oxidation. The steady rise in peroxide value during fat oxidation is indicative of the formation of hydro peroxides. According to some research, oils with added essential oil or other bioactive compounds have the lowest rates of peroxide value increase (Chandran et al., 2017). This study also demonstrated that the PV of all oil samples increased over the course of their storage periods as time went on (Figure 4.5). This may be because bioactive compound from

spices are effective radical scavengers, which in turn neutralizes free radicals and reduces their ability to form peroxides. According to the Codex Alimentarius Commission, oil with value of peroxide up to 10 meq/Kg is considered acceptable for human consumption. After storage, the peroxide value in all the treated samples (Figure 4.5) was less than 10 meq/Kg of oil, which is well within the acceptable range for use in food. Similar outcome was observed in a study, where the growth of peroxide value of vegetable oil was found to be slowed down when natural extracts were added to the oil (Ramadan and Wahdan, 2012).

5.4 Total Phenolic Content of natural and synthetic antioxidant rich oil

Higher phenolic percentage of spices and herbs is to be responsible for increase in value of TPC of treated oil samples. Synthetic antioxidants typically feature a high amounts of phenolic combinations as well. As a result, the TPC of enriched oils was greater than the pure sample. Table 4.2 show that the addition of the biochemical extract induced a shift in the phenolic level of the enriched mustard oil. All the variations on adding something to mustard oil improved upon the original oil, but adding clove extract to the oil produced the most noticeable changes. (Figure 4.6)

During the mixing phase, the interactions that take place between the oils and the added biochemical agents are in charge of bond formation between phenolics and spice and herb constituents. These interactions could provide an explanation for the differences that exist between the phenolic contents of pure mustard oils and enriched mustard oils. By neutralizing various free radicals, phenolic compounds (PCs) serve as an antioxidant. The transfer of hydrogen atoms, a single electron, consecutive proton loss electron transfer, and the chelation of transition metals are some of the mechanisms by which antioxidants work (Zeb, 2020). The most significant antioxidants found in nature are phenolics. Robards et al., reported that phenolics have a direct relationship with antioxidant capacity, and Amarowicz and Pegg agreed that phenolics are crucial dietary antioxidants.

5.5 Total Antioxidant Activity of natural and synthetic antioxidant rich oil

When compared to other food items, spices and herbs have ten times the ability to scavenge free radicals than fruits and vegetables (Yashin et al., 2017). The extract of spices is responsible for this phenomenon because it has a large quantity of phytochemicals, such as polyphenols, which are highly capable of scavenging harmful

free radicals and inhibiting the production of peroxides (Hamad et al., 2015). Some of the most important aspects that determine the effectiveness of an extract are the active materials found in spices, the concentration, and the process that was used to extract them (Dua et al., 2013). Furthermore, the availability of extracted lipophilic phenolic compounds can be related to better stability of oxidative nature of the treated oil samples (Žanetić et al., 2013). Since polar phenolic compounds are insoluble in fat, they did not dissolve in the oil even though other, more lipophilic, natural material components did disperse (Taha et al., 2014). Therefore, the antioxidant activity of the oil is solely due to the lipophilic components found in spices & herbs.

It is obvious that synthetic antioxidant addition will cause rise of antioxidant activity. But we can also see that the impact of natural antioxidant can also come close to synthetic one (Figure 4.7). This proves the both natural and synthetic antioxidant can be responsible for slowing down the rate of lipid oxidation. Wang et al., carried out a study in which they used 45 different essential oils in order to find out the antioxidant activity by analyzing the total phenolic content and the DPPH free radical scavenging activity of the oils. They discovered that having cinnamon and clove essential oil in the mixture produced the most effective antioxidant activity. They also stated that antioxidants, both synthetic & natural, can delay or slow down the fat oxidation method. Additionally, antioxidants can stop the oxidation of oil by acting as a reducing agent, a free radical scavenger and also a singlet oxygen scavenger (Shahid et al., 2018).

Chapter 06

Conclusion

It can be concluded from this research that the stability of oxidative nature and storage life of mustard oil are significantly impacted by the addition of bioactive enriched spice and herb extracts as natural antioxidants. It is to be noted that the extracts of clove a spice and extracts of asafoetida an herb contain a plethora of active substances that have antioxidant potential, such as polyphenols, flavonoids, and carotenoids, among others. These compounds not only exhibit potent antioxidant activity but also provide various health benefits, making the use of these extracts a more attractive alternative to embanox-2 a synthetic antioxidants. The outcome of the study proves that due to extract addition, oil shows great control in reducing peroxide value, acid value, iodine value and controlling other physiochemical properties. It also increases phenolic and antioxidative power of the oil as the obtained extracts are rich source of antioxidants. Due to these natural treatments, the mustard oil is enhanced and shows almost similar efficacy as the synthetic antioxidants in terms of reducing the oxidative degradation of mustard oil and prolonging its shelf-life. Thus, the findings of this study suggest that the use of spice and herb extracts could be a promising alternative to synthetic antioxidants in treatment of edible oils. In addition, the study also highlights the importance of natural antioxidants in the food industry and how they can play a crucial role in ensuring food safety and quality. The increasing consumer demand for healthier and more sustainable food options, combined with the potential health benefits of using natural antioxidants and use of analytical techniques, such as gas chromatography and mass spectrometry, to standardize and quantify the antioxidant activity of these natural extracts highlights the need for further research into the use of bioactive enriched spice and herb extracts in the food industry.

Chapter 07

Recommendations and Future aspect

Future research is needed to study the effectiveness of different types of spice and herb extracts and their optimal concentration for use in food products so that the use of natural antioxidants in the industry becomes the primary choice.

Here are some future perspectives of this study:

1. Evaluate the nutritional quality of various edible oils treated with bioactive enriched spice & herb extracts.
2. Establish the economic feasibility of using all sorts of bioactive enriched extracts in comparison to synthetic antioxidants.
3. Highlight the potential health benefits of using bioactive enriched extracts of plant source as natural antioxidants.

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Appendices

Appendix-I Certificate of Analysis of Embanox-2

RTC CHEMTRONICS

CERTIFICATE OF ANALYSIS

Embanox - 2

BATCH NO. :
DATE OF MANUFACTURING :
DATE OF EXPIRY :
ORIGIN :

Embanox is a synergistic mixture of the antioxidants BHA (Butylated Hydroxy Anisole) and BHT (Butylated Hydroxy Toluene); Vegetable Oil is added as a carrier.

Properties:
It prevents oils and fats from oxidation and foodstuffs containing there. It is applied in the food industries as an antioxidant for fats and oils.

Application:

: Vegetable oil
: Animal fats
: Fried Products
: Baked products

Chemical – Physical properties:

Appearance	: Oily liquid
Odour	: Phenolic (Slightly)
Taste	: Not available.
Colour	: Pale yellowish to golden.
Boiling Point	: 264°C (507.2°F)
Critical Temperature	: Not available.
Specific Gravity	: 0.94 (25 °C)
Refractive Index	: 1.47 (25 °C)

Solubility

Water	: Insoluble
Vegetable oils	: Unlimited

Doses: It is recommended to be applied in a dosage between 20 – 2000 ppm

Handling Procedure
Normal handling precautions for handling chemicals are recommended. Avoid contact with skin and eyes.

Storage
It must be stored under normal temperature conditions, not bellow 0°C – not above 30°C (Optimum 15°C).

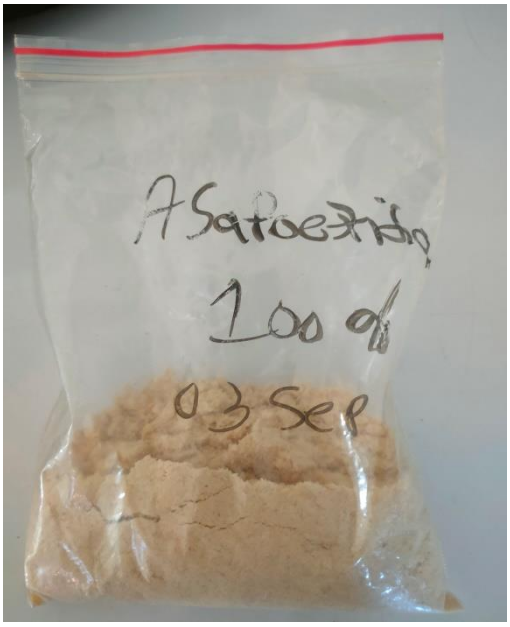
RT CHEMTRONICS GMBH

RT Chemtronics Chemikalien GmbH
DIN EN ISO 9001:2000
Huyssenallee 76
D-45128 Essen
Germany
Telefon + 49-201-24 531-0
Telefax + 49-201-24 531-50
Mail rtc@rtchemtronics.de
Dresdner Bank Essen
(BLZ 360 800 80) 0 428 816 800
S.W.I.F.T.-ADDRESS DRES DE FF 360
IBAN: DE28 3608 0080 0428 8166 00
Managing Director: G. Barkhofen
Reg. HRB Essen 10534
VAT Id. No. DE 811 530 101
St.-Nr. 112/5724/0441

Appendix – II Photo Gallery

a) Raw Sample

Clove grinded powder



Asafoetida grinded powder

Embanox-2 Antioxidant

b) Extracts

Extract before evaporation

Extract after evaporation

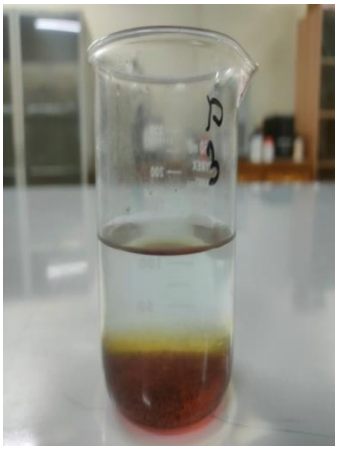
c) Treated Oils

Mustard Oil Samples

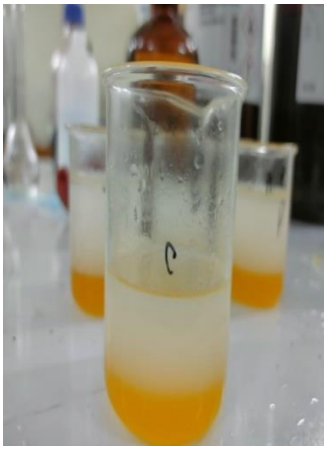
d) Experiments

Pycnometer Reading

Acid Test Endpoint



Iodine Test Endpoint



Peroxide Test Endpoint

UV-Visible spectrophotometer for TAA and TPC determination

Brief Biography

Mohammad Rihan Arefin has passed the Secondary School Certificate (SSC) Examinations in 2013 with Grade Point Average (GPA) 5.00 followed by Higher Secondary Certificate (HSC) Examination in 2015 with GPA 4.75. He received the B.Sc. (Hon's) in Food Science and Technology in 2019 (held in 2020) from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, he is a candidate for the degree of MS in Food Processing and Engineering under the Department of Food Processing and Engineering, Faculty of Food Science and Technology, CVASU. His career objective is to obtain and secure a challenging position as a Food Engineer. He has profound interest to work in a challenging environment where his skill can be put to good use for problem solving and come up with innovative solutions.