

**FORMULATION OF ACTIVE PACKAGING SYSTEM USING
CLOVE OIL MICROEMULSIONS FOR ENHANCING THE
SHELF LIFE OF FRESH FRUITS**

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**MASTER OF SCIENCE
FOOD QUALITY MANAGEMENT**

Supervised by: Dr. Anum Ishaq



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**Formulation of Active Packaging System using Clove Oil Microemulsions for
Enhancing the Shelf Life of Fresh Fruits**

Gulam Fizza

F2021324005

A thesis submitted in partial fulfillment of the requirement for the degree of

Master of Science

in

Food Quality Management

Supervised by: Dr. Anum Ishaq



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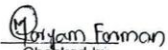
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(To be signed after viva voice exam)

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External Examination
Dr. Muneeb Khan

Chairperson
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ABSTRACT

The purpose of food packaging is undergoing significant transformations to meet the evolving demands of the market. Factors such as food safety, consumer preference for healthier options, the need for high-quality products, extended shelf life, convenience, transparent packaging, and sustainable materials have led to the emergence of innovative packaging technologies, including active packaging. This study focused on the development of an active packaging system using chitosan, known for its effective barrier properties against moisture and gas permeation. Firstly, stable microemulsions containing clove oil were formulated, with an average particle size of $142.2 \pm 2 \mu\text{m}$, and characterized for thermal stability and pH changes. Secondly, these microemulsions were incorporated into chitosan matrices to create the chitosan-based active packaging system. To address the perishability and post-harvest losses of tomatoes, the developed active packaging system was applied to tomatoes through various treatments involving active coatings and sprays.

Keywords: antimicrobial activity, clove oil microemulsions, edible coating, emulsified food packaging, shelf-life

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Chapter 1

INTRODUCTION

Forty percent of food produced worldwide is wasted throughout the supply chain and never obtains its desired target (Abd Ghafar, 2017). The amount of food lost due to inappropriate packaging during the supply chain might vary depending on the type of food, the packaging's quality, and supply chain conditions.

According to estimates from the Food and Agriculture Organization (FAO) of the United Nations, around 14 percent of food loss occurs during the post-harvest and processing stages, which includes the transportation and storage of food (Gustavsson et al., 2011). Several factors, such as inadequate storage facilities, poor handling methods, and packing, can lead to food loss. Packaging is especially important in this regard. Nearly 1.3 billion tons of food produced for human use disappear or disposed away every year. Every year, an enormous 1.3 billion tons of food enough to feed approximately 2 billion people are misplaced or thrown away globally. This poses a major environmental issue (Shafiee-Jood & Cai, 2016). This includes the waste of food generated during the processes of production, transit, storage, and consumption.

1.1 Problem statement

Protecting human health from the risks associated with microbial contamination requires maintaining the quality, safety, and nutritional integrity of food-related products while also prolonging their shelf life.

1.2 Objectives

- I. To formulate and characterize CMEs for their stability and antimicrobial properties

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Chapter 2

REVIEW OF LITERATURE

According to Gustavsson et al. (2011), around 40% of the food yielded worldwide was wasted or lost, with packaging being a major contributor. While coatings can aid to maintain the perishable foods quality like vegetables and fruits, packaging can aid to avoid food loss during transportation and storage. It is necessary to create new kinds of antibacterial, biodegradable, and ecologically friendly packaging material because certain coatings might include dangerous chemicals or additives that may adversely affect human health. Due to their nutritional value, tomatoes are a valuable commodity, yet a substantial amount is lost to spoilage. To meet dietary requirements, tomato quality must be improved, and quality is influenced by a variety of elements, including appearance, storage life, texture, and biochemical characteristics. An active packaging technique incorporating microemulsions of chitosan and clove oil has been created to prevent microbiological deterioration preserve freshness and improve the shelf stability (Shehata et al., 2021).

2.1 Clove oil as antimicrobial & antioxidant agent

De Silva et al, (2017) evaluated the strong anti-microbial activity of clove oil by applying the clove oil against 6 pathogenic species *Aeromonas hydrophila*, *A. caviae*, *Citrobacter freundii*, *Salmonella enterica*, *Edwardsiella tarda*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*. They found out that the clove oil obtained from *Cymbopogon clove* was effective against all the six species isolated from pet turtles. Researchers evaluate the anti-microbial activity of clove oil against different pathogenic bacteria. They also evaluated the antifungal activity of clove oil. Clove oil was found effective against all the gram-positive bacteria which were under study (Mukarram et al., 2021).

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CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Tomatoes were procured from the local market and were brought in the lab in proper packaging conditions. All the chemicals such as chitosan, sorbitol, decaglycerol, and acetic were purchased from Sigma Aldrich. USA. Clove oil was purchased from the local market.

Table 3.1: Treatment plan for the formulation of edible coatings

Treatments	Chitosan (g)	Acetic acid (mL)	Encapsulation of CMEs (mL)
T ₀	0	0	0
T ₁	1	2	3
T ₂	1	2	5

Notes: T₀= control tomatoes, T₁= coated tomato encapsulation of 3mL CMEs, T₂= coated tomato encapsulation of 5mL CMEs

3.2 Formulation of clove oil microemulsions

The microemulsion was prepared in two phases, the oil and aqueous phase, by following the procedure presented by (Iqbal et al., 2020). The oil phase consisted of clove oil. However, the aqueous phase was prepared by dispersing various concentrations of decaglycerol and distilled water. Both phases were mixed to prepare a course emulsion. Course emulsion was then treated with a high-speed homogenizer for 20 minutes at 10000 rpm for maximum possible solubility of the solution (Iqbal et al., 2020).

3.3 Characterization of microemulsions

3.3.1 Analysis of particle size

The particle size of microemulsion was analyzed using a laser particle size analyzer, as described by (Subongkot & Ngawhirunpat, 2017). To ensure accurate measurements, the system was washed with distilled water multiple times and particle size readings were taken in triplicates.

$$\text{Acid}\% = \text{Titer} \times 0.0064 (\text{citric acid factor}) \times \frac{100}{1\text{mL}} \text{ juice} \text{ --- (3.1)}$$

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Chapter 4

RESULTS AND DISCUSSION

4.1 Characterization of microemulsion

4.1.1 Analysis of droplet size and pH

Food-grade clove oil microemulsions were formulated using 1% (w/w) decaglyceride as a natural emulsifier containing 1 % (w/w) oil. The clove oil microemulsion appeared milky having a mean particle size (d_{av}) of 142.2 ± 2 . Fig. 4.1 shows that O/W microemulsions stabilized by decaglyceride exhibited slight monomodal but wider droplet size distribution and have a mean d_{av} of 142.2 ± 2 μm . Formulated clove oil microemulsions were stored at room temperature for over 60 days. The wider distribution of droplet size in a clove oil microemulsion can be attributed to the absence of a high-pressure homogenizer during the emulsification process.

Even though the high-pressure homogenizer was not utilized, which resulted in large particle size and wider size distribution of particles. However, the focus was to solubilize clove oil in chitosan coating which have been achieved in the form of microemulsions encapsulating clove oil as an active packaging material. The lack of a high-pressure homogenizer causes a reduction in shearing forces and turbulence, which are essential for reducing droplet size. As a result, the emulsion may have larger droplets with a wider variation of sizes, which could have an impact on its appearance, stability, and general performance (Lu & Gao, 2010).

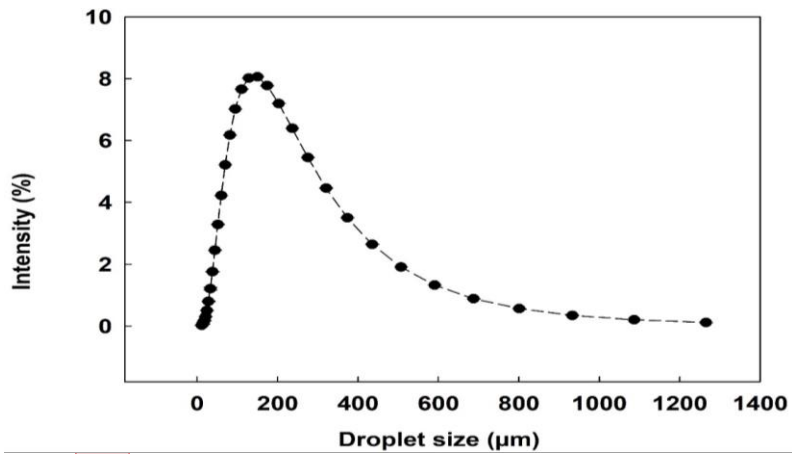
Alternative methods such as mechanical stirring, ultra-sonication, or low-energy emulsification techniques can create stable emulsions in place of high-pressure homogenizer, but may not effectively reduce larger droplets into smaller, more uniform sizes (Karunaratne et al., 2017).

Additionally, the functionality of the microemulsion may be impacted by a broader droplet size dispersion. Smaller droplets often have a higher interfacial area, which improves stability and effectiveness for specific applications. The performance of the microemulsion may be diminished with a wider variety of droplet sizes (Tammannavar et al., 2013).

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Figure 4.1: Particle size distribution of microemulsions stabilized by decaglyceride

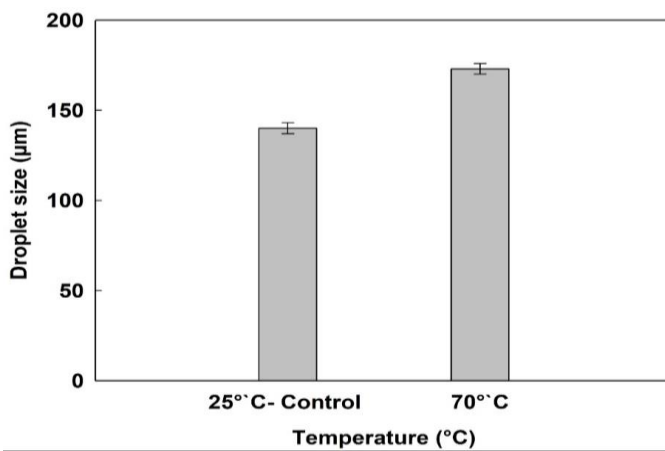


Figure 4.2: Effect of heat treatment on droplet size of CMEs

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Clove oil microemulsion's stability is affected by several variables, including pH level, surfactant concentration, temperature, and the size of the droplets. However, the optimum pH for clove oil microemulsion stability may change based on the precise formulation and ambient conditions employed. Because essential components like eugenol and eugenol acetate are more soluble at acidic pH values, stability is often improved by lower pH values (Peralta-Ruiz et al., 2020). Stability may be increased by adjusting pH to a slightly acidic range, such as pH-4 (Wang & Zhao, 2016). Nevertheless, it is important to consider that pH adjustments can also impact the physicochemical properties of the microemulsion, including droplet size.

4.1.2 Thermal stability

Heat impacted the particle size of the microemulsions, resulting in wider particle size distribution. A significant change was observed, however, the particle size remained stable as shown in Fig. 4.2, and this increase in particle size can be attributed due to thinning of the emulsified layer surrounding the droplets. However, at room temperature, no significant changes in droplet size were observed. Whereas, it was reported that minute effect of heat has been observed on particle size of emulsions that have been formulated by high pressure homogenizer (Iqbal et al., 2020).

In comparison, clove oil microemulsions formulated with natural emulsifiers demonstrated superior stability at ambient temperature compared to those stabilized using protein surfactants or emulsifiers (Mishra et al., 2016), which tend to coagulate as temperature increases. Protein-based surfactants tend to coagulate as temperatures increase since proteins can unfold, exposing hydrophobic and sulfhydryl groups that induce aggregation and result in microemulsion instability (Chaiyana et al., 2017). Different studies have reported varying temperature ranges for optimal stability of clove oil microemulsions. Some suggest that a temperature range of 25-30°C is ideal (Fu et al., 2007), while others propose refrigeration at 4°C to enhance stability (Subongkot & Ngawhirunpat, 2017). Notably, the microemulsion's stability can be compromised by extreme temperatures, whether excessively high or low.

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Table 4. 1: *Analysis of variance for total phenolic contents in clove oil*

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Chapter 5

SUMMARY

Growing consumer demands for ongoing food safety and quality monitoring and shelf-life extension led to the development of more sophisticated types of packaging and coating techniques, such as active packaging technologies. The commercial application of active packaging techniques will increase food product storage life, and level of safety and also maintain the nutrient value of food. In this way, active packaging technologies enhance food safety and protect food quality. The key challenge for scavenging and absorbing systems is to create effective products that can maintain their original qualities during distribution and storage.

5.1 Strength and limitation

5.2 Recommendation

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Abd El-Hack, M. E., El-Saadony, M. T., Shafi, M. E., Zabermawi, N. M., Arif, M., Batiha, G. E., Khafaga, A. F., Abd El-Hakim, Y. M., & Al-Sagheer, A. A. (2020). Antimicrobial and antioxidant properties of chitosan and its derivatives and their applications: A review. *International Journal of Biological Macromolecules*, *164*, 2726–2744. DOI: 10.1016/j.ijbiomac.2020.08.153

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APPENDICES

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