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**ALANYA ALAADDIN KEYKUBAT UNIVERSITY
GRADUATE SCHOOL OF EDUCATION
DEPARTMENT OF FOOD ENGINEERING**

**DEVELOPMENT OF A 3D PRINTED ACTIVE FOOD PACKAGING TO
EXTEND SHELF LIFE OF A TRADITIONAL TURKISH DESSERT,
AŞURE**

Master of Science

Evrin AKTURK

**Thesis Advisor
Associate Professor Sinan UZUNLU**

**ALANYA
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Food Engineering (MSc) (Thesis) (English)

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JÜRİ VE ENSTİTÜ ONAYI

Evrım AKTÜRK'ün "Development of a 3D Printed Active Food Packaging to Extend the Shelf Life of a Traditional Turkish Dessert, Aşure" başlıklı tezi 21/04/2025 tarihinde aşağıdaki jüri tarafından değerlendirilerek "Alanya Alaaddin Keykubat Üniversitesi Lisansüstü Eğitim-Öğretim Yönetmeliği"nin ilgili maddeleri uyarınca, Gıda Mühendisliği Anabilim Dalında Yüksek Lisans tezi olarak oy birliği/oy çokluğu ile kabul edilmiştir.

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ABSTRACT

DEVELOPMENT OF A 3D PRINTED ACTIVE FOOD PACKAGING TO EXTEND SHELF LIFE OF A TRADITIONAL TURKISH DESSERT, ASURE

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In this study a prototype food packaging tray was developed by using a 3D printer. This thesis aimed at determining the stability of a traditional Turkish dessert, aşure under cold storage conditions for 14 days by using the 3D printed active food packaging tray. Cinnamaldehyde (CNMA), a GRAS-listed food additive, was injected into the 3D-printed packages at 2% and 5% (v/w) concentrations through the package channels. Aşure samples were placed in the packages and vacuum sealed then stored at 4°C for 14 days. Physicochemical (pH, dry matter, CNMA release, color), microbiological (*Salmonella* spp., staphylococcal enterotoxins, total bacterial count), and sensory analyses were conducted at periodic intervals. For characterization of packages, oxygen permeability, oxygen transmission rate and mechanical properties (Young's modulus, tensile and flexural strength) were measured. No viable microorganisms were detected at either CNMA concentrations, and physicochemical results showed that there were no significant ($p>0.05$) changes. Sensory analysis indicated that CNMA's aroma odour correlated positively along with the concentration, without exhibiting negative perception on panelists. The findings demonstrated that 3D-printed packaging ensures controlled CNMA release, high oxygen barrier properties, and resistance to physical impact. This study highlights the potential of essential oils like CNMA in food preservation and suggests further research into innovative packaging solutions for extended shelf-life and microbial safety.

Keywords: Active food packaging, Cinnamaldehyde, Antimicrobial agents, Aşure, Functional packaging material.

ÖZET

GELENEKSEL BİR TÜRK TATLISI OLAN AŞURENİN RAF ÖMRÜNÜ UZATMAK İÇİN 3B YAZICI İLE ÜRETİLMİŞ AKTİF GIDA AMBALAJI GELİŞTİRİLMESİ

Evrin AKTÜRK

Gıda Mühendisliği Anabilim Dalı

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Bu çalışmada, 3D yazıcı kullanılarak aktif bir gıda ambalajı geliştirilmiş ve geleneksel Türk tatlısı aşurenin 14 günlük soğuk depolama süresindeki stabilitesi incelenmiştir. GRAS listesinde yer alan gıda katkı maddesi sinnamaldehit (CNMA), 3D yazıcıyla üretilen paketlere %2 ve %5 (h/a) konsantrasyonlarında enjekte edilmiştir. Aşure örnekleri bu ambalajlara yerleştirilmiş ve 4°C’de 14 gün depolanmıştır. Belirli aralıklarla fizikokimyasal (pH, kuru madde, CNMA salınımı, renk), mikrobiyolojik (*Salmonella* spp., stafilokok enterotoksinleri, toplam bakteri sayımı) ve duyu analizler gerçekleştirilmiştir. Ambalajların oksijen geçirgenliği, oksijen iletim oranı ve mekanik özellikleri (Genç Modülü, çekme ve eğilme dayanımı) belirlenmiştir. Sonuçlar, her iki CNMA konsantrasyonunda da canlı mikroorganizma tespit edilmediğini ve fizikokimyasal değişimlerin istatistiksel olarak anlamlı olmadığını göstermiştir ($p > 0.05$). CNMA’nın aroması konsantrasyonla pozitif ilişki göstermiş, ancak panelist algısı üzerinde olumsuz bir etki oluşturmamıştır. Bu çalışma, 3D yazıcıyla üretilmiş ambalajların kontrollü CNMA salınımı sağladığını, yüksek oksijen bariyer özellikleri sunduğunu ve fiziksel darbelere karşı dayanıklı olduğunu göstermektedir. CNMA gibi esansiyel yağların gıda muhafazasındaki potansiyelini vurgulayan bu araştırma, yenilikçi ambalaj çözümleri üzerine daha fazla çalışma yapılmasını önermektedir.

Anahtar Sözcükler: Aktif gıda ambalajlama, Sinnamaldehit, Aşure, Antimikrobiyal ajanlar, Fonksiyonel ambalajlama malzemeleri.

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LIST OF ACRONYMS/ABBREVIATIONS

ACRONYMS

%	Percentage
G	Gram
°C	Centigrade degree
ml	Milliliter
std	Standard deviation
mg/ml	Milligrams per milliliter

ABBREVIATIONS

ANOVA	Analysis of Variance
AOAC	Association of Official Agricultural Chemists - Official methods of analysis
BAM	Bacteriological Analytical Manual
BR13	2 % concentration of cinnamaldehyde
CNMA	Cinnamaldehyde
CRP	Controlled-Release Packaging
EC	European Commission
ELFA	Enzyme-linked fluorescence assay
EO s	Essential Oils
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FDM	Fused Deposition Modeling
GC	Gas Chromatography
GRAS	Generally Recognized as Safe
ISO	International Organization for Standardization

MIC	Minimum inhibitory concentration
MR19	5 % concentration of cinnamaldehyde
OTR	The Oxygen Transmission Rate
RH	Relative Humidity
Spp	Species plural
TRP	Transient Receptor Potential
TRPM5	TRP melastatin 5
TSI	Triple Sugar Iron Agar
TVC	Total Viable Count
UNEP	United Nations Environment Program
WHO	World Health Organization
VIDAS	VITEK Immunodiagnostic Assay System

1.INTRODUCTION

Food packaging is one of the major steps for food safety. A food package preserves the food's safety, quality, and freshness. It has many functions that help maintain the safety and quality of food, from preventing contamination to increasing shelf life. So packaging is crucial not only for food safety but also protection, transportation and storage issues (Adeyeye, 2019).

In ancient Egypt, materials such as animal skins, leaves, gourds, and nuts were commonly used for packaging (Rasmussen, 2012). Conversely, in ancient China, the invention of paper led to its subsequent use in food wrapping (Cartwright, 2017). Between 1700s and 1900s wood boxes and wooden barrels, cans, tin-coating iron cans, first samples of card box which were replaced to Tetra Pack packaging after many years and various types of packaging materials and methods were discovered respectively (Twede, 2005).

With recent advancements in technology and heightened consumer awareness, the industry is now prioritizing eco-friendly packaging that uses fewer additives and more natural, biodegradable materials. Companies are increasingly opting for sustainable alternatives to traditional plastic packaging, aiming to reduce environmental impact. This shift is driven by both regulatory pressures and the growing demand from consumers for greener products. Innovations in bio-based materials, such as biodegradable plastics and compostable packaging, are paving the way for a more sustainable future. These efforts not only contribute to reducing waste but also align with the global movement towards circular economy principles.

As reported in the Food Waste Index Report 2021 by the United Nations Environment Program (UNEP), approximately 931 million tons of food waste were generated globally in 2019. Of this total, households accounted for 61%, food services contributed 26%, and retail was responsible for 13%. These figures indicate that 17% of the total global food production might go to waste. Therefore, effective food packaging prevents the formation of food waste. Packaging materials development shows a harmony to its era.

With the developments in food packaging, the negative perspective from consumers not only environmental but also health concerns arising with the food additives. Therefore, the food industry has developed solutions to overcome using natural bioactive compounds instead of synthetic ones in food packaging.

The traditional functions of food packaging protection, preservation, and communication with consumers have historically been considered passive roles, primarily serving to contain and safeguard the product (Chauhan, 2022). However, recent advancements in packaging technology have shifted the focus towards active packaging, which plays a more dynamic role in enhancing food quality and safety. Active packaging systems engage with the food product and its surroundings to prolong shelf life, preserve freshness, and enhance overall quality (Jiang, 2023). This paradigm shift reflects a growing emphasis on innovative solutions that not only protect but also actively contribute to the overall value and sustainability of the packaging system.

Among the food additives which might be used in bio-based food packaging are classified according to their active substance/material; phenolic compounds, organic acids, bacteriocins and essential oils (EO). EOs can be counted as secondary metabolites of the plant which are commonly gained from the exact part of the plant such as leaves, bark or fruit (Burt, 2004). EOs indeed have a complex composition, typically containing between 20 and 60 different components. These components include; ketones, phenols, terpenes, aldehydes, carbohydrates, ethers and alcohols. This diversity in composition is what gives each essential oil its unique therapeutic properties and scent profile (Mishra et. al.2020).

The specific concentration of each component can diversify widely depending on the plant source and extraction method used. EOs are volatile and as a consequence it should be kept in airtight containers without sunlight in order to prevent compositional changes (Lacroix, 2007). The bacteria group in Gram-positive are more susceptible to bioactive agents than Gram-negative bacteria, while *Pseudomonas* species are the least susceptible (Ceylan, 2004). Due to the scientist's research, an essential oil with phenolic compounds shows greater antimicrobial activity compared to EOs rich in terpenoids (Cosentino, 1999). Garlic, onion, allspice, oregano, thyme, tarragon, cumin, cloves, lemongrass bay leaf, capsicums, rosemary plants and cinnamon can be exemplified as high inhibitors as including EOs (Billing, 1999). Essential oils, such as cinnamaldehyde from cinnamon, are widely used for their germicidal properties. They aid in prolonging the product lifespan of food products by preventing the growth of spoilage microorganisms (Sun, 2024).

Cinnamomum, commonly known as cinnamon, has indeed been utilized as a spice for centuries. Its essential oils are highly valued in various industries today (Bertacchi, 2021). In the food industry, cinnamon essential oil is used for its flavoring

and preservative qualities. It has been shown to inhibit pathogen expansion and lipid oxidation, which helps in maximizing shelf stability of food products. Additionally, its active compounds, such as cinnamaldehyde, contribute to its strong antimicrobial properties (Alonso, 2024).

The majority of cinnamon essential oil is cinnamaldehyde, as the secondary metabolite (Rao et al., 2014). CNMA is also GRAS and is legally approved by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). Therefore, CNMA can be used as a safe food additive in food (Food and Drug Administration, FDA). According to a recent study where cinnamaldehyde was encapsulated in a chitosan/alginate complex coacervate (CINA@CH/ALG) and applied to cotton fabric to provide bacteriostatic and mold-resistant characteristics. The size of the chitosan/alginate nano capsules was strongly affected by the chitosan-to-alginate weight ratios and the cinnamaldehyde volume fractions. The study showed that cinnamaldehyde has a significant effect on *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, *Aspergillus flavus* (Sultan, 2024).

Enhancing the dosage of cinnamon EO in coatings can indeed inhibit a wide range of microorganisms due to its germicidal properties. However, cinnamaldehyde, can also impact the quality, color, firmness, flavor, and odour of the coated fruit. The optimal concentration of cinnamon EO should effectively inhibit microbial growth while maintaining the food's desirable sensory properties of food (Basaglia, 2021; Han, 2018; Sarengaowa, 2022).

This study aimed to develop an innovative 3D printed food packaging material using polylactic acid (PLA), a biodegradable and food-safe polymer recognized for its environmental sustainability and suitability for food contact applications. PLA was chosen due to its favorable mechanical properties, renewability and its potential to serve a greener solution to petroleum-based polymers. To enhance the functionality of the packaging, cinnamaldehyde, a natural bioactive compound with well-documented antimicrobial properties derived from cinnamon, was employed.

The integration of cinnamaldehyde into the packaging material was intended to address key challenges in food preservation by providing active protection against microbial contamination. This bioactive approach not only inhibits spoilage but also extends the shelf life and maintains the sensory and nutritional quality of the packaged food.

In this study, a prototype food packaging tray by using a 3D printer which was recently patented (PCT/TR2024/050383) was developed. The idea was to make narrow channel double-walled structured sides at verticals and horizontal of a regular tetragon and injection of liquid CNMA from laterals.

Therefore, it was aimed to determine followings;

- To demonstrate effectiveness of cinnamaldehyde in a new food packaging tray obtained by a 3D printer,
- To show feasibility of designing an active antimicrobial food packaging tray by means of using a 3D printer,
- To determine the stability of a traditional Turkish dessert, aşure, which was compatible with cinnamon, at cold storage period for 14 days by using the 3D printed active food packaging tray.

2. LITERATURE

Food packaging regulations aim to safeguard consumers from unacceptable levels of contamination through the use of appropriate packaging methods. Higher food standards safety was emerged by development of active food packaging. So, concerns of chemical contaminants and microbial contamination were minimalized (Gokoglu,2019). Synthetic additives are artificially produced substances not derived from natural sources, incorporated into food to enhance its appearance, texture, flavor, expiry period, freshness, and nutrient content. Although they are regulated by strict safety and regulatory guidelines, they remain a concern for many consumers. Consumer's awareness and choice on minimized processed food and ready to eat food brought about that development on packaging. Active food packaging, by extending shelf life, offers several benefits: it expands global market opportunities, increases profitability, and reduces food waste.

Maintaining food quality and safety, and increasing shelf-life, are achieved through the innovative technology of active food packaging (Alamri, 2021). The European regulation (EC) No 450/2009 describes active packaging as “deliberately incorporating components that would release or absorb substances into or from the packaged food or the environment surrounding the food” (European Commission 2009).

2.1 Active Packaging

The food packaging industry has faced a significant shift from traditional petroleum-based materials, such as polypropylene, to more sustainable, bio-based alternatives. The shift away from traditional plastics is fueled by the critical necessity to alleviate environmental impacts and decrease carbon emissions (Yin,2024). Due to their ability to decompose naturally and their lower environmental footprint, materials derived from biological sources, including starch, cellulose, and polylactic acid (PLA), present viable alternatives. These materials not only provide a sustainable alternative but also enhance food preservation by incorporating natural antioxidants and moisture control mechanisms. Current investigations have revealed the potential benefits of bio-based packaging materials in Increasing the duration of product viability and strengthening food safety standards. For instance, D’Almeida and De Albuquerque (2024) emphasize the importance of transitioning to create efficient, safe, and sustainable packaging, the focus is on integrating bio-based materials and intelligent packaging technologies.

Active packaging can be provided in two different ways; absorbing systems and releasing systems. These systems actively modify the conditions within the packaging to prevent spoilage, inhibit microbial growth, and maintain the sensory properties of the food (Jiang, 2023). Absorbing systems majorly control moisture, ethylene and carbon dioxide by using sachets, desiccants were inserted into multi layered boxes and drip absorbent pads beneath of the scent. While, the releasing systems, the emitters are used to ensure food and its environment are free from undesirable substances. In addition, antioxidant releaser, carbon dioxide emitters and antimicrobial packaging systems are some of the other types of applications of releasing systems (Kruijf, 2002).

The framework of the packaging is mainly designed on processing conditions of foods, structural properties of packaging materials. The former is basically structured on food research, while the latter one is laid out on packaging research. The desired shelf life, which is influenced by storage conditions, packaging material interaction, and the composition of the food product, is determined by the target release rate of the active compound from the packaging material (Uzunlu, 2018).

These approaches, adapted from Han (2003b), which highlights diverse methods for integrating antimicrobial functions into food packaging to enhance food safety and shelf life (Figure 2.1).

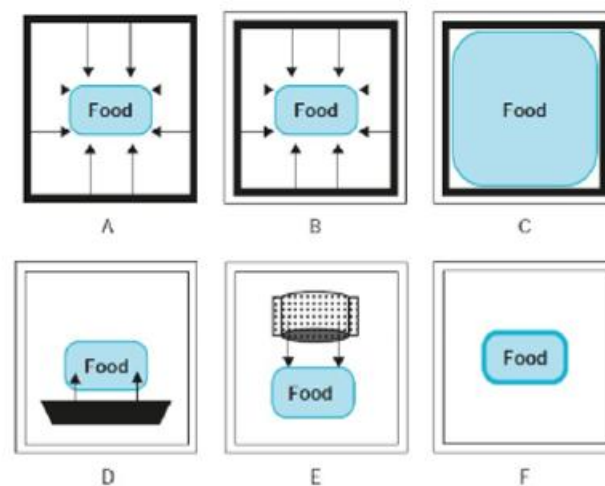


Figure 2.1 Several ways to integrate antimicrobial properties into food packaging A, utilizing specialized antimicrobial materials; B, applying coatings to existing packaging; C incorporating agents into polymer structures; D, using antimicrobial inserts; E, employing volatile release mechanisms; F, antimicrobial edible coating on foods (Han,2003b)

Incorporating natural antimicrobial compounds into biodegradable barrier materials for food preservation, consumable films, and coatings is a promising approach for the purpose of preventing microbial proliferation and prolonging food product

viability. This method leverages naturally derived compounds such as essential oils, herbal extracts, and enzymes, which are recognized for their antimicrobial defense. The packaging materials act as carriers for antimicrobial agents, which are gradually released into the food product. This controlled release helps to inhibit microbial growth, thereby enhanced product preservation and sustained quality, and ensuring the protection of food integrity.

Controlled release strategies are crucial in active packaging. These strategies ensure that antimicrobial substances are released at a rate that effectively inhibits microbial growth over an extended period. During the preceding years, a diverse array of antimicrobial agents has been functionally integrated into the packaging polymer to enhance food preservation efficacy and extend shelf life (Han ,2008). These antimicrobial agents can be incorporated into various biodegradable polymers like chitosan, polylactic acid (PLA), and starch-based materials, provides the dual benefit of food preservation the packaging and ecological friendly (Kamarudin, 2022). Recently, applied technology of antimicrobial preservation: direct or film/coating applications are exemplified in Table 2.1.

Table 2.1 Antimicrobial biopolymer food packaging systems enhanced with antimicrobial agents

Biopolymer Matrix	Antimicrobial agent	Observations	Reference
Polylactic acid	Pediocin	Polylactic acid (PLA) film, enhanced with active agents, limited the growth of <i>L. monocytogenes</i> in packaged raw pork	(Woraprayote, 2013)
Pectin and Polylactic acid	Nisin	The pectin-polylactic acid composite film, containing nisin as a bioactive antimicrobial compound, effectively suppresses <i>L. monocytogenes</i> proliferation in cultivation media and food matrices.	(Jin, 2009)
Chitosan	Lactic acid	A high level of antimicrobial activity against <i>Bacillus subtilis</i> is observed in chitosan-lactic acid films.	(Vartiainen,2004)
Alginate	Clove, cinnamon, and marjoram oil	Exhibited inhibitory activity towards <i>E. coli</i> , <i>S.aureus</i> , and <i>L.monocytogenes</i> .	(Alboofetileh, 2014)
Bio-based zein	Cinnamon essential oil	Suppression of <i>E. coli</i> and <i>S. aureus</i> growth	(Vahedikia, 2019)

Table 2.1 Antimicrobial biopolymer food packaging systems enhanced with antimicrobial agents (continue)

Biopolymer Matrix	Antimicrobial agent	Observations	Reference
Polyvinyl alcohol/Polyethylene glycol	Thyme essential oil	Biocidal impact against <i>E. coli</i> and <i>S. aureus</i>	(Min, 2021)
Nano-emulsion	Thyme and lemongrass oil	Antibacterial effect against <i>E. coli</i> and <i>L. innocua</i>	(Guerra-Rosas,20171")
Sodium caseinate	Matricaria recutita essential oil	Exhibition inhibitory activity towards <i>S. aureus</i> , and <i>E. coli</i>	(Aliheidari, 2013)
Chitosan/nanoclay nanocomposite	Rosemary essential oil	Complete decrease 1.2 to 2.1 log CFU/g for fresh poultry meat storage	(Souza, 2019)
Carboxymethyl cellulose/agar bio composite	Summer savory sensory oil	Demonstrated robust antibacterial activity against <i>B. cereus</i> and <i>E. coli</i>	(Abdollahi, 2019)

Mastromatteo et al. (2010) attempted to explain the release system by categorizing it into three main types:

2.1.1 Diffusion-limited release (Reservoir systems):

A depot system consists of a therapeutic compound encapsulated within a rate-regulating membrane (Figure 2.2). These membranes can be characterized by micro-porosity, macro-porosity, or non-porosity, with non-porous membranes representing the predominant selection. The efflux rate in these systems is governed by the membrane's dimension, exterior surface, and permeability coefficient. When the depot holds a surplus of the therapeutic compound, the efflux rate follows zero-order kinetics, maintaining a consistent release over time (Mastromatteo, 2010).

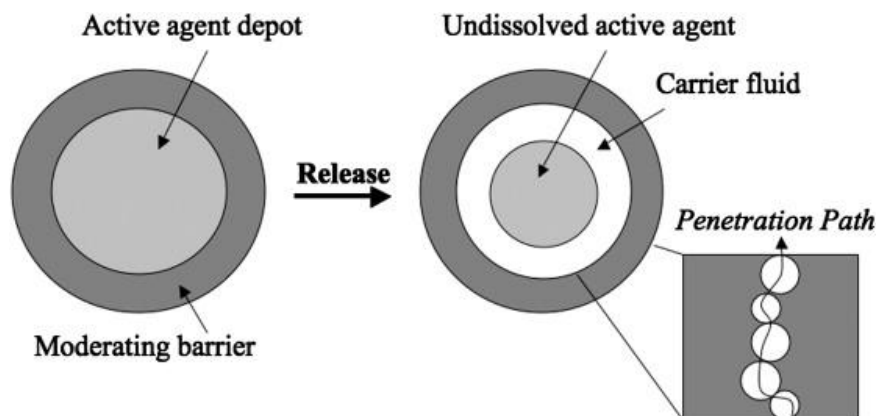


Figure 2.2 Reservoir system in active food packaging (Mastromatteo, 2010)

2.1.2 Swelling induced release:

The low diffusion coefficient of the active agent in swelling-controlled systems effectively impedes significant initial diffusion. The polymer matrix experiences volumetric expansion upon imbibing fluid from a compatible medium. The enhanced diffusion coefficient within the swollen matrix enables the active agent's release. In diffusion-controlled systems, the matrix remains unaffected during release. Swelling-controlled systems exhibit a membrane that undergoes a transition from a rigid to an elastic configuration. The more mobile Polymeric strands in this elastic configuration permits for faster diffusion of the active agent. The release rate is determined by the glass-to-rubber transition process (Crank, 1955; Peppas, 1984).

2.1.3 Biodegradation induced release:

Langer and Peppas (1983) categorized polymer erosion into two distinct mechanisms: surface (heterogeneous) and bulk (homogeneous). The two types of erosion, surface erosion and bulk erosion, differ in how they degrade the polymer material.

Surface erosion involves faster degradation at the surface, while bulk erosion involves uniform degradation throughout the material. The choice between these mechanisms depends on the desired release profile and the specific application requirements (Burkersroda, 1999).

A study was conducted on how electron beam irradiation, storage conditions, and pH levels of model food solutions impact the pattern of trans-cinnamaldehyde release from polyamide-coated low-density polyethylene films. The results highlighted that the release rate of trans-cinnamaldehyde was influenced by the storage temperature (4, 21, and 35 °C) and the pH (4, 7, and 10) of the food simulant solutions (10% aqueous ethanol). As anticipated, the antimicrobial diffusion rate was lower at the refrigerated temperature (4 °C), measuring 0.013 µg/mL/h, compared to higher temperatures, where it reached 0.029 µg/mL/h at 21 °C and 0.035 µg/mL/h at 35 °C. Irradiation reduced the diffusion rate of trans-cinnamaldehyde by 69% in irradiated films compared to non-irradiated controls so can be used to control the controlled elution of active agents, providing a foundation for the evolution of antimicrobial packaging systems (Han, 2008).

A study by Del Nobile (2008) investigated the capacity of thymol-containing zein films to suppress the growth of diverse microbial species, including *B. cereus*, *C. lusitaniae*, *Pseudomonas* spp., and *S. thermophilus*. The release behavior followed Fick's Second Law, with tests conducted at 25°C showing that the diffusion coefficient of

thymol was independent of its concentration. While the highest diffusion coefficient was observed at a 35% thymol concentration, it was not significantly different from the coefficient at 10%, suggesting that thymol concentration has little effect on the release kinetics.

2.2. Bioactive Packaging

Active food packaging has introduced an innovative dimension to packaging technology, leading to the development of bioactive food packaging. This advancement was driven by three key factors:

- Environmental issues are associated with the discharge of plastic waste into ecosystems.
- Materials with favorable environmental profiles, such as whey protein films, derived from by-products.
- Conventional food packaging techniques were found insufficient in some cases.

Bioactive packaging represents a significant advancement in food packaging technologies, offering solutions that go beyond traditional containment and protection. By incorporating bioactive agents such as antioxidants, antimicrobials, or enzymes, this innovative approach addresses growing consumer demands for sustainable and functional food packaging. food spoilage is not only minimized but also reduces the need for synthetic additives in food products, aligning with the global shift toward health-conscious and eco-friendly solutions (D’Almeida,2024).

With the awareness of consumers on natural based ingredients, the tendency of usage of bio natural materials, search and development, usage, and consumption has increased. Active food packaging materials can be produced by either biodegradable resources or synthetic polymers. Also, sometimes it is a blend composition. They are both used as raw materials in the packaging industry (Rahman, 2020).

2.2.1 Bioactive packaging ingredients/ additives

Bioactive packaging involves incorporating active ingredients or additives into packaging materials to enhance food preservation and safety. These components assist in maintaining food integrity and security, while simultaneously promoting environmental sustainability by lessening the reliance on artificial preservatives and packaging substrates. Some common bioactive ingredients/additives used in such packaging are summarized below.

2.2.1.1 Bionanocomposites

Nanocomposite materials are formulated from naturally occurring biopolymers, including chitosan, starch, cellulose, and alginate, among others, sourced from plant, microbial, and animal origins, which are plentiful in the environment. Their protective properties for packaging, such as tensile strength or resistance to gases, moisture, and microbial penetration, can be substantially augmented through the incorporation of nanofillers, such as nanoparticles (Wypij, 2023).

Despite the advantageous use of nanomaterials integrated with biopolymers in food and agricultural sectors, scientists posit a significant probability of nanomaterial migration into foodstuffs, necessitating comprehensive investigations into food safety and security. Given the paramount concern of food toxicity, rigorous evaluation of toxicological hazards, their control, and the regulatory frameworks governing these risks is imperative (Wypij, 2023).

Figure 2.3 illustrates the comparison between packaging without nanoclay and packaging incorporating nanoclay. The inclusion of active nanolayers (plastic composites) with a thickness of less than 100 nm in the polymer structure enhances the efficiency of antimicrobial agent transport within the packaging material (Yam, 2012).

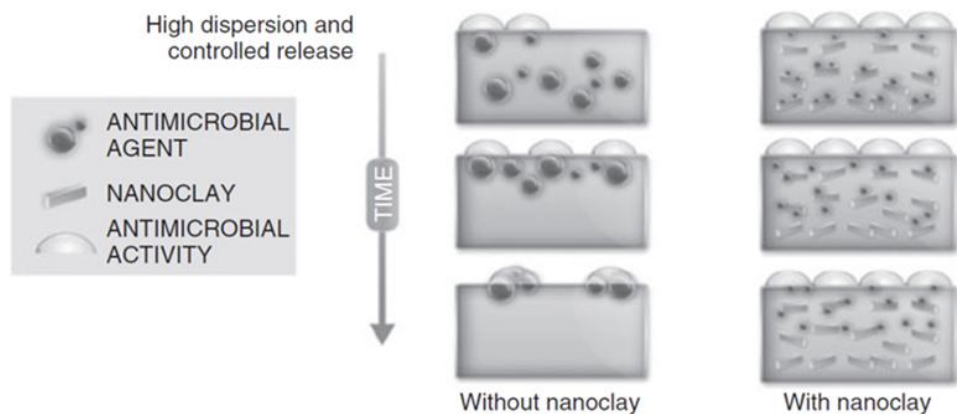


Figure 2.3 Schematics of the expected higher efficiency of active nano clays containing antimicrobial agents dispersed within packaging plastics and bioplastics (Yam, 2012)

2.2.1.2 Bioactives

The designation 'bioactive compounds' encompasses molecules that possess biological activity, including secondary metabolites present in plant matrices, which induce pharmacological effects in animals and humans and are potentially beneficial to health. Presently, biocidal inhibitors are incorporated into packaging matrices, such as films and trays, deposited as coatings on packaging surfaces, or encapsulated in sachets to combat deteriorating microorganisms and pathogens in food products. Consequently,

this method diminishes the quantity of active compounds required in food formulations (Nath et al, 2023).

Phenolic compounds, organic fatty acids, bacteriocins and essential oils are natural food additives. They are accepted as "GRAS" status which is an acronym for the phrase Generally Recognized as Safe by Food and Drug Administration (FDA). Some antimicrobial food packaging systems examples are shown in Table 2.2

Table 2.2 Antimicrobial food packaging systems, essential oils (Yildirim, 2018)

Active substances	Matrix/packaging application	Tested Microorganisms	Food application	Benefit	Reference
Cinnamon essential oil	Active multilayer polymeric composites	<i>E. coli O157:H7</i> , <i>S. cerevisiae</i>	Tomato puree	Effective inhibition of microbial proliferation: demonstrated high activity against <i>E. coli O157:H7</i> and <i>S.cerevisiae</i> .	Gherardi and others (2016)
Cinnamaldehyde	Chitosan-mediated reversible covalent immobilization	<i>S. aureus</i> , <i>E. coli</i> and in milk inoculated with <i>L. monocytogenes</i>	Milk	Expansion of microbiological product lifespan	Higuera and others (2015)
Carvacrol and thymol	A hybrid nanocomposite of clay and polyethylene	Gray mold	Strawberry	Effective inhibition of gray mold	Campos-Requena and others (2015)
Vanillin	Gelatin films sourced from starfish biomass	<i>Listeria monocytogenes</i>	Crab stick	Diminution in <i>L. monocytogenes</i> growth	Lee and others (2016)
Oregano	Pectin, consumable layers	Fungi	Tomatoes	Mitigation of fungal decay and amplification of antioxidant activity.	Rodriguez-Garcia and others (2016)

Cinnamaldehyde (CNMA), which is extracted from *Cinnamomum spp.*, is a natural antibacterial active ingredient (Faikoh, 2014). CNMA is also generally recognized as safe (GRAS) and adheres to the regulatory standards of Food and Agriculture Organization/World Health Organization (FAO/WHO) so it means CNMA is a reliable source of food preservative (Sun, 2020). Previous studies have shown that cinnamaldehyde (CNMA), a major component of cinnamon essential oil, interacts with bacterial cell membranes. This interaction can disrupt membrane integrity, inhibit ATPase activity, and interfere with energy metabolism. CNMA has a remarkable antifungal and antibacterial activity, however it has not been completely defined as the mechanism of inhibition (Smid, 1996). It has been shown that bacterial cell morphology, membrane integrity and permeability are damaged when CNMA is used at low concentration (0.31 mg/mL) in *Escherichia coli* and *Staphylococcus aureus*, bacteria that cause pathogenicity to humans through food consumption. Such adverse effects of cinnamaldehyde are predicted to be responsible for the reductions in biomass production rate and morphogenesis. Additionally, increasing cinnamaldehyde concentration linearly increases bacterial membrane damage (Shen, 2015).

In another study, Mondéjar-Lopez et al. (2024) encapsulated cinnamaldehyde in chitosan nanoparticles and incorporated it into a 2% chitosan film. A biphasic release profile has been demonstrated with sustained release over 5 days. The antimicrobial activity of the films was determined to be 4.85 log cfu/g for *Listeria monocytogenes* and a 1.26 log cfu/g reduction in the total number of coliforms within 20 days (Mondéjar-López et al. 2024). Another study tested the polylactic acid biofilm uploaded with cinnamaldehyde for cheese packaging to display an active food packaging material. The strongest antibacterial activity was seen in the PLA-PBATTiO₂-7%Cinn film against *S. aureus* and *E. coli*. Cheese packaged with PLA-PBAT-TiO₂-7%Cinn film showed minimal weight loss during 12 days of storage and increased its antibacterial activity against *E. coli*. The use of cinnamon oil-loaded TiO₂ in films has shown positive effects on the shelf life, quality and safety of the food product, and it has been stated that it has a high potential for commercial use (Sharma et al. 2023).

In similar studies (Uzunlu & Niranjana, 2017), 5% and 10% concentrations of cinnamaldehyde inactivated *S.aureus* and *E.coli* by 6 log cfu/g in films made with polycaprolactone at different temperatures (37°C, 20°C, 4°C). and durations (11 to 28 days). It was determined that approximately 80% of cinnamaldehyde was released in the first 21 hours, but then its stability was achieved over time. In another study, the

antimicrobial activity of cinnamaldehyde film with 1% concentration of polycaprolactone in manti packages was tested. As a result, it was determined that the active films showed a bacteriostatic effect against *E. coli* and *S. aureus* when inoculated at 5 log cfu/g to food samples for 28 days at 4°C (Uzunlu, 2019).

Cinnamaldehyde has been shown to exert significant antioxidant properties. A study has demonstrated that cinnamaldehyde can prevent chain initiation, scavenge free radicals, and quench singlet oxygen formation. These activities forefront its potential in reducing oxidative stress and protecting cells from damage (Friedman 2017).

There are several encapsulation techniques designed to enhance the water dispersibility and stability of cinnamaldehyde under severe conditions: liposomes, emulsions, biopolymer nanoparticles, complex coacervation, molecular inclusion, and spray drying. Each encapsulation technique employs different wall materials and mechanisms, which come with their own sets of advantages and drawbacks (Culas, 2024).

Liposomes are effective due to their high surface area and strong antimicrobial properties. However, their lipid membranes can be vulnerable to oxidation and hydrolysis, which can compromise their stability and effectiveness over time (Nadarajah, 2021).

Emulsions are a popular choice for encapsulating cinnamaldehyde due to their high dispersibility in water and strong antimicrobial properties. However, as it is mentioned, they do have some drawbacks like flocculation, aggregation, and gravitational separation (Muhoza, 2023).

Yet some of the adverse properties such as volatility, insolubility in water, easily decomposition and deterioration, sensitivity to light and temperature, and having a pungent odour limits the usage of CNMA in food technology (Yi ,2010). A study conducted on smoked horse meat sausages, sensory evaluation and antimicrobial properties of cinnamaldehyde were reported. The findings showed that the group with cinnamaldehyde named as 'KQ batch' which contained cinnamaldehyde achieved a higher appreciation score by the panelists in color, odour and taste criteria compared to the other batches (Yu et. al., 2024).

The combination of clove and cinnamon oils has shown promising results in inhibiting the growth of various bacteria, especially in the vapor phase. The synergistic effect mentioned, where the combined oils are more effective than when used separately, is particularly interesting and could have significant implications for food safety and

packaging (Goñi, 2009). However, the precise molecular targets and mechanisms by which CNMA affects foodborne pathogens are still being investigated.

2.3 Barrier and Mechanical Properties of Active Food Packaging

2.3.1 Barrier properties of active food packaging

The barrier properties of a packaging material refer to its capacity to impede or regulate the permeation of gases (e.g., oxygen, carbon dioxide), water vapor, volatile organic compounds, and other entities between the food product and the surrounding environment. The integration of active functionalities with robust barrier characteristics is paramount for efficacious food preservation strategies. While the barrier function mitigates external factors contributing to spoilage, the active component addresses specific deterioration mechanisms within the packaging headspace or the food itself.

The food packaging sector is seeking to substitute fluorinated synthetic polymers with natural and biodegradable materials that offer resistance to grease, water vapor, and gas permeation. For this reason, several natural, decomposable polymers, including proteins, lipids, and polysaccharide-based coatings, such as zein, chitosan-beeswax, chitosan, and hydroxy-propyl methylcellulose, have been shown to be effective. However, many of them are partially hydrophilic, which leads to a decline in their barrier capabilities under conditions of high moisture (Sanchez, 2014).

PLA, a biologically decomposable and eco-friendly polymer originating from lactic acid produced by the microbial conversion of carbohydrates from sources like corn, sugar cane, or potato (Vink, 2003), has been launched commercially as an excellent substitute for artificial polymers (Gruber, 2002).

Muller et al. (2017) incorporated cinnamaldehyde essential oil into PLA-starch dual-layer films. Given starch's superb oxygen impermeability and PLA's notable water vapor resistance, the resulting layered film exhibited outstanding barrier attributes. Laboratory studies demonstrated that PLA/S films containing CIN suppressed *E. coli* and *L. innocua* at concentrations exceeding their minimum inhibitory concentrations.

2.3.2 Mechanical properties of active food packaging

Polylactic acid (PLA) is a biologically decomposable thermoplastic and aliphatic polyester derived from replenishable and eco-friendly materials abundant in starch. Owing to its biodegradability, biocompatibility, and robust mechanical attributes, including tensile strength and Young's modulus similar to those of polystyrene (PS) and polyethylene terephthalate (PET), PLA has been regarded as a significant biopolymer.

Nevertheless, its diminished impact resistance has led to the exploration of numerous additives to enhance its characteristics. Substances like food and its byproducts, natural oils, organic matter, diverse fiber types, nanoparticles, and others have been integrated into the PLA structure with the goal of exhibiting superior mechanical performance (Acosta, 2025).

Boro et al. (2022) explored the use of clove essential oil together with alkali-treated halloysite nanotubes (NHNT) to improve the attributes of PLA film. They fabricated the film using a simple solution casting method and enhanced the halloysite nanotube surface area by treating it with an alkaline NaOH solution. The film labeled PCOH_{0.5}, containing 0.5 wt.% NHNT and 200 µL CEO, exhibited the most significant enhancements in properties compared to pure PLA. Specifically, its mechanical characteristics, including tensile strength, flexibility, and elastic modulus, showed increases of 20%, 682%, and 38%, respectively. The findings suggest that these PLA/CEO/NHNT nanocomposite films are effective and hold promise for their potential application as active food packaging.

In recent years, aiming for enhanced characteristics, Ordoñez et al. (2023) developed a PLA/Starch/PLA (PSP) three-layered film integrated with ferulic or cinnamic acid using electrospinning or solution pulverization. These triple-layered PSP structures displayed superior strength and impermeability compared to single-layered PLA and starch films and satisfied food packaging demands.

PLA has served as an alternative to synthetic resources, showcasing desirable mechanical and tensile features and presenting minimal oxygen permeability (Sanchez, 2014).

2.4 Aşure

As a Turkish tradition, aşure dessert, which is cooked each year on the days of Muharram according to the Hijri calendar, continues from the Ottoman Empire period to the present day. The origin of aşure dessert dates to ancient times and has an important place in many cultures. The etymology of the term 'aşure' traces back to the Arabic word "asherah" meaning "ten" and refers to the tenth day of the month of Muharram (Sacıkaralı, 2015).

The emergence of aşure dessert dates back to the time when Prophet Noah's ship survived the flood and landed on Mount Cudi. According to rumor, this dessert was made with a mixture of the last foods left on the ship (such as wheat, beans, chickpeas). For this

reason, aşure is considered a symbol of abundance and sharing (Sacıkaralı, 2015). Aşure took its place as an important dessert during the Ottoman period. In Evliya Celebi's Seyahatname, it is mentioned as a meal that should be done on the tenth day of Muharram. In the Alawite culture, aşure is cooked and shared in memory of Hussein, who was martyred in the Battle of Karbala. In summary, aşure is not only a dessert but also a symbol of cultural heritage and communal harmony. Its rich nutritional profile and historical significance continue to make it a cherished dish in Turkish cuisine and beyond.



Figure 2.4 Traditional presentation of aşure (Anonymous, 2024)

Traditionally, aşure is made by cooking a mixture of grains, legumes, and dried fruits. Common ingredients include wheat berries, chickpeas, white beans, raisins, dried apricots, and figs. These components are sweetened, often with sugar or honey, and flavored with spices such as cinnamon. As illustrated in Figure 2.4 is typically garnished with nuts like walnuts or almonds and pomegranate seeds, adding both texture and visual appeal (Sacıkaralı, 2015).

Although the literature on aşure is limited, research was conducted on milk desserts (pudding, milk pudding, water pudding, etc.), which are other listed foods of the Turkish Food Codex classified in “ready to eat dessert”.

In accordance with that study, bacterial load may increase during production, storage, transportation and presentation to the consumer if hygiene standards and cold chain are not readily applied. Therefore, it might result in deterioration of foods by means of reduced microbial quality. Public health might be under risk in such circumstances

(Sahiner, 2019). Up to date to our best of knowledge no literature has been found for aşure in the field of food engineering.



3.MATERIALS AND METHODS

3.1 Materials

The ingredients for aşure were as follows;

Aşure wheat (200 g), kidney beans (150 g), chickpeas (140 g), dried figs (10 pieces), dried apricots (10 pieces), raisins (300 g), table sugar (1000 g) were purchased from local markets in Alanya. Antalya, Turkiye.

Materials used for packaging and laboratory equipment;

Microbiological Media: Maximum Recovery Diluent (Merck, Germany), Plate Count Agar (Merck, Germany), Buffered Peptone Water

Embossed vacuum bag (240x240 mm), Thickness: 110 Micron, Weight: 100 gr / M² (Packtech, Turkey)

The 3D printed food packages material used in this study is an eco-friendly thermoplastic, polylactic acid (PLA), sourced from renewable agro-based materials. Polylactic acid (PLA) stands as the foremost manufactured biopolymer, presently favored for select food packaging applications involving biological contact, owing to its ability to decompose through biological and non-biological processes, transparency, rigidity, low-temperature thermal sealing, regulatory clearance as Generally Recognized as Safe (GRAS), and adequate protection against flavor and aroma transfer (Yadav, 2018). The provided filaments (ESUN, China) made of PLA +, have a diameter of 1.75 mm. The PLA+ filament's enhanced properties confer high toughness, high precision, high-speed printing, and no warping characteristics. Additional technical specifications provided by the manufacturer for the PLA+ filament, are detailed below Table 3.1.

Table 3.1 PLA+ filament (ESUN) technical properties

Property	Recommended Printing Parameter
Printing Temperature	210-230 °C
Printing Speed	40-100 mm/s
Density	1.23 g/cm ³
Tensile Strength	60 MPa
Elongation at break	20 %
Bending strength	74 MPa
Flexural Modulus	1973 MPa

The details of the 3D food packages produced by a 3D printer using Creality Ender- 5 Pro (Shenzhen, China) by Fused Deposition Modeling (FDM) printing technology. The 3D printer used in this research operates with a filament diameter of 1.75 mm and a standard nozzle diameter of 0.4 mm. It is capable of reaching a maximum

nozzle temperature of 260°C and a maximum heat bed temperature of 100°C. The standard printing speed is 50 mm/s and maximum is 100 mm/s. These specifications ensure high precision and quality in the printed samples (Figure 3.1).

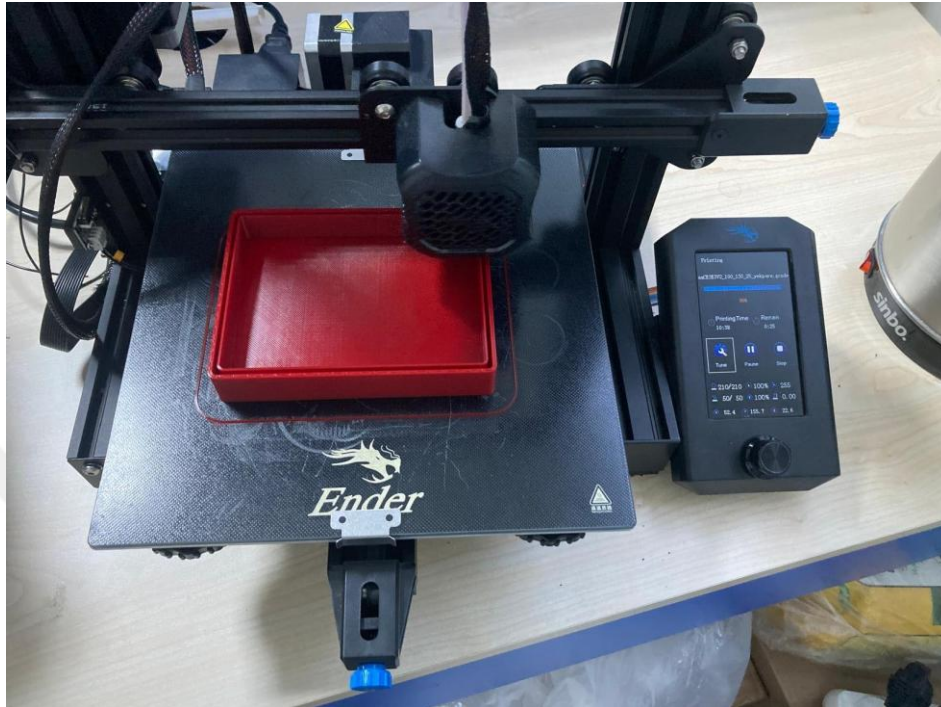


Figure 3.1 3D food packages printing process, Creaform Ender- 5 Pro

3.1.1 Design features of the 3D food packaging:

The 3D food pack comprises two nested trays, namely Tray A and Tray B which are manufactured using PLA material (Figure 3.5). There is an empty volume between the outer and inner trays (Tray A and Tray B), which was filled with an antimicrobial substance (Figure 3.1). Before placing ašure in the 3D food packages, each package was exposed to UV light for 20 minutes for sterilization.

The inner container, Tray, is the one in direct contact with the food. There is an empty space between Tray A and B (outer and inner containers) intended to be filled with an antimicrobial-antioxidant substance.

Each lateral face of Tray A forms a 4-degree angle with the nearest perpendicular plane. The corners of Tray A are rounded. The open mouth of Tray A has a lip (d) for sealing purposes. Inside the bottom of Tray, A, there are fifty-four supports (c) on which Tray B rests (Figure 3.4). Similarly, each lateral face of Tray B forms a 4° angle with the nearest perpendicular plane, and its corners are also rounded. To fill the empty space between Tray A and B with the antimicrobial-antioxidant substance and to allow air to escape from this space, there are four holes (d2) on the lips (d1) of Tray B (Figure 3.2,

Figure 3.3). In another configuration of the invention, to achieve the same purpose, the lips (d1) of tray B feature channels (not shown in figures) in the longitudinal and latitudinal planes.

The selection of the antimicrobial-antioxidant substance to be filled in the empty space between Tray A and B is based on the technical effects demonstrated by the substances listed in that section.



Figure 3.2 The side views of Tray A and B

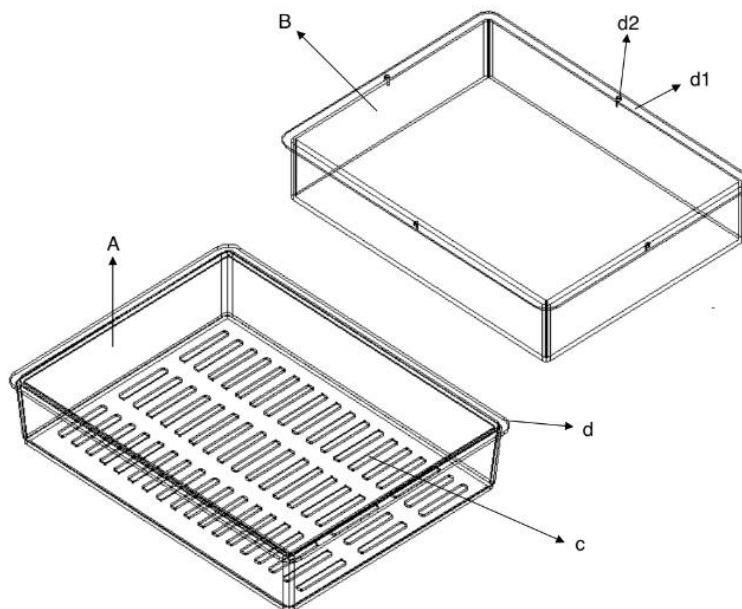


Figure 3.3 The perspective views of Tray A and B

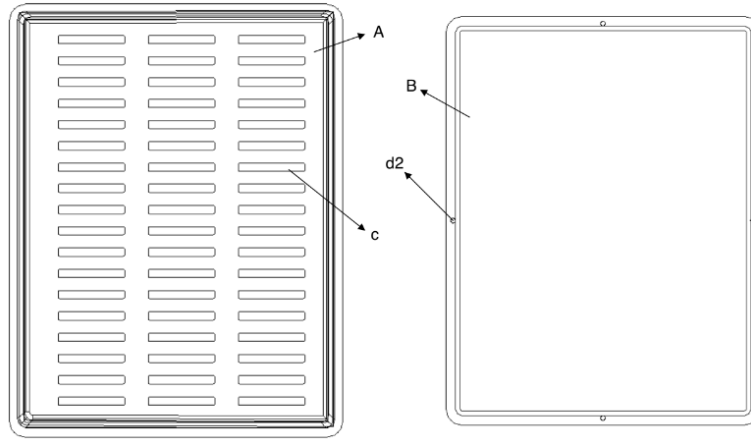


Figure 3.4 The top views of Tray A and B



Figure 3.5 A completed 3D printed food pack

3.2 Methods

3.2.1 Cooking procedure for aşure

Aşure was prepared in the direction of the guide of Edirne Provincial Directorate of Culture Tourism (Anonymous, 2023).

Wheat for Aşure, beans, and chickpeas were left in water overnight and were allowed to swell until morning. After they swelled, each legume was cooked separately under pressurized steam. First, wheat for Aşure was placed in a pressure cooker, twice the amount of water was poured over the wheat and cooked for 10 minutes, beans were cooked for 10 minutes under the same conditions, and chickpeas were cooked for 20 minutes. On the other hand, figs, dried apricots, and raisins were boiled in water approximately 5 cm above the area they covered in the pot and softened. Wheat for Aşure, beans, and chickpeas were combined in a separate pot and boiled together for 30 minutes

and sugar was added. Fruits were added 5–6 minutes before the end of the cooking process and the cooking process was completed (Anonymous, 2023).

For the physicochemical and microbiological analysis 2400 grams of aşure was cooked and placed into 16 trays. While, for sensory analysis 5400 grams of aşure was cooked and placed into 18 individual trays containing 300 grams.

Each individual package trays were vacuumed by using a household type vacuum (Kumtel, Model HVSE-01, Kayseri). In previous preliminary experiments, it was decided to add CNMA at concentrations of 2% (v/w) and 5% (v/w) to prevent consumers from encountering an unpleasant, pungent odour when they opened the package, while also providing antimicrobial activity in the gas phase. CNMA (Sigma W228613, Germany) was prepared from a concentration of 95% \geq purity, with Tween 80 and distilled water at different concentrations (%2 (v/w), %5 (v/w)). 1 mL of each concentration was taken and injected into the packaging designed by Uzunlu and Darıcık and was stored at 4°C for 14 days, and the following periodic analyses were performed.

3.2.2 Microbiological analysis

Microbiological analyses were planned to be studied in accordance with the Turkish Food Codex Microbiological Criteria Regulation Table 3.2 (dated 12.29.2011 and numbered 28157).

Table 3.2 Turkish Food Codex Microbiological Criteria Regulation

Food	Microorganisms/ toxins/ metabolites	Limits	Reference Method
1.13.4. All kinds of ready-to-eat (cooked) desserts (pudding, custard, cream, asure, water pudding, etc.)	Staphylococcal enterotoxin	Should not be present in 25 g	
	<i>Salmonella spp.</i>	Should not be present in 25 g	EN/ISO 6579

Turkish Food Codex Parameters are *Salmonella spp.*, Staphylococcal enterotoxins. Total Viable Counts (TVC) were studied to determine hygienic quality of the product. TVC analyses were performed on the 0th, 3rd, 7th and 14th days of the storage period, while *Salmonella spp.* and Staphylococcal enterotoxins were performed on 0th and 14th days.

3.2.2.1 Total viable counts

10 g of aşure samples were accurately weighed and transferred into bottles containing 90 mL of Maximum Recovery Diluent (MRD). Serial decimal dilutions were prepared, and appropriate concentrations were transferred onto Plate Count Agar (PCA)

in Petri dishes using a 100 µL pipette. The samples were spread evenly across the surface of the agar and incubated at 35°C for 48 hours. (BAM Chapter 3: Aerobic Plate Count)

3.2.2.2 *Salmonella* spp.

Salmonella spp. detection in aure samples was conducted following a combined protocol derived from ISO 6579 and FDA methodologies. A 25 g aliquot of each sample underwent pre-enrichment in 225 mL of Buffered Peptone Water (Merck, Germany) and was incubated at 37 °C for 16-20 hours. Subsequently, 1 mL of the pre-enrichment culture was transferred to 10 mL of Selenite Cystine Broth (Merck, Germany), and 0.1 mL to 10 mL of Rappaport Vassiliadis Broth (Merck, Germany) for selective enrichment. Both enrichment cultures were incubated at 35 °C for 24 hours. Following selective enrichment, loopfuls of each broth were streaked onto Hektoen Enteric Agar (Merck, Germany) and Xylose Lysine Deoxycholate Agar (Merck, Germany) plates and incubated at 35 °C for 20-24 hours. Presumptive *Salmonella* colonies were identified and five typical colonies were selected for confirmation. Confirmation was performed using Lysine Iron Agar (Merck, Germany) and Triple Sugar Iron Agar (TSI) (Merck, Germany) slants, incubated at 35 °C for 24 hours. Presumed-positive cultures from TSI slants were then transferred to Urea broth tubes and incubated at 35 °C for 24 hours. Final identification was achieved through API 20 E (Analytical Profile Index, Biomerieux) biochemical testing and serological polyvalent flagellar and somatic antigen assays. (Anonymous, 1996).

3.2.2.3 Staphylococcal enterotoxin

Staphylococcal enterotoxin determination was performed on the 0th and 14th days by VIDAS method based on service procurement at Antalya Food Control Laboratory Directorate (Ministry of Agriculture and Food). VIDAS is a reliable and easy-to-use automatic benchtop immunoanalyzer. It provides high-quality test results with enzyme-linked fluorescence assay (ELFA) technology.

3.2.3 Physicochemical analyses:

3.2.3.1 Headspace gas composition

CNMA release was determined by Gas Chromatography (GC) for 2% (v/w) and 5% (v/w) concentrations on days 3 and 14. GC/MS analysis was performed on a Thermo GC Ultra Gas Chromatograph/ ISQ instrument (Figure 3.6).

HP-5 ms (5% phenyl methyl siloxane) column (15 m) was used for the separation. Injection was performed by manual injection from the headspace (Li, 2012).

For this purpose, packages were transported under cold chain to Antalya at Akdeniz University Food Safety and Agricultural Research Center, and immediately analyzed using a GC-MS instrument (Thermo GC Ultra Gas Chromatograph/ ISQ).



Figure 3.6 GC-MS instrument (Thermo GC ultra-gas chromatograph)

In modeling the diffusion of CNMA, pore diameters ranging from 1 to 400 nm necessitate the consideration of both Fickian and Knudsen diffusion, as referenced in the literature (Desobry, 2005). The release of antibacterial agents from biopolymers is governed by a mass transfer process analogous to heat conduction. In practical applications, most diffusion processes result in unsteady diffusion, where the concentration varies over time. This behavior is typically modeled using Fick's Second Law of diffusion (Dury-Brun, 2007, Cussler, 2009; Bird et al., 2007).

Fick's Second Law describes how concentration changes over time due to diffusion (Equation 1.1)

$$\frac{\partial C}{\partial t} = D \cdot \frac{\partial^2 C}{\partial x^2} \quad (1.1)$$

- C = Concentration (mg/m³)
- t = Time (s)
- x = Position (m)
- D = Diffusion coefficient (1×10⁻⁹ m²/s)

This is a partial differential equation, and solving it analytically requires boundary and initial conditions. We'll model the system as a semi-infinite slab with a fixed diffusion path length (the package wall thickness), assuming the cinnamaldehyde diffuses from the inner surface (where it's initially concentrated) to the outer surface (headspace, where concentration is initially zero).

- Surface area (A): 0.061 m²
- Volume of cinnamaldehyde: 4 mL
- Initial concentration (C₀): 0.39 mg/mL
- Diffusion coefficient (D): 1×10⁻⁹ m²
- Diffusion path length (L): 2.96 mm=2.96×10⁻³ m
- Time (t): 14 days = 14×24×60×60=1,209,600 s

The package wall was modelled as a thin slab of thickness $L = 2.96 \times 10^{-3}$ m. The cinnamaldehyde is initially uniformly distributed inside the package, and diffusion occurs through the wall to the headspace. It was assumed:

- At t=0, the concentration inside the package (at x=0) was C₀=0.39×10⁶ mg/m³ and the concentration at the outer surface (at x=L) was 0 mg/m³ (headspace, assuming instantaneous removal).
- The package wall was a barrier through which diffusion occurs, and the cumulative mass released into the headspace was measured.
- This setup resembles a diffusion problem with a Dirichlet boundary condition (fixed concentration at the boundaries). The solution to Fick's Second Law for a slab with one side at C=C₀ (initially) and the other at C=0, over time, can be approximated using a series solution. However, for thin slabs and long times, a simplified cumulative release formula could be used.

The cumulative mass released (M_t) from a slab can be approximated using the following solution for diffusion out of a slab (Crank, *The Mathematics of Diffusion*, 1975) (Equation 1.2)

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \exp(-D(2n+1)^2 \pi^2 \frac{t}{L^2}) \quad (1.2)$$

- M_t = Mass released at time t
- M_∞ = Total mass available for release
- L = Slab thickness

- D = Diffusion coefficient

For long times (when Dt/L^2 was large), the series was quickly converged, and an approximation was made by taking the first few terms (often just the first term was found to dominate).

$$L^2 = (2.96 \times 10^{-3})^2 = 8.7616 \times 10^{-6} \text{ m}^2$$

$$Dt = (1 \times 10^{-9}) \times 1,209,600 = 1.2096 \times 10^{-3} \text{ m}^2$$

$$Dt = (1 \times 10^{-9}) \times 1,209,600 = 1.2096 \times 10^{-3} \text{ m}^2$$

$$\frac{Dt}{L^2} = \frac{1.2096 \times 10^{-3}}{8.7616 \times 10^{-6}} \approx 138.07$$

Since Dt/L^2 was much greater than 1, the diffusion process was considered well advanced, and most of the cinnamaldehyde was assumed to have been diffused out by 14 days. The first term ($n = 0$) of the series was used for a good approximation:

$$\frac{M_t}{M_\infty} \approx 1 - \frac{8}{\pi^2} \exp\left(-\frac{x^2 Dt}{L^2}\right)$$

$$\frac{x^2 Dt}{L^2} = x^2 \times 138.07 \approx 1364.49$$

$\exp(-1364.49) \approx 0$ (very small, essentially negligible)

$$\frac{M_t}{M_\infty} \approx 1 - \frac{8}{\pi^2} \times 0 \approx 1$$

It was indicated that nearly all the cinnamaldehyde had been diffused out by 14 days, which was aligned with the large value of Dt/L^2 .

$$M_\infty = 1.56 \text{ mg}$$

$$M_t \approx 1 \times M_\infty = 1.56 \text{ mg}$$

It was confirmed that, under these conditions, the entire 1.56 mg of 2% CNMA and 3.96 mg of 5% CNMA was released by 14 days. Convert PPM to Concentration (mg/m^3) (Equation 1.3)

Cinnamaldehyde's molar mass is 132.16 g/mol. At standard conditions (25°C, 1 atm), 1 ppm of a gas corresponds to a concentration in mg/m^3 using the formula (Cussler, 2009, Perry, 2008):

$$\text{Concentration} \left(\frac{\text{mg}}{\text{m}^3} \right) = \text{ppm} \times \frac{\text{molar mass} \left(\frac{\text{g}}{\text{mol}} \right)}{24.45 \left(\frac{\text{L}}{\text{mol}} \right)} \quad (1.3)$$

Where 24.45 L/mol 24.45L/mol is the molar volume of an ideal gas at 25°C and 1 atm.

$$\text{Concentration (mg/ m}^3\text{)} = \text{ppm} \times \frac{132.16}{24.45} \approx \text{ppm} \times 5.404$$

- 5% CNMA:

$$\text{Day 3 } 8.33 \text{ ppm} \times 5.404 = 45.015 \text{ mg/m}^3$$

$$\text{Day 14 } 9.08 \text{ ppm} \times 5.404 = 49.068 \text{ mg/m}^3$$

- 2% CNMA:

$$\text{Day 3 } 2.07 \text{ ppm} \times 5.404 = 11.186 \text{ mg/m}^3$$

$$\text{Day 14 } 2.31 \text{ ppm} \times 5.404 = 12.483 \text{ mg/m}^3$$

The package dimensions are:

- Length: 0.13 m
- Width: 0.10 m
- Height: 0.025 m

The total volume of the package is:

$$\text{Total volume} = 0.13 \times 0.10 \times 0.025 = 0.000325 \text{ m}^3 = 325 \text{ cm}^3 = 0.325 \text{ L}$$

In food packaging, the headspace is typically 10–20% of the total volume to allow for gas exchange and expansion so it was assumed a headspace of 15% of the total volume as a reasonable estimate:

$$\text{Headspace volume} = 0.15 \times 0.000325 \text{ m}^3 = 0.00004875 \text{ m}^3 = 48.75 \text{ cm}^3 = 0.04875 \text{ L}$$

Mass in Headspace (Equation 1.4)

$$\text{Mass in the headspace} = \text{Concentration} \left(\frac{\text{mg}}{\text{m}^3} \right) \times \text{Headspace volume} (\text{m}^3) \quad (1.4)$$

- 5% CNMA:

$$\text{Day 3 } 45.015 \text{ mg/m}^3 \times 0.00004875 \text{ m}^3 = 0.0021945 \text{ mg}$$

$$\text{Day 14 } 49.068 \text{ mg/m}^3 \times 0.00004875 \text{ m}^3 = 0.0023926 \text{ mg}$$

- 2% CNMA

$$\text{Day 3 } 11.186 \text{ mg/m}^3 \times 0.00004875 \text{ m}^3 = 0.0005453 \text{ mg}$$

$$\text{Day 14 } 12.483 \text{ mg/m}^3 \times 0.00004875 \text{ m}^3 = 0.0006086 \text{ mg}$$

3.2.3.2 pH measurements:

The measurements were conducted on the storage days of 0, 3, 7 and 14 using Milwaukee, Mi151 pH/ORP/Temperature Bench Meter. 10 g of sample weighed on 90 ml of distilled water beakers. Aşure sample was homogenized, then triplicate readings were taken and the mean value was expressed (AOAC, 2013).

3.2.3.3 Color analysis:

The color analysis was performed at the department's laboratory on days 0, 3, 7 and 14 by taking the average of three measurements in terms of CIE, L^* , a^* , b^* values (Konica Minolta/Data processor DP-400, Japan). CIE L^* , a^* , and b^* colorimetric parameters (lightness, redness, and yellowness, respectively) were measured in triplicate and subsequently analyzed.

3.2.3.4 Dry matter basis

Dry matter of asure was also performed on days 0, 3, 7 and 14 in the food department laboratory. According to the gravimetric method, 5 grams of sample from each concentration were placed in a pre-weighed drying container. Total weight of the sample and pre-weighed container was measured on a precision balance and recorded. The drying container was placed in a drying oven, and the temperature was set to 105°C. The drying process continued to constant weight. The attainment of constant weight was determined when the difference between two consecutive weightings, conducted at 1-hour intervals near the end of the drying process, fell to 5 mg or less. Once constant weight was reached, the lid of the drying container was closed, and the container was transferred to a desiccator to cool. After cooling, the drying container was weighed with its lid closed, using a balance with a minimum sensitivity of 2 mg. The amount of water in the sample was determined by the change in weight from the start to the end of the drying process. The remaining weight was calculated as the total dry matter. The kinetics of dehydration are influenced by the specific food substance (Cemeroglu, 2007).

3.2.3.5 Oxygen permeability

The oxygen transmission rate (OTR) was determined utilizing an oxygen permeation analyzer (8001, Systech Illinois Instruments Co., Johnsburg, IL, USA) (Figure 3.7). The experimental temperature for the samples was 23°C and 0% relative humidity (RH) in accordance with the ASTM D 3985 method (Salmas, 2021). Oxygen transmission rate (OTR) values were quantified in in cubic centimeters of O₂ per square meter per day (cc O₂/m²/day). These analyses were performed at the Department of Food Science and Technology, University of Patras, Greece.

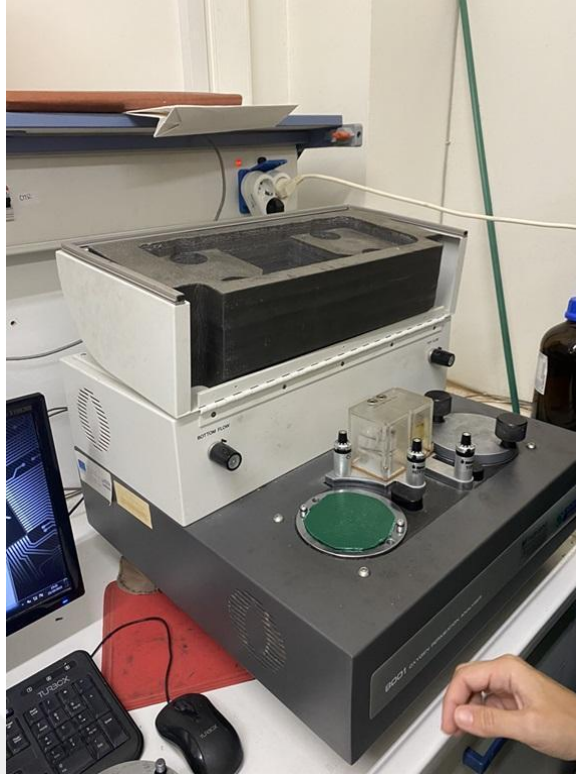


Figure 3.7 Oxygen permeability analysis of 3D printed food pack sample using an oxygen permeation analyzer

Gas permeability through polymers can be represented as follows (Equation 2.1):

$$\frac{J}{A} = P e_{gas} \cdot \frac{\Delta C}{\Delta x} \quad (2.1)$$

where J/A [$\text{mol}/(\text{cm}^2\text{s})$] indicates the specific amount of gas passing through the membrane, $P e_{gas}$ (cm^2/s) is the permeability coefficient, ΔC (mol/cm^3 STP) represents the pressure gradient across the two opposite sides of the membrane, and Δx (cm) denotes the membrane thickness (Equation 2.2);

$$P e_{gas} = \frac{J}{A \times \Delta C} \times \Delta x \quad (2.2)$$

By performing a dimensional analysis, the term $J/(A \Delta C)$ [cm^3 STP/ (cm^2s)] corresponds to the OTR measurements. Therefore, in the context of the study oxygen gas (Equation 2.3);

$$P e_{O_2} = OTR \times \Delta x \quad (2.3)$$

The oxygen permeability coefficient values (PeO_2) for the tested samples were determined by multiplying the OTR values by the average thickness of the 3D printed food packages (Yasuda, 1975).

3.2.3.5 Tensile properties

The tensile properties of 3D-printed food packages made from PLA were measured using the ASTM D638 method. A Shimadzu (AX- 5kNt model) instrument (Shimadzu, Japan) was used to measure the tensile properties of vertical walls of inner and outer layers of the 3D food pack (three different samples) at an across head speed of 2 mm. min⁻¹ (Figure 3.8). The samples were shaped like dumbbells with gauge dimensions of 10 x 3 x 0.22 mm (Figure 3.9). The stress, strain, and modulus of elasticity values were derived from the recorded force (N), deformation (mm), and gauge dimensions.



Figure 3.8 Shimadzu AX-G 5kNt instrument



Figure 3.9 Dumbbell shaped 3D printed food pack wall samples

3.2.4 Sensory analyses:

A panel was recruited from Alanya Aladdin Keykubat University to participate in a five-point hedonic test.

Ethical approval for this study was granted by the Faculty Research Ethics Committee at the University, under reference number 2024/01. A group of 16 untrained participants (3 males, 13 females) attended the panel disregarding their demographic characteristics (Figure 3.10). The panelists were asked to assess aşure samples for sensory scores of sweetness, cinnamon aroma, and cinnamon odour for samples with 2% and 5% CNMA concentrations.

The testing scale was a five-point hedonic category scale where 1 represented dislike extremely and 5 represented like extremely on the selected modality. Panelists were asked to put a random point on the panelist scale forms without indicating digit numbers. Aşure samples were cooked on the day of sensory assessment, and tested on days 0, 7, and 14 of cold storage. For day 0 prior to testing, samples were held for three hours to allow CNMA odour release in the package. Samples were presented in individual cups with randomized four-digit codes which were chosen randomly and thermally equilibrated to room temperature for 20 minutes preceding the sessions. Each panelist

carried out their own judgment on their specified time periods. A median score of 2.5 was regarded as a threshold standard for acceptable quality.



Figure 3.10 Sensory panel

3.2.5 Statistical analyses

Trials were performed in duplicate. Results were analyzed using a single-factor variance analysis (ANOVA) to assess the impact of CNMA levels (2% and 5%), relative to the untreated samples, throughout the storage period for each measured parameter. A Duncan multiple range test was then executed at a statistical significance threshold of $p < 0.05$ (Sokal & Rohlf, 1987).

4. RESULTS

4.1 Microbiological Analyses

4.1.1 Total viable counts

Total Viable Count (TVC) analyses were conducted on samples containing both for 2% (v/w) and 5% (v/w) concentrations were preserved at 4°C over a 14-day period, with evaluations performed on days 0, 3, 7, and 14. No microbial growth was detected in any of the samples throughout the duration of the study.

4.1.2 *Salmonella* spp.

For *Salmonella* spp. Detection, day 0 and day 14 samples of each concentration were taken to be tested. Following the ISO 6579 and FDA procedures four subsequent steps were applied. No *Salmonella* spp. was observed in any of the 2% (v/w) and 5% (v/w) concentration samples. However, for 2% (v/w) concentration sample on day 0, *Pseudomonas aeruginosa* and *Escherichia coli* were identified in accordance with the performed biochemical tests. These bacterial species were not detected in the subsequent storage days.

4.1.3 Staphylococcal enterotoxin

Samples with 2% (v/w) and 5% (v/w) concentrations stored on days 0th and 14th were analyzed using the VIDAS method. No staphylococcal enterotoxins were detected in each of the tested samples.

4.2 Physicochemical Analyses

4.2.1 Headspace gas composition

The data of the headspace gas composition tests analyzed on the 3rd and 14th storage days showed that the CNMA concentration increased over the storage period in both sample sets (2% (v/w) and 5% (v/w) (Table 4.2).

According to the Fick's Second Law diffusion modelling on Table 4.1 the outcomes were shown. Fick's Second Law governs the release, indicating that the driving force for diffusion is proportional to the concentration gradient.

Table 4.1 Theoretical CNMA Release Over 14 Days

CNMA Concentration	Initial Mass (mg)	Theoretical Release (mg)	Headspace Concentration (ppm)	Estimated Headspace Mass (mg, 0.1 L)
5% (0.99 mg/mL)	3.96	3.96	8.33 (Day 3) 9.08 (Day 14)	0.00219 (Day 3) 0.00239 (Day 14)
2% (0.39 mg/mL)	1.56	1.56	2.07 (Day 3) 2.31 (Day 14)	0.00055 (Day 3) 0.00061 (Day 14)

4.2.2 pH measurements

Samples containing 2% and 5% concentrations of CNMA were monitored on days 0, 3, 7, and 14 of storage. Based on the statistical analysis, no significant disparities ($p > 0.05$) were found among the storage days, as documented in Table 4.2.

4.2.3 Dry matter basis

The total dry matter amount of ašure which was for 2% (v/w) and 5% (v/w) concentrations were monitored over the 0th, 3rd, 7th, and 14th days of storage. According to the statistical analyses there were no significant ($p > 0.05$) changes during the storage period in both sample sets (Table 4.2).

4.2.4 Color analysis

2% (v/w) and 5% (v/w) concentrations of the samples tested on the storage days at 0, 3, 7 and 14. Collected data in terms of CIE, L^* , a^* , b^* , ΔE^* values were presented in Table 4.2. Based on the data; CIE L^* values for the 2 % concentration of CNMA slightly decreased on the 7th day, however subsequent days there were no significant ($p > 0.05$) changes. In case of the 5% concentration similar trend was observed, no significant changes ($p > 0.05$) were observed throughout the sampling days. CIE a^* values of the 5% concentration dramatically decreased from starting on day three, while there were no significant ($p > 0.05$) changes between 0th and 14th day of the 2% concentration sample set.

Table 4.2 Physicochemical Properties of Asure Samples with Cinnamaldehyde at 2% and 5% Concentrations During Storage Period

	CNMA %	Storage Days			
		0 th .	3 rd .	7 th .	14 th .
Concentration (ppm)	2%	0.10±0.0*	2.07± 0.58	N/A	2.31 ±0.49
	5%	0.10±0.0*	8.33±1.12	N/A	9.08±0.18
pH	2%	7.06±0.52	6.04±0.23	6.87±0.29	6.88±0.19
	5%	6.90±0.42	6.43±0.43	6.89±0.29	6.98±0.03
Dry Matter (%)	2%	47.35±6.43	48.00±0.84	44.50±0.42	44.75±1.90
	5%	45.00±4.94	50.40±2.40	45.45±0.21	46.00±3.11
COLOR L^*	2%	13.64 ±0.52 ^b	14.29±2.49 ^b	10.33±0.72 ^a	12.54±0.97 ^b
	5%	16.96±0.42 ^a	15.44±0.43 ^a	12.77±0.29 ^a	15.01±0.03 ^a
COLOR a^*	2%	1.67±0.66 ^{ab}	1.01±0.49 ^a	1.75±0.51 ^b	1.52±0.45 ^{ab}
	5%	1.68±0.30 ^b	0.99±0.09 ^a	1.11±0.35 ^a	1.14±0.47 ^a
COLOR b^*	2%	5.67±1.15 ^a	5.23±1.11 ^a	4.94±0.06 ^a	5.25±0.57 ^a
	5%	6.20 ±0.85 ^b	5.53±1.31 ^{ab}	4.66±0.24 ^a	5.85±1.15 ^{ab}
ΔE^*	2%	78.26±1.90	77.59±2.47	81.56±0.74	79.35±0.96
	5%	74.94±2.79	76.45±4.36	79.10±2.05	76.9±3.35

Values are means ± SD.

Values in rows with different letters indicate significant differences ($P < 0.05$).

No letters indicate no significant differences ($P > 0.05$) in rows.

N/A not assessed

* The concept of value is inherently self-ascribed

4.3 Barrier Properties

The package floor thickness, oxygen permeability coefficient values (PeO_2) and the oxygen transmission rate (OTR) values were presented below (Table 4.3).

Table 4.3 Package floor thickness and PeO_2 values of 3D printed food pack

Package Thickness (mm)	Floor	OTR ($mL m^{-2} day^{-1}$)	PeO_2 ($10^{-9} cm^2/s$)
2.96 ± 0.18		6.85 ± 0.01	2.35 ± 3.43

4.4 Tensile Properties

The mean value of the elastic modulus, E modulus (Young's Modulus), ultimate tensile strength (σ_{uts} (MPa)) and Strain at Break (ϵ_b %) are provided in Table 4.4.

Table 4.4 Modulus of elasticity (E), tensile strength (σ_{uts}), and % elongation at break (ϵ_b) of the 3D food pack

Samples	E Modulus (MPa)	σ_{uts} (MPa)	ϵ_b %
Outer layer	2055.53± 184.9	66.16± 15.39	13.3± 1.56
Inner layer	1454.66± 199.71	42.37± 8.40	17.83± 9.10

4.5 Sensory Analyses

The mean sensory data for the aure samples packed in food packages containing CNMA, evaluated by panelists on days 0, 7, and 14 of the storage periods, are shown in Table 4.4. The sensory scores of sweetness, cinnamon aroma, and cinnamon odour for samples with 2% and 5% CNMA concentrations, according to the five-point hedonic category scale panelists, reported no significant dissatisfaction with the samples they tasted.

Table 4.5 Mean scores of the sensory analysis dependent on concentration

Concentration	Sensory Parameters	Mean Scores		
		0 th day	7 th day	14 th day
%2 CNMA	Odour	2.34±1.32	2.85±1.32	1.76±1.36
	Aroma	2.58±1.36	2.79±1.27	2.73±1.76
	Sweetness	3.91±1.42	3.6±1.41	3.89±1.50
%5 CNMA	Odour	2.51±1.20	2.96±1.41	2.25±1.15
	Aroma	2.71±1.19	2.84±1.47	2.75±1.54
	Sweetness	3.71±1.49	3.72±1.25	3.56±1.57

Values are means ± SD
N=16 (total number of the panellists)

5. DISCUSSION

5.1 Antimicrobial Activity

Items containing elevated levels of sugars are notably prone to degradation by fungal microorganisms. Osmophilic yeasts pose a significant threat in food storage due to their ability to thrive in environments with high salt or sugar concentrations. These yeasts not only adapt to the restrictive conditions of high osmotic pressure but also exhibit remarkable resistance to common preservatives like sulfur dioxide, sorbic acid, benzoic acid, and acetic acid (Warth 1985). Yeasts are capable of evolving resistance mechanisms against preservatives following extended exposure to diminished concentrations (Jenie,2010).

The recipe for aşure, with nearly 50% of its total weight comprising sugar, classifies it as an osmophilic dessert. Such environments are particularly conducive to the presence of osmophilic yeasts. Yeasts are the most common osmophilic microorganisms encountered in non-ionic, high-osmolarity environments, such as foods rich in sugar. Osmotolerant yeasts are frequently associated with the microbial degradation of foods with elevated saccharide content, exemplified by jams, honey, concentrated fruit preparations, and chocolate sweets with soft interiors. Spoilage in these products is often characterized by gassing, slime formation, and off-flavors (Kim, 2014)

Cinnamaldehyde has been reported to influence microbial cell morphology and surface texture (Clemente, 2016). Additionally, Evidence indicates that it induces lesions in microbial cell envelopes, plasma membranes, and mitochondrial organelles, in addition to disrupting the morphogenesis of fungal spores and spore-bearing structures. The antimicrobial activity of cinnamaldehyde is predominantly attributed to its electrophilic carbonyl group's ability to react with microbial nucleophile groups, including protein sulfhydryl and amino groups (Balaguer, 2013).

Shen (2015) reported that exposure to the minimum inhibitory concentration minimum inhibitory concentration (MIC) of cinnamaldehyde (0.31 mg/mL) causes damage to cell morphology, membrane integrity, and permeability in *E. coli* and *S. aureus*. Additionally, higher concentrations of cinnamaldehyde result in more severe damage to bacterial membranes. In the present study, the lack of microbial viability can be attributed to the cinnamaldehyde concentration reaching 0.99 mg/mL for 5% CNMA concentration and 0,39 mg/mL for 2% CNMA concentration in the package atmosphere.

It is, therefore, the pre-determined concentrations of CNMA both in 2% and 5% efficiently inhibited the probable microbial growth in the sealed atmosphere.

Another study indicated that; CNMA vapor can inhibit the synthesis of ergosterol, the primary sterol derivative of the fungal plasma membrane, which is crucial for cell viability, material transport, membrane integrity, and fluidity in *Aspergillus niger* HY2. Additionally, it promotes malondialdehyde production and causes plasma membrane damage, leading to the leakage of intracellular contents. The reduction in mitochondrial membrane potential, adenosine triphosphatase activity, and succinate dehydrogenase activity further demonstrated impaired mitochondrial function (Niu et al., 2022). The application of CNMA nanoemulsions resulted in the highest inhibitory effect against *Salmonella typhimurium* and *Staphylococcus aureus*, demonstrating the lowest MIC values (Badr, 2022). The antimicrobial properties of cinnamon have been thoroughly documented in the literature. Studies have shown that cinnamon essential oil at concentrations of approximately 0.08% (v/v) is bacteriostatic, while at 0.1% (v/v) it is bactericidal against foodborne pathogens. Generally, Gram-positive bacteria exhibit greater sensitivity to these concentrations compared to Gram-negative bacteria (Bouhdid et al., 2010; De La Torre Torres et al., 2017; Mazzarrino et al., 2015; Smith-Palmer, 1998). This study demonstrated that cinnamaldehyde, within concentrations of 2% (v/w) and 5% (v/w), effectively inhibited the probable growth of viable bacteria on days 0, 3, 7, and 14, which aligns with the previous studies. Considering this knowledge, the absence of *Salmonella spp.* and the negative results in staphylococcal enterotoxin tests might be attributed to this mechanism.

5.2 Physicochemical Analyses

5.2.1 Headspace composition

To determine efficacy of essential oils in the vapor phase for antimicrobial activity testing for foods is an innovative approach (Lopez et al., 2005). As documented in earlier works antimicrobial sensitivity of bacterial types are noticeably different from direct contact testing methods such as disc diffusion tests (Goñi et al., 2009). The antimicrobial properties of EOs and their vapor-phase combinations are closely linked to the headspace composition. Phenolic compounds, in particular, have been extensively documented for their significant antimicrobial efficacy (Baydar et al., 2004; Lopez et al., 2007b). The headspace composition of cinnamon EO, and consequently the cinnamon–clove combination, contains various compounds at trace levels. Lopez (2005) conducted a study

exploring the potential of using natural compounds to create a protective atmosphere, thereby extending the shelf life of packaged foods while minimizing organoleptic changes. The study tested the antimicrobial properties of six different essential oils: cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*), basil (*Ocimum basilicum*), rosemary (*Rosmarinus officinalis*), dill (*Anethum graveolens*), and ginger (*Zingiber officinale*). These oils were evaluated against various microorganisms using solid and vapor diffusion tests. The EOs were examined against four Gram-positive bacteria, four Gram-negative bacteria, and three fungi. Among all EOs the results were remarkable about cinnamon and clove oil. Cinnamon and clove showed the strongest and similar inhibitions. Clove and cinnamon oils were the most effective, generating a unique atmosphere containing compounds like calamenene and thymol, known for their antimicrobial properties. Additionally, eugenol was found at high concentrations, contributing significantly to their effectiveness. In contrast, basil oil was ineffective due to the lack of relevant compounds like estragol. Despite having β -caryophyllene and R-humulene, rosemary oil showed no antimicrobial effect, indicating these compounds are less effective than eugenol (Lopez et al., 2005).

Notably, cinnamaldehyde, a well-documented powerful antimicrobial agent, is among these compounds (Lopez et al., 2007b; Valero, 2006). A recent study evaluated the antifungal effects of CNMA vapor against *Aspergillus niger* HY2. The results indicated that CNMA in the vapor phase was more effective than in liquid phase. CNMA vapor can inhibit the ergosterol synthesis of *A. niger* HY2, promote malondialdehyde production, and cause plasma membrane damage, leading to leakage of intracellular contents. The reduction in mitochondrial membrane potential, adenosine triphosphatase, and succinate dehydrogenase activities also revealed impaired mitochondrial function (Niu, 2022).

In the current study, the 2% concentration of CNMA sample was showed results of 2.07 ppm on the third day and 2.31 ppm on the fourteenth day. The other test sets (for day 3 and 14) results of 5% concentration of CNMA were 8.33 ppm and 9.08 ppm, respectively. These findings indicate no significant ($p > 0.05$) changes at 2% (v/w) and 5% (v/w) concentrations (Table 4.2).

The design of Controlled-Release Packaging (CRP) systems is grounded in the concept of target release rate, which is defined as the specific release rate or range of release rates of active compounds necessary to effectively inhibit microbial growth or lipid oxidation at the target site (Zhu, 2012). The release rates of active compounds must

strike a balance. A rapid release can result in an excessive amount of tocopherol being released compared to free radical production, causing the surplus tocopherol to form dimers or other products, making it unavailable for subsequent stages of lipid oxidation. On the other hand, a slow release will not permit sufficient amounts of tocopherol to effectively inhibit lipid oxidation (Balasubramanian, 2012).

Assessing the controlled release of active compounds in the headspace of a food pack is of paramount importance. This suggests that the food pack provided an effective barrier while maintaining an adequate release rate in the headspace atmosphere.

To the best of current knowledge, this is the first study of a prototype of a food pack concerned on target release rate, replacing instant addition of additives in food formulations. In the previous research (Uzunlu and Niranjana, 2017), a 5% concentration of CNMA demonstrated strong antimicrobial activity, while a 1% concentration (Uzunlu, 2019) effectively inhibited *E. coli* and *S. aureus* bacteria. Considering this data, current study showed that CNMA effectively stored at the headspace of the 3D printed food packages, therefore exhibiting no presence of microbial organisms during the shelf-life period. Aşure containing 3D printed food packages exhibited high barrier properties both for release of CNMA at the headspace and oxygen transmission rate.

At 5% of CNMA (0.99 mg/ml), the initial concentration is significantly higher than at 2% of CNMA (0.39 mg/ml). This means there is more CNMA available for diffusion in the system with a higher concentration. The 2% of CNMA starts with a lower concentration, limiting the total amount of CNMA available for release.

At 5% CNMA (0.99 mg/mL), the greater concentration difference leads to a faster initial release rate compared to 2% CNMA (0.39 mg/mL). The release from 2% CNMA is expected to be slower throughout the process, as the lower concentration difference results in a weaker diffusion driving force.

Over 14 days, the 5% CNMA releases a greater mass of CNMA due to its higher initial concentration. For the 2% CNMA, although the release rate is slower, its diffusion pattern is expected to exhibit a similar overall behavior, but with a proportionally lower total released mass.

The 5% of CNMA would provide stronger antibacterial activity because more CNMA is released into the environment over time. This may be suitable for applications where higher efficacy is needed. The 2% of CNMA could be used for situations where a slower, more controlled release is desired to avoid high concentrations or prolong the effectiveness of the agent.

Table 4.1 summarizing the Fick's Second Law data for the theoretical release of cinnamaldehyde (CNMA) over 14 days. It includes the initial mass, theoretical release, and experimental headspace concentrations for comparison. For both concentrations, the dimensionless time parameter ($Dt/L^2 \approx 138$) suggested nearly complete release within 14 days.

The headspace masses were much smaller than the theoretical release 3.96 mg and 1.56 mg respectively, suggesting that most CNMA was either absorbed by the food (aşure) or remains within the package matrix, consistent with a controlled-release system.

The slight increase from day 3 to day 14 (0.00219 mg to 0.00239 mg for 5% CNMA 0.00055 mg to 0.00061 mg for 2% CNMA) aligns with the experimental data showing a gradual rise in headspace concentration. The comparison was made to reveal that, although complete release of CNMA was predicted by Fick's Second Law, the measured headspace concentrations indicated that a slower, sustained release had been achieved, thereby demonstrating the effectiveness of the 3D-printed package in preserving antimicrobial activity over a 14-day period.

The parameters obtained from Fick's diffusion equation are theoretical values derived from empirical data. To enhance the applicability of Fick's diffusion models, future research should prioritize accurately determining the concentration of natural agents within active food packaging types.

5.2.2 pH

The pH value of aşure stored in package with 2% CNMA was found to be as 7.06 on day 0. The following measurements on the 3rd, 7th and 14th days were 6.04, 6.87 and 6.88, respectively. The pH value of the other samples with 5% of CNMA was 6.90. Subsequent days, the pH was measured as 6.43, 6.89 and 6.98, respectively. Both concentrations showed no dramatic changes ($p > 0.05$) from day 0 to day 14. All the data remained around neutral values. The stability of pH values at 4°C might be attributed to the refrigerated temperatures (Cetin, 2020).

In a study, the nano emulsifiers were prepared with cinnamon oil and CNMA exhibited pH values of 6.17 and 6.10, respectively. These findings are in line with the current study's pH range. Since these values remain within the neutral pH range, combined usage of CNMA could be considered as safe for food applications (Badr, 2022). Taking account of cinnamon EO possesses acidic properties, when added to milk rice pudding in direct contact, the milk content has promoted a decrease in pH (Ibrahim,

2024). However, in the current study, the barriers of 3D food packages prevent direct contact with the cinnamaldehyde and thus the food samples showed no decrease in terms of pH.

5.2.3 Dry matter mass

Aşure is a traditional dessert consisting of grains, legumes (chickpeas, kidney beans, wheat), and dried fruits (grapes, apricot, fig). Carbohydrate-rich foods, such as wheat, chickpeas, and dried beans, are classified as staple foods due to their high starch content, ranging from 39.6% to 62.85% (Turksoy, 2018). Starch, a high molecular weight oligosaccharide, forms a significant portion of the dry mass of food products. Therefore, traditional aşure contains fructose and saccharose from dried fruits, providing sweetness and a rich carbohydrate content from cereal legumes. Consequently, aşure has a dry matter basis of approximately half (Turksoy, 2018).

The dry matter content of the aşure samples stored in 3D printed food packages with cinnamaldehyde at 2% and 5% concentrations was monitored over 14 days. At both concentrations, the dry matter content showed no significant ($p>0.05$) change. At 2% concentration, the dry matter content slightly increased from 47.35% on day 0 to 48 % on day 3 but showed a decrease to 44.5 % on day 7, where reached to 44.75% on day 14. This decline suggests potential moisture change or redistribution within the packaging environment over time, likely due to the hygroscopic properties of the dessert. For the 5% concentration, a similar trend was observed, with dry matter increasing from 45 % on day 0 to 50.4 % on day 3, followed by a decrease to 45.45% on day 7 and stabilizing at 46 % on day 14. The higher variability in dry matter content at this concentration could be attributed to the increased release of cinnamaldehyde, potentially interacting with the dessert's moisture content.

The presence of cereal legumes, combined with the sweetness of dried fruits, increases the food's water-binding capacity, thereby reducing the amount of free water and increasing the amount of bound water. This reduction in free water helps prevent biochemical reactions and microbial growth, establishing a positive correlation between the food's water activity and its shelf life.

5.2.4 Color

The CIE L^* , a^* , b^* color measurement system is a standardized and approved method used to assess food color quality (Kato *et al.* 2000; Velu *et al.* 2006; Nasar-Abbas *et al.* 2009). The L^* , a^* , b^* color system was employed in this experiment, the L^* value

representing the lightness of the product color, the a^* value indicating redness, and the b^* value measuring the yellowness of the food product.

In the current study, the CIE L^* value showed no statistical change when comparing the initial and final days of the analysis. The 3D food packages containing different concentrations of cinnamaldehyde did not affect the brightness of the food, as they did not come into direct contact with the food.

CIE a^* values decreased in the storage period for both concentrations, as well as CIE b^* had the same trend (Table 4.2). Cocero et al. 2009 documented that lemongrass, cinnamon, and sandalwood essential oils exhibit a color range from light yellow to red-orange, while bergamot and chamomile EOs reveal green and blue hues, respectively.

Based on this knowledge, the observed decrease in the CIE b^* value of the aşure samples suggests that the yellowness of the dessert did not increase as a result of the indirect incorporation of CNMA EO. This indicates that the addition of CNMA did not significantly alter the color characteristics of aşure in terms of its yellowness.

The tendency to decrease about CIE a^* and b^* might be associated with approaching the end of its shelf life. To understand total variation of color parameters, ΔE^* showed no significance ($p > 0.05$). Although ΔE^* showed no statistical change, an increase was observed over the storage days, meaning a reduction in color lightness and an increase in darkening. Generally, these changes correspond to non-enzymatic darkening and may be related to the polymerization reactions of low molecular weight phenolic compounds into high molecular weight tannins (Reyes-Moreno, 2000).

A study was conducted on soybean grains to monitor the color, texture, and cooking quality of the bean testa during the storage period. Soybean (*Glycine max* L.) samples were stored for a duration of 12 months, with observations made at intervals of 0, 3, 6, 9, and 12 months. The samples were maintained at three different moisture contents (MC) of 9%, 11%, and 13%, and at three temperatures of 10°C, 20°C, and 30°C. Storing soybeans for 12 months at a higher moisture content (13%) and temperature (30°C) caused significant color changes and darkening of the soybean testa. Conversely, lower moisture content (9%) and temperature (10°C) resulted in minimal color change. Aligned with the current study's findings the refrigerated aşure samples showed minimal color changes. Moreover Yousif, (2014) indicated that storage conditions greatly influence soybean testa color quality, impacting consumer acceptability and market value, and cold storage is a preferable option to maintain a lighter color in grains, as suggested.

Terzi (2020) aimed to obtain protein isolate from *Phaseolus vulgaris L.* beans and investigated its effects on the quality characteristics of products such as cakes and plant-based patties. An increase in protein content in the cake samples was observed to correspond with an increase in ΔE^* . Similar results have been reported for cake samples containing bean, pea, and amaranth protein isolates. It has been suggested that this is due to the increased protein content, which raises the proportion of amino acids that can participate in the Maillard reaction (Shevkani, 2014). Taking account of a sure recipe the protein content seems to be high enough, when considering Maillard reaction in cooking procedures.

5.3 Barrier Properties

The thickness of the 3D food pack was approximately 2.96 mm. The value of oxygen transmission rate was 6.85 mL m⁻²/ day, it suggests that the packaging had a relatively consistent oxygen barrier performance. The 3D printed food packages made from PLA had adequate thickness and oxygen barrier properties which directly affect the packaging's performance in preserving food quality, as determined previously (Marano, 2022). Polypropylene (PP), with an oxygen transmission rate (OTR) typically ranging between 93 and 300 ml (STP) m⁻²/day, exhibits lower oxygen barrier properties compared to PLA. This makes PLA a promising alternative for food packaging applications, especially where enhanced oxygen barrier performance is required (Lu, 2018).

Therefore, using PLA as a packaging material, particularly for fresh foods, can be considered a more suitable option, as it limits oxygen transfer to a greater extent.

It was planned in the current thesis study that to introduce cinnamaldehyde in the gas phase, for preserving food quality would be a key concern in minimizing foodborne disease risks.

5.4 Tensile Properties

5.4.1 Elastic modulus

The Elastic Modulus (Young's Modulus) is a measure of the material's stiffness or resistance to elastic deformation when a stress is applied. Higher values of E Modulus indicate stiffer materials (Salem, 2022). This stiffness might be critical for maintaining the package's shape, protecting contents during handling, and stacking during storage and transportation. It may also include surface treatments for printability, water resistance, or enhanced barrier properties against oxygen or moisture, critical for food preservation (Marano, 2022). Outer and inner layers of the 3D Food Pack elastic modulus mean values

were measured as 2055.53 ± 1184.96 MPa and 1454.66 ± 199.71 MPa, respectively. The outer layer is significantly stiffer than the inner layer, with a higher E Modulus. This suggests that while the outer layer contributes to the structural rigidity of the food container, the less rigid inner layer is thought to enhance permeability, facilitating the diffusion of the antimicrobial agent.

5.4.2. Tensile strength (σ_{uts})

Tensile Strength (also called Ultimate Tensile Strength, or σ_{uts}) is the maximum stress that a material can withstand before breaking or failing. It is a measure of the material's resistance to breaking under tension. The outer layer is measured as a higher tensile strength (66.16 MPa) than the inner layer (42.37 MPa). In a comparative study, Bhasney (2020) conducted, PLA and PP materials tensile strength were measured as 47.2 MPa and 21.6 MPa respectively. PLA demonstrates more than double the tensile strength of PP, suggesting that it can endure significantly higher stress before breaking when subjected to tension. Among both materials, the ultimate tensile strength (UTS) values for PLA are consistent, aligning closely with previously reported values. This consistency underscores the reliability of the mechanical performance of PLA as a material, particularly in applications requiring high tensile strength. In sum, while both PP and PLA are suitable for packaging, PLA shows competitive tensile strength but may require additional optimization for ductility to fully match PP's versatility. This reinforces PLA's viability as a more sustainable alternative for rigid packaging applications.

5.4.3 Strain at break (ϵ_b)

Strain at Break (ϵ_b) is the elongation or deformation of a material experienced before it breaks. It is expressed as a percentage of the original length of the sample. The inner layer showed a higher strain at break (17.83%) compared to the outer layer (13.3%), meaning the inner layer can stretch more before breaking, indicating it is more ductile or flexible. The outer layer was less ductile and breaks at a lower strain. In sum, the outer layer seemed to be designed for more load-bearing and structural applications, being stiffer and stronger, but with less flexibility. On the other hand, the inner layer had better flexibility and elongation capacity, making it potentially more suitable for applications where bending or stretching is important, though it was weaker and less stiff. The flexibility and elongation capacity of the inner layer can be a double-edged sword. It can either promote gas transfer (for controlled release) or increase the loss of volatile compounds. The inner layer's greater flexibility and lower stiffness can prevent cracks

and maintain a secure seal, especially for vacuum-sealed or irregularly shaped products. The effect depends on the specific application, and balancing these properties with other barrier layers or material treatments may be necessary to achieve the desired result (Marano, 2022).

It is suggested that PLA exhibits high ultimate tensile strength (σ_m) and low elongation at break (ϵ_b), which can be attributed to its rigid and brittle nature. This behavior arises from PLA's semicrystalline structure and its inherent stiffness due to its molecular configuration. The high σ_m makes PLA suitable for applications requiring strength and rigidity, but its low ϵ_b limits its performance in scenarios requiring flexibility or toughness (Llanes, 2021).

5.5 Sensory Analysis

In the obtained data, no change was observed for the cinnamon odour at concentrations of 2% and 5%. The samples with 2% concentration had a close score to the acceptable threshold value of 2.5 on the first trial day and decreased below 2, by the 14th day. The samples with 5% cinnamaldehyde had the highest score as an expected consequence of higher concentration (Table 4.5). Both concentrations were more acceptable on the 7th day due to the release of cinnamaldehyde. After 14 days of storage, it was found that the cinnamon odour reduced in both experimental sets due to the volatile compounds of cinnamaldehyde. Antigo et al. (2017) conducted a study on a traditional dessert of Brazil, dulce de leche. Throughout the 210-day storage period, a sensory analysis was conducted, which included testing antimicrobial properties as well as evaluating odour and taste parameters. The results indicated that the cinnamon essential oil achieved the acceptance rating closest to the control group among the cinnamon, clove oil, and clove-cinnamon oil mixture samples. The product with cinnamon essential oil received an acceptance rating of 81.67 %. The mixture of cinnamon and clove stem essential oils had an acceptance rating of 78.22 %, while the sample with clove stem essential oil achieved an acceptance rating of 79.78 % (Antigo, 2017).

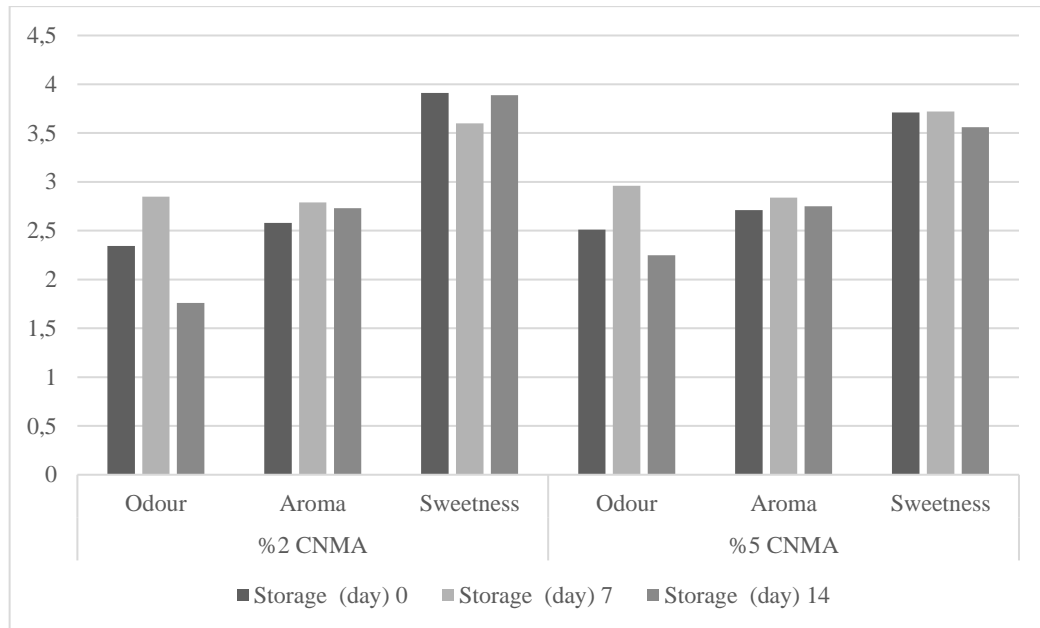


Figure 5.1 Sensory analysis scores dependent on concentrations

Panelists started the sensory analysis by evaluating the cinnamon odour and then continued by tasting and scoring the cinnamon aroma. The cinnamon aroma received a preference score proportional to its percentage intensity. Both concentrations achieved the highest aroma scores on the 7th day of storage, but by the 14th day, their scores decreased below the initial values. In terms of aroma, the samples containing 5% cinnamon experienced a greater loss in score on the 14th day when compared to the 7th day. However, the aroma score of the 5% sample was consistently higher than that of the 2% sample on each storage day. This can be explained as an intensity-dependent behavior. In a comparative study of milk rice pudding, the antimicrobial properties and sensory characteristics of clove oil and cinnamon essential oil were evaluated. Both samples were prepared at a concentration of 0.6%. Measurements indicated that cinnamon essential oil received a higher preference score in terms of flavor (Ibrahim, 2024). In Turkish culture, the dessert aşure is enriched with cinnamon spice and served. Therefore, the perceived cinnamon aroma or odour may contribute to its acceptance by consumers without causing no discomfort. To sum up, from a moderate to strong scale positive relationship between aroma and odour scores can be suggested for the 2% and 5% CNMA samples. Notably, there was a noticeable enhancement in aroma as the odour became stronger.

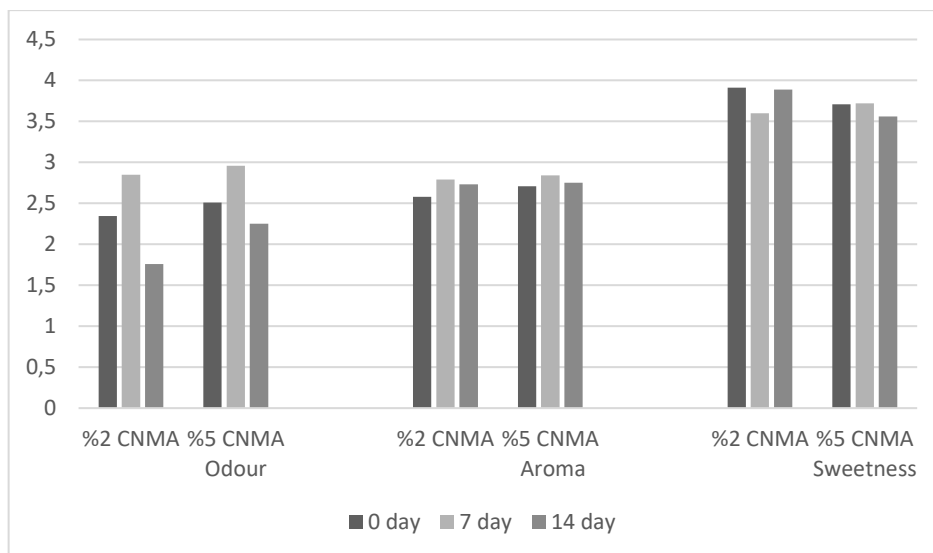


Figure 5.2 Sensory analysis scores dependent on parameters

In this study, the effect of cinnamaldehyde release on sweetness was evaluated. Panelists scored the highest acceptance on the hedonic scale for both concentrations. However, according to the panelists the 2 % concentration sample sets were more acceptable compared to the 5% of CNMA samples. A similar study demonstrated that among three 0.25%, 0.50% and 0.75% concentrations, the lowest one was the most preferable in terms of tasting dark chocolate with cinnamon essential oil (Dwijatmoko et al., 2016). Therefore, preferring a lower concentration can be more economical and preferable in terms of food quality.

A recent study by Matsumoto et al. (2023) demonstrated that transient receptor potential (TRP) channels are found in various tissues and cell types, including the taste cells and nerves in circumvallate papillae. TRP channels are thought to play a role in taste sensitivity, as the temperature at which food is tasted influences flavor perception. TRP melastatin 5 (TRPM5) in taste cells is crucial for the transmission of sweet, bitter, and umami flavors (Pérez, 2022). Also, cinnamaldehyde can only express spicy flavor through TRPA1 channel (Nkambeni et al. 2021). Findings from previous studies indicate that the perception of cinnamon and sweetness is detected by two different transient receptor potential channels (TRP). Therefore, the relationship between sweetness and cinnamon should be considered as a complex and indirect interaction, which is based on the reported studies. The rate of cinnamaldehyde release for both concentrations contributed no both in positive and negative in terms of sweetness index and perceived sensorial judgment.

6. CONCLUSION

Active food packaging represents a pivotal innovation for enhancing food safety and extending shelf life while addressing the need for environmentally sustainable materials. Unlike conventional systems, active packaging interacts dynamically with the food and its surrounding environment, offering added functionality such as antimicrobial protection.

In this study, a traditional Turkish dessert, *aşure*, was packaged using specially designed 3D-printed food packages where injected at two different concentrations with CNMA as an antimicrobial agent.

Among the findings;

- Microbial analyses confirmed that both 2% and 5% CNMA concentrations, with solution densities of 0.39 mg/mL and 0.99 mg/mL respectively, effectively prevented microbial growth at 4°C over a 14-day storage period, demonstrating the strong antimicrobial efficacy of CNMA in the packaging of *aşure*.
- Headspace gas composition analysis demonstrated a controlled release of CNMA, with higher concentrations releasing more active agents. This indicates the packaging's potential to maintain antimicrobial activity throughout storage. The findings revealed an increase in CNMA concentration during storage where it exhibited its antimicrobial property effectively.
- The theoretical models confirm that CNMA diffuses through the package, but the actual release is controlled, aligning with the design of the controlled-release packaging (CRP) system. This supports the observed antimicrobial efficacy over 14 days.
- pH measurements across all samples remained stable, with slight variations that were statistically insignificant ($p > 0.05$), suggesting that the packaging did not adversely affect the acidity of the food product.
- Similarly, dry matter content and colorimetric parameters (CIE L^* , a^* , b^*) exhibited minor fluctuations during the storage period, but none were statistically significant ($p > 0.05$), further affirming the structural and functional stability of the packaging.
- The 3D-printed food packages, with a base thickness of 2.96 mm and oxygen transmission rate measured at 6.85 mL m⁻²/day, demonstrates relatively consistent oxygen barrier performance.

- In terms of mechanical performance, PLA demonstrated superior tensile strength compared to conventional polypropylene (PP), affirming its potential for rigid packaging applications. However, its brittle nature, indicated by low elongation at break, may limit its flexibility, suggesting a need for further material modifications to enhance its versatility.
- Sensory evaluations revealed that the packaging system not negatively affected the sensory attributes of the aşure dessert, with the product receiving positive and acceptable scores. The traditional visual perception of the Aşure dessert is that it is not browned. Although it was not included in the sensory analysis of this study, its investigation in future analyses can be considered.
- During the storage period, sensory attributes such as perceived odour, aroma, and sweetness showed an increase on the 7th day. However, by the 14th day, these attributes had decreased compared to the 7th day, suggesting that the product was almost close to the end of its shelf life. This declining trend was less pronounced for aroma compared to odour. Among the parameters assessed during the sensory analysis evaluation days, sweetness received the highest score.

This study highlights the effectiveness of the specially designed active packaging system in ensuring microbial safety, maintaining sensory quality, and delivering environmental sustainability. The incorporation of cinnamaldehyde into the packaging system proved effective in antimicrobial protection, making it a strong candidate for broader use in food packaging applications.

Future research should focus on optimizing the material's flexibility, scaling up production, and exploring other active agents to expand its applicability across diverse food types and storage conditions.

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APPENDIX

T.C.

ALANYA ALAADDİN KEYKUBAT ÜNİVERSİTESİ REKTÖRLÜĞÜ

Fen ve Mühendislik Bilimleri Bilimsel Araştırma ve Yayın Etiği Kurul Kararı

TOPLANTI SAYISI	KARAR SAYISI	KARAR TARİHİ
01	01	25.01.2024

Karar Numarası: 2024/01

Doç. Dr. Sinan UZUNLU'nun Danışmanlığını yaptığı Yüksek Lisans öğrencisi Evrim AKTÜRK'ün Araştırmanın yürütücüsü olduğu 10.01.2024 tarihli ve 6074 E. No'lu “**Geleneksel Bir Türk Tatlısı Olan Aşurenin Raf Ömrünü Uzatmak İçin 3D Printer Teknolojisi Kullanılarak Geliştirilmiş Aktif Gıda Ambalajı**” başlıklı yüksek lisans tez çalışmasına ait etik kurul başvurusunun görüşülmesi istemi.

Doç. Dr. Sinan UZUNLU'nun Danışmanlığını yaptığı Yüksek Lisans öğrencisi Evrim AKTÜRK'ün Araştırmanın yürütücüsü olduğu 10.01.2024 tarihli ve 6074 E. No'lu “**Geleneksel Bir Türk Tatlısı Olan Aşurenin Raf Ömrünü Uzatmak İçin 3D Printer Teknolojisi Kullanılarak Geliştirilmiş Aktif Gıda Ambalajı**” başlıklı yüksek lisans tez çalışmasına ait başvurusunun fikri, hukuki ve telif hakları bakımından metot ve ölçeğine ilişkin sorumluluğun başvurusuna ait olmak üzere araştırma süresince uygulanmasının Fen ve Mühendislik Bilimleri Bilimsel Araştırma ve Yayın Etiği Kurulunca etik olarak uygun olduğuna oybirliği ile karar verildi. **25.01.2024**

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