Nutritional, microbiological, and feasibility evaluation of sorghum-based hummus

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Abstract

Sorghum is a drought-resistant cereal crop cultivated worldwide; however, it remains underutilized in human food applications. Hummus represents a familiar product format for incorporating alternative grains, offering an opportunity to enhance food security while developing sorghum-based foods with improved nutritional properties. Despite this potential, limited information is available on the nutritional performance, microbiological stability, and feasibility of sorghum-based hummus. Therefore, the objective of this study was to evaluate the nutritional quality, microbiological stability, and sensory acceptance of sorghum-based hummus formulations compared to traditional chickpea hummus.

Hummus formulations were prepared using chickpea, red sorghum, and white sorghum. To evaluate microbial shelf-life stability, a thymol-based emulsion was incorporated into selected formulations, and products were experimentally inoculated with *Listeria monocytogenes, Staphylococcus aureus,* and *Salmonella spp*. Microbial populations were monitored during refrigerated storage for 7 days. In vitro protein digestibility was assessed to compare the nutritional performance of the formulations, and sensory acceptance was evaluated through consumer testing. Data were analyzed using mixed-effects models and confidence interval estimation ($\alpha = 0.05$).

No significant differences were observed between thymol-treated and untreated samples across grain types ($p > 0.05$); therefore, microbiological analyses were restricted to day 7, confirming the product's stability under the evaluated conditions. Red and white sorghum hummus exhibited significantly higher protein digestibility than traditional chickpea hummus, with increases ranging from 4% to 7% ($p < 0.05$). Sensory evaluation indicated that chickpea hummus was preferred overall; however, consumers expressed a willingness to consider sorghum-based hummus when it was associated with added health benefits.

Sorghum-based hummus represents a promising alternative to conventional formulations, aligning nutritional benefits with food security goals. Our research demonstrated that sorghum could provide improved protein digestibility and maintain microbiological stability, supporting its feasibility as a value-added food product.

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Dedication

I dedicate this thesis to my parents. Thank you for teaching me the value of hard work, perseverance, and resilience. Your unwavering love and sacrifices have provided me with opportunities you never had, and I hope this accomplishment honors everything you have done for me.

Chapter 1 - Review of the Literature

1.1 Sorghum

Grain sorghum is a resilient, nutrient-dense grain from the *Gramineae* family, with a variety of benefits, but underutilized across the world due to various factors, including accessibility, dietary preferences, agricultural practices, and regulations. While this crop is a reliable source for ethanol and ideal food applications, there is still a need for innovation and optimization (Duff et al., 2019). Sorghum has the ability to persist through drought. Compared to traditional plants, such as corn, sorghum becomes dominant versus drying out in these conditions (Abah et al., 2020).

As previous mentioned, sorghum belongs to the *Gramineae*, or *Poaceae*, family, which falls alongside other grasses, such as wheat, millet, and maize (Swamy, 2023). Originally identified as Holcus, Sorghum is a grain cultivated for its grain, which is the input for food, feed, and ethanol. The grain is divided into five races based on inflorescence type: Durra, Bicolor, Caudatum, Kaffir, and Guinea. There are four main types of sorghum including sorghum viz., Grain, Forage, Sweet, and Biomass sorghum. Grain sorghum is typically what is utilized for human food, feed, and fuel (Hossain et al., 2022). These species have the ability to grow in variable climate and have been known to cross-pollinate, resulting in unique traits and patterns, creating vast opportunities for producers (Swamy, 2023).

Similar to corn, sorghum is composed of beneficial nutrients, such as protein, that range from 4.4 to 21.1%. Sorghum also contains linoleic acid, which is an essential, major fatty acid, that serves as a unique addition compared to other grains (Abah et al., 2020). Phenolic compounds, such as tannins and 3-deoxyanthocyanidins, are found in multiple varieties of sorghum, including red sorghum. Isolates of these compounds have been linked to benefit the gut microbiota and alleviate oxidative stress, inflammation, diabetes, and other noncommunicable diseases (Morais Cardoso et al., 2017). Compared to traditional grains, such as various wheat varieties, sorghum offers a gluten-free alternative to consumption while simultaneously providing functional nutrients to the consumer.

1.2 Global Sorghum Market

As the fifth most grown grain in the world, sorghum has the potential to expand markets globally. The current applications of ethanol production, human food, and animal feed, enable it to be a commodity that can impact communities substantially. Globally, 47.5% of sorghum is used for human food products, while 34.7% is used for animal feed. The remaining percentage accounts for product rendered non-food, seed, or lost in processing (Charyulu et al., 2024). The U.S. leads sorghum production at 17% across the world in which 40% of this production is used for ethanol (Mundia et al., 2019). Following the U.S., Nigeria, Ethiopia, Mexico, and Sudan are major producers of sorghum (George et al., 2022). With it being predominantly grown across certain regions, this opens the door to international trade.

The agricultural sector within Nigeria is a growing market with capacity for innovation related to crop commodities. Nigeria produces 6.9 million tons of sorghum annually, providing profit for small-scale farmers, 90% of total yield, and uses it as a staple dish within households. Nearly 45% of land within Nigeria, mostly Northern regions, is dedicated to cereal crop production. As a major producer of sorghum worldwide, Nigeria optimizes the grain to create value-added products, such as grain flour and fermented beverages. Despite being a staple commodity, the sorghum market is not being optimized due to production and productivity constraints (Ahmad Yahaya et al., 2022).

The United States agricultural market is constantly expanding due to domestic growth and globalization. Referred to as the sorghum belt, the central U.S. vastly contributes to the global market through trade. While traditional crops, like maize and wheat, continue to be produced in the U.S., sorghum has grown in popularity due to the emerging threats from climate change. Within the U.S., the crop remains below its yield potential due consumer preferences and lack of processing techniques, as well as social stigma of the crop primarily being applied as animal feed.

As climate patterns and consumer preferences continue to change rapidly, there is a need for product innovation to combat food insecurity in ways that optimize available resources. In most food-insecure regions, crop productivity is limited by little water, harsh soil properties, and low ecological stability. However, sorghum is persistent which facilitates a reasonable expansion for crop farmers across the globe (Hossain et al., 2022). Less input accompanied by high yield can give producers a competitive advantage in the market, while simultaneously promoting crop diversity. Crop optimization and food innovation are needed to address growing populations as climate change creates hotter surface temperatures and less rainfall. With the minimal input of resources, such as water, sorghum can create opportunities for producers in the Middle East. Historically, the Middle East has faced issues with water resources to supply to the population (Kaniewski et al., 2012). These conditions not only impact the populations' access to water, but also the capabilities within the agriculture sector. With the Middle East having desert to semi-arid climate patterns, sorghum could serve as a positive adaptive change for producers. In the Kingdom of Saudi Arabia, water requirements for crops have steadily increased annually (International Federation of Red Cross and Red Crescent Societies (IFRC), n.d.). Sorghum, requiring minimal water input, would allow producers to adapt to climate patterns, while creating a market that enhances sustainability.

Creating value-added products that incorporate sorghum, which complements agricultural resources within a region, can further increase production of the crop, which can lead to improved markets. Another consideration is the opportunity for enhanced country-to-country collaboration and trade. Sudan is one of the top producers of sorghum in Africa and a neighbor to Saudi Arabia (Hariprasanna and Rakshit, 2016). Current trade between these countries is agricultural products and machinery, creating an opportunity for new crops and food products.

1.3 Case Study: Sorghum Hummus as Value-added Product to Improve Food Security

Hummus, a food product consumed across the world, is formulated by mixing cooked, mashed chickpeas, tahini, lemon juice, and other ingredients to enhance the product, such as olive oil and spices. There are currently other dips, similar to hummus, that use beans to create a value-added product for consumers (Taylor et al., 2016). There is capacity for innovation within the food industry as consumers tend to seek nutrient-dense food products. Using cooked sorghum grain as a nutrient-dense, alternative to the dip creates a value-added product that can improve food security. As previously mentioned, sorghum is a cereal commodity that can impact agricultural markets and provide an adaptable crop in the midst of climate change. Therefore, using cooked, mashed sorghum in a manner similar to chickpeas in traditional hummus, creates a sustainable solution to alleviate food insecurity and provide consumers with a nutrient-dense dip.

The shelf-life of traditional, chickpea hummus is seven days after opening, under constant refrigeration. Creating sorghum hummus that has a longer duration under storage conditions would enhance accessibility to consumers, as well as producers. The longer a product can be purchased and free from spoilage microorganisms, the increased duration that consumers have to a nutrient-dense product. Preventing the growth of microorganisms is mandatory for food products, especially products that are Ready- to- Eat, like refrigerated dips. This is a critical parameter of food products to not only maintain the quality of the hummus, but protect the consumer from foodborne illness.

Prevention growth of microorganisms is necessary in order to maintain food safety and product integrity. The development of pathogenic bacteria, such as *Listeria monocytogenes*, *Salmonella spp*., and *Staphylococcus aureus*, are typically indicators of poor food safety practices or deviations during processing. Pathogenic organisms and spoilage microbes can be introduced at any point within a food system; therefore, require safe practices and innovation (Silva and Ribeiro, 2024). To prevent the growth of microorganisms, functional ingredients can be added to extend the shelf-life, or period of time a product's quality and safety are guaranteed, of food products.

One way to extend the shelf-life and prevent microbial growth of a product is by incorporating functional ingredients or compounds that have antimicrobial or anti-spoilage properties. Essential oils are a common application within food matrices that have an antimicrobial effect and serve as an intervention strategy within products. Essential oils are bioactive molecules that have a wide range of applications, including food, that are widely accepted by consumers and regulatory bodies. These additives can prevent or delay oxidation, which is the process that results in a food product spoiling or becoming inedible (Nogueira and Holanda e Silva, 2021).

1.4 Thymol, an Essential Oil with Antimicrobial Activity

Thyme essential oil is a common additive currently used in multiple industries for its natural preservation properties. It has been found effective as an organic pesticide that inhibits fungal growth, active viruses, and other bacteria. It is important to keep in mind that thymol is a natural compound in thyme, which contains properties that ward off pests; however, it should not be confused with traditional pesticides which are created from synthetic chemicals. Thymol also has many applications outside of natural pesticides including perfumes, food flavorings, mouthwash, pharmaceutical preparations, and cosmetics. It also has applications for meat products, fruits, vegetables, dairy, and other processed products, including sausage and cheese (Luo et al., 2025). A study utilizing 1mM thymol in soy sauce was effective at eliminating foodborne pathogens, such as *E. coli O157:H7, S. thyphimurium* and *L. monocytogenes* (Marchese et al., 2016).

Thymol, chemically defined as 2-isopropyl-5-methylphenol, is a phenolic compound derived from thyme (*Thymus vulgaris* or other plants from the Lamiaceae family) the commonly used kitchen herb. The compound is derived from several different plants varying in concentrations, which correspond with efficacy. Before the creation of thymol, thyme was used in various manners, such as a healing remedy and for therapeutic use. The compound was first discovered by a German chemist who was conducting extractions on thyme essential oil. Following this, a French chemist in the early 19th century discovered the compound's antibacterial effect after studying plant essential oils. Nearly 20 years later, the studied compound from the thyme essential oil was formally denoted as "thymol" (Liu et al., 2026).

There are several extraction methods used to obtain the essential oil, thymol, from thyme: Soxhlet, heat reflux, ultrasound-assisted, microwave-assisted, bead milling, and steam distillation. Over the past decade, eco-friendly extraction methods of natural extracts have been gaining popularity due to the decline in approval of traditional methods, many of which produced toxic waste. These alternative methods are referred to as "green techniques." The green technique that outputs the most volume, while maintaining key characteristics of thymol, is the microwave-assisted extraction method. This process uses a microwave reactor where thyme leaves and an ethanol/water ratio is placed in a Teflon reactor and warmed. The extract is obtained after two hours at a constant power, 78 °C, and 15 bars of vapor pressure. This method results in the largest quantity of thymol and retention of compounds (Adhar et al., 2022). Despite the innovation-forward approach of green techniques, most regions rely on steam distillation as a large-scale method.

Thymol ($C_{10}H_{14}O$) is a volatile, crystalline structure that has a similar scent to thyme; however, a strong flavor profile. It is soluble in a variety of organic solvents; however, less probable to dissolve in cold water. Thymol has a phenolic hydroxyl group, serving as an active antioxidant, which gives it the compound antioxidant effects. This process occurs during dehydrogenation, in which the effect is active through the process of neutralizing the highly reactive oxygen species, which are capable of oxidative damage. Thymol's lipophilic nature from the isopropyl group assists in the compound's permeability across membranes. (Liu et al., 2026).

Within the meat industry, thymol was not applied directly to the food item, but incorporated into film packaging to understand the compound's capabilities. This was done to chicken, beef, and seafood products. Using food packaging film as a mode of delivery, thymol was deemed effective at inhibiting spoilage microorganisms and minimizing protein degradation due to lipid oxidation (Luo et al., 2025).

Various applications in fruits and vegetables also deemed thymol effective as an antimicrobial agent against unwanted pathogens. Several applications made use of thymol through edible film packaging as well as a vapor to mitigate postharvest disease. Multiple of these concentrations are significantly higher (>40%) than what would be applied within a food matrix, as consumers are encouraged to wash produce before consuming (Luo et al., 2025).

Thyme essential oil has the potential to inhibit bacteria, molds, and other undesired growth within food systems. It is important to note that thymol applications are historically within films and not incorporated into food products as an ingredient. It displays potential to be utilized in different products; hence, the need for innovation of application within the food industry.

1.4.1 Mechanisms of Action of Nano-encapsulated Thymol Emulsion

Incorporating essential oils, like thymol, can enhance a products shelf-life; however, essential oils have low solubility and uncontrolled volatility (Nogueira and Holanda e Silva, 2021) These molecules have various applications dues to their composition for fatty acids, terpenes, triterpenes, and lipophilic contents that can inhibit microorganism growth, yet still remain limited with direct application to food products. Therefore, there is a need for technological innovation to make compounds soluble and controlled within products. One way to enhance properties of thymol and optimize functionality is to encapsulate the essential oil within a nanoemulsion. By encapsulating thymol into a nanoemulsion, the compound can be protected and controlled to complement product characteristics (Nogueira and Holanda e Silva, 2021). Controlled release and emulsification would allow for the bioactive properties of the essential oils to have increased solubility and extended release within food items.

Previous applications of essential oils in studies have been deemed effective. For example, 0.03% to 0.06% thymol nanoemulsion was effective at limiting the growth of Staphylococcus aureus when determining the Minimum Inhibitory Concentration (MIC). The compound was also effective at microbial reduction when applied onto strawberries and cucumbers (Luo et al., 2025). The applications of thymol span between various food products, as well as industries. The essential oil was found to be effective in multiple applications ranging from biofilm formation to food packaging. The phytochemical makeup and activity is highly dependent upon the source, as well as the vehicle.

Creating an emulsion is the process of homogenizing two or more liquids that are typically immiscible, or not easily combined, for example, oil and water. There are various technologies that use high speeds and/or pressure to make the desired liquids homogenous. A nano-encapsulated emulsion, or nanoemulsion, uses similar techniques to those of a regular emulsion to create a solution that has a droplet size from 10 to 1000nm (Jayari et al., 2022). The pH of the thymol nanoemulsion is 3.5, indicating the need for a more acidic application (Chang et al., 2012).

Using a thymol nanoemulsion enhances the bioavailability and stability of the essential oils itself. This allows the nanoemulsion to disrupt microbial cell membranes through increased surface area, while maintaining a controlled release. Main mechanisms of action are membrane depolarization, permeability alteration, leakage of intracellular components, and inhibition of bacterial enzymes. Thymol, like all essential oils, is hydrophobic, which allows molecules of the emulsion to break through the phospholipid bilayer of the plasma membrane within food products. This causes deterioration of the cytoplasmic membrane, hence complications with permeability. The lipophilic characteristics of essential oils are effective at attacking polysaccharide-dense plasma membranes, which are found in most living organisms, including unwanted pathogens in food products (Jayari et al., 2022).

These mechanisms of the thymol nanoemulsion lead to the inhibition of spoilage microbes, as well as limiting the growth of foodborne pathogens within food products. By incorporating thymol into a nano-encapsulated emulsion, the aim is to have a prolonged and enhanced antimicrobial effect within food products, while preserving the essential oil from oxidation.

As previously mentioned, thymol has antimicrobial and antifungal properties. The essential oil has been found useful at creating a bacteriostatic effect on Gram-negative and Gram-positive bacteria, including *Salmonella typhimurium, Staphylococcus aureus*, and *Listeria* innocua (Marchese et al., 2016). A bacteriostatic effect is possible due to the damage of lipid membrane, in turn allowing thymol to interact with the bacteria. For instance, thymol at a concentration of 20% can inhibit the growth of E. coli and 2.5mM inhibits *S. aureus, E. coli*, and *S. typhimurium*. Thymol as an antifungal agent in food products has been seen effective at similar ranges within various fungi (Marchese et al., 2016).

1.5 Implementation of Sorghum Hummus in the Middle East: A Regulatory Perspective

Sorghum hummus creates a value-added product that can improve food security. To positively impact Saudi Arabian markets, a regulatory analysis of involved entities and product requirements will allow for the optimization of the products' success. Most ingredients receive approval under Saudi food laws.

The Saudi Food and Drug Authority (SFDA) is the primary regulatory body responsible for overseeing the safety and quality of food products in Saudi Arabia. In addition to regulatory enforcement, the SFDA carries out legislative authority, issues technical requirements to govern production, and oversees the import/export of foods. Its scope includes the regulation of foods, whether fresh, processed, or semi-processed, as well as products intended for animal consumption. The SFDA also reviews food products, enforces current standards, and provides public education related to food safety. As a member of the World Trade Organization, Saudi Arabia aligns its food regulatory framework with international expectations, the Gulf Standardization Organization (GSO), and various other entities (Al-Mutairi et al., 2015). Sorghum-based hummus entering the market in Saudi Arabia must comply with SFDA legislation. Sorghum is categorized within cereals and grain products; therefore, the commodity is subject to multiple GSO technical standards. Labeling, health claims, microbiological criteria, and limits of pesticide usage standards would be applicable to the product as a whole. However, the added antimicrobial ingredient (thymol emulsion) would require additional compliance with SFDA.FD/GSO 2500 (Additives Permitted for Use in Foodstuffs), given its intended microbial control and inhibition function (Saudi Food and Drug Authority (SFDA), 2021).

Although the SFDA serves as the primary entity for food authority, additional regulatory bodies influence food safety in Saudi Arabia. The Saudi Standards, Metrology, and Quality Organization, previously known as the Saudi Arabian Standards Organization, is primarily concerned with the safety of consumers and the environment. The Codex Alimentarius Commission establishes requirements that include quality control and export certification. The Ministry of Municipality and Rural Affairs establishes food legislation, regulates inspections, oversees adulteration control, conducts sampling of food and water, and maintains registration of health premises. The Ministry of Health, which is slowly transferring food safety oversight to the SFDA, divides the General Department of Preventive Health and Environment Health

Administration. These organizations work with Municipalities and departments of agriculture to monitor drinking water, track foodborne outbreaks, mitigate pesticide usage, and update associated writings (Al-Mutairi et al., 2015). The Saudi Grains Organization and National Centre for Palms and Dates (NCPD) promote crop patterns suited to the local climate to reinforce nutrition security (Ahmed, 2024). The NCPD can serve as a primary partner with connections to the community to further promote the adoption of sorghum-based hummus.

While these entities do not hold primary authority, it is important to emphasize that these are supporting entities. A strong understanding of food safety through collaboration across the food system drives the success of new products. As stated previously, the SFDA classifies sorghum under cereal and whole-grain commodities while also including it in regulated food products; therefore, producers only need to uphold the standard of conformity documentation (SFDA, 2021). Sorghum ingredients must demonstrate compliance with relevant GSO standards for cereals, including identity, purity, and contaminant limits. Utilizing a thymol-based emulsion would require compliance with SFDA's GSO 2500 and would need to be declared on the label due to its composition, which includes dairy, a major allergen. Different authorities set varying standards for thymol, despite its international use as an antimicrobial agent. The incorporation of a thymol-based emulsion requires adherence to previously mentioned standards, which govern additives permitted for use in food. The hummus formulation utilizes thymol at four percent by weight; therefore, it is below the threshold of the maximum concentration allowed. Halal certification is also required as sodium caseinate, derived from dairy cattle, is used as an emulsifier. The SFDA evaluates final product safety under microbiological performance expectations outlined in SFDA.FD/GSO 1016, which guided the technical research for this project (SFDA, 2021). Together, these requirements ensure that ingredient additions provide technological benefit while maintaining regulatory compliance and consumer safety.

1.6 Implementation of Sorghum Hummus in the United States: A Regulatory Perspective

The United States (U.S.) predominantly uses sorghum as animal feed and fuel production due to a lack of exploration in food processing technologies that enhance palatability, limiting the potential health outcomes that consumers can benefit from. There are also limited applications due to anti-nutritional factors, such as tannins, phytic acid, trypsin inhibitors, and protein cross-linkers, which reduce protein, and mineral bioavailability, which indicate a need for processing methods that will create nutritional accessibility to these properties (Rashwan et al., 2021). In the U.S., sorghum is not a traditionally consumed as a staple crop; therefore, creating products that appeal to current consumer preferences is crucial to development.

To impact United States markets, doing a regulatory analysis of entities involved, and product requirements will allow for the optimization of the products' viability in the market. All ingredients are approved under a variation of the United States Department of Agriculture (USDA) and the Food and Drug Administration laws; however, understanding the mechanisms of product approval are beneficial for globalization.

The United States Food and Drug Administration (FDA) is the primary entity responsible for the oversight of food products within the United States; this would include sorghum products. The FDA prioritizes the public's health by ensuring the food supply through safety protocols, efficacy controls, and security measures (FDA, 2023). Regarding food products, there are several key dimensions that the FDA oversees including: toxic compound monitoring, safety protocols, import and export regulations, and nutritional labeling standards. While these are only several important factors that play a role in sorghum-product manufacturing, the Food Safety Modernization Act, which was signed into law in 2011, outlines key standards, rules, and regulations that must be followed to ensure product safety (FDA, 2024a). These rules prioritize prevention of contaminants within food systems to ensure superior quality of the public's health. As a member of the World Trade Organization, the United States aligns its food regulatory framework with international expectations, while holding import compliance to domestic standards. Like Saudi Arabia, the United States would also have to conform to the requirements established by the Codex Alimentarius Commission. Sorghum-based hummus is not traditionally a product sold in the United States; however, to build capacity for innovation, researchers must analyze its potential and the regulatory requirements associated with entering markets. Sorghum hummus would follow similar specifications related to chickpea hummus, but would require compliance with standards held for Ready-to-Eat Dips and Spreads. Fulfillment of the FDA's Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food Rule would be required as well for this product. Labeling, health claims, microbiological criteria, and limits of aflatoxins would be applicable. The addition of a thymol-emulsion would be permitted under the FDA's 21 CFR 172.515 (Synthetic flavoring substances and adjuvants), which recognizes the compound as a flavoring and natural preservative (FDA, 2026). Although the FDA serves as the primary entity for human food products, not including meat, poultry, eggs, or catfish, additional regulatory bodies create a synergistic effect on food systems in the United States.

The United States Department of Agriculture (USDA) is currently the primary authority over sorghum, as it is mostly used for animal feed, which is covered through various channels within the USDA. USDA Agricultural Marketing Services (AMS) leads programs that hold space for international and domestic marketing opportunities and the United States Standards for Grain Sorghum, which defines the requirements the grain must meet (USDA, FGIS 2026). The Federal Grain Inspection Service (FGIS) is a program within USDA AMS. FGIS establishes standards for quality, handling practices, and inspections for sorghum, creating and maintaining the grain's integrity (USDA, FGIS 2008). There are various non-governmental organizations, such as the United Sorghum Checkoff Program and National Sorghum Producers, that conduct research, promote safe practices, and educate communities on the benefits of sorghum. The United States Environmental Protection Agency predominantly oversees the use of pesticides, insecticides, etc. on crops (United States Environmental Protection Agency (EPA), 1993). While these entities do not hold primary authority, it is important to emphasize that these are sectors that enhance food safety and security in food systems.

Generally Recognized As Safe (GRAS) is a standard for any substance added to a food product that is proven to be safe by qualified experts (FDA, 2024b). Thymol is a compound that is GRAS and has historically been used in food products. The intended use varies between products; however, it is listed under 21 CFR 172.515 as it falls under synthetic flavoring substances and adjuvants. Other components of the emulsion, coconut oil and sodium caseinate, are approved under U.S. standards for food usage. Therefore, the emulsion being added to the product would not require any further steps. There are no documented levels of the maximum concentration of thymol allowed in a product; however, under 21 CFR 172.515, it is noted that the product must use the lowest quantity required to create the anticipated effect (FDA, 2026).

Despite the ease-of-access for the product to be approved, one of the most important requirements of this product is proper labeling. Sodium caseinate is a milk-derived protein and tahini, which is used in the hummus formulations, contain major allergens. The final product would need to be clearly labeled with the allergens. The product would require compliance with the FDA's sampling assignment for Ready-to-Eat Dips and Spreads to ensure product safety from pathogens commonly associated with similar products (FDA, 2023). Collaboratively, these requirements ensure that ingredient additions provide product benefit while maintaining regulatory compliance and consumer safety.

1.7 Conclusion

Sorghum, a crop with untapped potential, has the ability to impact markets globally within feed, fuel, and the human food sectors. Uses of the grain vary amongst regions; however, with innovative technology and novel processing techniques, it can alleviate food insecurity. Sorghum hummus, a value-added product, can maximize the grains palatability, potential, and utility of the product. Validating the safety of the product compared to similar products currently on the market will allow the optimization of product development. Essential oils, like thymol, offer antimicrobial effects that can enhance the products longevity resulting in increased consumer accessibility. Understanding regulatory frameworks and how products are approved in food systems are crucial to the success of the product. With the governments being obliged to improve agriculture sectors, sorghum-based hummus can improve food costs, uphold crop self-sufficiency desire, and provide employment to citizens. There are several challenges that accompany the adoption of sorghum-based products in Saudi Arabian markets, including consumer acceptance, fragmented input from regulatory entities, and the need for public health communication. Together, these findings demonstrate the potential of sorghum hummus as a sustainable, nutritious, and globally adaptable food product.

References

Abah, C. R., C. N. Ishiwu, J. E. Obiegbuna, and A. A. Oladejo. 2020. "Sorghum Grains: Nutritional Composition, Functional Properties and Its Food Applications." *European Journal of Nutrition & Food Safety*, June 22, 101–11. https://doi.org/10.9734/ejnfs/2020/v12i530232.

Adhar, Mariem, Basma HadjKacem, Sandrine Périno-Issartier, et al. 2022. "Thymol-Enriched Extract from *Thymus Vulgaris* L Leaves: Green Extraction Processes and Antiaggregant Effects on Human Platelets." *Bioorganic Chemistry* 125 (August): 105858. https://doi.org/10.1016/j.bioorg.2022.105858.

Ahmad Yahaya, Muhammad, Hussein Shimelis, Baloua Nebie, Chris O. Ojiewo, and Gideon Danso-Abbeam. 2022. "Sorghum Production in Nigeria: Opportunities, Constraints, and Recommendations." *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science* 72 (1): 660–72. https://doi.org/10.1080/09064710.2022.2047771.

Ahmed, Adam E. 2024. "Food and Nutrition Security in the Kingdom of Saudi Arabia, Vol. 2: Macroeconomic Policy and Its Implication on Food and Nutrition Security | Springer Nature Link." Accessed March 3, 2026. https://link.springer.com/book/10.1007/978-3-031-46704-2.

Al-Mutairi, Saad, Ian Connerton, and Robert Dingwall. 2015. "Food Safety Organisations in Saudi Arabia – Organisational, Historical and Future Analysis." *Food Control* 47 (January): 478–86. https://doi.org/10.1016/j.foodcont.2014.07.047.

Chang, Yuhua, Lynne McLandsborough, and David Julian McClements. 2012. "Physical Properties and Antimicrobial Efficacy of Thyme Oil Nanoemulsions: Influence of Ripening Inhibitors." *Journal of Agricultural and Food Chemistry* 60 (48): 12056–63. https://doi.org/10.1021/jf304045a.

Charyulu, D. Kumara, Victor Afari-Sefa, and Murali Krishna Gumma. 2024. "Trends in Global Sorghum Production: Perspectives and Limitations." In *Omics and Biotechnological Approaches for Product Profile-Driven Sorghum Improvement*, edited by Ephrem Habyarimana, Muhammad Azhar Nadeem, Faheem Shehzad Baloch, and Nusret Zencirci. Springer Nature. https://doi.org/10.1007/978-981-97-4347-6_1.

Duff, John, Austin Vincent, Doug Bice, and Ian Hoeffner. 2019. "Background on Grain Sorghum Usage." In *Sorghum: Methods and Protocols*, edited by Zuo-Yu Zhao and Jeff Dahlberg. Springer. https://doi.org/10.1007/978-1-4939-9039-9_17.

George, Toyosi T., Anthony O. Obilana, Ayodeji B. Oyenihi, Anthony B. Obilana, Damilola O. Akamo, and Joseph M. Awika. 2022. "Trends and Progress in Sorghum Research over Two Decades, and Implications for Global Food Security." South African Journal of Botany 151 (December): 960–69. https://doi.org/10.1016/j.sajb.2022.11.025.

Hariprasanna, K., and Sujay Rakshit. 2016. "Economic Importance of Sorghum." In *The Sorghum Genome*, edited by Sujay Rakshit and Yi-Hong Wang. Springer International Publishing. https://doi.org/10.1007/978-3-319-47789-3_1.

Hossain, Md. Saddam, Md. Nahidul Islam, Md. Mamunur Rahman, Mohammad Golam Mostofa, and Md. Arifur Rahman Khan. 2022. "Sorghum: A Prospective Crop for Climatic Vulnerability, Food and Nutritional Security." *Journal of Agriculture and Food Research* 8 (June): 100300. https://doi.org/10.1016/j.jafr.2022.100300.

International Federation of Red Cross and Red Crescent Societies (IFRC). n.d. *Kingdom Of Saudi Arabia (Ksa) Climate Fact Sheet*. https://prddsgofilestorage.blob.core.windows.net/api/documents/KSA_-_Climate_Fact_Sheet/KSA_Climate_Fact_Sheet_EN.pdf.

Jayari, Asma, Francesco Donsì, Giovanna Ferrari, and Abderrazak Maaroufi. 2022. "Nanoencapsulation of Thyme Essential Oils: Formulation, Characterization, Storage Stability, and Biological Activity." *Foods* 11 (13): 1858. https://doi.org/10.3390/foods11131858.

Kaniewski, David, Elise Van Campo, and Harvey Weiss. 2012. "Drought Is a Recurring Challenge in the Middle East." *Proceedings of the National Academy of Sciences* 109 (10): 3862–67. https://doi.org/10.1073/pnas.1116304109.

Liu, Shizhuo, Junyuan Lei, Hongyang Liu, et al. 2026. "Thymol: Properties, Synthesis, Mechanism of Action, and Applications." *Frontiers in Nutrition* 13 (March). https://doi.org/10.3389/fnut.2026.1774718.

Liu, Yao, Hui Yan, Bing Yu, et al. 2022. "Protective Effects of Natural Antioxidants on Inflammatory Bowel Disease: Thymol and Its Pharmacological Properties." *Antioxidants* 11 (10): 1947. https://doi.org/10.3390/antiox11101947.

Luo, Donglan, Xue Li, Xiaogang Wang, Sen Cao, Liangjie Ba, and Xiaohong Kou. 2025. "Research Progress on the Food Preservation Effects Exploiting the Antifungal Properties of Thymol." *Food Chemistry: X* 30 (August): 102954. https://doi.org/10.1016/j.fochx.2025.102954.

Marchese, Anna, Ilkay Erdogan Orhan, Maria Daglia, et al. 2016. "Antibacterial and Antifungal Activities of Thymol: A Brief Review of the Literature." *Food Chemistry* 210 (November): 402–14. https://doi.org/10.1016/j.foodchem.2016.04.111.

Morais Cardoso, Leandro de, Soraia Silva Pinheiro, Hércia Stampini Duarte Martino, and Helena Maria Pinheiro-Sant'Ana. 2017. "Sorghum (Sorghum Bicolor L.): Nutrients, Bioactive Compounds, and Potential Impact on Human Health." *Critical Reviews in Food Science and Nutrition* 57 (2): 372–90. https://doi.org/10.1080/10408398.2014.887057.

Mundia, Clara W., Silvia Secchi, Kofi Akamani, and Guangxing Wang. 2019. "A Regional Comparison of Factors Affecting Global Sorghum Production: The Case of North

America, Asia and Africa's Sahel." *Sustainability* 11 (7). https://doi.org/10.3390/su11072135.

Nogueira, Barradas Thaís, and de Holanda e Silva, Kattya Gyselle. 2021. "Nanoemulsions of Essential Oils to Improve Solubility, Stability and Permeability: A Review." *Environmental Chemistry Letters* (Dordrecht, Netherlands) 19 (2): 1153–71. https://doi.org/10.1007/s10311-020-01142-2.

Rashwan, Ahmed K., Hala A. Yones, Naymul Karim, Eman M. Taha, and Wei Chen. 2021. "Potential Processing Technologies for Developing Sorghum-Based Food Products: An Update and Comprehensive Review." *Trends in Food Science & Technology* 110 (April): 168–82. https://doi.org/10.1016/j.tifs.2021.01.087.

Saudi Food and Drug Authority (SFDA). 2021. "Food Clearance Conditions and Requirements."

Silva, Celia C. G., and Susana Chaves Ribeiro. 2024. "Microorganisms and Their Importance in the Food Industry: Safety, Quality and Health Properties." *Foods* 13 (10): 1452. https://doi.org/10.3390/foods13101452.

Swamy, K. M. 2023. "Origin ,Domestication, Taxonomy, Botanical Description, Genetics And Cytogenetics, Genetic Diversity And Breeding Of Sorghum (Sorghum Um Bicolor (L.) Moench)." Vl.15,I Ssue,10,.26251-26285.

Taylor C. Wallace 1,*, Robert Murray 2 and Kathleen M. Zelman 3. 2016. *The Nutritional Value and Health Benefits of Chickpeas and Hummus*.

U.S. Environmental Protection Agency (EPA). 1993. *EPA R.E.D. Facts: Thymol*. United States Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances. https://archive.epa.gov/pesticides/reregistration/web/pdf/thymol.pdf.

U.S. Food and Drug Administration (FDA). 2023. "FDA Releases Summary Report on Ready-to-Eat Dips and Spreads Sampling Assignment." *FDA*. https://www.fda.gov/food/hfp-constituent-updates/fda-releases-summary-report-ready-eat-dips-and-spreads-sampling-assignment.

U.S. Food and Drug Administration (FDA). 2024a. "Background on the FDA Food Safety Modernization Act (FSMA)." *FDA*. https://www.fda.gov/food/food-safety-modernization-act-fsma/background-fda-food-safety-modernization-act-fsma.

U.S. Food and Drug Administration (FDA). 2024b. "Generally Recognized as Safe (GRAS)." FDA, FDA. https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras.

U.S. Food and Drug Administration (FDA). 2026. "21 CFR 172.515 -- Synthetic Flavoring Substances and Adjuvants." Electronic Code of Federal Regulations. https://www.ecfr.gov/current/title-21/part-172/section-172.515.

USDA, FGIS. 2008. Subpart I -- United States Standards for Sorghum.

USDA, FGIS. 2026. “Federal Grain Inspection Service | Agricultural Marketing Service.” https://www.ams.usda.gov/about-ams/programs-offices/federal-grain-inspection-service.

Chapter 2 - Motive and Hypothesis Research Gaps

Sorghum-based hummus offers advantages across multiple domains, including agriculture sustainability, global markets, and consumer markets. Formulating products that align with consumers' preferences and incorporate added nutritional benefits creates an opportunity to address complex challenges, such as food insecurity, chronic illness, and sustainability in food systems. Food insecurity is a complex challenge that results in communities lacking resources and nutrients that are necessary for survival. This is an issue that impacts communities across the globe; therefore, product developers must focus on sustainability and optimization of existing commodities.

Sorghum is grown in various locations across the world, including the United States, Nigeria, and Saudi Arabia. The crop requires minimal resources, in regard to climate and rainfall, making it ideal for areas facing issues related to climate. This crop naturally maintains drought-tolerant characteristics that current staple crops, such as corn, wheat, and rice, have been selectively bred to sustain. Therefore, promoting crop diversity with sorghum allows farmers to have increased product yields with less labor and input of resources. Sorghum is also gluten-free, offering an alternative to those with gluten sensitivities.

Sorghum-based hummus allows consumers to be able to eat a food product in a familiar format that is already consumed globally. Sorghum possesses nutrients that can serve functional benefits, which may persuade consumers to be more inclined to consume the product, for example, increased digestion or inflammation relief. Increased awareness towards products that have direct benefits has gained popularity in the past decade, for example, probiotic sodas or protein-heavy desserts. Sorghum-based hummus would create a similar effect as it possesses functional properties.

Sorghum-based hummus would create an accessible product to communities across the world. This establishes a synergistic effect within food systems among producers, industries, wholesalers, and consumers, resulting in efficiency across all domains in regard to sorghum optimization.

One way to increase the accessibility and safety of sorghum hummus is to formulate a product with a longer shelf-life, as well as inhibition of bacteria. Ideally, incorporating encapsulated essential oils enhances the product's durability against foodborne pathogens, resulting in the longevity of the product. Previous applications of encapsulated thymol have been found active within products, but if the mechanisms within a homogenous product were effective, sorghum hummus could have a longer shelf-life and enhanced resistance against spoilage microorganisms (Marchese et al., 2016).

Therefore, the objective of this thesis is to evaluate microbiological stability, nutritional quality, sensory acceptance, and implementation feasibility of sorghum-based hummus. These objectives can be achieved through complementary laboratory, consumer, and regulatory investigations.

References

Marchese, Anna, Ilkay Erdogan Orhan, Maria Daglia, et al. 2016. "Antibacterial and Antifungal Activities of Thymol: A Brief Review of the Literature." Food Chemistry 210 (November): 402–14. https://doi.org/10.1016/j.foodchem.2016.04.111.

Chapter 3 - Nutritional, microbiological, and feasibility evaluation of sorghum-based hummus

3.1 Microbial Assay

Essential oils can enhance the shelf-life of products depending on the application; however, free oils are high volatility and low solubility. Encapsulation of essential oils within a nanoemulsion can enable the controlled release of the compounds within a product (Nogueira and Holanda e Silva, 2021). Thymol and limonene are plant-derived compounds that have antifungal and natural preservation properties, with existing application on food products (Han et al., 2019; Luo et al., 2025). Using additives with enhanced antimicrobial effect within food products can result in a prolonged shelf-life of a product.

3.1.1 Materials and Methods

Bacterial Strains and Inoculum Preparation

Listeria monocytogenes KSU 390-6, Staphylococcus aureus ATCC 25923, and *Salmonella spp. ATCC 142028* were the bacterial strains identified in this study. The strains were stored at -80°C to inhibit deactivation. Prior to the assay, the strains were streaked onto Tryptic Soy Agar (TSA: BD, Franklin Lakes, NJ, USA) and incubated at 37°C for 24h. One colony from the TSA plate was transferred into 10 ml of Tryptic Soy Broth (TSB; BD, Franklin Lakes, NJ, USA) and incubated overnight at 37°C (Hossain, 2024).

Media Preparation

On the day of the experiment, fresh TSA was prepared, autoclaved, and held at 45 °C until assay. The three overnight cultures were individually vortexed for five to ten seconds to ensure uniform dispersal. Subsequently, 20 µL of the corresponding overnight culture was added to 20 mL of liquid TSA, gently mixed and poured into a petri dish. Inoculated media was allowed to solidify. The same procedure was followed for all the bacteria selected (Hossain, 2024).

Antimicrobial Sample Screening

One-hundred microliters of two different compound-based emulsions were screened for antimicrobial activity in the initial study: thymol and limonene. Sodium caseinate (4% w/v), an emulsifier, was dispensed into deionized water while stirring. The emulsifier took approximately two hours to fully dissolve at room-temperature stirring. After fully dissolved, the active ingredient (thymol or limonene) was added. The emulsifier dispersed in water was mixed with the lipid core using IKA T18 basic, Ultra-Turrax at 10,000 rpm for three minutes. Emulsions were prepared using a high-pressure homogenizer (PandaSmart Homogenizer, GEA) for 3 cycles at 500 bars. The emulsions were stored at 4 °C until used (Kumar and Mehta, 2025).

Following preparation, each emulsion was dispensed into the respective wells on the inoculated TSA plate. Pure limonene (100%) and 50% thymol dissolved in coconut oil were considered controls and tested for comparison purposes. Inhibition halos were measured with a ruler after 24 h incubation (Hossain, 2024).

Data Analysis

All data was visually analyzed, then verified using RStudio Version 2026.01.0+392. A two-way additive ANOVA was conducted to evaluate the effect of pathogen strain and microbial treatment on halo growth zones (mm).

3.1.2 Results

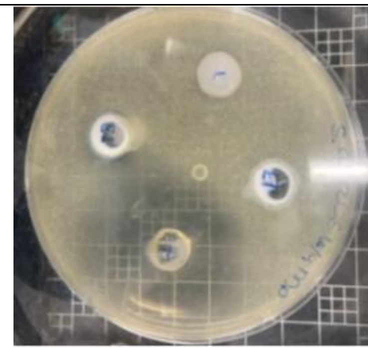
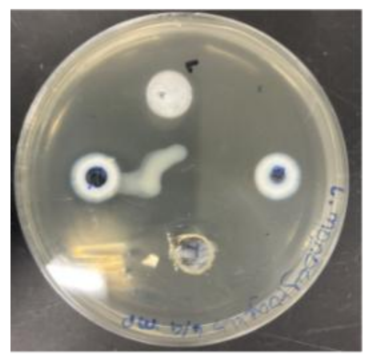
After 24 hours at 37°C, the microbial assay plates were analyzed. Halo formation was characterized by discoloration of the media, creating a "halo effect" around the well. The measurements include the well (10 mm).

A two-way additive ANOVA was conducted to evaluate the effect of pathogen strain and microbial treatment on halo growth zones (mm). The analysis revealed a highly significant main effect for the experimental treatments ($p<0.001$). However, no significant differences were observed across different pathogen strains ($p=0.314$).

As illustrated in Table 1, the limonene emulsion has little effectiveness against all pathogens. The 100% limonene had no effectiveness against the tested pathogens. Measurements were slightly larger than the well due to absorption of the liquids into the media.

However, the thymol emulsion displayed major differences compared to both limonene samples. The thymol emulsion measures 13.0 mm to 13. 5 mm in diameter across the assays, with the largest difference being observed against *Salmonella spp.*. The 50% thymol with coconut oil solution displayed the largest difference across all samples and pathogens. These measurements rand from 30 mm to 45 mm with *Salmonella spp.*, again to show the most change at 45 mm (Table 1).

Table 1: Microbial assay to determine antimicrobial efficacy against selected pathogens *Listeria monocytogenes KSU 390-6, Staphylococcus aureus ATCC 25923,* and *Salmonella spp. ATCC 142028.*

Bacteria	Halo Diameter*	Plate Image
Salmonella spp. ATCC 142028	E1: 13.0 mm	
	E2: 13.5 mm	
	L: ~11.0 mm	
	T: 41.0 mm	
Staphylococcus aureus ATCC 25923	E1: 12.0 mm	
	E2: 13.0 mm	
	L: ~11.0 mm	
	T: 30.0 mm	
Listeria monocytogenes KSU 390-6	E1: 12.5 mm	
	E2: 13.0 mm	
	L: ~11.0 mm	
	T: 35.0 mm	

*Halo diameter acronyms: E1 is a limonene emulsion, E2 is a thymol emulsion, L is 100% limonene, and T is 50% thymol dissolved in coconut oil.

The findings of the microbial assay allowed researchers to make the decision to proceed with thymol as the compound of interest.

3.1.3 Discussion

The preliminary study was performed to highlight the efficacy of the thymol emulsion and 50% thymol and coconut oil solution where the results indicated effectiveness. Both treatments displayed efficacy in the media; however, the emulsion later showed no evidence of increased or decreased bacterial growth within the tested hummus types. A limitation of thymol is its low water solubility, which inhibits homogeneity in aqueous media (Escobar et al., 2020); however, the objective of formulating a nanoemulsion was to overcome this natural barrier (Jayari et al., 2022).

Thymol nanoencapsulated by sodium caseinate has an optimal pH ranging from 3.5 to 4.6 (Pan et al., 2014; Chang et al., 2012). This leads researchers to question how halos formed in the inoculated Tryptic Soy Agar, a neutral medium (pH 7.0). One explanation for the large halo formation around the 50% thymol and coconut oil solution is the volatile nature of these compounds. Both of the compounds vaporize rapidly at incubation temperatures (Doghish et al., 2023). There is a possibility that the coconut oil and thymol evaporated into the space between the agar surface and petri dish lid, resulting in large bacterial inhibition. This result, in turn, led researchers to believe that the emulsion would have a similar effect against pathogens within a food matrix. However, volatile compounds within food products can lead to rapid product degradation, therefore, influencing researchers to proceed with the microbiological study utilizing the nanoemulsion.

There are several limitations to this study. First off, the nature of a microbial assay is qualitative. Therefore, a quantitative study would have further confirmed the findings of study (Gonzalez-Pastor et al., 2023). Another limitation is the concept of observer bias. The qualitative nature of the study is open to individual interpretations based on visual inspection.

References

Chang, Yuhua, Lynne McLandsborough, and David Julian McClements. 2012. “Physical Properties and Antimicrobial Efficacy of Thyme Oil Nanoemulsions: Influence of Ripening Inhibitors.” Journal of Agricultural and Food Chemistry 60 (48): 12056–63. https://doi.org/10.1021/jf304045a.

Doghish, Ahmed S., Amr M. Shehabeldine, Hesham A. El-Mahdy, et al. 2023. “Thymus Vulgaris Oil Nanoemulsion: Synthesis, Characterization, Antimicrobial and Anticancer Activities.” Molecules 28 (19): 6910. https://doi.org/10.3390/molecules28196910.

Escobar, Angélica, Miriam Pérez, Gustavo Romanelli, and Guillermo Blustein. 2020. “Thymol Bioactivity: A Review Focusing on Practical Applications.” Arabian Journal of Chemistry 13 (12): 9243–69. https://doi.org/10.1016/j.arabjc.2020.11.009.

Gonzalez-Pastor, Rebeca, Saskya E. Carrera-Pacheco, Johana Zúñiga-Miranda, et al. 2023. “Current Landscape of Methods to Evaluate Antimicrobial Activity of Natural Extracts.” Molecules 28 (3): 1068. https://doi.org/10.3390/molecules28031068.

Han, Yingjie, Zhichang Sun, and Wenxue Chen. 2019. “Antimicrobial Susceptibility and Antibacterial Mechanism of Limonene against Listeria Monocytogenes.” Molecules 25 (1): 33. https://doi.org/10.3390/molecules25010033.

Hossain, Tanim Jabid. 2024. “Methods for Screening and Evaluation of Antimicrobial Activity: A Review of Protocols, Advantages, and Limitations.” European Journal of Microbiology & Immunology 14 (2): 97–115. https://doi.org/10.1556/1886.2024.00035.

Jayari, Asma, Francesco Donsì, Giovanna Ferrari, and Abderrazak Maaroufi. 2022. “Nanoencapsulation of Thyme Essential Oils: Formulation, Characterization, Storage Stability, and Biological Activity.” Foods 11 (13): 1858. https://doi.org/10.3390/foods11131858.

Kumar, Amit, and S. K. Mehta. 2025. “Advancements in Essential Oil-Based Emulsions: Eco-Friendly Alternatives to Conventional Agrochemicals.” Current Opinion in Colloid & Interface Science 80 (December): 101964. https://doi.org/10.1016/j.cocis.2025.101964.

Luo, Donglan, Xue Li, Xiaogang Wang, Sen Cao, Liangjie Ba, and Xiaohong Kou. 2025. “Research Progress on the Food Preservation Effects Exploiting the Antifungal Properties of Thymol.” Food Chemistry: X 30 (August): 102954. https://doi.org/10.1016/j.fochx.2025.102954.

Nogueira, Barradas Thaís, and de Holanda e Silva Kattya Gyselle. 2021. “Nanoemulsions of Essential Oils to Improve Solubility, Stability and Permeability: A Review.” Environmental Chemistry Letters (Dordrecht, Netherlands) 19 (2): 1153–71. https://doi.org/10.1007/s10311-020-01142-2.

Pan, Kang, Huaiqiong Chen, P. Michael Davidson, and Qixin Zhong. 2014. “Thymol Nanoencapsulated by Sodium Caseinate: Physical and Antilisterial Properties." Journal of Agricultural and Food Chemistry 62 (7): 1649–57. https://doi.org/10.1021/jf4055402.

3.2 Microbiological Study

Sorghum hummus is a product that has not been on the market before; therefore, there is a need to understand how time and storage would affect the product. Analyzing how the product maintains quality under storage conditions. Hummus is naturally a high moisture food, with relatively low acidity, therefore refrigeration is necessary to maintain the quality of the product (Canadian Food Inspection Agency, 2018). Traditional hummus has a refrigerated shelf-life of seven days after opening, whereas there is no current information on sorghum hummus. The formulation used by Osaili et al. (2024) can be adapted to fit sorghum hummus to evaluate if sorghum can maintain quality during shelf-life similar to chickpea hummus. Prevention of hazardous microorganisms is a required parameter of all hummus types. By using a thymol emulsion as an experimental factor within the study, the potential extension of the shelf-life can be determined. By creating a product with a longer shelf-life, consumers will have more access to the nutrient-dense product.

3.2.1 Materials and Methods

Antimicrobial Selection and Hummus Preparation

Based on the results observed in the antimicrobial screening study, the following antimicrobial combinations were chosen to perform the hummus shelf-life study:

Table 2: Nanoencapsulated Thymol emulsion formulation added to experimental hummus.

Ingredient	Percentage
Sodium caseinate	80.0
Coconut oil	16.0
Thymol	4.0

Red sorghum, white sorghum, and chickpea hummus were all prepared identically. The grains were soaked in water at room temperature for 24h prior to cooking. Each of the grains were separately pressure-cooked in water using an Instant Pot 8-Quart DUO 7-in-1 Multi-Cooker on high for 45 minutes. After straining the water, the grains (75%) were made into a paste using a Hamilton Beach Stack & Press 2 Speed Food Chopper & Mini Food Processor (3 cups) to and combined with the following ingredients: sesame paste (12%), bottled water or thymol emulsion (12%) , salt (0.6%), and citric acid (0.3%) (Osaili et al., 2024).

Hummus Challenge Study

The 3 pathogenic strains selected for antimicrobial screening (*Listeria monocytogenes 390-6, Staphylococcus aureus 25923,* and *Salmonella spp. 142028)* were prepared and replicated as previously described. On the day of the experiment, overnight cultures were centrifuged at 4000 rpm for 10 min. The supernatant was discarded and the pellet dissolved in 6 mL of phosphate buffer saline (PBS; EMD Millipore, Burlington, MA, USA). A cocktail was obtained combining 1 ml from each prepared microbial solution. Total count was checked before starting experiments by serial dilution on TSA. The combined microbial cocktail (1 ml) was used to experimentally inoculate 100 g of each hummus type in triplicate (3 bags of 100 g portion). Inoculated samples were then massaged for one minute to ensure uniform pathogen distribution and stored at 4°C for seven days.

Sampling Plan

Samples (5 g) were taken at day 0 (just after inoculum), 3, 5, and 7 days and transferred to 90 mL of Buffered Peptone Water (BPW; BD, Franklin Lakes, NJ, USA) in a sterile, stomacher bag. The sample was then hand massaged for one minute and remaining population e verified by serial dilutions on selective media: Oxford Medium Base (MOX; BD, Franklin Lakes, NJ, USA) plus supplement (Listeria selective, Oxoid, Thermo Fisher Scientific, Waltham, MA, USA) for *L. monocytogenes*, Xylose Lysine Deoxycholate (XLD; Hardy Diagnostics, Santa Maria, CA, USA) for *Salmonella* , and Mannitol Salt Agar (MSA; Hardy Diagnostics, Santa Maria, CA, USA) for *S. aureus*. All plates were incubated at 37 °C for 24 hours and colonies were counted and expressed as CFU/g.

Data Analysis

All data was analyzed using RStudio Version 2026.01.0+392. Linear mixed-effect models were used to evaluate the effects of bacterial species, grain type, treatment, and storage time on bacterial growth, measured as $\log_{10}$ CFU/g (Equation 1). Batch was included as a random effect to account for the incomplete block design of the experiment.

Equation 1: Linear mixed-effects model used to evaluate bacterial growth.

$$y_{ijkl} \sim N(\mu_{ijkl}, \sigma^2)$$

$$\mu_{ijkl} = \mu_0 + B_i + T_j + G_k + BT_{ij} + BG_{ik} + TG_{jk} + BTG_{ijk} + \beta_{1ijk} \times x_{ijkl} + b_l$$

$$b_l \sim N(0, \sigma_b^2)$$

Model parameters are defined in Table 3.

Table 3: Definitions of parameters included in the linear mixed-effects model used to evaluate bacterial growth.

Symbol	Effect	Description
B	Bacteria	Effect associated with bacterial species
T	Treatment	Effect of the thymol emulsion treatment
G	Grain type	Effect of white sorghum, red sorghum, and chickpea
x	Sampling day	Continuous sampling time (Days 0, 3, 5, and 7)
b	Batch	Random variation attributable to experimental batches

Sampling day was initially evaluated as a continuous and categorical predictor. Model comparison using Akaike's Information Criterion (AIC) and Bayesian Information Criterion (BIC) modeling sampling day as a continuous covariate in the final model. Residual diagnostics conducted using the DHARMa package indicated that the final model adequately met assumptions of normality and homogeneity of variance.

Type III analysis of variance was used to assess the effects of the thymol emulsion treatment, while accounting for bacterial species, grain type, and sampling day. No significant interaction was observed over the sampling period of seven days ($p > 0.05$), indicating that treatment effects were consistent over time. Sampling day was therefore retained as a continuous covariate, and results are presented at Day 7 for interpretation. Temporal changes in bacterial growth were evaluated using estimated slopes derived from the mixed-effects model using emtrends function.

3.2.2 Results

Listeria monocytogenes KSU 390-6, Staphylococcus aureus ATCC 25923, and *Salmonella spp. ATCC 142028* populations were evaluated over a seven-day storage period using two treatments within each hummus type: a control group made with water and a treatment group made with thymol emulsion.

There was little variation between the pH values, indicating initial signs of no major pathogen growth or undesired characteristics of the product, such as lactic acid bacteria (Table 4). There were higher pH values in the red sorghum hummus and chickpea hummus; however, all hummus types remained stable at a pH of 5.0 by day 5 and day 7.

Table 4: Average pH values of tested hummus types (3) over 7 days.

Hummus Type	Treatment	Day 0	Day 3	Day 5	Day 7
White Sorghum	EO	5.17± 0.29	5.0	5.0	5.0
	Control	5.17± 0.29	5.0	5.0	5.0
Red Sorghum	EO	5.17± 0.29	5.17± 0.29	5.0	5.0
	Control	5.17± 0.29	5.17± 0.29	5.0	5.0
Chickpea	EO	5.25± 0.35	5.08± 0.12	5.0	5.0
	Control	5.25± 0.35	5.08± 0.12	5.0	5.0

As shown in Table 4, the addition of the essential oil (EO) treatment had no impact on the pH of any tested hummus type, outputting values identical to control groups across all sampling days. For all formulations, pH values steadily declined from sampling Day 0 and led to a pH of 5.0 by Day 5, remaining stable through Day 7.

Physical characteristics of the hummus were dependent upon the grain incorporated. Over the seven-day refrigeration period, there was no difference between the texture, color, and consistency of any of the hummus types (Table 5). Both sorghum hummus samples, red and white, visually exhibited a coarser consistency than the chickpea hummus.

Table 5: Pictures of control and experimental hummus types on day 0 and day 7.

Treatment	Day 0	Day 7
White Sorghum Control		
White Sorghum with EO*		
Chickpea Control		
Chickpea with EO*		

Red Sorghum Control		
Red Sorghum with EO*		

*EO is the experimental group treated with thymol emulsion.

Sampling day (0, 3, 5, and 7) was modeled as a continuous covariate, and estimated slopes were compared across thymol emulsion treatment, bacterial species, and grain type to assess whether rates of change over time differed among experimental conditions. Across bacterial species, grain types, and treatment conditions, estimated slopes did not differ significantly from zero, where 95% confidence intervals included zero. This indicates that there is no evidence of increased or decreased bacterial growth over the seven-day sampling period. Bacterial populations remained stable over time, confirming the product's stability under the conditions evaluated.

Pairwise comparisons indicated no significant differences between treatment and control across bacterial species and grain type combinations (adjusted $p > 0.05$; confidence intervals included zero), suggesting that thymol emulsion did not significantly affect bacterial growth under the conditions tested effects differed among bacterial species and grain type combinations (Figure 1).

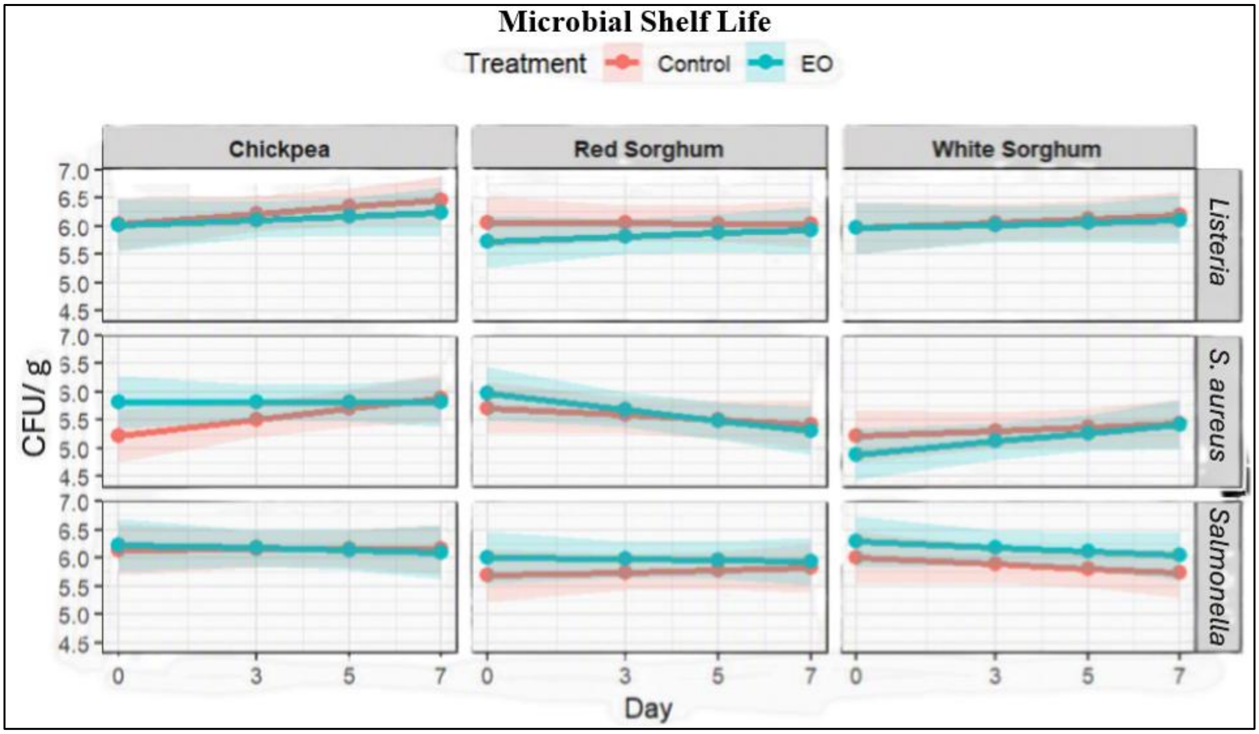


Figure 1: Bacterial growth trajectories ($\log_{10}$ CFU/g) over storage time (0, 3, 5, and 7 days) by bacterial species, grain type, and treatment based on the final mixed-effects model.

Estimated marginal means were used to evaluate the difference in bacterial growth ($\log_{10}$ CFU/g) between thymol emulsion treatment and the control with the pairing of bacterial species and grain type at Day 7 (Figure 2).

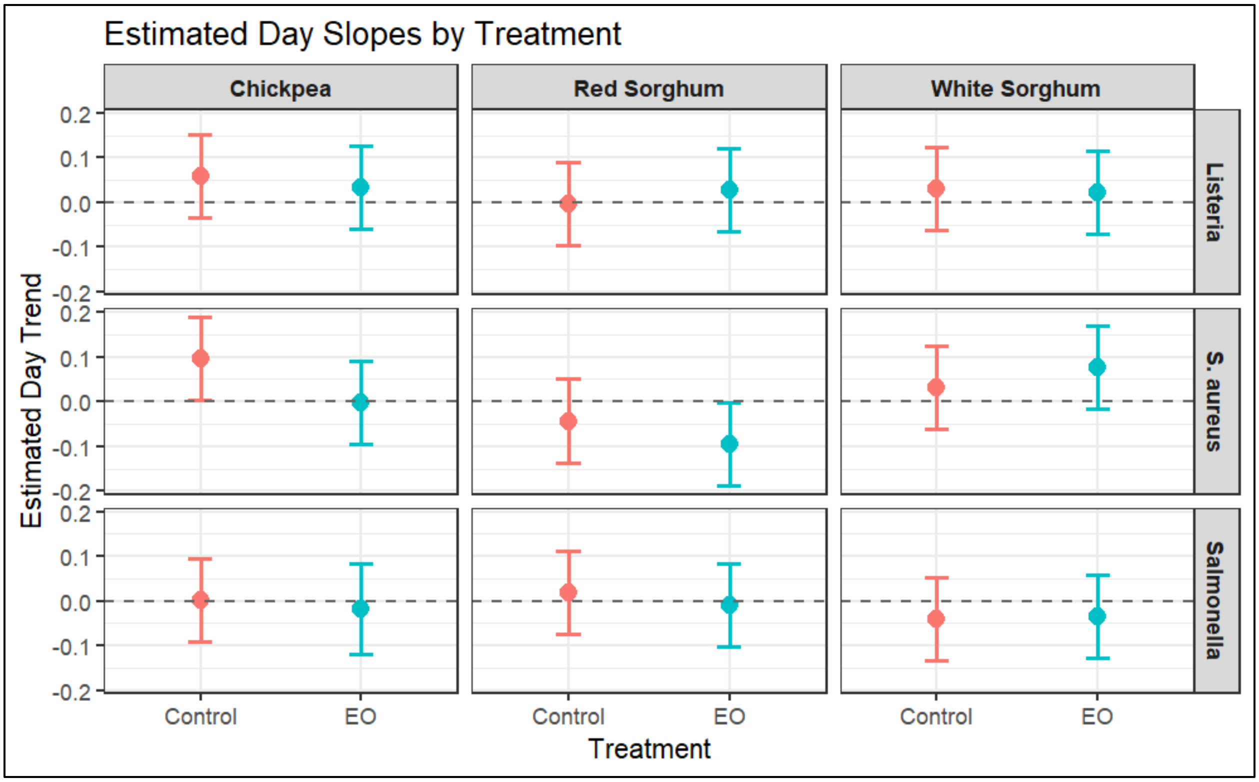


Figure 2: Estimated slopes of bacterial growth over time (emtrends) by thymol emulsion treatment, bacterial species, and grain type.

Model-based predictions of bacterial growth were consistent with the inferential results from both the Type III analysis and the emtrends analysis. Predicted $\log_{10}$ CFU/g values indicated stable bacterial populations over time, with minimal variation attributable to the thymol emulsion treatment.

Differences in bacterial growth were primarily associated with bacterial species and grain type, whereas temporal changes and treatment effects were insignificant under the experimental conditions.

3.2.3 Discussion

Physical characteristics of the hummus types remained constant during the sampling period. Visually, chickpea hummus was consistently smooth whereas both sorghum types had visual grains over the seven-day storage period. While each of the base materials for the hummus types were soaked overnight, this did not completely soften the dense interior of sorghum. This resulted in a denser, more coarse texture of red and white sorghum hummus.

The pH values of the hummus types were stable during the refrigeration period. While pH values were slightly elevated in the red sorghum hummus and chickpea hummus during initial sampling, all hummus types leveled out at a pH of 5.0 by day 5. The pH of traditional hummus is 5.65 ± 0.01, indicating that all hummus types fell under the typical range, which could be due to the amount of citric acid in the formulation (Goswami et al. 2025). In this study, the pH supports product stability through the duration of sampling.

The statistical analysis revealed that bacterial species, grain type, and treatment did not result in an increase or decrease of bacterial populations during the seven-day sampling period. As previously mentioned, nanoencapsulated thymol with sodium caseinate has an optimal pH of 3.5 to 4.6, indicating that all hummus types have a pH higher than this range (Pan et al., 2014; Chang et al., 2012).

A study incorporated a thymol, sodium caseinate nanoemulsion in milk and was effective at a neutral pH at inhibiting *Listeria monocytogenes,* with optimal activity within the first 48 hours at room temperature. The experimental design of this study has several differences than those tested here, such as applied food matrices, storage temperature, and use of soluble soybean polysaccharide (SSPS). In place of the SSPS, the hummus study used coconut oil, mainly composed of saturated fats, in the formulations. The milk study found that with increased fat, encapsulated and free thymol reduced in concentration, which could result in loss of antimicrobial activity (Pan et al., 2014). Therefore, once sodium caseinate was disrupted by the abnormal pH, thymol would have a high affinity for bonding with other polar, hydrophobic molecules, like coconut oil. Hence, there is a lack of antimicrobial activity. The lack of antimicrobial is further supported by the pH of the hummus. All hummus types had a pH ranging from 5.0 to 5.25, this neared sodium caseinates isoelectric point, therefore it could aggregate and result in separation of the emulsion (Rasnani and Mirhosseini 2011).

Overall, the study allows for the conformation of the product's stability under the evaluated conditions. This does not account for consumers or manufacturers storage over an extended period of time. A limitation of the microbiological study is that the processing technology utilized, a food processor, did not effectively puree each product. Red sorghum and white sorghum exhibited a coarser texture, which could lead to variation in replicability in experimental trial and in the industry setting, depending on machinery.

References

Canadian Food Inspection Agency. 2018. “Shelf Life Studies.” Reference material Guide. June 13. http://inspection.canada.ca/en/preventive-controls/shelf-life-studies.

Chang, Yuhua, Lynne McLandsborough, and David Julian McClements. 2012. “Physical Properties and Antimicrobial Efficacy of Thyme Oil Nanoemulsions: Influence of Ripening Inhibitors.” Journal of Agricultural and Food Chemistry 60 (48): 12056–63. https://doi.org/10.1021/jf304045a.

Goswami, Meena, Rishav Kumar, Xin M. Teng, et al. 2025. “Physicochemical, Nutritional, and Structural Characterization of a Novel Meat-Based Hummus.” Foods 14 (14): 2507. https://doi.org/10.3390/foods14142507.

Osaili, Tareq M., Anas A. Al-Nabulsi, Asma’ O. Taybeh, et al. 2024. “Garlic and Chitosan Improve the Microbial Quality of Hummus and Reduce Lipid Oxidation.” Foods 13 (24): 4074. https://doi.org/10.3390/foods13244074.

Pan, Kang, Huaiqiong Chen, P. Michael Davidson, and Qixin Zhong. 2014. “Thymol Nanoencapsulated by Sodium Caseinate: Physical and Antilisterial Properties.” Journal of Agricultural and Food Chemistry 62 (7): 1649–57. https://doi.org/10.1021/jf4055402.

Rasnani, N. M., & Mirhosseini, H. 2011. Effect of pH on physicochemical properties and stability of sodium caseinate-pectin stabilized emulsion. Retrieved https://www.researchgate.net/publication/235907032_Effect_of_pH_on_physicochemical_properties_and_stability_of_sodium_caseinate-pectin_stabilized_emulsion

3.3 Sensory Analysis

Currently, sorghum-based hummus is not a commercially available product. Sensory analysis enables consumer feedback before a product enters a commercial setting. Hence, enabling researchers to understand how consumers view a product and the potential it would have in a retail space (Ruiz-Capillas and Herrero, 2021). By completing an analysis for the hummus, an understanding of how traditional, chickpea hummus, compares to red and white sorghum hummus. The findings provide experimental clarity on the products developed in several aspects including measurable consumer perspective, understanding of the formulation's properties, and how future developments should proceed (Pop, 2023).

3.3.1 Materials and Methods

Study Design

Forty-one participants were given a total of nine samples. Three different emulsions were combined with each of the three grain types: distillers dried grains with solubles emulsion, 2% w/v (DDGS); thymol chitosan emulsion, 2% w/v (TC); thymol sodium caseinate emulsion, 4% w/v (TSC). All samples were prepared in 250 g batches as described above in paragraph two of Antimicrobial Selection and Hummus Preparation. Following preparation, 5 g of a sample was placed into a clear plastic 2oz portion cup with a lid and labeled with a unique number to indicate sample. All participants received samples 1-9, palate cleansers, and a paper survey. The palate cleansers provided were water and unflavored water crackers (Jadayil et al., 2026).

The paper survey consisted of demographic and preliminary questions to track previous traditional hummus consumption, 5-point hedonic scale questions for each sample (a total of nine), and binomial questions following each sample to determine the odds of consuming again.

Participants were briefed before the assessment of the purpose of the study, procedures, confidentiality, risks, benefits, contact information of researchers and the Institutional Review Board, and a reminder of voluntary participation. All participants verbally agreed to participate in the study. The paper results were collected as the study concluded.

Data Analysis

All data was analyzed using RStudio Version 2026.01.0+392. To evaluate consumer preferences across the various hummus types and treatments, a non-parametric Friedman test was performed on the sensory panel scores, followed by post-hoc pairwise Wilcoxon signed-rank tests utilizing a Bonferroni adjustment to identify specific differences between individual treatment pairs ($p<0.05$).

3.3.2 Results

There were a total of 41 participants that voluntarily participated within the tasting panel. Participants were required to be above the age of 18, a member of the Kansas State University system, have no allergies to dairy, shellfish, and sesame, and consent to participation. No participants withdrew from the study once initiated.

Roughly 61% of participants reported regular consumption for traditional hummus, where 37% of these participants recorded consumption once a month. Chickpea hummus formulated with distillers dried grains with solubles emulsion (CH DDGS) and red sorghum hummus with distillers dried grains with solubles emulsion (RSH DDGS) formulations received the highest overall proportion of positive responses, where over 50% of participants responded, “Somewhat like” and “Like a lot.” WS TSC, the emulsion used in the microbiological study, showed a higher proportion of negative responses (Figure 2). Nearly half of participants responded "Not like at all" or "Somewhat dislike" on white sorghum hummus formulation

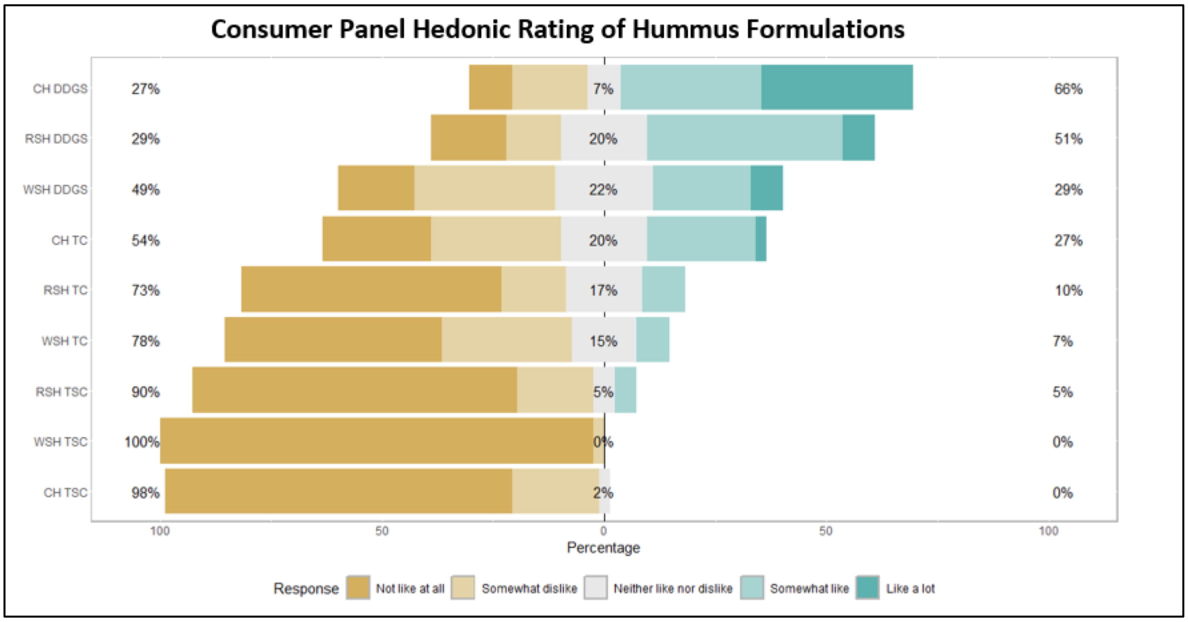


Figure 3: Distribution of consumer hedonic ratings for hummus formulations. Responses were measured using a 5-point hedonic scale ranging from "Not like at all" to "Like a lot." CH = chickpea-based hummus; RSH = red sorghum -based hummus; WSH = white sorghum-based hummus; DDGS = distillers dried grains with solubles emulsion; TC = thymol chitosan emulsion; TSC = thymol sodium caseinate emulsion. Colors represent increasing hedonic intensity from negative to positive responses.

Sensory panel evaluation revealed that consumer acceptance was heavily dictated by treatment type rather than underlying hummus substrate. Formulations utilizing the DDGS treatment achieved the highest overall preference, yielding a median score of 4.0 ("Somewhat like"), for both CH DDGS and RSH DDGS. Conversely, the incorporation of TC and TSC treatments resulted in sensory rejection. CH TC, a median "Somewhat dislike," demonstrated no statistically significant difference in preference ($p= 1.000$), where all TSC treatments had a median of "Not like at all." This sensory rejection was highly significant when compared to CH DDGS to TSC ($p<0.001$). The lack of significant differences among the TSC groups (PSH TSC versus CH TSC, p=1.000) confirms that the TSC treatment suppressed consumer preference regardless of the hummus base used.

On the survey, participants were permitted to handwrite any additional comments. Several participants noted the coarser texture in the sorghum hummus types and most reported the strong, unpalatable taste of the formulations using thymol.

3.3.3 Discussion

Chickpea hummus was highly ranked by consumers with the emulsion (DDGS) and thymol chitosan emulsion (TC). Hummus types formulated with a DDGS emulsion were preferred over all emulsion types. Consumers noted the distinct taste of formulations utilizing the thymol sodium caseinate emulsion (TSC), whereas DDGS did not receive similar feedback. Thymol has a notable strong, irritating taste, which corresponds with consumer feedback (Liu et al., 2022). While chickpea hummus was generally preferred, consumers expressed a willingness to consume red sorghum hummus if there were added health benefits. Consumers seek products that have higher nutritional profiles and express how “more digestible” ancient grains, like sorghum (Chambers and Chambers, 2025).

The panel was restricted to university participants; therefore, it may not accurately represent all consumers. The study did not include the hummus control, made with water versus emulsion. The purpose of utilizing different emulsions was to see the potential for future directions of the study. The consumer panel supports the use of DDGS as a future emulsion to incorporate into the hummus formulation; however, there is a need for research to determine the microbiological efficacy of this additive.

References

Chambers, Edgar, and Edgar Chambers. 2025. “Consumer Interest in Alternative Grains, Especially Sorghum: A Qualitative Investigation.” Foods 14 (22): 3918. https://doi.org/10.3390/foods14223918.

Jadayil, Seham Abu, Ali Alsaed, Leena Ahmad, et al. 2026. “Sensory and Microbial Quality Chickpea and Broad Bean Dips Produced in Jordan.” Preprint, In Review, April 9. https://doi.org/10.21203/rs.3.rs-8921419/v1.

Liu, Yao, Hui Yan, Bing Yu, et al. 2022. “Protective Effects of Natural Antioxidants on Inflammatory Bowel Disease: Thymol and Its Pharmacological Properties.” Antioxidants 11 (10): 1947. https://doi.org/10.3390/antiox11101947.

Pop, Mihaela Dana. 2023. “Sensory Evaluation Techniques of Food.” Universitatea Valahia Din Targoviste. Analele. Serie Agricultura (Warsaw, Poland) 15 (2): 58–62. https://doi.org/10.2478/agr-2023-0019.

Ruiz-Capillas, Claudia, and Ana M. Herrero. 2021. “Sensory Analysis and Consumer Research in New Product Development.” Foods 10 (3): 582. https://doi.org/10.3390/foods10030582.

3.4 Digestion Study

Several studies suggested that different sorghum varieties could contain improved protein digestibility. This characteristic might be beneficial to consumers seeking functional health benefits, such as improved gut microbiota and alleviation of oxidative stress, inflammation, diabetes, or other noncommunicable diseases (Morais Cardoso et al., 2017). By simulating the digestion process of hummus in a laboratory-setting, researchers can characterize the bioaccessibility of nutrients faster, cheaper, free of human participants, and in parallel (Di Matteo et al., 2026). Furthermore, understanding the functionality of sorghum hummus enables claims of added-health benefits, which can be preferred by consumers seeking alternative food products.

3.4.1 Materials and Methods

Sample Preparation

Hummus was made in batches as described above in paragraph two of Antimicrobial Selection and Hummus Preparation. The samples were then collected into 50 mL centrifuge tubes and frozen at -20 °C for 24h. After freezing, the samples were freeze-dried for 48h until moisture was removed.

Infogest Protocol

Two grams of each freeze-dried hummus sample were stirred in 8 mL of sterile deionized (DI) water and covered at 37 °C for thirty minutes. Then, 6.4 mL Simulated Salivary Fluid (SSF; Brodkorb et al. 2019), 40 µL of 0.3 M $CaCl_2$, and 1,56 mL DI water were added to the stirring solution. The pH was adjusted to 7.0 using 1 M NaOH or 1 M HCl as needed, then 0.099 g of salivary amylase (Sigma-Aldrich, St. Louis, MO, USA) was added and covered with aluminum foil for two minutes at 37 °C. After the allotted time, 4 mL of the sample was collected in microcentrifuge tubes and heated at 95 °C for 10 minutes. The remaining digest (~ 12 mL) was used for the gastric phase.

To the same sample, 10.92 mL of Simulated Gastric Fluid (SGF; Brodkorb et al. 2019), 0.6 mL of sterile DI water, and 6 µL of 0.3 M $CaCl_2$ were added. After adjusting the pH to 3.0M (using 1 M NaOH or 1 M HCl as needed), 0.015 g pepsin (Sigma-Aldrich, St. Louis, MO, USA) and 0.038 g gastric lipase were added, and the solution was again covered for 2h and maintained at 37 °C. The pH was monitored every twenty minutes and adjusted to a pH of 3.0 if necessary for 2h. Following this time, 4 mL of the sample were collected and heated at 95 °C for 10 minutes. The remaining digest was used for the intestinal phase.

Finally, 11 mL of Simulate Intestinal Fluid (SIF; Brodkorb et al. 2019), 40 µL of 0.3 M $CaCl_2$, and 8.7 mL of sterile DI water were added to the solution. The pH was adjusted to 7.0 using 1 M NaOH or 1 M HCl as needed. Once the pH was adjusted, 0.506 g of pancreatin (Sigma-Aldrich, St. Louis, MO, USA) was added solution and covered. The pH was monitored every 15 minutes and adjusted to pH 7.0 if needed for the next two hours at 37 °C. At the end of this time, 4 mL of the sample were collected and heated at 95 °C for 10 minutes. All samples were held at -20 °C until analysis (Brodkorb et al., 2019).

BCA (Bicinchoninic Acid) Protein Assay for Analysis

The in vitro digestion study allowed for the simulation of the physiological conditions of the upper gastrointestinal tract associated with human digestion to further understand the bioavailability of nutrients in the hummus types. To quantify the soluble protein following the digestion of red sorghum, white sorghum, and chickpea hummus, a BCA (Bicinchoninic Acid) Assay was performed (Thermo Scientific, Rockford, IL, USA) (He, 2011).

To prepare the buffer, 0.606g of Tris base was dissolved in 90 mL of sterilized DI water. pH was adjusted to 7.50 using 1 M NaOH or 1 M HCl as needed. Total volume was brough to 100 mL using sterilized DI water and mixed. The standard control was made with 200 µL of the buffer without a reducing agent and the sample control was prepared using 200µL of the same buffer with 4µL reducing agent. Protein standards were created by diluting one Albumin Standard (BSA) ampule into several microcentrifuge tubes, using the same buffer as the sample without the reducing agent. The following set of standards were prepared according to Table 6.

Table 6: Guide to prepare set of protein standards (assay range = 125-2,000µg/mL).

Vial	*Diluent volume (uL)*	*BSA source and volume (uL)*	*Concentration (ug/mL)*
A	0	200 of stock	2000
B	66	200 of stock	1500
C	100	100 of Vial A	1000
D	100	100 of Vial B	750
E	100	100 of Vial C	500
F	100	100 of Vial E	250
G	100	100 of Vial F	125

Reconstitution buffer was diluted 1:1 using sterilized DI water. 1mL of Reconstitution Buffer was added to one tube of Compatibility Reagent and vortexed at high speed for 30 seconds to dissolve. The following formula was used to determine the total volume of BCA working reagent (WR) needed:

Equation 2: Calculation to determine total volume of WR.

$$(\#\ control + 3\ standards + \#\ unknowns) \times (\#\ replicates) \times (volume\ of\ WR\ per\ sample) = total\ volume\ WR\ needed$$

9µL of each replicate of standard control, sample control, standard, and unknown sample were pipetted into the center of the microplate well. Then, 4µL of Compatibility Reagent was added to the sample in each well, covered, and placed on a plate shaker for one minute at medium speed. The plate was then incubated at 37°C for 15 minutes. Following incubation, 260 µL of the WR was added to each well. The plate was then covered and mixed on a plate shaker for one minute. The plate was incubated at 37°C for 30 minutes, then cooled at room temperature for five minutes. Using standard control as the blank, the absorbance of the standards, unknown samples, and sample controls were read at 562nm on a plate reader (He, 2011).

The initial raw material was characterized for crude protein content by multiplying its total nitrogen percentage by the standard conversion factor of 6.25 (Mariotti et al., 2008). The in vitro protein digestibility (IVPD) (%) of the samples was evaluated using the formula:

Equation 3: In vitro protein digestibility (%) calculation following BCA assay.

$$IVPD\ (\%) = (\frac{P_s}{P}) \times 100$$

where P_s represents soluble protein content after digestion (ug) and P represents protein content before digestion (ug) (Zhou et al., 2025).

3.4.2 Results

IVPD was determined by comparing sample absorbance values against a Bovine Serum Albumin (BSA) standard curve (Table 7).

Table 7: BCA assay results used to calculate factors of IVPD.

Hummus Type	Initial protein in sample (mg)	Total soluble protein (mg)
White sorghum	338.4573333	102.24
Red sorghum	352.9166667	96.60
Chickpea	482.5	111.86

Following the analysis, red sorghum hummus IVPD % showed a nearly 7% increase in protein digestibility compared to chickpea hummus. White sorghum hummus IVPD % fell between the values or chickpea and red sorghum hummus (Figure 3).

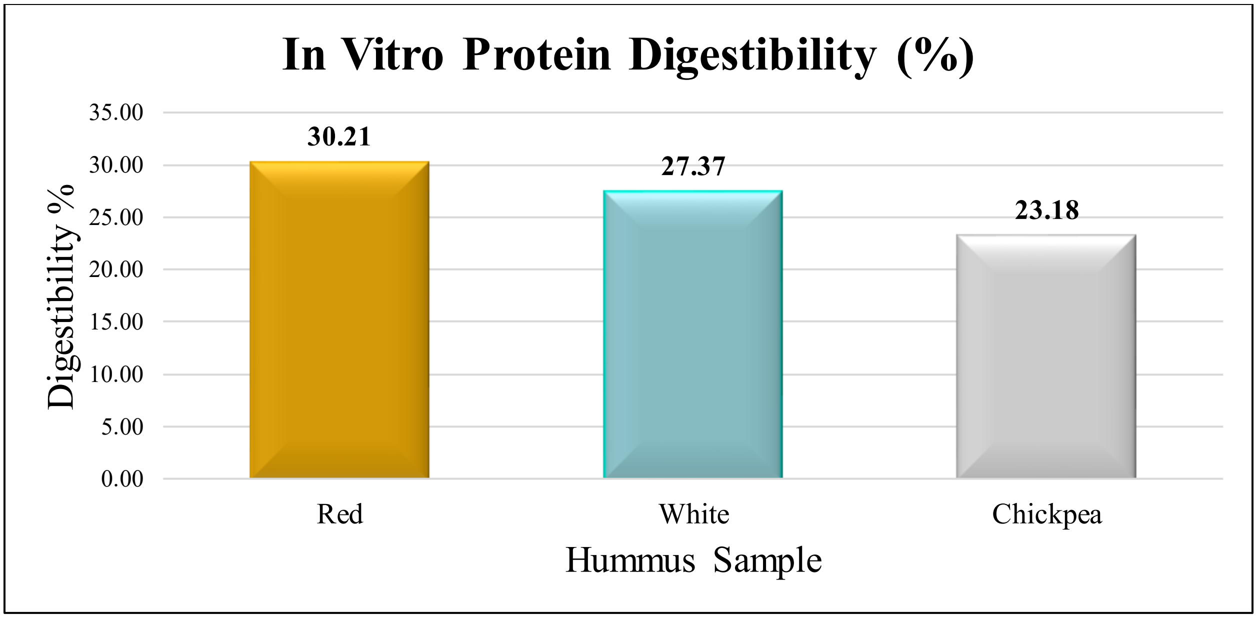


Figure 4: In Vitro Protein Digestibility (%) for red sorghum hummus, white sorghum hummus, and chickpea hummus samples. Calculated using soluble protein content after digestion (ug) and protein content before digestion (ug) derived from BCA assay.

3.4.3 Discussion

Red sorghum hummus had an increased digestibility percentage compared to chickpea hummus whereas a current study finds that raw chickpeas have a higher in vitro protein digestibility compared to raw sorghum (Bekele et al., 2021). By using high pressure to cook the red sorghum and chickpeas, the composition of the products was altered to where increased digestibility was possible. The formulation of the hummus types synergistically enhanced the composition of the cooked red sorghum, resulting in an enhanced digestibility compared to chickpea hummus. The processing steps of the sorghum, soaking and pressure cooking, enabled the breakdown of antinutritional components, such as tannins and phytic acid (Aguiar et al. 2023). Therefore, protein in sorghum is more readily available to digest.

While the thymol emulsion did not prove effective at decreasing pathogens within the product, there is still a need for research on it improving digestibility. In this study, the emulsion was not tested as it did not show major benefits to food safety. A limitation of this in vitro digestion study is that it reflects the mechanisms of a healthy human adult (Brodkorb et al., 2019).

References

Aguiar, Etiene Valéria, Fernanda Garcia Santos, Valéria Aparecida Vieira Queiroz, and Vanessa Dias Capriles. 2023. "A Decade of Evidence of Sorghum Potential in the Development of Novel Food Products: Insights from a Bibliometric Analysis." *Foods* 12 (20): 3790. https://doi.org/10.3390/foods12203790.

Bekele, Esayas K., Robert T. Tyler, Carol J. Henry, James D. House, and Matthew G. Nosworthy. 2021. "In Vitro Protein Digestibility of Direct-Expanded Chickpea–Sorghum Snacks." Legume Science 3 (4): e87. https://doi.org/10.1002/leg3.87.

Brodkorb, André, Lotti Egger, Marie Alminger, et al. 2019. "INFOGEST Static in Vitro Simulation of Gastrointestinal Food Digestion." Nature Protocols 14 (4): 991–1014. https://doi.org/10.1038/s41596-018-0119-1.

Di Matteo, Giacomo, Alessandra Cimbalo, and Massimo Frangiamone. 2026. "Editorial: In Vitro Digestion in the Study of Food." *Frontiers in Nutrition* 13: 1785006. https://doi.org/10.3389/fnut.2026.1785006.

He, Fanglian. 2011. "BCA (Bicinchoninic Acid) Protein Assay." *Bio-Protocol*, ahead of print, March 5. https://doi.org/10.21769/BioProtoc.44.

Mariotti, François, Daniel Tomé, and Philippe Patureau Mirand. 2008. "Converting Nitrogen into Protein--beyond 6.25 and Jones' Factors." *Critical Reviews in Food Science and Nutrition* 48 (2): 177–84. https://doi.org/10.1080/10408390701279749.

Morais Cardoso, Leandro de, Soraia Silva Pinheiro, Hércia Stampini Duarte Martino, and Helena Maria Pinheiro-Sant'Ana. 2017. "Sorghum (Sorghum Bicolor L.): Nutrients, Bioactive Compounds, and Potential Impact on Human Health." *Critical Reviews in Food Science and Nutrition* 57 (2): 372–90. https://doi.org/10.1080/10408398.2014.887057.

Zhou, Siyi, Yanan Wang, Qingqing Hu, Jianghua Li, Jian Chen, and Xiao Liu. 2025. "Enhancement of Nutritional Quality of Chickpea Flour by Solid-State Fermentation for Improvement of in Vitro Antioxidant Activity and Protein Digestibility." *Food Chemistry* 468 (March): 142418. https://doi.org/10.1016/j.foodchem.2024.142418.

3.5 Future Directions

The findings do not support the use of the nanoencapsulated thymol with sodium caseinate emulsion in hummus; however, there are several takeaways that can be further explored. The elevated pH of the hummus is not an ideal product for emulsions formulated with sodium caseinate, as it surpasses the isoelectric point, resulting in no effect on antimicrobial activity. Products with a pH below 4.6 or with less interfering food components would be ideal for the tested emulsion. An effective food matrix for the application of a thymol emulsion is milk or an acidic juice, as studies support the bacterial inactivity in these products (Xue et al., 2017). Following the results of the digestion study, red and white sorghum displayed an increase of in vitro protein digestibility percentage compared to chickpea hummus. Sorghum-based products are supported by current literature where processing techniques can improve the digestibility of the ancient grain. Soaking is a low-cost, that breaks down antinutritional factors and cooking improves the bioavailability of nutrients (Fernando et al., 2025). By soaking and pressure-cooking sorghum before utilizing the formulation of hummus, the grain was able to be made into a value-added product that shows signs of improved digestibility.

The consumer panel further supported not incorporating thymol emulsions in hummus, as it was least preferred overall by consumers. The use of sodium caseinate to encapsulate essential oils is promising to create an extended effect of antimicrobial activity, however thymol is pungent in taste and not preferred by consumers. By evaluating consumer preferences with new emulsions added to hummus, sorghum Distillers Dried Grains with Solubles (DDGS) presents an opportunity for future studies.

References

Fernando, W. M. A. D. Binosha, Rasheed A. Abdulraheem, Kalmee Pramoda Kariyawasam, et al. 2025. “Sorghum and Sorghum-Based Products: Nutritional Composition, Prebiotic Potential and Health Benefits in Gut Microbiota Interactions.” International Journal of Food Science 2025 (November): 7084868. https://doi.org/10.1155/ijfo/7084868.

Xue, Jia, P. Michael Davidson, and Qixin Zhong. 2017. “Inhibition of Escherichia Coli O157:H7 and Listeria Monocytognes Growth in Milk and Cantaloupe Juice by Thymol Nanoemulsions Prepared with Gelatin and Lecithin.” Food Control 73 (March): 1499–506. https://doi.org/10.1016/j.foodcont.2016.11.015.

Chapter 4 - Overall Conclusion

Collectively, microbiological, sensory, and nutritional evaluations demonstrated that sorghum hummus is a feasible value-added food product with potential applications in sustainable food systems. Sorghum hummus is a promising product with improved digestibility compared to traditional, chickpea hummus. Production of sorghum hummus in regions facing climate-related issues can alleviate food insecurity and enhance sustainability. Sorghum hummus has an equivalent food safety status compared to chickpea hummus; therefore, regulations will be similar in regions that already produce chickpea hummus.

These studies support that the emulsion, nanoencapsulated thymol with sodium caseinate, did not add any advantage in terms of food safety within the tested hummus formulations. The lack of efficacy was likely associated with physicochemical interactions between the nanoemulsion and the hummus matrix, including pH conditions outside the optimal stability range of sodium caseinate and partitioning of thymol into the lipid phase. Thymol has been successful in multiple products, including food, pesticides, and pharmaceuticals; however, is limited by specific food matrices (Luo et al., 2025).

Successful commercialization of sorghum hummus requires acceptable nutritional and microbiological performance, but also compliance with regulatory frameworks governing food safety, additives, and labeling. The implementation of sorghum hummus in the Middle East and United States would require conformity with current standards within each region. Understanding the process of adopting thymol into food products internationally allows for the exploration of regulatory requirements and trade opportunities (SFDA, n.d.; FDA, n.d.). The comprehension of regulatory systems enables a rounded approach to product development within nations. Regardless of location, food safety is a collaborative process across multiple entities.

Through international agencies and agreements, like the World Trade Organization Agreement on the agreement on the Application of Sanitary and Phytosanitary Measures, enables cross-sector collaboration globally.

Utilizing a transdisciplinary approach to complex issues, such as food insecurity, enables research motives to be curated to fit specific needs of a system. Sorghum hummus will not solve the global issues of food insecurity; however, it creates opportunity for further research to be shaped around the developing of the world and its current state.

Collectively, these findings support sorghum hummus as a sustainable, nutritionally valuable, and microbiologically stable food product that contributes to ongoing efforts to improve food security through innovative utilization of climate-resilient crops.

References

Food and Drug Administration (FDA) (Electronic Code of Federal Regulations). 2026. "21 CFR 172.515 -- Synthetic Flavoring Substances and Adjuvants." Accessed April 6, 2026. https://www.ecfr.gov/current/title-21/part-172/section-172.515.

Saudi Food and Drug Authority (SFDA). 2021. "Food Clearance Conditions and Requirements."

Luo, Donglan, Xue Li, Xiaogang Wang, Sen Cao, Liangjie Ba, and Xiaohong Kou. 2025. "Research Progress on the Food Preservation Effects Exploiting the Antifungal Properties of Thymol." *Food Chemistry: X* 30 (August): 102954. https://doi.org/10.1016/j.fochx.2025.102954.