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Review Article

Thymol as a Natural Preservative in Cottage Cheese Preservation

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Abstract: Food spoilage is a universal problem where the food item will lose its quality, texture, flavor, and it will be no more suitable for consumption. Biological contaminants are the main reason for food deterioration and damage. There exists a process called "Food Preservation" used to prevent, maintain, and protect food from damage and spoilage. It is essential also to extend the shelf life of the food product. Each type of food can be protected and preserved either physically or chemically. However, some of the chemical preservatives have drawbacks that can't be anymore safe to be used. A number of studies shed the light on the importance of a natural compound named "Thymol" to be used as a preservative in cottage cheese. The latter has limited shelf life due to its high moisture content (~75%) and relatively high pH (~5). Thymol is characterized by its phenolic group and it has many roles. Recently, many researchers tried to detect the effectiveness of thymol in extending the shelf life of the cottage cheese by improving its quality and protecting it from microorganisms. The studies assure that thymol protects the sensorial properties of cottage cheese and extends its shelf life without causing any risks.

Keywords: Food Spoilage, Food Preservation, Shelf Life, Food Quality, Thymol, Thyme and Cottage Cheese.

INTRODUCTION

Food spoilage is a serious and global issue where a food product becomes unsuitable for consumption. The spoiled food will lose its quality, texture, flavor, and it will be no more edible for humans. Many factors will deteriorate the food item but the dangerous ones are the biological contaminants such as bacteria, molds, and fungi. To prevent and reduce spoilage, food preservation is necessary. Food preservation is a process to keep food safe and undamaged. It is crucial to maintain, protect and extend the shelf-life of the food product. There are several physical methods that are used to maintain food for longer period. Chemical preservatives are also used to protect food from injury and deterioration. There are many chemical preservatives that are added to dairy products and especially to cheese. However, some of the preservatives have side effects that can't be anymore added to cheese. A new natural preservative called "Thymol" is added to cottage cheese to protect it from spoilage. Thymol is the main monoterpene phenol found in the oil extracted from plants belonging to the Lamiaceae family such as thyme. It is used as an antioxidant, anti-inflammatory, and it has antifungal and antibacterial property. By adding thymol to the cheese, the quality remains good for longer period and the shelf-life will be extended.

The objective of this paper is to shed the light on the effectiveness of thymol as a preservative in cottage cheese

Food Spoilage

Human societies have been always concerned by alterations in food availability, both temporary and spatially. The period of time during which the food remains edible depends on both, intrinsic and extrinsic factors such as temperature and humidity that affect the growth rates of microorganisms which lead to food spoilage and contamination [1]. Food spoilage is a process that causes food to be unpleasant or unacceptable to human consumption due to changes in sensory properties [2]. The onset of food spoilage is rather indefinite. In addition, food contamination is defined as a process by which a product is spoiled or adulterated due to the presence of some microorganisms, which lead to borne illness or sometimes to death. Complications could attributed to the existence of toxic substances, that turn the food to be no more suitable for consumption [3]. Worth to know that not all food products can spoil easily. Some types take more time to be marred while others are destroyed within few days. Food are classified on the basis of their stability as non-perishable, semi-perishable and perishable. Perishable food is defined as food which are likely to decay, spoil or go bad quickly unless a preservative method is used. This group

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includes dairy products, meat, fruits and vegetables. Sugar and flour are considered as non-perishable or relatively stable, therefore, they don't spoil unless they are handled incorrectly. Potatoes remain good and unspoiled for a long period if they are properly handled and stored and they are considered as semi-perishable or protectable food [4].

In this context, it is crucial to shed the light on Potentially Hazardous Food that favor the rapid growth of microorganisms. This type of food consists up of raw meat, rice, milk, sauces and many others [5].

Food can be biologically, chemically or physically contaminated but the former being more common and more dangerous. The biological contaminants have different routes throughout the supply chain to enter and make a food unfit for consumption. Thus, our aim will be focused on the biological contamination.

Infectious Microorganisms

The food borne infection is due to the ingestion of food containing live bacteria that could develop in the human's gastrointestinal tract [6]. *Salmonella, Escherichia coli, Campylobacter jejuni* and *Listeria monocytogeneous* are the major microorganisms that cause food borne infections (Table-1) [5].

Salmonella, a group of gram negative bacteria that are potential enteric pathogens. It is one of the major causes of food poisoning. *Salmonella* survive gastric acidity, invade mucosa of small and large intestine and produce toxins leading to acute inflammatory response. Most people suffer from diarrhea, fever and abdominal cramps when they are infected with *Salmonella*, an infectious state called salmonellosis. The symptoms lasts for 4-7 days and most individuals recover without treatment. But, many others can be hospitalized or even die due to this infection [7]. *Salmonella* can exist in contaminated eggs, poultry, meat, unpasteurized milk, cheeses, contaminated fruits and vegetables and others [8].

Escherichia coli is a gram negative bacteria that live normally in the intestine of the humans and animals. It includes a diverse group of bacteria. Some are harmless while others are pathogenic. The harmful types lead to diarrhea, abdominal pain and vomiting. Enterotoxigenic *Escherichia coli*, or ETEC, is the name given to a group of E.coli that produces special toxins that stimulate the lining of the intestines and cause excessive production of fluid, resulting in diarrhea [9]. The most pathogenic serotype is E.coli O157:H7 that causes bloody diarrhea and in severe cases, may lead to kidney failure. The symptoms appear 1-8 days after ingesting the contaminated food such as, undercooked ground beef, unpasteurized milk and juices, raw fruits and vegetables, contaminated water and many others [8]. E.coli is a marker for water contamination [9].

Campylobacteriosis is an infectious disease caused by *Campylobacter jejuni*. It is a gram negative bacteria. This type of bacterial infection leads to diarrhea, nausea, fever, vomiting and abdominal cramps. These symptoms appear 2-5 days after ingesting the contaminated food and lasts for a week. People get infected by *Campylobacter jejuni* when they ingest raw or undercooked poultry or from contamination of other food by these items [10].

Listeriosis is a serious infection caused by gram positive bacteria called *Listeria monocytogenes*. It can cause a variety of symptoms depending on the person and the part of the body that is affected. Fever, muscle aches, fatigue, diarrhea, headache and confusion are the major symptoms that mainly appear 9-48 hours after ingesting the contaminated food [11]. Some food are more risky than others as soft cheeses, raw milk and raw sprouts [12].

Table-1: Infectious Microorganisms

Organism	Gram state	Common name of illness	Onset time after ingesting	Food sources	Signs and symptoms
Salmonella enterica	Negative	Salmonellosis	6-48 hours	Poultry, eggs, meat, unpasteurized milk or juice, cheese, contaminated fruits and vegetables	Diarrhea, fever, vomiting, abdominal cramps
Escherichia coli	Negative	Hemorrhagic colitis	1-8 days	Undercooked beef, unpasteurized milk, raw fruits and vegetables and others	Bloody diarrhea, abdominal pain and vomiting
Campylobacter jejuni	Negative	Campylobateriosis	2-5 days	Raw and undercooked poultry, contaminated water	Diarrhea, fever, vomiting and others
Listeria monocytogenes	Positive	Listeriosis	9-48 hours	Soft cheeses made with unpasteurized milk	Fever, muscle aches, nausea and many others

Intoxicating Microorganisms

Foodborne intoxication is caused by ingestion of food containing bacteria-formed toxins. It is not necessary to consume the live microorganism [6]. *Staphylococcus aureus, Clostridium botulinum* and

Clostridium perfringens are the microorganisms that lead to intoxication (Table-2) [5].

Staphylococcus aureus is a gram positive bacterium. This type of bacteria multiplies rapidly at room temperature where a toxin is produced and causes illness. It can lead to several symptoms as nausea, loss of appetite, mild fever, severe abdominal cramps and vomiting. These symptoms appear 1-6 days after ingestion. The food sources of *Staph aureus* include bakery products, salads, milk and dairy products, meat, poultry and eggs [8].

Clostridium botulinum is a spore-forming gram positive bacterium that produces neurotoxin during growth which lead to botulsim, a life-threatening disease. Nausea, vomiting, blurred vision, neurological and gastrointestinal problems are the main symptoms. These symptoms appear 12-72 hours after ingestion. Death can occur if there is a respiratory failure. This bacterium exists mainly in canned food that are not well handled and in fermented fish [13].

Clostridium perfringens is also a spore-forming gram positive bacterium able to produce a specific toxin that can be detected by feces or other tests. The common food sources of this bacterium are beef, poultry, gravies and dried food. People that get infected with *Clostridium perfringens* do not have fever or vomiting. However, they suffer from abdominal cramps and diarrhea within 8-16 hours after ingestion [14].

Table-2: Intoxicating Microorganisms

Organism	Gram state	Common name of illness	Onset time after ingesting	Food sources	Signs and symptoms
Staphylococcus aureus	Positive	Staphylococcal food poisoning	1-6 hours	Cream pastries, potato and egg salads	Nausea and vomiting, abdominal cramps
Clostridium botulinum	Positive	Botulism	12-72 hours	Improperly canned food, fermented fish	Vomiting, diarrhea, blurred vision, difficulty in swallowing
Clostridium perfringens	Positive	Perfringens food poisoning	8-16 hours	Meats, poultry, gravy	Intense abdominal cramps

Fungi, Yeasts and Molds

Fungi as a group are of the most resilient spoilage microorganisms and are able to overcome the control strategies utilized by the food industry. Different fungal types are dispersed rapidly by water and air, survive under extreme conditions and increase in biomass sustainably. Fungi are not considered biological hazards, but many diverse fungi are opportunistic pathogens. Due to the various structural characteristics and survival mechanisms, fungal species can contaminate and damage processed food. Producers also faced fungal spoilage problems in raw ingredients and finished products (Table-3). The fungal growth in the processed food can lead to consumer dissatisfaction, food wastage and mistrust of food manufacturers and retailers [15].

Table-3: Common names of commercial food spoilage issues and the associated causative fungus and quality deviation

Issue	Description	Scientific Name
Machinery Mold	Associated with dairy, fruits, and vegetables and frozen food processing environments. Cause off-flavors and dextran production	*Geotrichum candidum*
Blown pack spoilage	Swollen container resulting from gas production, with fermentation and growth occurrence	Yeast spoilage, very rarely filamentous fungi
Cat hair	Defect on the surface of cheeses	*Mucor and Rhizopus spp.*
Mat	Mold growth at the top of beverages or other fluids (beer, pickles...)	Pellicle: aerobic growth of one or more fungi

Molds are able to grow in wide range of water activity, pH (especially in acidic medium) and temperature. Thus, molds can present in dairy products, breads, cereals, beverages and others. During germination and proliferation, molds can produce enzymes such as lipases, proteases. These enzymes transform the sensorial properties of food inducing off-flavor, changes in color and produce toxins that are dangerous to human being [16]. Another microorganism essential to food spoilage is yeast. Yeasts are widely present in nature and are associated with liquid food that contains sugars and acids in particular. Moreover, they adapt perfectly to adverse conditions such as acidity and dehydration. The presence and growth of yeast in canned food can lead to spoilage, usually in the form of alcohol production and large amounts of carbon dioxide that swells the container. If this happens, gross under processing, post-processing contamination or leakage must be suspected. Importantly, yeast growth in processed food does not lead to a public health problem [17].

Viral Foodborne Illnesses

Viral foodborne illnesses are caused by different types of viruses that can contaminate food at any stage of food supply chain. Norovirus, Rotavirus and Hepatitis A and E are the most viruses that lead to foodborne illness. Rotavirus, associated with gastroenteritis in children while, hepatitis A and E lead to inflammation of the liver [18].

Noroviruses (Norwalk Viruses) are the common cause of acute gastroenteritis. Norovirus illness, stomach flu or viral gastroenteritis, spreads easily. The symptoms such as diarrhea, vomiting, nausea and stomach pain appear 12-48 hours after ingestion. Diarrhea tends to be watery and non-bloody and it is more common in adults while, vomiting was diagnosed more in children. People who are infected with this virus can spread it directly to other people or can contaminate the food that they prepare. The sources of food include shellfish, ready-to-eat food touched by infected food workers (salads, cookies...) and others [8].

Dairy Products and Contamination

The dairy food chain is complex, that's why, microbial contamination has more opportunities to take place [19]. Due to the selective effects of manufacturing, formulation, processing, packaging, storage, distribution and handling practices, the type of spoiling microorganisms differs widely among dairy products (Table-4). The spoiling organisms are yeasts, molds, heterofermantative lactobacilli, aerobic psychrotropic gram-negative bacteria and spore-forming bacteria [20].

Fungal spoilage can cause changes in color and texture and leads to off-flavor and bad odors due to the metabolic by-products. Moreover, heterofermantative lactobacilli and spore-forming bacteria are the causes of gassing defects. Psychrotropic bacteria can produce large quantities of extracellular hydrolytic enzymes that affect the shelf life [20].

Coliforms were and still used as indicators for dairy product's contamination. Worth mentioning that *Staphylococcus aureus* is a predominant cause of food poisoning worldwide and especially in dairy products [19]. Each type of food has a shelf-life after which it begins to deteriorate and spoil [21].

Table-4: Dairy products and typical types of spoilage microorganisms or microbial activity

Food	Spoilage microorganism or microbial activity
Raw milk	Wide variety of microbes
Pasteurized milk	Psychrotrophs, microbial enzymatic degradation, spore formers
Concentrated milk	Spore-forming bacteria, osmophilic fungi
Dried milk	Microbial enzymatic degradation
Butter	Psychrotrophs, enzymatic degradation
Cultured buttermilk	Psychrotrophs, coliforms, yeasts, lactic acid bacteria
Cottage cheese	Psychrotrophs, coliforms, yeasts, molds, microbial enzymatic degradation
Yogurt	Yeasts
Cream cheese	Fungi, spore-forming bacteria
Soft, fresh cheeses	Psychrotrophs, coliforms, fungi, lactic acid bacteria, microbial enzymatic degradation
Ripened cheeses	Fungi, lactic acid bacteria, spore-forming bacteria, microbial enzymatic degradation

Cheeses belong to dairy products and are perishable food that could be rotten quickly. Cottage cheese is a type of cheeses that our study will be focused on (Figure-1). This product can be spoiled easily. Due to the high percentage of moisture content in this cheese, it makes it an excellent growth medium for psychrophilic bacteria, yeasts and molds. There are other factors causing contamination such as the type of milk, starter culture and equipments [22].

Some yeasts are beneficial for cheese ripening while others taint the cheese. For example, the growth of *D. hansenii* by *Y. lipolytica* diminishes the quality of cheese by affecting the appearance as well as the sensorial characteristics. So, yeasts can cause early blowing, off-flavor and brown discoloration to the cheese [23].

The extracellular enzymes mainly proteases and lipases derived from psychrotrophs lead to the deterioration of the cheese. There are different types of psychrotrophic bacteria that dominate the cottage cheese [24]. One of the dangerous types is the genus Pseudomons, the major cause of numerous defects in dairy products [25].

To reduce spoilage, to control contamination, and to increase the product shelf life, a food additive should be added. Food additives and preservatives are used to improve the shelf-life, taste, texture, nutrition and appearance [26].

Fig-1: Cottage Cheese

Food Preservation

The process of treating food to stop or slow down food spoilage, deterioration, and bad quality is referred to as Food Preservation. This latter has three main objectives: preserving the appearance, preserving nutritional characteristics and extending the time during which the food can be stored. Therefore, food preservatives are food additives used to retard the growth of microorganisms and to arrest spoilage by different mechanisms such as affecting cell permeability and interfering with enzymatic reactions that are essential for microbes to stay alive. Preservatives can be of physical or chemical nature (Figure-2) [27].

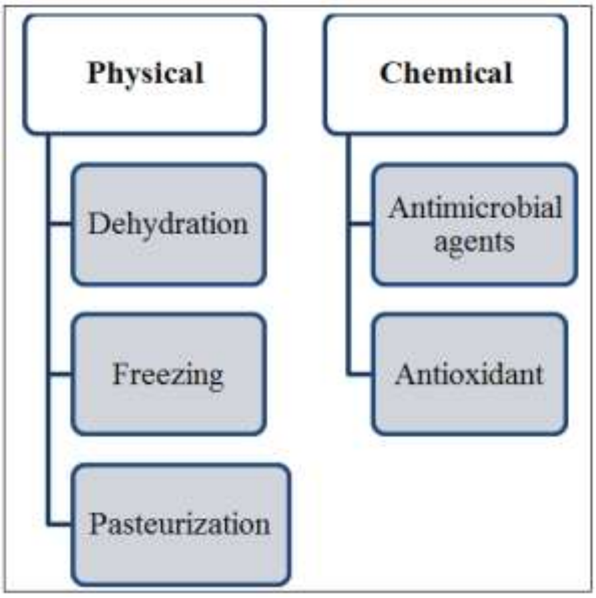

Fig-2: Types of Preservatives

Physical Preservatives

Physical methods of food preservation are those using physical treatments to restrain, damage, or eliminate undesirable spoiling microorganisms [21]. They can be classified into three types: dehydration, freezing and pasteurization.

A. Dehydration is used as a preservation technique to improve the shelf life of the food [28]. By reducing the moisture content of food, this prevents the growth of spoilage microorganisms and reduces the enzymatic reactions that take place inside the food texture [29]. There are different methods of food drying and most of them in their own way are unique [28].

B. Freezing is on one of the oldest and most widely used food preservation methods that enable food to preserve texture, taste and nutritional value better than any other method [30]. It is an effective way to make perishable food last longer by preventing the growth of bacteria, yeasts and molds. When the food is safely freezed, it is then protected from deterioration, and the quality remains good. For that purpose, the food item should be stored at (-17.78 °C) [31].

C. Pasteurization is used to ensure the protection and safety of food supply [32]. It reduces the bacterial count, inactivates enzymes and extends the shelf life. Pasteurization is not intended to kill all microorganisms that are present in the food. There are three different methods of pasteurization that differ in term of temperature used to save food (Table-5) [33].

- LHT (Low temperature hold method): milk is heated at 63°C or 30 minute before being cooled to 7°C. The heat process takes place in the plate heat exchanger and the cooling process is performed using chilled water [33].
- HTST (High-temperature short-time): milk is heated at 72°C and hold for 15 second [33].
- UHT (Ultrahigh-temperature): refers to pasteurization technique with a temperature of at least 138°C for 1 second approximately. It occurs in a continuous flow [33]. It involves several heating conditions and it is of two types: "direct" and "indirect". In direct heating systems, the product is held at high temperatures for shorter time and it involves the mixing of super-heated steam with milk [34]. However, in the indirect heating system a

heat exchanger transfers heat across a partition tool between the milk and the heating medium [35].

Table-5: Pasteurization Temperature

Temperature	Time	Pasteurization Type
63°C; 7°C cooled	30 min	LHT
72°C	15 sec	HTST
138°C	2 sec	UHT

Chemical Preservatives

As per the FDA standards, the term chemical preservative is defined as "any chemical that when added to food tends to prevent or retard deterioration" [36]. They are added in very low amounts in a way that they do not alter the organoleptic and physio-chemical properties of the food. They are added after the food has been processed and before being packaged [37]. Antimicrobial agents and antioxidants are considered as chemical preservatives.

A. Antimicrobial preservatives diminish the microbial spoilage of food by retarding the growth and multiplication of bacteria, yeasts and molds [27].

B. Antioxidants preservatives are chemicals used to prevent oxidation in which the latter leads to rancidity and off-flavor. Ascorbates (E300-E305), tocopherols (E306-E309), erythorbates (E310-E319), Lactates (E320-E329), phosphates (E340-E349) and succinates (E360-E369) are most effective antioxidants added to food and beverages [27].

Conditions for Preservatives to be FDA Approved

The use of preservatives in food is strictly studied, controlled and examined by FDA. Federal regulations require proof of food additives being safe for their intended use [38].

In order to use the chemical preservatives in food, the chemical should be: generally recognized as safe and not used to conceal destruction or inferiority or to make food look better or more valuable than it is (U.S. Food and Drug Administration, 1989 [57].

In addition to that, preservatives should be food grade, perform its intentional purpose and be used in accordance with good manufacturing practices and relevant with existing food additive regulations. Preservatives should never be replaced for sanitation and selection of good raw materials. The food must be labeled if it contains a preservative in which the statement must indicate the common or usual name of the preservative (U.S. Food and Drug Administration, 1989 [57].

Moreover, in order for the preservatives to be in accordance with good manufacturing practices, the use of preservatives should not affect badly the nutritive value of food or allow continued growth of food poisoning organisms while suppressing growth of others that would make spoilage obvious (U.S. Food and Drug Administration, 1989 [57].

Preservatives Used in Dairy Products

Preservation techniques are considered crucial in food industry especially in the field of dairy production. The safety of dairy products should be taken into consideration when any preservative is added [39].

The acceptable daily intake (ADI) represents the quantity of daily consumption of a substance without any risk even for a lifetime. The maximum permitted limit for food additive is calculated as mg/Kg of body weight according to ADI. Benzoate, sorbate and natamycin are the most used preservatives in dairy products and especially in cheeses and yogurt [39].

The first chemical preservative approved by FDA was sodium benzoate (E211). It's more soluble than potassium and calcium benzoate. This type of preservative is affected by pH. Hence, when pH of a medium increases, the effectiveness of sodium benzoate decreases. Sodium benzoate is used against bacteria, yeasts and fungi that exist in acidic medium. Potassium sorbate (E202) is characterized by its high stability, solubility and being easy to use. Thus, it is widely applied in food industry. It is considered as safe (GRAS). The presence of carboxyl group and conjugated double bond give sorbate a strong effect on antimicrobial activity. Natamycin (E235) has low solubility in water (approximately 40mg/Kg) [39].

There are several types of chemical preservatives that are added to the cottage cheese and each one has a maximum level to be added (Table-6) [40].

Table-6: Preservatives and their maximum level

INS no.	Name of Preservative	Maximum level
200	Sorbic acid	1000 mg/kg singly or in combinations with sorbic acid
202	Potassium sorbate	
203	Calcium sorbate	
234	Nisin	12.5 mg/kg
280	Propionic acid	-
281	Sodium propionate	-
282	Calcium propionate	-
283	Potassium propionate	-

- Salt is a traditional chemical preservative. It prevents the development of microorganisms in food by increasing the osmotic pressure to a specific level [37]. It could lead to hypertension.
- Sorbic acid and its salts are generally recognized as safe (GRAS) [27]. They are mainly tasteless and odorless in food. Their main effect on mold and yeasts [37]. However, in some cases, sorbates can lead to urticarial and contact dermatitis [27].

- Potassium sorbate is considered as safe (GRAS). It is present in cheeses and many other types of food [36]. It is naturally occurring antimicrobial compound. It can be a human skin toxicant or allergen (EWG's Skin Deep) [43]. However, the application of calcium sorbate is limited due to the stability. It is less used than potassium sorbate [37].
- Propionic acid is used as a cheese preservative where it is considered as safe [41]. It may interfere with the overall cellular metabolism [42].
- Nisin is a bacteriosin that is widely used in cheeses as a food preservative [44]**.** It is considered GRAS additive [36]. It is active mostly against Gram-positive bacteria as lactococci, bacilli, micrococci, *Listeria monocytogenes*, *Staphylococcus aureus* and *Clostridium botulinum* but has little or no activity against Gram-negative bacteria, yeasts or molds. The use of nisin in a free form in the cheese industry as Nisaplin is costly and has several disadvantages including low activity, stability and bioavailability. Furthermore, free nicin may interfere with the process of cheese making or decrease the quality of the cheese by inhibiting the growth of starters or non-starter lactic acid bacteria that are essential for ripening and flavor development [44]**.**

As indicated, most chemical preservatives have drawbacks. Some can affect our normal flora that exist in the gastrointestinal tract if added in high dose as Nisin, others can increase the blood pressure and lead to a chronic disease; hypertension. Therefore, a natural compound that can act as a preservative and have low bad effects than others must be formed. It must have low or no risks and it must be characterized by several functions that can protect the food from spoilage and injuries.

Thymol: A Preservative in Cottage Cheese

Thymol: Definition and Characteristics

The genus Thymus comprises over 350 aromatic plant species with little difference in the characteristics and chemical heterogeneity [45]. Volatile oil is the most valued component in thyme and especially in the leaves. It is formed of terpens, sesquiterpenes and several oxygenated derivative compounds such as phenol, ether, ester and all are responsible for the characteristic plant odor and flavor. The most biologically active constituent of thyme essential oil are thymol and its isomer carvacrol [45].

Thymol (2-isopropyl-5-methylphenol) is the main monoterpene phenol found in the essential oil extracted from plants belonging to the Lamiaceae family and other species such as *Lippia gracitis* [46]. It is a natural antimicrobial phenolic compound found in thyme, oregano, sage and the main flavor component in cloves and all spices [22]. It is colorless and has thyme's odor [47]. Thymol has moderate water solubility; 900 mg/l at 20°C [48] and low palatability [46]. It volatiles at 100°C [49]. It is rapidly absorbed from the gastrointestinal tract and eliminated within 24 hours without any changes [46]. The boiling point of thymol is 233°C and its melting point is 49.6°C [50]. Its flash point is 110°C and it is stable under storage conditions [51].

Roles of Thymol

Biological Activity of Thymol

Thymol has many therapeutic applications and potentials where it could be used as antioxidant [52], anti-inflammatory [53], antiseptic [54], antifungal and antibacterial [46]. It also has a beneficial effect on the cardiovascular system. Thymol was studied for its antioxidant activity *in vitro* in a liquid medium containing a purified fraction of triaglycerol of lard (TGL) and sunflower oil (TGSO). The result revealed that there was an inhibition for both TGL and TGSO oxidation due to the presence of thymol [46].

More recently, thymol has been shown to possess anti-inflammatory and antinociceptive activities *in vivo* [46]. This was evaluated by using paw edema and peritonitis methods for the anti-inflammatory property [46]. Another study was considered to inquire the effects of thymol on allergic inflammation in OVA-induced mice asthma. The authors showed that the pretreatment with thymol diminished the level of OVA-specific IgE and many other effects. Therefore, thymol may be used as a substitute agent for treating allergic asthma [55].

To examine the effect of thymol in high-fat diet (HFD)-induced obesity in murine model, a study was conducted [56]. Thymol reduced body weight gain, visceral fat-pad weights, lipids and others. Thus, thymol prevents HFD-induced obesity through several mechanisms [56].

With regard to the cardiovascular system, the phenolic type of monoterpenes has an effect in inducing hypotension and vasorelaxation [46]. Thymol induces cardiac arrhythmias in canine ventricular cardiomyocytes through the inhibition of k^+ and Ca^{2+} currents in a concentration dependent manner [46].

Recently, thymol has been found to have anti-cancer effect via different mechanisms of action (apoptosis, cell growth inhibition...) [46].

Antibacterial and Antifungal Activity of Thymol

To evaluate the effect of thymol on several types of microorganisms such as *Escherichia coli* (*E. coli*), *Psuedomonas*, *Proteus* (Gram negative bacteria) and *Staphylococcus aureus* and *Bacillus* (Gram positive bacteria), a study was performed. The findings indicate that thymol had bacteriostatic activities against the microorganisms mentioned above [46]. The antimicrobial action has been related to the effect of the phenolic compound on the cellular membrane leading to structural and functional damage to the lipid bilayer [22].

Another study was designed to detect the effect of the thyme essential oil on *E. coli* by using the time-kill curve method. The results showed that thymol could inhibit the growth of *E.coli*. This is due to the ability of thymol to induce the permeabilization and deplolaization of the cytoplasmin memebrane. The thymol ester derivatives have the most enhanced role against gram positive bacteria [46]. Also, the mechanism of action of thymol against *Salmonella ser. typhimurium* was studied. The minimum inhibitory concentration (MIC) of thymol against this type of bacteria was found to be 750mg/l [54].

Moreover, an investigation was performed to determine the function of thymol as a preservative in cosmetics in the presence of bacteria. The data was collected and showed that the encapsulated thymol was an effective preservative by which it inhibited the growth of microorganisms [46].

To evaluate the interaction of thymol with some antibacterial and antifungal drugs against an agent responsible for pythiosis in humnas and animals, a study was designed. This combination produced a synergism. Thus, thymol in combination with some antimicrobial agents may be an effective alternative treatment fot pythiosis [46].

In addition to that, thymol and its isomer carvacrol are used as alternative to antibiotics in poultry firming especially against *Clostridium perfringens* [46]. Also, thymol showed strong antibacterial effects against *E. coli*, *C. perfringens* and *Salmonella in vitro* [46].

More recently, soy sauce was combined with six essential oils. One of these oils is thymol. The study aimed at the examination of the antimicrobial activity of thymol against foodborne pathogens as *E. coli* and others. The results were positive, where thymol eliminated all the pathogens and worked in a synergistic way with the other oils to increase the antimicrobial activity toward the existing microorganisms [46].

Several investigations were designed to determine the effect of thymol on some fungal agents that lead to diseases. The findings showed that thymol has an antifungal property [46].

To summarize, thymol can be used in food and cosmetic industries for several purposes. However, we still need more investigations to detect *in vivo* the effectiveness of thymol in treatment of humans' infections [46].

Usage of Thymol in Cottage Cheese

Investigators conducted a study on the usage of thymol as a preservative on dairy products and mainly on cottage cheese. So, trials were made to determine the effect of thymol in enhancing the shelf life of the cottage cheese without affecting the quality. Three different levels; 30, 40 and 50 ppm of 30% thymol solution in butteroil were used to investigate the physio-chemical, microbiological and organoleptic properties of the cottage cheese. First, cottage cheese was produced via dual acidification technique (Figure-3) [22].

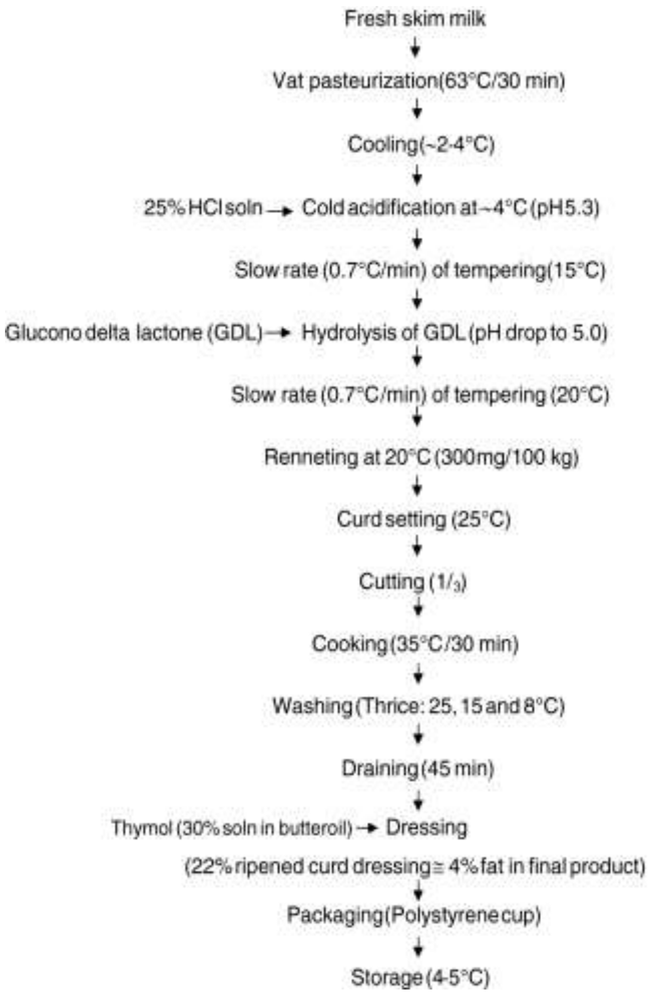

Fig-3: Flow diagram for manufacturing direct acidified cottage cheese employing dual acidification technique

Extraction of Thymol

The oil of the herb thyme can be extracted by steam distillation or traditional solid-liquid extraction methods such as soxhelt, or extraction under reflux. These methods are simple but they have several drawbacks such as a long extraction time and high solvent consumption [45]. To reduce these shortcomings, a new solvent extraction technique called Pressurized Liquid extraction (PLE) is used. PLE is based on the usage of solvents at temperature above their normal boiling point and at high pressures to let the extraction fluid in the liquid state during the whole process. By using this method, a faster extraction process can be obtained with higher yield and lower volumes of organic solvents [45].

Supercritical Fluid Extraction (SFE) is a valuable alternative to classical methods to get the oil from plants and herbs. A pure CO_2 is used as extraction solvent and based on the extraction conditions, several concentrations of thymol are achieved [45].

Ethyl lactate and D-limonene are called green extraction solvents. They are used to replace the toxic organic solvents. They are generally recognized as safe (GRAS). They are non-toxic, non-carcinogenic, non-corrosive and fully biodegradable [45].

To start with the extraction process, the thyme leaves should be dried and then ground in a cooled mill. Sieves are used to separate particles of size less than 250um. PLE and SFE are applied by using D-limonene, ethanol and ethyl lactate as solvents. Gas chromatography is used for analysis. As a result, the solubility of thymol is enhanced by increasing temperature regardless the type of solvent. The relative affinity of thymol in ethanol is much more than ethyl lactate and more than D-limonene. But, limonene still possess higher selectivity regarding the extraction of thymol from thyme leaves due to its lipophilic character. The highest concentrations of thymol were obtained by using SFE what make it the best method to be used [45].

After adding thymol, the samples were collected and analyzed for sensory, biochemical and microbiological quality assessment after each 4 days interval during the study. Sensory evaluation was carried by using the standard cottage cheese score card recommended by the ADSA (American Dairy Science Association) and was performed by 10 trained panellists. Flavor, body, texture, color and appearance are the attributes that were evaluated. In physiochemical analysis, moisture content and the acidity of the cottage cheese were determined as per the AOAC. The oven drying was used to determine the moisture content of the curd and microprocessor for pH. In addition to that, total fatty free acidity and soluble nitrogen were also measured. In microbiological analysis, enumeration of yeasts, molds, psychrotrophs (incubation at 5 ºC for 7 days) as well as coliform counts in the cheese was performed with a standard method. After that, the results were obtained and discussed (Figure 4, 5, 6, 7, 8 and 9) [22].

Changes in Cottage Cheese

Alterations in Sensory Quality

Flavor

The addition of thymol gave a distinguishing thymol flavor named as medicinal flavor to the product as the level of addition increased. Thymol at 30 ppm (sample T_1) gave a slight medicinal flavor. As the level increases from 30 (T_1) to 40 (T_2) to 50 ppm (T_3), the flavor increases too. Compared to the control, the control achieved a higher score for the flavor at the beginning. However, later on, there was deterioration in the flavor in the control sample with time. Off-flavor and bitterness were highly discovered in the control sample at 12 days. Compared to the treated samples, there was a delay in deterioration in the flavor as the level of thymol increases (Figure-4) [22].

Body and Texture

With progressing storage period, all samples showed a gradual deterioration in body and texture. The control sample exhibited a mealy and pasty texture at 12th day but the treated samples showed that result after 22 days of storage (Figure-4). Thus, thymol delayed the damage in the body and texture of the cottage cheese [22].

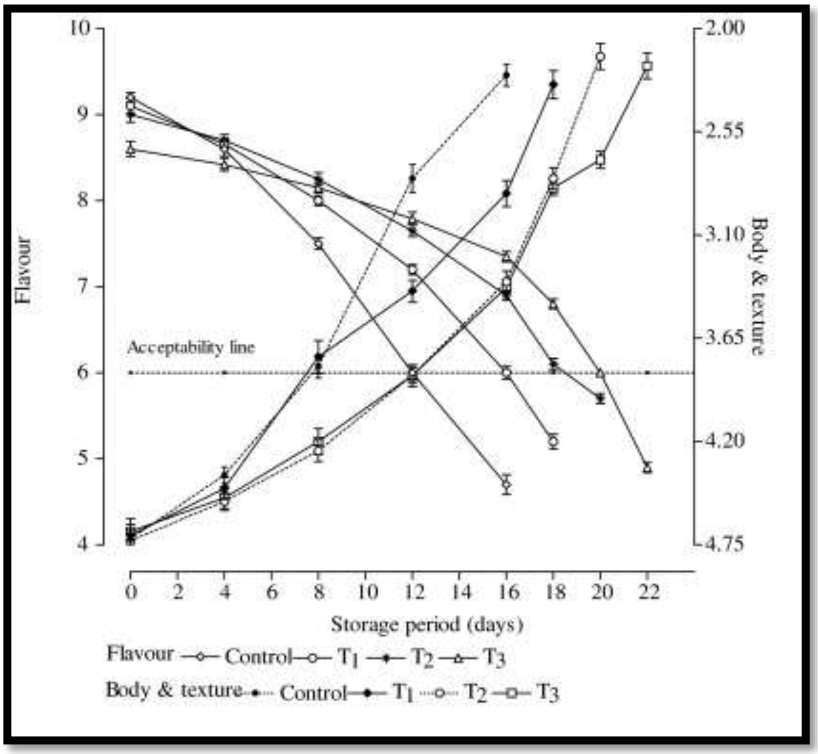

Fig-4: Effect of different concentrations of thymol on the flavor, body and texture of the acidified cottage cheese stored at 4-5°C. T1, T2 and T3 are the samples that are treated with 30, 40 and 50 ppm of thymol (30% sol in butteroil), respectively. n= 10 panelists

Color and appearance

The addition of thymol didn't affect the color and appearance of the treated samples. But, with time, there was a poor color in all samples. Deterioration in color and shape were observed first in the control sample (Figure-5) [22].

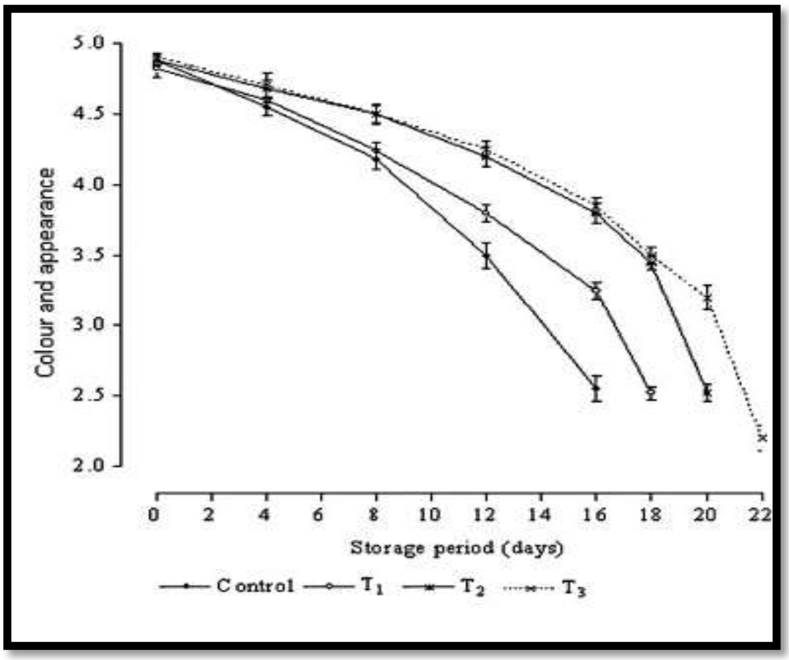

Fig-5: Effect of different concentrations of thymol on the color and appearance of acidified cottage cheese stored at 4-5°C

Modification in Physio-chemical Properties

Moisture

There was a slowly reduction in the moisture content in all samples during storage. At the early stage of storage, the samples showed a rapid moisture loss and then it declined gradually (Figure-6). The moisture loss is not related to the addition of thymol as indicated in the results [22].

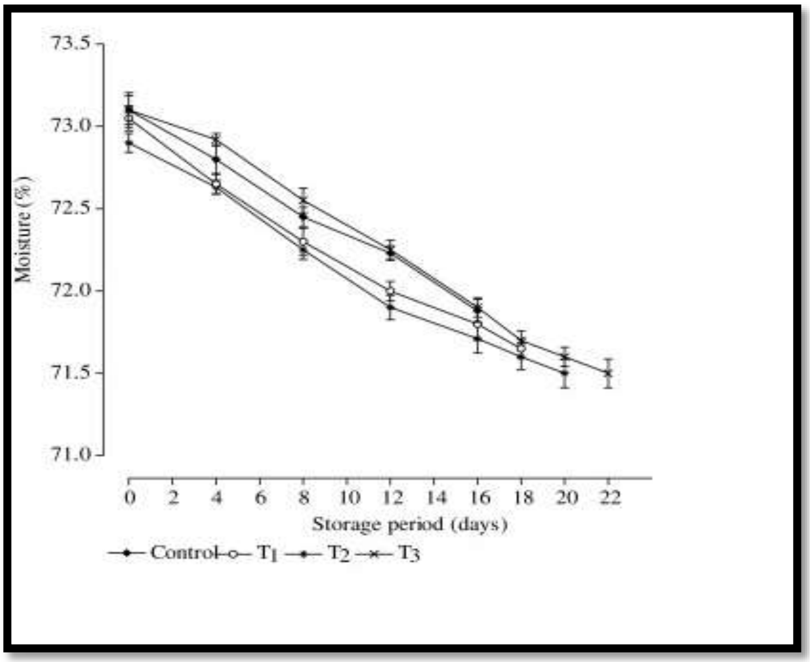

Fig-6: Effect of addition of different concentrations of thymol on the moisture content of the acidified cottage cheese stored at 4-5 °C

Acidity

Normally, the acidity of the fresh cottage cheese ranged between 0.61 and 0.62%. There was an increase in the acidity of the control sample more than the treated ones after 16 days of storage. The acidity in the treated samples declined as the level of thymol increases (Figure-7). Thus, the addition of thymol in the cheese delayed the acid development during storage. This is due to its antimicrobial activity against microorganisms causing spoilage [22].

PH

The pH of the fresh cottage cheese ranged between 4.98 to 5. Upon storage up to 12, 16, 18 and 20 days, there was a gradual decline in pH respectively in all samples. The pH in the control sample declined gradually after 12 days of storage compared to the treated samples (Figure-7). Hence, the treated samples showed delayed and small changes in pH. The addition of thymol at high concentration exerted a strong antimicrobial activity against the lactose fermenting organism, so this let the pH of sample T_3 to be changed slowly [22].

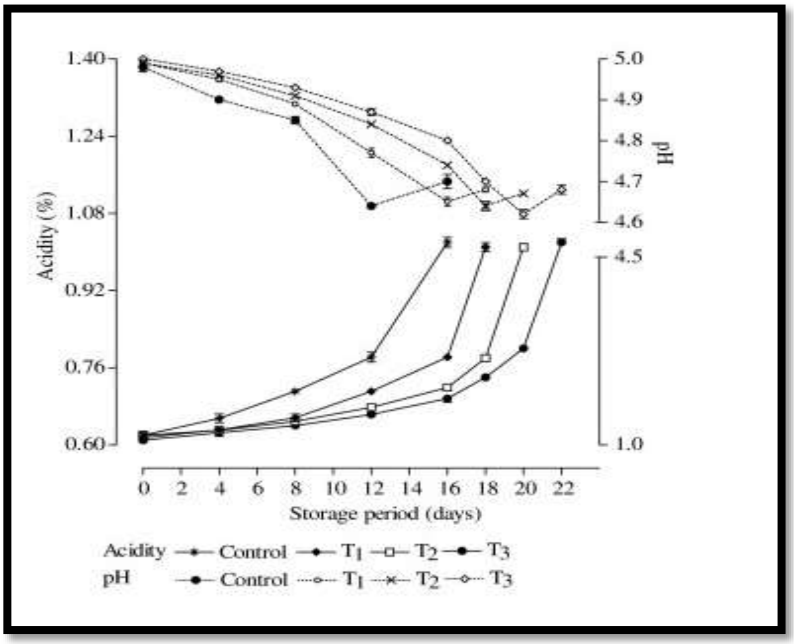

Fig-7: Effect of addition of different concentrations of thymol on the acidity and pH of the acidified cottage cheese stored at 4-5 °C

Free fatty Acid

The free fatty acid in fresh cottage cheese ranged between 0.13 to 0.134% oleic acid. At 16 days of storage, the FFA content sharply increased in the control sample compared to the treated ones. Upon 18 to 20 days of storage, there was no change in FFA content in the treated samples. But, up to 22 days, FFA rise sharply. With increasing the thymol concentration, the rate of FFA content decreases (Figure 8). This is due to the antimicrobial activity of thymol that reduces the effect of lipolysis that takes place normally in the cottage cheese [22].

Soluble Nitrogen

The control sample showed a rapid increase in soluble nitrogen (Proteolysis) compared to the treated samples. The level of thymol controlled the rate of proteolysis in cottage cheese during storage (Figure-8) [22].

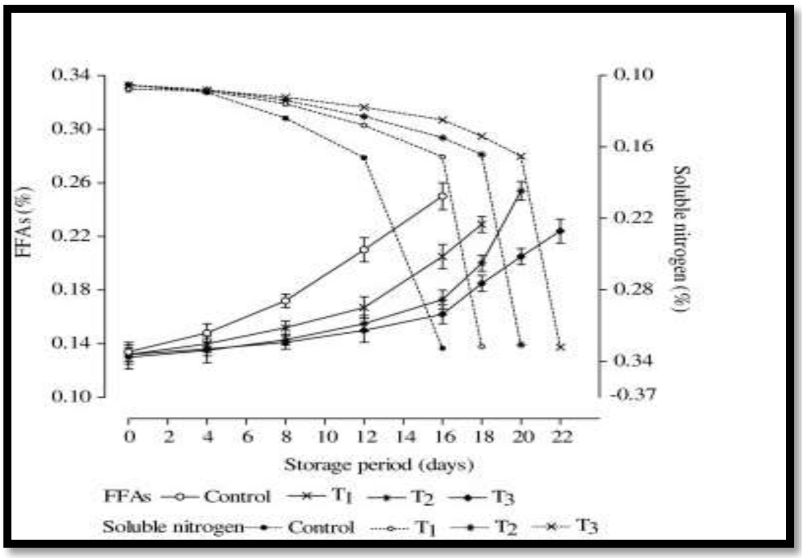

Fig-8: Effect of addition of different concentrations of thymol on the FFA and the soluble nitrogen of acidified cottage cheese stored at 4–5 °C

Changes in Microbiological quality

Psychrotrophs

The psychotropic count of the fresh sample was 3.841985 log cfu/g while in T_1, T_2 and T_3, the psychotropic count was 3.766413, 3.68842 and 3.574031 log cfu/g respectively. Thus, there was a reduction by 16, 30 and 46% in the amount of bacteria in the treated samples compared to the control. This reduction is due to that bactericidal effect of thymol. The control sample showed a sharp rise is psychotropic count compared to the treated samples. Upon storage for 18 and 20 days, samples T_2 and T_3 showed same growth of psychotropic count as the control sample. At the end of storage, each sample showed faster growth of psychrotrophs compared to early stage of storage (Figure-9) [22].

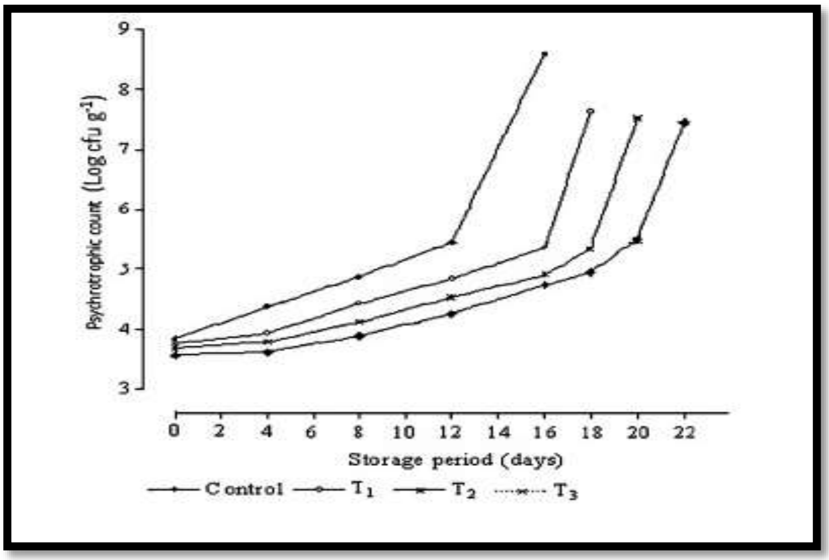

Fig-9: Effect of addition of different concentrations of thymol on the psychrotrophic count of the acidified cottage cheese stored at 4–5 °C

Yeasts and Molds

The average count of yeast and mold in fresh cottage cheese was 0 to 0.3103 log cfu/g, while there was no data conforming the occurrence of yeasts and molds in the treated samples up to 12 days. From day 12 to 16, there was a slightly increase in these microorganisms in the treated samples. The addition of thymol delayed and decreases the growth of yeasts and molds. Thus, thymol has an antifungal effect. As the level of thymol increases, the growth of yeasts decreases (Table-7) [22].

Table-7: Effect of different levels of thymol on yeasts and molds count (log cfu/g) of direct acidified cottage cheese during storage at 4-5 °C

Days	Control	Levels of thymol		
		T_1	T_2	T_3
0	0-0.30103	Negative	Negative	Negative
4	0.778151	Negative	Negative	Negative
8	1.113943	Negative	Negative	Negative
12	1.977724	0-0.69897	0-0.30103	Negative
16	2.64836	1.832509	1.681241	0-0.778151
18	ND	2.544068	2.049218	1.875061
20	ND	ND	2.824776	2.133539
22	ND	ND	ND	2.89487

Coliforms

At the beginning, the coliforms counts in all the samples were less than 1 log cfu/g (Table-8). As the period of storage increased, the coliforms count in control and T_1 sample increased also to become 1.447158 and 1.30103 log cfu/g respectively. At the end of storage, there was a gradually increase in the coliforms count in the control compared to the treated samples. On the 18th and 20th day of storage, the samples T_1 and T_2 showed a similar observation when compared with T_3 sample. The use of thymol was effective in controlling the coliforms counts in the cottage cheese [22].

Table-8: Effect of different levels of thymol on Coliforms (log cfu/g) of direct acidified cottage cheese during storage at 4–5 °C

Days	Control	Levels of thymol		
		T_1	T_2	T_3
0	<1	<1	<1	<1
4	1.447158	1.30103	<1	<1
8	1.875061	1.748188	1.380211	1.20412
12	2.491362	2.09691	1.778151	1.447158
16	3.875061	2.565848	2.041393	1.778151
18	ND	3.515874	2.352183	2.120574
20	ND	ND	3.164353	2.447158
22	ND	ND	ND	3.50515

As a conclusion, as increasing the level of thymol, cottage cheese undergo a minimal changes. The addition of 50 ppm instead of 40 ppm, enhanced the shelf life only by 2 days and gave more medicinal flavor to the cheese. While, the usage of 40 ppm improved the quality of the cottage cheese and it was effective to extend the shelf life from 2 to 20 days compared to the control sample [22].

CONCLUSION

Thymol has been proved to be a good preservative added to cottage cheese that can replace the other chemical preservatives. It imposes minimal risk and it has many advantages. It can delay the deterioration of the flavor and texture. It keeps the cheese in a good situation without any injury or destruction. It protects the cheese from spoilage microorganisms that can affect the quality badly. Thus, thymol extends the shelf-life of the cheese. More studies are needed to detect the effectiveness of thymol on other dairy products such as yogurt.

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