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Extracting and using some preservatives from natural sources as alternatives to industrial preservatives

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Abstract

Natural preservatives extracted from plants are added to food products to preserve food and prolong their shelf–life, due to their antimicrobial and antioxidant activities. Natural preservative materials from some spices and herbs including clove, thyme, ginger, and rosemary, were extracted using EtOH with concentration of 75% in water, and the effect of extraction time on the extracted yield have been studied at extraction time intervals 0.50, 1.0, 1.50, and 2.0 hours. The results revealed an increase in the extracted yield from clove and thyme with extend the extraction time. The higher percents of clove and thyme extracts amounted to 17.14% and 6.00%, respectively, and obtained after 2 hours of extraction. Whereas rosemary and ginger exhibited the higher extracted yield (30.0% and 7.77%) after 1.0 and 1.5 hours of extraction, respectively. Properties of the extracts of spices and herbs under study in preserving the quality of kareish cheese and extending its shelf–life have been investigated. The results indicated that the addition of clove, ginger, thyme, and rosemary water extract with concentrations 0.50%, 0.25%, 0.75% and 1.0%, respectively, will improve appearance, body texture, flavor of kareish cheese than those of other concentration. Furthermore, the plant water extracts will extend the shelf–life of kareish cheese from 14 to 23 days when stored at the refrigeration temperature (4 ± 2 °C). The higher shelf–lives, 20, 23, 21, and 23 days, were obtained by the clove, ginger, thyme, and rosemary water extracts with concentrations 0.50%, 0.75%, 1.0% and 1.0%, respectively. In vitro antibacterial activities of the thyme and rosemary extracts against Gram – positive bacteria: *Staphylococcus aureus* (ATCC 25923) and Gram – negative bacteria: *Escherichia coli* (ATCC 25922) have been evaluated. The results illustrated that the plant extracts behaved as good antibacterial agents towards the screened types of bacteria, and they can be used as natural food preservatives, and to increase the shelf life of food products.

Key Words:

Natural preservatives, spices, extraction, antibacterial activity, shelf–life

1. Introduction: Natural preservatives are compounds derived from plants and natural sources. Plant extracts or volatile oils are usually added to food products to preserve food, extend their shelf-life, and create new attractive taste (Rasooli, 2007, 111–136).

Synthetic preservatives are chemical substances that are added to food during the manufacturing process to stop the growth and activities of the microorganisms such as benzoates, nitrites, calcium lactate and sorbic acid, in addition of antioxidants that are used to slow down or prevent the oxidation of fats and oils in food (Ahmed et al., 2022, 1–14) such as formaldehyde, butylated hydroxy anisole (BHA), and butylated hydroxytoluene (BHT). Although the use of synthetic preservatives has undeniable benefits, such as prolonging shelf life and preserving food quality, they have potential detrimental effects on health (Sulieman et al., 2023, 1451) from breathing difficulties, cancer risks and heart diseases to behavioral changes (steetaramaiah et al., 2011, 583–99). As a result, there is a growing global reliance on natural preservatives to shield consumers from the health risks posed by synthetic preservatives.

Natural food preservatives have strongly been studied in recent years due to their antimicrobial and antioxidant activities, which make them potentially act as effective alternative to synthetic preservatives (Gould, 1996, 82–86).

Spices and herbs have long been used in foods not only for their flavoring, but also for their preservative properties (Davidson el al., 1983, 371–419). They are used for preventing food spoilage, inhibition the growth of pathogenic microorganisms (Tfouni and Toledo, 2002, 647–654), and extending shelf-life of food (Cortés-Rojas et al., 2014, 90–96). Moreover, they are used to slow or prevent changes in color, flavor or texture and delay rancidity (Saleh, 2018, 7–12). Antimicrobial plant's extracts are chemical substances derived from different parts of the plant such as leaf, flower bud, fruit, or rhizome, and they could be soluble in water or organic solvents. Over the years, spices like clove, cinnamon, thyme, mint, and fennel have become pronounced as natural food preservatives due to their antimicrobial and antioxidant activities (Cortés-Rojas et al., 2014, 90–96).

As a result, Considerable research has been carried to examine the antimicrobial, and antioxidant activities of many herbs, spices, and their extracts. The effectiveness of rosemary oil as antimicrobial agents in mozzarella cheese have been studied (Hoon et al., 2015, 75–84). Thyme is one of the most active substances and considered as powerful antibacterial agent (Piccaglia et al., 1993, 47–50). The research studies showed the efficiency of ginger extract in Killing salmonella and inhibition of fungi (Metwalli et al., 2011, 639–649).

Kareish cheese is one of the most popular dairy products of the Egyptian cities., it has high protein and low fat, and it contains an amount of sugar, some water, soluble vitamins in addition of calcium and phosphorus. The increasing demand for it by Egyptian consumers is mainly attributed to its

high protein content and low price. The quality and composition of kariesh cheese may vary due to many factors such as the quality and composition of the clotted skimmed milk, the method of manufacture, and the method of handling finished cheese. The shelf life of the fresh kareish cheese is limited to 7–11 days (Aldo et al., 2013, 1–6), depending upon the quality of the raw materials, processing conditions, and post processing conditions. The quality and safety of kareish cheese is the major area of concern for producers and consumers. Therefore, there is a need to develop and improve the kariesh cheese quality and increase its shelf life (Metwalli et al., 2011, 639–649).

Accordingly, this study aimed to extraction of natural preservative materials from some spices and herbs including clove, thyme, ginger, and rosemary, and to examine the properties of the plant extracts in preserving the quality of kareish cheese (is one of the dairy products) and extending its shelf–life, in addition to evaluate the efficiency of plant extracts to be use as antibacterial agents against gram–negative bacteria such as *Escherichia coli*, gram–positive bacteria such as *Staphylococcus* aureus.

2. The Theoretical Framework

This study will focus on extraction of the natural preservatives from some plants (clove, thyme, ginger, and rosemary) illustrated in Table 1, exploring their benefits in food preservation and as antibacterial agents.

Table (1): The common and scientific names of the plants.

Common name	Scientific name	Botanical family	Main bioactive compound	Used part
Clove	Syzygium aromaticum	Myrtaceae	Eugenol	Flower buds
Thyme	Thymus vulgaris	Lamiaceae	Thymol	Leaves
Ginger	Zingiber officinale Roscoe	Zingiberaceae	Gingerol	Rhizomes
Rosemary	Rosmarinus officinal	Lamiaceae	Carnosol	Leaves

Clove (Syzygium aromaticum) is the aromatic flower buds from the clove tree and belonging to the Myrtaceae family (Abd Rahim and Gengatharan, 2023, 100283). Clove oil consists of eugenol, eugenyl acetate, and β–caryophyllene, in addition to minor constituents such as methyl salicylate, chavicol, α–copaene, α–amorphene, and caryophyllene oxide, Figure 1. Clove extract boasts a rich assortment of bioactive elements, which include flavonoids, saponins, phenolic compounds, tannins, steroids, terpenoids, and alkaloids. These constituents offering antibacterial and antifungal traits (Afrendi et al., 2023, 1–8), which makes cloves a good candidate for use in food preservation.

Moreover, cloves have effective antioxidant activity and can increase the shelf life of food products by hindering lipid peroxidation (Abd Rahim and Gengatharan, 2023, 100283).

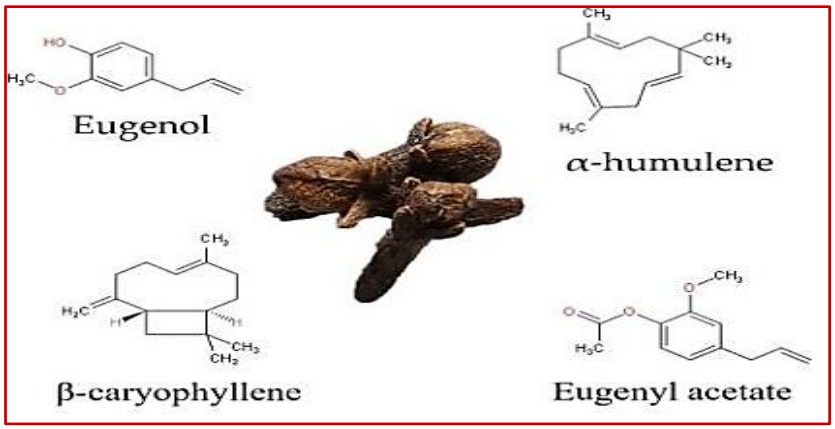

Figure (1): Some constituents of clove oil.

Thyme (Thyme vulgaris) is a member of the Lamiaceae family. It contains various monoterpene phenols, such as carvacrol (iso–propyl–ortho–cresol), thymol (2–isopropyl–5–methylphenol or iso–propylmeta–cresol), Figure 2, and *p*–cymene. Additionally, it contains other monoterpenes like α–pinene, 1,8–cineole, camphor, linalool, and borneol. Thyme's richness in phenolic compounds makes it a valuable natural preservative. The antioxidant effect of thyme is attributed to its essential oil (Chattopadhyay and Bhattacharyya, 2007, 239–247), which contains at least 60 bioactive compounds with potent antioxidant properties (Nieto, 2020, 96). The recent studies demonstrated that the thyme essential oil exhibits a broad scale antimicrobial activity against foodborne pathogens, and when it is used with bread and dairy products, it can improve food preservation (Maksimov, 2017, 19–22).

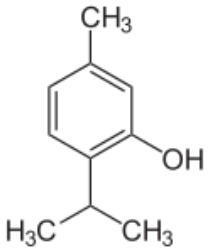

Figure (2): Structure of thymol.

Ginger (Zingiber officinale Roscoe) belongs to the Zingiberaceae family. Ginger roots contain numerous bioactive compounds, including phenolic and terpene compounds. The primary phenolic compounds found in ginger are gingerols, shogaols, and paradols. Among these, gingerols like 6–gingerol, 8–gingerol, and 10–gingerol are the major polyphenols in fresh ginger. Additionally, ginger also contains other phenolic compounds such as quercetin, zingerone, gingerenone–A, and 6–dehydrogingerdione, Figure 3. Ginger extract can be used as natural preservative and it demonstrated antioxidant effects (Metwalli, 2011, 639–649). The biomolecules in ginger

extract have appreciable biological activities and they exhibited antibacterial and antifungal activity against foodborne pathogens (Murthy et al, 2015).

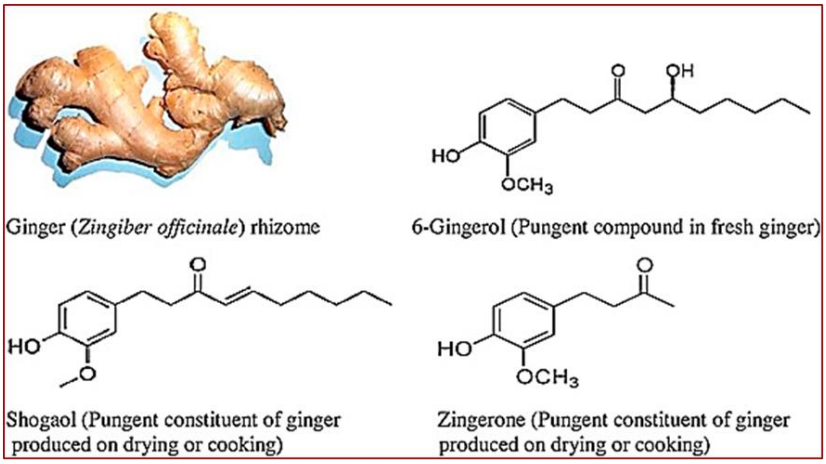

Figure (3): Structures of some phenolic compounds in ginger.

Rosemary (Rosmarinus officinal) is a medicinal plant belonging to the Lamiaceae family. It contains various beneficial phytochemicals with antioxidant and anti–inflammatory properties, including carnosic acid, carnosol, rosmarinic acid, ursolic acid, camphor, caffeic acid, and betulinic acid, Figure 4. Recognized for imparting flavor, rosemary leaves also serve as an important source of antioxidants, which are vital in combatting oxidative stress associated diseases that include cancer, and cardiovascular disorders (Boggula at el., 2019, 323–330). Rosemary is considered as a food preservative due to its high antioxidant and antimicrobial activities. These properties make it an inhibitor of the growth of microbes while reducing food spoilage through the oxidation process (Veenstra and Johnson, 2021, 1–10).

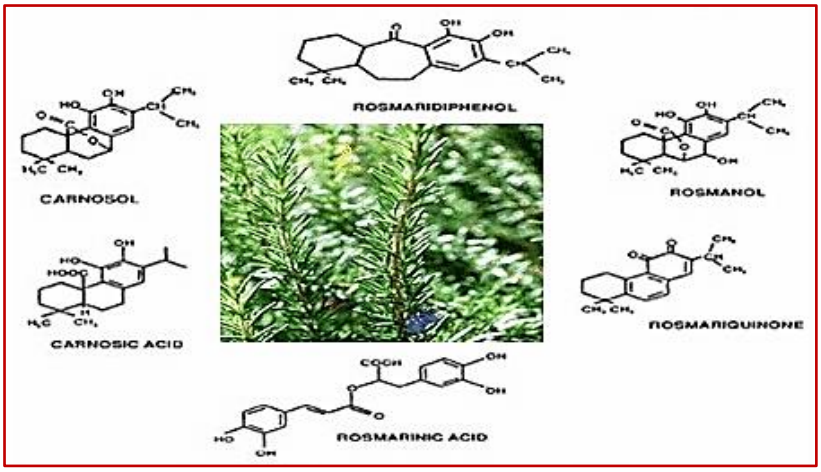

Figure (4): Structures of some antioxidant compounds in rosemary.

3. Materials and Methods used

3.1. Materials

Spices and herbs plants (clove, thyme, ginger, rosemary), Figure 5, were obtained from local agricultural nurseries in Cairo, Egypt. The Fresh prepared kareish cheese sample was purchased from the local market in Sharkia, Egypt, and it kept in refrigerator at temperature 4 ± 2 °C.

Figure (5): Spices and herbs plants (clove, thyme, ginger, rosemary) under study.

3.2. Methods

3.2.1. Extraction of natural preservative materials from spices and herbs

Natural preservative materials from spices and herbs plants under study were extracted using the method described by (Atta and Imaizumi, 1998, 475–480). The used parts (flower buds, leaves, or rhizomes) of the plants (clove, thyme, ginger, rosemary) under study were cleaned well and grinded using an electrical grinder. 10 g of the grinded sample was shaking with 100 mL of EtOH (75%) in a

250 mL conical flask at room temperature for 30 minutes. The suspension was allowed to stand for 1 hour and filtered through a piece of cotton cloth. The extraction procedures were repeated twice with the solid residue. The combined filtrate was concentrated to half of its volume via evaporation on a hot plate at 50 °C and left to cool to room temperature, Figures 6 and 7. The extracted material was filtered and dried in air at room temperature, Figure 8. The dried extract was weighed to determine the extracted yield and it kept in refrigerator at temperature 4 ± 2 °C until further use. The extraction process was repeated in the same manner with changing the shaking period (1.0, 1.50, and 2.0 hours) to study the effect of the extraction time on the extracted yield.

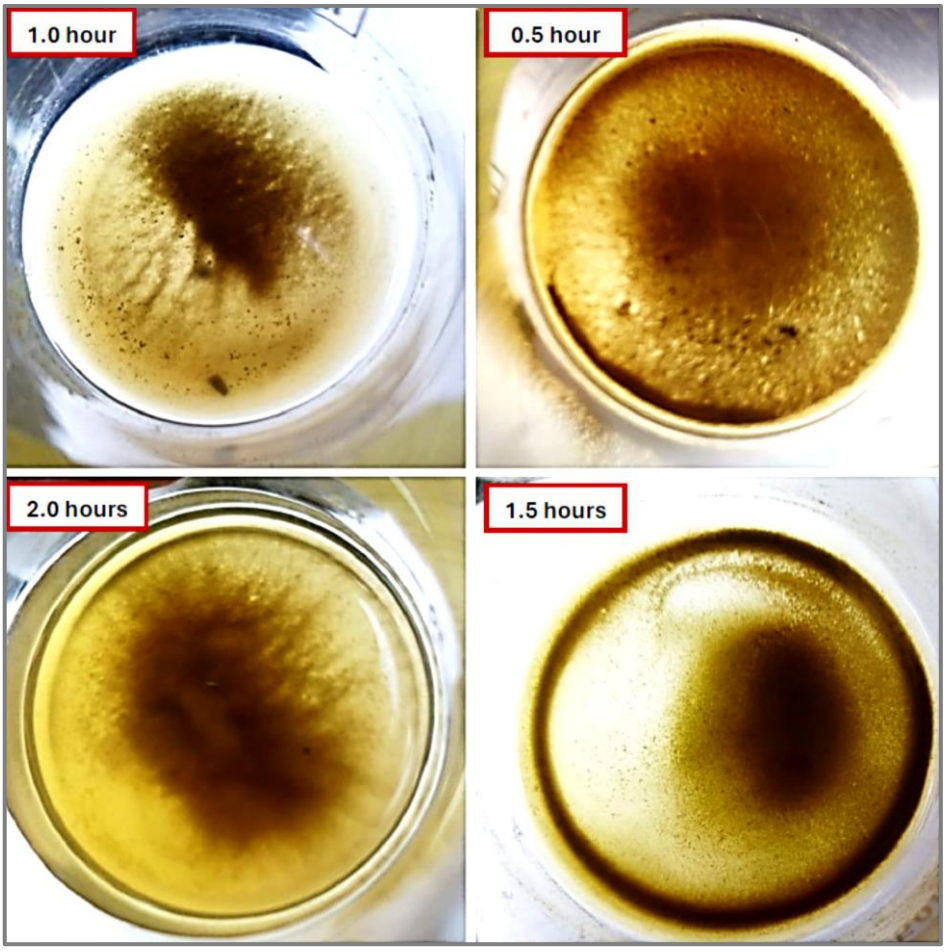

Figure (6): Clove extracts by EtOH (75%) with changing the shaking period.

Figure (7): Thyme extracts by EtOH (75%) with changing the shaking period.

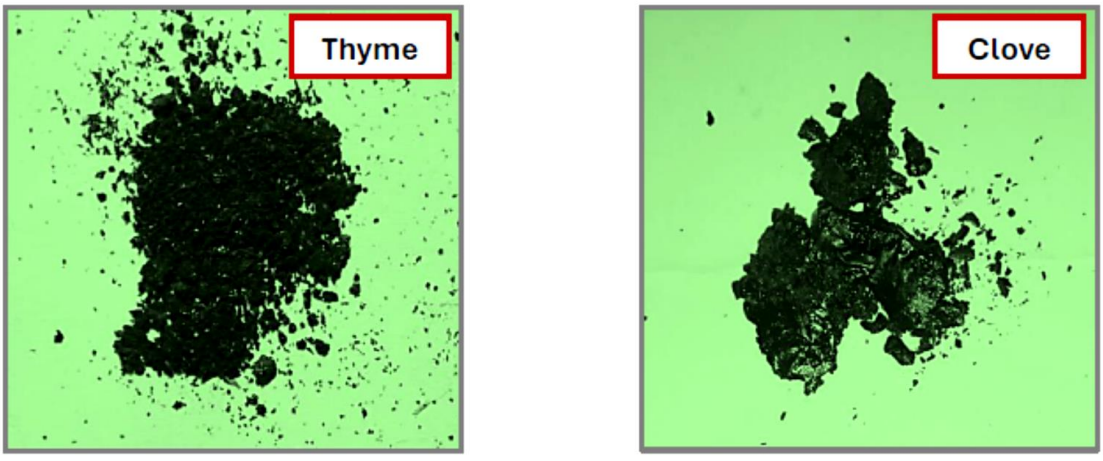

Figure (8): Dried clove and thyme extracts at room temperature.

3.2.2. Examination of properties of the plant extracts in preserving the quality of kareish cheese and extending its shelf–life

Properties of the extracts of spices and herbs under study in preserving the quality of kareish cheese and extending its shelf–life were investigated according to the following experimental procedures: 100 ml of clove, thyme, ginger, or rosemary water extract (0.50%) was added to a 250 mL glass beaker contains 50 g of a fresh kareish cheese. The beaker is kept in refrigerator at temperature 4 ± 2 °C. The sensory characteristics of kareish cheese samples (including flavor, body texture, and appearance) in addition to their shelf–life were evaluated by members of graduation project group – chemistry department – Faculty of education – Ain Shams University during 25 days of storage at the refrigeration temperature, according to the method that as described by Nelson and Trout, 1981, where the scores of judging were 60 for flavor, 30 for body texture and 10 for appearance. The previous procedures were repeated in the same manner with changing the concentration of plant water extracts, (0.25% and 0.75%) for both clove and ginger extracts, (0.75% and 1.0%) for both thyme and rosemary extracts, to study the effect of the extract concentration on extending the shelf–life of kareish cheese. The control kareish cheese sample was 50 g of fresh kareish cheese in 100 mL water and stored in refrigerator at temperature 4 ± 2 °C, Figures 9 and 10.

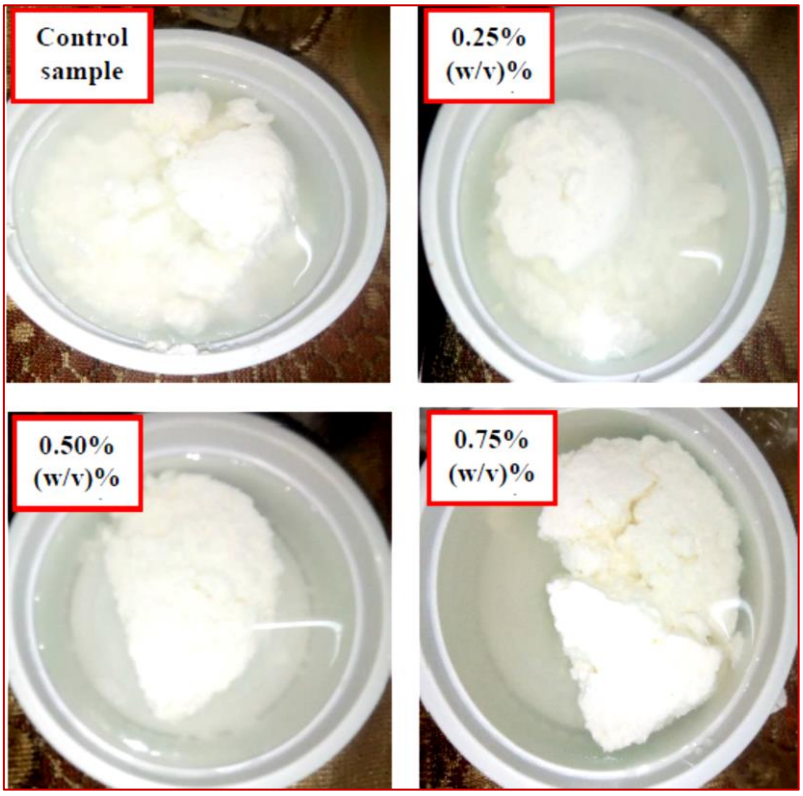

Figure (9): Kareish cheese samples in ginger water extract, at different concentrations.

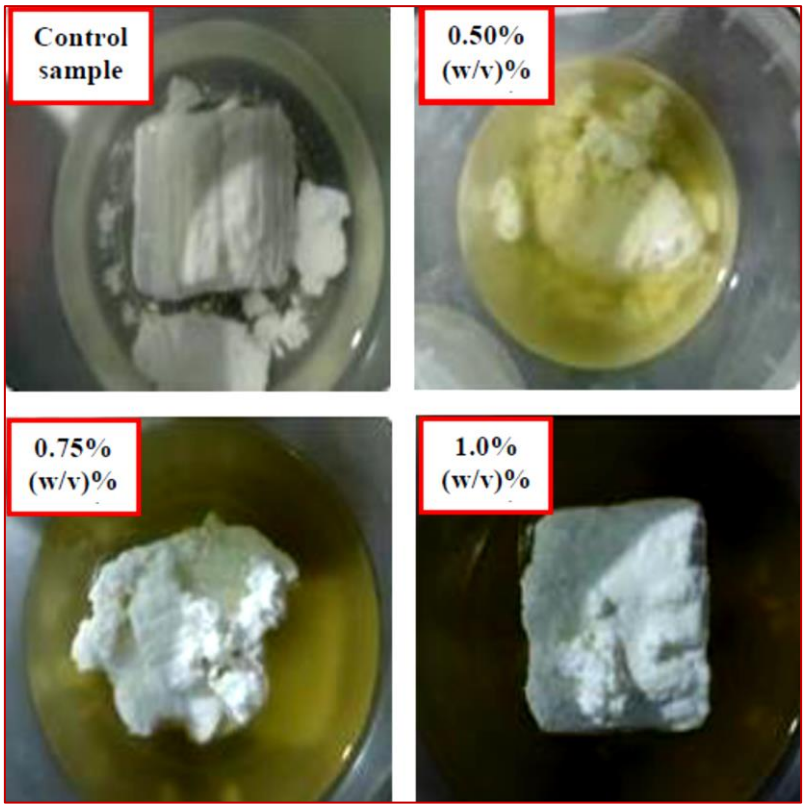

Figure (10): Kareish cheese samples in thyme water extract, at different concentrations.

3.2.3. Evaluation of antibacterial activities of the plant extracts

The diffusion agar technique (Rahman et al., 2001 and Khan et al., 2000, 915–922.) was followed to determine the activity of the plant extracts against Gram – positive bacteria: ***Staphylococcus aureus*** (ATCC 25923) and Gram – negative bacteria: ***Escherichia coli*** (ATCC 25922) at Regional Center for Mycology and Biotechnology, Al–Azhar University, Cairo, Egypt. The screened plant extracts were dissolved in DMSO, and prepared in concentration of 100 mg/mL, then 10 µL of each preparation was dropped on disk of 6 mm in diameter. The concentration is 1 mg/disk. Gentamycin was employed as reference in the case of the Gram–positive and Gram–negative bacteria.

4. Results and Discussion

4.1. Effect of extraction time on the extracted yield of spices and herbs

The effect of extraction time on the extracted yield of spices and herbs (clove, thyme, ginger, rosemary) under investigation was studied using ethanol with concentration of 75% in water and change the shaking period (0.50, 1.0, 1.50, and 2.0 hours) for the suspension of plant extract. The data obtained were depicted in Table 2 and represented in Figure 11. The results revealed an increase in the extracted yield from clove and thyme with

extend the extraction time. The higher percents of clove and thyme extracts amounted to 17.14% and 6.00%, respectively, and obtained after 2 hours of extraction. On the other hand, rosemary and ginger exhibited the higher extracted yield (30.0% and 7.77%) after 1.0 and 1.5 hours of extraction, respectively. The highest extracted yields (4.57% and 17.14%) at the time intervals 0.5 and 2.0 hours, respectively, were obtained from clove, while the highest extracted yields (30.0% and 16.0%) at the time intervals 1.0 and 1.5 hours, respectively, were obtained from rosemary.

Table (2): Effect of extraction time on the extracted yield from plants under study.

Extraction time (hour)	Extracted yield% = (weight of the dried extract/weight of the raw plant) x 100			
	Clove	Thyme	Ginger	Rosemary
0.5	4.57	2.66	4.44	4.00
1.0	8.00	4.00	4.44	30.0
1.5	13.71	4.66	7.77	16.0
2.0	17.14	6.00	3.33	4.00

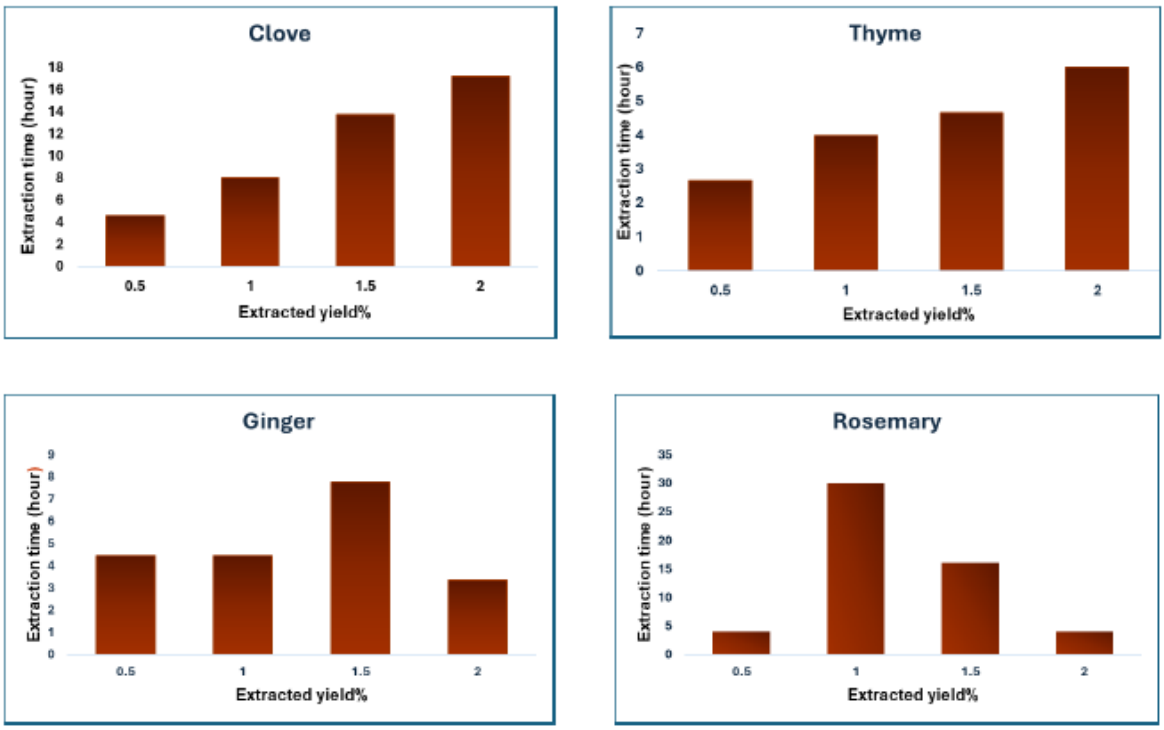

Figure (11): Variation of the extracted yield with extraction time.

4.2. Sensory evaluation and shelf–life of Kareish cheese

The quality of kareish cheese samples that were kept in both clove and ginger water extracts (0.25%, 0.50% and 0.75%), and in both thyme and rosemary water extracts (0.50%, 0.75% and 1.0%), and stored in refrigerator at temperature 4 ± 2 °C was evaluated for flavor, body texture, and appearance. Also, the shelf–lives of kareish cheese samples were evaluated at the same conditions. The data obtained were listed in Table 3 and demonstrated in Figure 12. Results of the sensory evaluation of kareish cheese gained higher score for appearance, body texture, and flavor than the control cheese sample. The results indicate that the addition of clove, ginger, thyme, and rosemary water extract with concentrations 0.50%, 0.25%, 0.75% and 1.0%, respectively, will improve the quality of kareish cheese (soft and moist texture and better flavor) than those of other concentrations. Furthermore, the plant water extracts will prolong the shelf–life of kareish cheese from 14 to 23 days when stored at the refrigeration temperature. The higher shelf–lives, 20, 23, 21, and 23 days, were obtained by the clove, ginger, thyme, and rosemary water extracts with concentrations 0.50%, 0.75%, 1.0% and 1.0%, respectively.

Table (3): Sensory evaluation and shelf-life of Kareish cheeses kept in some spices and herbs water extracts at the refrigeration temperature.

Sensory characteristics (scores of judging)	Control sample	spices and herbs water extracts											
		Clove			Ginger			Thyme			Rosemary		
		0.25%	0.50%	0.75%	0.25%	0.50%	0.75%	0.50%	0.75%	1.0%	0.50%	0.75%	1.0%
Flavor (60)	47	55	59	43	59	55	50	52	58	55	42	50	55
Body texture (30)	28	29	28	22	29	29	29	29	28	28	29	28	28
Appearance (10)	6	2	9	5	6	7	8	8	9	9	8	7	9
Total (100)	81	86	96	70	94	91	87	89	95	92	79	85	92
Shelf-life (day)	11	19	20	19	20	22	23	14	17	21	19	19	23

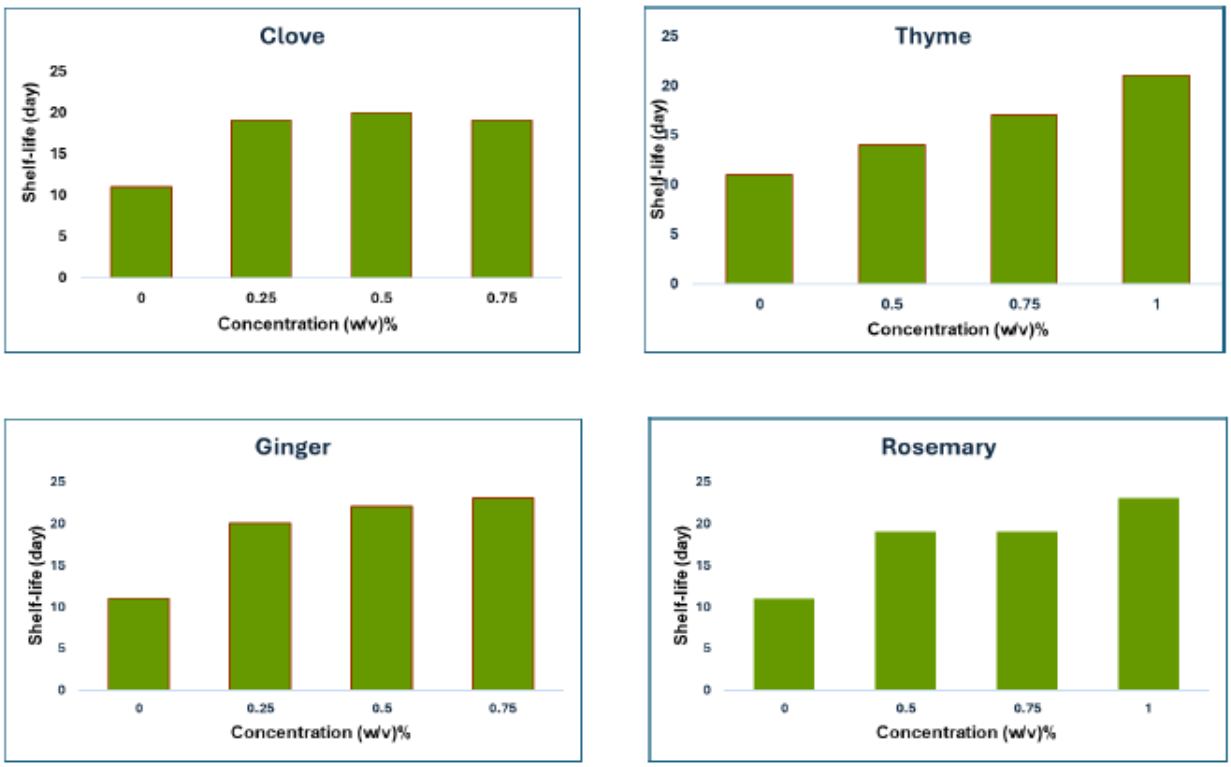

Figure (12): The shelf-life of kareish cheese in different concentrations of plant water extracts.

4.3. Antibacterial activities of the plant extracts

In vitro antibacterial activities of the thyme and rosemary extracts have been screened against Gram – positive bacteria: ***Staphylococcus aureus*** and Gram – negative bacteria: ***Escherichia coli***, in comparison with Gentamycin (inhibition zone = 24 and 30 mm, respectively), as standard reference, by applying the diffusion agar technique. The results obtained were recorded in Table 4. For Gram positive bacteria, the thyme and rosemary extracts under investigation showed moderate activities towards *S. aureus* with inhibition zone values 12 and 10 mm, respectively. For Gram negative bacteria, a moderate activity against *E. coli* appeared by both the thyme and rosemary extracts with inhibition zone values 14 and 13 mm, respectively. The results indicated that the thyme extract has slightly higher activity against the screened types of bacteria than rosemary extract. The antibacterial activities of thyme and rosemary extracts makes them act as good inhibitors of the growth of microbes. Thus, they can be used as natural food preservatives and to increase the shelf life of food products. The extent of the inhibitory effect of plant extracts is attributed to the presence of volatile oils, which have an aromatic nucleus containing polar functional group (Pandey et al, 2021, 1–18). The more hydroxylation, the greater the antimicrobial activity (Prastiyanto et al, 2020, 5644–5649).

Table (4): Antibacterial activities of thyme and rosemary extracts.

No.	Plant extract	Mean* of zone diameter ,nearest whole mm.	
		Gram – positive bacteria	Gram – negative bacteria
		S. aureus	*E. coli*
1	Thyme	12	14
2	Rosemary	10	13
	Control #	24	30

* = Calculate from 3 values.

#: Gentamycin in the case of Gram-positive bacteria and Gram-negative bacteria

5. Conclusion

EtOH (75%) acted as an effective solvent to extract natural preservatives from the studied spices and herbs (clove, thyme, ginger, and rosemary) plants. The relationship between extraction time and extracted yield of the spices and herbs have been studied. The results exhibited an enhancement of the extracted yield with increasing the extraction time. Clove displayed the highest extracted yields at the time intervals 0.5 and 2.0 hours, while at the time intervals 1.0 and 1.5 hours, rosemary displayed the highest extracted yields among those of the other studied spices and herbs. Efficiency of the extracts of studied spices and herbs to improving the sensory properties of kareish cheese and prolonging its shelf-life were evaluated. The results indicated that the clove, thyme, ginger, and rosemary extracts will enhance the quality of kareish cheese (soft and moist texture and better flavor) and will prolong its shelf-life when stored at the refrigeration temperature. The antibacterial activity study showed that the thyme and rosemary extracts acted as good antibacterial agents against *Staphylococcus aureus* and *Escherichia coli*, and they can be used as natural food preservatives.

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References and Sources

Abd Rahim, M. H., Gengatharan, A. (2023). The application of clove extracts as a potential function component in active food packaging materials and model food systems: A mini- review. Journal of Applied Food Research, 3(7), 100283.

Afrendi, E., Astuti, R. I., Batubara, I., Prastya, M. E., Wahyuni, W. T. (2023). Bioactivity of the Ethanol Extract of Clove (Syzygium aromaticum) as Antitoxin. International Journal of Food Science, 1-8.

Ahmed, Q. A., Bakr, M., Kamel, T. M. (2022). Side Effects of Preservatives on Human

Life. Scientific Research Journal, 2(5), 1–14.

Aldo, T., Fady, A. A., Ola A. H. O. (2013). History, Processing and Quality Enhancement of Traditional Egyptian Kariesh Cheese: A Review. Food science and technology, 1(1), 1–6.

Atta, M. B., lmaizumi, K. (1998). Antioxidant activity of nigella (Nigella sative L.) seeds extracts. Journal of Japan Oil Chemists' Society, 47(5), 475– 480.

Boggula, N., Kompelly, A., Kompelly, S., Vasodha, B. (2019). Rosmarinus officinalis L.: an update review of its phytochemistry and biological activity. Journal of Drug Delivery and Therapeutics, 9(1), 323–330.

Chattopadhyay, R. R., Bhattacharyya, S. K. (2007). Herbal spices as alternative antimicrobial food preservatives: An update. Pharmacognosy Reviews, 1(2), 239–247.

Cortés–Rojas, D. F., De Souza, C. R. F., Oliveira, W. P. (2014). Clove (Syzygium aromaticum): a precious spice. Asian Pacific Journal of Tropical Biomedicine, 4(2), 90–96.

Davidson, P. M., Post, O. L. S., Branen, A. L., Mccurdy, A. R. (1983). Naturally occurring and miscellaneous food antimicrobials. In: Branen AL and Davison PM (eds.) Antimicrobials in Food (PP. 371–419). New York: Marcel Dekker.

Gengatharan, A., Abd Rahim, M. H. (2023). The application of clove extracts as a potential functional component in active food packaging materials and model food systems: A mini review. Applied Food Research, 3, 100283.

Gould, G. W. (1996). Industry perspectives on the use of natural antimicrobials and inhibitors for food applications. Journal of Food Protection, 59(13), 82–86.

Hoon, H. J., Patel, D., Eun, K. J., Min, S. C. (2015). Microbial inhibition in mozzarella cheese using rosemary and thyme oils in combination with sodium diacetate. Food Science and Biotechnology, 24, 75–84.

Khan, K. M., Saify, Z. S., Zeesha, A. K., Ahmed, M., Saeed, M., Schick, M., Kohlbau, H. J., Voelter, W. (2000). Arzneim. Forsch., 50, 915–922.

Maksimov, O. (2017). Thyme Thymus vulgaris L. thymol CT essential oil as natural preservative. American Journal of Essential Oils and Natural Products, 5(2), 19–22.

Metwalli, S. A. H. (2011). Extended shelf life of kareish cheese by natural preservatives. Egyptian Journal of Agricultural Research, 89(2), 639–649.

Murthy, P. S., Gautam, R. (2015). Ginger oleoresin chemical composition, bioactivity and application as bio-preservatives. Journal of Food Processing and Preservation, 39(6), 1905–1912

Nelson, A. T., Trout, G. M., (1981). Judging Dairy Products 4th ed. USA: AVT Publishing Co. INC, Wesport. Connecticut.

Nieto, G. (2020). A Review on Applications and Uses of Thymus in the Food Industry. Journal of Plants, 9(8), 96.

Pandey, A. T., Pandey, I., kinase, A., Verma, A., Garcia-Canibbano, B. (2021). Validating anti-Infective activity of Pleurotus Opuntiae via standar-dization of its bioactive mycoconstitu-ents through multimodal. Biochemical Approach, 11(484), 1-18.

Piccaglia, R., Marotti, M., Giovanelli, E., Deans, S. G., Eaglesham, E. (1993). Antibacterial and antioxidant properties of Mediterranean aromatic plants. Industrial Crops and Products, 2 (1), 47-50.

Prastiyanto, M. E., Rukmana, R. M., Saraswati, D. K., Darmawatis, Maharani E. T. W, Tursinawati, Y. (2020). Anticancer Potential of Methanolic Extract From Pleurotus Species On Raji Cells and Antibacterial Activity Against Methicillin-Resistant Staphylococcus aureus. Biodiversitas, 21(12), 5644-5649.

Rahman, A. U., Choudhary, M. I., Thomsen, W. I (2001). Bioassay Techniques for Drug Development. Netherlands: Harwood Academic Publishers.

Rasooli, I. (2007). Food preservation-a biopreservative approach. Food, 1(2), 111-136.

Saleh, A. E. (2018). Keeping Quality of Kareish Cheese as Affected with Added Certain Spices and Herbs Oil as Antifungal Agent. Food and Dairy Sciences. Mansoura University, 9(1), 7-12.

Seetaramaiah, K., Smith, A. A., Murali, R., Manavalan, R. (2011). Preservatives in food products-review. International Journal of Pharmaceutical and Biological Science Archive, 2(2), 583-99.

Sulieman, A. M. E., Abdallah, E. M., Alanazi, N. A., Ed-Dra, A., Jamal, A., Idriss, H., Alshammari, A. S., Shommo, S. A. M. (2023). Spices as Sustainable Food Preservatives: A Comprehensive Review of Their Antimicrobial Potential. Pharmaceuticals, 16(10), 1451.

Tfouni, S. A. V., Toledo, M. C. F. (2002). Estimates of the mean per capita daily intake of benzoic and sorbic acids in Brazil. Food Additives & Contaminants, 19(7), 647-654.

Veenstra, J. P., Johnson, J. J. (2021). Rosemary (Salvia rosmarinus): Health-promoting benefits and food preservative properties. International journal of nutrition, 6(4), 1-10.