

Journal of Food and Dairy Sciences

Journal homepage & Available online at: www.jfds.journals.ekb.eg

Antimicrobial Activity of Methanolic Extract from Clove and the Test of Preservative Power of Ras Cheese

Osman, A. H. A.^{1*}; A. I. A. Mansour¹ and A. M. Ahmed²

¹Dairy Department, Faculty of Agriculture, Al-Azar University, Assiut Branch, P.O. Box 71524 Assiut, Egypt.

²Agronomy-Biochemistry Department, Faculty of Agriculture, Al-Azar University, Assiut Branch, P.O. Box 71524 Assiut, Egypt.



Cross Mark



ABSTRACT

The effect of methanolic extract from Clove (*Syzygium aromaticum* L.) and sodium sorbate (SS) on growth of certain fungal, bacteria strains and Ras cheese samples during storage were examined. Ras cheese made from standardized cow's milk (4% fat) with a modified process. During production curd cheese, four groups were formed: first group taken after salting for 12 days, without Clove extract (CE), as control, second group (T1) add CE to curd cheese, third group (T2) add SS to curd cheese, and fourth group (T3) add CE on surfaces of each cheese wheel. After completely salting process, the cheese ripened at 15±2°C and about 85% relative humidity for 3 months. All treatments evaluated microbiologically at fresh, and after one, two & three months. The samples we took from the exterior (Ex) and inside (In) of cheese wheel. The control samples had higher counts of yeast & fungal and TBC in S and M of cheese than that of other treatments. The counts of yeast & fungal and TBC of S and M of control samples increase with increasing of storage, while cheese treated with CE & SS decrease with increasing storage periods up to end of storage. Moreover, the coliform bacteria were not detected up to the end ripening. Generally, the results showed that the SS had higher antimicrobial effect than that with CE on growth of yeast & fungal and general microorganism especially the responsible for ripening cheese during storage.

Keywords: Ras cheese, Microbiological examination, Methanolic extract Clove, Fungal, Yeast and bacteria strains.

INTRODUCTION

Romy cheese is call an Egyptian Ras or hard cheese manufactured by cows and-buffalo's milk, or a combination the two. It is a popular dairy product in Egypt and the Arab world (El-Fadaly *et al.*, 2015). The depravation of Ras cheeses inside dairy produced labs in Egypt is familiar during the ripening process, where a layer of fungi and yeasts grow on the surfaces of the cheese wheels, due to the lack of care in the manufacturing and production. The processes and the failure to achieve the regular conditions of the ripening process leading to high economic losses, as well as, the production of some of these types of fungi toxic compounds that are not safe for health, called aflatoxins, which cause many diseases. When consume contaminated products, negative impact on the consumers health as a result of their exposure to mycotoxins, especially that produced by *Aspergillus* spp. (Mohsen, 2012). Therefore, the microbial growth decontamination and prevention on Ras cheese wheels surface is of great importance. Many methods used natural preservatives to reduce and inhibition of Mould and yeast growths on Egypt Ras cheese, several previous reports were focused on the antimicrobial properties of different plant essential oils specially Clove oil or methanolic extract from it in the production of hard and soft cheese (El-Fadaly, *et al.*, 2015).

Syzygium aromaticum, and well-known for its as Clove, belongs to family Myrtaceae, which is native to the Indonesian Maluku Islands but has recently been farmed

elsewhere in the world (Batiha *et al.*, 2019). The commercial component of the clove tree, which consists of leaves and buds, starts to produce flowering buds four years after planting. Following that, they are harvested either manually or with the aid of a natural phytohormone during the pre-flowering stage (Cortés-Rojas *et al.*, 2014). Traditional spice clove has been used to preserve food and has a number of pharmacological effects, including: It is abundant in sesquiterpenes, monoterpenes, hydrocarbons, and phenolic compounds, among other phytochemicals. The three most important phytochemicals found in clove oil are eugenyl acetate, eugenol, and -caryophyllene. Pharmacologically, it has been tested against a variety of pathogenic bacteria, parasites, and microbes, including hepatitis C and Plasmodium, Babesia, and Theileria. Numerous studies have shown that eugenol has antibacterial, antiviral, antifungal, anticancer, antiseptic, antidepressant, spasmodic, and anti-inflammatory properties that make it effective against a variety of pathogenic bacteria, including methicillin-resistant *S. aureus* and *S. epidermidis* (Batiha *et al.*, 2020). In this study, we examined the antimicrobial activities of methanolic Clove extract, and study its effect on microbial decontamination and preservation of Ras cheese during storage periods at 15±2°C and about 85% relative humidity for three months.

MATERIALS AND METHODS

Fresh whole cow's milk acquired from the Animal Herd Production Division, Al-Azhar University's Faculty of

* Corresponding author.

E-mail address: alihassan.2249@azhar.edu.eg

DOI: 10.21608/jfds.2022.172509.1077

Agriculture (Assiut Branch). Whole fresh milk that has been skimmed cow's using a laboratory centrifuge set at 3000 x g for 10 min at 20 °C, milk is made from defatted cream. Enzyme sources (Microbial rennet, 1 N): used as a powder that was purchased from DSM (France) and had a brand name (Fromase R 2200). 3g were dissolved to make of powder rennet in 100 ml distilled water; and used at a rate of 1.0 ml/L milk (0.03 g/L milk) Purchasing salt (commercial sodium chloride) from the El-Nasr Company for salt (Alexandria, Egypt). Calcium chloride obtained from Caso Co., Italy, imported by El-Zawaoui Co. El-Gesh Street, Cairo, Egypt. Fresh whole Clove samples from Edfu, Aswan, Egypt. Sodium sorbate from local market.

Preparation of methanolic extract:

Clove methanolic extract were prepared according to Wilson (1995) as follow:

Fresh whole clove was ground in a laboratory grinder (MJ-176P) and kept in opaque screw-tight jars until use. 25 g of chopped clove were combined with 150 ml of methanol, and the mixture was then stirred in a shaker incubator for 8 hours. It was then funnelled through Whatman paper and into a conical flask. The filtrate in the conical flask was extracted by using a rotavapor machine. In order to prevent the bioactive chemicals from being damaged by high temperatures, the filtrate was dried at a temperature below 50°C. The dark colored oily extract obtained is known as crude methanolic extract. Until the analysis was finished, the sample extract was stored at 4°C, the temperature of the refrigerator.

Ras cheese manufacture:

Ras cheese was prepared in accordance with Abdel Tawab (1963) with the following modifications:

Cow's milk (4% fat) heated to 75°C for 20 s, and then cooled to 40°C. Lactic acid starter {*Str. thermophilus*, *Lac. delbrueckii* spp. *bulgaricus* and *Lac. casei* spp. *casei* (1:1:1)} were used 1% and added at 30°C, Add rennet 0.03 g/l milk after completely blending, and then wait 30 minutes for the acid to develop. After coagulation within 45 min, The whey was then drained to the level of the curd, which had an acidity of 0.14%, after the curd had been sliced into small cubes and the temperature elevated to 45°C in 15 minutes. After cooling the curd, 2 kilogramme of salt was added for every 100 kg of milk. For the first two hours, the mould and press were loaded with 160 lbs. Overnight, the weight was increased to 1000 lbs for pressing. Dry salt was then applied to both sides of the cheese wheel to finish drying it. The procedure of salting certain sites took 12 days to complete. Keep portion without any additive as control samples, and then adding both CE 100mg/1kg and SS 1.5g/1kg curd cheese for the two treatments T₁ and T₂; respectively following a thorough salting procedure, cheese wheels received T3 treatment, which painted clove extract on the surface. All treatments make ripe at 15±2°C and about 85% relative humidity, and stored for 3 months, cheese wheels not waxed. Samples were taken initially at fresh and after 1st, 2nd and 3rd months for analysis.

Antimicrobial activity of Clove methanolic extract:

Determination of antifungal activities of Clove methanolic extract:

Potato dextrose agar (PDA) media (200 g of potato, 20 g of dextrose and 20g of agar) was thawed at 45°C and poured onto 9 cm Petri dishes. After cooling at room

temperature, the dishes were fertilized with uniform discs of the fungus. Two tested fungi genus; AF and AN against the tested Methanolic extract 1 mg/10 ml di-methylsulphoxid (DMSO). The experiment was made in triplicate for each fraction. The dishes were incubated for 7 days at 25±3°C, then, the inhibition zone was measured as mentioned by McKee et al. (1990).

Determination of antibacterial activities of Clove methanolic extract:

The sample was extracted from Clove with methanol and then the solvent was removed. Methanolic extract 1 mg/10 ml di-methylsulphoxid (DMSO) was added to the sterilized, cooled PDA-medium. In addition, the inoculation with *E. coli* and salmonella were done and all dishes were incubated at 37°C. After 2 days the dishes were examined and the growth was determined with compared to control (Fardiaz, 1989).

Qualitative examination of secondary metabolites of Clove methanolic extract:

Secondary metabolites such tannins and saponins in the Clove methanolic extracts were determined as described by Madankumar and Pari (2020).

Quantitative determination of secondary metabolites of Clove methanolic extract:

Secondary metabolites such as tannins and saponins in the Clove methanolic extract were determined as described in literatures (Zheng and He, 2014; Makkar et al., 2008).

Extraction and determination of total phenolic compounds (TPCs):

The total phenolic compounds were extracted from sample (0.5 g) by refluxing with 30 ml of methanol containing 1.0% HCl for 10 min and centrifugation at 5000 rpm for 10 min. Using a standard method developed by Singleton and Rossi (1965), the content of all phenolic components in the methanolic extracts was quantified as gallic acid equivalents.

Microbiological examination of Ras cheese:

Using the "poured dishes" technique, all cheese samples were serially diluted in one ml and inoculated into a dish containing potato dextrose agar (PDA) media (three replicates). Each plate received approximately 50 cc of PDA media at about 50°C, which was then properly mixed and allowed to solidify. The dishes were incubated for 5 days at 25°C. Each dish's formed colonies were counted after the incubation time. According to APHA (1998), the total bacterial count of Ras cheese was calculated using nutritional agar media (Difco, 2009), and the mean count of plates was recorded. It comprises of (g/L):

Beef extract 3 g; peptone 5 g; agar 15 g; distilled water 1 L and sterilized by autoclaving at 121°C for 15 min. The pH was adjusted to 6.8, dishes were incubated at 37°C for 48 h, before counting.

Coliform of cheese samples were determined according to American public Health Association (1992), using VRBA media, its consists of (g/L):

crystal violet (0.002 g), neutral red (0.03 g), glucose (10 g), peptone (7 g), sodium chloride (5 g), bile salts "No. 3" (1.5 g), agar (12 g), and purified water (1000 ml). The final pH was adjusted to 7.0 ± 0.2. All ingredients were allowed to soak for 15 min, and then boiled with shaking until completely dissolved. The suspension was autoclaved

at 121°C for 15 minutes to sterilise it. The dishes were incubated at 37°C for 48 hours after being poured.

RESULTS AND DISCUSSION

Yield of methanolic extracted from Clove:

According to Table 1, data received indicated that a high methanolic extract yield (25%) was recorded from the Clove. Our findings are fairly consistent with Rungnapa's (2019) and his colleagues who found that the aqueous and methanolic extract of dried Clove bud (20 g) were 23 and 34%; respectively.

Table 1. Yield of methanolic extracted from Clove.

Yield of methanolic extract (%)	25%
---------------------------------	-----

Qualitative examination of the secondary metabolites of Clove methanolic extract:

The Clove methanolic extract was examined for qualitative detection of secondary metabolites, and Table 2 displays the outcomes. It could be noticing that, the Clove contains three different groups of secondary metabolites including phenolic compounds, tannins and saponin.

Table 2. Screening of secondary metabolites in the Clove methanolic extract.

Sample	Total phenolic	Tannins	Total saponin
Clove	+	+	+

Quantitative determination of the secondary metabolites in the Clove methanolic extract:

Total Phenolic compounds, tannins and saponins in Clove methanolic extract were determined and the results are given in Fig. (1). each gram of the Clove methanolic extract was found to contain appreciable amounts of TPCs (240.25 ± 0.75 mg) and tannins (56.5 ± 0.56 mg) in addition to saponins (40.25 ± 1.67 mg). These substances, which are abundantly found in the plant world and have been reported to be effective against a variety of species, aid in the defence against predators and pathogens (Gawri and Upadhyay, 2012).

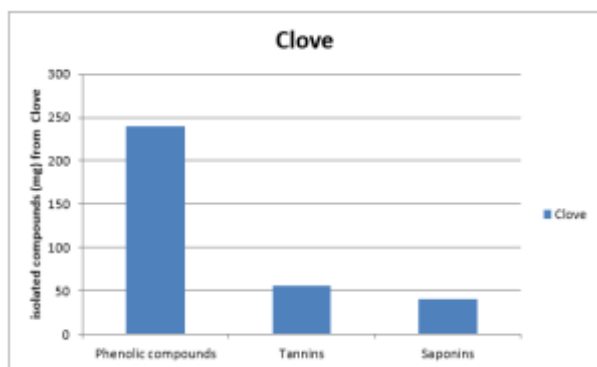


Figure 1. Levels of (TPCs), tannins and saponins in the Clove methanolic extract mean \pm SEM (n= 4)

Effect of Clove methanolic extract on growth of *Aspergillus flavus* (AF) and *Aspergillus Niger* (AN):

The impact of methanolic clove extract on fungal growth namely AF and AN are given in Fig 2. Our results showed existence of inhibition zones with different Clove methanolic extents and the higher concentrations led to wider zones. In addition, the data revealed that the growth of AF is less resistant than AN with another words the cells of AF is more sensitive. Similar studies were carried out by

Rungnapa *et al.* (2019) sought to examine the antifungal effects of betel leaf and clove bud aqueous and methanolic extracts against AF and to determine the viability of using the plant extract to prevent the growth of fungi while storing dried chili. The capability of antifungal aqueous and methanolic extracts of Clove bud and betel leaf showed a dose dependent manner, which methanolic extracts of both plants exerted 2-5 times higher antifungal activity against AF TISTR 3151 in PDA as compared with the aqueous extracts. Moreover, Yassin *et al* (2020) found that Clove ethyl acetate extract exhibited the highest antifungal activity against *C. tropicalis*, *C. albicans* and *C. glabrata*.

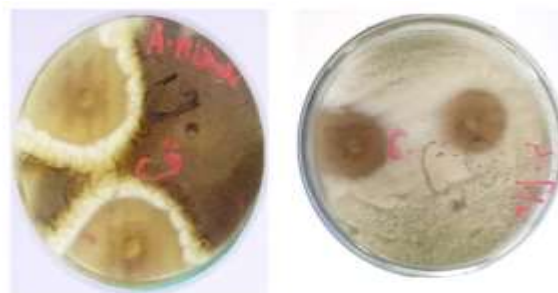


Fig. 2. Effect of Clove methanolic extract on fungal growth of AF and AN.

When used against *C. tropicalis*, *C. albicans*, and *C. guilliermondii*, clove oil was said to have the strongest antifungal effects (Kumar *et al.*, 2012). Strong antifungal activity was also demonstrated by encapsulated clove oil against *Fusarium oxysporum* (Estrada-Cano *et al.*, 2017) at a dosage over 0.070%, and antimicrobial effects on meat products (Kaur and Kaushal, 2019).

Effect of Clove methanolic extract on *E. coli* and *Salmonella* growth:

Results of effect Clove methanolic extracted on growth of *E. coli* and *Salmonella* are given in Fig. 3 showed that the methanolic extract of cloves revealed positive results on inhibition *E. coli* growth compared to *Salmonella* for antibacterial activity. Additionally, it is clear from the findings that when extract concentration grew, the diameter of inhibition also did so. The findings of the current study concur with those of Saeed *et al* (2013), they found that clove methanolic extract has good antibacterial action against *E. coli* utilising aqueous and methanol extracts. The strong antibacterial activity of clove extracts in ethanol and methanol against *Pseudomonas aeruginosa* and *E. coli* has been documented (Garba *et al.*, 2019).



Fig .3. Effect of Clove methanolic extract on bacterial growth of on *E. coli* and *Salmonella*.

Clove methanolic extract was demonstrated to have a higher inhibitory effect than pure eugenol at the same concentration on five bacteria responsible for urinary tract infections. This inhibition was broad spectrum (Gram-positive and Gram-negative) (Rosarior et al., 2021). Methanol extract has been shown to have antibacterial efficacy against *Klebsiella pneumonia* (Hemalatha et al., 2016).

The microbiological properties of Ras cheese samples during storage:

The information shown in Table 3 evidenced this, samples of Ras cheese treated with (Clove methanolic extract & sodium sorbate) and untreated (control) for

storage periods up to three months had different counts of TBC and yeast & fungal.

The findings demonstrated that, counts of TBC and yeast & fungal were quite high in control samples in all treatments, the counts of yeast and fungal of fresh samples were lower in T1 and T2 than that of control samples and T3, with counts of 2.74 & 3.10 and 4.05 & 4.05 Log cfu; respectively in samples taken from the middle. The TBC of cheese taken from exterior and inside of fresh cheese for C, T1, T2 and T3 were (4.01 & 5.91), (4.01 & 5.84), (3.74 & 4.11) and (4.00 & 5.21) Log cfu/g; respectively. The counts of surface lower than that of middle in all treatments. The surface of control samples had not any counts of yeast & fungal in all treatments.

Table 3 Microbiological analysis of Ras cheese wheel samples from both treated and untreated cheese during storage times of up to three months.

Parameters	Storage periods (Month)	C		T1		T2		T3	
		Ex **	In ***	Ex	In	Ex	In	Ex	In
Yeast & Fungal (Log cfu)	Fresh	ND*	4.05	ND	2.74	ND	3.10	ND	3.05
	1	3.53	4.72	2.53	2.62	2.12	2.64	2.29	3.72
	2	3.65	4.78	2.28	2.33	2.11	2.61	2.25	3.17
	3	3.71	4.84	2.23	2.12	2.03	2.04	2.21	3.31
TBC**** (Log cfu)	Fresh	4.01	5.91	4.01	5.84	3.74	4.11	4.00	5.21
	1	5.12	6.01	3.77	5.77	3.21	3.87	3.84	5.54
	2	5.12	6.11	3.44	5.61	3.11	3.84	3.53	5.64
	3	5.00	6.11	3.12	5.57	2.51	3.71	3.11	5.60
Coliform bacteria		ND							
ND*: Not detected,		Ex **: Exterior,	In ***: Inside,	TBC****: Total bacterial count					

Generally, control samples had higher counts of yeast & fungal as well as TBC in surface and middle of cheese than that of other treatments. The counts of yeast & fungal as well as TBC of the surface and middle of control samples increase with increasing of storage periods, while they decrease of cheese treated with Clove methanolic extract & sodium sorbate with increasing storage periods up to end of storage in all treatments. These results due to the inhibition effect Clove methanolic extract & sodium sorbate of growth yeast & fungal as well as TBC on surface and middle Ras cheese wheels during storage periods. These outcomes are consistent with those mentioned by **El-Fadaly et al. (2015)**. Moreover, TBC in surface and middle area of control samples during the ripening stage up to 3rd month were higher than those of other treatments of treated with extract Clove and sodium sorbate T1, T2 and T3 in all treatments. In addition that, not all treatments revealed the presence of coliform bacteria from beginning of manufacture up to the end ripening stage 3rd month. Generally, the results showed that the sodium sorbate had higher antimicrobial effect than that with extract Clove on growth of yeast & fungal and general microorganism especially the responsible for ripening cheese during storage. On the other hand, the controlled hygienic conditions resulted in less fungal growth on Ras cheese wheels surface which inhibited by the extract Clove or sodium sorbate.

CONCLUSION

Our study reported that, the first in vitro test there was antibacterial activity of extract Clove on growing pathogens microorganism. Moreover, there were inhibition effect of surface and middle Ras cheese using Clove methanolic extract & sodium sorbate of growth yeast & fungal as well as TBC.

REFERENCES

- Abdel-Tawab, G. (1963). Manufacturing Ras cheese from pasteurized milk. M.Sc. Thesis, Fac. Agric. Ain Shames Univ.
- American Public Health Association "APHA" (1992). Standard methods for the examination of dairy products. INC, 16th Ed., Am. Publ. Health Associate, Washington, D.C.
- APHA, American Public Health Association (1998). Standard Methods for the Examination of Water and Wastewater. 20th Ed. APHA, Inc. New York.
- Batiha, G.E.; Alkazmi, L.M.; Wasef, L.G.; Beshbishy, A.M.; Nadwa, E.H. and Rashwan, E.K. (2020). *Syzygium aromaticum* L. (Myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. *Biomolecules*, 10(2-17).
- Batiha, G.E.; Beshbishy, A.A.; Tayebwa, D.S.; Shaheen, M.H.; Yokoyama, N. and Igarashi, I. (2019). Inhibitory effects of *Syzygium aromaticum* and *Camellia sinensis* methanolic extracts on the growth of *Babesia* and *Theileria* parasites. *Ticks Tick. Borne Dis.* 10, 949-958.
- Cortés-Rojas, D.F.; de; C.R. Souza and W.P. Oliveira (2014). Clove (*Syzygium aromaticum*): A precious spice. *Asian Pac. J. Trop. Med.*, 4, 90-96.
- Difco (2009). Manual of Microbiological Culture Media Second Edition. Becton, Dickinson and Company parks, Maryland 21152. U.S A
- El-Fadaly H.A.; M.N.F.; Hamad S.M.L.; El-Kadi and Habib, A.A. (2015). Effect of Clove Oil on Physicochemical and Microbiological Characteristics of Egyptian Ras cheese (Romy) During Storage. *Inter., J. of Food Sci., and Nutr. Engineering*, 5(1): 15-23.

- Estrada, C.; Castro, M.A.; Castellanos, M.L.; Olivas, A.N.; Triana, A.G. and Ochoa, L.H. (2017). Antifungal Activity of Microencapsulated Clove (*Eugenia caryophyllata*) and Mexican Oregano (*Lippia berlandieri*) Essential Oils against *Fusarium oxysporum*. J. Microbiol. Biochem. Tech. 9, 567–571.
- Fardiaz, S. (1989). C.F. Antibacterial Activity Of Leaves Extracts of *Jatropha curcas*, Linn Against *Enterobacter Aerogenes*. International Journal of Scientific Research 3, 1,129-131.
- Garba, L.; Lawan, H.S.; Puma, H.U.; Abdullahi, M.M.; Yusuf, I. and Mukhtar, M.D. (2019). Phytochemical Screening and In Vitro Bacteriostatic Effects of *Syzygium aromaticum* (Clove) Extracts on Clinical Bacterial Isolates. J. Biochem. Microbiol. Biotechnol. 7(1), 5–9.
- Gawri, S and A. Upadhyay (2012). A comparative study on the antimicrobial activity and the presence of phytochemicals in the petioles and callus of *Jatropha curcas* L. Journal of phytology, 4, 18–20.
- Hemalatha, R.; Nivetha, P.; Mohanapriya, C.; Sharmila, G.; Muthukumaran, C. and Gopinath, M. (2016). Phytochemical Composition, GC-MS Analysis, In Vitro Antioxidant and Antibacterial Potential of Clove Flower Bud (*Eugenia Caryophyllus*) Methanolic Extract. J. Food Sci. Technol. 53(2), 1189-1198.
- Kaur, K. and S. Kaushal (2019). Phytochemistry and Pharmacological Aspects of *Syzygium aromaticum*: A Review. J. Pharmacognosy Phytochemistry 8 (1), 398-406.
- Kumar, A.; Thakur, S.; Thakur, V.C.; Kumar, A.; Patil, S. and Vohra, M.P. (2012). Antifungal Activity of Some Natural Essential Oils against *Candida* Species Isolated from Blood Stream Infection. J. Krishna Inst. Med. Sci. Univ. 1, 61-66.
- Madankumar, D. and S. Pari (2020). Phytochemical activity and quantitative analysis of senna alata and senna hirusta medicinal plants. Journal of shanghai jiaotong university (16), 336-343.
- Makkar, H.P.; K. Becker and G. Francis (2008). Protein concentrate from *Jatropha curcas* L. screw-pressed seed cake and toxic and anti-nutritional factors in protein concentrate, Journal of the Science of Food and Agriculture. 8(8), 1542-1548.
- McKee, E.E.; B.L. Grier; G.S. Thompson and J.D. McCourt (1990). Isolation and incubation conditions to study heart mitochondrial protein synthesis. 258(1), 492-502.
- Mohsen A. Z.; El-Hawary, M.Y. and Sadik, A.A. (2012). Impact of some inhibitors on fungi growth during ras Cheese storage. Mansoura J. Food and Dairy Science. 3(4):207-223.
- Rosario, V.L.; Lim, P.S.; Wong, W.K.; Yue, C.S.; Yam, H.C. and Tan, S.A. (2021). Antioxidant-rich Clove Extract, a strong Antimicrobial Agent against Urinary Tract Infections-Causing Bacteria In Vitro. Tlsr 32(2), 45-63.
- Runghana, B.; Sriyaha, F. and Kamnon, G. (2019). Antifungal activity of Clove and betel extract against *Aspergillus flavus* during dried chili storage. Asia Pacific Environmental and Occupational Health Journal, 5(2): 17-22.
- Saeed, M.; Nadeem, M.; Khan, M.R.; Shabbir, M.A.; Shehzad, A. and Amir, R.M. (2013). Antimicrobial activity of *Syzygium aromaticum* extracts against food spoilage bacteria. African journal of microbiology research, 7(41), 4848-4856.
- Singleton, V.L. and J.A. Rossi (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of enology and viticulture, 16(3), 144-158.
- Wilson, R.M.; Runciman, W.B.; Gibberd, R.W.; Harrison, B.T.; Newby, L. and Hamilton, J.D. (1995). The quality in Australian health care study. *Medical journal of Australia*, 163(9), 458-471.
- Yassin, M.T.; A.A. Al-Askar; A.A. Mostafa and M.A. El-Sheikh (2020). Bioactivity of *Syzygium aromaticum* (L.) Merr. & L.M. Perry Extracts as Potential Antimicrobial and Anticancer Agents. J. King Saud Univ. Sci. 32(8), 3273-3278.
- Zheng, J. and L. He (2014). Surface-enhanced raman spectroscopy for the chemical analysis of food. Comprehensive reviews in food science and food safety (13) 317–328.

النشاط المضاد للميكروبات لمستخلص القرنفل الميثانولي واختبار القوة الحافظة في الجبن الراس

علي حسن علي عثمان¹، علي ابراهيم علي منصور¹ و عبدالراضي محمد أمين أحمد²

¹قسم الألبان، كلية الزراعة، جامعة الأزهر، فرع أسبوط، مصر.
²قسم المحاصيل - كيمياء حيوية، كلية الزراعة، جامعة الأزهر، فرع أسبوط، مصر.

المخلص

يهدف البحث إلى دراسة تقييم النشاط الميكروبي لـ (مستخلص القرنفل الميثانولي) لبعض الميكروبات التي تنمو على الجبن الراس بعد الصناعة وخلال فترة التسوية فترة ما قبل التسويق والتي تؤدي إلى فساد المنتج وتؤدي الحالة الاقتصادية وإيضاً الإضرار بالحالة الصحية للمستهلك، ومقارنة النشاط المضاد لهذا المستخلص بأحد المركبات الكيميائية المستخدمة لهذا الغرض في هذا المنتج وهو (سوربات الصوديوم) داخل الحد المسموح به. قمنا أولاً بدراسة مدى تأثير مستخلص القرنفل بتركيز (10 ملجم/100 مل) على نمو نوعين من الفطريات هما (*Aspergillus niger*, *Aspergillus flavus*)، بالإضافة إلى نوعين من البكتيريا هما (*Salmonella*, *E. coli*) كان هناك تثبيط لنمو هذه الميكروبات مع اختلاف حجم التثبيط على بيئة نموها داخل الأطباق وكان التركيز المستخدم هو الأمثل لتثبيط هذه الميكروبات دون التأثير على الفلورا الطبيعية والمسؤولة عن تسوية الجبن. قمنا في الخطوة الثانية بدراسة نفس التركيز من مستخلص القرنفل بالإضافة إلى سوربات الصوديوم بتركيز 1.5 جم/100 مل خثرة جبن بعد التعبئة وقيل عملية التشكيل للخرثرة فكان هناك ثلاث معاملات إضافة إلى الكنترول على النحو التالي: الكنترول (C) بدون أي إضافات و T₁، T₂ بإضافة مستخلص القرنفل والسوربات على التوالي. وأخيراً T₃ طلاء المستخلص على السطح الخارجي لقرص الجبن بشكل كامل بعد الانتهاء من التمليح الجاف مباشرة 12 يوم. تم أخذ عينات لتقييمها ميكروبيولوجياً للجبن الطازج قبل التخزين للتسوية وعلى 1، 2، 3 أشهر من السطح ومن وسط القرص وقدر العد الكلي للبكتيريا والفطريات والخمائر في كل العينات. أظهرت النتائج أنه لم يستدل على بكتيريا القولون في كل المعاملات وخلال فترات التخزين المختلفة. وجد أيضاً تأثير للمادة الحافظة السوربات على المحتوى الميكروبي عموماً بشكل أكبر وخاصة المحتوى الميكروبي المسؤول عن تسوية الجبن مقارنة بتأثير مستخلص القرنفل الذي أعطى قدرة على التثبيط دون التأثير على عملية التسوية في المنتج بالإضافة إلى أنه مصدر آمن صحياً دون آثار سلبية على المستهلك.