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Lycopene and pine bark extract as nitrite replacers in Egyptian basterma

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ABSTRACT

The goal of this work was design to study the influence of lycopene and pine bark extracts as natural food additives as alternatives to nitrite, which poses serious health risks to consumers of processed foods containing this substance. These extracts were also found to be effective antimicrobial and antioxidant agents, particularly when used as an example in the current study for manufactured basterma. For this, Egyptian basterma were inoculated with 10^4 CFU g-1 of Staph. aureus and E. coli into raw meat separately, and then treated with a 1% concentration of lycopene and a 1% of pine bark extract. For the duration of the storage period, basterma was kept at room temperature for microbiological assessment and sensory analysis. Staph. aureus and E. coli levels decreased by 4 log and 2 log, respectively, on the fourteenth day. Additionally, Egyptian basterma sensory evaluation yielded a good, acceptable result. Therefore, as compared to the effects of nitrite, lycopene, and pine bark extract, they show strong antibacterial activity during product preservation. The findings provided here may indicate that lycopene and pine bark extracts offer protection against *Staph. aureus* and *E. coli*, which means they have the potential to replace nitrite as a natural preservative in the food production.

INTRODUCTION:

Being highly nutritious and having a suitable pH, meat and its byproducts, like basterma, are thought to be ideal medium for bacterial development and spoilage. According to these data, *Salmonella* enterica, *Staph. aureus*, *Escherichia coli*, and *Bacillus cereus* are the most common bacteria linked to food poisoning in

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developing nations (**Wannes 2010; Giacometti 2018**). Food poisoning and gastroenteritis in humans can be caused by the spherical, Gram-positive bacteria *Staph. aureus*, which is capable of producing extremely heat-stable enterotoxins **(Kotler and Sordillo 2010)**. While food-borne illnesses can be contracted from contaminated food or unsanitary environments, Escherichia coli is categorized as a gram-negative member of the Enterobacteriaceae family of bacteria that can cause a variety of nosocomial infections **(Makharita et al. 2020**). Meat and meat products' fat and protein can degrade due to microbial spoilage, autolytic enzymes, and lipid oxidation, which can have a significant negative effect on the environment and economy (**Jayasena & Jo 2013**). Meat's nutritional value is diminished by oxidative processes, which can result in a variety of hazardous substances that can pose a number of risks and lower the meat's sensory quality. These days, adding antibacterial and antioxidant compounds to meat and meat products is the meat industry's primary tactic to prevent lipid oxidation and microbial contamination (**Domínguez et al. 2019**). Furthermore, various additives, like nitrite, could be added to the meat to keep it at an appropriate hue. In addition to giving meat its distinctive reddish-pink color and flavor, nitrite also has antibacterial and antioxidant properties that it can use either on its own or in conjunction with other additives to achieve (**Sindelar & Milkowski 2011**). Because N-nitrous chemicals, like nitrosamines, are created during the meat processing process, the World Health Organization (WHO) has declared processed meats to be carcinogenic to consumers (**WHO 2015)**. Nitrite is also categorized as an oxidizing solid (hazard category 3). Therefore, various regulatory bodies have limited the use of nitrite in meat products (**FDA 2020a& b)** Finding ways to reduce the consumption of synthetic nitrite is a problem facing the meat business. Development of natural source alternatives and alternative preservation methods that are thought to be relatively healthier is of great interest. As a result, using natural plant extract as food additives is a trend in the food sector toward sustainable growth. Plant-based food additives have gained a lot of attention because to their benefits over synthetic ones, including environmental protection, health benefits, and green safety. These organic ingredients, which are found in plant roots, leaves, flowers, bark, and extract, give products their inherent hues and antibacterial qualities. Although natural pigments are carcinogenic, people are choosing them over synthetic colorants, which can be utilized in some applications, because they are more environmentally friendly (**Sivakumar et al. 2011**). The pigments known as carotenoids, which are found in large quantities in nature, give fruits and vegetables their appealing color (**Maiani et al. 2009; Doménech-Asensi et al. 2013**). Lycopene, one of these naturally occurring antioxidant carotenoids, has become important in halting the oxidation of lipids, oils, and food. The natural non-provitamin bioactive carotenoid lycopene is derived from a variety of fruits and vegetables, including watermelons, papaya sand, and tomatoes It gives a variety of fruits and vegetables their red to pink hue (**Chen et al. 2019**). Furthermore, lycopene has been linked to a wide range of biological processes. It possesses potent antioxidant, antibacterial, anti-inflammatory, and anti-proliferative qualities. Furthermore, the food industry today takes into account a number of bioactive components that have been isolated from various plant species. These compounds are primarily valued for their antibacterial and antioxidant properties; tannins, phenolic compounds, and other chemicals give these plant extracts their strength. These compounds are typically found in the bark, roots, leaves, and shoots of plants. The bark of Pinus pinaster L., a byproduct of the timber industry, is extremely rich in phenolic compounds, primarily flavonoids (such as taxifolin) and phenolic acids (such as ferulic, cinnamic, and ellagic acids). flavonols, such as narginin, and flavonoids, such as taxifolin (**Ferreira-Santos et al. 2020**). Pine bark extract can be used as a nutraceutical preparation for supplement formulation because it has been shown to have positive biological properties, such as anti-inflammatory, antiviral, anticancer, antibacterial, and antioxidant (**Gernandt et al. 2005**). In this way, the food industry might find the pine bark extract to be quite appealing.

The aim of this study is to evaluate the bactericidal and sensory acceptability of Egyptian basterma that has been prepared using a combination of two natural additives (lycopene and pine bark extract) in place of nitrite during storage.

MATERIAL AND METHODS

The experiment was performed at the Reference Lab for Safety Analysis of Food of Animal Origin, AHRI, ARC, Giza, Egypt.

Microbial Analysis on Egyptian Basterma:

Bacterial Strains Preparation:

The standard bacterial strains used were MRSA (ATCC 43300) and *E. coli* (ATCC 25922), Prior to use, all strains were cultivated in trypticase soy agar at 37 °C. The plates incubated overnight in an ambient atmosphere. Bacterial colonies were moved directly from the agar to the physiological NaCl solution, and their concentration was adjusted to 0.5 McFarland using a photometer (Gene-Trak Systems, Hopkinton, MA, USA). This was equivalent to the concentration of $1.5x10^8$ CFU mL -1, which was further diluted to correspond to the concentrations of 1.5×10^4 CFU mL -1

Raw materials:

The ingredients for basterma, which include fresh beef steak, seasoning mixture, coriander, pepper, and garlic, were bought from a local market in Cairo, Egypt. While pine bark extract and lycopene extract were bought from the MAKIN Company in the Egyptian province of Giza

Manufacturing of Egyptian basterma:

The Egyptian Organization for Standardization and Quality Control's (ES 4177 (2005)) specifications were followed in the manufacturing of the basterma

DESIGN FOR EXPERIMENT

Under complete aseptic conditions, recently beef muscles that were delivered to the lab in less than an hour from a nearby shop. The meat samples were divided into six groups (1 Kg each) as follows: Treatment 1(T1): Basterma contained nitrite only: Treatment 2 (T2): Basterma with 1% of both lycopene and pine bark extracts only. T1 and T2 were used for sensory evaluation after ripening of basterma on 14, 21, 30, 60 and 90 days. The other four groups; (T3, T4) were contaminated with *Staph. aureus* (4 log_{10} cfu/g) with the addition of nitrite (100 ppm) For T3, while T4 contained lycopene 1% and pine bark 1%. As for T5 & T6, they were treated as the previous two groups, with the replacement of *Staph. aureus* with *Escherichia coli.* ISO 6887-2:2017 was used in the preparation of the test samples, the initial suspension, and the decimal dilutions for the microbiological analysis. The populations of Coagulase positive Staph. aureus (ISO 6888 -1: 2021) and E. coli (ISO 16649-2:2001 part 2) were then determined by plating and incubating the samples over 0, 7, 14, 21, 30, 60, and 90 days of storage. The analyses were carried out three times.

Sensory Evaluation:

On 14, 21, 30, 60, and 90 days, various trained judges evaluated the various Egyptian basterma's sensory characteristics. The eleven judges on the panel were drawn from a variety of staff members, including men and women of various ages, in accordance with the methodology described by **Trindade et al. (2009).** The performance on the sensory characteristic scales' ranges from 1 (very poor) to 10 (outstanding).

Analytical statistics:

Using an average of three replications, the collected results were reported as mean ± standard deviation (SD). The T-test was employed to assess the differences in means. The statistical analysis program (SPSS Statistics Window version 20, Chicago, USA) was used to handle the data. The significance threshold was established at $P < .05$.

RESULTS

Table 1. Mean organoleptic scores for basterma formulated with nitrite (T1) and basterma treated with a mixture of 1% lycopene and 1% pine bark extracts (T2).

Groups		Day 14	Day 21	Day 30	Day 60	Day 90
appearance	T_1	9.5 ±0.5	9.2 ±0.3	9.0 ±0.0	8.3 ±0.6	7.7 ±0.6
	T_2	10.0 ±0.0	9.2 ±0.3	9.0 ±0.0	8.5 ±0.0	7.7±0.6
color	T_1	10.0 ±0.0	9.5±0.0	9.0 ±0.0	8.3 ±0.6	7.3 ±0.6
	T_2	9.7 ±0.6	9.3 ±0.3	9.0 ±0.0	8.2±0.3	7.3±0.6
Taste	T_1	10.0 ±0.0	9.0 ±0.0	8.5 ±0.5	7.5±0.5	7.0 ±0.0
	T_2	9.0 ±0.0	8.5 ±0.5	8.2 ±0.3	7.5 ±0.0	7.0 ±0.0
Odor	T_1	9.3 ±0.6	9.3 ±0.3	8.3 ±0.6	7.5 ±0.0	7.0±0.0
	T_2	9.7 ±0.6	9.0 ±0.0	8.0 ±0.0	7.8 ±0.3	7.2 ±0.3
Texture	T_1	9.7 ±0.6	9.0 ±0.0	8.3 ±0.6	7.5 ±0.5	6.8 ±0.3
	T_2	9.0 ±0.0	8.5 ±0.5	8.3±0.3	7.3 ±0.3	6.5 ±0.0
OA	T_1	9.0 ±0.0	8.5 ±0.5	7.8 ±0.3	7.3 ±0.3	6.7 ±0.6
	T_2	8.3 ±0.6	8.3 ±0.6	7.7 ±0.3	7.2 ±0.3	6.8 ±0.3

There are no significance differences (P>0.05) between the two treatments in any parameter in the same day.

Table 2. *Staph. aureus* and *E. coli* counts (mean count ± SD log cfu/g) in Egyptian basterma treated with mixture of lycopene and pine bark extract.

	S. aureus		*E. coli*	
	Control* (T3)	Treated* (T4)	Control* (T5)	Treated* (T6)
Day zero	4.0^{a} ±0.05	4.6^{b}±0.15	4.1^{a}±0.10	4.6^{a}±0.51
Day 7	2.3^{a} ±0.35	1.8^{a}±0.13	3.7^{a}±0.12	3.1^{a}±0.93
Day 14	2.3 ±0.24	<1	2.7^{a}±0.24	2.0^{b} ±0.03
Day 21	2.0 ±0.04	<1	2.5^{a}±0.20	2.0^{b}±0.06
Day 28	3.4 ±0.39	<1	3.6^{a}±0.58	2.0^{b}±0.05
Day 60	3.8 ±0.20	<1	4.1^{a}±0.17	1.9^{b}±0.05
Day 90	4.0 ±0.07	<1	4.4^{a}±0.43	1.8^{b}±0.05

* There are significances differences (P<0.05) between means having different superscripted letters in the same raw for control and treated samples and for each organism separately
* Control (T3, T5 basterma with nitrite). Treated (T4, T6 basterma contained mixture of lycopene and pine bark extract).

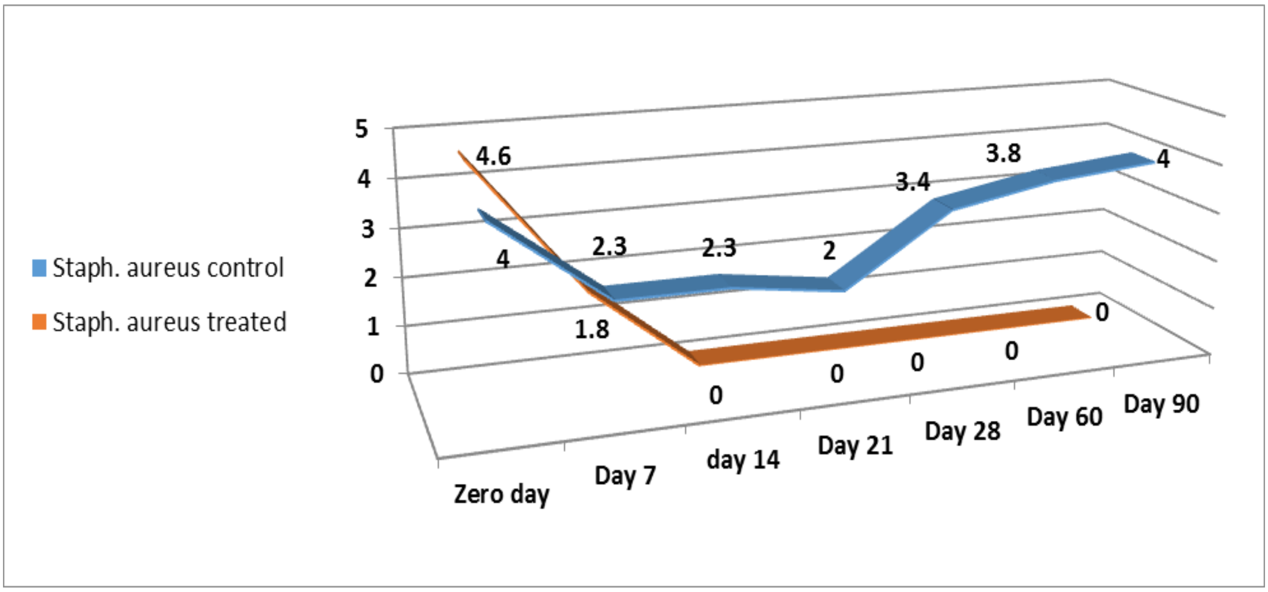

Fig. (1). Treated and control samples contaminated with *Staph. aureus*

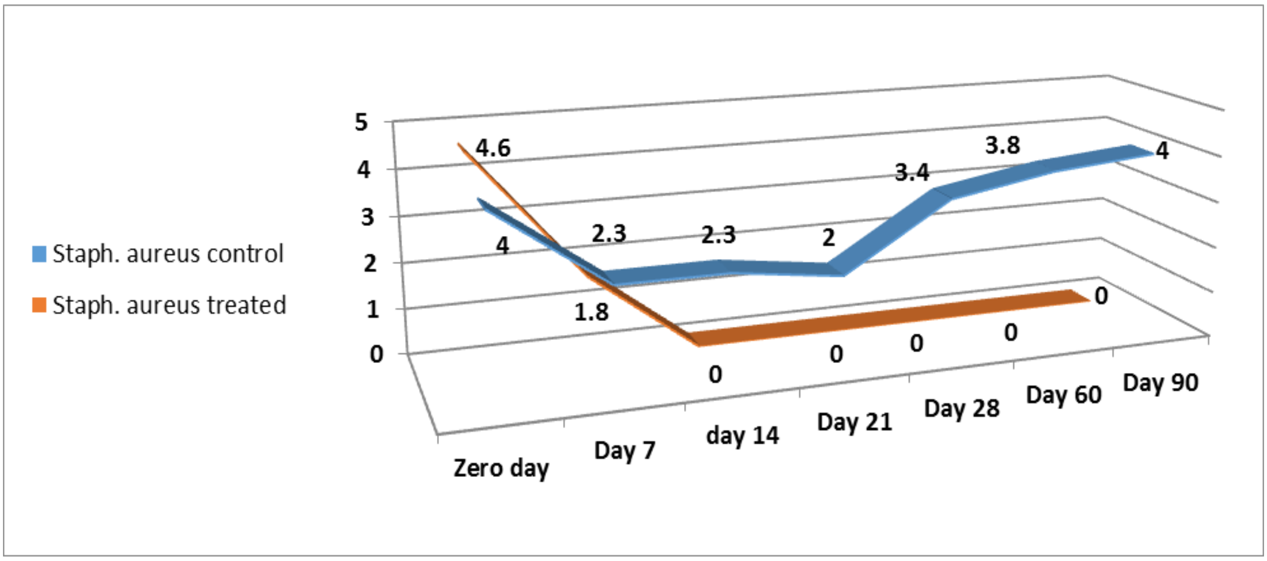

Fig. (2) Control and treated samples contaminated with *E. coli*

DISCUSSION

According to the present results in Table (1), it was observed that addition of a mixture of 1% pine bark and 1% natural lycopene extracts achieved a high acceptable score for all parameters. That was in agreement with results mentioned by **Darwish et al. 2019, as t**hey manufacture beef patties with 1% artificial color and varying concentrations of natural lycopene pigment (0, 0.5, 1.0, and 1.5%) and found that addition of 1% natural lycopene pigment yielded the best results across all other treatments. The results obtained are in line with the findings of **García et al. (2009)**, who discovered that adding 4.5% of tomato peels to beef burgers as a source of fiber and lycopene yielded the greatest overall acceptance score when compared to the non-treated control sample.

Therefore, depending upon the present and earlier results, the addition of 1% natural lycopene pigment and 1% pine bark extracts to Egyptian basterma improved its sensory parameters as the antioxidant effect of lycopene prevent the formation of free radicals which causes tissue damage through scavenging them, or by promoting their decomposition.

Alkaloids, tannins, saponins, flavonoids, and steroids are examples of plant phytochemical substances that are used in food preservation. These chemicals have been shown to be physiologically active and thus partially responsible for the antibacterial actions of plants. Since it has the ability to physically and chemically quench free radicals, it is the most important carotenoid that quenches singlet oxygen, according to **Stahl and Sies (1996)**. Due to its distinct molecular characteristics, lycopene may be able to shield certain cellular constituents from harm caused by extremely reactive oxygen species.

Natural preservatives are desperately needed, and a lot of research has been achieved to obtain novel naturally occurring antibacterial compounds, including plant extracts, for the safe preservation of food **(Mostafa et al. 2018).** For that reason, the present study, highlighted the antibacterial activity of a mixture containing two natural plant preservatives (lycopene 1% and pine bark extract 1%) against two food poisoning represents Gram-positive *Staph. aureus* (ATCC® 6538) and Gram-negative *E. coli* (ATCC® 25922), experimentally inoculated in Egyptian basterma. According to the aforementioned results in Table (2) and Fig. (1&2), natural plant preservatives exhibited potent antibacterial activities from zero day of incubation. There were significant differences (P<0.05) in the number of *Staph. aureus* bacteria in treated group in comparison with the control group (Fig. 1). The values obtained after 7 days were 1.8±0.13 CFU/g for treated group and 2.3±0.35 CFU gm −1 for control one. Additionally, there were no detectable bacteria in the treated groups with the extracts beginning from the 14th day of storage till the end of the total experimental days (90) even though the initial count of *Staph. aureus* was significantly reduced from 4.0-log CFU gm −1 to <1 log_{10} CFU/g. Concerning the lycopene and pine bark extracts' inhibitory actions on Gram-negative *E. coli* as shown in table (1 & Fig 2), there were no significant differences (P> 0.05) on days zero as well as the 7th day of storage. While from day 14 of preservation, clear significance differences (P<0.05) were observed between treated and control groups as the recorded values of *E. coli* were 2.7±0.24 for control and 2.0±0.00 for treated group; while the mean values of control and treated samples for *E. coli* recorded 2.5±0.20 & 2.0±0.06; 3.6±0.58 & 2.0±0.05; 4.1±0.17 & 1.9±0.05 and finally 4.4±0.43 & 1.8±0.00 at 21, 28, 60 and 90 experimental says, respectively. From the obtained results, it could be concluded that pine bark extract and lycopene show potent bactericidal activity against Gram-positive bacteria represented by *Staph. aureus* and bacteriostatic effect against Gram-negative represented in the present study by *E. coli*. This might be related to how Gram-positive and Gram-negative bacteria differ in their bacterial cell structures, as described by **Breijyeh et al. (2020)** wherein Gram-negative bacteria have a more sophisticated, yet thinner, cell wall than Gram-positive bacteria. It has been proposed that there are variations between Gram-positive and Gram-negative bacteria's antibacterial activity toward specific antimicrobial drugs., thereby reducing the killing efficiency of the antimicrobial compound. The data presented here are supported by many assay repetitions. According to **Nisca et al. (2021**), pine extracts more readily inactivate Gram-positive bacteria than Gram-negative ones. In fact, there haven't been many investigations on the inhibitory impact of pine bark extract on E. coli. Some have emphasized the antibacterial activity of phenolic-rich extracts from pine bark against E. Coli and Staph. aureus. **Ferreira -Santos et al. (2020)** have recently shown that 50 mg mL−1 aqueous and ethanolic extracts obtained from P. pinaster bark have an inhibitory effect against Staph. aureus (ATCC® 6538) when treated for 24 hours; however, they do not prevent the development of E. coli (ATCC® 9337). On the other hand, **Torras et al. (2005)** 20 µg mL−1 pine bark has been shown to have bacteriostatic activity against E. coli (ATCC® 9337) and Staph. aureus (ATCC® 6538), but no bactericidal impact. With a focus on proanthocyanins and catechins, these research groups have all hypothesized that phenolic chemicals could play a role in the antibacterial activity of pine bark extracts (**Ajiboye 2016; Mayer 2008**). Furthermore, it is appropriate to raise the possibility of complexation between phenolic compounds and bacterial proteins via hydrophobic and hydrogen bond interactions (**Torras et al., 2005**) and/or synergistic interactions between phenolic compounds and other extract constituents. Additionally, quinic acid, a component of polar extracts made from pine bark, might play a significant part in the antibacterial activity of these extracts (**Bai et al. 2018**). have recently shown that quinic acid has an antibacterial impact on 10 food-borne pathogens, including Staph. aureus ATCC 6538 (minimum inhibitory concentration of 2.5 mg mL^{-1}), which can harm the Staph. aureus cell membrane's normal function. Additionally, this chemical has demonstrated growth inhibition against E. coli ATCC® 11229 (minimum inhibitory concentration: 5 mg mL^{-1}). Furthermore, lycopene's antibacterial action has been demonstrated in multiple investigations to work on the tested microorganisms. These could be brought on by complex ingredients, since polyphenols, flavonoids, and vitamin E, for example, have antibacterial properties (**Hussain et al. 2008**). The combined antibacterial properties of pine bark and naturally occurring compounds in lycopene may have contributed to the bactericidal activity. Cell lysis could result from the interaction increasing the permeability of the cell membrane. Based on the encouraging findings of this investigation, lycopene and pine bark extracts have strong antibacterial properties.

CONCLUSION

The aforementioned results indicate that the experimental assay demonstrated a noteworthy effect of lycopene and pine bark extract's remarkable biological activities, such as its antioxidant and antibacterial qualities, which have a bactericidal impact on E. coli and Staph. aureus rather than the bacteriostatic activity of other preservatives that only prevent bacterial growth. As a result, this might be a significant new supply of natural antibacterial compounds that are utilized as food preservatives. Since there is insufficient research to support the use of these natural extracts against various pathogenic bacteria and food deterioration agents in meat, Therefore, more research must be conducted using these materials, as well as other materials that may give similar or better results for preserving food, prolonging its shelf life, and, at the same time, being employed as a food preservation agent instead of nitrite.

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