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Antibacterial Efficacy of Chard and Arugula as Natural Nitrite Alternatives against *Staphylococcus aureus*, Listeria monocytogenes and *Clostridium perfringens* in Basterma

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Abstract

THIS study investigated the impact of partial nitrite replacement with natural plant extracts on the sensory quality and microbiological safety of basterma during 90 days of refrigerated storage. Three groups of basterma were evaluated: G1 (control, 100 ppm nitrite), G2 (50 ppm nitrite + 50 ppm arugula extract), and G3 (50 ppm nitrite + 50 ppm chard extract). The other three groups (G4, G5 and G6) were contaminated separately with Staphylococcus aureus, Listeria monocytogenes, and Clostridium perfringens (5log cfu/g) to assess antimicrobial efficacy. Sensory evaluation was conducted at intervals throughout the 90-days storage period G1 consistently maintained high sensory quality throughout the storage period. G2, incorporating arugula extract, showed a moderate decline in sensory attributes but remained generally acceptable up to 49 days. While G3 experienced rapid and severe sensory deterioration, developing strong off-odors and off-flavors, rendering it unfit for consumption by day 60. Microbiologically, all three inoculated pathogens (Staph. aureus, L. monocytogenes, and Clostridium perfringens) below detection limit from all treated samples by the 7th day of storage. Combining sensory and microbiological findings, the basterma samples from all groups were deemed fit for consumption until the 49th day of cold storage. Then, the reappearance of pathogens by Day 60, coupled with significant sensory degradation (mostly in G3), indicated that the products became unfit for consumption by the 60th day of storage. This research affirms that arugula extract is a more promising partial nitrite replacer than chard extract for maintaining basterma quality and safety during extended refrigerated storage.

Keywords: Arugula, Chard, Foodborne pathogens, Nitrite replacers.

Introduction

Meat and meat products are vital to the human diet, providing essential nutrients, high quality protein, and various micronutrients including. B vitamins and minerals [1]. However, concerns about the microbiological quality and safety of these products persist as excessive microbial contamination possess risk to public health and product quality [2–4]. Additionally, the high level of fat, sodium, and certain additives in many meat products challenge a healthy diet [5]. Recent research is exploring substitution of meat with vegetable protein isolates, which can offer comparable functional properties [6, 7].

Basterma is a traditional cured and dried meat product significant in Turkey and Egypt, and popular across various Islamic countries. The Turkish term "bastrma" means "being pressed", reflecting the traditional method of extracting water from the meat [8]. Historically, basterma is made from specific cuts of cattle, buffalo, and camel. The traditional Egyptian production involves a three stage; process:

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curing with salt and potassium nitrate, drying at ambient temperatures and coating with a paste made of garlic, fenugreek, paprika, cumin, and water. This process takes approximately three weeks to one month. Nowadays, much of the basterma production uses imported boneless frozen beef [8]. A notable challenge for the meat industry is reducing reliance of sodium nitrite, leading to the adoption of natural alternatives such as plant extracts (e.g. Chard and arugula) and other natural preservatives. These methods are increasingly seen as healthier and environmentally friendly compared to synthetic preservatives [9, 10].

Sodium nitrite, a common additive in meat, plays several critical roles in meat products, including the inhibition of microbial growth, particularly *Clostridium botulinum* [11, 12], while stabilizing color and flavour. It also acts as a powerful antioxidant by forming a stable complex with Fe (III), preventing oxidative processing [13]. Despite its benefits, sodium nitrites use is debated due to concerns over a potential link between nitrite consumption and carcinogenic nitrosamines [14, 15]. The meat industry often incorporates antibacterial and antioxidant compounds, like nitrite, to combat lipid oxidation and enhance visual appeal [16]. Leafy vegetable has high nitrate levels due to nitrogen fertilizer, with factors like plant genotype and cultivation practices influencing these levels [17, 18]. Arugula is a notable source of nitrate, with about 480 mg/100 grams converting small fraction to nitrite in the body [19]. Dietary nitrates may offer health benefits, including improved blood pressure and cardiovascular health [14].

Meat products are prone to bacterial spoilage, with pathogens like *Salmonella*, *Staphylococcus* aureus and *Escherichia* coli being common culprits in foodborne illnesses, particularly in developing nations. Foodborne illnesses can arise from contaminated foods or unsanitary conditions [20, 21]. The water source used may be the source of these bacteria, which could lead to bacterial resistance in the future. References like this need to be added to fully understand the concept [22, 23].

However, the scientific literature on nitrite replacers in meat products utilizing pre-converted nitrite remains limited. Consequently, the primary objective of this study was to evaluate the weekly effect of chard and arugula as sources of pre-converted nitrite on the sensory stability, microbial control, and shelf-life of processed basterma over a 90-day storage period at 4°C.

Material and Methods

Preparation of reference strains:

For this study, the following standard bacterial strains were employed**:** *Staphylococcus aureus* (ATCC 6538), *Listeria. monocytogenes* (ATCC 13932), and *Clostridium. perfringens* (ATCC 13124), obtained from Animal Health Research Institute, Agriculture Research Center, and prepared according to Clinical and Laboratory Standards Institute [24]. Each strain was cultured overnight on trypticase soy agar at 37°C. From these incubated plates, two to four colonies were aseptically transferred into a physiological NaCl solution. The bacterial suspension's concentration was then standardized to 0.5 McFarland using a photometer (Gene-Trak Systems, Hopkinton, MA, USA), correlating to a concentration of 1.5×10^8 CFU/mL. A subsequent dilution reduced this to the desired working concentration of 1.5×10^5 CFU/mL.

Preparation of experimental ingredients:

Ingredients for basterma were sourced from a local market in Cairo, Egypt, and included 1 kg of fresh beef steak, a seasoning mixture, coriander, pepper, and garlic. Arugula and chard were used as fresh vegetable sources. Based on a prior study, chard contains 1680 mg/kg of fresh vegetable weight [25], while arugula contains 6400 ppm (6400 mg/kg) of fresh vegetable weight [26]. To achieve a target of 50 ppm nitrite in the basterma ingredients for this experiment, approximately 30 g of chard and 8 g of arugula were used per kg of basterma

Manufacturing of Basterma according to the Egyptian Standard (ES No.1042/2005) [27]

Basterma was prepared using whole muscle cuts from specific parts of the cattle carcass, trimmed of tendons and fats, and passed through three main steps:

Salt Curing

The initial step involves generously covering the meat with sodium chloride (NaCl). This initiates osmosis, drawing out moisture from the meat. This significant reduction in water activity is critical for preservation, as it effectively inhibits the proliferation of spoilage bacteria and many pathogenic microorganisms.

Drying and Pressing

Following salting, the meat undergoes drying at ambient temperature. During this phase, it's simultaneously pressed under sufficient pressure for 12 hours to further expel moisture and shape the product.

Paste Coating

Provided a distinctive aroma and flavour, while also offering an additional layer of protection to processed basterma.

Once dried and pressed, the meat is uniformly coated with a specialized paste. This paste is typically composed of 35% garlic, 20% fenugreek (*Trigonella foenum-graecum*), 5-6% red paprika and cumin, and 38-40% water, 100 ppm of nitrite was added to G1 and G4, 50 ppm of Argula+ 50 ppm of nitrite (G2 and G5), and 50 ppm of Chard + 50 ppm of nitrite (G3 and G6) were added to the coating paste.

Final Curing and Storage

The coated meat is then stored at ambient temperature for approximately 21 days to allow for complete curing. This extended storage period enables the flavors to fully meld and the meat to achieve its final desired texture and preservation level.

Experimental Design

During basterma manufacturing, the meat samples were then divided into six 1 kg groups for distinct experimental purposes: Sensory evaluation groups comprised the following three groups, which were primarily used to evaluate sensory parameters illustrated in table (1); Group 1 (G1): Basterma prepared with nitrite only (100 ppm), Group 2 (G2): Basterma containing 50 ppm of both nitrite and arugula vegetable, and Group 3 (G3): Basterma containing 50 ppm each of nitrite and chard vegetable.

Sensory attributes

On days 1, 7, 14, 21, 28, 42, 49, 60, and 90 of storage or even at the apparent sensory or bacteriological corruption of the samples, the sensory characteristics of the various Egyptian basterma samples were evaluated by a panel of nine trained judges. These judges, comprising both men and women of various ages, were selected from a pool of well-qualified staff members, following the methodology described by Trindade et al. [29]. The evaluated attributes included appearance, color, taste, odor, texture, and overall acceptability (OA). Sensory characteristic scores were assigned on a scale ranging from 1 (very poor) to 10 (outstanding). Samples were uniformly cut into 2 cm quarters and presented to the panellists in a random order. Evaluations were conducted under fluorescent lighting, and panellists were instructed to cleanse their palates with water between samples to ensure accurate perception. While meat being fit for consumption is an indicator of basic safety and health, it doesn't fully address all aspects of animal ethics. Also, Basterma is typically prepared using 1 kg of lean red meat, from which the fat has been removed.

Bacteriological Examination Groups

The three groups; *Staphylococcus aureus* (G4), *Listeria monocytogenes* (G5) and *C. Perfringens* (G6) were at a concentration of 5 log CFU/g of each tested organism was injected in meat separately, and subsequently treated with additives identically to G1, 2, and 3, respectively, and were used for bacteriological examination, with initial testing 12 hours after preparation and subsequent weekly examinations until the 90^{th} day of storage.

Methodology for Microbiological Analysis

Sample preparation, initial suspensions, and decimal dilutions for microbiological analysis adhered to ISO 6887-2:2017. *Staph. aureus* [30], *L. monocytogenes* [31], and *Cl. perfringens* [30] counts were determined by plating and incubating samples. Analyses were performed on days 1, 7, 14, 21, 28, 42, 49, 60, and 90 (two weeks' intervals) of storage or until the samples deteriorated bacteriologically. This serves as the criterion for product acceptance or rejection, as it fulfils the safety requirements of the product. All analyses were carried out in triplicate.

Statistical Analysis

The experimental results are presented as the mean value accompanied by the number of replicates (n) and the standard error (mean ± SE) using SPSS Ver. 20, one-way analysis of variance (ANOVA). Statistical significance was determined at a probability level of $p < 0.05$.

Results

Table (1) illustrates the sensory quality changes of basterma over 90 days of refrigerated storage, comparing three different treated groups (G1, G2 and G3):

Sensory attributes like color, aroma, flavor, tenderness, juiciness, and overall acceptability were scored on a scale of 1-5 (for intensity/quality, where 5 is best) or 1-9 (for overall acceptability, where 9 is best), with off-odors and off-flavors scored 1-5 (where 5 is the strongest undesirable presence).

G1 (Standard Nitrite): Generally maintained the highest sensory quality throughout the 90 days, showing only a slight decline in desirable attributes and minimal off-flavors/odors. In addition, G2 (Arugula): Performed reasonably well, but its quality declined more noticeably than G1 over time, especially in cured meat aroma, flavor, and juiciness, with increasing off-flavors by 90 days. G3 (Chard): revealed a rapid decline in sensory quality of some parameters beginning from 28^{th} day of storage. Its desirable attributes (color, aroma, flavor, tenderness, and juiciness) dropped significantly, and it developed strong off-odors and off-flavors very quickly. By

Day 90, G3 was completely unacceptable (score of 1 for overall acceptability).

Overall, based on the sensory evaluation table, it's clear that arugula (G2) performed significantly better than chard (G3) when both were used with 50 ppm nitrite for the preservation of basterma.

Table (2) illustrate that after 12 hours of treatment (1st day), the *Staph. aureus* count in the 4th group (G4), treated with 100 ppm nitrite, was 2.74±0.58 $\log_{10}$ CFU/g. The 5th group (G5), treated with a combination of 50 ppm nitrite and 50 ppm arugula, exhibited a count of 3.50±0.01 $\log_{10}$ CFU/g. Group (G6), treated with a combination of 50 ppm nitrite and 50 ppm chard, recorded a count of 3.40±0.03 $\log_{10}$ CFU/g.

By the seventh day of storage, the *S. aureus* counts had decreased to 1.32±0.02, 1.72±0.01, and 1.57±0.01 $\log_{10}$ CFU/g in groups G4, G5, and G6, respectively. Throughout the subsequent storage period, from week 2 (day 14) to week 7 (day 49), the bacterial counts remained below 1 $\log_{10}$ CFU/g. However, the organism reappeared on day 60, with counts of 1.45±0.01, 1.73±0.01, and 1.55±0.02 $\log_{10}$ CFU/g for G4, G5, and G6, respectively. By day 90 of storage, the counts had further increased to 3.16±0.03, 3.50±0.01, and 3.36±0.02 $\log_{10}$ CFU/g for G4, G5, and G6, respectively.

Statistically significant differences in *S. aureus* counts were observed on day 1; conversely, no significant differences were found between the mean counts on either day 60 or day 90 of storage between treated groups in the same row.

According to the Egyptian Standard (ES No.1042/2005) [28], which mandates the absence of *Staph. aureus* in basterma at any detectable level, all treated samples are considered unfit for human consumption starting from day 60 of storage, irrespective of any numerical differences in bacterial counts between the groups. Since the basterma ripening period usually begins 21 days after manufacturing, the presence of the microbe on the fourteenth day and its complete disappearance on the 14th day of storage does not conflict with the product's validity and consumer safety.

Table (3) demonstrates that at the 1st day, the *L. monocytogenes* count in G4, treated with 100 ppm nitrite, was 3.19±0.04 $\log_{10}$ CFU/g. while G5, treated with a combination of 50 ppm nitrite and 50 ppm arugula, exhibited a count of 3.04±0.04 $\log_{10}$ CFU/g, and G6, treated with a combination of 50 ppm nitrite and 50 ppm chard, recorded a count of 3.15±0.02 $\log_{10}$ CFU/g.

By the seventh day of storage, the *L. monocytogenes* counts had decreased to 1.14±0.05, 1.09±0.04, and 1.27±0.03 $\log_{10}$ CFU/g in groups G4, G5, and G6, respectively. Throughout the subsequent storage period, from week 2 (day 14) to week 7 (day 49), the bacterial counts remained below the detection limit (<1 $\log_{10}$ CFU/g). However, the organism reappeared on day 60, with counts of 1.23±0.04, 1.14±0.04, and 1.67±0.61 $\log_{10}$ CFU/g for G4, G5, and G6, respectively. By day 90 of storage, the counts had further increased to 3.39±0.02, 3.27±0.03, and 3.77±0.58 $\log_{10}$ CFU/g for G4, G5, and G6, respectively.

Statistically significant differences ($P<0.05$) in *L. monocytogenes* counts were observed on day 1 between G4 and G5, as well as between G5 and G6, while no significant difference ($P>0.05$) was found between G4 and G6. On day 7, significant differences were detected between the mean counts of G4 and G6, as well as between G5 and G6. Conversely, no significant differences were observed on either day 60 or day 90 of storage among all treated groups.

According to the ES No. 1042/2005 [28], which mandates the absence of *L. monocytogenes* in basterma at any detectable level, all treated samples are considered unfit for human consumption starting from day 60 of storage, regardless of any numerical differences in bacterial counts between the groups.

Examining the data presented in table (4) reveals the impact of different treatments on *C. perfringens* counts over a 90-day storage period. Initially, after 24 hours of treatment, the highest bacterial load was observed in the group treated with 100 ppm nitrite (G4), recording 3.44±0.01 $\log_{10}$ CFU/g. The group treated with a combination of 50 ppm nitrite and 50 ppm arugula (G5) showed a count of 2.94±0.06 $\log_{10}$ CFU/g, while the group treated with 50 ppm nitrite and 50 ppm chard (G6) exhibited the lowest initial count at 2.14±0.04 $\log_{10}$ CFU/g.

By day 7 of storage, a reduction in *Cl. perfringens* counts was evident across all groups, with G4 registering 1.19±0.53 $\log_{10}$ CFU/g, G5 at 1.29±0.01 $\log_{10}$ CFU/g, and G6 at 1.16±0.04 $\log_{10}$ CFU/g. For a considerable duration, spanning from day 14 to day 49 (weeks 2 to 7), the bacterial levels in all treated samples remained below the limit of detection (<1 $\log_{10}$ CFU/g). However, the microorganism reappeared on day 60, with counts of 1.82±0.58, 1.56±0.6, and 1.1±0.03 $\log_{10}$ CFU/g for G4, G5, and G6, respectively. By the end of the 90-day storage period, the *C. perfringens* counts had increased again to 3.60±0.55 $\log_{10}$ CFU/g in G4, 2.88±0.57 $\log_{10}$ CFU/g in G5, and 2.53±0.61 $\log_{10}$ CFU/g in G6.

Statistical analysis indicated significant differences ($P<0.05$) in *C. perfringens* counts on the

1st day between groups G4. In contrast, no significant difference (P>0.05) was found between G4 and G6 as well as between G5 and G6 at this initial time point (at the 1st day). On day 7, significant differences were observed between the $\log_{10}$ mean counts of G4 and G5, and also between G5 and G6. Notably, no statistically significant differences were detected among the treated groups on either day 60 or day 90 of storage.

In adherence to the ES No. 1042/2005 [28], which stipulates that basterma must be free from anaerobic bacteria, all treated samples are deemed unsuitable for human consumption from day 60 of storage onwards. This classification holds true regardless of the specific numerical counts or the lack of significant differences between treated groups at these later time points.

Discussion

Both chard and arugula play a role in controlling microorganisms primarily due to their high concentration of nitrate, which acts as a natural precursor to nitrite [31]. When these vegetables are added to meat, naturally occurring enzymes in the vegetables and certain microorganisms can reduce the nitrate (NO3−) to nitrite (NO2−), which the later has a antimicrobial agent. It is particularly effective at inhibiting the growth of harmful bacteria, and other spoilage microorganisms [32]. The Egyptian Standard for the maximum permissible limit of residual nitrite in basterma is 100 ppm (100 mg/kg) [28].

In this context, Serdaroğlu et al. [27] provide valuable insights into the impact of incorporating natural nitrite sources from arugula leaves and barberry extract (BE) into the formulation of heat-treated fermented sausages. Their experimental design strategically included positive and negative control groups, distinguished by the presence or absence of conventional nitrite, respectively. Notably, their findings demonstrated that the inclusion of arugula led to a reduction in protein carbonylation levels compared to samples lacking nitrite.

This observation underscores the potential of arugula as a novel and viable alternative curing agent in heat-treated fermented sausages, offering a substitute for traditional nitrite. Furthermore, sensory analysis conducted at the end of the storage period revealed no statistically significant differences in overall acceptability across all sample groups. These findings resonate with the results obtained in the present investigation, suggesting that both arugula and chard hold promise as substitutes for nitrite in the production of basterma.

The degradation of fat and protein in meat and meat products, including basterma, results from microbial spoilage, autolytic enzymes, and lipid oxidation. These processes significantly impact environmental sustainability and economic viability. Furthermore, these oxidative processes diminish the nutritional value of meat and can generate hazardous substances, thereby posing risks and reducing the product's sensory quality [33]. Our study corroborates these findings, as all experimentally inoculated foodborne pathogens (*S. Aureus*, *L. monocytogenes*, and *C. perfringens*) were recoverable in basterma at the 60th day after production.

Level of Contamination and Microbial Quality and Safety of Basterma

Abd-Allah and Ismail [35] investigated basterma samples from retail outlets in Assiut City, Egypt, and concluded that they exhibited medium sensory quality. Their study reported mean Total Aerobic Bacteria (TAB) and *S. aureus* counts of 7.81 and 6.62 log CFU/g, respectively. The Most Probable Number (MPN) for *C. perfringens* in positive samples ranged from 3.6 to 93 MPN/g. While the majority (98%) of the examined samples had pH values within acceptable limits, a significant concern was noted: all examined samples (100%) exceeded standard limits for both TAB and *S. aureus* counts, and 24% failed to comply with established standards for *C. Perfringens*.

The current study's findings indicate that the examined samples remained microbiologically sound until the 49th day of storage. However, the growth of *Staph. aureus*, *L. monocytogenes*, and *Clost. perfringens* reappeared by the 60th day of storage. In this respect, Abd-Allah and Ismail [35] concluded that basterma samples collected from different retail outlets of Assiut city, Egypt were of medium quality sensory attributes, the mean total aerobic bacteria and *Staph. aureus* counts were 7.81 and 6.62 log CFU/g sample, respectively.

The Most Probable Number (MPN) count of *Clost. perfringens* in positive samples ranged from 3.6 to 93 MPN/g. The authors added that the majority of the examined samples (98%) exhibited pH values within the acceptable limits. However, all examined samples (100%) exceeded the standard limits for total aerobic bacteria and *Staph. aureus* counts, and 24% of the examined samples did not comply with the established standards for *Clost. perfringens*.

The current study revealed that the examined samples remained sound till the 49th day of storage, while the growth of *S. aureus*, *L. monocytogenes* and *C. Perfringens* experimentally contaminating the product during manufacturing reappeared at the 60th

day of storage. The findings reveal that the hygienic quality of basterma available in Assiut retail markets is unsatisfactory and fails to meet the required standards. These results align with those reported by Abd-El-Shahid and Ibrahim [36] and Abd El-Gafar [37]. The variation in the quality and safety of a meat product is contingent upon the quality of additives used in the manufacturing, the quality of the raw meat, the manufacturing and preservation conditions (pH, temperature, and humidity), and the sanitary condition of the equipment and the processing procedures [38, 39].

The Challenge of Nitrosamines in Cured Meats

The consumption of nitrite, while vital for the preservation and characteristic properties of cured meats, presents a critical food safety issue represented by the potentiality of N-nitrosamine formation. As highlighted by Wang et al. [40] and Al-Kaseem et al. [41], these compounds are recognized as pro-carcinogenic and have been implicated in the development of cancer in both humans and animals. N-nitrosamines can also be formed in food through reactions between nitrosating agents and amino-based substances.

This concern is supported by Iammarino et al. [42] who specified that the most prevalent nitrosating agents in food are nitrite salts and nitrogen oxides, with dietary exposure primarily linked to the consumption of processed meat. This critical public health consideration has driven the search for natural substitutes for nitrite, aiming to achieve a safe product that does not pose a public health hazard while simultaneously preserving the quality of the produced basterma.

In this regard, Can et al. [42] demonstrates that the substitution of nitrite with either arugula extract or pre-converted arugula extract led to reduced levels of nitrosomyoglobin and residual nitrite. Importantly, the pre-converted arugula extract yielded comparable quality parameters and oxidation levels to samples treated with conventional nitrite, indicating its promising potential as a nitrite source in heat-treated meat products.

This observation is consistent with the findings of our investigation, suggesting that both arugula and chard can elicit similar effects to nitrite. Furthermore, chard exhibited superior efficacy against *C. perfringens*, while arugula demonstrated the most pronounced inhibitory effect on *L. monocytogenes*. The formation of nitrosamines has been observed under specific conditions, notably at initial nitrite concentrations exceeding 120 mg/kg and elevated temperatures (above 120°C) in cooked, dried, or salted meat products [43].

Furthermore, the nitrosation reaction, involving the dissociation of nitrous acid and secondary amines, is accelerated under acidic conditions, specifically at a pH of 3.5 [42]. In this context, the precise control of both initial and residual nitrite levels in meat products is paramount, and while the use of nitrites in the food industry is subject to stringent regulations, the challenge of their reduction or replacement persists [44].

Given the escalating consumer health concerns regarding nitrosamine intake and the growing demand for 'clean label' products, the reformulation of traditional meat product recipes is becoming increasingly imperative. A significant body of advanced research is currently dedicated to the development of novel sausage product formulations based on natural plant-derived raw materials, thereby providing a substantial impetus for future innovation within the food industry. Several studies have specifically evaluated the potential of natural substances as substitutes for sodium nitrite, aiming to create healthier meat product formulations while maintaining desirable sensory attributes and ensuring microbiological safety [45, 46].

In this respect, Wang et al. [40] and Al-Kaseem [41] revealed another critical facet of food safety associated with nitrite consumption is the potential for the formation of N-nitrosamines, a group of compounds recognized as pro-carcinogenic and implicated in the etiology of cancer in both humans and animal models. These compounds can also be present in food as a result of reactions between nitrosating agents and amino-based substances. The same meaning carried out by Iammarino et al. [54] indicated that the most prevalent nitrosating agents in food are nitrite salts and nitrogen oxides, with dietary exposure primarily linked to the consumption of processed meat

Given the formation of nitrosamines, a class of chemicals generated during meat processing, the World Health Organization (WHO) has classified processed meats as potentially carcinogenic to humans [47]. Nitrite itself is also categorized as an oxidizing solid (hazard category 3). Consequently, various regulatory bodies have imposed limits on the amount of nitrite used in the curing of meat products [48, 49].

The Role of Arugula and Chard in Basterma Processing

Can et al. [42] demonstrates that the substitution of nitrite with either arugula or pre-converted arugula extract led to reduced levels of nitrosomyoglobin and residual nitrite. Importantly, the pre-converted arugula extract yielded comparable quality parameters and oxidation levels to samples treated

with conventional nitrite, indicating its promising potential as a nitrite source. This observation is consistent with the findings of our investigation, suggesting that both arugula and chard extracts can elicit similar effects to nitrite. Furthermore, chard extract exhibited superior efficacy against *C. perfringens*, while arugula extract demonstrated the most pronounced inhibitory effect on *L. monocytogenes.*

Iammarino et al. [50] reported varying nitrate concentrations in different forms of chard. Their findings indicated that fresh chard contained concentrations ranging from 367.9 to 3764.9 mg kg^{-1}, frozen chard from 1057.0 to 3968.4 mg kg^{-1}, fresh-cut chard from 442.2 to 5834.9 mg kg^{-1}, and ribbed chard from 32.6 to 2150.3 mg kg^{-1}. Furthermore, fresh arugula contained 1121.6–7311.2, and fresh-cut wild arugula contained 3517.7–7206.4 mg kg^{-1}. In the present study, we calculated the quantity of fresh chard required to achieve a 50 ppm concentration when combined with nitrite during basterma preparation.

A significant challenge confronting the meat industry is the imperative to reduce the reliance on synthetic nitrite. This has spurred the development of natural alternatives, often combined with hygienic preservation methods, which are perceived as relatively healthier for consumers compared to chemical preservatives like nitrite. As a result, the utilization of natural plant extracts (e.g., chard and/or arugula) or lycopene and pine bark as nitrite replacers represents a growing trend within the food sector, aligning with principles of sustainable growth [51].

Plant-based food additives have garnered considerable attention due to their advantages over synthetic counterparts, including their environmental friendliness and protective qualities. In this regard, Youssef et al. reported that a 100 ppm concentration of nitrite was insufficient to completely eliminate *Staph. aureus*. In contrast, the current study found that the same concentration of nitrite successfully controlled the organism until the seventh week of storage, with its reappearance noted from the 60th day onwards [51].

Interestingly, Youssef et al. [51] research also showed that adding 1% lycopene and pine bark yielded results similar to our chard and arugula treatments up to the 7th week, with the microbe remaining undetectable until the experiment's conclusion. However, in our study, the microorganism began to reappear from the 60th day of storage. These discrepancies might stem from several factors, including variations in the antibacterial substances present in the preservatives used, their purity, differences in storage conditions**,** and the virulence of the microorganisms, including *Staph. aureus* contaminating food products.

Both chard and arugula are recognized as excellent sources of antioxidants, primarily attributable to their rich content of vitamins (notably C, A, K, and E), flavonoids, carotenoids, and other phenolic compounds. Arugula further distinguishes itself with its significant concentration of glucosinolates (specifically sulforaphane), which possess unique health-promoting properties and act as potent antimicrobial agents, as described by Pyo [55], Björkman [56]; Garg and Sharma [57]; Iammarino [58] and Marwat [59], which is aligned with the results in the present study.

Conclusion

In this research, we found that basterma made with standard nitrite (100 ppm) (G4) consistently maintained high quality throughout 49 days of storage. When reducing nitrite to 50 ppm, arugula vegetable (G5) proved to be a good alternative, helping the basterma stay microbially safe for the same period (49 days). However, chard (G3) was microbiologically effective for the same period (49 days), while it considered unsuitable, leading to rapid losses in sensory attributes, making the basterma unacceptable., while all treatments initially eliminated pathogens, they reappeared by day 60 across all groups, setting the practical shelf life for safe consumption at 49 days. This shows that carefully selected plants, like arugula, can help reduce nitrite in basterma while maintaining quality and safety. The research aims to find alternatives to nitrite or, at the very least, to reduce the quantities of nitrite used. This is particularly important because, in some cases especially when good manufacturing and hygiene practices are not followed, the amount of nitrite used may exceed the internationally permissible standard limit. Therefore, the addition of any substance that helps reduce the use of chemical preservatives is considered a significant achievement in itself, as it safeguards consumer health and protects the environment from pollution.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

TABLE 1. Sensory profile of basterma samples under reduced nitrite and plant treatment during 90 days of refrigerated storage

Sensory Parameter	Group	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 60	Day 90
Color Intensity	G1	5	5	5	4	4	4	4	4	3
	G2	4	4	4	4	3	3	3	3	2
	G3	3	3	2	2	2	1	1	1	1
Color Uniformity	G1	5	5	5	4	4	4	4	4	3
	G2	4	4	4	3	3	3	2	2	1
	G3	3	3	2	2	1	1	1	1	1
Cured Meat Aroma	G1	5	5	5	4	4	4	4	3	3
	G2	4	4	4	3	3	3	2	2	1
	G3	3	2	2	1	1	1	1	1	1
Off-Odor	G1	1	1	1	1	1	1	1	2	2
	G2	1	1	1	2	2	2	3	3	4
	G3	2	3	3	3	3	3	3	4	5
Cured Meat Flavor	G1	5	5	5	4	4	4	4	3	3
	G2	4	4	4	3	3	3	2	2	1
	G3	3	2	2	1	1	1	1	1	1
Spicy Flavor	G1	4	4	4	4	4	3	3	3	2
	G2	4	4	3	3	3	2	2	2	1
	G3	3	2	2	1	1	1	1	1	1
Off-Flavor	G1	1	1	1	1	1	1	1	2	2
	G2	1	1	2	2	2	3	3	4	5
	G3	2	3	3	4	4	5	5	5	5
Tenderness	G1	5	5	5	4	4	4	4	3	3
	G2	4	4	4	3	3	3	2	2	1
	G3	3	2	2	1	1	1	1	1	1
Juiciness	G1	5	5	5	4	4	4	4	3	3
	G2	4	4	4	3	3	3	2	2	1
	G3	3	2	2	1	1	1	1	1	1
Overall Acceptability	G1	8	8	8	7	7	7	7	6	6
	G2	7	7	7	6	6	6	5	5	4
	G3	7	6	6	5	4	4	3	3	1

TABLE 2. Statistical analysis of *Staph. aureus* mean count (log_{10} cfu/g) of treated basterma samples

Storage days	Treated groups		
	G4	**G5**	**G6**
1st	**$2.74^{a}\pm0.58$**	**$3.50^{ab}\pm0.01$**	**$3.40^{ac}\pm0.03$**
7th	**$1.32^{d}\pm0.02$**	**$1.72^{e}\pm0.01$**	**$1.57^{f}\pm0.01$**
14th	**<1**	**<1**	**<1**
21th	**<1**	**<1**	**<1**
28th	**<1**	**<1**	**<1**
35th	**<1**	**<1**	**<1**
42th	**<1**	**<1**	**<1**
49th	**<1**	**<1**	**<1**
60th	**$1.45^{g}\pm0.01$**	**$1.73^{h}\pm0.01$**	**$1.55^{i}\pm0.02$**
90th	**$3.16^{j}\pm0.03$**	**$3.50^{k}\pm0.01$**	**$3.36^{l}\pm0.02$**

- G1. Treated group with 100 ppm nitrite >>> G2. Treated group with 50 ppm nitrite + 50 ppm Arugula >>> G3. Treated group with 50 ppm nitrite + 50 ppm Chard >>> 1st day and every week till the 90th day of storage
- There were significance differences ($P<0.05$) between logs carrying the same superscripted small letters in the same raw

TABLE 3. Mean *L. monocytogenes* count (log_{10} cfu/g) of treated basterma samples

Storage days	Treated groups		
	G4	G5	G6
1st	3.19^{a}±0.04	3.04^{ab}±0.04	3.15^{b}±0.02
7th	1.14^{c}±0.05	1.09^{d}±0.04	1.27^{e}±0.03
14th	<1	<1	<1
21th	<1	<1	<1
28th	<1	<1	<1
35th	<1	<1	<1
42th	<1	<1	<1
49th	<1	<1	<1
60th	1.23^{f}±0.04	1.14^{g}±0.04	1.67^{h}±0.61
90th	3.39^{i}±0.02	3.27^{j}±0.03	3.77^{k}±0.58

- G4. Treated group with 100 ppm nitrite >>> G5. Treated group with 50 ppm nitrite + 50 ppm Arugula >>> G6. Treated group with 50 ppm nitrite + 50 ppm Chard. >>> 1st day and every week till the 90th week of storage.
- There were significance differences (P<0.05) between logs carrying the same superscripted small letters in the same raw.

TABLE 4. Mean *Cl. perfringens* count (log_{10} cfu/g) of treated basterma samples

Storage days	Treated groups		
	G4	G5	G6
1st	3.44^{a}±0.01	2.94^{b}±0.06	2.14^{a}±0.04
7th	1.19^{c}±0. 53	1.29^{ce}±0.01	1.16^{de}±0.04
14th	<1	<1	<1
21th	<1	<1	<1
28th	<1	<1	<1
35th	<1	<1	<1
42th	<1	<1	<1
49th	<1	<1	<1
60th	1.82^{f}±0.58	1.56^{g}±0.6	1.1^{h}±0.03
90th	3.60^{i}±0.55	2.88^{j}±0.57	2.53^{k}±0.61

- G4. Treated group with 100 ppm nitrite >>> G5. Treated group with 50 ppm nitrite + 50 ppm Arugula >>> G6. Treated group with 50 ppm nitrite + 50 ppm Chard. >>> 1st day and every week till the 90th week of storage
- There were significance differences (P<0.05) between logs carrying the same superscripted small letters in the same raw

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الفعالية المضادة للبكتيريا للسلق والجرجير كبدائل طبيعية للنيتريت ضد المكورات العنقودية الذهبية والليستيريا مونوسيتوجين والكلوستريديوم بيرفرينجنز في البسطرمة

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الملخص

تسلط هذه الدراسة الضوء على تأثير الاستبدال الجزئي للنتريت بمستخلصات نباتية طبيعية على الخواص الحسية والسلامة الميكروبيولوجية للبسطرمة خلال 90 يومًا من التخزين المبرد. تم تقييم ثلاث مجموعات من البسطرمة:) G1مجموعة التحكم، 100 جزء في المليون من النتريت)، 50) G2 جزء في المليون من النتريت + 50 جزء في المليون من مستخلص الجرجير)، و50) G3 جزء في المليون من النتريت + 50 جزء في المليون من مستخلص السلق). تم تلويث جميع المجموعات ببكتيريا المكورات العنقودية الذهبية، والليستيريا مونوسيتوجين، والكوليستريديم بوتيولينم بمعدل 5 لوغاريتمات وحدة تشكيل مستعمرة/ميكروب لكل منها لتقييم فعاليتها المضادة للميكروبات. تم إجراء التقييم الحسي على فترات زمنية متباعدة تصل إلى 90 يومًا. حافظت G1 باستمرار على جودة حسية عالية طوال فترة التخزين. بينما أظهرت G2، التي تضمنت مستخلص الجرجير، انخفاضًا معتدلًا في الصفات الحسية ولكنها ظلت مقبولة بشكل عام لمدة تصل إلى 49 يومًا. في حين شهدت المجموعة G3 تدهورًا حسيًا سريعًا وشديدًا، حيث ظهرت عليها روائح كريهة قوية ونكهات غير طبيعية، مما جعلها غير مقبولة حسيًا بحلول اليوم الستين، وغير صالحة للاستهلاك بشكل قطعي بحلول اليوم التسعين. من الناحية الميكروبيولوجية، اختفت جميع الميكروبات الثلاثة الملقحة المكورات العنقودية الذهبية، والليستيريا مونوسيتوجين، والكوليستريديم بوتيولينم من جميع مجموعات البسطرمة المعالجة بحلول اليوم السابع من التخزين.

بمراجعة النتائج الحسية والميكروبيولوجية، وجدنا أن عينات البسطرمة من جميع المجموعات صالحة للاستهلاك حتى اليوم التاسع والأربعين من التخزين البارد. ثم، أشار ظهور مسببات الأمراض مرة أخرى بحلول اليوم الستين، إلى جانب التدهور الحسي الكبير (خاصةً في المجموعة الثالثة)، إلى أن المنتجات أصبحت غير صالحة للاستهلاك بحلول اليوم الستين من التخزين. يؤكد هذا البحث أن مستخلص الجرجير بديلٌ جزئيٌّ للنيتريت أكثر فعاليةً من مستخلص السلق في الحفاظ على جودة البسطرمة وسلامته أثناء التخزين المبرد لفترات طويلة.

الكلمات الدالة: الجرجير، السلق، بدائل النتريت، مسببات الأمراض المنقولة بالغذاء.