CHAPTER 12

Microbiological stability of filled gingerbread: problems and technological solutions

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Abstract

Microbial stability remains a major challenge in extending the shelf life of bread and flour-based confectionery products. This issue is particularly relevant for filled gingerbread (pryaniki), where consistent product quality throughout the distribution period is essential for both consumer acceptance and manufacturer reputation. The present paper combines a narrative review of the scientific literature with a practical case study to examine the factors affecting microbial spoilage of filled gingerbread and to discuss technological approaches for improving their microbial stability.

The review summarizes current knowledge on the shelf life of bread and related bakery products, with filled gingerbread considered as a representative example of a multi-component system. In such products, the crumb and filling differ in composition and physicochemical properties, which can lead to internal moisture redistribution during storage. Literature data indicate that shelf-life limitations are associated not only with staling but also with moisture migration and post-baking contamination. Local increases in water activity may occur at the crumb-filling interface, creating microenvironments that favor the growth of xerotolerant molds and osmophilic yeasts. In addition, air and contact surfaces in cooling and packaging areas represent important contamination pathways.

The review is complemented by a case study of filled gingerbread, in which selected physicochemical and microbiological indicators were evaluated during storage. The combined analysis highlights the importance of a hurdle strategy that integrates control of component aw, hygienic zoning after baking, appropriate barrier packaging and headspace management, as well as formulation adjustments and complementary preservation measures.

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Keywords
Filled gingerbread, microbiological spoilage, xerophilic molds, water activity, shelf life, contamination control, bakery products.

12.1 Introduction

Gingerbread represents a significant share of flour confectionery products in Europe and Ukraine. These products have long cultural and gastronomic traditions and are strongly associated with festive baking, most often with Christmas. Gingerbread is a type of cookie distinguished mainly by a high content of spices (cloves, cinnamon, ginger, cardamom, nutmeg, and others).

The recipe, the combination of spices, and technological methods differ depending on regional preferences. In Northern European countries, gingerbread is often made from thin, crispy spiced dough (pepparkakor in Sweden, pepperkaker in Norway, piparkakut in Finland, peberkager in Denmark). Similar thin and crispy products with a pronounced aroma, known as speculaas (speculoos), are produced in the Netherlands and Belgium. Gingerbread products in Central Europe usually have a moderately dense and elastic structure and a characteristic aromatic profile (Polish pierniki, Czech perníky). The well-known Nuremberg gingerbread Lebkuchen is characterized by a minimal amount of flour and a high proportion of nuts. Softer and slightly more airy gingerbread products are traditional for Southern Europe, such as pan de jengibre, as well as polvorones and mantecados in Spain, and panpepato and panforte in Italy. These historically formed consumption traditions support stable demand for gingerbread and contribute to the development of modern production technologies.

Despite significant differences in formulation and processing technologies, gingerbread products share a common characteristic: an extended shelf life. This is mainly due to the antibacterial properties of spices [1, 2]. In general, spicy-aromatic components are added to the formulation of bakery and confectionery products to provide specific organoleptic characteristics, but they may also contribute to extending the shelf life [3, 4].

Unlike most flour confectionery products with a short sales period, gingerbread is considered a relatively stable product. However, even at relatively low values of water activity (a_w) and high osmotic concentration (sugars, invert syrups, honey components), it remains vulnerable to the development of xerotolerant and xerophilic mycobiota [5].

An important stage in the development of gingerbread technology and assortment was the use of marzipan, nut, and fruit-berry fillings. This made it possible to obtain new flavor-aroma profiles and texture characteristics of the products. At the same time, products with fillings have a higher risk of microbiological spoilage. This risk increases due to the non-uniform moisture profile in different parts of the product, especially at the phase boundary between crumb and filling. The filling often releases free moisture and locally increases aw in the contact zone with the baked layer, creating conditions for the growth of microorganisms.

Therefore, the aim of this work was to discuss the causes of microbiological spoilage in filled gingerbread and to identify possible technological solutions.

12.2 Factors affecting the microbiological stability of filled gingerbread

The main causes of spoilage in flour-based confectionery products are molds, while bacterial mechanisms (including spore-forming bacteria) are less typical for gingerbread and, as a rule, do not determine the shelf life under standard processing and packaging conditions. Molds are able to grow in the presence of oxygen even at relatively low values of *a_w* (down to ~ 0.62 and above), and the risk increases in packaged products due to moisture retention in the package headspace and on the product surface [6]. For gingerbread products, the presence of species adapted to reduced *a_w* values is critical. In particular, xerophilic fungi such as *Eurotium* spp. (now often considered in relation to the teleomorphic forms of *Aspergillus*) and *Wallemia sebi* have been identified in the production flow of gingerbread manufacturing; these microorganisms are associated with the spoilage of low-moisture sweet products and are able to survive technological processing conditions [5]. The resistance of xerophiles to preservatives may vary: in a model study with xerophilic isolates, propionic acid demonstrated higher effectiveness against spoilage compared with potassium sorbate, which is important when selecting fungicidal preservatives.

In fillings with higher *a_w* or acidity, osmophilic and acid-tolerant yeasts may dominate, whereas spore-forming bacteria (*Bacillus* spp.) are usually limited by the low water availability in the gingerbread matrix but may pose a risk in cases of local moisture increase or temperature control failures. Therefore, when assessing risks, the product should be considered as a multi-component system, and the parameters of individual components should be controlled separately [6, 7].

Another important aspect concerns the sources of contamination and the critical control points in the process after baking. In a study investigating the factors responsible for gingerbread spoilage, it was shown that the air of the production environment is the main source of spores, while the cooling area is characterized by increased mycological load [7]. In samples taken from production facilities, *Aspergillus* spp. and *Penicillium* spp. predominated, whereas *Aspergillus niger* and *Penicillium chrysogenum* were isolated from finished products. Importantly, challenge tests demonstrated that *A. niger* and *P. chrysogenum* were able to grow in gingerbread, with *A. niger* showing higher enzymatic activity (amylase/protease/lipase), which may accelerate the degradation of the product structure as well as its sensory and structural quality. Thus, for gingerbread products it is not only the "presence of spores" that matters, but also which particular species contaminate the product and how capable they are of degrading its structure.

The prediction of microbiological stability is largely based on water activity and the sorption behavior of the gingerbread matrix. Studies show that the relationship between moisture content and *a_w* in gingerbread has a characteristic S-shaped form, which is typical for many products with a high sugar content. This relationship is usually described using sorption isotherms. For their mathematical representation, the GAB model (Guggenheim-Anderson-de Boer) is often applied, as it allows estimating the ability of a product to absorb or release moisture at different levels of water activity. The model provides a reliable description of moisture behavior in food systems over a wide range of storage conditions and is therefore widely used in studies dealing with product stability and shelf-life prediction [8].

Importantly, the relationship between product moisture and *a_w* is not linear. It varies depending on product temperature, storage conditions, and formulation, particularly the sugar content. This occurs because sugars are able to bind water molecules effectively, thereby reducing the fraction of water that remains available within the product structure.

Certain thresholds of environmental relative humidity may lead to a sharp increase in the equilibrium moisture content of the product, effectively marking the point at which the system shifts toward a higher risk of microbial growth. For this reason, storage parameters for gingerbread, including relative humidity and temperature, as well as the barrier properties of packaging, should be aligned with the sorption isotherm specific to the given formulation rather than transferred by analogy from other flour-based confectionery products.

In multi-component products, moisture migration is one of the main factors limiting shelf life. In filled gingerbread products, average values of moisture and water activity may conceal local conditions at the phase boundaries. Moisture migration is not always governed solely by the *a_w* gradient; differences in water-binding mechanisms and matrix composition may lead to moisture transfer patterns that deviate from simple diffusion-based predictions [9]. The direction of initial moisture transfer depends not only on the *a_w* gradient but also on the affinity of the components for water and on the proportion of free water present. A component containing a higher fraction of free water may release moisture to another component with stronger water-binding capacity, even if their initial *a_w* values do not fully predict this transfer.

According to production studies, the average *a_w* of finished gingerbread products was approximately 0.655. However, the product is highly heterogeneous: in the gingerbread base the *a_w* can be very low (around 0.327), whereas in the fillings it may reach values close to 0.949 [7].

Packaging characteristics, particularly air and water vapor permeability, together with the relative humidity of the surrounding environment, further influence moisture exchange through the headspace inside the package. In filled gingerbread products, this means that the interface between the crumb and the filling may gradually reach higher moisture levels and *a_w* values, creating favorable conditions for the growth of microorganisms that would otherwise remain limited by the low water availability in the main gingerbread matrix.

Thus, microbiological spoilage of filled gingerbread products results from the combined influence of several factors arising from the interaction of: (i) product systems with different physicochemical properties (crumb–filling), (ii) post-baking contamination, (iii) *a_w* and sorption behavior, and (iv) internal moisture migration within the multi-component crumb–filling system.

The main factors influencing microbiological spoilage of filled gingerbread products are presented in **Fig. 12.1**.

Fig. 12.1 Key factors affecting the microbiological spoilage of filled gingerbread
Source: developed by the authors

12.3 Spoilage mechanisms and risk factors in filled gingerbread

The main form of microbiological spoilage in gingerbread is the growth of mold on the surface and in the near-surface layers. This process is often initiated by airborne spores and by cross-contamination during post-baking handling, cooling, and packaging. In a classic study of gingerbread production, the highest concentrations of fungal spores were associated with the cooling room, conveyor areas, and the packaging line. This finding highlights the importance of the processing environment as a source of contamination, not only the raw materials [5]. A more recent survey conducted at a commercial production facility identified the cooling and packaging areas as "hot spots" of airborne microflora. Disinfection of walls and ceilings with 95% ethanol reduced fungal counts from 1.66×10^3 to 80 CFU/m^3 in the cooling room and from 1.93×10^3 to 160 CFU/m^3 in the packaging area [7].

Control of spoilage in filled gingerbread should simultaneously (i) reduce the growth potential of microflora in the filling and in the baked layer and (ii) minimize post-baking contamination and oxygen availability.

Thermal lethality is not always sufficient against xerophilic molds. For example, *Eurotium* spp. have been reported to withstand heating at 75–85°C for 60 min, whereas *Wallemia sebi* can be inactivated after 30 min at 65°C [5].

Limiting water availability after baking is therefore critical. A commonly cited practical recommendation for long-term storage at room temperature is to maintain product moisture below approximately 12–15% (on a dry matter basis), which corresponds to about 60–64% relative humidity [8]. However, as discussed above, in filled gingerbread the average moisture level may vary significantly at the crumb-filling interface, particularly when the filling contains fruit components or dairy-derived ingredients that can support the growth of yeasts and molds [7].

The thermodynamic driving force for moisture migration in multicomponent systems is the tendency of a_w to equilibrate between domains (dough/crumb-filling-surface layer). From a kinetic perspective, the rate of this process depends on the effective diffusion of water and the geometry of contact between the domains. In practice, control can therefore be achieved either by reducing the Δa_w gradient through formulation adjustments or by limiting mass transfer through structural or barrier approaches [10].

Adjustment of water activity (a_w) through formulation can be achieved in several ways.

First, this involves increasing the proportion of soluble solids such as sucrose, glucose syrup, or invert sugar. Another approach is the use of moisture-binding and moisture-retaining agents, including glycerol and sorbitol, as well as the selection of hydrocolloids capable of binding water without releasing it during storage. The addition of up to 5% glycerol derived from hydrogenated cottonseed oil has been recommended in gingerbread formulations to improve product quality during the storage period [11].

For fillings, high-carbohydrate systems can reach inhibitory *a_w* values. Fruit-based and chocolate fillings with *a_w* values of about 0.74–0.77 and soluble solids content above approximately 65°Brix have been reported as microbiologically stable at room temperature over extended storage periods [12]. A similar heat-stable filling contained about 65% soluble solids and had a pH of 3.3–3.5, using low-methoxyl pectin to form stable gels compatible with thermal processing [13]. Such approaches simultaneously reduce *a_w*, limit bacterial growth through increased acidity, and slow moisture migration due to the presence of a structured gel network.

In products with a complex heterogeneous structure, not only the average moisture content but also the local *a_w* profiles near the crumb-filling interface are critical. Moisture redistribution in this area may create micro-zones with higher water availability. These zones are particularly susceptible to contaminant growth, especially xerotolerant molds, and are also associated with textural defects such as local softening of the crumb near the filling.

Moisture migration interacts with staling processes and related textural changes.

Recrystallization of sucrose and changes in the crystalline structure of gingerbread during storage can affect perceived hardness as well as the distribution of water within the product. The use of raffinose has been proposed as a way to reduce the intensity of sucrose crystallization and slow quality deterioration during storage [14].

In another study, replacing conventional wheat flour with waxy wheat flour improved moisture retention during storage. The experimental samples showed moisture losses of 0.08–0.18%, compared with 0.20–0.38% in the control after 25 days. The authors also suggested combining modified atmosphere packaging, freezing, and the use of antioxidants as additional approaches to extend shelf life [15].

From an engineering perspective, moisture dynamics in multicomponent products can be described using multilayer diffusion models together with the vapor permeability characteristics of the packaging material. For gingerbread products, such modelling may support the selection of appropriate barrier films and help manage humidity within the package headspace in order to prevent crumb softening or moisture accumulation that may promote mold growth.

However, in practice the most common trigger for mold development remains improper storage conditions, which create a favorable environment for fungal growth (**Fig. 12.2**).

Fig. 12.2 Surface mold growth on gingerbread (gingerbread with cherry filling – packaged in a cardboard box; third month of storage during the summer period; non-compliance with recommended temperature and humidity conditions in the retail network)
Source: author's photo

12.4 Study of microbiological spoilage of filled gingerbread

Soft gingerbread cookies filled with cherry-flavored fruit filling and coated with sugar glaze, produced by HD Bakery & Snacks ALC, Ukraine, were used in this study. These products comply with the safety requirements of the national standard of Ukraine DSTU 4187:2003 – Gingerbread Confectionery Products. The product formulation is presented in **Table 12.1**.

To investigate the possible causes of microbiological spoilage in gingerbread with cherry filling, two samples were examined: a fresh gingerbread sample (FGB) and a sample with an exceeded storage period (PGB). The latter had been stored for 120 days from the production date in the original packaging under conditions that did not meet the recommended storage regime. The recommended conditions were a temperature of (18 ± 5)°C and relative humidity not higher than 75%. During the experiment, the samples were kept under uncontrolled fluctuations of temperature ($0°C \leq T \leq 24°C$) and relative humidity ($55\% \leq RH \leq 90\%$). These conditions were chosen to stimulate the development of microbiological spoilage.

a_w of the gingerbread samples was measured using an AquaLab 3TE instrument. Each measurement was performed in triplicate, and the mean value was used for further analysis.

The moisture content (%) was determined by the thermogravimetric method at a drying temperature of 102 ± 2°C. The reported value represents the arithmetic mean of two parallel measurements. The difference between parallel determinations did not exceed 0.24%.

Table 12.1 Formulation of the "Cherry Orchard" gingerbread cookies

Raw material	Quantity, kg	Formula, % (to total)
Wheat flour	475.77	43.18
Sugar	281.75	25.57
Milk powder	24.05	2.18
Sodium bicarbonate	3.07	0.28
Ammonium carbonate	2.72	0.25
Thermostable cherry filling (flavoring filler with cherry flavor)	145.76	13.23
Vanilla-cream flavoring	0.94	0.09
Glucose-fructose syrup	112.20	10.18
Improver Probake SP	0.95	0.09
Palm oil	54.50	4.95
Water	0.13	0.01
Total	**1101.84**	**100.00**

The total number of mesophilic aerobic and facultative anaerobic microorganisms was determined using non-selective nutrient medium plate count agar (PCA) according to ISO 4833. Cultivation conditions: temperature 30 ± 1°C; incubation time 72 ± 3 hours.

For the determination of coliform bacteria, the selective culture medium Violet Red Bile Lactose Agar (VRBL agar) was used according to ISO 4832.

The number of yeasts and molds was determined on Sabouraud agar with incubation at 25 ± 1°C for 5 days according to DSTU 8447:2015.

Pathogenic microorganisms, including *Salmonella* spp., were detected according to DSTU EN 12824:2004.

According to the results of our study, the water activity of both samples did not exceed the levels considered critical for the microbiological stability of food products (**Table 12.2**).

Although the data obtained in our study generally align with the trends described by other researchers, we did not find evidence of a significant difference in water activity among the different layers of the gingerbread.

However, this did not guarantee the microbiological stability of the product under improper storage conditions (**Table 12.3**).

Table 12.2 Water activity and moisture content in "Cherry Orchard" gingerbread cookies

Analyzed sample	*a_w*			Moisture, %
	Filling	Filling-crumb interface layer	Crumb	
FGB	0.728	0.721	0.718	15.09
PGB	0.669	0.637	0.625	12.68

Table 12.3 Microbiological indicators of "Cherry Orchard" gingerbread cookies

Microbiological indicator	Requirement according to DSTU 4187:2003	Time of storage, day	
		0	120
Number of mesophilic aerobic and facultative anaerobic microorganisms, CFU per 1 g of product, not more than	5.0×10^3	5.0×10	2.0×10^3
Bacteria of the coliform group	Not permitted in 0.1 g	Not detected	Not detected
Yeasts, CFU per 1 g of product, not more than	5.0×10	0	< 5
Molds, CFU per 1 g of product, not more than	5.0×10	0	0
Pathogenic microorganisms, including *Salmonella* spp., in 25 g of product	Not permitted	Not detected	Not detected

The results of the microbiological analyses confirmed that the studied product complied with the established microbiological standards throughout the entire storage period and remained safe according to the tested indicators.

No mold growth was detected during the whole storage period, indicating an appropriate sanitary condition of the production process.

However, after 120 days of storage, the presence of yeasts was detected in the product (less than 5 CFU/g, which is significantly below the permissible level of 5.0×10^1 CFU/g). These microorganisms may potentially contribute to product spoilage during further storage.

Yeast growth usually stops at *a_w* values of about 0.88–0.90. Nevertheless, osmophilic yeasts are able to grow at much lower water activity levels, down to approximately *a_w* ≈ 0.60–0.65 [16].

The approximate *a_w* thresholds for the development of major groups of microorganisms can vary over a fairly wide range. Nevertheless, at *a_w* ≈ 0.60, the growth of bacteria and most fungi is effectively inhibited, and only spores are able to survive. In practice, achieving such a low water activity in filled gingerbread is quite difficult. For this reason, manufacturers usually rely on a combination of technological measures to ensure product stability during storage.

12.5 Process sanitation and control of the production environment

Since contamination often occurs after baking, maintaining good hygiene in the production environment is essential. Potential sources of contamination include the air in cooling rooms, conveyors, packaging areas, and open containers used for cooling sugar solutions [5]. Practical measures to reduce this risk include separating "raw" and post-baking zones, managing airflow and filtration in cooling rooms, prioritizing dry cleaning methods (to avoid creating moist niches), minimizing the time between product exposure and packaging, and implementing validated sanitation procedures for conveyors, trays, and other contact surfaces. The effectiveness of sanitation practices should be verified through regular microbiological monitoring of air and surfaces, as illustrated by the significant reduction in airborne CFU counts after ethanol disinfection in a production facility [7].

For filled gingerbread, sanitary control should also extend to the filling preparation system, including mixing tanks, pumps, and dosing equipment. High-moisture fillings may support the survival of yeasts and molds if product residues or biofilms are present. In addition, packaging operations should be designed to limit oxygen access and the deposition of spores, for example by sealing the product promptly after cooling and selecting packaging materials with suitable barrier properties.

12.6 Chemical approaches to prevent microbial growth

Preservatives remain a common tool in products intended for extended storage, although their selection should take into account the target microflora (xerophilic or common molds), as well as the pH and a_w of the product. Potassium sorbate and sodium propionate have shown the highest antifungal activity, and their combination with chitosan was reported to reduce fungal growth by more than 80% [7].

These findings support the concept of targeted application of preservatives, for example in the filling (where a_w is higher) and/or on the product surface, where spoilage typically begins, provided that regulatory limits are respected and consumer expectations are considered.

To reduce sensory impact and improve the stability of antimicrobial agents (such as essential oils, organic acids, or enzymes), microencapsulation is increasingly considered a promising approach. Encapsulation protects active compounds during processing and allows their controlled release over time. Review studies describe several microencapsulation strategies used in food systems, including spray drying, coacervation, lipid capsules, and polymer matrices, with the aim of protecting sensitive ingredients and controlling release kinetics [17, 18].

In filled gingerbread, encapsulated antifungal compounds may be incorporated into the filling, applied as a surface coating, or embedded into edible films, helping to maintain inhibitory concentrations during storage without causing a pronounced off-flavor or odor.

Edible coatings and biodegradable films are also considered promising solutions, as they can function both as surface barriers and as carriers of bioactive substances. Research on biodegradable films for bakery and confectionery products, using differential scanning calorimetry, has shown that edible coatings can improve product quality and enable the incorporation of bioactive compounds that would not withstand thermal processing [19].

For gingerbread products, such coatings may help reduce spore attachment, limit oxygen transfer at the surface, or deliver antifungal agents through controlled release. When combined with microencapsulation, edible films may serve as a multifunctional layer of active packaging [18, 19].

12.7 Packaging and physical treatment methods

Packaging influences product spoilage by controlling oxygen availability, moisture exchange with the surrounding environment, and the microclimate at the product surface. For low-moisture products, selecting films with low water vapor transmission rates (WVTR) helps prevent moisture uptake under conditions of high ambient humidity. At the same time, for products prone to drying and hardening, an excessively strong barrier may lead to undesirable textural effects, such as condensation, and therefore requires careful optimization. Approaches to shelf-life evaluation also show that predicted storage stability may depend significantly on the sorption isotherm model applied. For this reason, selecting appropriate packaging materials requires product-specific sorption data [20].

Non-thermal methods can be used as an additional step after baking, when the product becomes most vulnerable to secondary microbial contamination. UV-C treatment can reduce surface fungal spoilage, but its effectiveness depends on selecting an appropriate dose and ensuring adequate irradiation of the entire product surface [21]. Cold atmospheric plasma has also been reported to reduce microbial load, although overly intensive treatment may cause surface drying and lead to changes in product texture [22].

Ozonation is more commonly applied for sanitizing air and surfaces in cooling and packaging areas, where secondary contamination most often occurs. In food facilities equipped with ozone-based air treatment systems, lower bacterial and fungal counts in the air and reduced bacterial contamination on surfaces have been reported [23].

Ionizing irradiation may be useful for certain types of packaged products intended for long-term storage. However, its use is generally justified only in cases where maximum microbiological stability is prioritized over possible changes in product quality [24].

12.8 Practical strategies for improving microbiological stability of products and directions for further research

No single method can fully prevent spoilage in filled gingerbread. Effective control requires the combination of complementary "hurdles". Based on the data discussed above, a practical integrated strategy may include:

1) designing fillings with low *a_w* and/or low pH. For example, high-solids fruit gels with pH around 3.3–3.5 [12, 13];

2) the use of water-binding ingredients in the gingerbread base. For example, glycerol up to about 5%, provided technological and sensory acceptability [11], together with the selection of flour types and hydrocolloids that help reduce moisture loss and staling [15];

3) the targeted application of antifungal preservatives in critical microenvironments (filling, interface, and product surface), with the effectiveness of potassium sorbate and propionates confirmed at the production level [7];

4) minimizing post-baking contamination through zoning, sanitation, and environmental monitoring [5, 7];

5) selecting packaging materials and edible coatings based on product-specific sorption behavior and barrier requirements [8, 19, 20].

The analysis carried out indicates the need for further in-depth research on the factors determining the stability of filled gingerbread during storage. In particular, studying local profiles of *a_w* and moisture at the crumb-filling interface appears especially promising, as this zone often represents a critical area for microbial development. Measurements should therefore be performed throughout the storage period under different conditions of relative humidity and ambient temperature, which would allow a more accurate assessment of moisture redistribution within the product.

Another important direction involves mathematical modelling of moisture migration in multicomponent systems, taking into account not only the properties of the product itself but also the characteristics of the packaging. Such models should integrate formulation parameters, water activity of individual components, WVTR, and storage conditions (relative humidity and temperature). Further validation of these models with experimental data would allow better prediction of product quality changes and support the selection of appropriate packaging for specific types of gingerbread.

A promising technological approach is the use of encapsulation techniques or structured systems within the filling that can act as barriers to moisture migration. Future studies should focus on selecting suitable capsule shell compositions, evaluating their resistance to mechanical and thermal stresses during mixing, forming, and baking, and assessing permeability and structural stability of the capsules during storage of finished products.

Further research should also address the evaluation of combined hurdle strategies for ensuring microbiological stability, with particular attention to fungi of the genera Aspergillus and Penicillium, as well as xerophilic species.

In addition, the development of standardized challenge-test protocols for filled gingerbread would be useful for obtaining comparable results across different studies. Such protocols should define the selection of test microorganisms, incubation conditions, and criteria for evaluating product acceptability. The implementation of these approaches would contribute to more effective control of microbiological stability and improve the safety of filled gingerbread products.

Conflict of interest

The authors declare that there is no conflict of interest in relation to this paper.

Data availability

Manuscript has no associated data.

Use of artificial intelligence statement

The authors used the AI assistant Perplexity (Grok 4.1, Perplexity AI) for translation and literature source selection. The authors bear full responsibility for the final manuscript. Generative AI tools are not credited and are not responsible for the final results.

Authors' contributions

Sergiy Smirnov: Writing – original draft, Writing – review and editing, Investigation, Visualization, Validation.

Olesia Priss: Supervision, Conceptualization, Methodology, Writing – original draft, Investigation, Writing – review and editing, Formal analysis.

Svitlana Danylenko: Writing – original draft, Investigation, Visualization, Validation.

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References

1. Bumozah, M., Alonaydheel, N., ALQarni, M., Bujulaya, A., Algamdi, A., Alawadh, A. (2022). Utilizing Some Spices and Their Essential Oils As Flavoring Agents, And Preserving Agents For Biscuits. Journal of Survey in Fisheries Sciences, 9 (4), 135–137. https://doi.org/10.53555/sfs.v9i4.2572
2. Souza, E. L. de, Stamford, T. L. M., Lima, E. de O., Trajano, V. N., Barbosa Filho, J. M. (2005). Antimicrobial effectiveness of spices: an approach for use in food conservation systems. Brazilian Archives of Biology and Technology, 48 (4), 549–558. https://doi.org/10.1590/s1516-89132005000500007
3. Osokina, N., Kostetska, K., Gerasymchuk, H., Voziian, V., Telezhenko, L., Priss, O. et al. (2017). Substantiation of the use of spice plants for enrichment of wheat bread. Eastern-European Journal of Enterprise Technologies, 4 (11 (88)), 16–22. https://doi.org/10.15587/1729-4061.2017.108900
4. Silveira, M. P., Moreira, K. G., Santos, M. A. de A., Andressa, I., Araújo, M. A., Neves, N. de A. et al. (2025). Enhancing Wholemeal Bread Shelf Life Using Optimized Mixtures of Cinnamon, Clove, and Bay Leaf Essential Oils. Chemistry & Biodiversity, 23 (1). https://doi.org/10.1002/cbdv.202501988

5. Vytřasová, J., Přibáňová, P., Marvanová, L. (2002). Occurrence of xerophilic fungi in bakery gingerbread production. International Journal of Food Microbiology, 72 (1–2), 91–96. https://doi.org/10.1016/s0168-1605(01)00626-2
6. Noshirvani, N., Abolghasemi Fakhri, L. (2024). Advances in Extending the Microbial Shelf-Life of Bread and Bakery Products Using Different Technologies: A Review. Food Reviews International, 41 (1), 87–112. https://doi.org/10.1080/87559129.2024.2386029
7. Rahman, M. N., Islam, M., Hasan, Md. R., Alim, Md. A., Begum, R., Li, S. et al. (2025). Evaluation of Gingerbread Spoilage Factors: Study of Fungal Causative Agents and Tracing of Their Environmental Sources. Applied Research, 4 (5). https://doi.org/10.1002/appl.70039
8. Cervenka, L., Rezkova, S., Kralovsky, J. (2008). Moisture adsorption characteristics of gingerbread, a traditional bakery product in Pardubice, Czech Republic. Journal of Food Engineering, 84 (4), 601–607. https://doi.org/10.1016/j.jfoodeng.2007.07.006
9. Zardetto, S., Martello, A. D., Pasini, G. (2025). Moisture migration model of packed fresh-filled pasta during storage under different humidity conditions. Innovative Food Science & Emerging Technologies, 100, 103930. https://doi.org/10.1016/j.ifset.2025.103930
10. Labuza, T. P., Hyman, C. R. (1998). Moisture migration and control in multi-domain foods. Trends in Food Science & Technology, 9 (2), 47–55. https://doi.org/10.1016/s0924-2244(98)00005-3
11. Ruzibayev, A., Abdurakhimov, A., Calvo-Gomez, O., Akhmedova, S., Kurambayev, S. (2024). Purification of Crude Glycerol Derived from Hydrogenated Cottonseed and Its Use in Confectionary Products. Rural Sustainability Research, 51 (346), 81–93. https://doi.org/10.2478/plua-2024-0008
12. Miquelim, J. N., Alcântara, M. R., Lannes, S. C. da S. (2011). Stability of fruit bases and chocolate fillings. Food Science and Technology, 31 (1), 270–276. https://doi.org/10.1590/s0101-20612011000100041
13. Slashcheva, A. V., Bodnaruk, O. A., Zakusilo, T. I., Dengub, A. D. (2024). Technology of thermo-resistant filling with bioprotective action for confectionery products. Suchasni tekhnolohii kharchovykh produktiv, 49 (2), 5–12.
14. Kovalchuk, K., Bodak, M., Katruk, M., Gyrka, O., Tkachenko, A., Guba, L. et al. (2020). Influence of raw materials on the change of crystal structure of gingerbread in the storage process. Technology Audit and Production Reserves, 1 (3 (51)), 53–57. https://doi.org/10.15587/2312-8372.2020.195507
15. Yorhacheva, E. H., Makarova, O. V., Khvostenko, E. V. (2014). Stabilisation of gummy gingerbread quality during storage. Eastern-European Journal of

Enterprise Technologies, 2 (12 (68)), 138. https://doi.org/10.15587/1729-4061.2014.23775

16. Tapia, M. S., Alzamora, S. M., Chirife, J. (2007). Effects of Water Activity (aw) on Microbial Stability: As a Hurdle in Food Preservation. Water Activity in Foods, 239–271. https://doi.org/10.1002/9780470376454.ch10
17. Bernard, F. G., Kermasha, S., Alli, I., Mulligan, C. N. (1999). Encapsulation in the food industry: a review. International Journal of Food Sciences and Nutrition, 50 (3), 213–224. https://doi.org/10.1080/096374899101256
18. Calderón-Oliver, M., Ponce-Alquicira, E. (2022). The Role of Microencapsulation in Food Application. Molecules, 27 (5), 1499. https://doi.org/10.3390/molecules27051499
19. Shulga, O., Chorna, A., Kobylinskyi, S. (2017). Differential scanning calorimetry research of biodegradable films for confectionery and bakery products. Chemistry & Chemical Technology, 11 (4), 492–496. https://doi.org/10.23939/chcht11.04.492
20. Lee, D. S., Robertson, G. L. (2022). Shelf-life estimation of packaged dried foods as affected by choice of moisture sorption isotherm models. Journal of Food Processing and Preservation, 46 (3). https://doi.org/10.1111/jfpp.16335
21. Romano, R. C., Restuccia, C., Rutigliano, C. A. C., Spartà, S., Parafati, L., Barbagallo, R. N. et al. (2024). Effect of UV-C Treatment on Shelf Life of Soft Wheat Bread (Bun). Foods, 13 (6), 949. https://doi.org/10.3390/foods13060949
22. Starek-Wójcicka, A., Różyło, R., Niedźwiedź, I., Kwiatkowski, M., Terebun, P., Polak-Berecka, M. et al. (2022). Pilot study on the use of cold atmospheric plasma for preservation of bread. Scientific Reports, 12 (1). https://doi.org/10.1038/s41598-022-26701-1
23. Caggiano, G., Lopuzzo, M., Spagnuolo, V., Diella, G., Triggiano, F., D'Ambrosio, M. et al. (2022). Investigations on the Efficacy of Ozone as an Environmental Sanitizer in Large Supermarkets. Pathogens, 11 (5), 608. https://doi.org/10.3390/pathogens11050608
24. Yusof, S. C. M, Mohamad, A., Abdul Nasir, Mohd. H., George, C., Abdul Wahab, A. (2024). Determination of the microbiological quality and acceptance of selected irradiated perishable food products during storage. International Journal of Innovation and Industrial Revolution, 6 (19), 140–151. https://doi.org/10.35631/ijirev.619011