DOI: 10.22616/FoodBalt.2019.046

SHORT COMMUNICATION

SHELF LIFE ASSESSMENT OF MEAT PIES

Liga Skudra[1*], Karlis Loba[2], Daiga Kunkulberga[1]

[1] *Department of Food Technology, Faculty of Food Technology, Latvia University of Life Sciences and Technologies, Rigas iela 22, Jelgava, Latvia, e-mail: liga.skudra@llu.lv*

[2] *Institute of Food and Environmental Hygiene, Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies, K. Helmana iela 8, Jelgava, Latvia*

Abstract

Demand for ready-to-use and frozen baked goods is increasing. It provides the supply of fresh products as close as possible to the final consumer. One of the traditional and popular products in Latvia is meat pies. The purpose of the study was to determine the shelf life of ready-to-eat, frozen and defrosted meat pies by evaluating the possible impact of various factors on quality and microbiological parameters. The study analysed classical meat pies from yeast dough with smoked meat and onion filling from one Latvian bread producer. Each of the two types of meat pies were frozen after baking, packed in two different packaging materials and stored at -18 °C for five days. The defrosting of pies was carried out at room temperature (23±2 °C). Tests for frozen products was started immediately after their defrosting and every 24 hours during storage. Physicochemical and microbiological indicators were determined for meat pies. The identification of microorganisms present in the products by species was performed using API CHB/E biochemical test. Potential sources of microbial contamination at the plant throughout the production process were also evaluated. The results of the study showed that shelf life of baked, frozen and defrosted cut meat pies was 72 hours, for classic meat pies shelf life did not exceed 48 hours. The study did not identify any positive effects of the selected packaging materials on the extension of shelf life. The identified microorganisms, which reduce the storage time of the products and food safety, were *B. subtilis* and *B. licheniformis*.

Keywords: meat pies, frozen products, defrosting, shelf life

Introduction

Freezing is one of the ways to extend the shelf life of products, increase production efficiency, and offer retailers and consumers safe, qualitive and fresh food. Freezing process reduces the content of the liquid phase in the product, which prevents microbial and enzymatic processes (Symons, 1994; Vasafi et al., 2019). The use of frozen ready-to-use products at retail outlets gives traders the opportunity to offer fresh products on the day of purchase (Meziani et al., 2012).

Inappropriate storage of raw materials of baked goods can lead to impairment in the quality of the product. The shelf life of baked-frozen products is determined by the quality of production, the cleanliness of the premises and the hygienic conditions of storage. The average shelf life of meat-filled dough pies after defrosting is 2–3 days. Frozen products can be stored for up to 12 months at -18±3 °C (Symons, 1994). After defrosting, the products become wastage during storage, which is about 4–5% by weight of the product. When the product is in a closed packaging, wastage increases the moisture content in the packaging, which promotes favourable conditions for the development of micro-organisms. Properly selected packaging ensures the longest possible storage time (Gupta et al., 2016; Krämer, Prange, 2016). Spore-forming microorganisms affect health, including *Bacillus cereus* and *Bacillus subtilis* (Pepe et al., 2003; Benwart, 2004; Saranraj, Geetha, 2012; Madigan et al., 2014).

The speed of growth and development of the microorganisms in the product depends on storage temperature, composition of the product, water activity (a_w), pH of the product and air quality in the production plant. By varying these factors, the deterioration and shelf life of the product is affected (Krämer, Prange, 2016; Lelieveld et al., 2016; De Boeck et al., 2017).

Bacterial spores can be in the air. In this way, they can cause visible damage to the food. Spore damage causes the formation of an unusual flavour and taste. Product deterioration is facilitated by enzyme reactions with carbohydrates, fats and proteins in the product, thereby promoting enzymatic, lipolytic and proteolytic changes. If *Bacillus* bacteria are detected in the company during air analysis, these organisms are difficult to control and eliminate (Sorokulova et al., 2003; Smith et al., 2010; Viedma et al., 2011). *Bacillus* spp. endospores are more resistant to elevated temperatures, UV and gamma rays than their vegetative cells. They die in the crumb and crust of the product at 160 °C in dry air for 2 hours or at 150 °C in water vapour environment after 15 minutes (Parihar, 2013; Stewart, 2015).

Constructive design and layout of industrial premises, production flows, and outdoor air pollution in production premises can affect the quality of finished products. It is therefore important to carry out systematic hygiene control and monitoring. During baking, the temperature in the centre of the product is close to 100 °C, but the temperature exposure time does not allow the bacterial endospores to die. Therefore, special control should be given to rooms where dough and filling are prepared (Benwart, 2004). Pollution in a manufacturing plant may result from inadequate cleaning of premises or hygiene practices that do not comply with the production specification. Even if the equipment is easy to disassemble and clean, it does not exclude the possibility that microorganisms may form on them, as well as dust or flour particles from the air (Lelieveld et al., 2016). Good personal hygiene can prevent unwanted contamination of products with

microorganisms, including the pathogens. Bacteria that cause food poisoning can be found on the hands of every employee, even a healthy person (Chao, 2003; Gupta et al., 2016).
The aim of the study was to determine the shelf life of ready-to-eat, frozen and defrosted meat pies by evaluating the possible impact of various factors on quality and microbiological parameters.

Materials and Methods

Materials

The meat pies from the yeast dough with smoked meat and onion filling were obtained from a Latvian bakery. One pie has a weight of 15 g, of which 10 g is a yeast dough and 5 g of meat filling (smoked pork cheeks meat with fresh onions, salt and pepper). After baking, the pies were frozen at -30±2 °C temperature, packed and stored at -18±2 °C. The defrosting was performed at room temperature (23±2 °C).

Physicochemical analysis

The water activity (a_w) was determined using a_w meter Novasina LabSwift-AW according to standard ISO 18787:2017 (ISO, 2017).
Moisture content was determined using Kern MLB N Air-Owen according to standard AACCI 44-15.02.
pH was determined using Jenway 3510 pH meter according to standard AACC 02-52.01.

Microbiological analysis

Samples of products for analysis of microorganisms were prepared according to standard ISO 6887-4:2017 "Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 1: General rules for the preparation of the initial suspension and decimal dilutions" (ISO, 2017). Triplicate plates were prepared using pour plate method for enumeration of total plate count on Plate Count Agar (Ref. 01-161, incubation at 30 °C for 72 h).
Swabs from the hands were taken in accordance with standard ISO 18593:2018 "Microbiology of the food chain. Horizontal methods for surface sampling". Swabs were applied to agar media "Biolife" Endo Agar (Ref. Nr. 4014602, incubation at 30 °C for 72 h). Testing of *E. coli* was performed according to standard ISO 7251:2005 (ISO, 2005). Gram staining, followed by API identification system (Ref. 50300, API50 CH strips, API50 CHB Medium, incubation at 30 °C for 48 h) was used for identification of microorganisms.

Product packaging

Two packaging materials were used in the study – DuPont Teijin Films, Milar OL40, single layer polyester packaging bags with 40 µm thickness, size 200×400 mm (marked in the text - PL), and single layer transparent crystallized polypropylene packaging bags with thickness 25 µm, size 200×400 mm (marked in the text – PU). The weight of one package was 200 g±15 g.

Statistical analysis

All measurements were performed in triplicate and reported as mean and standard deviation. The standard deviation and confidence level was calculated using a Microsoft Office Excel v13.0 application. For statistical processing of total plate count (TPC) in graphs, the results are expressed as lg CFU g^{-1}. The closeness of the relationships was determined by the coefficient "r" within the range [-1; 1]. Pearson's correlation coefficient was used for the cross-analysis of results indicators. The values were considered to be significantly different when $p<0.05$.

Results and Discussion

Sanitary hygienic control of workers was carried out during production. Swabs from the both hands of 10 employees were taken. Six employees worked on packing of frozen products, four – on product forming. During the work there is no rotation of these employees in the different departments of bakery. The presence of intestinal bacteria was not detected on the staff's hands in the packaging and forming department.
Meat pies are a combined product that contains vegetable and animal raw materials. Such physical factors as water activity (a_w) and pH are decisive factors in the development of microorganisms as bacteria, moulds, microscopic fungi development in baked goods (Kilcast, Subramaniam, 2011). Water activity (a_w) can be one of the parameters for monitoring food safety and shelf life. The manufacturer can then obtain complete information on changes in the product during production (Jay et al., 2005).
Water activity (a_w) was determined in frozen meat pies after thawing and for the next three days during storage in two different packaging materials.

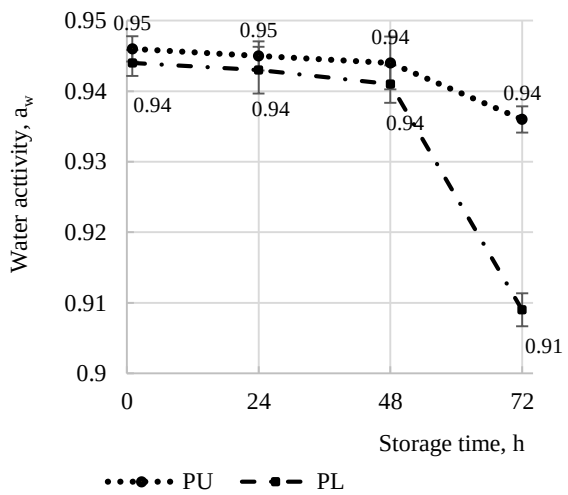

Figure 1. Water activity in meat pies during the storage time
PL – polyester packaging, PU – polypropylene packaging

The results of the study show that the water activity (a_w) of meat pies packed in PU packaging decreased by 1.06% (from 0.946 to 0.936) during storage, but in pies packed in PL packaging – by 3.71% (from 0.944 to

0.909). Comparing the packaging differences, we can say that for products packaged in PU-packaging, water activity (aw) was by 2.89% higher than in the PL-packaging (Fig. 1).

Moisture analysis showed that changes in moisture content during storage were not significant (p>0.05). Moisture content of pies during storage packed in PU increased by 0.58% (from 34.3 to 34.5%), but packed in PL – by 0.89% (from 33.7 to 34.0%). Changes in moisture content of less than 1% indicated that both types of packaging during storage prevent the release of moisture generated during defrosting, which contributes to the development of microorganisms.

Microorganism activity and product composition contribute to pH changes in the product. The pH of the product is one of the parameters for monitoring food safety and shelf life, so it should be periodically determined for each batch of products (Jay et al., 2005). In all samples, regardless of the type of packaging, there was a decrease in pH. During storage for meat pies in PU packaging, pH dropped from 6.06 to 5.70, while for meat pies packaged in PL packaging, the pH dropped from 6.14 to 5.81 (Fig. 2). Analysis shows that changes in pH during storage in different packaging material were not significant (p>0.05).

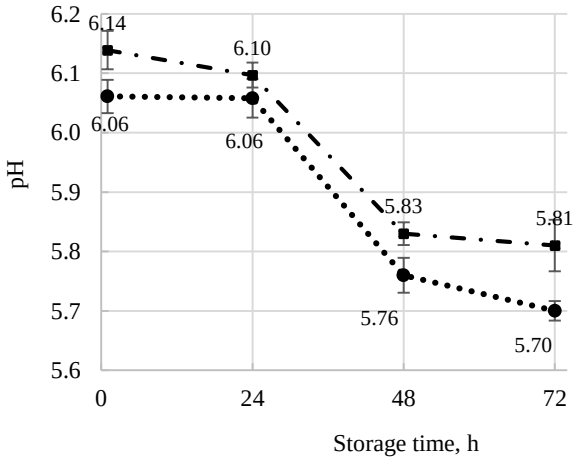

Figure 2. pH in meat pies during the storage time
PL – polyester packaging, PU – polypropylene packaging

The results show that the packaging material did not affect the growth rate of microorganisms. The TPC of the meat pies packed in PU packaging reached 3.53 lg CFU g^{-1} after 48 hours and in meat pies packed in PL packaging – 3.51 lg CFU g^{-1} (Fig. 3). According to the guidelines for assessing microbiological safety, the TPC for bakery is "satisfactory" below 10^4 CFU g^{-1}, "border line" between 10^4–10^6 CFU g^{-1} and "unsatisfactory" when more than 10^6 CFU g^{-1} (Health Protection Agency, 2009). The study followed the quality standards of the bakery for their products. They require that the size of the CFU indicator for meat-filled baked goods should not exceed 3.50 lg CFU g^{-1}. Therefore, the meat pies became "unsatisfactory" within 48 hours at ambient conditions after defrosting.

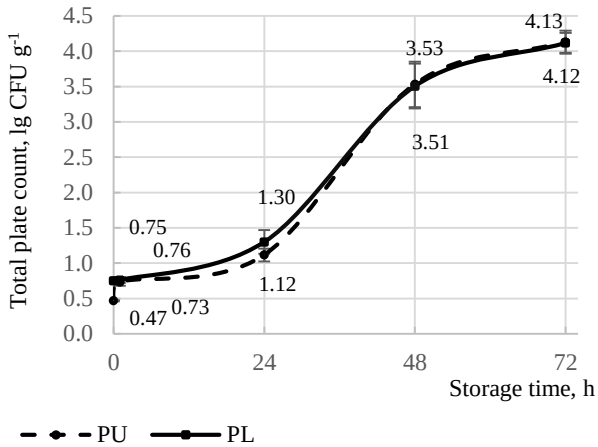

Figure 3. Total plate count of packaged meat pies during the storage time
PL – polyester packaging, PU – polypropylene packaging

If storage after defrosting exceeds 48 hours, then the product is considered unsafe for human consumption.

The close correlation exists between the total number of bacteria and pH, regardless of the packaging material. Pearson's correlation between these indicators was close and negative (Fig. 4). The correlation indicates if the total plate count decreases, the pH of the products is approaching the frozen and two-hour defrosted product parameters.

After microbial testing of products, it can be concluded that none of the product samples showed the presence of *E. coli*. All samples had bacteria from the *Bacillus* family, aerobic endospores. Bacterial endospores are found in products from raw materials, filling and forming process. No mould formation on the surface of the products was found during the defrosting-time of the finished products and throughout the storage. This can be explained by the fact that the oven temperature of 180 °C is high enough to destroy mould spores.

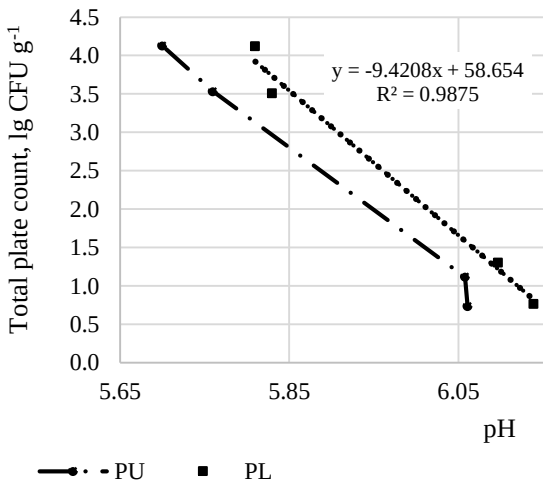

Figure 4. Pearson's correlation between total plate count and pH of meat pies in two packaging materials
PL – polyester packaging, PU – polypropylene packaging

As we know from literature, the deterioration in product quality is caused by *B. subtilis* and *B. licheniformis*. These bacteria form endospores that are heat resistant and are present in flour and air (Sorokulova et al., 2003). According to the results of the API test, after 72 hours of storage all samples of the meat pies showed signs of deterioration caused by the *Bacillus* species. Changes in quality also were observed. The samples had an unpleasant flavour, crust was unusual for the fresh product, it was soft and crumbled easily.

Packaging in which the products were defrosted and stored contributed to the growth of the bacteria because during defrosting packaging material did not allow the removal of moisture which is always released during defrosting. Similar results have been obtained by other scientists (Saranraj, Geetha, 2012).

Conclusions

Although water activity (a_w) in meat pies slightly decreased during three-day storage, it was high enough (0.946) for moisture to be available for the development of microorganisms.

The microbiological deterioration of the products was caused by *Bacillus* bacteria, of which *Bacillus subtilis* and *Bacillus licheniformis* were identified. Product contamination in the process of forming products with *Bacillus* bacteria more often is possible from air and raw materials.

In the study, comparing the packaging of products from polypropylene and polyethylene material, it can be concluded that both types of packaging are similar and do not prevent the development of microorganisms.

The shelf life of frozen meat pies after defrosting is up to 48 hours in the polypropylene or polyethylene packaging.

References

1. Benwart G. J. (2004) *Basic Food Microbiology* (2nd edn.). CBS Publishers & Distributors Pvt. Ltd. 773 p.
2. Chao T. S. (2003) *Workers personal hygiene in Food plant sanitation*. New York: Marcel Dekker Inc., p. 132–145
3. De Boeck E., Jacxsens L., Goubert H., Uyttendaele M. (2017) Ensuring food safety in food donations: Case study of the Belgian donation/acceptation chain. *Food Research International*, Vol. 100 (2), p. 137–149.
4. Gupta R. K., Dudeja P., Minhas S. (2016) *Food Safety in the 21st Century: Public Health Perspective*. Elsevier Science. 624 p.
5. *Health Protection Agency. Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods. London, 2009* [accessed on 15.01.2019.]. Available: https://www.gov.uk/govern-ment/publications/ready-to-eat-foods-microbiological-safety-assessment-guidelines https://www.iso.org /standard/63335.html
6. *International Organization for Standardization (ISO), 2017. Foodstuffs - Determination of water activity* [accessed on 14.01.2019]. Available: https://www.iso.org/standard/63379.html
7. *International Organization for Standardization (ISO). ISO 6887-4:2017 Microbiology of the food chain. -* [accessed on 14.01.2019]. Available: https://www.iso.org/obp/ui/#iso:std:iso:6887:-4:ed-2:v1:en
8. *International Organization for Standardization (ISO). ISO 7251:2005. Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of presumptive Escherichia coli* [accessed on 14.01.2019]. Available: https://www.iso.org/standard/34568.html
9. Jay J. M., Loessner M. J., Golden D. A. (2005) *Intrinsic and extrinsic parameters of foods that affect microbial growth.* New York: Springer Science & Business Med Inc., p. 39–60
10. Kilcast D., Subramaniam P. (2011) *Food and Beverage Stability and Shelf Life* (1st edn.). Woodhead Publishing. 864 p.
11. Krämer J., Prange A. (2016) *Lebensmittel-Mikrobiologie*. Stuttgart: Uni-Taschenbücher. p. 400.
12. Lelieveld H. L. M., Holah J., Mostert M. A. (2016) *Handbook of Hygiene Control in the Food Industry* (2nd edn.). London: Woodhead Publishing. 720 p.
13. Madigan M. T., Martinko J. M., Bender K. S., Buckley D. H., Stahl D. A., Brock T. (2014) *Brock Biology of Microorganisms* (14th edn). New Jersey: Pearson. 1032 p.
14. Meziani S., Kaci M., Jacquot M., Jasniewski J., Ribotta P., Muller J.M., Ghoul M., Desobry S. (2012) Effect of freezing treatments and yeast amount on sensory and physical properties of sweet bakery products. *Journal of Food Engineering*, Vol. 111 (2), p. 336–342.
15. Parihar H. S. (2013) *Bacillus cereus*. Encyclopedia of Toxicology (3rd edn.). p. 353–354.
16. Pepe O., Blaiotta G., Moschetti G., Greco T., Villan F. (2003) Rope-producing strains of *Bacillus* spp. from wheat bread and strategy for their control by lactic acid bacteria. *Applied and Environmental Microbiology*, p. 2321–2329.
17. Saranraj P., Geetha M. (2012) Microbial spoilage of bakery products and its control by preservatives. *International Journal of Pharmaceutical & Biological Archives*, Vol. 3(1), p. 38–48.
18. Smith J. P., Daifas D. P., El-Khoury W., Koukoutsis J., El-Khoury A. (2010) Shelf life and safety concerns of bakery products — A Review. *Critical Reviews in Food Science and Nutrition*, Vol. 44(1), p. 19–55.
19. Sorokulova I. B., Reva O. N., Smirnov V. V., Pinchuk I. V., Lapa S. V., Urdaci M. C. (2003) Genetic diversity and involvement in bread spoilage of *Bacillus* strains isolated from flour and ropy bread. *Letters in Applied Microbiology*, Vol. 37(2), p. 73–169.
20. Stewart G. C. (2015) The exosporium layer of bacterial spores: A connection to the environment and the infected host. *Microbiology and molecular biology reviews*, Vol. 79(4), p. 437–457.
21. Symons H. Frozen foods, **In:** Shelf life evaluation of foods (C. M. D. Man and A. A. Jones, eds.), Blackie Academic & Professional, London, 1994, p. 298–311.
22. Vasafi P.S., Hamdami N., Keramat J. (2019) Quality and microbial stability of part-baked “Barbari bread” during freezing storage. *LWT – Food Science and Technology*, Vol. 104, p. 173–179.
23. Viedma P. M., Abriouel H., Omar N. B., López R. L., Gálvez A. (2011) Inhibition of spoilage and toxigenic *Bacillus* species in dough from wheat flour by the cyclic peptide enterocin AS-48. *Food Control*, Vol. 22(5), p. 756–761.