Facultative thermophilic microorganisms in potato products in retort packaging

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Thermally treated potato products in retort packaging with an extended shelf-life (up to 90 days before opening) at room temperature (20 ± 1 °C) from two Latvian vegetable processing companies (A and B) were analysed in this research. The aim of this research was to determine the presence of thermotolerant microbial contamination in thermally treated potato products in retort packaging, to ensure their safety and suitability for immediate consumption. Samples from three production batches were used.

The total plate count was determined, and a microscopic examination of potato product samples was performed. Microscopic examination of microorganisms showed the presence of microbial vegetative forms in potato products from company B, but the standard investigation did not register them; therefore, Tryptone Glucose Yeast Extract Agar (TGYA), TGYA enriched with 1 % starch solution and TGYA enriched with 5 % starch solution were used for potential microorganism isolation. Samples were incubated at 25 °C; 37 °C; 41.5 °C; 50 °C; 55 °C and 60 °C temperatures under aerobic and anaerobic conditions, incubation time 12, 24 and 48 h. Facultative thermophilic Bacillus licheniformis bacteria which produce thermotolerant spores were isolated from thermally treated potato products in retort packaging from company B. The average colony count in company B samples was 1.27×10³ CFU/g (SD = 78.25).

The presence of microorganisms was not detected in potato products produced at company A, while facultative thermophilic and thermotolerant microorganisms were found in potato products produced at company B.

Key words: potato products, thermophilic and thermotolerant microorganisms, thermal treatment, retort packaging.

Introduction

With an increasing consumer demand for high quality food, manufacturing companies need to develop innovative solutions for an efficient processing of products in order to inactivate the undesirable product microflora that may endanger consumers’ health [1]. In the recent years, food infections have been diagnosed in increasing amounts. Food infectious agents, such as Salmonella spp., verotoxigenic E. coli, norovirus, enteroviruses, etc., enter the human body mainly via consumed meat, fresh fruit, vegetables, and water [2]. The demand for safe food with an extended shelf-life has increased greatly [3]. Raw potatoes are difficult to digest, therefore, several ways of cooking are used, such as boiling, baking, braising, grilling, microwave treatment, all of which improve the structure of potatoes and make them easier to use in the diet, but potatoes have a relatively short shelf life after these treatments [4]. Due to the growing demand for convenience and high quality products, potato processors are constantly searching for new and innovative solutions to effectively use potatoes [5]. One of the possibilities to extend potato shelf-life is thermal treatment in packaging (retort packaging). Heat treatment is a common food preservation method. Optimal heat treatment regimen for each type of food has to be selected in order to reduce unwanted quality losses and improve the desired increase in quality, while ensuring microbiological safety [6]. Currently, product processing in packaging is one of the leading processing technologies that provide for a long-term storage of the product at room temperature [7].

However, the quality focus should be on the product safety in thermally treated vegetables, as pathogenic microorganisms such as Clostridium spp. and Bacillus spp. can develop after thermal treatment [2]. Food processing companies are exposed to various risks of food contamination because soil (where the raw materials come from) is the source of pathogenic spore-producing bacteria. Contamination with spores during food production can occur in several ways: spores can move with the help of soil dust, food processing machines can come into contact with raw products, or an employee who is in breach of hygiene may become one of the sources of contamination. There are stages in the technological process of cooked potatoes where the product may be contaminated with the undesirable microflora. Potatoes are washed, peeled, cut, placed in packaging; packages are hermetically sealed and subjected to heat treatment. However, between pre-treatment and sterilization (in low-power food companies), the product is kept at relatively
high temperatures, and during this time facultative thermophilic bacteria are capable of forming spores or even multiply [8]. To avoid contamination of the product, it is important to understand which process stages are at high risk of food contamination with undesirable microflora [8].

One of the requirements in food processing is to neutralize biological threat and choose the appropriate type of treatment. The pathogenic spore producing microflora is most common in thermally treated vegetables [2]. Heat treatment above 100 °C normally kills off microorganism vegetative forms and partially or totally – pathogen spores [9]. "The Codex Alimentarius" states that in order to ensure a safe distribution of food products, manufacturers need to store heat-treated products at 37 °C for 10 to 14 days. There are several features that may indicate food spoilage in heat-treated products in packaging. Gas formation inside the package results in packaging bloating, forming of unusual flavour, colour and / or pH changes. If any of the features are observed during post-treatment incubation, the company is obliged to destroy the produced batches to prevent consumers from the risk of infection [9, 10].

In vegetables with a low acidity pH > 4.5, microbiological deterioration is observed at the incubation temperature of +40 °C when a rapid development and growth of thermophilic microorganisms take place. For example, facultative thermophilic bacteria Bacillus coagulans, Bacillus licheniformis, Anoxybacillus spp., Paenibacillus spp., Thermoaerobacter spp. and Clostridium thermohabitantium are able to survive 10 min of heat treatment above 100 °C [8]. The most frequent facultative thermophilic microorganism in heat-treated vegetables is B. licheniformis which is more resistant to higher temperature and pressure than other bacteria of the genus Bacillus [11].

Bacteria from the genus Bacillus are soil microorganisms; on average, 10 000 spores can be found in 1 g of soil. Bacillus species are capable of producing spores in adverse environmental conditions, and they can survive in an acid medium and at elevated temperature; furthermore, the higher the temperature in which Bacillus bacteria produce spores, the higher is their resistance to heat treatment [12].

Spores can persist for a long time in the soil and on the surface of products, as well as in the industrial environment. This can cause food spoilage and sometimes food toxicoinfection in people; therefore, an early detection of this microorganism is crucial in order to ensure food security and avoid economic losses. It is necessary to choose the most efficient process capable of providing for the safety of the product [13, 14, 15].

It is necessary to identify thermophilic spore-forming bacteria and their viability after heat treatment when the industry of heat-treated products in packaging in Latvia is developing. The aim of this research was to determine the presence of thermotolerant microbial contamination in thermally treated potato products in retort packaging, to ensure their safety and suitability for immediate consumption.

Materials and methods

The research was carried out at the laboratories of the Institute of Food and Environmental Hygiene, Faculty of Veterinary Medicine, Latvia University of Agriculture and Food and Environmental Investigations, Institute of Food Safety, Animal Health and Environment BIOR. The research was conducted according to the given research structure (Fig. 1).

**Fig. 1.** Research structure

Samples from three batches from two Latvian vegetable-processing companies (A and B) were acquired at different grocery stores in Latvia. Thermally treated potato products in retort packaging with an extended shelf-life (up to 90 days before opening) are stored at food shops at +6 to +10 °C, but the information provided on the product label specifies the storage temperature of +2 to +25 °C (A company) and +1 to +20 °C (B company). After purchase, thermally treated potato products were stored at +20 ± 2 °C for three days.

Overall samples from six different batches were studied. Initially, all samples were subjected to microscopic examination during which vegetative forms of microorganisms in the product were determined. Microscopic examinations were performed with an OLYMPUS BX43F microscope (Japan) at magnification 1000× (10 × 100).
The total plate count (aerobic and facultative anaerobic, mesophilic bacteria) was determined according to the EN ISO 4833:2003 standard “Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of microorganisms -- Colony-count technique at 30 °C” on the Plate Count Agar (PCA) agar medium.

Tryptone Glucose Yeast Extract Agar (TGYA), TGYA enriched with 1 % starch solution and TGYA enriched with 5 % starch solution were used for potential microorganism isolation from potato products. Samples were incubated at 25 °C; 37 °C; 41.5 °C; 50 °C; 55 °C and 60 °C temperatures under aerobic and anaerobic conditions, incubation time 12, 24 and 48 h.

Microorganism culture identification was carried out according to the biochemical identification method.

Results and discussion

Consumer demand for food products depends on the quality of the product and its convenient use [16]. Consumers must be sure that purchased products are safe to use in their diet and that there is no risk of infection with pathogenic microorganisms which may maintain their viability in the product after an incomplete technological process [1].

The production of thermally treated potato products in retort packaging is rapidly developing [17]. Potato preparation may take up to 40 minutes at home (pre-treatment plus preparation time). Nowadays, consumers’ daily life has become increasingly more dynamic; therefore, the demand for products and services that save time resources is increasing [18].

Thermally treated potato products in retort packaging (ready for use with an extended shelf-life) are commercially available at the market in Latvia, produced by two Latvian vegetable processing companies (A and B). After a three-day storage at +20 ± 2 °C, gas formation and deformation of the packages, which shows the gas-producing microorganism vegetative form presence in the product, was observed in all samples from company B. The optimal storage temperature for potato products from company B is +1 to +20 °C (indicated on the label). External changes, suggesting product quality changes, were not observed in potato product samples from company A (optimal storage temperature +2 to +25 °C). The microscopic investigation of B samples showed the presence of rod-shaped bacteria, while microorganisms were not detected in A samples. Despite the obtained results, A samples were also used in the subsequent microbiological examination to fully compare the quality of the products and their microbiological safety.

After incubation, TPC was <10 CFU/g, and it was impossible to isolate microorganism vegetative forms. As the microscopic investigation showed the presence of microorganisms in B samples, Tryptone Glucose Yeast Extract Agar (TGYA), TGYA enriched with 1 % starch solution and TGYA enriched with 5 % starch solution were used for the further research. Agar medium enrichment with starch was performed to adjust the nutrient feed of potato products.

In B potato product samples, the development of microorganisms under both aerobic and anaerobic conditions was observed after 12 h at +37 °C to +55 °C, which suggests that microorganisms were facultative anaerobic.

After 24 h, the microorganism count in B samples significantly increased at temperatures +37 °C to +60 °C under aerobic and anaerobic conditions; the most rapid microorganism growth was observed at +55 °C. In A samples, microorganism presence was not observed at any temperature and environment regimens.

The average colony count in company B samples was 1.27 × 100 CFU/g (SD = 78.25).

After 48 h microorganism colonies were observed in B samples in the culture medium incubated at +25 °C under aerobic and anaerobic conditions as well, which clearly demonstrates that these microorganisms are facultative thermophiles. The results showed that microorganism vegetative form isolation was possible with the TGYA medium and TGYA enriched with a starch solution. Visually assessing the growth media after the incubation period, it can be concluded that the microorganism has the ability to hydrolyse starch, and it is one of saprophytic bacteria which can possibly produce amylolytic enzymes.

Microorganism culture identification according to the biochemical identification method was carried out to determine which microorganism was present in B samples. The results showed that Bacillus licheniformis was present in thermally treated potato products in retort packaging from company B. Bacillus licheniformis is known as one of food infectious agents. The main symptoms of infection are nausea, vomiting, diarrhea, and abdominal pain. B. licheniformis is also associated with several clinical manifestations in humans, such as bowel disease, septicaemia, peritonitis, eye inflammation, etc. [19, 20, 21]. Food-borne illness cases initiated by B. licheniformis are mostly associated with the use of cooked meat, fresh and pasteurized vegetables, and dairy products in the diet [18]. The results obtained in this research have established that thermally treated potato products in retort packaging from company B are not safe for human consumption if stored at temperatures specified on the labelling, because at room temperature the microorganism vegetative forms which produce toxins begin to develop inside the packaging. This type of food is dangerous for the safety of consumers.

Conclusions

It can be concluded that potato products from company A meet the defined quality requirements and are suitable and safe for human consumption after comparison of thermally treated potato products in retort packaging from two Latvian vegetable processing companies (A and B).

Facultative thermophilic Bacillus licheniformis bacteria which produce thermostolerant spores were isolated from thermally treated potato products in retort packaging from company B. Potato products from company B do not meet the defined quality requirements and are not safe for human consumption if stored at
temperatures specified on the labelling (+1 to +20 °C). Consumption of this type of food can cause a serious toxico-infection.

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References

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FAKULTATYVŪS TERMOFILINIAI MIKROORGANIZMAI, RASTI BULVIŲ PRODUKTUOSE LAMINUOTOJE PAKUOTĖJE

Santrauka

Darbe tirti dviejų Latvijos daržovių perdirbimo įmonių (A ir B) prailginto vartojimo termino (daugiau kaip 90 dienų iki atidarymo) termiškai apdoroti laminuotoje pakuotėje esantys bulvių produktai, laikyti kambario temperatūroje (20 ± 1°C). Šio tyrimo tikslas – nustatyti termiškai apdorotų bulvių produktų laminuotoje pakuotėje mikrobiologinį užterštumą, įvertinti ją saugą ir tinkamumą tiesioginiam vartojimui.

Bulvių produktuose nustatytas bendras mikroorganizmų skaičius, taip pat mėgintai, tirti mikroskopu. Tyrimai mikroskopu parodė, kad bulvių produktuose, gautuose iš B įmonės, buvo vegetatyviniių mikroorganizmų, tačiau taikant standartinius nustatymo metodus mikroorganizmų nebuvo rasta, todėl potencialiems mikroorganizmams išskirti buvo naudotas triptono, gliukozės ir mielių ekstrakto agaras, papildytas 1 ir 5 % krakmolio tirpalu. Mėgintai 48 valandos inkubuoti 25; 37; 41,5; 50; 55 ir 60 °C temperatūroje aerobinėmis ir anaerobinėmis sąlygomis.

Gauti tyrimų rezultatai parodė, kad bulvių produktuose, gautuose iš A įmonės, mikroorganizmų nebuvo nustatyta, o bulvių produktuose, gautuose iš B įmonės, fakultatyvės termofilinės ir aukštiems temperatūriniams režimams atsparių Bacillus licheniformis bakterijų buvo rasta.