ISSN 1392 – 1231. CHEMINĖ TECHNOLOGIJA. 2015. Nr. 1 (66)

Facultative thermophilic microorganisms in potato products in retort packaging

A. Ruzaike, S. Muizniece-Brasava
Department of Food Technology, Faculty of Food Technology, Latvia University of Agriculture, Liela Str. 2, Jelgava Palace, LV-3001 Jelgava, Latvia
E-mail: aija.ruzaike@gmail.com

K. Kovalenko
Institute of Food and Environmental Hygiene, Faculty of Veterinary Medicine, Latvia University of Agriculture, K. Helmana Iela 8, LV-3004 Jelgava, Latvia

crossref http://dx.doi.org/10.5755/j01.ct.66.1.12363

Received 20 April 2015; Accepted 14 July 2015

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 1.27×100 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 extent 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., *Thermoanaerobacter* spp. and *Clostridium thermobutyricum* /*thermopalmarium* 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).

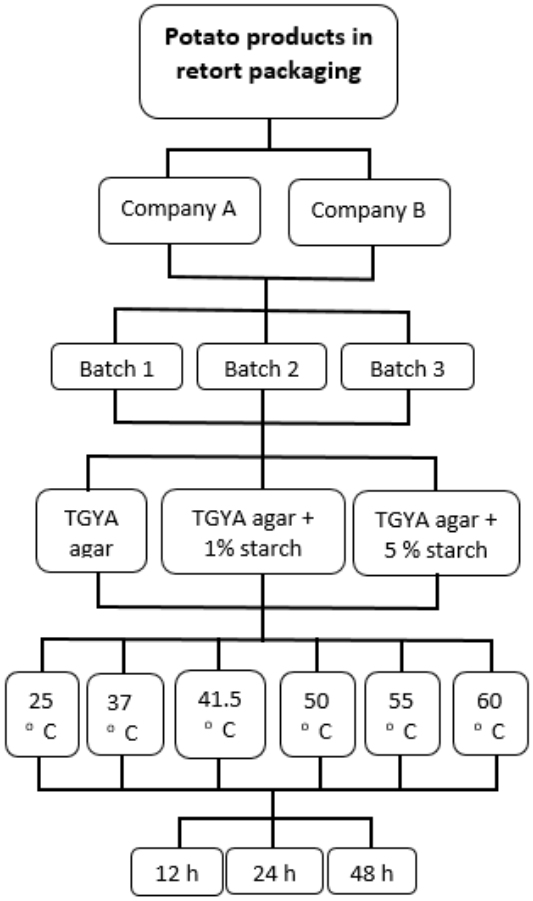

Fig. 1. Research structure

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. lichenisformis* 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 thermotolerant 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 toxicoinfection.

Acknowledgement

This research was supported by the project co-funded by local resources research and sustainable use programme "Agricultural Resources for Sustainable Production of Qualitative and Healthy Foods in Latvia (AgroBioRes) (2014–2017)", Project No. 4 "Sustainable use of local agricultural resources for qualitative and healthy food product development (FOOD)".

References

1. **Singh A. P., Singh A., Ramaswamy H. S.** Modification of a static steam retort for evaluating heat transfer under reciprocation agitation thermal processing // Journal of Food Engineering. 2015. Vol. 153. P. 63–72. http:/dx.doi.org/10.1016/j.jfoodeng.2014.12.001
2. **Nguyen-The C.** Biological hazards in processed fruits and vegetables – Risk factors and impact of processing techniques // LWT - Food Science and Technology. 2012. Vol. 49. N 2. P. 172–177. http:/dx.doi.org/10.1016/j.lwt.2012.07.002
3. **Valceschini E.** Poultry meat trends and consumer attitudes // EPC 2006 – 12th European Poultry Conference, Verona, Italy, 10–14 September, 2006, P. 1–10.
4. **Carini E., Curti E., Littardi P., Luzzini M., Vittadini E.** Water dynamics of ready to eat shelf stable pasta meals during storage // Innovative Food Science & Emerging Technologies. 2013. Vol. 17. P. 163–168. http:/dx.doi.org/10.1016/j.ifset.2012.09.010
5. **Abu-Ghannam N., Crowley H.** The effect of low temperature blanching on the texture of whole processed new potatoes // Journal of Food Engineering. 2006. Vol. 74. N 3. P. 335–344. http:/dx.doi.org/10.1016/j.jfoodeng.2005.03.025
6. **Lemmens L., Colle I., Knockaert G., Buggenhout S. V., Loey A. V., Hendrickx M.** Influence of pilot scale in pack pasteurization and sterilization treatments on nutritional and textural characteristics of carrot pieces // Food Research International, 2013. Vol. 50. P. 526–533. http:/dx.doi.org/10.1016/j.foodres.2011.02.030
7. **Ito R., Fukuoka M., Hamada-Sato N.** Innovative food processing technology using ohmic heating and aseptic packaging for meat // Meat Science. 2014. Vol. 96. N 2. Part A. P. 675–681. http:/dx.doi.org/10.1016/j.meatsci.2013.10.012
8. **Durand L., Planchon S., Guinebretiere M. H., André S., Carlin F., Remize F.** Contamination pathways of spore-forming bacteria in a vegetable cannery // International Journal of Food Microbiology. 2015. Vol. 202. P. 10–19. http:/dx.doi.org/10.1016/j.ijfoodmicro.2015.02.019
9. **André S., Zuber F., Remize F.** Thermophilic spore-forming bacteria isolated from spoiled canned food and their heat resistance. Results of a French ten-year survey // International Journal of Food Microbiology. 2013. Vol. 165. N 2. P. 134–143. http:/dx.doi.org/10.1016/j.ijfoodmicro.2013.04.019
10. **Ramos B., Miller F. A., Brandão T. R. S., Teixeira P., Silva C. L. M.** Fresh fruits and vegetables – An overview on applied methodologies to improve its quality and safety // Innovative Food Science and Emerging Technologies. 2013. Vol. 20. P. 1–15. http:/dx.doi.org/10.1016/j.ifset.2013.07.002
11. **Tola Y. B., Ramaswamy H. S.** Combined effects of high pressure, moderate heat and pH on the inactivation kinetics of *Bacillus licheniformis* spores in carrot juice // Food Research International. 2014. Vol. 62. P. 50–58. http:/dx.doi.org/10.1016/j.foodres.2014.02.006
12. **Fernández-No I. C., Guarddon M., Böhme K., Cepeda A., Calo-Mat a P., Barros-Velázquez J.** Detection and quantification of spoilage and pathogenic *Bacillus cereus*, *Bacillus subtilis* and *Bacillus licheniformis* by real-time PCR // Food Microbiology. 2011. Vol. 28. N 3. P. 605–610. http:/dx.doi.org/10.1016/j.fm.2010.10.014
13. **Baril E., Coroller L., Couvert O., Leguérinel I., Postollec F., Boulais C., Carlin F., Mafart P.** Modeling heat resistance of *Bacillus weihenstephanensis* and *Bacillus licheniformis* spores as function of sporulation temperature and pH // Food Microbiology. 2012. Vol. 30. N 1. P. 29–36. http:/dx.doi.org/10.1016/j.fm.2011.09.017
14. **Caamaño-Antelo S., Fernández-No I.C., Böhme K., Ezzat-Alnakip M., Quintela-Baluja M., Barros-Velázquez J., Calo-Mata P.** Genetic discrimination of foodborne pathogenic and spoilage *Bacillus* spp. based on three housekeeping genes // Food Microbiology. 2015. Vol. 46. P. 288–298. http:/dx.doi.org/10.1016/j.fm.2014.08.013.
15. **Nieminen T., Rintaluoma N., Andersson, M., Taimisto A.M., Ali-Vehmas T., Seppälä A., Priha O., Salkinoja-Salonen M.** Toxinogenic *Bacillus pumilus* and *Bacillus licheniformis* from mastitic milk // Veterinary Microbiology. 2007. Vol. 124. N 3–4. P. 329–339. http:/dx.doi.org/10.1016/j.vetmic.2007.05.015
16. **Chen Q., Anders S., An H.** Measuring consumer resistance to new food technology: A choice experiment in meat packaging // Food Quality and Preference. 2013. Vol. 28. N 2. P. 419–428. http:/dx.doi.org/10.1016/j.foodqual.2012.10.008
17. **Day L., Xu M., Øiseth S. K., Mawson R.** Improved mechanical properties of retorted carrots by ultrasonic pre-treatments // Ultrasonics Sonochemistry. 2012. Vol. 19. N 3. P. 427–434. http:/dx.doi.org/10.1016/j.ultsonch.2011.10.019
18. **Olsen N. V., Sijtsema S. J., Hall G.** Predicting consumers' intention to consume ready-to-eat meals. The role of moral attitude // Appetite. 2010. Vol. 55. N 3. P. 534–539. http:/dx.doi.org/10.1016/j.appet.2010.08.016
19. **Salkinoja-Salonen M., Vuorio, M. Andersson R., Kampfer P., Honkanen-Buzalski T.** Toxigenic strains of *Bacillus licheniformis* related to food poisoning // Applied and Environmental Microbiology. 1999. Vol. 65. N 10. P. 4637–4645.
20. **Logan N. A.** Bacillus and relatives in foodborne illness // Journal of Applied Microbiology. 2012. Vol. 112. N 3. P. 417–429. http:/dx.doi.org/10.1111/j.1365-2672.2011
21. **Postollec F., Mathot A. G., Bernard M., Divanac'h M. L., Pavan S., Sohier D.** Tracking spore-forming bacteria in food: From natural biodiversity to selection by processes // International Journal of Food Microbiology. 2012. Vol. 158. N 1. P. 1–8. http:/dx.doi.org/10.1016/j.ijfoodmicro.2012.03.004

A. Ruzaike, S. Muizniece-Brasava, K. Kovalenko

FAKULTATYVŪS TERMOFILINIAI MIKROORGANIZMAI, RASTI BULVIŲ PRODUKTUOSE LAMINUOTOJE PAKUOTĖJE

S a n t r a u k a

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 jų saugą ir tinkamumą tiesioginiam vartojimui.

Bulvių produktuose nustatytas bendras mikroorganizmų skaičius, taip pat mėginiai, tirti mikroskopu. Tyrimai mikroskopu parodė, kad bulvių produktuose, gautuose iš B įmonės, buvo vegetatyvinių 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 % krakmolo tirpalu. Mėginiai 48 valandas 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, fakultatyvinės termofilinės ir aukštiems temperatūriniams režimams atsparių *Bacillus lichneniformis* bakterijų buvo rasta.