MICROBIOLOGICAL SAFETY OF FUNCTIONAL READY TO EAT POTATO MEALS DURING THE STORAGE

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Abstract
Changes in consumer lifestyle, which requires a convenient food consumption, aids in the growing demand for ready to eat meals. It is important to ensure not only healthy but also microbiologically safe ready to eat meals. Thermal treatment is intended to ensure industrial sterility of the products. Commercially sterile food is processed in packaging, and the temperature is the key to reduce the amount of microorganisms in the food to a level where the food is free from viable forms of microorganisms, including spores.

The aim of this study was to determine the efficiency of thermal treatment on functional ready to eat meals in flexible packaging.

Five types of ready to eat meals were prepared for this study (control samples - potatoes and potatoes with chicken fillet, and functional ready to eat meals - potatoes with amaranth, quinoa, and bulgur). Ready to eat meals were filled in pouches of two different packaging materials (PA/PE and PET/ALU/PA/PP), hermetically sealed under vacuum and thermally treated in a pilot autoclave HST 50/100, ZIRBUS Technology GmbH (Germany) at 120 ± 0.5 °C for 10 minutes. After processing, the products were stored at 37 ± 2 °C for 14 days. pH, aw and aerobic and facultative anaerobic, mesophilic microorganisms were determined in product samples during storage.

The obtained results indicate that the selected processing technology – thermal treatment of the products in packaging – is capable of ensuring microbiological safety of the product. 14-day storage at +37 °C did not have a significant influence on physical and microbiological parameters of the products (p>0.05), proving the effectiveness of the selected technology.

Key words: functional food, microbiological safety, thermal treatment

1. INTRODUCTION
Ready to eat products in convenient packaging with shelf-life over one year are an important component of the diet for most of the population in the developed countries. Preservation process depends on the intensity of heat treatment to inactivate microorganisms and ensure product safety (Patras et al., 2009).

Thermal processing is a popular food preserving method and despite the development of new technologies, it is one of the most effective and widely used processing technologies to ensure food safety (Choi et al., 2013; Miri et al., 2008; Awuah et al. 2007; Chen, Ramaswamy, 2004; Farid et al., 2004). Sterilization by heat has been successfully used in the food industry for the past 100 years, ensuring product quality and extending product shelf-life (Choi et al., 2013). This method is based on the principle of heat conduction and convection, using steam or hot water for indirect heat transfer to the product (Pardo, Zufía, 2012).

The concept of thermal processing can be described by sterilization and pasteurization of the packaged food; the main difference between these two processes is the intensity of thermal processing (Farid et al., 2004; Awuah et al., 2007). Nowadays, product processing in flexible packaging is one of the leading processing technologies, which provides long-term storage of the product at room temperature (Ito et al., 2014). This type of processing technology has many advantages: it allows to effectively conduct the heat from the steam or water to the product through packaging, eliminating the repeated product contamination risk, it eliminates off-flavour formation and product oxidation, it prevents moisture and nutrient loss during evaporation, as well as the loss of volatile compounds during cooking and the growth of aerobic microorganisms in the product (Sansone et al., 2012).
The efficiency of sterilization may be affected by several factors: variable heat transfer to the product surface (sterilization temperature, surface permeability coefficient), variable product and packaging characteristics (product formulation, pH, water activity, salt content, product homogeneity, product thermo-physical properties, thermal diffusion, initial product temperature, amount of product in packaging, total product weight), variable initial product quality (initial microbiological contamination, etc.) (Spani et al., 2015; Awuah et al., 2007; Ávila et al., 2006).

The main purpose of sterilization by heat is to inactivate live microorganisms and their spores in the food (Barbosa-Cánovas et al., 2014; Lammens et al., 2013; Nguyen, 2012; Baucour et al., 2003). The main condition for sterilized products is the destruction of Clostridium botulinum spores (Miri et al., 2008). Product thermal treatment above 100 °C for a certain time period, destroys vegetative forms of microorganisms and partly or fully kills pathogen spores (André et al., 2013). However, optional thermotolerant bacteria Bacillus coagulans, Bacillus licheniformis, Anoxybacillus spp., Paenibacillus spp. Thermoanaerobacter spp. and Clostridium thermobutyricum / thermopalmarium, are able to survive thermal treatment at >100 °C for 10 min (Durand et al., 2015).

After thermal processing microbiological deterioration for sterilized products with low acidity (pH > 4.5) can be observed at incubation temperature +40 °C, when rapid development and reproduction of thermophilic microorganisms takes place (Durand et al., 2015). The Codex Alimentarius defines that in order to ensure the food is safe for distribution, manufacturers are required to subject heat treated products to 10-14-day storage at +37 °C in an incubator. The following product features may indicate spoilage of products processed in packaging: gas formation inside the package which results in bloated packaging, unusual flavour, colour or pH changes. If any of these features are observed during incubation, the company must destroy the faulty batch of products, in order to prevent the risk of infection (André et al., 2013).

For food producers and manufacturers, it is very important to choose a suitable heat treatment regimen and appropriate packaging materials for each type of product in order not to deteriorate its quality while ensuring microbiological safety during storage (Alonso et al, 2013; Lammens et al., 2013).

The aim of this study was to determine the efficiency of thermal treatment on functional ready to eat meals in flexible packaging.

2. MATERIALS AND METHODS

Experiments were carried out at the laboratories of Faculty of Food Technology and Faculty of Veterinary Medicine, Latvia University of Agriculture. Functional ready to eat meal samples were prepared and subjected to physical analysis (pH, water activity) at Packaging Material Properties Research Laboratory, Department of Food Technology. Microbiological parameters were determined at Molecular Biology and Microbiology Research Laboratory, Latvia University of Agriculture and the laboratories of Institute of Food and Environmental Hygiene, Faculty of Veterinary Medicine. A total of five different types of ready to eat meal samples were prepared for this study: control samples – potatoes and potatoes with chicken, potatoes with amaranth (Amaranthus L.), potatoes with bulgur (Triticum durum Desf.) and potatoes with quinoa (Chenopodium quinoa Willd.).

Peeled potatoes were cut by Robot Coupe vegetable preparation machine CL50 in equal-sized cubes (10x10 mm). Cut potatoes were mixed with chicken fillet, which was cut into medium-sized pieces, or amaranth, quinoa, or bulgur, then 0.5% salt was added to each sample. After mixing, products (300±10 g) were filled in 200x250 mm sized laminated pouches. Two different packaging materials suitable for thermal treatment were used: two-layer PA/PE laminated packaging material with 80 µm thickness and PET/ALU/PA/PP packaging material with aluminium layer, 110 µm thickness. After filling, pouches of functional ready to eat meals were hermetically sealed using chamber type vacuum packaging machine Multivac C350; hermetic sealing mode – vacuum, 20 MPa, sealing time for PA/PE packaging – 3.8 seconds, sealing time for PET/ALU/PA/PP packaging – 5 seconds. Vacuum sealed pouches were then thermally treated in a pilot autoclave HST 50/100, ZIRBUS Technology GmbH (Germany). Sterilization was carried out at 120 ± 2 °C for 10 min, the cooling temperature was 20 ± 2 °C. After thermal treatment,
sterilized products were stored at 37 ± 2 °C for 14 days. Physical analysis and microbiological parameters were determined at the production day and after 5, 10, 14-day storage.

Methods used to determine physical and microbiological parameters.

_Determination of pH_. pH was determined using the standard method – LVS ISO 1132: 2001 – with pH meter JENWAY 3510 using JENWAY 3 mol / KCl electrode.

_Determination of water activity aw_. Water activity was determined using standard method – ISO 21807: 2004 – with AquaLab LITE (Decgon Devices Inc.), accuracy of ± 0.015 aw.

_Microbiological parameters_. Microbiological parameters – aerobic and facultative anaerobic, mesophilic bacteria (hereafter referred to as TPC) – were determined according to the standard EN ISO 4833: 2003 “Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of microorganisms – Colony-count technique at 30 degrees C” on Plate Count Agar (PCA) medium.

_Data analysis_. The obtained data were processed using SPSS software package 16.0; differences among results were considered significant if p-value < 0.05. One-way analysis of variance (ANOVA). Factors were rated as significant if p-value < α0.05. For the interpretation of the results it was assumed that α = 0.05 with 95 % confidence.

3. RESULTS AND DISCUSSION

Consumers are becoming more demanding for high quality food products with excellent organoleptic, microbiological and health-promoting properties (Moronta et al., 2016). Preservation process depends on the intensity of thermal treatment to inactivate microorganisms and ensure product safety (Patras et al., 2009). Heat-resistance of microorganisms depends on the treatment temperature and time, however, such factors as pH, aw, etc. are capable of affecting heat-tolerance of microorganisms (Esteban et al. 2016).

Based on the previously mentioned factors that can affect the quality of products, functional ready to eat meals were analysed during storage to determine changes in pH, aw and TPC.

pH value is a unit defining ratio between acids and alkalis. Environment pH is one of the key factors in determining which microorganisms can develop in the product. Food infectious agents typically have an optimum around neutral environment - pH 6 to 7. pH characteristic to vegetables is from 4.2 to 6.5. pH of fresh potatoes ranges from 5.4 to 5.8 (Suryawanshi, 2008). It should be taken into account that if products have different pH, the bacterial resistance to thermal treatment can be variable (Garcia et al., 2007).

Changes in pH of potato meals and functional potato meals in PA/PE packaging during 14-day storage at 37 ± 2 °C were not significantly different (p>0.05) (Fig. 1). Thermally treated functional potato meals have pH close to neutral or weak acid, which is considered an optimal environment for the growth of food infection-causing agents, therefore thermal treatment intensity plays an important role in maintaining the quality of these foods.

Low acidity reduction was observed in a control sample with potatoes (P1, from pH 5.73 to 5.71) and in functional potato product with quinoa (P3, from pH 6.11 to 5.99). Slight pH increase was observed in the other three products – potatoes with chicken fillet (control sample) and functional potato products, i.e., with amaranth and bulgur – but not at a significant level (p> 0.05).
Figure 1. Changes in pH of ready to eat meals during storage in PA/PE packaging.

P1 – control sample-potatoes; P2 – potatoes with amaranth; P3 – potatoes with quinoa;
P4 – potatoes with bulgur, P5 – control sample-potatoes with chicken fillet.

Figure 2 shows changes in pH of potato meals and functional potato meals in PET/ALU/PA/PP packaging during 14-day storage at 37 ± 2 °C. A slight pH increase in the control sample - potatoes (F1, from pH 5.77 to 5.8), functional potato meal with quinoa (F3, from pH 5.87 to 5.9), and functional potato meal with bulgur (F4, from pH 6.14 to 6.16), but the change in pH are not considerably different (p>0.05). Significant differences were not observed between functional ready to eat meal samples in different packaging materials (p>0.05). It can be concluded that the time and 37±2 °C temperature of incubation, which is necessary to determine the efficiency of thermal treatment, does not significantly influence pH of functional potato meals and control meal samples during 14-day storage. This shows that constant product quality is maintained after thermal treatment.
Water activity is defined as the equilibrium fugacity of water vapour over a solution ($f$) the relative number of the fugacity of water vapour over pure water ($f_0$) ($aw = f/f_0$) (Toner, Catling, 2016). Water activity (aw) is the main factor affecting the viability and growth of microorganisms, as well as the resistance to thermal treatment (Syamaladevi et al., 2016; Liang et al., 2015). Product thermal treatment is an effective means to control the growth of pathogens in medium and high-moisture foods ($aw \geq 0.6$) (Syamaladevi et al., 2016). Water activity in the new functional products was determined at the day of production and 5, 10, 14 days after storage $+37 \pm 2 \^\circ C$. Significant differences in the changes of water activity were not observed during 14-day period ($p > 0.05$), therefore, the average water activity values are given in Table 1.

### Table 1. Water activity ($aw$) in ready to eat meals after thermal treatment

<table>
<thead>
<tr>
<th>Packaging</th>
<th>Products</th>
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<tbody>
<tr>
<td></td>
<td>Potatoes</td>
</tr>
<tr>
<td>PA/PE</td>
<td>0.952</td>
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<tr>
<td>PET/ALU/PA/PP</td>
<td>0.963</td>
</tr>
</tbody>
</table>

All thermally treated meals are high moisture foods, suggesting that the environment is favourable for the growth of microorganisms, therefore thermal treatment efficiency is essential to prevent the viability of microorganisms in the product after processing.

Low-acid products are categorised by having pH above 4.6 and water activity above 0.85 (Afuah et al., 2007). The results suggest that functional potato meals can be considered low-acid products, and the environment is favourable for the development of microorganisms.
Packaged food products are subjected to a specific duration of thermal treatment, which provides the decrease of microorganisms and their spores to a level that is sufficient for the product to be harmless even when it is stored for a longer period of time. The main purpose of the sterilization by heat is to inactivate live microorganisms and their spores in the food (Alonso et al., 2013).

Aerobic and facultative anaerobic, mesophilic microorganisms were determined in all products on the production day after thermal treatment (sterilization at 120 ± 2 °C for 10 min) and after 5, 10, 14-day storage.

The results showed that treatment intensity (temperature and time combination) was able to guarantee the required microbiological safety. Vegetative forms of microorganisms were not found in the products, which confirms that new functional ready to eat meals are safe for consumption.

4. CONCLUSIONS

Physical parameters of new functional potato products suggest that ready to eat meals are a favourable environment for microbial growth and survival (pH in the range of 5.69 to 6.11; aw ranges from 0.932 to 0.954), thus the intensity of thermal treatment plays a vital role. Sterilization of functional potato products at 120 ± 2 °C for 10 min is suitable to ensure the microbiological safety of the products during storage, indicating effective time-temperature combination. Significant differences in microbiological parameters between functional potato products in different packaging materials (PA/PE and PET/ALU/PA/PP) were not found (p>0.05).

ACKNOWLEDGEMENTS

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).

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