EFFECT OF HOT-FILL PROCESSING AT REDUCED TEMPERATURES ON TOMATO SAUCE MICROBIOLOGICAL STABILITY IN PLASTIC PACKAGING

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Abstract. Tomato sauce production technology implies cooking at 80-95 ºC. High-density polyethylene (HDPE) packaging material is not recommended for hot-fill process, mainly because of packaging deformation under the influence of high temperature. However, condiments could be cooled and then filled in a HDPE bottle, but the secondary contamination still can occur during the filling process. The aim of the study was to find out whether cooked tomato sauce can be filled in HDPE bottles at reduced temperatures. Two commercial products were hot-filled at 65, 70 and 75 ºC, and incubated at 30 ºC for 12 weeks for accelerated shelf life prediction, assuming the temperature coefficient (Q10) is 2. Microbiological tests were performed after each 2 weeks. Based on target bacteria kinetic factors obtained from literature, and time-temperature data obtained during the hot-fill process, theoretical evaluation of heat treatment processing effectiveness was performed, resulting in prediction that no bacterial growth should be observed during the sample incubation. Untreated samples showed 4 ± 0.2 $\log_{10}$ g^{-1} total plate count (TPC), 2.4 ± 0.2 log10 g^{-1} yeasts and moulds count, and various mesophilic lactic acid bacteria and sulphite-reducing bacteria count. After heat treatment, both samples showed stable TPC during the incubation at 30 ºC for 12 weeks, which allowed us to conclude, that expected shelf life of chosen commercial products is at least 6 months. Study result brings us in-depth knowledge about commercial product microflora heat resistance and behaviour during the accelerated shelf-life storage, as well as expands our understanding of industrial pasteurization process designing.

Keywords: ketchup; shelf life; storage; food contamination, HDPE package.

1. Introduction

In cookery, a sauce is a liquid or creamy food, served on different kinds of dishes. Sauces are widely used in modern cuisine to add flavour, moisture, and appearance to a dish. Tomato sauce is one of the most popular sauces in the world [1]. During the industrial production of tomato sauce, various preservatives are added to reduce the growth of yeast and bacteria in the product, which extends the shelf life of the product. The most popular preservatives used in this product are potassium sorbate and sodium benzoate. In recent years, demand for preservative-free products has raised, therefore forcing to adapt manufacturers to produce preservative-free products with an appropriate shelf life.

The main ingredients for tomato sauces and ketchups are tomato paste, sugar, spices, salt and acidity regulators. Acidity regulators are the most important and are commonly used to prevent spoilage and microbial contamination of products. Pasteurization of tomato sauces and ketchup is used to ensure the quality and safety of the product during its shelf life.

Most of raw materials for food production come from soil, which is the primary habitat for many bacteria varieties. From here they enter the water and contaminate the air. However, air is an environment that does not support the reproduction of microorganisms, this is determined by lack of nutrients and lack of moisture. Consequently, microorganisms can enter food in different ways: from soil, air, water, animals and animal products, plants and plant products, food handling equipment, food processing and storage equipment. Water is used in the production of most products, therefore compliance of its quality with microbiological indicators is extremely important [2].

Food products are mainly contaminated with microorganisms during production and storage. However, in most cases, the cause of contamination is unknown. At the same time, there are several studies that show the sources of contamination of the microorganism in the production premises. In 2004, the results of a joint study made specifically for the Scandinavian and Nordic countries conducted in poultry, meat and fish processing enterprises showed the presence of *Listeria spp.* in 11 out of 13 factories. Ladders and floors were identified as one of the most contaminated sites [3].

Thermal processing operations in the canning industry aim to ensure adequate destruction of expected spoilage organisms and pathogens in the product based on reliable microbial thermal-death-time information. According to the Cabinet of Ministers Regulation No. 461 “Requirements for Food Quality Schemes, Procedures for Their Implementation, Operation, Monitoring and Control”, the microbiological indicators for tomato sauces and ketchups are mesophilic aerobic and facultative

anaerobic microorganism, mould and yeast cell count safe food production must comply with the Food Chain Supervision Law. Pathogens that can cause serious health problems, are strict in limits [4]. Latvian Regulation No. 461 (2014) stipulates that total mesophilic aerobic and anaerobic microorganism plate count should not exceed 1 x 10^4 CFU g^{-1} for tomato sauces and ketchups, but mould and yeast cells not more than 1 x 10^2 CFU g^{-1}. The pH should be between 2.5 and 4.5 according to the regulation [5].

The primary public health concern associated with low-acid canned food is the formation of botulinum toxin in the container, produced by mesophilic spore-forming bacteria *Clostridium botulinum* [6]. However, production of botulinum toxin does not happen at pH < 4.6 [7]. In acid or acidified canned foods the threat to public health is caused by *Escherichia coli* O157:H7 [8] and *Listeria monocytogenes* [9].

The most commonly used packaging for filling tomato sauces is glass jars. The glass has high barrier properties and protects tomato sauces from the environment, but glass packaging is not convenient for handling in terms of the logistics [10]. Nowadays, ketchups and tomato sauces are also packaged in HDPE packages that are easy to transport. Plastic packaging is flexible, lightweight, has a variety of shapes and materials, making it a good substitute for traditional glass packaging. Polymer materials have good barrier properties against oxygen and moisture, and they protect the product from changes in physicochemical and organoleptic properties. These are important factors in maintaining quality and safe food [11]. The production technology of tomato sauces implies heat treatment of 90-95 ºC during the cooking process. Unfortunately, not all plastic packaging materials are designed for filling at such high temperatures. HDPE packaging material is suitable for filling at moderate temperatures, mainly because of packaging deformation under the influence of high temperature. The sauce can be cooled and then filled in a HDPE packaging, but the secondary contamination still can occur during the filling process. The aim of the study was to find out whether cooked tomato sauce can be filled in HDPE bottles at reduced temperatures.

2. Materials and methods

2.1. Product description

In this study, samples of the two commercial products were used – "7 garden herb sauce" (K_{GH}) and "Ketchup R" (K_R) (Table 1).

Table 1

Chosen product description

Tomato sauce	Sample code	Ingredients	Total solids, %	pH
7 garden herb sauce	K_{GH}	Water, tomato paste 18,6%, sugar, celery root, modified corn starch-thickener, salt, sun dried tomatoes, herbs 0,7% (celery powder, dried parsley, dried leeks, dried oregano, ground coriander, ground bay leaves, dried dill), sweet pepper powder, acidity regulators (acetic acid, citric acid), ground chili pepper, garlic powder	21 ± 1	4.1 ± 0.1
Ketchup R	K_R	Water, tomato paste 18%, sugar, modified corn starch-thickener, salt, acidity regulator – acetic acid, spices	26 ± 1	4.0 ± 0.1

The production process for K_{GH} begins with swelling the celery root, cooking the dried tomatoes and grinding it all. For "Ketchup R" black and allspice is scalded in boiling water. The production process below is the same for both products – pouring the required amount of water into the boiler, adding tomato paste, salt, sugar, spices, dissolved starch, (for "7 garden herb sauce" ground celery and tomatoes), mixing everything together, heating the product to 90-95 ºC, adding acetic acid, stirring and leaving to stand for 3-5 minutes. The product is tested organoleptically (colour, taste, consistency), pH and dry matter content is determined, and then fed to filling into the packaging.

2.2. Analysis methods

Microbiological analyses were performed in an external accredited laboratory J.S. Hamilton. Used analytical methods are shown in Table 2.

Table 2

Analysis methods

Determined indicators	Method
Total plate count (TPC)	LVS EN ISO 4833-1:2014
Yeasts and moulds	LVS ISO 21527-2:2008
Mesophilic lactic acid bacteria count (LAB)	LVS ISO 15214:1998
Salmonella spp., presence	LVS EN ISO 6579-1:2017
Sulphite-reducing clostridia count (SRB)	ISO 15213:2003

2.3. Study framework

For product initial microorganism contamination definition before heat treatment, aggregate samples were prepared by mixing all ingredients together, except adding water, and analysed according to the study framework (Figure 1).

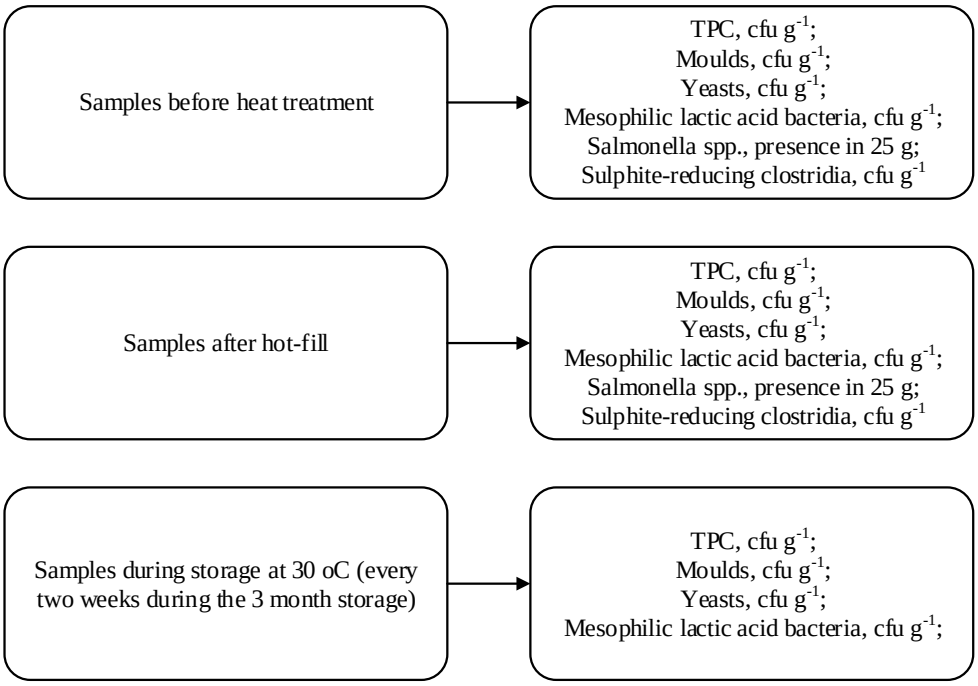

Fig. 1. **Study framework**

Product samples were collected immediately after the cooking process, cooled off and filled in the 0.5 L HDPE bottles at different temperatures, i.e. 65, 70 and 75 ºC. The temperature during the cooling process was measured in the samples filled at 65 and 75 ºC with wireless thermocouples (Tecnosoft, Italy), located inside of the packaging beforehand.

Samples were incubated at 30 ºC, according to the Q10 principle, and analysed every 2 weeks, one piece of each filling temperature at a time. The value of Q10 at 30 ºC was set as 2, as it is generally accepted in the industry [12]. Therefore, if the product shows microbiological stability during the storage time, then the expected shelf life of the food product is 6 months.

2.4. Data processing

Data processing was performed using the MS Office Excel 16.0 software. Stationary tests (Augmented Dickey-Fuller test (ADF) and Kwiatkowski-Phillips-Schmidt-Shin (KPSS)) and two factor analysis with replication (ANOVA) were used. Significance level was set at $\leq 5\%$.

3. Results and discussion

3.1. Theoretical evaluation

For the samples filled at 65 ºC it took 90 min to cool till the 40 ºC, and 150 min respectively for the samples filled at 75 ºC (Figure 2).

Based on the time-temperature data, pathogenic bacteria destruction rates were evaluated (Table 3). Expected log reduction for chosen bacteria varied from 32 to 89 log10 for the samples filled at 65 ºC and 154 to 711 for the samples filled at 75 ºC.

Generally, it is assumed that bacterial spores would be inhibited by pH < 4.6 and only acid tolerant microorganisms, such as lactic acid bacteria, acetic acid bacteria, yeasts, and moulds may develop [13]. *L. monocytogenes* is generally considered as the most heat resistant vegetative pathogen, with $D^{70} < 1$ min, while yeasts, lactic acid bacteria and moulds have lower D values [14].

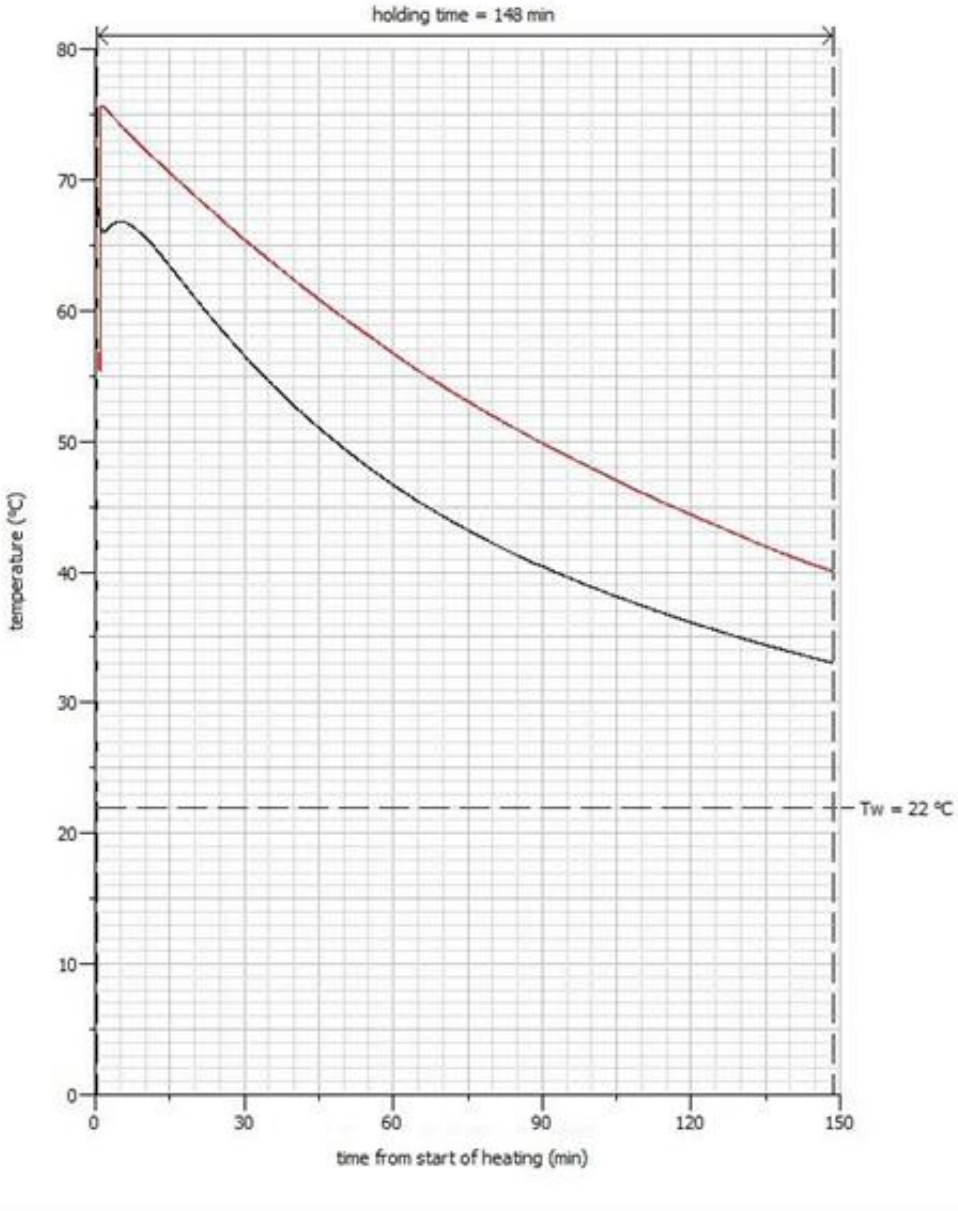

Fig. 2. **Time-temperature data inside of the product during the cooling process**

Since most of the mesophilic spore-forming bacteria cannot grow in acidified products, but are able to withstand chosen heat treatment conditions - while the product pH is maintained below 4.5, heat-stable pathogenic spore-forming bacteria are of no concern [15].

Table 3

Kinetic characteristics of target bacteria thermal destruction

Bacteria	T_{ref}	z	D	6D, min	Fo (65 °C)	Fo (75 °C)	log10 (65 °C)	log10 (75 °C)
L. monocytogenes[1]	63.0	11.4	1.20	7.20	39.0	184.5	32.5	153.7
Salmonella[2]	63.0	10.0	0.54	3.24	39.5	228.9	73.1	423.9
E. coli[3]	63.0	8.4	0.47	2.82	41.7	334.2	88.7	711.1

[1]Data obtained from [16]; [2]Data obtained from [17]; [3]Data obtained from [18]

3.2. Product microbial analysis

Non-heat-treated samples (Fig. 3A; 3B) TPC in K_R and K_{GH} wereevaluated as 3.7 and 4.3 log10 CFU g^{-1} respectively. K_R sample contained 2.4 log10 yeasts and 2.3 log10 lactic acid bacteria. K_{GH} samples contained 2.4 log10 moulds and 1.7 log10 sulphite-reducing clostridia. After the heat treatment and during the storage at 30 °C for 12 weeks, yeasts, moulds, mesophilic acidophilic bacteria or sulphite-reducing bacteria were not detected in both products.

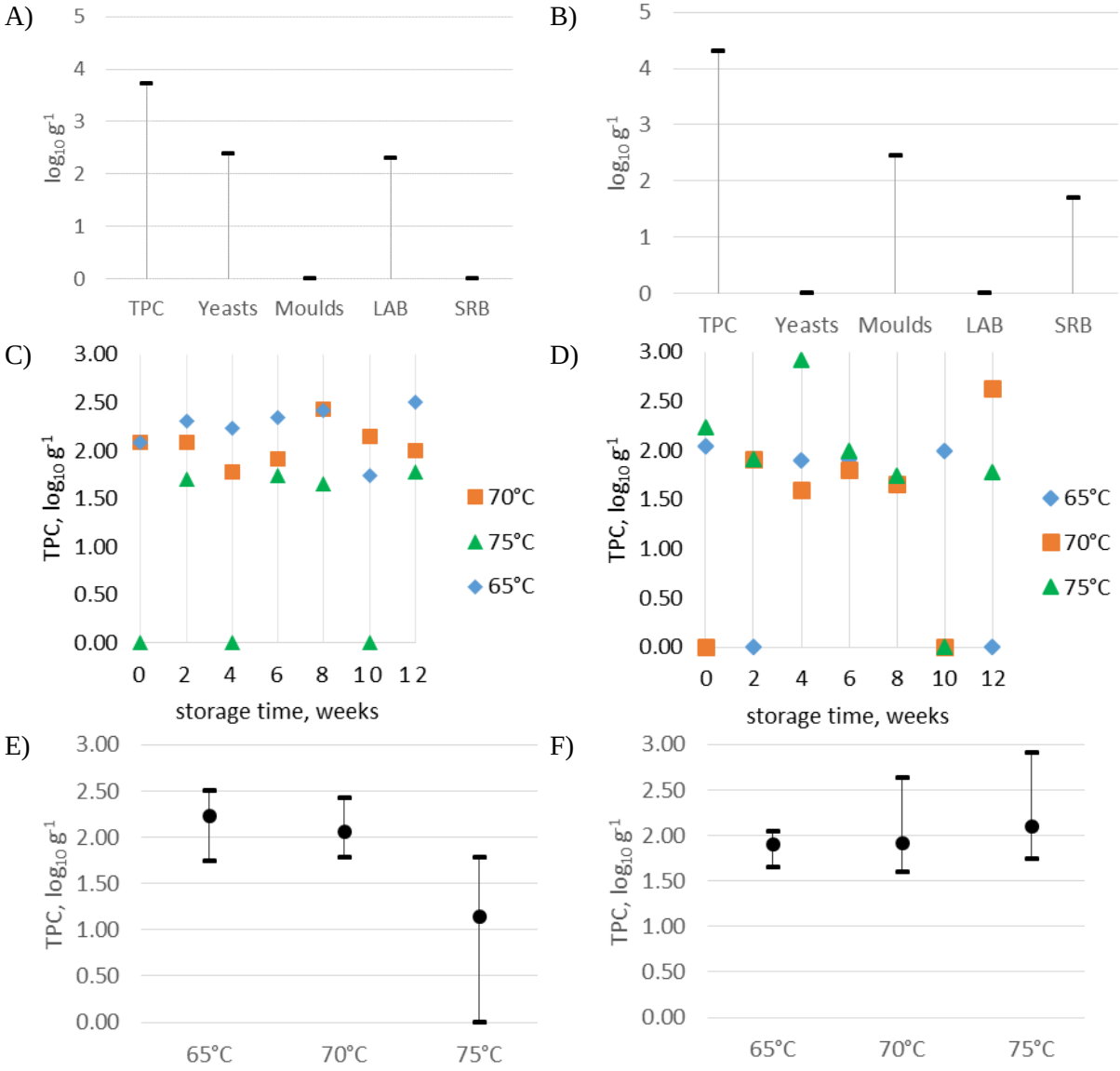

Fig. 3. **Product sample analysis results prior to heat treatment (A, B); TPC during 12-week incubation at 30 °C (C, D); average TPC during 12-week incubation at 30 °C (E, F)**

For *Salmonella spp.* evaluation, qualitative analysis was performed, determining the presence or absence of bacteria in the sample, which was not identified. K_R product TPC during the storage was stable for each sample (Fig. 3C; 3D), growth was not detected. It would be necessary to mention, that each sample was independent from others, i.e. each sample was filled in separate container and tested just once during the incubation. Therefore, each sample had different initial contamination rates at the beginning of the study, and each sample should be evaluated independently from the others. To determine whether time series is stationary or not, ADF test was performed. Statistical analysis showed that sample TPC was not stationary or time-trending, but rather exhibits a random type of behaviour. Such statement allows us to conclude that no bacteria growth was observed, and to assume that TPC observations are linked to the initial bacteria contamination.

In comparison with K_R, K_{GH} product samples showed higher TPC in some samples (Fig. 3E; 3F), but lower mean value. Such fact could be explained by the product composition differences. While K_R product is a homogenous thick liquid, consisting mainly from tomato paste and water, K_{GH} product contains dried spices in relatively large amounts (Table 1). Since spices are generally considered as a heat stable spore-forming bacteria contaminant [19], it is fair to suggest that K_{GH} TPC results should be less homogenous in comparison with the K_R TPC results. Statistical data analysis showed that there is no significant statistical difference ($p > 0.05$) between product types or filling temperature effectiveness.

3.3. Expected shelf life

Temperature coefficient (Q10) was used to predict the expected shelf life of the product. The value of Q10 at 30 ºC is set as 2. In this study, the samples were incubated in dark at 30 ºC for 12 weeks, expecting the shelf life to be 6 months, if bacteria growth would not be detected. In this work, none of the studied samples showed microbial growth characteristics during the incubation time, which allows us to assume, that the expected shelf life of the product is at least 6 months, concerning the microbial quality retention factor. However, it is unclear, how the product will behave after 6 months of storage, regarding the non-enzymatic quality parameter retention, affected by light exposure and packaging natural gas permeability.

4. Conclusions

1. The study has potential limitations. During the sample incubation process, each testing was performed on an independent sample with different initial TPC. However, descriptive statistics allowed us to make conclusions on product behaviour during the storage and to extrapolate its shelf life for the next 3 months.
2. The technology developed in the study made it possible to fill tomato sauce and ketchup in HDPE packaging using a reduced temperature of 65 ºC, thus preventing packaging deformations that occur at an elevated temperature of 80 ºC. During the accelerated storage of the product, the results of microbiological analyses confirm that the product is safe for consumers. The total number of microorganisms in the product, yeasts, moulds and mesophilic lactic acid bacteria were within allowed limits. *Salmonella spp.* and sulphite-reducing bacteria were not found in the product.
3. Study result brings us in-depth knowledge about commercial product microflora heat resistance and behaviour during the accelerated shelf-life storage, as well as expands our understanding of industrial pasteurization process designing.
4. For more comprehensive research in terms of ASLT, it would be necessary in the future to analyse the product quality factor retention during the storage time, and use mathematical modelling methods for accelerated shelf life prediction.

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