

PASTEURISATION OF TOMATO JUICE BY HIGH HYDROSTATIC PRESSURE TREATMENT OR BY ITS COMBINATION WITH ESSENTIAL OILS

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Laboratory batches of fresh tomato juices were treated in several experimental trials by high hydrostatic pressure alone or in combination with various concentrations of oregano, thyme or dill seed oils. Lactic acid bacteria formed the dominating component of the spoilage microbiota during post-processing storage at 15 °C causing spoilage of the untreated samples within 4 days. One tenth of a percent oregano or thyme oils at least doubled the microbiological shelf life, while their respective concentrations of 0.5% alone, or 400 MPa 5–20 min high hydrostatic pressure treatment alone resulted in microbial stability for at least two weeks. Two hundred MPa for 10 min resulted only in an approx. 3 days delay of spoilage, whereas 0.1% thyme oil increased the efficiency of this moderate UHP-treatment, resulting in a microbiologically stable product for at least 3 weeks at the storage temperature applied.

Keywords: essential oils, high hydrostatic pressure, tomato juice

Nowadays there is a strong demand for producing minimally processed foods (fresh-like commodities treated with quality-friendly, mild treatment(s)) and products preserved without artificial preservatives. In the frame of the INCO-COPERNICUS (EU IC CT97-1000), "PLANTCHEM" project, feasibility studies for preservation of vegetable food products with natural antimicrobials were carried out. Parallel with these experiments preliminary studies on the efficiency of a new, non-thermal processing method, ultra-high hydrostatic pressure (UHP) treatment has been performed by us.

Conventional methods of thermal processing often produce a number of undesirable changes in foods, such as loss of colour, flavour and functionality, which may be avoided by using alternative minimal processing strategies. The aim of such studies is to examine the processing parameters required for safe and high quality fresh, or fresh-like products in which the nutritional status, health protective compounds and the natural taste and colour are preserved. High hydrostatic pressure has the benefit of giving microbial inactivation, while still retaining fresh flavour and colour of treated

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fruit juices. UHP treatment in the range of 100 to 500 MPa, usually for 5 to 20 min, can extend the microbiological shelf-life of foods by reducing the number of spoilage microorganisms and activities of certain enzymes. Investigations of some non-sporeforming pathogenic bacteria, including *Listeria monocytogenes* and *Salmonella* spp., showed that they are relatively sensitive to these high pressures (PATTERSON et al., 1995). They indicated however that the nature of the food substance could also affect the sensitivity of microorganisms to UHP treatment. For example, milk during UHP treatment at 20 °C offered more protection to *Escherichia coli* O157:H7 and *Listeria monocytogenes* than poultry meat. Recently, combinations of high hydrostatic pressure and other agents have been investigated to enhance the lethal action of pressure on microorganisms under moderate conditions (MAGGI et al., 1996; PATTERSON & KILPATRICK, 1998; KALCHAYANAND et al., 1998).

Since early times, essential oils have been used for providing flavour and fragrance in the perfumery, pharmaceutical, cosmetic and food industries. Essential oils are mixtures of volatile compounds, mostly terpenes and their oxygenated derivatives, produced in small quantities as secondary metabolites by many plants known as aromatic or medicinal herbs. It is well established that they have certain antimicrobial properties (FARAG et al., 1989; ICMSF, 1998). Among the most active constituents of spices, having wide spectrum of antimicrobial effects are thymol and carvacrol of thyme (BEUCHAT & GOLDEN, 1989). However, it is only relatively recently that much attention has been given to their potential application as food preservatives. Application of essential oils as biopreservatives is frequently hindered by their strong organoleptic effect in the range of their effective antimicrobial concentration.

In the experiments to be presented here those essential oils (thyme oil, oregano oil and dill seed oil) were selected for application either alone or in combination with UHP treatment, which in our previous experiments (MOHÁCSI-FARKAS et al., 1999) had good *in vitro* antimicrobial effects, and using them in low concentrations in tomato juice did not jeopardize seriously the sensorial acceptability of the product.

1. Materials and methods

1.1. Experimental materials

Fresh, ripe tomato fruits purchased from the local retail market were used to produce juice.

The essential oils selected for these studies (oregano, *Oregano vulgare* spp. *vulgare*; thyme, *Thymus vulgaris*; and dill seed, *Anethum graveolens*) hydro-distilled from plant materials were obtained in the frame of the PLANTCHEM project from the Department of Food Technology, Kaunas University of Technology, Kaunas, Lithuania. According to their analyses, the main constituents of the essential oils used were the following:

- oregano: sabinene, p-cymene, Z-beta-ocimene, E-beta-ocimene, limonene, 1,8-cineole, beta-caryophyllene, germacrene D;

- thyme: thymol, p-cymene, gamma-terpinene, carvacrol;
- dill seed: carvone, limonene, 1,8-cineole.

1.2. Preparation and packaging of experimental batches of tomato juices

Tomato fruits were triturated removing seeds and skin then the fresh juice was divided into approx. 50 ml-portions and sealed into small laminated plastic pouches under vacuum (with or without previous addition of the selected essential oils in various concentrations).

1.3. High hydrostatic pressure and combination treatments

High hydrostatic pressure treatment was carried out with a “FoodLab 9000” high pressure rig (Stansted Fluid Power Ltd., Stansted, U.K.). The temperature during the pressure-decompression cycle remained within the 5–40 °C range.

In several experimental trials different batches of tomato juices, produced from separate purchases of tomato fruit, the following treatments were applied:

- untreated control (initial pH=3.9–4.1);
- 200 MPa for 10 min;
- 400 MPa for 5 min;
- 400 MPa for 10 min;
- 400 MPa for 20 min;
- 0.05% oregano oil;
- 0.1% oregano oil;
- 0.5% oregano oil;
- 0.05% thyme oil;
- 0.1% thyme oil;
- 0.5% thyme oil;
- 0.05% dill seed oil;
- 0.05% oregano oil + 200 MPa, 10 min;
- 0.05% oregano oil + 400 MPa, 5 min;
- 0.1% oregano oil + 400 MPa, 5 min;
- 0.1% oregano oil + 400 MPa, 20 min;
- 0.5% oregano oil + 400 MPa, 20 min;
- 0.05% thyme oil + 200 MPa, 10 min;
- 0.1% thyme oil + 200 MPa, 10 min;
- 0.1% thyme oil + 400 MPa, 10 min;
- 0.5% thyme oil + 400 MPa, 20 min;
- 0.05% dill seed oil + 200 MPa, 10 min.

1.4. Storage and sampling

The experimental batches were stored at 15 °C and duplicate samples per treatment were tested directly after treatments, and periodically during storage, up to maximum

four weeks for visual appearance, colour and microbiological quality. Total aerobic plate counts (TPC) and bacterial spore counts were determined on CASO agar (Merck No. 1.05458). Lactic acid bacteria (LAB) were plated in double-layer MRS agar (Merck No. 1.10660). Yeast and mould counts were analysed on OGY agar (Merck No. 1.10877) after surface spreading. Plates were incubated for 3–5 days at 30 °C for total plate counts and lactic acid bacteria and for 5 days for yeast and moulds at 25 °C.

2. Results and discussion

In the first experimental series, the effects of addition of 0.1 and 0.5% of oregano oil, resp., without or with 400 MPa for 20 min were investigated. Figs 1 to 4 show the effects of these treatments on the total aerobic plate counts, the LAB counts, yeast counts and mould counts, respectively. The bacterial spore counts were in almost all samples below the detection level of 10^2 CFU ml⁻¹. Use of thyme at the same concentration levels as those of oregano oil resulted in similar effects alone, or in combination with high hydrostatic pressure (results are not shown).

These results proved that lactic acid bacteria formed the dominating component of the spoilage flora of the tomato juice and caused spoilage at 15 °C within 4 days. One tenth of a percent oregano or thyme oils at least doubled the microbiological shelf life, while their respective concentrations of 0.5% alone, or 400 MPa, 20 min high hydrostatic pressure treatment alone were able to increase microbiological stability for at least two weeks.

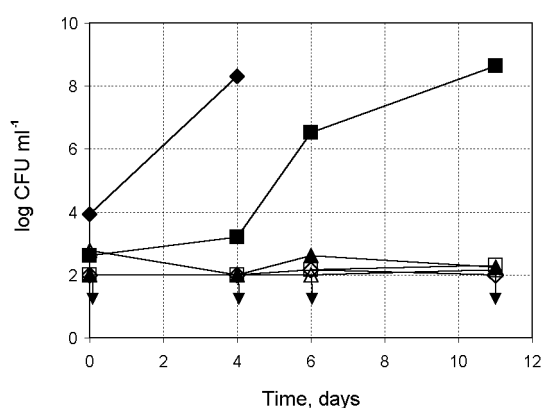


Fig. 1. Changes of total viable bacterial cell counts in tomato juice treated at various concentrations of oregano oil, and/or a high hydrostatic pressure treatment of 400 MPa for 20 min, and stored at 15 °C, as compared to those of the untreated control. ◆: Control; ■: oregano 0.1%; ▲: oregano 0.5%; ○: HP control; ◻: HP+oregano 0.1%; △: HP+oregano 0.5%

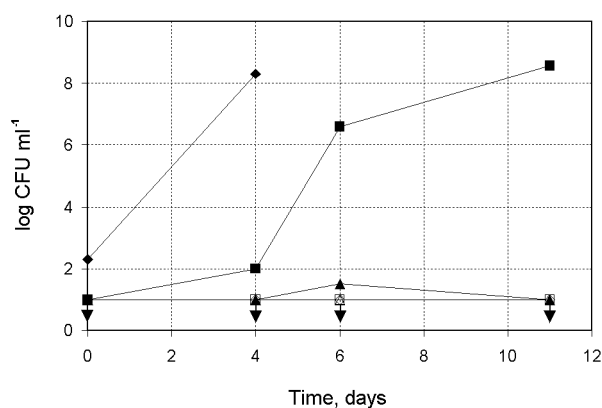


Fig. 2. Changes of counts of viable lactic acid bacteria in tomato juice treated at various concentrations of oregano oil, and/or a high hydrostatic pressure treatment of 400 MPa for 20 min, and stored at 15 °C, as compared to those of untreated control. ◆: Control; ■: oregano 0.1%; ▲: oregano 0.5%; ◇: HP control; □: HP+oregano 0.1%; Δ: HP+oregano 0.5%

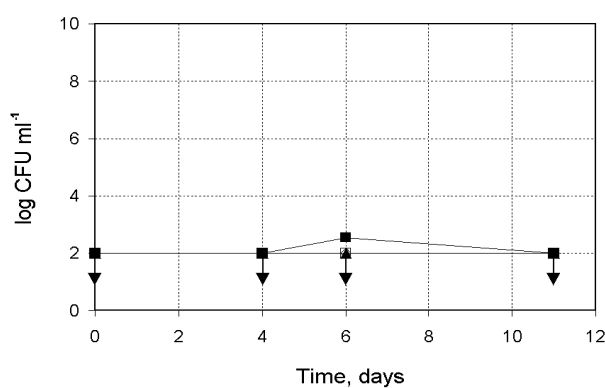


Fig. 3. Counts of viable bacterial spores in tomato juice treated at various concentrations of oregano oil, and/or a high hydrostatic pressure treatment of 400 MPa for 20 min, and stored at 15 °C, as compared to those of untreated control. ◆: Control; ■: oregano 0.1%; ▲: oregano 0.5%; ◇: HP control; □: HP+oregano 0.1%; Δ: HP+oregano 0.5%

In order to reduce sensory effect of the essential oils, in a second series of experiments reduced concentrations of oregano such as 0.05% was also applied and its effects were compared to those of a shortened UHP treatment (400 MPa, 5 min) applied alone, or, with combination of the essential oil addition. On the basis of the experience with the first experimental series, total plate counts and LAB counts were monitored only.

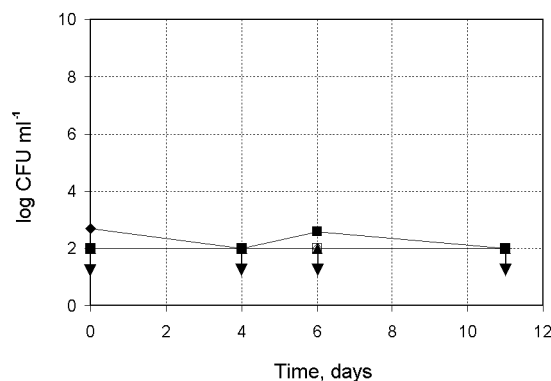


Fig. 4. Counts of viable yeast cells in tomato juice treated at various concentrations of oregano oil, and/or a high hydrostatic pressure treatment of 400 MPa for 20 min, and stored at 15 °C, as compared to those of untreated control. ◆: Control; ■: oregano 0.1%; ▲: oregano 0.5%; ◇: HP control; □: HP+oregano 0.1%; Δ: HP+oregano 0.5%

The results are shown in Fig. 5. Although the tomato juice treated in these experiments was originally highly contaminated by bacteria, a significant retardation of lactic acid spoilage was found even at 0.05% level of oregano oil, and microbiological stability of the tomato was achieved for at least four weeks at 15 °C by a short UHP treatment, even without the addition of the essential oil.

In a third experimental series dill seed, oregano oil or thyme oil in 0.05% concentrations, resp., were applied as single treatments, or in combination with a relatively mild, single UHP treatment of 200 MPa for 10 min. The low concentrations of essential oils had only very little effect on the microbial counts. The growth of the survivors during storage and the efficiency of the UHP treatment, resulting in at least 2.5 log-cycles reduction of the viable bacterial contamination of the juice, could not be increased by such low levels of essential oils, resulting only in a temporary delay of spoilage (Fig. 6).

In the fourth experiment, with a somewhat less contaminated juice than in that of the previous experiment (total plate count less than 10^4 CFU ml⁻¹, instead of 10^5 CFU ml⁻¹), 0.1% thyme oil extended considerably the microbiological shelf life, and increased the efficiency of the 200 MPa, 10 min UHP treatment, resulting in a microbiologically stable product for at least 3 weeks of storage at the temperature applied (Fig. 7).

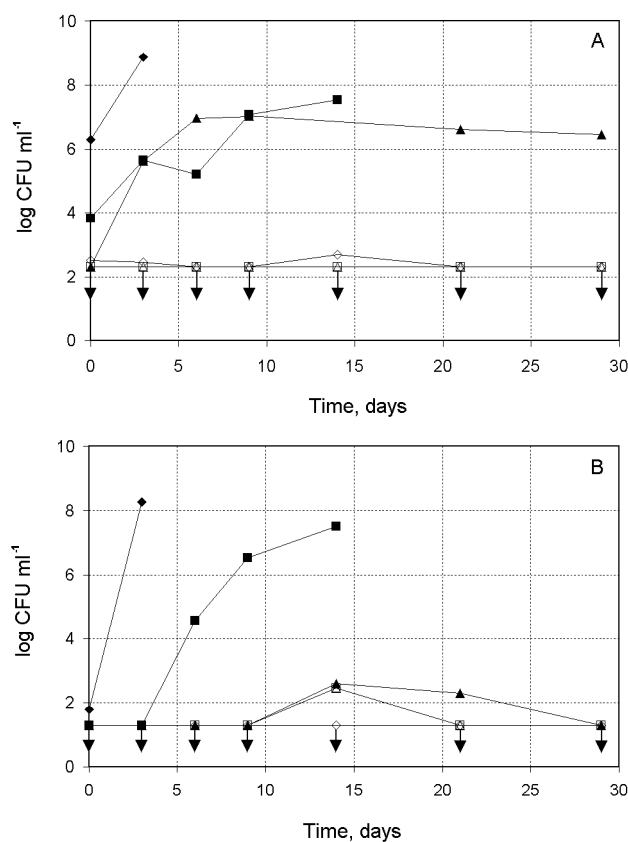


Fig. 5. Changes of total aerobic bacterial counts (A diagram) and lactic acid bacterial counts (B diagram) of tomato juice treated with low concentrations of oregano oil, and/or a high hydrostatic pressure treatment of 400 MPa for 5 min, as compared to those of the untreated control. ◆: Control; ■: oregano 0.05%; ▲: oregano 0.1%; ◇: HP control; □: HP+oregano 0.05%; Δ: HP+oregano 0.1%

The visual appearance remained essentially unchanged during storage, while microbial counts were below the spoilage level.

PORETTA and co-workers (1995) reported improved colour and increased viscosity properties of ultra-high pressure (500–900 MPa)-treated tomato juices as compared to their conventional heat-processed counterparts. However, their UHP-samples showed a large increase of hexanal concentrations and a rancid taste.

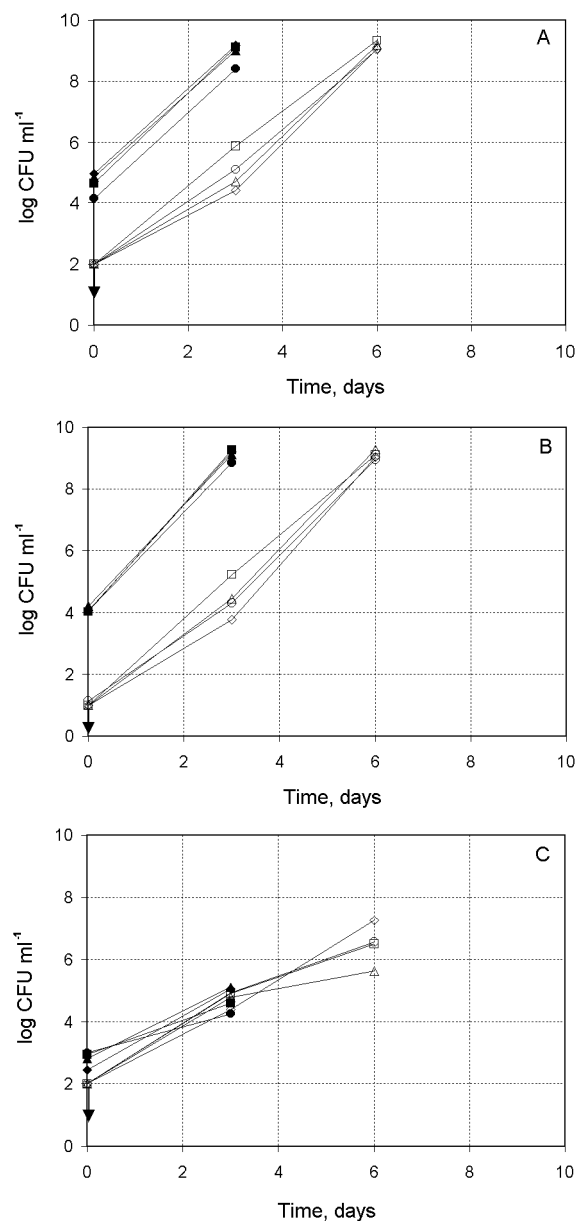


Fig. 6. Changes of total aerobic bacterial counts (A diagram), lactic acid bacterial counts (B diagram), and yeast counts (C diagram) of tomato juice treated with 0.05% concentration of various essential oils, and/or a high hydrostatic pressure of 200 MPa for 10 min, as compared to those of the untreated control. ◆: Control; ■: dill seed 0.05%; ▲: oregano 0.05%; ●: thyme 0.05%; ◇: HP control; □: HP+dill seed 0.05%; Δ: HP+oregano 0.05%; ○: HP+thyme 0.05%

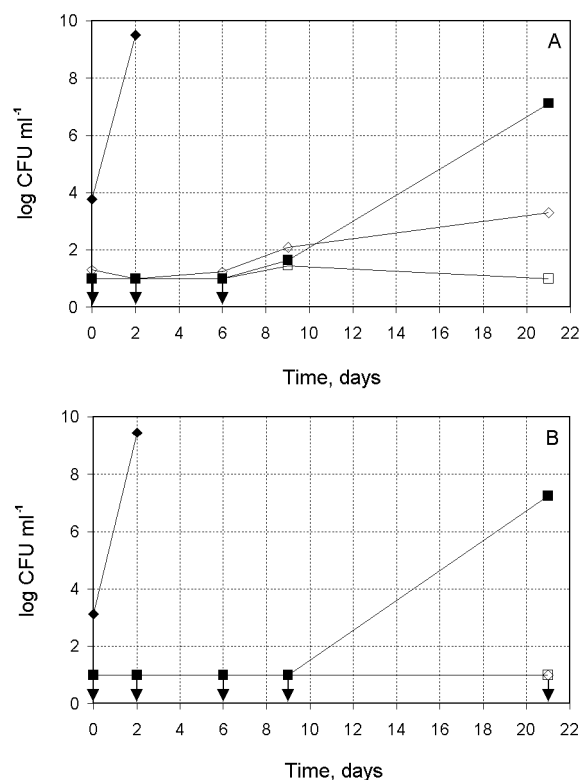


Fig. 7. Changes of total aerobic bacterial counts (A diagram) and lactic acid bacterial counts (B diagram) of tomato juice treated with 0.1% thyme oil, and/or a hydrostatic pressure of 200 MPa for 10 min, as compared to those of the untreated control. ◆: Control; ■: thyme 0.1%; ◇: HP control; □: HP+thyme 0.1%

3. Conclusions

The very strong flavours of volatile oils limit their utility for food preservation, but combining selected essential oils with UHP treatment, these non-thermal preservative agents made it possible to reduce the concentration requirements of essential oils and/or the severity requirement (pressure or duration) of the high hydrostatic pressure treatment for pasteurising tomato juice. However, assessment of the utility of essential oils as preservative agents requires an item-by-item approach. Further studies are required in the future to estimate the effect of UHP treatment alone or in combination with biopreservatives on not yet studied other quality and nutritional parameters (including biologically active antioxidant constituents), and activities of certain enzymes to establish optimal conditions and full technological feasibility of non-thermal pasteurisation of tomato juice.

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