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### High hydrostatic pressure: Can we trust published data?

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Abstract. There are numerous new technologies whose implementation in food industry is hampered by the fact that people hesitate to invest in expensive systems which they cannot be sure will work or at least are questionable in terms of a given product. Until recently, preservation by HHP, high hydrostatic pressure, was such a technology, and still is today in some branches of the food industry. Investigations were conducted to answer the question of whether the literature, the laboratory, and the industrial (or at least pilot plant) measurements and results agree with one another. We compared the literature data with two HHP systems which were significantly different in terms of treatment capacity, but their efficiency in killing microbes was studied under the same treatment parameters. Our results show that in nearly all cases only minimal differences exist between the data in the literature and the measurements taken on the two appliances.

#### 1 Introduction

HHP, or high hydrostatic pressure, is one of the "gentle" food preservation methods, as it does not use heat. In high hydrostatic pressure technology, food products are preserved by being subjected to between 100 and 1000 Mpa hydrostatic pressure. In HHP treatment, food products are vacuum-sealed in flexible packages and placed in liquid. Hydrostatic pressure is applied quickly and evenly (isostatically) according to the Pascal method (*Dalmadi & Farkas*, 2006); thus, neither the size nor the shape of the container play a role in the effects of the pressure treatment (*San Martin et al.*, 2002; *Farr*, 1990; *Lechowich*, 1993).

The advantage of this method, in contrast to traditional heat treatment, is killing microbes and inactivating enzymes without the undesirable effects of high temperature. This method does not change the product's taste, texture or colour and is less harmful to its nutritional value. Product freshness is better preserved as well. Additives may be reduced or eliminated completely. Moreover, with this method, new technological and functional properties may be developed (*Rico et al.*, 2007; *Oey et al.*, 2008).

Even in the area of liquid products, HHP technology is applied with increasing frequency (*Table 1*, *Knorr et al.*, 2011). Until now, rather small-capacity laboratory equipment has been used for testing the many advantageous properties of high hydrostatic pressure treatment of foods. The aim of our experiments was to determine the laboratory and pilot plant systems' degree of ability to kill microbes and, further, to discover how those results are comparable with the data in the literature.

Country	Year	Product
Japan	1993	Rice wine
France	1994	Citrus juice
Mexico	2000	Citrus juice and concentrate
Lebanon	2001	Fruit juices
Portugal	2001	Apple and citrus flavour fruit juices
Czech Republic	2004	Broccoli, apple, beet and carrot juices
USA	2005	Fibre fruit juices
Northern Ireland	2006	Apple, wild strawberry and ginger juices

Colostrum

Table 1: Examples of high-pressure processed products commercially available in different countries

#### 2 Materials and methods

2009

#### HHP treatment

New Zealand

Our experiments were carried out according to the parameters in published articles (matrix, treatment time, pressure during treatment and microbes). The Budapest Corvinus University Department of Refrigeration Technology and Animal Products owns two HHP systems: one laboratory type (Stansted Food Lab 900, plunger press system) and one pilot plant type (RESATO FPU 100-2000). Thus, it was possible to apply measurements derived from the literature, using similar parameters, on two separate machines.

#### Microbiological tests

We intended to examine each change in total viable cell count and the death of each important microbe specific to a given product. For our tests, we prepared inoculate with microbes grown on slant agar, after which we infected the samples with amounts which produced measurements similar to those found in the literature.

# Preparation of Salmonella Enteritidis, inoculation of samples and colony counting

The Salmonella enterica subsp. enterica, serotype Enteritidis NCAIM B2052 species used in the experiments was obtained from the National Collection of Agricultural and Industrial Microorganisms (NCAIM). We prepared inoculate from microbes grown throughout 24 hours on meat broth slant agar, after which we added two streaks of preparation to each 10 ml of sterile peptone

water (the diluting liquid), and then this was administered in various amounts to the samples. Following HHP treatment, dilution plating was completed with selective XLD and Harlequin Salmonella agar. Plates were incubated for 48 hours at 37 °C, after which the growth of colonies was determined with a colony counter.

# Preparation of *Listeria monocytogenes*, inoculation of samples and colony counting

The inoculation and colony counting of the samples with L. monocytogenes was carried out similarly to Salmonella Enteritidis, except that Brain Heart agar was used for the slant growth and plate pouring was done with PALCAM agar.

# Preparation of *Escherichia coli*, inoculation of samples and colony counting

Inoculation and colony counting of samples with *E. coli* was similar to that of *Salmonella Enteritidis*, but plate pouring was carried out with ChromoCult Coliform agar.

# Preparation of Staphylococcus aureus, inoculation of samples and colony counting

Inoculation and colony counting of samples with *S. aureus* was completed in a similar way to *Salmonella Enteritidis*, but here plate pouring was done with Baird Parker agar.

# Preparation of $Pseudomonas\ aeruginosa$ , inoculation of samples and colony counting

The inoculation and colony counting of *P. aeruginosa* samples happened in a similar way to that of Salmonella with the exception that Cetrimide agar was used for plate pouring.

#### Determination of aerobic viable cell count

A series was prepared from both the treated and untreated (uninfected) samples. They were diluted at a ratio of  $10^{-1}$  with sterile water, after which we used Nutrient agar plate pouring to determine the microbe levels in the samples. Plates were incubated for 48 hours at  $37\,^{\circ}\text{C}$  and a colony counter was used to determine how many colonies had appeared.

#### 3 Results and discussion

It can be seen from our results that the levels of microbe death in certain foods varied only slightly between the laboratory and the (pilot) plant, while they agreed with the data in the literature (Figure 1). Slight variations in the total viable cell count test could be attributed to deviations in the microflora composition; variations in the test of given phyla may derive from differences of serotype; moreover, it can be said that the homogeneity of the food used as matrix was imperfect (water and fat content).

It is apparent from the data that results taken from the literature and laboratory systems allow us to make conclusions about how the material's microbiological condition will change during processing in a plant.

Figure 1 shows literature data (the measurements which we tried to reproduce) and correlation to results from pilot plant systems. It is clear that there is a close correlation, ( $r^2 = 0.96$ ). The  $r^2$  value was 0.99 for Salmonella, 0.92 for Listeria, 0.98 for E. coli, 0.77 for S. aureus, 0.96 for Pseudomonas aeruginosa and 0.87 for total viable cell count.

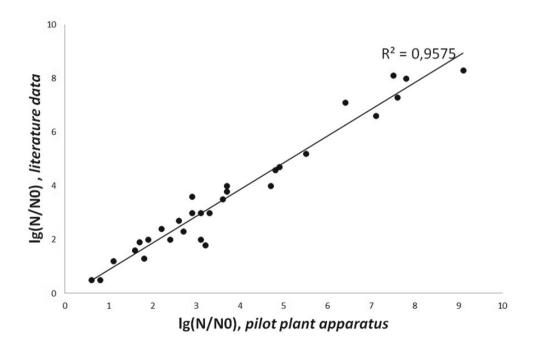


Figure 1: Correlation between the literature and the industrial results

Table 2: Comparison of literature data and our results

	p	$\mathbf{t}$	LAB	PPA	LIT.	REF.
Salmonella						
(Enteritidis) meat broth	345	600	3.3	3.1	3.0	Alpas et al., 2000
phosphate buffer	350	600	0.9	1.1	1.2	Tholosan et al., 2000
boiled ham	400	600	2.0	1.7	1.9	Aymerich et al., 2005
(Enteritidis) ham	500	600	6.5	6.4	7.1	Yuste et al., 2000
broth	500	600	8.2	7.8	8.0	Mackey et al., 1994
Listeria monocytogenes						
phosphate buffer	375	900	2.2	2.4	2.0	Patterson et al., 1995
boiled ham	400	600	2.5	2.2	2.4	Aymerich et al., 2005
turkey breast	400	60	0.7	0.6	0.5	Chen, 2007
ham	450	600	3.0	2.9	3.6	Morales et al., 2006
turkey breast	500	60	4.1	3.7	3.8	Chen, 2007
Escherichia coli						
liquid whole egg	300	600	0.8	0.8	0.5	Ponce et al., 1998
(O157-H7) meat broth	345	600	7.7	7.5	8.1	Alpas et al., 2000
liquid whole egg	450	600	3.3	3.6	3.5	Ponce et al., 1998
phosphate buffer	500	300	7.5	7.6	7.3	Hauben et al., 1997
phosphate buffer	600	300	8.7	9.1	8.3	Hauben et al., 1997
Staphylococcus aureus						
sheep's milk	345	600	4.2	3.7	4	Alpas et al., 2000
water	400	1800	5.0	5.5	5.2	Arroyo et al., 1999
sheep's milk	450	1800	3.0	3.2	1.8	Arroyo et al., 1999
milk	600	900	2.2	1.9	2.0	Mackey et al., 1994
turkey meat	600	900	3.2	2.9	3.0	Mackey et al., 1994
Pseudomonas						
sheep's milk	250	1800	7.0	7.1	6.6	Trujillo et al., 2002
(aeruginosa) water	300	1800	5.1	4.8	4.6	Arroyo et al., 1999
(fluorescens) water	300	1800	4.5	4.9	4.7	Arroyo et al., 1999
broth	345	600	4.5	4.7	4.0	Alpas et al., 2000
corned beef	500	300	3.4	3.1	2.0	Rubio et al., 2007
Viable cell count						
chicken liver	200	1200	1.5	1.8	1.3	Tuboly, 2009
sliced pineapple	270	900	1.5	1.6	1.6	Aleman et al., 1997
liquid egg	300	600	2.4	2.7	2.3	Németh et al., 2012
sliced pineapple	340	900	2.9	3.3	3.0	Aleman et al., 1997
ham in brine	600	360	3.0	2.6	2.7	Garriga et al., 2004
n: Mpa treatment pressure.	Mpa					

p: Mpa treatment pressure, Mpa

LAB: lg(N/N0) value in a laboratory apparatus

P.P.A.: lg(N/N0) value in a pilot plant apparatus

LIT.: lq(N/N0) value from literature data

REF.: literature which was used and which belongs to the LIT

### 4 Conclusions

In summary, we can say that the microbe-reducing effect of the HHP treatment – for the purposes of a processing plant – can well be calculated from literature data and from laboratory measurements. This very advantageous characteristic is due to the fact that with HHP technology the same pressure

t: treatment time

bears on every point of the treated material simultaneously, regardless of the volume or size of the product.

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