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## Microbiological evaluation of raw dog foods underwent HPP (High pressure processing) treatment

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### Abstract

The study includes three raw dog foods (diets) consisting of different types of meat. After production, each of them underwent additional high-pressure processing (HPP). The aim of the study is to perform a microbiological analysis of raw food known as „Raw meat-based diets” (RMBDs) and “Biologically Appropriate Raw Food” or “Bones and Raw Food” (BARF) that has undergone HPP treatment. The diets were tested for pathogenic microorganisms - *Salmonella enterica*, *E. Coli*, *L. monocytogenes* and Total Plate Count (TPC).

Microbiological analysis indicates that the three types of diets that have undergone HPP treatment are edible up to the 30th day of storage up to 0-4 °C.

Our results showed that there is a significant increase in shelf life without the use of preservatives or other additives. The end product is safe, not only for the dog but also eliminates the risk of asymptomatic carriage of some zoonoses, which can infect the owners.

**Keywords:** BARF/ RMBDs, HPP (High pressure processing), shelf life, pathogens, TPC (Total plate count)

### Introduction

#### Dog and BARF

In recent years, the dogs and cats feeding with raw foods has become increasingly popular among the pet owners (Freeman and Michel 2001) [38]. According to (Freeman *et al.*, 2013) [14], this practice presents a potential risk for the human and animal health. The most studies on the raw food use are associated with the risk of microbial contamination (Lenz *et al.*, 2009) [23]. The manifestation of the clinical symptoms in healthy animals in the presence of microbial contamination of raw foods is not the main problem. The excretion of pathogens by their faeces is the real risk for human health (Carter and Quinn 2000; Lenz *et al.*, 2009) [7, 23].

Particular attention is paid to *Salmonella enterica*, *Escherichia coli*, *Listeria*, *Clostridium* and *Campylobacter spp.*, as these microorganisms can be linked to the farm animals that may be used as source of raw materials for this type of food (Jenkins *et al.*, 2016) [18].

Dogs, as generation of predators, have many physiological and anatomical adaptive mechanisms that allow them to tolerate relatively high levels of microorganisms in their diet. As a consequence, usually they do not have clinical symptoms, when they consume the food with the high number of bacterial contaminants (Lenz *et al.*, 2009) [23]. When these microorganisms are excreted with the dog's faeces, there is a real risk to human health, because these pathogens can cause various diseases in humans (Lenz *et al.*, 2009) [23]. The presence of *Salmonella enterica* in the faeces of clinically healthy dogs, regardless of their diet, varies between 1.0 and 18.0%, but it is suggested that much higher levels of *Salmonella enterica*, are required to spread the infection (Sanchez *et al.*, 2002) [27].

There is evidence in the scientific literature of clinical cases of salmonellosis in domestic animals caused directly or indirectly by feeding them with raw food (Striver *et al.*, 2003; Morley *et al.*, 2006) [34, 25].

The results of a survey by Joffe and Schlesinger (2002) [19] with a group of dogs showed that not a small percentage of dogs fed BARF, gave a positive fecal sample for *Salmonella enterica*, and nearly 80% of the raw foods tested contained *Salmonella enterica*.

## HPP and BARF

Various processing technologies are available to reduce meat products contamination with microorganisms (Delmore *et al.*, 1998) [11]. These treatments include hot water, organic acids, chlorine, steam, pasteurization and high pressure treatment (HPP) (Belk 2005; Sofos 2005; Skandamis *et al.*, 2008) [5, 32, 30].

Inactivation of the vegetative bacteria in the food, using a high-pressure treatment, has been proven by various authors (Cheftel 1995; Smelt 1998, Farkas and Hoover 2000; Yuste *et al.*, 2001, Adamcova *et al.* 2019) [9, 31, 13, 37, 1].

HPP is a type of preserving technology for different types of foods, which uses high pressure without heat treatment (Hugas *et al.* 2002) [17].

During the HPP, the food is treated with a high hydrostatic pressure of 100-1000 MPa. The product temperature and exposure time can be adjusted by varying in temperature from 0°C to 100 °C and the time can be last from a few seconds to 20 minutes (Yaldagard *et al.*, 2008) [36]. The survey of other authors also noted that the use of HPP decreases a microbial load and this technology is widely used in the production of meat products (Hugas *et al.* 2002 and Simonin *et al.* 2012) [17, 29].

A study by Argyri *et al.* (2019) [4] on the raw chicken meat, confirms that the HPP - treated product has a significantly longer shelf life and minimal number microorganisms. The same author, but in another team, proves again the effectiveness of the technology by examining chicken inoculated with *Salmonella enterica*. Their results show that HPP technology deactivates salmonellosis bacteria regardless of their amount in the product (Argyri *et al.* 2018) [3].

Similar are Garriga *et al.* (2004) [15] results who research meat and meat products in vacuum packs after high pressure treatment (600 MPa for 6 min). They proved that HPP processing is an effective method of slowing down the growth of microorganisms involved in meat degradation and reducing the safety risks associated with *Salmonella enterica* and *L. Monocytogenes*.

Ananth *et al.* (1998) [2] conduct a similar survey with raw pork meat. The authors find that a pressure of 414 MPa for 13 minutes at 25°C inactivates the most common pathogens in foods such as *Listeria*, *Salmonella* and *Coli* bacteria. In addition to the deactivation of the bacteria, this treatment of the product extends the shelf life of the HPP treated meat.

Kruk *et al.* (2011) [21] also trace the effect of HPP processing on *E. coli*, *Listeria monocytogenes* and *Salmonella typhimurium*, in chicken meat. Using different pressures, the authors prove that the microorganisms present in the meat are eliminated, the shelf life is prolonged and the meat is safe for consumption.

The aim of this study is to evaluate microbiologically three raw diets for dogs, before and after technological treatment with HPP

## Materials and Methods

The research was conducted with 210 samples of three different dog raw foods. The ingredients of the diets are presented in Table No 1.

**Table 1:** Diet composition

Diet No 1	Ingredients Beef meat, chicken by-products, spinach, apples, eggs with shell, yoghurt, olive oil
Diet No 2	Mechanically separated chicken meat, beef liver, apples, zucchini, eggs without shell, cottage cheese, olive oil
Diet No 3	Beef meat, mechanically separated chicken meat, pork meat, beef liver, chicken hearts, carrots, eggs without shell, yoghurt, olive oil.

Our raw food is produced under the same conditions and packaged in special vacuum packs (withstanding high pressure). In addition, all production safety requirements are kept. All raw materials used are fit for human consumption.

After the raw food production, control samples 40 of each diet were left and the remaining amount of samples was treated with HPP. The three diets were tested on the day of production (day 0) for *Salmonella enterica*, *E. coli*, *L. monocytogenes* and Total Plate Count, then cooled at 0-4°C and stored at these temperature conditions throughout the experiment.

Samples (40 controls of 40 each diet ) were taken from each diet that had not undergone HPP treatment and were tested for Total Plate Count on days 0, 5, 10, and 15.

Also, 30 samples were taken from each diet that were treated by HPP for 3 minutes at 6000 bar and then stored at 0-4°C throughout the experiment. The microbiological tests were performed on days 0, 15 and 30 of the date of production for *Salmonella enterica*, *Listeria monocytogenes*, *Escherichia coli* and Total plate count.

The microbiological studies have been carried out in an accredited laboratory in accordance with ISO / IEC 17025: 2017 using the following test methods for each microorganism:

- ***Salmonella enterica*:** Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Detection of *Salmonella* spp. (ISO 6579-1:2017).
- ***Listeria monocytogenes*:** Microbiology of the food chain - Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. - Part 1: Detection method (ISO 11290-1:2017).
- ***Escherichia coli*:** ISO 16649-2:2014 - Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* - Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide (ISO 16649-2:2014);
- **Total Plate Count:** Microbiology of the food chain - Horizontal method for the enumeration of microorganisms - Part 1: Colony count at 30 degrees C by the pour plate technique (ISO 4833-1:2013).

HPP treatment is carried out with "AVURE AV-20M high-pressure treatment equipment" for storage microbiological reduction. The machine is capable of adjusting the pressure up to 660 MPa and with an operating temperature of 0-29 °C. The food was packed in individual high-pressure vacuum packs. The packaging used meets the requirements of Regulation (EC) No. 1935/2004. The food is packed in high-barrier polypropylene films meeting the criteria "Oxygen Transmission Rate" (OTR) < 5 and "Water Vapor Transmission Rate" (WVTR) < 5. The parameters during the HPP treatment of all samples are as follows: All samples were in the cooled state, duration of 3 minutes at a pressure of 6000 bar., also described by Yaldagard *et al.* (2008) [36].

## Statistical analysis

All results were processed statistically using Microsoft Excel

for Windows. The obtained results are presented as mean value with standard deviation ( $X \pm SD$ ), after applying statistical analysis with Student T-test. We considered statistically significant differences at  $p < 0.05$ .

## Results and Discussion

To survey the impact of technology on food shelf life, we conducted microbiological analyzes in dynamics before HPP treatment.

Table No. 2 presents the results obtained for the total plate count (TPC) for all three raw food diets before HPP treatment. The data showed that for the period from day 0 to

day 5, all diets had low microbial counts, indicating that the number of microorganisms was below acceptable levels and the food was safe and fit for consumption by dogs. After the 10th and 15th day of the production of the raw food, the total number of microorganisms increases and is above the permissible values and the food is not fit for consumption. In general, it is clear that after the 5th day of production, the food cannot be used, because the TPC is significantly increased, and in addition, visible signs of spoilage and a significant change in all organoleptic indicators began to be observed.

**Table 2:** The TPC of the three diets (not treated with HPP) (n-120)

Day Diet	Day 0 CFU ( $10^4$ )g	Day 5 CFU ( $10^4$ )g	Day 10 CFU ( $10^7$ )g	Day 15 CFU ( $10^9$ )g	Limits
Diet No 1	1,2 $\pm$ 0,42	9,2 $\pm$ 0,47	1,1 $\pm$ 0,29	1,3 $\pm$ 0,39	< 5.10 <sup>6</sup>
Diet No 2	2,8 $\pm$ 0,33	3,9 $\pm$ 0,39	1,8 $\pm$ 0,33	1,2 $\pm$ 0,42	< 5.10 <sup>6</sup>
Diet No 3	3,9 $\pm$ 0,29	7,4 $\pm$ 0,32	1,3 $\pm$ 0,47	1,2 $\pm$ 0,45	< 5.10 <sup>6</sup>

The remaining samples of the diets have been treated by HPP for 3 min at 6000 bar, and then stored in at 0-4 °C throughout the test period. Each diet was examined on day 0, day 15, and day 30 of production date for *Salmonella enterica*, *L. monocytogenes*, *E. coli* and Total Plate Count.

Table No. 3 presents the results of the microbiological studies of diet No 1 before and after the HPP processing in dynamics. On the day before HPP treatment (day 0), *Salmonella enterica* and *L. monocytogenes* were not detected in the food, which is normal considering that we are using raw materials suitable

for human consumption. The amount of *E. coli* is below the permissible value as well as the TPC - 2.8.104 $\pm$ 0.33 CFU/g. The results showed the absence of *Salmonella enterica* and *L. monocytogenes* until the end of the experiment (lasting 30 days). On day 15, the TPC decreased to <1.103 CFU/g as a result of HPP treatment, compared to day 0 (2.8.104 $\pm$ 0.33 CFU/g). The HPP treatment significantly reduces the TPC, and on the 30th day, it is 4.9.104 $\pm$ 0.46, which is below the permissible limit (of < 5.106 CFU /g).

**Table 3:** Diet No 1 before and after HPP treatment (n -30)

Microorganisms	Before treatment with HPP	After treatment with HPP		Limits	Test conditions
	Day 0	Day 15	Day 30		
<i>Salmonella enterica</i> cfu/10g	Absence	Absence	Absence	Absence	T=(37,0 $\pm$ 1,0) °C, T=(41,5 $\pm$ 0,5) °C
<i>Listeria monocytogenes</i> cfu/25g	Absence	Absence	Absence	Absence	T=(37,0 $\pm$ 1,0) °C, T=(41,5 $\pm$ 0,5) °C
<i>Escherichia coli</i> cfu/g	< 10	< 1	3,6.10 <sup>1</sup> $\pm$ 0,21	< 500	T=(37,0 $\pm$ 1,0) °C, T=(44,0 $\pm$ 1,0) °C
Aerobic colony count cfu/g	1,2.10 <sup>4</sup> $\pm$ 0,42	< 1.10 <sup>3</sup>	1,5.10 <sup>5</sup> $\pm$ 0,38	< 5.10 <sup>6</sup>	t=(30,0 $\pm$ 1,0)°C

The results of microbiological tests showed that diet No 1 (beef) is suitable for consumption until the 30th day when stored in refrigerated conditions (0-4 °C). Bermudez-Aguirre and Barbosa-Canovas (2011) [6] reported an increase in shelf life after HPP treatment which our data confirm. Researchers also have discovered that HPP processing is an effective technology for reducing bacterial contamination and ensuring the microbiological safety of foods (Cheftel and Culioli, 1997; Huang *et al.*, 2014; Sheen *et al.*, 2015) [8, 16, 28].

The microbiological studies of diet No. 2 (chicken), which underwent HPP processing, are presented in Table No 4. On the day before processing (day 0), *Salmonella enterica* and *L. monocytogenes* were not detected, as in the first diet. Until the end of the experiment /day 30/, the presence of *Salmonella enterica* and *L. monocytogenes* was also not detected. On the 15th day of storage, *E. coli* values were < 1 CFU/g in the

HPP-treated food compared to the beginning of the experiment (<10 CFU/g) before treatment. HPP also called cold pasteurization, reduces *E. coli* levels. On the 30th day, the values for *E. coli* were 3.6.101 $\pm$ 0.21, which is below the acceptable level (of <500 CFU/g).

On day 15, the TPC decreased to <1.103 CFU/g compared to the day 0 - 1.2.104 $\pm$ 0.42 CFU/g before HPP treatment. The results showed a significant reduce the TPC, which on the 30th day is 1.5.105 $\pm$ 0.38 CFU/g at a limit of < 5.106 CFU/g. It confirms Hugas *et al.* (2002) [17] and Simonin *et al.* (2012) [29] statements that HPP reduces the microbial load of food and extends shelf life. The obtained results correspond with those of Argyri *et al.* (2019) [4], who prove that raw chicken meat treated with HPP has a significantly longer shelf life and minimal amounts of microorganisms.

**Table 4:** Diet No 2 before and after HPP treatment (n -30)

Microorganisms	Before treatment with HPP	After treatment with HPP		Limits	Test conditions
	Day 0	Day 15	Day 30		
<i>Salmonella enterica</i> cfu/10g	Absence	Absence	Absence	Absence	T=(37,0 $\pm$ 1,0) °C, T=(41,5 $\pm$ 0,5) °C
<i>Listeria monocytogenes</i> cfu/25g	Absence	Absence	Absence	Absence	T=(37,0 $\pm$ 1,0) °C, T=(41,5 $\pm$ 0,5) °C
<i>Escherichia coli</i> cfu/g	< 10	< 10	< 10	< 500	T=(37,0 $\pm$ 1,0) °C, T=(44,0 $\pm$ 1,0) °C
Aerobic colony count cfu/g	2,8.10 <sup>4</sup> $\pm$ 0,33	<1.10 <sup>3</sup>	4,9.10 <sup>4</sup> $\pm$ 0,46	< 5.10 <sup>6</sup>	T=(30,0 $\pm$ 1,0) °C

- The presence of *Salmonella enterica* and *L. monocytogenes* was not detected, both before and after

HPP treatment and in diet No 3 (mix) (Table No 5). We observed again that the TPC did not exceed the initial amounts of < 10 CFU/g.

- A reduced microbial count on day 15 (<1.10<sup>3</sup> CFU/g) due to HPP treatment, compared to day 0, which again confirms the claim that the technology used significantly reduces the amount of microorganisms. On the 30th day of food storage, the TPC was 6.4.10<sup>5</sup>±0.43, which is

below the permissible limit (< 5.10<sup>6</sup> CFU/g). Our results are similar to those documented by other authors (Bermúdez-Aguirre and Barbosa-Cánovas, 2011) [6]. The HPP significantly increases the period of reliability, achieving significant decontamination of the product.

- Our study sustains Stewart and Cole's (2001) [33] claim that HPP technology not only extends shelf life but also improves food safety.

**Table 5:** Diet No 3 before and after HPP treatment (N -30)

Microorganisms	Before treatment with HPP	After treatment with HPP		Limits	Test conditions
	Day 0	Day 15	Day 30		
<i>Salmonella enterica</i> cfu/10g	Absence	Absence	Absence	Absence	T=(37,0±1,0) °C, T=(41,5±0,5) °C
<i>Listeria monocytogenes</i> cfu/25g	Absence	Absence	Absence	Absence	T=(37,0±1,0) °C, T=(41,5±0,5) °C
<i>Escherichia coli</i> cfu/g	< 10	< 10	< 10	< 500	T=(37,0±1,0) °C, T=(44,0±1,0) °C
Aerobic colony count cfu/g	3,9.10 <sup>4</sup> ±0,29	< 1.10 <sup>3</sup>	6,4.10 <sup>5</sup> ±0,43	< 5.10 <sup>6</sup>	T=(30,0±1,0) °C

All three diets' microbiological tests meet the requirements of Regulation 142/2011, which imposes zero tolerance regarding salmonellosis bacteria.

Our research data on raw dog food also confirm the thesis of Koutchma (2014), for a safe end product with a longer shelf life, without the use of preservatives and without significant change in the nutritional and organoleptic characteristics after HPP treatment.

### Conclusions

The data of the current study demonstrate that HPP treatment for 3 min at 6000 bar of BARF, significantly reduced the presence of *E. coli* and delay the increase of the TPC and the risk of food spoilage. After the HPP treatment of the BARF and storage at a temperature of 0-4 °C we can achieve a shelf life of up to 30 days. Last but not least we have decontamination of raw food without heat treatment or the use of various types of preserving products and additives. Our results suggest that the technology could be successfully implemented in BARF manufacturing, producing a safe product for the health of both pets and their owners.

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