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ORIGINAL RESEARCH PAPER

PROCESSING SLIME FROM SNAIL (*HELIX ASPERSA MAXIMA*): A PRELIMINARY STUDY ON THE EFFECT ON MICROBIAL LOAD

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Abstract: Slime from earth snails (*Helix aspersa maxima*) is a sub-product often used in the cosmetic industry. Due to its origin, a decontamination process ought to be considered. After exposure to UV radiation, no significant ($P > 0.05$) reduction of the initial microbial load of slime was observed, while treated at 60 °C, a decrease of 4.0 log $CFU \cdot mL^{-1}$ was obtained (when compared to non-treated slime). A similar reduction was attained for pressurized (500 MPa) slime. The sequential combination of temperature and pressure treatment had a similar effect ($P > 0.05$) on the microbial load when compared to 400 MPa (30 min), but the reduction was higher when compared to the less intense treatments (60 °C, 15 min; 400 MPa, 15 min). Overall, the results suggest that pressure (≥ 400 MPa) is a promising technology for processing snail slime, which can have positive effects on its microbial load, for further use in the cosmetic industry.

Keywords: *high pressure, microorganisms, snail (Helix aspersa maxima), temperature, UV radiation*

INTRODUCTION

The land snail *Helix aspersa*, often known as the garden snail, is a gastropod mollusk belonging to *Helicidae* family [1]. It is quite ubiquitous in Europe and the American continent, inhabiting temperate, tropical and even desert natural habitats, as well as being intensively reared in captivity for human consumption [2, 3]. Besides being considered a delicacy, snails possess a remarkable nutritional content (very low fat and high protein content per weight) [3], and are often found commercially available as live or processed [4].

The EU is the world's biggest importer of terrestrial snails, given the tradition of their consumption in Mediterranean countries, including Portugal. Heliciculture as a production sector was commercially developed in the last decades of the 20th century and has been legislated in Portugal since 2007 [5]. Snails for human consumption are defined in paragraph 6.2, Annex I of Regulation (EC) No. 853/2004 [6] as terrestrial gastropods of the species *Helix pomatia* Linné, *Helix aspersa* Müller, *Helix lucorum* and species of the family *Achatinidae*. The rising threat of foodborne pathogens also reaches snails' commercialization and distribution. Predominant bacterial species of the *Enterobacteriaceae* family, Gram-positive *Staphylococcus aureus* (*S. aureus*) and *Listeria* spp. have already been detected in snails [7 – 9]. *Aeromonas hydrophila* and *Escherichia coli* (*E. coli*) are among the foodborne disease agents that can also be detected [10 – 12]. *Salmonella enterica* was also detected in live edible *Helix pomatia* snails directed to the European Union [9]. The absence or the scarcity of information regarding this specific sector does not guarantee consumer safety and, at the same time, could discourage the business of farming and marketing. Veterinary controls are the last part of the productive chain. Clear rules are needed for the labeling, traceability and the origin of the snails that reach the market. In Portugal, like in any other Mediterranean countries, this issue is of considerable concern. Even though snails can represent a risk, the type of treatment that snails are usually submitted to must be considered because correct cooking can reduce it. Most of the snails are introduced in the food-processing industry and subjected to cooking and subsequent freezing as snail meat, usually placed back in the shell [3, 7, 8, 11].

One of the complications in commercial processing of snail meat has been the naturally produced mucus or "slime" secreted by the snails, used in their locomotion, defense, water retention and other physiological activities [13]. When snails are considered for their meat commercialization, slime can be an impediment, but if it is regarded as a sub-product with special properties, it could address the needs of the pharmaceutical and cosmetic industries. The slime contains important amounts of collagen, elastin and other biochemical components, such as allantoin, vitamins, and glycolic acid, useful in the pharmaceutical and cosmetic industries [1, 14, 15], as well as in medicine [16]. Little information can be found in open literature regarding the procedures and processing conditions to obtain the extract of slime for the cosmetic and pharmaceutical industries. The general information that could be gathered is that slime is collected, settled, filtered, concentrated and then dried according to various processes, either at low temperature under vacuum evaporation, or freeze dried or spray dried. The resulting aqueous solutions can then be retained aseptically within sterile vials after filtration through specific filters, or by adding antiseptic substances, the most commonly glycerin [14]. The several stages of the process require economical (equipment, maintenance and

operational) costs. For these reasons, along with the richness of its composition which could be easily altered by temperature, other emerging technologies such ultraviolet (UV)-C irradiation and high hydrostatic processing which are being applied with success in the food industry [17, 18], could be considered to treat the "raw" slime, respecting the original, "natural" characteristics of the product and, at the same time, possibly guarantee its safety.

The use of UV radiation is one of several physical processes which can be used for sterilization or sanitization of surfaces, as UV radiation application in the decontamination of food products is still not widely used due to their low degree of penetration, but it is known that it can be easily applied to liquid and solid food products, that will come into contact with food. As a physical preservation method, UV irradiation is gaining a positive consumer image [19]. Ultraviolet light has been documented to be effective in reducing various bacterial populations on egg shell surfaces including total aerobic plate count [20], *Salmonella* Typhimurium and *E. coli* [21]. High pressure processing (HPP) within the range of 400 to 600 MPa, at refrigeration or ambient temperatures, has been the most successful alternative technology adopted by the food industry for the pasteurization of liquid and solid foods generally having a low impact on their functional properties and nutritional value and, at the same time, assuring their food safety [22]. Recently the combination of temperature (high or low) with pressure has been extensively studied because of synergistic effects of HPP, especially as regards the combination of different intensities of pressure [19].

The objective of this study was to evaluate the effect of several treatments (low temperature, UV-C, pressure and temperature-pressure) on the microbial load of slime of *Helix aspersa maxima (H. aspersa maxima)* snail to ensure that their marketing could be practicable and, to our present knowledge, this is the first paper in this research field.

MATERIALS AND METHODS

Sample collection

The experimental work was carried out from March to June 2016. The collection and delivery of the samples (eggs, baby snails, slime) from *Helix aspersa maxima* snails was carried out by a Northern Portuguese producer. The samples were stored at 4 ± 1 °C until processing and further microbiological analysis.

Processing conditions

UV-C exposure

Slime from *Helix aspersa maxima* snail (*ca.* 10 g), in the liquid state, was transferred, in two independently assays, to one sterile transparent polystyrene Petri dish (60 mm of diameter), with no lid on, and placed inside of a camera (75 × 70 × 45 cm^3) designed by University of Algarve (Portugal), with four germicidal UV lamps (TUV 15W/G15 T8, Philips, Holland). The UV radiation lamps presented a peak emission at 254 nm (UV-C), and before use, they were turned on for 60 min to stabilize the radiation emission. Samples were placed inside the chamber in a specific position [17] allowing an UV-C radiation intensity of 13.44 $W \cdot m^{-2}$, according to the conditions used in the

protocol described by authors with the same chamber for similar objectives [23, 24]. The samples were placed 30 cm below the UV-C lamps and, at different pre-set time intervals (0, 30 and 60 min), samples (*ca.* 1 mL each) were taken.

Temperature and high pressure processing

Aliquots of slime (*ca.* 10 g) were transferred to low permeability polyamide-polyethylene bags (PA/PE-90, Albipack - Packaging Solutions, Águeda, Portugal). The bags are made of polyamide (PA) and polyethylene (PE), which are a popular packaging frequently used in the food industry, and often used for our pressurized food material. PA/PE films are composed of at least one layer of PA and one layer of PE. Their composition can, however, be more complicated in some cases. The attributed properties from the polyamide point of view are an improvement on the mechanical properties (high tear and puncture resistance) and low gas barrier as well as grease and aroma permeability; while from the polyethylene, water vapor impermeability is considered, acting as a sealant layer and stabilizes the film. The bags (7 × 15 cm) were heat sealed manually with care to avoid as much as possible to leave air inside. Each bag containing the slime was afterward inserted in a second PA/PE-90 bag that was heat sealed under vacuum. For the thermal treatment, a controlled (T ± 1 °C) water bath (Julabo FP40, Seelbach, Germany) was used previously stabilized at 62 °C. In order to guarantee that 60 °C was attained at the center of the sample, preliminary assays were performed. A slime sample was put in the thermostatic water bath, with a thermocouple inserted and the time-temperature was followed. The assay was done in triplicate. A 75 s period for come-up time was set and further considered as time 0. For high pressure processing, the slime samples were double-vacuum-sealed in PA/PE-90 bags in order to avoid any possible contamination that might occur of the compression fluid. The pressurization fluid used was water, at a controlled temperature of 18 °C. Pressure build-up took place at a compression rate *ca.* 300 MPa min^{-1}, while decompression was nearly instantaneous. Pressure stability was studied within the range of 300 to 500 MPa (5 - 60 min, 20 °C), in a hydrostatic press from Hiperbaric (Model 55-L Burgos, Spain). For the sequential temperature-pressure treatment, the samples were initially treated at 60 °C (15 min), followed by pressurization at 400 MPa (20 °C, 15 min). Non treated samples were maintained at atmospheric pressure (0.1 MPa), under refrigeration (4 °C). The effect of all the applied treatments was determined by quantification of total aerobic mesophilic microorganisms [25]. All the experiments were carried out in two independent replicates.

Microbial analysis

One gram of each snail slime sample was weighed aseptically into a sterile tube with 9 mL of ¼-strength Ringer's solution (Lab M, LAB100Z, United Kingdom) and homogenized (by vortexing, up to 60 s). Serial decimal dilutions in sterile ¼-strength Ringer's solution were prepared for microbial enumeration of total aerobic mesophilic microorganisms in Plate Count Agar (PCA, Pronadisa, Cat nº 903, Spain), incubated at 30 ± 1 °C for 72 ± 3 h [25]; *E. coli* in Triptone Bile X-glucuronide (TBX, Bio-Rad, Cat nº 3564035, USA) incubated at 44 ± 1 °C for 48 ± 3 h [26]; coagulase-positive staphylococci in Baird Parker Agar (BPA, Biokar Diagnostics, Cat nº 43531, France) incubated at 37 ± 1 °C for 48 ± 3 h [27]; *Pseudomonas* spp. in Pseudomonas Agar Base

(Pronadisa, Cat nº 1153, Spain) incubated at 30 ± 1 °C for 48 ± 3 h [28]; *Enterobacteriaceae* in Violet Red Bile Glucose Agar (VRBGA, Biokar Diagnostics, BK011HA, France) incubated at 37 ± 1 °C for 24 ± 1 h [29]; *Lactobacillus* spp. on De Man Rogosa and Sharpe Agar (MRS agar, Lab M, LAB093, United Kingdom) incubated at 30 ± 1 °C for 72 ± 3 days [30]; and yeasts and molds on Rose Bengal Chloramphenicol Agar (Pronadisa, BK151HA, Spain) incubated at 25 ± 1 °C for 5 ± 1 days [31]. Detection of *Listeria monocytogenes* and *Salmonella* spp. was performed according to the International Standards, ISO 11290-2/Amd. 1:2004 [32] and ISO 6579:2002 [33], respectively. The same microbiological parameters were analyzed for eggs and baby snails. However, a 25 g of each sample were added to 225 mL of Buffered Peptone Water (BPW, Merck, Cat nº 1072280500, Germany), homogenized in a stomacher (Lab-Blender 400, Seward Medical, London, United Kingdom) for 1 min, and followed by plating, in duplicate, the appropriated decimal dilution.

Statistical analysis

The results were expressed as log colony forming units (CFU) per gram of eggs or baby snails (log CFU·g^{-1}) or log CFU per milliliter (log CFU·mL^{-1}) in case of slime. For treated samples, bacteria counts were transformed into logarithmic reduction using the equation: Log (N/N_0), where N is the logarithmic CFU·g^{-1} (or CFU·mL^{-1}) at a given treatment condition and N_0 is the initial log CFU·g^{-1} (or CFU·mL^{-1}). All experimental data of microbial load after the applied treatments was carried out to assess statistically significant differences among the samples. The differences were analyzed using the One-Way Analysis of Variances (ANOVA) using IBM SPSS Statistics, 22.0 (IBM Corporation, USA).

RESULTS AND DISCUSSION

Initial microbial load of samples

The favorable habitat for snails' development is normally composed of soggy soil, not compacted, and the presence of their own excrements around these animals can lead to elevated cross contamination. As such, the microbial load of non-processed eggs from *H. aspersa maxima* was firstly assessed, followed by baby snails and slime, as shown in Table 1.

All samples (eggs, baby snails and slime) presented high counts of total aerobic mesophilic bacteria, an important indicator of hygienic quality in foods. High counts of total aerobic mesophilic bacteria (6.8 log CFU·g^{-1}) and yeasts and molds (5.6 log CFU·g^{-1}) have already been reported by Temelli *et al.* [8] in live snails. Juvenile snails could also be susceptible to microbial contamination since their structural organs are still developing and, as a consequence, their associated biochemical reactions (e.g., several constituents that might be absent in slime) are not completely effective. Scarce literature has been found associated to the microbial load of slime from *H. aspersa*. However, Castro *et al.* [34] mentioned that slime prior to gamma radiation treatment presented an initial value of 5.2 log CFU·mL^{-1} for total aerobic mesophilic bacteria counts. Also found in literature was a raw materials specification sheet of

certified commercialized organic snail slime [35] which mentioned that when one gram of lyophilized snail slime from *H. aspersa* Müller is dissolved in one liter of water, it may present ≤ 2.9 log CFU·mL^{-1} and ≤ 2.0 log CFU·mL^{-1} in total aerobic mesophilic bacteria and yeasts, respectively.

Table 1. *Bacterial counts for different H. aspersa maxima snail samples (eggs, baby snail, slime)*

Microorganisms	Sample		
	Eggs [log CFU·g^{-1}]	Baby snail [log CFU·g^{-1}]	Slime [log CFU·mL^{-1}]
Total aerobic mesophilic bacteria	8.0 ± 1.0	8.4 ± 0.9	7.3 ± 0.9
Pseudomonas spp.	7.8 ± 0.8	8.8 ± 1.0	6.7 ± 0.9
Enterobacteriaceae	4.8 ± 0.0	6.0 ± 0.0	5.9 ± 0.9
Yeasts and moulds	4.8 ± 0.9	6.0 ± 0.0	5.7 ± 0.9
Staphylococcus spp.	4.3 ± 0.0	6.2 ± 1.0	6.3 ± 1.0
E. coli	n.d.	n.d.	n.d.
Lactobacillus spp.	n.d.	n.d.	n.d.
Listeria spp.	+[a]	+[a]	+[b]
Salmonella spp.	-[a]	-[a]	-[b]

n.d. – not detected (< 10 CFU·mL^{-1}, or < 1 CFU·g^{-1}); presence (+) or absence (-) in 25 g ([a]) or 1 mL ([b]) of sample

Snails can also harbor pathogenic bacteria capable of potential public health significance [36]. However, no *E. coli* and *Salmonella* spp. were detected in any samples. Even though no counts were obtained for *Listeria* spp., its presence was detected in all samples. The occurrence of *Listeria* spp. in live land *Helix pomatia* [8] and *H. aspersa* [9] snails has already been reported, and an indicator of a primary contamination source from raw material in the heliciculture plant. A recent study related with the assessment of current application of food safety legislation for primary production to snail farming performed in Portugal concluded that the growth conditions for their optimization were positive for contamination and microbial development, especially towards agents that are often present in soils and served by water [5]. No *Lactobacillus* spp. was detected in any of the samples.

When *Pseudomonas* spp. counts in snail slime was compared to baby snails, slightly lower counts ($P < 0.05$) were observed (Table 1). A possible explanation could be due to the presence of antimicrobial substances in adult snail slime. The antimicrobial properties of the slime collected from African giant land snails (*Achatina fulica*) were first described in the 1980s [37], which exhibited positive antibacterial activity for both Gram positive bacteria, *Bacillus subtilis* and *S. aureus*, and Gram negative bacteria, *E. coli* and *Pseudomonas aeruginosa (P. aeruginosa).* More recently, Pitt *et al.* [38] indicated that *H. aspersa* slime had a strong antibacterial effect against several strains of *P. aeruginosa* and a weak effect against *S. aureus*.

From the point of view of microbial quality, the snail slime to be incorporated into cosmetic products was considered as animal-derived cosmetic products (Category 2 - other products) according to the Guidelines on Microbiological Quality of the Finished Cosmetic Product [39]. The accepted criteria for total aerobic mesophilic bacteria and yeasts and molds were ≤ 10^3 CFU·g^{-1} (or mL^{-1}), while for *E. coli*, *P. aeruginosa*, and *S. aureus* should be absent in 1 g (or 1 mL). According to our results, if raw snail slime

is to be considered for direct (without any extraction procedure regarding specific components) incorporation in cosmetic products, a technological process should be considered in order to guarantee the safety of the product. The different processes considered were UV-C exposure, thermal and high hydrostatic pressure. To the authors' knowledge this is the first paper that studies the effect of these processes regarding the microbial load of *H. aspersa maxima* slime.

Effect of UV radiation, heat and high hydrostatic pressure conditions on the microbial load

After an UV-C radiation, the results related to total aerobic mesophilic bacteria indicated that there were no statistically ($P > 0.05$) differences between the control (no treatment, 7.3 log CFU·mL^{-1}) and after 60 min of slime exposure (7.3 log CFU·mL^{-1}). Snail slime is a viscous opaque matrix and as such the UV treatment can be limited due to low transmittance, which restricts the dose delivery and, consequently, an efficient microbial inactivation. Moreover, complex interactions may also occur between microorganisms and surface materials, such as shielding effect from incident UV, efficacy of UV light depends on surface structure or topography [19]. The correct choice and/or design of the UV reactor, its flow characteristics and UV source can reduce the interference of high UV absorption and viscosity associated to this kind of matrix. Castro *et al.* [34] have chosen a more energizing radiation (2, 3 and 5 kGy) to treat snail slime for the cosmetic industry. When gamma radiation (Co^{60}) at 3 kGy was applied, the initial microbial load of total viable counts reduced to values lower than the detection limit (1.0 log CFU·mL^{-1}), without significantly changing the physico-chemical properties. The same authors added that no changes in odor and color were also observed in all irradiated samples when compared to the control.
As it can be seen in Figure 1, there was a fast decline within the first 30 min of the heat treatment, followed by a slow decrease until the end of the treatment. As pointed out by Corradini and Peleg [40], bacterial heat inactivation only rarely follows a first-order kinetics. Apparently, there are two types of microbial populations in snail slime with different temperature sensitivities.

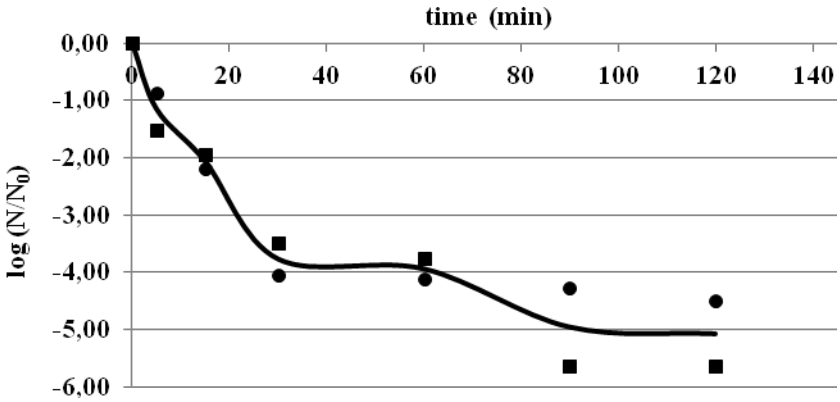

Figure 1. *Effect of low thermal treatment (60 °C) on the total aerobic mesophilic bacteria in slime from Helix aspersa maxima snail (the results (●, ■) represent two independent replicates)*

At 60 °C and after 15 min of exposure, yeasts and moulds were the most sensitive microorganisms, since there was decrease to values lower than the detection limit (≤ 10^3 CFU·mL^{-1}), while *Staphylococcus* spp. and *Pseudomonas* spp. presented a reduction of 1.5 to 1.7 log CFU·mL^{-1}. It was only after 120 min of thermal treatment that counts in snail slime samples decreased by *ca.* 5 log CFU·mL^{-1} to values close to the detection limit (1 log CFU·mL^{-1}). However, when visual analysis of heated slime was done, the colour samples clearly changed from greyish to brown after 15 min of temperature exposure (Figure 2).

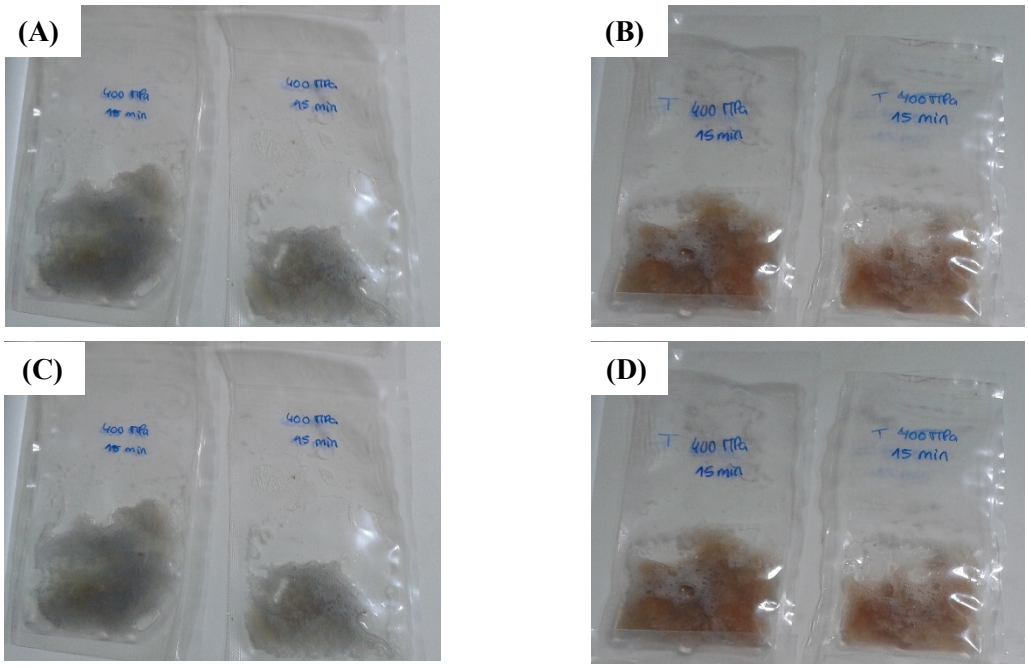

Figure 2. *Visual aspect of slime samples: (A) non-treated; (B) heat treated (60 °C, 15 min), before pressurization; (C) pressure treated (400 MPa, 15 min); (D) heat treated (60 °C, 15 min), followed by pressure (400 MPa, 15 min)*

Other properties (e.g., proteins, antimicrobials) should also be addressed to consider the possible application of this treatment for such an extended period, or eventually applying slightly higher temperatures to reduce the holding period. For example, the antibacterial factor found in the body surface of the African giant snail (*Achatina fulica*) was heat resistant at 50 °C but its activity was lost after heating at 75 °C for 5 min [41].
The effect of mild to high pressure treatments (300 - 500 MPa, 5 - 60 min) on the initial total aerobic mesophilic bacteria counts of snail slime is presented in Figure 3.
Short pressure treatments (5 min) were not effective (*ca.* 0.8 to 1.8 log reduction for 300 and 400 MPa, respectively) in reducing the total aerobic mesophilic bacteria of slime, with the exception of 500 MPa (*ca.* 3.8 log reduction when compared to non-treated samples). An increase in the holding period at 300 MPa did not increase the reduction of the initial microbial load ($P > 0.05$), with the exception of 60 min. However, the inactivation of the microbial load increased with pressure intensity. The pressure range is considered one of the important factors that influence microorganisms' inactivation and recently reviewed by Syed *et al.* [42]. To our knowledge this is the first time that the effect of pressure on microbial inactivation of snail *H. aspersa* slime has

been reported. Wang *et al.* [18] applied HHP treatments (100 - 500 MPa, 20 min, at room temperature) to vacuum-packed mud snails (*Bullacta exarata*) to ensure its safety.

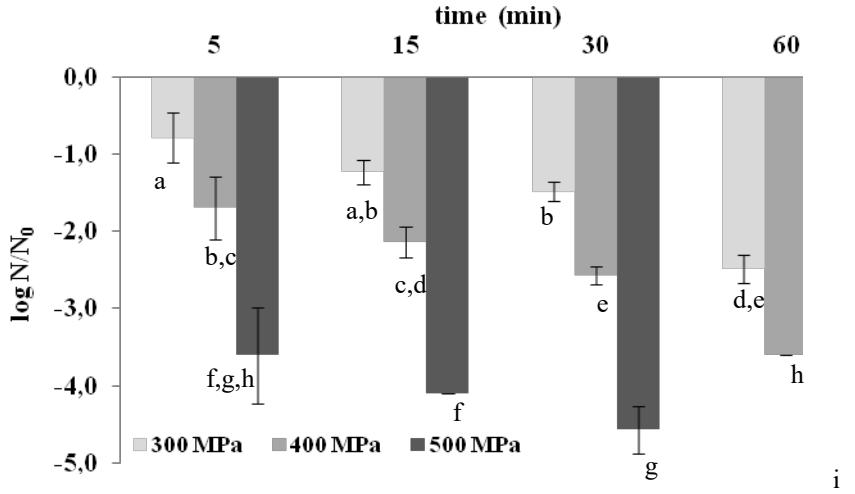

Figure 3. *Effect of high hydrostatic pressure (300 - 500 MPa, 5 - 60 min, 20 °C) on the total aerobic mesophilic bacteria counts from Helix aspersa maxima slime (different letters mean significant different results (P < 0.05))*

A significant decrease in the microbial load of mud snails, up to *ca.* 3.6 log CFU·mL^{-1} regarding the initial value, was observed when pressures ≥ 300 MPa (20 min) were applied.

At 400 MPa, an increase in the holding period, from 5 to 60 min, caused a significant (P < 0.05) decrease in the *Staphylococcus* spp., from 3.1 ± 0.2 log CFU·mL^{-1} to values lower than the detection limit, while *Pseudomonas* spp. reduced up to *ca.* 4 log CFU·mL^{-1} (Table 2).

Table 2. *Bacterial counts for different H. aspersa maxima snail slime after a pressure treatment of 400 MPa, for 5 and 60 min*

Microorganisms	**Slime snail [log CFU·mL^{-1}]**		
	0 min	**5 min**	**60 min**
Total aerobic mesophilic bacteria	7.3 ± 0.9	5.5 ± 0.1	7.3 ± 0.9
Pseudomonas spp.	6.7 ± 0.9	5.4 ± 0.2	3.8 ± 0.4
Yeast and molds	5.7 ± 0.9	n.d.	n.d.
Staphylococcus spp.	6.3 ± 1.0	3.3 ± 0.2	n.d.

n.d. – not detected (< 10 CFU·mL^{-1}, or < 1 CFU·g^{-1})

Even though *S. aureus* is considered one of the most pressure-resistant non-sporulating foodborne pathogens, the microorganism's pressure resistance is also highly dependent on the strain, and their growth conditions, the food matrix and experimental conditions [42]. According to Table 2, yeasts and molds present in slime were the most barosensitive population, since at 400 MPa and 5 min, their initial counts were reduced to values lower than the detection limit of the enumeration technique.

High hydrostatic pressures (HPP) are often applied in the food industry within the range of 400 to 600 MPa. When 500 MPa pressure treatments were applied to slime, an

increase of 100 MPa can lead to a decrease in the processing holding period, from 60 to 5 min, since a similar ($P > 0.05$) reduction in viable count was obtained (*ca.* 3.6 log CFU·mL^{-1}) (Figure 3). In food, the pressure treatment cost per kilogram of food depends on the operating pressure intensity and pressure holding time. For example, an increase from 400 to 500 MPa (at a certain time and temperature) can imply an increase of US $0.017/kg of processed cheese [43]. But prolonged periods of pressure treatments are also not very convenient: a 5 min holding time at a constant pressure would imply costs of US $0.159/kg, while 10 min would cost US $0.21/kg, 15 min US $0.263/kg, and 20 min US $0.316/kg [43]. Also, and when compared to the thermal treatment applied for 120 min, the treatment of 500 MPa for 60 min gave similar results ($P < 0.05$) but in contrast to thermally treated samples, no visual changes were observed for all the slime samples pressurized at 500 MPa.

Considering the obtained results for pressurized slime (Figure 3), a pressure treatment of 400 MPa and 15 min was chosen to combine with a mild thermal treatment (60 °C, 15 min). A comparison between the effect of temperature and pressure treatments alone and the chosen sequential combination on the total aerobic mesophilic bacteria slime from *H. aspersa maxima* snail is shown in Figure 4.

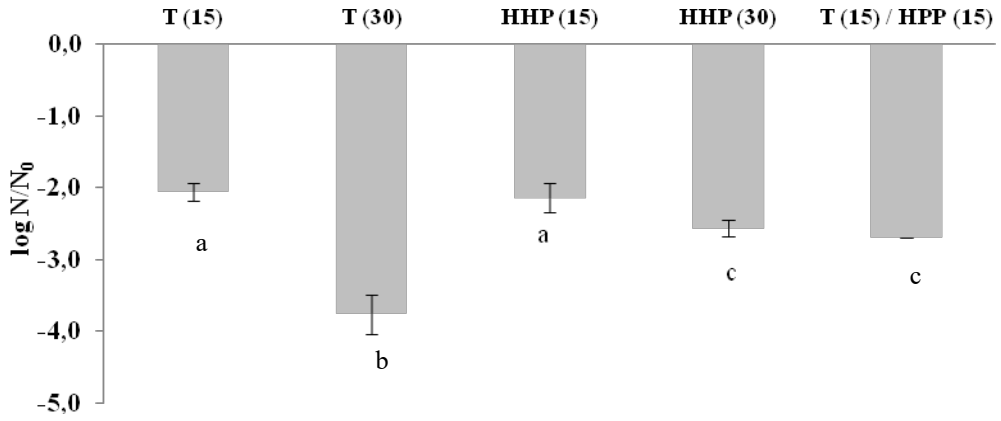

Figure 4. *Comparison between temperature (60 ºC) and high hydrostatic pressure treatments (400 MPa, 20 ºC) alone with the sequential treatment of temperature and high hydrostatic pressure (60 ºC, 400 MPa) on the total aerobic mesophilic bacteria slime from Helix aspersa maxima snail*
(different letters mean significant different results (P < 0.05))

The sequential combination of temperature and pressure had a similar effect ($P > 0.05$) on the total aerobic mesophilic bacteria when compared to 400 MPa applied for 30 min; but the reduction was higher when compared to the less intense treatments, such as thermal (60 °C, 15 min) and pressure treatment (400 MPa, 15 min) alone. No additional visual changes were observed when temperature treatment was followed by pressure as can be seen in Figure 2.

Overall, the higher reduction of the total aerobic mesophilic bacteria slime from *H. aspersa maxima* snail was obtained for temperature alone (60 °C, 30 min), but detrimental changes in the visual aspect of slime were also observed.

Other pressure combinations should also be studied and together with the effect on the total aerobic mesophilic bacteria other aspects should also be considered.

CONCLUSIONS

The microbial load of eggs and baby snails was considered unsatisfactory, showing that the conditions for growth optimization in the snail farm were positive to primary contamination, possibly from soil, water or even feeding, and could lead to additional microbial development. Under the UV-C applied conditions, no reduction on the microbial load of slime was obtained. Prolonged periods of thermal treatment are needed to reach values close to the detection limit, but detrimental visual changes could also be detected. High hydrostatic pressure (≥ 400 MPa) was demonstrated to be a promising treatment for the reduction of microbial load in snail slime, and without visual changes noticed. However, further studies concerning other physico-chemical (*e.g.*, antimicrobials, proteins) characteristics, and more intense pressure treatments (high intensity, short period), should also be addressed in order to maximize the regenerative properties of slime.

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