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High pressure inactivation of *Pseudomonas* in black truffle – comparison with *Pseudomonas fluorescens* in tryptone soya broth

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Pseudomonas is one of the most common genera in black Perigord truffle. Its inactivation by high pressure (100–500 MPa/10 min) applied on truffles at sub-zero or low temperatures was studied and compared with those of *Pseudomonas fluorescens* in tryptone soya broth. Pressurization of truffles at 300 MPa/4 °C reduced the bacterial count of *Pseudomonas* by 5.3 log cycles. Higher pressures of 400 or 500 MPa, at 4 °C or 20 °C, allowed us to slightly increase the level of destruction to the value of ca. 6.5 log cycles but did not permit us to completely inactivate *Pseudomonas*. The results showed a residual charge of about 10 CFU/g. Pressure-shift freezing of truffles, which consists in applying a pressure of 200 MPa/–18 °C for 10 min and then quickly releasing this pressure to induce freezing, reduced the population of *Pseudomonas* by 3.3 log cycles. The level of inactivation was higher than those obtained with conventional freezing. Endogenous *Pseudomonas* in truffle was shown to be more resistant to high pressure treatments than *P. fluorescens* used for inoculation of broths.

Keywords: high pressure; truffle; inactivation; *Pseudomonas fluorescens*; *Pseudomonas*

1. Introduction

Tuber melanosporum, well known as the Perigord black truffle, has important culinary and commercial value because of the flavors it imparts to foods [1]. Truffles used as fresh products are minimally processed and stored at low temperature (4 °C), which permits preservation of their organoleptic properties and extends their shelf-life [2]. Truffles used for the production of a wide variety of food products such as oil, cheese, pasta and sauce are immediately frozen or sterilized in an autoclave at 100 °C for 3 h or at 120–130 °C for 30 min. Unfortunately, in these conditions, the sensory characteristics of the truffles are highly degraded [2,3].

High pressure (HP) processing could be a promising alternative to stabilize black truffles [3–5]. Generally, pressurization at refrigeration, ambient or moderate temperature allows inactivation of pathogenic and spoilage micro-organisms in foods with fewer changes in texture, color and flavor

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as compared with conventional technologies [6]. A recent study showed that the treatment of black truffles by high pressure at low or sub-zero temperatures did not induce extensive modifications in major volatile compound concentrations [5]. The present work focuses on the impact of such treatments on spoilage micro-organisms in truffles.

Microbial charge of freshly collected truffles is particularly extensive. The total mesophilic bacteria, including a large proportion of *Pseudomonas*, showed a range of 10^7 – 10^8 CFU/g [7–9]. Our objective was to study high pressure inactivation of *Pseudomonas* in black truffle (100–500 MPa/10 min) at sub-zero or low temperatures (-18°C , 4°C or 20°C). Preliminary experiments were carried out on *Pseudomonas fluorescens* in suspension in tryptone soya broth.

2. Materials and methods

Fresh black truffles were harvested in Perigord (Dordogne, France). Fruit bodies were brushed, grated and mixed with great care. The strain used for preliminary experiments was *Pseudomonas fluorescens* CIP 69.13 (from the Institut Pasteur, Paris, France). The strain was grown in tryptone soya broth (TSB, Difco, Detroit, MI) at 37°C for 18 h.

Samples consisting of 25 g of grated truffles or 25 mL of *P. fluorescens* suspensions were vacuum-packed in a sealed plastic bag and kept at 4°C for a few hours before being hydrostatically pressurized in a 3-L stainless steel vessel using an hyperbar experimental apparatus from FRAMATOME (described previously in detail [10]). HP treatments were carried out at pressures ranging from 100 to 500 MPa. Temperatures of pressure-transmitting medium were -18°C (only for pressurizations at 200 MPa), 4°C or 20°C . The treatment time was 10 min, excluding the pressure increase and release times.

HP treatments performed at 200 MPa/ -18°C were followed by pressure release in 1 min 40 s, inducing significant ice nucleation in samples. Complete freezing was obtained by maintaining samples at -18°C for a few hours before analyses. Such treatments are called pressure-shift freezing (PSF). In order to compare the effects of PSF with those of a classical freezing, some untreated samples (initially at 4°C) were placed in deep freeze and stored at -18°C for a few hours (the same time of storage as for pressurized samples).

Controls and samples pressurized at low temperature were kept at 4°C for a few hours before analyses.

The number of surviving *Pseudomonas* was enumerated as CFU by surface plating on a cetrimide selective medium and counting after a 10 day incubation at 30°C . Bacterial counts were subjected to analysis of variance using R software. Significance of differences was defined at $p < 0.05$.

3. Results and discussion

3.1. Effect of high pressures at low temperature

Table 1 shows the effect of high pressures at 4°C or 20°C on viable counts of *Pseudomonas fluorescens* in tryptone soya broth and *Pseudomonas* spp. in black truffles.

Pressurization of *P. fluorescens* suspensions at 300 MPa/ 4°C led to the complete destruction of bacterial population. Between 100 and 300 MPa, the level of inactivation increased with pressure. At 200 MPa, it was higher at 4°C than at 20°C . These results are in accordance with those of Lopez-Caballero et al. [11], indicating that the efficiency of HP in killing *P. fluorescens* decreased as the pressurization temperature increased between 5°C and 35°C .

Table 1. Effect of high pressure at low temperature (4 °C or 20 °C/10 min) on viable counts of *Pseudomonas fluorescens* in tryptone soya broth and *Pseudomonas* spp. in black truffles.*

	<i>Pseudomonas fluorescens</i> in tryptone soya broth		<i>Pseudomonas</i> spp. in black truffles	
	4 °C	20 °C	4 °C	20 °C
Control	6.75 ± 0.21 ^a	6.75 ± 0.21 ^a	7.58 ± 0.30 ^d	7.58 ± 0.30 ^d
100 MPa	6.75 ± 0.24 ^a	6.71 ± 0.23 ^a		
200 MPa	3.40 ± 0.28 ^b	4.81 ± 0.25 ^c		
300 MPa	ND [†]	ND	2.25 ± 0.31 ^e	1.63 ± 0.33 ^{e,f}
400 MPa			1.28 ± 0.40 ^f	1.10 ± 0.38 ^f
500 MPa			1.10 ± 0.37 ^f	1.33 ± 0.36 ^f

Notes: Values with different superscript letters are significantly different ($p < 0.05$) ND, not detected *Values represent mean \log_{10} CFU/ml or CFU/g of unpressurized control samples ($n = 12$) and pressurized samples ($n = 3$) [†]No CFU was detected in 1 mL of cell suspension from each of the samples tested

The application of high pressures on black truffles induced less extensive reductions of the *Pseudomonas* spp. population. Pressurization of truffles at 300 MPa/4 °C reduced the bacterial count of *Pseudomonas* by 5.3 log cycles. Higher pressures, 400 or 500 MPa, at 4 °C or 20 °C, allowed a slight increase in the level of destruction to the value of ca. 6.5 log cycles but did not permit complete inactivation of *Pseudomonas*. The results showed a residual charge of about 10 CFU/g.

3.2. Effect of high pressures at sub-zero temperature

PSF at 200 MPa and –18 °C led to the complete inactivation of *P. fluorescens* in tryptone soya broth (Table 2). The killing effect was considerable as compared with conventional freezing and higher than those obtained with pressurization at 200 MPa and 4 °C.

PSF was also able to inactivate endogenous *Pseudomonas* in black truffles. A 3.5 log reduction was obtained with PSF at 200 MPa and –18 °C, while no significant reduction was observed with conventional freezing (Table 2). However, it clearly appeared that endogenous *Pseudomonas* in truffle was more resistant to PSF than *P. fluorescens* inoculated in broths (as TSB) or foods (as smoked salmon mince, for example [12]).

Even if some species of endogenous *Pseudomonas* may be naturally more resistant to pressure than *P. fluorescens*, it can be supposed that truffles exert a protective effect on endogenous *Pseudomonas* at the time of pressurization, more particularly at sub-zero temperatures.

Table 2. Effect of pressure-shift freezing (PSF, *i.e.* pressurization at –18 °C/200 MPa/10 min and depressurization in 1 min 40 s inducing freezing) on viable counts of *Pseudomonas fluorescens* in tryptone soya broth and *Pseudomonas* spp. in black truffles.*

	<i>Pseudomonas fluorescens</i> in tryptone soya broth	<i>Pseudomonas</i> spp. in black truffles
Control	6.75 ± 0.21 ^a	7.58 ± 0.30 ^c
Classical freezing	5.15 ± 0.23 ^b	7.22 ± 0.31 ^c
PSF	ND [†]	4.26 ± 0.32 ^d

Notes: Values with different superscript letters are significantly different ($p < 0.05$) *Values represent mean \log_{10} CFU/ml or CFU/g of unpressurized control samples ($n = 12$) and processed samples ($n = 3$) [†]No CFU was detected in 1 mL of cell suspension from each of the samples tested

4. Conclusion

Pressurization of black truffles at low or sub-zero temperatures permits us to significantly inactivate the endogenous *Pseudomonas* population. The ability of high pressure processing to stabilize black truffles has now to be confirmed by a preservation study.

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