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THE EFFECT OF HIGH PRESSURE PROCESSING ON MAJOR STRUCTURAL PROTEINS OF RAINBOW TROUT FISH FILLETS

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ABSTRACT. Fresh rainbow trout fillets are very perishable food products. Therefore, they cannot be stored at refrigeration temperatures for a long period of time. High pressure processing (HPP) can improve the quality of the fillets through microbial load control. As a result of this, the shelf-life of the product is extended. However, some physicochemical changes can appear. The aim of this study was to assess the degree of protein denaturation in rainbow trout fillets treated with various levels of high pressure. The results showed that protein denaturation is definitely higher for the fillets treated with higher pressure levels, in the range of 400 MPa/3 min – 600 MPa/6 min and lower for the fillets treated with lower pressure levels, in the range of 100 MPa/3 min – 200 MPa/6 min. The use of lower pressure levels is beneficial to the structural quality preservation of the fillets, but less effective concerning the microbial inactivation. Maintaining a good structural and nutritional quality of the product is not very useful in this case, because it cannot be combined with other great advantages offered by the HPP tools, namely microbial control and spoilage decline. Therefore, further studies should focus on readjusting (e. g. minimizing) the holding time and other possible parameters, without lowering the high levels of pressure.

Keywords: *high-pressure processing, microbial load, protein denaturation, rainbow trout fillets*.

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INTRODUCTION

Fish meat is much more appreciated by consumers all over the world nowadays due to it's important role in maintaining a healthy lifestyle (Pieniak et al. 2009). It provides high-quality protein, omega-3 and -6 polyunsaturated fatty acids and a variety of minerals and vitamins, which can prevent or help improving the symptoms of some illnesses (Soumia et al. 2013).

However, the main disadvantage of this type of meat is related to it's spoilage characteristic. Refrigeration on ice can provide freshness for a short period of time and many times this cold chain is broken during transportation by the buyers, leaving room for a rapid decay (Sotelo et al. 1995; Trebar, 2017). Freezing ensures the safety of the product for longer periods of time, but it often interferes with the sensorial quality, especially with colour, aroma and texture.(Gökoğlu et al. 2015).

In this context, current researchers are trying to test some of the more advanced alternative processing technologies such as high pressure processing (HPP), which can potentially offer the benefit of preserving fish meat safe, with good sensorial quality and nutritional value. HPP has been used in the past for foodstuffs like smoothies, guacamole, meat products, shellfish (Bolumar et al. 2016; Heinz et al, 2010), but recently a growing interest for fish meat and fish meat products was observed (Truong et al, 2015).

While some of the nutritional and sensorial components in fish remain stable after HPP, some encounter changes. Many studies show that the colour of the fish muscle changes after HPP, becoming whiter (cooked aspect). However, the change in colour can be overcomed if fish products (e.g. fish fillets) are meant to be further processed (thermal treatments or smoking treatments). Another concern is related to the level of protein denaturation after HPP (Kramer et al. 2013; Mazorra-Manzano et al. 2018; Teixeira et al. 2013). Even though HPP appeared to be a promising preserving tool for at least three decades (Balny, 1993) and optimization of the method in order to improve this aspect is still to be discovered, analyses are stll conducted with diverse parameters and samples. The breaking of structural muscle proteins can lead to nutritional value loss (Lanier, 1998), but it can also influence texture and water holding capacity changes in negative ways (Skipnes et al. 2007).

Differential scanning calorimetry (DSC) is the main technique used to determine the level of protein denaturation in fish products (Kramer et al. 2013). DSC curves are dependent on the species of analyzed fish and

therefore, the degree of protein denaturation can be different (Schubring et al. 2005).

The majority of the studies conducted until today were on wild-catch, on species such as salmon, mackerel, cod, heering and ocean perch (Kramer et al. 2013; Schubring et al. 2005), while very few studies focused on the species coming from aquaculture. A very popular species among fish farmers and consumers from Europe (Timberg et al. 2011) is rainbow trout (*Oncorhynchus mykiss*), a moderately fat fish, with exceptional tenderness and aroma.

The aim of this paper was to present the effects of HPP on major structural proteins of a widely appreciated farmed fish, the rainbow trout.

RESULTS AND DISCUSSION

The thermal properties of rainbow trout proteins and the amplitude of their denaturation was measured within a range of 20 to 95°C DSC heating procedure and a scanning rate of 5 °C/min.

The two major structural proteins, myosin and actin, were illustrated in the DSC curves from the graphs of Fig 1. for each high pressure processed sample. The first peak was represented by myosin and the second one, by actin.

For the samples processed with pressure levels of 100 and 200 MPa for 6 min (Fig 1. C. and E.) pressurization time the peaks corresponding to myosin and actin were slightly lower than the peaks of the samples processed with 100 and 200 MPa at 3 min pressurization time (Fig 1. B. and D.). Apparently, these fish fillets suffered more protein denaturation. The effect of denaturation is therefore correlated with the processing time, proportionally increasing with the increment of the processing time. However, Ko Wen-Ching et al. 2006 relates the results obtained for samples treated at high pressure in between 100 and 300 MPa with reversible protein denaturation, dissociation, or precipitation, while Arnaud et al. 2015 considers that an average high pressure treatment in the range of 100-200 MPa (≈ 150 MPa) “does not oxidize or denature protein more than the natural trends”.

An important difference is spotted between the samples treated with 100 and 200 MPa and the samples treated with 400 (Fig 1. F. and G.) and 600 MPa (Fig.1 H. and I.). The DSC profile change is most of the time attributed to considerable protein denaturation (Iso Shin-ichi, 1994; Arnaud et al. 2015). Protein denaturation is more substantial for the fillets

pressurized with the high-pressure levels. This situation is demonstrated through the variation of enthalpy values (ΔH). In the same times in which the enthalpy values decrease, the peaks of myosin and actin tend to proportionally become very low, to almost nonexistent (Table 1.) This corresponds to a very high degree to almost complete degradation of myosin and actin proteins. In the present case, actin seems to be more affected by a higher level of pressure than myosin.

Comparing to the untreated sample (Fig 1. A.), the degree of protein denaturation in the samples previously HPP treated with low pressure levels (100 and 200 MPa for 3 and 6 min) was low. This was validated by their enthalpy values which were very close to the enthalpy values of the control (Table 1.)

Some samples received an additional peak or so called shoulder before the myosin peak. This situation is more obvious for the fillets treated at 100 MPa/3 min and 100 MPa/6 min (Fig 1. B and C.). These additional peaks could symbolize sarcoplasmic and connective tissue problems (Schubring R., 2008).

The overall results revealed that high levels of pressure such as 400 MPa/3 min, 400 MPa/6 min, 600 MPa/3 min and 600 MPa/6 min have a negative impact on the main proteins of rainbow trout fillets.

Table 1. Transition temperatures and enthalpies calculated from differential scanning calorimetry (DSC) curves (Perkin Elmer DSC 2920) taken on heat-treated rainbow trout fillets dependent on the heating temperatures used

No	Treatment	Ton (°C)	Tmax (°C)	ΔH (J/g)	Ton (°C)	Tmax (°C)	ΔH (J/g)
1.	Fresh untreated	37.7	43.6	1.2550	71.25	75.35	0.4993
2.	HPP 100 MPa (3 min)	39.75	46	0.9141	70	74.7	0.5401
3.	HPP 100 MPa (6 min)	39.7	46.1	1.0226	70	74.9	0.5350
4.	HPP 200 MPa (3 min)	36.65	46.6	0.9063	70.8	75.3	0.4502
5.	HPP 200 MPa (6 min)	39.1	45.7	0.4875	70.5	75.3	0.4751
6.	HPP 400 MPa (3 min)	38	45.5	0.3799	65	68.7	0.1492
7.	HPP 400 MPa (6 min)	38	45.8	0.3949	70	73	0.1109
8.	HPP 600 MPa (3 min)	37.3	45.25	0.4523	67.95	75.8	0.1711
9.	HPP 600 MPa (6 min)	35.05	46.7	0.479	70.2	75.95	0.2108

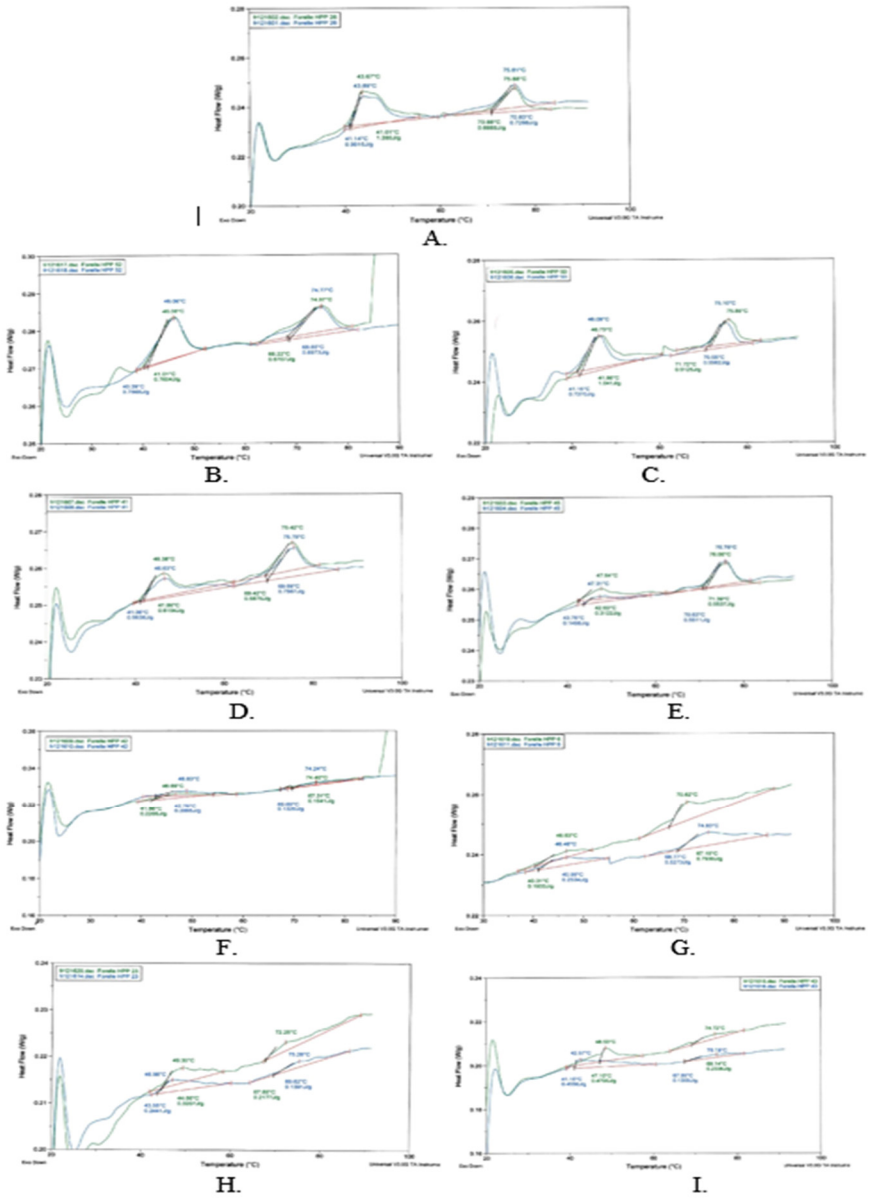

Fig 1. The variation of protein denaturation in untreated and HPP treated samples[1]

[1] Legend: A. untreated sample; B. Pressure level 100 MPa/3 min; C. Pressure level 100 MPa/6 min; D. Pressure level 200 MPa/3 min; E. Pressure level 200 MPa/6 min; F. Pressure level 400 MPa/3 min; G. Pressure level 400 MPa/6 min; H. Pressure level 600 MPa/3 min; I. Pressure level 600 MPa/6 min

CONCLUSIONS

A remarkable protein denaturation process was observed in the samples treated with pressure levels in the range of 400 - 600 MPa. Obviously, this denaturation of proteins in rainbow trout fillets leads to a lower nutritive quality of the product. Therefore, despite the great benefit - a significant microbial inactivation followed by shelf life prolongation - offered by HPP when using levels such as 400 MPa and 600 MPa, it is important to consider this aspect in any research with application in the food industry and attempt to improve it.

The use of lower pressure levels is beneficial to the structural quality preservation of the fillets, but less effective concerning the microbial inactivation and shelf-life extension.

Further studies should consider readjusting the holding time for high pressure levels especially by reducing it as much as possible (from 3-6 min to maximum 1 min) in an attempt of reducing the protein denaturation process and maintaining the strong advantage of spoilage and pathogen control.

EXPERIMENTAL SECTION

Sample Collection

A total of 56 rainbow trout fish were purchased on the same day of catch from an intensive fish farming system in Osnabrück, Germany during January to February 2017. The fish was immediately slaughtered and cut in fillets. The skin was also removed on site. The skinless fillets were transported on ice to the German Institute of Food Technologies (DIL), Quakenbrück. The samples where vacuum-packed and the HPP analysis was conducted in less than 4 h post-harvest, at the time of delivery to the laboratory. All samples were stored at refrigeration temperature (4°C). The following day, within 24 h post-harvest, the samples were analyzed by DSC in order to investigate the effects of HPP on major structural proteins. Each sample was analyzed in duplicate.

Sample preparation and analytical techniques

Through DSC, the rainbow trout fillets were studied in order to observe the thermal transition temperatures for the denaturation of proteins, namely myosin and actin (Hastings R. J., 1985). This analysis in comparison with others used in the past, offers the advantage of not destroying in any chemical or mechanical way the fish proteins (Uddin Musleh, 2001).

The device used for measuring the thermal stability of rainbow trout samples collected from the same muscle part of the fillets was DSC 2920 Modulated DSC TA Instrument (USA). The fish samples consisting in ca. 10 mg pieces were weighted into a 60 µl stainless steel pans (PerkinElmer, Germany) and sealed. The samples previously HPP treated were heated from 20 to 95°C, at a scanning rate of 5 °C/min, with a sealed empty pan as reference. Each sample was measured in duplicate. Results are presented as average curves (e.g. Fig. 1.). The average curves are used to record the onset and transition temperature (Ton and Tmax) and to calculate the transition enthalpy (ΔH) expressed as J/g of the sample material from the peak area (Table 1.).

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