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COMBINED EFFECT OF VARIOUS NITRITE CONCENTRATION AND HIGH PRESSURE TREATMENT ON FUNCTIONAL CHARACTERISTICS OF RAW MEAT BATTER

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Abstract

The quality of meat products is basically determined by the meat batter. However, relatively few studies deal with the preservation of the quality of meat batter and the possibility of reducing added additives. In order to reduce the amount of additives in these products, however, the shelf life should not be shortened, other minimal processing technologies are required such as high hydrostatic pressure (HHP) management.

The aim of this study was to investigate the combined effect of nitrite concentration and high pressure treatment on objective color, water holding capacity (WHC) and protein solubilization of raw meat batter, containing 50, 75, 100 and 125 ppm nitrite, respectively. The applied pressure treatment were 450 and 600 MPa. Meat batter samples were measured using objective color measurement (CIELab) and to investigate water holding capacity pressing test was used. Meat proteins were tested by gel electrophoresis (SDS PAGE). The effect of the nitrite and the pressure on color and water holding capacity was evaluated by two-way analysis of variance (ANOVA) at p 0.05 significance level.

High pressure and nitrite concentrations had effect on the objective color of raw meat batters. The nitrite reduction and high pressure decreased the redness (a*) of the samples. High pressure increased the batters lightness (L*) but nitrite reduction decreased it and resulted in darker color. Yellowness (b*) of the raw meat batters were not influenced by the high pressure. The 450 and 600 MPa pressure treatments significantly enhanced the water holding capacity of raw meat batters. However, reducing the amount of nitrite used for making the batter can adversely affect water holding capacity of the raw batter. Based on the statistical results, there was no interaction between the applied pressure and the amount of nitrite used. High pressure had impact on the albumins (60 - 70 kDa) and sarcoplasmic proteins (100 - 250 kDa). Pressure treatment at 450 MPa or above decreased the intensity of protein bands. The high pressure treatment had no effect on myoglobin (16.9 kDa) solubilization. The nitrite reduction didn't affect protein denaturation.

Overall, it can be said that by increasing the pressure the amount of nitrite can be reduced in the meat batter.

***Key words**: Meat batter, High hydrostatic pressure, Color, Water holding capacity, Two-way analysis of variance.*

1. Introduction

Today, the composition and origin of the products to buy has become important to consumers. The number of additives, the number of E-numbers, and the long shelf-life have become more and more authoritative for customers. In order for industry to produce products that meet expectations, while reducing additives, it is necessary to develop and disseminate minimal processing technologies. High hydrostatic pressure (HHP) treatment is a good option, and so far it has proven its many benefits.

The product after the meat was chopped and adding the required additives is called meat batter. Basically, the characteristics of the meat paste determine the quality of meat products, which can have a positive effect on HHP treatment. According to the results of Cheftel and Culioli, [1], the gelation induced by HHP results in a smoother, more even, more elastic, better water retention and less rigid white structure than heat

treatment. The application of high pressure processing (HPP) has increased rapidly during the last 30 years. High hydrostatic pressure is a non-thermal technology, and consists of submitting the foods to pressures above 100 MPa (Cruz *et al.*, [2]). The high hydrostatic pressure treatment retains more original properties of foods than other technologies, like heat treatment, so it is known as 'minimal processing technology', too. (Rastogi *et al.*, [3]). A lot of studies have shown the efficacy of this technology in inactivation of pathogenic microorganisms and increasing the shelf-life of meat products (Vercammen *et al.*, [4]). According to Messens *et al.*, [5], high pressure can modify the functional properties of meat products by protein denaturation, aggregation or gelation. Due to changes in protein structure this method submits a process to tenderize meat or produce innovative meat products (Jung *et al.*, [6]; Sun and Holley, [7]).

High pressure treatment have effect on fresh meat color. The lightness (L* value) increases above 250 MPa and the redness (a* value) decrease at 400 to 500 MPa. It is resulting in a gray-brown color (Tintchev *et al.*, [8]). These changes are acceptable and depending on the water content and a_w value of meat products (Ferrini *et al.*, [9]).

An important additive in the production of meat products is nitrite, which contributes to the achievement of the desired color, flavor, stock and microbiological and antioxidant effects. However, research has shown nitrite's health-damaging effect. According to Cassens, [10], and Greer and Shannon, [11], nitrite in meat products may react with certain amines to produce carcinogenic nitrosamines in the food. Taking into account the health risk of nitrite, it is necessary to reduce the amount used, which is a challenge for the industry.

In this sense the high pressure treatment could be used to improve the functional properties and safety of meat products with reduced additive (e.g. nitrite) content. Impact of sodium chloride and phosphates under high pressure was investigated by Villamonate *et al.*, [12], Crehan *et al.*, [13], and Tintchev *et al.*, [14]. However, there is no clear relationship between the applied sodium nitrite amount and high hydrostatic pressure treatment. Therefore the aim of this study was to investigate the effect of sodium nitrite and high hydrostatic pressure on color, water holding capacity and protein solubility of raw meat batter.

2. Materials and Methods

2.1 Preparation of meat batter

The meat batter was prepared from pork shoulder and back-fat. The shoulder was ground (diameter 5 mm) and mixed with salt (NaCl; 2% weight of meat), polyphosphate (tetrasodium pyrophosphate, $Na_4P_2O_7$; 0.4% of meat), Na ascorbate (sodium L ascorbate; 0.5% weight of meat), ice (70% weight of meat), pork backfat (40% weight of meat), white pepper (0.2% weight of meat), pepper (0.3% weight of meat) and garlic powder (0.1% weight of meat) until homogeneous batter was reached. The amount of added nitrite (sodium nitrite, $NaNO_2$) was 0.005% (50 ppm), 0.0075% (75 ppm), 0.01% (100 ppm) and 0.0125% (125 ppm) weight of meat. The raw meat batters were vacuum packed and relaxed for 4 - 6 ^{0}C for 30 minutes.

2.2 Pressure treatment

Pressure treatment of the vacuum packed meat batter was pressurized at 450 MPa and 600 MPa for 5 minutes in Resato FPU-100-2000 (Resato International B.V, Netherlands) high pressure equipment. The pressure gradient was 100 MPa/min. Pressure treatment was carried out at room temperature and sample temperature variations due to adiabatic heat were considered as intrinsically included inside pressure factor. Value 0 MPa means unpressurized meat batters.

2.3 Color measurement

Objective color was measured with Minolta ChromaMeter CR-400 (Konica Minolta Inc., Japan) at 3 different points on the surface of meat batter. The results of the color measurement were evaluated in the CIE Lab system, in which the three color factors were lightness (L *), redness (a *) and yellowness (b *).

2.4 Water holding capacity determination

The water holding capacity of the raw meat batter was measured according to Grau, [15]. An amount of sample between 200 and 300 mg was exactly weighed on an analitycal scale and put on a known weight 2500 mm^2 (50 x 50 mm) area filter paper. The filter paper and the sample were placed between two glass plates and pressed for 5 minutes under 0.5 kg weight, then the filter papers were dried. After cutting the appeared spot (extracted from meat batter) from the paper, the remaining paper was weighted on an analytical scale. The released water from sample was calculated with the following formula:

$$\frac{\text{area of spot } [\text{mm}^2]}{\text{weight of sample } [\text{mg}]} = \text{water holding capacity } [\frac{\text{mm}^2}{\text{mg}}]$$

Three replicates were analyzed from each pressure treatment-nitrite content combinations.

2.5 Electrophoresis (SDS-PAGE) protein solubility

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE) was used for the separation

of proteins. Bio-Rad Mini Protean (Bio-Rad, Munich, Germany) electrophoresis cell was used for the measurement. The range of molecular standard (Precision Plus Protein Standards All Blue, Bio-Rad, USA) was 250 - 10 kDa. The samples were made according to Laemmli, [16]. SDS-treated proteins separate by size in the polyacrylamide gel. By using known molecular weight calibrating standard proteins, the molecular weight of the examined protein can be determined. The gels after fixation and staining were analyzed using Gel Doc XR Scanner (Bio-Rad, Germany). The densities of the bands were quantified by using Quantity One software program (Bio-Rad).

2.6 Statistical analysis

Numerical results of objective color and water holding capacity were analyzed using IBM SPSS 22.0 software. Statistical analysis was performed by two-factor variance analysis (Anova). The investigated factors were HHP treatment (0, 450, 600 MPa) and nitrites (50, 75, 100 and 125 ppm). Difference was considered to be statistically significant at $p < 0.05$.

3. Results and Discussion

3.1 Color measurement

In the Table 1 are shown the lightness (L *), redness (a *) and yellowness (b *) color factors of pressurized meat batters prepared with various nitrite concetrations. Appearance of meat batter is basically determined by its lightness and redness.

Table 1. Effect of 50, 75, 100, 125 ppm nitrite and 0, 450 and 600 MPa pressure treatment on lightness (L*) and redness (a*) of raw meat batter internal surface

Pressure [MPa]	Nitrite [ppm]	L*	a*
0	50	$59,95^{d} \pm 1,01$	$8,79^{b,c} \pm 0,19$
	75	$62,2^{b,c} \pm 0,21$	$8,64^{b,c,d} \pm 0,44$
	100	$62,75^{b,c} \pm 0,50$	$9,59^{a,b} \pm 0,41$
	125	$65,83^{a} \pm 0,66$	$9,9^{a} \pm 0,77$
450	50	$61,86^{d} \pm 0,26$	$7,99^{c,d,e} \pm 0,17$
	75	$63,15^{b,c} \pm 0,56$	$8,36^{c,d,e} \pm 0,28$
	100	$63,19^{b,c} \pm 0,83$	$8,61^{b,c,d,e} \pm 0,17$
	125	$65,16^{a} \pm 0,72$	$8,84^{b,c} \pm 0,32$
600	50	$62,31^{b,c} \pm 0,38$	$7,65^{d,e} \pm 0,22$
	75	$64,06^{a,b} \pm 0,99$	$7,59^{e} \pm 0,29$
	100	$63,92^{a,b} \pm 0,50$	$8,27^{c,d,e} \pm 0,19$
	125	$65,85^{a} \pm 0,62$	$8,35^{c,d,e} \pm 0,18$

Average and standard deviation (n=9)

The different letters in the coloumns represent a significant difference ($p<0.05$)

The HHP treatments increased the lightness (L *) of the batter, a significant difference can be seen in the pressure treatment at 600 MPa. Nitrite reduction decreased the L* value of meat batter. Based on statistical results 125 ppm nitrite content increased significantly the lightness both at 450 and 600 MPa. Nitrtire reduction resulted in decrease of redness (a*) too. The redness (a*) of samples 100 and 125 ppm nitrite concentrations was significantly higher than 50 and 75 ppm nitrite. High pressure treatment of meat pigment (myoglobin) caused partial denaturation and discolouration (Defaye *et al.*, [17]). In this study th ered color of samples treated at 450 and 600 MPa is lower than unpressurized meat batter. The red color of HHP treated samples at the same nitrite concentrations is therefore lower than that of untreated samples.

3.2 Water holding capacity

Table 2 shows the released water of pressurized meat batters prepared with varying nitrite concentrations.

Table 2. Effect of 50, 75, 100, 125 ppm nitrite and 0, 450 and 600 MPa pressure treatment on released water of raw meat batter

Pressure [MPa]	Nitrite [ppm]	WHC [mm^3/mg]
0	50	$3,135^{a} \pm 0,05$
	75	$3,046^{a,b,c} \pm 0,04$
	100	$3,031^{a,b,c} \pm 0,05$
	125	$2,972^{c} \pm 0,02$
450	50	$3,099^{a,b} \pm 0,01$
	75	$2,997^{b,c} \pm 0,03$
	100	$2,964^{c} \pm 0,08$
	125	$2,956^{b,c} \pm 0,02$
600	50	$2,959^{c} \pm 0,01$
	75	$2,962^{c} \pm 0,02$
	100	$2,939^{c,d} \pm 0,03$
	125	$2,836^{d} \pm 0,02$

Average and standard deviation (n=3)

The different letters in the coloumn represent a significant difference ($p<0.05$)

he nitrite concentration had an effect on amount of released water of pressurized samples. Examining the effect of HHP treatment, it was found that both the 450 MPa and the 600 MPa treatments significantly improved the water retaining capacity of the meat batter. Due to high hydrostatic pressure treatment, the saroplasmic proteins are denaturated, which has a good effect on the water holding capacity of the batter (Marcos *et al.*, [18]). Nitrite reduction slightly increased the amount of released water in case of batter. The water holding capacity of meat batter prepared with 50 ppm nitrite was significantly lower than batters with higher nitrite content. Based on the statistical results there was no interaction between the HHP treatment and the amount of nitrite on the water holding capacity.

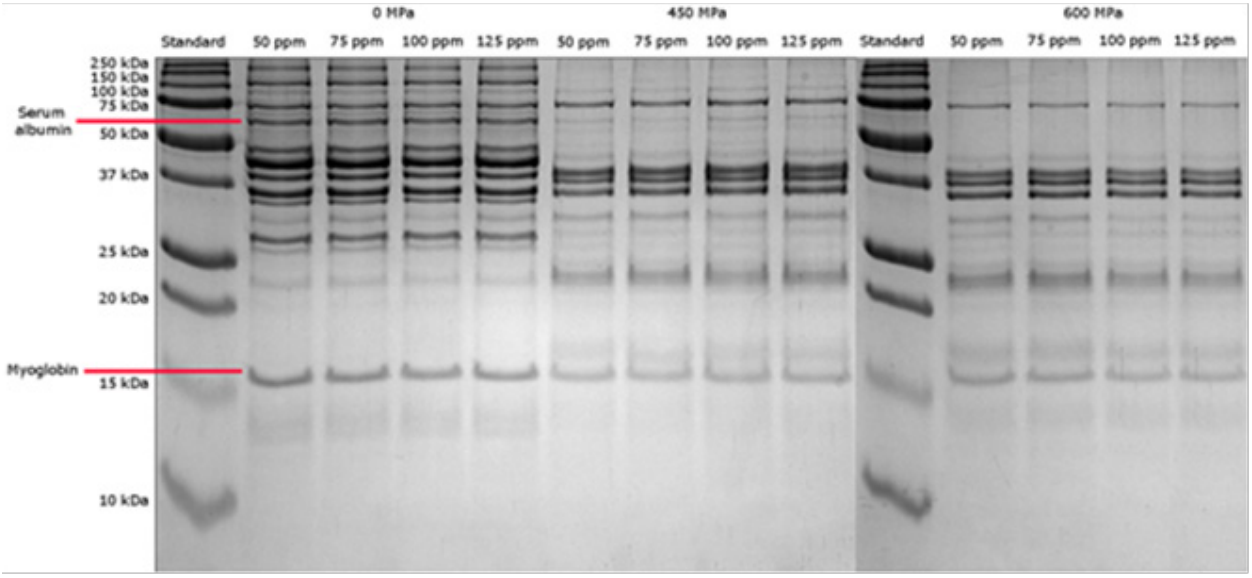

Figure 1. Separation image of SDS polyacrylamide gel electrophoresis prepared with different amounts of nitrite and HHP treated meat

3.3 Protein solubility

The results of SDS-PAGE of proteins of raw meet batter can be seen in Figure 1.

The analysis of protein solubility is essential, because the change of solubility affects the functional properties and qualitative parameters, too (Joo *et al.*, [19]). According to Marcos *et al.*, [18], sarcoplasmic and myofibrillar proteins change during the high pressure treatment. This change is due to aggregation and denaturation (Galazka *et al.*, [20]; and Iwasaki *et al.*, [21]). Following high hydrostatic pressure, there was a significant difference in solubility of protein sin the samples. As a result of HHP treatments, the intensity of the sarcoplasmic proteins band significantly decreased, and the sarcoplasmic proteins weighing more than 100 kDa were probably denatured and / or aggregated. HHP treatment also had an effect on albumin in the 60 - 70 kDa range, but 450 MPa treatment significantly reduced the intensity of the albumin band. The intensity of the myoglobin band at 16.9 kDa was only slightly reduced by the pressure treatment, so the HHP treatment had less effect on myoglobin solubility. The nitrite reduction had no effect on the solubility of the proteins either in the untreated (0 MPa) or HHP treated samples.

4. Conclusions

- Based on color changes due to HHP treatment, both the amount of nitrite and the high of pressure applied affect the color of the meat batter. Increasing the concentration of nitrite and handling at higher pressure also gives the product lighter color (L *). Pressure treatment causes the red color (a *) of the batter to show a decreasing tendency. Increasing the a nitrite content, in line with practical experience, made the meat batter redder. So as a result of the nitrite reduction and the effect of the HHP treatment, a decrease in the red color and the formation of lighter color of meat batter was observed which may be related to the oxidation of myoglobin.

- Due to the high hydrostatic pressure treatments, the saroplasmic proteins were denatured. The envolved protein network can result in improved water holding capacity of treated meat batters.

High pressure had effect on the albumins (60 - 70 kDa) and sarcoplasmic proteins (100 - 250 kDa) but had no on myoglobin (16.9 kDa) solubilization. The nitrite reduction didn't have impact on protein denaturation.

- However, further experiments need to be carried out to determine the amount of myoglobin forms (oxy-, deoxy, and metioglobin) by UV-VIS spectrophotometry and the denaturation of proteins by differential scanning calorimetry (DSC).

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