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Response surface methodology study on the effects of sodium chloride and sodium tripolyphosphate concentrations, pressure level and holding time on beef patties properties

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ABSTRACT

A Box-Behnken design was applied to evaluate the effects of sodium chloride (NaCl, 0–2%) and sodium tripolyphosphate (STPP, 0–0.5%) concentrations, pressure level (100–300 MPa) and holding time (1–5min) on technological parameters, physicochemical and texture properties of beef patties. Patties were manufactured with lean beef (80%w/w), fat (10%w/w), water (10%w/w) and NaCl and STPP concentrations according to the design and were subjected to high hydrostatic pressure (HHP) treatments according the design. Raw and cooked patties pHs were modified by pressure level, NaCl and STPP concentration. Cooking loss increased when pressure level increased and additives concentrations decreased. NaCl and STPP concentrations and pressure level significantly modified colour parameters of raw patties; however, they had no effects on cooked patties. Moreover, texture parameters values increased with pressure level.

1. Introduction

The high daily intake of sodium is associated with the increase in blood pressure, thereby increasing the risk of cardiovascular disease (strokes, heart attacks and heart failure) and renal disease (Aburto et al., 2013; He & MacGregor, 2009). The World Health Organisation has declared a policy of prevention of cardiovascular diseases consisting of a gradual reduction in salt (NaCl) intake to reach a value of less than 5 g per day. In this regard, meat industry has an important challenge in reducing the sodium chloride content of their products, which are a major source of this additive in the diet (Desmond, 2006). Different alternatives have been proposed to minimize the adverse effects of the reduction of NaCl content in meat products. One of them is the use of alkaline phosphates such as sodium tripolyphosphate (STPP). This salt increases the meat tissue pH (Trout & Schmidt, 1986) and the ionic strength releasing sites of negative charges on meat proteins, which induces an increase in water retention (Desmond, 2006). The addition of phosphates enhances the functionality of NaCl, due to the synergic action of both salts in the depolymerisation of the thick filaments (Offer & Knight, 1988). Another alternative may be the application of high hydrostatic pressure (HHP), which causes denaturation, solubilisation, and aggregation of proteins (Simonin, Duranton, & de Lamballerie, 2012) depending on the system, the applied pressure and temperature and the duration of pressure treatment (O'Flynn, Cruz-Romero, Troy, Mullen, & Kerry, 2014). Moreover, HHP induces changes in the non-covalent interactions of proteins such as depolymerisation of F-actin and myosin at pressures of 100–300 MPa (Buckow, Sikes, & Tume, 2013).

HHP has been applied to reduce NaCl content in different meat products (Macfarlane, McKenzie, Turner, & Jones, 1984; Sikes, Tobin, & Tume, 2009). Iwasaki, Noshiroya, Saitoh, Okano, and Yamamoto (2006) concluded that the rheological properties of pork patties were improved by pressurisation, and suggested the possibility of developing low-salt batters using pressures below 200 MPa. In addition, Villamonte, Simonin, Duranton, Chéret, and de Lamballerie (2013) suggested that HHP may allow the manufacturing of meat products with optimal technological properties with less NaCl and without polyphosphates. In addition, Speroni, Szerman, and Vaudagna (2014) indicated that the application of 200 or 300 MPa to meat patties induced the formation of different types of aggregates of myofibrillar

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proteins, thus technological and textural properties could be modified. Although several studies involved the evaluation of the effects of pressure level or NaCl concentration on beef products, very little is known about the effectivity of HHP treatments in combination with the addition of STPP to reduce the content of NaCl. Moreover, the effects of HHP treatments on water retention and weight losses of meat products are arguable. For that reason, the aim of this work was to study simultaneously the effects of the addition of NaCl and SSTP, pressure level and holding time on technological parameters, and physicochemical and textural properties of beef patties using response surface methodology.

2. Materials and methods

2.1. Materials

Fresh beef shoulder clods from British breed steer carcasses 48 h post-slaughter (pH 5.4–5.7) were obtained from a local supplier (COTO CICSA, Argentina). Meat pieces were vacuum-packed in Cryovac BB2800CB bags (permeability to: O_2 30 $cm^3\,m^{-2}\,24\,h^{-1}\,bar^{-1}$; CO_2 150 $cm^3\,m^{-2}\,24\,h^{-1}\,bar^{-1}$; water vapour 20 g $24\,h^{-1}\,m^{-2}$; Sealed Air Co., Argentina) and stored at 1.0 ± 1.0 °C for 48 h. Meat pieces were defatted, and fat was conserved for patty preparation. Then, meat and fat were vacuum-packed in Cryovac BB2800CB bags and refrigerated at 1.0 ± 1.0 °C for 24 h.

The salts used were food grade NaCl (Dos Anclas, Argentina) and STPP (N 15–16 Chemische Fabrik Budenheim R.A Oetker, Budenheim).

2.2. Product manufacturing

Patties were prepared with the following composition: lean beef meat, from 77.5 to 80% (w/w), modifying this percentage according to the concentrations of salts; fat, 10% (w/w); water, 10% (w/w) and the concentrations of salts (NaCl, STPP) according to the experimental design (Table 1). Patties were prepared in 3 different batches, each one including 1 repetition of the centre point. Meat and fat were separately minced using a 4 mm plate in a meat grinder (mod. T215GA, The Hobart MFG.Co., Troy, Ohio, USA), using approximately 1700 g for each formulation. During mincing, temperature was monitored using a puncture thermometer (Testo, model 230, USA) and it was lower than 8 °C. After mixing lean meat and fat by hand, according to the percentages mentioned above, the mixture was minced again in the same equipment indicated above. Then, STPP was added and manually mixed. Finally, NaCl (previously dissolved in water at 8 °C) was incorporated and the batter was hand-mixed until obtaining a uniform distribution (approx. 5 min). Then, 140 g of batter were formed into patties between greaseproof papers using a manual patty press (100 mm diameter). Two smaller patties (50 mm diameter, 15 mm height, approx. 35 g each one) were obtained from each patty using a stainless steel punch since the diameter of canister of the HHP equipment (High Pressure Iso-Lab System, model FPG9400:922, Stansted, UK) is 70 mm. Then, patties were vacuum-packed in Cryovac BB2800CB bags and stored at 1.0 ± 1.0 °C for 24 h.

2.3. High hydrostatic pressure treatments

Vacuum-packed patties were subjected to HHP treatments (Table 1) in a High Pressure Iso-Lab System Stansted Fluid Power Ltd. (model FPG9400:922, Stansted, UK), with a vessel working volume of 2 L (maximum pressure: 900 MPa; temperature range: −20 to 120 °C). A mixture of propylene glycol and distilled water (30:70 v/v) was used as compression fluid. Pressurisation rate applied was 300 MPa min^{-1}. Conditioning temperature of vessel and initial temperature of compression fluid were 5 °C. The adiabatic heating induced a temperature increase that reached a maximum (10 °C) at 300 MPa measured in the compression fluid. After HHP treatments, all patties were stored at −40 °C until further testing, with the exception of those in which raw pH and colour parameters were measured, which were kept at 1.0 ± 1.0 °C.

Table 1
Coded and real values for sodium chloride (NaCl) and sodium tripolyphosphate (STPP) concentrations, pressure level and holding time established according to Box-Behnken design.

	Coded Values				Real Values			
	NaCl	STPP	Pressure Level	Holding Time	NaCl (%)	STPP (%)	Pressure Level (MPa)	Holding Time (min)
1	−1	−1	0	0	0	0	200	3
2	1	−1	0	0	2	0	200	3
3	−1	1	0	0	0	0.50	200	3
4	1	1	0	0	2	0.50	200	3
5	0	0	−1	−1	1	0.25	100	1
6	0	0	1	−1	1	0.25	300	1
7	0	0	−1	1	1	0.25	100	5
8	0	0	1	1	1	0.25	300	5
9	0	0	0	0	1	0.25	200	3
10	−1	0	0	−1	0	0.25	200	1
11	1	0	0	−1	2	0.25	200	1
12	−1	0	0	1	0	0.25	200	5
13	1	0	0	1	2	0.25	200	5
14	0	−1	−1	0	1	0	100	3
15	0	1	−1	0	1	0.50	100	3
16	0	−1	1	0	1	0	300	3
17	0	1	1	0	1	0.50	300	3
18	0	0	0	0	1	0.25	200	3
19	0	−1	0	−1	1	0	200	1
20	0	1	0	−1	1	0.50	200	1
21	0	−1	0	1	1	0	200	5
22	0	1	0	1	1	0.50	200	5
23	−1	0	−1	0	0	0.25	100	3
24	1	0	−1	0	2	0.25	100	3
25	−1	0	1	0	0	0.25	300	3
26	1	0	1	0	2	0.25	300	3
27	0	0	0	0	1	0.25	200	3

2.4. Cooking procedure

Patties were cooked in an electric grill (George Foreman, mod. GR2144P, China) at 165–180 °C, until reaching a temperature of 75 °C at the centre (end of cooking). The temperature was monitored with a type T flexible thermocouple (SILSE S.A., Buenos Aires, Argentina) coated with high temperature resistant ceramic, and data were recorded using a digital multimeter Fluke Model Hydra 2625A (John Fluke Mfg. Co., Inc., USA).

2.5. pH measurement

The pH values of patties were measured after HHP treatment (raw pH) and after cooking (cooked pH). Slurries (5 g of sample: 25 ml of distilled water standardised at pH 7) were prepared with a laboratory blender (StomacherTM, Colworth, UK). The pH measurement was performed in duplicate with a pH-meter (Thermo Orion 710A+, Beverly MA, USA) equipped with a combination pH electrode (Thermo Orion Model 8102BN ROSS Electrode, Beverly MA, USA) and a ATC-Probe (Thermo Orion, Beverly MA, USA). The pH measurement was performed in duplicate.

2.6. Cooking loss

Patties were weighed before and after cooking using a precision balance (± 0.01g, Vibra AJ, Shinko Denshi Co. Ltd., Japan), cooled (25 °C), and dried with a towel paper to retire water and fat released during cooking. Cooking loss (CL) was calculated as follows:

$$CL = \left[\frac{(m_1 - m_2)}{m_1}\right] \times 100$$

where m_1 is the mass of the patty before cooking, after HHP treatment, and m_2 is the mass of the patty after cooking. Measurements were carried out in 12 patties per treatment.

2.7. Expressible moisture

Expressible moisture (EM) was measured in cooked patties samples (1.5 ± 0.2 g) according to Szerman et al. (2012). Measurements were carried out in 2 patties per treatment (per triplicate). EM was calculated as follows:

$$EM\,(\%) = \frac{(m_1 - m_2)}{m_1} \times 100$$

where m_1 and m_2 are the mass of the sample before and after centrifugation, respectively.

2.8. Colour parameters

L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) in the CIEL*a*b* system were measured in raw and cooked HHP-treated patties, using a chroma meter CR-400 (Konica Minolta, Japan) with illuminant D65 and 2° observer. The instrument was calibrated using a white standard calibration plate (Minolta). After cutting a slight slice (1–2 mm) of the external surface, each patty was placed on a white tray and colour parameters were measured on 4 different points of the surface, located at 5 mm from the border and 90° among them. For each treatment, three patties were analysed.

2.9. Shear force and work of shearing

Cooked patties were cooled to room temperature (25 °C) and cut in half to obtain two pieces of 75-mm height from each one. They were weighed on a digital scale (AJ Vibra, Shinko Denshi Co. Ltd., Japan). The shear force and work of shearing were measured using a ten-blade Kramer cell attached to a texture analyzer (Stable Micro Systems Model TA.XT plus, UK) with a 50 kg load cell. The speed conditions followed were 1 mm·s^{-1} for pre-test and test, and 10 mm·s^{-1} for post-test. Force-deformation curve data were recorded. Results were expressed as N·g^{-1} (force per gram) and J·g^{-1} (work per gram).

2.10. Texture profile analysis

Texture profile analysis (TPA) was performed on 8 cylindrical samples (1.5 cm diameter and 1.5 cm height) cut through a cork borer from three cooked patties for each treatment studied, equilibrated at room temperature (25 °C). Texture parameters were determined by a double compression test using a cylindrical probe (3.5 cm diameter) attached to a texture analyzer (Stable Micro Systems model TA.XT plus, UK) with a 50 kg load cell. Samples were compressed to 50% of its original height, at a speed of 0.5 mm·s^{-1}. The evaluated parameters, calculated using Texture Exponent 32 software (v 5.1.1.0), were hardness (N), springiness, cohesiveness and chewiness (N).

2.11. Experimental design and statistical analysis

Response surface methodology was used to study the simultaneous effects of NaCl and STPP concentrations, pressure level and holding time on technological parameters, and physicochemical and textural properties of beef patties. The experiment was based on a 4-factor-3-level Box-Behnken design (Box & Behnken, 1960) with 3 replicates at the centre point. Table 1 shows the coded and real values of factors and their levels. Each combination of factors (Table 1), named treatment, was applied on 18 patties. The experiment was carried out in three blocks; each of them consisted of 9 treatments.

The following full quadratic equation system containing 15 coefficients was used to describe the observed responses:

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \beta_{ii} x_i^2 + \sum_{i<j} \sum \beta_{ij} x_i x_j + \varepsilon$$

where, Y is the response variable; β_0 is the constant, β_i, β_{ii} and β_{ij} are the coefficients for linear, quadratic and interaction effects respectively, and x_i are the coded independent variables, which are linearly related to NaCl and STPP concentrations, pressure level and holding time.

The significance of the equation coefficients for each response variable –obtained by multiple regression analysis-was assessed using the F test with $p < 0.05$. Independent variables that were found significant at $p < 0.05$ in the full model were retained in the reduced models. Those reduced models were used to generate responses surfaces and contour plots. All procedures were carried out using the statistical package SAS (version 8, SAS Institute Inc., 2004; Cary, NC) and Minitab 15.0 (*trail version*).

3. Results and discussion

3.1. pH values

Table 2 shows the estimated regression coefficients for raw (pH_R) and cooked beef patties pH (pH_C) obtained from responses by multiple linear regression analysis.

The model for pH_R was significant ($p < 0.05$) and explained the 99.2% of the observed responses ($R^2 = 0.992$). STPP concentration and pressure level had a significantly ($p < 0.05$) positive linear effects, which indicated that the increase of those factors increased pH_R values (Table 2). Neither NaCl concentration nor holding time significantly modified pH_R. Interactions had no significant effects (Table 2).

The increase of pH values of meat after HHP at pressures above 200 MPa is a well-known phenomenon (Cheah & Ledward, 1996; Mandava, Fernandez, & Juillerat, 1994; McArdle, Marcos, Kerry, & Mullen, 2010; Szerman et al., 2011). This increase has been attributed

Table 2
Regression coefficients and analysis of variance of the regression models for raw pH (pH_R), cooked pH (pH_C), cooking loss (CL) and expressible moisture (EM) of beef patties submitted to HHP.

Terms		pH_R	pH_C	CL	EM
Constant		6.159	6.224	34.81	17.91
Lineal	NaCl	−0.008	−0.036*	−12.66*	4.45*
	STPP	0.172*	0.133*	−7.13*	3.04*
	Pressure	0.060*	−0.004	5.74*	−3.48*
	Time	0.012	0.005	0.71	−1.62
Quadratic	$NaCl^2$	0.009	0.033*	−3.44*	−0.67
	$STPP^2$	−0.075*	−0.067*	1.64	0.49
	$Pressure^2$	0.004	−0.002	−0.80	0.25
	$Time^2$	0.012	0.014	−2.87	1.88
Interactions	NaCl x STPP	0.000	−0.004	−5.78*	0.88
	NaCl x Pressure	0.006	0.008	2.94	−0.96
	NaCl x Time	0.000	0.005	0.51	0.63
	STPP x Pressure	−0.024	0.007	2.68	−0.93
	STPP x Time	−0.001	−0.001	1.62	−0.86
	Pressure x Time	0.007	0.003	1.98	−2.05
R^2		0.992	0.984	0.963	0.850
p-value		≤0.05	≤0.05	≤0.05	≤0.05

Reduced equations for technological parameters (coded values).
pH_R = 6.17 + 0.06Presure + 0.172STPP - 0.08$STPP^2$
pH_C = 6.233 + 0.133STPP - 0.036NaCl - 0.069$STPP^2$ + 0.030$NaCl^2$
CL = 33.72–12.66NaCl - 7.13STPP + 5.74Presure - 3.03$NaCl^2$ - 5.78NaClx STPP
EM = 18.77 + 4.45NaCl + 3.04STPP - 3.48Presure
NaCl, sodium chloride concentration (%); STPP, sodium tripolyphosphate concentration (%); Pressure, pressure level (MPa); Time, holding time (min).
* Signification level at $p < 0.05$.

to different mechanisms that occurred during processing. Among them, the loss of free protons caused by the redistribution of ions that is facilitated by the increased ionisation that occurs at elevated pressures (Macfarlane, McKenzie, Turner, & Jones, 1981) and the decrease of available acidic groups as a result of conformational changes associated with protein denaturation (Mandava et al., 1994). Moreover, it is a recognised fact that alkaline phosphates increase meat pH, which depends on its type and concentration (Sofos, 1989).

Concerning pH_C values, the model was significant ($p < 0.05$) and explained the 98.4% of the observed responses ($R^2 = 0.984$). NaCl concentration had a significant ($p < 0.05$) negative linear effect. Conversely, STPP concentration had a positive linear effect ($p < 0.05$) on this parameter. Both variables had a significant quadratic effect ($p < 0.05$). In accordance with these results, Szerman, Barrio, et al. (2011) and Szerman, Guibaldo, et al. (2011) observed that the pH values of cooked patties increased as STPP concentration increased and NaCl concentration decreased. Pressure level and holding time had no significant effects. Cooking denatures meat proteins; therefore, the slight conformational changes generated by the application of pressure are no longer important for the modification of pH values.

3.2. Cooking loss and expressible moisture

Table 2 shows the estimated regression coefficients for CL and EM obtained by multiple regression analysis.

The model for CL was significant ($p < 0.05$) and explained the 96.3% of the observed responses ($R^2 = 0.963$).The increase of the NaCl and STPP concentrations significantly ($p < 0.05$) diminished CL, and a synergic effect between them was observed since NaClxSTPP interaction was significant (Table 2, Fig. 1a). Therefore, the combined use of salts was more effective for reducing CL. Pressure level had a positive linear effect (Table 2, Fig. 1b and 1.c). Holding time had no significant effect.

It is well known that the addition of NaCl and STPP to meat products reduces CL. In this regard, Offer and Knight (1988) and Ruusunen and Puolanne (2005) reported that the effect of NaCl on water holding capacity, and consequently on CL, is mainly related to the extraction of myofibrillar proteins. In addition, protein extraction is enhanced by the increase of the ionic strength of meat tissue. Trout and Schmidt (1986) suggested that the ability of NaCl to increase ionic strength to values higher than 0.1 M allows that phosphates act modifying protein hydrophobic interactions. Besides, the synergic action of both salts in the depolymerisation of the thick filaments (Offer & Knight, 1988) enhances protein extraction.

Several authors described that the addition of NaCl and/or STPP to comminute products in combination with HHP treatments reduced the CL (Iwasaki et al., 2006; Macfarlane et al., 1984; Villamonte et al., 2013). In the present work, salt concentrations and HHP treatments had no significant interactions. Salts, acting synergically, diminished CL; however, the application of HHP treatments between 100 and 300 MPa increased this parameter.

The model for EM was significant ($p < 0.05$) and explained the 85.0% of the observed responses ($R^2 = 0.850$). The increase of NaCl and STPP concentrations significantly ($p < 0.05$) increased EM (Fig. 2a); whereas, pressure level decreased it (Fig. 2b). Quadratic terms and interactions had no significant effects ($p > 0.05$).

Speroni et al. (2014) concluded that pressure levels between 200 and 300 MPa had an important role in myofibrillar protein denaturation. HHP induced the formation of different types of aggregates, which depend on whether proteins were still forming myofibrils (resulting in insoluble aggregates) or whether they had been already extracted by STPP and/or NaCl (soluble aggregates). According to this, both types of aggregates may play different roles immobilizing free water. The extraction of myofibrillar proteins achieved with the incorporation of salts favoured the retention of water after cooking, which indicates that the soluble aggregates had higher water retention than the insoluble ones. Nevertheless, the effect of pressure was similar for both types of aggregates possibly due to an increase in protein-protein interactions, in detriment of water binding. In addition, Mandava et al. (1994) reported that HHP caused the denaturation of meat proteins, limiting their extraction and functionality. These proteins have hydrating properties, due to protein-protein and protein-water interactions, which decrease after denaturation.

3.3. Colour parameters

Table 3 shows the estimated regression coefficients, obtained by multiple regression analysis, for L*, a* and b* parameters of raw HHP-treated patties.

The model for L* parameter was significant ($p < 0.05$) and explained the 92.9% of the observed responses ($R^2 = 0.929$). NaCl concentration and pressure level significantly ($p < 0.05$) affected this parameter; thus, L* increased as NaCl concentration decreased and pressure level increased. NaClxSTPP interaction had a significant ($p < 0.05$) effect.

Several authors observed similar effects on meat products added with NaCl and HHP-treated (Carlez, Veciana-Nogues, & Cheftel, 1995; Sikes et al., 2009; Szerman et al., 2011). Carlez et al. (1995) suggested that the increase in L* values after treating beef minced meat at pressures between 200 and 300 MPa could be mainly due to the denaturation of globin or to the displacement of hemo group. Other authors proposed that those changes were associated to the modification of the conformation or coagulation of proteins (Jung, Ghoul, & de Lamballerie-Anton, 2003) and damage of porphyrinic ring (Goutefongea, Rampon, Nicolas, & Dumont, 1995). Hughes, Oiseth, Purslow, and Warner (2014) summed up that the changes in the lightness of meat, subjected to HHP, could be a consequence of the modifications in structure that involve changes in myofibrillar packing, alterations in the refraction of the sarcoplasm, muscle fibre diameter reduction and changes in light scattering properties of the meat. Both alterations in the microstructure and myoglobin, indicative of the inter-relationship between colour perception and structural attributes of the tissue, modify lightness of meat.

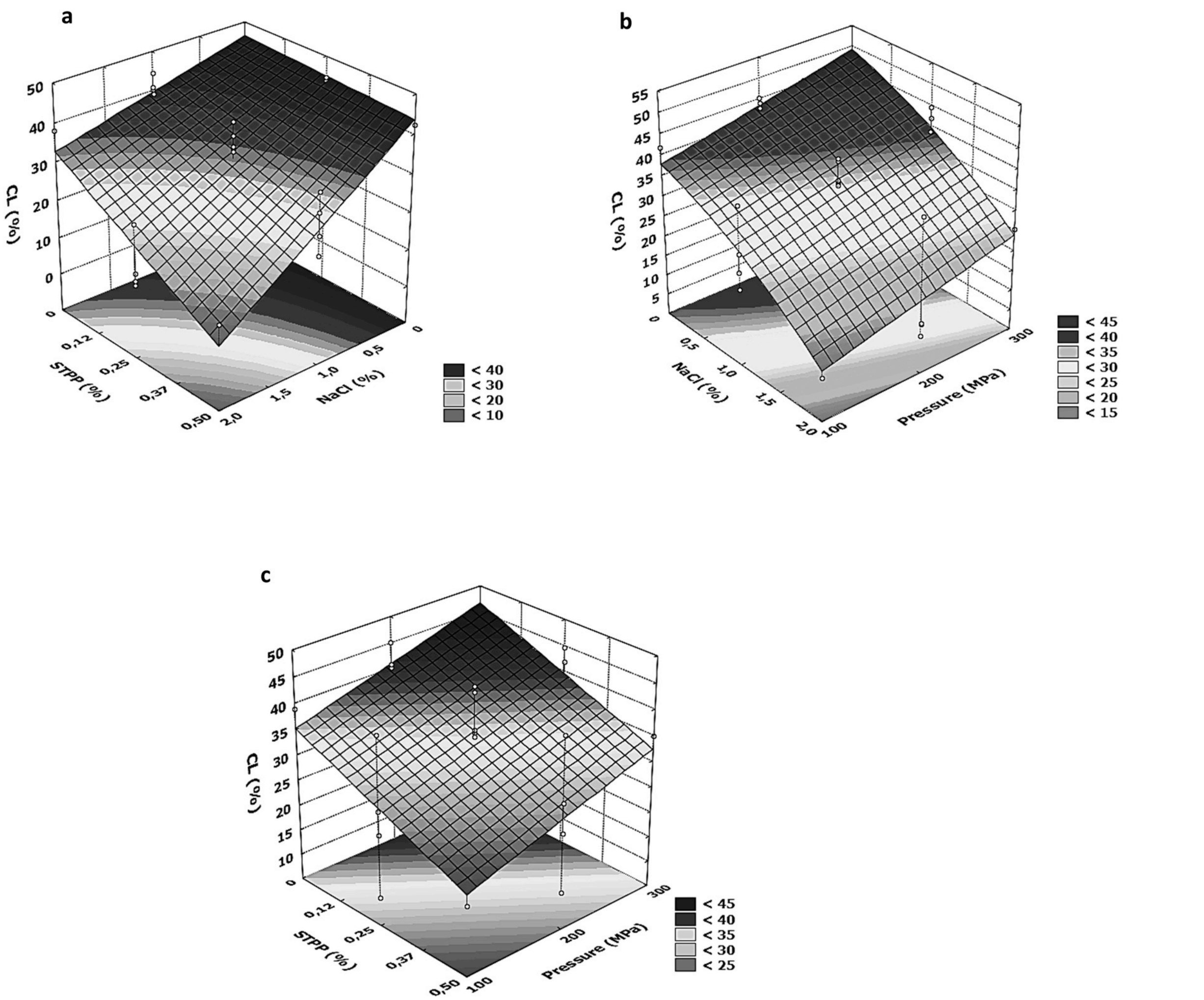

Fig. 1. Response surfaces. Insert a: Effect of sodium tripolyphosphate (STPP) and sodium chloride (NaCl) concentrations on cooking loss (CL; pressure level: 200 MPa; holding time: 3 min). Insert b: Effect of NaCl concentration and pressure level on CL (STPP concentration: 0.25%; holding time: 3 min). Insert c: Effect of STPP concentration and pressure level on CL (NaCl concentration: 1.0%; holding time: 3 min). Experimental values (-o-).

The effect of NaCl on L* parameter was associated to a higher water retention; therefore, meat surface looked darker.

The regression model for a* parameter was significant ($p < 0.05$) and explained the 95.0% of the observed responses ($R^2 = 0.950$). The increase of NaCl concentration significantly ($p < 0.05$) diminished a* values, whereas the increase of STPP concentration significantly ($p < 0.05$) increased them. STPPxPressure interaction had a significant ($p < 0.05$) effect.

The regression model for b* parameter was significant ($p < 0.05$) and explained the 88.3% of the observed responses ($R^2 = 0.883$). The concentration of NaCl had significant ($p < 0.05$) negative lineal and quadratic effects. STPPxPressure interaction had a significant ($p < 0.05$) effect. At pressures higher than 350 MPa, the reduction in redness and the increase in yellowness (Jung et al., 2003; Marcos et al., 2010) is postulated to be associated with ferric metmyoglobin formation and alterations in myoglobin hemo and porphyrin ring structure, which could reduce solubility (Carlez et al., 1995).

The regression models for L*, a* and b* parameters for cooked HHP-treated patties were non-significant ($p > 0.05$, data not shown). Due to the denaturation of meat proteins during cooking the effects of salt addition and pressure level observed in raw HHP-treated patties were masked. As it was explained, most of the changes in colour parameters were due to modifications of myoglobin conformation caused by HHP treatment, which after cooking were completed denatured.

3.4. Kramer shear force and work of shearing

Table 4 shows the estimated regression coefficients for KSF (N/g) and WS (J/g) of cooked HHP-treated patties.

For KSF, the model was significant ($p < 0.05$) and explained the 82.9% of the observed responses ($R^2 = 0.829$). KSF values significantly ($p < 0.05$) diminished with the increase of STPP concentration and increased with the increase of pressure level and holding time. NaCl concentration had a significant ($p < 0.05$) quadratic effect. Interactions had no significant ($p > 0.05$) effects.

The model for WS was significant ($p < 0.05$) and explained the 82.7% of the observed responses ($R^2 = 0.827$). The increase of STPP concentration significantly ($p < 0.05$) diminished WS and the increase of holding time increased it. NaCl concentration had a significant ($p < 0.05$) quadratic effect. Interactions had no significant ($p > 0.05$) effects.

Carballo, Fernandez, Carrascosa, Solas, and Jimenez Colmenero (1997) reported that the increase of pressure level (100–300 MPa) and holding time (5–20 min) increased shear force and work of shearing values of beef patties. Macfarlane et al. (1984) suggested that the effect of HHP on texture could be due to the modification of the conformation of myofibrillar proteins, which induce an increase on the bounding of meat particles. This increase of the bounding forces would be provoked by the increase of protein-protein interactions. Thus, Hong, Park, Kim,

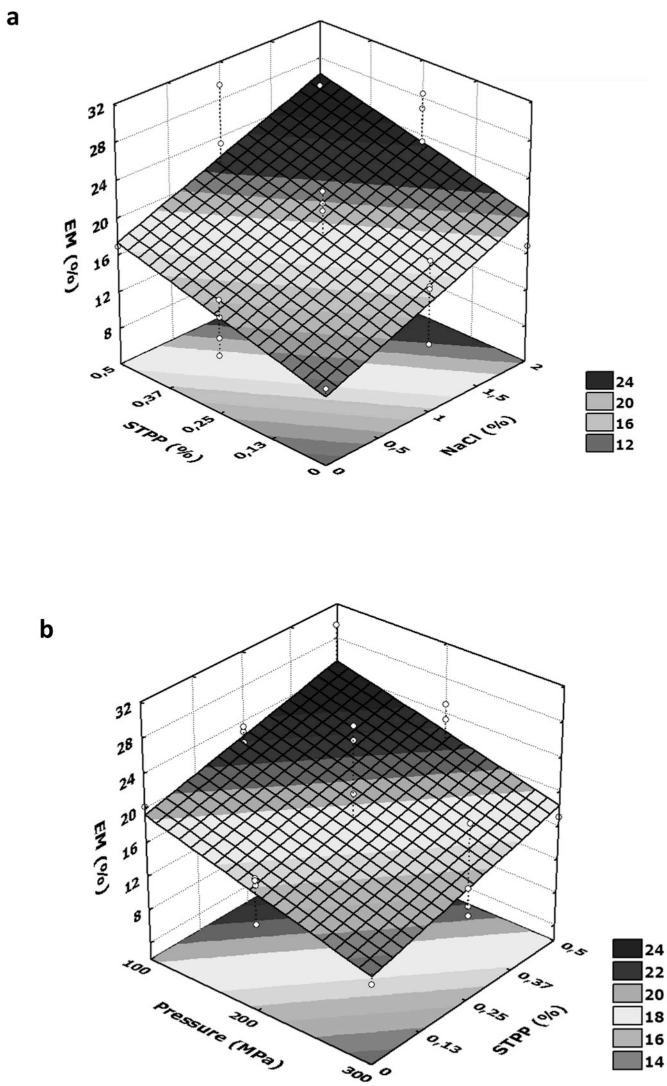

Fig. 2. Response surfaces. Insert a: Effect of sodium tripolyphosphate (STPP) and sodium chloride (NaCl) concentrations on expressible moisture (EM; pressure level: 200 MPa; holding time: 3 min). Insert b: Effect of STPP concentration and pressure level on EM (NaCl concentration: 1.0%; holding time: 3 min). Experimental values (-o-).

and Min (2006) and Macfarlane et al. (1984) observed that the union force among meat particles increased when the pressure level and the holding time increased.

3.5. Texture profile analysis

Table 4 shows the estimated regression coefficients obtained by multiple regression analysis for hardness, springiness, cohesiveness and chewiness parameters. Regression models were significant ($p < 0.05$) for all the studied variables.

STPP concentration had a significant ($p < 0.05$) negative lineal effect on hardness and chewiness. On the contrary, pressure level had a significant ($p < 0.05$) positive lineal effect on those textural parameters. NaCl concentration significantly ($p < 0.05$) increased springiness. Also, STPPxPressure and PressurexTime interactions had significant ($p < 0.05$) effects on that parameter. Cohesiveness significantly ($p < 0.05$) increased with the increase of pressure level. NaClxNaCl and TPFSxTPFS had significant effects ($p < 0.05$) on that parameter.

The texture of cooked patties is intimately related to their microstructure, which depends on the interactions among meat particles. Myofibrillar proteins have an important role, since the change in conformation and functionality influence this interaction. The application of HHP treatments modifies myofibrillar proteins, which form aggregates stabilized by disulphide and hydrophobic bonds, improving the bound among meat particles (Jiménez-Colmenero, Carballo, Fernández, Barreto, & Solas, 1997). Because of that, hardness, cohesiveness and chewiness values were higher in HHP-treated patties. Sikes et al. (2009) observed that hardness and chewiness values were higher in sausages treated at 200 MPa than in the unpressurized ones; meanwhile, cohesiveness were lower. STPP enhanced the extraction of myofibrillar proteins, leading to greater water retention. As a consequence a lesser firm structure, with a gel-like characteristic, was obtained and hardness and chewiness values were lower. NaCl only modified springiness parameter, which indicates that the presence of this salt generated a more elastic structure.

Table 3
Regression coefficients and analysis of variance of the regression models for CIE L*a*b* colour parameters of beef patties submitted to HHP before cooking.

Terms		L*	a*	b*
Constant		41.57	14.17	10.65
Lineal	NaCl	−1.60*	−5.04*	−1.21*
	STPP	−0.34	3.19*	0.34
	Pressure	3.06*	−0.05	−0.41
	Time	0.67	−0.27	−0.09
Quadratic	$NaCl^2$	1.43*	1.97*	1.23*
	$STPP^2$	0.63	1.69*	0.58
	$Pressure^2$	1.51*	0.76	0.24
	$Time^2$	0.27	0.56	0.51
Interactions	NaCl x STPP	1.82*	−1.29	0.36
	NaCl x Pressure	−0.58	0.08	−0.44
	NaCl x Time	0.21	0.12	0.05
	STPP x Pressure	−0.51	−2.20*	−0.80*
	STPP x Time	−0.35	−0.32	−0.06
	Pressure x Time	0.94	−0.40	−0.11
R^2		0.929	0.950	0.883
p-value		≤0.05	≤0.05	≤0.05

Reduced equations for CIE L*a*b* colour parameters (coded values):
$L^* = 42.12–1.48NaCl + 3.02Pressure + 1.12NaCl^2 + 1.31Pressure^2 + 1.61NaClxSTPP$
$a^* = 16.45–5.13NaCl + 3.15STPP$
$b^* = 11.37–1.20NaCl + 0.95NaCl^2$
NaCl, sodium chloride concentration (%); STPP, sodium tripolyphosphate concentration (%); Pressure, pressure level (MPa); Time, holding time (min).
* Signification level at $p < 0.05$

4. Conclusions

In this work, we simultaneously studied the effects of NaCl and STPP concentrations, pressure level and holding time on physicochemical, technological and textural properties of beef patties. Holding time had no significant effects on most of the parameters studied, which indicated that only 1 min at pressures between 100 and 300 MPa was enough to modified meat proteins properties. The addition of NaCl and STPP to beef patties increased water-holding capacity, and consequently, diminished cooking loss. However, this parameter increased when pressure level increased, being this effect more important in patties formulated with the higher concentrations of additives. Colour parameters had significant differences in HHP-treated patties; however, the cooking standardised them. Texture parameters values increased with pressure level, which may indicate higher protein-protein interactions. Therefore, under the studied conditions, it would not be possible to reduce the additives concentration by applying HHP treatments without affecting the water holding capacity and the parameters related to this property (cooking loss and expressible moisture). Consequently, optimal results can be obtained at lower pressures (100 MPa) and higher additives concentrations.

Table 4
Regression coefficients and analysis of variance of the regression models for Kramer shear force (KSF) and work of shearing (WS), hardness, springiness, cohesiveness and chewiness of cooked HHP-treated beef patties.

Terms		KSF (N/g)	WS (J/g)	Hardness (N)	Springiness	Cohesiveness	Chewiness (N)
Constant		44.32	158.43	31.02	0.730	0.640	14.52
Lineal	NaCl	−1.60	−5.00	−1.966	0.010*	0.005	−0.820
	STPP	−2.15*	−7.96*	−3.796*	−0.004	0.010	−1.552*
	Pressure	2.82*	5.64	3.782*	−0.001	0.021*	2.193*
	Time	2.64*	9.42*	−0.935	−0.004	−0.008	−0.5496
Quadratic	$NaCl^2$	−5.30*	−22.20*	−3.106	−0.003	−0.023*	−2.032*
	$STPP^2$	−1.10	−2.20	2.231	0.007	−0.038*	0.271
	$Pressure^2$	−1.72	−7.90	−0.834	−0.008	0.007	−0.435
	$Time^2$	−3.16	−9.65	−0.843	0.006	−0.010	−0.289
Interactions	NaCl x STPP	−0.64	−4.13	−0.474	0.005	0.011	0.384
	NaCl x Pressure	0.27	−1.70	−1.438	−0.009	0.012	−0.353
	NaCl x Time	0.81	3.97	−0.969	−0.014	−0.005	−0.812
	STPP x Pressure	−0.42	0.80	−3.763	−0.023*	0.014	−1.768
	STPP x Time	0.54	4.90	2.198	0.001	0.012	1.363
	Pressure x Time	2.39	4.48	3.631	0.023*	0.009	2.104
R^2		0.829	0.827	0.831	0.827	0.826	0.824
p-value		≤0.05	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05

Reduced equations for textural parameters (coded values).
KSF = 41.14–2.15STPP + 2.82Pressure + 2.64 Time - $4.10NaCl^2$
WS = 147.89–7.96STPP + 9.42Time - $18.25NaCl^2$
Hardness = 29.880–3.796STPP + 3.725Pressure.
Springiness = 0.734 + 0.012NaCl - 0.0275STPPxPressure + 0.023PressurexTime
Cohesiveness = 0.640 + 0.023Pressure - $0.021NaCl^2$ - $0.036STPP^2$
Chewiness = 14.270 + 2.193Pressure - 1.552STPP - $1.941NaCl^2$
NaCl, sodium chloride concentration (%); STPP, sodium tripolyphosphate concentration (%); Pressure, pressure level (MPa); Time, holding time (min).
* Signification level at $p < 0.05$

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