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Model system evaluation of the effects of pea and pH on the emulsion properties of beef

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Abstract

The effects of dried ground pea (0 - 1%) and pH (4.80 - 7.20) on the emulsion properties of beef were investigated using the model system. The study was designed according to the central composite rotatable design using the Response Surface Methodology. Pea had significant effects on emulsion activity and stability. The effects of pH on emulsion capacity, stability, activity, density, viscosity and apparent yield stress were significant. In addition, the interaction of both factors (pea and pH) caused significant effects on emulsion density, apparent yield stress of emulsion and emulsion gel. Pea addition increased emulsion activity at low concentrations and decreased at high concentrations. This study suggests that pea can improve the properties of emulsion type meat products and may be considered as an alternative additive in such products.

Keywords: Beef, emulsion, pea, pH, response surface
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Introduction

Pea products are valuable low-cost plant products. It is considered as an alternative to animal proteins and has the potential to contribute to food producers because it is sustainable and cheap (Laudadio & Tufarelli, 2012; Munialo *et al*., 2014). Pea is a source of protein which consists of vicillin (7S), legumin (11S), and albumins (2S), of which 7S and 11S are the most abundant (O'Kane *et al*., 2005; Söderberg, 2013). Its composition contains approximately 25% protein, 58% starch, and 12% fiber (Polesi *et al*., 2011; Söderberg, 2013). As a valuable component of functional food, interest in peas has been increasing and has led to studies involving antioxidant activity in pea seeds (Laudadio & Tufarelli, 2011). Pea proteins and carbohydrates in fiber form are the focus of attention as human nutrients and peas with high levels of lysine are important in terms of eliminating the necessary amino acid deficiency in diets (Tian *et al*., 1999; Chel-Guerrero *et al*., 2007; Barac *et al*., 2015). Pea products can be added to the formulations of food products to improve product quality and technological properties. The technological properties of pea proteins have a positive effect on emulsification (Franco *et al*., 2000; Gharsallaoui *et al*., 2009; Barać *et al*., 2015). Pea proteins contain soluble proteins with good gel formation, water-binding, and emulsification properties required for meat products (Tarté, 2009). Protein solubility and emulsion properties of meat products are closely related (Schut, 1976; Barać *et al*., 2015). However, protein solubility is closely related to extrinsic factors, such as pH, ionic strength, and temperature (Söderberg, 2013; Bolontrade *et al*., 2013). Water holding, and oil absorption capacities of pea products increase relative to the protein content of the flour, the protein fraction, and isolate (Sosulski & McCurdy, 1987).

Not only do the technological properties of proteins in food emulsions play an important role, but also their interactions with polysaccharides. In meat proteins, myofibrillar proteins with high ionic strength have high solubility (Schut, 1976). Their interactions with non-meat proteins and polysaccharides affect the emulsion properties of meat systems. By adding emulsifiers and/or thickening agents, such as proteins and polysaccharides, the activation energy of the system can be overcome, and the emulsions can be made kinetically stable (Sun *et al*., 2007). To make pea products more versatile as a food ingredient, it is important to understand the relationship between the physico-chemical properties of these products and meat

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emulsions. Therefore, the aim of this study was to determine the effects of peas, and the relationships of peas with pH, on the emulsion properties of beef.

Materials and Methods

The day after slaughter, two-years-old beef was taken from a local butcher as a *Semimembranosus* muscle to be used as the meat source. Sunflower oil was obtained from a local market. Pea flour (protein 24%, fat 2%, carbohydrate 52.5%, fibre 12%) was obtained from Kimbiyotek (Istanbul, Turkey). Analytically pure chemicals were used.

To produce the meat homogenate preparation, beef was ground by a laboratory type grinder (Tefal, LeHachoir 1500, France) with a small perforated (3 mm diameter) plate. The meat samples were divided into 25 grams of equal parts and packaged using polyethylene packing material and stored at -20 ºC until analysis. About 100 ml 0.45 M NaCl solution, 25 g ground meat and pea flour (according to Table 1) were comminuted at 18,000 rpm for 60 seconds in a blender (Waring 80011S, USA).

The emulsion capacity (EC) can be defined as the maximum amount of oil that proteins can bind. A model system was used for the determination of EC as reported by Ockerman (1985). The end point of the oil addition was determined using a microprocessor according to the electrical conductivity method reported by Webb *et al.* (1970). A 12.5 mL of homogenate, 37.5 mL of NaCl solution (0.45 M) and 50 mL of sunflower oil were placed in a blender (Kenwood KM010, UK). The oil was added at a rate of 0.4 to 0.5 ml / sec at 6500 *rpm* until the emulsion was broken. The water was circulated around the oil-containing double-walled burette to keep the added oil at a constant temperature (20 °C). A microprocessor was used to monitor the electrical conductivity of the emulsion during emulsification. Immediately when the conductivity dropped, the addition of oil was stopped. The sum of the added oil before and after emulsification was determined as EC.

To prepare the emulsion, a 32.5 ml of homogenate, 97.5 ml NaCl solution (0.45 M), and 100 ml of sunflower oil were placed in a blender (Kenwood KM010, UK). About 80 ml oil was added at a rate of 0.4 - 0.5 ml/sec at 6500 *rpm*. After the oil addition was finished, the emulsion was subjected to a further 5 sec of emulsification.

The emulsion stability (ES) was performed according to the model system method reported by Ockerman (1985) and Zorba *et al*. (1993). The tube containing 10 g of emulsion was heated at 80 °C in a water bath (Wisd WiseBath WB-11, Germany) for 30 min. The emulsion was then cooled to 25 °C in a cold-water bath. The emulsion was then held at ambient conditions for 1 hour to stabilize the emulsion and then centrifuged at 350 x *g* for 20 min. The oil and water separated from the emulsion were measured and ES calculated with the following equation:

$$ES(\%) = 100 - (SW + SO)$$

$$SW(\%) = ml\ of\ separated\ water\ x\ 10$$

$$SO\ (\%) = ml\ of\ separated\ oil\ \ x\ d\ (specific\ gravity)\ x\ 10$$

In oil-containing samples, emulsion density (ED) is an important property and ED measurement is one of the simplest methods. It is one of the analyses that can be done in many laboratories (Mcclements 1999). Emulsion density was determined using a 20-ml pipette with enlarged mouth. Emulsion density was calculated after 20 ml emulsion weighing.

The emulsifying activity (EA) was measured using the method reported by Neto *et al*., (2001). About 10 g of emulsion was centrifuged at 350 *g* for 20 min. The height of emulsified layer and total content in the tube were measured and calculated as follows:

$$EA = \frac{\text{(Height of emulsified layer in the tube)} \quad \text{x 100}}{\text{Height of the total contents in the tube}}$$

The emulsion viscosity (EV) was analysed with a rheometer (Brookfield DV3T). A spindle of type RV3 (viscosity range = 100 - 200.000 mPa.s) was set to rotate at 15 rpm. The measurements were taken after the rheometer was tested using a calibration fluid (Brookfield 4700 cP, 25 ºC). Prior to EV measurement, each sample was standardized to 25 ºC.

The study was designed as a central composite rotatable design. This study was carried out in two replications. A quadratic model was used to determine the combined effect of pea and pH. A total of 10 combinations including centre points (two replicates) were designed in random order. Encoded and actual levels are given in Table 1.

Table 1 Central composite rotatable design of two independent variables

Run order	Codified levels		Actual levels	
	Pea (X_1)	pH (X_2)	Pea (%)	pH
1	0	-1.41	0.50	4.80
2	-1	-1	0.15	5.15
3	1	-1	0.85	5.15
4	-1.41	0	0.00	6.00
5	0	0	0.50	6.00
6	0	0	0.50	6.00
7	1.41	0	1.00	6.00
8	-1	1	0.15	6.85
9	1	1	0.85	6.85
10	0	1.41	0.50	7.20

For each factor, the variance is divided into quadratic, linear and interacting components and is represented using a second order polynomial equation. The equation is

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_{ii}^2 + \sum_{\substack{i=1 \\ i<j}}^{k} \sum_{j=1}^{k} \beta_{ij} x_i x_j$$

Where: Y is the estimated response,
β_0, β_i, β_{ii}, β_{ij} are constant coefficients,
k is the number of factor variables, and
X_i, X_j, are defined as the independent variables.

Results and Discussion

The variance analysis results of emulsion capacity are given in Table 2.

Table 2 Analysis of variance of the linear, quadratic and interaction effects of pH and pea on emulsion properties of beef

Source of variation	DF	EC R^2 = 0.94 F-value	ES R^2 = 0.90 F-value	ED R^2 = 0.74 F-value	EA R^2 = 0.88 F-value	AYSe R^2 = 0.92 F-value	AYSg R^2 = 0.91 F-value	EV R^2 = 0.89 F-value
Model	5	46.134**	24.141**	7.902**	21.036**	30.604**	29.541**	22.111**
X_1 (Pea)	1	0.067	5.1431*	1.120	16.062**	1.048	2.633	0.538
X_2 (pH)	1	7.420*	1.2416	15.954**	12.355**	0.483	5.372*	0.495
X_1*X_2	1	0.268	0.3677	6.812*	0.512	14.464**	6.268*	0.934
X_1*X_1	1	0.150	5.2350*	0.554	15.174**	0.412	1.802	0.354
X_2*X_2	1	19.998**	11.3686**	18.302**	42.548**	39.323**	0.752	21.658**
Lack of fit	9	1.096	0.996	0.797	1.701	1.523	0.346	2.024
Error	14							
C. Total	19							

*P <0.05 significance level, **P <0.01 significance level, DF: Degree of freedom, EC: Emulsion capacity, ES: Emulsion stability, ED: Emulsion density, EA: Emulsion activity, EV: Emulsion viscosity, AYSg: Apparent yield stress of emulsion gel, AYSe: apparent yield stress of emulsion

While the linear and quadratic effects of pH (Table 2) on EC were significant (*P* <0.05, *P* <0.01, respectively), the effect of peas was not significant (*P* >0.05). Aluko *et al*. (2009) and Adebiyi and Aluko (2011) stated that the emulsifying capacity of a pea protein isolate is better than soy protein isolate at different concentrations of isolate and at the pH 5.0 and 7.0. With the increase in pH, the EC first increased slowly and then rapidly (Figure 1). The pH plays an important role during emulsification as it is closely related to the physico-chemical properties of the emulsion. The solubility of the proteins increases as pH is removed from the isoelectric point (Kurt, 2010).

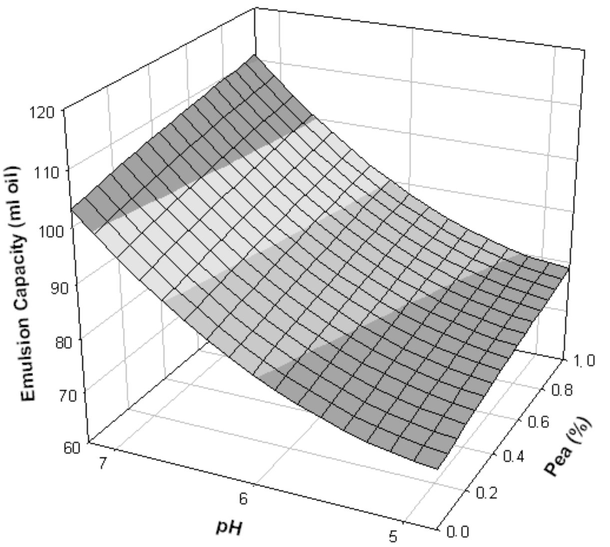

Figure 1 Effects of pH and pea on the emulsion capacity of beef

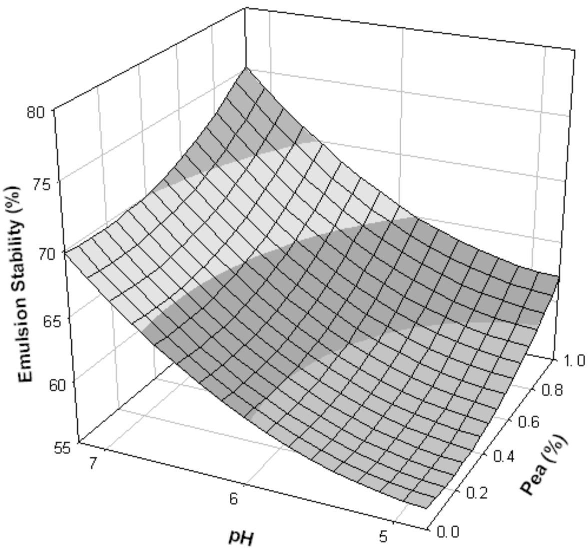

Figure 2 Effects of pH and pea on the emulsion stability of beef

Another important emulsion characteristic is the ES (Schut, 1976). While the linear and quadratic effects of peas on ES were significant (*P* <0.05), the quadratic effect of pH was found to be significant (*P* <0.01, Table 2). Pea-addition increased emulsion stability and this increased further as the pea concentration increased (Figure 2). Moreover, ES increased with increasing pH and this increasing tendency was greater with decreasing acidic medium. The ES values reached a critical point (55.91) where the pea level was 0.22% and the pH was 5.02. This critical point was the minimum as a predicted solution. The effect of peas may be related to the protein and polysaccharide content of peas. pH causes interactions at the interface between polysaccharides and proteins (Khalloufi *et al*., 2009). The relationship of pH to the isoelectric point of proteins is also related to the net charge on the surface of the droplet that plays a role in ES (Laplante *et al*., 2006). The reduction of electrostatic repulsion enables the droplets to become close to each other and to be prone to coalescence (McClements, 1999*;* Srinivasan *et al*., 2000). As shown in Figure 2, ES increased rapidly as pH moved away from the isoelectric point of the meat proteins. Moreover, the main pea proteins have very low emulsion stability in the pI (isoelectric point) range (Barać *et al*., 2015). Varying pH, ionic species, and ionic strength cause pea proteins to form different network structures (Munialo *et al*., 2014). Thus, proteins can form a strong network structure, which can hold oil droplets and increase emulsion stability (Smith, 1988).

The linear and quadratic effects of pH on ED were found to be significant (*P* <0.01, Table 2). The effect of pH and pea interaction was also significant (*P* <0.05). The ED values reached a critical point (0.913) where the pea level was 0.10% at pH 6.59. This critical point was a maximum as a predicted solution (Figure 3). The ED can be affected by the size of fat globules and the air entering the emulsion (Kurt & Ceylan, 2017). This might be related to the behaviour of adsorbed proteins due to the interaction of pH and proteins.

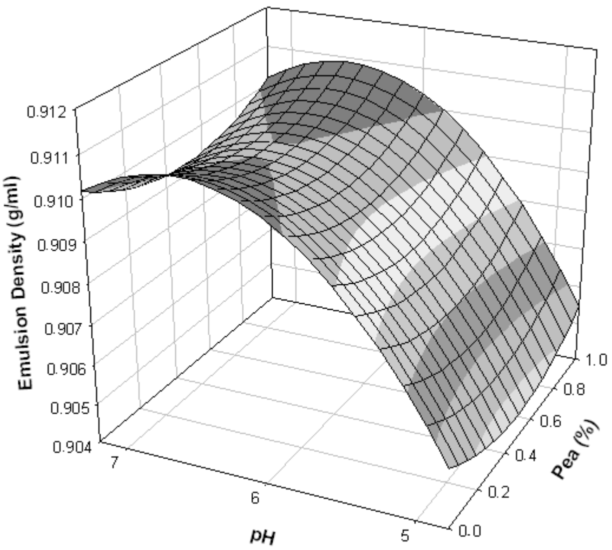

Figure 3 Effects of pH and pea on the emulsion density of beef

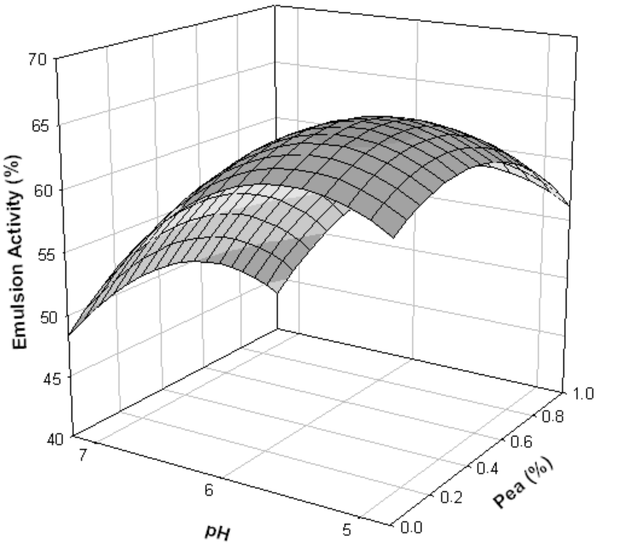

Figure 4 Effects of pH and pea on the emulsion activity of beef

According to the ANOVA, the linear and quadratic effects of pH and peas on EA were found to be significant (P <0.01, Table 2). In particular, both pH and peas increased EA values to a critical point (68.48) where the pea level was 0.42% and the pH 5.66 (Figure 4). There is a significant relationship between EA and pH values (Neto *et al*., 2001) affecting the solubility of protein and polysaccharide and leading to differences in net electrical charge. Barać *et al.* (2015) suggested that the emulsification activity is a minimum in the isoelectric point range of major pea proteins. Moreover, they reported that as the pH is moved away from pI, the emulsifying properties improve due to intensive dissociation, which is more evident in the case of legumin (Neto *et al*., 2001). Khalloufi *et al.* (2009) reported that by controlling the interaction between charged polysaccharides and proteins, it is possible to increase the thickness of the surface layer surrounding the droplets and to form multi-layered surfaces. Moreover, peas contain significant amounts of fibres that improve the emulsion volume due to the hydration and swelling properties of the fibres, which can affect emulsion activity (Rubilar *et al*., 2010).

One of the features of emulsions playing an active role in the formation and processing of emulsions is the rheological property. The pH value and peas significantly affected the rheological properties of beef emulsion (Table 2). The quadratic effect of pH and its interaction with peas on the AYSe was found to be significant (P <0.01); however, the linear effect of pH and its interaction with peas on the AYSg was found to be significant (P <0.05). On the other hand, only the quadratic effect of pH was found to be significant (P <0.01) on EV. As shown in Figure 5, AYSe values are reduced around the isoelectric point of meat proteins and are increased by increasing pH. Conversely, the AYSg values decreased with increasing pH (Figure 6). It can be stated that proteins, pea polysaccharides and fibres are effective on AYS values. In addition, it can be said that the heat treatment is effective on the AYSg values being different from the AYSe values. On the other hand, the increase in pH ensured a significant quadratic increase in the EV. As Figure 7 shows, EV has increased rapidly with an increase in pH up to 6, then this increase is slowed down. Barać *et al.* (2015) reported that isoelectric point (pI) of pea proteins was around 4.5 and that the solubility of pea protein isolates was poor at pH 5.0, but better at pH 7.0 and 8.0. Boye *et al.* (2010) reported that the solubility of pea protein concentrate was highest between pH 1 and 3 and 7 to 10. Pea protein isolates contain considerable soluble proteins with good emulsification and gel formation properties that can be needed for processed meat applications (Tarté, 2009). In addition, peas contain significant amounts of starch, which affects emulsifying and foaming properties of meat (Barać *et al*., 2015). The solubility in protein/polysaccharides can affect the rheological properties of meat emulsions and these effects are closely related to pH (Kurt, 2010). In a study, Zhao *et al.* (2015) reported that the structure of proteins and polysaccharides, as well as their interactions, are considerable for their effect on the rheological properties of emulsions. Further, Elizalde *et al.* (1988) stated that the rheological behaviour of protein solutions is closely related to molecular size, shape, hydration grade, elasticity and intramolecular interactions. Moreover, the hydrophilic and lipophilic balance of proteins can play an important role on the rheological properties of emulsion. Differences in the hydrophilic and lipophilic balance of proteins and polysaccharides allow much lower energy input, reducing fat and water interface tension (Elizalde *et al*., 1988; Krause, 2002). Pea contains a considerable number of polysaccharides that can affect rheological behaviour of meat emulsions. Thebaudin *et al.* (1997) reported that insoluble fibres can affect the rheological characteristics of foods due to their water retention and swelling properties. The viscosity and apparent yield stress of emulsion are affected by the coalescence of fat globules or by the ability of the protein matrix to retain fat. In addition, there may be differences in the behaviour of proteins in meat emulsions due to fiber source. Polysaccharides can form a stronger gel network by allowing aggregation of incompatible proteins (Samant *et al*., 1993).

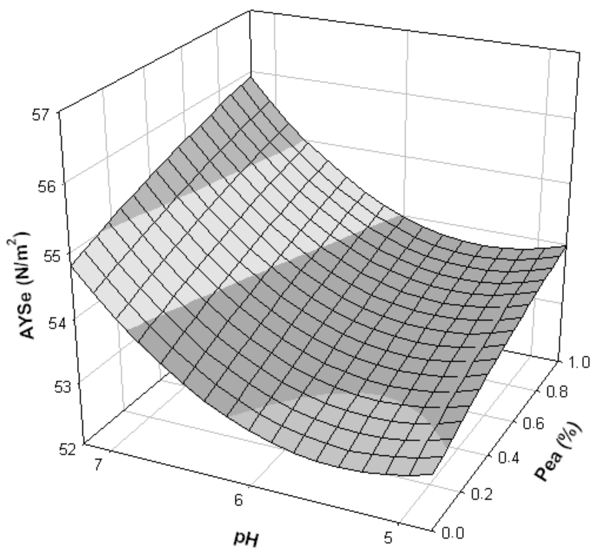

Figure 5 Effects of pH and pea on the apparent yield stress of emulsion

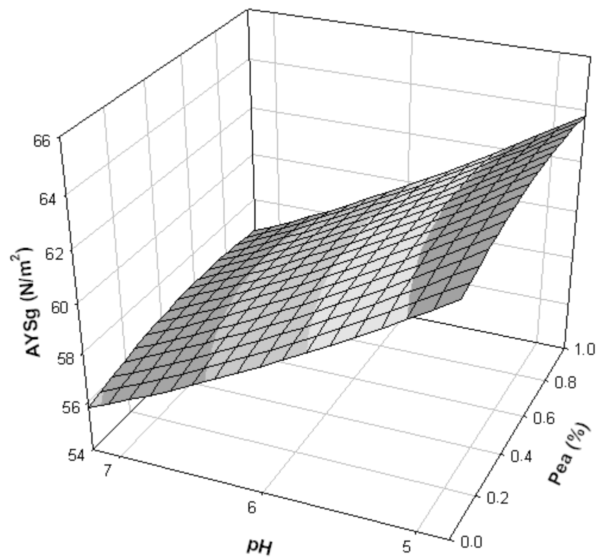

Figure 6 Effects of pH and pea the apparent yield stress of emulsion gel

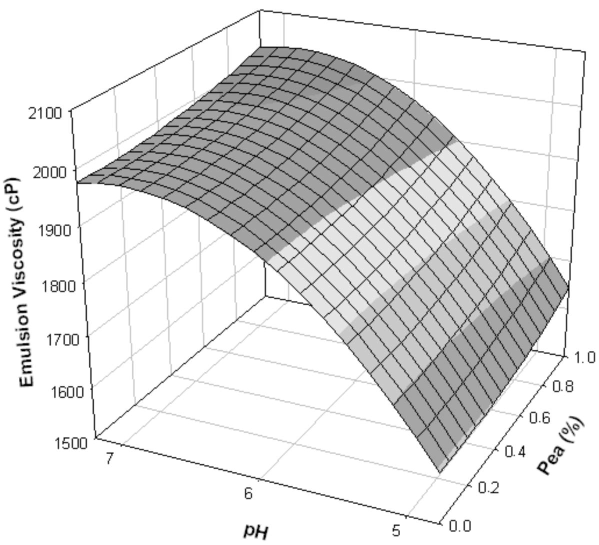

Figure 7 Effects of pH and pea on the emulsion viscosity of beef

The effects of pH and peas, and their interactions, on beef emulsion properties were given mathematically as predicted equations in Table 3. These model equations are useful to separately determine the effects of different levels of factors and their interactions on parameters. Thus, the factor levels and parameter values at any point of the response surface can be determined.

Table 3 Predicted model equations for effects of pea (X_1) and pH (X_2) on emulsion properties of beef

$$Y_{EC} = 185.427 - 242.787X_1 + 406.055X_2 + 75.630X_1X_2 - 193.001X_1^2 + 167.766X_2^2$$
$$Y_{ES} = 713.989 + 1254.398X_1 + 98.069X_2 - 52.311X_1X_2 + 672.561X_1^2 + 74.686X_2^2$$
$$Y_{ED} = 0.4842 – 0.6144X_1 – 0.3690X_2 – 0.2364X_1X_2 - 0.2296X_1^2 - 0.0995X_2^2$$
$$Y_{EA} = -1415.232 - 2784.151X_1 - 388.546 X_2 - 77.521 X_1X_2 - 1438.106X_1^2 -181.466X_2^2$$
$$Y_{AYSe} = 8.435 - 98.703X_1 - 10.663 X_2 - 57.180X_1X_2 - 32.875X_1^2 -24.200X_2^2$$
$$Y_{AYSg} = -172.547 - 410.759X_1 - 93.362X_2 - 98.839X_1X_2 - 180.574X_1^2 -8.793X_2^2$$
$$Y_{EV} = 7643.120 + 12778.621X_1 -1951.901X_2 + 2626.576X_1X_2 + 5507.334X_1^2 -3248.054X_2^2$$

EC: Emulsion capacity, ES: Emulsion stability, ED: Emulsion density, EA: Emulsion activity, EV: Emulsion viscosity, AYSg: Apparent yield stress of emulsion gel, AYSe: apparent yield stress of emulsion

Conclusion

Peas and pH improved the emulsion properties of beef. While the peas improved the stability and activity of the beef emulsion, the pH affected all the emulsion properties investigated. In addition, the interaction of peas with pH had beneficial effects on the apparent yield stress of the emulsion, as well the emulsion gel (AYSe, AYSg). Thus, peas can be used as a functional ingredient to improve emulsion properties in emulsified meat products.

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Authors' Contributions

ŞK designed the study, conducted statistical analyses of the data and edited the draft version of the manuscript. HGC contributed to laboratory analysis with ŞK.

Conflict of Interest Declaration

Authors declare that there is no conflict of interest for this study.

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