Optimization of the Use of Selected Non-Phosphate Water Retention Additives in Minced Beef Using Response Surface Methodology

Xiaolan Shang*, Jie Qiao and Yujie Liu

College of Life Science, Langfang Teachers University, Langfang 065000, China

*Corresponding author e-mail: iris381@163.com

Abstract. This study looked to determine what the optimum cooking loss for minced beef was when three different non-phosphate water retention additives (L-Arginine, sodium carbonate, and sodium citrate) were combined; the optimum value was determined using a Box–Behnken response surface design method. The optimum value was found to be 8.26%, and it was obtained when 0.29% L-Arginine, 0.45% sodium carbonate, and 0.24% sodium citrate were added to the beef.

1. Introduction

Using their plentiful resources, the meat industry has managed to provide consumers with more tender, juicy, and flavorful meat products [1]. During the thermal treatment of beef, there will be a certain degree of cooking loss, the cooking loss of shin cuts reached 47% and wing rib cuts reached 55% [2]. A key issue for the processing of beef products, then, is how to ensure that water is retained within the muscle tissues of cooked beef. The most commonly used method for reducing cooking loss in beef is to add phosphates to it [3-4]; this is beneficial for many reasons, including increasing the pH level [5] and ionic strength of the products [6] and accelerating the dissociation of act myosin [7] and chelate metal ions [8].

Although phosphates have been demonstrated to be useful when applied to meat products, there are potential drawbacks to using them. At high concentrations (i.e., 0.4–0.6%) they produce a metallic astringent flavor [9], and they may cause short-term (e.g. abdominal distress and diarrhea) and long-term (e.g., increased bone calcium mobilization) health problems; this is in spite of the maximum permissible concentration level being 0.5% [10]. For this reason, non-phosphate water retention additives are increasingly being used instead of phosphates.

One example of a non-phosphate water retention additive that has been studied is that of potato starch [11]; it was found that if 4% potato starch was added to Alaska pollock and North Pacific hake, the water holding capacity (WHC) of these fish varied from 92.7 to 96.6% and from 92.9 to 94%, respectively. In another study, Khan et al. used protein hydrolysates instead of phosphates as water retention additives[12]; they found that using the protein hydrolysates from the fish-scrap of three different types of fish (including horse mackerel, white croaker, sardines, and flying fish) resulted in the WHC of Waniros lizardfish increasing. Two further studies [13-14] indicated that the water resistance and overall quality of meat products could be improved by the addition of polysaccharides to the products. In these studies, it was found that the gel properties of the meat products were changed primarily by physical and chemical interactions between the polysaccharides and Myofibrillar protein.
L-Arginine is an alkaline amino acid [15] that has a pH of 10.76. Thus, adding L-Arginine to meat products increases the pH of the meat products and shifts the isoelectric point of the meat products away from that of L-Arginine. It has also been reported that L-Arginine can improve the WHC of meat products. Zhou et al., for example, found that the WHC of pork sausages increased when 0.8% L-Arginine was added to them [16], but decreased when only 0.2% was added. Qin et al., meanwhile, found that L-Arginine could reduce the cooking losses of chicken sausages and was conducive for the formation of the three-dimensional gel structure of the sausages [17]. Sodium carbonate and sodium citrate are examples of strong-base weak-acid salts; due to their high anionic content, they can be used to increase the ionic strength, and therefore pH value, of beef. These characteristics are also conducive for the gel formation of myosin, and the improvement of the WHC of beef products [18].

The application of a formulation of L-Arginine, sodium carbonate, and sodium citrate on WHC has not yet been reported upon. We will determine this by finding the formulation that results in optimum water retention in a sample of minced beef. Once this has been established, the cooking loss of the sample will be used to evaluate the effectiveness of the formulation. Our study was able to produce a reliable method for developing non-phosphate water retention additives that will can be used to retain water in meat products.

2. Materials and Methods

2.1. Preparation of the minced beef samples
The minced beef used for the present study was obtained from a local slaughterhouse (Langfang, Hebei, PR China; pH 5.53 ± 0.15). It was chopped for 3 min with a chopping machine (ZB-40, Shenyang JiXiang Food Machinery Co., Ltd., China), all treatments contained 1.0% sodium chloride (meat weight basis), and stored at 4 °C for 10 h before use. The L-Arginine, sodium carbonate and sodium citrate of different contents were added as a powder form.

2.2. Cooking loss (CL)
The value of WHC could be determined using the method of cooking loss. 10 g samples were heated in a water bath at 80 °C for 10 min until they had reached an internal temperature of 72 °C; following this they were cooled to 4 °C and stored at that temperature until they were analyzed. The formula used to assess CL was ((m1 − m2) / m1) × 100%, where m1 is the weight of a sample before cooking and m2 is the weight of a sample after cooking [19].

2.3. Response surface methodology
Response surface methodology (RSM) is a modeling tool used to optimize processing operations in the meat industry, as it can be used to evaluate the influence of individual factors and their interactions. Prior to the experiment, a Box–Behnken design [20] was performed in order identify the three independent variables that had the strongest effect on water retention: these were found to be L-Arginine content (%), x1), sodium carbonate content (%), x2) and sodium citrate content (%), x3). Following this, the three variables were tested in 17 experiments in which the content of these additives were varied in order to determine the optimum combination of the three for water retention (Table 1). Each experiment was performed in triplicate, with the average CL (%) being assigned as a response value, y.

2.4. Statistical analysis
The results were expressed as mean ± standard deviation (S. D., n = 3). The software SPSS 16.0 (Pearson, US) was used to carry out a one-way analysis of variance (ANOVA), and a Tukey’s test was used to determine the significance of the measurements made for the CLs. The Box–Behnken design and modeling was performed using the Design-Expert 8.0 software (Stat-Ease, US).
3. Results and discussion

3.1. Effect of L-Arginine content on CL
The effect of varying the amount of L-Arginine in the samples on CL was assessed for 0–0.40% L-Arginine content. The results of this assessment were made using fixed amounts of sodium chloride, sodium carbonate, and sodium citrate (0.1%, 0.4%, and 0.2%, respectively), and they are shown in Fig. 1. It was found that CL decreased as the L-Arginine content increased up until 0.20%, after which point CL has no significant increasing even though the L-Arginine content increased. Our results were not consistent with those of Zhou et al. [16], who found that L-Arginine content of 0.2%, 0.4% and 0.6% reduced the CL of pork sausages, and the degree of decrease increased gradually with L-Arginine content increasing. Thus, our results indicated CL value was lowest at 0.2%. The reason needs to be further studied.

![Fig. 1. Effects on cooking loss of minced beef with different concentration L-Arginine](image)

Note: Means ± SD (n=3). Mean values having different capital letters are significantly different, p<0.01; Mean values having different lower case letters are significantly different, p<0.05. The same below.

3.2. Effect of sodium carbonate content on CL
The effect of varying the amount of sodium carbonate in the samples on CL was assessed for 0–0.8% sodium carbonate content. The results of this assessment were made using fixed amounts of sodium chloride, L-Arginine, and sodium citrate (0.1%, 0.2%, and 0.2%, respectively), and they are shown in Fig. 2. CL decreased as sodium carbonate content increased up until 0.4% sodium carbonate, at which point CL plateaued at about 12%. We believe that this occurred because alkali treatments cause protein denaturation, and the heating caused the proteins to aggregate; this was beneficial for protein cross-linking and the formation of high-strength gels that could lock moisture into the gel network structure and reduce CL [21-22]. Rigdon [23] used a strong alkaline electrolyte solution as a non-phosphate water retention additive in pork, but they found that the WHC of the pork did not increase; they theorized that the high pH value of the strong alkaline electrolyte solution (11.5) might have damaged the structure of the proteins in the pork, which hindered their water retention.
3.3. Effect of sodium citrate content on CL
The effect of varying the amount of sodium citrate in the samples on CL was assessed for 0–0.4% sodium citrate content. The results of this assessment were made using fixed amounts of sodium chloride, L-Arginine, and sodium carbonate (0.1%, 0.2%, and 0.4%, respectively), and they are shown in Fig. 3. Although CL was found to decrease as the sodium citrate content increased, the degree of reduction was not as significant as that found for either L-Arginine or sodium carbonate.

3.4. Analysis of the RSM
A RSM was utilized in order to determine the optimum production process for the cooking of beef using three different parameters (namely, the contents of L-Arginine, sodium carbonate, and sodium citrate). The optimization process was conducted using a Box–Behnken design that drew data from 17 experiments, each of which used different conditions based on the three parameters mentioned. The results showed that CL was different for the different treatment conditions used (Table 1). The ANOVA was carried out in order to test whether the regression model was feasible or not. The date shown in Table 2 indicated the regression model was feasible. The value of the adjusted R2 (0.9888) obtained suggested that a total of 98.88% of the results obtained for CL could be attributed to contents of the three non-phosphate additives. The R2 value (0.9951) indicated that there was a good correlation between the experimental values and the predicted values. The lack-of-fit measures fails in experimental domain, and so these points are not included in the regression model. The polynomial for CL (represented by y) can be represented by the following regression equation:
\[ y = 11.69 - 6.99x_1 - 0.93x_2 - 0.46x_3 + 0.63x_1x_2 - 0.15x_1x_3 - 2.500e^{-3}x_2x_3 + 3.74x_1^2 + 0.77x_2^2 + 0.68x_3^2 \] (1)

**Table 1.** Codes and levels of factors for response surface method experiment.

| Run | L-Arginine (x_1) | Sodium carbonate (x_2) | Sodium citrate (x_3) | Actual value (%) | Predicted value (%) |
|-----|------------------|------------------------|----------------------|------------------|---------------------|
| 1   | 0.2              | 0.2                    | 0.1                  | 14.25            | 14.53               |
| 2   | 0.2              | 0.6                    | 0.1                  | 12.98            | 12.08               |
| 3   | 0.1              | 0.4                    | 0.1                  | 23.61            | 23.41               |
| 4   | 0.2              | 0.6                    | 0.3                  | 12.03            | 11.75               |
| 5   | 0.2              | 0.4                    | 0.2                  | 12.30            | 11.69               |
| 6   | 0.1              | 0.4                    | 0.3                  | 23.02            | 22.80               |
| 7   | 0.2              | 0.4                    | 0.2                  | 10.80            | 11.60               |
| 8   | 0.3              | 0.4                    | 0.1                  | 9.52             | 9.74                |
| 9   | 0.3              | 0.6                    | 0.2                  | 8.84             | 8.92                |
| 10  | 0.2              | 0.4                    | 0.2                  | 11.75            | 11.49               |
| 11  | 0.2              | 0.4                    | 0.2                  | 11.62            | 11.69               |
| 12  | 0.3              | 0.2                    | 0.2                  | 10.02            | 9.52                |
| 13  | 0.1              | 0.2                    | 0.2                  | 24.83            | 24.75               |
| 14  | 0.2              | 0.2                    | 0.3                  | 13.31            | 13.61               |
| 15  | 0.1              | 0.6                    | 0.2                  | 21.14            | 21.64               |
| 16  | 0.2              | 0.4                    | 0.2                  | 12.00            | 11.69               |
| 17  | 0.3              | 0.4                    | 0.3                  | 8.82             | 8.52                |

**Table 2.** ANOVA (analysis of variance) of items of regression equation.

| Source of variation | Sum of squares | Degree of freedom | Sum of squares | Mean square | F-value | P-value | Significance |
|---------------------|---------------|-------------------|---------------|-------------|---------|---------|-------------|
| Model               | 457.48        | 9                 | 51.94         | 158.39      | <0.0001 | **      |
| $x_1$               | 390.00        | 1                 | 390.00        | 1101.08     | <0.0001 | **      |
| $x_2$               | 0.88          | 1                 | 0.88          | 20.99       | 0.0025  | **      |
| $x_3$               | 1.69          | 1                 | 1.69          | 5.16        | 0.0573  |         |
| $x_1x_2$            | 1.58          | 1                 | 1.58          | 4.80        | 0.0045  |         |
| $x_1x_3$            | 0.093         | 1                 | 0.093         | 0.28        | 0.1018  |         |
| $x_2x_3$            | 2.500E-005    | 1                 | 2.500E-005    | 7.633E-005  | 0.0003  |         |
| $x_1^2$             | 59.03         | 1                 | 59.03         | 180.00      | <0.0001 | **      |
| $x_2^2$             | 2.49          | 1                 | 2.49          | 7.60        | 0.0282  | *        |
| $x_3^2$             | 1.04          | 1                 | 1.04          | 5.92        | 0.0452  | *        |
| Residual            | 2.30          | 7                 | 0.33          |             |         |         |
| Lack of fit         | 1.03          | 3                 | 0.34          | 1.08        | 0.4528  |         |
| Pure                | 1.27          | 4                 | 0.32          |             |         |         |

Note: The marker ** represents p<0.01, The marker * represents p<0.05
The interactions between L-Arginine and sodium carbonate, L-Arginine and sodium citrate, and sodium carbonate and sodium citrate on CL are shown in Fig. 4, 5, and 6, respectively. From these figures, it can be seen that the effects of $x_1$ and $x_2$ on CL were significant ($p < 0.01$), while that of $x_3$ was not ($p > 0.05$). It can also be seen that the effects of $x_{12}$, $x_{22}$, and $x_{32}$ on CL were all significant ($p < 0.01$), while the interaction effects ($x_1x_2$, $x_1x_3$, and $x_2x_3$) were not ($p > 0.05$).

Design-Expert 8.0 was used to solve Eq. (1), which enabled the optimal CL value using the three different additives tested to be obtained. The condition for achieving the lowest CL was found to be 0.29% L-Arginine, 0.45% sodium carbonate, and 0.24% sodium citrate. The CL predicted under this condition was 8.26 $\pm$ 0.12%. In order to validate this prediction, three independent replicates were prepared. The average CL value was found to be 8.41 $\pm$ 0.13% ($n = 3$), which meant that the values of the optimized condition agreed well with the experimental results. The good correlation between these results confirmed that the model we developed adequately reflected the optimum CL.

**Fig. 4.** Cross interaction between L-Arginine content and sodium carbonate content on cooking loss of minced beef

**Fig. 5.** Cross interaction between L-Arginine content and sodium citrate content on cooking loss of minced beef
4. Conclusion

We determined what combination of three non-phosphate additives, namely L-Arginine, sodium carbonate, and sodium citrate, would result in the optimum CL of a cooked beef product. The optimum value was found to be 8.26%, and it was achieved using 0.29% L-Arginine, 0.45% sodium carbonate, and 0.24% sodium citrate.

Acknowledgments

The study was supported with funds provided by Young science foundation project of Hebei Provincial Education Department (QN2016164) and Doctoral foundation of Langfang Teacher's University (LSLB201602). The authors declare that there is no conflict of interest regarding the publication of this article.

References

[1] Garrido MD, Egea M, Linares MB, Borrisser-Pairó F, Rubio B, Viera C, Martínez B. 2017. Sensory characteristics of meat and meat products from entire male pigs. Meat Sci 129: 50–53.
[2] Schönfeldt HC, Strydom PE. 2011. Effect of age and cut on cooking loss, juiciness and flavour of South African beef. Meat Sci 87: 180–190.
[3] Vasavada MN, Dwivedi S, Cornforth D. 2006. Evaluation of Garam Masala spices and phosphates as antioxidants in cooked ground beef. J Food Sci 71: 292–297.
[4] Kılıç B, Şimşek A, Claus JR, Atlgan E. 2014. Encapsulated phosphates reduce lipid oxidation in both ground chicken and ground beef during raw and cooked meat storage with some influence on color, pH, and cooking. Meat Sci 97(1): 93–103.
[5] Hellendorn EW. 1962. Water-binding capacity of meat as affected by phosphates. Food Technol 16: 119–124.
[6] Chang CC, Regenstein JM. 1997. Water uptake, protein solubility, and protein changes of cod mince stored on ice as affected by polyphosphates. J Food Sci 62: 305–309.
[7] Patterson BC, Parrish JrPC and Stromer MH. 1988. Effect of salt and pyrophosphate on the physical and chemical properties of beef muscle. J Food Sci 53: 1258–1265.
[8] Whiting RC. 1987. Influence of various salts and water soluble compounds on the water and fat extrudation and gel strength of meat batters. J Food Sci 52: 1130–1132.
[9] Steinhauser JE. 1983. Food phosphates for use in the meat, poultry and seafood industry. Dairy and Food Sanitation 3: 244–247.
[10] Bell RR, Draper HH, Tzeng DYM, Shin HK, Schmidt GR. 1977. Physiological Responses of human adults to foods containing phosphate additives. J Nutr 107: 42–50.
Tabilo-Munizaga G, Barbosa-Canovas GV. 2005. Pressurized and heat-treated surimi gels as affected by potato starch and egg white: microstructure and water-holding capacity. LWT-Food Sci Technol 38: 47–57.

Khan MAA, Hossain MA, Hara K, Osatomi K, Ishihara T, Nozaki Y. 2003. Effect of enzymatic fish-scrap protein hydrolysate on gel-forming ability and denaturation of lizard fish Saurida wanieko surimi during frozen storage. Fisheries Sci 69: 1271–1280.

Velde FV. 2008. Structure and function of hybrid carrageenans. Food Hydrocolloid 22: 727–734.

Pietrasik Z. 2003. Binding and textural properties of beef gels processed with κ-carrageenan, egg albumin and microbial trans-glutaminase. Meat Sci 63: 317–324.

Witte MB, Barbul A. 2003. Arginine physiology and its implication for wound healing. Wound Repair Regen 11: 419–423.

Zhou CL, Li J, Tan SJ, Sun J. 2014. Effects of L-Arginine on Physicochemical and Sensory Characteristics of Pork Sausage. Advance Journal of Food Science and Technology 6: 660–667.

Qin H, Xu P, Zhou CL, Wang YJ. 2015. Effects of L-Arginine on water holding capacity and texture of heat-induced gel of salt-soluble proteins from breast muscle. LWT-Food Sci Technol 63: 912–918.

Ke S. 2006. Effect of pH and salts on tenderness and water-holding capacity of muscle foods. University of Massachusetts Amherst.

Andrés SC, Zaritzky NE, Califano AN. 2009. Innovations in the development of healthier chicken sausages formulated with different lipid sources. Poultry Sci 88: 1755–1764.

Yang HJ, Khan MA, Han MY, Yu XB, Bai XJ, Xu XL, Zhou GH. 2016. Optimization of textural properties of reduced-fat and reduced-salt emulsion-type sausages treated with high pressure using a response surface methodology. Innov Food Sci Emerg 33: 162–169.

Pérez-Mateos M, Lanier TC. 2007. Comparison of Atlantic menhaden gels from surimi processed by acid or alkaline solubilization. Food Chem 101: 1223–1229.

Tadpitchayangkoon P, Yongswatdigul J. 2009. Comparative study of washing treatments and alkali extraction on gelation characteristics of striped catfish (Pangasius hypophthalmus) muscle protein. J Food Sci 74: C284–C291.

Rigdon M, Hung YC, Stelzleni AM. 2017. Evaluation of alkaline electrolyzed water to replace traditional phosphate enhancement solutions: Effects on water holding capacity, tenderness, and sensory characteristics. Meat Sci 123: 211–218.