Optimization of textural and technological parameters using response surface methodology (RSM) for the development of beef products for older consumers

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ABSTRACT

Red meat has a tougher texture compared with many other food products, therefore consumption is often reduced among older adults. Acidic treatments had a positive effect on WBSF values (reduced the WBSF values from 23.35 N for control to 14.83 N), and texture parameters and a combination with apple fibre and rice starch may improve the health profile of a meat product with benefits for consumers, particularly for older population. A novel meat product with a softer texture (apple fibre 0.15 %, rice starch 0.30 % and citric acid 0.16 M) was optimized and successfully validated in this study. The results obtained for the objective measurements of tenderness were confirmed by consumers' tenderness results (*P* <

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0.05) moreover texture-optimized beef samples were found to be more acceptable by older consumers compared with control.

PRACTICAL APPLICATIONS

Meat processors have an important role in increasing the availability of suitable meat products for older consumers, by developing products that will meet their nutritional and textural needs. In this study, a novel meat product with a softer texture was developed, successfully validated and the product was found to be acceptable by older consumers. Meat processors could consider using rice starch, apple fibre and citric acid as feasible alternative to traditional ingredients used for beef injection, in order to obtain a softer product, that appeal to older consumers with difficulties in mastication.

Keywords: beef; older consumers; response surface methodology; tenderness

1. INTRODUCTION

The aging process is correlated with a regular and continuous loss of muscle mass progressing with a lower strength and physical endurance (Eom et al., 2015; Deutz et al., 2014). Protein is a significant macronutrient required by the body for building, repairing, and maintaining adequate muscle function (Deutz et al., 2014; Hoffman and Falvo, 2004). Beef is a valuable dietary source of high quality protein which makes it highly relevant to the diet of the older population (Williams, 2007). According to Rothenberg et al. (2007), van der Bilt (2002) and Palmer et al. (2000), in the older population, the decline of masticatory and/or swallowing functions are common and affects their food choices and their ability to eat. Schimmel et al. (2015) reported that complete denture wearers present a significant oral disability, which often leads to a gradual deterioration of their individual dietary habits. Moreover, in a study by Millwood and Heath (2000), it was mentioned that older population, denture wearers, indicated meat as difficult to chew. Also it was reported that older adults are more apprehensive about food texture (Forde and Delahunty, 2004), compared to younger adults, as they commonly have difficulty consuming hard, crunchy, dry, and stringy textured foods, such as meat products and celery (Hildebrandt et al., 1997). Therefore, their intake of important protein and micronutrient sources, such as red meat, can decline.

There is a necessity to exploit the possibilities to develop texture-optimized beef products which appeal to older consumers that could contribute to enhance their health status.

Beef products have potential for fortification with ingredients that have technological and health benefits. Considering the proven contribution of organic acids to the partial denaturation of meat proteins and their positive effect on meat tenderness (Chang et al., 2010; Ke at al., 2009; Aktas et al., 2003), they were selected for examination in this study. Furthermore, Goni et al. (1989) suggested that apple pulp is a typical source of dietary fibre, while Verma and Banerjee, (2010); Fernández-Ginés et al. (2003), and Wolfe & Liu (2003), indicated that due to the high concentration of flavonoids, polyphenols and carotenes in apple fibres, they have superior quality compared with other dietary fibres. Therefore, dietary fibres such as fruit fibres (citrus fibres, apple fibres) could be used as functional ingredients in meat products. As outlined by (Resconi et al., 2016; Fernández-Ginés et al., 2005) dietary fibres can be successfully added to meat products to improve their functional, technological and nutritional properties.

Researchers have examined the use of healthier ingredients, such as rice starch as a replacement for phosphate (usually perceived as an artificial ingredient or additive) in injected meat products and noted similar water retention levels and tenderness enhancement as is obtained using phosphate (Resconi et al., 2015; Joly & Anderstein, 2009). While some studies have examined the influence of fortification with health-promoting ingredients on beef tenderness (Pogorzelska-Nowicka et al., 2018; Resconi et al., 2015), there is a knowledge deficit in understanding the interaction of specific ingredients like organic acids, fruit fibres and starch on meat tenderness, particularly in the development of targeted meat products for older consumers.

A screening study followed by a response surface methodology (RSM) approach was considered in order to analyze and optimize the interactions between the ingredients: citric acid, apple fibre and rice starch on beef texture, to facilitate the optimization of texture modified beef products targeted at older consumers. Developing texture-optimized beef steaks using these ingredients might benefit older consumers by contributing on enhancing their health status.

2. MATERIALS AND METHODS

2.1. Experimental design of screening studies

2.1.1. Acids

The aim of this study was to identify a suitable food grade acid that might be used for inclusion in beef products. As citric and malic acids are two major organic acids associated to fruits we decided to use them for this study. Five treatments along with one control were applied: citric acid at low (CAl) and high concentrations (CAh), 0.1M and 0.5M, respectively; malic acid at low (MAl) and high concentration (MAh), 0.1M and 0.5M, respectively; combination of Cal and MAl (CM); the muscles assigned to control were injected with water (C).

2.1.2. Rice starch and fruit fibres

Three treatments along with one control were applied to beef muscles [*M. semitendinosus* (*ST*)] muscles: apple fibre and rice starch (0.35% and 0.70%, respectively: AR), citrus fibre (from Fiberstar, USA) and rice starch (0.35% and 0.70%, respectively: CR), mixture of apple/citrus fibre and rice starch (0.17%, 0.17%, 0.70%, respectively: ACR); the control muscles were injected with water (C). The aim of this study was to identify a suitable combination of fruit fibres and rice starch for inclusion in beef products, in order to improve the technological parameters.

2.2. Ingredient optimization using Response Surface Methodology

A D-optimal RSM experiment was designed using Design Expert software (v. 10.0.3, Stat-Ease Inc., USA). Three numerical factors were used: Citric acid, from All in All Ingredients - Ireland, added at a concentration of 0.15 - 0.35 M in the injection solution; Apple fibre (Healy Group, Ireland), added at a level of 0.15 - 0.4 % in the injection solution; Rice starch, from Beneo - Belgium, added at a level 0.3 - 0.8 % in the injection solution.

Under these conditions the generated experimental design had 19 runs, divided in 2 blocks with each run/treatment representing a different combination of the three numerical factors, including 11 model points, 4 replicate points and 4 lack of fit points. Each combination was applied to *ST* muscles. Each run represents a combination of the three ingredients for the injection formulation solution as described in Table 1.

2.3. Product processing

Beef *ST* from Holstein-Friesian steers were purchased from a local commercial processor on day 1 *post mortem* and aged for 7 days at 3°C. After aging, the muscles were trimmed of surface fat. Muscles were pumped to 115 % of their green weight, with corresponding solutions using a 21-needle brine injector (Inject-O-MAT type PSM-21, Dorit Maschinen, Handels AG, Switzerland). Muscles were tumbled (Dorit Vario-Vac VV-T-50, Dorit Food Processing Equipment Ltd., Switzerland) for 2h continuous at 7 rpm (2 - 4°C). Tumbled muscles were vacuum packed in pouches [O_2 permeability (23°C - 0 % RH)] 100 cc/m^2/24 h (LietPak, Lithuania) using a VG 400 Ilpra machine (Vigevano, Italy), and steam cooked (Fessmann cooker, T1800, Germany) to a core temperature of 72°C (≈4 h). Cooked muscles were subsequently chilled (2 - 4 °C, overnight) before being sub-sampled and vacuum packed for subsequent analyses: Warner-Bratzler shear force measurements (WBSF), Texture profile analysis (TPA), cook loss, color and proximate composition analysis. Sampling location within the muscle and the size of the samples was kept consistent between analyses and treatments.

2.4. Texture analysis

2.4.1. Warner-Bratzler shear force measurements

The analyses were carried out on cooked samples according to AMSA guidelines (AMSA, 1995) and Wheeler et al. (1996), at room temperature (20 ± 2°C). Samples were sheared perpendicular to the fibre direction using the Instron Universal testing machine, Model 4464 (Instron Ltd., UK), load cell of 500 N, cross head speed 250 mm/min, V-notch blade and analyzed in Bluehill®2 Software. Maximum peak force recorded during the test was reported as shear force. Seven cores (1.25 cm diameter) per steak were analyzed and following exclusion of the highest and lowest values, the average of five measurements was recorded for each sample.

2.4.2. Texture Profile Analysis (TPA)

Cooked samples were analyzed according to the method described by Bourne (1978) and Keenan et al. (2015). Cylindrical cores of diam. 25 × ht. 20 mm have been used for running the analysis, the plunger had 11.5 cm height. Three cores per treatment were obtained and axially compressed to 90% of their original height in a two-cycle compression test using an Instron Universal Testing Machine Model 4464 (Instron Ltd., High Wycombe, UK). Force time deformation curves were obtained using a 2 kN load cell applied at a cross speed of 500 mm/min. TPA recorded the following attributes: hardness (N) - peak force required for first compression cycle (‘first bite’); springiness/elasticity (mm) - distance the sample recovers after first compression (the time between the end of the first bite and the start of the second bite), (AMSA, 1995); cohesiveness (cohesion force) - ratio of positive peak force area during the second compression to the peak force area during the first compression; chewiness (N x mm) defined as hardness x cohesiveness x springiness.

2.5. Cook loss

Cook loss was determined using the equation: cooking loss = 100 (m_i–m_f) / m_i, where m_i is the weight of the beef muscle measured before cooking and m_f the weight of the beef muscle after cooking.

2.6. Color analysis

Measurements were taken using the CIE L*a*b* system with a dual beam xenon flash spectrophotometer (Hunter Lab Ultra Scan Pro, Inc., Reston, VA). The illuminant (D65, 10°) consisted of an 8° viewing angle and a 9.9 mm port size. Before each series of measurements, the instrument was calibrated using black glass and a white tile. Means of readings at three locations on each side of the thermal treated sample were assessed.

2.7. Proximate composition analysis of cooked samples

Compositional analysis was carried out on cooked samples thawed at 4°C overnight. The samples were homogenized in a Robot Coupe (R101, Robot Coupe SA, France). Intramuscular fat and moisture concentrations of minced samples were determined using the Smart System 5 microwave moisture drying oven and NMR Smart Trac rapid Fat Analyzer (CEM Corporation USA) using AOAC Official Methods 985.14 & 985.26, 1990. Protein concentration was determined using a LECO FP328 (LECO Corp., MI, USA) Protein analyzer based on the Dumas method and according to AOAC method 992.15, 1990.

2.8. Microstructure analysis

Treated beef samples (2 cm^3) were snap frozen in iso-pentane and liquid nitrogen (according to Meng et al. 2014) and stored at -80 °C. Sections were cut (20 µm thickness) using a Leica CM1950 cryostat (Leica Biosystems, Nussloch, Germany) after equilibration to specimen chamber temperature (-25 °C). Confocal scanning laser microscopy (CSLM) was used in conjunction with differential staining to visualize the impact of the treatments on the meat fibre structure and integrity. The staining technique was based on that developed by Fitzgerald *et al* (2014). Briefly, sections were sequentially stained with three fluorescent agents (Sigma Aldrich, Ireland): Fast green FCF (0.1% aqueous wt/wt) to label protein (633 nm excitation); fluorescein iso-thiocyanate (FITC, 0.1% wt/wt in ethanol) to label starch (514 nm excitation); and Fluorescent brightener FB28 (0.125% wt/wt aqueous) to label cellulosic material (405 nm excitation). After 5 min, sections were examined under a Leica SP5 confocal microscope (Leica Microsystems GmBH, Mannheim, Germany). Triple channel imaging was used to highlight the meat proteins, starch and cellulosic material which were pseudo-colored red, green and blue, respectively.

2.9. Statistical analysis of data

2.9.1. Screening trials

Product technological data were analyzed using ANOVA in Genstat 14.1 (Rothamsted Experimental Station, Hertfordshire, U.K.) and Fisher's LSD test, with the level of significance set as $P < 0.05$.

2.9.2. Response surface methodology

Statistical analysis was performed using Design Expert software. Each model was selected, i.e., linear, quadratic, by evaluating the regression coefficient (R^2). Models were considered significant when P values < 0.05. Automatic reduction algorithms were applied to reduce the number of insignificant terms in the models. The lack of fit test obtained from the analysis of variance (ANOVA) – that describes the variation in the data around the fitted model was also performed (Patel et al., 2011). It was previously reported by Noordin et al. (2004), that a not significant lack of fit indicates an effective model. However, a significant lack of fit suggests the possibility of interactions between the regresses and responses that are not considered by the model. By dividing the lack of fit mean square by its error mean square we obtained the F values and the results of the lack of fit test are presented in Table 2 and 3.

2.10. Consumer studies

2.10.1 Recruitment

Panelists of varying age cohorts were recruited for this study, they were chosen in compliance with the following criteria: community dwelling, healthy, not on a pre-described diet, did not have a food allergy, did not have any difficulties swallowing, and were regular consumers of beef steak. The subjects were recruited by the Agri-Food and Biosciences Institute, Belfast, Northern Ireland, from active retirement groups from Northern Ireland region, taking into account demographic information (age group, former occupation) and product related questions (labels, openability of packaging). The assessor cohorts were derived from various socio-economic backgrounds. All participants indicated their gender in the questionnaire. They also signed a declaration indicating they did not have any difficulties swallowing food. Cooked optimized beef steaks were evaluated by a panel comprised of 116 adults aged 65 to 91 years old.

2.10.2. Consumer tests

Consumer acceptance tests were carried out on 116 older consumers. Ages ranged from 65 to 91 years and categories ranged as follows: 65 – 69 (n = 45), 70 – 74 (n = 27), 75 – 79 (n = 28), 80 – 84 (n =13), 85 - 90 (n= 3), 90+ (n=1). The tests were carried out in the sensory facility in AFBI, Northern Ireland. The samples were assigned random three digit codes and consumer acceptance test were carried out on triplicate batches presented to the panelists in duplicate. Assessors were asked to rinse their mouths with water in between each sample. The panelists were asked to rate the coded and randomized order samples. The following attributes were assessed: liking of aroma, tenderness, juiciness, liking of flavor and overall liking.

The following products were presented to 116 older consumers:

- Beef steaks injected with water – control;
- Texture-optimized beef steaks.

Statistical analysis carried out was ordered logistic regression (proportional odds model) with random effects. It was fitted using maximum likelihood estimation using the meologit command in Stata (v14.2).

3. RESULTS AND DISCUSSION

In this study, the possibility of using organic acids, fruit fibres and starch on improving beef tenderness and functional properties has been assessed. Screening studies of acid, fibre and starch combinations, followed by an optimization using RSM were conducted. Two food grade acids (citric and malic acid) of different concentrations and their combination were used in an injection solution in order to facilitate the optimization of texture-modified beef products targeted at elderly consumers. Two fruit fibres and their mixture, in combination with rice starch were used in an injection process of beef *ST* to facilitate the optimization of a suitable combination of fibre and starch that might improve the technological and textural parameters of beef products. From the first screening study citric acid was selected and from the second apple fibre and rice starch. To the authors' knowledge, there are no commercially available processed (injected) beef products which have been fortified with a combination of dietary fibre (apple fibre), rice starch and citric acid. Tenderness and technological parameters of beef products were evaluated. Texture and color measurements were performed, as well as the impact of the selected ingredients at microstructural level. Furthermore, consumer studies were completed, in order to evaluate the acceptability of texture enhanced meat products by older consumers.

3.1. Texture analysis

3.1.1. Warner-Bratzler Shear Force

All acidic treatments resulted in significantly reduced WBSF values (*P* < 0.05) from 23.35 N to 14.83 N for a mixture of citric and malic acid. For fruit fibres and rice starch treatments they decreased in a numerical fashion, but not significant from 26.09 N for control to 21.71 N for a combination of apple fibre and rice starch but the difference was not significant. For the WBSF values a linear model was significant (*P* = 0.002) for the RSM study, with WBSF values negatively correlated with citric acid concentration. In Figure 1 it can be observed that increasing the concentration of citric acid in the injection solution led to a beef product with an improved tenderness. No main or interaction effects were observed for rice starch and apple fibre. Ke et al. (2009) studied the impact of citric acid on the tenderness of beef muscle, and reported similar results, suggesting that the most effective (from a texture perspective), acidic marination for beef was the one with citric acid. As reported by Chang et al. (2010), the increase in meat tenderness was caused by the physical weakening of muscle structure that resulted in the swelling of myofibrillar proteins (actin and mysosin).

3.1.2. Texture profile analysis

All treatments resulted in significantly reduced values for TPA hardness (*P* < 0.05) from 145.86 N for control to 99.75 for a treatment with a mixture of CA and MA. For chewiness all treatments, except MA1, resulted in highly significant reduced values. The same softening effect caused by organic acids was reported by Chang et al. (2010), they explained that the increase in meat tenderness was caused by the physical weakening of muscle structure, that resulted in the swelling of myofibrillar proteins (actin and mysosin). However, for cohesion force and springiness no effect was observed.

Even though no significant effect was measured for the other TPA parameters, their values decreased in a numerical fashion when compared with control samples.

For the RSM study, TPA parameters hardness and chewiness were each fitted with linear models. All three showed similar response surfaces and again, only citric acid was a significant factor. These parameters were minimized with maximal citric acid concentration as can be observed in Figure 2a and 2b for chewiness (P = 0.001). For hardness, a linear model was found to be significant (P = 0.001). A higher concentration of citric acid resulted in lower values for hardness as it can be observed in Figure 3. The explanation could stand in the fact that citric acid contributes to the partial denaturation of meat's proteins during cooking, which makes the substrate accessible for the absorption of different added ingredients. The same softening effect caused by organic acids was reported by Chang et al. (2010), who explained that the increase in meat tenderness was caused by the physical weakening of muscle structure, acid inclusion caused dramatic heterogeneous changes in the myofibrillar ultrastructure. No significant effect was observed for cohesion force and springiness.

Other authors (Schilling et al., 2001; Motzer et al., 1998) have reported a reduction in hardness and chewiness when a modified food starch was used for the production of hams. These effects were attributed to the retention of moisture by the starch causing this weakening effect, which is beneficial for tenderness improvement of our products.

3.2. Cooking loss

Cooking loss was not significantly affected by the combination of ingredients that was used for this study (Table 3). Cooking loss ranged from 32.46 to 37.97 %, a positively quadratic correlation was observed for the inclusion of with citric acid, apple fibre and rice starch in the injection solution of beef muscles. When combinations of fruit fibres and rice starch were used for injection of beef muscles, reduced cooking loss values were observed, when compared with, thus indicating water has a higher affinity to the fibre and starch. Other studies (Resconi et al., 2016; Verma et al., 2010) reported a reduction in cooking loss in meat products with starch inclusion, which may be attributed to the retention of moisture by the starch. Moreover, the use of citric acid in marination has the benefit of increasing water-holding capacity.

3.3. Color analysis

Both citric and malic acid had a high effect of the on the redness and yellowness of the cooked samples. At higher concentration of citric acid, redness values decreased, leaving the product very brown with a burnt undesirable appearance (Table 5). For the fibre and starches treatment of *ST* muscles, no effect was observed on the color of cooked steaks.

On the other hand, for the RSM study, for lightness (L*) and yellowness (b*) values, a linear model was fitted, the values weren't significant. However, for redness (a*) the values were significant (P = 0.01). For higher concentrations of citric acid, lower values for redness were registered (as presented in Figure 4). Kim et al. (2015) reported that an increase in the level of citric acid showed a tendency to reduce the redness of the juice resulted after sous-vide cooked meat samples. The decrease in the red color of meat products during storage might be explained by the binding of the heme iron of myoglobin with citric acid or the prevention of the formation of pink pigments by acidification process as explained by Kieffer et al., 2000.

The same effects were observed in the screening studies, at higher concentrations of malic and citric acid, a decrease trend for the redness values were observed, leaving the product very brown with an burnt undesirable appearance.

Sammel and Claus (2003), reported that citric acid decreased the redness (a*) values of cooked ground turkey, moreover a reduction in a* values when irradiated turkey rolls were treated with 0.3% citric acid was reported by the same authors (Sammel and Claus, 2006).

Stackhouse et al. (2016), studied the effect of citric acid enhancement on cooked beef strip loin color, and reported that citric acid decreased the redness values. Furthermore, Desmond and Troy (2001) studied the effect of lactic and citric acid on low-value beef and reported that a decrease in the redness of frankfurters was observed when compared with one of the controls. Arganosa and Marriott (1989) studied the effect of citric acid reported on cooked restructured steaks' colour, and reported that the acid treated samples were whiter when compared to controls. The authors stated that the acid treatment appeared to enhance the conversion of myoglobin to metmyoglobin, which has a lower degree of color intensity.

The reduction in the redness might be attributed to the rice starch inclusion; other authors (Resconi et al., 2015) reported a reduction in redness with rice starch inclusion in a linear manner.

3.4. Proximate composition analysis of cooked beef samples

Protein, fat and moisture of cooked beef muscles were determined. Even though the lack of fit wasn't significant but the model only tended to be significant for protein (P = 0.0597) and fat content (P = 0.06). A slightly decreased trend was observed for protein content when the concentration of citric acid was elevated. Further, an interaction between the rice starch and citric acid (P = 0.02) was observed and reported (Figure 5a and 5b). Both rice starch and apple fibre, have technological functionality and have almost inert chemical interactions with meat's protein that resulted in no significant effect on the protein content (Tarte, 2009). Although RS was expected to contribute in retaining moisture content, it did not have a significant effect on moisture content of the samples. However, CA treatment significantly enhanced moisture content of the samples by increasing water holding capacity of the beef matrix (Figure 6a and 6b).

3.5. Formulation optimization

Based on the results presented, the optimization tool from Design Expert® predicted the optimal values for the three ingredients. Optimization was done by using an objective method of desirability (Keenan et al., 2014; Meyers et al., 2009). The goal was to minimize the concentrations of AF, RS, CA and the values of the responses critical for the older consumers such as WBSF, TPA hardness and chewiness. The software suggested a combined factor of AF 0.15 %, RS 0.30 % and CA 0.16 M to achieve the above mentioned goal with a desirability level of 0.63 (Table 4). Although the values of the WBSF, TPA hardness, and chewiness could be further reduced by increasing CA concentration, the overall appearance of the products was unacceptable at the concentrations of above 0.16 M (Table 5). The other two factors namely AF and RS did not have significant effect on the targeted responses. Prior to the response surface optimization study, a screening study was carried out to test the extent of effectiveness of CA on WBSF, TPA hardness and chewiness. This screening study has found that WBSF, TPA hardness and chewiness values were reduced by 30.36 %, 15.34 %, 32.78 % and 29.27 %, when CA treatment was used. The optimized factors further reduced these values while keeping the key sensory parameters acceptable. The factors, however, did not affect other relatively less important responses such as springiness, cohesion force, cooking loss, protein, and fat contents. Hence, the models for these responses were not significant. For this reason, these responses were not taken into consideration while optimizing the three factors used in the present study.

3.6. Validation of the optimized formulation

The applicability and accuracy of the described models were tested using three model performance indices such as bias, accuracy and average mean deviations. Even though optimization is a very important tool in predicting the most suitable combinations of ingredients, to confirm that the presented model can be used to predict appropriate results it is important to validate it. According to Tiwari et al. (2008), the average mean deviation measures the errors of fitting data into the model showing the proximity of predicted and experimental values. The experimental values for the responses WBSF, TPA hardness, and chewiness were in close agreement with the predicted values at optimum factors conditions. The average mean deviations between predicted and experimental values for these responses were in the range of 3.0 to 5.7 %, except for TPA hardness which had 15% deviation. In a response surface design with three factors and a range of responses, a 15% deviation for a response may be considered acceptable. A large variability among the means of TPA hardness values was also observed which partly contributed to this deviation.

Bias and accuracy are two important factors that are used to understand the correlation between the predicted values from the optimization and the experimental values from the results. As showed by O'Shea et al. (2015), the bias factor is an important tool to describe the reliability of the model and the accuracy factor is used as a tool to see the difference between the predicted values from the model and the experimental values. Although a perfect model will have bias and accuracy values of 1.0 where predicted and experimental values are identical, it is not realistic in most cases as experimental conditions and instrument's performance in separate measurements are never identical (Ross et al., 2000). The bias and accuracy factors of the presented model for WBSF, TPA hardness, chewiness and moisture content were in the range of 0.94 to 1.01 and 1.01 to 1.06 showing a high degree of reliability of the models.

3.7. Microstructure analysis

The microstructure images of treated and untreated meat samples are presented in Figure 7 (a-d). The presence of rice starch (pseudo-colored green rounded particles) plus fibrous cell wall material (pseudo-colored blue) exclusively in the connective tissue regions of the muscle fibres in all three treated samples can be observed. An agglomeration of rice starch particles can be seen as a gelatinized structure. As described by Resconi et al., 2015, usually rice starch gel is located in free spaces, i.e. where the connective tissue was present or between myofibres. Alternatively, these gaps may have originated during tumbling and/or cooking (Katsaras and Budras, 1993). The size of the rice starch and fruit fibres were visible macroscopically and but they haven't affected the acceptability of product appearance, as perceived by consumers.

CSLM represents a modern technique compared with the more traditional fluorescence microscopy, and it allows 3D imaging and clear visualization of the fibre structure of muscle tissues (Damez & Clerjon, 2008). Straadt et al. (2007) used CSLM to investigate the variations in myofibres and myofilaments related to ageing and cooking muscle, respectively and stated that CSLM is a promising technique to investigate meat quality at microstructural level (Straadt et al., 2007). An important characteristic of confocal microscopy is three-dimensional imaging of food structures. In this study, imaging analysis by CSLM demonstrated to be an efficient tool for observing textural changes in meat and was effectively applied to various meat products including beef steak which had been subjected to various injection formulation treatments.

Chen (2016) and Nyberg et al. (2015) described the importance of developing and enhancing new food products adapted to older consumers, in order to promote health and prevent malnutrition. It is important to assess the technological performance of such products. In this optimizing study, fruit fibres, food grade acids and rice starch have been assessed in relation to developing texture modified meat products (beef steaks) targeted at older consumers. Providing a basis for optimizing beef steaks that require reduced mastication effort might lead to increased consumption by older consumers.

<u>3.8. Consumer acceptability of rice starch, apple fibre and citric acid for texture enhancement of injected beef products targeted at older consumers</u>

The existing innovation in meat products development research targets the overall population, while formulations specifically addressing the needs of older consumers are still lacking. For meat products, it would be important to consider age-related issues with texture (for example difficulties in chewing). Texture-optimized beef samples were found to be more acceptable by older consumers when compared with control. Significant differences were observed for tenderness, when the tenderized beef steaks were compared with control ($P < 0.05$). For the other attributes, even if the results weren't statistically significant, all had higher acceptability scores when compared with controls, as presented in Figure 8. The results obtained for the objective measurements of tenderness were confirmed by consumers' tenderness results moreover overall liking scores were numerical higher for the fortified samples. Even though, age subgroups (65-69, 70-74, 75-79 and 80+ years) did not show significant result for juiciness and overall liking, the same trend has been observed: higher scores for the texture-optimized steaks, when compared with control.

4. CONCLUSIONS

D-optimal design, RSM was found to be an effective technique to investigate the synergistic effect of the proposed ingredients for beef injection on beef tenderness.

The presented results suggest that the model was reliable and fitted well to the experimental data. Apple fibre and rice starch don't have a negative effect on meat tenderness, but they might improve the health profile of a meat product with benefits for consumers, particularly for older population. Citric acid had a positive effect on texture parameters, and a combination with the other two ingredients may result in a product that might be suitable for consumption by older population. The optimization (AF 0.15 %, RS 0.30 % and CA 0.16 M) of a novel meat product with a softer texture was developed and successfully validated in this study and the texture-optimized beef steaks were found to be acceptable by older consumers. Overall, it can be concluded that rice starch, apple fibre and citric acid could represent feasible alternative to traditional ingredients used for beef injection and the final products are suitable for consumption by older population.

ETHICAL STATEMENTS

Conflict of Interest: The authors declare that they do not have any conflict of interest.

Ethical Review: The sensory evaluation component of the study was approved by the Agri-Food and Biosciences Institute, Belfast, Northern Ireland.

Informed Consent: Written informed consent was obtained from all study participants.

AUTHORSHIP CONTRIBUTIONS

C. Botinestean designed the study, analyzed the data and drafted the manuscript. M. Hossain assisted with the response surface methodology and statistical analysis. A.M. Mullen, M.A.E. Auty, J.P. Kerry contributed to the interpretation of results, R.M. Hamill contributed to conception/designing the study and revised the manuscript.

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Table 1. Ingredient combinations according the response surface experimental design (D - optimal)

Run	AF (%)	RS (%)	CA (M)
1	0.400	0.800	0.150
2	0.213	0.550	0.250
3	0.275	0.800	0.350
4	0.400	0.300	0.350
5	0.150	0.550	0.150
6	0.400	0.300	0.350
7	0.150	0.300	0.250
8	0.400	0.300	0.150
9	0.150	0.300	0.150
10	0.400	0.800	0.150
11	0.150	0.800	0.350
12	0.275	0.300	0.150
13	0.150	0.300	0.350
14	0.150	0.800	0.150
15	0.400	0.550	0.350
16	0.150	0.300	0.350
17	0.150	0.800	0.150
18	0.400	0.800	0.250
19	0.338	0.550	0.200

AF - apple fibre; RS - rice starch; CA - citric acid

Table 2. The response surface models of WBSF and TPA parameters for injected beef

	WBSF (N)	**Hardness (N)**	**Chewiness (N x mm)**	**Cohesiveness (-)**	**Springiness (mm)**
Model	Linear	Linear	Linear	Linear	Linear
***P* value**	0.0020	0.0016	0.0407	0.1161	0.1318
***P* value lack of fit**	0.3082	0.8163	0.8876	0.7041	0.9260
R^2	0.5634	0.4028	0.3147	0.1929	0.1764
F values:					
AF	0.01	0.07	0.39	0.42	0.54
RS	2.67	3.54	0.91	1.13	6.36
CA	24.25	20.14	7.42	5.87	0.11
NS - not significant; AF - apple fibre; RS - rice starch; CA - citric acid					

Table 3. The response surface models of cook loss (%), colour parameters (L^*, a^* and b^*), protein, fat and moisture for injected beef

	Cook loss (%)	**Lightness (L^*)**	**Redness (a^*)**	**Yellowness (b^*)**	**Protein (%)**	**Fat (%)**	**Moisture (%)**
Model	Quadratic	Linear	Linear	Linear	2FI	Quadratic	Linear
***P* value**	0.1502	0.0685	0.0166	0.0562	0.0597	0.0639	0.0117
***P* value lack of fit**	0.9283	0.5416	0.7113	0.4607	0.9330	0.8207	0.9885
R^2	0.2570	0.3157	0.4022	0.2797	0.3124	0.7175	0.4329
F values:							
AF	2.28	1.26	1.081E-003	0.020	0.32	7.04	1.34
RS	0.41	0.63	0.01	1.29E-003	0.06	11.25	1.27
CA	0.49	7.45	13.27	8.92	1.80	16.07	13.35
AF x RS	6.498E-003	-	-	-	4.583E-003	2.04	-
AF x CA	0.15	-	-	-	1.49	3.06	-
RS x CA	4.91	-	-	-	7.80	0.10	-
AF^2	5.26	-	-	-	-	16.47	-
RS^2	0.35	-	-	-	-	1.38	-
CA^2	2.34	-	-	-	-	1.79	-

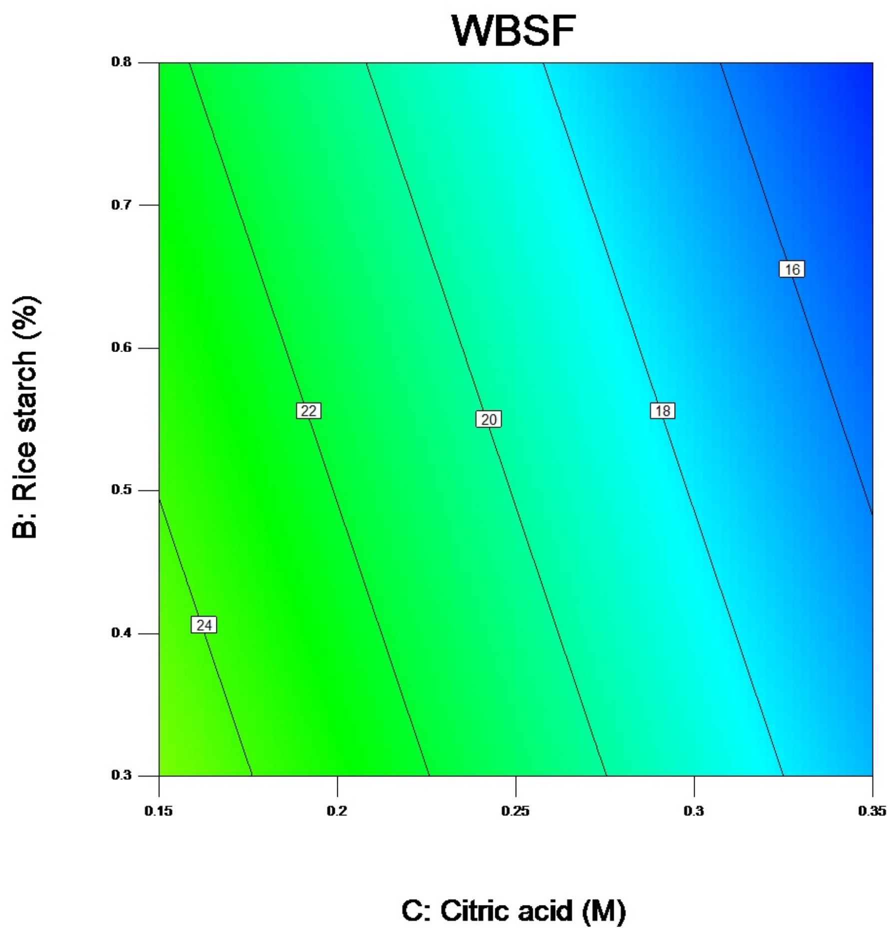
NS - not significant; AF - apple fibre; RS - rice starch; CA - citric acid; 2FI - 2 factorial interaction

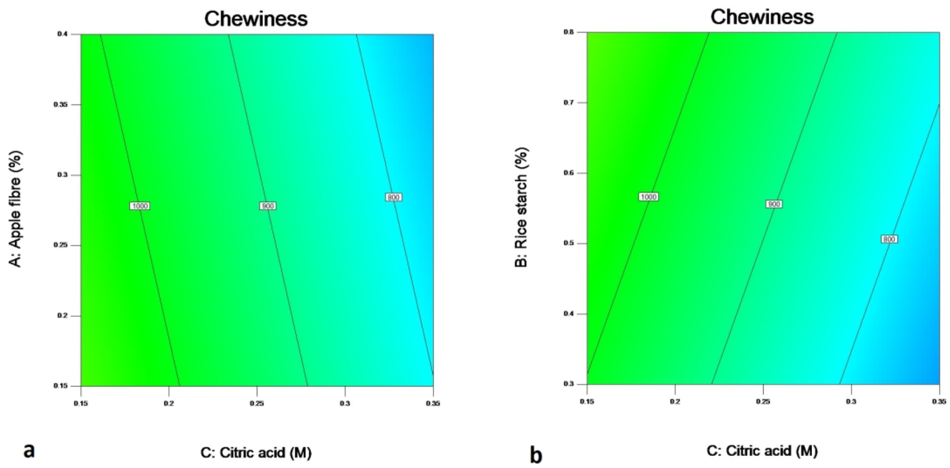
Table 4. Predicted, experimental values, accuracy & bias factors and mean deviation of the optimised formulation: 0.16 M CA, 0.15 % AF and 0.30 % RS

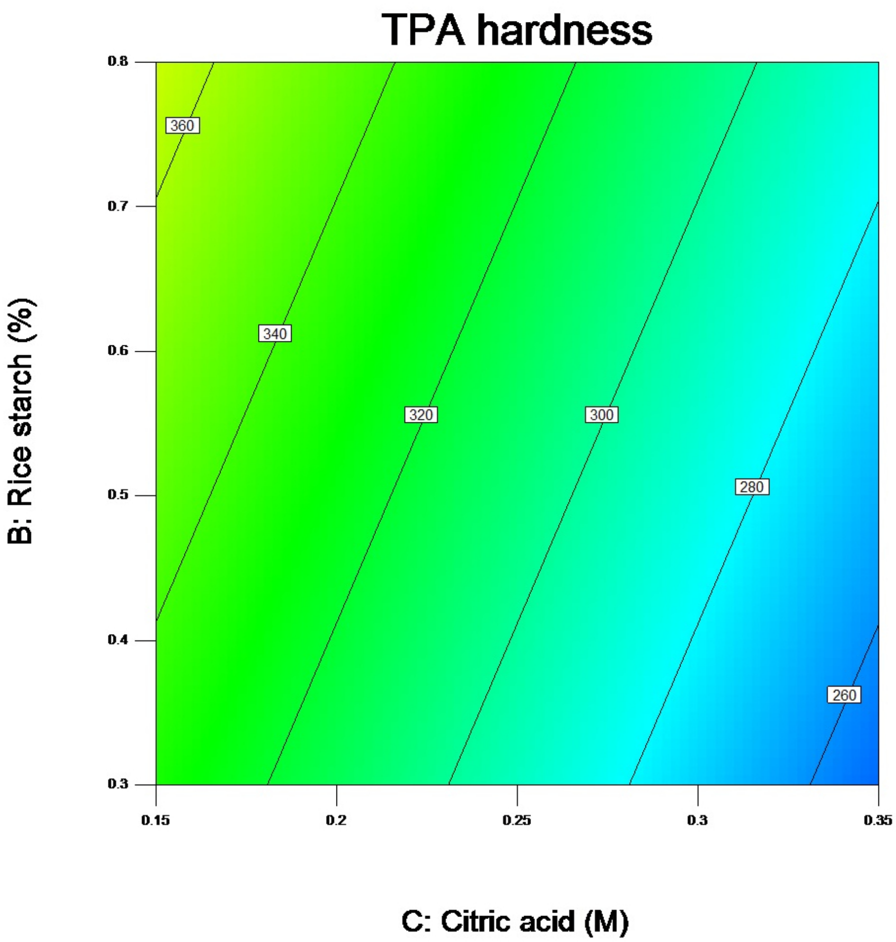
Response	Predicted	Experimental	Bias Factor	Accuracy Factor	Average mean deviation (Σ)
WBSF (N)	26.629	25.81 ± 2.76	1.01	1.01	3.08
Hardness (N)	343.780	395.45 ± 41.39	0.94	1.06	15.03
Chewiness (N x mm)	1075.739	1124.78 ± 157.10	0.98	1.02	4.56
Cohesiveness (-)	0.848	0.8451 ± 0.0092	1.00	1.00	0.34
Springiness (mm)	6.642	6.87 ± 0.10	0.99	1.01	3.43
Cook loss (%)	33.735	33.71 ± 0.25	1.00	1.00	0.07
L*	53.401	53.73 ± 2.21	1.00	1.00	0.62
a*	5.442	5.59 ± 0.13	0.99	1.01	2.72
b*	12.861	13.43 ± 0.49	0.98	1.02	4.42
Protein (%)	30.893	30. 01 ± 0.35	1.01	1.01	2.86
Fat (%)	1.765	3.08 ± 0.55	0.79	1.27	4.50
Moisture (%)	67.262	66.68 ± 0.92	1.00	1.00	0.87

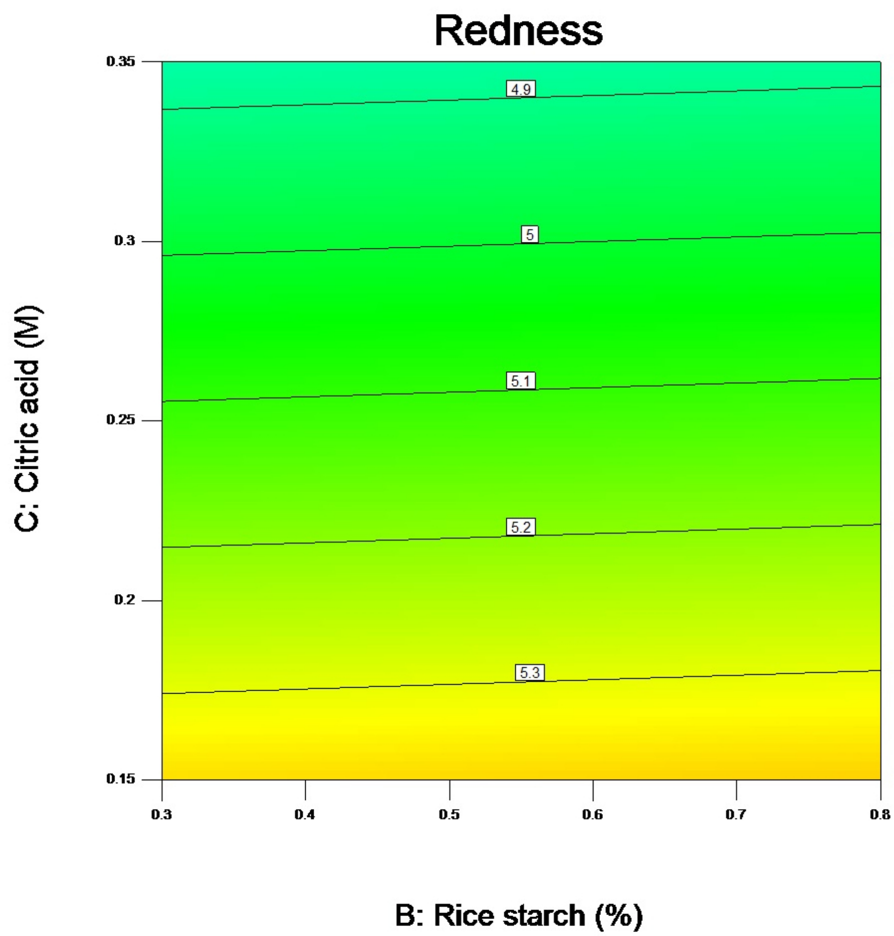
Table 5. Effect of acidic treatments on colour parameters: lightness (L*), redness (a*), yellowness (b*), and cooking loss (%) of *ST* beef steaks

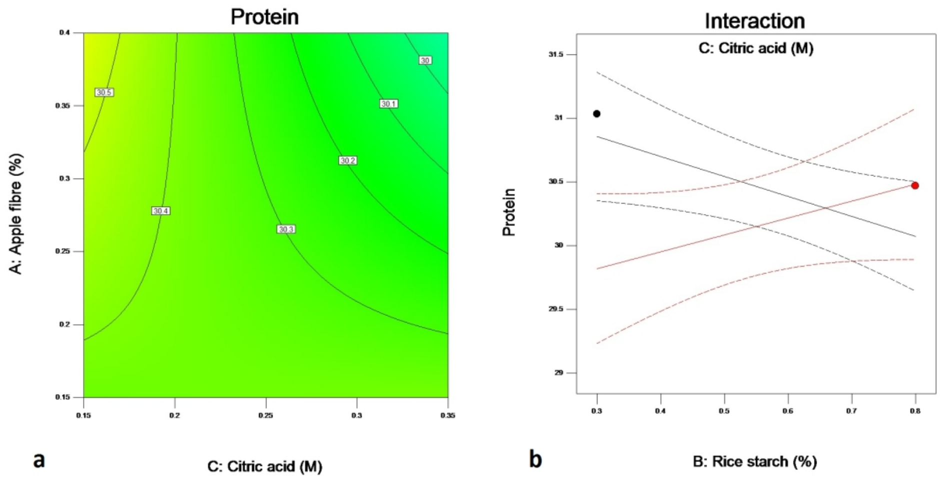
Acidic treatments	Lightness	Redness	Yellowness
C	63.53 ± 2.84	5.60 ± 0.20^{a}	16.91 ± 0.23^{a}
CAl	56.09 ± 0.23	4.02 ± 0.01^{b}	11.41 ± 0.24^{b}
CAh	52.21 ± 0.55	4.55 ± 0.18^{bc}	11.68 ± 0.69^{b}
MAl	55.89 ± 4.99	4.86 ± 0.20^{cd}	13.34 ± 1.53^{ab}
MAh	56.87 ± 2.83	5.22 ± 0.12^{ad}	13.87 ± 1.06^{ab}
CM	54.45 ± 3.41	4.44 ± 0.21b^{c}	12.34 ± 0.88^{b}
P value	0.090	<.001	0.007
SEM	2.101	0.118	0.635
a, b, c, d, e – means within column that do not share a common letter are significantly different (*P*<0.05); * *P*≤0.001			

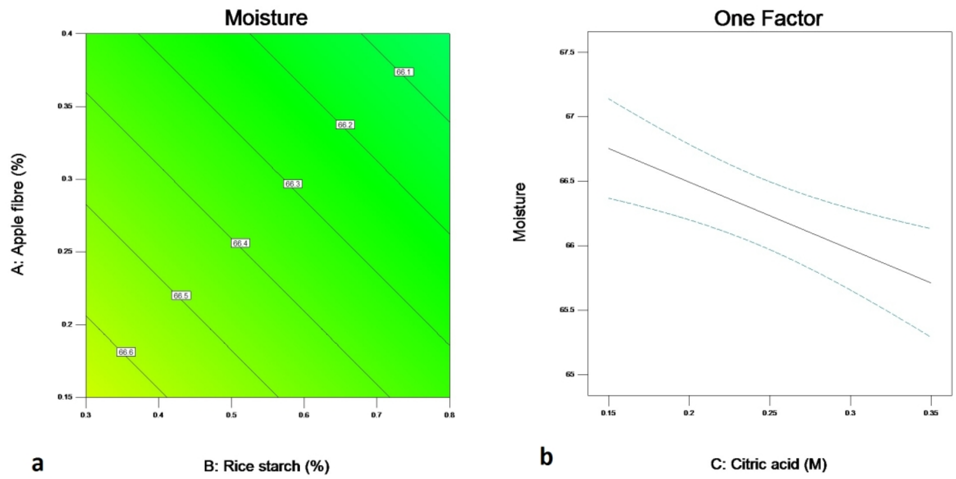

WBSF
B: Rice starch (%)
0.8
0.7
0.6
0.5
0.4
0.3
24
22
20
18
16
0.15
0.2
0.25
0.3
0.35
C: Citric acid (M)

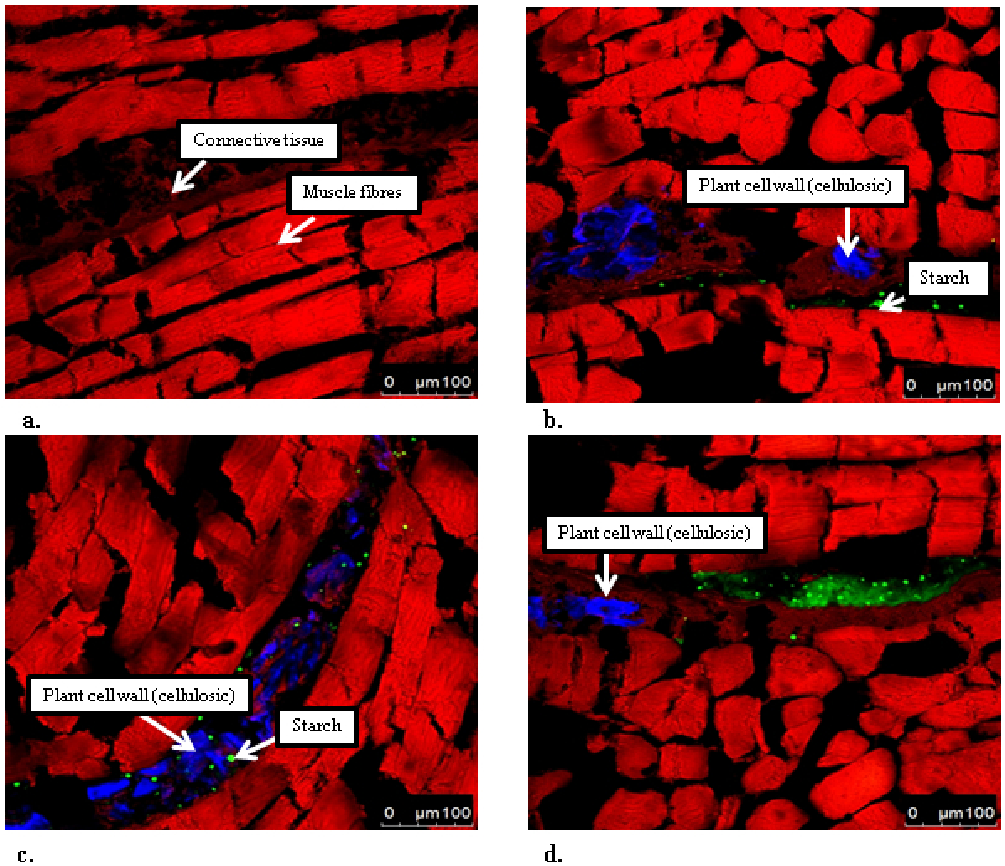

Chewiness
A: Apple fibre (%)
C: Citric acid (M)
1000
900
800
a
Chewiness
B: Rice starch (%)
C: Citric acid (M)
1000
900
800
b


TPA hardness
B: Rice starch (%)
C: Citric acid (M)
0.8
0.7
0.6
0.5
0.4
0.3
0.15
0.2
0.25
0.3
0.35
360
340
320
300
280
260

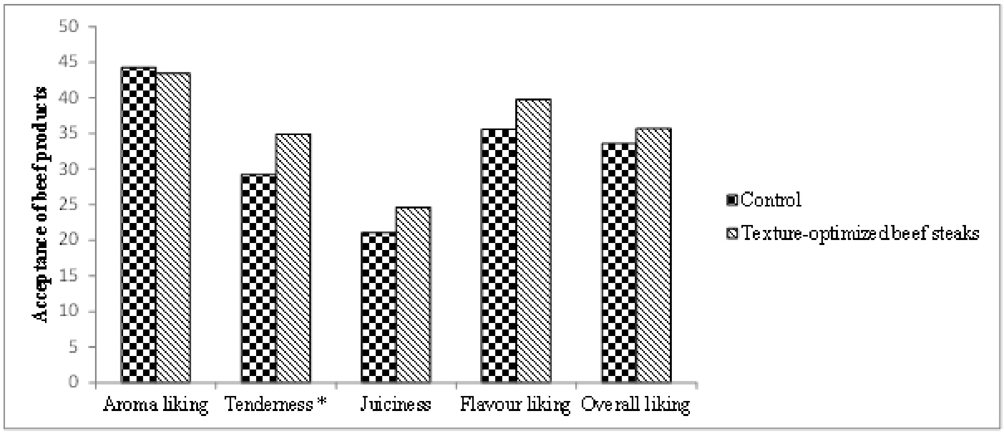

Redness
4.9
5
5.1
5.2
5.3
0.35
0.3
0.25
0.2
0.15
C: Citric acid (M)
0.3
0.4
0.5
0.6
0.7
0.8
B: Rice starch (%)


Protein
A: Apple fibre (%)
C: Citric acid (M)
30.5
30.4
30.3
30.2
30.1
30
a
Interaction
C: Citric acid (M)
Protein
B: Rice starch (%)
b


Moisture
A: Apple fibre (%)
B: Rice starch (%)
66.1
66.2
66.3
66.4
66.5
66.6
a
One Factor
Moisture
C: Citric acid (M)
b



Connective tissue
Muscle fibres
0 µm 100
a.
Plant cell wall (cellulosic)
Starch
0 µm 100
b.
Plant cell wall (cellulosic)
Starch
0 µm 100
c.
Plant cell wall (cellulosic)
0 µm 100
d.


Acceptance of beef products
50
45
40
35
30
25
20
15
10
5
0
Aroma liking
Tenderness *
Juiciness
Flavour liking
Overall liking
Control
Texture-optimized beef steaks