Optimising the acceptability of reduced-salt ham with flavourings using a mixture design

Gonzalo Delgado-Pando, Paul Allen, Joe P. Kerry, Maurice G. O'Sullivan, Ruth M. Hamill

PII: S0309-1740(19)30177-9
DOI: https://doi.org/10.1016/j.meatsci.2019.05.010
Reference: MESC 7839

To appear in: *Meat Science*

Received date: 8 March 2019
Revised date: 10 May 2019
Accepted date: 11 May 2019

Please cite this article as: G. Delgado-Pando, P. Allen, J.P. Kerry, et al., Optimising the acceptability of reduced-salt ham with flavourings using a mixture design, Meat Science, https://doi.org/10.1016/j.meatsci.2019.05.010

Optimising the acceptability of reduced-salt ham with flavourings using a mixture design

Gonzalo Delgado-Pando[1], Paul Allen[1], Joe. P. Kerry[2], Maurice G. O'Sullivan[2] & Ruth M. Hamill[1,*] ruth.hamill@teagasc.ie

[1]Teagasc Food Research Centre Ashtown, Dublin 15, Ireland

[2]School of Food & Nutritional Sciences, University College Cork, Cork, Ireland

[*]Corresponding author at: Teagasc Food Research Centre *Ashtown,* Dublin 15, Ireland.

Abstract

The objective of this study was to optimise the acceptability of reduced-salt cooked ham containing a mixture of glycine and yeast extract as flavourings by using response surface methodology. Twelve different formulations were prepared with varying levels of salt and the two flavourings, according to a mixture design. The sensory properties were assessed along with the instrumental texture and colour. A multiple factor analysis showed that higher scores in tenderness, saltiness and juiciness were positively correlated, whereas instrumental hardness and chewiness were negatively correlated with acceptability. Response surface plots and optimisation software allowed the inference of two optimised formulations: HO1 with 1.3% salt and yeast extract content of 0.33%; and HO2 with 1.27% salt, 0.2% yeast extract and 0.16% glycine. A panel of 100 consumers found no significant differences in overall acceptability when both were compared to a control (1.63% salt). These results show it is possible to manufacture consumer accepted cooked ham with up to 20% salt reduction.

Keywords: multiple factor analysis, response surface, meat product, salt reduction

1. Introduction

At the 66th World Health Assembly (2013) the Member States adopted a global target regarding salt intake: by 2025 a 30% reduction should be achieved (WHO, 2013). Since then, salt reduction strategies including development of reduced-salt products have been given added momentum all over the world (Trieu et al., 2015). Meat products contribute to 15-25% of the salt/sodium in the diet, and thus, have been widely targeted in developing of reduced-salt analogues (Kloss, Meyer, Graeve, & Vetter, 2015). Salt reduction in meat products is a complicated task, however, as salt not only plays a role providing the typical salty taste and flavour, but it further acts as preserving agent and it is also essential in the adequate development of the desired physicochemical characteristics (Barat & Toldrá, 2011; Desmond, 2006).

The major impact of salt reduction on the sensory properties of the product is clear, and for this reason, the consumer's response to reformulation must be taken into account, throughout the whole optimisation process. Optimisation can be defined as the different steps needed to obtain the best result under a particular set of circumstances (Gacula, M.C., 1993). In the context of a reduced-salt product, the circumstances are both the salt and ingredients' level needed to achieve the most acceptable product in its class, in this case among analogues with reduced-salt content.

In order to systematically determine what is best in the relevant setting, several sensory factors have to be taken into account, such as flavour, colour, texture, etc. The use of Response Surface Methodology (RSM) provides statistically robust yet intuitive and easy to use, graphical solution and aids in achieving a single formulation, simultaneously optimised combining multiple attributes. This methodology has been successfully applied to product optimisation in different food products like cheese, wine, coffee, juices, burgers and rashers (Abdullah & Cheng, 2001; Baugreet, Kerry, Allen, & Hamill, 2017; Delgado-Pando, Allen, Kerry, O'Sullivan, & Hamill, 2018a; Dooley, Threlfall, & Meullenet, 2012; Khetra, Kanawjia, & Puri, 2016; Kumar, Ravi, & Saraswathi, 2010; Mendes, de Menezes, Aparecida, & da Silva, 2001).

Hedonic or affective tests provide a holistic evaluation of the acceptability of single or multiple products based on appearance, aroma, mouthfeel, taste, flavour and texture. These tests assess the sensory appeal of a product and how much it is liked. Therefore, the use of hedonic acceptance testing is the gold standard for measuring acceptability and optimising a product (Lawless & Heymann, 2010). The selection of the consumer panel for the hedonic test is a key point in the process, as the success of the optimisation will rely on the results from this test. Optimisation processes have relayed on hedonic tests with as little as 25 panellists (Mendes et al., 2001) but larger numbers (36-108) are often used (Abdullah & Cheng, 2001; Dooley et al., 2012; Kumar et al., 2010). In any case, an acceptance test is not enough to fully understand how the changes in the formulation impact the acceptability; there is a need of further testing to improve the diagnostic of how the differences in the formulation affect consumer appeal. One of the most utilised tests are the Just-About-Right (JAR) scales which help in indicating if an attribute is too weak or too strong relative to the consumer's perception (Popper & Kroll, 2005). An alternative is the use of descriptive sensory testing to identify which sensory attributes are important for the acceptance. The main disadvantage is that a different panel must be trained with the use of reference standards for the attributes to measure, which entails an increase of effort and time. A variation of these type of analysis has been proposed by Richter, de Almeida, Prudencio, and de Toledo Benassi (2010) and it is called Ranking Descriptive Analysis (RDA). RDA is a simple and fast method where the panellists rank the products based on specific attributes previously selected by them (Fellendorf, Susann, Kerry, Hamill, & O'Sullivan, 2018; Kang, Kang, Lee, & Chang, 2018; Mamede & Benassi, 2016). RDA is a variant of Flash Profiling. It is more time consuming but gives a more comprehensive description of the samples (Mamede & Benassi, 2016). As opposed to this, other profiling techniques such as Check-All-That-Apply (CATA) have less power than quantitative data and therefore require a rather large number of panellists (Valentin, Chollet, Lelièvre, & Abdi, 2012).

The most frequently used approach for developing reduced-salt meat products is based on salt substitution and/or inclusion of flavour enhancers (Desmond, 2006). Nonetheless, the increasing market shift towards clean label food product formulations makes it necessary to keep the extraneous additives and ingredients to a minimum. Some flavourings can be used in very small quantities and can enhance specific flavours, like umami and salty taste in meat, or mask undesirable flavours (Dermiki et al., 2013). However, their role in maintaining the physicochemical properties of the product changes from one product to another and from one formulation to another. In addition, flavourings can work in a synergistic way with some flavourings, enhancing their flavouring power, but also in the opposite way masking the desired flavours or increasing the unwanted ones. For this reason, mixture designs represent an appropriate choice when the response is dependent on the proportion of the ingredients and synergistic or antagonist effects are expected (Gacula, M.C., 1993).

One of the most utilised flavourings by the food industry is yeast extract. This flavouring can be used as masking agent or flavour enhancer, as it provides precursors of several volatile compounds and interacts with other additives. It also contains a number of umami taste compounds and when added to cooked meat it can enhance the umami taste and saltiness perception (Campagnol, dos Santos, Wagner, Terra, & Pollonio, 2011; Dermiki et al., 2013). The use of yeast extract as flavourings in reduced-salt foodstuffs has been successfully studied in several products such as cheese and fermented sausages (Campagnol et al., 2011; Khetra et al., 2016). Glycine is the simplest amino acid present in food, and is usually applied in the preparation of processed-meat flavourings (Wong, Abdul Aziz, & Mohamed, 2008). It has been reported that the reaction between cysteine and reducing sugars to form meat-like flavours can be accelerated by glycine (Zhao et al., 2019). For this reason, glycine has been utilised as an effective flavouring in reduced-salt formulations of meat products like corned beef, white and black pudding and dry-cured pork loins (Fellendorf, Susann et al., 2018; Fellendorf, S., O'Sullivan, & Kerry, 2016a, b; Gou, Guerrero, Gelabert, & Arnau, 1996).

In the present study we aim to optimise a reduced-salt cooked ham containing a mixture of glycine and yeast extract as flavourings by using RSM on a mixture design. We also aim to evaluate the adequacy of using a hedonic test, a RDA and instrumental measurements in the optimisation process.

2. Materials and Methods

2.1. Experimental design and sample preparation

Reduced-salt cooked ham formulations were prepared using different mixtures of sodium chloride and two flavour enhancers: glycine and low sodium yeast extract. Ham recipes also contained dextrose, phosphates, sodium ascorbate and sodium nitrite. All ingredients were food grade and purchased from All in All Ingredients Ltd (Dublin, Ireland).

For the optimisation study, a mixture I-Optimal experiment with 3 lack of fit points was built by Design Expert v10 (Stat Ease Inc., USA). The model comprised 12 different points (Table 1) with sample H9 replicated two times (H10 and H11), and sample H12 being the control (1.63% salt no flavourings). Minimum and maximum levels were: 0.82 to 1.63% for salt, 0 to 0.7% for glycine and 0 to 0.3% for yeast extract. The levels were selected according to previous trials (Delgado-Pando et al., 2018b) and manufacturer recommendations. For the validation study, three different ham samples were produced: an optimised control ham (CO) where salt content was 1.63% (with glycine and yeast, both 0%), and two optimised products

HO1 (1.33% salt, 0.3% yeast extract) and HO2 (1.27% salt, 0.16% glycine and 0.20% yeast extract).

For the ham preparation, four full deboned pork legs were purchased from a meat supplier (Rosderra Irish Meats, Edenderry, Ireland) and brought to the meat processing facility at Teagasc Food Research Centre Ashtown. The fat was trimmed and the legs were cut in small pieces prior to mincing using a ø 32mm disc (La Minerva Food Service Equipment, Italy). The meat was vacuum-packed and stored under chilling regime until it was used the following day. Portioned meat was added to a tumbler (Dorit Maschinen, Handels AG, Switzerland). Brines were prepared by adding the specific amount of water, salt, glycine and yeast extract (Table 1) as per calculations of 120% of meat green weight. Sodium nitrite (0.015%) and phosphates (0.3%) were weighed and dissolved in part of the water then added to the mix. Dextrose (0.5%) and sodium ascorbate (0.05%) were the last ingredients added to the brine. The brine was thoroughly mixed before being added to the meat. Each formulation was tumbled for 60 min at 6 rpm on intervals of 15 min work/rest periods under chilling conditions and then vacuum-packed and stored at 2 °C overnight. The next morning, the formulations were put in stainless steel moulds and cooked under steam conditions (HR 100%) at 85 °C to a core temperature of 72 °C. A chill shower during 30 min was applied and the cooked hams were stored for 24 h at 2±1 °C. The hams were sliced and stored in vacuum bags under chilling storage until further use. Each of the formulation was manufactured twice.

2.2. Colour and texture measurements

Colour was analysed using an Ultrascan XE spectrophotometer (Hunter Associates Laboratory, Inc, USA). White standard plate (X=93.5, Y=0.3140, Z=0.3318) was used for calibration. Reflectance spectra was registered using a D65 illuminant an 10 ° angle and results were given in CIELAB system where L* is defined as brightness, a* as redness and b* as yellowness. The cured colour ratio was calculated following the equation: ratio=650 nm/570 nm (AMSA, 2012). For each formulation three different slices were measured three times each.

Texture profile analysis was carried out using an Instron Universal Testing Machine (5542) using a 25 mm circular flat probe and a 500 N load cell (Instron Ltd., High Wycombe, UK). Slices of 20 mm thick were used, three per formulation. Ten cores (25 mm diameter) were axially compressed to 50% of the original height (speed 5 cm/min) in a two cycle compression. Hardness and chewiness were calculated from the data.

2.3. Sensory evaluation

The experiment was conducted in a standardised test room conforming to International Standards (ISO, 1988). Slices of cooked ham were served at 10±1°C on white plastic plates in a monadic way. Sample presentation was randomised according to William Latin squares to balance the first-order carryover effects and coded with random three digits numbers.

The sensory acceptance test for the optimisation study was conducted using untrained assessors (n = 25, 15 females) in the age range of 21–65, chosen on the basis that they were weekly consumers of cooked ham (Stone, Bleibaum, & Thomas, 2012). In this study, a large number of samples required sensory testing therefore we conducted a sample size analysis to identify an efficient design with sample size resulting in sufficient power to detect differences in the consumer reaction towards the products under study. Based on a previous sensory analysis for the same type of products, sample size for confidence interval estimation was calculated according to Gacula, M.C. and Singh (1984). From this previous analysis we observed an estimate of the standard deviation of acceptability of 12.4% with 19 observations. We then calculated the sample size needed to estimate the mean acceptability score with a maximum variation of ±7.5% with a 95% degree of confidence interval and alpha of 0.05. The panellists were asked to evaluate on a 9-point hedonic scale the liking of flavour and overall acceptability of the ham samples and water was provided to cleanse the palate between samples. The assessors then participated in a ranking descriptive analysis (RDA) (Richter et al., 2010) in which, for each consensus attribute (tenderness, juiciness, saltiness and off-flavour), samples were ordered by increasing intensity on a linear scale with two anchor points (Meilgaard, Civille, & Carr, 2007). Due to high number of samples to test and to avoid sensory fatigue, the sensory analysis was split in two different sessions according to a balanced incomplete block design (Meilgaard et al., 2007). Therefore, the 12 formulations were randomly allocated to two blocks of six samples. At the end of the test, each panellist attended two sessions (30 minutes each) and evaluated all 12 formulations. Compusense five software (Compusense Inc., West Gelph, Canada) was used for data acquisition.

In addition, and to overcome the limitations of the sensory panel size, a verification study was performed. For this study, 100 consumers (66 females) in the age range of 18-61 were recruited (regular consumers of cooked ham) and then participated in a consumer acceptance test (Lawless & Heymann, 2010) to evaluate the acceptance of the two optimised samples (HO1 and HO2) and the control (CO) on a 9-point hedonic scale.

2.4. Statistical Analysis

All statistical analysis were performed in R (version 3.5.1) using R Studio (R, Core Team 2018). One way ANOVA and Tukey post-hoc test were performed to ascertain the effect of formulation on physicochemical properties. All the sensory data were transformed to 0-100 scale. For the hedonic attributes in the optimisation and validation experiments, the analysis of variance was performed considering panellists as a random factor (Lawless & Heymann, 2010). The package *lmerTest* based on lme4 was used to evaluate the mixed effects ANOVA using the Satterthwaite approximation and multiple comparison using *multcomp* package (Hothorn, Bretz, & Westfall, 2008; Kuznetsova, Brockhoff, & Christensen, 2017). For the sensory intensity attributes Friedman test was used and post hoc comparisons were run using the exact inference method proposed by Eisinga, Heskes, Pelzer, and Te Grotenhuis (2017) within the *PMCMRplus* R package (Pohlert, 2018). Sensory and instrumental data were also analysed using *FactomineR* and *factoextra* R packages (Kassambara & Mundt, 2017; Le, Josse, & Husson, 2008) by means of a multiple factor analysis. The I-Optimised mixture models were analysed using Design Expert Software (v10, Stat-Ease Inc., Minneapolis, USA).

3. Results

3.1. *Influence of formulation on physicochemical properties and sensory attributes*

The differences in formulation (Table 1) significantly affected both sensory and physicochemical properties with the exception of instrumental colour characteristics (Table 2, Fig. 1). Instrumental lightness (F=1.61, P=0.16), redness (F=1.94, P=0.09), yellowness (F=2.15, P=0.06) and cured colour ratio (F=1.11, P=0.39) were not significantly affected by formulation. The cooked hams all presented a noticeable cured colour (1.7-2.0) (AMSA, 2012).

With regards to instrumental texture parameters, the control sample (H12) had the lowest hardness and chewiness, and H1 and H3 (lowest salt content) were the ham samples with the highest hardness and chewiness values, respectively. Hence, it could seem that salt was the main driver of the instrumental texture. However, the use of lower salt levels with diverse combination of the two flavourings also produced hams with no significant differences in hardness (H4, H5, H8, H9) and chewiness (H5, H6, H8, H9) (Table 2).

The hedonic test showed that sample H9 (as average of H9, H10 and H11) was the most liked in terms of both flavour and overall acceptability (Table 2). Control sample (H12) hedonic scores were not significantly different than H9, H8 and H4. Sample H2 obtained the lowest mean score in likeness of flavour and H7 the lowest mean score in overall acceptability. Both hedonic sensory attributes were highly correlated between each other ($r=0.82$, $P<0.001$) and when analysed by t-test paired comparison, there were no significant differences between them ($t=-1.53$, $P=0.13$). Therefore, flavour seems to be a strong component of the overall acceptability of the samples.

The results from the RDA pointed out that panellists found significant intensity differences between formulations in tenderness ($\chi^2= 45.14$, $P<0.01$), juiciness ($\chi^2= 34.12$, $P<0.01$) and saltiness ($\chi^2= 66.62$, $P<0.01$), but not in off-flavour ($\chi^2= 6.94$, $P=0.64$). This indicates that the use of the two flavourings—glycine and yeast extract—did not significantly produce an increase or decrease in the off-flavour intensity at the tested levels (Table 1). Tenderness values ranged from 62.14 to 80.98, juiciness from 48.64 to 63.42, saltiness from 35.88 to 61.94 and off-flavour from 20.54 to 30.26 (Fig.1). The highest values for tenderness, juiciness and saltiness were found in the control sample (H12). When analysing the tenderness scores by all-pairs Friedman test comparison (p-values adjusted by Bonferroni), H1, H2, H7 were ranked significantly less tender than samples H19 and H12. These three samples are the ones that had higher glycine content (Table 1). Sample H7 was significantly less juicy than samples H4, H6, H8, H9 and H12. This sample, as mentioned earlier, was also the less tender and the one with lowest score in overall acceptability. With regards to saltiness intensity samples H1, H2 and H3 were perceived less salty than samples H6, H8, H9, and H12. This is in agreement with the differences in salt content of these samples. However, salt content did not explain perceived saltiness in full, as samples with low salt content, such as H5 and H7, were perceived as salty as any other (no significant differences). In order to delve into the differences between the reformulated samples (H1-H9) and the control (H12), a post-hoc pairwise many-to-one test was also performed using the same method proposed by Eisinga et al. (2017). Significant differences for the sensory intensity terms with respect to sample H12 are shown in Fig.1; off-flavour is not displayed as no significant differences were found between samples. The comparison against the control revealed that samples H1, H2, H7 and H8 were significantly less tender and samples H1-H3 and H7 less salty. In terms of juiciness only sample H7 was perceived as less juicy than the control.

3.2. *Relationship between sensory properties and instrumental characteristics*

To better understand the relationship between the instrumental measurements and the sensory characteristics of the samples, a multiple factor analysis (MFA) was carried out. This multivariate analysis highlights the common structures of a set of variables (sensory, instrumental) observed for the same individuals—ham samples in this case—balancing out the weight of each of the datasets, and allowing to observe hidden patterns and relationships among them (Pagés & Husson, 2005). For our study we used three different datasets: a dataset comprised of the hedonic sensory responses, a dataset from intensity of sensory responses and a dataset from the instrumental measurements; all of them where included as active groups in the analysis.

The results showed that the first component was dominant with an eigenvalue (inertia) of 2.64 and a 58.47% of variance explained, while the second axis explained a further 17.26% of variance and had an eigenvalue of 0.78. The following components showed a drop in the eigenvalues and therefore only the first two components where considered for analysis. The contribution of each of the datasets to the first component was balanced: 30.84, 35.96 and 33.20% for hedonic, intensity and instrumental, respectively, consequence of the weighing strategy of the MFA. Nonetheless, the instrumental dataset was the main contributor (74.6%)

to the second component. The RV coefficients showed that the correlation between instrumental and intensity characteristics was strong (RV=0.73), whereas it was moderate between instrumental and hedonic (RV=0.46) and hedonic and intensity attributes (RV=0.63).

With the exception of off flavour, the intensity attributes were positively correlated with the hedonic scores, which means high scores in tenderness, saltiness and juiciness were correlated with higher acceptability and flavour liking (Fig. 2). All of these parameters were on the rightmost part of the first component, whereas hardness and chewiness appeared on the opposite side. This shows a strong but negative correlation between instrumental texture and tenderness intensity, and also with the rest of sensory attributes. The lower the score in hardness and chewiness the better the overall acceptability and organoleptic perception. Instrumental colour values of a*(yellowness), b* (redness) and in a lesser extent the cured colour ratio were also negatively correlated with the overall acceptability (Fig. 2). We can also observe an absence of correlation between lightness and off flavour and the hedonic attributes.

The three partial points (one for each of the groups) for each ham sample are represented in Fig.3. With some exceptions, there is coherence between the hedonic, intensity and instrumental characteristics. The first component sets apart hams H1, H2, H3 and H7 from H4, H8, H9 and H12 irrespective the point of view. Therefore, samples H4, H8 and H9 were the ones more similar to the control (H12). The intensity attributes were in agreement with the instrumental along the first component, with no big differences for any of the samples. The biggest differences were found in sample H6, where hedonic and instrumental parameters appeared in discordance. This is explained by the fact that the low values for hardness and chewiness in this sample were expected to positively impact on sensory hedonic characteristics, but it was assessed with low overall acceptability scores (Table 2). Some differences also appeared in the second component due to the influence of off flavour and instrumental colour characteristics.

3.3. *Response surface methodology for the optimisation of the ingredients mixture*

Response surface methodology (RSM) was carried out for optimising the mixture according to the selected variables (Myers, Montgomery, & Anderson-Cook, 2009). From the MFA we observed that all of the studied variables, with the exception of lightness and off flavour, were highly correlated with the overall acceptability of the hams. For each of these parameters a model was constructed, selecting those significant, not aliased and with higher hierarchy. From the colour characteristics only significant associations were found for yellowness (b*), the rest of colour parameters were left out of the analysis. Analysis of variance was performed and the non-significant terms were dropped, the ANOVA was then recalculated. The selected models (Table 3) were those of higher statistical power, lower variance inflation factor for the coefficients and adequate signal to noise ratio. Because the mixture model does not contain an intercept term, the linear terms incorporate the overall average response and are tested together.

With respect to the overall acceptability, the model showed that yeast was the ingredient with the higher relative impact, meaning it provided more acceptability to hams when compared to the other two ingredients alone (Table 3). Salt proportion was also important, for the same amount of yeast extract the overall acceptability increased when the salt proportion was higher—meaning lower glycine content (Fig. 4a). It is noteworthy that the maximum levels of acceptability were in the higher ranges of salt content but not at the highest point, as yeast extract had a higher impact, indicating that salt reduction could be possible with the use of flavourings as replacers. The maximum corresponded to a binary mixture of 1.33% salt and 0.3% yeast extract.

The pure salt mixture produced the hams with better liking of flavour, whereas yeast extract appeared to exert a negative impact when used alone (Table 3). However, when yeast extract was in combination with either salt or glycine, a synergistic effect was observed as reflected by the positive and significant quadratic terms. This means that the values for the mean response were higher when combinations of these two ingredients were used than when using the mean for each pure mixture. The maximum flavour liking appeared at around 1.45% salt and 0.18% of yeast extract, although high flavour values (>70) were also observed for ternary mixtures (Fig. 4b). We have to point that the model presented a significant lack of fit. Even though alternative models were tested, no improvement was found. The plot of residuals against predicted values did not show any particular pattern, thus the model was accepted. With respect to the intensity sensory attributes, the tenderness model depicted a similar shape to the liking of flavour model. Salt was the main contributor, the higher the salt the higher the tenderness. A negative linear term was also found for yeast extract, indicating that when used alone higher concentrations meant lower tenderness. A synergistic effect between salt and yeast extract and glycine and yeast extract was also found (Table 3). In this case the maximum tenderness intensity appeared for the binary mixture 1.51% salt, 0.12% yeast extract (Fig. 5a). Salt content and yeast extract were the main contributors to the juiciness intensity of the hams (Table 3). Nonetheless, a small synergistic effect between glycine and salt was also observed. The highest juiciness values were found at the highest salt concentration, almost irrespective of the use of flavouring as replacers; the maximum appeared at 1.58% salt and 0.05% glycine (Fig. 5b). At salt concentrations below 1.25%, flavourings had a more active role on juiciness intensity. As it was expected, salt content was the main driver of the saltiness intensity, with no much influence of the two flavourings; maximum appeared at the salt pure mixture (1.63% salt)(Table 3, Fig. 5c).

Mixture models for the instrumental attributes were all linear, no significant synergistic or antagonistic effects were found (Table 3). In the case of yellowness, yeast extract was the component with higher relative impact. This is in agreement with its appearance, as it presents a beige colour and gives yellowish solutions in water. The maximum yellowness value was found at a ternary mixture of 0.82% salt, 0.51% glycine and 0.3% yeast extract (Fig. 6a). With respect to the textural parameters, the harder and chewier hams were those with higher proportion of glycine (Table 3). For the same proportion of glycine, the hardness values seldom varied with salt and yeast extract concentrations (Fig. 6b). Nonetheless, for the same amount of glycine, lower salt content—higher yeast extract—produced higher chewiness values; maximum chewiness appearing at 0.82% salt, 0.7% glycine and 0.11% yeast extract (Fig. 6c).

From this study two predicted optimal formulations were selected for further validation. The selection was done using the numerical and graphical optimisation from the software package Design Expert. The first optimised mixture was calculated through optimisation of hedonic components only, setting the following targets: a minimum score of 70 for both acceptability and liking of flavour, minimising the salt content, and maximising the overall acceptability. This optimised ham (HO1) would have a salt content of 1.3% and a yeast extract content of 0.33%. For the second optimised ham (HO2) the hedonic parameters were not included. We looked for further salt reduction, maximising tenderness and juiciness intensity while minimising yellowness, hardness and chewiness. HO2 would have a composition of 1.27% salt, 0.16% glycine and 0.20% yeast extract.

3.4. *Validation of optimised products*

Both optimised products (HO1 and HO2) were validated along with the control sample (CO, 1.63% salt) by overall acceptability in a consumer acceptance test. An initial model was specified with the inclusion of sample, gender and age as fixed factors, the consumers as random effect and all possible interactions among them as explained by Kuznetsova et al. (2017). It turned out that only the effect of consumers and sample were significant ($\chi^2=15.02$ $p<0.001$, $F=3.10$ $p<0.05$, respectively) and therefore were the ones included in the final model. No significant differences in acceptability were found between the three samples, although median value was significantly higher for sample HO2 (Fig.7). The mean overall acceptability for CO sample was 58.8, while it was 64.6 for HO1 and 63.9 for HO2. The mixture model for overall acceptability in actual components (as %) was:

Acceptability = 42.733*NaCl + 26.047*Glycine + 50.115*Yeast Extract

From this equation the predicted values (mean±SD) for CO, HO1 and HO2 were 69.65±5.21, 71.87±5.16 and 68.46±4.65 respectively. Even though the observed values were below the predicted ones, they still fell within the 95% confidence interval of the predicted value.

4. Discussion

This study shows that the use of a flavouring mixture could be a valid strategy for salt reduction in formed hams. The ingredient levels, at which the mixture was analysed, affected both sensory and physicochemical properties of the hams. This is in connection with the different roles salt has in the meat products. Changes in salt content not only affect the flavour, but also other properties like textural and appearance, thereby affecting physicochemical and sensory qualities alike (Barat & Toldrá, 2011; Lorenzo et al., 2015a; Lorenzo, Cittadini, Bermúdez, Munekata, & Domínguez, 2015b).

The initial quality of a food product, especially of meats and meat products, is perceived from its colour (Lawless & Heymann, 2010). One of our main concerns when selecting the ingredients levels in the mixture was the effect they would have on the colour of the final product. The upper limit established for yeast extract addition was in part based on its effect on ham colour, as this ingredient presents a light beige tonality. Furthermore, salt reduction can also affect the ham colour (Greiff, Mathiassen, Misimi, Hersleth, & Aursand, 2015). The finding of no significant differences in the instrumental colorimetric characteristics (Table 2) meant not only an adequate level of yeast extract, but also adequate mixture combination able to overcome the changes in colour due to salt reduction.

The sensory assessment of the product is a key part of any optimisation process as it gives an idea of what the consumers perceive. Instrumental characteristics are, to some extent, correlated with the sensory attributes, but they fail when trying to give the full interpretation of the sensory perception as experienced by the human brain (Lawless & Heymann, 2010). Keeping in mind the main objective of the optimisation process—obtaining the best product under the given criteria—we consider that a consumer or affective test would be our only choice. However, this type of test would not tell us what the drivers of these differences are. In our study we also acquired information on how the changes in formulation were perceived in terms of intensity of certain sensory attributes, and how these could be related not only to acceptance and liking but to the instrumental characteristics (Fig. 2, 3). The use of MFA to combine the results from a RDA, a hedonic test and instrumental measurement of physicochemical characteristics of ham, succeeded as an appropriate methodology for establishing the relationship between the products' characteristics and their acceptance. This approach could be an alternative to the well-established JAR scales in consumer optimisation research (Popper & Kroll, 2005), as JAR scales have been criticised for being challenging to consumers and for presenting differences when compared to hedonic scores (Shepherd, Smith, & Farleigh, 1989; van Trijp, Punter, Mickartz, & Kruithof, 2007). With this multifactorial approach we proved we were able to discern which product characteristics were important in terms of acceptability. The ones with higher correlation with the overall acceptability were selected and their sensory and technological performance verified in the validation study, where sample HO2, optimised by only taking into account intensity sensory attributes and instrumental characteristics, was as acceptable as the ham with normal salt content and obtained the highest median acceptability score from the three products.

Our affective testing involved an unusual number of consumers (25) due to the prioritisation of time and resources—consumers had to taste 12 different samples—over the higher accuracy a bigger group would have given. The risks of taking this approach were minimised by both the verification study—with a bigger sample size (n=100)—and the use of a second optimised product (HO2) not based on the hedonic characteristics as mentioned before. Nonetheless, the optimised product based solely on the hedonic testing (HO1) obtained the highest acceptability mean score in the verification study and was not significantly different from the control. These results indicate that the sample size was enough for the optimisation of this type of product.

Both instrumental and sensory characteristics helped us with the analysis of the effect of the changes in the formulation and lead us closer to obtaining a product with high acceptability. However, we cannot ascertain the weight each attribute carries in the acceptability of the product and thus easily select those more important. A graphical solution of the optimisation process is given by RSM, where multiple attributes can be measured (Gacula, M.C., 1993). Our RSM approach was based on I-Optimal mixture design where the design space was built for minimising the average prediction variance and the levels of each variable were restricted and dependent on each other. It is not surprising that the only models with significant lack of fit were the ones corresponding to the hedonic test: overall acceptability and liking of flavour (Table 3). The loss of accuracy in the hedonic tests (as explained before) was reflected in the models, where some data were not well predicted by them. Notwithstanding this lack of fit, the prediction values for acceptability fell within the 95% confidence interval of the results obtained in the verification study. It is noteworthy the synergistic effects found in three of the models. Yeast extract proved to increase the liking of flavour and tenderness intensity of the hams when in combination with salt or glycine. The umami taste compounds from yeast extract might have influenced the flavour perception of the hams creating this synergistic effect with salt. Myrdal Miller et al. (2014) observed a similar effect of umami-rich mushrooms on reduced-salt cooked meats. Glycine is known for its role in the Maillard reactions and its relation to sweet and pleasant taste (Wong et al., 2008). In our study, when combined with yeast extract it increased the liking of flavour at higher levels than when each of them was used alone. With respect to changes in the textural properties, although salt was the main factor contributing to an adequate textural perception, glycine also played an important role of increasing hardness and chewiness of the hams. Salt impacts texture by affecting the water holding capacity of the meat products and also improving the cohesiveness of meat batters (Ruusunen & Puolanne, 2005). When in combination with other ingredients the interpretation presents an extra challenge. Glycine might interact with water and salt creating physically bonded ion-pairs affecting the role of water and both chloride and sodium ions in the textural properties of the ham.

The validation study allowed us to test if the optimisation based on RSM worked. The prediction from the RSM overestimated the acceptability values for the three samples, being bigger for the control. Even though these discrepancies existed, the differences were not statistically significant as we pointed out earlier. The age and sex of the panellists did not have an impact on the perceived acceptability, meaning the likeability of the hams was not dependent on these factors, and thus there was not a specific profile for liking these widely consumed products. The consumer panel confirmed that a 20% salt reduction on a cooked ham product would be as accepted as the full-salt counterpart when utilising certain amount of flavourings—yeast extract and/or glycine—in the mixture.

5. Conclusions

Salt reduction in cooked ham products can be achieved with the use of a mixture of yeast extract and/or glycine without impacting the acceptability of the product. The results show it is possible to produce reduced-salt hams without the need of using high quantities of additives and/or replacers to maintain the quality. The addition of these flavourings in percentages below 0.4% was sufficient to obtain a product with similar quality to the full-salt version. The use of a small scale hedonic test, a RDA and instrumental analysis all later analysed by means of a MFA, was an adequate alternative to ascertain the relationships between the acceptability of a product and the sensory and physicochemical properties. Through the mixture design approach and the RSM we were able to optimise the reduced-salt ham according to our desired attributes and increase our understanding of the synergistic effect of yeast extract and glycine on key consumer sensory parameters such as liking of flavour, tenderness and juiciness. Future work on microbial safety, shelf life and other quality parameters will ensure the commercial viability of these salt-reduced products.

Acknowledgements

This work was supported by the Food Institutional Research Measure (FIRM) of the Irish Department of Food, Agriculture and the Marine as part of the project titled “PROSSLOW; Development of consumer accepted low salt and low fat Irish traditional processed meat (Ref: 11F 026)”.

References

Abdullah, A., & Cheng, T. C. (2001). Optimization of reduced calorie tropical mixed fruits jam. *Food Quality and Preference, 12*(1), 63-68

AMSA. (2012). Meat Color Measurement Guidelines. American Meat Science Association. *Illinois, USA*

Barat, J. M., & Toldrá, F. (2011). 13 - Reducing salt in processed meat products. In J. P. Kerry & J. F. Kerry (Eds.), *Processed Meats* (pp. 331-345): Woodhead Publishing.

Baugreet, S., Kerry, J. P., Allen, P., & Hamill, R. M. (2017). Optimisation of protein-fortified beef patties targeted to the needs of older adults: a mixture design approach. *Meat Science, 134*, 111-118

Campagnol, P. C. B., dos Santos, B. A., Wagner, R., Terra, N. N., & Pollonio, M. A. R. (2011). The effect of yeast extract addition on quality of fermented sausages at low NaCl content. *Meat Science, 87*(3), 290-298

Delgado-Pando, G., Allen, P., Kerry, J. P., O'Sullivan, M. G., & Hamill, R. M. (2018a). Interaction of salt content and processing conditions drives the quality response in streaky rashers. *LWT, 97*, 632-639

Delgado-Pando, G., Fischer, E., Allen, P., Kerry, J. P., O'Sullivan, M. G., & Hamill, R. M. (2018b). Salt content and minimum acceptable levels in whole-muscle cured meat products. *Meat Science, 139*, 179-186

Dermiki, M., Mounayar, R., Suwankanit, C., Scott, J., Kennedy, O. B., Mottram, D. S., Gosney, M. A., Blumenthal, H., & Methven, L. (2013). Maximising umami taste in meat using natural ingredients: effects on chemistry, sensory perception and hedonic liking in young and old consumers. *Journal of the Science of Food and Agriculture, 93*(13), 3312-3321

Desmond, E. (2006). Reducing salt: A challenge for the meat industry. *Meat Science, 74*(1), 188-196

Dooley, L., Threlfall, R. T., & Meullenet, J.-F. (2012). Optimization of blended wine quality through maximization of consumer liking. *Food Quality and Preference, 24*(1), 40-47

Eisinga, R., Heskes, T., Pelzer, B., & Te Grotenhuis, M. (2017). Exact p-values for pairwise comparison of Friedman rank sums, with application to comparing classifiers. *BMC Bioinformatics, 18*(1), 68

Fellendorf, S., Kerry, J. P., Hamill, R. M., & O'Sullivan, M. G. (2018). Impact on the physicochemical and sensory properties of salt reduced corned beef formulated with and without the use of salt replacers. *LWT, 92*, 584-592

Fellendorf, S., O'Sullivan, M. G., & Kerry, J. P. (2016a). Effect of using ingredient replacers on the physicochemical properties and sensory quality of low-salt and low-fat white puddings. [journal article]. *European Food Research and Technology, 242*(12), 2105-2118

Fellendorf, S., O'Sullivan, M. G., & Kerry, J. P. (2016b). Impact of ingredient replacers on the physicochemical properties and sensory quality of reduced salt and fat black puddings. *Meat Science, 113*, 17-25

Gacula, M. C. (1993). *Design and analysis of sensory optimisation*. Trumbull, USA: Food & Nutrition Press.

Gacula, M. C., & Singh, J. (1984). *Statistical methods in food and consumer research*. London: Academic Press.

Gou, P., Guerrero, L., Gelabert, J., & Arnau, J. (1996). Potassium chloride, potassium lactate and glycine as sodium chloride substitutes in fermented sausages and in dry-cured pork loin. *Meat Science, 42*(1), 37-48

Greiff, K., Mathiassen, J. R., Misimi, E., Hersleth, M., & Aursand, I. G. (2015). Gradual Reduction in Sodium Content in Cooked Ham, with Corresponding Change in Sensorial Properties Measured by Sensory Evaluation and a *Multimodal Machine Vision System*. *PLoS ONE, 10*(9), e0137805

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous Inference in General Parametric Models. *Biometrical Journal, 50*(3), 346-363

ISO. (1988). Sensory analysis. General guidance for the design of test rooms. Ref. no. Genève, Switzerland: International Organization for Standardization (ISO 8589:1988).

Kang, M. S., Kang, Y. R., Lee, Y., & Chang, Y. H. (2018). Effect of mixed culture inoculation on chemical and sensory properties of aronia (Aronia melanocarpa). *Lwt-Food Science and Technology, 98*, 418-423

Kassambara, A., & Mundt, F. (2017). factoextra: Extract and Visualize the Results of Multivariate Data Analyses *R package version 1.0.5.999. http://www.sthda.com/english/rpkgs/factoextra*.

Khetra, Y., Kanawjia, S. K., & Puri, R. (2016). Selection and optimization of salt replacer, flavour enhancer and bitter blocker for manufacturing low sodium Cheddar cheese using response surface methodology. *LWT - Food Science and Technology, 72*, 99-106

Kloss, L., Meyer, J. D., Graeve, L., & Vetter, W. (2015). Sodium intake and its reduction by food reformulation in the European Union — A review. *NFS Journal, 1*, 9-19

Kumar, S. B., Ravi, R., & Saraswathi, G. (2010). Optimization of Fruit Punch Using Mixture Design. *Journal of Food Science, 75*(1), S1-S7

Kuznetsova, A., Brockhoff, P., & Christensen, R. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software, 82*(13), 1-26

Lawless, H. T., & Heymann, H. (2010). *Sensory Evaluation of Food. Principles and Practices*. New York (USA): Springer.

Le, S., Josse, J., & Husson, F. (2008). FactoMineR: An R Package for Multivariate Analysis. *Journal of Statistical Software, 25*(1), 1-18

Lorenzo, J. M., Bermúdez, R., Domínguez, R., Guiotto, A., Franco, D., & Purriños, L. (2015a). Physicochemical and microbial changes during the manufacturing process of dry-cured lacón salted with potassium, calcium and magnesium chloride as a partial replacement for sodium chloride. *Food Control, 50*, 763-769

Lorenzo, J. M., Cittadini, A., Bermúdez, R., Munekata, P. E., & Domínguez, R. (2015b). Influence of partial replacement of NaCl with KCl, CaCl2 and MgCl2 on proteolysis, lipolysis and sensory properties during the manufacture of dry-cured lacón. *Food Control, 55*(Supplement C), 90-96

Mamede, M. E. D., & Benassi, M. D. (2016). Efficiency assessment of Flash Profiling and Ranking Descriptive Analysis: a comparative study with star fruit-powdered flavored drink. *Food Science and Technology, 36*(2), 195-203

Meilgaard, M., Civille, G. V., & Carr, B. T. (2007). *Sensory evaluation techniques*. Florida, USA: CRC Press.

Mendes, L. C., de Menezes, H. C., Aparecida, M., & da Silva, A. P. (2001). Optimization of the roasting of robusta coffee (C. canephora conillon) using acceptability tests and RSM. *Food Quality and Preference, 12*(2), 153-162

Myers, R. H., Montgomery, D. C., & Anderson-Cook, C. M. (2009). *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*. Hoboken, New Jersey: John Wiley & Sons, Inc.

Myrdal Miller, A., Mills, K., Wong, T., Drescher, G., Lee, S. M., Sirimuangmoon, C., Schaefer, S., Langstaff, S., Minor, B., & Guinard, J.-X. (2014). Flavor-Enhancing

Properties of Mushrooms in Meat-Based Dishes in Which Sodium Has Been Reduced and Meat Has Been Partially Substituted with Mushrooms. *Journal of Food Science, 79*(9), S1795-S1804

Pagés, J., & Husson, F. (2005). Multiple factor analysis with confidence ellipses: a methodology to study the relationships between sensory and instrumental data. *Journal of Chemometrics, 19*(3), 138-144

Pohlert, T. (2018). PMCMRplus: Calculate Pairwise Multiple Comparisons of Mean Rank Sums Extended *R package version 1.0.1. https://CRAN.R-project.org/package=PMCMRplus*.

Popper, R., & Kroll, D. (2005). Just-about-right scales in consumer research. *Chemo Sense, 7*(3), 3-6

R. (Core Team 2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Richter, V. B., de Almeida, T. C. A., Prudencio, S. H., & de Toledo Benassi, M. (2010). Proposing a ranking descriptive sensory method. *Food Quality and Preference, 21*(6), 611-620

Ruusunen, M., & Puolanne, E. (2005). Reducing sodium intake from meat products. *Meat Science, 70*(3), 531-541

Shepherd, R., Smith, K., & Farleigh, C. A. (1989). The relationship between intensity, hedonic and relative-to-ideal ratings. *Food Quality and Preference, 1*(2), 75-80

Stone, H., Bleibaum, R., & Thomas, H. (Eds.). (2012). *Sensory evaluation practices* (Vol. 4). USA: Elsevier Academic Press.

Trieu, K., Neal, B., Hawkes, C., Dunford, E., Campbell, N., Rodriguez-Fernandez, R., Legetic, B., McLaren, L., Barberio, A., & Webster, J. (2015). Salt Reduction

Initiatives around the World – A Systematic Review of Progress towards the Global Target. *PLOS ONE, 10*(7), e0130247

Valentin, D., Chollet, S., Lelièvre, M., & Abdi, H. (2012). Quick and dirty but still pretty good: a review of new descriptive methods in food science. *International Journal of Food Science & Technology, 47*(8), 1563-1578

van Trijp, H. C. M., Punter, P. H., Mickartz, F., & Kruithof, L. (2007). The quest for the ideal product: Comparing different methods and approaches. *Food Quality and Preference, 18*(5), 729-740

WHO. (2013). Sixty-sixth World Health Assembly Retrieved 23rd August 2017, from https://www.who.int/mediacentre/events/2013/wha66/journal/en/

Wong, K. H., Abdul Aziz, S., & Mohamed, S. (2008). Sensory aroma from Maillard reaction of individual and combinations of amino acids with glucose in acidic conditions. *International Journal of Food Science & Technology, 43*(9), 1512-1519

Zhao, J., Wang, T., Xie, J., Xiao, Q., Du, W., Wang, Y., Cheng, J., & Wang, S. (2019). Meat flavor generation from different composition patterns of initial Maillard stage intermediates formed in heated cysteine-xylose-glycine reaction systems. *Food Chemistry, 274*, 79-88

Figure Captions

Figure 1. Intensity attributes scores from the Ranking Descriptive Analysis for the different cooked hams (n=25). Superscript star denotes statistical difference with respect to control sample (H12). *p<0.05, **p<0.01, ***p<0.001

Figure 2. Multiple Factor Analysis (MFA) scores and loadings plot.

Figure 3. Multiple Factor Analysis partial point plot. Continuous line represents hedonic characteristics, short-dashed lines instrumental characteristics and long-dashed lines intensity characteristics.

Figure 4. Response surface of hedonic properties of cooked hams. a) Overall Acceptability, b) Flavour likeness.

Figure 5. Response surface of intensity properties of cooked hams. a) Tenderness, b) Juiciness, c) Saltiness.

Figure 6. Response surface of instrumental characteristics of cooked hams. a) b* (Yellowness), b) Hardness, c) Chewiness.

Figure 7. Notched boxplot of overall acceptability of optimised hams. The notch displays the confidence interval around the median. CO: control sample (1.63% salt); HO1: 1.3% salt, 0.33% yeast extract; HO2: 1.27% salt, 0.16% glycine and 0.20% yeast extract.

Table 1. Processing conditions according to mixture I-Optimal design

Formulation	NaCl (%)	Glycine (%)	Yeast Extract (%)
H1	0.82	0.70	0.11
H2	1.08	0.55	0.00
H3	0.82	0.51	0.30
H4	1.11	0.22	0.30
H5	1.02	0.41	0.20
H6	1.44	0.19	0.00
H7	0.92	0.70	0.11
H8	1.32	0.01	0.30
H9	1.23	0.27	0.14
H10	1.23	0.27	0.14
H11	1.23	0.27	0.14
H12	1.63	0.00	0.00

Table 2. Hedonic ratings (n=25), instrumental colour and texture characteristics of ham formulations

Sample	Flavour (%)	Overall Acceptability (%)	L*	a*	b*	Cured ratio	Hardness (N)	Chewiness (N)
H1	59.02bcd	61.34bc	66.70	9.71	9.44	2.01	204.78a	683.88ab
H2	55.08d	59.72bc	66.21	9.41	8.32	2.02	166.88b	708.46a
H3	57.46cd	59.78bc	67.40	9.06	9.33	1.97	159.33bc	694.21ab
H4	68.34abc	67.80ab	68.43	8.59	8.76	1.92	135.30bcde	532.74cd
H5	59.08bcd	62.60bc	66.90	9.62	8.93	2.04	136.20bcde	548.43bcd
H6	59.04bcd	59.80bc	65.34	8.97	7.79	1.97	145.05cbd	537.83cd
H7	57.40cd	56.24c	66.48	9.65	8.93	2.06	168.52b	661.90abc
H8	69.40ab	69.20ab	65.13	9.55	8.35	2.05	125.22cde	517.76d
H9[1]	71.61a	71.66a	65.67	9.06	8.62	2.01	118.89de	473.58d
H12	70.76a	69.00ab	66.28	8.55	8.00	1.95	109.64e	419.73d
SEM[2]	1.96	1.64	0.31	0.12	0.15	0.01	8.06	29.17

[1] Sample results H9 comprise average from H9, H10 and H11

[2] Standard error of the mean

For each attribute different lowercase letters denotes significant differences at P<0.05

Table 3. Analysis of variance and beta coefficients of pseudo components of mixture models for selected hedonic, intensity and instrumental characteristics.

	Overall Acceptability	Flavour	Tenderness	Juiciness	Saltiness	b*	Hardness	Chewiness
Model type	Linear	Quadratic	Quadratic	Quadratic	Linear	Linear	Linear	Linear
F-value	4.90	7.56	13.88	26.20	10.20	13.64	8.95	6.11
Model p-value	0.036	0.011	0.002	<0.001	0.005	0.002	0.007	0.021
Adj-R^2	0.42	0.71	0.82	0.87	0.63	0.70	0.59	0.48
P-value (lack of fit)	0.09	0.02	0.42	0.71	0.69	0.88	0.96	0.95
ß-coefficients:								
A-NaCl	69.65*	68.40**	81.01**	63.25**	60.40**	7.74**	114.61**	446.98*
B-Glycine	56.14*	47.33**	50.97**	48.51**	37.21**	9.00**	191.96**	724.24*
C-Yeast Ex.	75.63*	-58.37**	-58.85**	62.15**	48.63**	9.80**	106.69**	515.56*
AB		-	-	16.93*				
AC		215.13*	188.92**	-				
BC		209.25*	257.18**	-				

ns: p>0.05, *:p≤0.05, **:p≤0.01. Significance for the each of the linear ß-coefficients is the same and is based on the p-values of the linear mixture.

Highlights

- Cooked ham with up to 20% salt reduction was optimised with flavourings
- Yeast extract and glycine successfully acted as flavourings in cooked ham
- Response Surface Methodology was used for optimisation
- Multiple Factor Analysis correlated physicochemical and sensory properties
- No differences were found in a consumer acceptance test