EFFECTS OF CALCIUM, MAGNESIUM AND PH DURING SALT CURING OF COD (*Gadus morhua* L.)

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ABSTRACT

The quality of heavily salted cod was investigated as influenced by adjusted levels of calcium, magnesium and pH in the salt. The investigation was carried out as a multivariate study consisting of two separate 2^3 full factorial designs. The chemical composition, waterholding capacity, weight and protein yields, colour and firmness were used as quality indicators or responses to the salt mixtures tested. The pH of the salt was positively correlated with the muscle pH of the cured fillet. The pH of the muscle correlated negatively with the relative protein content, the lightness values and the sensory firmness of the cured muscle. The protein yield after the salt curing was also negatively correlated with the muscle pH. Calcium ions increased both the lightness and firmness and magnesium ions increased the lightness of the cured fillet.

INTRODUCTION

Heavily salted cod are traditional products from the North-Atlantic fisheries and are highly regarded as ripened fish products in many countries. Today, thoroughly washed split or filleted cod are usually pickle-salted or brine-cured for a short week and then salt ripened, i.e. kench cured in stacks, for at least 10 days. In brine curing, the fish is added to pre-formed brines of saturated sodium chloride while in pickle salting, fish and salt are alternate layered in tubs and since the salt extracts moisture from the fish, a brine is formed within a day. During the kench curing, the liquid which exudes from the fish, is allowed to drain away from the stack [1,2,3,4]. The salt-ripened products have a water content of 55-60 % (w/w) and a salt content of 20-25 % (w/w) [3,4]. The water content can be further reduced by drying and when it becomes less than 50 % (w/w), so called dried salt-cured cod (klipfish) is obtained. After rehydration for 24-48 hours, the fish is either consumed immediately, stored chilled or frozen and then finally used for preparation of different kinds of baccalao dishes.

Most consumers prefer a firm and white surface of the salted fish although in some countries an ivory surface is favourable. These sensory properties appear to be affected by the salt type used in the curing process. Solar salts, the most common salt used, often have calcium and magnesium ions as major impurities. The content of these cations in solar salts may vary from 0.1 to 1.5 % [5] and it is generally believed that they contribute to the whitening of the muscle surface and stiffening of the flesh texture [2,6]. The nature of the increased whiteness is not known, but may be due to increased protein precipitation or interaction between the divalent cations and the muscle surface. The texture of fish flesh is characterised by the fibrous proteins and their functional properties such as; waterholding capacity, gelation, cohesion-adhesion and elasticity. The relative contribution to the texture of the myofibrillar proteins and the connective

tissue depends on *antemortem* and *postmortem* factors and on processing conditions of the fish [7,8].

Both pH and ionic strength are important factors that influence waterholding properties of muscle proteins [9,10,11]. Due to seasonal variations in feed intake, spawning activities and migrations, the ultimate *post mortem* muscle pH of cod varies [12,13]. It is known that this pH is important for quality properties such as; texture [12,13], filet gaping [14,15], waterholding capacity in the fresh fish [16,17,18] and susceptibility to oxidation during salt curing [19]. In general, a low ultimate muscle pH in the fish gives an inferior texture/firmness, more gaping and lower waterholding capacity. A very low muscle pH post mortem has also been suggested to cause denaturation of proteins leading to the rather extreme whiteness sometimes observed in fresh fillets [20].

In spite of the large influx of salt into the cod muscle during the curing process weight is lost due to the extensive dehydration [21]. The exudate from the fish contains soluble muscle proteins and it has been recommended that this should be reduced as much as possible [22]. The physical and chemical nature of the muscle surface may affect the drainage of soluble protein during the process. It is not known if interaction effects between calcium, magnesium and pH during the salting process influence the sensory properties, weight and protein yields.

The aim of the present work was to investigate the effects of adjusted levels of calcium, magnesium and pH in the salt on the quality of the cured cod product. The chemical composition, waterholding capacity, weight and protein yields, colour and firmness were used as quality indicators of the salt ripened fillets.

4

MATERIALS AND METHODS

Fish

The Cod (*Gadus morhua* L) used in this study was caught in September and February by trawling in the coastal waters off Tromsø, Northern Norway. The fish with average gutted weight of 2-6 kilos were stored in ice for 4-5 days before processing to skin-on fillets (weight: 500-1600 g) to be used in the salt curing experiments. The variation in size of the fillets varied similar in the test groups.

Salt preparations

Mixtures of solid salts in batches of 40 kilos were prepared according to a multivariate experimental design. Sixteen different salt mixtures of pro analysis (p.a.) quality were tested in two separate 2^3 full factor designs. Experiment 1 had combinations no 1 to 9 while experiment 2 had combinations no 3 and 9 to 16 (Table 1). The design variables were calcium, magnesium and pH. Calcium and magnesium were added as solid $CaCl_2$ x 2 H_2O p.a. and $MgCl_2$ x 6 H_2O p.a. to solid NaCl p.a. and mixed thoroughly. The aimed pH levels of the salt mixtures were low level 4 and high level 8. Based on preliminary investigations salt mixtures producing brines with different pH values were obtained by either adding 0.48 to 2.38 mmol kg^{-1} citric acid ($C_6H_8O_7$ x H_2O p.a.) to lower the pH or 58.8 to 107.1 mmol kg^{-1} sodium bicarbonate ($NaHCO_3$ p.a.) to increase the pH. In the preliminary study, batches of 1 kilo of solid salt mixture were made. All chemicals used for salts preparation and chemical analyses were delivered from Merck, Darmstadt, Germany.

Salt curing of the fish

In the salt curing experiments, which were carried out at 4°C, 15-20 fillets were used with each of the 16 salt combinations. The fish were pickle-salted by stacking fillets and solid salt (weight ratio 1:1) in layers in plastic tubs of 40 litres. After pickling for 5 days, the fillets were removed from the brine, cleaned of used salt and kench curing by restacking with fresh salt mixtures. After 9 days of kench curing, the fillets were again restacked with fresh salt. The kench curing lasted totally for 23 days. The weight of the individual fillets was recorded for the fresh fillets and when the fillets were regarded salt ripened after 28 days of salting.

Colour measurements

The instrumental colour of the muscle surface was determined by using a Minolta Chromameter, CR-200 (Minolta Camera Co. Ltd., Osaka, Japan). The detector was placed at the dorsal and the ventral side of the central line in the muscle surface of the fillet (Figure 1) and the L* a* b* modus was recorded, obtaining a mean value and standard deviations. Prior to the measurements, excessive salt was carefully removed from the muscle surface. The colour was determined on 15-20 fillets from each combination.

Sensory evaluation

Sensory evaluation was performed in a cold store room with standard artificial lightning and absence of sunlight. The fillets were distributed on a white nonglossy plate of wood prior to evaluation. The sensory lightness and firmness of 5 blind-coded salt cured cod fillets from each salt combination were evaluated by four or five employees experienced in grading salt cured cod. A numeric scale from 1 to 10,

describing the intensity of lightness and firmness was used. The firmness of the fillet was determined by pressing a fingertip onto the muscle surface.

Texture measurements

The instrumental shear force value was determined by using a KGS Systems texture analyser (KGS Systems, Tromsø, Norway) with a one blade Kramer shear force cell. After the sensory analysis, a loin part (6 cm width x 20 cm length) of the skin and boneless fillets were manually excised with a knife and used in the texture measurements (Figure 1). The steel cutting blade (8.3 cm wide and 1 mm thick) of the Kramer cell had an inclined edge and a flat centre. A 100 kg measuring load cell (type U1, FNr B71949, Hottinger Baldwin Messtechnik, Darmstadt, West Germany) was used to cut each piece of fillet transversally 6 times. The texture analyser was run at a speed of 0.94 mm s^{-1}, and the shear force values are presented as maximum peak height, giving units of N or kgm s^{-2}. The shear force was determined on 6 fillets from each combination of salts.

Chemical analysis

Waterholding capacity (WHC), pH, water, protein, ash, Ca and Mg determinations were performed on pooled samples. Each of the pooled samples was prepared by coarsely homogenising the fillets including the loin part used in the texture measurements, in a tap water cooled Stephan mixer, UM12 (Hameln, West Germany) for 3 x 5 seconds. Samples for WHC and pH determinations were taken from this homogenate. The mince was further homogenised in a Dito Sama (K55) Food Processor (Abusson, France) for approximately 1 minute providing samples for determination of water, protein, ash, Ca

and Mg content. The samples were either analysed immediately (WHC and pH) or kept in sealed plastic bags and stored for 1 to 4 weeks at –80°C before analysis.

The WHC was measured on coarsely homogenised muscle tissue at 4-5°C by determining the weight of the liquid lost after a low speed centrifugation (i.e. 210 x g in 15 minutes) as described earlier [23]. The WHC was expressed as weight of water retained (g Kg^{-1}). Mean values were calculated from 4-6 replicates for each sample.

The pH of fresh muscle tissue was measured in a 1 : 1 mixture of muscle homogenate and 0.15 M KCl as described earlier [24], while the pH of cured muscle was recorded in a 1 : 5 weight ratio of muscle homogenate and distilled water. The pH of salt cured muscle was measured after 5 x dilution in distilled water to avoid interference from Na-ions. From each of the 16 different salt combinations, the solid salt samples dissolved in distilled water (4 %, w/w) were used to determine the pH. A PHM 80 Radiometer (Copenhagen, Denmark) with a glass electrode was used for both muscle and salt samples and 3–6 replicates of each pooled sample were made to obtain a mean value.

The water content in the muscle samples was determined by drying to constant weight at 105ºC [25], and the protein content of muscle samples was determined as Kjeldahl protein [26]. The ash content of muscle samples was determined by standard procedure [27]. There were used 3-6 replicates for each sample to determine the water, protein and ash content.

The concentration of Ca and Mg in the minced muscle samples was determined as described [28] using a Perkin Elmer 3110 atomic absorption spectrometer (Perkin Elmer Co. Ltd., Norwalk, CT, USA). The method was modified by including 1% (w/w) Lanthan-111-oxide (La_2O_3 p.a.) in the extract prior to the analysis as recommended for

samples with chemical interference [29]. Four or five extracts from each sample were made and average and standard deviation calculated from these values.

Experimental design and data analysis

Adjustments of the Ca, Mg and pH levels of the salt were assumed to have multivariate and additive effects on the cured cod products. To obtain estimates of the effects of the Ca, Mg and pH levels of the salt with sufficient precision from limited numbers of experiments, the two experiments of the present work were performed as 2^3 full factor designs. The design variables were calcium, magnesium and pH of the salt. The response-variables were: instrumental and sensory lightness (InsLight and SensLight), instrumental shear force (IShearF) and sensory firmness values (SensFirm), waterholding capacity (WHC), pH, Ca, Mg, water, protein and ash contents of the cod muscle.

The designed pH values of the salt and the data from the response variables analysed at the end of the curing process (day 28) (Table 1 and 2) were used as data-matrix in a principal component analysis (PCA). The PCA was made to visualise the multivariate pattern of the designed pH level in the salt and the response variables (Table 3 and Figure 5). Principal component analysis (PCA) and partial least squares (PLS) regression were performed on the mean values from each salt combination. All variables were means centred and scaled to unit variance prior to analysis, both in PCA and PLS. Multivariate regressions were performed using PLS algorithms [30]. The calibration samples were arranged in two matrices, one containing the independent and one the dependent variables (Table 4). Significant effects were found by calculation of the regression coefficients (Bw) when $p < 0.05$ (Tables 5, 6 and 7). Both multivariate principal component analysis and partial least square regression analysis were validated

using full-scale cross-validation [30]. UNSCRAMBLER (Version 7.5, Camo ASA, Oslo, Norway) was used as computer software program.

RESULTS AND DISCUSSION

Chemical composition

The chemical composition and sensory properties of cured fish muscle describe the final product quality. The chemical composition of the fresh and cured cod fillets is shown in Table 2. Cured cod fillets had a water content in the range of 546-563 g Kg^{-1}, an ash content in the range of 218-240 g Kg^{-1} and a waterholding capacity (WHC) in the range of 632- 828 g Kg^{-1}. These values are generally in accordance with levels reported earlier for heavily cured cod [1,2,3,4,21,31]. By multivariate analysis (PCA and PLS) of the data, water, ash and waterholding capacity values were found influenced by the adjusted Ca, Mg and pH levels of the salt. These effects are presented and discussed below.

Muscle pH, Ca and Mg levels

To obtain a visualisation of the raw data prior to the multivariate data analysis, three scatter plots were made. Two-dimensional plots with two y-axis, were made from the analytical results of the cured fillets at day 28 (Figures 2, 3 and 4). Adjustments of the salt gave different pH, Ca and Mg levels of the cured muscle. The pH of the salt was positively correlated with the muscle pH and negatively correlated with the Ca and Mg content of the cured muscle (Table 5). A positive correlation between the pH of the salt and the pH of the cured muscle could be expected since the pH of the salt combinations varied from 4.24 to 8.41 (Table 1). The buffering capacity of the fresh cod muscle, which had a pH of 6.56-6.65, was insufficient to prevent a salt driven change in muscle pH. The direction and to some degree the extent of the change in muscle pH was dependent on the pH of the salt. Salt curing induces conformational changes of the

11

proteins and normally a reduction in the muscle pH is observed [19,31,32,33]. At high concentrations, it is assumed that the salt ions will compete with the internal electrostatic bonds of the proteins, cause conformational changes and dehydrate the proteins [34]. The net result is reduction in the waterholding capacity and increase in the amount of acidic groups of the cured proteins leading to an acidic shift in both the isoelectric point and the muscle pH [32,35].

It is shown in Figures 2-4, that the muscle pH was clearly negatively correlated with the concentration of Ca and Mg in the muscle. This can be explained by the lower solubility of Ca^{2+} and Mg^{2+} ions at higher pH values, and by the increased precipitation caused by the CO_3^{2-} ions added to the salt to adjust the pH.

The systematic variation (50%) of the Ca, Mg and pH levels of the cured muscle explained 59% of the systematic variation of the rest of the muscle response variables (Table 6). The muscle pH level was significantly ($p<0.05$) and negatively correlated with the protein content, the lightness and the sensory firmness values of the cured muscle. Lowering of the muscle pH probably caused denaturation and precipitation of both the muscle proteins on the muscle surface and deeper into the flesh and increased the water loss from the muscle during the process.

As expected, the Ca content of the muscle showed a significant ($p<0.05$) and positive correlation with the sensory lightness and firmness values of the cured fillet. This may be due to the crosslinking effect from the Ca-ions and their ability to denature and precipitate the proteins. The Mg content of the cured fillets, showed a significant ($p<0.05$) and positive correlation only with the lightness values. Gordon and Barbut [36,37] have found that the divalent chloride salts gave similar texture of meat batters except for differences in hardness and cohesiveness.

Weight and protein yield

Lost protein ends up in the brine or in the used dry salt and represents both waste of valuable fish proteins and a possible environmental problem. It was therefore of interest to test if the adjusted levels of Ca, Mg and pH of the salt affected these parameters. It is shown in Table 7 that 76% of the systematic variations of the weight and protein yields, explained 44% of the systematic variations of the X-variables. The weight yields of cured fillets were positively correlated with the water content and the instrumental lightness value, but negatively correlated with the instrumental shear force value and the protein content of the cured fillets. When the weight yield was high (>72%), the cured fillets were characterised by a "watery" and soft texture and a relative low protein content. When the weight yield was low, the fillets were oppositely characterised. According to Hamm [31] the ultimate uptake of water in salt cured meat depends mainly on the NaCl concentration and the pH of the brine. In our experiments, the protein yield of cured fillets was negatively correlated with the muscle pH. At low muscle pH the drainage of proteins from the fillets might have been reduced both due to the aggregation and denaturation of the proteins themselves and to the formation of a firmer fillet surface.

The weight yields (WY) of the cured fillets varied in the range of 69-74g 100g^{-1} (w/w) (Table 2) estimated as weight percentage of the fresh fillet prior to curing. These values are most probably too high because it was very difficult to remove excess salt on the surface of the cured products. However, weight yields in the same range have been reported earlier for heavy cured cod fillets [21,30]. The observed weight loss of the fillets during salt curing is mainly due to dehydration and protein leaching from the muscle. Most of the weight loss occurs in the kench curing steps. During these steps the muscle undergoes salting out effects as described earlier [34].

The protein yields (PY) based on the protein content and the weight yield of the cured products varied from 91 to 100 g 100g^{-1} (w/w) (Table 2). These yields are slightly too high due to the overestimated weight of the cured product. In industrial production, the fillets in the lower parts of the kench curing stacks will be exposed to a heavy weight. The liquid and protein loss from the fillets may therefore be even higher.

Colour and texture

The pH of the salt was negatively correlated with the sensory lightness and sensory firmness the cured muscle (Table 5). The significant and negative correlation between the pH of the salt and the sensory lightness values of the cured muscle is related to the salting-out and denaturation of the proteins. Salting-out is often described as a loss of stable hydrophilic surface, causing the exposed hydrophobic areas of proteins to interact, aggregate and precipitate [34]. At the isoelectric point (IP) the muscle proteins have minimum solubility and the light scattering property of the muscle is at maximum [38,39]. Curing with acidic salts probably shifted the muscle pH closer to the IP of the proteins resulting in more denatured muscle proteins and a whiter appearance of the fillet surface. As expected from earlier reports [2], increased Ca and Mg levels of the muscle also increased the lightness values of the cured fillets (Table 6).

Lowering of the pH of the salt increased significantly the sensory firmness value and the Ca and Mg content of the cured muscle (Table 5). The sensory firmness measured in this study reflects the physical strength of the cured fillet. A large reduction in the water content was observed in the cured cod and it may be deduced that the measured increase in the sensory firmness was due to denaturation of the proteins and a reduction in hydration of the fibrous proteins. These changes have apparently been amplified by Ca and Mg -ions possibly by crosslinking polypeptide chains [32,33] and by

lowering of the muscle pH (Table 5) [35]. Precipitation of sarcoplasmic proteins has recently been reported to contribute substantially to the increase in firmness of salt-vinegar cured mackerel meat [40]. The ability of calcium and magnesium to increase the firmness of cured cod surfaces has been suggested earlier [2,4]. Specific anionic effects of the added citric acid may also have contributed to denaturation of the muscle proteins [32].

Principal component analysis of the data

The results from the principal components analysis (PCA) are presented as a biplot of scores and loadings (Figure 5) and as the residual variance (Table 3). It is shown in the biplot that the PCA model explained 59% of the total variation of all variables. The total residual variance (both calibrated and validated), of the PCA model declined with increasing number of principal components (Table 3). The loadings of the biplot show how the variables caused differences in the different cured products (Figure 5). The systematic variation (77%) in the pH level of the salt explained 47% of the systematic variation of the response variables.

Conclusion

In this multivariate study it has been shown that the pH of the salt was positively correlated with the final muscle pH of the cured fillets. The results show that the pH of the salt cured cod fillets is of major importance for the quality of such products. The pH was negatively correlated with the relative protein content, the lightness value and the sensory firmness of the surface of the product. The protein yield after salt curing was also negatively correlated with the muscle pH. The presence of calcium ions increased

both the lightness and firmness while magnesium ions increased the lightness of the cured fillet surface.

To get a salt cured product of white colour and firm texture with a minimal protein loss during the process, the calcium and magnesium contents of the salt should be high (800 and 400 mg Kg^{-1} respectively) and the pH of the salt should be low (<6).

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Figure legends:

Figure 1. Schematic illustration of a salt cured fillet indicating where the texture and the instrumental colour measurements were performed.

Figure 2. Scatter-plot of the pH, Ca and Mg content (mg Kg^{-1}) and instrumental shear force (Kg ms^{-2}) of the cured muscle.

Figure 3. Scatter-plot of the pH, Ca and Mg content (mg Kg^{-1}) and instrumental lightness (L*) of the cured muscle.

Figure 4. Scatter-plot of the pH, Ca and Mg content (mg Kg^{-1}) and sensory score of the cured muscle. Sensory score values were given on a numeric scale from 1 to 10 to describe the intensity of the parameter (lightness and firmness).

Figure 5. The principal component analysis (PCA) of the designed salt levels of Ca, Mg and pH and the cured muscle response variables (instrumental and sensory lightness, instrumental shear force, sensory firmness, water holding capacity, pH, Ca, Mg, water, protein and ash content). The data are presented as a bi-plot, with both scores and loadings on the same plot.
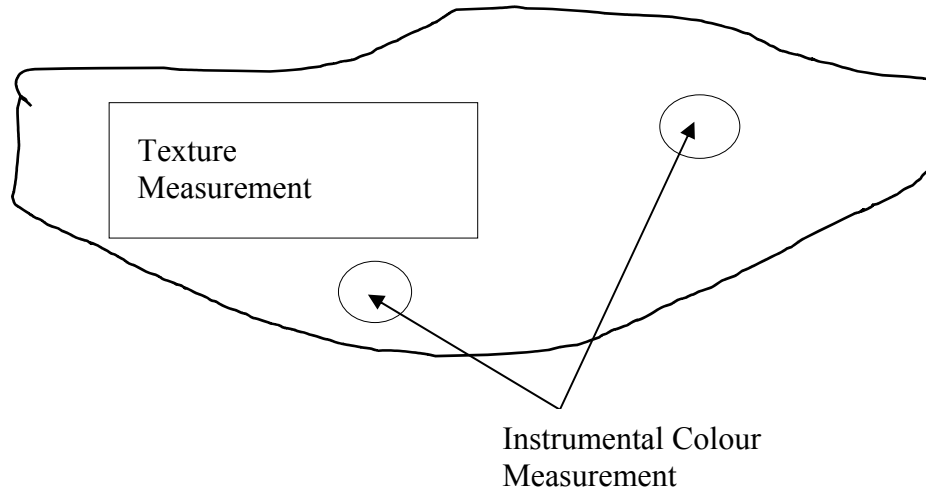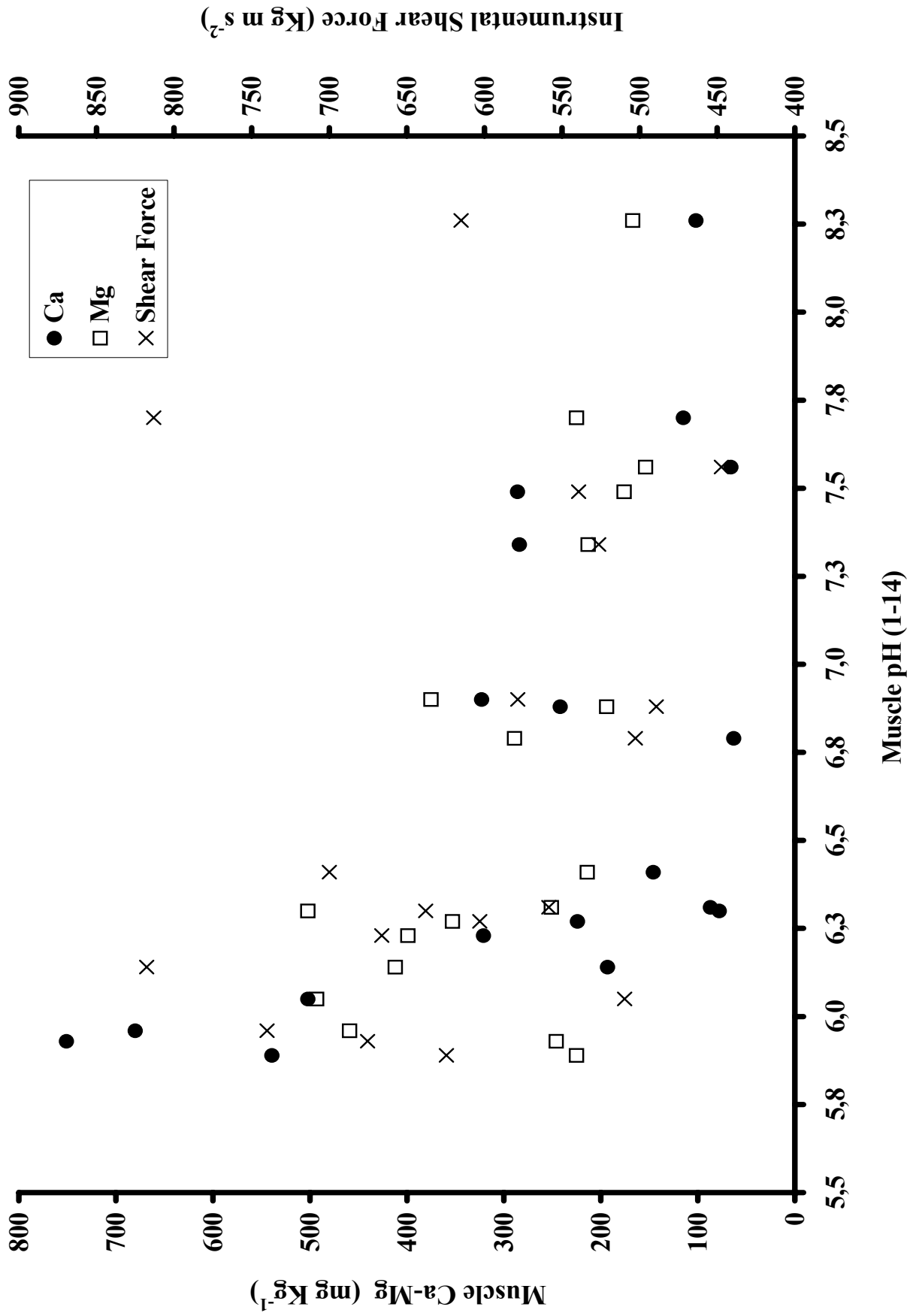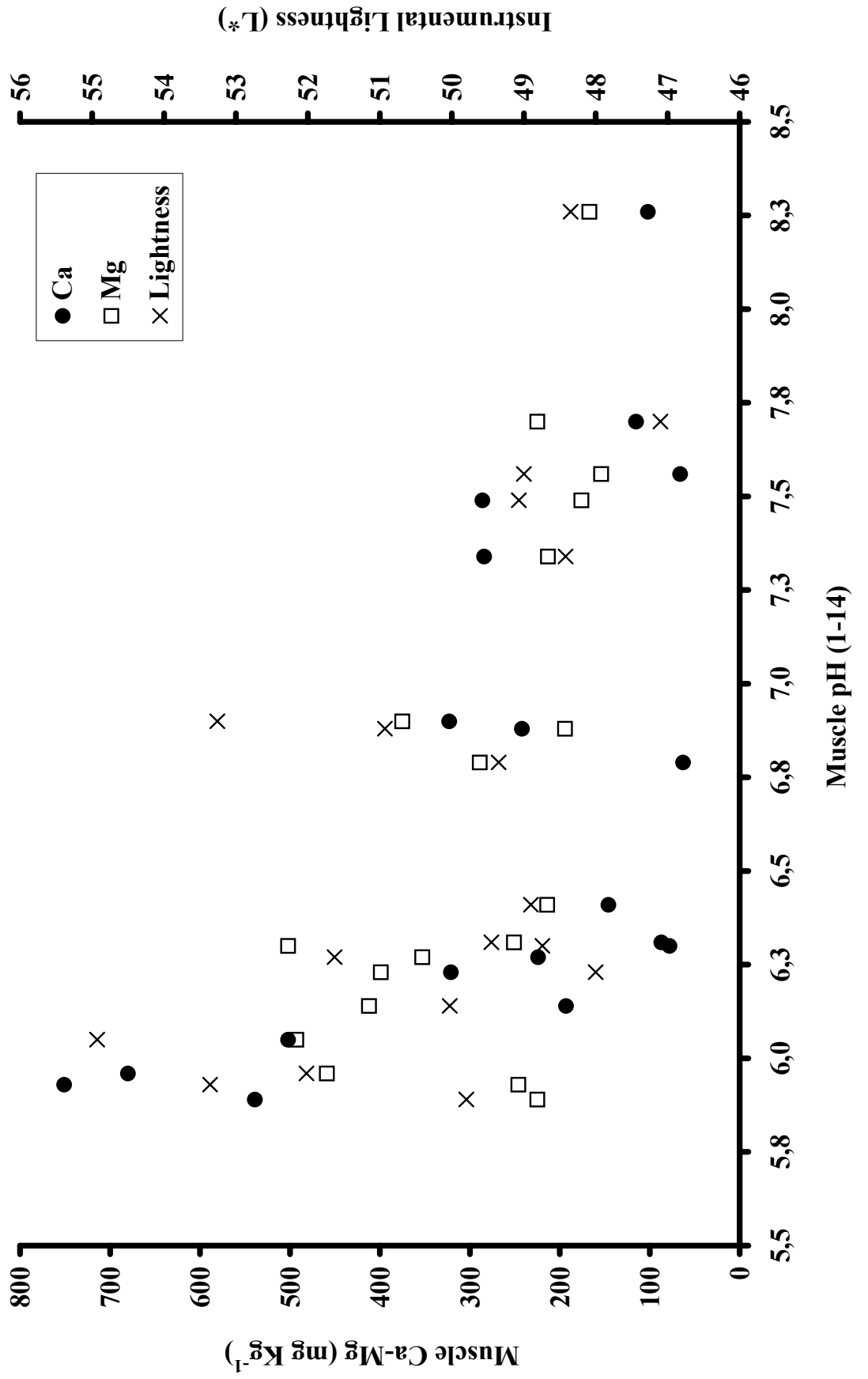
Figure 1

Texture
Measurement

Instrumental Colour
Measurement

Figure 2

Figure 3

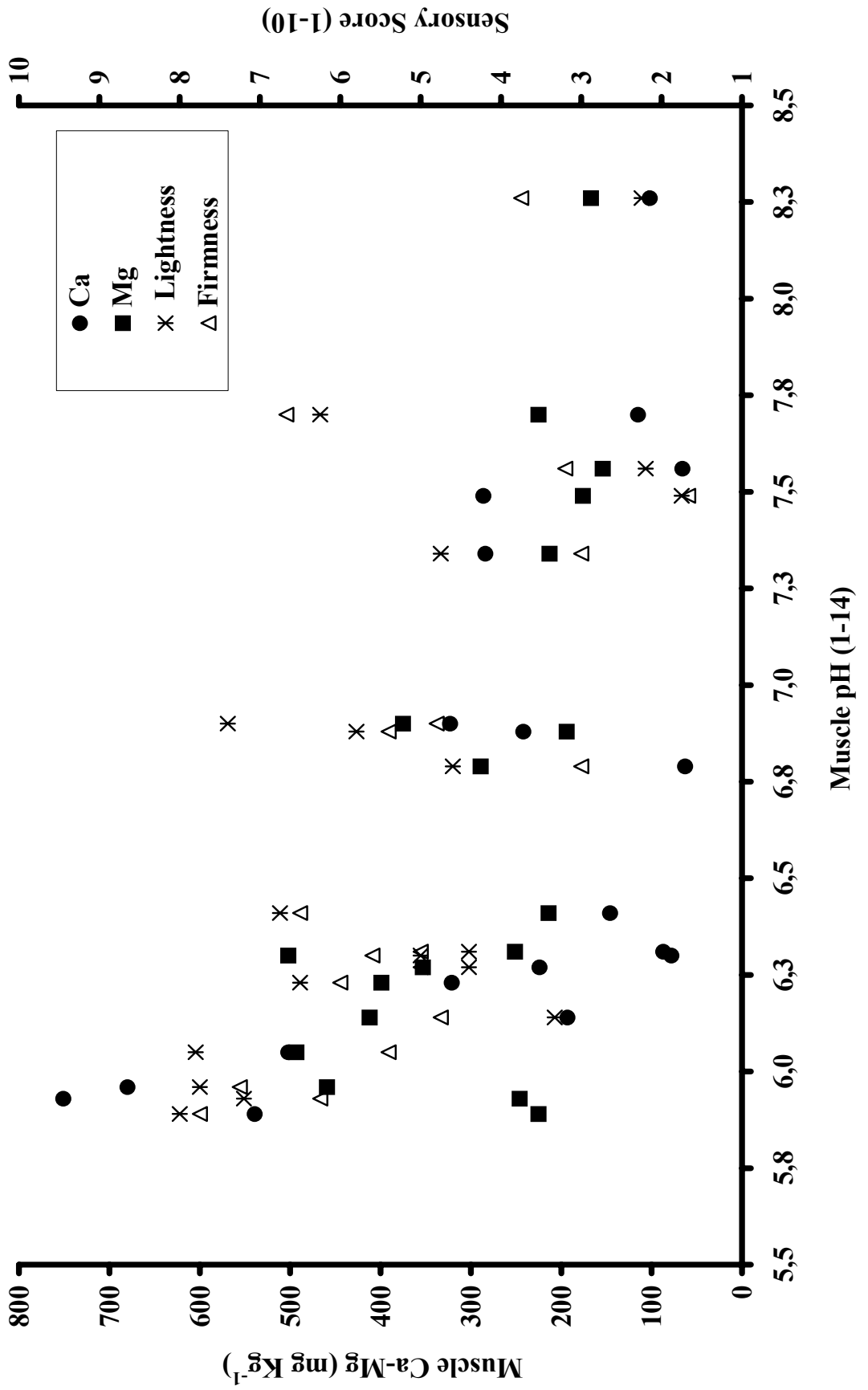**Figure 4**

Figure 5

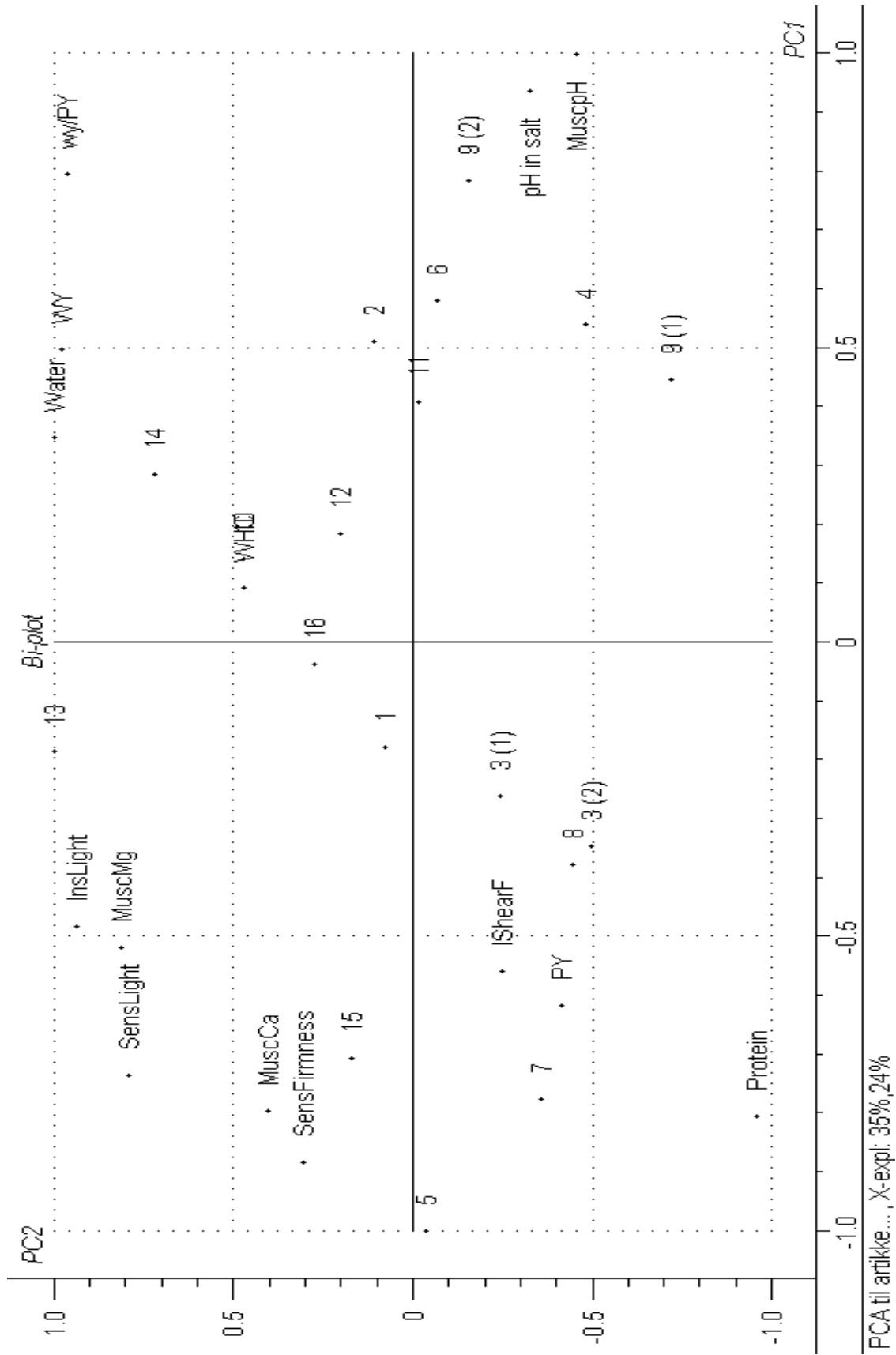

PCA til artikke..., X-expl: 35%,24%

Table 1.
Designed levels of Ca, Mg and pH in 16 different salt combinations. Mean values of muscle-Ca, muscle-Mg and muscle pH contents determined in the cured cod products after 28 days of salting, (w/w wet weight). Pooled muscle samples (n=5 fillets) from each salt combination were analyzed in 3-6 replicates.

Combination [a] no	Salt preparation			Salt cured fillets		
	Ca^{2+} in salt (mg Kg^{-1})	Mg^{2+} in salt (mg Kg^{-1})	PH in salt	Muscle-Ca (mg Kg^{-1})	Muscle-Mg (mg Kg^{-1})	Muscle-pH
1	2000	1000	6.55	321 ±35.0	329 ±4.0	6.23
2	100	2000	8.62	115 ±9.0	225 ±17.0	7.70
3 (1)	100	100	5.35	146 ±14.0	214 ±13.0	6.41
4	4000	100	8.25	286 ±16.0	176 ±15.0	7.49
5	4000	2000	4.24	680 ±57.0	459 ±11.0	5.96
6	4000	2000	7.99	284 ±30.0	213 ±9.0	7.34
7	4000	100	6.13	539 ±35.0	225 ±7.0	5.89
8	100	2000	6.48	193 ±30.0	412 ±10.0	6.14
9 (1)	100	100	7.96	102 ±15.0	167 ±14.0	8.26
10	4000	2000	5.86	224 ±16.0	353 ±8.0	6.27
11	100	4000	8.41	63 ±7.0	289 ±9.0	6.79
3 (2)	100	100	5.01	87 ±4.0	251 ±8.0	6.31
12	8000	100	7.81	242 ±21.0	194 ±7.0	6.88
13	8000	4000	4.28	502 ±58.0	493 ±13.0	6.05
14	8000	4000	7.88	323 ±32.0	375 ±8.0	6.90
15	8000	100	5.65	751 ±25.0	246 ±8.0	5.93
16	100	4000	5.20	78 ±11.0	502 ±16.0	6.30
9 (2)	100	100	7.76	66 ±13.0	154 ±6.0	7.56

[a] : (1) and (2) = experiment number 1 (combination no 1-9) and 2 (combination no 3, 9-16)

Table 2.

Effects of the 16 different salt combinations on cured muscle after 28 days of salting; weight yield (WY)/protein yield (PY), water holding capacity (WHC), water, ash and protein content. Raw material means fresh cod fillet prior to salt curing. Weight and protein yields were estimated from n=15-20 fillets in each combination. Pooled muscle samples (n = 5 fillets) from each salt combination were analyzed in 3-6 replicates regarding WHC, water, ash and protein contents.

Combination [a] No.	WY[b]/PY[c] (g 100g⁻¹)	WHC (g Kg⁻¹)	Water Content (g Kg⁻¹)	Ash Content (g Kg⁻¹)	Protein Content (g Kg⁻¹)
Raw material (1)	100/100	834 ±49.5	824 ±0.4	11±0.4	162 ±21.7
Raw material (2)	100/100	829 ±23.5	815 ±2.5	11 ±0.2	166 ±2.5
1	71/95	675 ±38.8	563 ±2.2	221 ±4.7	220 ±8.5
2	72/91	647 ±15.7	563 ±1.2	232 ±0.7	208 ±4.4
3(1)	70/94	632 ±5.5	553 ±1.3	232 ±1.7	222 ±5.4
4	70/94	696 ±13.4	552 ±1.4	230 ±1.2	220 ±2.1
5	69/96	661 ±2.6	546 ±1.4	225 ±1.8	228 ±4.0
6	70/91	669 ±7.5	557 ±0.3	231 ±1.5	212 ±3.1
7	69/97	670 ±6.0	549 ±2.5	227 ±1.1	232 ±1.5
8	70/97	666 ±11.7	549 ±4.1	230 ±1.4	228 ±11.0
9(1)	70/95	672 ±6.1	548 ±4.0	235 ±3.0	223 ±4.0
10	74/95	785 ±4.8	558 ±0.4	228 ±0.4	211 ±5.5
11	74/99	774 ±22.4	554 ±0.7	240 ±0.5	218 ±12.5
3(2)	70/99	799 ±8.7	547 ±0.8	223 ±1.9	232 ±3.3
12	73/98	793 ±16.5	566 ±0.7	222 ±3.9	220 ±3.9
13	72/92	746 ±22.6	562 ±4.5	234 ±2.1	208 ±1.9
14	74/94	725 ±9.9	563 ±1.2	230 ±1.4	208 ±1.9
15	73/100	828 ±35.0	550 ±1.3	218 ±0.4	226 ±4.1
16	72/95	756 ±13.2	560 ±1.3	221 ±2.4	216 ±3.0
9(2)	73/94	795 ±0.4	546 ±3.4	238 ±1.1	211 ±1.7

[a] (1) and (2) = experiment number 1 (combination no 1-9) and 2 (combination no 3, 9-16)
[b] WY = cured muscle weight x 100 / fresh muscle weight
[c] PY = PC/PF x WY
PF = protein content of fresh muscle
PC = protein content of cured muscle

Table 3.
The reduction in residual variance (calibrated and validated) of weighted data (1/Standard Deviation) with increasing number of principal components used in the principal component analysis (PCA) and the total explained validated variance (%) of the data-set. The PCA was performed on the designed pH levels of the salt and the data from the response variables.

Principal component no	Res Xcal[a] Tot(PCs)	Res Xval[b] Tot (PCs)
PC no 0	0.944	1.059
PC no 1	0.617	0.877
PC no 2	0.386	0.689
PC no 3	0.224	0.514
Total explained variance (%)	76.3	51.4

[a] Res Xcal = Residual variance calibrated
[b] Res Xval = Residual variance validated

Table 4.
Multivariate PLS models used to find significant effects from the designed pH level of the salt on the response variables of cured cod muscle and significant interactions in between the response variables.

X-matrix: All response variables	PC[a]	X-matrix: All response variables except of Muscle Ca, Muscle Mg and Muscle pH	PC[a]	X-matrix: All response variables except of WY[b], and PY[c]	PC[a]
Y-matrix: pH in salt(A)	2	Y-matrix:	2	Y-matrix:	2
		Muscle Ca (A)		WY (A)	
		Muscle Mg (B)	2	PY (B)	2
		Muscle pH (C)	2		
		A x B	2	A x B	2
		A x C	2		
		B x C	2		
		A x B x C	2		

[a] PC = number of principal components used in the PLS model
[b] WY = weight yield = cured muscle weight x 100 / fresh muscle weight
[c] PY = protein yield = PC/PF x WY
PF = protein content of fresh muscle
PC = protein content of cured muscle

Table 5.

Regression coefficients (Bw) of PLS analysis of the effects of the designed pH level of the salt (Y-Var+ Interactions) on the response variables of the cured muscle (X-Vars + Interactions).

	PC no 1	PC no 2
Y-variable:	pH in salt	pH in salt
X-variables:		
Muscle Mg	-0.154 *	-0.361 *
Muscle pH	0.204 *	0.337 *
Muscle Ca	-0.116 *	-0.018 *
Muscle Water	0.052	0.132
Instrumental shear force	-0.059	0.0432
WHC[a]	-0.024	-0.135
PY[b]	-0.057	0.092
WY[c]	0.069	0.133
Muscle Protein	-0.090	-0.018
Sensory Lightness	-0.121 *	-0.060 *
Instrumental Lightness	-0.121	-0.204
Sensory Firmness	-0.147 *	-0.155 *
	X-Vars, Y-vars + Interactions (PC1)	X-Vars, Y-vars + Interactions (PC2)
Total explained variance (%)	32, 63	15, 14

* = Significant effect at level p <0.05

[a] WHC = water holding capacity
[b] WY = weight yield = cured muscle weight x 100 / fresh muscle weight
[c] PY = protein yield = PC/PF x WY
PF = protein content of fresh muscle
PC = protein content of cured muscle

Table 6.

Regression coefficients (Bw) of PLS-analysis of the effects of the muscle Ca, Mg and pH levels (Y-vars + Interactions) on the rest of the response variables (X-Vars + Interactions).

Y-variables: X-variables:	PC no 1			PC no 2		
	Muscle pH	Muscle Ca	Muscle Mg	Muscle pH	Muscle Ca	Muscle Mg
Muscle Water	0.001	-0.092	-0.001	-0.006	0.034	0.079
Instrumental shear force	-0.107	0.097	0.052	-0.110	0.109	0.080
WHC[a]	-0.033	0.030	0.016	-0.034	0.033	0.023
PY[b]	-0.119	0.108	0.058	-0.111	0.070	-0.029
WY[c]	0.034	-0.030	-0.016	0.029	-0.009	0.032
Muscle Protein	-0.115 *	0.104	0.056	-0.105 *	0.061	-0.042
Sensory Lightness	-0.276 *	0.249 *	0.134 *	-0.283 *	0.281 *	0.208 *
Instrumental Lightness	-0.265 *	0.238	0.128 *	-0.280 *	0.306	0.283 *
Sensory Firmness	-0.235 *	0.212 *	0.114	-0.237 *	0.218 *	0.127
	X-Vars, Y-Vars + Interactions (PC1)			X-Vars, Y-vars + Interactions (PC2)		
Total explained variance (%)	27, 43			32, 7		

* : = Significant effect at level $p < 0.05$

a. WHC = water holding capacity

b WY = weight yield = cured muscle weight x 100 / fresh muscle weight

c PY = protein yield = PC/PF x WY

PF = protein content of fresh muscle

PC = protein content of cured muscle

Table 7.
Regression coefficients (Bw) of a PLS-analysis of the effect of the protein yield (PY)and weight yield (WY) (Y-Vars + Interactions) on the rest of the response variables (X-Vars + Interactions).

Y-variables:	PC no 1		PC no 2	
	WY[b]	PY[a]	WY[b]	PY[a]
X-variables:				
Muscle Mg	0.041	-0.035	0.035	-0.042
Muscle pH	0.131	-0.110 *	0.016	-0.239 *
Muscle Ca	-0.090	0.075	-0.072	0.095
Muscle Water	0.226 *	-0.189	0.231 *	-0.182
Instrumental shear force	-0.110 *	0.092	-0.217 *	-0.285
WHC[c]	0.055	-0.046	0.370	0.309
Muscle Protein	-0.348 *	0.291	-0.270 *	0.378
Sensory Lightness	-0.005	0.004	0.016	0.028
Instrumental Lightness	0.047	-0.039	0.189	0.121
Sensory Firmness	-0.104	0.086	-0.107	0.082
	X-Vars, Y-Vars + Interactions (PC1)		X-Vars, Y-Vars + Interactions (PC2)	
Total explained variance (%)	24, 56		20, 20	

*:   = Significant effect at level $p < 0.05$

[a] WY   = weight yield = cured muscle weight x 100 / fresh muscle weight

[b] PY   = protein yield = PC/PF x WY

PF   = protein content of fresh muscle

PC   = protein content of cured muscle

[c] WHC   = water holding capacity