Sodium reduction in processed cheese spreads and the effect on physicochemical properties

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ABSTRACT

The effect of a reduced amount of emulsifying salt on the chemical and textural properties of processed cheese was studied. Reducing the amount of emulsifying disodium phosphate salt added resulted in a slight reduction in the pH, lightness and viscosity of the processed cheese. When replacing disodium phosphate with potassium phosphate or potassium citrate alternatives, the processed cheese had slightly higher pH compared with the reference sample. When 15% of sodium in phosphate salt was replaced by the same molar amount of potassium in phosphate form or as citrate salts, the viscosity of the cheese was similar to the reference sample and most of the samples were evaluated as acceptable. However, when 30% of sodium in phosphate form was replaced by either potassium phosphate or citrate a slightly lower viscosity of the processed cheese was indicated and this could be due to the slightly higher pH.

1. Introduction

The intake of sodium in modern western diet is excessive, with potentially harmful effects on health. In industrial countries about 70–85% of dietary salt is obtained through processed food consumption (Kloss, Meyer, Graeve, & Vetter, 2015). Public health and regulatory authorities (FSA, 2004; WHO, 2006) have published advisory guidelines for reduction of salt intake down to 5 g day^{-1} or lower. Increased awareness of sodium content in food has led the food industry and food scientists to work to reduce the amount of sodium in processed food products. The amount of sodium present in processed cheese is usually higher (325–798 mg 50 g^{-1}) than present in natural cheese (95–697 mg 50 g^{-1}) due to addition of NaCl and emulsifying salts (Agarwal, McCoy, Graves, Gerard, & Clark, 2011; Johnson, Kapoor, McMahon, McCoy, & Narasimmon, 2009). Therefore, the processed cheese industry is looking for technological solutions for low salt processed cheesed formulations without changing product quality or sensory profile.

Processed cheese is produced by adding emulsifying salts (e.g., sodium phosphates, citrates) to natural cheese (Gouda, Cheddar). In combination with heating and shearing, the emulsifying salts break calcium phosphate bridges that crosslink *para*-casein molecules, resulting in soluble casein forming a network structure that is responsible for processed cheese viscosity. During heating, *para*-caseinate emulsifies oil and forms a stable oil-in-water emulsion, a process referred to as creaming. This process forms a homogenous product with an extended shelf life, but leads to relatively high levels of sodium compared with hard cheese because the addition of emulsifying (sodium) salts.

The major sources of sodium in processed cheese are emulsifying salts (44–48%), the cheese (28–37%) used for processed cheese production and added salt (15–24%) (Johnson et al., 2009). There are 13 types of emulsifying salts that can be used in processed cheese manufacture, either singly or in combination with each other (Lucey, Maurer-Rothmann, & Kaliappan, 2011); disodium phosphate is one of the main types. Trisodium citrate is also common choice of an emulsifying salt for processed cheese, but usually for slices or sliceable blocks, not spreads.

Options available to reduce the amount of sodium in processed cheese are reduction of sodium in the hard cheese (raw material) or in the sodium content of the emulsifying salt by changing the emulsifier salt system. The amount of emulsifying salt needed to produce processed cheese depends on the cheese raw material, the type of emulsifying salt used, the processing conditions and the type of processed cheese product.

Different emulsifying salts contribute differently to processed cheese quality, sensory and rheology. Lower meltability of cheese produced using phosphate salts compared with that produced with citrate salt was explained by the phosphate anion being a more functional Ca^{2+}chelant than the citrate anion in the development of the internal structure of the protein matrix (Gupta, Karahadian, & Lindsay, 1984). Potassium analogs of phosphate and citrate emulsifier salts yielded similar emulsifying properties, although the potassium salts seem to cause slightly less emulsification at equivalent concentrations. However, potassium salts at high levels impart a bitter taste to the product (Gupta et al., 1984). The amount and type of emulsifying salts affect the pH of the product (Lucey et al., 2011). An increased amount of emulsifying salt will increase the product pH, leading to a more open and looser processed cheese network, better water binding capacity and emulsification (Lu, Shirashoji, & Lucey, 2008; Mulsow, Jaros, & Rohm, 2007). However, the pH effect is also dependent on the type of emulsifying salts involved, as well as the other ingredients used for processed cheese production.

Processed cheese filled into tubes is an important product in Norway and other Scandinavian countries. The textural and rheological properties of this product are particularly important as the product has to be easy to squeeze out of the tube without being runny. When changing composition or reducing the amount of emulsifying salt, textural and rheological properties of the processed cheese can be changed. Such changes may be compensated for by changing the production process. Increasing creaming time leads to increased viscosity. However, prolonged processing time may lead to collapse of the protein-gel network structure (Lee, Buwalda, Euston, Foegeding, & McKenna, 2003). Increase in processing temperature leads to a significant reduction in viscosity of the cheese mass (Dimitreli & Thomareis, 2004) and, in the final product, usually results in reduced fat globule diameter, accompanied by an increase in firmness. However, the temperature effect may also depend on the fat and other components amount in the product.

The formulation of a balanced processing cheese mix will depend on the composition of the raw material cheese, emulsifying salts and their interaction. In Norway, processed cheese in tubes is based on Gouda-type cheese, which comprises 60% of the final product mass. This is high compared with the amounts usually used for similar products in Europe, where ingredients such as proteins, starch and polysaccharides are used to improve the texture.Therefore, to obtain the fine emulsified system using higher amount of hard cheese, it can be that more or different emulsified salt and optimal processing conditions should be applied compared with the studies discussed above. In the present work, effects on physiochemical and sensory properties of processed cheese when disodium phosphate is reduced or exchanged with dipotassium phosphate and trisodium and tripotassium citrates were investigated. Sodium reduction in combination with changes in process parameters on physiochemical and sensory properties of processed chese based was also investigated.

2. Material and methods

2.1. *Chemicals and emulsifying salts*

Sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), copper sulphate ($CuSO_4$), potassium sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$), sodium chloride (NaCl), potassium chloride (KCl) were from Merck, Darmstadt, Germany; potassium sorbate was from Brenntag, Jiangsu, China. Disodium phosphate dihydrate (Na_2HPO_4 $2H_2O$) was from Joha, Ladenburg, Germany, dipotassium phosphate (K_2HPO_4) and tripotassium citrate monohydrate ($K_3C_6H_5O_7$ H_2O) were from VWR, Leuven, Belgium; trisodium citrate dihydrate ($Na_3C_6H_5O_7$ $2H_2O$) was from Merck.

2.2. *Raw material and processed cheese composition*

Ingredients used for processed cheese preparation were Gouda-type natural cheese was used for processed cheese preparation with 26% fat, 27% protein and 1.2% NaCl as declared by the supplier, aged for 12 weeks, rework of processed cheese, potassium sorbate and water. In total, 15 different recipes were used in the study, as summarised in Table 1. The exact amounts of the ingredients cannot be given because of the commercial interests; the 100% concentration of emulsifying salt was 15.3 mmol 100 g^{-1}.

2.3. *Processed cheese preparation*

The processed cheese samples were prepared in a Stephan-Geiger homogeniser-grinder (UM 5, 1996) with the capacity for 2 kg of product and stirring speed 21 rpm and heated by both indirect and direct steam injection. The process was as follows: water, cheese cut into cubes (3 × 3 × 3 cm), emulsifying salt, rework and potassium sorbate were initially mixed at room temperature for 15 s. The blend was heated up to 60 °C using both direct and indirect steam while stirring constantly at 21 rpm. After the mass reached 60 °C, it was stirred for 1.5 min to get creaming reaction. The temperature was controlled using thermometer integrated with the Stephan-Geiger homogeniser-grinder. In the experiments with varying process conditions (experiment B, see below), the creaming time used was 1.5, 3 or 4.5 min, whereas creaming temperature was 45, 55 or 65 °C. After the creaming reaction, the temperature or processed cheese mass was raised to 95 °C and held for 6 min. The processed cheese mixture was vacuumed and hot filled into 175 g tubes (Tectubes, 40 mm × 174 mm). Within 10 min, all the manufactured and sealed samples were placed and stored at 4 °C until analysed.

2.4. Experimental design

The study involved two experiments, A and B. Experiment A comprised 20 samples produced from recipes 1–12 (Table 1); experiment B comprised 24 samples made from recipes 1–5, 9–10 and 13–15 (Table 1). Both experiments were run over two days. The amounts and composition of emulsifying salts used in the experiments, and the total calculated amount of sodium in the processed cheese are given in Tables 2 and 3 for experiment A and B, respectively.

In experiment A, Na content was reduced to 85% and 70% in three different ways: (i) without replacement, (ii) with partial or full replacement of disodium phosphate dihydrate (Na-P) with dipotassium phosphate (K-P) and (iii) with replacement with tripotassium citrate (K-C) and trisodium citrate (Na-C). The latter did not give Na reduction, but was included to compare effects of different citrates (sodium and potassium). Experiment A is summarised in Table 2.

The purpose of experiment B was to test if changes in process parameters could compensate for changes in rheological/textural properties caused by changing the amounts and composition of emulsifying salt. This experiment was conducted as a fractional factorial design with two levels of the factors: creaming time, creaming temperature, Na-P, K-P and K-C. A reference sample (no reduction of Na-P) and a centre point were added to the experiment. The levels of each of the parameters are given in Table 3. To check repeatability, two replicates were made of the centre point, the reference and three of the design points (Table 3).

2.5. Chemical composition analysis.

2.5.1. Dry matter

Dry matter in processed cheese sample was determined using HR73 Halogen Moisture Analyzer (Mettler Toledo, Germany). The results are expressed in g 100 g^{-1} dry matter. The pH of each sample was measured with pH meter (Mettler Toledo).

2.5.2. Sodium and potassium content

The sodium and potassium contents were determined using Dual Star™ pH/ISE meter (Thermo Fisher Scientific, Waltham, MA, USA) with a Na-selective electrode (Orion Ross® Sodium Ion Selective Electrode; Thermo Fisher Scientific) and K-selective electrode (Orion Ross® Potassium Ion Selective Electrode; Thermo Fisher Scientific).

Sample preparation was followed according to the method of Kivikari (1996) and its modification by Greiff et al. (2014). The extracts were prepared by homogenising 7.5 g of processed cheese in 250 mL plastic bottle with ultrapure water using an Ultraturrax T-25 (IKA, Labortechnik, Staufen, Germany) at 9000 rpm for 1 min, warmed to 90 °C for 30 min, cooled to room temperature and ultrapure water added to approximately 250 g weight in total; the total weight was noted. Samples were filtered through a cellulose filter paper (Whatman no. 1, Whatman International Ltd., Maidstone, UK). The extracts were analysed at room temperature using the Na- or K-selective electrode.

The direct calibration method was used for measuring. For sodium and potassium determination a calibration curve was done with four standards (1, 10, 100 and 1000 ppm) of analytical grade NaCl or KCl, respectively. Sodium ionic strength adjustor or potassium ionic strength adjustor (Thermo Fisher Scientific), respectively was added to all solutions to make sure that standards and samples had similar ionic strengths. Three parallel determinations were made for each sample.

2.6. Physical and rheological characteristics

2.6.1. Acceptance test (quality control)

When final product was obtained (after two weeks of storage at 4 °C), an acceptance test of the product was performed. In this test, textural properties of the processed cheese spreads, were evaluated by pressing out the cheese spread from the tubes in stripes on aluminium foil. After 5–10 min the expansion of the stripes were evaluated by visual inspection, and the cheese was classified as accepted (yes) if there was no appearant expansion, and non-accepted (no) if the stripe had expanded. In addition four samples were labelled "maybe", these were treated as not accepted in the analyses. The acceptance test was done by a trained operator. This acceptance test is also used as quality control in standard production.

2.6.2. Viscosity measurement

Viscosity of the processed cheese was determined using Brookfield DV-II+ viscometer with helipath stand (Brookfield engineering labs, USA). The viscosity was determined at three points under different determination conditions explained in Table 4. When viscosity was determined, 60 g of the sample was transferred in 100 mL beaker. For sample obtained after cooking (Vcp-hot), the viscosity was recorded for 4 min and only one determination was performed per sample. For viscosity determination of final processed cheese (Vcp-4C and Vcp-20C), the determination was performed for 2 min in duplicate and the viscosity value is given as an average of two determinations.

2.6.3. Objective measurement of expansion of processed cheese stripes

The aim of this method was to measure expansion of processed cheese stripes by computer vision. The application of the test set up was similar to the application in acceptance test (2.5.1). The test set up consisted of two USB 3.0 cameras (Point Grey Grasshopper3 GS3-U3-23S6C-C), a line laser (Z-LASER 450 nm) and a moveable platform actuated by a stepper motor (Fig. 1A). The platform moves the stripes past the laser line where the first camera measure the height profile of each stripe and then stops the platform in front of the second camera resulting in a time lapse image. The process is continuously repeated during the test. The line scanner creates a depth image consisting of 400 scan lines. These data can subsequently be converted to a 3D representation of the stripes (Fig. 1B). The expansion of each stripe is measured for each of the 400 scan lines and compared with the original expansion during the first scan. The expansion u for stripe i at time t is defined as:

$$u_i(t) = \frac{1}{400}\sum_{n=1}^{400}\frac{b_{in}(t)}{b_{in}(0)}$$

where $b_{in}(t)$ is the measured width of stripe i at scan line n at time t and $b_{in}(0)$ is the original width of the same stripe at the same scan line. The width b is measured from the scan line as shown in Fig. 1C. During the experiment, 20 stripes were scanned in groups of four for an entire hour. The expansion was evaluated measuring three different features: the final expansion after one hour of measurement (FinalExpansion), the expansion obtained after 5 min of measurement (Expansion-5min) and the time that takes the expanding stripe to reach 1-1/e of its final expansion (TimeConstant).

2.6.4. *Rheological measurements*

Rheological measurements were performed 1 month after production using StressTech rheometer (Reologicá Instruments AB, Sweden) at 20 °C and 5 °C with a 40 mm diameter stainless steel plate. The samples were spooned on the plate of the rheometer and the excess cheese was removed using a wooden spatula after the rheometer geometry was in place. The samples were equilibrated for 2 min before measurements commenced. Two type of tests were given: (i) oscillation strain sweep with the maximum loading force 4.158×10^4 Pa and (ii) deformation strain from 0.001 to 1 and constant frequency 1 Hz. The storage modulus, G′, the loss modulus, G″, the complex modulus, G* were recorded as well as the loss factor tan δmax. The results of this test were (all at 20 °C and 5 °C): (i) yield stress (YS-20 and YS-5, respectively); (ii) yield strain (YN-20 and YN-5, respectively); (iii) standard viscosity sweep (V50-20 and V50-5); (iv) shear rate (SR-20 and SR-5, respectively); (v) shear stress (SS-20 and SS-5, respectively).

2.6.5. *Colour evaluation*

Colour measurement was done using Minolta Chroma meter CR-400 (Konica-Minolta, Osaka, Japan). Data were stored in L*a*b* values. Parameter L* refers to the lightness of the samples, and ranges from black (L = 0) to white (L = 100). A negative value of parameter a* indicates green, while a positive one indicates red–purple colour. Positive value of parameter b* indicates yellow while negative value indicates blue colour. The Minolta CR-400 Chromameter D65 calibration plate was used for calibration. Measurements were replicated three times.

2.6.6. Sensory evaluation by projective mapping

To obtain more information on perceived sensory properties, projective mapping (PM) (Pages, 2003; Risvik, McEwan, Colwill, Rogers, & Lyon, 1994) was performed by a trained sensory panel of nine assessors at Nofima AS, Norway, on a set of nine batches from Experiment B. All samples were presented simultaneously to each panellist. The panellists were then requested to taste the samples, and then organise the samples on a web-based table cloth in such a way that two samples were placed near each other if they seemed identical, and distant from each other if they were different. Asssesors were also asked to describe samples or groups of samples with suitable attributes. For sensory evaluation, panellists were given approximately 11 g of cheese from each sample, the samples were served at room temperature in white plastic cups coded by random three-digit numbers. One of the samples were served in two replicates. The assessors had unsalted crackers and lukewarm water for rinsing the palate between samples. Each assessor evaluated all samples at individual speed on a computer system for direct recording of data, EyeQuestion Software (Logic8 BV, the Netherlands).

2.7. Statistical analysis

2.7.1. Multivariate analysis of textural properties

Explorative analysis of textural properties of the processed cheese where done by principal component analysis (PCA) on rheological, viscosity and the expansion measurements for all samples. As a tool for interpretation of the PCA models, experimental factors were included as supplementary variables to obtain the correlation loadings for these variables. No systematic difference between samples run on different days could be discovered in PCA plots for neither of the two experiments, and day effect was neglected for further analyses.

The textural properties comprises a number of highly correlated measurements (viscosity, rheology and objective expansion measurements), it is therefore natural to use the multivariate structure in the data for the hypothesis texting. For experiment A, the textural properties were tested by PC-ANOVA (Luciano & Næs, 2009), whereas in experiment B 5050-MANOVA (Langsrud, 2001, 2002; Langsrud, Jorgensen, Ofstad, & Naes, 2007) was applied. In PC-ANOVA, a PCA is first applied then the scores of the first components are analysed by ANOVA using the same models as for the set of univariate responses. 5050-MANOVA is a method for multivariate ANOVA in designed experiments with highly correlated responses. Rotation tests (Langsrud, 2005) were applied to control for multiple testing issues by controlling the False Discovery Rate (FDR), and to determine which of the responses were significantly affected by the experimental factors.

2.7.2. Hypothesis testing

For experiment A, significant effects of Na-reduction (part a) and Na-reduction with full replacement by K-P (part b) was tested by one way ANOVA on pH, dry matter and brightness/colour measurements and on PC1-PC3 of the textural responses. Part c was analysed as a full factorial experiment with factors Na-P (70, 85), Na-C (0, 15) and K-C (0, 15) (recipes 2, 3 and 7–12, Table 1), but due to large variation between replicates, no consistent results were found, and the analyses are not discussed further.

In experiment B, main effects of the factors creaming time, creaming temperature, Na-P, Na-K and Na-C were estimating using ANOVA for univariate responses (pH, dry matter and brightness/colour measurements) whereas 5050-MANOVA was employed for the textural quality, which is a multivariate response. In all cases only the fractional part of the design (Table 3) were included in the analyses. We report the false discovery rate (FDR) values from the rotation test, as well as the *p*-values from the univariate t-tests.

3. Results

To reduce sodium content in the processed cheese several approaches were tested. The approaches investigated in experiment A were: (i) reduction of emulsifying salt, (ii) replacement of Na-P with K-P and (iii) use of citrates. The main focus in experiment B was to see if process parameters (creaming time and temperature) could be adjusted to compensate for less sodium without replacement, with K-P or with K-C.

3.1. Dry matter

Dry matter content of the samples ranged from 38.2% to 40.2% and 37.8% to 39.7% in experiment A (Table 2) and B (Table 3), respectively. No significant differences ($p > 0.05$) within each experiment could be identified.

3.2. Effects on pH

Effects of emulsifying salt reduction on pH are summarised in Fig. 2 for both experiments, see also Tables 2 and 3. Fig. 2a shows a clear decrease in pH between 100% and 85% Na-P for experiment A, results for 70% Na-P are, however, not conclusive as the two replicates are very different. The overall test of difference is therefore not significant ($p = 0.31$). In experiment B, on the other hand, there was a significant ($p < 0.001$) increase in pH from 70% to 85% Na (Fig. 2b).

Fig. 3 shows results when Na-P is partially replaced by K-P. In experiment A, the reference (100% Na-P) had lower pH than 70% Na-P, 30% K-P. The average for 85% Na-P, 15% K-P was, however, higher than both reference (100% Na-P) and 70% Na-P, 30% K-P. The differences were only near significant (p = 0.08, Table 5).

In experiment B (Table 6), it was confirmed that K-P replacement can have a positive effect on pH, as pH was significantly higher in the sample with 15% K-P compared with the reference ($p < 0.001$, Fig. 3).

Due to large experimental error, it was difficult to make any conclusions regarding the effect of K-C in experiment A; in experiment B, K-C increased pH significantly ($p < 0.0001$; Table 6, Supplementary material Fig. S1).

In summary, the results indicate that reduction in Na-P decrease pH, but that other emulsifiers such as K-P and K-C can increase pH. The observed changes in pH for different emulsifiers are, however, small when compared with differences in average pH from experiment A (6.16) and B (5.94). Hence other factors such as raw material composition may influence the pH more than the sodium content.

3.3. Effects on textural properties

Effects on textural properties were studied using PCA of the rheology, viscosity and expansion measurements. The scores plots (Fig. 4) show that accepted and non-accepted samples (section 2.5.1) are located on the left and right side of the scores plots, respectively. One exception is the sample with 85% Na-P and 15% of both citrates from experiment A that is located far to the left in Fig. 4 top, this sample was described as “too thick”. All samples with one or both of the citrates are on the left side, indicating that adding citrates had a positive effect of improving the textural composition. It is clear from Fig. 4 that there is no clear border between accepted and products that are too thin, indicating that there are several options that may give acceptable quality in Na reduced products. There are, however, some variations between replicates.

Fig. 5 shows the correlation loading plot from experiment B. A similar correlation pattern between the variables was observed for both experiments, hence the plot for experiment A is not shown. The first component (explaining 54% of the variability) is related to viscosity (V50 and Vcp), the loss modulus (G) and yield stress (YS) on the left side, and expansion measurements from the objective imaging (Exp5, ExpF) together with shear rate (SR) on the right side. Hence this component is related to the fluidity of the samples, and shows that the non-accepted samples on the right side of Fig. 4 have too low viscosity and floats out too much during the expansion test. The variables contributing most to the second component (explaining 15%) are yield strain (YN), shear stress (SS) and as well as the variabes from the objective imaging (TC, Exp5, ExpF). These variables seem to be more related to between replicate variation than the design.

For most of the variables, measurements at 5 and 20 °C lie close together, hence processed cheese taken from the fridge and when kept in room temperature for a period have similar properties. The largest variation between the two temperatures are observed for YN and SS. PC-ANOVA (experiment A) and 5050-MANOVA (experiment B) were applied to test effects of salt reduction, replacers and process conditions. In experiment A, the first component which was related to textural properties, was significalty affected by level of Na-P, but not by K-P and K-C (Table 5). In experiment B, a model with main effects of all five factors (Na-P, K-P, K-C, creaming time and creaming temperature) was fitted. The *p*-values for the effects are summarized in Table 6, whereas Table 7 provides the FDR adjusted *p*-values for the different measurements. 5050-MANOVA indicated significant effect of all three emulsifying salts (Na-P, K-P and K-C), but unsure effect of creaming time (p =0.13) and no effect of creaming temperature ($p>0.7$). All the salts had a significant effect ($p < 0.003$). A second model without creaming temperature, and all two factor interactions indicated significant interactions between creaming time and K-C and between the emulsifiers ($p < 0.1$).

3.4. Effects on brightness and colour

Small differences in brightness (L) and colour measurements on the yellow-blue scale (b) could be detected when comparing samples with 100%, 85% and 70% Na-P without replacement. In experiment A , Na –reduction without replacement had a significant effect on b (Table 5), with lower values (less yellow, more green) for samples with more Na. In experiment B, the main-effect of K-P was significant (Table 6) with respect to L and a values (higher values when K-P added). Results may indicate that amount and type of emulsifing salt have an influence on the appeareance, giving less bright and less yellow products with Na-P reduction. The differences are, however, small compared with differences between the two experiments, hence further studies are needed to see if the changes will be detectable by consumers.

3.5. Projective mapping

PCA of textural measurements showed that a number of samples are similar according to the measured variables, but judged differently in the acceptance test (Fig. 4). Therefore a projective mapping (PM) was performed on nine selected samples from Experiment B to gain more insight to how the instrumental measurements are related to the perceived quality. The samples selected for PM was B1 (reference), B4 (centre point), B7 , B8, B9, B16, B19, B21 and B22 (Table 3). Sample B4 was replicated in the PM. Of these samples B8, B16, B21, B22, have 70% Na, whereas B7, B9 and B19 have 85% Na. Sample B22 (70% Na, 15% K-C) was clearly different also in the PM, and was commented on as thin by the panel, otherwise the panel detected small differences between the samples. The words used by the panellists indicated, however, that samples with 70% Na were grainy, whereas the reference and those with 85% were perceived as smooth and creamy.

4. Discussion

4.1. Amount and type of emulsifying salt

The processed cheese recipe used in this study contained 1.1% of Na in the wet weight. The Na ions sources in the processed cheese is the cheese used as raw material (0.40%) and emulsifying salts (0.71%). To reduce the Na content in the final product, only the emulsifying salt Na amount was reduced in this study. Therefore, in the sample where total Na content is reduced by 30%, the emulsifying salt Na amount was reduced by 48% compared with 100% sample to get 30% reduction of Na in the final product. To verify if the theoretical calculated Na content in the processed cheese was similar to the analytical values, Na content was determined in the processed cheese samples. The Na content given in the produced samples (see Table 2 and Table 3) were similar to the calculated ones.

Emulsifying salts usually are basic and therefore by increasing their amount in the processed cheese, the pH of the cheese increases (Table 2 and Table 3). The optimal pH value for the production the processed cheese is between 5.6 and 6.1. Higher pH value (pH > 6.1) causes that the cheeses are soft and may have microbiological problems, on the other hand lower pH value (pH < 5.4) leads to harder cheese (Lee & Klostermeyer, 2001). The slightly lower pH of the final product in the experiment B (Table 3) could be due to fact that cheese used for production was from another production batch and the buffer capacity and pH of the cheese could be different. When replacing sodium emulsifying salts with potassium emulsifying salts, slightly higher pH of the processed cheese was obtained (Fig. 3). Higher pH was also obtained in the processed cheese when replacing sodium with potassium equivalent salts in the El-Bakry, Duggan, O'Riordan, & O'Sullivan (2011) and Kapoor and Metzger (2008) studies. The solubility of potassium phosphate is higher compared with sodium phosphate and this could be the reason of slightly higher pH of the cheese containing potassium phosphate and citrate. Therefore, to replace sodium emulsifying salts by potassium emulsifying salts, the pH adjustment needs to be considered.

Increasing emulsifying salt amount led to slight increase in lightness (Table 2 and 3). In the Award, Abdel-Hamid, El-Shabrawy, & Singh (2004) study, an increased lightness was explained by more soluble proteins adding more emulsifying salt that results in a more shinny and less dark processed cheese.

Viscosity of the processed cheese was influenced by both the amount and type of the emulsifying salt used for processed cheese production (Table 7) to some extent. Lower viscosity was obtained in samples when viscosity was measured at 20 °C compared with 4 °C and this is in agreement with the observations of Dimitreli, Thomareis, & Smith (2005). Viscosity measurements at 20 °C (Vcp-20C), 4 °C (Vcp-4C) and after cooking (Vcp-hot) contributed all to the same description of the differences between the samples, and due to this, measurements of viscosity at one of this temperatures gives enough information about the differences between samples in further experiments.

The viscosity dependence on the emulsifying salt amount and type had the same tendency for the measurements at all three measuring temperatures. Adding less emulsifying salt, the processe cheese has lower viscosity (Table 7) as less calcium can be bound and casein solubilized. In the Guinee and O'Kennedy (2012) study the minimum amount of 0.75 g 100 g^{-1} emulsifying salt (disodium orthophosphate) was obtained to get the homogeneous processing cheese made from Cheddar type cheese. However, in this study a Gouda type cheese was used, also the ingredient such as sodium hydroxide, lactic acid was not added and most probably therefore more emulsifying salt had to be used to get homogeneous mass.

When 15% of sodium in phosphate form is replaced by the same amount of potassium in phosphate or citrate salts, the viscosity of the cheese was similar to the reference sample and most of the samples were evaluated as accepted (Fig. 4). Similar effect of citrate and phosphate on the softness of the processed cheese was also obtained in other studies (Caric, Gantar, & Kalab, 1985). However, when 30% of Na in phosphate form was replaced by either potassium phosphate or citrate a slightly lower viscosity (thinner) of the processed cheese was indicated. When adding potassium or citrates emulsifying salts a slight increase in pH (Fig. 3, Supplementary material Fig. S1.) was obtained. A slight increase in pH may lower protein-protein interaction and therefore slightly increased flowability and decreased the viscosity of the product. Moreover, a weaker binding of the water by potassium compared with sodium (Ohtaki & Radnai, 1993) also could lead to increased flowability of the cheese. Therefore replacing 30% of Na by replacing sodium emulsifying salts with potassium emulsifying salts or phosphate salts added citrate without adjustment of other processing parameters (e.g., creaming time) would lead to the product with lower viscosity.

4.2. Creaming time

Due to the theory of processed cheese (Lee et al., 2003), the casein strands are dispersed by action of mechanical shear and emulsifying salts during the initial phase of cooking, and calcium phosphate bridges are broken and enhances protein hydration. The swelling of protein units increase the dispersed phase volume as the proteins unfolded and spread out increasing protein-protein interaction. During creaming proteins re-associate to form a network structure which reflect in the observed viscosity increase (Lee et al., 2003). Therefore, creaming time is important to make a good emulsion and protein interaction. In the current study, the viscosity of the processed cheese increased with creaming time (Fig. 5, experiment B bottom). However, over-creaming can collapse the gel-like network and cause too compact structure, associated with product defects like grain, dry and brittle (Mulsow et al., 2007).

In the Hoffmann and Schrader (2015) study the viscosity of the spreadable cheese was decreasing with increased processing time (5 to 9 min) and this was attributed to the fact that high cutter speed caused a viscosity peak and a new network formation within the first 5 min of manufacture and prolong processing just break the network formed. Therefore optimal creaming time should be set depending on the composition of the processed cheese.

Yield stress and strain reflects to the force that is needed before processed cheese starts to flow. Shear stress and shear rate is a force that is needed be to applied to deform the sample. Shear stress and shear rate decreased with increasing creaming time, potassium citrate and phosphate levels. It could be that the hardness of the processed cheese was actually reduced by increasing the creaming time (more expanded protein-protein network structure), even that the product was less flowable. However increasing sodium content (emulsifying salt amount) in the processed cheese the shear rate was reduced while shear stress increased. Yield stress and yield strain increased with increasing the creaming time and emulsifying salt amount. This may indicate that increasing the creaming time and emulsifying salts, the formed protein-protein network structure was more stable and therefore it was needed more forces to get it to flow. Similar results were observed by Beykont and Kilic-Akyilmaz (2014) that cheese hardness increase with increased emulsifying salt concentration.

5. Conclusions

Reducing the amount of sodium phosphate emulsifying salt reduced the sodium content in the final product, but also resulted in lower pH and viscosity. Replacing 15% of sodium phosphate with potassium phosphate or citrate gave no changes in pH, viscosity and rheological properties in the final processed cheese. Addition of citrate (both sodium and potassium) improved the textural properties. This indicated that 15% of sodium can be reduced by replacing sodium salts by potassium salts. However, 30% replacement of sodium by potassium phosphate and citrate led to slightly lower pH and viscosity of the processed cheese. Prolonged creaming time (from 1.5 min to 4.5 min) slightly improved the viscosity of the processed cheese. However, creaming temperature (45–65 °C) was not important for viscosity of the final product, but this is also could be due to some difficulties to control it as it increases during creaming time.

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Table 1

Amount and composition of emulsifying salts used in the experiments and total calculated amount of sodium (Total [Na]). [a]

Recipe	Code	Amount of melting salt (mmol 100 g^{-1})				Total [Na] (mmol 100 g^{-1})
		Disodium phosphate	Dipotasium phosphate	Trisodium citrate	Tripotasium citrate	
1	100% Na-P	15.3				43.9
2	85% Na-P	11.7				36.7
3	70% Na-P	8.0				29.5
4	85% Na-P; 15% K-P	11.6	3.6			36.5
5	70% Na-P; 15% K-P	8.0	3.6			29.3
6	70% Na-P; 30% K-P	7.9	7.2			29.1
7	70% Na-P; 15% Na-C	8.0		2.4		36.6
8	85% Na-P; 15% Na-C	11.6		2.4		43.8
9	70% Na-P; 15% K-C	8.0			2.4	29.3
10	85% Na-P; 15% K-C	11.6			2.4	36.4
11	70% Na-P; 15% Na-C; 15% K-C	7.9		2.4	2.4	36.4
12	85% Na-P; 15% Na-C; 15% K-C	11.5		2.4	2.4	43.4
13	77.5% Na-P; 7.5 %K-P; 7.5% K-C	10.0	1.8		1.8	33.3
14	85% Na-P; 15%K-P; 15% K-C	11.7	3.5		2.4	36.6
15	70% Na-P; 15%K-P; 15% K-C	7.9	3.5		2.4	29.1

[a] Abbreviations are: Na-P, disodium phosphate dihydrate (Na_2HPO_4 $2H_2O$); K-P, dipotassium phosphate (K_2HPO_4); K-C, tripotassium citrate monohydrate ($K_3C_6H_5O_7$ H_2O); Na-C, trisodium citrate dihydrate ($Na_3C_6H_5O_7$ $2H_2O$).

Table 2

Overview of experiment A. [a]

Recipe	Sqc.	Short name	Acceptance test	pH	Dry matter (%)	$[Na]_T$ (%)	$[Na]_M$ (%)	Colour		
								L	a	b
1	Ref.	100% Na-P	yes ?	6.18	39.34	1.01	0.97	86.5	-3.2	16.6
1	A8	100% Na-P	yes	6.2	39.4	1.01	0.99	86.6	-3.1	16.7
1	A19	100% Na-P	yes	6.14	38.96	1.01	0.97	86.3	-3.2	15.8
2	A11	85% Na-P	no	6.08	39.3	0.84	0.82	86.4	-3.3	16.8
2	A9	85% Na-P	no	6.05	38.97	0.84	0.84	86.7	-3.3	16.8
2	A18	85% Na-P	no	6.01	38.71	0.84	0.80	86.2	-3.2	16.9
3	A1	70% Na-P	no	5.97	38.84	0.68	0.65	86.2	-3.3	17.8
3	A13	70% Na-P	no	6.17	39.08	0.68	0.65	85.1	-3.4	18.0
4	A2	85% Na-P, 15% K-P	no	6.25	38.93	0.85	0.78	86.7	-3.2	16.2
4	A15	85% Na-P, 15% K-P	yes	6.37	39.22	0.85	0.79	86.1	-3.4	17.2
4	A17	85% Na-P, 15% K-P	yes	6.3	39.38	0.85	0.79	86.5	-3.1	16.4
5	A7	70% Na-P, 15% K-P	no	6.03	38.21	0.68	0.63	87.5	-2.9	16.0
5	A12	70% Na-P, 15% K-P	no	6.21	38.84	0.67	0.66	86.5	-3.3	16.5
6	A16	70% Na-P, 30% K-P	no	6.26	38.32	0.68	0.62	86.4	-3.2	16.4
6	A3	70% Na-P, 30% K-P	no	6.29	39.19	0.68	0.65	86.3	-3.3	16.8
11	A6	70% Na-P, 15% Na-C, 15% K-C	yes	6.1	39.24	0.85	0.80	86.1	-3.3	17.2
9	A14	70% Na-P, 15% K-C	no	6.28	38.86	0.68	0.68	86.2	-3.2	17.0
8	A4	85% Na-P, 15% Na-C	yes	6.07	39.14	1.02	1.01	86.9	-3.0	16.5
12	A20	85% Na-P, 15% Na-C, 15% K-C	no	6.22	39.05	1.02	0.95	85.9	-3.3	17.1
10	A5	85% Na-P, 15% K-C	yes	6.11	40.21	0.85	0.83	86.8	-3.1	16.6
7	A10	70% Na-P, 15% Na-C	no	6.29	39.33	0.85	0.83	86.4	-3.3	17.0

[a] The amount and composition of emulsifying salt for each recipe is given in Table 1. The sequence (Sqc.) of the experiments follows the order: A1–A20. Acceptance test, pH, Dry matter, theoretical calculated and measured sodium amounts ($[Na]_T$ and $[Na]_M$, respectively) and colour were measured in the final products. Creaming time and creaming temperature were constant 1.5 min and 60 °C, respectively. Standard deviation for measured sodium amount $[Na]_M$ <0.02%, colours: L <0.3; a <0.2; b<0.2.

Table 3

Overview of experiment B. [a]

Recipe	Sqc.	Creaming		Short name	Acceptance test	Start temp (°C)	Max temp (°C)	pH	Dry matter (%)	$[Na]_T$ (%)	$[Na]_M$ (%)	Colour		
		Time (min)	Temp (°C)									L	a	b
1	B1	1.5	60	100% Na-P	no	55	75	5.95	39.1	1.01	1.00	86.39	-3.39	16.55
1	B13	1.5	60	100% Na-P	yes	55	78	5.91	38.8	1.01	1.01	86.02	-3.64	15.49
2	B10	1.5	60	85% Na-P	no	55	79.7	5.83	38.4	0.84	0.75	84.83	-3.88	16.57
2	B7	4.5	75	85% Na-P	yes	65	84.4	5.85	38.6	0.84	0.78	85.84	-3.54	16.35
3	B23	1.5	75	70% Na-P	no	65	84.4	5.85	39.0	0.68	0.65	84.55	-3.84	17.65
3	B3	1.5	75	70% Na-P	no	65	84	5.77	38.6	0.68	0.65	84.27	-4.12	17.72
3	B5	4.5	60	70% Na-P	no	55	84.8	5.82	39.1	0.68	0.67	85.66	-3.59	17.89
4	B19	4.5	60	85% Na-P; 15%K-P	yes	55	81.9	6.05	38.8	0.85	0.80	86.73	-3.21	16.38
4	B20	1.5	75	85% Na-P; 15%K-P	maybe	65	83	6.00	39.0	0.85	0.81	86.02	-3.40	16.63
5	B11	4.5	75	70% Na-P; 15% K-P	yes	65	83.9	5.89	39.4	0.68	0.67	86.07	-3.49	16.52
5	B12	1.5	60	70% Na-P; 15% K-P	no	55	78.7	5.90	38.2	0.68	0.67	85.13	-3.81	16.71
5	B16	1.5	60	70% Na-P; 15% K-P	maybe	55	68.9	5.93	37.8	0.68	0.66	86.25	-3.42	16.65
5	B21	4.5	75	70% Na-P; 15% K-P	yes	65	85.7	5.93	38.6	0.68	0.66	86.35	-3.42	16.75
9	B14	1.5	60	70 Na-P; 15% K-C	no	55	78.9	5.86	38.3	0.68	0.70	85.20	-3.81	16.33
9	B22	4.5	75	70% Na-P; 15% K-C	no	65	87.3	5.90	39.1	0.68	0.67	85.50	-3.87	16.01
10	B17	1.5	75	85% Na-P; 15% K-C	maybe	65	82.9	5.99	38.9	0.85	0.83	86.12	-3.39	16.91
10	B18	4.5	60	85% Na-P; 15% K-C	yes	55	79.7	5.98	38.8	0.85	0.82	86.45	-3.29	16.51
13	B15	3	67.5	77.5% Na-P; 7.5% K-P; 7.5% K-C	maybe	60	82.8	5.95	38.5	0.77	0.78	86.21	-3.42	16.83
13	B4	3	67.5	77.5% Na-P; 7.5% K-P; 7.5% K-C	yes	60	85	5.89	38.9	0.77	0.76	86.12	-3.38	17.21
14	B2	1.5	60	85% Na-P; 15% K-P; 15% K-C	yes	45	72	6.16	39.3	0.84	0.80	85.83	-3.50	17.96
14	B9	4.5	75	85% Na-P; 15% K-P; 15% K-C	yes	65	85.5	6.06	39.5	0.84	0.77	85.87	-3.63	15.77
15	B24	4.5	60	70% Na-P; 15% K-P; 15% K-C	yes	55	83.7	6.03	39.7	0.67	0.68	85.79	-3.60	15.62
15	B6	4.5	60	70% Na-P; 15% K-P; 15% K-C	yes	55	80.3	6.04	38.9	0.67	0.64	85.89	-3.49	16.97
15	B8	1.5	75	70% Na-P; 15% K-P; 15% K-C	yes	65	83	6.00	39.4	0.67	0.67	85.68	-3.44	17.12

[a] The amount and composition of emulsifying salt for each recipe is given in Table 1. The sequence (Sqc.) of the experiments follows the order: B1-B24. Acceptance test, pH, Dry matter, theoretical calculated and measured sodium amounts ($[Na]_T$ and $[Na]_M$, respectively) and colour were measured in the final products. Standard deviation for measured sodium amount $[Na]_M$ <0.02%, colours: L <0.3; a <0.2; b<0.2. Samples with bold labels indicate the fractional part of the design, remaining samples are references, centre points and replicates of single corners of the factorial design.

Table 4

Viscosity determination conditions using Brookfield DV-II+ viscometer.

Abbreviation: measuring point	Sample temperature	Spindle	Shear rate
Vcp-hot: viscosity determined after processed cheese was cooked	70±5 °C	Disc type - RV4	140 rpm. Because of very low viscosity 200 rpm was used in initial experiment for sample 1 and 13 and main experiment for samples 4, 5, 10 and 12.
Vcp-4C: determined on final processed cheese after 2 weeks of storage at 4 °C temperature and measured at 4 °C	4 °C	T – bar type. T-C	1.5 rpm. Because of high viscosity the following changes were made: in initial experiment for samples 6 and 20, 0.8 rpm and for sample 13, 0.6 rpm; in main experiment for samples 6 and 9, 0.2 rpm; for sample 2, 0.4 rpm and for samples 7 and 24, 0.7 rpm.
Vcp-20: final processed cheese after 2 weeks of storage at 4 °C and product then stored for 30 min at 20 °C before analysis	20 °C	T – bar type. T-C	6 rpm. Because of high viscosity the following changes were made: in initial experiment for samples 6 and 20, 2 rpm and for sample 13, 3 rpm; in main experiment for sample 2, 1 rpm, for sample 6, 0.9 rpm and for sample 9, 0.7 rpm. Because of very low viscosity the following changes were made: in main experiment for sample 16, 10 rpm and for sample 18, 4 rpm.

Table 5

Summary experiment A. [a]

Response	*p*-Value for overall difference between 100% Na. 85% Na. 70% Na No replacement (Experiment A. part a)	Explained variance for PCA (%)	*p*-Value for overall difference between (100.0). (85. 15) and (70.30) (Experiment A. part b)	Explained variance for PCA (%)
pH	0.311		0.085	
Dry matter	0.700		0.481	
Colour L	0.242		0.923	
Colour a	0.186		0.897	
Colour b	0.017		0.663	
PC1	0.025	38.8	0.155	44.4
PC2	0.063	27.0	0.792	22.8
PC3	0.823	12.7	0.799	14.9

[a] The first column represents *p*-values from one-way ANOVA including samples with full Na level and reduction without replacement. Textural properties were tested by ANOVA on the three first principal components from PCA on rheological, viscosity and expansion measurements. The PCA was conduced only for samples included in the test, and the explained variance is given in the second column. The third column gives *p*-values from one-way ANOVA on Na reduction with replacement, here also the PCA was done only for samples included in the test and the explained variance is given in the last column.

Table 6

Results experiment B. [a]

Response	Method	*p*-Values for main effects				
		Creaming time	Creaming temp	Na-P	K-P	K-C
pH	ANOVA	0.491	0.207	**0.0003**	**0.000003**	**0.0001**
Dry Matter	ANOVA	0.214	0.156	0.296	0.296	**0.036**
Colour L	ANOVA	0.074	0.571	0.190	**0.042**	0.777
Colour a	ANOVA	0.309	0.491	0.155	**0.043**	0.862
Colour b	ANOVA	0.258	0.485	0.405	0.983	0.629
Multivariate texture	5050-MANOVA	0.131	0.706	**0.003**	**0.005**	**0.012**

[a] ANOVA was used for each of the responses pH, Dry Matter, Colour L, Colour a and Colour, whereas 5050-MANOVA was applied for the set of variables describing texture (multivariate texture). For both univariate and multivariate analyses a model with main effects of creaming time, creaming temp, Na-P, K-P and K-C was applied. *p*-Values < 0.1 highlighted as bold font.

Table 7

FDR (false discovery rate) adjusted *p*-values for rotation test for each of the effects. [a]

Parameter	Creaming		Na-P	K-P	K-C
	Time	Temp			
Viscosity					
Vcp-hot H	0.529	0.774	**0.055**	**0.017**	**0.026**
Vcp-4C	0.494	0.935	0.129	**0.034**	**0.076**
Vcp-20C	0.494	0.939	0.252	**0.058**	**0.076**
Rheology					
V50-5	0.270	0.774	**0.004**	**0.026**	**0.026**
SS-5	0.270	0.945	0.610	0.293	0.319
SR-5	0.494	0.818	**0.071**	**0.040**	0.341
V50-20	0.257	0.935	**0.050**	**0.034**	**0.076**
SS-20	0.494	0.935	0.685	0.273	0.319
SR-20	0.494	0.774	0.208	0.229	0.341
G-5	0.556	0.889	**0.009**	**0.017**	**0.076**
YS-5	0.494	0.935	**0.004**	**0.071**	**0.076**
YN-5	0.525	0.818	0.129	0.992	0.248
G-20	0.257	0.774	**0.013**	**0.022**	**0.026**
YS-20	0.378	0.935	**0.075**	0.120	**0.076**
YN-20	0.410	0.774	**0.050**	0.101	**0.086**
Objective measurement of expansion of processed cheese stripes					
TimeConstant	0.525	0.774	0.610	0.157	0.248
FinalExpansion	0.494	0.818	0.303	**0.017**	0.108
Expansion-5min	0.659	0.854	0.288	**0.017**	0.215

[a] Results experiment B. Rheology results are: yield stress at 20 °C and 5 °C, YS-20 and YS-5, respectively; yield strain 20 °C and 5 °C, YN-20 and YN-5, respectively; standard viscosity sweep 20 °C and 5 °C, V50-20 and V50-5, respectively; shear rate at 20 °C and 5 °C, SR-20 and SR-5, respectively; shear stress at 20 °C and 5 °C, SS-20 and SS-5, respectively; storage modulus at 20 °C and 5 °C, G-20 and G5, respectively. Viscosity measurements: Vcp-hot, viscosity measured after processed cheese was cooked; Vcp-4C and Vcp-20, final processed cheese at 4 °C and 20 °C temperature. *p*-Values < 0.1 highlighted as bold font.

Figure legends

Fig. 1. Objective measurement of expansion of processed cheese stripes. A - Test set up consisted of two USB 3.0 cameras, a line laser and a moveable platform. B- 3D representation of the stripes. C - height profile measurements.

Fig. 2. Effects of Na reduction on pH in processed cheese from experiment A (left side) and experiment B (right side). Mean values of pH for different sodium phosphate-concentrations are given by circles, the error bars shown represent the mean plus/minus three standard errors of the mean (SEM), whereas the asterisks and crosses show individual measurements. For experiment B, the fractional part of the design is shown with asterisks, whereas the replicates and centre points are shown with crosses. In experiment A the overall test for difference is not significant ($p=0.31$), whereas in experiment B the main effect of Na-P is highly significant ($p<0.001$).

Fig. 3. Effects of Na-reduction and partial replacement with K-P on pH for experiment A (left side) and B (right side). Circle and error bars show the mean value for each level plus/minus 3 x SEM (standard error of the mean). Observations are shown as asterisks. In experiment A observations with (70,15) are shown as triangles, whereas in B observations that are not part of the factorial experiment are shown as crosses. The x-axis indicates level of K-P, the Na-level is 100 (K-P=0), 85 (K-P=15) and 70 (K-P=30), except for the triangles where Na-P is 70% (recipe 5, Table1). In experiment A the overall test is near significant ($p=0.08$), but no significantly different pairs after Tukey HSD post hoc test. In experiment B, the main effect of K-P is highly significant ($p<0.001$).

Fig. 4. Score plot, experiment A (top), experiment B (bottom). The symbols indicate different emulsifying systems as indicated by the legend, whereas the colour indicate if the sample was accepted or not in the acceptance test. Red = accepted, blue = not accepted. One sample (B23 with 70% Na and no replacement (see Table 3) was removed from the data before PCA. The reason for this was that the sample was extremely thin and non-homogenous. As a result, this sample dominated the second component completely. The results of PCA is therefore shown without this sample to highlight differences among the other samples.

Fig. 5. Correlation loading plot for experiment B. Experimental factors included as supplementary variables (blue text, italics). Due to space limits, the variable names are shortened: Rheology measurements: G =loss modulus, SS = Shear Stress, SR = Shear Rate, YS = Yield Stress, YN = Yield Strain, V50= Viscosity sweep. Vcp stands for viscosity. Vcp-H is viscosity measured after processed cheese was cooked (H=hot), The stars indicate measurements taken at 20 °C, the rest is taken at 5°C, with the exception of viscosity measurements which was taken at 4 °C. The correlation loadings obtained with experiment A was comparable to this and are not shown. The first component is most influenced by viscosity measurement and Yield Stress on the left side and expansion measurements and Shear Rate on the right side. Measurements taken at 5 and 20 °C are consistent for viscosity, Yield Stress and Shear Rate, whereas measurement on Yield Strain and Shear stress show differences between the temperatures.

Figures TC Edits

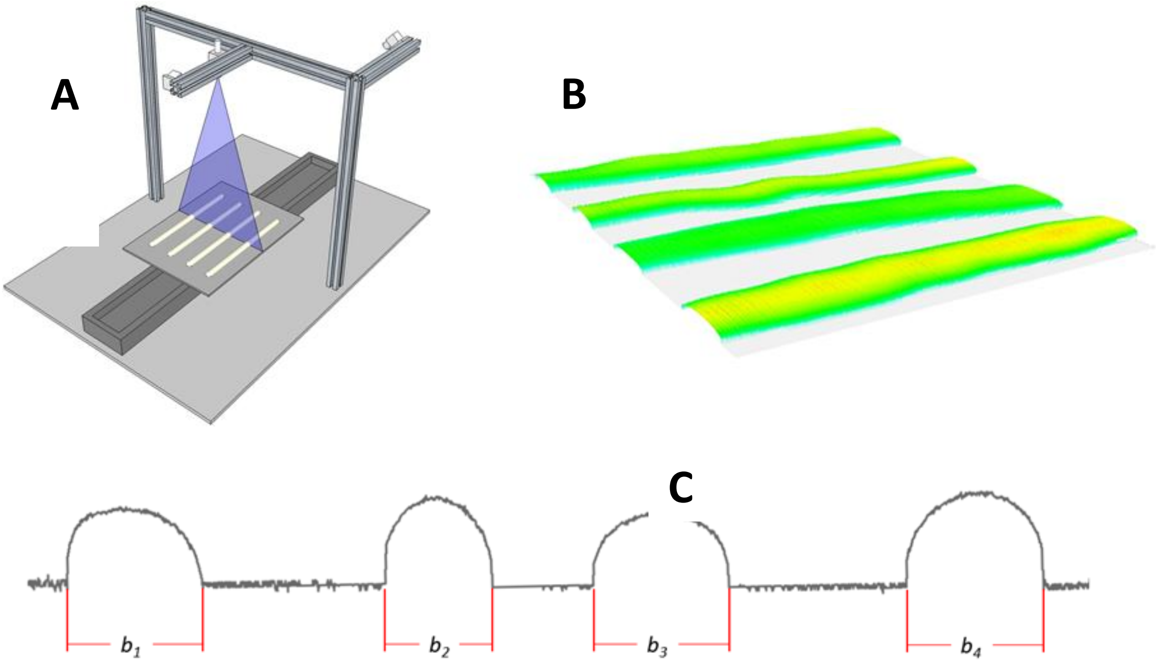
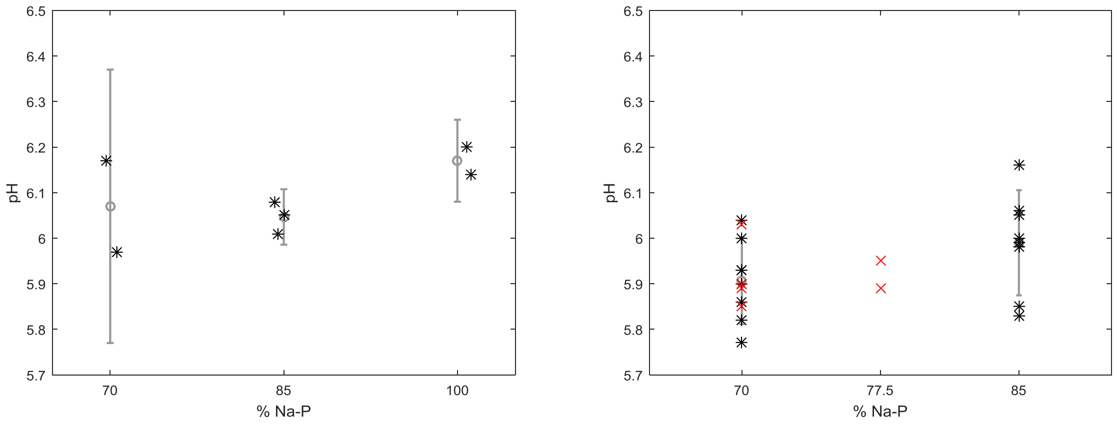


Figure 1



6.5
6.4
6.3
6.2
6.1
6
5.9
5.8
5.7
pH
70
85
100
% Na-P
6.5
6.4
6.3
6.2
6.1
6
5.9
5.8
5.7
pH
70
77.5
85
% Na-P

Figure 1.

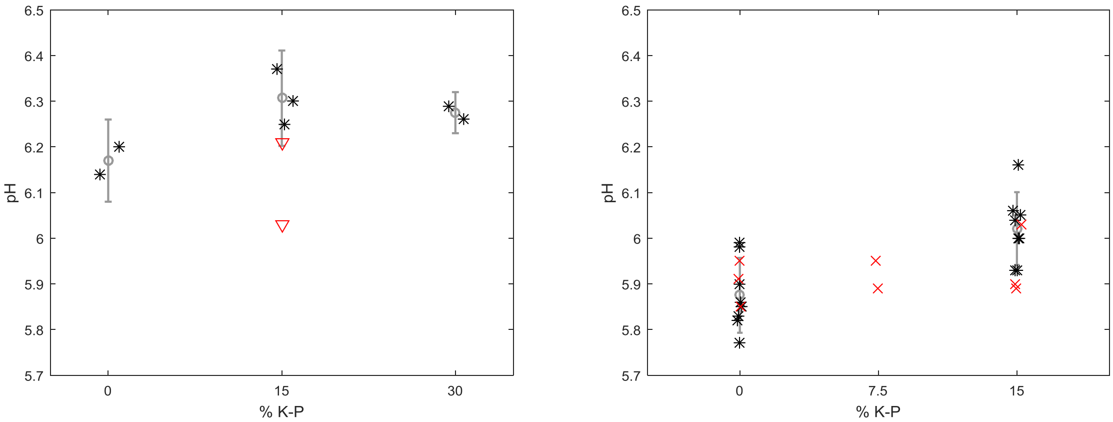

6.5
6.4
6.3
6.2
6.1
6
5.9
5.8
5.7
pH
0
15
30
% K-P
6.5
6.4
6.3
6.2
6.1
6
5.9
5.8
5.7
pH
0
7.5
15
% K-P

Figure 2

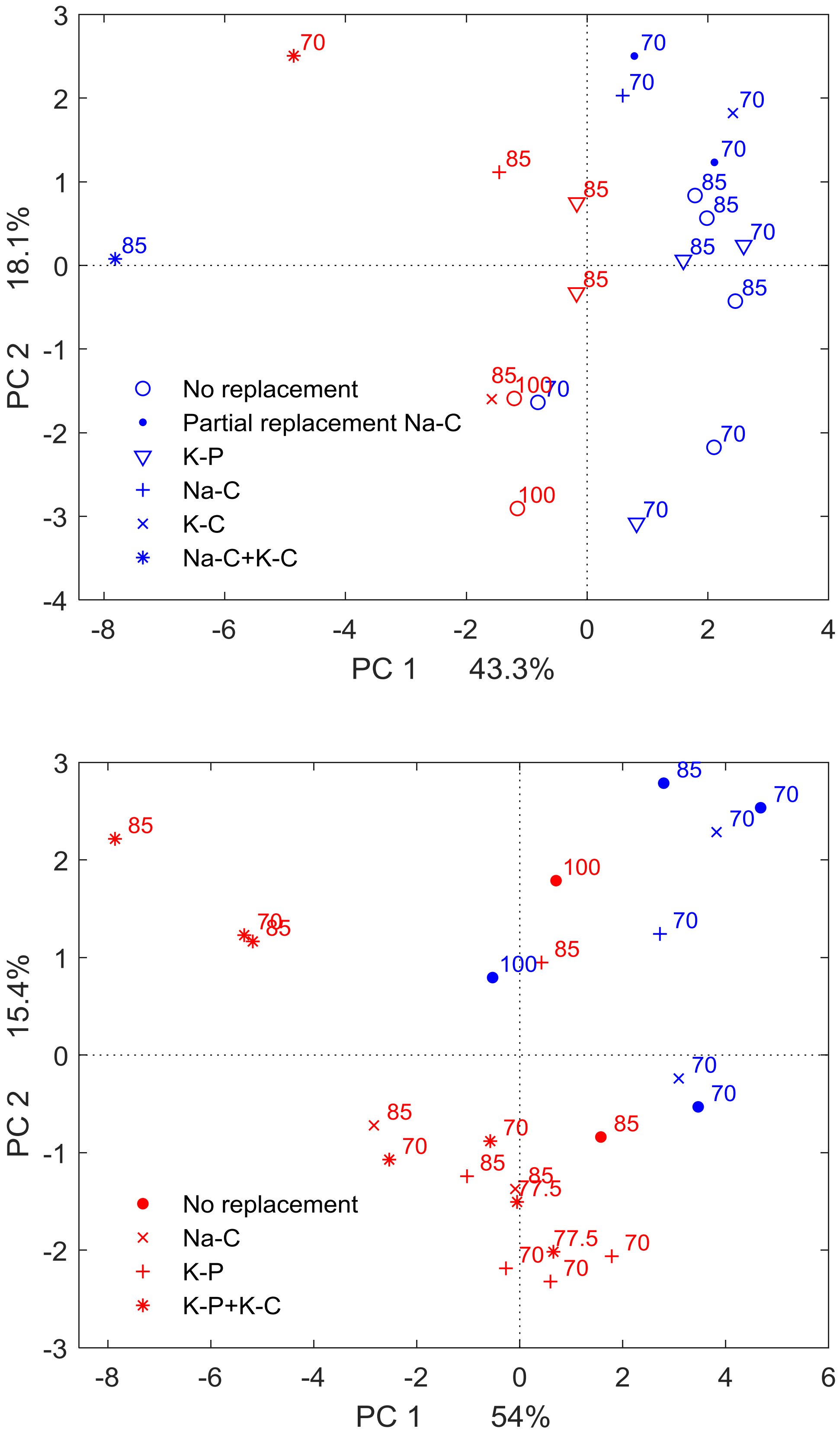

PC 1 43.3%
PC 2 18.1%
No replacement
Partial replacement Na-C
K-P
Na-C
K-C
Na-C+K-C
PC 1 54%
PC 2 15.4%
No replacement
Na-C
K-P
K-P+K-C

Figure 3

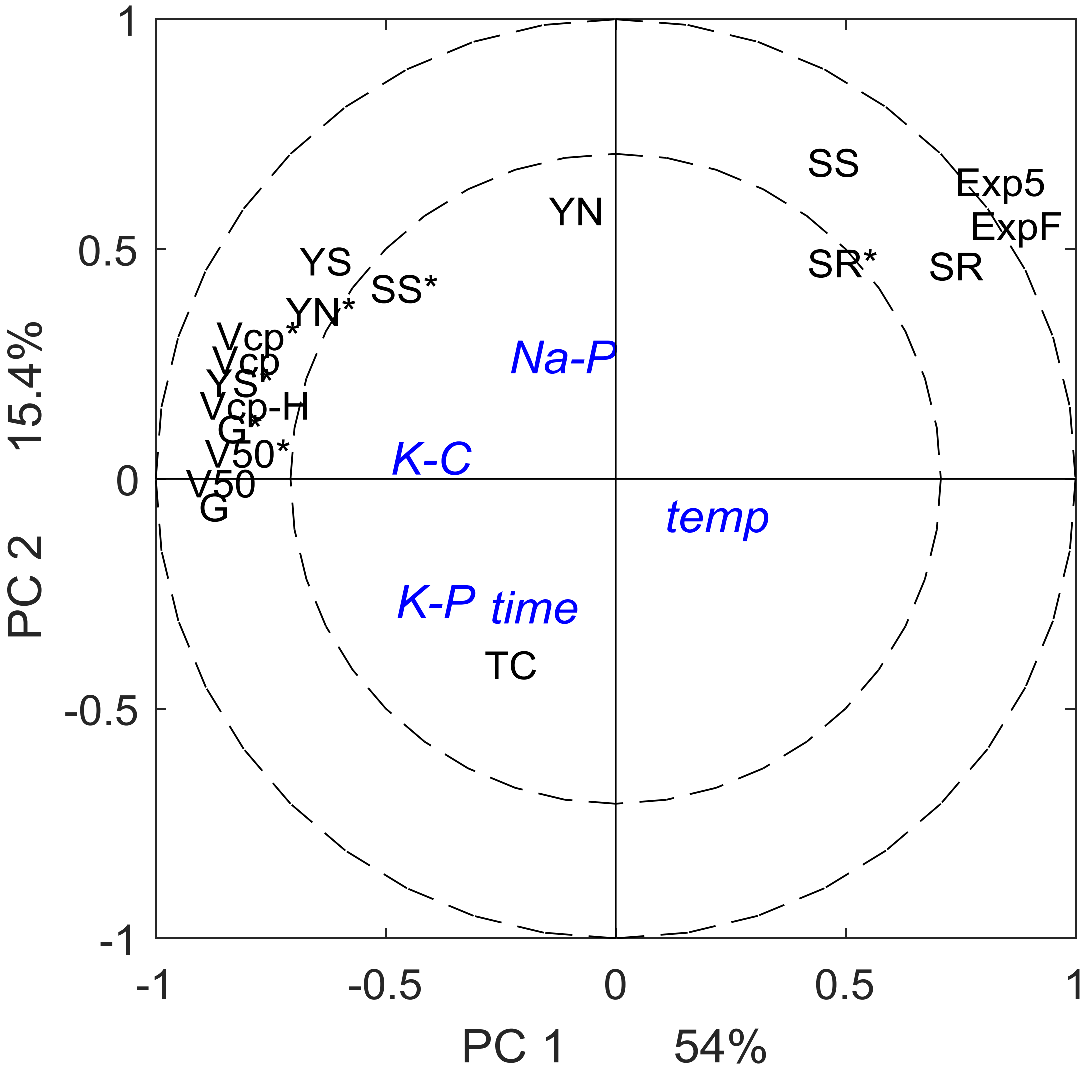

SS
Exp5
YN
ExpF
YS
SR*
SR
SS*
YN*
Vcp*
Vcp
YS*
Vcp-H
G*
V50*
V50
G
Na-P
K-C
temp
K-P
time
TC
1
0.5
0
-0.5
-1
-1
-0.5
0
0.5
1
PC 1
54%
PC 2
15.4%

Figure 4

Supplementary Material

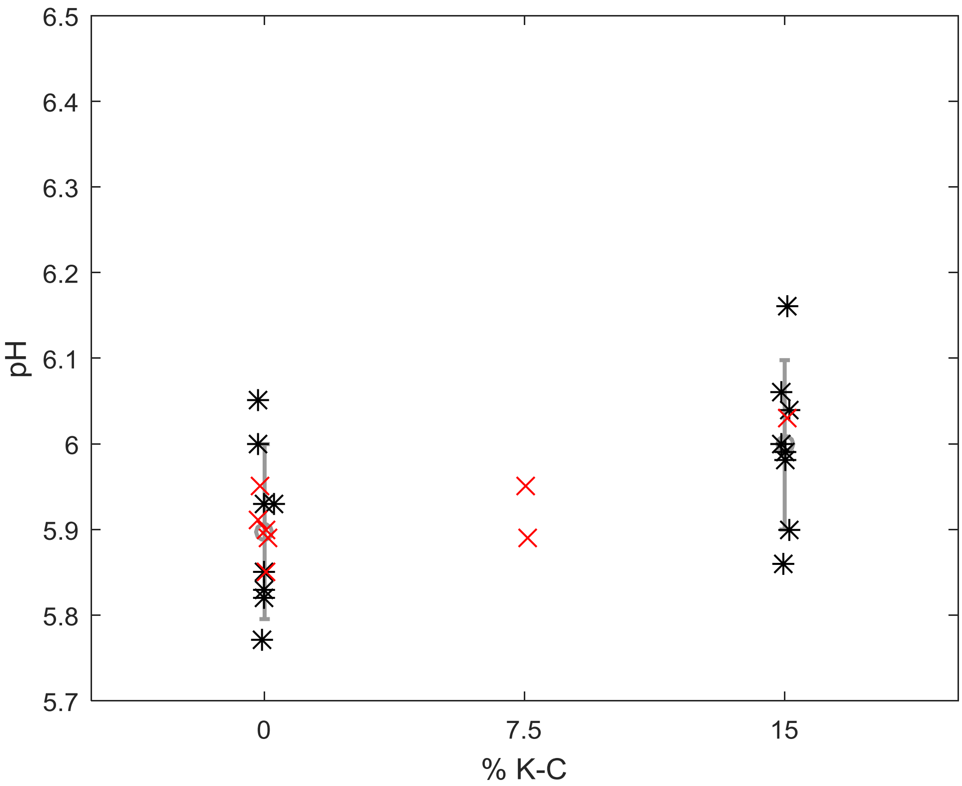


Fig. S1. Effect of K-C in experiment B.: Circle and error bars show the mean value for each level plus/minus 3*sem (standard error of the mean). Observations are shown as asterisks. Observations that are not part of the factorial experiment are shown as crosses. The x-axis indicates level of K-P. The p-value for the main effect of K-C on pH was <0.01