# Report

## Test of Meat Protein Extract in restructured ham

## Trial III

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**Summary**

*Background*

Previous trials have suggested that Meat Protein Hydrolysates (MPE) manufactured by use of enzymatic hydrolysis, improves taste and texture of cooked cured ham. In this trial Novozymes would like to test the use of two different MPEs, manufactured by use of Protamex (P) and Protamex + Flavourzyme (PF) respectively.
The MPEs added are without salt, and in a spray dried version in order to assure proper solubility. In addition it is tested if MPE influences the microbial shelf life of the meat products.
The design variables are a) salt content in hams, b) MPE type, c) amount of added MPE, and d) meat content.
The response variables are cooking loss, sliceability, sensory properties, slice coherency, proximate composition and total plate counts.

*Conclusion*

Brines made with MPE-P and MPE-PF appear visually different, a fact which does not seem to have any practical importance. No differences were noticeable neither on the raw meat batters nor the cooked sliced products. Although the cooking losses in all samples were low, there was a clear positive (low cooking losses) concentration dependent effect of addition of MPE. The sensory properties were fully acceptable for all products. In fact, it appears that it's possible to produce a ham product with MPE with 5 % less meat and only 1,7 % salt which is fully acceptable from a sensory point of view. MPE addition also improves the adhesiveness of cooked cured ham. It is to be noted that formulations for salt reduced products must be evaluated individually to determine the food safety. Although the hydrolysates contain a moderate level of bacteria and spores, there is no indication, that the use of MPE influences the shelf life of the ham products.

## Materials and methods

*Layout*

Table 1. Experimental design and relevant variables

| Batch | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| **Meat (%)** | 75 | 75 | 75 | 75 | 75 | 75 | 70 | 70 | 70 |
| **Brine (% yield)** | 25 | 25 | 25 | 25 | 25 | 25 | 30 | 30 | 30 |
| **MPE-P[1] (%)** | | | 5 | | | | | | |
| **MPE-PF[1] (%)** | | | | 5 | 5 | 5 | 10 | 10 | 10 |
| **Salt[2] (%)** | 2,5 | 1,8 | 2,0 | 2,0 | 1,8 | 1,6 | 2,0 | 1,8 | 1,6 |

[1] Included in brine

[2] Added amount in the product

*Raw material*

Topside ham muscle, chopped thru two kidney-plates.

- Approx. chunk size 3 x 3 x 3 cm.
- 15 kg of meat in each batch.

The MPE was from pork raw material without the traditional addition of salt (Carnad, Løgstør). The MPE was spray dried at Novozymes as preliminary trial had shown that this improved its solubility. The MPE was delivered in two different versions: MPE-P manufactured by use of Protamex alone and MPE-PF produced by use of Protamex and Flavourzyme in combination

*Brine composition*

See page 12

*Tumbling*

The brines (incl. MPE) were prepared one day prior to tumbling. Meat and brine was added batch-wise to each chamber in a three-chamber tumbler. Tumbling was done under vacuum for 6 hours, 6 RPM, 5 minutes rotation, 5 minutes rest.

*Stuffing*

The batters were stuffed in impermeable casings (4 x 3.5 kg / batch).

*Heat Treatment*

The raw ham was pasteurized on racks in a cooking cabinet at 80°C until a core temperature of 75°C.They were then chilled until 2°C.

*Setting*

After chilling, the hams were stored for 6 days at 5°C before slicing and analyses, resembling a typical setting period in the industry.

*Slicing*

Two hams from each batch were sliced for sensory analyses and evaluation of sliceability (2 mm slices) and for adhesion test (5 mm slices). Packages of 100g were vacuum-packed and stored at 5°C.

## Analyses

*Sliceability and cooking loss*

Before slicing, the hams in casings were peeled, and the liquid removed from the surface to determine cooking loss.
In order to determine the sliceability 50 slices of 2 mm thickness from each batch were made and the number of non-perfect (incoherent) slices was registered. The slicer was disinfected between batches in order to avoid carry over effects of bacteria.

*Sensory assessment*

The sensory properties of hams (2 mm slices) from each batch were assessed by seven people experienced in judging meat products and/or products with MPE addition. The samples were randomized and coded. A 5-point scale vas used (1=low, 5 =high), with the reference samples no.1 having the designated value 3 for all attributes evaluated. The assessment included 6 products characteristics; Color, aroma, taste, saltiness, firmness and coherency.

*Adhesion*

10 slices of 5 mm thickness from each batch were tested for adhesion properties at 5°C in a texture analyzer with tensile grips. From the center of each slice, samples of 4 x 6 cm were cut with a small incision on each of the longest sides. The exact protocol is obtainable upon request.

*Chemical composition*

Protein, fat, water, salt and pH were determined in duplicate for each batch. The exact protocols are obtainable upon request.

*Microbial analysis*

Five packages of batches no. 2, 3, 7, 8, 9 were stored at 5°C. Two weeks later the packages were punctured and placed at 8°C in order to simulate consumer behavior (opening of retail package, temperature abuse in consumer refrigerator). The packages were stored for another 2 weeks at 8°C and then analyzed for total plate counts.

## Results and discussion

*Visual appearance*

**MPE-P** **MPE-PF**

Figure 1. The appearance of brines no. 3 and no. 4.

Figure 1 shows that the brines appeared somewhat different. While the MPE-P brine was dark and transparent, the MPE-PF brine was lighter and opaque. The differences do not seem to have influenced the product quality. The MPE solubilities and rheological properties do not appear to be unsuited for multi needle injection.

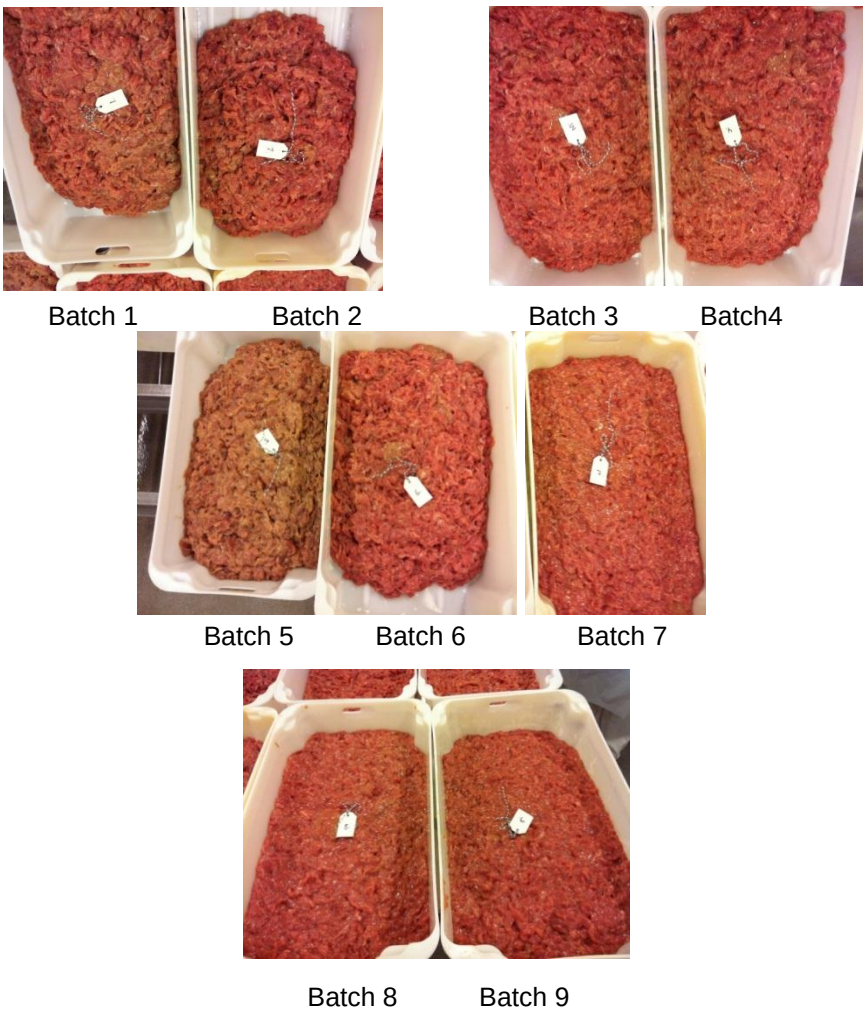


Figure 2. Meat batters after tumbling

Figure 2 shows the meat batters after tumbling. They appear equally “sticky”, indicating that the protein extraction has taken place apparently to a similar level for all batches. The fact that batch 5 is somewhat more brown is explained by the fact, that the vacuum had left this specific chamber. As there is sufficient exudate and no foam in the batter it was concluded that the vacuum was lost during and after the tumbling had taken place, having no importance to the testing. In accordance with previous experiences the color was regained during cooking.

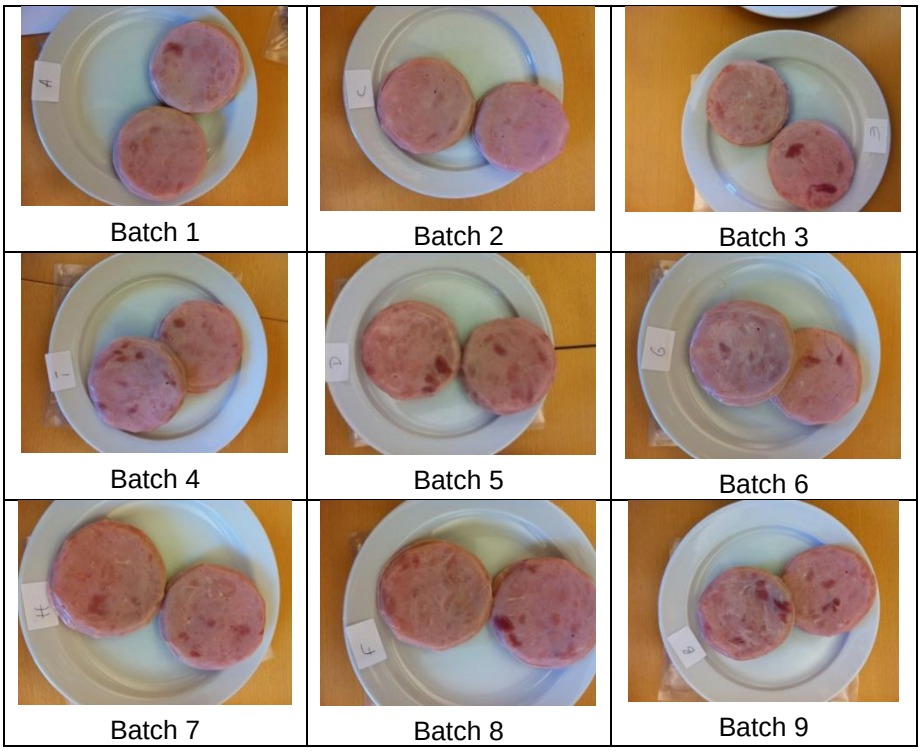


Figure 3. Appearance of ham slices from each batch.

Figure 3 shows the meat samples after cooking and slicing. As confirmed by the sensory evaluation (see below) no visual differences between batches can be observed (The dark spots are caused by differences in the raw material muscle fiber composition and are of no relevance to the present test).

*Sliceability, cooking loss*


Figure 4. Cooking loss in cooked ham with and without MPE. Error bars are std. err.

As can be observed in figure 4, lowering the salt content increases the cooking loss. It is also evident that the addition of MPE-P and MPE-PF decreases the cooking loss. Comparing batch 6 and 9 it appears, that the there is a concentration effect, as ham with only 1,6 % salt may be manufactured without increased cooking loss when 10 % MPE protein is used, while it is not possible with addition of 5 % MPE. For the moderately salt reduced batches (batch no. 2 vs. no. 5 and no. 8) it appears that 5 % MPE addition is sufficient. Comparing batch no. 3 and no. 4 it appears that MPE-PF has a markedly higher effect compared to MPE-P. Hence, the addition of MPE has a positive, and concentration dependent, effect on the cooking loss. It should however be noted that the cooking losses observed are low; In the manufacturing industry cooking losses of 1-2 % are common.

Phosphate is known to significantly improve water binding and in this trial phosphate was added to the level of 0,3 % in the final product. When interpreting the results it could be hypothesized speculated, if the high water binding capacity is due to the added tripolyphosphate. It may be hypothesized, that the effect of MPE on cooking loss may be more pronounced if no phosphate is used in the brines.

*Sensory assessment*

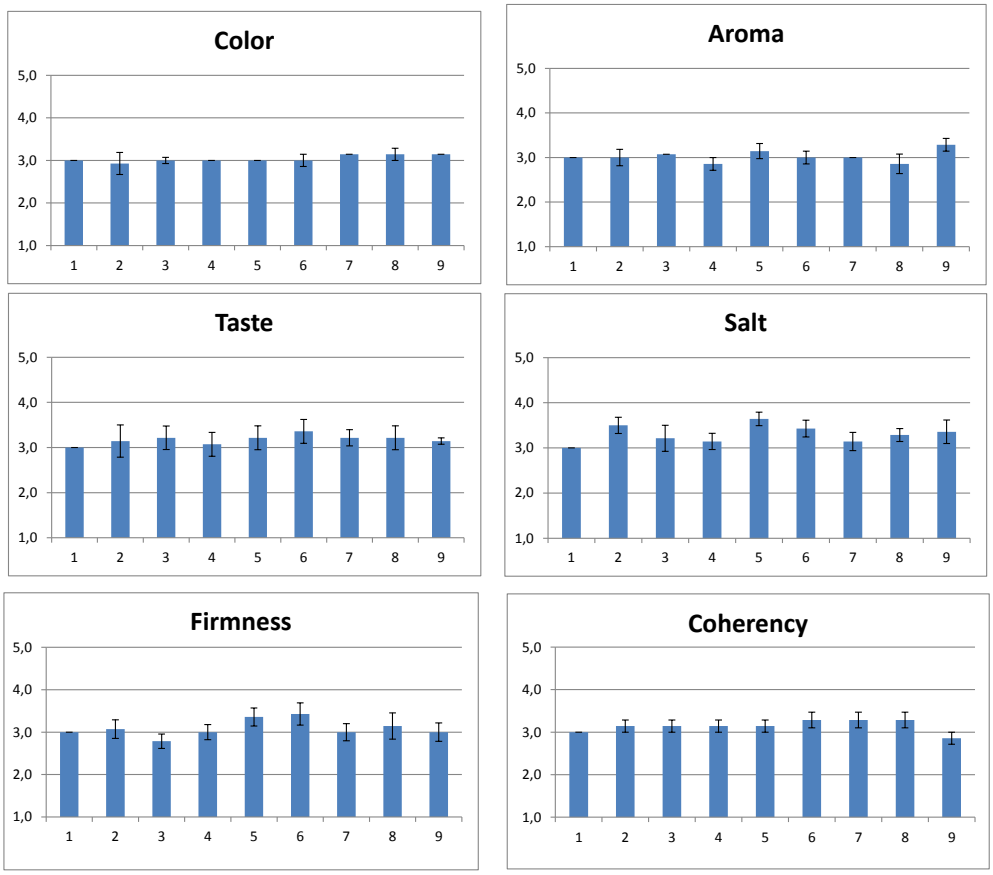


Figure 5. Sensory evaluations. Error bars are std. err. of the mean

Color: The samples are not significantly different. There is a very slight tendency for assessors to disagree on batch 2. The other differences are so small that the overall conclusion is, that the color is not affected by the various treatments.

Aroma: Sample 9 is marginally better, while the other differences are too small to be mentioned.

Taste: There are minor disagreements (std. err.) on the evaluation on the samples but they are all at the same level. All samples including the 1,8 % reference sample without MPE, are (numerically) evaluated as more tasty than the reference 1. This could indicate that lower salt content “reveals” the meat taste.

Salt: There is a tendency for samples with 1,8 and 1,6 % salt to be perceived as more palatable compared to the reference. As this includes sample 2 (without MPE) the interpretation is thus, that the less salty samples are preferred.

Firmness: None of the samples were perceived as too soft. There is a slight tendency for samples 5 and 6 to be a bit firmer. The differences are however minor.

Adhesion: Sample 9 is apparently a bit less coherent and sample 6-8 a bit more coherent. There is no obvious explanation for this, but the differences are so small that they must be considered unimportant.

Generally it appears that the assessors prefer the salt reduced products regardless of MPE addition. On the other hand the products with MPE and low meat content are considered fully acceptable compared to the reference. Apparently by use of MPE-PF it is possible to manufacture a product with 5 % less meat and only 1,6 % salt which is slightly more palatable than reference products with both 2,0 and 1,8% salt. In fact, if sample 2 is considered the (low salt) reference then no distinguishing between 1,6 %, 1,8 % and 2,0 salt is possible. It is however a bit surprising that no clear product defects are present in sample 2. This is probably due to the effect of the phosphate in the brines.

*Adhesion*

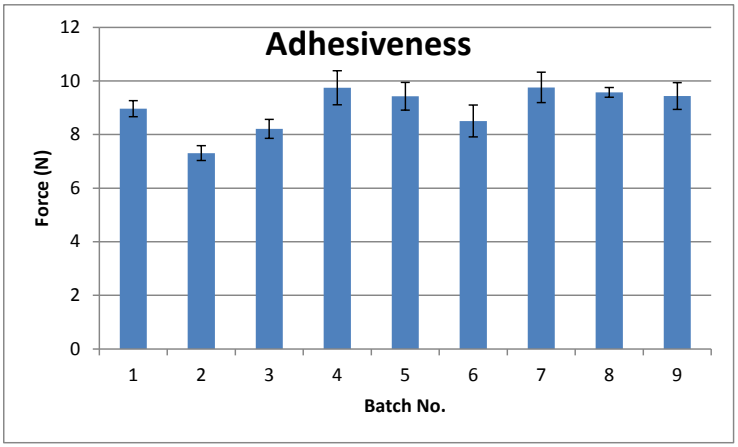


Figure 6. Measurement of adhesiveness / coherency by Texture Analyzer. Error bars are std. err.

The texture measurements for adhesiveness show, that sample 2 has a looser structure, while samples containing MPE-PF are slightly more coherent.
The difference between sample 6 and 9, both containing 1,6 % salt suggests, that the amount of added protein makes a difference. Apparently the addition of MPE improves the adhesiveness significantly.

*Chemical composition*

Table 2. Proximate composition of hams with MPE

| Batch No. | Fat | | pH | | Protein | | NaCl | Water | | NaCl/Water |
|---|---|---|---|---|---|---|---|---|---|---|
| | % | | | | % | | % | % | | % |
| 1 | 1,5 | ± 0.61 | 6,1 | ± 0.08 | 17,5 | ± 0.53 | 2,61 | 77,5 | ± 0.35 | 3,37 |
| 2 | 1,4 | ± 0.41 | 6,1 | ± 0.08 | 18,3 | ± 0.53 | 1,91 | 77,8 | ± 0.35 | 2,46 |
| 3 | 1,5 | ± 0.41 | 6,1 | ± 0.08 | 20,6 | ± 0.53 | 2,15 | 75,1 | ± 0.35 | 2,86 |
| 4 | 1,7 | ± 0.41 | 6,1 | ± 0.08 | 20,1 | ± 0.53 | 2,12 | 75,0 | ± 0.35 | 2,83 |
| 5 | 1,6 | ± 0.41 | 6,0 | ± 0.08 | 20,1 | ± 0.53 | 1,94 | 75,4 | ± 0.35 | 2,57 |
| 6 | 2,0 | ± 0.41 | 6,1 | ± 0.08 | 20,2 | ± 0.53 | 1,73 | 75,4 | ± 0.35 | 2,29 |
| 7 | 1,6 | ± 0.41 | 6,0 | ± 0.08 | 21,6 | ± 0.80 | 2,14 | 73,8 | ± 0.35 | 2,90 |
| 8 | 1,5 | ± 0.41 | 6,1 | ± 0.08 | 21,8 | ± 0.53 | 1,95 | 74,0 | ± 0.35 | 2,64 |
| 9 | 1,8 | ± 0.41 | 6,1 | ± 0.08 | 21,7 | ± 0.80 | 1,76 | 74,0 | ± 0.35 | 2,38 |

The data for proximate composition are generally as expected. The fat content is low and at a similar level. The protein content reflects a normal level of 17-18 % protein in ham plus the protein added from MPE. Also the pH is at the expected level. The salt content is on average 0,13 % higher for all samples than what was intended. An additional chemical test off the hydrolysates showed that the chloride content of the MEP-P and the MPE-PF was equivalent to 1,35 % and 0,63 % NaCl respectively yielding a contribution to the salt content in the hams of approximately 0,01% salt.

The theoretical contribution to chloride from the meat itself is in the order of 0,13% salt, which is exactly the degree of displacement in all the samples, why the effect of the different treatments may still be evaluated. It is however to be noted, that the differences in texture and cooking loss decrease as the salt content increases. The differences mentioned regarding these variables would hence have been somewhat more pronounced if the salt content had been 0,14 % lower.

The NaCl/ water ratio gives a good indication of the potential safety of the products. As an example a ham type product stored at 5°C, with 3,5% salt in the water phase, pH 6,1 and 60 ppm nitrite added will limit growth to max. 2 log *listeria monocytogenes* for approx. 20 days if vacuum packed and 30 days if MAP packed (30 % $CO_2$). Most of the products in this trial are hence not sufficiently stable and lactate, or the like, should be added. This emphasizes, that although MPE may compensate for other quality parameters, it is not given that the safety is assured in the salt reduced products.

*Shelflife*

Table 3. Total plate counts on ham samples after 2 weeks of storage at 5°C in 30% $CO_2$ followed by 2 weeks storage at 8°C (ambient atmosphere)

| Batch | 2 | 3 | 7 | 8 | 9 |
|---|---|---|---|---|---|
| Replicate 1 | <1 | 2,0 | <1 | <1 | <1 |
| Replicate 2 | <1 | 1,9 | 4,7 | <1 | <1 |
| Replicate 3 | <1 | 1,8 | 2,4 | <1 | 1,6 |
| Replicate 4 | 2,7 | 1 | <1 | <1 | <1 |
| Replicate 5 | 2,0 | <1 | 1,3 | <1 | 1,5 |
| Average | 2,4 | 1,7 | 2,8 | <1 | 1,5 |
| Std Dev* | 0,3 | 0,4 | 1,4 | 0,0 | 0,1 |
| *) values <1 not incl. | | | | | |

As mentioned 5 packages of batches no. 2, 3, 7, 8, 9 were stored at 5°C. Two weeks later the packages were punctured and placed at 8°C for another 2 weeks, in order to simulate consumer behavior (opening of retail package, temperature abuse in consumer refrigerator). They were then analyzed for total plate counts after growth on PCA agar at 20° for 5 days.

The results are shown in table 3. Batch 2, without MPE contains from <1 to 2,7 log cfu/g, which is considered low after 4 weeks of storage. The same applies to batch 3 containing MPE- P which has <1 to 2 log cfu/g. In samples 7, 8, 9 the picture is the same: low growth and no effect of doubling the amount of MPE. The only exception is replicate 2 for batch 7 which is moderately high. This is considered an outlier and a result of recontamination during the slicing and packaging of the samples. Microscopy on the two MPE powders reveal that the contained a variety of bacteria, including bacteria spores. However after dissolving the powders and heating them to 70°C for 20 min., less than 10 spores/ ml. could be found.

Although the MPE powders contained some bacteria and bacterial spores, there is no indication, that the use of MPE influences the shelf life of the ham products.

## Conclusion

Brines made with MPE-P and MPE-PF appear visually different, a fact which does not seem to have any practical importance. No differences were noticeable neither on the raw meat batters nor the cooked sliced products. Although the cooking losses in all samples were low, there was a clear positive (low cooking losses) concentration dependent effect of addition of MPE. The sensory properties were fully acceptable for all products. In fact, it appears that it's possible to produce a ham product with MPE with 5 % less meat and only 1,7 % salt which is fully acceptable from a sensory point of view. MPE addition also improves the adhesiveness of cooked cured ham. It is to be noted that formulations for salt reduced products must be evaluated individually to determine the food safety. Although the hydrolysates contain a moderate level of bacteria and spores, there is no indication, that the use of MPE influences the shelf life of the ham products.

**Brines**

| | Brine 1 25% gain, 2,5% salt | | Brine 2 25% gain, 1,8% salt | |
|---|---|---|---|---|
| | % | kg | % | kg |
| Water | 85,84 | 3,219 | 89,33 | 3,350 |
| Vacuum salt | 7,49 | 0,281 | 4,0 | 0,150 |
| Nitrite salt | 5,01 | 0,188 | 5,01 | 0,188 |
| Phosphate | 1,49 | 0,056 | 1,49 | 0,056 |
| S.ascorbate | 0,16 | 0,006 | 0,16 | 0,006 |
| MPE-P | | | | |
| Total | 99,99 | 3,750 | 99,99 | 3,75 |
| | Brine 3 25% gain, 2,0% salt | | Brine 4 25% gain, 2,0% salt | |
| | % | kg | % | Kg |
| Water | 77,20 | 2,895 | 77,20 | 2,895 |
| Vacuum salt | 4,99 | 0,187 | 4,99 | 0,187 |
| Nitrite salt | 5,01 | 0,188 | 5,01 | 0,188 |
| Phosphate | 1,49 | 0,056 | 1,49 | 0,056 |
| S.ascorbate | 0,16 | 0,006 | 0,16 | 0,006 |
| MPE-P | 11,15 | 0,418 | 0 | |
| MPE-PF | | | 11,15 | 0,418 |
| Total | 100 | 3,750 | 100 | 3,745 |
| | Brine 5 25% gain, 1,8% salt | | Brine 6 25% gain, 1,6% salt | |
| | % | kg | % | kg |
| Water | 78,19 | 2,932 | 79,17 | 2,969 |
| Vacuum salt | 4,00 | 0,150 | 3,01 | 0,113 |
| Nitrite salt | 5,01 | 0,188 | 5,01 | 0,188 |
| Phosphate | 1,49 | 0,056 | 1,49 | 0,056 |
| S.ascorbate | 0,16 | 0,006 | 0,16 | 0,006 |
| MPE-PF | 11,15 | 0,418 | 11,15 | 0,418 |
| Total | 100 | 3,750 | 99,99 | 3,750 |
| | Brine 7 30% gain, 2,0% salt | | Brine 8 30% gain, 1,8% salt | |
| | % | kg | % | Kg |
| Water | 70,56 | 3,175 | 71,42 | 3,214 |
| Vacuum salt | 4,33 | 0,195 | 3,47 | 0,156 |
| Nitrite salt | 4,33 | 0,195 | 4,33 | 0,195 |
| Phosphate | 1,31 | 0,059 | 1,31 | 0,059 |
| S.ascorbate | 0,13 | 0,006 | 0,13 | 0,006 |
| MPE-PF | 19,33 | 0,870 | 19,33 | 0,870 |
| Total | 99,99 | 4,500 | 99,99 | 4,500 |

| | Brine 9 30% gain, 1,6% salt | |
|---|---|---|
| | % | Kg |
| Water | 72,29 | 3,253 |
| Vacuum salt | 2,76 | 0,117 |
| Nitrite salt | 4,18 | 0,195 |
| Phosphate | 1,31 | 0,059 |
| S.ascorbate | 0,13 | 0,006 |
| MPE-PF | 19,33 | 0,870 |
| Total | 100 | 4,500 |