WHAT ARE THE MICROSTRUCTURAL IMPLICATIONS IN AN EMULSION SAUSAGE WHEN LOWERING THE SALT AND FAT CONTENT?

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Abstract – In order to lower the NaCl and the fat content in emulsion sausages we must learn more about how salt and fat influence the capacity of the meat proteins, i.e. the sarcoplasmic (SP) and the myofibrillar (MP) proteins, to form emulsions and gels. The solubility and the degree of aggregation of the proteins in the soluble phase and the swelling ability in the solid phase were studied at different salt solutions (0.15, 0.40 and 0.80 M) and pH (5.5 and 5.8). The emulsifying properties of the meat proteins were characterised, gel-emulsions were formed by heating the emulsions and the texture was determined.
SP aggregates with salt addition, whereas MP solubilizis, but the amount solubilised is minute. The MP mainly consists of insoluble particles that swell somewhat at 0.8M NaCl and during heating they contract.
The emulsifying capacity seems to be better using the SP, giving droplets of 25-50 µm, whereas the MP gives oil droplets from 20 up to 220 µm.
At the lower oil concentrations of 5-10% the MP at salt concentrations from 0.40 M and above give rise to 4 times better gel-emulsions than SP, but at the higher oil concentrations of 20-40% good gels are achieved even with SP.

Key Words – emulsifying and gelling capacity, salt and fat content, sarcoplasmic and myofibrillar proteins

I. INTRODUCTION

Sodium chloride (NaCl) is added to products like emulsion sausages to increase its quality. Lately, this has been questioned as excessive sodium consumption is associated with hypertension, which is a major risk factor for stroke and cardiovascular diseases. Moreover, the fat content of about 20% in such products is considered to be too high in order to lower the calorie intake and to avoid obesity. In order to lower both the NaCl and the fat content in such products we must learn more about how salt and fat influence the microstructural behaviour of the sausages. The meat proteins, i.e. the sarcoplasmic and the myofibrillar proteins, are essential in forming this microstructure.

Strictly speaking, emulsion-type sausages are not true emulsions as they are a combination of an emulsion and a mixture of insoluble compounds, such as meat fibers and other insoluble meat protein and fat particles [1]. Moreover, the properties of an emulsion sausage are strongly dependent upon the formation of a meat protein matrix, a gel, which governs its texture and water-holding characteristics. This meat protein gel is influenced by a number of factors, where the amount and type of myofibrillar proteins extracted from the muscle has been considered to be of prime importance [2]. The type of protein matrix formed is also related to the dispersed or aggregated state of the proteins prior to denaturation [3]. The gel forming ability of the system is also dependent on the amount and type of insoluble material such as meat fibers and particles and fat droplets and cells, if they participate in the gel matrix. The influence of the amount and type of the insoluble material on the gel forming ability in an emulsion sausage has been less studied and it becomes especially important when there is a need to lower the fat content in low-fat sausages.

Therefore we have in this investigation varied the oil content from 5 to 40% in meat protein stabilised emulsions and also heated them in 72°C for 17 min to form a gel-emulsion to simulate the ultimate sausage microstructure. We have also

separated the two main meat proteins, the sarcoplasmic proteins (SP) and the myofibrillar (MP) in pork muscle (*M. longissimus dorsi*) and studied their susceptibility to different pH:s (5.5 and 5.8) and salt solutions (0.15, 0.40 and 0.80 M). This was done with regard to the solubility of the proteins, the degree of aggregation of the proteins in the soluble phase and the swelling ability of the solid phase of the proteins. The capacity of these proteins to form emulsions and gel-emulsions has then been evaluated.

II. MATERIALS AND METHODS

Extraction and separation of sarcoplasmic and myofibrillar proteins

The extracting solution used was 0.15 M NaCl solution of which pH was adjusted to be 5.5 or 5.8 by 0.2 M HCl or 0.2 M NaOH. To extract the sarcoplasmic proteins, 70 g of ground meat was homogenized with 700 mL extracting solution for 30 sec. and the homogenate was centrifuged at 1000g for 10 min. The supernatant, which is the sarcoplasmic fraction, was then filtered and collected and kept at 4 °C. The pellet was resuspended in 400 ml extracting solution and filtered through a mesh in order to remove the connective tissue and to achieve the rest as the myofibrillar proteins.

Determination of protein content in the protein solution

As the obtained protein solutions are composed of protein, salt and water, the protein content can then be estimated by subtracting the ash content from the dry matter. The determination of ash and dry matter content in the protein solutions (pH 5.5 and 5.8) was done by following the method described by AOAC (2000).

Laser light scattering and light microscopy

The degree of meat protein aggregation in protein solutions/suspensions at pH 5.5 and 5.8 in NaCl solutions of 0.15, 0.40, 0.60 and 0.80 M before and after heating (72°C for 17 min) was measured as particle size distribution (PSD) by the use of integrated-laser light scattering unit (Hydro 2000SM, Mastersizer 2000, Malvern Instruments, UK). The volume-weighted diameter (d_{43}) was used to characterize the size of the aggregates. The structure of the protein aggregates was examined under light microscope (Olympus BX 50F4, Japan).

Emulsion and gel-emulsion formation

Total amount of 20 ml emulsions were made by adding rapeseed oil to the protein solution at different concentrations – namely, 5%, 10%, 20%, 30% and 40%. Emulsification was performed using Ultra turrax (Ystral, Bergius Trading AB, Germany) at 22k RPM for 30 sec. Gel-emulsions were formed by heating the emulsions at 72°C for 17 min and the texture was determined using a penetrometer, giving stress-penetration curves, where the stress (Pa) at 15% penetration was measured.

III. RESULTS AND DISCUSSION

Table1. Protein content in the total and the soluble part of the sarcoplasmic (SP) fractions.

Fraction	At pH 5.5		At pH 5.8	
	% Total protein content in the solution	% Protein content in the soluble part	% Total protein content in the solution	% Protein content in the soluble part
Sarcoplasmic proteins in 0.15 M NaCl	0.71 ± 0.01	0.71 ± 0.01^{aA}	0.71 ± 0.02	0.71 ± 0.02^{aA}
Sarcoplasmic proteins in 0.4 M NaCl		0.52 ± 0.04^{bcA}		0.56 ± 0.06^{bA}
Sarcoplasmic proteins in 0.6 M NaCl		0.56 ± 0.02^{bB}		0.63 ± 0.04^{abA}
Sarcoplasmic proteins in 0.8 M NaCl		0.49 ± 0.03^{cA}		0.52 ± 0.01^{bA}

Values are expressed as mean ± SD. Means in rows and columns with different superscript letters are significantly different (P<0.05). Capital and lower case letters refer to rows (pH) and columns (concentration of NaCl), respectively.

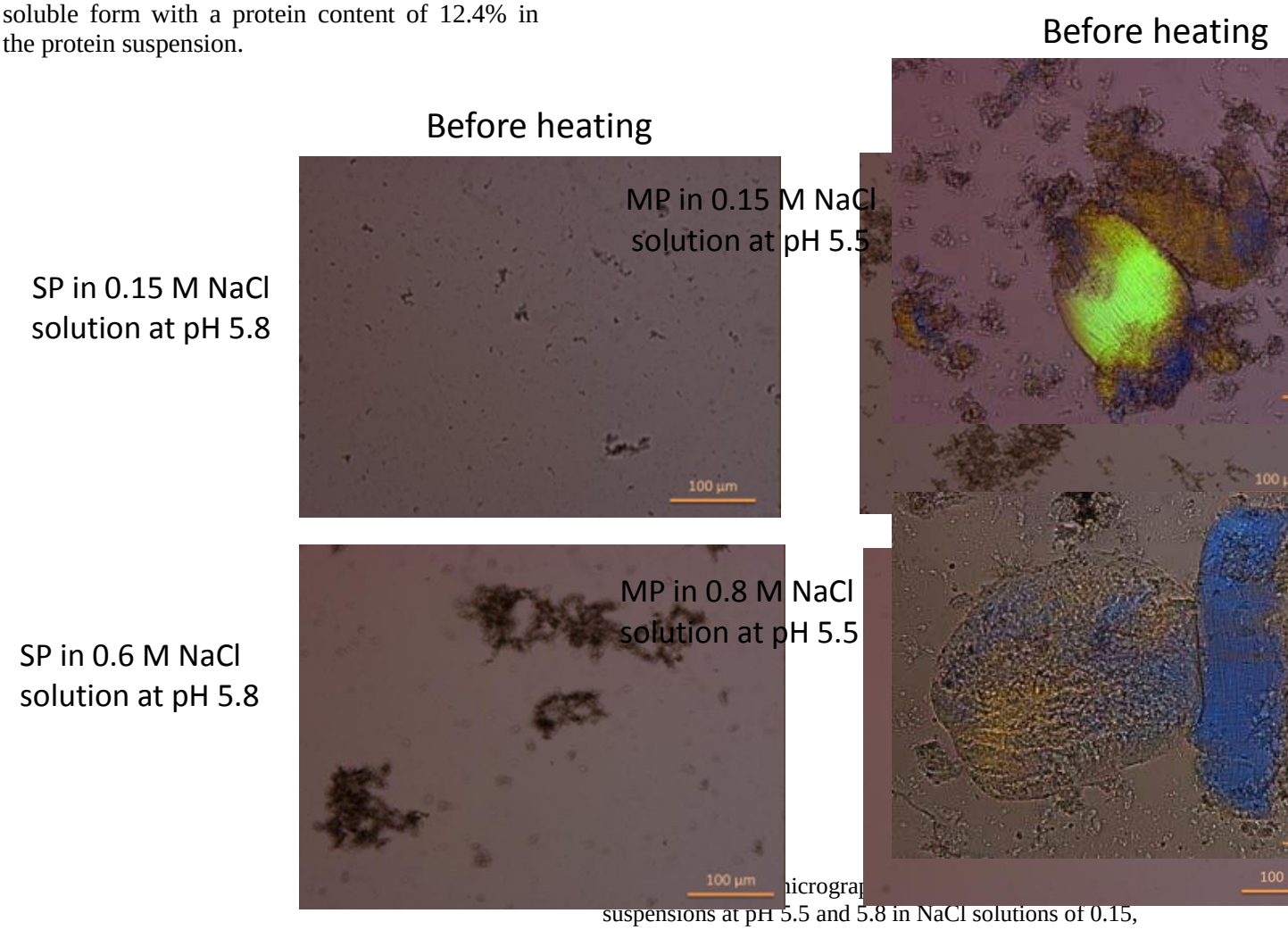
Table2. Protein content in the total and the soluble part of the myofibrillar (MP) fractions.

Fraction	At pH 5.5		At pH 5.8	
	% Total protein content in the solution	% Protein content in the soluble part	% Total protein content in the solution	% Protein content in the soluble part
Myofibrillar proteins in 0.15 M NaCl	12.43 ± 0.27	0.10 ± 0.02^{bA}	11.21 ± 0.15	0.13 ± 0.01^{cA}
Myofibrillar proteins in 0.4 M NaCl		0.20 ± 0.02^{aA}		0.21 ± 0.02^{bA}
Myofibrillar proteins in 0.6 M NaCl		0.23 ± 0.02^{aA}		0.27 ± 0.02^{aA}
Myofibrillar proteins in 0.8 M NaCl		0.23 ± 0.01^{aA}		0.26 ± 0.02^{aA}

Values are expressed as mean ± SD. Means in rows and columns with different superscript letters are significantly different (P<0.05). Capital and lower case letters refer to rows (pH) and columns (concentration of NaCl), respectively.

As revealed from Table 1 the protein content of SP in the supernatant is 0.7%. These proteins aggregate on salt addition, lowering the amount of soluble proteins down to 70% of the original at 0.80 M NaCl. MP proteins (Table 2), however, solubilize, but the amount solubilized is minute increasing only from 0.8% of the original value in 0.15 M NaCl to 1.8 % in 0.8 M NaCl at pH 5.5, whereas at pH 5.8 the increase in solubility is somewhat higher going from 1.2% to 2.3%. Evidently, most of the MP proteins are in the non-

soluble form with a protein content of 12.4% in the protein suspension.



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suspensions at pH 5.5 and 5.8 in NaCl solutions of 0.15, 0.60 and 0.80 M before and after heating.

The question is then how these two types of meat proteins aggregate at different pH, salt concentrations and on heating. The SP protein aggregate size (d_{43}, results not shown) increased from 28 to 52µm from 0.15 to 0.8 M NaCl and grew even further on heating as seen in the micrographs in the upper part of Fig. 1. The MP proteins, which consist of mainly insoluble particles, as seen in the micrographs in the lower part of Fig. 1, had an average particle size of 130 µm (d_{43}, results not shown) at pH 5.8 up to 0.6 M NaCl and swelled to around 140 µm at 0.8M NaCl. On heating, however, the suspended particles of myofibrillar proteins contracted down to about 115 µm. None of the protein suspensions formed a gel on heating, probably because of the 10 times dilution of the original meat needed to separate the two types of meat proteins.

The results of the studied emulsifying properties of the SP proteins can be seen in Fig.2. The SP proteins are good emulsifiers, but oil droplet sizes (d_{43}) as large as 25-50 µm were achieved with the increase of oil from 5 to 40% (v/v). For the emulsion stability checked after 5 days the oil droplets had increased to almost double the original size at 0.15M NaCl and pH 5.8 but remained stable at 0.6 M NaCl. This suggests that SP proteins give more stable emulsions when being more aggregated, which indicates the phenomenon of particle stabilised emulsions [4].

MP in 0.4 M NaCl

Oil content	D [4, 3] - Volume weighted mean (µm)	
	D-day	5 days after
5% oil	66.02 ± 9.07 ^{aA}	63.38 ± 12.77 ^{abA}
10% oil	89.57 ± 34.85 ^{aA}	42.56 ± 1.33 ^{cA}
20% oil	110.10 ± 62.26 ^{aA}	73.85 ± 1.42 ^{aA}
30% oil	62.34 ± 7.51 ^{aA}	51.10 ± 4.78 ^{bcA}
40% oil	51.30 ± 8.81 ^{aB}	67.46 ± 4.56 ^{abA}

SP in 0.15 M NaCl

Oil content	D [4, 3] - Volume weighted mean (µm)	
	D-day	5 days
5% oil	24.70 ± 0.12 ^{bB}	39.32 ± 0
10% oil	28.24 ± 0.79 ^{bB}	46.63 ± 1
20% oil	32.23 ± 0.06 ^{bB}	71.54 ± 2
30% oil	47.08 ± 11.19 ^{aB}	99.96 ± 2
40% oil	49.41 ± 0.76 ^{aB}	112.91 ± 4.49 ^{aA}

MP in 0.8 M NaCl

Oil content	D [4, 3] - Volume weighted mean (µm)	
	D-day	5 days after
5% oil	236.38 ± 5.56 ^{aA}	90.45 ± 10.11 ^{bB}
10% oil	220.91 ± 16.45 ^{aA}	44.59 ± 3.90 ^{cB}
20% oil	109.22 ± 18.69 ^{bA}	48.30 ± 1.67 ^{cB}
30% oil	28.74 ± 0.33 ^{dB}	89.27 ± 6.63 ^{bA}
40% oil	62.93 ± 2.85 ^{cB}	129.80 ± 8.76 ^{aA}
40% oil	46.32 ± 7.05 ^{aA}	

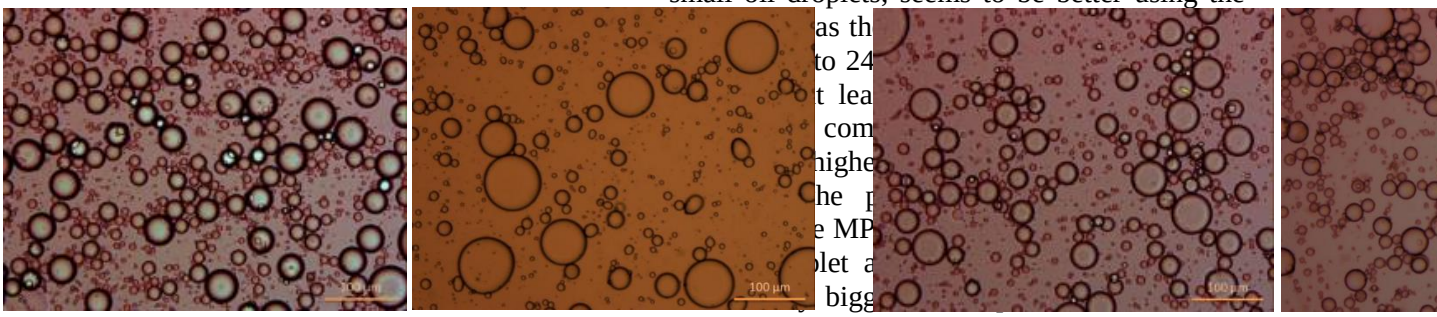

Figure 2: Particle size (d_{43}) of the emulsions made of SP proteins at pH 5.8 in 0.15 and 0.6 M NaCl at production (D-day) and after 5 days storage.

Table 3: Particle size (d_{43}) of the emulsions made of MP proteins at pH 5.8 in 0.4 and 0.8 M NaCl at production (D-day) and after 5 days storage.

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the MP stabilised emulsions. Moreover, an optimum in oil droplet size is obtained with increasing amounts of oil, being 20% for 0.4 M NaCl and 5% for 0.8 M NaCl. This suggests that the higher the amounts of oil the more viscous the suspensions that in turn lowers the rate of coalescence. On the other hand the more oil the higher the probability for the oil droplet to meet each other leading to coalescence, which is why there is an optimum. The reason this optimum decreases with salt addition is probably due to

the fact that the viscosity of the suspension is higher the more salt added. The emulsion stability of the MP stabilised emulsions after 5 days is higher compared to the SP stabilised emulsions, which can be dependent on the fact that the viscosity and the protein content is higher for the former.

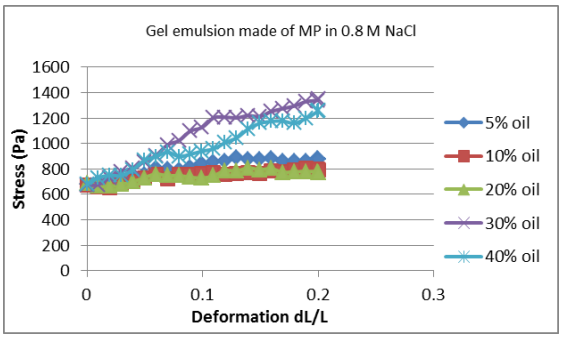

Figure 3: A stress (Pa)-deformation curve for gel-emulsions stabilized by MP proteins in 0.8 M NaCl for emulsions made with varying oil conc. from 5-40%.

Gel-emulsions were formed by heating the emulsions and the gelling ability was evaluated using a penetrometer giving stress-deformation /penetration curves (an example is given in Fig.3). The stress (Pa) at 15% (dL/L=0.15) deformation was then compared for all the gel-emulsions studied and the results can be seen in Fig. 4. At the lower oil concentrations of 5-10% the MP proteins at salt concentrations from 0.4 M and above gave in general rise to 4 times better gel-emulsions.

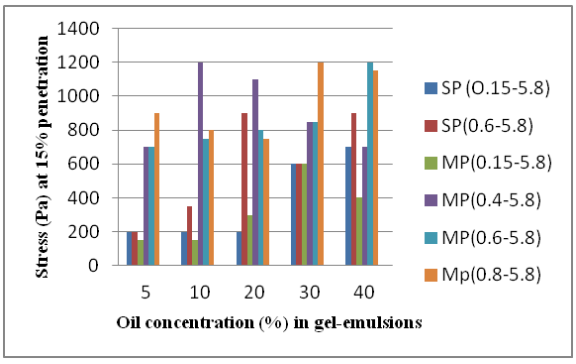

Figure 4: The stress (Pa) at 15% penetration into gel-emulsions at varying oil concentration from 5-40 % made of SP and MP proteins at pH 5.8 and different salt concentrations of 0.15-0.8 M.

But at the higher oil concentrations of 20-40 % the mean free distance between the oil droplets is so low that good gels are achieved even with SP proteins.

IV. CONCLUSION

In this study it is shown that it is difficult to make gel-emulsions, like sausages, with a good texture having a low fat content, especially when SP proteins and MP proteins below 0.4 M NaCl are used as emulsifiers.

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