EFFECT OF SALT AND LIVER/FAT RATIO ON MICROSTRUCTURE AND MACROSCOPIC PROPERTIES OF SPREADABLE LIVER PASTE

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This paper reports the effect of two liver/fat ratios (35/35 and 20/50) and two salt levels (0 and 1.8 %) on the microstructure (dynamic viscoelastic properties and light microscopy) and macroscopic properties (emulsion stability and texture) of spreadable liver paste. This study was performed in order to obtain detailed insight into the structural organization of spreadable liver paste and gain knowledge on the relationship between its microstructure and macroscopic properties. Liver paste was characterized as a weak gel-like emulsion with G´ greater than G˝. Addition of salt led to a microstructure with smaller fat globules and increased fat binding properties. This resulted in a harder, stronger and more stable product. The effect may be attributed to solubilisation of salt soluble proteins, making more liver proteins available to act as emulsifier. Although G' and G'' were not affected, a lower liver/fat ratio decreased the linear viscoelastic region with the formation of a less stable and more heterogeneous microstructure with bigger fat globules and fat channels throughout the structure of the product.

Key Words – light microscopy, meat emulsion stability, rheological properties, texture

I. INTRODUCTION

Liver paste products are traditional meat products manufactured and consumed all over the world, however in Europe they are mainly consumed in France, Denmark, Spain and Belgium. Generally, these products are classified according to their processing temperature: they can be processed raw (e.g. liver sausage) or precooked (e.g. spreadable liver paste) [1]. This article is about spreadable liver paste, a warm processed fine emulsion-like meat product which has the characteristics of an oil-in-water (O/W) emulsion. This product is mainly composed of liver, fat, water, salt and a small amount of auxiliary components. Analyzing the open literature, it becomes clear that only a limited number of publications has studied the composition of spreadable liver paste and its effect on the microstructural aspects [2, 3] or the macroscopic properties of spreadable liver paste (e.g. Cheong & Fischer [4]), however none of these authors paid attention to explain the observed changes in macrostructure. In other words, none of these studies investigated the microstructure in relationship with the macroscopic properties of spreadable liver paste. This will aid to control its functionality, improve existing products and help in the development of new strategies to obtain products with a healthier image (low-fat and/or low-salt liver paste, liver paste with healthier
lipid formulation, etc.). Therefore, the objective of this work is to investigate the effect of two composition variables (liver/fat ratio and salt) on the microstructure and macroscopic characteristics of spreadable liver paste. This allows to gain knowledge on the relationship between these different structural levels, as this has not been studied before. To study the effect of both composition variables and study the effect of more and less stable products, a high (35/35, conventional formulation) and low (20/50) liver/fat ratio was chosen. 1.8 % salt was selected because liver paste products commonly contain 1.8 % salt. Katsaras et al. [2] claimed that a liver protein structure can develop without salt addition. Therefore, a second salt level of 0 % was chosen.

MATERIALS AND METHODS

A. Manufacturing of liver paste

The spreadable liver pastes, further called liver pastes, were processed in the pilot plant of the research group 'Technology and Quality of Animal Products' (KU Leuven Technology

Campus Ghent). Fresh pork liver and fresh pork back fat were obtained from a local industrial slaughterhouse. Pork liver was ground through a 8 mm plate and pork back fat was chopped into small cubes (30 cm^3). mixed thoroughly and both were packed separately into plastic bags and frozen (−18 °C) until use. The day before preparation of liver paste, the raw materials were thawed overnight in a refrigerator at 4 °C. The formulation of the different experimental liver pastes is presented in Table 1.

Table 1: Recipe (% ingredients) for liver pastes with different liver/fat ratios and with and without salt

Ingredient (%)	35/35 NS	35/35 S	20/50 NS	20/50 S
Liver	35	35	20	20
Back fat	35	35	50	50
Water	30	30	30	30
NaCl	0	1,8	0	1,8
Na nitrite	0.012	0.012	0.012	0.012
Na ascorbate	0.05	0.05	0.05	0.05
Glucose	0.5	0.5	0.5	0.5
Spices	0.8	0.8	0.8	0.8

The content of liver and back fat and the salt level were varied, resulting in four different formulations:
high and low liver/fat ratio, without and with salt (35/35 NS, 35/35 S, 20/50 NS and 20/50 S).The liver pastes were prepared by first pre-chopping the frozen liver (-3°C) with sodium chloride (only for the liver pastes containing salt) and sodium nitrite. The liver batter was stored at 4°C until further processing. Secondly, the scalded back fat (boiled at 100°C during 20 minutes) was chopped together with part of the broth. Finally, when the temperature reached 51°C, the liver batter with the remaining ingredients were added and mixed to obtain the liver paste batter. When processing was complete, the liver paste batters were filled into cans and heated at 76°C (core temperature 74°C). After 1.5 h cooking, the cans were cooled to 4°C and stored at that temperature for 1 week, after which they were analyzed.

B. Dynamic Viscoelastic Properties

Rheological measurements were performed using an AR2000ex stress controlled rheometer (TA instruments, New Castle, US) equipped with a 40 mm parallel plate-plate system. To prevent slippage of the samples, the upper and lower plate were both crosshatched and the gap between the plates was set to 1000 µm . The AR 2000 was supplemented with an efficient Peltier temperature control system and upper heated plate to control the sample temperatures precisely. Oscillation experiments were conducted at 4°C. The extent of the linear viscoelastic region (LVR) was determined by performing a stress sweep between 1 and 10000 Pa at 1 Hz. The critical shear stress, σ_c, an indication of the onset of the non-linear region where the structure starts to deform under applied stress was calculated from the stress sweep curves as the point at which the complex modulus (G*) deviates more than 5 % from a constant G* (plateau) value. Dynamic frequency sweep tests were performed at a stress of 8 Pa (within LVR determined previously) between 0.1 and 10 Hz. Storage modulus (G', a measure of elastic property) and loss modulus (G", a measure of viscous property) were obtained directly from the software (Rheology Advantage, TA version 5.7).

C. Light microscopy of Liver Paste

Microscopic analyses were performed by Histalim, Montpellier, France. From each liver paste sample, six subsamples were cut and paraffin-embedded according to the NF V 04-417 standard [5]. Three µm paraffin sections were stained with picro-indigo carmine. Specimens were observed using a Leica DM4000 B/M microscope (Leica Microsystems GmbH, Wetzlar, Germany) and a digital camera Infinity 2-1C (Lumenera, Ottowa, Canada), at a 50-fold magnification. The most representative micrograph of each liver paste variant was selected.

D. Texture

Texture Profile Analysis (TPA) of the liver pastes was performed using a Lloyd Texture Analyzer (Model LF plus, Lloyd Instruments Ltd, Fareham, Hampshire, England). Cans (diameter 7 cm, height 5 cm, 250 g) with liver paste were axially penetrated to 40% of their originally height. Force-distance deformation curves were derived using a 100 N load cell and a cylindrical probe (diameter 6 mm) at a constant crosshead speed of 100 mm/min. The textural parameters were directly obtained from the recorded force-distance curves: hardness

(results shown), cohesiveness, springiness, gumminess, chewiness, adhesiveness (results not shown).

E. Emulsion stability

The methodology to study emulsion stability of the liver paste products was based on the procedure by Hughes, Cofrades and Troy [6]. The raw liver paste batter (30 g) was placed in a pre-weighted centrifuge tube (CT). The sample was heated in a cooking chamber for 30 min at 70 °C and centrifuged for 3 min at 4025 g. The supernatant, a mixture of water and fat, was poured into a pre-weighed crucible, dried overnight at 100 °C and weighed. The percentage of total expressible fluid (%TEF) (mixture of fat and water from the supernatant) and fat (% Fat) were calculated as follows: % TEF = weight supernatant/sample weight x 100%; % Fat = weight dried supernatant/sample weight x 100%

G. Statistical analysis

A 2 x 2 factorial design was set up to analyses the effect of liver/fat ratio and salt by analysis of variance (ANOVA) via the general linear model. Statistical analysis was performed using SPSS version 16.0. The level of significance for all tests was set at $P < 0.05$.

II. RESULTS AND DISCUSSION

A. Microstructure: rheology and light microscopy

The stress sweep experimental data (Table 2) indicate that a higher liver/fat ratio and addition of salt in liver paste resulted in a significantly higher value of σ_c, which implies a better resistance of the system to external stress.

Table 2: Effect of salt and liver/fat ratio on σ_c (critical stress), G´ (1Hz) and G˝ (1 Hz) of liver paste

Liver paste	Stress sweep data	Frequency sweep data	
	σ_c (Pa)	G' (1Hz)	G" (1Hz)
35/35 NS	44.82^{a1} ± 4,70	18077^{a1} ± 1470	4141^{a1} ± 358
20/50 NS	28.21^{a2} ± 1,50	17897^{a1} ± 1312	3869^{a1} ± 314
35/35 S	69.52^{b1} ± 4,82	22886^{b1} ± 580	4961^{b1} ± 115
20/50 S	40.74^{b2} ± 7,21	23708^{b1} ± 1495	4788^{b1} ± 492

The effect of salt was more pronounced for 35/35 while the effect of a higher liver/fat ratio was greater when the liver paste contained salt. As a result, with salt and a higher liver/fat ratio, stronger emulsions with more network interactions and a higher stability were formed.

In general, all liver pastes showed characteristic spectra of weak gels with a slight frequency dependence of both moduli, comparable to the liver paste batter, within the frequency range studied. As shown in Table 2, no interaction could be determined between both variables. Although tan δ hardly varied (results not shown), G´ and G˝ were both significantly influenced by the addition of salt, higher G´ and G˝ values were obtained when salt was added to the formulation, irrespective of the liver/fat ratio. As for the effect of liver/fat ratio, no differences in G´ and G˝values (1 Hz) between the liver pastes 35/35 and 20/50 were found, regardless of the salt content. Both raw materials (liver and fat) are thus important for the structure of liver paste and influence the structure of liver paste.
Figure 1 shows the light micrographs of the liver paste emulsions.

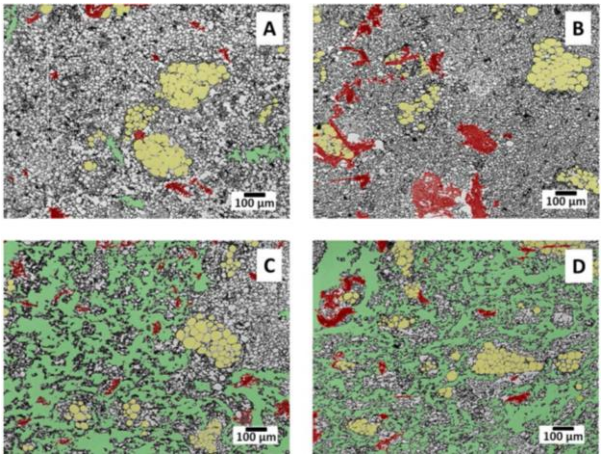

Figure 1: Light micrographs of liver paste 35/35 NS (a), 35/ 35 S (b), 20/50 NS (c) and 20/50 S (d). Color codes: white=fat droplets; yellow=fat adipocytes; green=fat coalescence; red=insoluble parts of connective tissue fragments, nerves; and Bar=100 µm.

Addition of salt in liver paste 35/35 (35/35 S) (Figure 1B) resulted in smaller and more uniform

fat globules with no fat channels. This effect can be attributed to the extraction of the salt soluble liver proteins, probably caused by a screening of the attracting charged groups of the proteins and the chaotropic effect of the Cl- anions. As a result, with salt, more liver proteins are available to act as an emulsifier which leads to a finer emulsion.
Decreasing the liver/fat ratio resulted in the formation of a more heterogeneous microstructure consisting of non-emulsified, characterized by fat globule coalescence and fat channels (green zones) and emulsified zones, characterized by irregularly shaped fat globules surrounded by a protein layer. (Figure 1). Decreasing the liver/fat ratio resulted in bigger fat globules as (only for the salt containing variant).

B. Macroscopic properties: stability and texture

Addition of salt caused significantly lower values of % TEF and % Fat (results not shown). These results indicate that in the presence of salt liver proteins had better emulsifying properties. The decrease in % Fat caused by salt can mainly be explained based on the microstructural characteristics of the products, as discussed above. A lower liver/fat ratio (20/50) resulted in a strong increase of % TEF which could be attributed to an increase in % Fat. Again, the effect on % Fat can be explained by Stokes' law and the microstructural features as discussed above. Addition of salt led to significantly higher values for hardness (results not shown) and was more pronounced for liver paste 20/50. There is no literature available on the effect of salt on the texture of liver paste. However, the increase in hardness with the addition or increasing concentration of salt was also observed in frankfurters [7]. A lower liver/fat ratio did not have an important impact on the texture (hardness) of liver paste, although the hardness of the liver pastes without salt were slightly decreased. Also for the effect of liver/fat ratio on texture, there is no literature available on liver paste. The effect of fat reduction has been studied by Delgado-Pando et al. [3] who showed that reducing the fat content in spreadable liver paste results in a decrease of the textural parameters.

III. CONCLUSION

Addition of salt led to smaller fat globules which was also reflected in the higher emulsion stability and especially the increased fat binding properties of liver paste resulting in a harder, stronger product with more network interactions. It is assumed that with salt, more proteins are available to act as an emulsifier and for gel formation. As for the effect of liver/fat ratio, a decrease resulted in the formation of a more heterogeneous emulsion with bigger fat globules because less proteins were dissolved in the continuous phase and available for adsorption at the oil/water interface. Although less stable emulsions were formed with a lower liver/fat ratio, no differences in G, G'' and hardness could be obtained between the liver pastes 35/35 and 20/50. Apparently, both liver protein gelation and fat crystallization contribute to the hardness of liver paste.

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