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SPEGEPØLSER-SMAG

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Manuskript nr. 296E

Investigation of Free Fatty Acid Content of Danish Dry Sausages 1)

by

Zbigniew Duda 2)
Department of Meat Technology
Agricultural College of Poznan
Poland

The Danish dry sausages contain large amounts of fatty tissue, mainly pork fat (40-60%). During manufacture and storage periods biochemical changes in the fat tissue will occur. It is thought that these transformations play a prominent role in the formation of flavour components in the sausage.

Decomposition of fat by microorganisms has been investigated by several authors.(1, 2, 3). E. Numi and Niinivaara (5) have been studying the lipolytic properties of 199 microbial strains and have found that many of the examined species cause hydrolysis of pork fat at pH 5.5 and 5.0, and with a salt concentration of 3 and4 per cent, respectively. These are the conditions prevailing in the dry sausage during the ripening and storage period.

Pezacki and collaborators (6) and Andersen (7) (unpublished data) show that the fatty tissue in the dry sausages during manufacturing and storage period undergoes hydrolytic changes causing an increase of the concentration of free fatty acids. Maillet and Henri (8) found that the fatty acid content of dry sausage increased from its initial value of 0.45 to 2-4 per cent and even to 5-7 per cent. The present studies were undertaken to provide additional information on the effects of a variety of manufacturing methods on the total amounts of free fatty acids. Three different types of Danish dry sausage were investigated. Gas-liquid-chromatography was used in the determination of the changes in the amount of the individual free fatty acids (C_{12}- C_{18}) which were isolated from crude fat extracted from the sausages.

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SLAGTERIERNES FORSKNINGSINSTITUT
9. marts 1965

SPEGEPØLSER-SMAG

Manuskript nr. 296E

A. Sampling

The sausages investigated were of three different types and they were prepared under commercial conditions.

Type 1 from Factory A
Type 2 from Factory B
Type 3 from The Meat Trade School

The recipes and the manufacturing process of the investigated sausages are shown in Table 1.

The amounts of free fatty acids were estimated just after stuffing the sausages, thereafter tank curing (if this is done) and then drying and smoking. During a storage period of 12 weeks 3 estimations were made.

Determination of changes of free fatty acids in sausages of type 1 was performed on one production only, while the changes in free fatty acid of type 2 and 3 were investigated 3 times each, i.e., for three production series in succession.

B. Analytical Procedure

Reagents: Chloroform, methanol, acetone, ethyl ether, petroleum ether, formic acid and sulphuric acid, isopropanol, and potassium hydroxide, all analytical grade.

Ethyl- and petroleum ether were redistilled in glass.

Isopropanol-KOH solution was prepared according to the method of Keeney(9). For the separation columns was used Mallinckrodt silicic acid, 100 mesh, labelled "Suitable for chro analysis by the method of Ramsey and Patterson".

Analytical Sample Preparation. 250-350 grammes of sausage after removing the casing were minced twice in an ordinary mincer with a plate aperture of 3 mm diameter and then thoroughly mixed.

Basic Analysis. Moisture, Fat, and Dry Matter. Moisture was determinated by drying to constant weight at 105 C.

Fat was determinated by the Gerber method.

The percentage dry matter was estimated as difference.

SLAGTERIERNES FORSKNINGSINSTITUT
9. marts 1965

SPEGEPØLSER-SMAG

Manuskript nr. 296E

Extraction of Crude Fat. 100-150 grammes of minced sausage stuffing were extracted for 2-4 hours with chloroform in a Soxhlet apparatus. After extraction the chloroform phase was dried by filtering through anhydrous sodium sulphate. The solvent was removed on a boiling water bath, at first under a weak - and towards the end of the evaporation - under a vigorous stream of nitrogen. Having removed the solvent the crude fat was quickly cooled.

Isolation of Free Fatty Acids from Crude Fat. Preparation of the column and isolation of the free fatty acids were according to the method described by McCarthy and Duthie (10). 3-5 grammes of the sample of crude fat, dissolved in a small quantity of ethyl ether (4-6 ml), were placed on the column and thoroughly washed into the packing by several small portions of ethyl ether. The solvent was removed from the free fatty acids were then transferred by ethyl ether to weighed small round-bottom flasks (50 ml), provided with a standard taper joint. The solvent was evaporated again in the same manner. After cooling in a desiccator (10-15 min.) the fee fatty acids were weighed on an analytical balance.

Esterification of the Fatty Acids. The methyl esters of the free fatty acids were prepared by acid-catalized esterification according to the method described by Luddy et al. (11).

Extraction of the Esters by Petroleum Ether. Extraction of methyl esters was done according to procedure by Luddy et al. (11). The solvent and the rest of the washing water were evaporated on the water bath (70-75 C) under a stream of nitrogen. The methyl esters were then transferred to small tubes, the air was removed by a stream of air nitrogen, and the tubes were closed by a rubber stopper. The samples were kept 1-3 days in freezer (-12 - -14°C) until submission to the gaschromatography.

Gas-chromatographic Separation of the Methyl Esters. The Fractometer Perkin-Elmer Corp. Model 116E with a thermal conductivity detector was employed. A 2 m x 6.35 mm OD aluminium column packed with 20 per cent butandiolsuccinat as polyester on Chromosorb W was used. The carrier gas (helium) pressure in the column was regulated at 2 atm. and gave a flow rate of 111 ml/min. The temperature was 195 C and the injected amount of sample was 1 µ.

SLAGTERIERNES FORSKNINGSINSTITUT
9. marts 1965

SPEGEPØLSER-SMAG

Manuskript nr. 296E

C. Results of the Investigation

Content of Free Fatty Acids in Sausages after Different Production and Storage Periods.

This may be looked at in several ways:

1. The changes in free fatty acids (FFA) in the crude fat, which was extracted from three kinds of Danish Dry Sausages during the manufacture and storage period, are given in Figure 1.

The highest increase in the level of FFA in the crude fat was found in sausage No 2. This increase was particularly intense in the 19 days' processing period, i.e., during tank curing and smoking and is almost 0.272 g per day. During the first 8 weeks' storage period the increase in FFA in the crude fat in sausage No. 2 was 8 times slower than during the manufacturing (0.034 g/day). The last 4 weeks' storage period, however, was characterized by an unexpected large increase in the amount of FFA, which was difficult to explain. This increase was actually only two times smaller than during the manufacturing period, i.e., 0.136 g/day and it was 4 times higher than the daily increase in FFA in the previous 8 weeks' storage period.

The two other types of sausages, marked Nos 1 and 3 were charaterized by a relatively uniform increase in contents of FFA in crude fat during the processing and 12 weeks' storage period.

The increase in amount of FFA in sausage No. 3 in the manufacturing period was 0.087 g/day and in the storage period about 0.050 g/day and in the case of sausage No.1 - 0.144 g/day and 0.053 g/day. In sausages Nos. 1 and 2 about 1% and 1.5-2.5% of FFA were found after tank curing and smoking and drying periods.

When sausage No. 1 (tank cured) was compared with sausage No. 3 (dry cured) the level of FFA in all phases of manufacture and storage was about 1% higher.

It is rather difficult to draw conclusions about the method of manufacture since sausage No. 1 was investigated for only one production, i.e., no standard deviation could be calculated.

However, the size of the standard deviation of sausage No. 3 suggests that the two methods of manufacturing are not statistically significantly different with respect to the influence of the method of manufacture on the concentration of FFA in the final product.

When the period of tank curing was increased from 5 to 8 days and the period of smoking from 7 to 11 (as in sausage No. 2) a great increase in FFA was produced.

SLAGTERIERNES FORSKNINGSINSTITUT
9. marts 1965

SPEGEPØLSER - SMAG

Manuskript nr. 296E

The level of FFA in sausage No. 2 during the manufacturing period was nearly 2 to 4 times larger (2.2 - 5.6%) when compared with the other experimental sausages. The difference in the amount of FFA in the manufacturing time between sausage No. 2 and Nos. 1 and 3 would be statistically significant if the standard deviation were about 1 σ . In the storage period the difference in level of FFA in crude fat between sausages which were manufactured by different methods becomes smaller - particularly during the first 7 to 8 weeks' storage.

Maximum concentration, i.e., more than 11% of FFA was detected in sausage No. 2 after 12 weeks' storage. After this storage period sausages Nos. 1 and 3 showed 5.7% and 7.0% FFA, respectively, i.e., about two times smaller.

The reason for the very rapid increase in concentration of FFA in sausage No. 2 is not easy to explain because the initial composition of the forcemeat, the population of lipolytic bacteria and activity, and concentration of tissue and bacterial lipase were unknown.

It must be noted that the very fast increase in FFA observed in sausage No. 2 was mainly due to the large amount, detected in one of the series, i.e., first series (13.00% FFA in crude fat). This could possibly be due to an experimental error.

2. The accumulation of FFA in experimental sausages expressed as the amount in fat free dry matter (FFDM) is shown by Figure 2.

The character of the changes occuring is rather similar to that of the changes shown in Figure 1, i.e., in crude fat. However, the biggest amount of FFDM in sausage No. 1 corresponds with the lowest amount of FFA, both in manufacturing and storage period. The level of FFA expressed as FFDM was rather larger in sausage No. 2, i.e., about 42 $\pm$ 7% after 12 weeks storage. In sausages Nos. 1 and 3 in this same period the level was about 14.0% and 18.0% $\pm$ 4% , respectively. It must be emphasized that in sausage No. 2 the level of FFA expressed as amount in FFDM was at least 2 to 3 times higher after 4 and 8 weeks' storage than in sausages Nos. 1 and 3. However, the standard deviation was so large that it could not be concluded that there was any significant difference in effect of method of manufacturing on the accumulation of FFA.

3. The dynamic increase in level of FFA in the sausages with reference to the average amount of these compounds in the forcemeat is represented in Figure 3.

The amount of FFA in sausage No. 2 showed a 13 $\pm$ 1 times increase in the manufacturing period over the amount detected in forcemeat. On the other hand sausages Nos. 1 and 3 show an increase of only about 5 $\pm$ 0.4 times in the manufacturing period.

These results indicate that there is a high statistically significant difference between sausages No. 2 and Nos. 1 and 3, and thus evidence for the influence of manufacturing method and probable initial composition of stuffing on the dynamic of increase of the FFA in this kind of sausage.

After 12 weeks of storage the FFA content of sausage No. 2 had increased by 27 ± 6 times and Nos. 1 and 3, 14 and 20 ± 4 times over the initial amount of FFA in the stuffing. It is therefore concluded with high probability that the storage period of the finished product does not significantly influence the FFA-level in sausages which were manufactured by the different methods.

Investigation of Individual FFA in Sausages

In preliminary gaschromatography investigations as many as 18 different methyl esters of FFA isolated from crude fat were detected. The results of one of these preliminary analyses are presented in the Figures 4a and 4b.

However, only 7 of these were present in all samples in significant amounts, i.e., more than 1% and therefore only these were estimated quantitatively.

The results of the changes of the following acids: miristic (14 : 0), palmitic (16 : 0), palmitoleic (16 : 1), stearic (18 : 0), oleic (18 : 1), linolic (18 : 2), and anachidonic (20 : 4) during the manufacturing and storage period are shown in Figure 5. As expected oleic and palmitic acids mainly contributed to the amount of FFA in raw sausages, the amount of oleic being 2.5 to 4.0 times bigger than the amount of palmitic - both in processing and storage period.

On the basis of the results it must be concluded that no one of the 7 individual FFA undergoes any essential quantitative change during the manufacturing and storage period of the sausage.

Thus it seems that decomposition of triglycerides in raw sausages is independent both of the kind of fatty acid and of the method of manufacturing. However, this conclusion requires confirmation by a variance analysis which, due to lack of time, has not yet been carried out.

The changes in the amount of unsaturated and saturated fatty acids during the manufacturing and storage periods are shown in Figure 6.

The results indicate that the increase in content of saturated fatty acids mainly occurs during storage period. During the curing in brine the amount of saturated fatty acids showed a decrease. As the amount of saturated fatty acids increased during the storage period the level of unsaturated fatty acids proportionately decreased. This may perhaps be due to oxygenation of the unsaturated fatty acids released during the storage period or selective, enzymatic, hydrolytic processes. These proposals requiee further experimental confirmation.

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SLAGTERIERNES FORSKNINGSINSTITUT
9. marts 1965

SPEGEPØLSER-SMAG

Manuskript nr. 296E

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Zbiegniev Duda/KB

SLAGTERIERNES FORSKNINGSINSTITUT
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SPEGEPØLSER-SMAG
Manuskript nr. 296E

Table 1. Recipes and Manufacturing Process of Sausages

	Sausages types		
	1	2	3
Raw products:	27% pork[a] 18% veal[a] 55% fat ?	45% {pork[b] / veal[b]} 55% fat	36.2% pork 13.8% veal 50.0% fat
Salt and spices:			
Sodium chloride	5.00 g/kg		44.80 g/kg
Sodium nitrite	0.24 g/kg		0.27 g/kg
Pepper	3.60 g/kg		2.50 g/kg
Garlic powder	0.90 g/kg		1.09 g/kg
Artificial colour	0.22 g/kg		0.22 g/kg
Kristalpur	3.00 g/kg		-
Casings:	Artificial protein Diameter 65 mm	Ditto Diameter 55 mm	Ditto Ditto
Tank curing:	5 days, 5-8 °C, 23 °Bé	8 days, 6-8 °C, 24 °Bé	Not
Drying:	4 days, 18 °C RH 90-70%	7 days, 20 °C RH 90-80%	7 days, 18 °C RH 75%
Smoking:	5 days, 18 °C RH 90-70%	4 days, ? ?	7 days, 20 °C RH 95-80%
Storage period:	12 weeks, 18 °C RH 65-75%	Ditto	Ditto

a) Presalted with addition 28 g/ kg of Sodium chloride

b) Presalted with addition ca. 30 g/kg of sodium chloride

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XI. KONGRES DER EUROPÄISCHEN FLEISCHFORSCHER
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Zb. D u d a
Poznań /Polen/
Institut für Fleischtechnologie der Landwirtschaftlichen Hochschule.

2

Untersuchungen über den Gehalt an freien Fettsäuren der dänischen Rohwürste.

Z u s a m m e n f a s s u n g

In der vorgelegten Arbeit wurde der Gehalt an freien Fettsäuren in den Rohwürste gewichtmässig festgestellt und ihre qualitative und quantitative Zusammensetzung mittels der Gaschromatographie ermittelt. In dieser Weise wurden die hydrolitische Veränderungen der Fette in drei Arten dänischen Rohwürste binnen 12 Wochen langer Aufbewahrung untersucht. Es wurde festgestellt

a. Der Gehalt an freien höheren Fettsäuren /fh F/ ist vor allem von ihren Ausgangsgehalt in Wurstbrät abhänigg. Auch die Herstellungsart der Rohwürste übt einen Einfluss auf diesen Qualitätsmerkmal aus.

b. Mit dem Verlauf des Herstellungsprozesses und der Aufbewahrung wird der Gehalt an fh F immer grösser. Er wächst nämlich vom 0,5% im Wurstbrät bis zu 5 -12% des Fettgewichtes während der Versuchsanfbewahrung der Rohwürste.

c. Die Veränderungen am Gehalt der fh F beeinflüssen jedoch nicht unerwünscht die organoleptischen Eigenschaften der Rohwürste.

d. Mittels der Gaschromatographie wurde die Anwesenheit von 18 Verbindungen nachgewiesen unter denen 7 folgende höhere Fettsäuren aufgefunden wurden : Miristin-, Palmitin-, Palmitoleïn-, Oel-, Stearin-, Linolen - und Arachidonsäure.

e. Die Gehaltsveränderungen einzelner Fettsäuren, welche während des Herstellungsprozesses und Aufbewahrung der Rohwürste stattfinden, sind nicht statistisch gesichert.

f. Während des Herstellungsprozesses und der Aufbewahrung wird jedoch immer kleiner der summarische Gehalt an unsättigten freien Fettsäuren und wächst gleichzeitig ähnlicher Gehalt an freien gesättigten Fettsäuren der Rohwürste. Diese Tatsache deutet, dass mit den hydrolitischen zugleich Oxydationfettveränderungen in Rohwurst stattfinden.

g. Die Versuchsergebnisse erklären die Art und Weise der biochemischen Fettveränderungen in Abhängigkeit von Herstellungstechnologie der Rohwürste z.B. im Falle der Anwendung von Eintauchen der Würste in Salzlake während ihrer Reifungszeit.

h. Die geschilderten Versuchsergebnisse sind keine Endergebisse. Weitere Untersuchungen müssen vor allem die Tatsache berücksichtigen, dass die Fettveränderungen die Quelle der Geruchs- und Geschmackstoffe der Rohwürste sein können.