L/4

Specificity of Lipolysis during Dry Sausage Ripening.

D. Demeyer, J. Hoozee and H. Mesdom*.
Laboratorium voor Voeding en Hygiene
(Dir. Prof. Dr. J. Martin)
R.U.G.
Bosstraat, 1
9230 Melle
Belgium.

Summary.
The amounts of total and individual fatty acids present in Triglycerides (T.G.), Free Fatty acids (FFA), Diglycerides (D.G.), Monoglycerides (M.G.) and Polar Lipids (P.L.) were determined at various stages of dry sausage ripening using a combination of thin layer and gas chromatography. Total FFA increased from 1 to 5 % of total F.A., D.G. fatty acids from 0.5 to 4 %, whereas T.G. fatty acids showed a corresponding decrease. The rate of liberation of F.F.A. was in the order 18:2 > 18:1 > 18:0 > 16:0 while M.G. and D.G. were enriched in 16:0. These results suggest specificity of lipolysis

Résumé.
La quantité d'acides gras totaux et individuels dans les Triglycérides (T.G.), acides gras libres (F.F.A.), diglycérides (D.G.), Monoglycérides (M.G.) et lipides polaires (P.L.) a été déterminée au cours de la maturation du saucisson sec, en utilisant une combinaison de chromatographie sur couche mince et en phase gazeuse. Les F.F.A. augmentaient de 1 à 5 % des acides gras totaux, les acides gras des D.G. de 0.5 à 4 %, tandis que les acides gras des T.G. diminuaient en proportion. La vitesse de libération des F.F.A. suivait l'ordre 18:2 > 18:1 > 18:0 > 16:0, tandis due les D.G. et les M.G. étaient enrichis en 16:0. Ces résultats indiquent une lipolyse spécifique.

---

* Present adress: FAO - UNDP - P.00 Box 913 - Khartoum - Sudan.

Zusammenfassung

Die totalen und einzeln Fettsäuren in Triglyzeride (T.G.) freien Fettsäuren (FFA), Diglyzeride (D.G.), Monolyzeride (M.G.) und polairen Lipide wurden bestimmt, während der Herstellung und Aufbewahrung von schnellreifenden Rohwurst, mittels einer Kombination von Dunnschicht und Gaschromatographie. F.F.A. Konzentration erhöhte von 1 bis 5 % der totalen Fettsäuren, und die D.G. Fettsäuren von 0.5 bis 4%. Fettsäuren in T.G. nahmen im gleichen Verhältenis ab. Die Geschwindigkeit der Lipolyse nahm ab in die Ordnung 18:2 > 18:1 > 18:0 > 16:0 während man für die D.G. und M.G; eine Bereicherung von 16:0 nachwiesen konnte. Diese Resultate suggerieren eine spezifische Lipolyse.

Zusammenfassung

Die totalen und einzeln Fettsäuren in Triglyzeride (T.G.) freien Fettsäuren (FFA), Diglyzeride (D.G.), Monolyzeride (M.G.) und polairen Lipide wurden bestimmt, während der Herstellung und Aufbewahrung von schnellreifenden Rohwurst, mittels einer Kombination von Dunnschicht und Gaschromatographie. F.F.A. Konzentration erhöhte von 1 bis 5 % der totalen Fettsäuren, und die D.G. Fettsäuren von 0.5 bis 4%. Fettsäuren in T.G. nahmen im gleichen Verhältenis ab. Die Geschwindigkeit der Lipolyse nahm ab in die Ordnung 18:2 > 18:1 > 18:0 > 16:0 während man für die D.G. und M.G; eine Bereicherung von 16:0 nachwiesen konnte. Diese Resultate suggerieren eine spezifische Lipolyse.

Introduction.

In recent years, an extensive literature demonstrating lipolysis during dry sausage ripening has accumulated. Maillet and Henry (1960) stated that the free fatty acid (FFA) content may reach values up to 5 - 7 % in the finished product. Values between 1 and 7 % were reported by Stanculescu et al (1970) (Wurziger and Ristow,(1966) Ten Cate (1960) Terplan (1969) Duda (1966), Mihalyi and Körmendy (1967), Canteni et al (1967 b) and Wahlroos and Niinivaara (1969).

Evidence indicating selective liberation of unsaturated fatty acids has been occasionally reported (Duda, 1966) (Wahlroos and Niinivaara, 1969). In these studies however, determination of total FFA is by titration of a lipid extract while individual FFA are identified by Gas chromatography after extraction of FFA alkali salts with aqueous or ethanolic alkali. This method can cause artefactual generation of FFA by hydrolysis of esters (Meinertz, 1971) (Laurell, 1957), while alkaline extracts are selectively enriched in short-chain and unsaturated fatty acids (Stoll, 1972) (Demeyer and Hoozee, unpublished results).

In this paper, we report a systematic study of lipolysis during dry sausage ripening, using a combination of thin-layer and gas chromatography to quantitate individual lipid classes.

Materials and Methods.

Preparation of sausages :

Two batches of ten sausages each were prepared by a local butcher, according to usual practice (composition as shown in table 1). They were kept at partial vacuum and 23°C for 5 days (batch A) or 3 days (batch B), transferred to a room at 22°C and 90-95 % R.H. untill the 8th day, after which temp. was decreased to 19° C and R.H. to 85 - 90 % until the 12th day. They were then kept at 18° C and 75 % R.H. until the end of the experiment.

Table 1. Composition of sausage mixture.

Cooled, deboned and chopped pork	3 kg
Cooled, deboned and chopped beef	4 kg
Cooled and chopped lard	3 kg
Salt (NaCl)	25 g
Coloring salts (KNO_3 + $NaNO_2$)	2 g
Sugars[1]	7 g
Soy protein concentrate	8 g
Smoke concentrate	1 g
Spices	3.5 g
Polyphosphates	1 g

[1] A commercial preparation, containing oligosaccharides with small amounts of ascorbic acid, glucono-delta-lacton and salt was used. In batch A, 5 g of glucose was added to 7 g of the preparation.

A sausage was transported to the laboratory at different stages of the ripening period, the casing removed and the sample ground in a meat grinder before analysis.

Analytical Methods:

Samples were analyzed for Dry Matter (D.M.), crude protein, and crude fat by conventional methods.

Quantitative determination of lipid classes:

Lipids were extracted with chloroform/Methanol 2/1 v/v and lipid classes separated and quantitated by a combination of thin layer and gas chromatography as described by Christie et al (1970) and elsewhere (Demeyer et al, in preparation).

Determination of carbonyl compounds:

Total aldehydes were determined using the benzidine reaction (Tóth, 1970) and the amounts were calculated using a molar absorption of 1.370 (Holm et al, 1957)

and an average molecular weight of 91.
For batch A, total carbonyl compounds were also isolated as 2.4 - Dinitrophenylhydrazones (DNP-hydrazones) as described by Hansen and Keeney (1970). The concentration of carbonyl compounds was calculated using a mean molar absorption of 22.500

Results and Discussion.

Fig. 1 shows a 15 to 20 % increase in D.M. content during the ripening process. Average values for crude protein content in D.M. were 27.8 $\pm$ 1.0 % (mean $\pm$ S.E., 40 determinations on 8 different stages in the ripening process) for batch A and 34.6 $\pm$ 0.2 % for batch B (35 determinations on 7 different stages).
Crude fat content was determined for batch A, and found to be higher than the total fatty acid content as determined on the lipid extract using margaric acid as I.S. (Table 2)

This discrepancy is obviously, at least partly due to the presence of glycerol and lipids containing no fatty acids such as cholesterol in the crude fat. Fatty acid distribution in lipid classes, is expressed as % of the total obtained by addition. These data, for various stages of the ripening process, are presented in fig. 2.

It is clear that a continuous decrease in triglyceride fatty acids during ripening occurs, with corresponding increases in FFA, diglycerides and, less outspoken, monoglycerides. Polar lipid fatty acids only decrease later in the ripening prodess. At the end of this process, FFA represent approximately 5 % of the total fatty acids present in the sausage, or less than 2 % in the sausage. No difference was observed between the two batches, although Micrococci counts tended to be higher in batch A (De Ketelaere et al, 1973).

Fig. 1 Increase in Dry Matter content (%D.M.) with time.

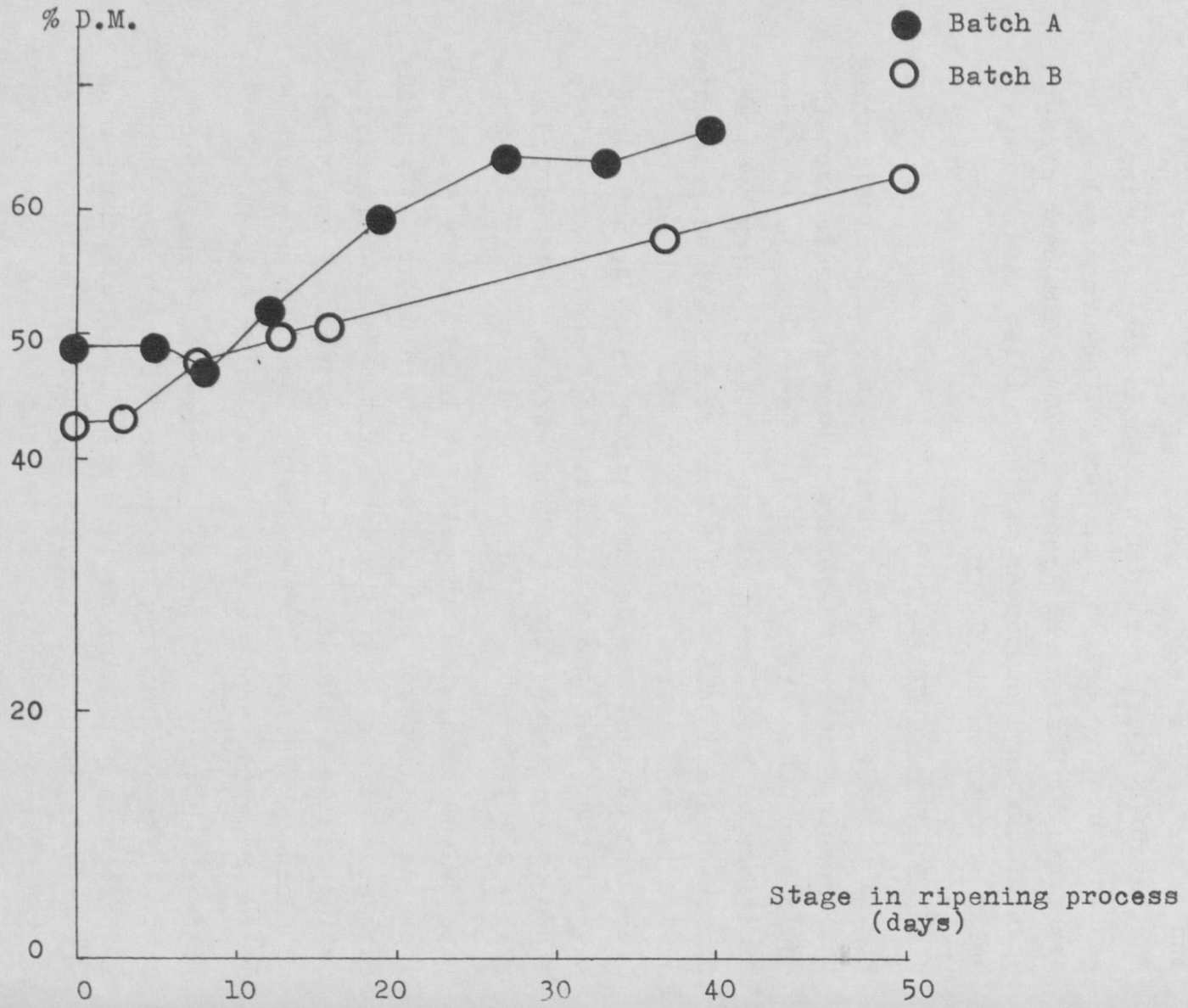
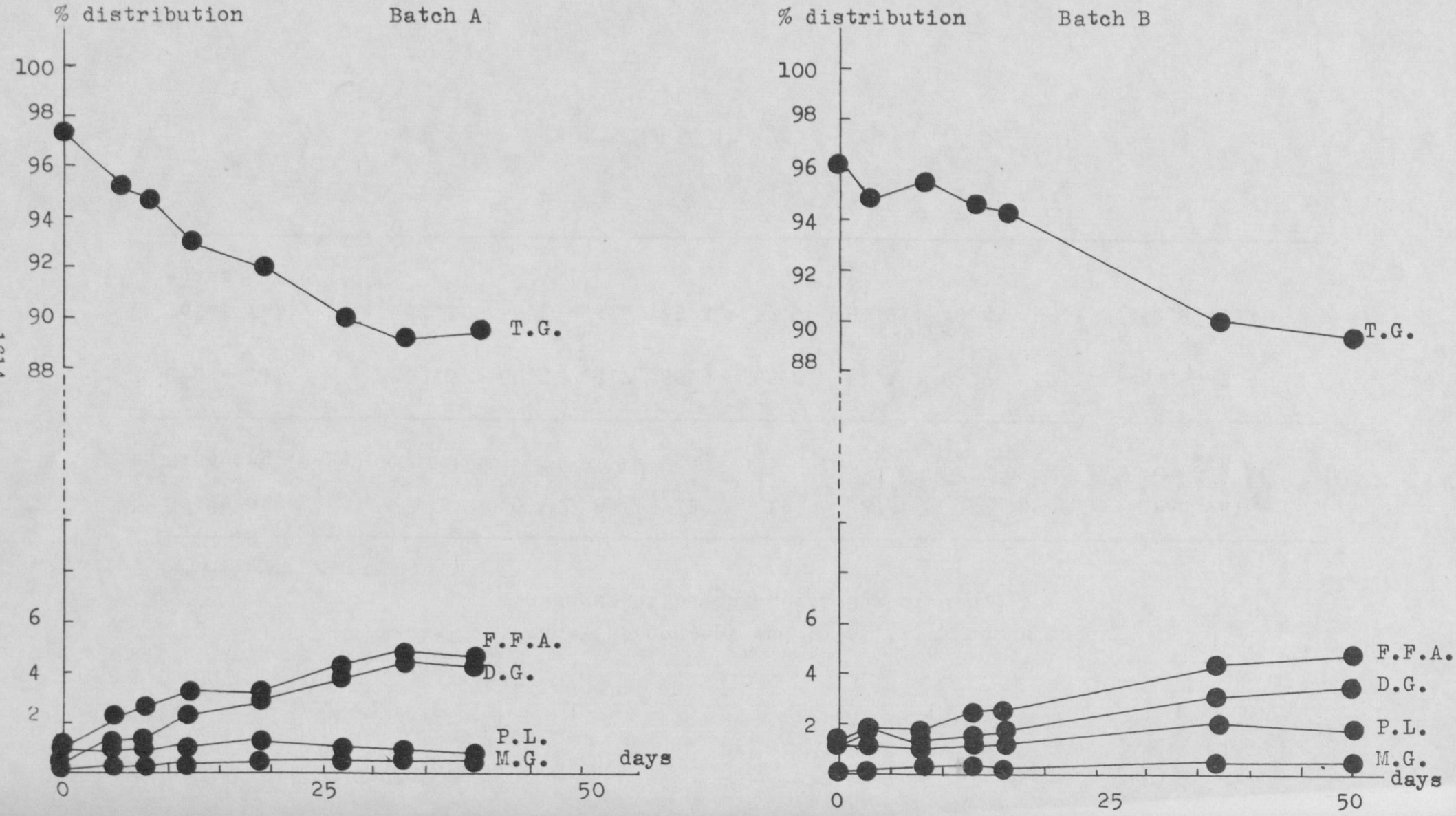


Table 2. Crude fat content and total fatty acids in dry sausages of batch A (g/100 g of D.M.)

Stage of Ripening (days)	0	5	8	12	19	27	33	40	Mean value ± S.E.
Crude fat	63.2	60.5	63.2	68.9	65.8	64.1	63.7	67.1	64.5± 2.9
Total fatty acids	51.1	56.7	54.6	49.4	53.7	51.2	46.4	58.1	52.6± 1.3

Fig. 2 Changes in fatty acid distribution over lipid classes (%) during the ripening process.

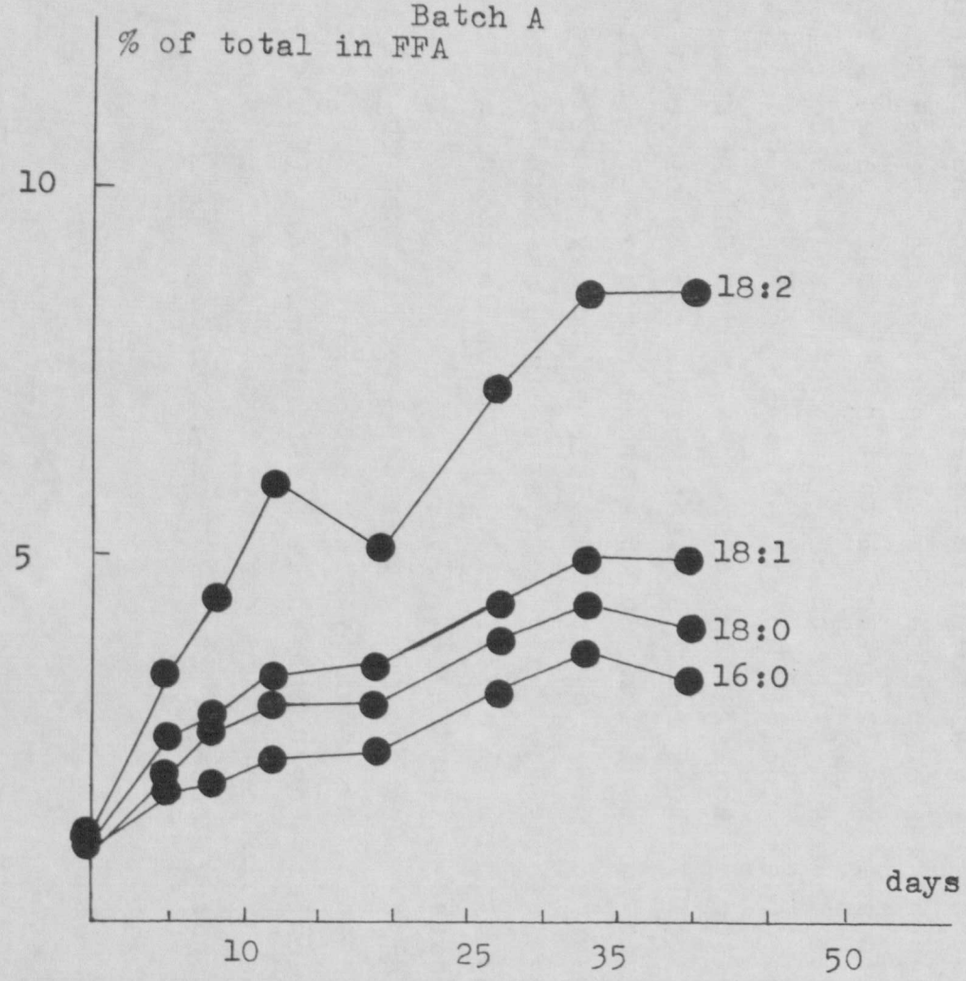


Micrococi are generally accepted to be the major group of microorganisms responsible for lipolysis in dry sausage (Cantoni et al, 1967 a) (Nurmi and Niinivaara, 1964), but evidence is available for production of lipases by Lactobacilli (Coretti, 1965) (Oterholm et al, 1968). Lipases are known to hydrolyze preferentially the outer fatty acids of a triglyceride molecule (Alford et al, 1971). The accumulation of Diglycerides, together with FFA, suggests that preferentially one of the outer positions is attacked. It is known, that pig fat triglycerides show a particular fatty acid distribution pattern, most of the stearic acid (ca 60 %) being present at position 1, palmitic acid (60 - 80 %) at position 2, and octadecenoïc acids (50 - 60 %) at position 3 (Brockerhoff, 1966). If the lipases specifically attack position 1 or position 3 bound acids, the fastest rate of lipolysis should be observed, either for stearic, acid or for octadecenoïc acids. The % distribution over the lipid classes of total palmitic, stearic, oleic and linoleic acid present was calculated for all samples and showed that linoleic acid was liberated into the FFA fraction at a faster rate than all other acids. Rate of lipolysis decreased in the order linoleic > oleic > stearic > palmitic acid. (Fig. 3) As the molecular weights of these acids only differ slightly these results clearly indicate specificity of lipolysis for position 3 of the triglycerides. The difference observed for linoleic and oleic acids may be related to a specificity for fatty acid structure, as both positional and structural specificity are known to occur in microbial lipases (Alford et al, 1971).

Diglycerides and monoglycerides are enriched in palmitic acid, as shown by their mean fatty acid composition, calculated over the whole ripening period. (Table 3)

The evolution of the carbonyl compounds during the ripening process, is illustrated in fig. 4.

Fig. 3 % of total palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2) acid present in FFA.



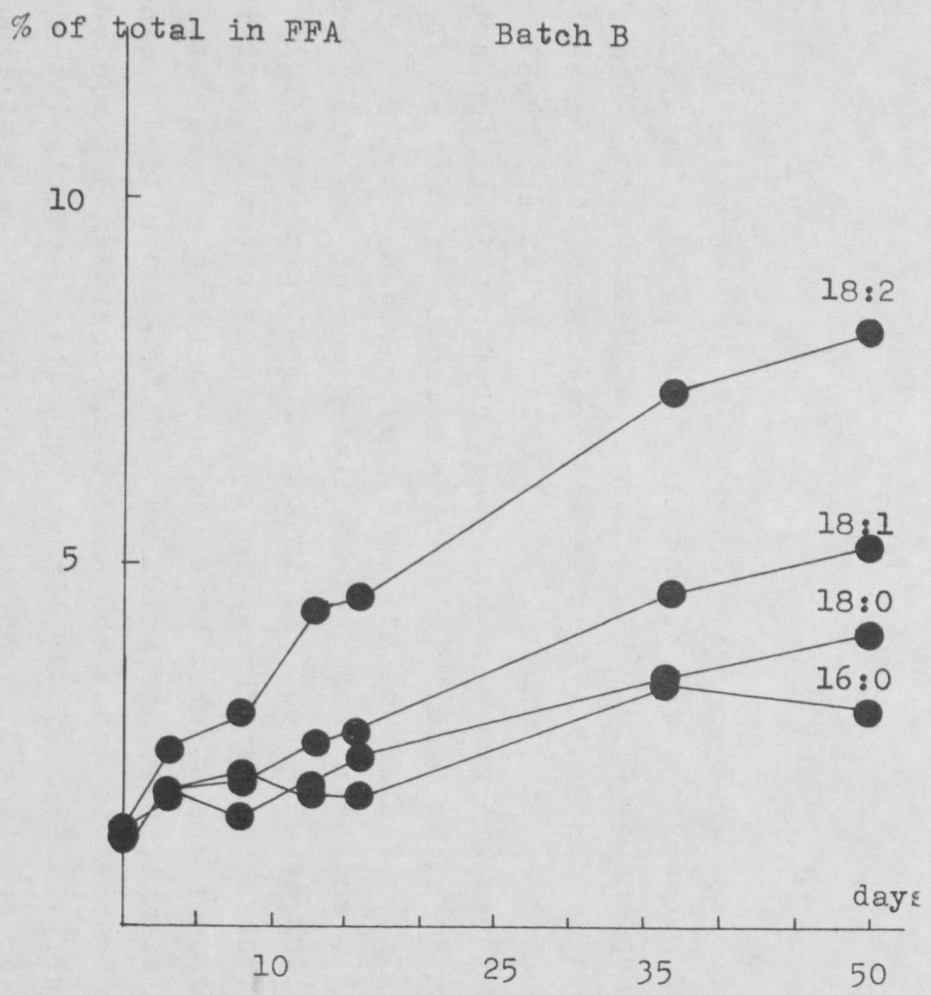


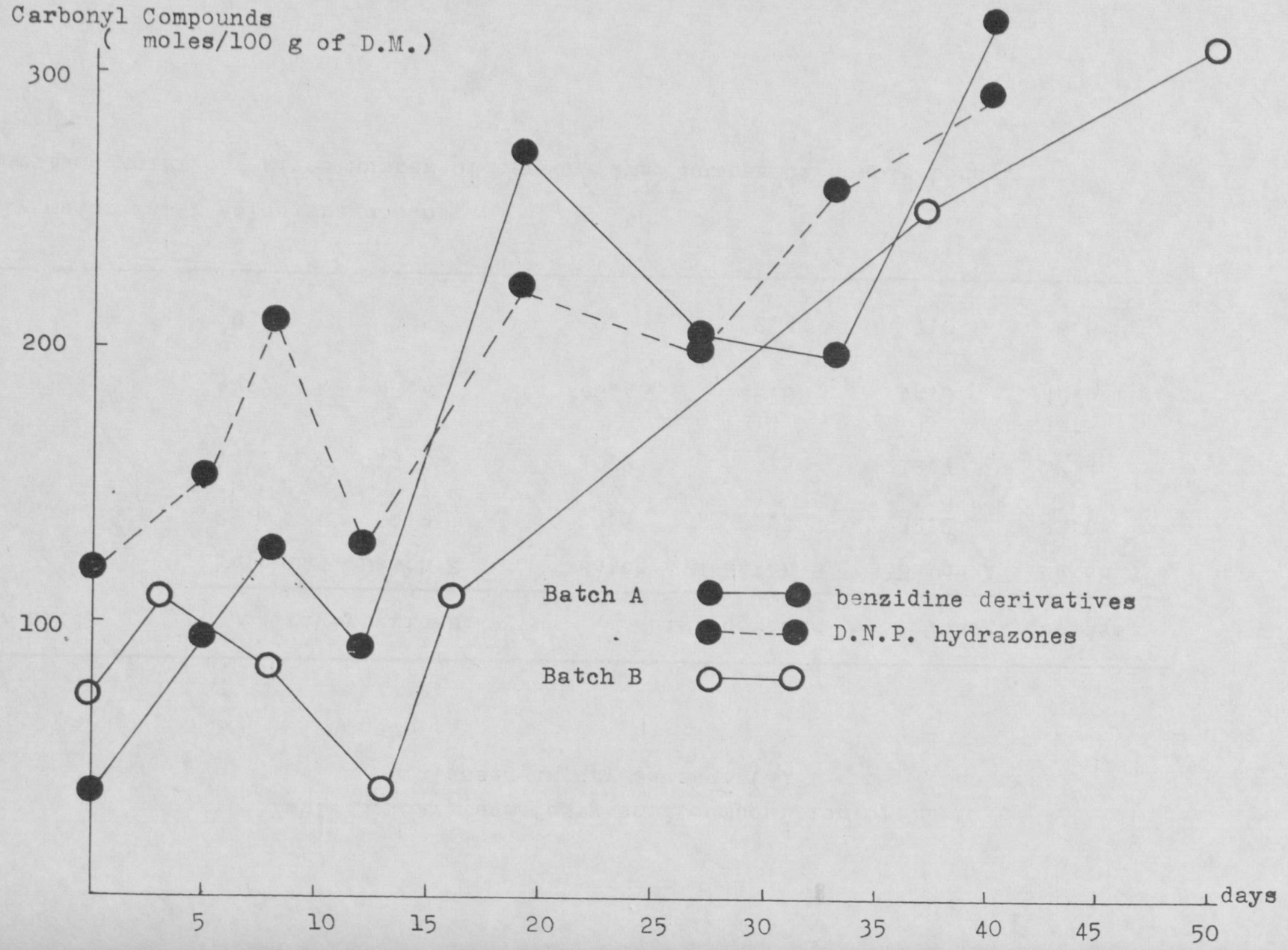
Table 3. Average fatty acid composition of lipid classes in dry sausage (%)[1].

Fatty Acid[2]	Triglycerides		Diglycerides		Monoglycerides	
	Batch A	Batch B	Batch A	Batch B	Batch A	Batch B
16:0	25.8	24.9	29.5	25.3	32.2	28.0
18:0	14.2	13.0	10.2	9.5	12.7	12.4
18:1	45.9	48.4	46.4	48.9	44.3	48.6
18:2	9.6	7.5	9.2	8.4	7.0	6.6

1 Only major fatty acids are shown

2 Shorthand notation gives number of carbon atoms: number of double bonds.

Fig. 4. Changes in Carbonyl concentration during ripening.



For batch A, results obtained using the benzidine reaction, are lower than those obtained with DNP-hydrazine, in the first week of the ripening period, indicating the presence of a large proportion of ketone compounds. Later in the ripening process, similar amounts are found using both methods. In both batches, carbonyls increase during the first week of ripening, decrease after smoking and again increase to final values of about 300 µmoles/100 g of D.M. or about 150 µmoles/100 g of sausage. This is somewhat lower than values reported by Langner (1972): 16.7 to 143.4 mg/100 g of sausage or 200 - 1400 µmoles/100 g of sausage. The initial increase is probably due to compounds formed in carbohydrate fermentation, most intensive in this period, (De Ketelare et al, 1973) while the increase in the last stages may be due to further metabolism of lipid peroxides, as suggested by Cerise et al (1973). The decrease, observed after smoking, was also reported by Langner et al (1970).

Acknowledgement:

This research was supported by a grant form the I.W.O.N.L. (Brussels - Belgium).

References.

Alford J.A., Smith J.L. and Lilly H.D. 1971. Relationship of Microbial Activity to changes in Lipids of Foods. J. Appl. Bact. 34,133.

Brockerhoff H. 1966. Fatty Acid Distribution patterns of Animal depot fats. Comp. Biochem Physiol. 19,1

Cantoni C., Molnar M.R., Renon P. and Giolitti G. 1967 a. Lipolytic Micrococci in Pork fat. J; Appl. Bact. 30, 190

Cantoni,C., Molnar M.R., Renon P. and Giolitti G. 1967 b Untersuchung über die Lipide von Dauerwürsten. Die Nahrung 11, 341

Cerise L., Bracco U., Horman I., Sozzi T. and Wuhrmann J. 1973. Veränderungen des Lipid anteils während des Reifungsprozessess von Salami aus reinem Schweinefleisch. Fleischw. 53,223

Christie W.W., Noble R.C. and Moore J.H. 1970 Determination of Lipid classes by a Gas chromatographic Procedure. Analyst 95,940

Coretti K. 1965. Vorkommen und Bedeutung lipolytischer Mikroorganismen in Dauerwürsten. Fleischw. 45,21

De Ketelaere A;, Demeyer D., Vandekerckhove P. and Vervaeke I. 1973. Stoichiometry of carbohydrate fermentation during Dry Sausage ripening. J. Food Sci. (Submitted).

Duda Z. 1966. Untersuchungen über den gehalt an freien fettsäuren in rohwürsten. Fleischw. 46,974

Hansen A.P. and Keeney P.G. 1970. Comparison of Carbonyl Compounds in moldy and non-moldy Cocoa Beans. J. Food Sci. 35,37

Holm U., Ekbom K. and Wode G. 1957. Determination of the extent of oxidation of Fats. J. Am. Oil Chem. Soc. 34,606

Langner H.J. 1972. Aromastoffe in derRohwurst. Fleischw. 52,1299

Langner H.J., Heckel U. and Malek E. 1970. Aromastoffe in der reifenden Rohwurst. Fleischw. 50,1193

Laurell S. 1957. Turnover rate of unesterified fatty acids in human plasma. Acta Physiol. Scand. 41,158

Maillet J. and Henry M. 1960. Cited by Nurmi and Niinivaara (1964).

Meinertz H. 1971. Analytical Procedures for free fatty acids. Progr. biochem. Pharmacol. 6,317

Mihalyi V. and Körmendy L. 1967. Changes in protein solubility and associated propterties during the ripening of Hungarian dry Sausages. Food Technol. 21,108

Nurmi E. and Niinivaara F.P. 1964. Lipolytic changes of Fats in Dry Sausage. Proc. 10th Europ. Meet. Meat. Res. Work. Roskilde, Denmark.

Oterholm A., Ordal Z.J. and Witter L. D. 1968. Glycerol ester hydrolase activity of lactic acid bacteria. Appl. Microb. 16,524

Stanculescu C., Sandulescu C. and Sbircea C. 1970. Variation of compounds resulted from the principal biochemical changes, on zones, during the ripening of raw Rumanian sausage. Proc. 16th Europ. Meet. Meat. Res. Work. Varna, Bulgaria, p. 1042.

Stoll U. 1972. Qualitative and quantitative Untersuchung der freien fettsauren in depotfetten und fetthaltigen erzeugnissen. Fette - Seifen - Anstrichmitt. 74, 385.

Ten Cate L. 1960. De lipasen der snijworst. Tijdschr. Diergeneesk. 85, 999.

Terplan G. 1969. Biologisch, Chemische und physikalische Vorgänge bei der Herstellung von gepökelten und gereiften Fleischwaren. Gerhard Röttger Verlag, München, Germany.

Toth L. 1970. Bestimmung des Ranziditäts grades von Fetten in Speck und Wurstwaren mit hilfe der Benzidin-Reaktion. Fleischw. 50,849

Wahlroos O. and Niinivaara F.P. 1969. Chemical changes in Lipids and Sulfur-containing substances during ripening of raw Sausage. Proc. 15th Europ. Meet. Meat. Res. Work., Helsinki, Finland, p. 240.

Wurziger J. and Ristow R. 1966. Zur beurteilung der "Sauerranzigkeit bei Rohwursten". Fleischw. 46, 971.