UNIVERSITÀ DI MILANO
ISTITUTO DI ISPEZIONE DEGLI ALIMENTI
DI ORIGINE ANIMALE

231
17

Giovanni Giolitti & Giovanna Massacra.

Lipolytic bacteria in row sausages

INTRODUCTION

The ripening process of sausages has still many unknown aspects with regards to the detailed knowledge of the controlling phae= nomena. Many investigations carried out have shown that in row sausages various species of both bacteria and yeasts can be found Niniivaara, Gianelli, Zanzucchi e Delindati, Giolitti, have thouroughly rewieved the rilevant literature also with referen= ces to the microorganisms producing the flavour; readers are re= ferred to the papers of these Authors for the bibliography.
It should be recalled that recently Cate (b) has noted the im= portance of Streptococci D in the production of the flavour and reconfirms the rapid disappearence of gram-negative rods with survival of Streptococci D up to the 16th - 18th day. The Lacto= bacilli on the other hand remain during the whole of the ripe= ning period at a more or less constant level with respect to the maximum value which is reached at about the 4th day.
In conclusion many microorganisms belonging to the most variing species ~~play~~ sem to play a role in the production of flavour, but it may be considered that the Cocci and Lactobacilli have a predominant importance.
Even though the evolution of the microbial flora in sausages is fairly clear and has fairly concordant findings, the regulation of this equilibrium and the produntion of flsvour is still not well known. With regards to the characteristics of the microor= ganisms in crude sausages it should be recalled that the produc= tion methods are different in different countries. Some methods (smoking, addition of nitrates, nitrites or a mixture of these) may have a noticeable influence on the microbial flora (e.g. in smoked sausages gram-negative bacteria disappear rapidly within two days (Cate, a) whereas in Italian salami with nitrates and no smoking procedure they survive sixteen to eighteen days

IX^th CONFERENCE OF EUROPEAN MEAT RESEARCH WORKERS
Budapest, September 4-11, 1963

(Gianelli). According to some Authors (Buttiaux) the production of lactic acid from carbohydrates electively selects Lactobacilli.

With regards to the production of flavour Coretti considered the transformation of glicides, lipids and protids of the sausage to be responsible. Later Cate(c) attributed the flavour to an increase of fatty acids which he considered due to a lipolytic action of the initial flora of the sausage and in particular to Pseudomonas, Achromobacter, Alcaligenes. At about the same time one of us (Giolitti) had advanced a similar hypothesis.

Since the production of flavour is a phenomenon of great interest concerning which the data is very imprecise, we have thought it opportune to reexamine the problem.

METHODS

Sausages Milano type especially prepared were used, containing pork and bovine meat, 3,5% salt, 0,025% $NaNO_3$, 20% total fats when prepared.

From the moment of preparation up to the 60th day of ripening the following microbiological and chemical examinations were carried out:

a) Microbiological investigations: representative samples of sausage (20 gm) including both the surface beneath the skin and the center were taken aseptically after having separated the skin with sterile forceps. Samples were omogenized in a mixer with distilled water in the proportion 1:5. Successive decimal dilution were made in salt tryptone solution (NaCl 8 gm, Tryptone 1 gm, distilled water 1,000 ml). Total counts were made on tryptose agar, Lactobacilli were counted on Rogosa,Mitchell and Wiseman medium, lipolytic microorganisms on Rath medium and microorganisms capable of growing on native proteins of pork and bovine meat on the medium previously described by one of us (Giolitti).

Counts were made after 72 hours incubation at 30°C. (for the lipolytic organisms for further 72 hours after the addition of fat with Victoria blue to indicate the liberation of fatty acids) Turner's medium was also used but it gave poor results insofar that Nile blue exerts an inhibitory effect on the moltiplication of the microbes.

b) Chemical investigations: in this first stage of our researches we have determined only the moisture content, pH, Eh, total fats and free fatty acids. The total fats have been determined accor= ding to the method of Ostrander and Dugan, in an atmosphere of nitrogen. The free fatty acids were estimated (from an aliquot of the chloroform extract, freed from chloroform by careful distil= lation of the solvent at low temperature and under reduced pres= sure, the residual beeing desolved in petrol ether boiling point 40° - 70°) at the same time with either potentiometric method (ASTM D 664-58) or with a titration with sodium ethilate 0.05N (J. Ass. Off. Agric. Chem 1927, 10, 50, 411). pH was measured with a combined calomel and glass electrode, the Eh with a combi= ned calomen and platinum electrode with saturated Kcl according to the method indicated by one of us (Giolitti, b), using the pH - Red-ox meter "Analytical Measurments".

RESULTS

Results are given in the following tables

TABLE I
CHEMICAL INVESTIGATIONS

	DAYS OF EXPERIMENT						
	1	4	10	20	30	50	60
Moisture %	57	_	40,9	39	35	33	31
Total Fats %	19,4	20,5	21,5	25	31,8	31,7	37
Sodium ethilate 0.05N per 100gm of fats	12,3	31,5	41,4	44,1	68,3	86,8	92,5
HOH 0.1N, mg per 1gm of fats (potentiometric titration	_	1,01	1,69	1,87	3,64	4,50	4,82
pH	5,60	5,40	5,3	5,38	5,40	5,35	5,35
Eh (SCE)	-30mV	_15mV	+30mV	+60mV	-120mV	+200mV	+135mV

TABLE II

Microbiological Investigations

X 1000 !

DAYS OF EXPERIMENT

	1	4	10	20	30	50	60
Total Count	3,350	20,000	155,000	185,000	125,000	57,000	72,000
Lactobacilli	1	1,650	94,000	84,000	89,000	56,000	62,000
Lipolytic bacteria	120	1,350	2,300	30,000	77,000	30,000	34,000
Bacteria growing on native pork and bo= vine meat proteines	1,245	4,000	18,500	54,000	43,000	59,000	-

- = not determinated

the figures have to be multiplied per thausand

DISCUSSION

From the results given in the tables it seems possible to draw some conclusions regarding some of the phenomena which occus in sausages.

Firstly the progressive regular increase of free fatty acids should be noted; this fact was previously observed by Cate (c) and was considered by him to be related to lipolysis due to the initial flora present in sausages: from our results it seems evident that lipolysis occurs during the whole ripening process and it is due to a flora which noticeably increases up to the 30th day. Isolates taken at various periods have shown that the lipolytic microorganisms are initially both gram-negative and gram-positive, but the former disappear within the first four days of ripening.

The gram-positive lipolytic flora remains and is prevalently re= presented by Coccaceae. There exist a close relationship between presence of free fatty acids and the number of lipolytyc bacte= ria.

The production of fatty acids (and of derivates with a clearly appreciable flavour) does not seem however to be due to a lipase action produced by the initial flora, but rather to a lipase pro= duced by cocci which actively multiply during at least half of the ripening process and which subsequently mantain a constant and elevated numerical level.

It can be considrered that liberation of fatty acids and of their break-down products has a determining effect on the behaviour of the microbial flora during ripening and may contribute to the understanding of some phenomena of ripening.

That gram-negative rods disappear during the early periods of ripening (from two to sixteen days) is confirmed by many resear= ches. With regards to the action of fatty acids on gram-negative though such action is not completeli understood, an inhibitory

action on Neisseriaceae, Enterobacteriaceae and Parvobacteria=ceaewx has been shown (Walker, Ley and Mueller, Spector, Pollock) and moreover Dervichian et Mousset have also shown that the pH of the medium (as in the case of sausages) influence the effect of the fatty acids in that the action on gram-negative take pla=ce at a lower pH than for gram-positive. It should be noted that the disappearence of gram-negative rods coincides - at least with regards to the Italian sausages unsmoxked - with a certain concentration of free fatty acids and breakdown products of the=se.

On the other hand it has been thoroughly demonstarated (Williams et Al., Kitay and Snell, Hutchings and Boggiano, Withehill et Al. Hassinen et Al.) that fatty acids are growth factors for various Lactobacteriaceae and other microorganisms: our results have shown a fairly evident relationship between concentration incre=ases of free fatty acids and the growth curve of Lactobacilli. Moreover it should be recalled that many investigations have shown fatty acids to be important as substitutes of biotin for the multiplication of Lactobacilli (Skeggs and Wright, Krehl et Al., Williams and Fieger, Axelrod et Al.).

From these first researches we consider that free fatty acids and some of the breakdown products of them have a considerable influence both on the behaviour of the microbial flora in sausa=ges and on the production of flavour.

Bibliography

Axelrod A.E., Mitz M.A., Hofmann K.: J/ Biol. Chem. 175, 265,948

Buttiaux R.:Quoted by Cate (a).

Cate, L., Ten (a):Tijdschr. Diergeneesk. 85, 743, 1960

Cate, L., Ten (b):Tijdschr. Diergeneesk. 85, 859, 1960

Cate, L., Ten (c): Tiidschr. Diergeneesk. 85, 999, 1960

Dervichian D.G., Mousset H.: Ann. Inst. Pasteur, 77, 703, 1949

Gianelli F.: Atti Soc. It. Sc. Vet. 7, 631, 633, 646, 1953

Hassinen J.B., Durbin G.T., Bernhart F.W., Arch. Biochem. 25,91, 1950

Hutchings B.L., Boggiano E.: J. Biol. Chem. 169, 229, 1947

Kitay E., Snell E.E., J. Bact. 60 , 49, 1960

Krehl W.A., Strong F.M., Elvehjem C.A.,: Ind. Eng. Chem. Anal. Ed. 15, 471, 1943

Ley H.L., Mueller J.H.,: J. Bact. 52, 453, 1946

Niinivaara F.P., Acta Agralia Fennica 85, 1955

Ostrander J., Dugan L.R., Am. Meat Institute Foundation, Bull. no. 50, 1961

Pollock M.R.,: Symposia Soc. Expl. Biol. 3, 193, 1949

Rogosa M., Mitchell I.A., Wiseman R.F.,: J. Bact. 62, 132, 1951

Skeggs H.R., Wright L.D., J. Biol. Chem. 156, 21, 1944

Spector H.,: Arch. Biochem. 11, 167, 1946

Turner R.H., J. Inf. Dis. 44, 126, 1929

Walker J.E., J. Inf. Dis. 38, 127, 1926

Withehill A.R., Oleson J.J., Subba Row Y.,: Arch. Biochem. 15, 31, 1947

Williams V.R., Broquist H.P., Snell E.E., J. Biol. Chem. 170, 619, 1947

Zanzucchi A,Delindati G.,: Ind. Ital. Conserve, 30, 167, 1955
32, 10, 1957

Giolitti G.: Arch. Vet. Ital. 11, 23, 1960

Giolitti G.: Atti Soc. Ital. Scienze Vet. 16, in press