42

127

SEVENTH MEETING OF EUROPEAN MEAT RESEARCH WORKERS
WARSZAWA, SEPTEMBER 18th to 23rd, 1961

PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF MICROORGANISMS-CRITERION OF THEIR SUITABILITY FOR BIOLOGICAL MEAT CURING.

by

Dr St. Stawicki and M.Machoń.

Polish Meat Research Institute, Warsaw
Livestock Division, Poznań.

Dr St.Stawicki and M.Machoń.

Polish Meat Research Institute-Livestock Division,Poznań.

Physiological and biochemical properties of microorganisms -criterion of their suitability for biological meat curing.

Reviewing the respective literature with regard to the microbiology of brines we more often meet with the opinion that for the improvement of organoleptic features of cured meat and particularly for the colour some groups of microorganisms are responsible/7,16,18 20,22,24,25,29,37/.

Buttiaux discerns in the brine three fundamental desirable groups of these bacteria pertaining to:micrococcus,Vibrio cost.and Achromobacter /2,7/.The greatest influence he attributes to the Vibrio saccharose-positive species Henry et al.also stress the importance of this bacterium /2,17/.

The process of salting and by the same of curing is characterized by a series of physical and chemico-enzymatic changes occuring in the meat tissue/39/.Their depends in a high degree not only on the kind of the tissue but also on the temperature,salt concentration hydrogen ions and first of all on the activity of the microorganisms.

The process where enzymatic reactions play a dominant role,the importance of microorganisms rises proportionally.For they are one of the most active re-

presentatives of these reactions.One of the most classical statements of this kind comes from Fournaud,who thinks that during the curing the quantity of microorganisms is probably the most important factor to observe in the reaction enzym/substrate.Such a relationship appears especially explicit with respect to reactions which lead to the formation of nitrites.In a brine, which contains less than 10^6 denitrifying bacteria in 1 ml.the nitrite formation is practically equal zero. Nitrite formation which practically influences the curing process of meat,begins no sooner till the quantity of denitrifying bacteria in 1 ml.of the brine exceeds one million.We may presume that the quantity index of bacteria acts similarly in microbiological processes which lead to the formation of the aroma in ham./12/.

The steadily grounded view on the importance of microorganisms in the process of meat curing became the fundamental reason for the more and more large elaboration of methods of biological curing by means of pure bacterial cultures /7,25,26,30,31,32,36,37/.Using pure cultures of specific physiological and biochemical properties under given conditions,we may obtain a product of desirable organoleptic features.

For that reason the problem of the use of pure bacterial cultures for the curing of meat,takes one of the

foremost places in the microbiological themes of the Institute.

Investigations,which have been carried out till now in the Institute have shown that the use of only one bacterial strain caused only some simultaneous improvements of organoleptic features.For instance the improvement of colour does not always correlate with the improvement of flavour or tenderness of the tissue resp. vice versa.

In the light of literature this fact seems to be perfectly comprehensible.It is difficult to require that one bacterial species should possess all the physiological and biochemical properties indispensable for the meat tissue to adopt general high organoleptic features.This could only perform a whole of microorganisms suitably selected with regard to quantity and quality.

For that reason the curing by means of so-called bacterial mixtures-although considerably more difficult- seems to be the most reasonable.

The difficulty arises from the variability of strains /5,6,11,23,33,34/ and in consequence the elaboration of a medium where the desirable microorganisms would find the best conditions for their growth and where their physiological and biochemical properties would shift in the direction advantageous for the curing process/4,13/ Using bacterial mixtures one must not disregard the phenomenon of symbiose and antagonism amid the micro-

organisms /3,4,9,10,15,21,28,38/.

Thus the work using bacterial mixtures demands first of all an exact determination of the physiological and biochemical properties of the particular strains in the mixture according to the conditions of breeding and medium.

We are of the opinion that such course of investigattions gives the only scientific basis for the appropriate elaboration of a method for the meat curing on an industrial scale.To draw conclusions as to the physiological and biochemical properties of strains only feron their appurtenance to a given species is insufficient.For such a characteristic concerns only standard media,which cannot be compared with the specific medium of the brine.Besides it is not always suitable when using strains for given industrial purposes.

Taking the a/m.into consideration and tending to the elaboration of methods for the biological curing of meat and first of all with bacterial mixtures,a special attention has been drawn to the formation of physiological and biochemical properties of the particular strains in relation to the changes of the medium and conditions of breeding.

Hereafter some examples,which confirm our opinion in this regard.

<u>Strains used in investigations.</u>

Six bacterial strains received from France from Mr

Dr Buttiaux and Mlle Fournaud were characterized.[x/] They pertained to three groups of the following symbols:

SL, S0, V0..... Vibrio cost.

2030, F_{44}..... Flavobacterium

2060..... Achromobacter

Direction of investigations.

In the present paper results obtained from investigations on the formation of phases of the growth of the afore-mentioned strains and their behaviour in retion to the ability and direction of the decomposition of the three fundamental sugars: glucose, lactose and saccharose, were taken into account.

The determination of the phases of growth was done on a medium, whose composition was near the pump pickle for hams /symbol E/.

The difference depended upon the decrease of the salt content to 6.5% what meant for the said bacteria an optimal osmotic pressure. In order to improve the growth it was carried out on a pork extract supplemented with 0.3% of yeast extract. The pork extract created conditions, which were near the medium of brine during the curing of meat.

The phases of growth for the particular strains were determined in optimal conditions of the breeding temperature namely: at 22°C for strains with symbols

2030, F_{44} and 2060 and at 30°C for SL, SO and VC/7, 12, 19/.

The biochemical properties in relation to sugar decomposition were checked on 5 media. Besides the medium used to determine the phases of growth, media generally used for this purpose were applied and prepared on pepton water supplemented with 0.5% and 6% NaCl/symbols A and B/ /5/ and on partially modified optimal media according to Buttiaux /7/ and Fournaud/12/ /symbols C, D/.

The exact composition of the media is shown on table 1.

Investigations were carried out at two temperatures. Besides the optimal ones for the afore-said particular groups of strains, there were also used for all a temperature near the conditions of curing i.e. within the limits of 6-8°C.

The determination of the phases of the growth of the particular strains in relation to the medium and temperature of the breeding has the following practical argumentation. The curing process is timely limited. Neither the temperature nor the medium of the brine, where the process is going on, favours the rapid increase of the population. From literature data as well as from our experiments it turned clearly out that positive results of curing are connected with the strain attaining an amount of cells within limits at least 10^7-10^9 per 1 ml. To get such an amount of desi-

Table 1

Index of media used

Symbol of medium	Components											
	Duplicate extract of pork meat in ml.	Yeast extract in g	peptone in g	Tryptone (Difco) in g	Tryptose (Difco) in g	Saccha-rose in g	Potassium nitrate in g	Sodium nitrite in g	Sodium chloride in g	Sodium carbonate in g	Distilled water in ml.	pH
A	—	—	10	—	—	—	0,1	—	5	0,2	1000	7,2 - 7,4
B	—	—	10	—	—	—	0,1	—	60	0,2	1000	7,2 - 7,4
C	77	3	—	—	15	20	—	—	60	—	923	7,2 - 7,4
D	637	—	—	5	—	8	8	—	65	—	363	7,2 - 7,4
E	1000	3	—	—	—	3,3	3,3	1,5	65	—	—	6,2 - 6,4

rable bacteria in the basin, one must know with regard to given breeding conditions the formation of the generation depending on the initial quantity of the used cells as an inoculum.

The analysis of the phases of growth allows beyond that an exact determination under existing conditions of the stop-phase the so-called "lag-phase", upon which at a high degree depends at a given time a strong increase of the strain. It is well known that depending on particular phases the strain has a different biochemical activity. The evolutional cycle, whose exponents are the phases of growth, is by many authors regarded as being specific for the given strain and thus an important factor not only for the determination of species but even for their particular mutants /1, 14, 35/. Practically based on the phases of growth there is easier to elaborate a right direction for the biological process of meat curing.

The biochemical investigations were exclusively confined to glucose, lactose and saccharose as sugars, which could be practically used. A special interest in their use was directed to the formation of pH of the medium.

The applied methodic of investigations.

The phases of growth were determined as follows: with 1 ml. suspension, containing 10^8 of bacterial cells 9 ml. of a brine medium E was inoculated and breeding at

an optimal temperature for the given strain was carried out.The suspension of the a/m.concentration of cells was obtained by dilution of the fundamental suspension which was acquired by rinsing with a 6% NaCl solution the breed,which grew up on a slanting constant medium.

The content of bacterial cells in the suspension was determined by two ways:by microscopical computation from dyed preparations /3/ and by quantitative dissemination.The formation of the phases of growth was checked every 12 hours by quantitative dissemination till the phase of loss distinctly appeared.Media for biochemical investigations were also disseminated with bacterial suspension obtained in the a/m.way.The breeding was conducted 5 days at temperatures of 22°C and 30°C and 10 days at temperatures of 6-8°C.After the termination of the breeding the decomposition of sugar was determined by measurements with the Danish pH-meter.

Results.

Diagram I shows the phases of growth of two strains of Vibrio.Differences between the two are distinctly evident.One of them marked with symbol VCI shows four phases of growth.Till 24 hours the phase of growth stoppage is marked,during the next 2 days the logarithmic phase,from 72-132 hours the stational phase and then the phases of losses.The maximal amount of bacterial cells is 300 millions per 1 ml.xx/

During quantitative investigations carried out every 12 hours the strain V.C.II does not show the phase of stoppage and the stational phase. After 24 hours of breeding the amount of cells attains the maximum /250 millions per 1 ml/ and then an impetuous phase of losses ensues. After 108 hours the content of living cells reaches only 5 millions/ml.

Considerable differences in the biochemical properties of these strains were also found. One the most important difference is that the strain C.C.I. compared with the V.C.II showed less ability of acidification of the medium regardless its composition and temperature of breeding.

These are typical examples of strain variances within one species which must seriously be taken into account in the biological process of meat curing.

The phases of growth presented on diagram II as to the strain F_{44} of the Flavobacterium species, are typical for the physiological changes occuring under the influence of only insignificant variances in the composition of the medium.

In the brine medium E supplemented with pepton/ table 1/ after 60 hours of breeding the amount of cells reached 20 millions and after 120 hours 10 billions in 1 ml. Replacing pepton by yeast extract caused a prolongation of the stoppage phase till 60 hours of breeding and the maximal content in 1 ml of the me-

dium was reached after 168 hours and amounted only one billion.

Diagram III shows the formation of the phases of growth of strains of the Vibrio species. It appears that the strain with symbol V.C.I. deviates with respect of its properties from the remaining two ones with symbol SL and SO. His maximal cell concentration namely in 1 ml. amounts to about 300 millions and the stational phase begins after 3 days and lasts almost 6 days, whereas the stational phase with the two remaining ones begins already after 24 hours and the maximal cell concentration in 1 ml. of the medium amounts to about 55 millions for the strain SO and 150 millions for the SL.

Diagram IV shows the phases of growth of the Flavobacterium and Achromobacter species'. Among them, too, are individual differences in growth but smaller than amid the strains of the Vibrio species.

On the whole it must be stated that strains of the Flavobacterium and Achromobacter species on the brine medium show a stronger increase /growth/ than strains of the Vibrio species bred on the same medium. For instance strains with symbol 2060 and F_{44} attain the highest cell concentration by 10 billions whereas strain 2030 -by 1 billion in 1 ml.

The behaviour of the investigated strains in relation to the decomposition of glucose, lactose and saccha-

rose depending on the medium and temperature shows table 2.

The obtainedd results allow the following conclusions:

1. None of the investigated strains showed an increase on a standard medium i.e.in pepton water supplemented with 0.5% NaCl /symbol A/.
2. All strains of the Vibrio species/symbols SL,SO,V.C.I. and V.C.II/ regardless the medium,decomposed with a strong acid formation-glucose and saccharose.Media supplemented with lactose underwent under their influence an alcalisation process except strain marked V.C.II which acidified the medium also in the presence of lactose.
3. Strains of the Flavobacter and Achromobacter species /symbols2030,F_{44} and 2060/ regardless the medium did not decompose,with the formation of acid,none of the three used sugars.They had on the other hand the ability of a strong alcalisation of the medium.
4. Some relationship between the acidification resp.alcalisation of the medium and the temperature of the breeding was found.Proportionally to the rise of the temperature both these processes became more distinct. They were weakest at a temperature of 6-8°C.

In summing up the results presented in this paper, which but are only a fraction of the current investigations it must be said,that the most important factor for the evaluation of strains as to their suitability for meat curing,is their physiological and biochemical characteristic.

x/ Acknowledgements.

We use this opportunity to express our best thanks to Mr Dr R.Duttiaux and Mlle Fournaud of the Institut Pasteur de Lille for afore-mentioned strains they have kindly given us gratuitly.

xx/ Diagrams were made basing on the quantity of bacteria obtained by dissemination by means of the quantitative method on plates.

Literature cited:

1.Bisset K.A. -The cytology and life history of bacteria Livingstone,Edinburgh /1950/

2. Borys A. -Report from a visit to France.

3.Dowling R.,Wynne E.S.-J.inf.dis.89 297/1951/

4.Duczin F.I.-Mikrobiologija 22 ,304 /1953/

5.Burbianka M,Pliszka A:Mikrobiologiczne badania produktów żywnościowych,PZWL,Warszawa /1957/

6.Burri- Zbl.Bakter.II, 28 ,321 /1910/

7.Buttiaux R.-Technique simple d'examen bacteriologique de saumures de jambon et ses résultats 2nd Intern.Symp.on Food Microb.Cambridge 137/1957

8.Byatt P.H.,Jann G.H. - J.Bact.55 787/1948/

9. Sall A.J. Charlton C. - J.Bact. 70 56 /1955/

10. Doktor W.M.,Couch J.R.- Arch.Biochem.a.Biophys.51 530 /1954/

11. Eddy B.P.- The effects of salt concentration and temperature on nitrite production.2nd Inter. Symp.on Food Microbiol.Cambridge /1957/

12.Fournaud J.et al. Étude bacteriologique des saumures de jambon de Paris.Mise au point de milieux selectifs.Principaux genres de bactéries rencontrées.V-ème Reunion des Instituts de Recherches sur les Viandes,Paris,7-12 Septemb. /1959/

13.Gibbons N.E.-The effect of salt on the metabolism of halophilic bacteria. 2nd Intern.Sympos.on Food Microbiology Cambridge /1957/

14.Hardley P.- J.Inf,Dis.60 129 /1937/

15. Harmon L.G., - J.Dairy Sci. 38 1189/1955/ Nelson F.E.

16. Henry M., Goret P., Joubert L. -Sur une bacterie hautement denitrificante des saumures des vaindes. 2nd Intern. Symp. on Food Microbiol. Cambridge, 10,4 /1957/

17. Henry M., Goret P., Joubert L. -Description et caractères biologiques d'une espèce bacterienne non encore décrite, isolée des saumures en "fermentation active" Son rôle dans l'évolution des saumures. C.R. Soc. Biol. 148 900 /1954/

18. Hornsey H.C., Mallows J.H. -Beef curing brines. I. Bacterial and chemical changes occuring in rapidly developing short life brines. J. Sci. Fd. Agric. 12,573 /1954/

19. Instruction from Lille -Typewriting received with strains in 1960

20. Iwańska W. - "Gospodarka Mięsna" 7/8,22 /4959/

21. Jakubowska J. -Zeszyty Problemowe Postępów Nauk Rolniczych 18 42 /1960/

22. Kazakow A.M. -Mikrobiologia mięsna WPLS Warszawa/1955/

23. Kunicki-Goldfinger Wł. -Acta Microbiol. 3 ,199/1954/

24. Leistner L. - Bakterielle Vorgänge bei der Pökelung von Fleisch. I. Der Keimgehalt von Pökellaken. Die Fleischwirtschaft 2 /1958/

25. Leistner L. -Bakterielle Vorgänge bei der Pökelung von Fleisch. II. Günstige Beeinflussung von Farbe, Aroma und Konservierung des Pökelfleisches durch Mikroorganismen. Die Fleischwirtschaft 4 /1958/

26. Leistner L. -Die Anwendung von Impfkulturen für Pökellaken. Sonderdruck aus "Archiv für Lebensmittelhygiene" 2 ,/1950/

27. Machoń M., Stawicki St. -Badania nad morfologią, fizjologią i biochemią szczepu 2030 stosowanego do peklowania mięsa./Just printed/

28. Nakhimowskaja N. -Mikrobiologija 8,116 /1939/

29. Niinivaara F.P. -Über den Einfluss von Bakterienreinkulturen auf die Reifung und Umrötung der Rohwurst. Zschr. f. Leb. Unt. u. Forsch. 4 145 /1957/

30.Niinivaara F.P.,Durchmen O.,Vatiovaara U. -Rohwurstherstellung unter Verwendung von Bakterienkulturen Acta Agric.Fenn.83,230 /1955/

31.Niinivaara F.P.,Pohja M.S. -Über die Reifung der Rohwurst.I.Mitteilung."Die Veränderungen der Baterienflora während der Reifung.Zschrf. f.Leb.Unt.u.Forsch.104,413-422/1956/

32.Niven Jr.,C.F. -Dacteria in Meat Processing.A.M.I.F. Circular 12,17 /1954/

33.Pelczar and Reid -Microbiology McGRAW-HILL Book Co. INCNew-York,Toronto,London /1958/

34.Penso G.,Ortali V.,Gori G.B. -Contribution to the morphological and biochemical study of halophilic bacteria. 2nd Int.Symp.on Food Microb.Cambridge /21, 1957/

35,Pohja M.S.,Niinivaara F.P. -Über die Reifung der Rohwurst III.Mitteilung.1.Über die antagonistische Wirkung eines Mikrokokkenstammes gegen die in der Rohwurst vorkommenden Bakterienstämme.Zschr.f.Leb. Unt.u.Forsch.4 106 296-301 /1957/

36.Raseta J. -Acta Veterinaria,Vo.VIII Faxc.1,Beograd1958

37.Ristić M.,Roseg D. -Ispitivanje mogucnosti poboljsanja boje bekona dodavanjem salenuri nekih mikroorganisma sa jakim reduktivnim sposobnostima.III.Sawetovanje o problemima industriaska proizwodnje mesa. 41-46,30 i 31 Oktobra /1959/ Institut za Technologji Mesa FNRJ.

38.Rogers L.A. - J.Bact.16 321 /1928/

39.Trawiński A. - Higiena i przetwórstwo mięsa.PWRiL.Warszawa,370-387 /1957