

Spoilage of sausage due to Clostridia

By

Dr. J. Takács and Dr. K. G. Narayan

Central Laboratory of Veterinary Meat Control Service Budapest, Hungary

/Head: Dr. J. Takács Candidate of Vet. Science/

10-H

The stuffing material /raw meat, spices etc/ of encased sausages may itself contain clostridia or may get contaminated with these during processing. The contamination of raw meat of the stuffing material may be external through the faeces and soil during slaughter and handling or internal through the circulatory system invaded by the intestinal flora. As the members of the genus Clostridium form heat resistant spores, produce toxins, cause food-poisoning and spoilage, the bacteriological examination of meat and meat products must include their determination too. It was decided therefore to examine the sausages for Clostridia.

Materials and Methods

Materials consisted of raw meat, ingredients, /other than meat/ semifinished and finished products of sausages.

5 g of material was suspended in 45 ml of saline and mixed thoroughly in a mortar with pestle. A tenfold dilution was made till 10^{-3} in all cases. Whenever some organoleptic changes were observed the dilution was increased to 10^{-6} . From each dilution 1,0 ml was inoculated into modified semisolid sulphite agar medium /9/.

Medium

The basic medium contained 1,0 % /w/v/ beef cream /8/, 0,5 % /w/v/ peptone /Richter/, 2,0 % /v/v/ yeast extract /1/ and 0,25 % /w/v/ agar. It was sterilized by autoclaving at 120° C for 20 minutes. The pH after autoclaving was $7,4 \pm 0,1$.

To each litre of the basic medium 0,1 % /w/v/ sodium sulphite $[\text{Na}_2\text{SO}_3 \cdot 7 \text{H}_2\text{O}/\text{SO}_2$ content ca. 23,0 %/] and 0,05 % /w/v/ iron citrate $[\text{Fe C}_6 \text{H}_5 \text{O}_7 \cdot 5 \text{H}_2\text{O}/$ were added. The solutions of these salts were freshly prepared and sterilized by filtration through G_5 or H_5 filter prior to use.

Results

Results are tabulated in table 1.

Table 1.

Percentage incidence of clostridia in sausages

Name and number of the material examined	Percentage found						
	negative	positive					
		Titre per gramm of the material					
		10^2	10^2	10^3	10^4	10^5	10^6
Raw meat 392	92,6	7,4					
Ingredients /other than meat/ 145	96,5	3,5					
Semi finished products 212	79,6	9,8	0,9	5,6	3,7		0,4
Finished products 6012	99,7	0,2	0,04	0,04	0,02		

The most interesting of all the positive materials was a liver sausage described below. It had a sour and H_2S smell with a pH 5,3. The enormous amount of gas produced in the specimen bottle raised the cork and consequently the material gushed out of it. On bacteriological examination it was found to contain 30 millions clostridia and 100 thousand Coliform bacteria/g. The total number of aerobes and facultative anaerobes was 600 millions. The *Clostridium* species was isolated and identified. The biochemical behaviour of the isolated clostridium are presented in table 2.

Table 2.

Biochemical behaviour and toxin examinations

<u>Biochemical behaviour</u>	
Glucose	Acid. gas
Maltose	Acid. gas
Lactose	Acid. gas
Sucrose	Acid. gas
D-Mannitol	No acid. no gas
H ₂ S	+
Indole	-
Nitrate reduction	+
Gelatin liquefaction	+
Blood agar	Beta-hemolysis
Motility	Nonmotile
Gram's stain	+
<u>Toxins</u>	
alpha	+
beta	-
epsilon	-

According to the biochemical behaviour and toxin production, the isolated clostridia proved to be Cl. perfringens type A. This strain did not form heat-resistant spores and so did not belong to the Hobbs' /3/ group of food-poisoning Cl. perfringens type A.

Discussion

The stuffing materials, semi finished and finished products of sausage may contain clostridia. Therefore the number and titre of clostridia should be de-

terminated besides the incidence of pathogens of the Enterobacteriaceae-family, number of bacteria which indicate faecal pollution [Coliform bacteria, Enterococcus /Lancefield Groupe D/, Proteus] and the number of aerobes and facultative anaerobes. The number of clostridia also furnishes data depending upon which the product can be judged whether it is fit or unfit for human consumption. The findings agree with the authors /2, 5, 7/ who suggested that foods with more than 100 clostridia/g are unfit for human consumption. This is further supported by our observations that the products containing more than 100 clostridia/g were usually associated with organoleptic changes. In the Hungarian sausages the number of clostridia exceeded 100/g in 0,06 % of cases only.

The liver constitutes a nutritive medium for the growth and multiplication of bacteria, particularly clostridia. The proteolytic and saccharolytic enzymes of bacteria find suitable substrates like glycogen and proteins in liver.

Therefore liver containing sausages are the most susceptible to spoilage. The saccharolytic enzymes of Cl. perfringens played a great role in the production of enormous amount of gas and stinking sour fermentation of the liver sausage reported here.

The spores of Cl. botulinum can germinate at temperatures as low as 10-12° C /6/. The salt and nitrates in the meat products do not inhibit the germination of spores and the multiplication of clostridia /4, 6/. Further, with the exception of finely chopped sausages, sausage made of chitterlings, liver sausages and head cheese-sorts, others are not to be stored and sold between 3-10° C temperature. Hygienic principles, proper cooking and cooling should be strictly followed. The meat must origin from properly rested animals, well prepared for slaughter in order to avoid the internal contamination. Strict hygiene during slaughter and processing must be followed to avoid outside faecal contaminations.

Summary

In examinations for the occurrence of clostridia in 392 samples of raw meat, and 145 of ingredients /other than meat/ used for sausage production 212 of semifinished and 6012 of finished sausage products less than 100 clostridia/g was detected in 7,4 %, 3,5 %, 9,8 % and 0,2 % cases, respectively. The number of clostridia in the semi-finished and the finished products was 100/g in 0,9 and 0,04 % cases and more than 100/g in 9,7 and 0,06 % cases, respectively. The organoleptic changes were usually associated with the samples showing more than 100 clostridia/g. One instance of stinking sour fermentation of liver sausage with ~~enormous~~ ^{sour} gas production and ~~strong~~ ^{sour} smell has been described. 300 millions of clostridia/g were detected in it. Clostridium was isolated and identified to be Cl. perfringens type A.

The necessity of examining meat and meat products for the occurrence of clostridia in the determination of bacteriological quality has been discussed. Clostridia indicate soil or faecal contamination and may cause food spoilage and food poisoning.

Порча колбасных товаров от клостридий

Я. Такач и К.Г. Нараян

Из 1 г-а 392 образцов сырого мяса, 145 образцов подсобного материала (не мясо) при изготовлении мясных изделий, 212 образцов полуготовых мясных изделий и 6012 образцов готовых для продажи мясных изделий при изучении на засоренность клостридиями удалось изолировать на питательной среде меньше 100 клостридийных бактериальных телец в 7,4-, 3,5-, 9,8- и 0,2 %-ах случаев соответственно. В образцах полуготовых и готовых для продажи мясных изделий в 0,9 и 0,04 %-ах случаев соответственно клостридийный титер равнялся 100/г-ам и тот же титер наблюдался выше 100/г в 9,7 и 0,06 %-ах случаев соответственно. Если образец содержал больше 100/г клостридий, как правило, уже органолептически удавалось выявить порчу. В одном случае в образцах одного ливерного мясного изделия при наличии большого количества других видов бактерий обнаружено 30 миллионов/г клостридий, благодаря ферментационной деятельности которых наступило кислое брожение, сопровождавшееся образованием большого количества газа и кислых запахов. Изолированный вид идентифицирован как тип Cl. perfringens A.

На основании своих наблюдений авторы приходят к заключению, согласно которому при бактериологической оценке мяса и мясных изделий считают нужным и изучение засоренности клостридиями. Наличие бактерий, указывая на засоренность продукта почвой, играет роль не только в порче мяса но может быть причиной заболевания потребителя.

Zusammenfassung

Im Laufe der bakteriologischen Untersuchungen auf Clostridien gelang es bei 392, aus zur Herstellung von Fleischerzeugnissen bestimmtem Rohfleisch, entnommenen Proben, 145 Zusatzstoffen /ohne fleischlichen Ursprung/, 212 halbfertigen- und 6012 verkaufsfertigen Fleischprodukten in 7,4 %, 3,5 %, 9,8 % und 0,2 % der Fälle, in bezug auf 1 g Untersuchungsmaterial, weniger als 100 Clostridien auszuküchten. In den Proben der halbfertigen und verkaufsfertigen Fleischprodukte stellten die Verfasser in 0,9 und 0,04 % der Fälle einen niedrigeren Clostridientiter als 100/g fest, sie fanden mehr als 100 Clostridien/g in 9,7 und 0,06 % derselben Proben. Wenn eine Probe mehr Clostridien enthielt als 100/g, waren in der Regel organoleptische Veränderungen vorhanden. In einer Leberwurst-Probe waren - mit anderen in grosser Zahl vertretenen Bakterien zusammen - 30 Millionen Clostridien/g anwesend, die mit ihrer Fermentbildungstätigkeit, unter Entwicklung von grosser Gasmenge und sauerem Geruch, einer sauren Gärung beitrugen. Die isolierte Clostridienart erwies sich bei der Identifizierung als Cl. perfringens typus A.

Nachher kam es zur Diskussion, dass zum Zweck der bakteriologischen Beurteilung von Fleischerzeugnissen auch auf das Vorkommen der Clostridien, Rücksicht genommen werden soll, inwieweit diese Keime die Darmkot- und Bodenkontamination bezeichnend, nicht nur im Verderben der Lebensmittel, sondern auch im Schaden an der Gesundheit des Verbrauchers Rolle spielen.

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