MICROFLORA OF CHINESE-STYLE SAUSAGE AND THEIR BIOCHEMICAL CHARACTERISTICS

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INTRODUCTION

Chinese-style sausage is one of semi-fermentation sausages.The ingrdients, manufacturing conditions, and flavor of the products are different from those of the sausages produced in other areas. Little information about microbial ecology and distribution of Chinese-style sausage is available. However, no any desirable strains of microorganisms are used in sausage manufacturing(fermentation).Therefore, isolation,identification of microorganisms were carried out to study microbial ecology of the sausage and the action of the isolated and selected organisms on sausage. The desirable microorganisms are used as a starter culture for manufacturing Chinese-style sausage and controling quality of the products.

MATERIALS AND METHODS

Sample for screening
Sausage products were collected as the samples for screening the organisms from the northern,central and southern parts of the island.
Isolation and identification
all the samples were determined the total aerobic count by concentional plate techniques using plate count agar(Difco) and incubated at 37°C for 48 hours.The fungi were enumerated on potato dextrose agar and incubated at 25°C for 5 days. From each plate, the colonies were picked at random and propogated on the special media for isolation and identification of organisms.
Micrococci and Staphylococci were enumerated on Mannitol salt agar and incubated at 30°C for 3 days. Total anaerobic agar and lactic bacteria were enumerated with Brewer anaerobic agar and APT agar & incubated at 37°C for 2 days.

Biochemical test for identification
All isolates were examined for Gram reastion morphology and motility(phase contrast microscope) and biochemical characteristics.
Alcohol utilization was determined with Carr's medium described by Paul (1980), and incubated at 37°C for 7 days .VP test,motility,nitrate reduction,sugars utilization,proteolysis(gelatin),and lipolysis(tributyrin) were determined according to the procedures described by Jean(1976).
Test organisms
Eight strains of organisms obtained from CCRC(FIRID,Taiwan) and 10 strains isolated from sausage were used as testing organisms.10^7-10^8 cfu/g were inoculated in the aseptic Longissimus dorsi of pork and incubated at 35° C (1,2,3 days), 25°C(3,7 days) and 7° C (7-21 days).
The inoculated pork was determined pH value,microbial counts, amino nitrogen ,and electrophoretic pattern of muscle proteins.
Preparation of sausage
A formula of lean:fat ratio at 3.5:1, containing NaCl 1.5%,sucrose 8%, MSG 0.8%,pepper powder 0.1%, five spices 0.1%,$NaNO_2$ 0.01%, and rice wine 3 % which were based on the weight of pork was used to prepare sausage for chemical analysis.
Chemical analysis
5 grams sample was blended with 50 ml of dist.water for 30 seconds used for pH determination.
Amino nitrogen was determined according to Sorensen method from A.O.A.C. Acid value was measured by the procedure of SDS-polyaclamide gradient gel decribed by Laemmli(1970) was used to measure the action of microorganisms on the protein in the sausage. Ethanol and organic acids were determined by enzymatic method of Boehringer Mannheim , GmbH, Mannheim,W.Germany. Volatile compounds were distilled by the rotating evaporator and absorbed with dichloromethane, and the dislillate was analyzed by Gas Chromatography.

RESULTS

Microbial counts
The microbial growth on the sausage products in Taiwan were varied with

localities,processing conditions and raw materials.Generally,the microbial counts were at range from 10^5 to 10^8 cfu/g.The microbial counts in the sausage obtained from the southern part were higher than from the nouthern and central parts. This may be due to the southern part is higher ambient temperature and humidity which were better for bacterial growth.

799 strains of organisms isolated by mannitol salt agar and APT agar were Gram(+),their physiological characteristics was shown in Table 2. 99.5% of these organisms were cocci, and only 0.5% were rods in morphology. Among them,Staphylococci were detected in the proportion of 28.4%, Micrococci were 46.3% , and lactic acid bacteria were 25.3%. This phenomena of microflora differed from western-style fermented sausage, in Chinese-style sausages the processing is accomplished in a very short time. Thus, Micrococci became a dominant of microflora in this experiment. The result agreed with the report of Savić et al(1988). As Lücke (1986) reported that Micrococci were the dominant at first day during fermentation in the fermented sausage, at this time, lactic acid bacteria were still not proliferated.

In addition, the diameter of Chinese-style sausage was small in size(approximately 30 mm) and the product was punctured & hung in the air for drying. This conditions of processing and storage were favor for micrococci growth. This were 35.2% of this organism having reducing nitrate ability,45.5% of the organism having lipolyzing ability and 73.3% of the organisms able to hydrolyze protein. However, very few of the isolates were capable of conversion ethanol to acetic acid, only 1% were detected (Table 3). This result indicated that most of the isolated organisms were unable to utilize ethanol.Thus, it may be noted that souring of sausage was not caused by the organisms fermenting ethanol (added wine).

A considerable difference in utilization of sugars such as fructose,lactose,sucrose,dextrose and maltose by the isolates was noted(shown in Table 4). Most of the isolated organisms were capable of utilizing fructose,sucrose, dextrose,and maltose more than lactose. As Acton et al(1977) reported : this might be due to the higher molecular wt of sugar to be converted into glucose -6-phosphate or fructose-6-phosphate requiring more metabolizing energy. However,the ability to utilizing the sugars was also influenced by strains and their enzyme system transporting ways.

49 strains from 200 strains isolated lactic acid bacteria could produced acetoin,and 73 strains could reduced nitrate,and only 7 strains had both abilities(Table 5). The further use of this 7 strains in the sausage making needed more work.

The action of the isolates on muscle protein

The 18 strains of test organisms obtained from CCRC and the products from local market,were inoculated in the aseptic pork,and incubated at 35°C, 25°C and 7°C. Microbial numbers of the sample incubated at 35°C increased to 10^9 - 10^{11} at the first day,and declined to 10^7-10^{10} at 3rd day.The microbial numbers of the sample incubated at 25°C increased to 10^8-10^{10} at 3rd day, and declined to 10^8-10^9,then tended to be stable while the number was 10^7-10^8 at 7th day,and then declined to 10^4-10^7. Pseudomonas fragi grew very well at 7°C, and the number was log 9.47 at 7th day and kept growing until 14th day.Micrococcus varin, P.fluorescens, Staphylococcus and M.rosens, could more adapt to the low temperature condition than other species could.L.plantarum and P. acidilactics were inoculated and incubated at 35° C,and found that the pH value tended to decline. The acid may be produced at this time,and P.fragi grew slower,the pH was between 6.22 and 7.47 and the at 25°C the microbial growth had the same trend. However , P.fragi grew at 25°C for 3 days the pH increased to 8.11,but at 7°C for 14 days the pH reached 8.00. The increase in pH might be due to the microbial counts declined from log 9.39 to 5.0. It was absorved in the effect of incubation temperature on the microbial growth from the amino nitrogen content. When the bacteria grew at 7°C,the amino nitrogen producing rate was slower,but different from the bacteria grew at 25°C and 35°C. This aspect may be caused by the mesophilic bacteria such as lactic acid bacteria

spp. and Micrococci spp. action on muscle protein,and produced more amino nitrogen.
The abililty to attack the muscle proteins could be detected from the electrophorotogram. No remarkable change was detected in the sarcoplasmic protein fraction on the electrophoretogram. However, myofibrillar proteins were utilized preferentially by the spoilage bacteria - P.fragi and P.fluorescens. The result was found the components of higher molecular weight disappeared or degradated and produced lower molecular weight components on the electrophoretogram(fig. 1).
The moisture content of the sausage prepared in lab was lowered from the initial level of 34 % to 21 % in the first week storage, and then remained at 15 % after 2 wk storage. The product was hung in gas atmospheres without packaging. Therefore, the moisture decreased with the storage time extended. The pH values of the product changed remarkably, within the first week of storage,from 6.70 declined to 6.04,and reached to 5.88 after 3 week storage. Increasing rate of amino nitrogen was not fast, and ethanol content did not change considerably. Acid value of the sausage inncreased with the storage time increased and all the values were higher than the initial value of raw meat (see Table 6).Lactic acid content also increased from 0.07 g/100g,approximately double of the initiail content. The increase in lactic acid and fatty acid content and decreased in moisture content may be a major cause to bring pH value of the sausage declined. It was also noted that a significant difference in volatile flavor compounds concentration between the products before drying and after drying. Many volatile flavor compounds increased significantly after the product dried.(see fig. 2)

DISCUSSION

Taiwan is located on higher temperature and humidity subtropical area which is favor to the microbial growth.Thus, the sausage products contain higher bacterial counts. The variation of the distribution of microorganisms on the sausages depends on the localities.

It was found that the micrococci were the dominant of the microflora on the sausages produced in Taiwan. Their ability to grow faster than the competing species lactic acid bacteria. The additions with high levels of sucrose and rice wine are some of the charactiristics of Chinese-style sausage produced in this island.
The acids are not produced too much became of the natural and shout time fermentation for the sausage processing. However,the acids do not orgin from the wine which is added into the products. It was observed that the isolates were able to utilize ethanol.
The acidic flavor or tangy flavor were still not acceptable to the consumers in Taiwan.Therefore , it is necessary to select the organisms with lower ability to produce acids,and homofermenting.

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Fig 2. Gas Chromatography of sausage. (——: 1 day after curing, ---: 21 days after drying)

Table 1. Microorganisms isolated from the sausage produced from the different parts of Taiwan.

Bacterial group	North	Middle	South
Total aerobic flora	1.1-201	18.4-53	43.6-830
Total anaerobic flora	5.0- 78	0.2-37	11.2-550
Mold	1.0- 93	1.0-17	3.1-330
Micro.&Staphy.	3.0-158	3.0-52	1.1-230
Lactic acid bacteria	5.0-116	10.0-17	10.0-8

colony x 10^6

Table 2. Physiological characteristics of 790 strains isolated from sausage.

	No. of strains	%
Staphylococci	225	28.4
Micrococci	365	46.3
Lactic acid bacteria	200	25.3
Cocci	786	99.5
Rods	4	0.5
Motility	0	0

Table 3. Sensitivity of some enzymatic activity test of isolates from sausage.

No. of strains	225	365	200	790
No. & %	Staphy.	Micro.	L.A.B.	Total
Nitrate reduction	66/29.3	149/40.8	63/31.5	278/35.2
Lipolysis	121/58.2	155/42.5	83/41.5	359/45.5
Proteolysis	166/73.7	244/66.8	169/84.5	579/73.3
Alcohol oxidation	0/0	4/ 1.1	4/2.0	8/1.0

Table 4. Utilization of sugars by isolates from sausages. (No.&%)

No. of strains	225	365	200	790
No. & % Acid from:	Staphy.	Micro.	L.A.B.	Total
FRUCTOSE	202/89.5	294/80.5	143/71.5	639/80.5
LACTOSE	78/34.7	149/40.8	88/44.0	315/9.9
SUCROSE	204/90.7	255/69.9	121/60.5	580/73.4
DEXTROSE	200/88.8	340/93.2	156/78.0	716/88.1
MALTOSE	212/94.2	291/79.7	159/79.5	662/83.8

Table 5. Production of acetoin & nitrate reductase by L.A.B.isolated from sausage.

	positive No.
acetoin production	49
Nitrate redustase	73
Acetoin production) Nitrate redustase	7

Table 6. Changes of acid value, amino nitrogen, ethanol, lactic acid and pH of the sausage during storage.

	before curing	1 day after curing	after drying			
			0d	7d	14d	21d
Moisture (%)		55	34	21	15	15
Acid value	23.00	25.24	36.07	45.07	46.56	55.72
Amino nitrogen(mg/g)	1.4	1.4	2.2	2.9	3.0	3.4
pH	6.77	6.78	6.70	6.04	6.03	5.88
Ethanol(g/100g)	0.39	0.21	0.18	0.22	0.22	0.22
Lactic acid(g/100g)	0.07	0.07	0.08	0.11	0.15	0.19
Malic acid	0.08	0.09	0.08	0.08	0.08	0.08
Formic acid	0.05	0.04	0.05	0.04	0.04	0.05

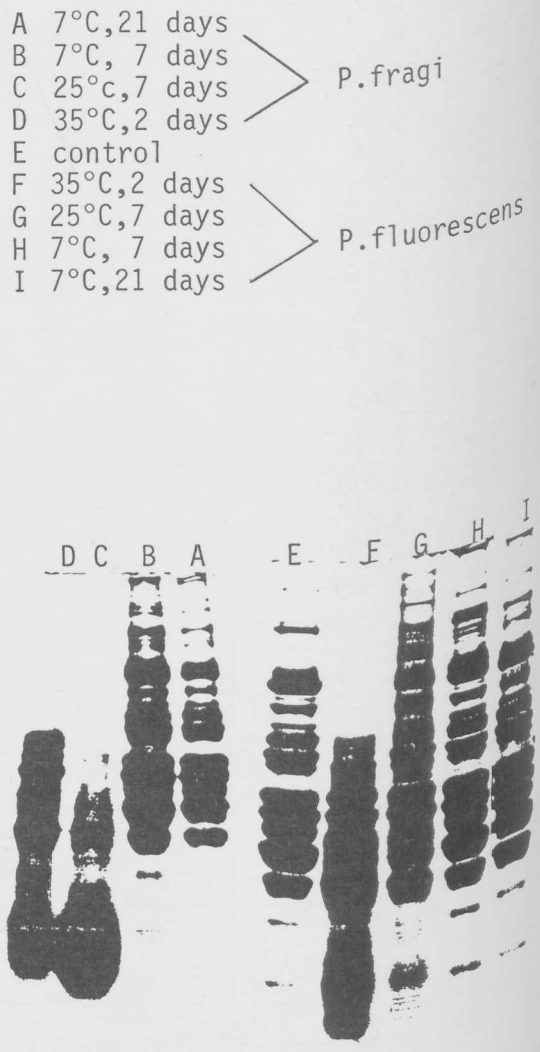

Fig 1. Acrylamide grdient Gel for myofibllar protein from M.Longissimus dorsi after inocubated with *Pseudomonas fragi* and *P.fluorescens*.