Optimization of Processing Conditions for Fermented Hams

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INTRODUCTION

Fermented meat products (sausages, hams etc) are appreciated in many parts of the world for their attractive flavor and good stability (Frey, 1981; Bartholomew and Blumer, 1977a; Gotoh, 1981). The manufacturing process for these products is based on salting, drying and fermentation (maturation). Apart from the development of the characteristic flavor,the main effect of fermentation is the lowering of pH, through the action of relatively salt-resistant lactic acid bacteria. The resulting acidity is essential for the control of pathogens and spoilage microorganisms (Bartholomew and Blumer, 1977b; Bacus and Brown, 1981). When maturation is carried out through the microflora naturally occuring in the meat, the process is lengthy and often unpredictable. Maturation periods of 3 to 6 months are mentioned (Eakes and Blumer, 1975; Christian and Blumer,1971), while even longer periods of up to 18 months seem to be necessary for the maturation of Parma hams, according to Baldini and Raczynski (1979). Faster maturation may be achieved through inoculation with starter cultures, but even then the necessary processing time is 7 to 8 weeks at least (Bartholomew and Blumer, 1980a). An alternative process, widely practiced in industry, is based on artificial acidification of sausages, mainly with glucono-delta-lactone (GDL), as described by Nestorow et al. (1982), Emerson (1981), Kissinger (1978) and others.

The purpose of our work was to study the effect of various starter culture-substrate combinations on the

maturation of fermented raw hams, the ultimate objective being the development of an industrially feasible method for the manufacture of this product at the minimum processing time and with good results as to final product quality.

MATERIALS & METHODS

Preparation of the Hams

Twenty two hams weighing 2-3 kg each were taken at random, out of a shipment of chilled (6-8^{o}C) young pig carcasses received at a commercial meat processing plant, 24 hours after slaughter. Fat and sinew were trimmed so as to leave only a subcutaneous fat layer of 3 mm. The hams were coded according to the treatment they were going to receive (see Table 1.). After measuring the tissue pH, the hams were injected with the appropriate type of brine at the rate of 7% by weight. The brines, prepared with tap water, containd 20% Nacl, 15% sugar (sucrose, glucose or lactose), 0.15% $NaNO_2$, 0.4% Na erythorbate and 20% starter suspension. In one variation the brine contained 14% glucono-delta-lactone (GDL) and no starter. Each treatment was applied in duplicate.

Four different freeze-dried starter cultures, obtained from Laboratorium Wiesby GmbH, Niebul, W. Germany were used for the preparation of the starter suspensions. - These were:

Culture No. 1 : Lactobacillus plantarum L 2-1

Culture No. 2 : Pediococcus pentosaceus P 8

Culture No. 3 : Micrococcus Kristinae-Varians

Culture No. 4 : Mixed cultures LPM 1 (a commercial starter containing L. plantarum and M. kristinae-varians).

The suspensions were prepared as follows: 5g of the freeze-dried culture were aseptically transferred into bottles containing 100 ml APT broth (Difco) and indubated in a shaker bath (30^{o}C, 100 stroked per minute) for 24 hours, as described by Bartholomew and Blumer (1980a), then diluted with the respective brines

so as to give the following concentrations when injected into the hams (in cells per g. of meat): Lactobacillus 2.6 x 10^7, Pediococcus 1.05 x 10^7, Micrococcus 3.9 x 10^7, LPM 1 2.6 x 10^7 Lactobacilli and 4.5 x 10^7 Micrococci.

After injection, a dry mixture consisting of 80% NaCl, 19.3% sugar (sucrose, glucose of lactose, according to the type of sugar used in the brine), 0.4% Na erythorbate and 0.3% $NaNO_2$ was applied to the surface of the hams at the rate of 35 g per kg meat. The hams were then put into separate polyethylene bags, stacked and stored at 6°C. After 3 days, the hams were weighed and a second dose of the dry mixture was applied at the same rate. On the ninth day the hams were removed from the plastic bags, folded in two and put into mesh sleeves so as to form cylindrical pieces. The sleeves were tightly tied on both ends, dipped into a 10% potassium sorbate solution(to prevent mold growth) and hung at 6°C. At the eleventh day the hams were smoked at 28°C, 75% relative humidity for 24 hours, then allowed to dry at 17°C and 70% relative humidity.

Analytical Procedures

Sampling: In order to determine penetration, cylindrical samples were cut from the hams by introducing into them a sharp-edged tube. The cylinders were then cut into 1 cm thick sections representing various depths within the hams. Moisture and salt were determined according to AOAC (1980). Nitrites and nitrates were determined as per Israel Standards ISO 2918 and ISO 3091 respectively.

Tissue pH was measured using a digital pH meter (Knick Portames 351) equipped with a sharp-tip cobined glass electrode. The values given are the average of readings taken in three different locations, as recommended by Hofmann (1979).

Water activity was measured using a Lufft hygrometer, as described by Rödel et al. (1979).

Sensory Evaluation

On tne 39th day, hams were finely sliced and kept under regrigeration until tested a few days later.

The tasting panel consisted of ten meat processing plant workers. Panel members were instructed to evaluate all the samples as to the following attributes according to Bartholomew and Blumer (1980b) and Kemp et al. (1981): saltimess, sourness, juiciness, aged flavor, color and overall acceptability.

Microbiological Determinations

Bacterial counts were made following Johnston and Eliott (1976) with minor changes. Plating media and incubation conditions were:

For total count - APT agar, 30°C, 48h ;

for enterococci - M Enterococcus agar, 37°C, 48 h ;

for Lactic acid bacteria - Rogosa SL agar, 30°C 48-72 h ;

On this last medium, white colonies were counted as Pediococci and yellow ones as Lactobacilli. The difference between total and lactic acid bacteria served as an estimation of the number of Micrococci. At the same time, Micrococci were also counted on plate count agar containing 5% salt, as recommended by Baross (1976). these colonies were yellow in agreement with Baird-Parker (1979).

RESULTS AND DISCUSSION

The different parameters associated with ripening are given in Table 2, for the various hams on the 39th day. Table 3 gives the microbial counts at the 7th and 39th day.

The sensory evaluation scores are summarized in Table 4.

The strongest and fastest pH decrease occured in the Lactobacillus-glucose combination, in agreement with Paradis and Mungal (1980), and with glucono-deltalactone. The Pediococcus inoculated hams, however, showed smaller and slower pH decrease, in spite of the fact the respective bacteria counts were not essentially different.

The criteria for satisfactory ripening of fermented raw hams are a weight loss of at least 18% (Kemp et al., 1981), pH 5.2 and a_w 0.95. These conditions were met by all the treatments tested, within a processing period of 39 days. This is considerably shorter than the usual processing time of raw hams reported in the literature.

The rate of weight loss was faster when the pH also decreased more quickly and stayed close to the isoelectric point (pH 5.1 for actinomyosin) or elow it (Reimers, 1982).

Measured water activity usually matchd the values calculated in the light of composition, following the equation proposed by Demeyer (1979). Deviations from the equation were larger in the case of the combinations containing glucose and GDL(due to the stronger water activity reducing effect of these compounds in comparison with disaccharides).

Measurements of salt, nitrite and nitrate conecentrations at various depths and different periods led to the following conclusions:

a) - The ratio of "total nitrite-nitrate" content (the sum of nitrite and nitrate expressed as nitrite) to salt concentration was essentially constant, indicating similar diffusivity for all these substances. Thus, salt content alone may be taken as an approximative indication of nitrite-nitrate penetration.

b) - The concentration of salt near the surface was considerably lower when external fat tissue was present.

c) - Salt concentration gradients disappeared rapidly. Equalization was complete by the 18th day. This initial period comprises treatments at relatively high temperatures (smoking at 28°C and ripening at 17°C). Yet spoilage did not occur, apparently due to the rapid decrease of water activity and pH as explained above.

d) - The combined average nitrite-nitrate content of the hams decreased approximately by 80% after 7 days, and by 88% after 39 days. This is in agreement with findings by Lee et al. (1978). At the end of the processing period of 39 days, more than 80% of residual nitrite-nitrate was in the form of

nitrate, substantiating the argument against the use of nitrate in this type of products. The nitrate- reducing capacity of Lactobacillus and Micrococcus was found to be significantly higher than that of Pesiococcus.

e) - The average coefficent of diffusion of salt in meat, calculated assuming unsteady state diffusion in a slab, was 4.98×10^{-6} $cm^2 sec^{-1}$.

f) - Results of microbiological tests confirm essentially the dynamics of microorganism growth described for fermented sausages (Johnston and Eliott, 1976) and fermented hams (Johnson, 1980). The main exception was the high incidence of Enterococcus (up to 10^5 per gram in the final product). Nevertheless, none of the hams showed any sign of spoilage. In this connection, it is appropriate to note that according to ICMSF (1978), many lactic acid bacteria frequently grow on media such as the one used for counting Enterococci.

The results of sensory evaluation indicate a marked preference for the following combinations: Lactose-Pediococcus, glucose-Lactobacillus, glucose-Pediococcus, lactose-Micrococcus ane sucrose-mixed cultures. Thus, it seems that in the formulation of fermented raw ham glucose, lactose and cultures of Lactobacilli and Pediococci should be included. A combination of the two bacteria mentioned is already used commercially (Paradis and Mungal, 1980a). The importance of Micrococci was not confirmed, in agreement with Bacus (1982). The mean overall acceptability score for the sucrose formulations (3.72+1.44) was significantly lower than those of glucose (4.37+1.16) and lactose (4.30+1.33) at p 0.01 and p 0.05. respectively.

The reaction of the panel with respect to saltiness seems to indicate that the application of salt (as the dry mixture) should be somewhat reduced, say to 6% instead of 7%.

In conclusion, good quality fermented hams can be produced in a total processing time shorter than 40 days, using the techniques of inoculation and processing described.

TABLE 1: *Starter Cultures and Sugars used in the hams*

No.	Ham Code	Sugar	Starter
1	GL	Glucose	Lactobacillus
2	SL	Sucrose	"
3	LL	Lactose	"
4	GP	Glucose	Pediococcus
5	SP	Sucrose	"
6	LP	Lactose	"
7	GM	Glucose	Micrococcus
8	SM	Sucrose	"
9	LM	Lactose	"
10	SX	Sucrose	Mixed cultures
11	SD	Sucrose	None(1)

(1) In this variant (Code SD) the brine contained 14% glucono-delta-lactone and no starter.

TABLE 2: Various parameters associated with maturation as measured on the 39th day of processing

Ham code	pH drop	Weight loss%	% Moisture	% NaCl	a_w measured	a_w calculated
GL	1.60	27.8	53.8	7.90	0.873	0.894
SL	1.04	23.1	61.3	7.05	0.905	0.912
LL	0.87	23.1	56.9	7.37	0.894	0.902
GP	0.65	23.5	60.6	8.01	0.885	0.902
SP	0.86	24.7	57.2	6.96	0.901	0.908
LP	0.71	23.1	52.4	6.97	0.906	0.902
GM	0.67	26.7	55.3	8.38	0.878	0.888
SM	1.22	24.6	60.4	6.48	0.916	0.917
LM	0.68	26.1	51.5	7.79	0.874	0.886
SX	1.11	25.3	60.0	7.07	0.909	0.910
SD	1.13	28.9	53.1	6.82	0.888	0.904

TABLE 3 : Results of microbial counts (log units of cells per gram of meat)

Micro-organism	Total Plate Count			Lacto-bacilli			Pedio-cocci			Entero-cocci			Coli-forms			Staph. aureus			Clostri-dium perfrin-gens			Micro-cocci (estimate)			Micro-cocci on TPC agar+5%NaCl
Set	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	III
GL	7.03	7.49	7.31	6.93	7.73	7.39	< 3	< 3	< 3	2.01	5.66	5.44	<1	<1	<1	1.88	1.88	< 2	<1	<1	< 1	6.34	-	-	5.04
SL	7.57	7.41	7.50	7.57	7.42	7.51	< 3	< 3	< 3	1.85	<1	3.88	1.30	0.88	<1	<1	<2	< 2	<1	<1	< 1	-	5.78	5.87	6.49
LL	7.41	7.42	7.75	7.35	7.45	7.79	< 3	< 3	< 3	<1	<1	4.95	0.88	1.10	<1	<2	3.74	1.88	<1	<1	< 1	6.52	-	-	6.28
GP	7.69	7.56	7.47	<3	<3	<3	7.62	7.54	7.09	<1	<1	1.10	2.51	1.90	<1	<2	<2	< 2	1.18	<1	< 1	6.86	6.21	7.23	7.08
SP	7.92	7.85	7.64	<3	<3	<3	7.85	7.67	7.97	<1	<1	<1	1.18	2.16	<1	<2	<2	< 2	<1	<1	< 1	7.09	7.38	7.54	5.41
LP	7.39	7.60	7.63	<3	5.71	<3	7.35	7.48	7.54	<1	<1	<1	1.10	<1	<1	<2	<2	2.24	<1	<1	< 1	6.33	6.98	6.91	6.74
GM	7.26	7.22	7.35	7.22	7.13	7.18	< 3	< 3	< 3	5.01	1.10	5.19	1.51	0.81	<1	<2	<2	< 2	<1	<1	< 1	6.20	6.49	6.87	6.54
SM	7.12	7.77	7.89	7.05	7.75	7.41	< 3	< 3	< 3	4.24	6.41	5.58	<1	<1	<1	<2	<2	< 2	<1	<1	< 1	6.29	6.51	7.72	7.72
LM	7.74	7.76	7.30	7.69	7.36	7.22	< 3	< 3	< 3	5.54	5.46	5.45	1.18	1.24	<1	<2	<2	< 2	<1	<1	< 1	6.78	7.54	6.54	6.15
SX	7.87	7.41	7.93	7.82	7.46	.80	< 3	< 3	< 3	1.81	<1	3.74	0.88	<1	<1	<2	<2	< 2	<1	<1	< 1	6.91	-	6.75	7.10
SD	4.88	7.20	7.73	4.88	7.18	7.72	< 3	< 3	< 3	2.24	<1	2.24	<1	<1	<1	<2	<2	< 2	<1	<1	< 1	-	5.85	5.89	5.81

Sets I, II and III refer to the 7th, 18th and 39th days, respectively.

The (-) sign indicated that TPC was lower than lactic acid bacteria count.

TABLE 4: Organoleptic Scores

Ham code	Average Scores[1] Saltiness	Sourness	Juiciness	Aged flavor	Color	Overall acceptability[2]
GL	4.75±0.99	3.75±1.04	3.35±0.90	4.1±0.97	3.75±0.60	4.4^{ac} ± 0.96
SL	4.15±1.78	3.7± 1.70	4.7 ±1.15	3.45 ±1.34	2.5 ±0.81	3.75^{ab}±1.61
LL	3.9 ±1.60	3.25±1.34	3.6 ±0.82	3.9 ±0.77	4.1 ±0.88	3.85^{ab}±1.55
GP	4.25±1.26	3.6±1.39	3.55±1.00	4.15 ±1.88	3.55±0.6	4.4^{ac}± 1.30
SP	4.15±1.52	3.8±1.16	3.8 ±1.10	3.7 ±1.25	3.9 ±0.98	3.8^{ab}± 1.35
LP	4.55±1.13	3.6±1.87	3.65±1.10	3.65 ±0.96	4.45±0.87	4.7^{C} ± 1.55
GM	4.75±1.37	4.15±1.14	3.1 ±0.96	4.35 ±0.83	4.25±0.66	4.3^{ac}± 1.23
SM	4.25±1.27	3.65±1.16	2.8 ±1.16	4.75 ±1.21	4.45±0.73	3.55^{b}± 1.35
LM	4.6 ±1.53	3.95±1.15	3.3 ±1.09	4.3 ±1.03	4.0 ±0.62	4.35^{ac}± 1.00
SX	3.9 ±0.89	3.8 ±1.03	3.75±1.28	3.7 ±0.91	3.1 ±0.97	4.15^{abc}±1.50
SD	3.7 ±1.19	3.65±1.41	3.2 ±1.04	4.0 ±1.41	4.25±1.13	3.9^{abc} ±1.57

(1) Meaning of Scores (on a scale of 1 to 7)
Saltiness: 1 = extremly bland, 4 = ideal, 7 = extremely salty
S Sourness : 1 = absolutely not sour,4 = ideal, 7 = extremely sour
Juiciness: 1 = extremely dry, 4 = ideal, 7 = extremely soggy
Aged Flavor:1 = extremely fresh, 4= ideal, 7 = extremely aged
Overall acceptability: 1 = very bad, 4 = satisfactory, 7 = excellent

(2) Any two results carrying different letters are significantly different at the 95% confidence level.

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Fermented hams were processed in eleven variations and tested for their pH decrease, weight loss, salt and water contents, water activity, nitrite and nitrate contents, bacteria counts and various organoleptic criteria. Nine of the variations were combinations of three sugars (sucrose, glucose and lactose, which were added to the meat at a level of about 2.25%) versus three bacteria (P. pentosaceus, L. plantarum and M. kristinae-varians) which were added to the meat at a level of about 10^7 cells/gr. The two other variations were: Use of a commercial bacteria mixture that contained the two latter bacteria, and chemical acidulation with GDL without adding any bacteria. In these two latter cases, sucrose was also added at a level of about 2.25%.

On the 39th day from the beginning of processing, the last series of tests were carried out, after which it was realised that this period,which is the shortest ever mentioned in the literature for this type of product, was enough for all the individual hams to pass the criterion of 18% weight loss plus at least one of the following criteria: $a_w < 0.91$ or $a_w < 0.95$ with $pH < 5.2$. the combination which gave the largest decrease in pH (1.6 units) was the combination glucose+Lactobacillus, but the combinations sucrose+Micrococcus, sucrose+GDL, sucrose +LPM1 (the commercial mixture) and sucrose+Lactobacillus, too, gave decreases larger than one pH unit. On the other hand, no one of the five combinations that won the highest organoleptic scores contained sucrose, and a statistical test also showed its inferiority in comparison with other sugars at a significance level of $p < 0.05$. The main conclusions from this work are consequently, that it is possible to produce fermented hams in less than forty days when one

uses Lactobacillus and Pediococcus bacteria at the a.m. level and the substrates for fermentation are(a mixture of) glucose+lactose at a level of about 1% each. All this is true provided that the detailed proces is used - a process that also consists of smoking and dipping in a 10% potassium sorbate solution.

It was not proved that there was any need for Micrococcus bacteria in addition to using the two bacteria mentioned earlier. However, using each of the three bacteria seperately, including the Micrococcus, helped in obtaining hams with no sign of spoilage.

Two other issues which were examined by doing this work, were the possibility to calculate the assumed water activity of the product by determining its water and salt contents, and a comparison between the diffusion coefficient (D) value - which was calculated according to the salt concentrations - to the values appearing in the literature. Both these actions were successfully carried out: The deviation between the calculated and the measured values of water activity rarely exceeded one hundredth of unity. The diffusion coefficient (mean value for salt in ham) was determined as $4.98 \cdot 10^{-6} cm^2 sec^{-1}$ at 6±2 C.