Dry Fermented Sausages inoculated with Debaryomyces hansenii

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Background: Dry fermented sausages are commonly produced in European countries. These sausages are produced by drying and the addition of bacterial starter cultures, but without thermal processing. During fermentation, lactic acid bacteria grow rapidly and inhibit other spoilage bacteria¹⁾, and the taste becomes a little sour. These sour tasting meat products have not generally been acceptable for Japanese consumers. Recently, many Japanese travel abroad and have the opportunity to eat fermented meat products. Young Japanese eat more meat and are gradually developing a taste for sour meat products. Meat processor in Japan have noticed this change in consumer preferences and are trying to make similar tasting products.

Objectives: We conducted this study to gain knowledge about the effects of manufacturing of dry fermented sausages: starter, proteolysis and the effects of using *Debaryomyces hansenii* inoculation to inhibit mold on the surface of sausages.

Methods: Lean pork meat (85%) and pork back fat (15%) were frozen and then cut for dry fermented sausages. The contents of the curing mixtures were as follow; 2% salt, 1% glucose, 0.6% sugar, 0.007% sodium nitrite, 0.066% potassium nitrate, 1% pepper, 0.3% onion, 0.2% garlic. Five types of bacterial mix starter cultures (P2M120, PLM230, S51, Lyo2M, SP318) were used. The surface of the sausages was inoculated with *Debaryomyces hansenii* at day 0 (Experiment 1) or day 3 (Experiment 2).

Temperature (20 to 15°) and humidity (85 to 65%) were control factors of the fermentation and drying processes.

Bacterial counts were determined using standard plate count agar, MRS agar, Chromocult agar, DHL agar, Vorgel Johnson agar. A 10 gram sample of sausage for bacterial counts was homogenized with 90ml of sterilized saline and after microbial test, the pH of the filtrate of homogenate was taken with a pH meter. Proximate analysis was performed on water, fat, ash and protein contents.

Peptides and free amino acids samples were obtained from the homogenate filtrate by adding an equal volume of 4% TCA solution. Peptide content in 2% TCA solubles was determined by Lowry method and free amino acids were analyzed using a JASCO amino acid analyzer (Model 8000). The nitrite ion was determined using a solution of sulfanilamide and naphthylethylenediamine solution at OD 540 nm.

A panel comprised of university stuffs and students performed sensory evaluation.

Results and Discussions: Total bacterial count was 1.2×10^{4} /g in the control sausage and 10^{6} ~ 10^{7} /g in the experimental sausages (P2M120, PLM230, S51, Lyo2M, SP318) at day 0 (Experiment 1). At 3 and 7 days, the bacterial counts were to 10^{8} - 10^{9} /g in the control and experimental sausages. Lactic acid bacteria also increased to 10^{7} - 10^{9} /g at 7-42 days in the control and experimental sausages, and the curve of the lactic aid bacteria count was similar to previous results²) but, Coliform groups were observed until the end of ripening, and they did not disappear in the control sausage.

The pH curve during ripening in Experiment 1 was different, and pH decline stopped at 7 days and then gradually increased to 6.2-6.7 on the final products. This was due to the inoculation of *Debaryomyces hansenii*, because these phenomenon were not observed previously²).

In Experiment 2, the time of *Debaryomyces hansenii* inoculation was delayed until after day 3. Lactic acid bacteria curves (Fig.1) were also similar to the ones in Experiment 1, but changes in pH were different from Experiment 1. The lowest pH was 4.6 on the SP318 at 3 days. Afterward, the pH of samples increased to 5.8-6.3 at 42 days (Fig 2). These values were 0.4 lower than that of Experiment 1. Coliform groups disappeared at 7 days in all sausages. These two experiments, suggested that *Debaryomyces hansenii* degrades or digests the lactic acid, because pH values increased after inoculation in Experiment 1 and 2.



Total free amino acids content increased 6-8 times more than the first measurement. On the contrary, peptides ^{content} did not increase as much; only 1.3-1.7 times. Dry matter of dry fermented sausages doubled with drying. These facts indicate that amino peptidases had higher activity than that of proteinases in dry fermented ^{sausages}.

Sensory evaluation was carried out on the samples of Experiments 1 and 2. The sour taste was week especially ⁱⁿ the samples of Experiment 1, and the difference of taste among the 5 samples was not clearly distinguishable. ^{In} Experiment 2, the taste difference was slightly distinguishable compared with Experiment 1. The SP318 ^{sausage} scored higher overall than the others on the sensory evaluation.

Conclusion: The total and lactic acid bacterial counts were similar in Experiments 1 and 2. However, pH ^{curves} and Coliform groups were different between Experiments 1 and 2. In Experiment 1, pH was 6.2-6.7 at 42 ^{days} and Coliform groups remained at the end of ripening.

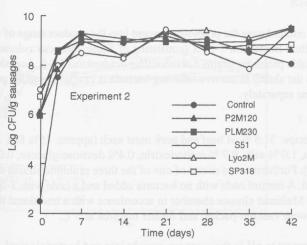
In Experiment 2, with *Debaryomyces hansenii* inoculation after 3 day, the pH dropped rapidly to 4.6 on the SP318 and $4.8 \sim 5.0$ on other experimental sausages at 3 and 7 days, respectively. The final pH at 42 days was $5.8 \sim 6.3$ on the control and experimental sausages. Coliform groups disappeared at 7 days in all sausages.

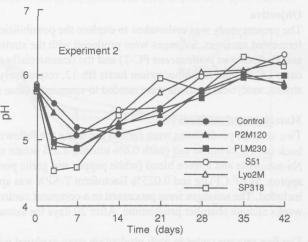
Protein, fat and ash content of the final products were double of the original amount. The peptide content was ^{the} highest in the SP318 sausage, next was the PLM230 sausage. The total free amino acids content was the ^{highest} in the Lyo2M sausage. The SP318 sausage scored higher overall than the others on the sensory ^{evaluation}.

Literature:

¹⁾ Schillinger, U. and Lucke, F.K., Fleischwirtsch., 70:1296-1299, 1990.

²⁾ Mikami, M., H. Kawasima and M. Sekikawa. Anim. Sci. Tech.(Jpn), 69:53-61, 1998.





 $^{\rm Fig.~1}$ Changes in lactic acid bacterial count in the fermented $^{\rm sausages}$ inoculation with Debaryomyces hanseni during ripening

Fig. 2 Changes in pH of the dry fermented sausages inoculated with *Debaryomyces hansenii* during ripening

Table 1. Sensory evalution of the dry fermented sausages inoculated with *Debaryomyces hansenii*

	Control	P2M120	PLM230	S51	Lyo2M	SP318
Flavor	2.91 ± 0.95	2.96±0.88	3.17±0.98	2.95±0.98	3.17±0.98	3.04±0.86
Order	2.70 ± 0.97	2.43±1.04	3.04 ± 0.93	2.65 ± 1.30	3.04±1.06	3.13±0.92
Colour	3.43±0.90	2.74±1.09	3.57±0.95	3.74±0.81	3.57±1.04	3.30±1.18
Sour	2.96±0.77	2.95±0.88	2.87 ± 0.81	2.91 ± 0.90	3.00±0.80	2.91±0.67
Overall	2.96±0.71	2.91 ± 0.67	3.00±0.80	3.04±0.64	3.09±0.73	3.30±0.70

X ± SD, 5: excellent, 4: very good, 3: good, 2: fair, 1: poor