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THE EFFECT OF FREEZING ON THE MICROFLORA OF TRIPE

DONDURMANIN İŞKEMBENİN MİKROFLORASI ÜZERİNE ETKİSİ

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SUMMARY: Tripe is mostly consumed as a traditional soup called "Tripe Soup" in Turkey. Tripe produced for local consumption is cleaned, scalded, washed and then sold in retail markets in the country. Few differences were detected in bacterial flora of frozen tripe samples stored at -18°C. Cleaned frozen tripe can be stored at -18°C for up to 2 years at least.

ÖZET: Ülkemizde daha çok çorba şeklinde tüketilen bir ürün olan işkembe, mezbahadan temizlenmiş olarak alınmış ve küçük parçalara bölünerek dondurulmustur. İşkembeler 2 yıl süre ile -18°C'de depolanmıştır. Belirli aralıklarla yapılan mikrobiyolojik ve duyusal analizler, temizlendikten sonra dondurulan işkembelerin -18°C'de en az 2 yıl bozulmadan depolanabilecegini ortaya koymustur.

INTRODUCTION

The bovine digestive tract includes a rumen, reticulum, omasum and abomasum. The rumen and reticulum are most commonly used in the preparation of seam and honeycomb tripe. Tripe is mostly consumed as a traditional soup called "Tripe Soup" in Turkey. The rumen contents of a 500 kg cow weigh about 70 kg and at the peak of the digestive cycle bacteria and protozoa constitute approximately 10 % of the volume. The rumen fluid of a cow fed on hay contained $1.7x10^9$ anaerobic bacteria per ml including species with a wide variety of metabolic abilities (SWINGLER, 1982).

In general the position of the tripe within the carcase makes difficult its hygienic removal. In the preparation of tripe the rumen and reticulum are emptied, washed to remove bacteria from the surface. The largest contributors to the microbial load are the intestines and stomachs. On the other hand offals are generally capable of high metabolic activities involving substantial protein turnover, and they can therefore, be expected to undergo autolysis relatively rapidly compared with muscle. The edible offals are often frozen in cartons prior to distribution and sale (LOWRY and GILL, 1985 ; PIERSON and DUTSON, 1988).

The limited data available on the compositions of tripe microflora and its behaviour on freezing (LOWRY and GILL, 1985).The objective of this study was to determine the shelf-life of frozen tripe and the growth patterns of microorganisms in tripe stored at -18°C.

MATERIALS AND METHODS

Preparation and storage of samples

An 8 kg scalded clean tripe was obtained from the municipal abattoir in İzmir and immediately transported to the laboratory. The tripe was cut into 2x2 cm square pieces aseptically and was packed in perforated plastic bags. Each pack contained 100 g of tripe. Samples were frozen and stored at -18°C. Bacterial enumeration and organoleptical tests were made for various periods up to 2 years.

Microbiological samples

Each time one pack was withdrawn at random during the storage period and was thawed at +4°C. From each stored pack 10 g of subsamples were removed aseptically and homogenized for 2 min in a Stomacher Model 400 (Seward Medical, London) with 90 ml of 0.1 % peptone water. From this, serial dilutions using the same diluent were prepared.

Microbiological methods

Aerobic plate count was determined by the pour plate method using plate count agar (PCA,Oxoid CM 325). The plates were incubated at 20 and 5° C for 3 and 7 days.

PCA was employed for the enumeration of mesophilic anaerobic bacteria using pour plate technique. The plates were placed in an anaerobic jar and the anaerobic conditions were provided by using gas generating kits (Oxoid B 38). Incubation was at 35° C for 3 days.

Enterobacteriaceae count was determined by the pour-overlay method using violet red bile glucose agar (Oxoid CM 485). The plates were incubated at 35° C for 24 h.

The enumeration of lactic acid bacteria was made on rogosa agar (Oxoid CM 627) using the pour plate method. The plates were incubated at 30° C for 5 days in an anaerobic jar using gas generating kits (Oxoid BR 38).

Total coliforms were estimated by using a three tube replication of lauryl sulfate tryptose broth (LSTB, Oxoid CM 45) in a most probable series. The tubes were incubated at 35° C for 48 h. Tubes showing growth and gas production were confirmed on eosin methylene blue agar (EMB,Difco B 76). Positive LSTB tubes were used to inoculate tubes of EC broth (Difco B 314) which were incubated at 44.5° C for 48 h.Tubes showing growth and gas production were considered as presumed positive for fecal coliforms (ICMSF, 1978) and the most probable number of total and fecal coliforms were computed by using published tables.

For isolation of *Salmonella*, 25 g samples were put into sterile blending jars and 225 ml of lactose broth (Oxoid CM 137) was added. Blended samples were kept at 37° C for 24 h.Tetrathionate brilliant green and selenite cystine broth was used for enrichment. Inoculated tubes were incubated at 43 and 37° C for 24 h. After inoculation, a loopful from each tube was streaked on XLD (Oxoid CM 469) and Brilliant Green Agar (BGA,CM 263) plates. These plates were incubated at 37° C for 24 h and checked for typical colonies (FDA, 1978 ; ICMSF, 1978).

Shelf-life determination

A three member panel evaluated the tripe samples for colour and odor.

RESULTS AND DISCUSSION

The results of the total plate counts in tripe samples are illustrated in Fig. 1. Initially aerobic plate counts were $9.0x10^3$ /g and $1.5x10^3$/g at 20 and 5 °C respectively.During the storage very few changes in total plate counts were noted. Numbers of bacteria at 5° C increased slowly and anaerobic plate count decreased. The level of lactic acid bacteria in samples increased during the storage (Table 1). In model systems, the rate of inactivation decreases with decreasing frozen storage temperatures viability declining much more rapidly at temperatures of -5° C or above than at temperatures between -10 and -20° C (INGRAM and MACKEY, 1976 ; WILSON et al., 1981).SULZBACHER (1950), showed that during the freezing of packaged meat -4° C and even -18° C, numbers of psychrotrophic bacteria increase. However, much of the increase observed in that study can be attributed to microbial growth during slow cooling of the meat.Growth would have exceeded the subsequent lethal effects of freezing.No bacterial growth is possible at normal commercial frozen storage temperatures, i.e. -12° C and below. The holding temperature can affect the number of organisms surviving prolonged frozen storage.On the other hand somewhat unexpectedly, fluctuation of temperatures within the freezing range (-20° C to -4° C) has been reported to cause numbers not to decrease below the levels observed for storage at the lowest temperature alone (LOWRY and GILL, 1985). SISON et al. (1980), who stored beef loins for up to six months at -18 and +27° C. Storage at -18° C did not change microbial counts. The fluctuating temperature system caused substantial increases in microbial counts.It is well known that freezing can cause sublethal injury and death to many microbial species in foods. Similar results with our study were reported by HANNA et al.(1982), who showed that freezing of edible offal (livers, kidneys and hearts) did not cause significant change in aerobic plate count.

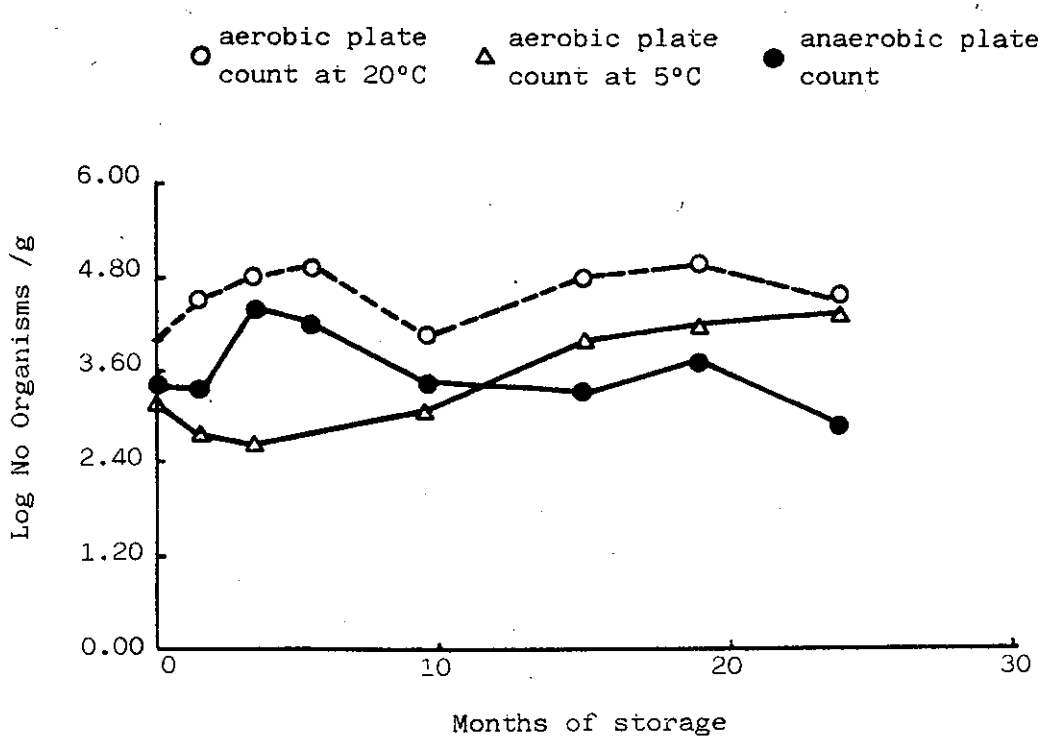

Fig 1. Growth of total plate count during storage of frozen tripe

Initially *Salmonella* were not detected in tripe. At the begining of the storage *Enterobacteriaceae* and coliform counts were low in tripe and after 15 months storage these groups bacteria were not detected (Table 1). SULZBACHER (1950) have reported that coliforms almost completely eliminated in unprotected pork samples held at -4°C. According to COX et al.(1983), in chicken patties, after thirty days of storage at -10°C, no *E.coli* or *S.aureus* were detected, even though prior to freezing, levels of $2(\log_{10})/g$ were observed. Low levels of *Salmonella* were found prefreezing but were still present after thirty days of frozen storage.

During two years storage unpleasent off-odor and bad colour were not detected in samples. The initial levels of bacterial contamination were not too high and few differences were detected during storage. Cleaned tripe can be stored at -18°C for up to 2 years at least.

Table 1. Growth of Microorganisms on the Frozen Tripe Held at -18°C

Analyses	Storage period (month)							
	0	1.5	3.5	5.5	9	15	19	24
Lactic acid bacteria (cfu/g)	20	<10	10	<10	<10	220	<10	3200
Enterobacteriaceae (cfu/g)	240	<10	<10	<10	80	<10	<10	<10
Coliform (MPN/g)	9	4	<3	<3	<3	<3	<3	<3
Fecal coliform (MPN/g)	<3	<3	<3	<3	<3	<3	<3	<3
Salmonella/25g	- *	- *	- *	- *	- *	- *	- *	- *

* - negative

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