MICROBIAL LIPOLYSIS IN BEEF ADIPOSE TISSUE UNDER REFRIGERATION

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SUMMARY

Samples of beef adipose tissue held under refrigeration for fourteen days were analyzed for free fatty acids (FFA) and lipid content. Simultaneously psychrotrophic and lipolytic counts were performed at 0, 2, 4, 7, 9, 11 and 14 days of storage. Psychrotrophic and lipolytic microorganisms reached counts of 8.20 log CFU/cm² and 9.30 log CFU/cm² respectivily. Highest concentrations of FFA observed were 34.44 and 26.42 µmoles/ gram of lipid which matched with highest psychrotrophic counts. Calculated rate of increase of FFA, from a linear regression curve, was of 1.35 µmoles/g/day. For samples with no apparent microbial growth the rate of increse of FFA was of 0.7 µmoles/g/day

INTRODUCTION

The spoilage of refrigerated food products, containing significant amounts of fat components by psychrotrophic microorganisms, has been known for a long time. An important mechanism of this spoilage is fat lipolysis that give rise to quality changes. Lipolysis includes the hydrolysis of triglyceride to fatty acids and the oxidative changes of the triglyceride or of the component fatty acids after preliminary hydrolysis.

Most microorganisms can grow and produce lipases on different oils and fats, including animal fats (Alford et al, 1971, Andersson, 1980). Among the psychrotrophic microorganisms the genus Pseudomonas plays an important role in fat lipolysis of refrigerated foods, including meat products, because of the stability and activity of their lipases at low temperatures. In meat the hydrolytic activity of the lipase of P. fragi added to the surface of meat has been reported (Bala et al, 1979).

Authors (Gill and Newton, 1980), have suggested that microbial lipolysis is not necessary for adipose tissue spoilage, but information about lipolytic activity of microorganisms on the surface of beef adipose tissue is scarce. Previous observations in our laboratory show that oxidative changes of lipids of beef adipose tissue under refrigeration, mesured as TBA number, were not significant, although there was an increase in free fatty acids concentrations(FFA).

This work was initiated to determine the importance of the hydrolysis due to the natural contaminant flora on the spoilage of refrigerated beef adipose tissue.

MATERIALS AND METHODS

Preparation of adipose tissue samples

Bovine brisket fat, from the first to the fifth sternebrae, was collected from a recently, two hours, slaughtered bovine. Twenty eight samples of adiposse tissue (4.90 cm², 3 mm depth) were aseptically taken at random using a steel borer. Half of these samples (14) were summerged into a sterile tetracycline solution (400ppm) to delay microbial growth, and placed into Petri dishes with a filter paper humidified with the antibiotc solution.

These will be referred as treated samples. The other half of the samples (14) were placed into Petri dishes with a filter paper humidified with sterile water. These samples were considered as controls.

All samples were stored at 5°C ± 1°C for fourteen days. At day 0, 2, 4, 7, 9, 11 and 14 two control samples and two treated were analyzed. Each sample was divided into halves. Bacterial counts were performed on one half, while on the other half the concentration of FFA and lipid content were determined.

Bacterial counts

Samples were homogenized, for one minute, in 0.1% peptone water with a Stomacher (Lab Blender 400). The appropriate dilutions were spread by duplicate on the surface of Plate Count Agar (PCA, Difco) for pychrotrophic counts and incubated for 10 days at 5°C. Lipolytic counts were done on Tributyririne Agar (TA, Merck), incubated for 72 hours at 25°C.

Analysis of free fatty acids (FFA)

One gram of adipose tissue was homogenized for 3 to 5 minutes in an Omnimixer with 25 ml of cloroform. On this extract the FFA were determined by the Regouw (1971) method modified by Margaría and García (1990). The content of the lipids extracted was also determined by weight.

Data analysis

A linear regression analysis was performed using the data obtained.

RESULTS and DISCUSSION

Bacterial counts and FFA concentrations are shown in Table 1. The initial contamination with the natural spoilage flora was low, from 2.60 to 3.08 log CFU/cm² for lipolytic counts at 25°C and less than 2.3 log CFU/cm² for psychrotrophic counts. On control samples maximun values of 8.20 and 9.30 log 10/cm², on day fourteen, were reached for psychrotrophic and lipolytic counts respectivily. On treated samples counts were less than 2.3 log 10/cm² all along the storage time.

Maximun values of FFA, 34.44 and 26.42 umoles/g, were detected for samples with the highest psychrotrophic counts, 8.08 and 8.2 log CFU/cm² at the beginning of the stationary phase. This may be related to the observed maximun in lipolytic activity of Pseudomonas at this phase (Andersson, 1980). In previous determinations in our laboratory Pseudomonas was the main psychrotrophic microorganism growing in the surface of beef adipose tissue under refrigeration. In adition, lipolytic enzymes may be subjected to catabolic repression while carbohydrates are still used in the adipose tissue (Gill and Newton, 1980).

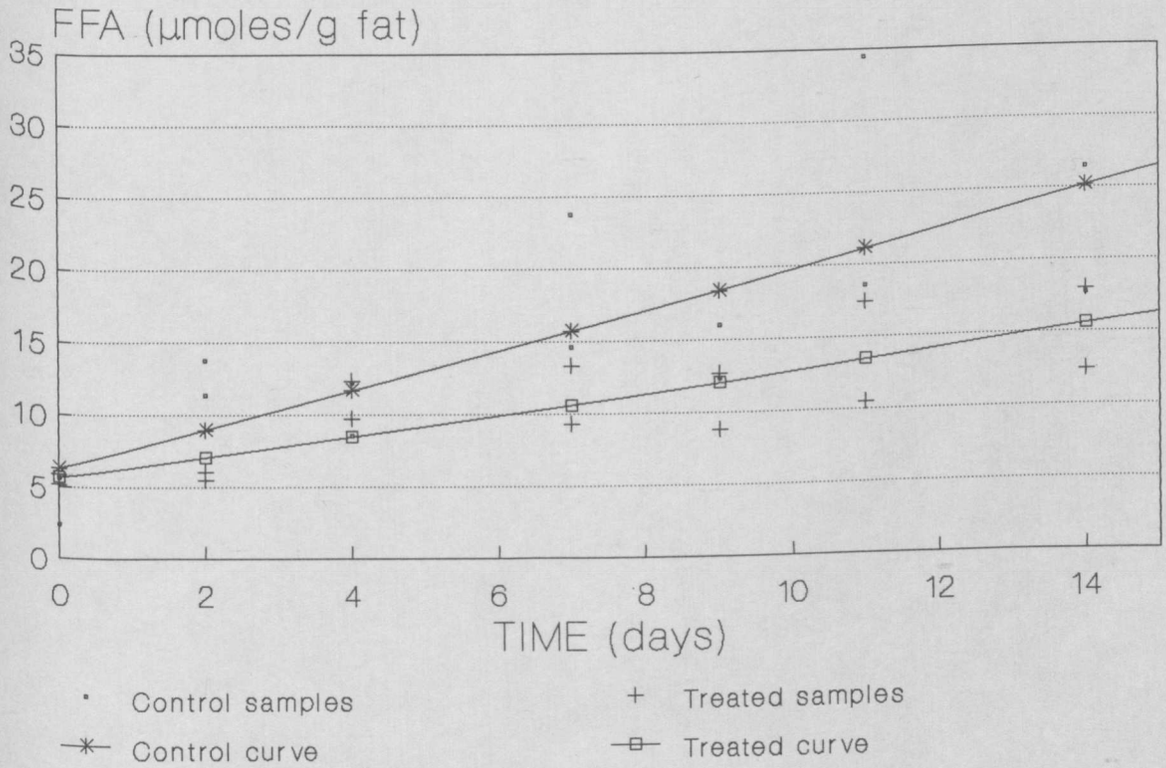
As a parameter to mesure de importance of microbial growth in the hydrolysis of fat, a rate of increase in FFA during storage time was calculated for control and treated samples. Linear regression curves (Figure 1) show a slope of 1.35 umoles of FFA/g/day for control samples against an increase of 0.7 umoles of FFA/g/day for treated samples. According to these curves, at day fourteen, the FFA concentration would be a 61% more for control samples

Table 1. Bacterial growth and FFA concentration in beef adipose tissue under refrigeration.

	a-Control samples			b-Treated samples
Storage time (days)	Lipolytic counts (log CFU/cm2)	Psychrotrophic counts (log CFU/cm2)	FFA (umoles/g fat)	FFA (umoles/g fat)
0	2.60	2.30	2.34	5.18
0	3.08	2.30	5.59	5.84
2	6.60	2.30	13.73	5.50
2	4.15	2.30	11.37	6.11
4	5.73	3.70	8.53	9.73
4	6.30	4.78	12.18	12.30
7	7.90	6.08	14.60	9.31
7	6.90	7.78	23.75	13.36
9	7.00	5.00	16.00	8.80
9	6.90	5.00	12.34	12.70
11	7.78	8.08	34.44	17.40
11	7.78	6.60	18.55	10.50
14	8.30	7.90	17.68	12.38
14	9.30	8.20	26.42	18.01

* for treated samples psychrotrophic and lipolytic counts were less than 2.3 log 10/cm2 all along the storage time

Fig 1-FFA in refrigerated beef fat



than for treated ones. The correlation coeficient was greater for treated samples (r=0.80) than for controls (r=0.75), which show a greater dispersion. The variability in microbial counts between control samples, and the non linearity of microbial growth may explain this finding.

CONCLUSIONS

The results obtained in this assay show that the increase of FFA in beef adipose tissue under refrigeration is greatly caused by the activity of microbial lipases of psychrotrophic microorganisms growing on the surface. The contribution to the hydrolysis of lipids by microbial growth seems to be more important after reaching high counts.

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