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Effect of Microorganisms on Meat Proteins at Low Temperatures

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Most of the information on the titled subject has come from studies on the spoilage of meats rather than from studies in which specific proteins were subjected to the activities of microorganisms. The ecologic parameters of low-temperature fresh meat spoilage are such that the spoilage organisms consume nonprotein nitrogenous constituents and the simpler proteins preferential to those of the myofibrillar type. The predominant spoilage organisms under these conditions consist of several genera of gram-negative bacteria and there is no evidence of protein breakdown by this flora at the time of incipient spoilage. Myofibrillar proteins such as tropomyosin, α-actinin, troponin T, and actomyosin are attacked by the psychrophilic spoilers only after frank spoilage has occurred following prolonged storage. Prior to this point, the most dramatic change brought about by the spoilers is an increase in hydration capacity of meat proteins. Because the increased hydration capacity is related to increases in amino sugars and amino sugar complexes of bacterial origin and because these compounds possess the inherent capacity to increase protein hydration, it is postulated that they are necessary precursors to the ultimate activities of bacterial proteases and possibly meat cathepsins.

A careful search of the literature reveals only a few studies in which the effect of microorganisms on meat constituents was sought. Most of what is known about the effect of the spoilage flora on meat muscle proteins has come from studies on the effect of the spoilage flora on intact meats. Our present knowledge of the effect of microbes on meats is, therefore, limited mainly to the information which can be obtained by this general approach. The research of microbiologists on meats during the past 80 years has been prompted by concerns for the detection and prevention of microbial spoilage along with interests in meat preservation and the possible toxic effects of consuming spoiled meats. These aspects have been reviewed elsewhere (Ingram and Dainty, 1971; Jay, 1972). Studies on the mechanism of spoilage have received attention only recently.

This report is a summary review of our state of knowledge of the specific effects of microorganisms on meat proteins. Since most of this information is derived from studies on meat spoilage, it is desirable to view the

meat spoilage process from the standpoint of the ecological parameters that affect the growth and activity of microorganisms in meats.

CHEMICAL COMPOSITION OF MEATS

The approximate composition of meats such as beef and pork is presented in Table I. While the protein content ranges from 16 to 22% with an average of 18.5%, it should be noted that nonprotein nitrogenous substances constitute 1.5% and carbohydrates approximately 1.0%. When provided with complex and simple nutrient sources, microorganisms will invariably or always utilize the simpler constituents preferential to the more complex ones such as proteins. The protein-sparing actions of free amino acids, nucleotides, and related compounds in spoiling beef have been demonstrated (Jay and Kontou, 1967). Once the simple nitrogen sources have been exhausted, the simpler proteins such as those from the sarcoplasm are uitilized (Jay, 1966). Due to the generally low level of carbohydrates in meats, spoilage bacteria effect the deamination of amino acids and use the remaining molecules as energy sources with a consequent increase in NH_3 and H_2S in the spoiling meats. With the increase in NH_3, the usual postmortem beef pH of 5.6 to 5.8 begins to

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Table I. Approximate Composition of Mammalian Skeletal Muscle (Percent Fresh Weight Basis)[a]

	Percent		Percent
WATER (range 65 to 80)	75.0	NON-PROTEIN NITROGENOUS SUBSTANCES	1.5
PROTEIN (range 16 to 22)	18.5		
Myofibrillar	9.5		
myosin	5.0	Creatine and Creatine phosphate	0.5
actin	2.0		
tropomyosin	0.8	Nucleotides	
troponin	0.8	(Adenosine triphosphate (ATP), adenosine diphosphate (ADP), etc.)	0.3
M protein	0.4		
C protein	0.2	Free amino acids	0.3
α-actinin	0.2	Peptides	
β-actinin	0.1	(anserine, carnosine, etc.)	0.3
Sarcoplasmic	6.0	Other nonprotein substances	
soluble sarcoplasmic and mitochondrial enzymes	5.5	(creatinine, urea, inosine monophosphate (IMP), nicotinamide adenine dinucleotide (NAD),	
myoglobin	0.3	nicotinamide adenine dinucleotide	
hemoglobin	0.1	phosphate (NADP))	0.1
cytochromes and flavo- proteins	0.1	CARBOHYDRATES AND NON- NITROGENOUS SUBSTANCES	
Stroma	3.0	(range 0.5 to 1.5)	1.0
collagen and recticulin	1.5	Glycogen (variable range 0.5 to 1.3)	0.8
elastin	0.1	Glucose	0.1
other insoluble proteins	1.4	Intermediates and products of	
LIPIDS (variable range: 1.5 to 13.0)	3.0	cell metabolism (hexose and triose phosphates, lactic acid, citric acid, fumaric	
Neutral lipids (range: 0.5 to 1.5)	1.0	acid, succinic acid, acetoacetic acid, etc.)	0.1
Phospholipids	1.0	INORGANIC CONSTITUENTS	1.0
Cerebrosides	0.5	Potassium	0.3
Cholesterol	0.5	Total phosphorus (phosphates and inorganic phosphorus)	0.2
		Sulfur (including sulfate)	0.2
		Chlorine	0.1
		Sodium	0.1
		Others (including magnesium, calcium, iron, cobalt, copper, zinc, nickel, manganese, etc.)	0.1

[a] From "Principles of Meat Science", by John C. Forrest, Elton D. Aberle, Harold B. Hedrick, Max D. Judge, and Robert A. Merkel, W. H. Freeman and Company, San Francisco, Calif., Copyright 1975.

increase and may reach an ultimate of over 8.0 in putrid beef. The general composition of meats is such, then, that trichloroacetic acid (Cl₃CCOOH) insoluble proteins are spared by the simpler nitrogenous constituents.

MEAT SPOILAGE FLORA

Most all studies on the spoilage of meats have employed refrigerator-range temperatures, 5-7 °C. This temperature range is restrictive to all but a small number of the many genera of bacteria, yeasts, and molds that can be found in fresh meats such as ground beef and pork. When meats are allowed to undergo natural spoilage at temperatures of 20 °C and above, a larger variety of microorganisms grow, most of which cannot grow at the 5-7 °C range. Zeetti (1937) was one of the first to show that several species of Clostridium and Micrococcus proliferate and effect the destruction of meat incubated at 18 to 22 °C but once incubation temperatures were lowered to the refrigerator range these types were inhibited. The effectiveness of C. perfringens and related organisms in destroying meat proteins at higher temperatures has been reported (Miller and Price, 1971; Ingram and Dainty, 1971).

The organisms that grow on fresh meats at low temperatures have been described by many investigators (Kirsch et al., 1952; Brown and Weidemann, 1958; Ayres, 1960; Jay, 1967). Those that predominate and presumably bring about the measurable changes in low-temperature spoiled meats belong to the gram-negative bacterial genera of Pseudomonas, Acinetobacter, Aeromonas, and Alcal-

igenes. Most investigators now agree that Pseudomonas spp. constitute the single most predominant and important group of bacteria in the refrigerator spoilage of fresh meats and poultry. A few gram-positive bacteria of the genera Streptococcus and Lactobacillus may increase in numbers during the spoilage process but yeasts and molds generally do not. The four genera of gram-negative bacteria noted above are aerobic types and under the conditions of low-temperature incubation and consequent high humidity, surface growth is favored. The consequent higher surface solubility of gases (O_2 in particular) also favors the faster growing aerobic types.

The overall result of the low-temperature spoilage of fresh meats is the production of a slimy surface by a few gram-negative, aerobic bacteria with the suppression of yeasts, molds, and most gram-positive bacteria. Incipient spoilage is measurable when the total numbers/gram reach approximately 10^8. Ayres (1960) found the number of bacteria/cm² for off-odor development on dressed chicken and packaged beef to be around $10^{7.5}$ and around 10^8 for slime development on these products. Dainty et al. (1975) found that slime and off-odor development occurred on beef slices when the microbial numbers were $4 \times 10^8/cm^2$. With numbers of bacteria of this magnitude, fresh meats may be presumed to be in a state of incipient spoilage with no evidence that protein breakdown has occurred by either Kjeldahl determinations of Cl₃CCOOH-insoluble protein (Jay, 1966) or by gel electrophoresis (Dainty et al., 1975).

EFFECT OF BACTERIA ON SPECIFIC MEAT PROTEINS

Most of the specific meat proteins on which data exist for the action of spoilage bacteria at low temperatures are presented in Table II. With respect to the myofibrillar proteins studied, only tropomyosin has been shown to be broken down by bacteria under conditions of natural spoilage (Dainty et al., 1975). When pure cultures of Pseudomonas were employed, several investigators have noted some effects upon some of the myofibrillar proteins (Borton et al., 1970; Tarrant et al., 1971; Dainty et al., 1975). Tarrant et al. (1973) reported that a partially purified enzyme of P. fragi was capable of degrading several of the myofibrillar components as well as effecting the loss of the dense material from the Z-line of the myofibril. Somewhat surprisingly, the enzyme was least effective on the less complex sarcoplasmic proteins than upon the more complex myofibrillar.

In an attempt to determine what effect spoilage bacteria had on the breakdown of actomyosin, we extracted this protein from beef in rigor and effected a partial purification of same by repeated precipitations. It may be seen from Table III that the Pseudomonas spp. were among the most effective actomyosin degraders with some of the 20 strains destroying up to 82%.

In regards to the sarcoplasmic proteins, it may be noted from Table II that the number of electrophoretic bands was reduced after 27 days at 7 °C (Jay, 1966). On the other hand, Dainty et al. (1975) found that a mixed spoilage flora had no effect on several identifiable sarcoplasmic components while a strain of Pseudomonas destroyed gel electrophoresis bands of some of the same components.

With respect to the stroma proteins, there is no evidence for any breakdown of these as meats undergo spoilage. In the case of collagen, it is known that the clostridia are among the most efficient producers of collagenases but these organisms are all but excluded by the low temperatures of fresh meat storage.

There is also a paucity of information on the fate of lipids in spoiling meats. It is generally believed that these

Table II. Effect of Bacteria on Specific Meat Constituents

Constituents	Fate	Days exposed/ temp, °C	Measuring technique	Organisms	Reference
Myosin, α-actinin, actin, troponins C, I, T	Not affected	18/5	Gel electrophoresis	Mixed flora[a]	Dainty et al. (1975)
Tropomyosin	Destroyed	18/5	Gel electrophoresis	Mixed flora	Dainty et al. (1975)
α-Actinin, troponin T, tropomyosin	Destroyed	9/5	Gel electrophoresis	Pseudomonas MR 175	Dainty et al. (1975)
0.6 M extracts of porcine LD[b] muscle	No band changes	20/2, 10	Starch-urea and disc-urea gels	P. cerevisiae L. mesenteroides M. luteus	Borton et al. (1970)
0.6 M extracts of porcine LD muscle	Some bands destroyed	20/2, 10	Starch-urea and disc-urea gels	Pseudomonas fragi	Tarrant et al. (1971) Borton et al. (1970)
KCl extracts ($\mu = 0.55$) of beef SM[c] muscle	No band changes	31/7	Cellulose acetate electrophoresis	Mixed flora	Harris (1968)
Beef sarcoplasmic fraction	Reduced quantity	27/7	Biuret/optical density	Mixed flora	Jay (1966)
Creatin kinases, phosphoglucomutase, metmyoglobin, myoglobin, albumen of beef sarcoplasm	Not affected	18/5	Gel electrophoresis	Mixed flora	Dainty et al. (1975)
Creatin kinases, phosphoglucomutase, metmyoglobin of beef sarcoplasm	Bands destroyed	9/5	Gel electrophoresis	Pseudomonas MR 179	Dainty et al. (1975)
A and I bands, H-zone, N-line of beef myofibrils	Destroyed	10/6	Electron microscopy	Mixed flora	Walker (1968)
Z-line of beef myofibril	Not affected	21/6	Electron microscopy	Mixed flora	Walker (1968)
Free amino acids and nucleotides of beef	Consumed	15/7	Paper chromatography	Mixed flora	Jay and Kontou (1967)

[a] Normal mixed spoilage flora. [b] Longissimus dorsi. [c] Semimembranous.

Table III. Effect of Bacteria on the Breakdown of Partially Purified Beef Actomyosin at 5 °C for 14 Days[a]

Bacteria	Source of strains	No. of strains	Mean % breakdown	Range
Pseudomonas spp.	Spoiled beef	20	61.8	38-82
Aeromonas spp.	Spoiled beef	3	58.7	60-65
Achromobacter spp.	Spoiled beef	3	57.0	47-65
Alcaligenes spp.	Spoiled beef	2	42.0	38-46
Flavobacterium spp.	Spoiled beef	2	28.5	0-57
Corynebacterium sp.	Spoiled beef	1	54.0	
Streptococcus sp.	Spoiled beef	1	71.0	
Proteus sp.	Spoiled beef	1	62.0	
Sarcina sp.	Spoiled beef	1	18.0	
P. fragi	Stock culture	1	76.0	
Alcaligenes fecalis	Stock culture	1	66.0	
Proteus vulgaris	Stock culture	1	51.0	
P. taetrolens	Stock culture	1	29.0	

[a] Pure cultures were inoculated into filter-sterilized actomyosin solutions at pH 7.4 and actomyosin breakdown was measured by loss of Cl$_3$CCOOH-insoluble protein.

compounds undergo both hydrolytic and oxidative changes and that the former may be due to bacterial action since the pseudomonads represent some of the best producers of bacterial lipases. Some of the apparent effects of spoilage organisms on lipids have been discussed by Pearson (1968).

MICROORGANISMS AND THE HYDRATION OF MEAT PROTEINS

Apart from the malodorous compounds that are associated with the refrigerator spoilage of fresh meats, the most dramatic change that occurs as they undergo microbial spoilage is the increase in hydration capacity of the proteins. This increased hydration may be measured by any one of the following methods: extract-release volume (ERV), the filter-paper press method for determining water-holding capacity, measurement of the viscosity of meat homogenates, or by use of the meat swelling technique of Wierbicki et al. (1962). These methods have been compared to bacterial numbers as a means of assessing the microbial quality of beef (Shelef and Jay, 1969a). While the increased hydration is related to the concomitant increase in pH of spoiling meats, it cannot be ascribed solely to the increased pH. This relationship has been discussed further elsewhere (Shelef, 1974).

The increased hydration capacity that accompanies the growth and activities of the spoilage flora at low temperatures is further substantiation for the lack of any significant breakdown of meat structural proteins by spoilage bacteria. When the ERV technique is employed to measure changes in hydration capacity as fresh beef undergoes refrigerator spoilage, the initially high volumes of extract, typically 40 ml, decrease to 0 as the bacterial numbers increase generally from around 10^6 to 10^{10}/g over a 10–14-day period. However, when pure cultures of bacteria that are capable of producing more effective

Table IV. Effect of Amino Sugars and Streptomycin upon the Water-Holding Capacity of Beef Muscle

Compounds	in.^{2} free H$_2$O area^a	% change
Control beef	2.93	
D-Glucose	2.53	
Galactose	2.94	
Glucosamine-HCl	1.39	−53
Galactosamine-HCl	1.14	−61
Lycosamine	1.00	−66
Mannosamine	0.80	−73
Streptomycin-SO$_4$	0.72	−75

^{a} Determined by the method of Wierbicki and Deatherage (1958).

Table V. Effect of Various Buffered Additives on the ERV of Beef Psoas Muscle

Additives	ERV
Control (no additives)	49
D-Glucose	52
N-Acetylglucosamine	52
2-Deoxy-D-glucose	49
Inosine	49
D-Glucosamine-HCl	20
Streptomycin-SO$_4$	6
Neomycin-SO$_4$	5

proteases are inoculated into beef or pork and incubated at temperatures permissive of growth, the increased hydration capacity (or decrease in ERV) is not noted. *Clostridium perfringens* has been shown to cause an increase in ERV when inoculated into fresh pork and incubated at 10 °C (Miller and Price, 1971). Indeed, the clostridia will bring about the complete hydrolysis of meat proteins and the subsequent liquefaction of fresh meats if incubated at 30 °C or above.

Among the attempts made to gain a better understanding of the mechanisms by which spoilage bacteria bring about the increased hydration capacity of meat proteins was the finding that 5 M urea in phosphate buffer at pH 5.8 reduced the ERV of beef from 48 ml to 0 in the total absence of microbial growth (Shelef and Jay, 1969a). An instant decrease in ERV can be achieved also by the addition of CaCl$_2$ or proteases such as papain. It has been pointed out by Hamm (1960, 1963) that agents such as these decrease the cohesion between adjacent molecules resulting in an enlarged network with a consequent increase in swelling or water binding. Further analyses of spoiled meats in our laboratory revealed the existence of larger quantities of amino sugars and amino sugar containing polymers than could be found in fresh meats. The ability of these compounds to increase hydration when added to fresh meats was first demonstrated in 1969 (Shelef and Jay, 1969b). A further assessment of this ability to increase beef hydration is presented in Table IV where glucosamine-HCl, galactosamine-HCl, lyxosamine, and mannosamine produced a 53 to 73% increase in water holding while D-glucose and galactose were without effect. Free glucosamine base produced a similar effect suggesting that the hydration change was not caused by the Cl⁻. Meat pH remained unchanged following the addition of these compounds. The same general effect can be observed when ERV is employed, whereas compounds structurally related to glucosamine, such as N-acetylglucosamine, do not affect beef muscle hydration (Table V).

In view of the findings that the hydration capacity of meat proteins increases as meats undergo normal microbial spoilage at low temperatures and that the amino sugar complexes of bacterial origin bear a causal relationship to the hydration changes, the following hypothesis may explain the overall mechanisms involved. Some part of the increased hydration is due to the increase in pH away from the isoelectric point of meat proteins. This increase in pH results from microbial growth and activity involving mainly the release of NH$_3$ from amino acids and related simple substances. Because of the toxic nature of NH$_3$ and its increased solubility in meats at low temperatures, the production of amino sugar complexes by the spoilage flora would relieve the environment of this toxic substance. The

resulting complexes may then associate with the insoluble structural proteins of meat and by their capacity to increase hydration thereby render the once refractive proteins more susceptible to attack by microbial proteases. In view of the conflicting reports on the capacity of muscle cathepsins to degrade muscle proteins (Davey and Gilbert, 1966; Martins and Whitaker, 1968), a muscle protein hydrating step such as that achieved by amino sugars may be necessary for the complete activity of this class of proteases. Further research on these hypotheses is being continued.

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