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BACTERIA-INDUCED BIOCHEMICAL CHANGES IN CHICKEN SKIN STORED AT 5°C

SUMMARY—The effect of psychrotolerant bacteria on the water-holding capacity as measured by the extract release volume (ERV), pH and protein degradation in chicken skin stored at 5°C was determined using strains of Achromobacter *and pigmented and nonpigmented* Pseudomonas. *All organisms caused a rapid decrease in ERV to about 50% of the original value during the early log phase of growth before development of off-odor. Changes in pH and content of extractable nitrogenous materials occurred during bacterial growth but were not pronounced until after the count was greater than 10^8 cells/g of skin and a faint off-odor was detectable. The content of extractable materials decreased during the early log phase, corresponding to the period of rapid increase in pH; later, during the late log or stationary phases of growth, it rapidly or gradually increased, depending on the organism.*

INTRODUCTION

AT ABOVE-FREEZING temperatures the shelf-life of whole chicken is often limited by the activity of psychrotolerant strains of *Pseudomonas* and *Achromobacter* growing on the surface of the skin (Lochhead et al., 1935; Ziegler et al., 1954). The activity involves the production of off-odor and off-flavor compounds, presumably resulting from the breakdown of free amino acids and possibly skin proteins. Biochemical changes leading to the production of off-odor and off-flavor in poultry meat stored aseptically have been studied (van den Berg et al., 1963, 1964; Khan et al., 1964; Khan, 1965), but little is known about the biochemical changes that occur in the skin or muscle resulting from bacterial growth.

This paper reports results of a study to determine biochemical changes in skin proteins caused by psychrotolerant strains of *Pseudomonas* and *Achromobacter*. This included measurement of changes in the extractable protein and nonprotein nitrogenous materials as well as in the water-holding capacity, as measured by Extract Release Volume (ERV) according to Jay (1964a; Jay, 1964b), and pH of skin stored at 5°C. The extent and rates of the changes for 9- and 24-week-old birds were compared. The two ages of skin were studied to determine whether possible differences in composition known to occur in mammalian skin (Carmichael et al., 1967) would affect biochemical changes induced by psychrotolerant bacteria. The work is part of a larger project dealing with biochemical and quality changes in poultry meat during storage.

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MATERIALS & METHODS

SKIN WAS OBTAINED from 9- and 24-week-old broiler-type chickens (Ottawa Meat Control Strain) raised and processed in the laboratory. The birds were slaughtered by cutting the jugular vein and carotid arteries, scalded (59°C) for 1 min, plucked by hand and rubbed free of loose pieces of the epidermal layer of skin and parts of feathers with a sterile damp cloth. The skin was removed, cooled to about 5°C, stretched on wooden blocks and the subcutaneous fat removed with a sharp knife. The defatted skin was then cut into small pieces and frozen by immersion in liquid nitrogen. The frozen skin was ground to a coarse powder by passing it twice through a hand-operated meat grinder continually cooled with liquid nitrogen. The resulting slurry was placed in a large beaker and stirred thoroughly to provide a homogeneous mixture of skin from the different birds. After removal of the nitrogen by evaporation the skin was stored in plastic bags at -100°C until used. For each age, skin from enough birds to provide the material required for all subsequent tests was processed at one time.

Tests were made with pure cultures of psychrotolerant bacteria isolated from the skin of commercially processed poultry (Clark et al., 1969). Included were one strain each of pigmented *Pseudomonas* (strain 1), nonpigmented *Pseudomonas* (strain 47) and *Achromobacter* (strain 89), and a mixed inoculum of 10 strains of *Achromobacter*, 5 strains of nonpigmented *Pseudomonas* and 2 strains of pigmented *Pseudomonas*. The single cultures were judged to be typical of the three types that cause deterioration of refrigerated poultry meat (Ayres et al., 1950; Thornley et al., 1960; Barnes et al., 1968). The relative proportion of each type in the mixed inoculum was the same as that found on commercially processed poultry in Canada (Clark et al., 1969). 24-hr-old cells grown on Standard Methods agar (SM agar, Difco) at 20°C and prepared as described previously (Clark, 1968) were used to make the inoculum in all tests.

In most tests, ground freshly thawed skin was irradiated (500,000 rads), spread thinly over the bottoms of Petri plates and inoculated by the spray-chamber method (Clark, 1963) to give about 200,000 cells/g. Preliminary work showed that a 500,000-rad dosage was sufficient to destroy all psychrotolerant bacteria in the ground skin. The inoculated skin from all plates was pooled, mixed thoroughly to spread the inoculum uniformly and then distributed in 6-g samples into Petri plates for incubation. One sample for each inoculum was analyzed immediately after inoculation; the others were incubated at 5°C in a water-saturated atmosphere together with controls consisting of irradiated but uninoculated skin. Incubated samples were removed for analysis at regular intervals over 21 days.

In a few tests, analyses were also made with samples irradiated but not inoculated or incubated. This was done to determine initial pH and composition differences between the two ages of skin and to provide a basis for comparison with inoculated skin.

All samples were analyzed for bacterial count, extract release volume, pH and content of total extractable nitrogen. The extractable nitrogen fraction was further analyzed for its content of nonprotein nitrogen and ninhydrin- and phenol-reagent-positive materials. To determine bacterial count, 0.5 g of skin was comminuted with 100 ml of 0.1% peptone in a Waring Blendor and the resulting suspension plated on SM agar using the surface method (Clark, 1967). For determination of ERV, 5 g of skin was ground with 20 ml of glass-distilled water in a Sorval Omni Mixer for 2 min (50-ml capacity cup, 5,000 rpm); the resultant mixture was filtered through Whatman No. 1 filter paper and the volume of filtrate collected during 15 min of filtration, as described by Jay (1964a), was measured. pH changes in the skin were measured electrometrically on the ERV extracts.

To determine changes in the content of extractable nitrogenous materials, 5 g of skin was extracted with 35 ml of 0.2 M NaCl at 2–3°C (Jackson et al., 1958) for 24 hr on a shaker. The suspension was centrifuged at 10,000 rpm at 2–3°C for 20 min and the residue washed with 10 ml of extractant and recentrifuged. The supernatant and washing were combined, made up to 50 ml and filtered (No. 1 Whatman). Preliminary tests comparing 4 extractants, 0.3 M sodium phosphate buffer (Orekhovich et al., 1948), citrate buffer (Orekhovich, 1948), KCl-borate buffer (Khan, 1962) and 0.2 M NaCl (Jackson, 1958), showed that extraction of soluble protein and nonprotein nitrogen materials was highest with 0.2 M NaCl. The total nitrogen content of the extract and the nonprotein nitrogen content of the protein-free extract (treated with trichloracetic acid, 5% w/v final concentration) were determined by a micro-Kjeldahl method. The contents of ninhydrin-positive materials and phenol-reagent-positive materials in TCA-treated extracts were mea-

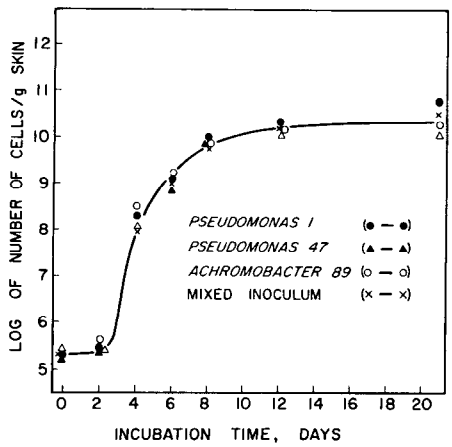

Fig. 1—Growth of psychrotolerant bacteria in comminuted skin of 9-week-old chickens.

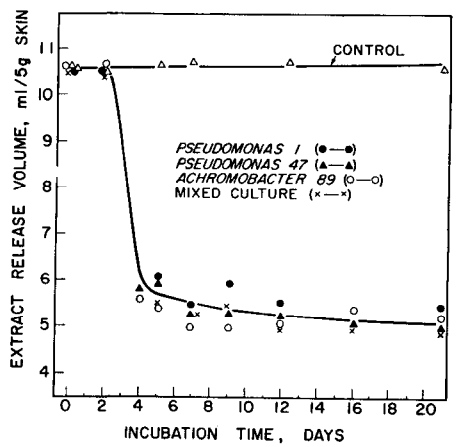

Fig. 2—Effect of bacteria on extract release volume of skin of 9-week-old chickens.

sured by the methods of Rosen (1957) and Folin et al. (1927), respectively, and were expressed as tyrosine equivalents.

RESULTS & DISCUSSION

RESULTS OF analysis of uninoculated samples showed that skin from 9-week-old birds contained more extractable nitrogenous material (29–39% more, depending on the specific material measured) and had lower ERV and pH values (8–10% lower) than skin from 25-week-old birds (Table 1). The differences in extractability were probably related to the effect of age on the solubility of neutral-salt-soluble collagenous proteins. Evidence obtained for bovine skin has shown that the solubility of these proteins in a sodium chloride solution decreases with age (Carmichael et al., 1967). The higher ERV value for older skin also indicates that solubility of skin collagen decreased with bird age. With decreased solubility the amount of inter- and intramolecular cross-linking among the α chains of the collagen molecule increases (Banga, 1966; Carmichael et al., 1967); as a result, sites capable of binding water are reduced. The effect of age on pH is not understood but the change was similar in extent to that reported for human skin; the skin of children is about 0.5 of a pH unit lower than that of adults (Behrendt et al., 1955; Behrendt et al., 1964).

The effect of bird age on the chemical composition of the skin, however, had no effect on the rate of bacterial growth (Clark, 1968) nor on the pattern of bacteria-induced biochemical changes measured. Therefore, only the results for skin of 9-week-old birds (broiler age) are presented in detail. Rates of growth of the individual test organisms and of the mixed culture in the ground skin were about the same (Fig. 1) and led to off-odor after about 4 days of incubation, coinciding with a total count of about 10^8 cells per gram of skin.

Results of tests with inoculated skin showed that all inocula caused a rapid decrease in the extract release volume during the early log phase of growth (Fig. 2). The decrease reached a maximum of about 50% of the original value after 4 days, corresponding to a bacterial count of about 10^8 per g. The test gave consistent and similar results for the relatively small sample used [5 g compared to 25 g used by Jay (1964a)] and for all types of bacteria studied. Therefore, the results show that the ERV test indicates the bacterial quality of whole poultry as it has been shown to do for ground beef (Jay, 1964b). It is noteworthy that with beef the ERV decreased with incubation time to zero (Jay, 1964a). This difference in extent of decrease is no doubt a reflection of the types and relative amounts of the proteins involved. Bacteria probably increase the water-holding capacity of tissues by enzymatically breaking the inter- or intramolecular linkages of proteins, thereby increasing the content of free end-groups, which are known to bind water (Ling, 1965).

Figure 3 shows that pH changes began to occur in inoculated skin after the log period of growth, but that these changes were not pronounced until after the count had reached 10^8 per g and a faint but distinct off-odor was noticeable. The pH then rose rapidly for all organisms, particularly for *Pseudomonas* 47 and *Achromobacter* 89. This period of increase corresponded to a rapid increase in the extent of off-odor, presumably caused mostly by ammonia and other decomposition products. The relatively slower rate of pH increase for the mixed inoculum compared to that for *Pseudomonas* 47 and *Achromobacter* 89 would appear to indicate a dominant effect of the pigmented pseudomonads in the mixture even though their proportion initially was low (about 12%). The small pH change obtained before off-odor development (0.1–0.2 of a pH unit), similar to that reported for skin of poultry contaminated naturally during processing (Fromm et al., 1965), indicates that a pH test on skin is of doubtful value for assessment of quality of whole poultry.

Figures 4 and 5 show that the bacteria caused changes in the nitrogenous constituents of the skin but, as with the pH test, the changes were not large until after 4 days of incubation and development of off-odor. All three types of bacteria caused a decrease in the contents of total extractable nitrogen, nonprotein nitrogen and phenol-reagent-positive- and ninhydrin-positive materials during the log

Table 1—Effect of age on pH, ERV and content of extractable nitrogenous material of uninoculated chicken skin.

Material	Skin from 9-week-old birds (14 samples)		Skin from 25-week-old birds (10 samples)	
	Avg	Range	Avg	Range
Total extractable nitrogen (mg/g)	2.8	2.5–3.0	1.8	1.7–2.0
Nonprotein nitrogen (mg/g)	0.85	0.78–0.95	.57	0.35–0.75
Ninhydrin-positive material (mg tyrosine/g)	6.8	5.9–8.2	4.8	3.6–6.5
Phenol-reagent-positive material (μg tyrosine/g)	172	160–185	109	70–180
ERV (ml/5 g)	10.8	9.4–11.6	11.4	9.9–12.0
pH of ERV extract	6.6	6.5–6.7	7.2	7.1–7.5

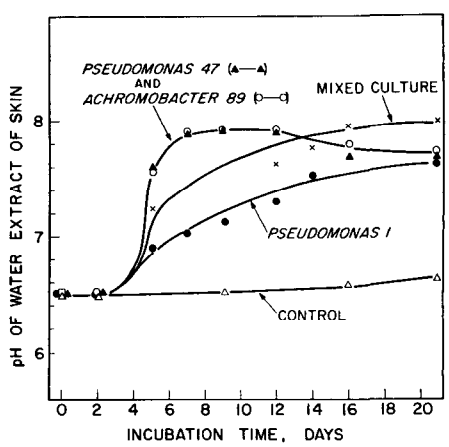

Fig. 3—Effect of bacteria on pH of skin of 9-week-old chickens.

phase, particularly between the 4th and 6th day of incubation, corresponding to the period of rapid increase in pH. After this period, values for *Pseudomonas* 47 and *Achromobacter* 89 remained stationary or increased gradually to the original values; those for *Pseudomonas* 1 increased rapidly, giving maximum values after 12–16 days. These results suggest that the organisms utilize the low-molecular-weight nitrogenous compounds during the period of rapid growth and subsequently replace such compounds through breakdown of proteins, rapidly or gradually, depending on the organism. Further studies are presently under way to determine the compounds affected.

The changes in pH in inoculated skin during incubation appeared not to affect markedly the results of the various tests. Figures 2 and 3 show that most of the changes in ERV values occurred before a measurable change in pH. Preliminary work in which skin samples were extracted (24 hr at 5°C) with buffered 0.2 M NaCl at various pH values between 6.5 and 8.0 showed that over this range the total extractable nitrogen content varied less than ± 4% from the average. Other workers have also shown with other tissues that pH between the values 6.5 and 8.0 has little effect on the solubility of protein and nonprotein nitrogenous materials in NaCl solutions (Dyer et al., 1950; Yasui et al., 1964).

Since cells of the test organisms could not be separated from the skin samples before extraction and centrifugation, part of the observed changes in composition could have resulted from the extractable metabolic end-products and autolyzed bacterial cells present. However, preliminary tests in which 1-g quantities of cells were extracted under the conditions used in the tests with skin, showed that less than 2% of the nonprotein nitrogen and total extractable nitrogen in the tests with inoculated skin could be accredited to the bacterial cells.

CONCLUSIONS

THE RESULTS showed that psychrotolerant spoilage bacteria cause a large increase in the water-holding capacity of chicken skin before development of off-odor. This change appears to be independent of the type of organism and of their relative ability to degrade proteins. Bacteria-induced changes in the pH and content of nitrogenous materials in skin begin as the organisms start to grow, but are not large until the cell count is above 10^8 per g and off-odor is produced. Therefore, ERV appears to be the most promising test among those studied for assessment of the microbial quality of whole poultry (skin intact). Since psychrotolerant bacteria grow and produce off-odor more quickly on the skin than on other poultry tissues (Lochhead et al., 1935; Ziegler et al., 1954), a bacteriological quality test involving the skin of whole birds would in most circumstances apply to the whole carcass. Further work is required to determine whether similar results for the ERV test are obtained for leg and breast muscle exposed in cut-up poultry.

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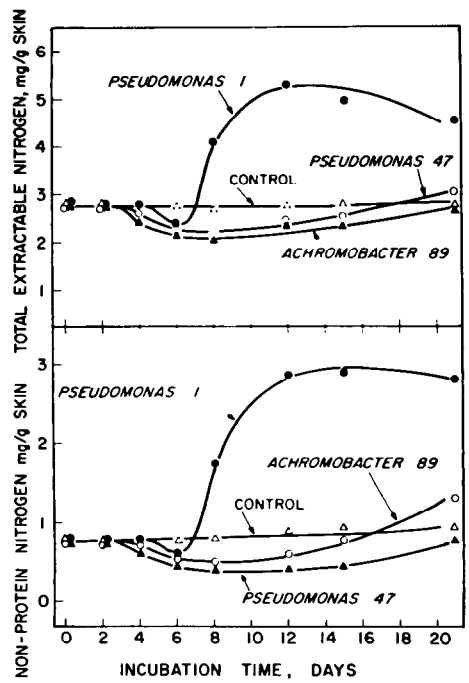

Fig. 4—Effect of bacteria on the content of extractable nitrogenous materials in skin of 9-week-old chickens.

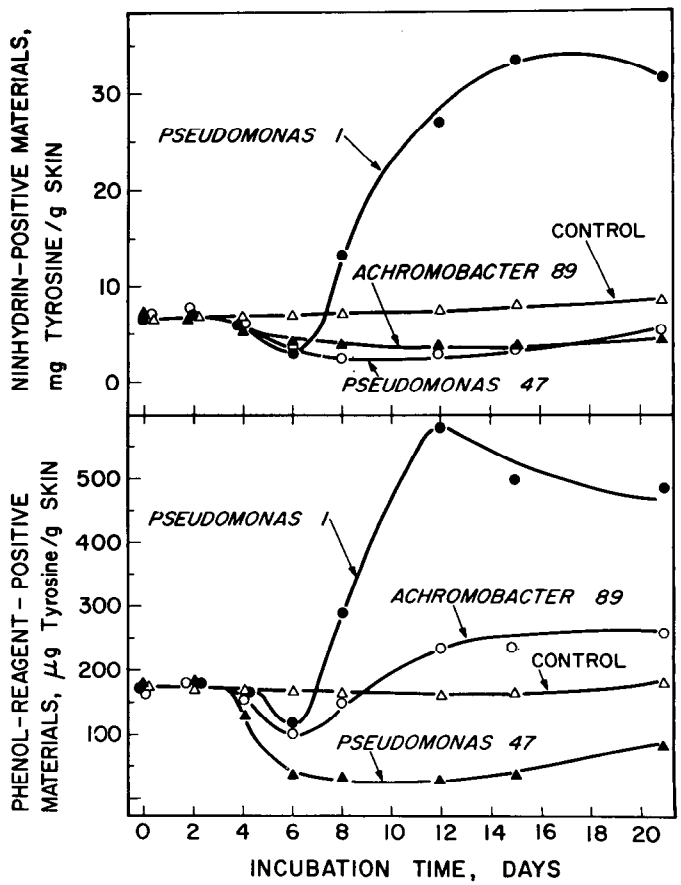

Fig. 5—Bacteria-induced changes in the content of phenol-reagent-positive- and ninhydrin-positive materials in skin of 9-week-old chickens.

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PHYSICAL AND CHEMICAL PROPERTIES OF EPIMYSIAL ACID-SOLUBLE COLLAGEN FROM MEATS OF VARYING TENDERNESS

SUMMARY—Components of the molecular structure of epimysial acid-soluble collagen (ASC) from meats of varying tenderness were studied by several methods. The ASC was studied since it contains an appreciable amount of intramolecular cross-links but is still soluble. Though the amount of total collagen in epimysial tissue was found to have no correlation with meat tenderness, the molecular studies indicated some correlation of the type of epimysial ASC with meat tenderness. Sucrose density-gradient ultracentrifugation analyses of denatured epimysial ASC suggested that this type of collagen contains a lesser degree of cross-links when obtained from tender meat samples. Viscosity measurements were found to be correlated to tenderness of meat in a manner similar to the results obtained by ultracentrifugation, in that the intrinsic viscosity of epimysial ASC from tender meat was lower than that from tough meat, indicating differences among the relative sizes of the collagen molecules from the different samples. Partial amino acid analyses of the epimysial ASC samples via gas chromatography showed no differences in the levels of aspartic, serine or hydroxyproline, amino acids which may be involved in the ester type of cross-links. Results of chemical estimation of the ester type of cross-links indicated that the proportions of esters in the various ASC samples were similar. The amount of lysine was found to be significantly higher ($P < 0.05$) in epimysial ASC from tough meat compared to tender meat, suggesting an increased potential of the aldehydic-type of cross-link, which is formed via an aldol condensation of two aldehydes derived biosynthetically from lysine. This was strengthened by the results obtained in the chemical estimation of the aldehydic-type of cross-link, in that the epimysial ASC of tough meat contained significantly more aldehyde than did that of tender meat ($P<0.05$).

INTRODUCTION

AS THE INQUIRY into the causes of tenderness or toughness of meat continues, investigators have concluded that this is a complex problem and cannot be simply resolved by the study of a single moiety, since many factors contribute to variations in beef tenderness. These factors may be divided into three broad classifications: ante-mortem, post-mortem and structural factors. Ante-mortem factors include physiological factors, such as age and inheritance, and feeding and management practices; whereas, post-mortem factors include temperature and length of storage time after slaughter, methods of trimming and cutting and cooking methods. The structural factors of tenderness are now being investigated more thoroughly and this study is centered upon some of the molecular implications of the structural factors. The structural factors may be said to include the molecular structure of the connective tissue protein, collagen, and how it influences the degree of tenderness of a muscle.

A few researchers have deduced from their investigations that tenderness or toughness is concerned with molecular cross-links in the collagen of connective tissue. Goll et al. (1962) studied the rate of connective tissue solubilization brought about by the action of collagenase in the biceps femoris muscle obtained from aged cow, cow, steer and veal age groups. The study indicated the occurrence of more frequent or stronger cross-links within and among the tropocollagen molecules of collagen from the older age group, since a decreasing rate of solubilization occurred as age increased. Herring et al. (1967) found results similar to those of Goll et al. (1962), collagen solubility decreasing significantly with advancing maturity of beef even though the collagen content was not different.

Cover et al. (1962) suggested that a tightening of the protein structure during heating (denaturation) occurs as new stable intermolecular cross-links are formed between the peptide chains of collagen. An alternate way that this may occur is for the tightening effect to occur intramolecularly along the peptide chains of collagen, thereby making the chains more resistant to cleavage.

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